DEVELOPMENT AND EVALUATION OF TRANSDERMAL DELIVERY SYSTEMS USING DIFFERENT GRADES OF ETHYL CELLULOSE.

By

YOGESH GULABRAO JAWARKAR
B. Pharm.

Dissertation Submitted to the
Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore

In partial fulfillment
of the requirements for the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Under the guidance of

Dr. V. Rama Mohan Gupta
Professor

DEPARTMENT OF PHARMACEUTICS

NET PHARMACY COLLEGE

RAICHUR-584103

KARNATAKA

2007

| DATTA PRASANNA |

DEDICATED
TO

MY BELOVED PARENTS,
LATE GRANDMOTHER
&
ELDER TWIN BROTHERS

Rajiv Gandhi University of Health and Sciences, Karnataka, Bangalore

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled **"Development and evaluation of transdermal delivery systems using different grades of ethyl cellulose"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. Rama Mohan Gupta, Professor, NET Pharmacy College, Raichur.**

Date:

Place: Raichur

Yogesh Gulabrao Jawarkar
B. Pharm.
Reg. No.: 05PU401

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **“Development and evaluation of transdermal delivery systems using different grades of ethyl cellulose”** is a bonafide research work done by **Mr. Yogesh Gulabrao Jawarkar** in partial fulfillment of the requirement for the degree of “**Master of Pharmacy” in Pharmaceutics.**

Date:

Place: Raichur

Dr. V. Rama Mohan Gupta

Professor

Department of Pharmaceutics

NET Pharmacy College,

Raichur - 584 103.

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"Development and evaluation of transdermal delivery systems using different grades of ethyl cellulose"** is a bonafide research work done by **Mr. Yogesh Gulabrao Jawarkar** in partial fulfillment of the requirement for the degree of "**Master of Pharmacy" in Pharmaceutics.**

Date:

Place: Raichur

Dr. C. Mallikarjuna Setty
Professor
Department of Pharmaceutics
NET Pharmacy College,
Raichur - 584 103.

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"Development and evaluation of transdermal delivery systems using different grades of ethyl cellulose"** is a bonafide research work done by **Mr. Yogesh Gulabrao Jawarkar** under the guidance of **Dr. V. Rama Mohan Gupta, Professor, NET Pharmacy College, Raichur.**

Seal & Signature of the HOD	**Seal & Signature of the Principal**
Dr. C. Mallikarjuna Setty	**Dr. V. Rama Mohan Gupta**
Date:	**Date:**
Place: Raichur	**Place: Raichur**

COPYRIGHT

Declaration by the Candidate

I hereby declare that the Rajiv Gandhi University of Health Sciences, Karnataka shall have the rights to preserve, use, and disseminate this dissertation/ thesis in print or electronic format for academic/ research purpose.

Date: **Yogesh Gulabrao Jawarkar**
Place: **Raichur** **Reg. No.: 05PU401**

ACKNOWLEDGEMENTS

It has been a privilege to work under the guidance of **Professor, Dr. V. Rama Mohan Gupta, Department of Pharmaceutics**. I express my sincere gratitude to him who has enlightened my ideas and who have been highly concerned about the progress of my project and without whom this work could not reach to the present stage.

I am grateful to **Dr. V. Rama Mohan Gupta**, **Principal, NET Pharmacy College, Raichur,** for providing the necessary infra-structural facilities to carry out the research work successfully.

I wish to thank **Prof. Dr. C Mallikarjuna Setty, Head Dept of Pharmaceutics,** for his guidance and constant encouragement from the beginning to final stage of this work. It gives me great pleasure to thank him for his valuable guidance, inestimable encouragement, dedicated involvement, optimist towards puzzling obstacles and unwavering support, without whom, this work would have not reached the present status.

I am especially thankful to the teaching staff of our college, **Dr. Udupi RH, Mr. Raviprakash, Mr. N.G. Ragavendra Rao, Mr. P. Srinivas Rao, Mr. Nagraj, Mr. Bimachary,** assistant **Mr. Subbanacharya, Mr. Sugurayya** and Digital library incharge **Mr.Veeresh** for their co-operation throughout my work.

I am special thank to **Dr. Martin Lucas** and **Dr. Chowdary,** Assistant Professor, Department of Anatomy .NET Medical College, Raichur, for providing Human cadaver skin.

I am very thankful to Hemdeep org pvt Ltd Lab. For providing me furosemide and Colorcon Asia, Goa for their gift samples of ethylcellouse.

I am gratified to my college friends**, Mr. Pratap Tanpure, Mr. Inayat**

Pathan, Mr. Dhaval Rathod, Mr. Dhaval Patel, Mr. Udit Meghnathi, Ms. Radhika Reddy and Mr. Giri Prasad, for sharing their ideas and extending support during the course of study.

I indebted to my Juniors, **Mr. Kulkarni, Mr. Anand, Mr. Suhas, Mr. Yagnesh, Mr. Moulesh, Mr. Ravi and Mr. Murali, Mr. Vijay** for their moral support constant encouragement from the beginning.

I remember my friends **Tushar, Mahesh, Prashant, Sharnu, Srisail, Girish, Vitthal, Bapu, Habib, Rahul, Navanath, Lodhe, Pralhad, Balu, Anna** who by their honest opinions and diligence kept me lively.

I remember my lovingly **Ku. Aaditi, Ku. Swarali, Chi. Abhidnya,** and **Chi. Pradhaney.**

I express my sincere gratitude to my ever loving, affectionate **Late dear Aaji, my dear Annaji and Aai,** and **Sister Mrs. Darshana** and **brother in-law Shri Kishor Nathe, Sister Mrs. Kalpana** and **brother in-law Prof. Rajendra Mehare, dear twin brothers Mr. Nilesh and Mr. Shailesh,** and **Sister in-law Mrs. Vrushali N. Jawarkar** who has been solid pillar of everlasting support and encouragement.

A word of thanks to all those gentle people, who have helped directly or indirectly during the time of need.

Date: **Yogesh Gulabrao Jawarkar**

Place:

LIST OF ABBREVIATIONS

Conc.	Concentration
%	Percent/Percentage
°C	Degree centigrade
g	Gram
HCl	Hydrochloride
EC	Ethyl cellulose
PVP	Polyvinyl pyrrolidone
hr	Hours
KH_2PO_4	Potassium dihydrogen orthophosphate
mcg	Microgram
mg	Milligram
min	Minute
rpm	Rotation per minute
ml	Milliliter
NaOH	Sodium hydroxide
PG	Propylene glycol
SD	Standard deviation
v/v	Volume by volume
Vs	Versus
w/v	Weight by volume
w/w	Weight by weight
max	Wavelength maximum

ABSTRACT

In case of oral administration of furosemide due to transient high blood concentration it can induce the side effects such as polyurea, dizziness, dry mouth, nausea and gastric disturbances. Aim of the present study was therefore to develop matrix type (ethyl cellulose + PVP) transdermal patches for furosemide using volatile oils (viz clove oil, lemon grass oil, menthol oil, eucalyptus oil) containing terpenes and combination of these terpenes with propylene glycol (PG). Effect of terpenes and PG on the in vitro permeation of drug through diffusion barriers including cellophane membrane and human cadaver skin was investigated. Patches were evaluated for drug content, thickness, moisture uptake, moisture content and folding endurance. Incorporation of PVP, volatile oils and PG enhanced the moisture uptake capacity and directly influenced the permeation of drug. Incorporation of volatile oils enhanced the release rate of furosemide. Among the penetration enhancers used, clove oil found to be more effective. Comparison of steady state flux (J) value produced with different barriers revealed that release was more with cellophane membrane than human cadaver skin. FTIR studies demonstrated that there was no interaction between drug and exipients used in the study. Stability studies ($40 \pm 2^{o}C$ / $75 \pm 5\%$ RH) for six month revealed no degradation of the drug.

In another set of work, surfactants were used as penetration enhancers to enhance the permeation of furosemide. Patches were evaluated for drug content, thickness, moisture uptake, moisture content and folding endurance. Incorporation of surfactants enhanced the moisture uptake capacity and influenced the permeation of the drug. The thickness of the patches prepared did not change. Incorporation of surfactants enhances the release rate of furosemide. Among the surfactant used span-

20 found to be the more effective and comparison of steady state flux (J) values produced revealed that release was more with cellophane membrane than human cadaver skin. FTIR studies demonstrated that there was no interaction between drug and exipients used in the study. Stability studies (40 ± 2°C / 75 ± 5% RH) for six months revealed no degradation of the drug.

Key Words: Transdermal patches, furosemide, penetration enhancers, In-vitro skin permeation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

In the field of the dermal or transdermal drug delivery the skin represent the application site and sometimes also the target. Skin until the early 1990s, it was believed to be an impervious barrier- designed to protect the body from foreign microorganism, including chemical and drugs. This view changed with a serendipitous finding that polar compound, such as dimethyl sulphoxide, are rapidly absorbed into the blood stream after its exposure to skin. This discovery led to active research to develop transdermal methods for systemic drug administration. Today, there are a host of drug for combating virtually every disease or condition known to man and a verity of means by which these drug are delivered to the human body for therapy such as tablets, capsule, injections, aerosols, creams, ointment, liquid etc, often referred to as conventional drug formulations. Therapy with such formulation involves attainment and maintenance of drug concentration in body within a therapeutically effective rang by introduction of fixed dosage of the drug, at regular intervals, into the body. After the administration of one dose, the drug concentration rises to high level, system wide, at least initially. With the passage of time, the concentration diminishes owing to natural metabolic processes and second dose must be administered to prevent the concentration from dropping below the minimum effective level. The disadvantages of this kind of therapy are:

1 Drug concentration in the body follow a peak and trough profile leading to greater chances of adverse effect or therapeutic failure.

2 Therapy is inefficient and costly since large amount of drug are lost in the vicinity of the target organ and close attention is required to monitor therapy to avoid overdosing.

It is recognized that continuous intravenous (i.v.) Infusion is a superior mode of drug administration as compared to the oral route not only to bypass hepatic "first-pass" metabolism but also to maintain a constant and prolonged drug level in the body. A closely monitored i.v infusion can provide the dual advantage of direct entry of drug in systemic circulation and the control of circulating drug level. However, such mode of the administration involved certain risk, which necessitates hospitalization of the patient for close medical supervision of drug administration.

It was realized and later demonstrated that the benefits of i.v infusion could be closely duplicated without its hassles by using the skin as the port of entry of drug. This is known as transdermal administration and the drug delivery systems are known as transdermal therapeutic system or transdermal drug delivery system or popularly as transdermal patches.

Transdermal therapeutic system are defined as self-contained, discrete dosage form which, when applied to the intact skin, deliver the drug (s), through the skin, at a controlled rate to the systemic circulation[1]. Table describes various transdermal drug delivery systems available commercially.[2]

Various transdermal drug delivery system available commercially.[2]

Sr.No	Therapeutic agent	TDDS	Design
1	Clonidine	Catapres-TTS	Four-layer patch
2	Estradiol	Estraderm (Novartis)	Four-layer patch
3	Estradiol	Vivelle (Novartis)	Three- layer patch
4	Fentanyl	Duragesic (Janssen)	Four-layer patch
5	Nicotine	Prosstep (Lederic)	Multilayer round patch
6	Testosterone	Testoderm (Alza)	Three- layer patch
7	Nitroglycerine	Transderm-Nitro (Novartis)	Four-layer patch
8	Scopolamine	Transderm-Scop	Four-layer patch
9	Nicotine	Habitrol (Novartis Consumer)	Multilayer round patch
10	Nicotine	Nicoderm CQ (Smithline Beecham)	Multilayer round patch
11	Nitroglycerine	Deponite (Schwarz Pharma)	Three- layer patch
12	Testosterone	Androderm (Smithline Beecham)	Five-layer patch
13	Estradiol	Climara (Novartis)	Three- layer patch

ADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM OVER OTHER ROUTES. [2, 3, 4]

- Avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH, enzymatic activity and drug interactions with food, drink, or other orally administered drugs.

- Avoid the first-pass effect, that is, the initial pass of drug substances through the systemic and portal circulation following gastrointestinal absorption, they're by digestive and liver enzymes.
- The system are non invasive, avoiding the inconvenience of parentral therapy.
- They provide extended therapy with single application, thereby improving patient compliance over other dosage forms requiring more frequent dose administration.
- Drug input can be terminated simply by removal of the patch.
- The activity of drug having short half live is extended through the reservoir of drug present in the therapeutic delivery system and its controlled release characteristic.
- The ability to modify the properties of the biological barrier to absorption (i.e. stratum corneum) by using flux enhancer and iontophoretic device.

DISADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM OVER OTHER ROUTES.[2, 3]

- Only relatively potent drugs are suitable candidates for transdermal delivery
- Same patients may develop contact dermatitis at the site of application due to one or more of the system component.
- The limitations of transdermal drug delivery are principally associated with the barrier function of skin, which severely constrain the absolute amount of a drug that is absorbed across a reasonable area of skin during a dosing period.

FACTORS THAT INFLUENCE TRANSDERMAL DRUG DELIVERY. [5,6]

The effective transdermal drug delivery can be formulated by considering three factors as drug, skin and vehicle. So the factor affecting can be divided into classes as biological factors and physiochemical factor.

A) Biological factors:

Skin condition: Acid and alkalis, many solvents like chloroform, methanol damage the skin cell and promote penetration. Disease state of patient alters the skin condition. The intact skin is better barrier but the above-mentioned conditions affect penetration.

Skin age: The young skin is more permeable than older. Children are more sensitive for skin absorption of toxins. Thus, skin age is one of the factors affecting penetration of drug.

Blood supply: Change in peripheral circulation can affect transdermal absorption. Capillaries and blood vessels present beneath the stratum corneum take up drug permeated through the skin. Injury or skin disease can change peripheral blood circulation.

Regional skin site: Thickness of skin, nature of stratum corneum, and density appendages vary site to site. These factor affecting penetration significantly.

There are differences in structure and chemistry of human stratum corneum from one region of the body to another. Despite greater thickness, plantar and palmer are not good diffusion barrier. Thus, stratum corneum shows some regional variation, which affect skin permeability. Table show regional variation of stratum corneum with respect of thickness[7]

Sr no	Skin region	Thickness (µm)	Permeation rate (mg/cm ^{2}/hr)	Diffusivity (cm 2/sec*10 ^{10}
1	Abdomen	15.0	0.34	6.0
2	Volar forearm	16.0	0.31	5.9
3	Back	10.5	0.29	3.5
4	Forehead	13.0	0.85	12.9
5	Scrotum	5.0	0.70	7.4
6	Back of hand	49.0	0.56	32.3
7	Palm	400.0	1.14	535.0
8	Plantar	600.0	3.90	930.0

Skin metabolism: Skin metabolizes steroid, hormones chemical carcinogens and some drugs. So skin metabolism determine efficacy of drug permeated through the skin.

Species difference: The skin thickness, density of appendages, and keratinization of skin vary species to species, so affect the penetration

B) Physicochemical factors:

Skin hydration: In contact with water, the permeability of skin increases significantly. Hydration is most important factor increasing the permeation of skin. So use of humectant is done in transdermal drug delivery.

Temperature and pH: The permeation of drug increase ten fold with temperature variation. The diffusion coefficient decrease as temperature falls. Weak acid and weak base dissociate depending on the pH and pKa value. The proportion of unionized drug determines the drug concentration in skin. Thus, temperature and pH are important factor affecting drug penetration.

Diffusion coefficient: Penetrations of drug depends on diffusion coefficient of drug. At a constant temperature the diffusion coefficient of drug depends on properties of drug, diffusion medium and interaction between them.

Drug concentration: The flux is proportional to the concentration gradient across the barrier and concentration gradient will be higher if the concentration of drug is more across the barrier.

Partition coefficient: The optimal K partition coefficient is required for good action. Drug with high K are not ready to leave the lipid portion of skin. Also, drug with low K will not be permeated.

Molecular size and shape: Drug absorption in inversely related to molecular weight, small molecules penetration faster than large one. Because of partition coefficient domination, the effect of molecular size is not known.

Criteria for the selection of drug in transdermal drug delivery system.[1, 5]

Physicochemical properties:

1. Drug should have a molecular weight less than approximately 1000 Daltons.
2. Drug should have affinity for both lipophilic and hydrophilic phase. Extreme partitioning characteristics are not conducted to successful drug delivery via the skin.
3. The drug should have a low melting point; this correlates with good ideal solubility.

Biological properties:

1. The drug should be potent with a daily dose of the order of a few mg/day.
2. The half-life of the drug should be short.
3. The drug must not include a cutaneous irritation or allergic response.
4. Drug, which degrade in the GI tract or are inactivated by hepatic first-pass effect are suitable candidate for transdermal drug delivery.
5. Drug, which have to be administrated for a long period of time or which cause adverse effect to non-targeted tissues can also, be formulated for transdermal drug delivery system.

Component of transdermal drug delivery system.[2]

The main components to a transdermal patch.

1) Release liner:

Release liner protects the patch during storage. The liner is removed prior to use.

2) Drug reservoirs:

The most important part of TDDS is drug reservoir. It consists of drug particles dissolved or dispersed in the matrix.

3) Adhesive:

Adhesive serve to adhere the component of the patch together along with adhering the patch to the skin. The adhesive must possess sufficient adhesion property so that the TDDS should remain in place for a long time. Pressure sensitive adhesive are commonly used for transdermal patch to hold the skin. Commonly used adhesive are silica adhesive, poly isobutylene adhesive, and polyacrylate-based adhesive.

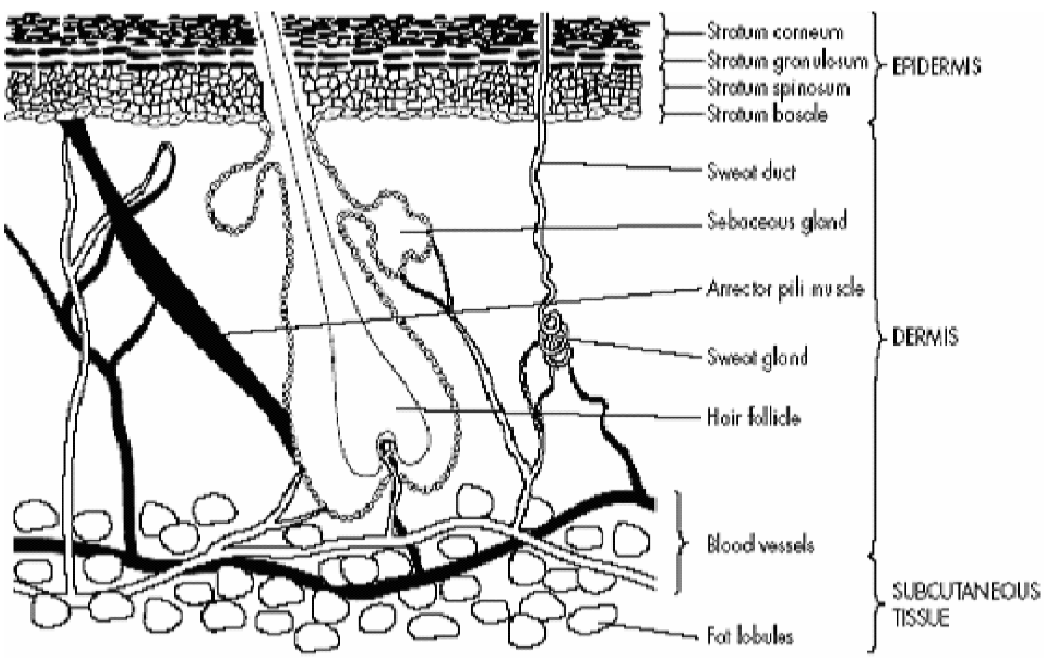
4) Membrane:

Membrane Control the release of the drug from the reservoir and multi-layer patches. It may or may not be split or crack on bending or stretching. Some of rate controlling membrane are polyethylene sheets, ethylene vinyl acetate copolymer, and cellulose acetated.

5) Backing:

Backing protects the patch from the outer environment. The backing layer should be impermeable to drug and penetration enhancer. It serve a function of holding the entire system and protect drug reservoir from atmosphere. The commonly used backing material is polyester, aluminized polyethylene.

