"FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF TRANSDERMAL PATCH OF REPAGLINIDE"

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IN

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BIOPHARMACEUTICS

BY

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CERTIFICATE

This is to certify that ***Mr. Maulik R. Patel*** *has prepared his thesis entitled "****Formulation, Optimization and Characterization of Transdermal Patch of Repaglinide****", in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmaceutics and pharmaceutical technology, Institute of Pharmacy, Nirma University.*

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DECLARATION

*I declare that the thesis "**Formulation, Optimization and Characterization of Transdermal Patch of Repaglinide**" has been prepared by me under the guidance of Dr. Renuka D. Mishra, Assistant Professor, Department of Pharmaceutics and pharmaceutical technology, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.*

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"God, grant me the serenity to accept the things I cannot change, courage to change the things I can, and the wisdom to know the difference."

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"Dream your dreams and May they come true!"

Maulik R. Patel

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LIST OF ABBREVIATIONS

Short name	Abbreviation
°C	Degree centigrade
µg	Microgram
ABS	Absorbance
BP	British Pharmacopoeia
Conc.	Concentration
CPR	Cumulative Percentage Release
TDDS	Transdermal Drug Delivery System
FTIR	Fourier Transfer Infra Red
HPC	Hydroxy Propyl Cellulose
EC	Ethyl Cellulose
mg	Milligram
PEG-400	Poly Ethylene Glycol 400
PG	Propylene Glycol
PhEur	European Pharmacopoeia
PVA	Poly Vinyl Alcohol
DBP	Dibutylpthalate
I**PM**	Isopropyl myristate
RH	Relative Humidity
SD	Standard Deviation
TS	Tensile Strength
USP	United States Pharmacopeia
UV	Ultra Violet
SEM	Scanning electron microscope
W/V	Weight by volume
W/W	Weight by weight

ABSTRACT

The purpose of the study was development of matrix-type transdermal therapeutic system containing Repaglinide. Repaglinide has half life of 1 hour, and 56 % bioavailability in the body.Total daily dose of Repaglinide is 4 mg (e.g., 1 mg four times daily); hence, it requires frequent dosing. Transdermalpatch of Repaglinide was prepared to sustain the release and improve bioavailability of drug and patient compliance.Drug purity was confirmed by using FTIR and melting point determination. Differentformulations were prepared using various concentrations of HPC-EF and Duro-Tak 87-9301 as polymer using solvent casting method. All formulation carried 20 % of PEG 400 as plasticizer and IPM as penetration enhancer. Prepared formulations were evaluated for various parameters like thickness, tensile strength, folding endurance, % elongation, %moisture content,%moisture uptake, % drug content, in vitro- ex vivo drug release, and in-vivo study. Batch H5 containing 10% w/v of HPC-EF and 10% w/v of IPM showed maximum in-vitro (92.41%) and ex-vivo (90.86%) drug release at 24 hr, as compared to batch D5 (80% w/w of Duro-Tak 87-9301 and 10% of IPM) as polymer.Higuichi model showed maximum r^2 value (0.954) so diffusion could be best expressed by Higuchi's equation for the release of drug that depends mostly on diffusion characteristic.In vitro and ex-vivo correlation study was performed which indicated in-vitro diffusion profile was similar to ex-vivo diffusion profile.Batch B2 (5 % of PVA) showed maximum tensile strength and % percentage elongation so it was selected as backing layer. Adhesive layer was prepared using 2 % w/w of Duro-Tak 87-9301 as PSA. In vivo study (skin irritation study and patch adherence study) was carried out on human skin and results showed that no skin irritation was observed.

1. AIM OF INVESTIGATION

Diabetes is usually a chronic disease in which there are high levels of sugar in the blood. There are two major types of diabetes. The causes and risk factors are different for each type:

Type 1 diabetes (insulin dependent diabetes) can occur at any age, but it is most often diagnosed in children, teens, or young adults. In this disease, the body makes little or no insulin. Daily injections of insulin are needed. The exact cause is unknown. **Type 2 diabetes** (noninsulin-dependent diabetes mellitus**)** makes up most diabetes cases. It most often occurs in adulthood. However, because of high obesity rates, teens and young adults are now being diagnosed with it. Many people with type 2 diabetes are unaware about their condition. Management strategies are needed to tackle health issues such as obesity, lack of exercise and incorporation of drugs. As the understanding of pathophysiology of diabetes is becoming clearer and new therapeutic agents such as Repaglinide are becoming available, the management of diabetes may become feasible in future. Repaglinide is a short-acting oral hypoglycemic agent used as a glucose regulator in the management of type -II diabetes mellitus. It possesses low oral bioavailability (56 %) due to hepatic first pass metabolism after oral administration and poor absorption in the upper intestinal tract. It has a very short biological half-life of 1h, which makes frequent dosing necessary to maintain the drug within the therapeutic blood level for longer period. Moreover it produces hypoglycemia; causes gastrointestinal adverse effects including abdominal pain, diarrhea, constipation, nausea and vomiting after oral administration. As diabetes is a chronic disease hence the treatment is intended over a prolonged period of time.

Transdermal delivery systems (TDDS) may provide a useful drug therapy with regard to patient compliance. Transdermal delivery can bypass the first pass metabolism and deliver the drug in a rate-controlled manner, which is desirable in

anti-diabetic therapy. With this view an attempt will be made to deliver Repaglinide in effective therapeutic concentration in TDDS form. The aim of this study is to enhance the bioavailability of Repaglinide and reduce the dosage frequency by formulation of transdermal patch and thereby improving therapeutic efficacy. In the present study, transdermal patch of Repaglinide will be developed by using Hydroxypropyl cellulose (HPC-EF), Duro-Tak 87-9301 as film forming agent, Poly ethylene glycol (PEG-400), Propylene glycol (PG) as plasticizer and Isopropyl myristate (IPM) as permeation enhancer. The patches will be formulated by solvent casting method. Transdermal patches are evaluated for mechanical properties such as thickness, tensile strength, folding endurance, % elongation, moisture content, moisture uptake and in vitro and ex-vivo diffusion**.**

2. INTRODUCTION

2.1INTRODUCTION TO SKIN

The skin constitutes one of the largest interfaces between the body and the environment. On one hand, the function of human skin is to protect our body against chemical, physical, and microbial injury, loss of water, and other endogenous substances; on the other hand, it is involved in the thermoregulation of the body and serves as an excretory organ.

This bifunctional nature of skin depends on its highly differentiated structure, with the main barrier function being located in the outermost skin layer.

2.1.1Anatomical structure of human skin:[1]

The multitude of different functions of the human skin can only be achieved by a unique anatomical structure of the different skin layers. These are as follows:

- Epidermis consisting of:

-- Stratum corneum(outermost layer)

-- Viable epidermis

- Dermis
- Subcutis or subcutaneous fatty tissue

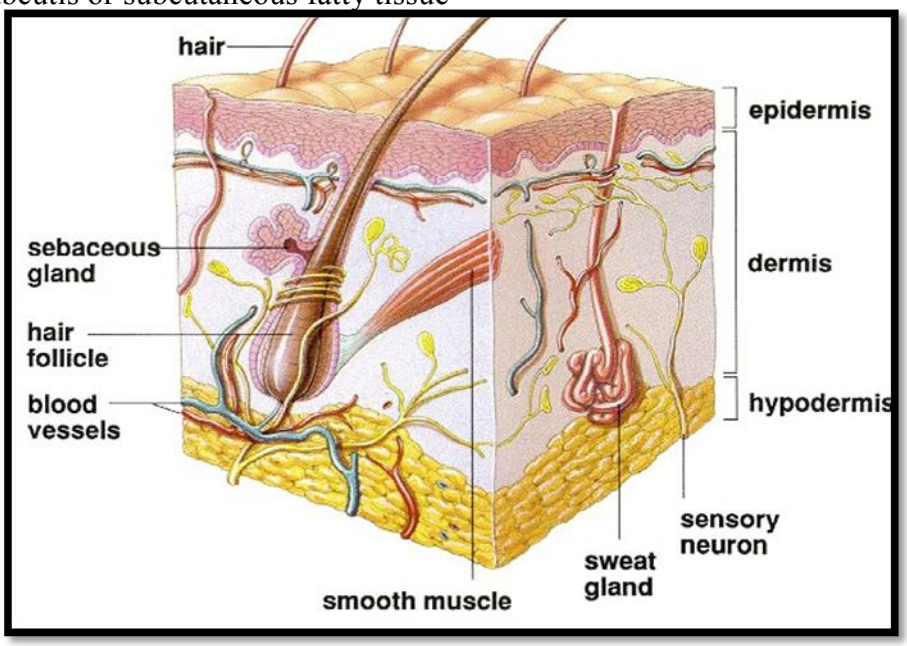


Figure 2.1- ***Anatomical structure of human skin***

Epidermis:

Because of practical reasons, the human epidermis can be generally divided into two main layers, the subcutaneous and the viable epidermis.

The Subcuteneousconsists of separated nonviable cornfield almost non-permeable corneocytes embedded into a continuous lipid bilayer madeof various classes' oflipids, for example, ceramides, cholesterol, cholesterol esters, freefattyacids, and triglycerides. Structurally, this epidermis layer is best described by the so-called brick-and-mortar model. The SCis crucial for the barrier function of the skin, controlling percutaneous absorption of dermally applied substances andregulating fluid homeostasis.

The thickness of the SCis usually 10-25 μm,withexception the soles of the feet and the palms, which swells several-fold when hydrated.

Dermis:

Depending on the body site, the thickness of the dermis ranges from 3 to 5 mm. The dermis consists of a matrix of connective tissue composed of collagen, elastin, and reticulin and is interspersed by skin appendages such as sweat glands, pilosebaceous units and hair follicles. Furthermore nerves, lymphatic andblood vessels are located in this skin layer. Blood vessels are founddirectlybeneaththe stratum germinativumof the viable epidermis supply removing metabolites.

Subcutis or subcutaneous fatty tissue:

The subcutaneous fat layer acts mainly as a heat insulator and a mechanical cushion and stores readily available high-energy chemicals.

Skin appendages:[1]

Skin appendages can be distinguished into hair follicles with their associated sebaceous glands, eccrine sweat glands, apocrine sweat glands, and nails.

Hair follicles:

Hair follicles with their associated sebaceous glands are present all over the skin surface with the exception of lips, palms, and soles. Furthermore, hair follicles intersperse down to the sub cutis offering permeation pathways deep into the skin.

Eccrine glands:

Eccrine glands can be found on the entire body surface of humans except for the lips, external ear canal, and clitoris. These glands play an important role in thermoregulation which is necessary for fluid and electrolyte homeostasis.

2.1.2Fundamentals of skin permeation[2]

Until the last century the skin was supposed to be impermeable with exception to gases. However, in the current century the study indicated the permeability to lipidsoluble drugs like electrolytes. Also it was recognized that various layers of skin are not equally permeable i.e. epidermis is less permeable than dermis. After a large controversy, all doubts about stratum corneum permeability were removed and using isotopic tracers, it was suggested that stratum corneum greatly hamper permeation.

Stratum corneum as skin permeation barrier

The average human skin contains 40-70 hair follicles and 200-250 sweat ductsper square centimeters. Especially water-soluble substances pass faster through these ducts; still these ducts don't contribute much for skin permeation. Therefore, mostneutral molecules pass through stratum corneum by passive diffusion. Thus, thestratumcorneum acts as a passive, but not inert, diffusion medium.Series of steps in sequence:

1. Sorption of a penetrant molecule on surface layer of stratum corneum.
2. Diffusion through it and viable epidermis, and finally
3. The molecule is taken up into the microcirculation for systemic distribution.

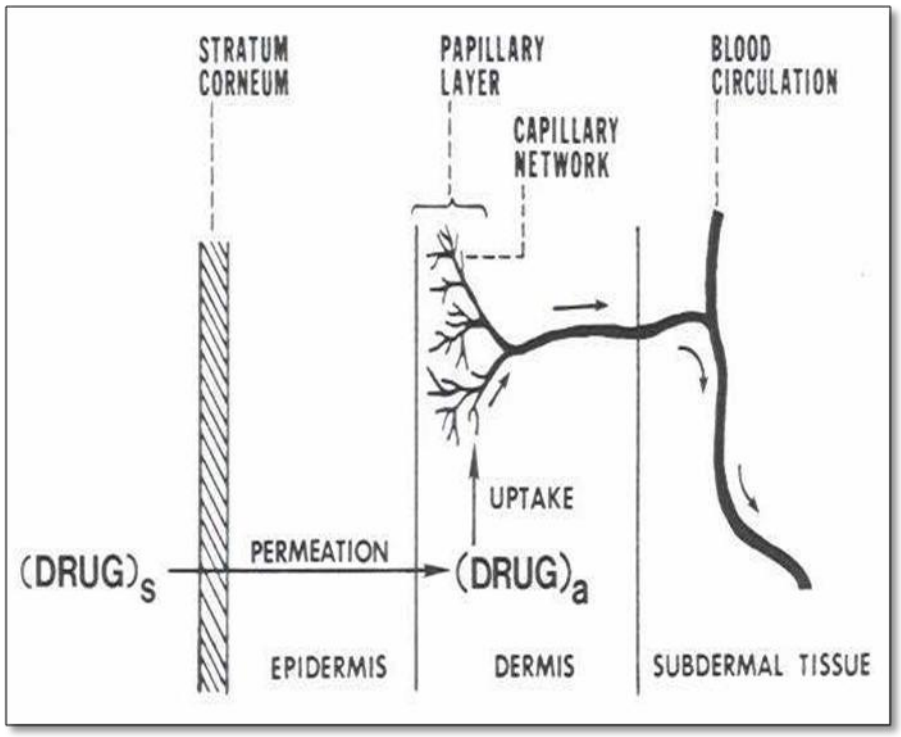


Figure 2.2- A multilayer skin model showing sequence of Transdermal Permeation of drug for systemic delivery.

Intracellular verses transcellular diffusion

Intracellular regions in stratum corneum are filled with lipid rich amorphousmaterial. In dry stratum corneum intracellular volume may be 5% to 1% in fullyhydrated stratum corneum.

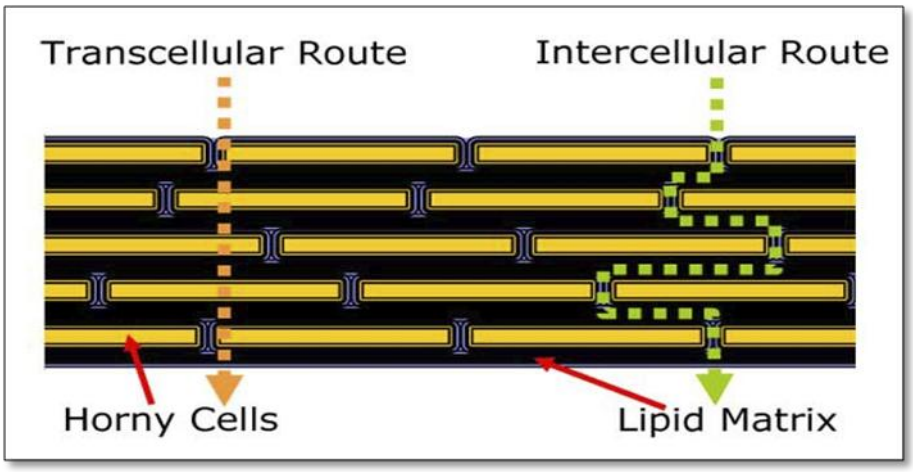


Figure 2.3-Transport of drugs through stratum corneum

2.1.3Permeation pathways[3]

Percutaenous absorption involves passive diffusion of the substances through the skin. A molecule may use two diffusional routes to penetrate normal intact skin, the appendage route and the epidermal route.

Appendageal route

Appendageal route comprises transport via sweat glands and hair follicles withtheir associated sebaceous glands. These routescircumvent penetration through the stratum corneum and are therefore known as"shunt" routes. This route is considered to be of minor importance because of itsrelatively small area, approximately 0.1 % of the total skin area.

Epidermal route

For drugs, which mainly cross-intact horney layer, two potential micro routes of entry exists, the transcellular (intracellular) and intercellular pathways.

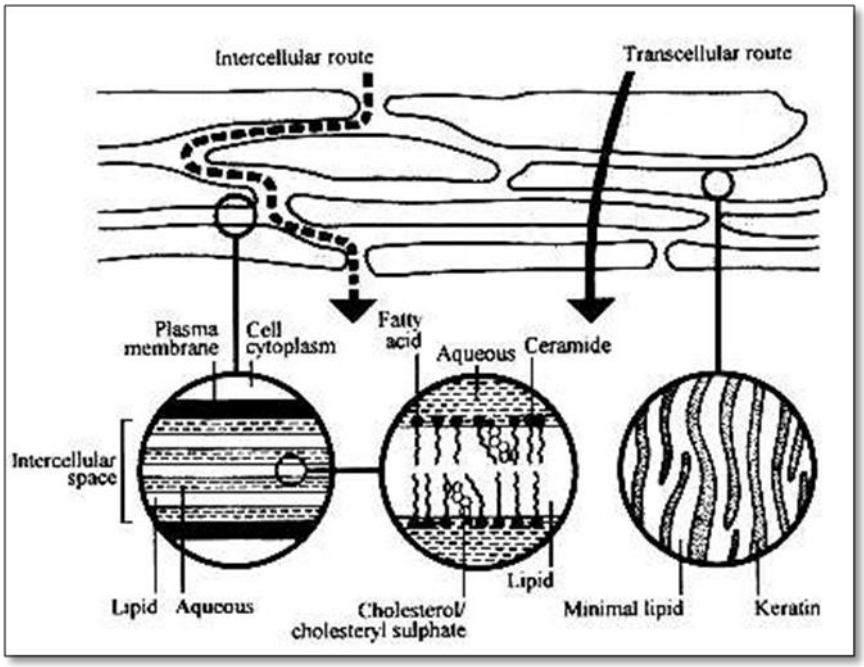


Figure2.4 - Epidermal routes for drug permeation

i) Transcellular

Transcellular pathway means transport of molecules across epithelial cellular membrane. These include passive transport of small molecules, active transport of ionic and polar compounds, and endocytosis and transcytosis of macromolecules.

ii) Paracellular

Paracellular pathway means transport of molecules around or between the cells. Tight junctions or similar situations exist between the cells. The principal pathway taken by a permeant is decided mainly by the partition (logk).

Most permeants permeate the stratum corneum by both routes. However, the tortuous intercellular pathway is widely considered to provide the principal route and major barrier to the permeation of most drugs.

2.2 Introduction to Transdermal drug delivery

Currently, transdermal drug delivery system (TDDS) is one of the most promising methods for drug application. Increasing number of drugs are being added to the list of therapeutic agents that can be delivered to systemic circulation via skin.[1]A transdermalpatch is a medicated adhesive patch that is placed on the skin to deliver a time-released dose of medication systemically for treating illnesses. Since early 1980s, this dosage form of transdermal therapeuticsystemhasbeenavailable commercially.[2]Transdermal drug delivery system (TDDS) allows delivery of selected drug into the systemic circulation via permeation through skin layers at a controlledrate.[3]An essential prerequisite for the development of TDDS is that the drug must be capable of passing through skin at a sufficiently high rate to achieve therapeutic plasma concentrations. However, the outermost layer of skin, stratum corneum (SC), forms major barrier to most exogenous substances including drugs.[4]

The discovery of TDDS is a breakthrough in the field of controlled drug delivery systems. The ability of TDDS to deliver drugs for systemic effect through intact skin while bypassing first pass metabolism has accelerated transdermal drug delivery research in the field of pharmaceutics. Over a decade of such extensive research activities, many transdermal patches have been developed and successfullycommercialized.[5]

Preparation of TDDS consists of three basic designs: membrane control or reservoir patches (RPs), matrix or monolithic patches (MPs),

andDruginadhesivepatches(DIAPs).[6]

TDDS avoids problems such as gastrointestinal irritation, metabolism, variations in delivery rates and interference due to the presence of food. It is also suitable for unconscious patients. The technique is generally non-invasive well accepted by patients and can be used to provide local delivery over several days.[7]

Transdermal patches are generally occlusive i.e., they do not allow water to be releasedfromskinsurface, and this is often the reason for skin irritation. On otherhandocclusion generally increases drug transport because it augments the water content of the stratum corneum, although the effect is not same for different permeants.[8]

2.2.1 Advantages of Transdermal Drug Delivery System (TDDS) [9]

The advantages of transdermal delivery over other delivery systems are as follows:

1. Avoidance of first pass metabolism of drugs.
2. Reduced plasma concentration levels of drugs, with decreased side effects.
3. Reduction of fluctuations in plasma levels of drugs, Utilization of drug candidates with short half-life and low therapeutic index.
4. Easy elimination of drug delivery in case of toxicity.
5. Reduction of dosing frequency and enhancement of patient compliance.
6. Transdermal medications deliver a steady infusion of a drug over an extended period of time. Adverse effects or therapeutic failure frequently associated with intermittent dosing can also be avoided.
7. Transdermal delivery can increase the therapeutic value of many drugs via avoiding specific problems associated with the drug. E.g. GI irritation, lower absorption, decomposition due to 'hepatic first pass' effect.
8. The simplified medication regimen leads to improved patient compliance and reduced inter and intra-patient variability.

2.2.2Limitation of TDDS [9]

The drug must have some desirable physicochemical properties for penetration through stratum corneum and if the drug dosage required for therapeutic value is more than 10 mg/day, the transdermal delivery will be very difficult if not impossible.Skin irritation or contact dermatitis due to the drug, excipients and enhancers of the drug used to increase percutaneous absorption is another limitation. Clinical need is another area that has to be examined carefully before a decision is made to develop a transdermal product The barrier function of the skin changes from one site to another on the same person, from person to person and with age.

Table-2.1 Limitation of TDDS[9]

Potency of the drug	Daily systemic dose should be ≤ 20 mg
Lipophilicity of drug	Log P should be in the range 1-4
Molecular size	< 500 Da
Irritation	Drug should not be direct irritate to skin
Melting point	Should be < 200° C
Immunogenicity	Drug should not stimulate immune reaction to skin
Half life	should be 10 hr or less
Oral bioavailability and therapeutic index	should be low

2.2.3Factors influencing transdermal drug delivery[10]

The effective transdermal drug delivery can be formulated by considering three factors namely Drug, Skin, and the vehicles. So the factors affecting can be divided into biological and physicochemical factors.

1.Biological factors

Skin condition – Acids and alkalis; many solvents like chloroform, methanol damage the skin cells and promotes penetration. Diseased state of patient alters the skin conditions. The intact skin is better barrier but the above mentioned conditions affect penetration.

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

Blood supply – Changes in peripheral circulation can affect transdermal absorption.

Regional skin site – Thickness of skin, nature of stratum corneum, and density of appendages vary site to site. These factors affect significantly penetration.

Skin metabolism –Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. So skin metabolism determines efficacy of drug permeated through the skin.

Species differences – The skin thickness, density of appendages, and keratinization of skin vary species to species, so affects the penetration.

2.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 humectants is done in transdermal delivery.

Temperature and pH – The permeation of drug increases ten fold with temperature variation. The diffusion coefficient decreases as temperature falls. Weak acids and weak bases dissociate depending on the pH and pKa values. The proportion of unionized drug determines the drug concentration in skin. Thus, temperature and pH are important factors affecting drug penetration.

Diffusion coefficient – Penetration 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 gradientacross the barrier and concentration gradient will be higher if the concentration of drug as more across the barrier.

Partition coefficient – The optimal partition coefficient is required for good action. Drugs with high K do not freely leave the lipid portion of skin. Also, drugs with low K will not be permeated.

Molecular size and shape – Drug absorption is inversely related to molecular weight; small molecules penetrate faster than large ones. Because of partitioncoefficient domination, the effect of molecular size is not known.

2.2.4BASIC COMPONENTS OF TDDS[11]

- Polymer matrix / Drug reservoir
- Drug
- Permeation enhancers
- Pressure sensitive adhesive (PSA)
- Backing laminates
- Release liner
- Other excipients like plasticizers and solvents

Polymer matrix

Polymers are the backbone of a transdermal drug delivery system. Systems for transdermal delivery are fabricated as multilayered polymeric laminates in which a drug reservoir or a drug–polymer matrix is sandwiched between two polymeric layers: an outer impervious backing layer that prevents the loss of drug through the backing surface and an inner polymeric layer that functions as an adhesive and/or rate-controlling membrane.

The polymers utilized for TDDS can be classified as…

Natural Polymers: e.g. cellulose derivatives, zein, gelatin,shellac, waxes, gums, natural rubber and chitosan etc.