Introduction to human skin.[7]



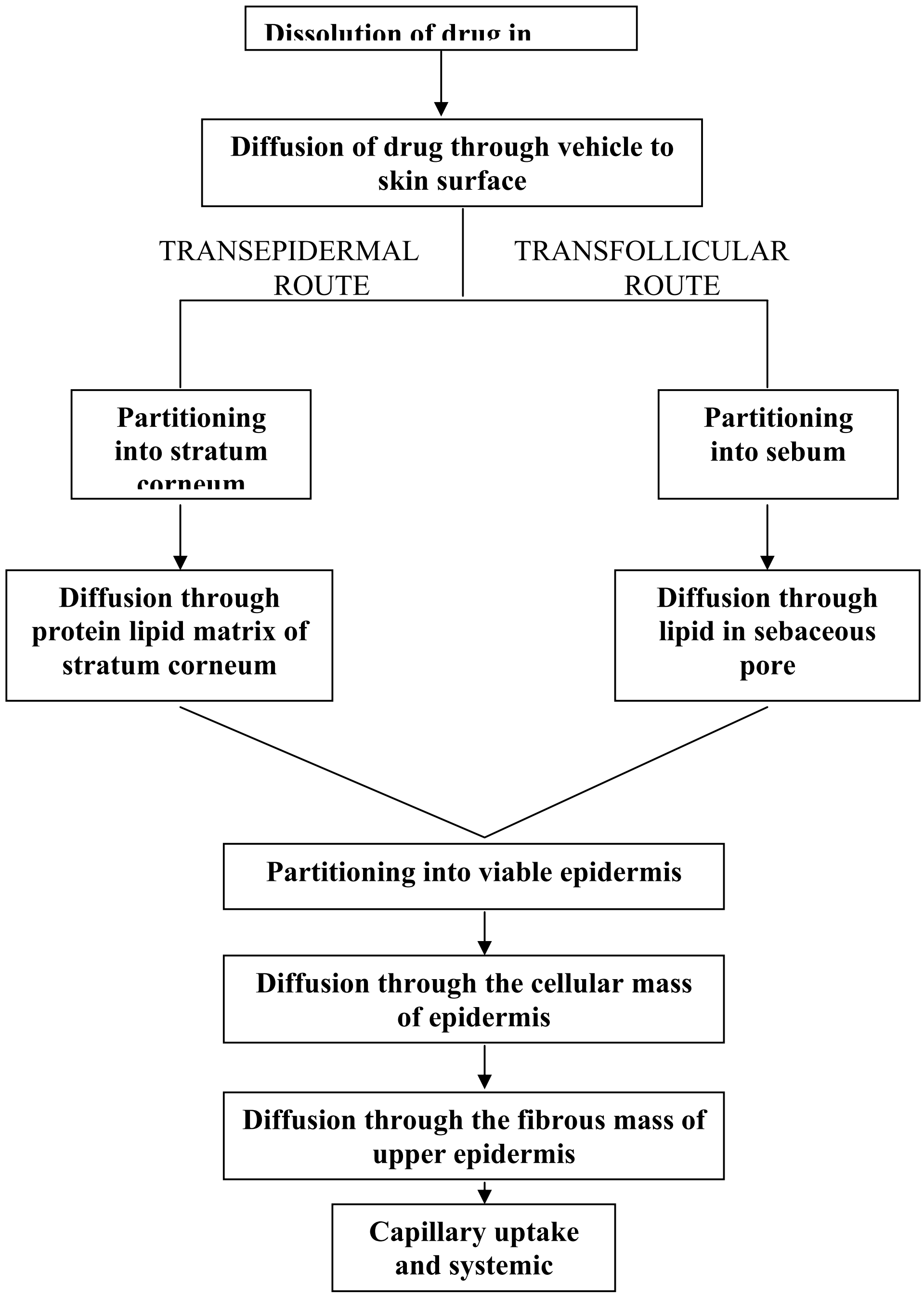
A diagrammatical cross section through human skin.

The skin of an average adult body covers around 2 square meters of surface area and receives approximately one third of all blood circulating through the body. It is one of the most readily accessible organs of the human body. With a thickness of only a fraction of a millimeters. The skin separates the underlying blood circulation network from the outside environment and serves as a barrier against physical, chemical and microbial attack, acts as a thermo blast in maintaining body temperature. Play a role in the regulation of blood pressure and protect against the penetration of ultraviolet rays. As shown in the above figure, anatomically skin consists of three layers, the outermost is the epidermis next is the dermis and the innermost layer is the subcutaneous fat. The epidermis itself is composed of the stratum corneum or horny layer (about 10 Micrometer thick), which is a layer of compressed and overlapping keratinized cell that form a flexible, tough, coherent membrane. This layer contains dead cell with form a flexible, tough, coherent membrane. This layer contains dead cells with keratin filaments in matrix of proteins with lipids and water-soluble substances. The epidermis is more resistant to the diffusion of chemicals than the other layers of the skin. This layer usually delays and frequently prevents the subsequent penetration of drugs to the underlying layers. The thickness depends upon the location on the body. The thickness and penetration properties depend upon the hydration of stratum corneum. Normally the Stratum corneum contains about 20% water. The cells of the stratum corneum are produced from the cells of the stratum granulosum, which in turns are formed from the Single layer of the cells called the stratum basal and both of these are living and located in the epidermis. Below the epidermis is the dermis, a matrix of connective tissue approximately 4 mm thick. Nerves, blood vessels and lymphatic are contained in the dermis. The innermost layer of the skin is the subcutaneous tissue, which contains adipose cells and collagen. The skin also contains appendages which are contained within the dermis and subcutaneous fat tissue same of these penetrate epidermis.

Recently it is becoming evident that the benefits of intravenous drug infusion can be closely duplicated without its hazards by using the skin as the site of drug administration to provide continuous transdermal drug infusion into systemic circulation. The process of drug infusion into systemic circulation known as percutaneous absorption process.

Percutaneous absorption process.[7]

This is the most important process from which we know that, how the drug release from topical and transdermal formulation.



Events governing percutaneous absorption.

Penetration enhancers.[8, 9]

The success of topical or transdermal system depend on the ability of the drug to permeate through skin in sufficient quantities to achieve desired therapeutic effect. The success of topical or transdermal systems depend on the ability of the drug to permeate through skin in sufficient quantities to achieve desired therapeutic effect. It has been increasingly recognized that every drug cannot be administered transdermal because of the barrier properties of stratum corneum. To reduce the resistance of the stratum corneum and it biological variability, penetration enhancers are incorporated into skin preparations. An ideal penetration enhancer can be defined as a chemical, with the unique property in relation to skin that it reversibly reduces the barrier resistance of the horney layer without damaging the viable cells. For delivering a drug via transdermal route, an extensive application area may be needed for desire therapeutic effect. One way to reduce device size is to incorporate penetration enhancer, which will improve the permeation characteristic of the skin.

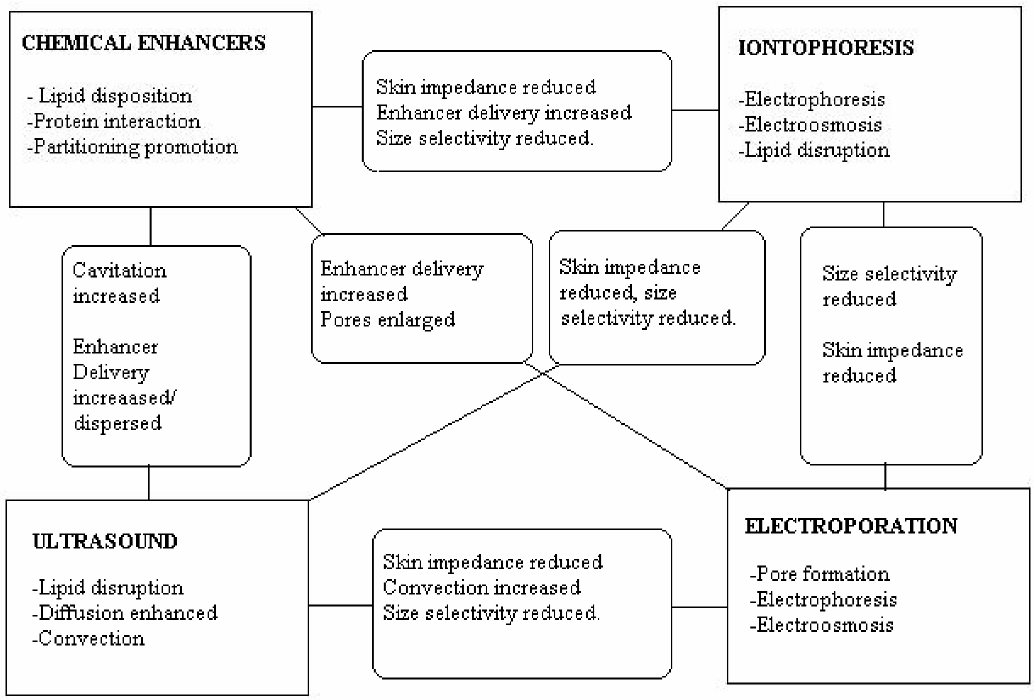
The attributes of ideal enhancers.[6]

- It should be pharmacologically inert, possessing no action of itself at receptor sites anywhere in the body.
- It should be non-toxic, non-irritant and non-allergic.
- Onset of action should be rapid.
- Duration of action should be predictable and suitable for the drug used.
- Upon removal of enhancer, the horney layer should immediately and fully recover its normal barrier property.

- The barrier function of the skin should reduce in one direction only. Endogenous material should not be lost of the environments by diffusion out of skin.
- It should be chemical and physically compatible with all drug and adjuvant to be formulated in topical preparation and device
- If penetration enhancer is liquid and to be used at high volume fraction, it should be a suitable solvent for drugs.
- It should spread well on the skin, with a suitable skin “feel”
- It should be inexpensive, odorless, tasteless and colorless to be cosmetically acceptable.

Mechanism of action of penetration enhancers.[6]

- Reduction of the resistance of stratum corneum by altering it physicochemical properties.
- Alteration of hydration of stratum corneum.
- Effecting a change in the structure of the lipids and lipoproteins in the intercellular channels.
- Change in carrier mechanisms in the transport of ionisable drugs.



Suggested mechanism of action of transdermal penetration enhancers (in main rectangular boxes) and possible synergistic action between methods as illustrated in connecting boxes (rounded rectangular). [10]

Classification of penetration enhancer.[2, 4]

The methods employed for modifying the barrier properties of the stratum corneum to enhance drug penetration through the skin can be categorized as follow

A. Physical enhancer.[10]

Ultrasound: This technique, used originally in physiotherapy and sports medicine, applies a preparation topically and massages the site with an ultrasound source. The procedure was extended to transdermal drug delivery studies. The ultrasonic energy (at low frequency) disturbs the lipid packing in stratum corneum shown in above figure by cavitation. Shock waves of collapsing vacuum cavities increase free volume space in bimolecular leaflets and thus enhance drug penetration into the tissue.

Phonophoresis used to probe the relative contribution of the follicular route to the penetration of hydrophilic permeants. A problem is the need to validate the technique for effectiveness and safety in patients. As yet, it is not readily suitable for home use.

Iontophoresis: The electrical driving of charged molecules into tissue passes a small direct current (approximately 0.5 mA/cm^2) through a drug-containing electrode in contact with the skin. A grounding electrode elsewhere on the body completes the circuit. Three main mechanisms enhance molecular transport: (a) charged species are driven primarily by electrical repulsion from the driving electrode; (b) the flow of electric current may increase the permeability of skin; and (c) electroosmosis may affect uncharged molecules and large polar peptides. Electroosmosis may even be the main force driving peptides and proteins through skin.

Skin electroporation: (electropermeabilization) creates transient aqueous pores in the lipid bilayers by application of short (micro-to millisecond) electrical pulses of approximately 100-1000 V/cm. These pores provide pathways for drug penetration that travel straight through the horny layer. During the pulse, drugs transport via iontophoresis and/or electroosmosis. Significant movement can also occur between pulses by simple diffusion due to relatively persistent changes in the stratum corneum lowering its resistance. Fluxes increases 10-104 fold for neutral and highly charged molecules of up to 40 kDa.

Magnetophoresis:

Limited work probed the ability of magnetic fields to move diamagnetic materials through skin.

B. Chemical method.[10]

Chemical penetration enhancers: substances temporarily diminishing the barrier of the skin, known also as accelerants or sorption promoters, can enhance drug flux. For safety and effectiveness, the best penetration enhancer is water. Most substances penetrate better through hydrated stratum corneum than through dry tissue, hence the value of occlusive patches. Thus, any chemical which is pharmacologically inactive, non-damaging and which promotes horny layer hydration, is a penetration enhancer. Examples include the natural moisturizing factor and urea.

Technologies for developing transdermal drug delivery system.[1]

The technologies can be classified in four basic approaches.

1) Membrane permeation controlled system

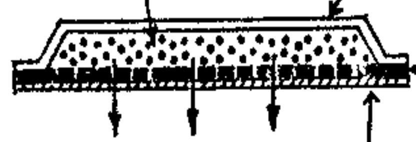
2) Adhesive dispersion type system

3) Matrix diffusion controlled system

4) Micro reservoir type or micro sealed dissolution controlled system

1. Membrane permeation-controlled systems

In this type of system, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug-impermeable or non-porous with a defined drug permeability property and the drug molecules are permitted to release through the rate-controlling membrane. In the drug reservoir compartment, the drug solids are either dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium to form a paste-like suspension. A thin layer of drug compatible, hypoallergenic adhesive polymer may be applied to the external surface of the rate controlling membrane to have intimate contact with the skin. The rate of drug release from this type of delivery system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate limiting membrane and adhesive.



The intrinsic rate of drug release (dQ/dt) from this drug delivery system is given by

$$\frac{dQ}{dt} = \frac{C_R}{1/P_m + 1/P_a}$$

Where CR= drug concentration in reservoir compartment

Pa=permeability coefficient of adhesive layer

Pm=Permeability coefficient of rate controlling membrane

Pm and Pa are given as

$$P_m = \frac{K_{m/r}\, D_m}{h_m} \quad \textbf{and}$$

$$P_a = \frac{K_{a/m}\, D_a}{h_a}$$

Where Km/r=Partition coefficient for the interfacial partitioning of drug from reservoir to membrane

Ka/m=Partition coefficient for the interfacial partitioning of drug from membrane to adhesive layer.

Dm=Diffusion coefficient in the rate controlling membrane

Da=Diffusion coefficient in the adhesive layer.

hm= Thickness of rate controlling membrane

Ha=Thickness of adhesive layer

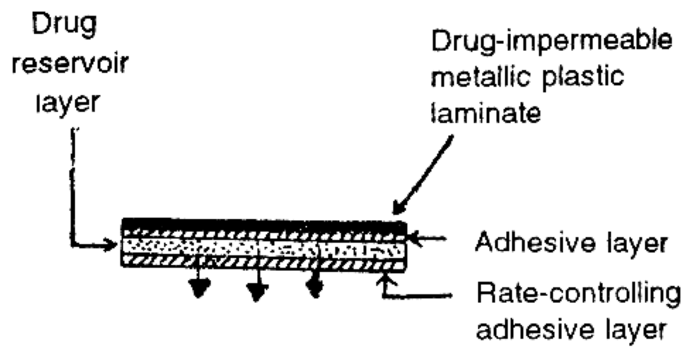
Substituting the values of Pm and Pa in equation (1) gives

$$\frac{dQ}{dt} = \frac{K_{m/r} K_{a/m} D_m D_a}{K_{m/r} D_m h_a + K_{a/m} D_a h_m} C_R$$

This equation defines the intrinsic rate of drug release from a membrane-moderated drug delivery system.

2. Adhesive dispersion-type system:

This is a simplified form of the membrane permeation-controlled system. The drug reservoir is formulated by directly dispersing the drug in an adhesive polymer and then spreading the medicated adhesive by solvent casting or hot melt, on to a flat sheet of drug impermeable metallic plastic backing to form a thin drug reservoir layer. On top of the drug reservoir layer, thin layers of non-medicated, rate controlling adhesive polymer of a specific permeability and constant thickness are applied to produce an adhesive diffusion-controlled delivery system.



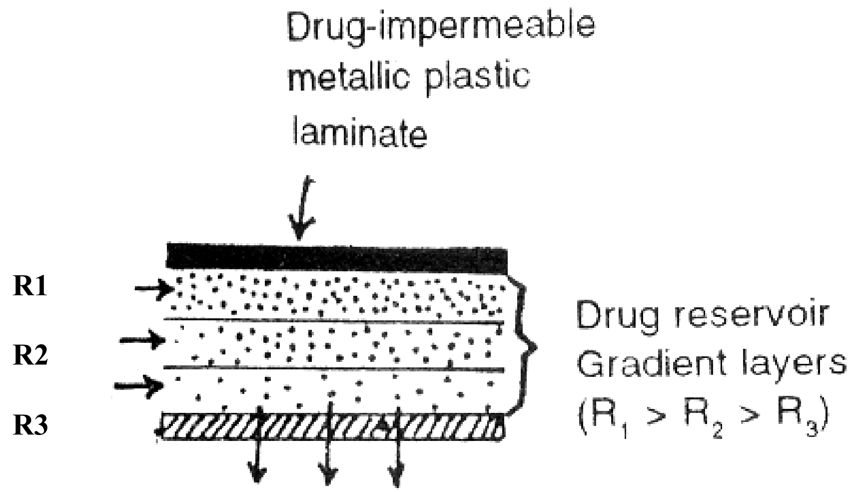
The rate of drug release from this system is given by the equation.

$$\frac{dQ}{dt} = \frac{Ka/r}{h} \times C_R$$

Where, Ka/r = Partition coefficient for the interfacial partitioning of the drug from the reservoir layer to adhesive layer

3. Matrix diffusion-controlled systems:

In this approach, the drug reservoir is prepared by homogeneously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The resultant medicated polymer is then moulded into a medicated disc with a defined surface area and controlled thickness. The dispersion of drug particles in the polymer matrix can be accomplished by either homogeneously mixing the finely grounded drug particles with a liquid polymer or a highly viscous base polymer followed by cross-linking of the polymer chains or homogeneously blending drug solids with a rubbery polymer at an elevated temperature. The drug reservoir can also be formed by dissolving the drug and polymer in a common solvent followed by solvent evaporation in a mould at an elevated temperature and/or under vacuum. This drug reservoir containing polymer disc is then pasted onto an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc.



The rate of drug release from this type of system is given by the equation.

$$\frac{dQ}{dt} = \left(\frac{AC_pD_p}{2t}\right)^{1/2}$$

Where

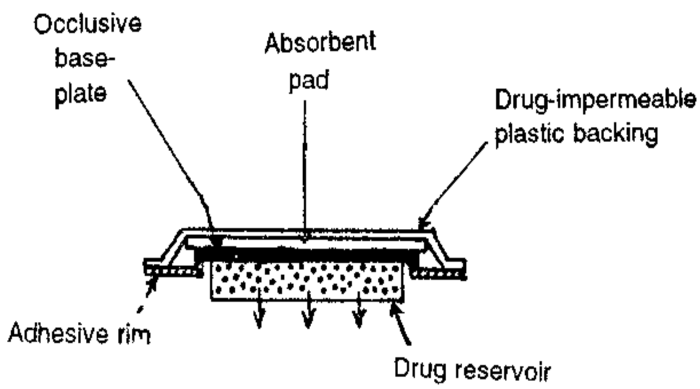
A=initial drug loading dose dispersed in the polymer matrix

Cp=solubility of the drug in the polymer

Dp=diffusivity of the drug in the polymer

4. Micro reservoir type or micro sealed dissolution controlled systems:

This can be considered a combination of the reservoir and matrix diffusion type drug delivery systems. Here the drug reservoir is formed by first suspending the drug solids in an aqueous solution of a water-soluble liquid polymer and then dispersing the drug suspension homogeneously in a lipophilic polymer by high energy dispersion technique to form several discrete, unleachable microscopic spheres of drug reservoirs. The quick stabilization of this thermodynamically unstable dispersion is accomplished by immediately cross-linking the polymer disc with a constant surface area and a fixed thickness. Depending upon the physicochemical property of the drug and the desired rate of drug release, the device can be further coated with a layer of biocompatible polymer to modify the mechanism and rate of drug release. Positioning the medicated disc at the center and surrounding it with an adhesive rim produce a transdermal therapeutic system.



The micro reservoir system has been claimed to follow the zero order release of drugs without danger of dose dumping.

The rate of drug release of drugs from this type of systems is given by the equation.

$$\frac{dQ}{dt} = \frac{D_pD_dmK_p}{D_ph_d+D_dh_pmK_p}\left(nS_p \quad \frac{D_1S_1\,(1-n)}{h_1}\left\{\frac{1}{K} + \frac{1}{K_m}\right\}\right)$$

Where

m=a/b

a=ratio of drug concentration in the bulk of the elution medium over drug solubility in the same medium.

b= ratio of the drug concentration at the outer edge of the polymer coating over the drug solubility in same polymer concentration.

n= ratio of the drug concentration at the inner edge of the interfacial barrier over solubility in the polymer matrix.

Di= drug diffusivity in the liquid layer surrounding the drug particle with thickness

Dp= drug diffusivity in the polymer coating membrane surrounding the polymer matrix with thickness of hp

Dd = drug diffusivity in hydrodynamic diffusion layer surrounding the polymer coating with thickness of hd

Ki = partition coefficient for the interfacial partitioning of the drug from the liquid compartment of the polymer matrix

Km = partition coefficient for the interfacial partitioning coating membrane

Kp= partition coefficient for the interfacial partitioning of the drug from the polymer coating membrane of the elution solution.

S_l = solubility of drug in liquid compartment

Sp= solubility of the drug in the polymer matrix.

General clinical consideration in use of transdermal drug delivery system.[2]

The patient should be advised of the following general guideline. The patient should be advised of the importance of using the recommended site and rotating location within the site. Rotation location is important to allow the skin to regain its normal permeability and to prevent skin irritation.

1 Transdermal drug delivery should be applied to clean, dry skin relatively free of hair and not oily, inflamed, irritation, broken, or call used .wet or moist skin can be accelerate drug permeation. Oily skin can impair the adhesion of patch. If hair is present at the site, it should be care fully cut, not wet shaved, nor should a depilatory agent to be used, since later can remove stratum corneum and affect the rate and extent of drug permeation.

2. Use of skin lotion should be avoided at the application site, because lotion affect the hydration of skin and alter partition coefficient of drug.

3. The release liner should be removed with care. The transdermal drug delivery system should be pressed firmly against skin site with the heel of hand for about 10 seconds.

4. A transdermal drug delivery system should be placed at a site that will not subject it to being rubbed off by clothing or movement. Transdermal drug delivery system should be left on when showering, bathing or swimming.

5. A TDDS should be worn for full period stated in the products instruction followed by removal and replacement with fresh system.

6. The patient or caregiver should clean the hands after applying a TDDS. Patient should not rub eye or touch the mouth during handling of the system

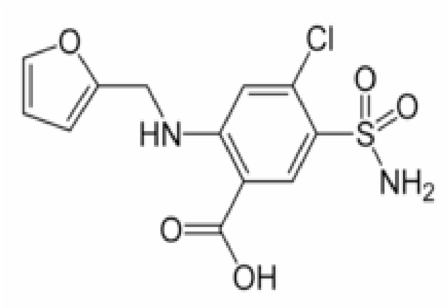
7. If the patients exhibit sensitivity to a TDDS or if under skin irritation results, the patient should seek reevaluation. The used patch discarded in a manner safe to children and pets.

FUROSEMIDE [11 - 14]

Molecular formula: $C_{12}H_{11}ClN_2O_5S$

Relative molecular mass: 330.8

Graphic Formula:



Chemical name: 4-Chloro-N-furfuryl-5-sulfamoyl anthranillic acid.

Melting point: 210°C.

Description: - A white or almost white crystalline powder; odorless.

Solubility: - practically insoluble in water, soluble in 75 parts of ethanol; slightly soluble in ether R; very slightly soluble in chloroform R.

Category: Diuretic

Storage: Furosemide should be kept in a well-closed container, protected from light.

Doses :Each tablet for oral administration contains 20 mg, 40 mg, or 80 mg of furosemide.Furosemide Oral Solution is available in two strengths: 40 mg per 5 mL, and 10 mg per mL.

Mechanism of Action/Effect: Furosemide acts by inhibiting the Na-K-2Cl symporter in the thick ascending loop of Henle. It also has inhibitory activity on carbonic anhydrase. it also abolishes the corticomedullary osmotic gradient and blocks negative as well as positive free water clearence

Indications:

Edema:

Furosemide is indicated in adults, infants and children for the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and renal disease, including the nephrotic syndrome. Furosemide is particularly useful when an agent with greater diuretic potential is desired.

Hypertension:

Oral furosemide may be used in adults for the treatment of hypertension alone or in combination with other antihypertensive agents. Hypertensive patients who cannot be adequately controlled with thiazides will probably also not be adequately controlled with furosemide alone

PHARMACOKINETICS:

Absorption: Approximately 60 to 70% of an oral dose of furosemide is absorbed. Food may slow the rate of absorption but does not appear to alter the bioavailability or diuretic effect. Absorption is reduced to 43 to 46% in patients with end-stage renal disease, and is probably reduced also in patients with edematous bowel caused by congestive heart failure or nephrotic syndrome; parenteral administration may be preferable in these patients. Following table show the pharmacokinetic parameter of Furosemide.

Pharmacokinetic parameter of Furosemide

Half-life	Up to 2 hour
Onset of action	Oral—20 to 60 minutes.
Elimination	Renal (88%);
Duration of action	6 to 8 hours.
Time to peak effect	1 to 2 hours
Protein binding	Very high (91 to 97%)
Biotransformation	Hepatic

Precautions to Consider Cross-sensitivity and/or related problems: Patients sensitive to sulfonamides (including thiazide diuretics) may be sensitive to furosemide.

Carcinogenicity/Tumorigenicity:

Furosemide was tested for carcinogenicity by oral administration in one strain of mice and one strain of rats. A small but significantly increased incidence of mammary gland carcinomas occurred in female mice at a dose 17.5 times the maximum human dose 600 mg. There were marginal increases in uncommon tumors in male rats at a dose of 15 mg/kg (slightly greater than the maximum human dose) but not at 30 mg/kg.

Mutagenicity:

Mutagenicity studies have not been conducted.

Pregnancy/Reproduction:

Pregnancy:

Pregnant women should be advised to contact physician before taking this medication, since routine use of diuretics during normal pregnancy is inappropriate and exposes mother and fetus to unnecessary hazard. Diuretics do not prevent development of toxemia of pregnancy, and there is no satisfactory evidence that they are useful in the treatment of toxemia. Diuretics are indicated only in the treatment of edema due to pathologic causes or as a short course of treatment in patients with severe hypervolemia.Furosemide crosses the placenta. Studies in humans have not been done.Studies in rabbits and mice have shown that furosemide causes an increased incidence of hydronephrosis in the fetus. In rabbits, unexplained maternal deaths and abortions have occurred at doses 2 to 8 times the maximum recommended human dose.FDA Pregnancy Category C.

Breast-feeding:

Furosemide is distributed into breast milk; it is not known whether bumetanide or ethacrynic acid is distributed into breast milk.

Pediatrics:

Caution is required in neonates because of the prolonged half-life of furosemide. Usual pediatric doses may be used, but the dosing interval should be extended.

Geriatrics:

Although appropriate studies on the relationship of age to the effects of loop diuretics have not been performed in the geriatric population, the elderly may be more sensitive to the hypotensive and electrolyte effects. In addition, elderly patients are at greater risk of developing circulatory collapse and thromboembolic episodes. Elderly patients are also more likely to have age-related renal function impairment, which may require adjustment of dosage or dosing interval in patients receiving loop diuretics.

DRUG INTERACTIONS AND/OR RELATED PROBLEMS:

Furosemide may increase the ototoxic potential of aminoglycoside antibiotics, especially in the presence of impaired renal function. Except in life threatening situations, avoid this combination.

Furosemide should not be used concomitantly with ethacrynic acid because of the possibility of ototoxicity.

Patients receiving high doses of salicylates concomitantly with furosemide, as in rheumatic disease, may experience salicylate toxicity at lower doses because of competitive renal excretory sites.

Furosemide has a tendency to antagonize the skeletal muscle relaxing effect of tubocurarine and may potentiate the action of succinylcholine.

Lithium generally should not be given with diuretics because they reduce lithium's renal clearance and add a high risk of lithium toxicity.

Furosemide may add to or potentiate the therapeutic effect of other antihypertensive drugs. Potentiation occurs with ganglionic or peripheral adrenergic blocking drugs Furosemide may decrease arterial responsiveness to norepinephrine. However, norepinephrine may still be used effectively.

Simultaneous administration of sucralfate and furosemide tablets may reduce the natriuretic and antihypertensive effects of furosemide.

Patients receiving both drugs should be observed closely to determine if the desired diuretic and/or antihypertensive effect of furosemide is achieved. The intake of furosemide and sucralfate should be separated by at least two hours

One study in six subjects demonstrated that the combination of furosemide and acetylsalicylic acid temporarily reduced creatinine clearance in patients with chronic renal insufficiency. There are case reports of patients who developed increased BUN, serum creatinine and serum potassium levels, and weight gain when furosemide was used in conjunction with NSAIDs.

Literature reports indicate that coadministration of indomethacin may reduce the natriuretic and antihypertensive effects of furosemide in some patients by inhibiting prostaglandin synthesis. Indomethacin may also affect plasma renin levels, aldosterone excretion and renin profile evaluation. Patients receiving both indomethacin and furosemide should be observed closely to determine if the desired diuretic and/or antihypertensive effect of furosemide is achieved

ADVERSE REACTIONS:

Adverse reactions are categorized below by organ system and listed by decreasing severity.

Gastrointestinal System Reactions:

Pancreatitis ,jaundice, (intrahepatic cholestatic jaundice),anorexia, oral and gastric irritation, cramping, diarrhea, constipation, nausea, vomiting .

Systemic Hypersensitivity Reactio ns:

Systemic vasculitis, interstitial nephritis, necrotizing angiitis.

Central Nervous System Reactions:

Tinnitus and hearing loss, paresthesias ,vertigo, dizziness, headache, blurred vision, xanthopsia.

Hematologic Reactions:

Aplastic anemia (rare), thrombocytopenia, agranulocytosis (rare), hemolytic anemia leukopenia ,anemia.

Cardiovascular Reaction:

Orthostatic hypotension may occur and be aggravated by alcohol, barbiturates or narcotics.

Whenever adverse reactions are moderate or severe, furosemide dosage should be reduced or therapy withdrawn.

OVERDOSAGE:

The principal signs and symptoms of overdosage with furosemide are dehydration, blood volume reduction, hypotension, electrolyte imbalance, hypokalemia and hypochloremic alkalosis, and are extensions of its diuretic action.

The acute toxicity of furosemide has been determined in mice, rats and dogs. In all three, the oral LD50 exceeded 1000 mg/kg body weight while the intravenous LD50 ranged from 300 to 680 mg/kg. The acute intragastric toxicity in neonatal rats is 7 to 10 times that of adult rats.

The concentration of furosemide in biological fluid associated with toxicity or death is not known.

Treatment of overdosage is supportive and consists of replacement of excessive fluid and electrolyte losses. Serum electrolytes, carbon dioxide level and blood pressure should be determined frequently. Adequate drainage must be assured in patients with urinary bladder outlet obstruction (such as prostatic hypertrophy).

Hemodialysis does not accelerate furosemide elimination

CONTRAINDICATIONS:

Furosemide is contraindicated in patients with anuria and in patients with a history of hypersensitivity to furosemide.

WARNINGS:

In patients with hepatic cirrhosis and ascites, furosemide therapy is best initiated in the hospital. In hepatic coma and in states of electrolyte depletion, therapy should not be instituted until the basic condition is improved. Sudden alterations of fluid and electrolyte balance in patients with cirrhosis may precipitate hepatic coma; therefore strict observation is necessary during the period of diuresis. Supplemental potassium chloride and, if required, an aldosterone antagonist are helpful in preventing hypokalemia and metabolic alkalosis.

If increasing azotemia and oliguria occur during treatment of severe progressive renal disease, furosemide should be discontinued.

Cases of tinnitus and reversible or irreversible hearing impairment have been reported. Usually, reports indicate that furosemide ototoxicity is associated with rapid injection, severe renal impairment, doses exceeding several times the usual recommended dose, or concomitant therapy with aminoglycoside antibiotics, ethacrynic acid, or other ototoxic drugs. If the physician elects to use high dose parenteral therapy, controlled intravenous infusion is advisable (for adults, an infusion rate not exceeding 4 mg furosemide per minute has been used).

BRAND NAMES:

Some of the brand names under which furosemide is marketed include: Aisemide®, Beronald®, Desdemin®, Discoid®, Diural®, Diurapid®, Dryptal®, Durafurid®, Errolon®, Eutensin®, Frusetic®, Frusid®, Fulsix®, Fuluvamide®, Furesis®, Furo-Puren®, Furosedon®, Hydro-rapid®, Impugan®, Katlex®, Lasilix®, Lasix®, Lodix®, Lowpston®, Macasirool®, Mirfat®, Nicorol®, Odemase®, Oedemex®, Profemin®, Rosemide®, Rusyde®, Salix®, Trofurit®, Urex

MATERIALS:

ETHYLCELLOUSE: [15]

Non proprietary name: BP: ethylcellouse

USP NF: ethylcellouse

Synonyms: Ethocel

Functional category: Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity-increase agent.

Description: Ethylcellouse is a tasteless, free flowing white to light tan- colored powder.

Solubility: Practically insoluble in glycerin, propylene glycol, and water. Freely soluble in chloroform, methyl acetate, and tetrahydrofuran.

Viscosity: viscosity of ethylcellouse is measured in typically at 25 C using 5% w/v ethylcellouse dissolved in a solvent blend of 80% toluene: 20%ethanol (w/w)

Application in pharmaceutical formulation:

Use	**Concentration use in formulation (%)**
Micro encapsulation	10.0-20.0
Sustained release tablet coating	3.0-20.0
Tablet coating	1.0-3.0
Tablet granulation	1.0-3.0

Stability and storage condition:

Ethylcellouse is stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions. Although it is more sensitive to acidic materials then are cellulose esters.

POLYVINYL PYRROLIDON: [15]

Non –proprietary names: BP: Povidone, JP: Povidone, PhEUR: Povidonum, USP: Povidone

Synonyms: E1201; kollidon; plasdone; poly [1-(2-oxo-1-pyrrolidinyl) Ethylene]; poyvidone; PVP; 1-vinyl-2-pyrrolidone polymer

Chemical name: 1-ethenyl-2-pyrrolidinone homopolymer

Functional categories: Disintegrant, dissolution aid, suspending agent, tablet binder

Description: Povidone occurs as a fine, white to creamy a white, colored, odorless or almost odorless, hygroscopic powder. Povidone with K-values equal to or lower than 30 are manufactured by spray drying spheres. Povidone K-90 and higher K-value povidone are manufactured by drum drying and occur as plates.

Solubility: Freely soluble in acids, chloroform, ethanol, ketones, methanol, and water practically insoluble in ether, hydrocarbons, mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution, which is a function of the K value.

Application in pharmaceutical formulation:

Although povidone is used in a variety of pharmaceutical formulation, it is primarily used in solid dosage forms. In tabletting, povidone solutions are used as binders in wet granulation processes. Povidone is also added to powder blends in the dry form and granulated in situ by the addition of water, alcohol, or hydro alcoholic solutions. Povidone is used as a solubilizer in oral and parentral formulations and has been shown to enhance dissolution of poorly soluble drugs from solid dosage forms. Povidone solutions may also be used as coating agents. Povidone is additionally used a suspending, stabilizing or viscosity-increasing agent in a number of topical and oral suspensions and solutions. The solubility of a number of poorly soluble active drugs may be in increased by mixing with povidone. Special grades of pyrogen-free povidone are available and have been used in parentral formulations.

PVP used in different concentration were given below.

USE	CONCENTRATION (%)
Carrier for drugs	10-25
Dispersion agent	Up to 5
Eye drops	2-10
Suspending agent	Up to 5
Tablet binder, tablet diluent, or coating agent	0.5-5

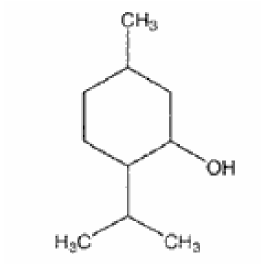
MENTHOL OIL: [15]

Non proprietary name: BP: Racementhol

USP: menthol

Synonyms: Hexahydrothymol, dl menthol, racemic menthol.

Structural formula:



Functional category: Flavoring agent, therapeutic agent.

Solubility: very soluble in ethanol; chloroform, and ether, very slightly soluble in glycerin, practically insoluble in water.

Application in pharmaceutical formulation:

Menthol has been investigated as a skin penetration enhancer and is also used in perfumery, tobacco product and as a therapeutic agent.

Use	Concentration use in formulation (%)
Inhalation	0.02-0.05
Oral suspension	0.003
Tablet	0.2-0.4
Topical formulation	0.05-10.0

Stability and storage condition:

Menthol should be stored in a well-closed container at a temperature not exceeding 25 C. Since it sublimes readily.

LEMON GRASS OIL: [16]

Synonyms: Verbena

Botanical: Family: Graminaceae

Genus: Cymbopogen

Species: Citratus

Chemical constituents: Citral 75-85%

Characteristic: Color: Reddish- yellow or brownish red

Odor: Strong, citrus like

Taste: Bitter

Specific gravity: 0.895-0.908

Solubility: insoluble in water, soluble in alcohol, ether, chloroform, slightly soluble in glycerol.

Pharmaceutical use: synthesis of vitamin, penetration enhancer

CLOVE OIL: [16]

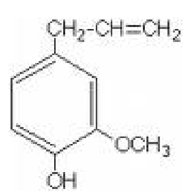
Botanical: ***Family:*** Myrtaceae

***Genus*:** Eugenia

***Species*:** Caryophyllata

Chemical constituents: Eugenol 82-90%

Structural formula:



Characteristics: color: colorless to yellowish or bluish

Odor: pepper like

Taste: bitter

Specific gravity: 0.88-0.91

Solubility: insoluble in water, soluble in alcohol, ether, and chloroform.

EUCALYPTUS OIL: [16]

Botanical: ***Family***: Myrtaceae

Genus: Eucalyptus

Species: globules

Chemical constituents: Cineol (eucalyptol) 70-90%

Characteristics: **color:** colorless or pale yellow

Odor: camphoraceous

Taste: spicy pungent, cooling

Specific gravity: 0.905-0.925

Solubility: insoluble in water, soluble in alcohol, ether, chloroform, oil, fats

PROPYLENE GLYCOL: [15]

Non proprietary name: BP: propylene glycol

USPNF: propylene glycol

Synonyms: methyl ethylene glycol, propane-, 2-diol

Description: Propylene glycol (PG) is the clear, colorless, viscous, practically odorless liquid with sweet slightly taste resembling that of glycerin. PG is widely used as a penetration enhancer in topical preparation, either alone or in combination with other penetration enhancer, with high diffusing property as proved by its flux profile, as well as miscible with water.

Mechanism of action: Its mechanism of action is to partition into the stratum corneum and increase permeant solubility in and thus permeate flux through stratum corneum. Most commercial formulation contain PG usually in a rang of 5-20%.

Application in pharmaceutical formulation:

Use	Dosage	Concentration use in formulation (%)
Humectants	Topical	15
Preservative	Solution, semi solid	15-30
Solvent or co solvent	Aerosol	10-30
	Oral solution	10-25
	Parentral	10-60
	Topical	5-8

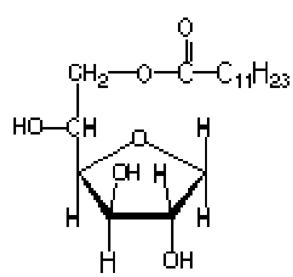
SORBITAN MONOLAURATE (SPAN-20): [15]

Chemical Name: Sorbitan Monododecanoate

Empirical Formula: C18H34O6

Molecular Weight: 346

Structural Formula:



Functional Category

Emulsifying agent, nonionic surfactant, solubilizing agent, wetting agent and dispersing /suspending agent.

Application in pharmaceutical formulation or technology

Sorbitan monoesters are a series of mixtures of partial esters of sorbitol and its mono- and dianhydrides with fatty acids. Sorbitan diesters are a series of mixtures of partial esters of sorbitol and its monoanhydride with fatty acids.

Sorbitan esters are widely used in cosmetics, food products, and pharmaceutical formulations as lipophilic nonionic surfactants. They are mainly used in pharmaceutical formulations as emulsifying agents in the preparation of creams, emulsions, and ointments for topical application. When used alone, sorbitan esters produce stable water-in-oil emulsions and microemulsions but are frequently used in combination with varying proportions of a polysorbate to produce water-in-oil or oil-in-water emulsions or creams of varying consistencies. Sorbitan monolaurate, sorbitan monopalmitate and sorbitan trioleate have also been used at concentrations of 0.01–0.05% w/v in the preparation of an emulsion for intramuscular administration.

Description: Yellow viscous liquid.

Pharmacoepial specifications:

Acid Value: • 7

Density: 1.01(g/cm3)

HLB Value: 8.6

Hydroxyl Value: 159 –169

Moisture Content : ≤ 0.5

Pour Point: 16 – 20(°C)

Saponification Value: 159 –169

Solubility: Sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water, although insoluble, they are generally dispersible.

Surface tension of 1% aqueous solution (mN/m): 28

Stability and Storage Conditions: Sorbitan esters should be stored in a well-closed container in a cool, dry place.

POLYSORBATE 80 (TWEEN 80): [15]

Chemical name: Polyoxyethylene 20 sorbitan monooleate.

Empirical formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310

Synonyms: Tween 80.

Functional Category: Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

Applications in Pharmaceutical Formulation or Technology: Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides.

Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for *p*-glycoprotein. Polysorbates are also widely used in cosmetics and food products.

Description: Polysorbates have a characteristic odor and a warm, somewhat bitter taste.

Typical Properties:

Acid value: 2.0 %

Acidity/alkalinity: pH = 6.0 – 8.0 for a 5 % w/v aqueous solution.

Flash point: 149°C

HLB value: 15

Hydroxyl value: 65 - 80

Moisture content: 3.0

Saponification value: 45 - 55

Surface tension for 0.1% w/v solutions 20(°C) : 42.5 mN/m

Viscosity (dynamic): 425 mPas.

Stability and Storage Conditions:

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

POLYSORBATE 20 (TWEEN 20): [15]

Chemical name: Polyoxyethylene 20 sorbitan monolaurate.

Empirical formula: $C_{58}H_{114}O_{26}$

Molecular weight: 1128

Synonyms: Tween 20.

Functional Category: Emulsifying agent; nonionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

Applications in Pharmaceutical Formulation or Technology: Polyoxyethylene sorbitan fatty acid esters (polysorbates) are a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5, or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides.

Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. They have been found to be useful in improving the oral bioavailability of drug molecules that are substrates for *p*-glycoprotein. Polysorbates are also widely used in cosmetics and food products.

Description: Polysorbates have a characteristic odor and a warm, somewhat bitter taste.

Typical Properties:

Acid value: 2.0 %

Acidity/alkalinity: pH = 6.0 – 8.0 for a 5% w/v aqueous solution.

Flash point: 149°C

HLB value: 16.7

Hydroxyl value: 96 -108

Moisture content: 3.0

Saponification value: 40 - 50

Viscosity (dynamic): 400 mPas.

Stability and Storage Conditions:

Polysorbates are stable to electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. The oleic acid esters are sensitive to oxidation. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Also, in common with other polyoxyethylene surfactants, prolonged storage can lead to the formation of peroxides.

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

DIBUTYL PHTHALATE: [15]

Non proprietary name: BP: Dibutyl phthalate,

PhEur: Dibutylis phthalas

Synonyms: Araldite 502, benzenedicarboxylic acid, benzene-*o*-dicarboxylic acid di-*n*-butyl ester, butyl phthalate

Chemical Name: Dibutyl benzene-1,2-dicarboxylate.

Functional Category: Film-former, plasticizer, solvent

Description: Dibutyl phthalate occurs as an odorless, oily, colorless, or very slightly yellow-colored, viscous liquid

Solubility: Very soluble in acetone, benzene, ethanol (95%), and ether; soluble 1 in 2500 of water at 20°C.

Applications in pharmaceutical formulations: Dibutyl phthalate is used in pharmaceutical formulations as a plasticizer in film-coatings. It is also used extensively as a solvent particularly in cosmetic formulations such as antiperspirants, hair shampoos and hair sprays. In addition to a number of industrial applications, dibutyl phthalate is used as an insect repellent, although it is not as effective as dimethyl phthalate.