Synthetic Elastomers: e.g. polybutadiene, hydrinrubber,polyisobutylene, silicon rubber, nitrile, acrylonitrile, neoprene,butylrubberetc.

Synthetic Polymers: e.g. polyvinyl alcohol, polyvinylchloride, polyethylene, polypropylene, polyacrylate, polyamide, polyurea, polyvinylpyrrolidone, polymethylmethacrylate*etc*. The polymers like cross linked polyethylene glycol, eudragits, ethyl cellulose, polyvinylpyrrolidone.

Drug

The most important criteria for TDDS is that the drug should possesses right physicochemical and pharmacokinetic properties. Transdermal patches offer advantage of drugs which undergo extensive first pass metabolism, drugs with narrow therapeutic window, or drugs with short half-life which causes non- compliance due to frequent dosing. Recently approved drugs for TDDS includerivastigmine for Alzheimer's and Parkinson dementia, rotigotine for parkinson, methylphenidate for attention deficit hyperactive disorder and selegiline for depression.

Permeation enhancers

The enhancement in absorption of oil soluble drugs is apparently due to the partial leaching of the epidermal lipids by the chemical enhancers, resulting in the improvement of the skin conditions for wetting and for transepidermal and transfollicular penetration.

Table-2.2 Classification of penetration enhancers

Terpenes(essential oils)	Limonene,carvone etc.
Pyrrolidones	N-methyl-2-pyrrolidone(NMP), azoneetc
Fatty acids and esters	Oleic acid, linoleic acid, lauric acid etc.
Sulfoxides	Dimethyl sulfoxide(DMSO)
Alcohols, Glycols, and Glycerides	Ethanol, Propylene glycol, Octyl alcohol etc.
Anionic, cationic, non-ionic Surfactants	Sodium lauraylsulphate, Trimethyl ammonium bromide, Synperonic NP series
Solvents	Ethanol, Lauryl alcohol, Linolenyl alcohol, Octanol
Azone or Lauracapram and its derivatives	1-dodecylazacycloheptan-2-one

Pressure sensitive adhesive

A PSA maintains an intimate contact between patch and the skin surface. It should adhere with not more than applied finger pressure, be aggressively and permanently tacky, and exert a strong holding force.

Desirable feature of pressure sensitive adhesive[12]

It should adhere to the skin aggressively, should be easily removed.

It should not leave an unwashable residue on the skin.

It should not irritate or sensitize the skin.

It should be physically and chemical compatible with the drug, excipients and enhancers of the device of which it is a part.

Permeation of drug should not be affected.

The delivery of simple or blended permeation enhancers should not be affected.

Backing laminate

The primary function of the backing laminate is to provide support. Backing layer should be chemical resistant and excipient compatible because the prolonged contact between the backing layer and the excipients may cause the additives to leach out or may lead to diffusion of excipients, drug or penetration enhancer through the layer.

Release liner

During storage release liner prevents the loss of the drug that has migrated into the adhesive layer and contamination.

2.2.5TYPES OF PATCHES[9]

A. Single-layer Drug-in-Adhesive

The adhesive layer of this system also contains the drug. In this type of patch the adhesive layer not only serves to adhere the various layers together, along with the entire system to the skin, but is also responsible for the releasing of the drug. The adhesive layer is surrounded by a temporary liner and a backing.

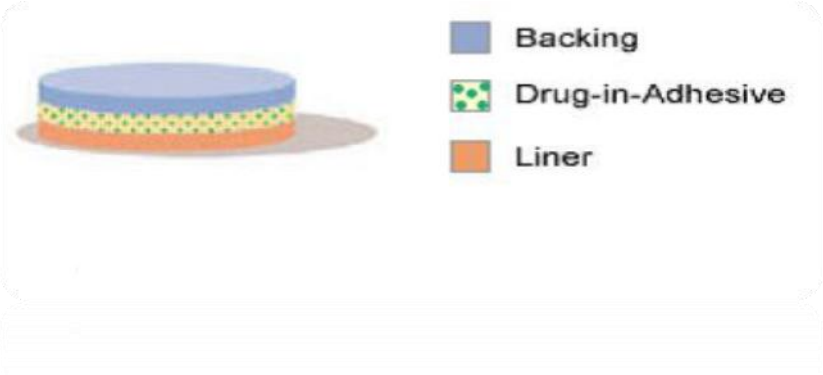


Figure 2.5 - Single-layer Drug-in-Adhesive

B. Multi-layer Drug-in-Adhesive

The multi-layer drug-in adhesive patch is similar to the single-layer system in that both adhesive layers are also responsible for releasing of the drug. The multi-layer system is different however in that it adds another layer of drug-in-adhesive, usually separated by a membrane (but not in all cases). This patch also has a temporary liner-layer and a permanent backing.

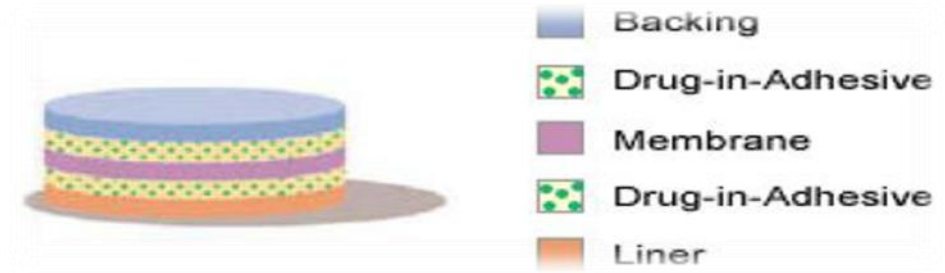


Figure 2.6- Multi-layer Drug-in-Adhesive

C. Reservoir

Unlike the Single-layer and Multi-layer Drug-in adhesive systems the reservoir transdermal system has a separate drug layer. The drug layer is a liquid compartment containing a drug solution or suspension separated by the adhesive layer. This patch is also backed by the backing layer. In this type of system the rate of release is zero order.

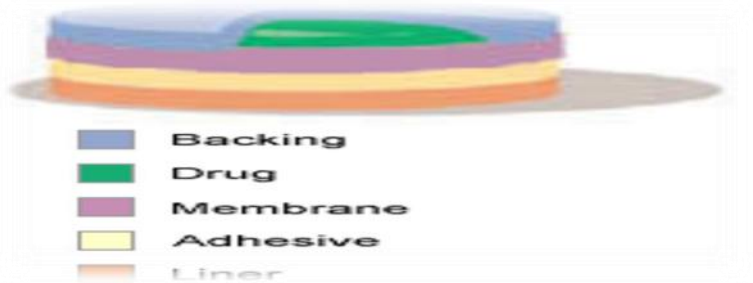


Figure 2.7- Reservoir

D. Matrix

The Matrix system has a drug layer of a semisolid matrix containing a drug solution or suspension. The adhesive layer in this patch surrounds the drug layer partially overlaying it.

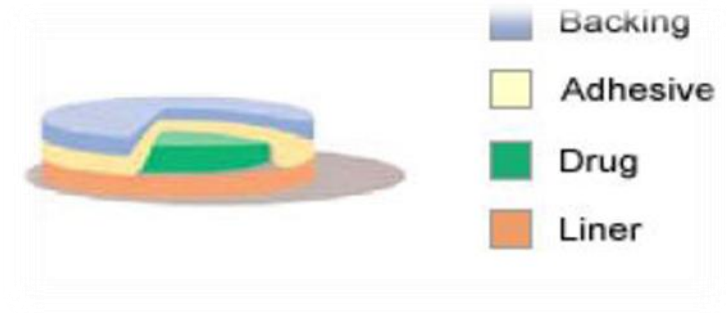


Figure 2.8- Matrix type patch

2.2.6 Transdermal Market [15]

The market for transdermal products has been in a significant upward trend that is likely to continue for the future. An increasing number of TDD products continue to deliver real therapeutic benefit to patients around the world. More than 35 TDD products have now been approved for sale in the US, and approximately 16 active ingredients are approved for use in TDD products globally.

Table- 2.3 Transdermal Market

PRODUCT NAME	DRUG NAME	COMPANY NAME	USE
Alora	Estradiol	TheraTech/Proctol and Gamble	Postmenstrual syndrome
Androderm	Testosterone	TheraTech/GlaxoSmithKline	Hypogonadism in males
Catapres-TTS	Clonidine	Alza/BoehingerIngelheim	Hypertension
Climaderm	Estradiol	Ethical Holdings/Wyeth-Ayerest	Postmenstrual Syndrome
Climara	Estradiol	3M Pharmaceuticals/Berlex Labs	Postmenstrual Syndrome
CombiPatch	Estradiol/Norethindrone	Noven , Inc./Aventis	Hormone Replacement therapy
Deponit	Nitroglycerin	Schwarz-Pharma	Angina pectoris
Estraderm	Estradiol	Alza/Norvatis	Postmenstrual Syndrome
Fematrix	Estrogen	Ethical Holdings/Solvay Healthcare Ltd.	Postmenstrual Syndrome
FemPatch	Estradiol	Parke-Davis	Postmenstrual Syndrome
Habitraol	Nicotine	Novartis	Smoking cessation
Minitran	Nitroglycerin	3M Pharmaceuticals	Angina pectoris
Nicoderm	Nicotine	Alza/GlaxoSmithKline	Smoking

			Cessation
Nicotrol	Nicotine	Cygnus Inc./McNeil Consumer Products, Ltd.	Smoking Cessation
Nitrodisc	Nitroglycerin	Roberts Pharmaceuticals	Angina pectoris

2.3INTRODUCTION TO DRUG[16, 17, 18]

Drug name: REPAGLINIDE

Synonyms:REPAGLINIDUM

REPAGLINIDA

Structure -

Mol.formula: $C_{27}H_{36}N_2O_4$

Mol weight:452.586 g/mol

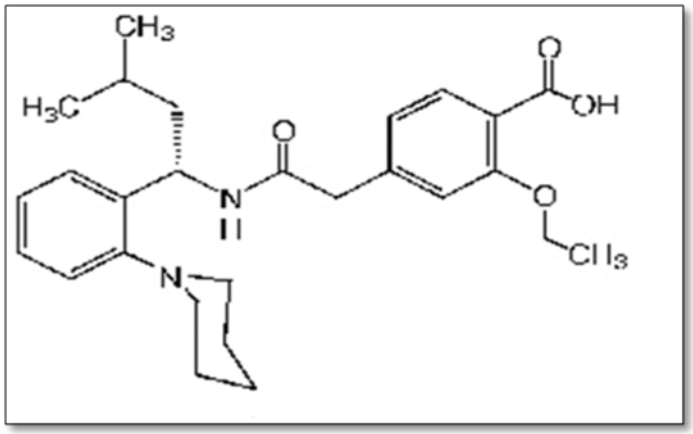


Figure 2.10- Structure of Repaglinide

Appearance:A white crystalline powder.

Solubility: Practically insoluble in water, freely soluble in dichloromethane & methanol.

Melting point: 130-131^{0}C

Storage : Protected from light.

Ionization constants or dissociation constants of Repaglinide:

Acid dissociation constant (pK_{a1}) = 3.96 ± 0.11

Base dissociation constant (pK_{a2}) = 6.20 ± 0.05

PHARMACOKINETIC PROPERTY

Absorption:

Repaglinide is absorbed rapidly and completely from the gastrointestinal tract, and peak blood levels are obtained within 1 hour. The mean absolute bioavailability is 56%.

Distribution:

Distributes into erythrocytes. Distributes into milk in rats; Protein drug binding 98%

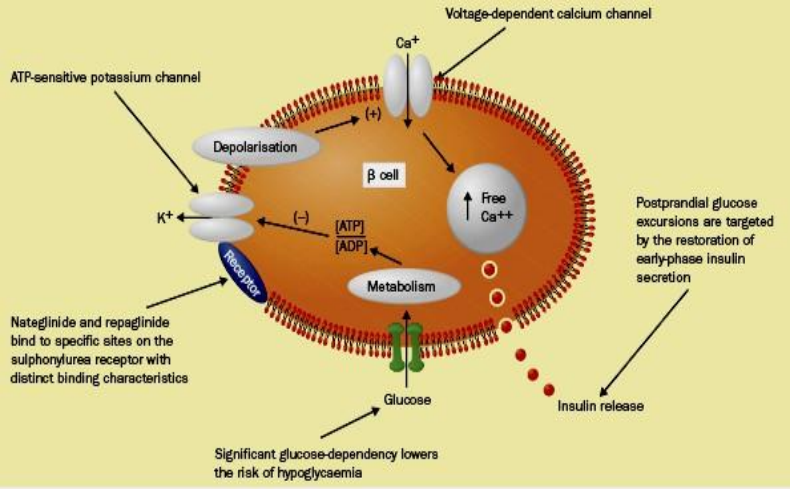


Figure: 2.11 Mechanism of action of Repaglinide

Metabolism:

Repaglinide is mainly metabolized in the liver by the CYP3A4 isoform of the P450 cytochrome enzyme to inactive derivatives. A small proportion (about 10%) of the drug is metabolized by the kidney. Metabolism is complete within 96 h.

Excretion:

Excreted into bile and feces (90%) as metabolites. Small amount excreted in urine (8%) principally as metabolites.

Mechanism of action:

Like sulfonylureas, repaglinide stimulates insulin release by closing ATP-dependent

potassium channels in pancreatic β cells. A high-affinity repaglinide binding site with lower affinity for glibenclamide has been identified with TC3 inslinma cells. By contrast with glibenclamide, repaglinide does not stimulate insulin secretion from TC3 insulinoma cells in the absence of glucose. Glucose metabolism, which depletes stores of ATP, or the binding of repaglinide to sites on the sulphonylureareceptor, causes the closure of ATP-sensitive potassium channels and the depolarization of the plasma membrane. Subsequent influx of calcium ions results in secretion of insulin.

Indications and usage:

Repaglinide is indicated as an adjunct to diet and exercise to lower the blood glucose in patients with type-II diabetes mellitus (NIDDM) whose hyperglycemia cannot be controlled satisfactorily by diet and exercise alone.

Dosage and administration:

Repaglinide is given by mouth up to 30 minutes before meals, in usual initial doses of 0.5 mg; initial doses of 1 or 2 mg are usually given to patients who have had previous hypoglycaemic treatment. The dose may be adjusted 3 to 4 times in a day. Repaglinide is also given with metformin or a thiazolidinedione in type 2 diabetes not adequately controlled by monotherapy.

2.4INTRODUCTION TO POLYMER

2.4.1 DURO-TAK 87-9301[19]

State: Liquid

Colorless

Solubility: Insoluble in water, Soluble in ethanol and methanol.

It is an acrylate polymer without any functional group.

It is pressure sensitive adhesive which exhibits excellent transdermal drug permeation, applied in an organic solvent solution.

2.4.2ETHYL CELLULOSE (EC):[20]

Nonproprietary names:BP:Ethylcellulose

PhEur: Ethylcellulosum

Synonyms: Aquacoat; Ethocel; Surelease

Chemical name: Cellulose ethyl ether

Empirical formula:EC is an ethyl ether of cellulose, a long-chain polymer consisting of anhydroglucose units joined together by acetal linkages.

Structural formula:

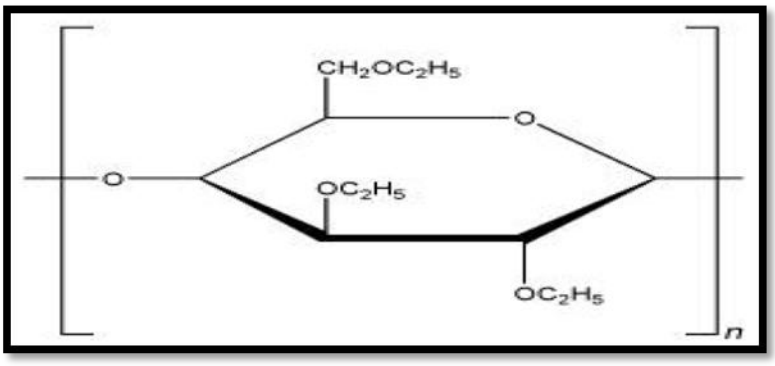


Figure 2.12- Structure of Ethyl cellulose

EC is an ethyl ether of cellulose, a long -chain polymer consisting of anhydroglucose units joined together by acetallinkages. Each anhydroglucose units has three replaceable hydroxyl groups which are substituted to the extent of 2.25-2.60 ethoxy groups per unit equivalent to ethoxy content of 44 - 51%.

Category: Coating agent; tablet binder; viscosity-increasing agent.

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

Solubility: Practically insoluble in glycerin, propylene glycol and water. EC that contains not less than 46.5% of ethoxygroups is freely soluble in chloroform, methyl

acetate, tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). EC that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

Viscosity: Various grades of EC which differ ethoxyl content and degree of polymerization may be used to produce 5% w/v solutions, in organic solvents, with viscosities of 6 -110 mPas.

2.4.3 POLYVINYLALCOHOL[20]

Synonyms: Polyvinyl alcohol; PVA; Polyvinol; ethanol homopolymer

Appearance: White free-flowing granules.

Mol. formula : $(C_2H_4O)_x$

Structure:

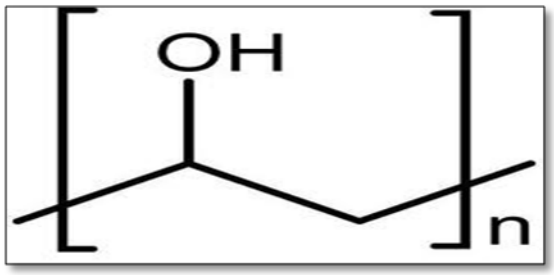


Figure 2.13- Structure of polyvinyl Alcohol

Odor: Mild odor.

Solubility: Moderately soluble.

Particle size: More than 95% through 14~120mesh screen.

TYPICAL PROPERTIES:

Specific gravity: 1.19 - 1.31

pH: Aqueous solution is neutral or slightly acid.

Density: 1.19-1.31 g/cm³

Melting point : 200°C

Boiling point : 228°C

Flash point: 79.44°C

Stability:

Stable under ordinary conditions of use and storage.

Storage:

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage.

Application:

Polyvinyl Alcohol is traditionally used for tablet film coating, as binder and as thickener in many pharmaceutical applications.

Especially its excellent film formation, high adhesion force, high film strength and superior moisture barrier function.

2.4.4HYDROXYPROPYLCELLULOSE(EF)[20]

Trademarks: Klucel (Hercules); Lacrisert (Merck & Co.)

Appearance: White or off-white granular solid, odourless.

Mol. wt.:Average Mw ~370,000

Structure:

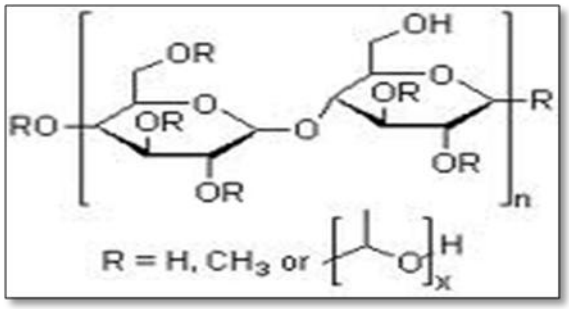


Figure 2.14- Structure of Hydroxypropyl cellulose

Solubility: Soluble in water below 38 °C

Insoluble in water above 45° C

Soluble in polar organic solvents such as: Methyl Alcohol -Ethyl Alcohol - Isopropyl Alcohol (95%) etc.

Particle size: Min 85 % through 30 meshes.

$_{p}$H range: 5.0 - 8.5

Stability: Stable. Incompatible with strong oxidizing agents.Combustibleform powder.

Storage:

Keep container dry. Keep in a cool place. Ground all equipment containing material. Keep container tightly closed. Keep in a cool, well-ventilated place. Combustible materials should be stored away from extreme heat and away from strong oxidizing agents.

Use:

As emulsifier, stabilizer, whipping aid, protective colloid, film former or thickener in foods; as binder in ceramics and glazes; in hair; in vacuum-formed containers and blow-molded bottles; as suspending agent in PVC polymerization. Pharmaceuticaid (tablet coating agent).

2.5INTRODUCTION TO PLASTICIZER

2.5.1 DIBUTYL PHTHALATE:[20]

Synonyms:

1,2-benzenedicarboxylic acid dibutyl ester; n-butyl phthalate; DBP; dibutyl benzene-1,2- dicarboxylate; di-n-butyl phthalate; phthalic acid dibutyl ester.

Empirical formula: $C_{16}H_{22}O_4$.

Molecular weight: 278.35

Structural formula:

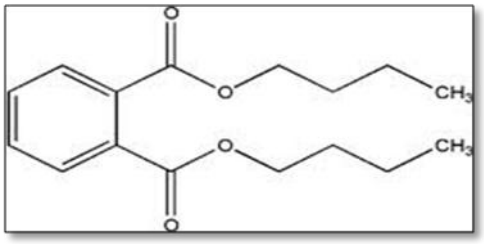


Figure 2.15- Structure of Ethyl cellulose

Description:

DBP occur as a clear, colorless or faintly.

TYPICAL PROPERTIES:

Boilingpoint:340^{0}C

Density:1.05 g/cm^3

Solubility: Very soluble in acetone, benzene, ethanol (95%).

Viscosity (dynamic): 15 mPas at 25^{0}C.

Applications in pharmaceutical formulations or technology: DBP is used as a plasticizer in film coatings. It is also used as an insect repellant, primarily for the impregnation of clothing.

2.5.2POLYETHYLENE GLYCOL (PEG) 400[20]

Nonproprietary names:BP: Macrogols

JP: Macrogol400

PhEur: Macrogola

USPNF: Polyethylene glycol

Synonyms: Carbowax, Carbowax Sentry, Lipo, Lipoxol, Lutrol E,PEG, Pluriol E, Polyethylene glycol (PEG).

Molecular weight: 380-420

Structure:

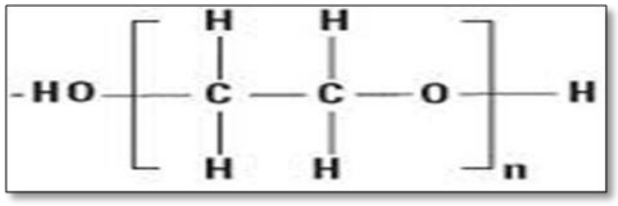


Figure 2.16- Structure of Polyethylene glycol

Description:

PEG grades 200- 600 are liquids while grades 1000 and above are solid at ambient temperatures. Liquid grades (PEG 200-600) occur as clear, colorless, or slightly yellow colored, viscous liquids. They have slight, but characteristic odor and bitter, slightly burning taste.

TYPICAL PROPERTIES:

Flash point: 238^0C

Freezing point: Density: 4-8^0C

Density:1.11-1.14 g/cm^3 at 25^0C for liquid PEGs.

Solubility: All grades of PEG are soluble in water and miscible in all proportions with other Polyethylene glycols (PEGs) (after melting if necessary). Aqueous solutions of higher molecular weight grades may formgels.Liquid PEGs are soluble in acetone, alcohols, benzene, glycerin and glycols.

Stability and storage conditions:

PEGs are chemically stable in air and in solution although grades with a molecular weight less than 2000 are hygroscopic. PEGs do not support microbial growth, nor do they become rancid. PEGs and aqueous PEG solutions can be sterilized by autoclaving, filtration or gamma irradiation.

APPLICATIONS IN PHARMACEUTICAL FORMULATIONS AND TECHNOLOGY:

PEGs are widely used in a variety of pharmaceutical formulations including parenteral, topical, ophthalmic, oral and rectal preparations. Solid grades are

generally employed in topical ointments with the consistency of base being adjusted by the addition of liquid grades of polyethylene glycol.

Mixtures of PEGs can be used as suppository bases. Aqueous PEG solutions can be used as water-miscible either as suspending agents or to adjust the viscosity and consistency of suspending agents. When used in conjunction with other emulsifiers, PEG scan act as emulsion stabilizers.

PEG300 & 400 has been vehicle for parenteral dosage forms. PEGs can also be used to enhance the aqueous solubility or dissolution characteristics of poorly soluble compounds by making solid dispersions. In film coatings, solid grades of PEG can be used alone for the film coating of tablets or can be useful as hydrophilic polishing materials.

Solid grades are also widely used as plasticizers in conjunction with film forming polymers. The presence of polymers especially liquid grades, in film coat tends to increase their water permeability.

3.0LITERATURE REVIEW

3.1 Literature review on Transdermal patch.

Panigrahiet al[3]proposed pseudo latex transdermal delivery system for delivery of terbutaline sulfate. The effect of pH and organic ester penetration enhancers on permeation kinetics of terbutaline sulfate through mice abdominal skin and human cadaver skin has been reported. Three permeation enhancer namely methyl laureate, isopropyl lanolate and isopropyl myristate were studied. Amongstthem, more pronounced enhancing effect was obtained with isopropyl myristate with respect to permeation flux, permeability coefficient and diffusion coefficient.