2. OBJECTIVES OF THE STUDY

The present study is planned with the following objectives:

- To prepare the transdermal patches of furosemide using ethyl cellulose. (20 cps)
- To carry out FTIR study.
- To study the effect of various penetration enhancers.
- To evaluate the patches for:
    - Drug content, thickness, moisture content, moisture uptake and folding endurance study.
    - In vitro permeation (using standard cellophane membrane and human cadaver skin) study
- Stability study.

3. REVIEW OF LITERATURE

1. Cho et al[17] prepared the ethylene vinyl acetate (EVA) matrix containing furosemide was prepared by the casting method and release pattern were observed. The release of drug from the matrix was studied as a function of temperature and drug concentration. Plasticizer such as the citrates and pthalates were added for preparing the membrane to increase the flexibility of the EVA matrix. Solubility of furosemide was highest when the concentration of PEG 400 was 40%(v/v). The release rate of drug increase when increase in temperature and drug load. In plasticizer diethyl phthalate showed the best enhancing effects in drug release. The application of an EVA matrix containing a plasticizer might be useful in the development of a controlled drug delivery system.

2. Gregorious et al[18] was examined the in vitro skin permeation of furosemide, a commonly used loop diuretic through human epidermis. In order to estimate the effects of the type, the concentration of enhancer and the concentration gelling agent on the cumulative amount of furosemide permeated through human epidermis using a 3^3 factorial design. In order to further increase the amount of the drug permeated, the combination of two enhancers. Azone ® and oleyl alcohol at three concentration levels was employed, using optimization technique. The higher amount of furosemide permeated when Azone ® was used at 5.0-6.5% (v/v) and oleyl alcohol at 7.5-9%(v/v) in the gels used. These formulations seen to be suitable for transdermal delivery of furosemide for pediatrics use.

3. Shin et al[19] prepared ethylene-vinyl acetate (EVA) copolymer matrix containing triprolidine and studied the effect of penetration enhancers like glycol (diethylene glycol, tetra ethylene glycol), fatty acid (lauric acid, myristic acid, capric acid) and non-ionic surfactant (polyoxyethylene-2-oleyl ether, polyoxyethylene-2-steary ether, polyxythylene-23-lauryl ether) and reported that penetration enhancer could be important for controlling transdermal delivery of triprolidine

4. Verma et al[20] prepared propranolol patch from eudragit grade RL 100, RLPM, RLPO and RSPO. The patches were uniform with respect to drug content, weight and thickness. The drug content in vivo was examined by plasma and urine analysis. The patch produced no irritation and the formulation was reported to be safe.

5. Devi et al[21] investigated in vitro and in vivo permeation of Diclofenac diethyl amine from ethyl hexyl acrylate and vinyl acetate pressure sensitive adhesive system. The patch was able to maintain the therapeutic drug rang in plasma for 24 hours. The patch did not produce any irritation to skin.

6. Funke et al[22] investigated the possibility of applying a highly lipophilic drug, the antiestrogen transdermally by polyacrylate- based matrix transdermal delivery system. In vitro release as well as in vitro permeation of antiestrogen through excised skin of hairless mice was found to be independent of concentration of both drug and enhancer. They concluded that the highly lipophilic antiestrogen could be administered transdermally by pretreating the

skin with the fluid permeation enhancer combination propylene glycol- lauric acid then applying a matrix transdermal delivery system.

7. Zhao et al [23] investigated the effect of enhancer (5% terpenes; i.e., eugenol, limonene, and menthone) in combination with 50% propylene glycol in water (50 % PG) on the in vitro percutaneoue absorption of tamoxifen through the porcine epidermis, on biophysical changes in the stratum corneum lipids. On macroscopic barrier properties, and on binding on drug to the stratum corneum. The enhancer in combination with 50 % PG significantly increase the permeability coefficient of tamoxifen in comparison with that of the control (50% PG in water). They concluded that the epidermis was enhanced with 5% eugenol/50% PG or 5% limonene/50% PG compared with 50 % PG alone.

8. Murthy et al[24] prepared transdermal formulation containing theophylline and salbutamol sulfate were formulated using hydroxy propyl methyl cellouse. The formulation was subjected to pharmacodynamic studies in guinea pigs. Pharmacokinetics studies were carried out in healthy volunteers. They concluded that the formulation of transdermal drug delivery system for simultaneous delivery of theophylline and solbutamol sulfate is feasible and the system is capable of maintaining the therapeutic level of drug in the blood.

9. Bharkatiya et al[25] prepared transdermal films of propranolol hydrochloride using different polymer along and in combination such as ethylcellouse, cellulose acetate, polyvinyl pyrrolidone, acrycoat L100, acrycoat S100. The

physicochemical parameter like weight variation, thickness, folding endurances, drug content, tensile strength and stability were evaluated. They concluded that release of drug from all formulation followed the diffusion controlled higuchi model and zero order release kinetics. The formulations were most stable at room temperature.

10. Mutalik et al[26] prepared glipizide matrix transdermal system using the combination of ethylcellouse/polyvinylpyrrolidone and eudragit RL100/eudragitRS100. Systems were evaluated for various in vitro and in vivo parameters. They concluded that matrix transdermal patches of glipizide exhibited better in vivo performance than oral glipizide administration in mice as well reversing the diabetic complication.

11. Monti et al[27] reported, six terpene- containing essential oil for their capacity to promote permeation of estradiol. Through hairless mouse skin in vitro. Cajuput, cardamom, Melissa, myrtle, niaouli and orange oil, all used at the 10 % w/w concentration in propylene glycol.

12. Gao et al[28] studied the effect of terpenes as penetration enhancer (eg carvon, 1,8 cineole and thymol) on the in vitro percutaneous absorption of the model hydrophilic compound 5- fluorouracil through porcine epidermis. The terpenes (5%w/v) significantly ($p<0.01$) increased the permeability coefficient of 5-fluorouracil in comparison to the control. Concluded that thymol enhanced the permeability absorption of 5- fluorouracile by increasing the stratum corneum lipids. Fluidity and perturbing the barrier integrity of the epidermis. However,

the other two terpenes (eg carvone and 1,8 cineole) enhanced the percutanecus absorption by perturbing the barrier integrity of the epidermis.

13. Guyot et al[29] prepared three transdermal delivery system of propranolol hydrochloride using three polymer i.e. hydroxypropylmethylcellulose, polyisobutylene and ucecryl. The influence of different factor such as polymeric material, matrix thickness, drug content, thickness of adhesive layer and dissolution enhancer was studied. The square root model more closely described the kinetic of drug release from most matrix type. They concluded that best release modulation was obtained with ucecryl TDDS. This device avoided the pronounced burst effect and whatever its formulation, result were increase as compared with other polymeric TDDS.

14. Aqil et al[30] prepared monolithic matrix type transdermal drug delivery system of metoprolol tartrate. Four formulation were developed, which differed in the ratio of matrix forming polymer were composed of eudragit RL100 and polyvinylpyrollidone K-30 with the following ratio 2:8, 4:6, 6:4, 8:2. The in vitro drug release and skin permeation performance formulation 8:2 was found to be better than other three formulations. They concluded that metoprolol tratrate could be administered transdermally through the matrix type TDDS. The drugs remain intact and stable in the transdermal drug delivery system during storage.

15. Arellano et al[31] investigated enhancing effect of naturally occurring terpenes on the in vitro percutaneous absorption of diclofenac sodium from carbopol gels containing propylene glycol. Permeation experiments were performed on

excised abdominal rat skin. They concluded that acyclic alcohol were found to be the best enhancer for diclofenac sodium, being geraniol, with an almost 20 fold increase, the most outstanding penetration enhancer.

16. Arora et al[32] prepared matrix type transdermal patches containing diclofenac diethyl amine using different ratio of polyvinyl pyrollidone and ethylcellulose by solvent evaporation technique. All the prepared formulations were subjected to physical studies (moisture content, moisture uptake and flatness), in vitro release studies and in vitro skin permeation studies. They concluded that diclofenac diethyl amine can be formulated in to transdermal matrix type patches to sustain its release characteristic and the polymeric composition (PVP/EC, 1:2) was found to be the best choice for manufacturing.

17. Fang et al[33] studied to evaluate the in vitro and in vivo transdermal iontophoresis of various diclofenac sodium polymer formulations. The excised rat skin, human cadaver skin as well as cellulose membrane were used to examine the in vitro drug permeation where as the microdialysis technique was used to moniter the drug concentration in vivo. They concluded that synergistic effect is obtained when combining iontophoresis and cardamom oil for transdermal diclofenac delivery.

18. Aqil et al[34] prepared monolithic matrix type transdermal drug delivery system of pinacidile monohydrate. Evaluated in vitro for drug release and skin permeation and in vivo by monitoring the effect of the TIDES on blood pressure of methyl perednisoione acetate induced hypersensitive rate. They

concluded that a single patch application of pinacidile transdermal drug delivery system can effectively control hypertension in rat for 2 days.

19. Ceschel et al[35] prepared topical formulation (microemulsion, hydrogels and microemulsion hydrogels) for application to the buccal mucosa. Optimizing the permeability of the S.desoleana essential oil by mean of an enhancer, the diethylene glycol monoetyl ether transcutol. They concluded that best formulation is the microemulsion-gel with diethylene glycol monoethyl ether at 20 % of concentration, which allow the highest Kp across the buccal mucosa for all of the essential oil components.

20. Cornwell et al[36] investigated the mechanism through which the terpenes, d-limonene, 1-8 cineole and nerolidol, increase the permeability of human stratum corneum and propylene glycol/terpene synergy using differential scanning calorimetry (DSC), small-angle X ray diffraction and enhancer uptake studies. They concluded that OG/terpenes synergy may produce enhanced lipid bilayer disruption. Enhancer uptake studies showed that PG does not significantly increase terpene delivery to the stratum corneum.

21. Moghimi et al[37] investigated the interaction of two terpenes (1,8-cineole and (+) limonene) with the matrix. Terpenes (5-40%w/w) were added to the matrix and their effect studied by hot – stage light microscopy (25-160°c) and differential scanning calorimetry (19-100°c). Results showed that cineol breake the model matrix into a dispersed system in which a lamellar mesomorphic structure is in equilibrium with an isotropic liquid at 25°c and

30°c, in good correlation with the effect of cineol on the intercellular lipids of the stratum corneum. Increase the concentration of limonene caused initially a decrease in the oily streake, then a lamellar to viscous. Isotropic phase transition and finally, changed matrix to a dispersed system of crystalline and mesomorphic phase in a continuous liquid phase at 25°c and 32°c

22. Mukhergee et al[38] prepared transdermal matrix patches containing the drug diclofenac diethylamine with various polymeric combinations of poly vinyl pyrolidone and ethyl cellulose and to study the mechanisam of release of drug from the patches and the skin permeation.In vitro skin permeation studies with rat skin ,using modified keshary-chien diffusion cell,were carried out to establish the most favorable polymeric combination and impact of span 20 was also studied.

23. Gabiga et al[39] studied the percutaneous absorption of isosorbide dinitrate (ISDN) from a transdermal therapeutic system with or without penetration enhancers. The concentration of isosorbide and its metabolites was determined in rat plasma during a 48 h application of TTS. Effect of Penetration enhancers such as oleic acid, propylene glycol, polyethylene glycol 400 and isopropyl myristate on percutaneous permeation of ISDN was studied. They concluded the most effective penetration enhancers for ISDN are oleic acid and propylene glycol.

24. Mehdizadeh et al[40] prepared drug in adhesive and reservoir formulation of fentanyl transdermal patch and evaluated with respect to drug release and adhesive properties. The result state that release kinetics obeyed the square

root of time or higuchi modle, indicating the diffusional controlled release mechanism. Amount of fentanyl needed for each 10 cm 2 three day drug in adhesive patch should be 33 mg.

25. Murthy et al[41] prepared matrix type transdermal delivery system of terbutaline sulfate using hydroxypropyl methylcellulose and analyzed the practical application of magnetophoresis for terbutaline sulfate transdermal delivery. They concluded that efficacy of a magnetic field to act as a permeation enhancer was demonstrated. Substitution of chemical enhancer by magnetic field in transdermal delivery system appear to be possible

26. Devi et al[42] develop transdermal free films and patches of ketorolac tromethamine. Polyvinyl pyrrolidone and polyvinyl alcohol comparison of various permeation enhancer and enhancement technique were done. They concluded that volatile oil brought about a dramatic increase in the amount of drug permeation.

27. Amnuaikit et al[43] develop film formulation of propranolol hydrochloride containing enhancers for transdermal use. Prepared by employing ethylcellulose and polyvinyl pyrrolidone as a film former. They concluded that 10% cineole and 10% w/w PG and cineole successfully improved the skin penetration of propranolol hydrochloride.

28. Shin et al have[44] studied the permeability of triprolidine through ethylene-vinyl acetate (EVA) copolymer membrane. The release rate of drug from ethylene-vinyl acetate (EVA) matrix was dependent on the volume fraction of PEG

400, temperature and drug loading. The amount of drug released at any drug loading was linear with square root of time.

29. Parsaee et al[45] have compared the diclofenac lotion and lipogel with conventional topical dosage forms (such as emulgel and lipogel). The results revealed that release rates were superior with lotions and lipogel

30. Mukherjee et al[46] designed a suitable matrix type transdermal delivery of dexamethasone using polymer combination of providone (PVP) and ethyl cellulose (EC) and Eudragit® with PVP, reported that PVP: EC combination provide the slower and more sustained release of dexamethosone than the PVP: Eudragit® formulation during skin permeation study

31. Khatun et al[47] have studied the effect of two plasticizer (PEG-1500, PEG-4000) and two release modifier (PVP, HPMC 15cps) on in vitro drug release from Naproxen loaded Eudragit® RS film and reported that drug release was function of drug load, PEG molecular weight and physicochemical properties of the release modifier incorporated.

32. Sarisuta et al[48] investigated the effect of polymer grade and type of surfactant on the release of clonidine hydrochloride from ethyl cellulose film .The effect of tween 80 (Polysorbate 80) and span 80 (Sorbian monooleate) surfactant on release characteristics of clonidine hydrochloride from ethyl cellulose 10 and 20 Cps matrix film containing castor oil as a plasticizer were investigated .The release rate of drug from these films in water at $37^{o}c$ were found to increase with the addition of surfactant.

33. William et al[49] reported the Penetration enhancers improve the transdermal drug delivery by many modes of action at different potential sites.

34. Aqil M et al[50] prepared monolithic matrix type transdermal drug delivery system of pinacidil monohydrate by film casting techniques on mercury substrate and in vitro drug release studies, skin permeation studies were done. Four formulations were developed which differed in the ratio of matrix forming polymers, eudragite RL 100 and PVP K-30. They concluded that formulation B_4 was found to be better than other three formulations and it was selected as optimized formulation.

35. Krishna et al[51] prepared three transdermal formulations containing propranolol hydrochloride in hydrophilic polymer without a rate controlling membrane(H1), with 20 micron thick ethylene vinyl acetate (H2), with 65 micron thick ethylene vinyl acetate (H3).Patches were evaluated for in vitro drug release .they concluded that zero order profile was seen with patch H2 and H3 and matrix diffusion profile was observed with H1 patch.

36. Baby et al[52] investigated the effect of surfactant which is often used to enhance the physical stability of many topical pharmaceutical dosage form and cosmetic product. DSC evaluated study the intraction of surfactants on the stratum corneum of shed snake skins as model membrane.

37. Nokhodchi et al[53] studied the effect of surfactant on the penetration of lorazepam through rat skin. The percutaneous permeation of lorazepam was

investigated in rat skin after application of water: propylene glycol (50:50%v/v). The enhancing effect of various surfactants (sodium lauryl sulfate cetyltrimethyl ammonium bromide, bezalkonium chloride or tween 80) with different concentration on permeation of lorazepam was evaluated using Franz diffusion cells fitted with rat skins. The permeation profile of lorazepam in presence of cationic surfactant, CTAB reveals that an increase in concentration of CTAB result in an increase the flux of lorazepam in comparison with control. But an increase in concentration of CTAB or bezalkonium chloride from 0.5 to 1%w/w or from 1to 2.5%w/w resulted in a reduction in ER respectively.

38. Castellano et al[54] proved that span 20 has same enhancer effect as azone on in vitro percutaneous penetration of lipophillic compound. The purpose of this work is to study the interaction of span 20 with stratum corneum lipids monolayers and to compare them with azone. To quantify and compare the effect of span 20 and azone the compressibility of enhancer lipid model mixed monolayer was calculated and expressed as a function of mole fraction of enhancer present on the film.

39. Shokri et al[55] studied the effect of surfactant on the skin penetration of diazepam. The effect of various surfactant (sodium lauryl sulfate, cetyl trimethyl ammonium bromide, benzalkonium chloride or tween 80) with different concentration on skin permeability was evaluated. The in vitro permeation experiment with rat skin revealed that the surfactant enhancer varied in their ability to enhance the flux of diazepam. Benzalkonium chloride

which possessed the highest lipophillicity(log p =1.9)among cationic surfactant.

40. Dehghan et al[56] investigation was carried out with a view to formulate and evaluate polymeric matrices and membrane systems for their potential use as transdermal drug delivery devices. Polymer such as Ethylcellulose, Polyethylene glycol 6000, polyvinyl pyrrolidon, Hydroxypropyl methylcellulose, Eudragit RLPM and Eudragit RSPM were employed. Plasticizer such as Glycerol and Dibutylphthalate were incorporated. Invitro drug release were investigated for different polymeric systems, the individual data were then analyzed for release characteristics i.e. zero order, first order, square root time relationship. The rate of release of ephedrine hydrochloride from HPMC matrix system was found to be faster than from HPMC+EC: PEG6000 membrane system than from HPMC+EC: PVP membrane system. The release from HPMC matrix system fallowed a pattern close to square root of time type release, for HPMC+EC: PEG6000 and HPMC+EC: PVP fallowed a pattern close to zero order type release.

41. Devi et al[57] prepared transdermal patches of verapamil hydrochloride were prepared using four different polymers. Eudragit RL 100(ER100), Eudragit RS100 (ERS100), Hydroxypropyl methylcellulose 15Cps (HPMC) and Ethyl cellulose (EC) of varying degrees of hydrophilicity. In vitro release studies showed zero-order release of drug from all the patches and mechanism of release was diffusion mediated. Further release and permeation of drug from most satisfactory formulation was evaluated through different biological

barrier (shed snake skin, rabbit skin and rat skin) to get an idea of the drug permeation through human skin. The pharmacokinetic parameter calculated from blood levels of drug revealed a profile typical of sustained release formulation.

42. Godwin et al[58] investigated the influence of drug lipophilicity on terpenes as transdermal penetration enhancers. Percutaneous absorption enhancing effects on the skin of hairless mice of 1 monoterpenes were investigated using three different model drugs (caffeine, hydrocortisone, triamcinolone, acetonide (TA)) with varying lipophilicities. The combination of terpenes in PG provided significant enhancement of the permeation of caffeine through mouse skin. Overall, the results indicate that the combination of terpenes with PG can significantly increase the transdermal penetration of the hydrophilic drug caffeine and the polar steroid hydrocortisone.

43. Yamne et al[59] studied the effect of propylene glycol/water co-solvent systems and terpene penetration enhancers (1,8-cineole, menthone.(+)-limonene and nerolidol) on the absorption rate of the model hydrophilic permeant, 5-flurouracil were investigated using excised human skin. Co-application of each terpene with the drug, but that saturation, in propylene glycol co-solvent systems increased drug flux significantly. Terpenes activity depended on the propylene glycol content in the vehicles. From the DSC result indicated that these terpenes are probably able to disrupt stratum corneum lipids.

44. Krishnaiah et al[60] indicated that hydroxypropyl methyl cellulose (HPMC) gel drug reservoir system prepared with 70:30 v/v ethanol-water solvent system containing 6% w/w of limonene was effective in promoting the in vitro transdermal delivery of nicorandil. The in vitro permeation of nicorandil from a limonene-based HPMC gel drug reservoir was studied across excised rat skin (control). EVA 2825 membrane, adhesive-coated EVA 2825 membrane and adhesive coated EVA 2825 membrane-excised rat skin composite to account for their effect on the desired flux of nicorandil.

45. Ghosh et al[61] studied the comparison of skin permeability of drugs in mice an human cadaver skin. The permeation rate in mice skin is higher than that in the cadaver skin and the meeting of the target flux in mice skin does not guarantee its good permeability in human skin.

46. Bendas et al[62] studied the in vitro penetration of topical glucocorticoids (GC) betamethason 17-valerate (BMV), hydrocortisone 17-butyrate (HCB) and hydrocortisone (HC) into an artificial lipid acceptor and excised human skin using binary hydrogels with varying propylene glycol content. PG penetrates rapidly into the artificial acceptor and into excised human skin. It acts as both cosolvent and enhancer. In case of hydrocortisone the enhancer effect is to be a solvent drag effect of PG.

47. Heard et al[63] studied the simultaneous skin permeation of drug and penetration enhancers. Formulation of mefanamic acid in PEG 4000 incorporating various proportion of ethanol or 1,8-cineol were prepared and applied to porcine ear

skin for infinite conditions. Receptor phase assayed by HPLC and 1,8-cineole or ethanol by GC. Concentration dependent permeation profiles were obtained for both ethanol or 1,8-cineole in addition to concentration dependent enhancement of mefanamic acid. The close connection between rates of excipient and solute permeation is generally referred to as the ‘pull’ (or ‘drag’) effect.

48. Sridevi et al[64] developed acrylate based transdermal drug delivery system (TDDS) for glibenclamide and evaluated it for its pharmacodynamic performance in male wistar rats. In methods of preparation using polymeric matrix of polymethylmethacrylate and ethyl cellulose was evaluated for hypoglycemic activity and shown that TDDS significantly sustained the hypoglycemic activity for 24 hrs, in normal rats when compared to oral administration where the effect declined after 8 hrs.