ThusharaBindu D et al[21] prepared transdermal patch of ibuprofen using gelatin, sodium benzoate and glycerin as main ingredients. In order to increase its permeability, permeation enhancers like olive oil, coconut oil and sun flower oil were used and the patches were characterized by evaluating physicochemical parameters like thickness, weight variation, folding endurance, drug content, breaking strength and ex vivo release study. From this study, it was concluded that olive oil is the better permeation enhancer for Ibuprofen transdermal patch.

Nicolibetal[22] investigated the in vitrokinetics of release and permeation of caffeine frombioadhesive transdermal films made of polyethylene membrane impregnated with isopropylmyristate. These films were not self-adhesive but became adhesive when applied to wet skin. The data obtained from present work suggest that release of caffeine from transdermal bioadhesive films was controlled either by the permeability characteristics of the skin or by the film itself, depending on drug loading.

H.S. Gwak et al., [23] studied effect of vehicles and penetration enhancers on transdermal

delivery of Ondansetron across dorsal hairless mouse skin. Among vehicles used, water and ethanol showed high permeation fluxes as 48.2 ± 23.7 & 41.9 ± 17.9 μg/cm2/hr. respectively. The highest flux was achieved at 40% of DGME combinations with PGMC & ethanol (80:20) and PGMC & PG (60:40) increased permeation by six- & two-fold respectively, compared to PGMC alone.

JainSunitaet al [24]developed matrix diffusion type of TDDS of captopril employing different ratios of polymers, EC and HPMC as (3:1) and (2:2). The *in vitro* skin permeation and *in vitro* dissolution studies showed that captopril release was more in matrices containing EC: HPMC as 2:2 compared to 3:1. Captopril from matrix containing EC: HPMC in ratio 2:2 was able to penetrate through rabbit abdominal skin. The *in vivo* study shows that the prepared matrices were free from any irritating effect and stable for three months.

Gannu R et al[25] prepared matrix type TDDS of nitrendipine by solvent evaporation technique. Ten formulations composed of Eudragit RL100 and HPMC 15 cps in the ratios of 5:0, 4:1, 3:2, 2:3, 1:4 was taken in A set and Eudragit RS100 and HPMC 15 cps in the same ratios was taken in B set. All formulations contained 6% w/vcarvone as penetration enhancer and 15% w/v of propylene glycol as plasticizer in dichloromethane and methanol as solvent system. The maximum drug release in 24 h for A series formulations was 89.29% (2:3) and 86.17% for B series (1:4).

Barhate SD et al[26] prepared carvedilol patches by solvent casting method using combination of PVA and PVP K30 along with glycerine, PEG 400 and PG asplasticizers. It was observed that the patch with PVA: PVP in the ratio 8:6 along with used plasticizers was a promising controlled release transdermal drug delivery system for carvedilol. The in vitrodrug skin permeation studies of the formulated transdermal patches revealed that the drug permeation from formulation containing 20% w/w and 40% w/w of PEG 400 were 91.50% and 94.21%, respectively.

Pandit JK et al[27] carried out *in vitro* iontophoretic delivery of glipizide across the pig skin. Matrix formulations of Eudragit E 100: NE 40D polymers (100:0, 70:30, 60:40, 50:50% w/w) with20% w/w of triacetine and 5% w/w of glipizidewere prepared by film casting method with different solvents(methanol, 2-propanol and acetone). In vitro releases of glipizide from the films were studied by vertical Franzdiffusion cells in HEPES buffer (pH 7.4) for 78 h.The target flux of glipizide was calculated to be 0.4147 µmol h^{-1}. As the highest flux obtained was 0.2727 µmol cm^{-2} h^{-1}, the author says that glipizide is a promising candidate for iontophoretic delivery.

Mutaliketal[28] preparedglipizide matrix transdermal systems for usingthe combinations of ethyl cellulose/polyvinylpyrrolidone-K30 (PVP) and Eudragit RL-100 (ERL)/Eudragit RS-100 (ERS). The systems were evaluated for various in vitro and in vivo parameters. The drug release was influenced by PVP and ERL 100 content of the patches. The in vivoresults revealed that the patches successfully prevented the severe hypoglycemia in the initial hour; they were also effective on chronic application.

Sankar V et al[29]investigated ethyl cellulose films for the permeation of the nifedipine drug through the film using castor oil and glycerol as the plasticizers. It was found that the drug release from the patches containing the glycerol as the plasticizer was more than that from the one containing castor oil.

Gattani SG et al[30]investigated transdermal films of chlorpheniramine maleate using different polymer combinations and concluded that hydrophilic polymer showed higher release than the lipophilic and hydrophilic-lipophilic combination.

Ubaidulla U et al[31] developed a matrix-type transdermal therapeutic system containing carvedilol with different ratios of hydrophilic and hydrophobic polymeric combinations by the solvent evaporation technique and reported that the developed transdermal patches

increased the efficacy of carvedilol for the therapy of hypertension by using different polymer ratios.

SaxenaM et al[32] prepared transdermal patches of metoclopramide hydrochloride using polyvinyl alcohol and polyvinylpyrrolidone. The combination of PVA: PVP in the ratio 1:4 containing 20 mg of drug showed the required sustained release effect.

Manvi FV et al[33]formulated transdermal patch of ketotifenfumarate usingcombination of eudragit L100: hydroxyl propylmethylcellulose and ethyl cellulose: Hydroxyl propylmethylcellulose as polymers along with permeation enhancers such asethyl sulfoxide and propylene glycol. Polyethylene glycol was used as a plasticizer. Itwas found that there was decrease in drug release rate from EL100: HPMC films incomparison to EC: HPMC was found, due to the hydrophobic nature of the polymer.

H.S. Gwak, et al[34]found feasibility of developing anOndansetron transdermal system using Duro-Tak 87-2100 and Duro-Tak 87-2196 aspressure sensitive adhesives (PSA). Effect of vehicles, propylene glycolmonocaprylate (PGMC)-diethylene glycol monoethyl ether (DGME)-propyleneglycol (PG) co solvents with 3% oleic acid, was studied & found that DGME inPGMC-DGME co solvent system decreased release rate as its concentration wasincreased. Also as amount of PSAs increased, the permeation flux was decreased.Overall fluxes from PSAs were significantly lower compared to those obtainedfrom solution formulations.

G Parthasarathyet al[35]developed transdermal drug delivery system of Naproxen with Ethylcellulose and Hydroxy propyl methyl cellulose polymer in various concentrations. Tramsdermal films were fabricated by matrix technique with various polymer proportions using dibutylphthalate as plasticizer. These transdermal drug patches were characterized for their thickness, tensile strength, content uniformity, in-vitro release. The release profiles were found to be varied with various concentrations of Ethylcellulose Polymer. The sample

of patches prepared with 2:8 and 8:2 ratios of Ethyl cellulose and Hydroxy propyl methyl cellulose shows highest and lowest in-vitro release of Naproxen respectively.

GilhotraRituMehra et al[36]prepared matrix type transdermal drug delivery system of celecoxib by the film casting method and characterized by physicochemical, in vitro by drug release studies, skin permeation studies and in vivo studies. Eight formulations were developed, which differed in the ratio of matrix forming polymers (PVA and HPMC) individually or in combination. All the eight formulations carried 2% m/m of celecoxib, eucalyptus oil or isopropyl alcohol as a permeation enhancer (10% v/v), plasticizer (20% w/w of polymer). Cumulative amounts of the drug released in 12 h from the eight formulations were ranged from 96.40±0.04 to 99.68±0.05 %. In vitro drug release was found to follow zero order kinetics and Higuchi kinetics.

G. Fetih et al[37] developed suitable film formulations of ketorolac tromethamine (KT) for transdermal use by using polyvinyl alcohol (PVA), sodium carboxymethylcellulose (NaCMC), and chitosan were used as film-forming polymers, and investigated the effect of film composition and permeation enhancers on the in-vitro release and skin permeation of the drug. The adhesive hydrophilic polymers plastoid® E35L (PL E35) and polyvinyl pyrrolidone (PVP) were added to improve bioadhesion. The permeation enhancers used were oleyl alcohol (OA), sodium glycocholate (NaGC) and propylene glycol (PG).Skin permeation of the drug was greatly improved by the addition of permeation enhancers, the rank of their effectiveness was: sodium glycocholate (Na GC) >oleyl alcohol (OA) > propylene glycol (PG). The results obtained showed that these polymeric films can be a promising therapeutic system for the transdermal delivery of ketorolac.

3.2 Literature review on Repaglinide.

PrajapatiShailesh T. et al [38]prepared Transdermal Patch of Repaglinide using solvent casting method. Transdermal patch of Repaglinide was prepared to sustain the release and improve bioavailability of drug and patient compliance. Different formulations were prepared by varying the grades of HPMC and concentration of PVP K30 by solvent casting method. The prepared formulations were evaluated for various parameters like thickness, tensile strength, folding endurance, % elongation, % moisture content, % moisture uptake, % drug content, in vitro drug release, in vitro permeation, and drug excipient compatibility. In vitro release data were fitted to various models to ascertain kinetic of drug release

SreeVidyanikethanet al[39]developed sustained release formulation of Repaglinide. The natural polymers like pectin, guar gum, xanthan gum were utilized in the formulation of matrix tablets containing Repaglinide by wet granulation technique and evaluated for its drug release characteristics. Pectin, guar gum and xanthan gum are hydrophilic and rate controlling polymers. Formulation was optimized on the basis of acceptable tablet properties hardness, friability, drug content, weight variations, swelling behavior and in vitro drug release.

Gupta Ashish et al[40]prepared microspheres of Repaglinide as model drug for prolongation of drug release time. An attempt was made to prepare microspheres of Repaglinide by Quasi emulsion solvent diffusion technique, with a view to deliver the drug at sustained or controlled manner in gastrointestinal tract and consequently into systemic circulation. The microspheres were formulated by using various concentration of HPMC, Ethyl cellulose and Eudragit RSPO as a retarding agent to control the release rate. The prepared microspheres were evaluated for Flow behavior, Compatibility study, Drug Entrapment Efficiency, In-vitro Dissolution, Scanning Electron Microscopy and Particle size analysis.

Nazir Imran et al[41]investigated Sustained release dosage form using microsphere of repaglinide by utilizing sodium alginate, olibanum gum and pectin in different ratios by using ionic-gelation method. Excellent results were found in rheological behavior and release studies. Microspheres size and percentage yield was found in the range of 694 μm to 727 μm and 73% to 75% respectively. SEM revealed that microspheres were discrete, spherical and free flowing. Entrapment efficiency was variable, ranges from 55% to 75%. Uniform drug release was observed in drug release kinetics, followed Higuchi model.

Chandra JagdishR.et al[42]evaluated poly (ε-caprolactone) microspheres of Repaglinide by using the solvent evaporation technique. The microspheres were prepared with different drug-to-carrier ratios: (1:3), (1:4), (1:5), and (1:6). The microspheres were then evaluated for particle size, SEM, FT-IR study, percentage yield, drug entrapment, stability studies, and for *in vitro* release kinetics. Scanning electron microscopy (SEM) revealed that microspheres were spherical with a nearly smooth surface morphology. The percentage yield and drug entrapment efficiency were high for all the formulations. The *in vitro* release study showed that Repaglinide release from all the formulations was slow and sustained over 12 h. Application of the *in vitro* drug release data to various kinetic equations indicated zero order release from Repaglinide microspheres.

Troy Purvis et al[43]produced rapidly dissolving formulation of poorly water-soluble drug Repaglinide using an innovative new technology, ultra rapid freezing (URF) and investigated the influence of different types and levels of excipients on Repaglinide stability. It was found that URF process yielded fast-dissolving formulations that were physically and chemically stable, and resistant to alkali.

4.0 EXPERIMENTAL WORK

MATERIALS AND EQUIPMENT USED

4.1 MATERIALS USED

Table 4.1-List of materials used

Drug	Repaglinide
Polymer	Duro-Tak87-9301 Hydroxypropylcellulose Polyvinylalcohol (PVA) Ethyl cellulose (EC)
Plasticizer	Polyethyleneglycol (PEG) Propylene glycol (PG) Dibutylpthelate (DBP)
Permeation enhancer	Isopropylmyristate (IPM) Dimethylsulfoxide (DMSO)
Solvent	Water Ethanol Acetone Isopropylalcohol(IPA)
Backing layer	3M Scotch-pack
Release liner	Saint-Gobain release liner Silicone coated release liner

4.2 LIST OF EQUIPMENT USED

Table-4.2 List of equipment used with company name

EQUIPMENTS	COMPANY NAME
Magnetic stirrer with hot plate	EIE Instrument Pvt Ltd., Ahmedabad
Q T S Texture Analyser	Brookfield Engineering laboratories
Digital Tensinometer	EIE Instruments, Ahmedabad
Thermonik Tablet Tester, DTH – 250	Campbell electronics, Mumbai
Sonicator Bath	EIE Instruments, Ahmedabad
UV/VIS Double beam spectrophotometer	Shimdzu UV 1800 corporation, Japan
Dissolution test apparatus USP	Electrolab TDT-08L, Mumbai
Hot air oven	EIE Instruments Pvt. Ltd., Ahmedabad
Humidity chamber	Nova Instruments Pvt. Ltd.Ahmedabad
FTIR	Jasco FTIR 6100 Type-A, Japan
Digital Balance	Citiweigh, Tejas exports, Ahmedabad

4.3 EVALUATION OF TRANSDERMAL PATCHES[13, 14]

(A)Physicochemical evaluation

(B)In vitroevaluation

(C)In vivoevaluation

(A) Physicochemical Evaluation:

(1) Thickness

Thickness of the films were measured by Thermonik Tablet Taster, DTH – 250. Patches were tested for three different positions by keeping the patch in between two jaws of machine and average thickness was calculated.

(2) % Elongation

It indicates the elasticity nature of the patch. Texture analyser was used to calculate the % elongation of the patch. Elongation was measured when the films breaks. Generally elongation of film increases as the plasticizer content increases.

$$\%\text{Elongation} = \frac{\text{Increase in length}}{\text{Original leangth}} \text{X100} \_\_\_\_\_ (1)$$

(3) Uniformity of weight

Weight variation is studied by individually weighing 10 randomlyselected patches and calculating the average weight. The individual weight should not deviate significantly from the average weight.

(4) Drug content determination

A 2 × 2 cm^2 patch was cut into small pieces and put in a 100 ml simulated saliva fluid. It is then shaken in a mechanical shaker for 2 hrs. To get a homogenous solution and filtered. Sample solutions were prepared by diluting to different concentrations and determined spectroscopically.

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in patch}}{\text{Theoretical amount of drug in patch}} \times 100 \text{----------(2)}$$

(5) Moisture content

The prepared films are weighed individually and kept indesiccators.These are then taken out and exposed to 84% relative humidity using calcium chloride at room temperaturefor 24 h. The films are weighed again after aspecified interval until they show a constantweight. The percent moisture content iscalculated using following formula.

$$\% \text{ moisture content} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100 \quad \text{---------- (3)}$$

(6) Moisture Uptake

Weighed films are kept in adesiccators at room temperature for 24 h. Theseare then taken out and exposed to 84% relativehumidity using saturated solution of Potassiumchloride in a desiccator until a constant weight isachieved. % moisture uptake is calculated asgiven below.

$$\textbf{\% moisture uptake} = \frac{\text{Increase in weight}}{\text{Initial weight}} \times 100 \quad \text{---------- (4)}$$

(7) Folding Endurance

Evaluation of foldingendurance involves determining the foldingcapacity of the films subjected to frequent extreme conditions of folding. Folding endurances determined by repeatedly folding the film at the same place until it break. The number of times the films could be folded at the same place without breaking is folding endurance value.

(8) Tensile strength

The mechanical properties of films were evaluated using a QTS Texture Analyzer. The peak load was used to evaluate by the help of probe Dual Grip Jig. The Texture expert software recorded the data when the probe started withdrawing from the film. The peak load and the area under load distance curve obtained from the texture profile were used to

assess the tensile strength and % elongation at of the films. Each measurement was repeated three times.

$$\text{Tensile Strength} = \frac{\text{Force at break (kg)}}{\text{Innitial cross sectional area of sample}} \times 100 \quad \text{---------- (5)}$$

Figure 4.1- Assembly of texture analyzer

(9) Tack properties

It is the ability of the polymer to adhere to substrate with little contact pressure. Tack is dependent on molecular weight and composition of polymer as well as on the use of tackifying resins in polymer.

(B)*In vitro* evaluation

In vitro release studies:

The Paddle over Disc:(USP apparatus 5/ PhEur 2.9.4.1).This method is identical to the USP paddle dissolution apparatus, except that the transdermal system is attached to a disc or cell resting at the bottom of the vessel which contains medium at 32 ±5°C.

The Cylinder modified USP Basket :(USP apparatus 6 / PhEur 2.9.4.3). This method issimilar to the USP basket type dissolution apparatus, except that the system is attached tothe surface of a hollow cylinder immersed in medium at 32 ±5°C.

In vitro permeation studies

In Vitro drug release studies were performed using Franz diffusion cell with a receptor compartment of capacity of 60 ml. Cellophane membrane was used for the determination of drug from the prepared transdermal matrix-type patches. Cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The prepared transdermal patch was placed on the cellophane membrane. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a hot plate magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads and the temperature was maintained at 32±0.5◦C. The samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically. The receptor phase was replenished with an equal volume of phosphate buffer at each sample withdrawal.

Ex-vivo permeation studies

Ex-vivo diffusion study was carried out using Franz diffusion cell. Abdominal skin of Guinea pig was used. Hair from the abdominal region was removed carefully by using hair removing cream; the dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels, equilibrate for an hour in phosphate buffer pH 7.4 before starting the experiment, and were placed on a magnetic stirrer. The cell was maintained at 32±0.5◦C using a thermostatically controlled heater. The isolated Guinea pig skin was mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Sample volume of 2ml was removed from the receptor compartment at regular interval, and an equal volume of fresh medium was

replaced. Samples were filtered through whatman filter and were analyzed using Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan).

(c) In vivo evaluation

In vivoevaluations are the true depiction of the drug performance.The variables which cannot be taken into account during *invitro* studies can be fully explored during *in vivo* studies. *Invivo* evaluation of TDDS can be carried out using:

• Animal models

• Human volunteers

Animal models

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

Human models

The final stage of the development of a transdermal device involvescollection of pharmacokinetic and pharmacodynamic data followingapplication of the patch to human volunteers. Clinical trials havebeen conducted to assess the efficacy, risk involved, side effects,patient compliance etc.

4.4 IDENTIFICATION OF REPAGLINIDE

4.4.1 MELTING POINT DETERMINATION

Melting point is the temperature at which the pure liquid and solid exist in the equilibrium. In the practice it is taken as equilibrium mixture at an external pressure of 1 atmosphere; this is sometime known as normal melting point. Melting point apparatus was used for melting point determination in the present study.

***T*able -4.3 Melting point determination of Repaglinide**

Actual melting point	**130-131˚C**
Observed melting point	**129-130˚C**

Result: The melting point of Repaglinide was found to be 129-130˚C.

Conclusion: The melting point determined is within the range of standard value, hence, it is concluded that the drug sample having intimate physical property as standard drug according to B.P.

4.4.2 IR Spectra of Repaglinide:

IR spectra of Repaglinide in KBR pellets at moderate scanning speed between 4000-400 cm^{-1} was carried out using FTIR (Jasco FTIR 6100 TYPE A, Japan). All the powder samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture.

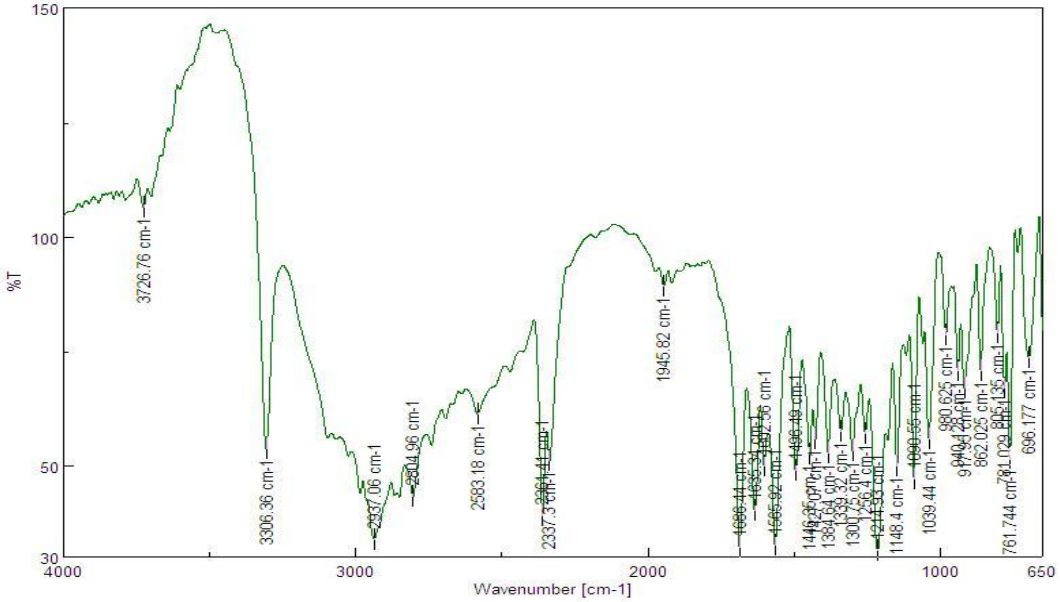


Table 4.2Comparison of reference and test IR frequency of Repaglinide

Functional Group	Standard frequency (cm^{-1})	Observed frequency (cm^{-1})
N- H	3306.36	3500-3100
C-O	1211.96	1300-1000
C-N	1300.75	1350-1100
COOH	2937.06	3400-2400
CONH2	1688.44	1680-1630

Discussion: The sample spectrum of Repaglinide was compared with standard frequency for each functional group.An observed spectrum was found to have similar peak values representing wave numbers as standard. Thus, it can be concluded that procured Repaglinide sample was a pure drug.

4.4.3 DETERMINATION OF ABSORPTION MAXIMA AND PREPARATION OF STANDARD CALIBRATION CURVE FOR REPAGLINIDE.

4.4.3.1 PREPARATION OF STANDARD SOLUTIONIN METHANOL:

PROCEDURE:

100 mg of Repaglinide was accurately weighed into a 100 ml volumetric flask and then volume was made up to 100 ml with methanol to get a concentration of 1000 µg/ml. From the above solution 10 ml was pipetted in a 100 ml volumetric flask and the volume solution was made up with methanol to get a concentration of 100 µg/ml .From thissolution,workingstandard solutions were prepared.

PREPARATION OF WORKING STANDARD SOLUTIONS:

From above solution aliquots of 1, 2, 3, 4, 5 ml were pipetted out into a series of 10 ml volumetric flasks and the volume was made with methanol to get a concentration ranging from 10-50 µg/ml. The absorbance of the resulting solutions was then measured using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance v/s concentration in µg/ml

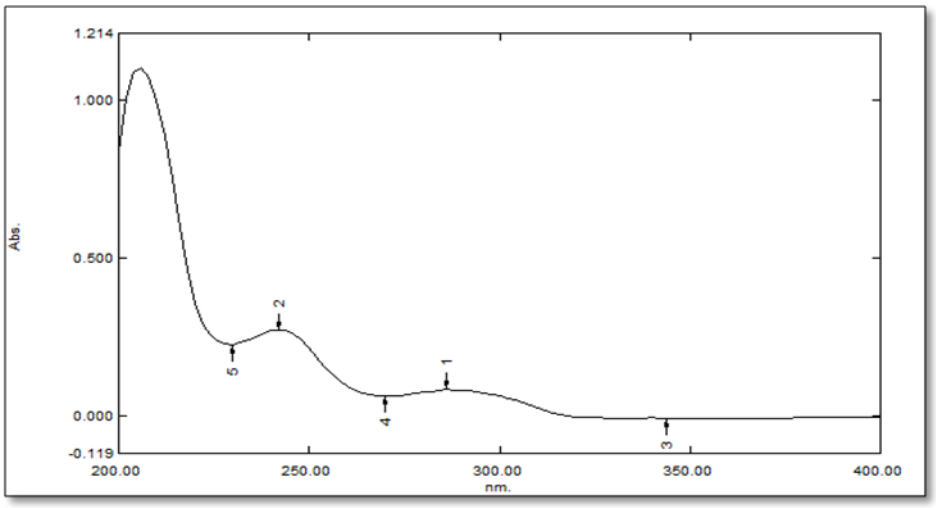


Figure 4.3- UV Absorbance spectra of Repaglinide

Table 4.5 Standard curve of Repaglinide using 95% methanol at 242 nm

SR NO	CONCERATION (mcg/ml)	ABSORBANCE (nm)			MEAN ABS.
1	0	0	0	0	0
2	10	0.2	0.197	0.2	0.199
3	20	0.426	0.413	0.418	0.419
4	30	0.629	0.629	0.601	0.618
5	40	0.847	0.836	0.831	0.838
6	50	0.981	0.971	0.979	0.997

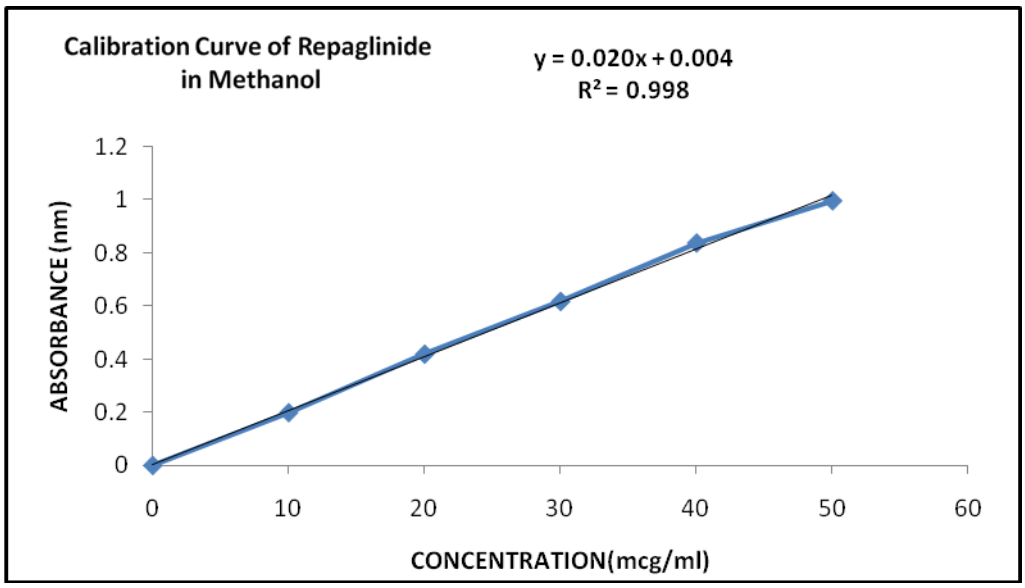


Figure 4.4: standard curve of Repaglinide in methanol at 242 nm

Regression Analysis

Table 4.6 -Regression analysis for 95 % of methanol

Regression parameter	Value
Correlation coefficient	0.998
Slope	0.020
Intercept	0.004

RESULT:

The correlation coefficient of calibration curves of Repaglinide in 95% of methanol was 0.998 and it was very close to 1. The linear graph obtained and the values of correlation coefficient showed that the Beer- Lambert's law was obeyed in the drug concentration range of 10-50 µg/ml.

4.4.3.2 PREPARATION OF STANDARD CALIBRATION CURVE FOR REPAGLINIDE USING 30 % METHANOLIC PHOSPHATE BUFFER (MIPB) Ph 7.4:

PROCEDURE:

1. **PREPARATION OF STANDARD SOLUTION:**

100 mg of Repaglinide was accurately weighed into a 100 ml volumetric flask and dissolved in small volume of MIPB pH 7.4 with sonication. The volume was made up to 100 ml with MIPB to get a concentration of 1000 µg/ml. From the above solution 10 ml was pipetted in a 100 ml volumetric flask and the volumes made up with MIPB to get a concentration of 100 µg/ml. From this, working standard solutions were prepared.

2. **PREPARATION OF WORKING STANDARD SOLUTIONS:**

From above aliquots of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 4.0, and 4.5 ml were pipetted out into

a series of 10 ml volumetric flasks and the volume was made with MIPB to get a concentration ranging from 5-45 µg/ml.

The absorbance of the resulting solutions was then measured using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance v/s concentration in µg/m

Table 4.7Standard curve of Repaglinide using methanolic phosphate buffer 7.4 at 283 nm

SR NO	CONCENTRATION (mcg/ml)	ABSORBANCE (nm)			MEAN
1	0	0	0	0	0
2	5	0.119	0.119	0.122	0.12
3	10	0.2	0.202	0.2	0.2
4	15	0.325	0.327	0.33	0.327
5	20	0.422	0.426	0.426	0.423
6	25	0.534	0.538	0.539	0.537
7	30	0.652	0.651	0.652	0.651
8	35	0.772	0.774	0.780	0.775
9	40	0.869	0.871	0.868	0.869
10	45	0.981	0.986	0.989	0.985

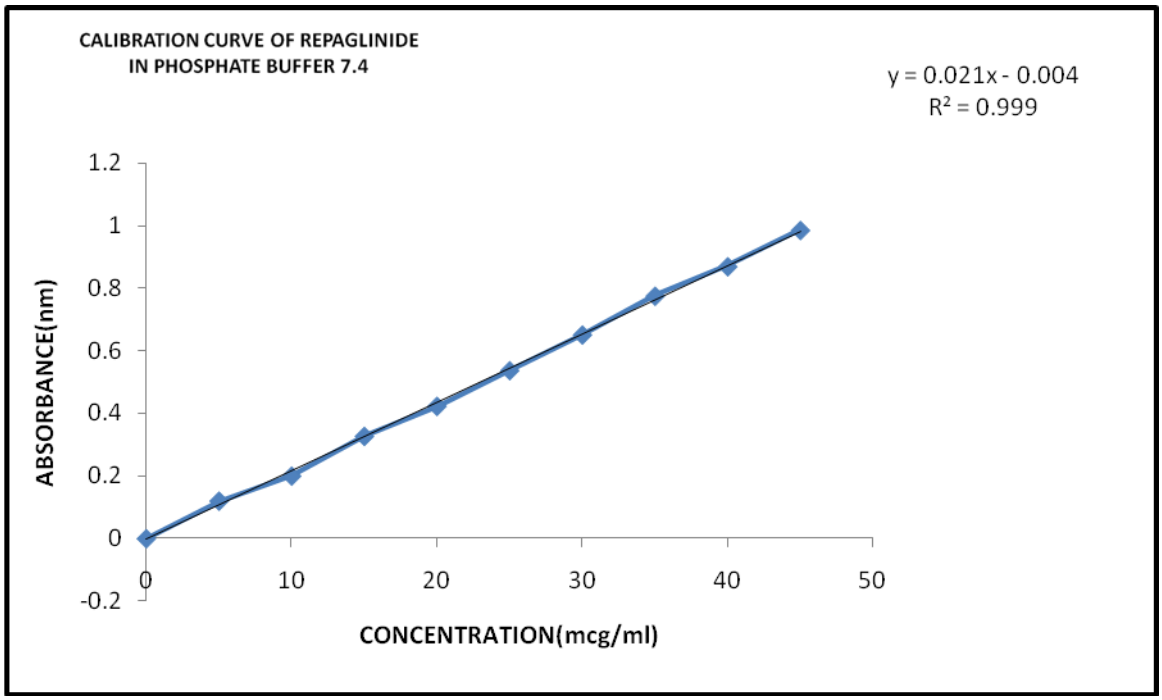


Figure 4.5-Standered curve of Repaglinide in phosphate buffer 7.4

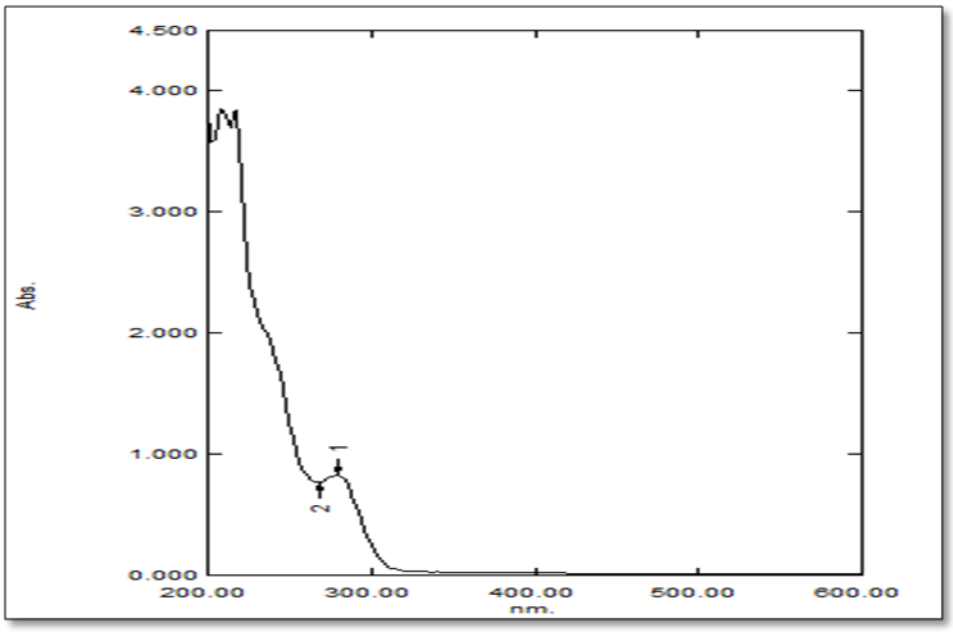


Figure 4.6- UV Absorbance spectra of Repaglinide in 7.4 phosphate buffer

Regression Analysis

Table 4.8 -Regression analysis for 7.4 phosphate buffer

Regression parameter	Value
Correlation coefficient	0.999
Slope	0.021
Intercept	0.004

RESULT:

The correlation coefficient of calibration curves of Repaglinide in 30 % methanolic 7.4 phosphate buffer was 0.998 and it was very close to 1. The linear graph obtained and the values of correlation coefficient showed that the Beer- Lambert's law was obeyed in the drug concentration range of 5-45 µg/ml.

4.5 PREPARATION OF TRANSDERMAL PATCH USING DURO-TAK 87-9301 AS POLYMER

(4.5.1) Preliminary Trials

(A) Optimization of Casting Surface

Initial trials for optimizing casting surface were carried out using 70%w/w and 90 %w/w of film as forming agent like Duro-Tak 87-9301 and ethanol as solvent. All the formulations were casted on glass, plastic, teflonpetridish, silicon coated liner and mercury surface. All the casted films were evaluated for the peel ability.

Table 4.9Optimization of Casting Surface

Batch no	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Duro-Tak 87-9301 (% w/w)	70	90	70	90	70	90	70	90	70	90
Casting surface	Glass	Glass	Plastic	Plastic	Mercury	Mercury	Teflon	Teflon	Slicon coated liner	Slicon coated liner
Film separation	No	No	No	No	No	No	No	No	No	No

Conclusion:

From the results of batches C1- C10 where Duro-Tak 87-9301 was used as polymer, it was concluded that film separation could not be achieved in any batch using different casting surface such as plastic, glass, teflon Petridis and silicon coated release liner. Therefore Scotch-Pack was used as backing layer where the separation of film is not required as the evaluation can be carried out directly using backing layer.

(B) Optimization of concentration of polymer and permeation enhancer

Transdermal patches of Repaglinide were prepared using solvent casting method. Different concentration of Duro-Tak 87-9301 was accurately weighed and dissolved in 20 ml of ethanol. Repaglinide was dissolved in the above solution and mixed until clear solution was obtained. Different concentration of Isopropyl Myristate (IPM) was used as permeation enhancer. The uniform solution was casted on the scotch pack backing layer and dried at 80 ^{0}C temperature for 8 h. After 8 h, the dried patches were taken out for further evaluation studies.

Calculation of drug dose

Total Surface area of petridish: πr^2

$= 3.14 \times (4.5)^2$

$= 63.58\ cm^2$

Size of patch= $2 \times 2 = 4\ cm^2$

Number of patches: $63.58 \div 4 = 15.89$

Dose of Repaglinide= 4 mg

Dose of Repaglinide per petridish= $15.89 \times 4 = 81.64$ mg

Table 4.10Optimization of concentration of polymer and permeation enhancer

Batch No	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**
Repaglinide (mg)	63.58			63.58			63.58		
Duro-Tak 87-9301 (%w/w)	70			80			90		
IPM (%w/v)	5	10	15	5	10	15	5	10	15
Ethanol (ml)	20			20			20		

Each batch contains 4mg drug in 4 cm^2 area

Evaluation parameters of batches D1 to D9

Table 4.11 Evaluation parameters of batches D1 to D9

Batch No	Thickness (mm)	Moisture content (%)	Moisture uptake (%)	Avg. weight (mg)
D1	0.36	6.90	3.65	146
D2	0.40	8.88	4.07	147
D3	0.41	14.3	3.35	156
D4	0.43	9.74	3.59	167
D5	0.45	14.86	3.46	170
D6	0.49	15.89	4.25	172
D7	0.56	8.14	2.70	187
D8	0.57	7.30	3.15	191
D9	0.50	8.38	4.23	192

Discussion:

It was found that batch D6 containing 80% w/w of Duro-Tak 87-9301 and 15 % w/v of IPM showed highest moisture content and moisture uptake.Thickness of patches was varying from 0.36 to 0.57mm and average weight was varying from 146mg to 192 mg as the amount of Duro-Tak 87-9301 was increased from 5 to 15 %. Thus, it was concluded that as the amount of Duro-Tak 87-9301 increased, thickness of patch also increased. To check the amount of drug permeability through the semi-permeable membrane, in-vitro diffusion was performed in batches D1 to D9.

4.5.2 DIFFUSION STUDY OF BATCHES D1 TO D9

(A) IN- VITRO DIFFUSION STUDY OF BATCHES D1 TO D9

In-vitro diffusion study of batches containing 70% Duro-Tak 87-9301 with varying % of IPM.

In-vitro diffusion studyof batches D1 to D3:

Table 4.12 In-vitro diffusion study of batch D1

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.16	7.48	14.95	0.03	0.90	0.00	0.90	22.43
2	2	0.17	8.05	16.10	0.03	0.97	0.03	1.00	24.89
3	3	0.18	8.33	16.67	0.03	1.00	0.06	1.06	26.55
4	4	0.18	8.57	17.14	0.03	1.03	0.10	1.12	28.10
5	5	0.21	9.57	19.14	0.04	1.15	0.13	1.28	31.96
6	6	0.22	10.33	20.67	0.04	1.24	0.17	1.41	35.20
7	7	0.24	11.10	22.19	0.04	1.33	0.21	1.54	38.52
8	8	0.26	12.29	24.57	0.05	1.47	0.25	1.73	43.20
9	9	0.28	13.05	26.10	0.05	1.57	0.30	1.87	46.71
10	10	0.32	15.00	30.00	0.06	1.80	0.36	2.16	53.88
11	24	0.48	22.62	45.24	0.09	2.71	0.42	3.13	78.23

Table4.13 In-vitro diffusion study of batch D2

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution Factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.19	8.95	17.90	0.04	1.07	0.00	1.07	26.86
2	2	0.25	11.76	23.52	0.05	1.41	0.04	1.45	36.18
3	3	0.29	0	0	0	1.62	0.08	1.70	42.50

4	4	0.30	1	1	0.19	1.69	0.14	1.82	45.56
5	5	0.31	14.43	28.86	0.06	1.73	0.19	1.92	48.11
6	6	0.33	15.62	31.24	0.06	1.87	0.25	2.12	53.12
7	7	0.35	16.48	32.95	0.07	1.98	0.31	2.29	57.26
8	8	0.38	17.95	35.90	0.07	2.15	0.38	2.53	63.33
9	9	0.40	18.90	37.81	0.08	2.27	0.45	2.72	67.99
10	10	0.47	22.10	44.19	0.09	2.65	0.53	3.18	79.45
11	24	0.51	24.19	48.38	0.10	2.90	0.61	3.52	87.94

Table 4.14 In-vitro diffusion study of batch D3:

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.15	7.10	14.19	0.03	0.85	0.00	0.85	21.29
2	2	0.18	8.52	17.05	0.03	1.02	0.03	1.05	26.28
3	3	0.21	9.81	19.62	0.04	1.18	0.06	1.24	30.99
4	4	0.23	10.81	21.62	0.04	1.30	0.10	1.40	34.97
5	5	0.24	11.19	22.38	0.04	1.34	0.14	1.49	37.20
6	6	0.27	12.71	25.43	0.05	1.53	0.19	1.72	42.89
7	7	0.27	12.62	25.24	0.05	1.51	0.24	1.75	43.87

8	8	0.29	13.81	27.62	0.06	1.66	0.29	1.95	48.70
9	9	0.31	14.57	29.14	0.06	1.75	0.35	2.09	52.37
10	10	0.30	14.29	28.57	0.06	1.71	0.40	2.12	52.97
11	24	0.41	19.52	39.05	0.08	2.34	0.46	2.80	70.11

Comparative in vitro diffusion study of batch D1 to D3

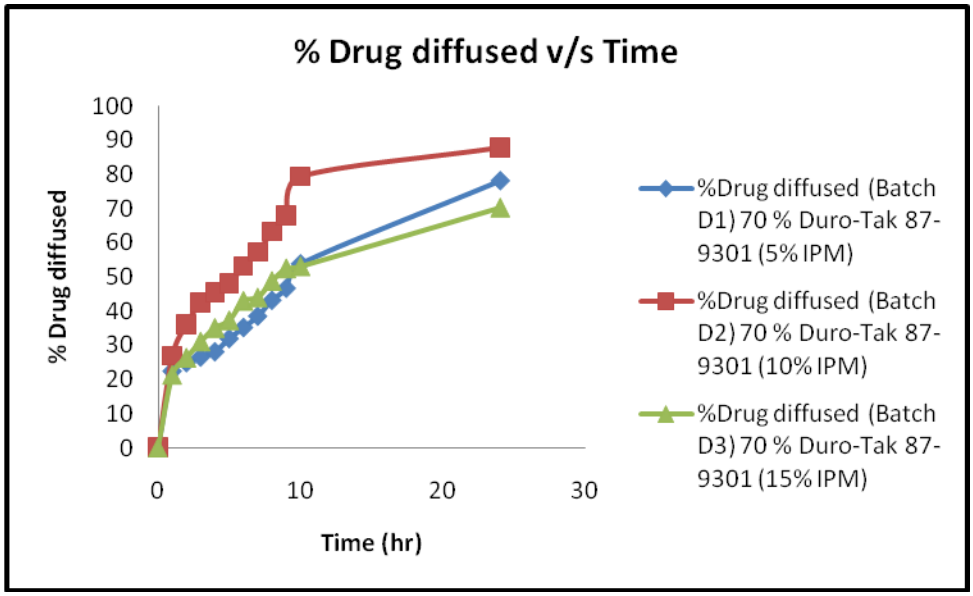


Figure 4.7 Comparative in vitro diffusion study of batch D1 to D3

Result:

The result indicated that patch containing 70 %Duro-Tak 87-9301 and 10% IPM (Batch D2) showed higher release of drug (87.94%) in 24 hr as compared to batch D1 and D2.

In-vitro diffusion study of batches containing 80% Duro-Tak 87-9301 with varying % of IPM.

In-vitro diffusion study of batchesD4 to D6:

Table 4.15 In-vitro diffusion study of batch D4

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	5.95	11.90	0.02	0.71	0.00	0.71	17.86
2	2	0.16	7.29	14.57	0.03	0.87	0.02	0.90	22.45
3	3	0.17	7.81	15.62	0.03	0.94	0.05	0.99	24.75
4	4	0.19	8.95	17.90	0.04	1.07	0.08	1.16	28.96
5	5	0.20	9.48	18.95	0.04	1.14	0.12	1.26	31.43
6	6	0.22	10.24	20.48	0.04	1.23	0.16	1.39	34.66
7	7	0.22	10.29	20.57	0.04	1.23	0.20	1.43	35.83
8	8	0.26	12.33	24.67	0.05	1.48	0.24	1.72	43.00
9	9	0.29	13.38	26.76	0.05	1.61	0.29	1.90	47.38
10	10	0.37	17.38	34.76	0.07	2.09	0.34	2.43	60.71
11	24	0.50	23.38	46.76	0.09	2.81	0.41	3.22	80.45

Table 4.16 In-vitro diffusion study of batch D5

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.57	11.14	0.02	0.67	0.00	0.67	16.71
2	2	0.16	7.43	14.86	0.03	0.89	0.02	0.91	22.84
3	3	0.17	7.81	15.62	0.03	0.94	0.05	0.99	24.73
4	4	0.19	8.81	17.62	0.04	1.06	0.08	1.14	28.51
5	5	0.22	10.24	20.48	0.04	1.23	0.12	1.35	33.68
6	6	0.26	12.33	24.67	0.05	1.48	0.16	1.64	40.99
7	7	0.36	16.76	33.52	0.07	2.01	0.21	2.22	55.50
8	8	0.36	16.71	33.43	0.07	2.01	0.28	2.28	57.04
9	9	0.40	19.05	38.10	0.08	2.29	0.34	2.63	65.71

10	10	0.42	19.62	39.24	0.08	2.35	0.42	2.77	69.33
11	24	0.54	25.57	51.14	0.10	3.07	0.50	3.57	89.15

Table 4.17 In-vitro diffusion study of batch D6

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.43	12.86	0.03	0.77	0.00	0.77	19.29
2	2	0.17	8.00	16.00	0.03	0.96	0.03	0.99	24.64
3	3	0.22	10.48	20.95	0.04	1.26	0.06	1.31	32.87
4	4	0.27	12.76	25.52	0.05	1.53	0.10	1.63	40.78
5	5	0.31	14.71	29.43	0.06	1.77	0.15	1.92	47.91
6	6	0.32	14.81	29.62	0.06	1.78	0.21	1.99	49.67
7	7	0.38	17.95	35.90	0.07	2.15	0.27	2.42	60.58
8	8	0.38	17.81	35.62	0.07	2.14	0.34	2.48	61.94
9	9	0.40	19.05	38.10	0.08	2.29	0.41	2.70	67.44
10	10	0.42	19.76	39.52	0.08	2.37	0.49	2.86	71.49
11	24	0.50	23.71	47.43	0.09	2.85	0.57	3.41	85.32

Comparative in vitro diffusion study of batch D4 to D6

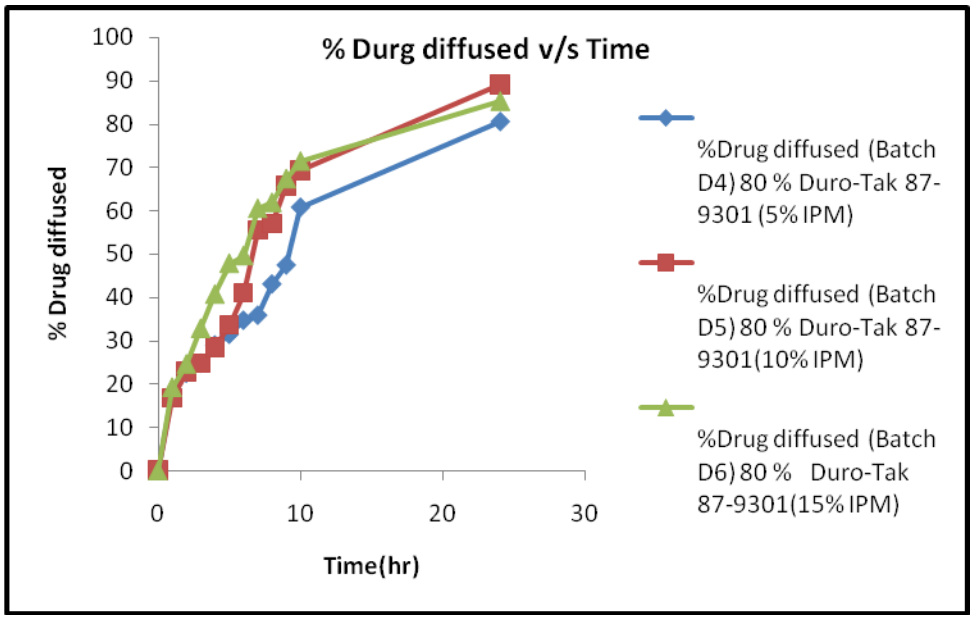


Figure 4.8 Comparative in vitro diffusion study of batch D4 to D6

Result:

The batch D5 (80 % Duro-Tak 87-9301 and 10 % IPM) showed 89.15 % drug release in 24 hours which was higher than batch (80.32%) and D6 (85.32%).

In-vitro diffusion study of batches containing 90% Duro-Tak 87-9301 with varying % of IPM.