49. Kulkarni et al[65] developed various polymeric membrane systems and polyvinyl pyrrolidone, ethyl cellulose, eudragit RS 100 and ethylene vinyl acetate, containing verapamil hydrochloride with glycerol and dibutyl phthalate as plasticizer. It indicated that the order of permeation of the drug from different polymeric membranes was polyvinyl pyrrolidone > ethyl cellulose > eudragit RS 100 > ethylene vinyl acetate. The drug release mechanism followed nearly zero order kinetics.

50. Gattani et al[66] developed monolithic transdermal films of ondansetron hydrochloride using different hydrophilic and lipophilic polymer combinations

such as polyvinyl alcohol, polyvinyl pyrrolidone, eudragit RLPM: RSPM and combination of hydrophilic-lipophilic polymers. Ethyl cellulose: polyvinyl pyrrolidone. In vitro drug release study through human cadaver skin indicates that hydrophilic polymer showed higher release than lipophilic and hydrophilic-lipophilic combination.

51. Sanker et al[67] prepared drug free polymeric films of ethyl cellulose (EC) as rate controlling membrane. The permeability characteristics of free films were studied using nifedipine as model drug. The in vitro permeation of the drug through the rat skin was slightly lesser than artificial membrane. So by using volatile oil treatment was employed. The permeation brought about by volatile oil treatment was in following order, no treatment < lemon oil < clove oil < eucalyptus oil < wintergreen oil.
52. Bhattacharya et al[68] prepared transdermal patches of combination of ethyl cellulose/poly vinyl pyrrolidone and eudragit RS 100/RL 100 and their drug release kinetics and skin permeation profiles were evaluated. Hydrophobic biocompatible substance viz isopropyl myristate, isopropyl palmitate and linoleic acid and also combination of isopropyl myristate and linoleic acid were used as permeation enhancers in the film. EC: PVP films showed initial higher release compared to RS100: RL100 films, which are due to initial hydration and swelling of the film.

53. Charoo et al[69] investigated the enhancing effects of lemon oil on the transdermal penetration of flurbiprofen through rat skin. The maximum flux achieved by isopropyl alcohol (IPA): propylene glycol (PG) (70:30 % v/v)

solvent mixture was further increased by lemon oil. Lemon oil produced more pronounced change in stratum corneum and the epidermis as compared with the control groups.

54. Hosny et al[70] investigated the effect of changing the eudragit RL 100 ratio and the influence of different penetration enhancers in various concentration on the release of prazosin from carboset 525: Eudragit RL 100 polymeric film using Franz diffusion cells. The addition of various enhancers n-decyl alcohol, azone, and cineole significantly enhanced the prazosin release from these polymeric film.

55. Hendradi et al[71] investigated the effect of mixed micelle formulations including terpenes on the transdermal delivery of diclofenac. The inhibitory action of diclofenac formulated in mixed micelles of lecithin with cholate or deoxycholate was observed on the rat hind paw edema induced by carrageenan. The hind paw edema was quickly inhibited when cyclic monoterpened such as d-limonene or l-menthol was included in formulations.

56. Farinha et al[72] studied the release of naproxen through synthetic membranes mounted in modified Franz-type diffusion cells, saturated solutions or commercially available topical formulations containing 10% naproxen. The release characteristics of the two gel formulations containing naproxen at the same concentration 10% w/w through different membranes have allowed the identification of a set of experimental conditions which characterize the release process of the drug from the product.

57. Krishnaiah et al[73] investigated the effect of carvone on the permeation of nicardipine hydrochloride across the excised rat abdominal epidermis from 2% w/w hydroxy propyl cellulose (HPC) gel system. Fourier transform infrared data indicated that carvone increased the permeability of nicardipine hydrochloride across the rat epidermis by partial extraction of lipids in the stratum corneum. The results shows carvone may be useful for enhancing the skin permeability of nicardipine.

4. METHODOLOGY

Instrumentation

Sl. No	Instruments / Equipments	Model and Manufacturer/ supplier
1.	Digital balance	Shimadzu AX-200 corporation, Japan.
2.	Electronic balance	Oriental
3.	UV-Visible spectrophotometer	UV-1700 Shimadzu corporation, Japan
4.	Diffusion cell	Modified- Keishery chein cell
5.	Distillation apparatus	Qualiagens fine chemicals, Bangalore, India
6.	Rotary water bath shaker	Remi equipment, Bangalore
7.	Magnetic stirrer	Remi equipment, Bangalore
8.	FTIR	8400S Shimadzu corporation, Japan
9.	Digital pH meter 7007	Digisun electronics, Hyd
10.	Desiccator	Tarson, Culcutta
11.	Micrometer	Mitoyoto, Japan
12.	Glass ware	Borosil A-grade
13	Stability chamber	Lab care Mumbai

Materials

Sl. No	Excipients	Source
1.	Furosemide	Gift sample, Hemdeep org Pvt Ltd Ankleshwar.
2.	Ethylcelloluse	Colorcon Asia Pvt Ltd
3.	Polyvinyl pyrrolidone K-30	NR Chem.Pvt limited, Mumbai
4.	Menthol oil	Rajesh Chemical. Co. Mumbai
5.	Lemon grass oil	Rajesh Chemical. Co. Mumbai
6.	Clove oil	Rajesh Chemical. Co. Mumbai
7.	Eucalyptus oil	Rajesh Chemical. Co. Mumbai
8.	Span 20	NR Chem.Pvt limited, Mumbai
9.	Tween 80	S.D. Fine Chemicals Pvt limited, Mumbai
10.	Tween 20	S.D. Fine Chemicals Pvt limited, Mumbai
11.	Propylene glycol	S.D. Fine Chemicals Pvt limited, Mumbai
12.	Potassium di hydrogen ortho phosphate	S.D. Fine Chemicals Pvt limited, Mumbai
13.	Chloroform	S.D. Fine Chemicals Pvt limited, Mumbai
14	Acetone	S.D. Fine Chemicals Pvt limited, Mumbai
15	Sodium hydroxide	S.D. Fine Chemicals Pvt limited, Mumbai
16	Cellophane paper	Sigma Chemical Co.USA.

ESTIMATION OF FUROSEMIDE:

Determination of UV absorption maxima for furosemide.

Solution of furosemide was prepared in phosphate buffer pH 8.0 and was scanned for absorbance between 200-400 nm using double beam UV/VIS spectrophotometer (Shimadzu, UV-1700, Japan). Furosemide exhibited maximum UV absorption at 277.5nm. The output from the equipment is shown in figure.1

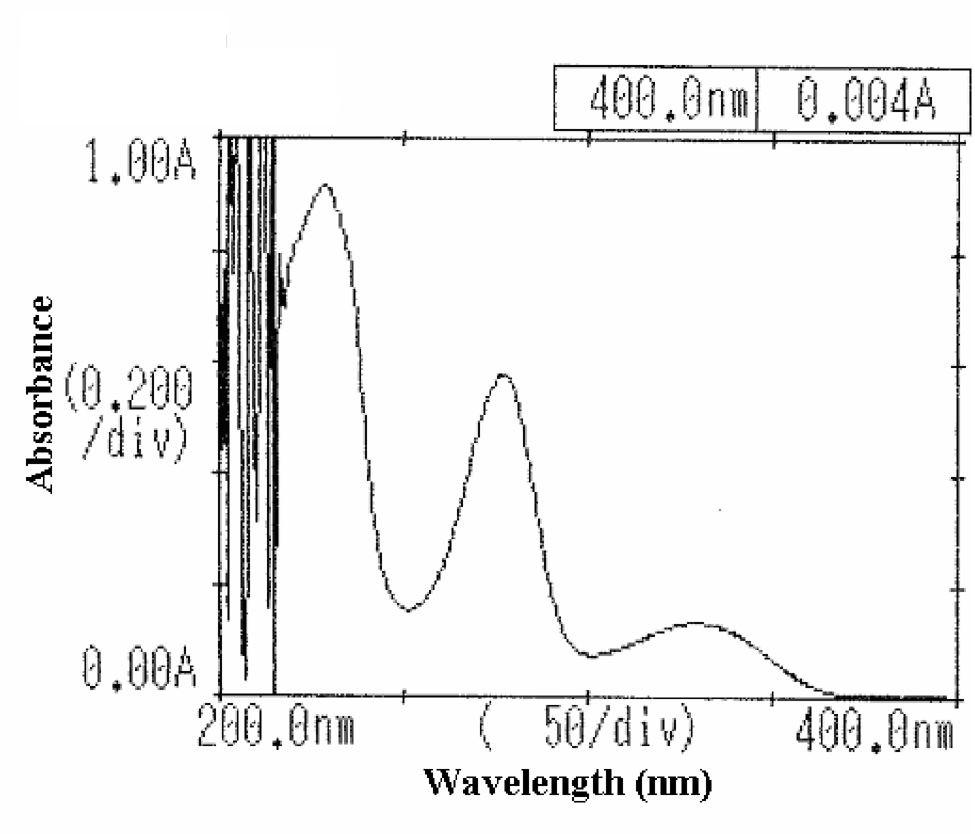


Figure 1: UV absorption maxima of furosemide.

Preparation of calibration curve in phosphate buffer pH 8.0

Preparation of standard solution.

20mg of furosemide was dissolved in 100ml of phosphate buffer pH 8.0, 10ml of solution was withdrawn and volume was made up to 100ml with buffer. From the stock solution, aliquot of 1,2,3,4,5,6 and 7 ml was pipetted out in 10ml volumetric flasks. The volume was made up to the mark with phosphate buffer pH 8.0. Absorbance of each solution was measured at 277.5 nm using UV/VIS spectrophotometer against phosphate buffer pH 8.0 as blank. The assay was performed in triplicate and results of experiment are shown in table 1. The standard calibration curve yield straight line with $R^2 = 0.9999$, indicating that the drug obeys Beer's law in the concentration range of 2-14 mcg/ml.

Table 1: Calibration curve data for furosemide in phosphate buffer pH 8.0

Sl.No	Concentration (mcg/ml)	Absorbance Mean (± SD), n=3
1	0	0
2	2	0.138±0.002
3	4	0.278±0.002
4	6	0.417±0.001
5	8	0.548±0.003
6	10	0.679±0.002
7	12	0.816±0.001
8	14	0.948±0.001

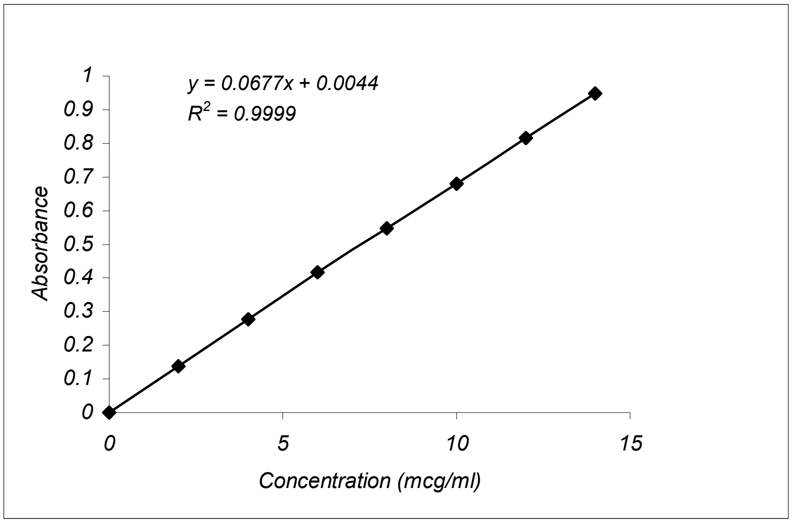


Figure 2: Calibration curve for furosemide in phosphate buffer pH 8.0

Preparation of TDDS

Matrix type transdermal patches containing furosemide were prepared using ethyl cellulose (table 2) by solvent evaporation technique in petri dish. The polymer was weighed and dissolved in acetone–chloroform (3:2) solvent system, then add PVP. Di-n-buthylphthalate (10 %w/w of polymer) was used as a plasticizer. Drug and enhancer were added to the polymer solution. The resultant homogeneous solution was poured into a petri dish. Controlled solvent evaporation was achieved by inverting funnel over the petri dish for 24 hr. The dry film were wrapped in aluminium foil and kept in desiccator until used. The composition of different patches is given in table 2.

Table 2. Composition of transdermal patches.

Formulation	Ethyl cellulose (mg)	PVP (mg)	Enhancer (%w/w of polymer)	Drug (mg/cm^2)
F1	800	---	---	3.70
FP2	800	50	---	3.70
FP3	800	50	Clove oil 10% w/w	3.70
FP4	800	50	Lemon grass oil 10% w/w	3.70
FP5	800	50	Menthol oil 10 % w/w	3.70
FP6	800	50	Eucalyptus oil 10% w/w	3.70
FP7	800	50	Clove oil 10% w/w + Propylene glycol 10%w/w	3.70
FP8	800	50	Lemon grass oil 10%w/w + Propylene glycol 10%w/w	3.70
FP9	800	50	Menthol oil 10 %w/w + Propylene glycol 10%w/w	3.70
FP10	800	50	Eucalyptus oil 10% w/w + Propylene glycol 10%w/w	3.70
FP11	800	50	Span 20 5% w/w	3.70
FP12	800	50	Tween 80 5% w/w	3.70
FP13	800	50	Tween 20 5% w/w	3.70
FP14	800	50	Span 20 10% w/w	3.70
FP15	800	50	Tween 80 10% w/w	3.70
FP16	800	50	Tween 20 10% w/w	3.70

Partition coefficient of drug:

The partition coefficient study was performed using n-octanol as oil phase and phosphate buffer pH as aqueous phases. The two phases were mixed (1:1) and were saturated with each other on a mechanical water bath shaker at 32˚C for 24 h. The saturated phase was separated by centrifugation at 2000 rpm. Equal volume of (10 ml each) of the two phases were taken in conical flask and to each, 100 mg of drug was added. Flask was shaken at 32˚C for 6 h to achieve a complete partitioning. The two phases were separated and they were then analyzed for respective drug content.[32]

The partition coefficient of drug Ko/w was calculated using the following formula

$$K_{o/w} = \text{concentration in octanol} / \text{concentration in pH } 8.0$$

Solubility study:

The solubility studies were performed in phosphate buffer solution 8.0 by adding excess amount of drug in each case and keeping on a water bath shaker for 24 hr at 32˚C. After 24 hr, solution was analyzed spectrophotometerically at 277.5nm.

Physicochemical properties of the film:

The films were evaluated for the following

Thickness:

The thickness of patch was determined using a micrometer (Mitoyoto, Japan). Film was measured at three different places of each patch and mean value was calculated.

Determination of drug content in film:

The uniformity of drug distribution was determined by taking known weight of the films at different places of the patches. The patches were dissolved in 2 ml of methanol and subsequently diluted with phosphate buffer pH 8.0. After appropriate dilution, solutions were analyzed spectrophotometerically for furosemide at 277.5nm.

Moisture content:

The prepared films were weighed individually and kept in a desiccator containing activated silica [32] at room temperature (30˚C) till a constant weight was attained. The percentage of moisture content was calculated as a difference between initial and final weight with respect to final weight.

Moisture uptake:

A weighed film kept in a desiccator at room temperature (30˚C) for 24 h was taken out and exposed to 84% RH in a stability chamber (Lab-Care, Mumbai) until a constant weight of film was obtained. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight [32]

Folding endurance:

This was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking/craking gave the value of folding endurance.[57]

In vitro permeation study:

Preparation of cadaver skin:

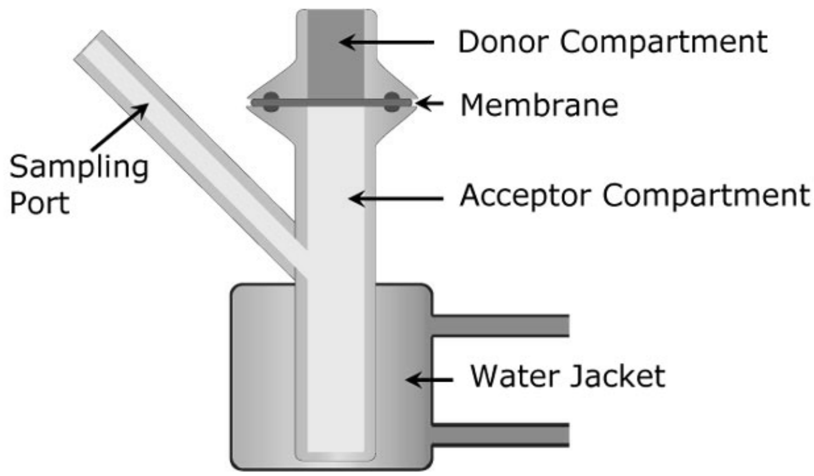
Sample of whole adult human skin (40 age) were obtained from breast reduction operation (provided by Navodaya Medical College, Raichur). Subcutaneous fat was carefully trimmed and then rinsed with normal saline. Skin was sealed in aluminium foil and plastic bag and stored at –20 ºC until used.[33]

Cellophane membrane:

The artificial membrane was presoaked in phosphate buffer pH 8.0 for 45 min before experiment[33].

Procedure:

Permeation studies were performed for different formulations across cadaver skin, artificial membrane using phosphate buffer pH 8.0 as in vitro fluid in receptor compartment of modified diffusion cell at 32˚C. This whole assembly was kept on magnetic stirrer and the solution was stirred continuously using a magnetic bead. The sample was withdrawn at different time interval and replaced with equal volume of diffusion media. Samples were analyzed in UV spectrophotometer at 277.5 nm.



FTIR:

The IR spectra were recorded for furosemide, drug loaded polymeric film, and drug loaded polymeric film with PVP[38] using FTIR spectroscopy from KBr pellets. The scanning range was 400-4000/cm.

Stability studies:

The stability studies were conducted according to ICH guidelines by storing the transdermal patches at 40±2˚C / 75% RH in stability chamber (Lab-Care, Mumbai) for six months[50]. The samples were withdrawn after 180 days and analyzed for drug content by a UV spectrophotometer.

Statistical analysis of data:

The results of permeation study, drug content, thickness were analyzed by one-way ANOVA with Turkey post *t*-test using Graph Pad Prism software-5 version (Graph Pad software, San Diego, CA, USA) and ($p < 0.005$) were considered statistically significant.

5. RESULTS AND DISCUSSION

In case of oral administration of furosemide due to transient high blood concentration, it can induce the side effect such as polyurea, dizziness, dry mouth, nausea and gastric disturbances. So considered for transdermal route for administration. Transdermal patch offers numerous advantages such as constant and prolonged plasma drug level, minimization of inter- and intra patient variability, reduced frequency of dosing and easy termination of medication.

In order to maintain the sink condition during permeation study, solubility of furosemide was determined in phosphate buffer pH 8.0. Solubility of drug was found to be 6.12 ± 0.6 mg/ml. To learn the partioning of drug between skin and in vitro study fluid (pH 8.0), the partition study were performed in triplicate and logarithmic value was found to be 1.446 ± 0.07. These result indicate that drug possesses lipophilic character.

Physicochemical parameters:

Thickness of patches:

Table 3 shows thickness of the various patches. Thickness of patches varied (from 0.101 to 0.110 mm). However, incorporation of any of the essential oil and propylene glycol (PG) (10% w/w) did not show any significant ($p>0.05$) change in the thickness of film.

Moisture content studies:

The results of moisture content studies of different formulations are shown in table 3. The moisture content of the menthol oil formulation and menthol oil / PG formulation (FP5 and FP9 respectively) was higher than other formulation containing other essential oils. Moisture content in the formulations was found to be in the following order: menthol oil > lemon grass oil> clove oil > eucalyptus oil.

Moisture uptake studies:

Table 3 shows moisture uptake of various formulations. Incorporation of essential oils (10 %) tended to increase the moisture uptake of patches depending on the type of essential oil in the patch. Moisture uptake (%) of menthol oil patches (FP5 and FP9) were more than other formulations containing other essential oils. Moisture uptake in the formulations was found to be in following order: menthol oil> lemon grass oil> clove oil > eucalyptus oil.

Drug content analysis:

Drug content for all formulations are shown in table 3. The drug content of all the formulations was ≥ 98.47 % with low standard deviations (≤1.4%) indicating that drug was uniformly distributed throughout the patch.

Folding endurance:

Folding endurance of all the formulations are shown in table 3. Folding endurance was found to be higher in formulations FP5 and FP9 than other formulations. Folding endurance in the formulations was found to in following order: menthol oil> lemon grass oil> clove oil > eucalyptus oil.

Table 3. Physicochemical properties of transdermal patches.

Formulation	Drug content (%) ± SD, n=4	Thickness (mm) ± SD, n =6	Moisture uptake capacity (%) ±SD, n=4	Moisture content (%) ± SD, n=4	Folding endurance (No. of folds),n=6
F1	98.83 ± 0.6	0.101 ± 0.005	1.3 ± 0.3	1.1 ± 0.3	2
FP2	98.47 ± 1.2	0.113 ± 0.0050	2.4 ± 0.2	1.9 ± 1.21	4
FP3	99.20 ± 1.3	0.116 ± 0.0058	3.7 ±1.5	2.8 ± 1.1	5
FP4	98.54 ± 0.83	0.110 ± 0.0056	5.5 ± 0.1	3.3 ± 0.2	6
FP5	98.83 ± 1.2	0.116 ± 0.016	7.5 ± 0.2	5.5 ± 0.43	8
FP6	99.12 ± 0.71	0.112 ± 0.002	3.5 ± 1.2	2.6 ± 0.5	4
FP7	98.60 ± 0.8	0.117 ± 0.001	6.8 ± 1.1	4.2 ± 1.2	22
FP8	99.61 ± 1.2	0.118 ± 0.001	8.5 ± 0.51	5.2 ± 0.6	24
FP9	98.94 ± 1.4	0.115 ± 0.004	12.11 ± 0.15	8.5 ± 0.2	27
FP10	98.99 ± 0.75	0.120 ± 0.002	5.6 ± 1.11	3.2 ± 1.13	21

In vitro permeation studies.