In-vitro diffusion study of batchesD7 to D9:

Table 4.18In-vitro diffusion study of batch D7

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	5.95	11.90	0.02	0.71	0.00	0.71	17.86
2	2	0.16	7.29	14.57	0.03	0.87	0.02	0.90	22.45
3	3	0.17	7.81	15.62	0.03	0.94	0.05	0.99	24.75

4	4	0.19	8.95	17.90	0.04	1.07	0.08	1.16	28.96
5	5	0.20	9.48	18.95	0.04	1.14	0.12	1.26	31.43
6	6	0.22	10.24	20.48	0.04	1.23	0.16	1.39	34.66
7	7	0.22	10.29	20.57	0.04	1.23	0.20	1.43	35.83
8	8	0.26	12.33	24.67	0.05	1.48	0.24	1.72	43.00
9	9	0.29	13.38	26.76	0.05	1.61	0.29	1.90	47.38
10	10	0.37	17.38	34.76	0.07	2.09	0.34	2.43	60.71
11	24	0.42	19.95	39.90	0.08	2.39	0.41	2.81	70.17

Table 4.19 In-vitro diffusion study of batch D8

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.57	11.14	0.02	0.67	0.00	0.67	15.14
2	2	0.16	7.43	14.86	0.03	0.89	0.02	0.91	20.34
3	3	0.17	7.81	15.62	0.03	0.94	0.05	0.99	22.65
4	4	0.19	8.81	17.62	0.04	1.06	0.08	1.14	25.23
5	5	0.22	10.24	20.48	0.04	1.23	0.12	1.35	30.38
6	6	0.26	12.33	24.67	0.05	1.48	0.16	1.64	37.18
7	7	0.36	16.76	33.52	0.07	2.01	0.21	2.22	52.40

8	8	0.36	16.71	33.43	0.07	2.01	0.28	2.28	54.035
9	9	0.40	19.05	38.10	0.08	2.29	0.34	2.63	62.09
10	10	0.42	19.62	39.24	0.08	2.35	0.42	2.77	67.89
11	24	0.48	22.71	45.43	0.09	2.73	0.50	3.22	80.58

Table 4.20 In-vitro diffusion study of batch D9

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.57	11.14	0.02	0.67	0.00	0.67	16.71
2	2	0.15	7.10	14.19	0.03	0.85	0.02	0.87	21.84
3	3	0.17	7.86	15.71	0.03	0.94	0.05	0.99	24.84
4	4	0.19	8.86	17.71	0.04	1.06	0.08	1.14	28.62
5	5	0.21	9.62	19.24	0.04	1.15	0.12	1.27	31.80
6	6	0.24	11.10	22.19	0.04	1.33	0.16	1.49	37.19
7	7	0.25	11.86	23.71	0.05	1.42	0.20	1.62	40.58
8	8	0.27	12.67	25.33	0.05	1.52	0.25	1.77	44.20
9	9	0.29	13.57	27.14	0.05	1.63	0.30	1.93	48.18
10	10	0.38	17.81	35.62	0.07	2.14	0.35	2.49	62.25
11	24	0.405	21.90	43.81	0.09	2.63	0.42	3.05	69.12

Comparative in vitro diffusion study of batch D7 to D9:

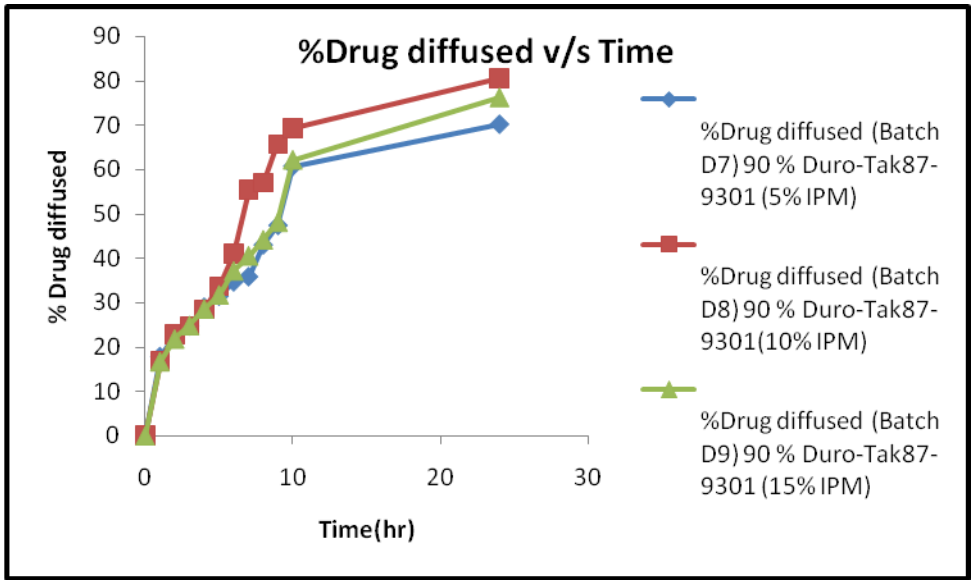


Figure 4.9 Comparative in vitro diffusion study of batch D7 to D9:

Result:

Table 4.21 Result of in-vitro diffusion study at 24 hr.

Batch No:	% Drug Diffused at 24 hr.
D1	78.23
D2	87.94
D3	70.11
D4	80.45
D5	89.15
D6	85.32
D7	70.17
D8	80.58
D9	69.12

Discussion:

In Vitro drug release studies were performed using a Franz diffusion cell with different concentration from 5 to 15 % w/v of Duro-Tak 87-9301 as polymer and 5 to 15 %w/v of IPM as permeation enhancer. As shown in Table 4.45, as concentration of Duro-Tak 87-9301 increases from 5 to 15 % w/v, there was increase in release of drug but higher release was not obtained as concentration of Duro-Tak87-9301 was further increased from 10 to 15 %w/v.Thus further increased in concentration of polymer had no effect on drug release. Similar types of results were observed with IPM. Up to certain concentration of IPM drug release was increased and further increased in concentration of IPM had no effect on drug release. Hence it was observed that batch D7 (90 % w/w of Duro-Tak 87-9301 and 5% IPM) showed minimum release of drug (70.17%) and batch D5 (80% w/w of Duro-Tak 87-9301and 10% IPM) showed maximum release of drug (89.15%). So batch D5 was selected for further studies.

(B) *EX-VIVO* DIFFUSION STUDY OF BATCH D1 TO D9

Ex-vivo diffusion study of batches containing 70% Duro-Tak 87-9301 with varying % of IPM.

Ex-vivo diffusion study of batch D1 to D3:

Table 4.22 Ex-vivo diffusion study of batch D1

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.17	7.86	15.71	0.03	0.94	0.00	0.94	23.57
2	2	0.18	8.38	16.76	0.03	1.01	0.03	1.04	25.93
3	3	0.19	8.62	17.24	0.03	1.03	0.06	1.10	27.48

4	4	0.20	9.24	18.48	0.04	1.11	0.10	1.21	30.20
5	5	0.21	9.86	19.71	0.04	1.18	0.14	1.32	32.98
6	6	0.23	10.90	21.81	0.04	1.31	0.18	1.48	37.11
7	7	0.25	11.57	23.14	0.05	1.39	0.22	1.61	40.20
8	8	0.27	12.67	25.33	0.05	1.52	0.27	1.79	44.64
9	9	0.29	13.57	27.14	0.05	1.63	0.32	1.94	48.62
10	10	0.33	15.67	31.33	0.06	1.88	0.37	2.25	56.27
11	24	0.49	23.19	46.38	0.09	2.78	0.43	3.22	80.40

Table 4.23 Ex-vivo diffusion study of batch D2

Sr no	**Time (hr)**	**Abs. (nm)**	**mcg/ml**	**Dilution factor**	**mg/2ml**	**mg/60ml**	**Error**	**Final Conc**	**% Drug Diffused**
0	0	0	0	0	0	0	0	0	0
1	1	0.18	8.48	16.95	0.03	1.02	0.00	1.02	25.43
2	2	0.25	11.62	23.24	0.05	1.39	0.03	1.43	35.70
3	3	0.29	13.62	27.24	0.05	1.63	0.08	1.71	42.87
4	4	0.30	14.19	28.38	0.06	1.70	0.13	1.84	45.94
5	5	0.31	14.76	29.52	0.06	1.77	0.19	1.96	49.08
6	6	0.34	16.05	32.10	0.06	1.93	0.25	2.18	54.41
7	7	0.36	17.05	34.10	0.07	2.05	0.31	2.36	59.01
8	8	0.40	18.71	37.43	0.07	2.25	0.38	2.63	65.72
9	9	0.42	20.00	40.00	0.08	2.40	0.46	2.86	71.45
10	10	0.48	22.76	45.52	0.09	2.73	0.54	3.27	81.73
11	24	0.52	24.52	49.05	0.10	2.94	0.63	3.57	89.29

Table 4.24 Ex-vivo diffusion study of batch D3

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.16	7.38	14.76	0.03	0.89	0.00	0.89	22.14
2	2	0.19	8.62	17.24	0.03	1.03	0.03	1.06	26.60
3	3	0.22	10.05	20.10	0.04	1.21	0.06	1.27	31.74
4	4	0.24	11.19	22.38	0.04	1.34	0.10	1.45	36.18
5	5	0.25	11.90	23.81	0.05	1.43	0.15	1.58	39.44
6	6	0.28	13.10	26.19	0.05	1.57	0.20	1.77	44.20
7	7	0.30	14.00	28.00	0.06	1.68	0.25	1.93	48.22
8	8	0.31	14.67	29.33	0.06	1.76	0.30	2.06	51.62
9	9	0.34	15.81	31.62	0.06	1.90	0.36	2.26	56.52
10	10	0.35	16.38	32.76	0.07	1.97	0.43	2.39	59.81
11	24	0.45	21.14	42.29	0.08	2.54	0.49	3.03	75.74

Comparative ex-vivo diffusion study of batch D1 to D3:

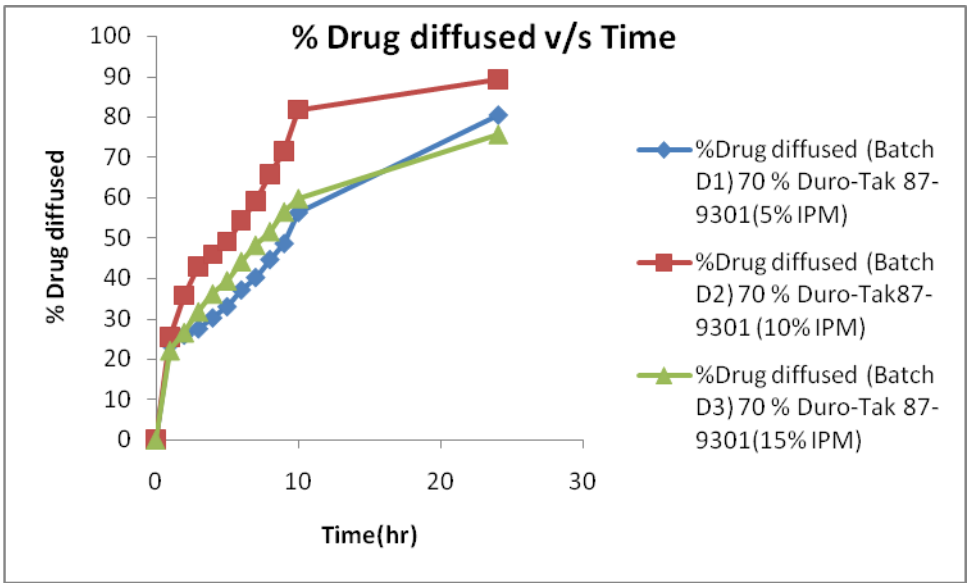


Figure 4.10 Comparative ex-vivo diffusion study of batch D1 to D3:

Result:

The result indicated that patch containing 70% w/w of Duro-Tak 87-9301 and 10% IPM (Batch D2) showed higher release of drug (71%) in 24 hr as compared to batch D1 (80.40%) and D3(75.74%).

Ex-vivo diffusion study of batches containing 80% Duro-Tak 87-9301 with varying % of IPM.

Ex-vivo diffusion study of batch D4 to D6:

Table 4.25 Ex-vivo diffusion study of batch D4

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	6.19	12.38	0.02	0.74	0.00	0.74	18.57
2	2	0.16	7.62	15.24	0.03	0.91	0.02	0.94	23.48
3	3	0.17	8.00	16.00	0.03	0.96	0.06	1.02	25.38
4	4	0.20	9.24	18.48	0.04	1.11	0.09	1.20	29.90
5	5	0.22	10.14	20.29	0.04	1.22	0.12	1.34	33.53
6	6	0.23	10.71	21.43	0.04	1.29	0.16	1.45	36.26

7	7	0.25	11.76	23.52	0.05	1.41	0.21	1.62	40.48
8	8	0.29	13.52	27.05	0.05	1.62	0.25	1.88	46.94
9	9	0.30	14.00	28.00	0.06	1.68	0.31	1.99	49.72
10	10	0.38	17.86	35.71	0.07	2.14	0.36	2.51	62.69
11	24	0.50	23.52	47.05	0.09	2.82	0.44	3.26	81.47

Table 4.26 Ex-vivo diffusion study of batch D5

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	5.95	11.90	0.02	0.71	0.00	0.71	17.86
2	2	0.16	7.62	15.24	0.03	0.91	0.02	0.94	23.45
3	3	0.19	8.81	17.62	0.04	1.06	0.05	1.11	27.79
4	4	0.20	9.48	18.95	0.04	1.14	0.09	1.23	30.67
5	5	0.24	11.19	22.38	0.04	1.34	0.13	1.47	36.76
6	6	0.28	13.19	26.38	0.05	1.58	0.17	1.76	43.88
7	7	0.33	15.43	30.86	0.06	1.85	0.22	2.08	51.91
8	8	0.37	17.48	34.95	0.07	2.10	0.29	2.38	59.60
9	9	0.41	19.29	38.57	0.08	2.31	0.36	2.67	66.77
10	10	0.43	20.38	40.76	0.08	2.45	0.43	2.88	71.99
11	24	0.56	26.29	52.57	0.11	3.15	0.52	3.67	91.74

Table 4.27 Ex-vivo diffusion study of batch D6

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.48	12.95	0.03	0.78	0.00	0.78	19.43
2	2	0.18	8.29	16.57	0.03	0.99	0.03	1.02	25.50

3	3	0.22	10.24	20.48	0.04	1.23	0.06	1.29	32.19
4	4	0.27	12.57	25.14	0.05	1.51	0.10	1.61	40.21
5	5	0.31	14.67	29.33	0.06	1.76	0.15	1.91	47.76
6	6	0.34	16.00	32.00	0.06	1.92	0.21	2.13	53.22
7	7	0.38	17.86	35.71	0.07	2.14	0.27	2.42	60.40
8	8	0.39	18.43	36.86	0.07	2.21	0.34	2.56	63.90
9	9	0.41	19.43	38.86	0.08	2.33	0.42	2.75	68.74
10	10	0.42	19.95	39.90	0.08	2.39	0.50	2.89	72.25
11	24	0.52	24.62	49.24	0.10	2.95	0.58	3.53	88.25

Comparative ex-vivo diffusion study of batch D4 to D6:

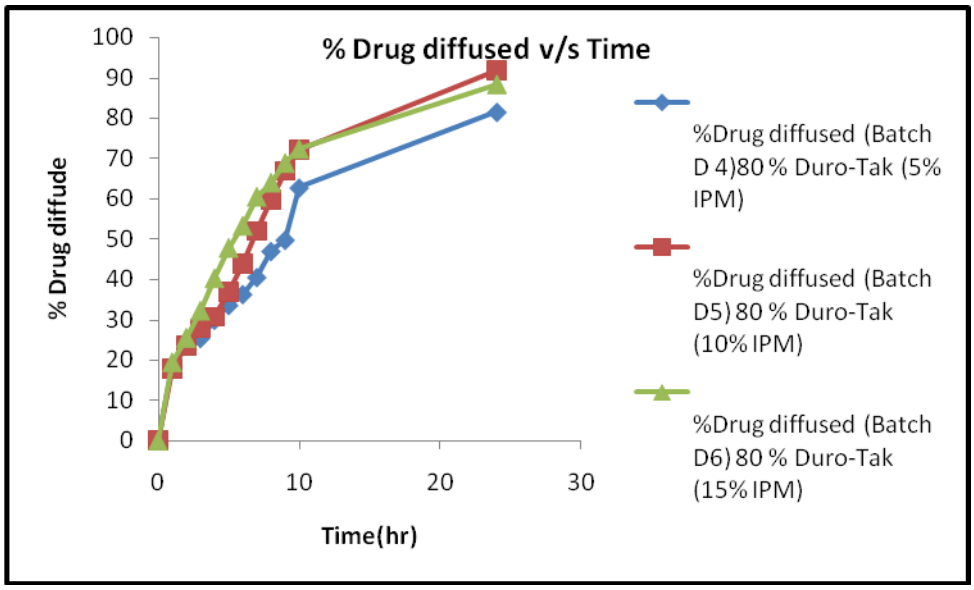


Figure 4.11 Comparative ex vivo diffusion study of batch D4 to D6

Result

Batch D5 (10 % w/w of Duro-Tak 87-9301 and 10 % IPM) showed 75 % the drug release in 24 hours which was higher than batch D4(81.47%) and D6 (88.85%).

Ex-vivo diffusion study of batches containing 90% Duro-Tak 87-9301 with varying % of IPM.

Ex-vivo diffusion study of batch D7 to D9:

Table 4.28 Ex-vivo diffusion study of batch D7

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	6.19	12.38	0.02	0.74	0.00	0.74	18.57
2	2	0.17	7.67	15.33	0.03	0.92	0.02	0.94	23.62
3	3	0.18	8.14	16.29	0.03	0.98	0.06	1.03	25.81
4	4	0.19	9.00	18.00	0.04	1.08	0.09	1.17	29.20
5	5	0.21	9.71	19.43	0.04	1.17	0.12	1.29	32.24
6	6	0.23	10.76	21.52	0.04	1.29	0.16	1.45	36.36
7	7	0.24	11.38	22.76	0.05	1.37	0.21	1.57	39.29
8	8	0.27	12.71	25.43	0.05	1.53	0.25	1.78	44.43
9	9	0.30	13.90	27.81	0.06	1.67	0.30	1.97	49.27
10	10	0.35	16.52	33.05	0.07	1.98	0.36	2.34	58.52
11	24	0.44	20.57	41.14	0.08	2.47	0.42	2.89	72.31

Table 4.29 Ex-vivo diffusion study of batch D8

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.48	10.95	0.02	0.66	0.00	0.66	16.43
2	2	0.16	7.38	14.76	0.03	0.89	0.02	0.91	22.69
3	3	0.18	8.33	16.67	0.03	1.00	0.05	1.05	26.29
4	4	0.20	9.29	18.57	0.04	1.11	0.08	1.20	29.98
5	5	0.23	10.76	21.52	0.04	1.29	0.12	1.41	35.33

6	6	0.29	13.57	27.14	0.05	1.63	0.16	1.79	44.84
7	7	0.34	16.05	32.10	0.06	1.93	0.22	2.14	53.62
8	8	0.37	17.19	34.38	0.07	2.06	0.28	2.35	58.66
9	9	0.41	19.14	38.29	0.08	2.30	0.35	2.65	66.23
10	10	0.43	20.14	40.29	0.08	2.42	0.43	2.85	71.15
11	24	0.50	23.43	46.86	0.09	2.81	0.51	3.32	83.02

Table 4.30 Ex-vivo diffusion study of batch D9

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	6.10	12.19	0.02	0.73	0.00	0.73	18.29
2	2	0.16	7.43	14.86	0.03	0.89	0.02	0.92	22.90
3	3	0.18	8.14	16.29	0.03	0.98	0.05	1.03	25.78
4	4	0.20	9.24	18.48	0.04	1.11	0.09	1.20	29.88
5	5	0.24	11.00	22.00	0.04	1.32	0.12	1.44	36.09
6	6	0.26	12.00	24.00	0.05	1.44	0.17	1.61	40.19
7	7	0.27	12.62	25.24	0.05	1.51	0.22	1.73	43.25
8	8	0.29	13.57	27.14	0.05	1.63	0.27	1.89	47.37
9	9	0.32	15.19	30.38	0.06	1.82	0.32	2.14	53.58
10	10	0.40	18.81	37.62	0.08	2.26	0.38	2.64	65.96
11	24	0.48	22.48	44.95	0.09	2.70	0.46	3.15	78.84

Comparative ex-vivo diffusion study of batch D7 to D9:

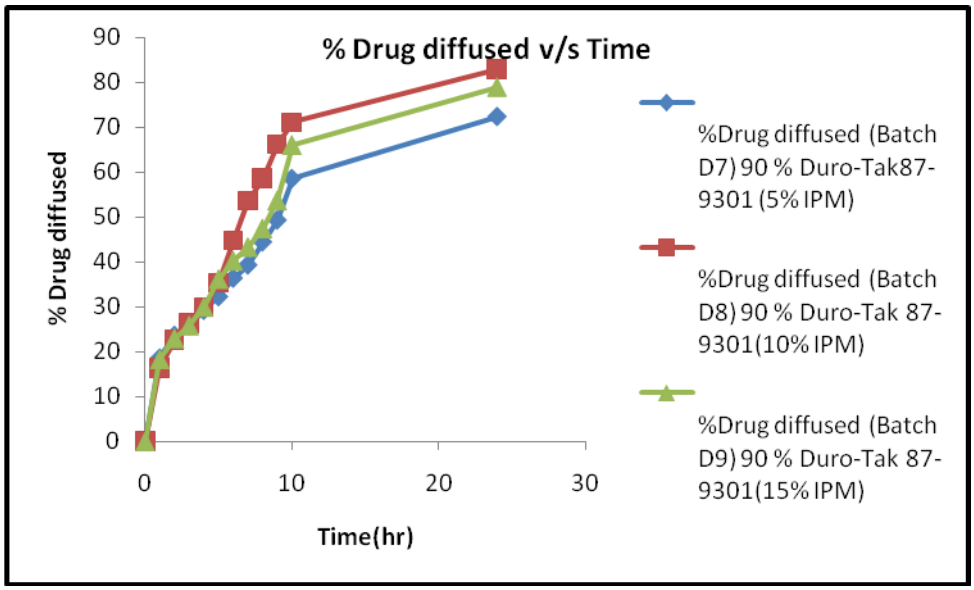


Figure 4.12 Comparative ex vivo diffusion study of batch D7 to D9

Result:

Table 4.31 Result of ex-vivo diffusion study at 24 hr.

Batch No	% Drug diffused in 24 hr.
D1	80.40
D2	89.29
D3	75.74
D4	81.47
D5	91.74
D6	88.25
D7	72.31
D8	83.02
D9	78.84

Discussion:

After carrying out the in-vitro diffusion studies, ex-vivo permeation studies were carried out for batches D1 to D9. The study was carried out using Guinea pig skin and the batch D5 containing80 % Duro-Tak 87-9301 and 10% IPM showed drug diffusion for 24 hours up to the extent of 91.74 % which was higher than all other formulation.

4.5.3 KINETIC MODELING OF DRUG RELEASE

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, andKorsmeyer-Peppas release model.

Batch D2, D5 and D8 showed maximum release of drug 87.94%, 89.15% and 80.58 % respectively as compared to all other batches so it was selected for kinetic model fitting.

Higuchi model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. In a general way it is possible to simplify the Higuchi model as (generally known as the simplified Higuchi model):

$f_t = Q = K_H \times t^{1/2}$---------- (6)

Where,

K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems.

Higuchi model release data

Table 4.32 Higuchi model release data

Time (hr)	Square root of time	Batch D2 %Drug diffused 70 % Duro-Tak 87-9301 (5% IPM)	Batch D5 %Drug diffused 80 % Duro-Tak 87-9301(10% IPM)	Batch D8 %Drug diffused 90 % Duro-Tak 87-9301 (10% IPM)
0	0	0	0	0
1	1	26.86	16.71	15.14
2	1.4	36.18	22.84	20.34
3	1.7	42.50	24.73	22.65
4	2	45.56	28.51	25.23
5	2.2	48.11	33.68	30.38
6	2.4	53.12	40.99	37.18
7	2.6	57.26	55.50	52.40
8	2.8	63.33	57.04	54.035
9	3	67.99	65.71	62.09
10	3.1	79.45	69.33	67.89
24	4.8	87.94	89.15	80.58

Higuchi model release pattern

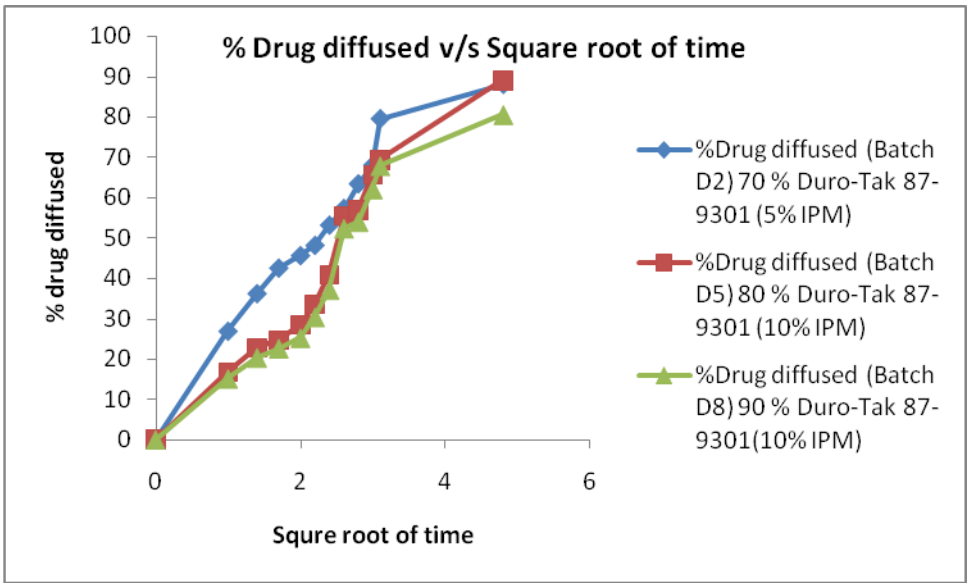


Figure 4.13 Higuchi model release pattern

Higuchi model fitting was performed for batches D2, D5, D8 and r^2 value was found to be 0.963, 0.938, and 0.961 respectively.

Zero Order Kinetics

This model is applicable when the release rate from a system is independent upon the concentration of drug in the system.

$Q_t = Q_0 + K_0t$ ---------- (7)

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in solution, which is zero,

K_0 = Zero order rate constant.

System is said to follow zero order when the plot of Q_t/t gives a straight

Zero order release data

Table 4.33 Zero order release data

Sr no	Time (hr)	Batch D2 %Drug diffused 70 % Duro-Tak 87-9301 (5% IPM)	Batch D5 %Drug diffused 80 % Duro-Tak 87-9301 (10% IPM)	Batch D8 %Drug diffused 90 % Duro-Tak 87-9301 (10% IPM)
0	0	0	0	0
1	1	26.86	16.71	15.14
2	2	36.18	22.84	20.34
3	3	42.50	24.73	22.65
4	4	45.56	28.51	25.23
5	5	48.11	33.68	30.38
6	6	53.12	40.99	37.18
7	7	57.26	55.50	52.40
8	8	63.33	57.04	54.035
9	9	67.99	65.71	62.09
10	10	79.45	69.33	67.89
11	24	87.94	89.15	80.58

Zero order release pattern

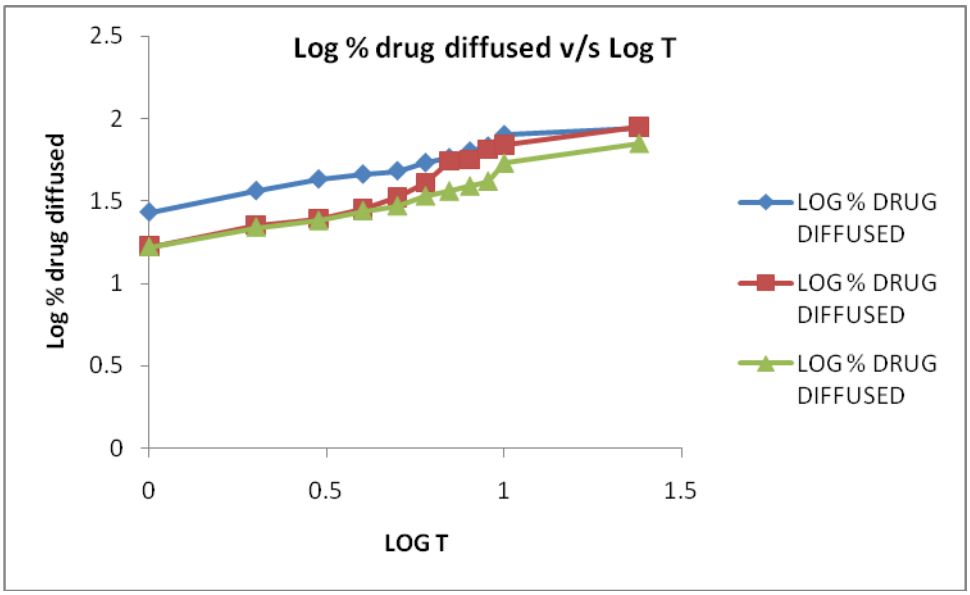


Figure 4.14 Zero order release pattern

Discussion:

Zero order model fitting was performed for batches D2, D5, D8 and r^2 value was found to be 0.708, 0.814, and 0.747 respectively.

First Order Kinetics

This model is applicable when the release rate from a system is dependent upon the concentration of drug in the system.

$$\text{Log } Q_t = \text{Log } Q_0 + K_t/2.303 \text{ ---------- (8)}$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in solution, which is zero,

K_t = First order rate constant.

System is said to follow first order kinetics when the plot of Log $(Q_0-Q_t)/t$ gives a straight line with a slope of $k_t/2.303$ and an intercept of Log Q_0 at t =0.

First Order release data

Table 4.34 First Order release data

Sr no	Time (hr)	Batch D2 Log % drug retained 70 % Duro-Tak87-9301 (5% IPM)	Batch D5 Log % drug retained 80 % Duro-Tak 87-9301 (10% IPM)	Batch D8 Log % drug retained 90 % Duro-Tak 87-9301 (10% IPM)
0	0	2	2	2
1	1	1.86	1.86	1.93
2	2	1.81	1.8	1.90
3	3	1.77	1.76	1.90
4	4	1.75	1.74	1.87
5	5	1.75	1.72	1.84
6	6	1.72	1.67	1.80
7	7	1.69	1.63	1.68
8	8	1.65	1.56	1.66
9	9	1.62	1.51	1.58
10	10	1.51	1.31	1.51
11	24	1.25	1.08	1.29

First Order release pattern

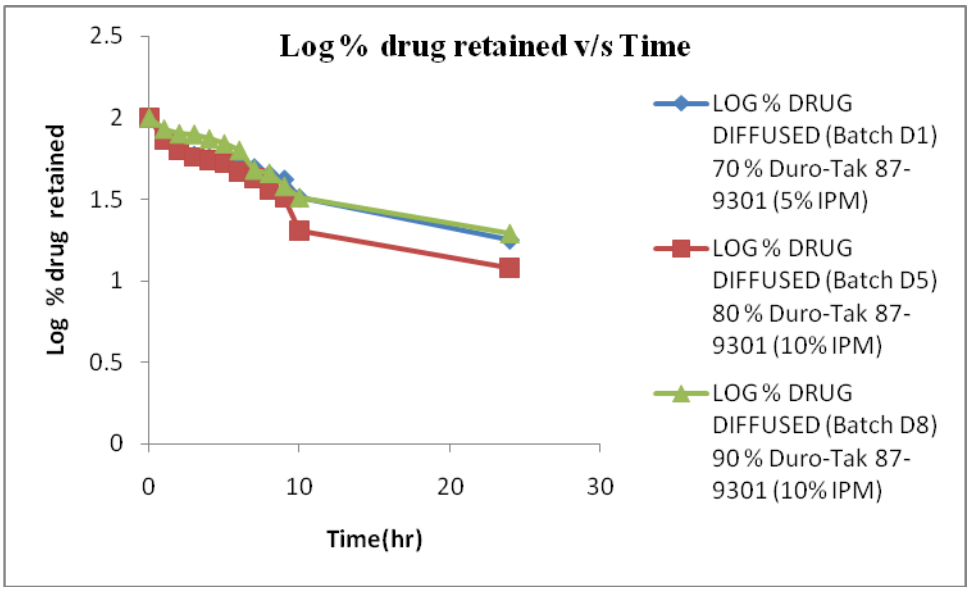


Figure 4.15 First Order release pattern

Discussion:

First order model fitting was performed for batches D2, D5, D8 and r^2 value was near to 1 found to be 0.932, 0.805, and 0.974 respectively.

Korsmeyer-Peppas model

The drug release data as fitted to the peppas model for predicting the mechanism of drug release from the system.

$M_t/M_\infty = K\ t_n$---------- (9)

Where,

M_t = Amount of drug released at time t,

M_∞ = Amount of drug released at infinite time,

K = Peppas release rate constant,

n = Slope of the line called as release exponent having different value according to the model fit equation representing the release mechanism of drug. System is said to follow Peppas model when the plot of Log (M_t/M_∞) / Log t gives a straight line with a slope of n and intercept of Log K.

Release data for KorsmeyerPeppas model

Table 4.35 Release data for KorsmeyerPeppas model

Sr no	Time (hr)	Log T	Log % drug diffused 70 % Duro-Tak 87-9301 (5% IPM)	Log % drug diffused 80 % Duro-Tak 87-9301 (10% IPM)	Log % drug diffused 90 % DURO-Tak 87-9301 (10% IPM)
1	1	0	1.43	1.22	1.22
2	2	0.30	1.56	1.35	1.34
3	3	0.48	1.63	1.39	1.38
4	4	0.60	1.66	1.45	1.44
5	5	0.70	1.68	1.52	1.47
6	6	0.78	1.73	1.61	1.53
7	7	0.85	1.76	1.74	1.56
8	8	0.90	1.8	1.75	1.59
9	9	0.95	1.83	1.81	1.62
10	10	1.00	1.9	1.84	1.73
11	24	1.38	1.94	1.95	1.85

Release pattern for Korsmeyer-Peppas model

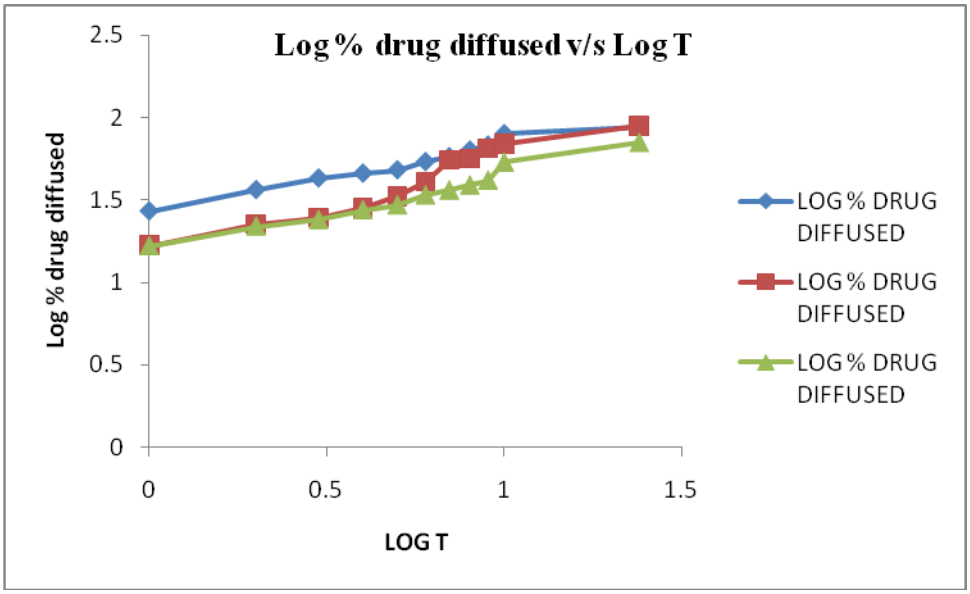


Figure 4.16 Release pattern for KorsmeyerPeppas model

Discussion:

KorsmeyerPeppas model fitting was performed for batches H2, H5, H8 and r^2 value was found to be 0.939, 0.932, and 0.911 respectively.

Result of kinetic model fitting

Table 4.36 R^2Values of various kinetic models

Batch no.	Higuchi model (R^2)	Zero order (R^2)	First order (R^2)	Pappas model (R^2)
D2	0.963	0.708	0.932	0.939
D5	0.938	0.814	0.905	0.932
D8	0.961	0.747	0.875	0.911

Table4.37 SSRValues of various kinetic models

Batch no.	Higuchi model (SSR)	Zero order (SSR)	First order (SSR)	Pappas model (SSR)
H2	221.39	6745.54	563.36	548.85
H5	242.22	3112.75	498.94	312.75
H8	346.59	2984.62	621.78	593.62

Table 4.38f Values of various kinetic models

Batch no.	Higuchi model (f)	Zero order (f)	First order (f)	Pappas model (f)
H2	24.25	227.98	63.37	155.74
H5	43.48	155.74	125.84	153.70
H8	82.50	225.14	108.50	116.98

Discussion:

The in vitro permeation data were fitted to different equations and kinetic models to explain permeation profiles (Table 4.60). The coefficient of correlation of each of the kinetics was calculated and compared. The in vitro permeation profiles of all the different formulations

of transdermal patches did not fit to zero order, first order and Korsmeyer's equation behavior. Higuichi model showed maximum r^2 value (0.954), minimum f (50.03) and SSR (270.32) value as compared to all other batches and it was near to 1. So there could be (batch D2, D5, D8) best expressed by Higuchi's equation for the release of drug from a homogeneous polymer matrix type delivery system that depends mostly on diffusion characteristic.

4.5.4 In-Vitro & Ex-Vivo Correlation of D5 (80 % Duro-Tak87-9301and 10% IPM)

Batch D5 showed highest in-vitro (89.15%) and ex-vivo (91.74%) drug release at 24 hr. as compared to all other batches. Thus it was selected for in-Vitro & Ex-Vivo Correlation study.

Table 4.39 In-Vitro & ex-Vivo Correlation of D5

Time	%Drug diffused	
	EX-VIVO	IN-VITRO
0	0	0
1	17.86	16.71
2	23.45	22.84
3	27.79	24.73
4	30.67	28.51
5	36.76	33.68
6	43.88	40.99
7	51.91	55.50
8	59.60	57.04
9	66.77	65.71
10	71.99	69.33
24	91.74	89.15

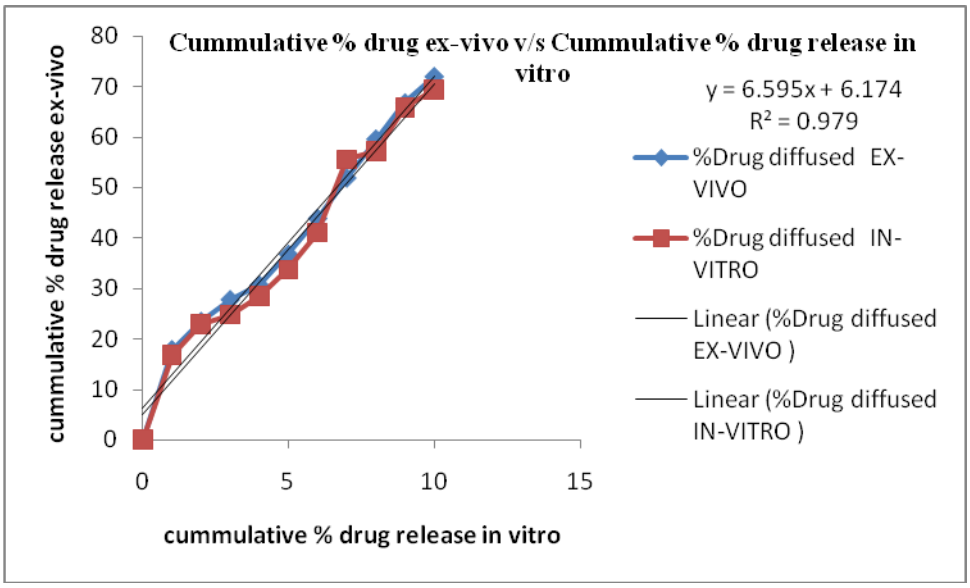


Figure 4.17In-Vitro & ex-Vivo Correlation of batch D5

Discussion

In-vitro and *ex-vivo* correlation was carried out to compare linearity of the in- vitro and ex-vivo release of drug. The release is governed by factors related to both *in vitro* and *ex-vivo* characteristics of the drug. The cumulative percentage of drug release both in *in vitro* and *ex-vivo* was plotted. Fig. 36 shows in-vitro and ex-vivo correlation. The coefficient of correlation (r^2) obtained was 0.979 which reveals that the correlation is positive and near to 1 and this indicates in-vitro diffusion profile is similar to ex-vivo diffusion profile.

4.6 PREPARATION OF TRANSDERMAL PATCH USING HPC-EF AS POLYMER

4.6.1 Preliminary Trials

(A) Optimization of Casting Surface

Initial trials for optimizing casting surface were carried out using 5%w/v of film forming agent like HPC-EF, PEG-400 as plasticizer and water as solvent. All the formulation were casted on glass, plastic and Teflon Petridish. All the casted films were evaluated for the peel ability.

Table 4.40Optimization of Casting Surface

Batch no	Type Of Casting Surface	Conc.of Polymer HPC-EF (% W/V)	Conc. Of plasticizer PEG-400 (% W/V)	Peel ability*
A1	Glass	5	20	+
A2	Plastic	5	20	++
A3	Teflon	5	20	+++

*+++ Easily Separable, ++ Partial Separable, + Difficult to separate

Conclusion:

From the above result it can concluded that teflon as a casting surface has a promising result compared to glass and plastic surface as the film had better peel ability whenteflon was used as a casting surface.

(B) Optimization of the amount of Plasticizer:

From literature survey, it was found that 5 % w/v of HPC-EF can be used for preparation of transdermal patch. So HPC-EF (5%w/v) was selected as film forming agent for optimization of the amount of plasticizer. Different types of plasticizers like Propylene Glycol (PG), Polyethylene Glycol (PEG 400) were tried and films were evaluated for mechanical properties.

Table 4.41 Optimization of the amount of Plasticizer

Batch No	Concent-ration of PG (% W/V)	Concent-ration of PEG 400 (%W/V)	Thickness (mm)	Tensile Strength (N/cm^2)	% Elongation	Folding endurance
A4	10	-	0.22	0.713	8.32	78
A5	20	-	0.35	0.789	8.61	>100
A6	30	-	0.47	0.742	8.10	>100
A7	-	10	0.23	0.888	9.79	93
A8	-	20	0.35	0.898	14.80	>100
A9	-	30	0.49	0.894	13.47	>100

Conclusion:

Different concentration of PG and PEG-400 varying from 10% to 30% w/v of the polymer were taken. Batch A8 containing 20%w/v of PEG-400 had highest tensile strength and acceptable mechanical properties compared to all other batches. So it was selected in further studies.

4.6.2 Optimization of concentration of polymer and permeation enhancer

Transdermal patches of Repaglinide were prepared using solvent casting method. Different amount of HPC-EF was accurately weighed and dissolved in 20 ml of ethanol. Repaglinide was dissolved in the above solution and mixed until clear solution was obtained. PEG 400 (20% w/v of total polymer) was used as plasticizer and different concentration varying from 5 to 15 % w/v of Isopropyl Myristate (IPM) were used as permeation enhancer. Uniform solution was casted teflonpetridish and dried at room temperaturefor 24 h. An inverted funnel was placed over the petridish to prevent fast evaporation of the solvent. After 24 h, the dried patches were taken out for further evaluation studies.

Calculation of drug dose

Surface area:πr^2

$= 3.14 \times (5.1)^2$

$= 81.67\ cm^2$

Size of patch: $2 \times 2 = 4\ cm^2$

Number of patches: $81.64 \div 4 = 20.41$

Dose of Repaglinide= 4 mg

Dose of Repaglinide per petridish= $20.41 \times 4 = 81.67$ mg

Table 4.42Optimization of concentration of polymer(HPC-EF) and permeation enhancer(IPM)

Batch No	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Repaglinide (mg)	81.67			81.67			81.67		
HPC-EF (%w/v)	5			10			15		
PEG-400 (% w/v)	20			20			20		
IPM (%w/v)	5	10	15	5	10	15	5	10	15
Ethanol (ml)	20			20			20		

Each batch contains 4mg drug in 4 cm^2 area

<u>Evaluation parameters of batch H1 to H9</u>

Table 4.43 Evaluation parameters of batch H1 to H9

Batch No	**Tensile strength (N/cm^2)**	**% Elongation**	**Folding endurance**
H1	0.890	10	>100

H2	1.079	14.706	>100
H3	0.812	10.345	>100
H4	0.896	12.90	>100
H5	1.169	16.67	>100
H6	0.827	13.79	>100
H7	0.814	14.81	>100
H8	0.912	15.42	>100
H9	0.867	14.95	>100

Table 4.44 Evaluation parameters of batches H1 to H9

Batch No	Thickness (mm)	Moisture content (%)	Moisture uptake (%)	Avg. weight (mg)
H1	0.21	9.85	8.75	80.33
H2	0.24	4.70	5.31	91.33
H3	0.30	6.59	4.96	92
H4	0.34	6	3.84	108
H5	0.37	7.54	1.81	111
H6	0.38	8.73	3.60	111.66
H7	0.48	6.72	8.33	127.33
H8	0.49	7.39	7.84	129
H9	0.71	7.71	6.79	130

Discussion:

It was found that batch H5 containing 10% w/v HPC-EF and 10 % w/v IPM showed maximum tensile strength and % Elongation as compared to all other batches. Folding endurance was found to >100 for all batches H1 to H9 which was acceptable for

transdermal patch. Thickness of patches was varying from 0.21 to 0.71 mm as the amount of HPC-EF was increased from 5 to 15%. Batch H2 showed minimum moisture content and batch H5 showed least moisture uptake compared to all other batches so these batches H2 and H5 can be used for formulation of transdermal patch.

Here, batches H1 to H6 (5 to 10 % w/v of HPC-EF) showed better peel ability compared to batches H7 to H9 (15 % w/v of HPC-EF).

4.6.3 DIFFUSION STUDY OF BATCH H1 TO H9

(A) IN- VITRO DIFFUSION STUDY OF BATCH H1 TO H9

In vitro diffusion study of batches containing 5 % w/v of HPC-EF with varying % of IPM.