Permeation study was carried out using standard cellophane membrane and human cadaver skin as permeation barriers. The permeation profile of the drug across these two barriers are shown in the figure 3-6. Table 4 shows the steady state flux values of various formulations through different permeation barriers. The steady state permeation flux was determined across the linear portion of cumulative amount of permeation (Q) verses time (t) plot. Comparison of flux values of F1 and FP2 indicated significant ($p<0.05$) increase in the permeation of the drug; so addition of PVP shows higher flux compared to formulation without PVP. As PVP acts as antinucleating agent, which retards the crystallization of drug and improve the solubility of the drug in matrix[74] further, to improve the permeation of the drug, different essentials oils were added to the formulations (FP3-FP6). Addition of essential oils increased ($p< 0.05$) the permeation of drug and the flux was higher with clove oil (FP3). For lipophillic drugs terpenes increases in partioning are likely due to bulk solvent effect.[36, 75]

PG in combination with penetration enhancers, found to be highly effective. In our study formulations (FP7- FP10) showed an increase in flux with addition of PG.

Table 4. In vitro flux of furosemide through various membrane barriers.

Formulation	Cellophane membrane (mcg /cm^2/hr) ±SD, n=3	Cadaver skin (mcg cm^2/hr) ± SD, n=3
F1	24.007 ± 3.92	18.75 ± 2.94
FP2	52.196 ± 7.85۞	40.76 ± 6.93۞
FP3	206.4 ± 4.08*	184.24 ± 4.83*
FP4	182.11 ± 3.30*	154.64 ± 8.11*
FP5	146.26 ± 1.68*	116.95 ± 2.63*
FP6	103.14 ± 4.89*	66.45 ± 3.53*
FP7	240.38 ±11,34•	218.54 ± 10.28•
FP8	214.39 ±19.87•	180.67 ± 12.20•
FP9	168.24 ± 25.49•	155.77 ± 7.89•
FP10	116.43 ± 11.05•	100.28 ± 9.02•

۞($p<0.05$) when compared with F1
***** ($p<0.05$) when compared with FP2
• ($p<0.05$) when compared with FP2

In vitro permeation profiles through human cadaver skin as permeation barrier are shown in figures 5 and 6. Comparison of flux values of F1 and FP2 indicated significant ($p<0.05$) increase in permeation of the drug with PVP. Similarly, flux value increases significantly ($p<0.05$) with addition of essential oils. Propylene glycol (PG) /terpenes synergy may produce enhanced lipid bilayer disruption.[36] Terpenes have been given the designation of generally recognized as safe (GRAS) by the FDA.[76] The mechanism by which terpene increase stratum corneum permeability by disrupting intercellular lipid bilayers.[77, 78] Terpenes, neat or in combination with propylene glycol (PG) or ethanol have been extensively investigated as skin permeation enhancers for harmone and other drugs.[79 - 81] However in the present study clove oil (eugenol), lemongrass oil (citral) menthol oil (menthol) and eucalyptus oil (cineole) were effective in enhancing the skin permeation alone and in combination with propylene glycol. Among these formulations FP3 and FP7 were found to show higher flux values than other. The result also indicated that flux values are always more in cellophane membrane than human cadaver skin.

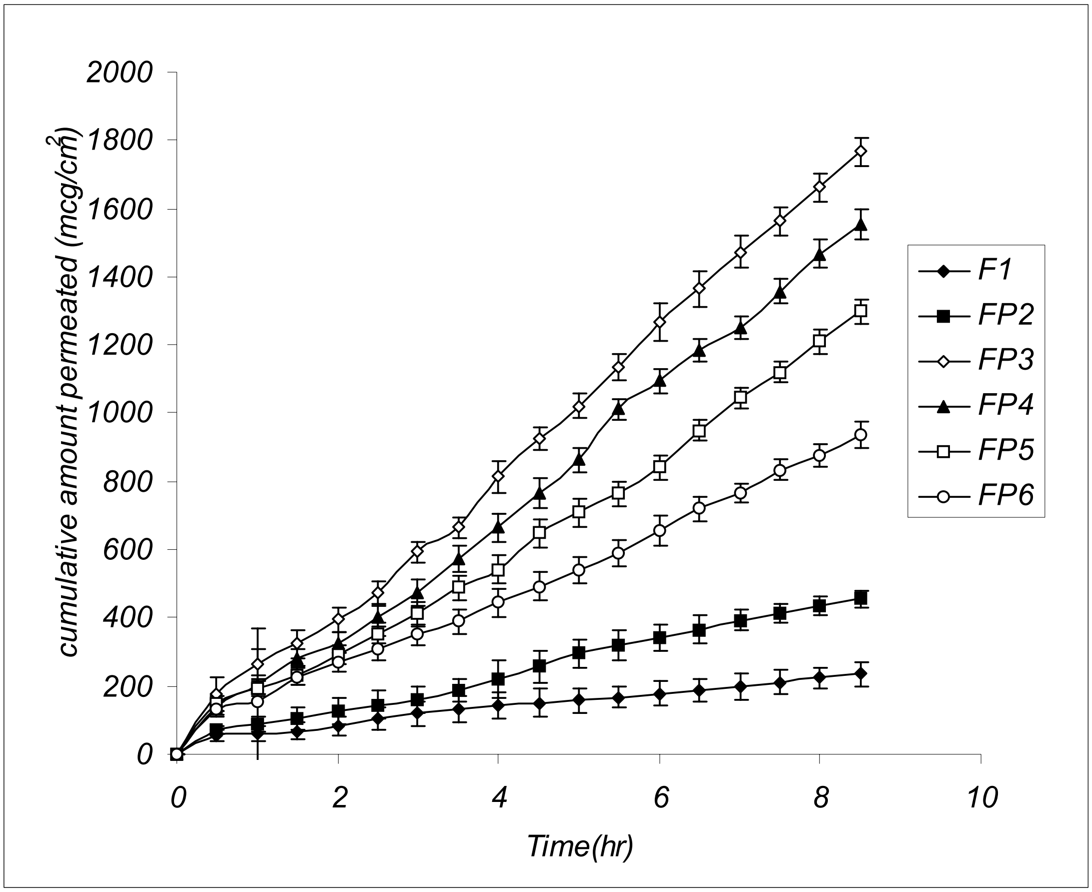


Figure 3. Effect of essential oils on the permeation of furosemide through cellophane membrane.

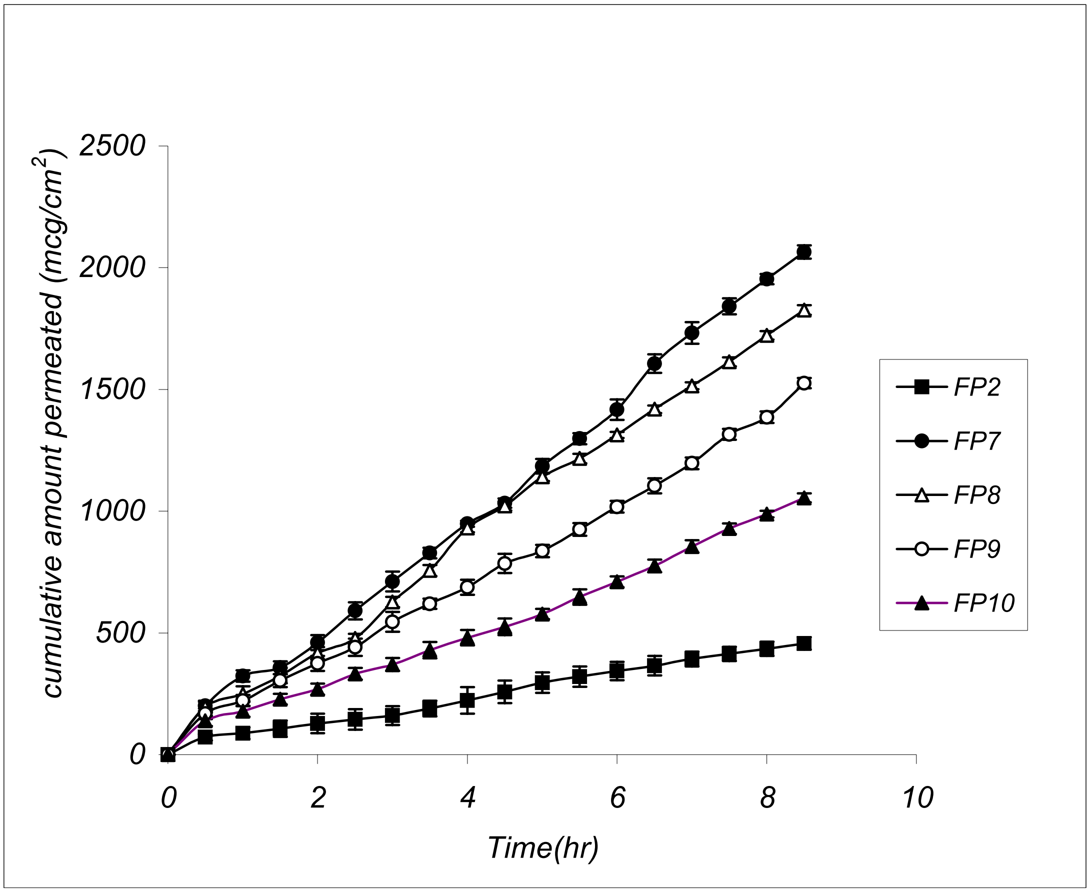


Figure 4. Effect of combination of essential oils with propylene glycol on the permeation of furosemide through cellophane membrane.

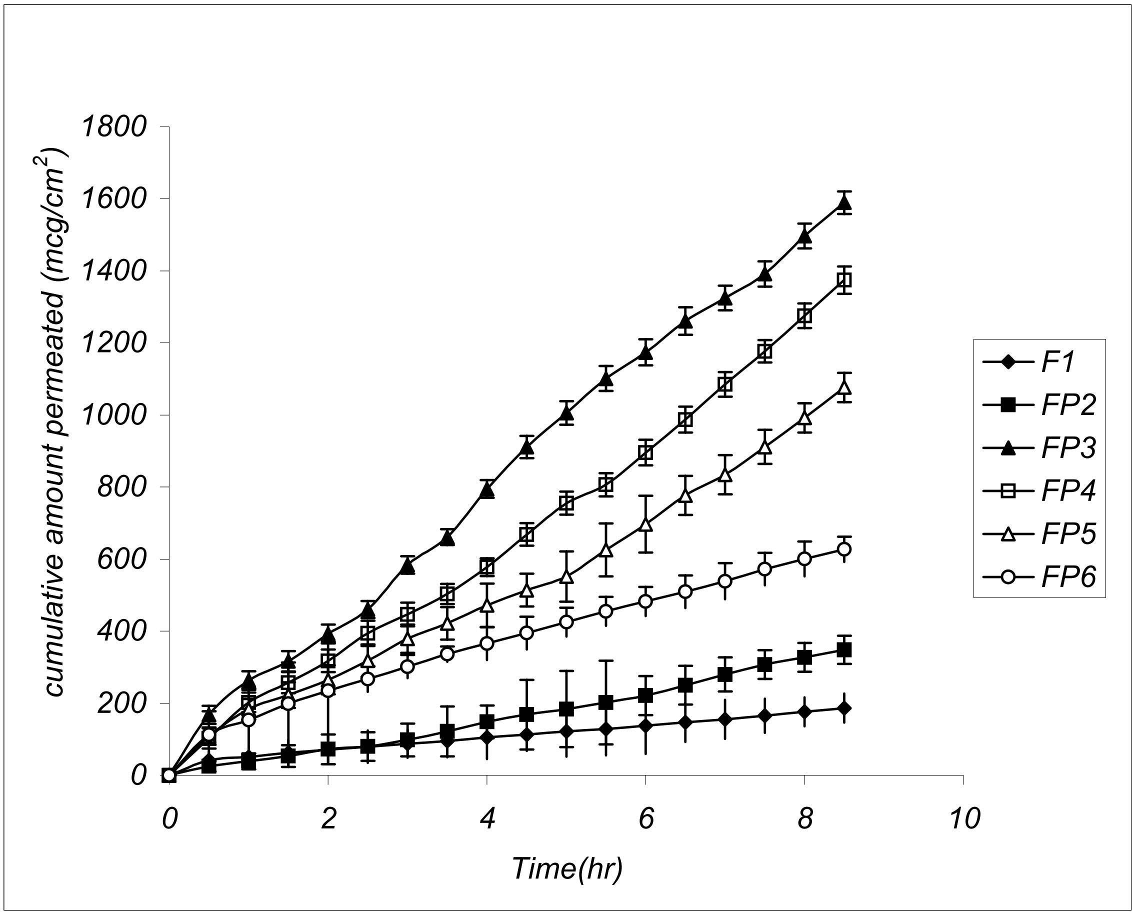


Figure 5. Effect of essential oils on the permeation of furosemide through human cadaver skin.

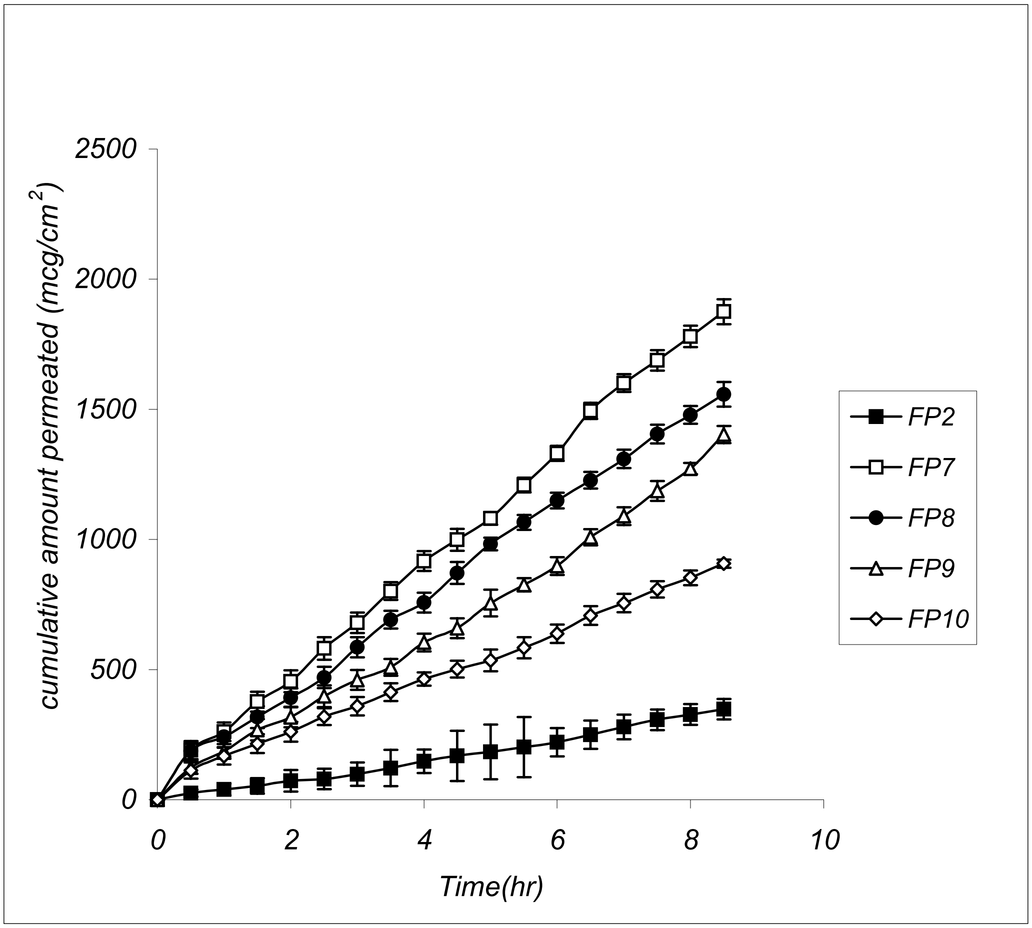


Figure 6. Effect of combination of essential oils with propylene glycol on the permeation of furosemide through human cadaver skin.

Drug-excipient interaction studies:

Drug-excipient interactions were studied by FTIR technique. Figure 7 shows the IR spectra of furosemide, drug and polymer, drug, polymer and PVP. IR spectra of drug (A) shows characteristic band at 3351-3397 1/cm (NH_2), 3283.58 1/cm (-NH), 3050 1/cm (aromatic C-H stretching), 2980 1/cm (O-H of COOH group), 2800 1/cm (-CH streching of-CH_2), 1671.20 1/cm (-CO of -COOH), 1582-1451 1/cm (C=C ring streching) and 581 1/cm (CL). From the spectra of different formulations with that of drug implied that all the excipients are compatible with furosemide. Changes in area of peaks occur simply due to mixing of components without any physical - chemical interactions[38].

Analysis of release data:

For matrix controlled release systems, the Higuchi equation is described as per[40]

$$Q = (2ADCs\ t)^{1/2} \ldots\ldots\ldots\ldots (1)$$

Where Q is the cumulative amount of drug released per unit area of the matrix, A is the total drug concentration in the matrix dissolved and undissolved, D is the diffusion coefficient of the drug in the matrix, Cs is the solubility or saturation concentration of the drug in the matrix, and t is time. Table 5 summarizes correlation coefficient of the data analysis. The high correlation (R^2>0.9627) value indicates that release of furosemide from the patches was in compliance with Higuchi diffusion model. This relationship observed in system in which the drug is either fully dissolved or suspended and thus membrane used has no effect on the release kinetics of the drug.

Table 5. R^2 values of Higuchi model of furosemide patches.

Formulation	Cellophane membrane (R^2)	Human cadaver skin (R^2)
F1	0.9920	0.9991
FP2	0.9822	0.9660
FP3	0.9826	0.9914
FP4	0.9754	0.9639
FP5	0.9677	0.9643
FP6	0.9688	0.9935
FP7	0.9811	0.9829
FP8	0.9912	0.9913
FP9	0.9727	0.9627
FP10	0.9652	0.9807

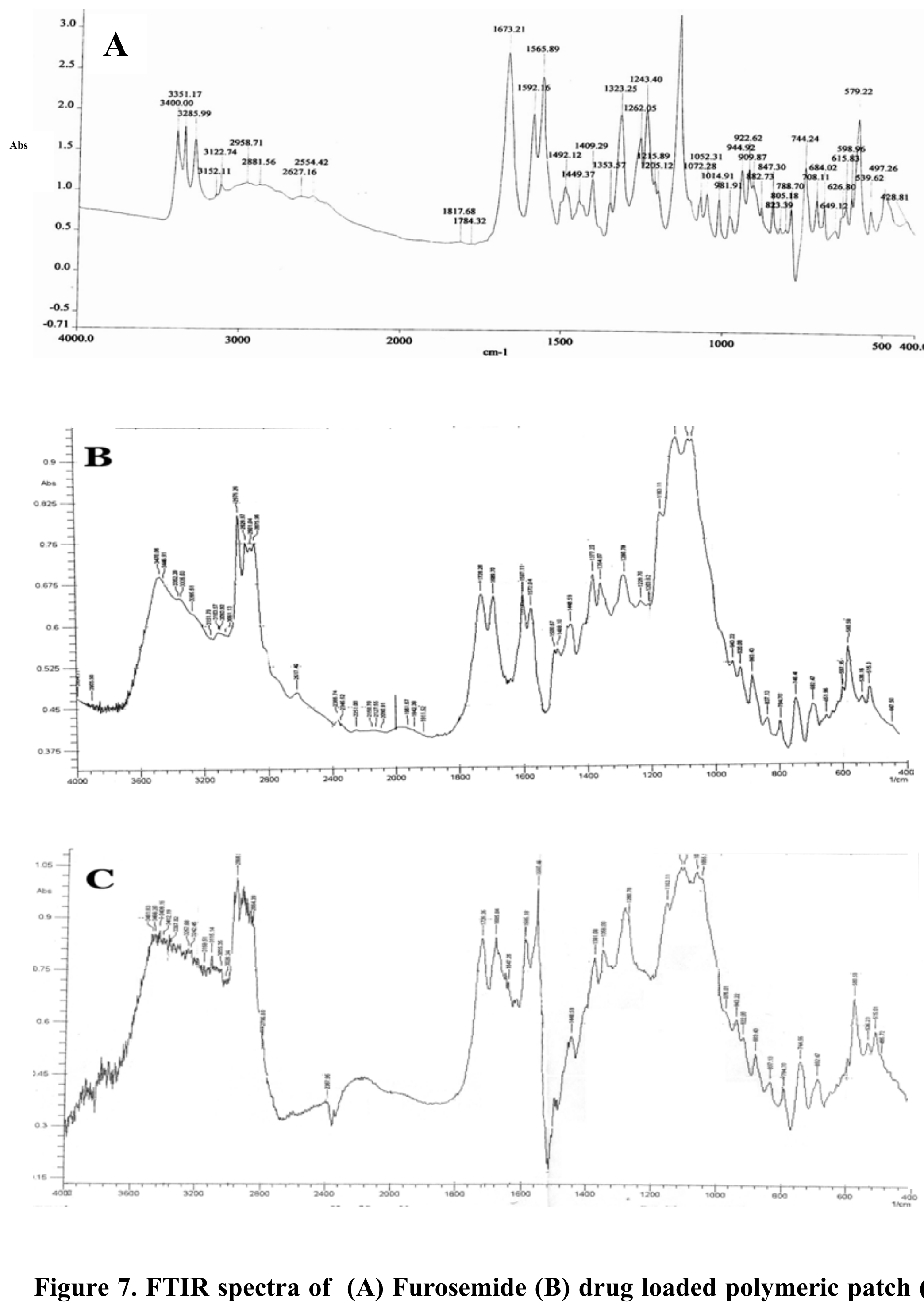


Figure 7. FTIR spectra of (A) Furosemide (B) drug loaded polymeric patch (C) drug loaded polymeric patch with PVP

In the second part of study, surfactant such as Span 20,Tween 80, and Tween 20 have been used to study the effect on the permeation of drug.