In-vitro diffusion studyofbatches H1 to H3:

Table 4.45 In-vitro diffusion study of batch H1

Sr. no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.09	4.19	8.38	0.02	0.50	0.00	0.50	12.57
2	2	0.11	5.14	10.29	0.02	0.62	0.02	0.63	15.85
3	3	0.19	8.81	17.62	0.04	1.06	0.04	1.09	27.36
4	4	0.22	10.38	20.76	0.04	1.25	0.07	1.32	32.96
5	5	0.26	12.05	24.10	0.05	1.45	0.11	1.56	39.00
6	6	0.29	13.57	27.14	0.05	1.63	0.16	1.79	44.77
7	7	0.30	13.95	27.90	0.06	1.67	0.22	1.89	47.27

8	8	0.32	15.00	30.00	0.06	1.80	0.27	2.07	51.81
9	9	0.32	15.10	30.19	0.06	1.81	0.33	2.14	53.60
10	10	0.35	16.29	32.57	0.07	1.95	0.39	2.35	58.68
11	24	0.41	19.33	38.67	0.08	2.32	0.46	2.78	69.45

Table4.46 In-vitro diffusion study of batch H2

Sr no	Time (hr.)	Abs. nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.52	11.05	0.02	0.66	0.00	0.66	16.57
2	2	0.24	11.24	22.48	0.04	1.35	0.02	1.37	34.27
3	3	0.29	13.62	27.24	0.05	1.63	0.07	1.70	42.53
4	4	0.32	15.05	30.10	0.06	1.81	0.12	1.93	48.18
5	5	0.33	15.52	31.05	0.06	1.86	0.18	2.04	51.11
6	6	0.34	16.00	32.00	0.06	1.92	0.24	2.16	54.10
7	7	0.35	16.48	32.95	0.07	1.98	0.31	2.28	57.12
8	8	0.36	16.95	33.90	0.07	2.03	0.37	2.41	60.20
9	9	0.44	20.62	41.24	0.08	2.47	0.44	2.92	72.90
10	10	0.42	19.81	39.62	0.08	2.38	0.52	2.90	72.53
11	24	0.51	23.86	47.71	0.10	2.86	0.60	3.47	86.65

Table 4.47 In-vitro diffusion study of batch H3:

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.17	7.90	15.81	0.03	0.95	0.00	0.95	23.71
2	2	0.26	12.19	24.38	0.05	1.46	0.03	1.49	37.36
3	3	0.29	13.57	27.14	0.05	1.63	0.08	1.71	42.72
4	4	0.32	14.95	29.90	0.06	1.79	0.13	1.93	48.22
5	5	0.30	14.05	28.10	0.06	1.69	0.19	1.88	47.00
6	6	0.34	15.81	31.62	0.06	1.90	0.25	2.15	53.70
7	7	0.35	16.52	33.05	0.07	1.98	0.31	2.30	57.42
8	8	0.36	16.90	33.81	0.07	2.03	0.38	2.41	60.21
9	9	0.37	17.52	35.05	0.07	2.10	0.45	2.55	63.76
10	10	0.42	19.57	39.14	0.08	2.35	0.52	2.87	71.66
11	24	0.50	23.62	47.24	0.09	2.83	0.60	3.43	85.76

Comparative in-vitro diffusion study of batches H1 to H3

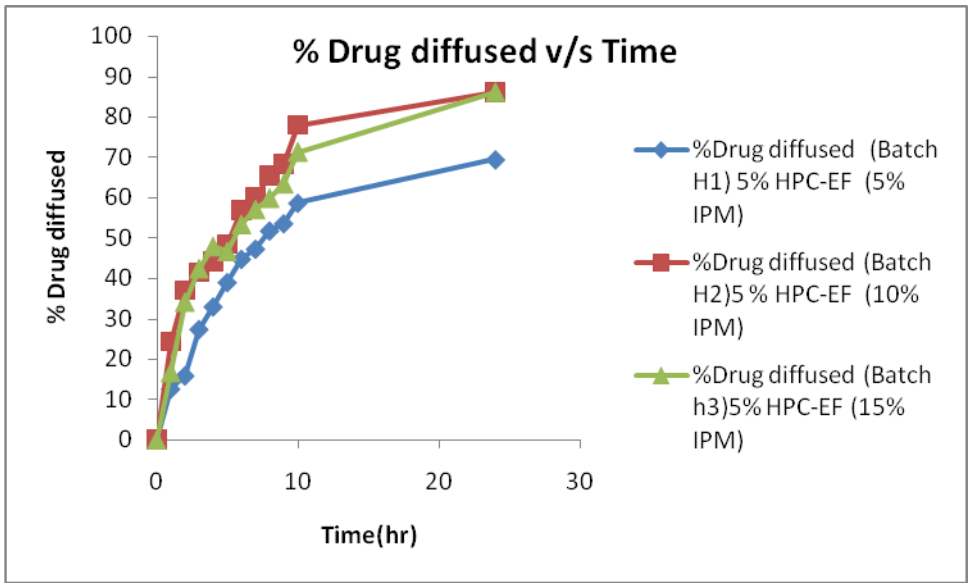


Figure 4.18Comparativein-vitro diffusion study of batches H1 to H3

Result:

The result indicated that patch containing 5% HPC-EF and 10% IPM (Batch H2) showed higher release of drug (86.65%) in 24 hr as compared to batch H1(69.45%) and H3 (85.76%).

In vitro diffusion study of batches containing 10 % w/v of HPC-EF with varying % of IPM.

In-vitro diffusion study of batches H4 to H6:

Table 4.48 In-vitro diffusion study of batch H4

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.11	4.90	9.81	0.02	0.59	0.00	0.59	14.71
2	2	0.12	5.67	11.33	0.02	0.68	0.02	0.70	17.49
3	3	0.16	7.57	15.14	0.03	0.91	0.04	0.95	23.77

4	4	0.20	9.24	18.48	0.04	1.11	0.07	1.18	29.53
5	5	0.24	11.19	22.38	0.04	1.34	0.11	1.45	36.31
6	6	0.27	12.76	25.52	0.05	1.53	0.15	1.69	42.14
7	7	0.32	14.86	29.71	0.06	1.78	0.21	1.99	49.70
8	8	0.35	16.57	33.14	0.07	1.99	0.26	2.25	56.33
9	9	0.38	17.86	35.71	0.07	2.14	0.33	2.47	61.85
10	10	0.41	19.43	38.86	0.08	2.33	0.40	2.73	68.35
11	24	0.51	24.05	48.10	0.10	2.89	0.48	3.37	84.15

Table 4.49 In-vitro diffusion study of batch H5

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.172	8.00	16.00	0.03	0.96	0.00	0.96	24.00
2	2	0.21	9.81	19.62	0.04	1.18	0.03	1.21	30.23
3	3	0.279	13.10	26.19	0.05	1.57	0.07	1.64	41.07
4	4	0.317	14.90	29.81	0.06	1.79	0.12	1.91	47.80
5	5	0.342	16.10	32.19	0.06	1.93	0.18	2.11	52.87
6	6	0.347	16.33	32.67	0.07	1.96	0.25	2.21	55.19
7	7	0.363	17.10	34.19	0.07	2.05	0.31	2.36	59.11

8	8	0.391	18.43	36.86	0.07	2.21	0.38	2.59	64.82
9	9	0.419	19.76	39.52	0.08	2.37	0.46	2.83	70.66
10	10	0.436	20.57	41.14	0.08	2.47	0.53	3.00	75.07
11	24	0.543	25.67	51.33	0.10	3.08	0.62	3.70	92.41

Table 4.50 In-vitro diffusion study of batch H6

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.57	13.14	0.03	0.79	0.00	0.79	19.71
2	2	0.15	6.95	13.90	0.03	0.83	0.03	0.86	21.51
3	3	0.17	7.90	15.81	0.03	0.95	0.05	1.00	25.07
4	4	0.22	10.24	20.48	0.04	1.23	0.09	1.31	32.86
5	5	0.21	9.81	19.62	0.04	1.18	0.13	1.30	32.60
6	6	0.23	10.76	21.52	0.04	1.29	0.17	1.46	36.43
7	7	0.26	12.19	24.38	0.05	1.46	0.21	1.67	41.80
8	8	0.28	13.14	26.29	0.05	1.58	0.26	1.83	45.87
9	9	0.34	16.00	32.00	0.06	1.92	0.31	2.23	55.76
10	10	0.39	18.38	36.76	0.07	2.21	0.37	2.58	64.50
11	24	0.53	25.05	50.10	0.10	3.01	0.45	3.45	86.34

Comparative in-vitro diffusion study of batches H4 to H6

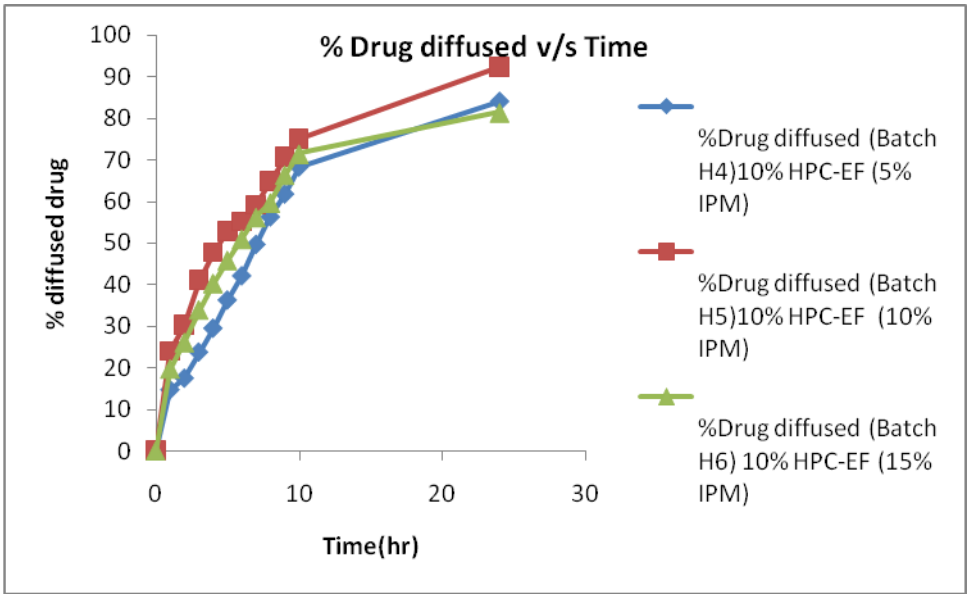


Figure 4.19 Comparative in-vitro diffusion study of batches H4 to H6

Result:

Batch H5 (10 % HPC and 10 % IPM) showed 92.41% the drug release in 24 hours which was higher than batch H4(84.15 %) and H6(86.34 %).

In vitro diffusion study of batches containing 15 % w/v of HPC-EF with varying % of IPM.

In-vitro diffusion study of batches H7 to H9:

Table 4.51 In-vitro diffusion study of batch H7

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution Factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0

1	1	0.11	5.05	10.10	0.02	0.61	0.00	0.61	15.14
2	2	0.15	7.10	14.19	0.03	0.85	0.02	0.87	21.79
3	3	0.18	8.19	16.38	0.03	0.98	0.05	1.03	25.79
4	4	0.19	9.05	18.10	0.04	1.09	0.08	1.17	29.18
5	5	0.22	10.10	20.19	0.04	1.21	0.12	1.33	33.22
6	6	0.26	12.33	24.67	0.05	1.48	0.16	1.64	40.95
7	7	0.30	14.05	28.10	0.06	1.69	0.21	1.89	47.32
8	8	0.32	14.81	29.62	0.06	1.78	0.26	2.04	51.01
9	9	0.33	15.38	30.76	0.06	1.85	0.32	2.17	54.21
10	10	0.35	16.57	33.14	0.07	1.99	0.38	2.37	59.32
11	24	0.49	23.24	46.48	0.09	2.79	0.45	3.24	80.98

Table 4.52 In-vitro diffusion study of batch H8

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution Factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.17	7.67	15.33	0.03	0.92	0.00	0.92	23.00
2	2	0.19	8.81	17.62	0.04	1.06	0.03	1.09	27.20
3	3	0.22	10.24	20.48	0.04	1.23	0.07	1.29	32.36

4	4	0.27	12.52	25.05	0.05	1.50	0.11	1.61	40.24
5	5	0.29	13.67	27.33	0.05	1.64	0.16	1.80	44.92
6	6	0.32	15.10	30.19	0.06	1.81	0.21	2.02	50.58
7	7	0.35	16.48	32.95	0.07	1.98	0.27	2.25	56.23
8	8	0.39	18.57	37.14	0.07	2.23	0.34	2.57	64.16
9	9	0.40	18.95	37.90	0.08	2.27	0.41	2.69	67.16
10	10	0.44	20.86	41.71	0.08	2.50	0.49	2.99	74.77
11	24	0.51	24.10	48.19	0.10	2.89	0.57	3.462857	86.57

Table 4.53 In-vitro diffusion study of batch H9

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution Factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.57	13.14	0.03	0.79	0.00	0.79	19.71
2	2	0.15	7.10	14.19	0.03	0.85	0.03	0.88	21.94
3	3	0.17	7.95	15.90	0.03	0.95	0.05	1.01	25.22
4	4	0.19	8.67	17.33	0.03	1.04	0.09	1.13	28.16

5	5	0.21	10.00	20.00	0.04	1.20	0.12	1.32	33.03
6	6	0.23	10.95	21.90	0.04	1.31	0.16	1.48	36.89
7	7	0.26	12.14	24.29	0.05	1.46	0.20	1.66	41.55
8	8	0.28	13.19	26.38	0.05	1.58	0.25	1.84	45.91
9	9	0.34	16.05	32.10	0.06	1.93	0.31	2.23	55.80
10	10	0.39	18.33	36.67	0.07	2.20	0.37	2.57	64.26
11	24	0.52	24.57	49.14	0.10	2.95	0.44	3.39	84.81

Comparative in-vitro diffusion study of batches H7 to H9:

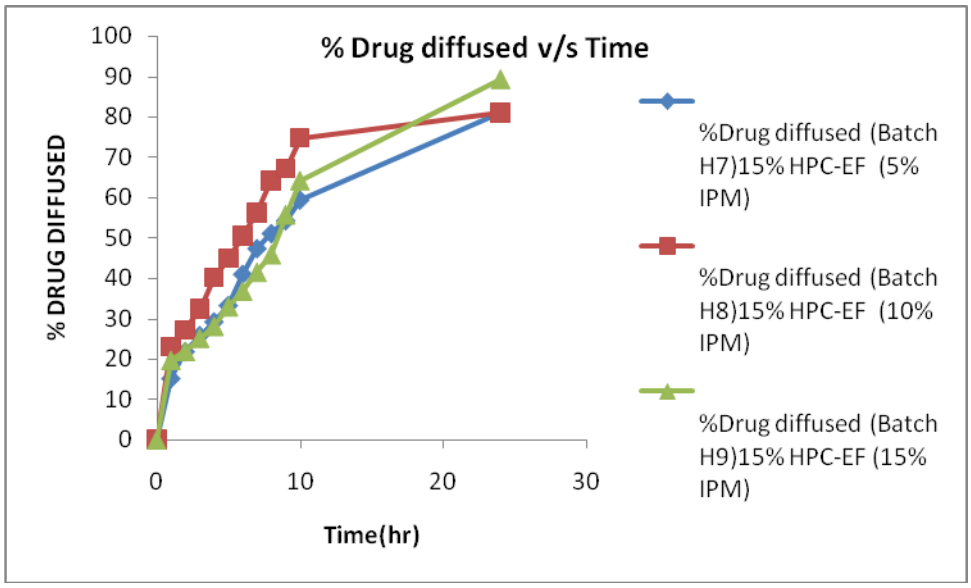


Figure 4.20Comparative in-vitro diffusion study of batches H7 to H9:

RESULT:

Table 4.54 Result of in-vitro diffusion study at 24 hr.

Batch No:	% Drug Diffused at 24 hr.
H1	69.45
H2	86.75
H3	85.76
H4	84.15
H5	92.41
H6	86.34
H7	80.98
H8	86.57
H9	84.81

Discussion:

In Vitro drug release studies were performed using a Franz diffusion cell with different concentration from 5 to 15 % w/v of HPC-EF as polymer and 5 to 15 %w/v of IPM as permeation enhancer. As shown in Table 4.23, as concentration of HPC-EF increased from 5 to 15 % w/v, there was increase in release of drug but higher release was not obtained as concentration of HPC-EF was further increased to 5 to 15 % w/v. Thus further increase in concentration of polymer had no effect on drug release. Similar types of results were observed with IPM. Up to certain concentration of IPM drug release was increased and further increased in concentration of IPM from 10 to 15 % w/v had no effect on drug release. Hence it was observed that batch H1 (5%HPC and 5% IPM) showed minimum release of drug (69.45 %) and batch H5 (10% HPC and 10% IPM) showed maximum release of drug (92.41%). So batch H5 was selected for further studies.

(B) EX-VIVO DIFFUSION STUDY OF BATCH H1 TO H9

Ex-vivo diffusion study of batches containing 5 % w/v of HPC-EF with varying % of IPM.

Ex-vivo diffusion study of batches H1 to H3:

Table 4.55 Ex-vivo diffusion study of batch H1

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution Factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.71	11.43	0.02	0.69	0.00	0.69	17.14
2	2	0.14	6.43	12.86	0.03	0.77	0.02	0.79	19.86
3	3	0.16	7.24	14.48	0.03	0.87	0.05	0.92	22.93
4	4	0.18	8.24	16.48	0.03	0.99	0.08	1.07	26.65
5	5	0.20	9.29	18.57	0.04	1.11	0.11	1.22	30.62
6	6	0.22	10.24	20.48	0.04	1.23	0.15	1.38	34.40
7	7	0.24	11.10	22.19	0.04	1.33	0.19	1.52	38.00
8	8	0.26	12.24	24.48	0.05	1.47	0.23	1.70	42.54
9	9	0.33	15.33	30.67	0.06	1.84	0.28	2.12	53.05
10	10	0.38	17.95	35.90	0.07	2.15	0.34	2.50	62.44
11	24	0.52	24.43	48.86	0.10	2.93	0.42	3.35	83.66

Table 4.56 Ex-vivo diffusion study of batch H2

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.57	13.14	0.03	0.79	0.00	0.79	19.71
2	2	0.15	7.10	14.19	0.03	0.85	0.03	0.88	21.94
3	3	0.17	7.95	15.90	0.03	0.95	0.05	1.01	25.22
4	4	0.19	8.67	17.33	0.03	1.04	0.09	1.13	28.16
5	5	0.21	10.00	20.00	0.04	1.20	0.12	1.32	33.03
6	6	0.23	10.95	21.90	0.04	1.31	0.16	1.48	36.89
7	7	0.26	12.14	24.29	0.05	1.46	0.20	1.66	41.55
8	8	0.28	13.19	26.38	0.05	1.58	0.25	1.84	45.91
9	9	0.34	16.05	32.10	0.06	1.93	0.31	2.23	55.80
10	10	0.39	18.33	36.67	0.07	2.20	0.37	2.57	64.26
11	24	0.54	25.48	50.95	0.10	3.06	0.44	3.50	87.52

Table 4.57 Ex-vivo diffusion study of batch H3

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.10	4.76	9.52	0.02	0.57	0.00	0.57	14.29

2	2	0.12	5.67	11.33	0.02	0.68	0.02	0.70	17.48
3	3	0.14	6.57	13.14	0.03	0.79	0.04	0.83	20.76
4	4	0.16	7.43	14.86	0.03	0.89	0.07	0.96	23.99
5	5	0.18	8.48	16.95	0.03	1.02	0.10	1.11	27.87
6	6	0.21	9.81	19.62	0.04	1.18	0.13	1.31	32.72
7	7	0.24	11.24	22.48	0.04	1.35	0.17	1.52	37.99
8	8	0.28	13.19	26.38	0.05	1.58	0.22	1.80	44.97
9	9	0.36	17.05	34.10	0.07	2.05	0.27	2.31	57.86
10	10	0.40	18.76	37.52	0.08	2.25	0.34	2.59	64.70
11	24	0.49	23.14	46.29	0.09	2.78	0.41	3.19	79.72

Comparative ex-vivo diffusion study of batches H1 to H3:

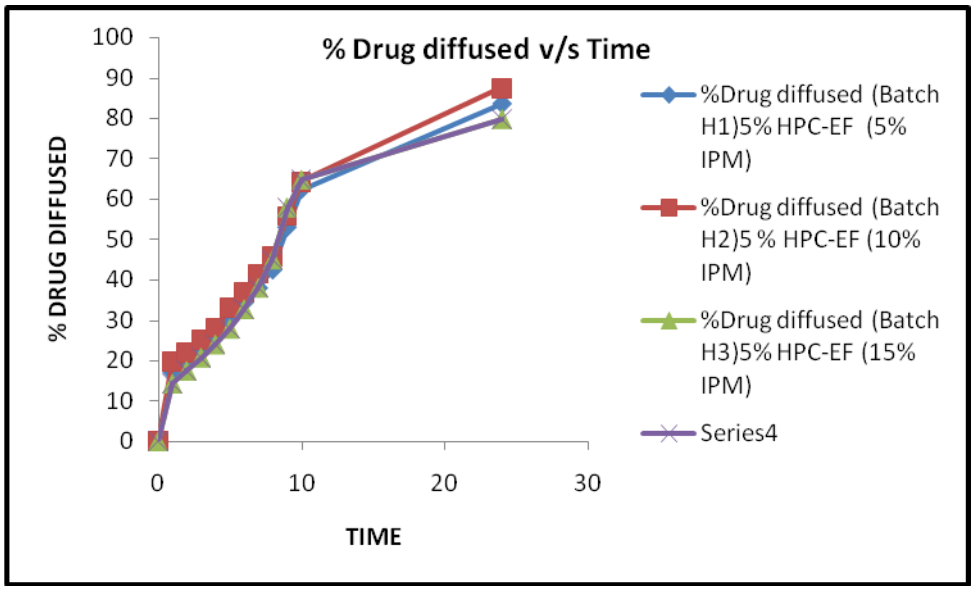


Figure 4.21 Comparative in vitro diffusion study of batches H1 to H3:

Result:

The result indicated that patch containing 5% HPC-EF and 10% IPM (Batch H2) showed higher release of drug (87.52%) in 24 hr as compared to batch H1(83.66 %) and H3(79.72 %).

Ex-vivo diffusion study of batches containing 10 % w/v HPC-EF with varying % of IPM.

Ex-vivo diffusion study of batches H4 to H6:

Table 4.58 Ex-vivo diffusion study of batch H4

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.13	5.76	11.52	0.02	0.69	0.00	0.69	17.29
2	2	0.15	6.81	13.62	0.03	0.82	0.02	0.84	21.00
3	3	0.17	7.81	15.62	0.03	0.94	0.05	0.99	24.69

4	4	0.20	9.29	18.57	0.04	1.11	0.08	1.20	29.90
5	5	0.24	11.33	22.67	0.05	1.36	0.12	1.48	36.97
6	6	0.27	12.81	25.62	0.05	1.54	0.16	1.70	42.53
7	7	0.30	14.00	28.00	0.06	1.68	0.22	1.90	47.38
8	8	0.35	16.43	32.86	0.07	1.97	0.27	2.24	56.07
9	9	0.38	17.90	35.81	0.07	2.15	0.34	2.49	62.14
10	10	0.41	19.52	39.05	0.08	2.34	0.41	2.75	68.79
11	24	0.51	23.90	47.81	0.10	2.87	0.49	3.36	83.88

Table 4.59 Ex-vivo diffusion study of batch H5

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.11	5.14	10.29	0.02	0.62	0.00	0.62	15.43
2	2	0.13	5.86	11.71	0.02	0.70	0.02	0.72	18.28
3	3	0.15	6.90	13.81	0.03	0.83	0.04	0.87	21.57
4	4	0.19	8.95	17.90	0.04	1.07	0.07	1.15	27.84
5	5	0.23	10.81	21.62	0.04	1.30	0.11	1.40	33.51

6	6	0.26	12.33	24.67	0.05	1.48	0.15	1.63	38.12
7	7	0.30	14.00	28.00	0.06	1.68	0.20	1.88	43.26
8	8	0.38	17.81	35.62	0.07	2.14	0.26	2.39	54.69
9	9	0.42	19.62	39.24	0.08	2.35	0.33	2.68	60.24
10	10	0.48	22.71	45.43	0.09	2.73	0.41	3.13	72.60
11	24	0.61	28.86	57.71	0.12	3.46	0.50	3.96	90.86

Table 4.60 Ex-vivo diffusion study of batch H6

Sr no	Time (hr.)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.1	4.57	9.14	0.02	0.55	0.00	0.55	13.71
2	2	0.12	5.52	11.05	0.02	0.66	0.02	0.68	17.03
3	3	0.14	6.48	12.95	0.03	0.78	0.04	0.82	20.44
4	4	0.16	7.43	14.86	0.03	0.89	0.07	0.96	23.94
5	5	0.18	8.38	16.76	0.03	1.01	0.10	1.10	27.54

6	6	0.21	9.81	19.62	0.04	1.18	0.13	1.31	32.67
7	7	0.24	11.24	22.48	0.04	1.35	0.17	1.52	37.93
8	8	0.28	13.14	26.29	0.05	1.58	0.21	1.79	44.77
9	9	0.3	14.10	28.19	0.06	1.69	0.27	1.96	48.94
10	10	0.34	16.00	32.00	0.06	1.92	0.32	2.24	56.07
11	24	0.5	23.62	47.24	0.09	2.83	0.39	3.22	80.52

Comparative ex-vivo diffusion study of batch H4 to H6:

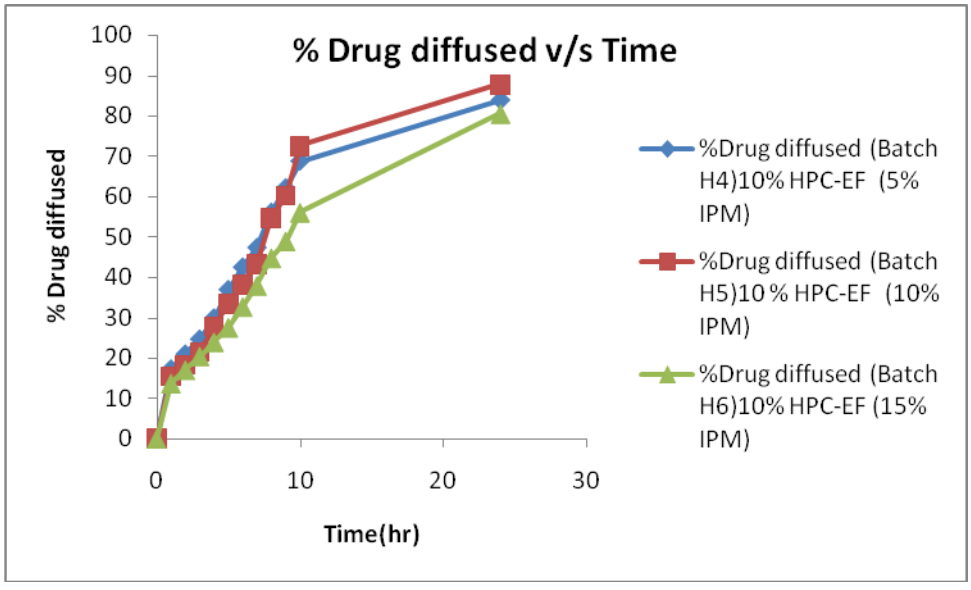


Figure 4.22 Comparative ex-vivo diffusion study of batches H4 to H6

Result

Batch H5 (10 % HPC and 10 % IPM) showed 90.86 % the drug release in 24 hr. which was better than batch H4(83.88 %) and H6(80.52%).