Physicochemical parameters:

Thickness of patches:

Table 6 shows thickness of the various patches. Thickness of patches varied between 0.11 to 0.12 mm. However, incorporation of any surfactant (5-10% w/w) did not show any significant ($p>0.05$) change in the thickness of patches.

Moisture content studies:

The results of moisture content studies of different formulations are shown in table 6. Incorporation of surfactant increased the moisture content of the patches and the moisture content in the Tween 80 formulation (FP12 and FP15) was higher than any other formulations. Since Tween 80 is hygroscopic in nature.[15] Moisture content in the formulation was found to be in the following order: Tween 80 > Tween 20 > Span 20.

Moisture uptake studies:

Table 6 shows patch moisture uptake of various formulations. Incorporation of various surfactants (5 -10% w/w) tended to increase the moisture uptake of patches. Moisture uptake (%) of Tween 80 patches (FP12 and FP15) were found to be higher than any other formulation containing other surfactants. Moisture uptake in the formulations was found to be in following order: Tween 80 > Tween 20 > Span 20

Folding endurance studies:

The results of folding endurance of different formulations are shown in table 6. Incorporation of surfactants increased the folding endurance and folding endurance was found to be higher in Tween 80 formulations (FP12 and FP15) than any other formulations. Folding endurance in the formulations was found to be in following order: Tween 80 > Tween 20 > Span 20.

Drug content analysis:

Drug content for all formulations are shown in table 6. The drug content of all the formulations was ≥ 98.16 % and showed very low standard deviations (≤1.52%) indicating that drug was uniformly distributed throughout the patches.

Table 6. Physicochemical properties of transdermal patches.

Formulation	Drug content (%)±SD, n=4	Thickness (mm)±SD, n=6	Moisture uptake capacity (%) ±SD, n=4	Moisture content (%) ±SD, n=4	Folding endurance (No. of folds),n=6
FP2	98.47 ± 1.2	0.113 ± 0.004	2.4 ± 0.2	1.9 ± 1.21	4
FP11	99.15 ± 0.33	0.111 ± 0.002	5.1 ± 1.12	3.2 ± 1.1	22
FP12	98.21 ± 1.51	0.115 ± 0.003	8.7 ± 1.11	6.5 ± 0.66	32
FP13	98.55 ± 0.84	0.116 ± 0.005	6.4 ±1.2	4.2 ± 0.11	24
FP14	99.93 ± 1.52	0. 120 ± 0.002	6.2 ±1.3	4.5 ± 1.2	31
FP15	98.16 ± 0.22	0.122 ± 0.001	9.5 ± 1.23	7.1 ± 0.12	42
FP16	99.85 ± 0.88	0.121 ± 0.002	7.2 ± 0.22	5.4 ± 0.12	35

In vitro permeation studies:

In vitro permeation studies carried out using standard cellophane membrane and human cadaver skin as permeation barriers for above formulations. Table 7 shows steady-state flux values of different formulations. The steady-state permeation flux was determined across the linear portion of cumulative amount of permeation (Q) versus time (t) profiles. Formulation FP11 to FP13 were prepared using Span 20, Tween 80, Tween 20 at (5% w/w.) respectively. The flux value of the formulation FP11 to FP13 increased significantly ($p<0.05$), when the concentration of the surfactants increased (10% w/w). The permeation profile of furosemide through cellophane membrane is shown in the figures 8-9. Flux value increased significantly ($p<0.05$) as the concentration of penetration enhancer increased. In addition, surfactant might have increased the solubility of drug in the film and resulted in increased drug release. Addition of surfactant into the film formulation could result in increased hydrophilicity of the film[48]. Among the surfactants used span 20 gives the highest flux.

Table 7. In vitro flux of furosemide through various membrane barriers.

Formulation	Cellophanemembrane (mcg /cm^2/hr) ±SD, n=3	Human cadaver skin (mcg /cm^2/hr) ±SD, n=3
FP2	52.196 ± 4.75	40.76 ± 2.21
FP11	205.75 ± 3.60*	174.02 ± 4.2*
FP12	183.78 ± 6.76*	154.62 ± 3.91*
FP13	162.4 ± 3.15*	130.43 ± 7.79*
FP14	288.93 ± 5.51$^\#$	233.91 ± 4.69$^\#$
FP15	244.45± 6.72$^\#$	201.58 ± 7.89$^\#$
FP16	216.98 ± 4.12$^\#$	172.54± 8.67$^\#$

* ($p<0.05$) when compared with FP2

$^\#$ ($p<0.05$) when compared with FP2

In the present study human cadaver skin was also used as permeation barrier to study the effect of surfactant as penetration enhancers on permeation of furosemide. Figures 10-11 shows the permeation profiles of furosemide through human cadaver skin. As the concentration of surfactants increased (10%w/w), the flux value also increased significantly ($p<0.05$). There are two possible mechanisms by which the rate of drug transport is enhanced using nonionic surfactants. Initially, the surfactants may penetrate into the stratum corneum, increase fluidity and eventually solubilize and extract lipid components. Secondly, penetration of the surfactant into the intercellular matrix followed by interaction and binding with keratin filaments may results in a disruption within the corneocyte.[82, 83] As compared to the cationic surfactants the non ionic surfactant have little damage to the skin and their effect on transdermal flux is comparatively small This could be a reason for the increase in flux in higher concentration.[84]

Thus in the present study, comparison of flux values (table 7) indicated the efficiency of surfactants in the following order: Span 20 > Tween 80 > Tween 20. The flux value was found to be higher in cellophane membrane as compare to human cadaver skin.

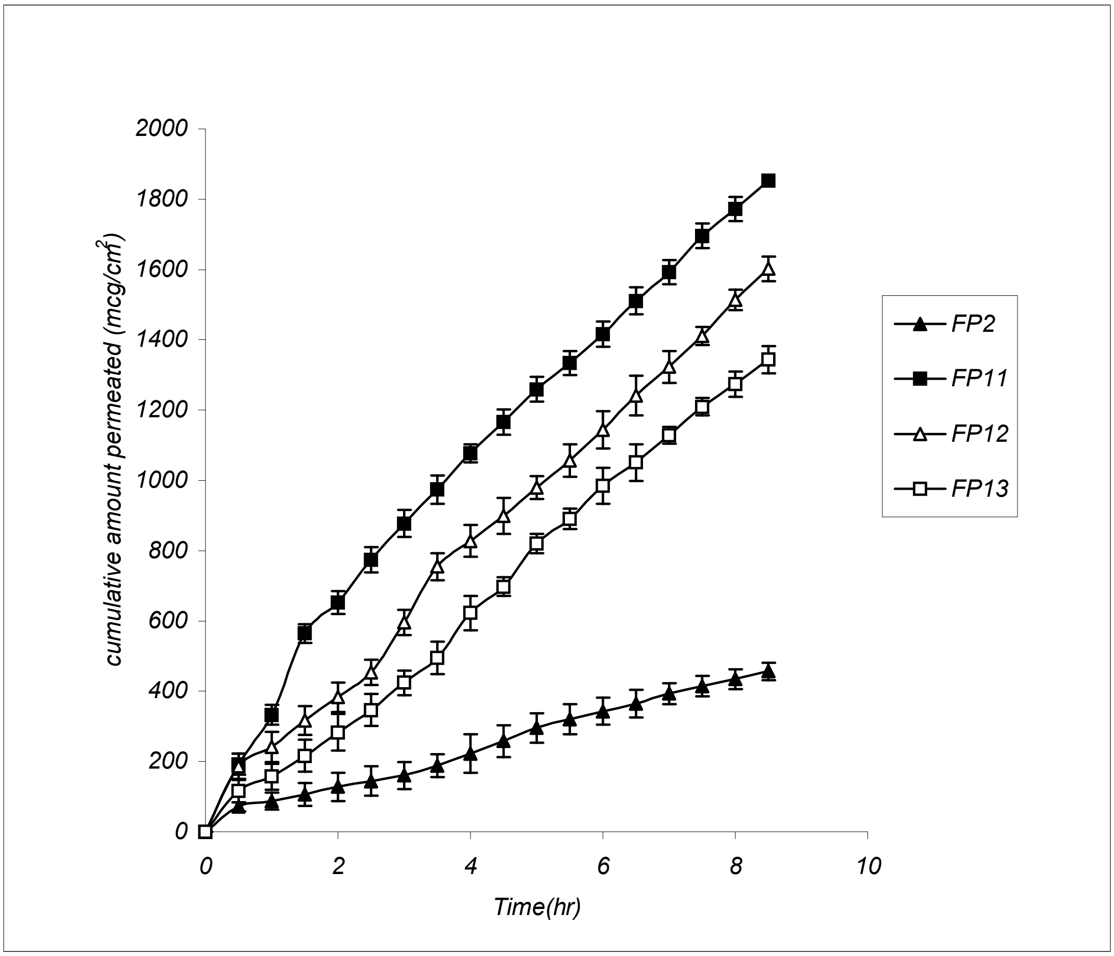


Figure 8. Effect of surfactants (5%) on the permeation of furosemide through cellophane membrane.

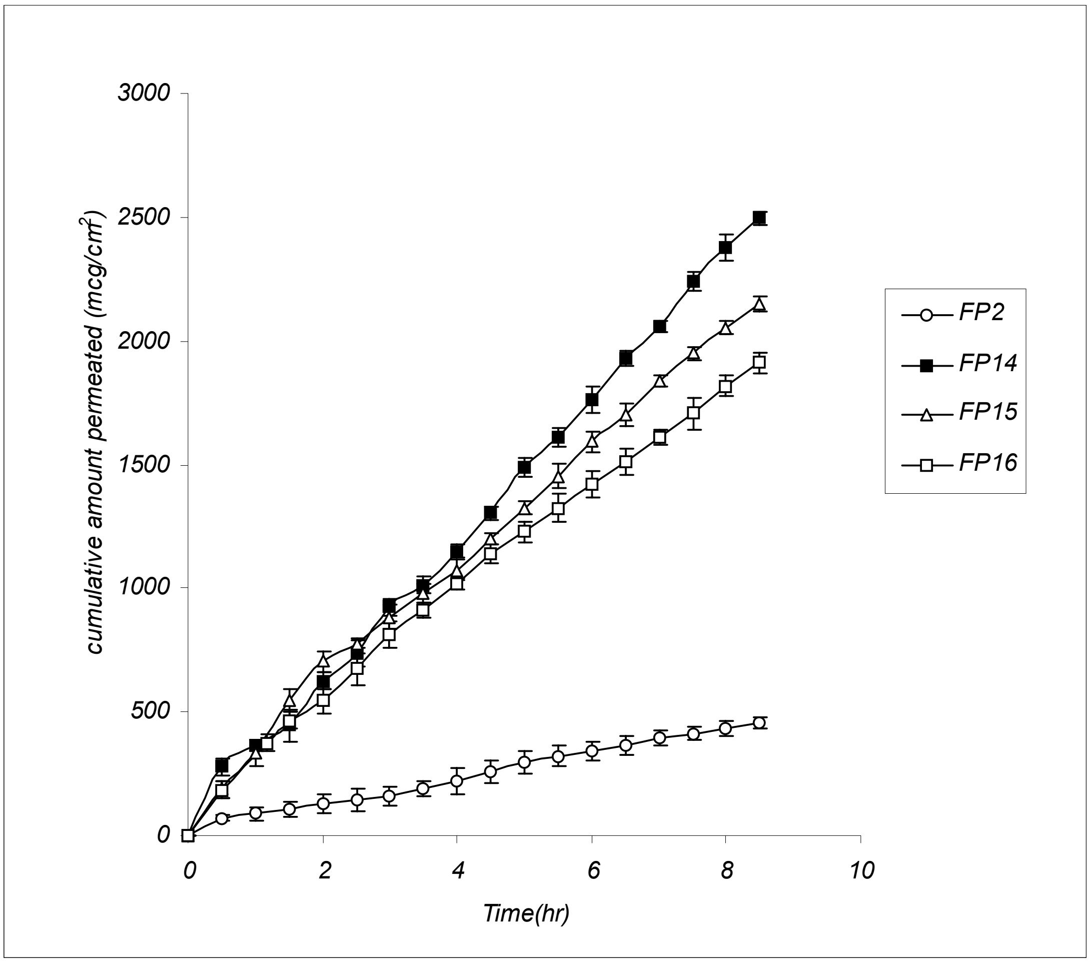


Figure 9. Effect of surfactants (10%) on the permeation of furosemide through cellophane membrane.

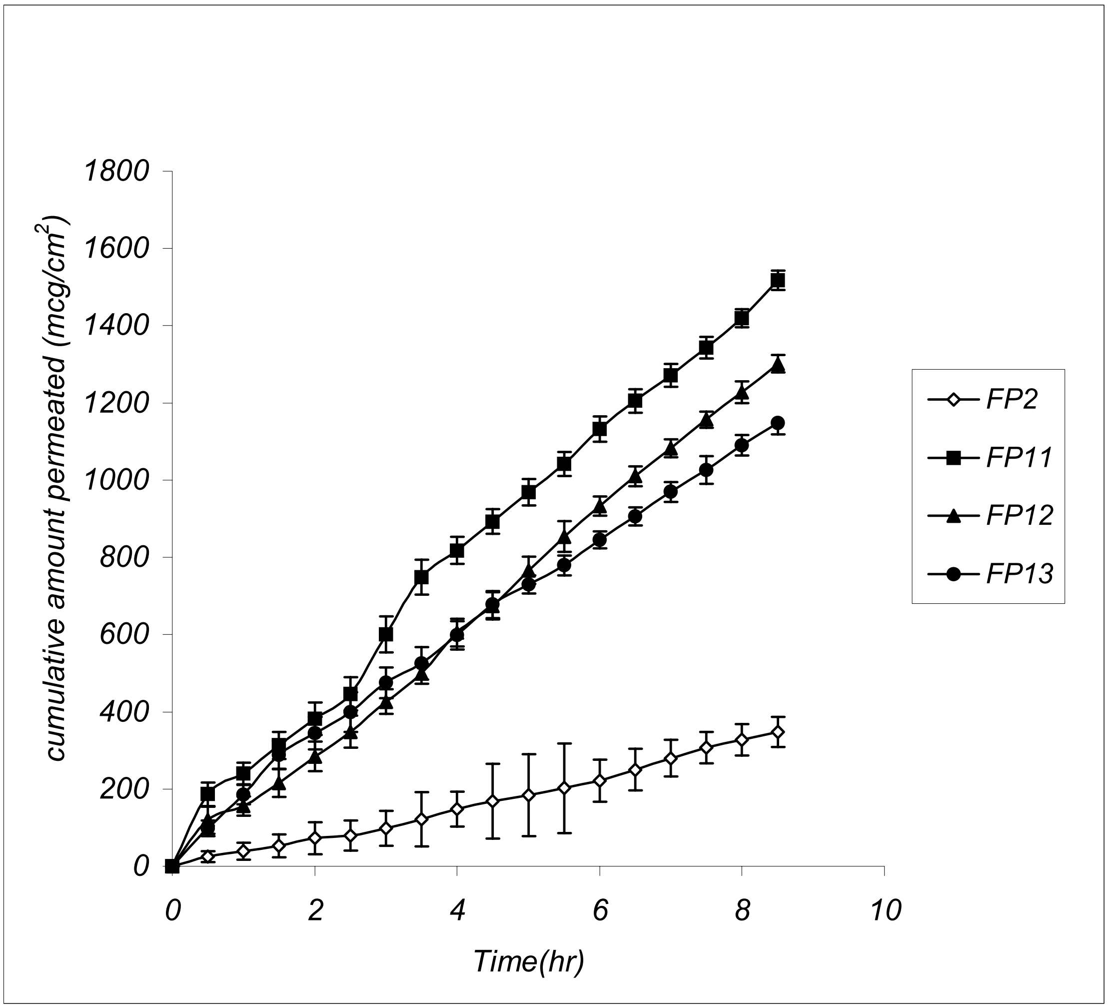


Figure 10. Effect of surfactants (5%) on the permeation of furosemide through human cadaver skin.

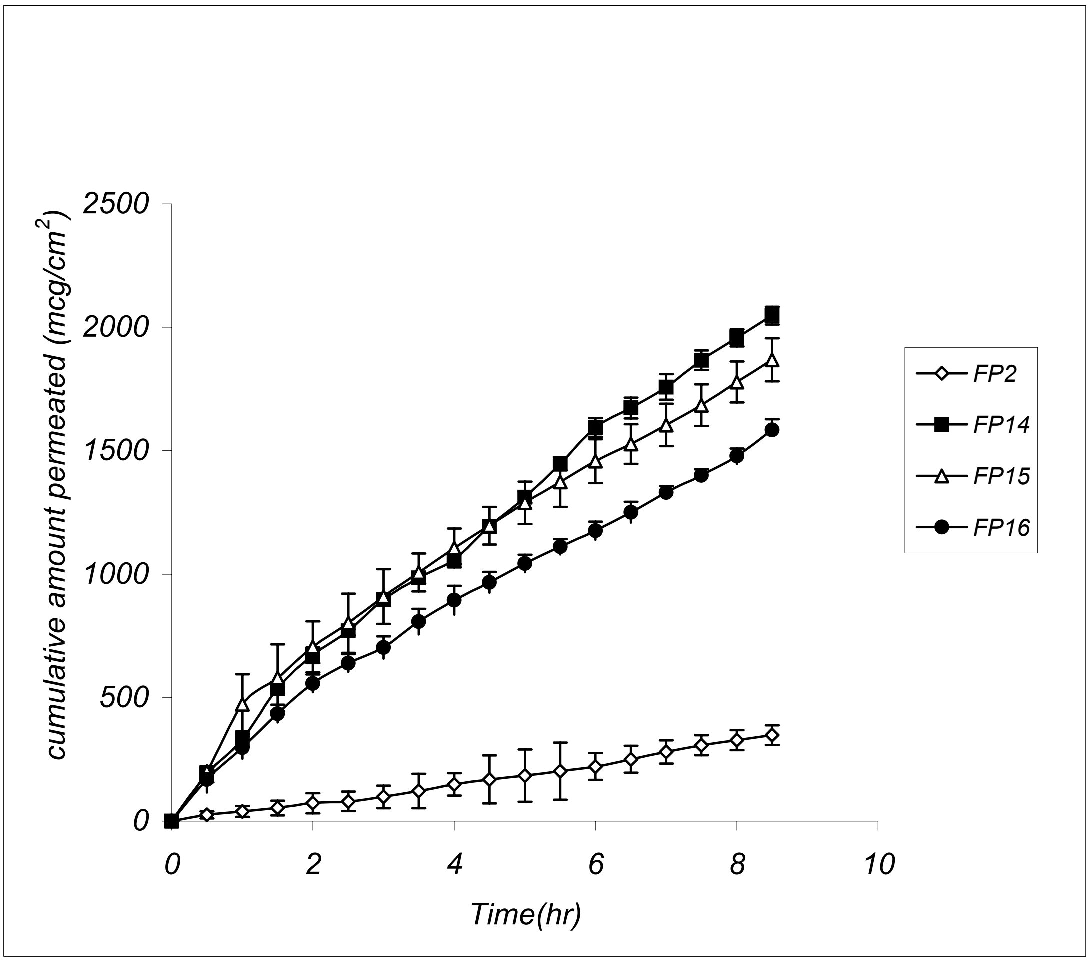


Figure 11. Effect of surfactants (10%) on the permeation of furosemide through human cadaver skin.

Drug-polymer interaction:

IR spectra of patches (figure12) showed all the characteristic bands corresponding functional groups of furosemide. Changes in area of peaks occur simply due to mixing of components without any physical chemical interactions.

Analysis of release data:

Table 8 summarizes correlation coefficient data of the formulation. The high correlation (R^2>0.9841) values indicate that release of furosemide from the patches was in compliance with Higuchi diffusion model. This relationship observed in system in which the drug is either fully dissolved or suspended and thus membrane used has no effect on the release kinetics of the drug.

Table 8. R^2 values of Higuchi model of furosemide patches

Formulation	Cellophane membrane (R^2)	Human cadaver (R^2)
FP2	0.9822	0.9660
FP11	0.9945	0.9943
FP12	0.9905	0.9868
FP13	0.9864	0.9891
FP14	0.9841	0.9881
FP15	0.9797	0.9966
FP16	0.9958	0.9927

Stability study:

Table 9 shows drug content of the formulations before and after stability study. These formulations were stored at 40±2˚C / 75%RH in stability chamber (Lab-Care, Mumbai) for six months. After six months, formulations were tested for drug content. Drug content of the patches after stability studies was found to be 99.9 % to 99.1 % and did not show any significant variations. These result indicate that drug remain stable after stability studies.

Table 9. Stability study data of patches containing furosemide.

Formulation code	**% Drug content before stability test (±SD), n=3**	**% Drug content after 180 days stability test (±SD), n=3**
F1	98.83 ± 0.6	98.12 ± 0.21
FP2	98.47 ± 1.2	98.01 ± 1.12
FP3	99.20 ± 1.3	98.87 ± 0.87
FP4	98.54 ± 0.83	98.10 ± 0.54
FP5	98.83 ± 1.2	98.35 ± 1.14
FP6	99.12 ± 0.71	98.99 ± 1.10
FP7	98.60 ± 0.8	98.01 ± 0.12
FP8	99.61 ± 1.2	99.12 ± 1.51
FP9	98.94 ± 1.4	98.15 ± 1.2
FP10	98.99 ± 0.75	98.01 ± 1.16
FP11	99.15 ± 0.33	98.99 ± 1.19
FP12	98.21 ± 1.51	97.99 ± 1.41
FP13	98.55 ± 0.84	98.12 ± 1.11
FP14	99.93 ± 1.52	99.01 ± 1.41
FP15	98.16 ± 0.22	97.99 ± 0.11
FP16	99.85 ± 0.88	98.15 ± 0.17

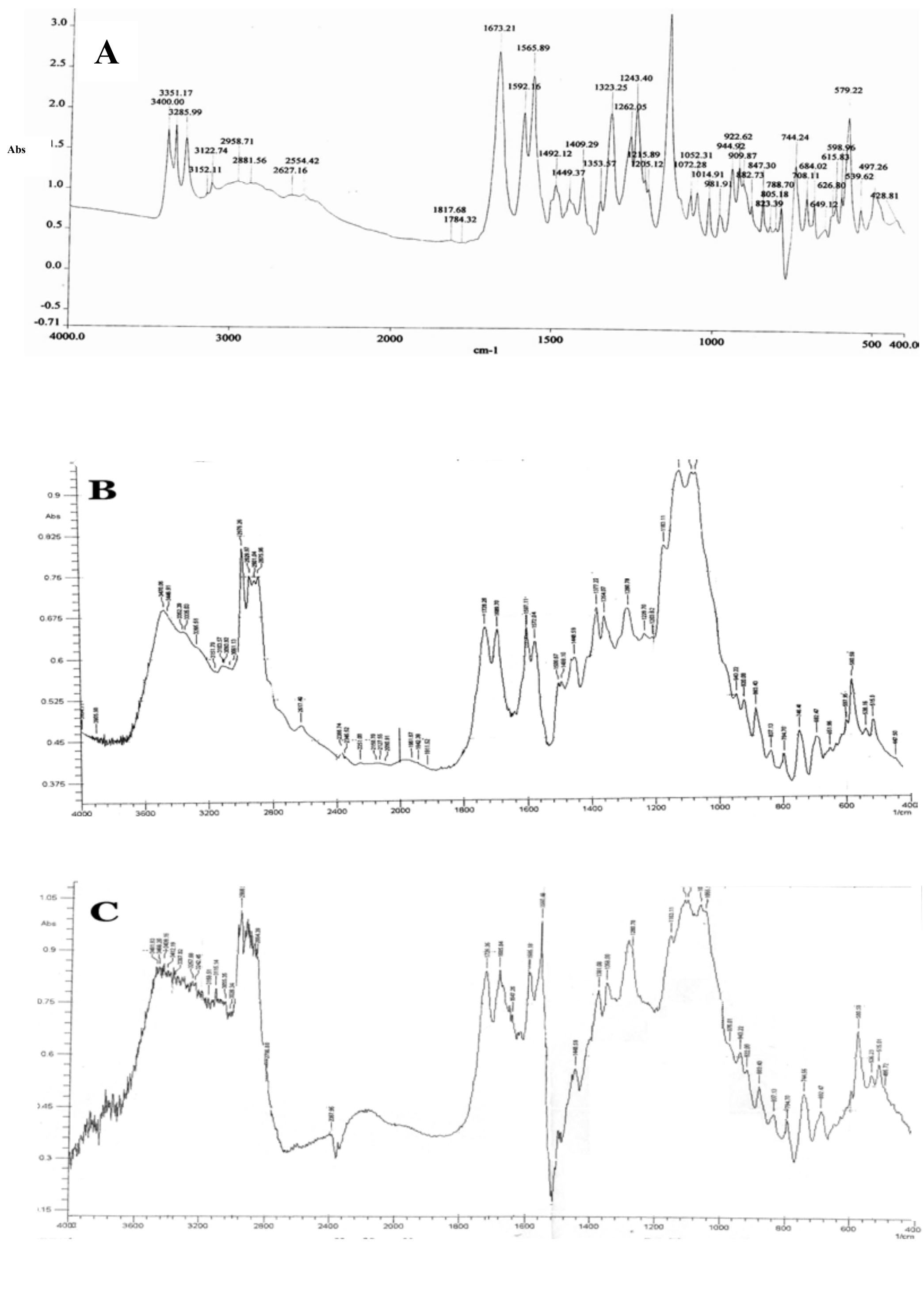


Figure 12. FTIR spectra of (A) Furosemide (B) drug loaded polymeric patch (C) drug loaded polymeric patch with PVP

6. SUMMARY AND CONCLUSION

Oral administration of furosemide due to transient high blood concentration, it can induce the side effects such as polyurea, dizziness, dry mouth, nausea and gastric disturbances. Therefore, matrix type (ethyl cellulose + PVP) patches for furosemide using essential oils (viz clove oil, lemon grass oil, menthol oil, eucalyptus oil) and combinations of this essential oils with propylene glycol (PG) were developed.

The patches were prepared by solvent evaporation method. FTIR studies showed no signs of interaction as the basic peaks of the drug were not disturbed. Patches were evaluated for physicochemical parameters such as thickness, moisture content, moisture uptake, drug content and folding endurance. Thickness of patches did not vary with essential oils and propylene glycol. Both moisture content and moisture uptake capacities increased with essential oils and greater with menthol oil and propylene glycol. A moisture absorption study reveals that ethyl cellulose + PVP films retain their shape for longer duration of time at higher humidity conditions. Drug content was found to be high (≥98.47) and uniform. Folding endurance was found to be greater with menthol oil and propylene glycol.

Permeation studies were carried out using standard cellophane membrane and human cadaver skin. Flux of clove oil and combination of clove oil with propylene glycol patches was found to be higher than other formulations.

In another part of work, surfactants (Span 20, Tween 80, Tween 20) were used as penetration enhancers. Patches were evaluated for physicochemical parameters such as thickness, moisture content, moisture uptake, drug content and folding endurance. Thickness did not change with incorporation of surfactants. Moisture content, moisture uptake and folding endurance values increased with the addition of surfactants and was greater with Tween 80. In vitro permeation studies were conducted using standard cellophane membrane and human cadaver skin. As the concentration of surfactants increased (5-10%w/w), the flux value also increased. The flux value was found to be higher with patches containing span 20. FTIR studies did not show any interactions between drug and polymer. There was no significant change in drug content after stability studies at 40 ± 2°C / 75% RH for six months.

From this study it is concluded that the clove oil, clove oil with propylene glycol and Span 20 as penetration enhancers can be effectively used to enhance the transdermal permeation of furosemide in ethyl cellulose matrix patches.

7. BIBLIOGRAPHY

1) Jain NK. Controlled and Noval Drug delivery. 1 st ed. New Delhi: CBS Publishers and Distributors; 1997.

2) Ansel HC, Allen LV, Popovinch JNG. Pharmaceutical dosage forms and drug delivery system. 7 th ed. Philadelphia: Lippincott Williams and Wilkins; 1994.

3) Vyas SP, Khar RK. Controlled drug delivery: Concepts and advances. I st ed. Vallabh prakashan; 2002.

4) Kanikkannan N, Kandimalla K, Lamba SS, Singh M. Structure activity relationship of chemical penetration enhancers in transdermal drug delivery. Current Medicinal Chemistry 1999; 6:593-08.

5) Aulton ME.Pharmaceutic, The science of dosage forms design. 2nd ed. Harcourt Publishers limited; 2002.

6) Chien YW. Novel drug delivery system. 2nd ed. New York: Marcel Dekker, Inc; Vol 50. 1992.

7) Banker SG. Modern pharmaceutics. 4th ed. France: Marcel Dekker, Inc; Vol 121.1983.

8) The pharmaceutical press. The pharmaceutical codex 12 th ed. Great Britan: Vignesh publisher; 1994.

9) Fang JY, Sung KC, Lin HH, Fang CL. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies. Int J Pharm 1999; 178:83-92.

10) Barry BW. Novel mechanism and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001; 14: 101-14.

11) The International Pharmacopoeia 2003, Vol 2-4. Quality specification for pharmaceutical substances.

12) Furosemide drug information, professional, http://www.drug.com.

13) Frusemide, http://www.drugbank.com.

14) Furosemide, http://www.wikipedia.com.

15) Rowe RC, Sheskey PJ, Weller PJ. Handbook of Pharmaceutical excipients. 4th ed. London: The Pharmaceutical Press; 2003.

16) Secondini O. Handbook of perfumes and flavors. Chemical publishing co, Inc; New York. 17-73.

17) Cho CW, Choi JS, Shin SC.Controlled release of furosemide from the ethylene vinyl acetate matrix. Int J Pharm 2005:299:127-33.

18) Agyralides GG, Dallas PP, Rekkas DM.Development and in vitro evaluation of furosemide transdermal formulation using experimental design techniques. Int J Pharm 2004; 281:35-43.

19) Shin S C, Lee H J. Controlled release of triprolidone using ethylene-vinyl acetate membrane and matrix system. Int J Pharm 2002; 54:201-06.

20) Verma P R P, Iyer S S. Transdermal delivery of propranolol using mixed grades of eudragit: Design and in vitro and in vivo evaluation. Drug Dev Ind Pharm 2000; 26(4): 471-76.

21) Devi K, Paranjothy KLK. Pharmacokinetic profile of a new matrix type transdermal delivery system: Diclofenac Diethyl Ammonium patch. Drug Dev Ind Pharm1999; 25(5): 695-700.

22) Funke AP, Gunther C, Muller RH, Lipp R. In vitro release and transdermal fluxes of a highly lipophilic drug and of enhancers from matrix TDS. J Control Rel 2002; 82:63-70.

23) Zhao K, Singh J.Mechanism(s) of in vitro percutaneous absorption enhancement of Tamoxifen by enhancers. J Pharm Sci 2000; 89:771-80.

24) Murthy SN, Rani S, Hiremath R. Formulation and evalution of controlled release transdermal patches of theophylline-salbutamol sulfate. Drug Dev Ind Pharm 2001; 27(10): 1057-62.

25) Bharkatiya M, Nema RK, Gupta GD, Gaud RS. Designing and evalution of propranolol hydrochloride transdermal patches. Res Dev 2005; 113-16.

26) Mutalik S, Udupa N, Kumar S, Agarwal S, Subramanian G, Ranjith AK. Glipizide matrix transdermal systems for diabetes mellitus: Preparation, in vitro and preclinical studies. Life Sci 2006;

27) Monti D, Chetoni P, Burgalassi S, Najarro M, Saettone M F, Boldrrini E. Effect of different terpenes containing essential oils on permeation of estradiol through hairless mouse skin. Int J Pharm 2002; 237:209-14.

28) Gao S, Singh J. Mechanism of transdermal transport of 5- flurouracil by terpenes: carvone, 1,8-cineole and thymol. Int J Pharm 1997; 154:67-77.

29) Guyot M, Fawaz F. Design and in vitro evaluation of adhesive matrix for transdermal delivery of propranolol. Int J Pharm 2000; 204:171-82.

30) Aqil M, Sultana Y, Ali A. Matric type transdermal drug delivery of metoprolol tartrate: in vitro characterization. Acta Pharm 2003; 53:119-25.

31) Arellano A, Santoyo S, Martin C, Ygartua P. Enhancing effect of terpenes on the in vitro percutaneous absorption of diclofenac sodium. Int J Pharm 1996; 130:141-45.

32) Arora P, Mukherjee B. Design, development, physicochemical, and in vitro and in vivo evaluation of transdermal patches containing diclofenac diethyl ammonium salt. J Pharm Sci 2002; 91:2076-89.

33) Fang JY, Sung KC, Lin HH, Fang CL. Transdermal iontophoretic delivery of diclofenac sodium from various polymer formulations: in vitro and in vivo studies. Int J Pharm 1999; 178:83-92.

34) Aqil M, Ali A, Sultana Y, Dubey K, Najmi AK, Pillai KK. In vivo characterization of monolithic matrix type transdermal drug delivery system of pinacidil monohydrate: a technical note. AAPS PharmSciTech 2006; 7(1): 1-4.

35) Ceschel GC, Maffei P, Moretti MDL, Demontis S, Peana AT. In vitro permeation through porcine buccel mucosa of Salvia desoleana and Picci essential oil from topical formulations. Int J Pharm 2000; 195:171-77.

36) Cornwell PA, Barry BW, Bouwstra JA, Gooris GS. Modes of action of terpenes penetration enhancers in human skin; diffraction and enhancer uptake studies. Int J Pharm 1996; 127:9-26.

37) Moghini RH, Williams AC, Barry BW. A lamellar matrix model for stratum corneum intercellular lipids. Effects of terpene penetration enhancers on the structure and thermal behaviour of the matrix. Int J Pharm 1997; 146:41-54.

38) Mukherjee B, Kanupriya, Mahapatra S, Das S, Patra B. Sorbitan monolaurate 20 as a potential skin permeation enhancer in transdermal patches. J Applied Res 2005; 5:96-108.

39) Gabiga H, Krzysztof C, Stanislaw J. Effect of penetration enhancers on isosorbide dinitrate penetration through rat skin from a transdermal therapeutic system. Int J Pharm 2000; 199:1-6.

40) Mehidizadeh A, Toliate T, Rouini M, Abashzadeh S, Dorkoosh F. Design and in vitro evaluation of new drug in adhesive formulation of fentanyl transdermal patches. Acta Pharm 2004; 54: 301-17.

41) Murthy SN, Hiremath SRR. Physical and chemical permeation enhancer in transdermal delivery of terbutaline sulphate. AAPS PharmSci Tech 2001; 2(1) 1-5.

42) Devi K, Paranjothy KLK.Development and evaluation of free films and transdermal patches of ketorolac tromethamine using polymers and pressure sensitive adhesives. The Eastern Pharmacist 1998; 97-100.

43) Amnuaikit C, Ikeuchi I, Ogawara K, Higaki K, Kimura T. Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use. Int J Pharm 20056; 289:167-78.

44) Shin SC, Lee HJ. Controlled release of triprolidine using ethylene- vinyl acetate membrane and matrix systems. Eur J Pharm Biopharm. 2002; 54: 201-06.

45) Parsaee S, Sarbolouki MN, Parnianpour M. In vitro release of diclofenac diethylammonium from lipid based formulations. . Int J Pharm 2002; 241: 185-90.

46) Mukherjee B, Mohapatra S, Gupta R, Patra B, Tiwari A, Arora P. (2005). A comparison between povidone-ethylcellulose and povidone eudragit

transdermal dexamethasone matrix patches based on in vitro skin permeation. Eur J Pharma Biopharm. 2005; 59: 475-83.

47) Khatun M, Ashraful Islam SM, Akter P, Quadir MA, Reza MS. Controlled release of Naproxen Sodium from Eudragit® RS 100 transdermal film. Dhaka University J Pharm Sci. 2004; 3: 1-10.

48) Sarisuta N, Saowakontha R, Ruangsuksriwong C. Effect of surfactant on release characteristic of clonidine hydrochloride from ethylcellulose film. Drug Dev Ind Pharm.1999; 25(3): 373-7.

49) Williams AC, Barry BW. Penetration enhancers. Adv. Drug Deliv Revi; 2004:56; 603-18.

50) Aqil M, Ali A. Monolithic matrix type transdermal drug delivery system of pinacidil monohydrate: in vitro characterization. Eur J Pharma Biopharm. 2002; 54: 161-64.

51) Krishna R, Pandit JK. Transdermal delivery of propranolol**.** Drug Dev Ind Pharm 1994; 20(15): 2459-65.

52) Baby AR, Lacerda ACC, Velasco MVR. Evaluation of the intraction of surfactants with stratum corneum model membranefrom Bothrops jararaca by DSC. Int J Pharm 2006; 317:7-9.

53) Nokhodhi A,Shokri J, Dashbulaghi A,Zaden D,Ghafourian T,JalaliM.The enhancement effect of surfactant on the penetration of lorazepam through rat skin. Int J Pharm 2003; 250:359-369.

54) Castellano A, Ivars C, Carballo, Dominguez M. The influence of span® 2oon stratum corneum lipids in Langmuir monolayers: comparison with Azone®. Int J Pharm 2000;203:245-253.

55) Shokri J, Nokhodchi A, Dashbolaghi A, Zaden D, Ghafourian T, Jalali M. The effect of surfactants on the skin penetration of diazepam. Int J Pharm 2001; 228: 99-107.

56) Dehghan M, Parakh S, Deshpande SG. Studies on polymeric system for transdermal drug delivery. Indian Drugs 1993; 30(12): 616-20.

57) Devi V, Saisivam S, Maria GR, Deepti PU. Design and evaluation of matrix diffusion controlled transdermal patches of verapamil hydrochloride. Drug Dev Ind Pharm 2003; 29(5): 495-503.

58) Godwin DA, Michniak BB. Influence of drug lipophilicity on terpenes as transdermal penetration enhancers. Drug Dev Ind Pharm 1999; 25(8): 905-15.

59) Yamne MA, William AC, Barry BW. Terpene penetration enhancers in propylene glycol/water co-solvent system: Effectiveness and mechanism of action. J Pharm Pharmacol 1995; 47: 978-87.

60) Krishanaiah YSR, Chandrasekhar DV, Rama B, Jayaram B, Satyanarayana V, Al-Saidan SM. In vivo evaluation of limonene-based transdermal therapeutic system of nicorandil in healthy human volunteers. Skin Pharmacol Physiol 2005; 18: 263-72.

61) Ghosh B, Reddy LH, Kulkarni RV, Khanam J. Comparison of skin permeability of drugs in mice and human cadaver skin. Ind J Exp Bio 2000; 38: 42-5.

62) Bendas B, Schmalfub V, Neubert R. Influence of propylene glycol as cosolvent on mechanisms of drug transport from hydrogels. Int J Pharm 1995; 116: 19-30.

63) Heard CM, Kung D, Thomas CP. Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect. Int J Pharm 2006; 321: 167-70.

64) Sridevi S, Chary MG, Krishna DR, Diwan PV. Pharmacodynamic evaluation of transdermal drug delivery system of glibenclamide in rats. Ind J Pharmacol 2000; 32: 309-12.

65) Kulkarni RV, Doddayya H. In vitro permeation of verapamil hydrochloride from polymeric membrane systems across rat and human cadaver skin. Ind J Pharm Sci 2002; 593-597.

66) Gattani SG, Gaud RS, Chaturvedi SC. Formulation and evaluation of transdermal films of ondansetron hydrochloride. Indian Drugs 2006; 43(3): 245-50.

67) Sanker V, Johnson D, Sivanand V, Ravichandran V, Raghuramma S, Velrajan G et al. Design and evaluation of nifedipine transdermal patch. Indian J Pharm Sci 2003; 65(5): 510-15.

68) Bhattacharya A, Ghosal SK. Effect of hydrophobic permeation enhancer on the release and skin permeation kinetic from matrix type transdermal drug system of ketotifen fumarate. The Eastern Pharmacist 2003; 507: 109-12.

69) Charoo NA, Anwer A, Kohli K, Pillai KK, Rahman Z. Transdermal delivery of flurbiprofen permeation enhancement, design, pharmacokinetic and pharmacodynamic studies in albino rats. Pharm Dev Tech 2005; 10: 343-51.

70) Hosny EA, Hady SS, Niazy EM. Effect of film composition and various penetration enhancers concentrations on prazosin release from acrylic polymeric film. Pharm Acta Helvetiae 1998; 72: 247-54.

71) Hendradi E, Obata Y, Isowa K, Nagai T, Takayama K. Effect of mixed micelle formulations including terpenes on the transdermal delivery of diclofenac. Biol Pharm Bull 2003; 26(12); 1739-43.

72) Farinha A, Toscano C, Campos R, Bica Antonio, Hadgraft J. Permeation of naproxen from saturated solutions and commercial formulations through synthetic membranes. Drug Dev Ind Pharm 2003; 4: 489-94.

73) Krishnaiah YSR, Satyanarayana V, Bhasker P. Enhanced percutaneous permeability of nicardipine hydrochloride by carvone across the rat abdominal skin. Drug Dev Ind Pharm 2003; 29: 191-202.

74) Megrab NA, William AC and Bony BW, J control release 1995; 36(277): 109-12.

75) Williams Ac, Barry BW. The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophillic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int J pharm 1991;74:157-68.

76) Elfbaum SG, Laden K. The effect of dimethyl sulfoxide on percutaneous absorption:a mechanistic study .J Cutaneous Pathol 1968;259-64.

77) Barry BW, Williams AC. Human skin penetration enhancement: the synergy of propylene glycol with terpenes. Proc Int Symp Control Rel Bioact Mater.1989; 16:33-34.

78) Cornwell PA, Barry BW .The effect of a series of homologous terpene alcohols on lipid structure of human stratum corneum as assessed by differential scaning calorimetry. In scott,R.C., Guy, R.H., Hadgraft,J. and Bodde, H.E.(Eds), Prediction of Percutaneous Penetration, IBC Technical Services, London.1991;2:394-400.

79) Barry BW, Williams AC. Terpenes as skin penetration enhancers. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical skin permeation enhancement. Marcel Dekker, New York.1993; 95-111.

80) Kobayashi D, Matsuzawa T, Sugibayashi K, Morimoto Y, Kimura M. Analysis of combined effect of 1-menthol and ethanol as skin permeation enhancers based on two-layer skin model Pharm res 1994; 11:96-103.

81) Cornwell PA, Barry BW. Effect of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int J Pharm 1995; 117:101-12.

82) Breuer MM, The interaction between surfactants and keratinous tissues. J Soc cosmet 1979; 30:41-64.

83) Walters KA, Walker M, Olejnik O, Non ionic surfactant effects on hairless mouse skin permeability characteristics. J pharm pharmacol.1987; 40:525-29.

84) Hwang CC, Danti AG. Percutaneous absorption of flufenamic acid in rabbits: effect of decyl methyl sulphoxide and various surface-active agents. J pharm sci 1983; 72:857-60.