Ex-vivo diffusion study of batches containing 15 % w/v of HPC-EF with varying % of IPM.

Ex-vivo diffusion study of batches H7 to H9:

Table 4.61 Ex-vivo diffusion study of batch H7

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.12	5.71	11.43	0.02	0.69	0.00	0.69	17.14
2	2	0.14	6.43	12.86	0.03	0.77	0.02	0.79	19.86
3	3	0.16	7.24	14.48	0.03	0.87	0.05	0.92	22.93
4	4	0.18	8.24	16.48	0.03	0.99	0.08	1.07	26.65
5	5	0.20	9.29	18.57	0.04	1.11	0.11	1.22	30.62
6	6	0.22	10.24	20.48	0.04	1.23	0.15	1.38	34.40
7	7	0.24	11.10	22.19	0.04	1.33	0.19	1.52	38.00
8	8	0.26	12.24	24.48	0.05	1.47	0.23	1.70	42.54
9	9	0.33	15.33	30.67	0.06	1.84	0.28	2.12	53.05
10	10	0.38	17.95	35.90	0.07	2.15	0.34	2.50	62.44
11	24	0.52	24.43	48.86	0.10	2.93	0.42	3.35	84.66

Table 4.62 Ex-vivo diffusion study of batch H8

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.14	6.57	13.14	0.03	0.79	0.00	0.79	19.71
2	2	0.15	7.10	14.19	0.03	0.85	0.03	0.88	21.94
3	3	0.17	7.95	15.90	0.03	0.95	0.05	1.01	25.22
4	4	0.19	8.67	17.33	0.03	1.04	0.09	1.13	28.16
5	5	0.21	10.00	20.00	0.04	1.20	0.12	1.32	33.03
6	6	0.23	10.95	21.90	0.04	1.31	0.16	1.48	36.89
7	7	0.26	12.14	24.29	0.05	1.46	0.20	1.66	41.55
8	8	0.28	13.19	26.38	0.05	1.58	0.25	1.84	45.91
9	9	0.34	16.05	32.10	0.06	1.93	0.31	2.23	55.80
10	10	0.39	18.33	36.67	0.07	2.20	0.37	2.57	64.26
11	24	0.54	25.48	50.95	0.10	3.06	0.44	3.50	87.52

Table 4.63 Ex-vivo diffusion study of batch H9

Sr no	Time (hr)	Abs. (nm)	mcg/ml	Dilution factor	mg/2ml	mg/60ml	Error	Final Conc.	% Drug Diffused
0	0	0	0	0	0	0	0	0	0
1	1	0.10	4.76	9.52	0.02	0.57	0.00	0.57	14.29

2	2	0.12	5.67	11.33	0.02	0.68	0.02	0.70	17.48
3	3	0.14	6.57	13.14	0.03	0.79	0.04	0.83	20.76
4	4	0.16	7.43	14.86	0.03	0.89	0.07	0.96	23.99
5	5	0.18	8.48	16.95	0.03	1.02	0.10	1.11	27.87
6	6	0.21	9.81	19.62	0.04	1.18	0.13	1.31	32.72
7	7	0.24	11.24	22.48	0.04	1.35	0.17	1.52	37.99
8	8	0.28	13.19	26.38	0.05	1.58	0.22	1.80	44.97
9	9	0.36	17.05	34.10	0.07	2.05	0.27	2.31	57.86
10	10	0.40	18.76	37.52	0.08	2.25	0.34	2.59	64.70
11	24	0.49	23.14	46.29	0.09	2.78	0.41	3.19	79.72

Comparative ex-vivo diffusion study of batches H7 to H9:

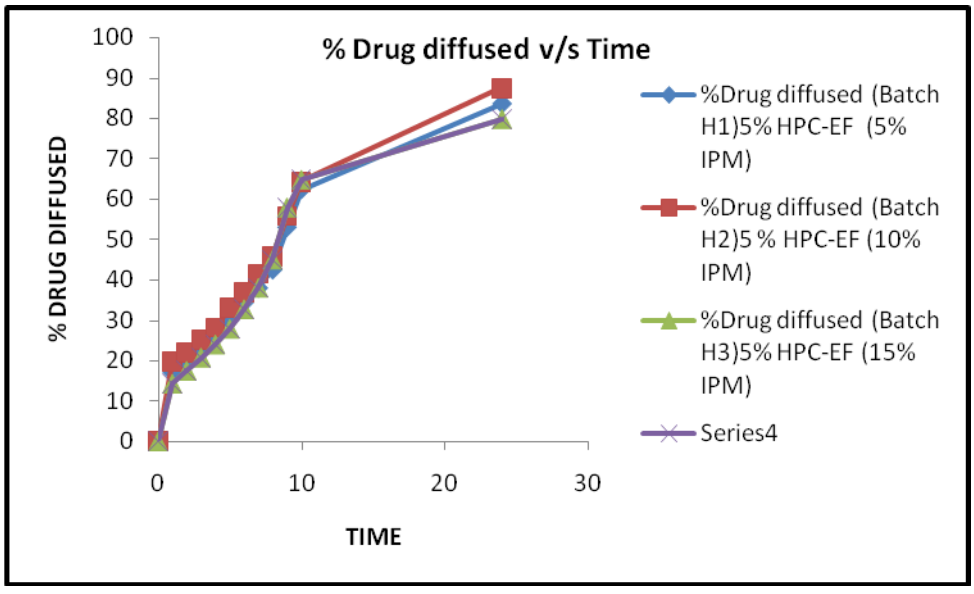


Figure 4.23 Comparative ex-vivo diffusion study of batches H7 to H9

RESULT:

Table 4.64 Result of ex-vivo diffusion study for H1 to H9 at 24 hr.:

Batch No	% Drug diffused at 24 hr.
H1	83.66
H2	87.52
H3	79.72
H4	83.88
H5	90.86
H6	80.52
H7	84.66
H8	87.52
H9	79.72

Discussion:

Batch H5 (10% HPC and 10% IPM) showed drug diffusion for 24 hours up to the extent of 90.86 % which was highest amongst all remaining batches.

4.6.4 KINETIC MODELING OF DRUG RELEASE.

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Batch H2, H5 and H8 showed maximum release of drug 87.52%, 90.86% and 87.52 % respectively as compared to all other batches so it was selected for kinetic model fitting.

Higuchi model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1961. In a general way it is possible to simplify the Higuchi model as (generally known as the simplified Higuchi model):

$f_t = Q = K_H \times t^{1/2}$---------- (10)

Where

K_H is the Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.This relationship can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems.

Higuchi model release data

Table 4.65 Higuchi model release data

Time (hr.)	**Square root of time**	**Batch H2 %Drug diffused 5 %HPC-EF (15% IPM)**	**Batch H5 %Drug diffused 10 %HPC-EF(10% IPM)**	**Batch H8 %Drug diffused 15 %HPC-EF(15% IPM)**
0	0	0	0	0
1	1	16.57	24	23
2	1.4	34.27	30.23	27.2
3	1.7	42.53	41.07	32.36
4	2	48.18	47.8	40.24

5	2.2	51.11	52.87	44.92
6	2.4	54.1	55.19	50.58
7	2.6	57.12	59.11	56.23
8	2.8	60.2	64.82	64.16
9	3	72.9	70.66	67.16
10	3.1	72.53	75.07	74.77
24	4.8	86.65	92.41	86.57

Higuchi model release pattern

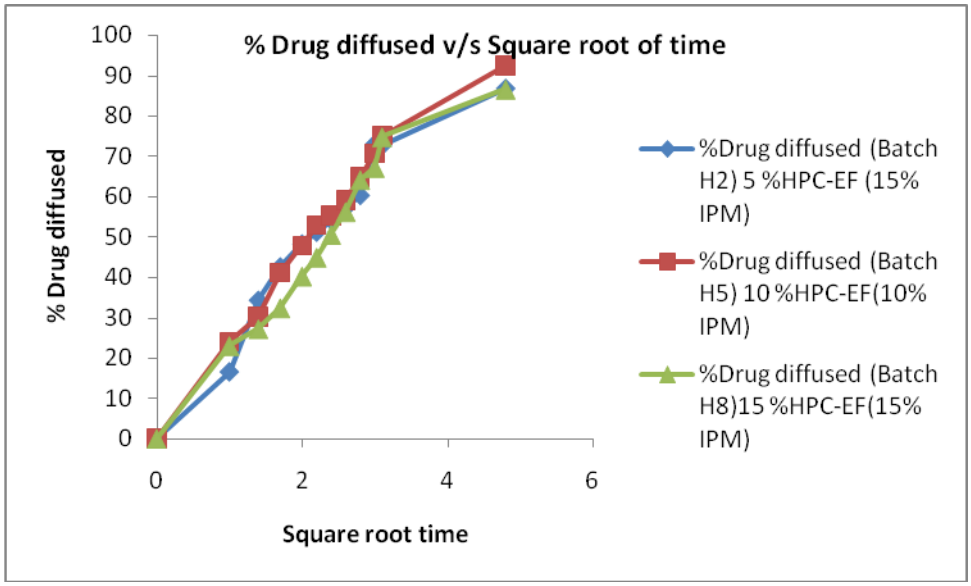


Figure 4.24Higuchi model release pattern

Discussion:

Higuchi model fitting was performed for batches H2, H5, H8 and r^2 value was found to be 0.935, 0.976, and 0.953 respectively.The r^2 value was nearest to 1 for batch H5.

Zero Order Kinetics

This model is applicable when the release rate from a system is independent upon the concentration of drug in the system.

$Q_t = Q_0 + K_0t$ ---------- (11)

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in solution, which is zero,

K_0 = Zero order rate constant.

System is said to follow zero order when the plot of Q_t/t gives a straight

Zero order release data

Table 4.64 Zero order release data

Sr no	Time (hr)	Batch H2 %Drug diffused 5 %HPC-EF (15% IPM)	Batch H5 %Drug diffused 10%HPC-EF(10% IPM)	Batch H8 %Drug diffused 15 %HPC-EF(15% IPM)
0	0	0	0	0
1	1	16.57	24	23
2	2	34.27	30.23	27.2
3	3	42.53	41.07	32.36
4	4	48.18	47.8	40.24

5	5	51.11	52.87	44.92
6	6	54.1	55.19	50.58
7	7	57.12	59.11	56.23
8	8	60.2	64.82	64.16
9	9	72.9	70.66	67.16
10	10	72.53	75.07	74.77
11	24	86.65	92.41	86.57

Zero order release pattern

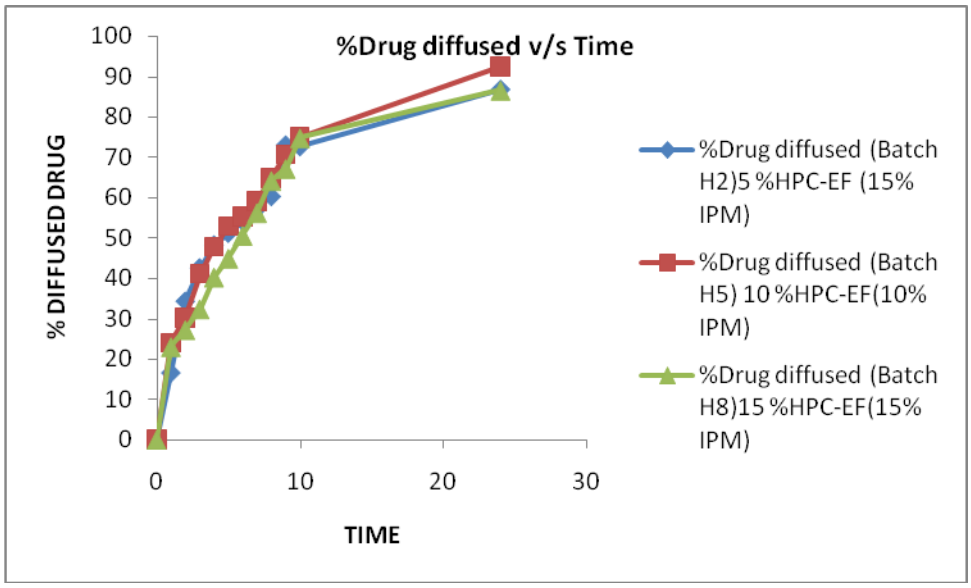


Figure 4.25 Zero order release pattern

Discussion:

Zero order model fitting was performed for batches H2, H5, H8 and r^2 value was found to be 0.690, 0.741, and 0.749 respectively.These values indicate r^2 not near to 1 thus zero order release pattern does not fit the release pattern.

First Order Kinetics

This model is applicable when the release rate from a system is dependent upon the concentration of drug in the system.

$$\text{Log } Q_t = \text{Log } Q_0 + K_t/2.303 \text{ ---------- (12)}$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in solution, which is zero,

K_t = First order rate constant.

System is said to follow first order kinetics when the plot of Log $(Q_0-Q_t)/t$ gives a straight line with a slope of $k_t/2.303$ and an intercept of Log Q_0 at t =0.

First Order release data

Table 4.66 First Order release data

Sr no	Time (hr)	Batch H2 Log % drug retained 5 %HPC-EF (15% IPM)	Batch H5 Log % drug retained 10 %HPC-EF(10% IPM)	Batch H8 Log % drug retained 15 %HPC-EF(15% IPM)
0	0	2	2	2
1	1	1.92	1.88	1.89
2	2	1.82	1.84	1.86

3	3	1.76	1.77	1.83
4	4	1.77	1.72	1.78
5	5	1.72	1.67	1.74
6	6	1.67	1.65	1.69
7	7	1.65	1.61	1.64
8	8	1.6	1.55	1.55
9	9	1.43	1.47	1.52
10	10	1.44	1.4	1.4
11	24	1.12	0.88	1.13

First Order release pattern

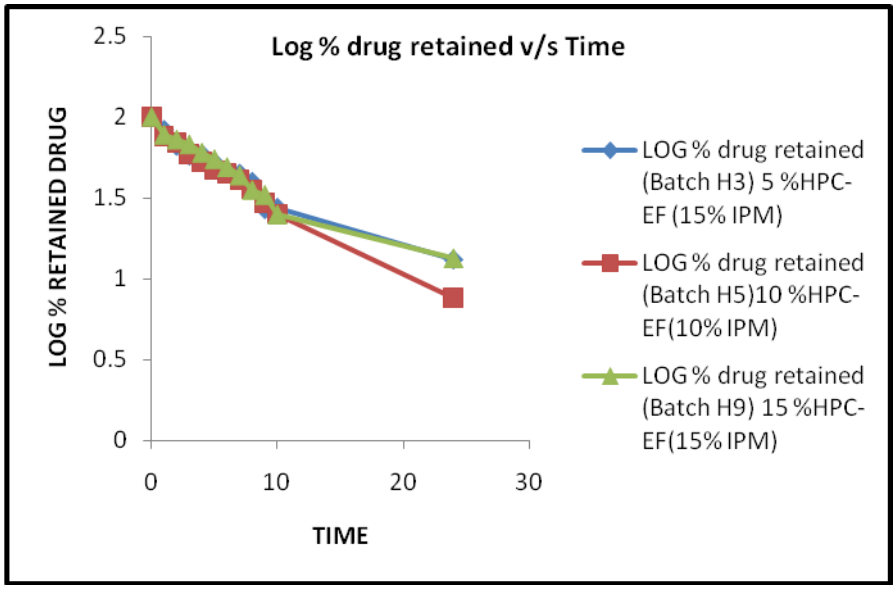


Figure 4.26First Order release pattern

Discussion:

First order model fitting was performed for batches H2, H5, H8 and r^2 value was found to be 0.925, 0.981, and 0.947 respectively. These values indicate r^2 not near to 1 thus first order release pattern does not fit.

Korsmeyer-Peppas model

The drug release data as fitted to the peppas model for predicting the mechanism of drug release from the system.

$M_t/M_\infty = K\ t_n$---------- (13)

Where,

M_t = Amount of drug released at time t,
M_∞ = Amount of drug released at infinite time,
K = Peppas release rate constant,

n = Slope of the line called as release exponent having different value according to the model fit equation representing the release mechanism of drug. System is said to follow Peppas model when the plot of Log (M_t/M_∞) / Log t gives a straight line with a slope of n and intercept of Log K.

Release data for KorsmeyerPeppas model

Table 4.67Release data for KorsmeyerPeppas model

Sr No	Time (hr)	Log T	Log % drug diffused 5 %HPC-EF(15% IPM)	Log % drug diffused 10 %HPC-EF(10% IPM)	Log % drug diffused 15 %HPC-EF(15% IPM)
1	1	0	1.21	1.38	1.36
2	2	0.30	1.53	1.41	1.43

3	3	0.48	1.62	1.61	1.51
4	4	0.60	1.68	1.67	1.6
5	5	0.70	1.7	1.73	1.65
6	6	0.78	1.73	1.74	1.7
7	7	0.85	1.75	1.77	1.74
8	8	0.90	1.77	1.81	1.8
9	9	0.95	1.86	1.84	1.82
10	10	1	1.86	1.87	1.87
11	24	1.38	1.95	1.96	1.93

Release pattern for Korsmeyer-Peppas model

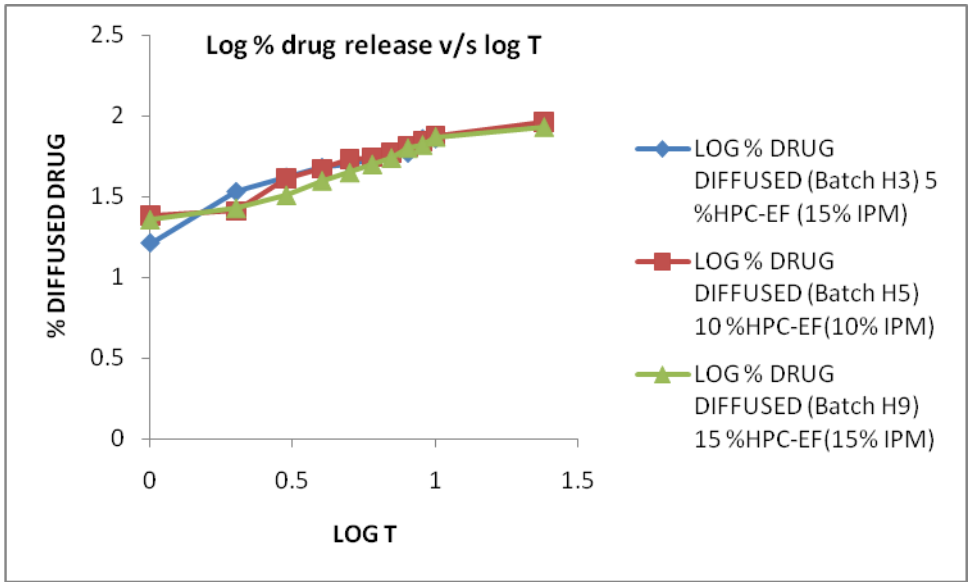


Figure 4.27Release pattern for KorsmeyerPeppas model

Discussion:

KorsmeyerPeppas model fitting was performed for batches H2, H5, H8 and r^2 value was found to be 0.915, 0.965, and 0.0.947 respectively.These values indicate r^2 not near to 1 as compared to Highichi model, thus Peppas model pattern does not fit.

Result of kinetic model fitting

Table 4.69 R^2 Values for various kinetic models

Batch no.	Higuchi model (R^2)	Zero order (R^2)	First order (R^2)	Pappas model (R^2)
H2	0.935	0.690	0.925	0.915
H5	0.976	0.741	0.981	0.965
H8	0.953	0.741	0.911	0.947

Table 4.70SSR Values for various kinetic models

Batch no.	Higuchi model (SSR)	Zero order (SSR)	First order (SSR)	Pappas model (SSR)
H2	283.78	6548.26	400.05	539.80
H5	141.83	6330.76	337.80	287.71
H8	259.18	5245.28	388.72	306.22

Table 4.71f Values for various kinetic models

Batch no.	Higuchi model (f)	Zero order (f)	First order (f)	Pappas model (f)
H2	22.27	144.10	109.41	107.43
H5	28.67	556.92	166.64	275.37
H8	25.91	180.91	120.24	180.71

Discussion:

The in vitro permeation data were fitted to different equations and kinetic models to explain permeation profiles (Table 4.39). The coefficient of correlation of each of the kinetics was calculated and compared. The in vitro permeation profiles of all the different formulations of transdermal patches did not fit to zero order, first order and Korsmeyer's equation behavior. Higuichi model showed maximum r^2value(0.940), minimum f value (25.61) and SSR value (228.26) as compared to all other batches so these could be best expressed by Higuchi's equation for the release of drug from a homogeneous polymer matrix type delivery system that depends mostly on diffusion characteristic.

4.6.5 In-Vitro & Ex-Vivo Correlation of H5 (10% HPC and 10% IPM)

Batch H5 showed highest in-vitro(92.41%) and ex-vivo(90.86%) drug release at 24 hr. as compared to all other batches. Thus it was selected for in-vitro & Ex-vivo Correlation study.

Table 4.72 In-Vitro & ex-Vivo Correlation of H5

Time (hr)	%Drug diffused	
	EX-VIVO	IN-VITRO
0	0	0
1	15.430	24
2	18.280	30.230

3	21.570	41.070
4	27.840	47.800
5	33.510	52.870
6	38.120	55.190
7	43.260	59.110
8	54.690	64.820
9	60.240	70.660
10	72.600	75.070
24	90.860	92.410

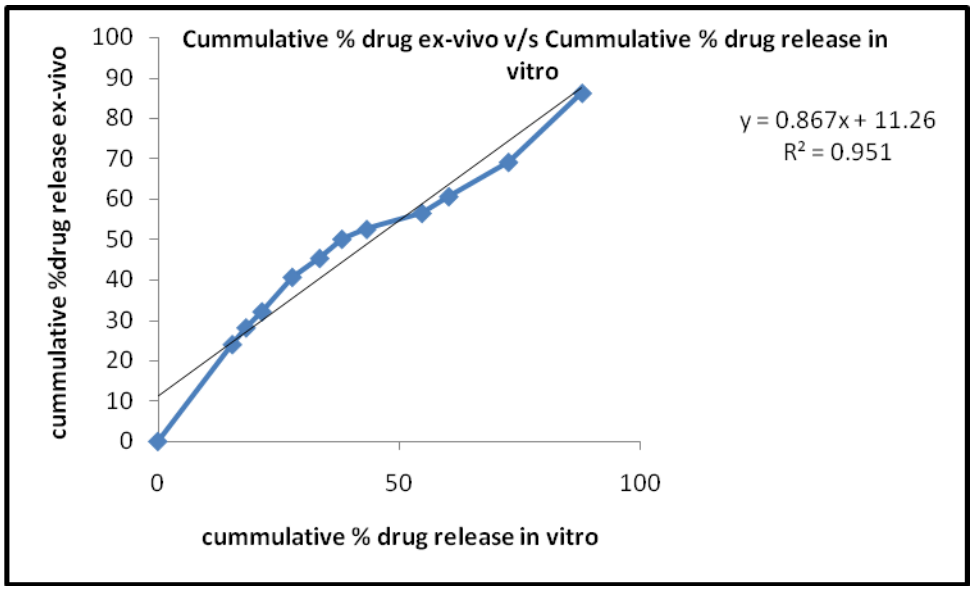


Figure 4.28 In-Vitro & ex-Vivo Correlation of H5

Discussion

In-vitro and *ex-vivo* correlation was carried out to compare linearity of the in- vitro and ex-vivo release of drug. The release is governed by factors related to both *in vitro* and *ex-vivo* characteristics of the drug. The cumulative percentage of drug release both in *in vitro* and *ex-vivo* was plotted. Fig. 25 shows in-vitro and ex-vivo correlation. The coefficient of correlation (r^2) obtained was 0.951 which reveals that the correlation is positive and near to 1 and this indicates in-vitro diffusion profile is similar to ex-vivo diffusion profile.

4.6.6 Drug content determination for batch H5

Here. Batch H5 was optimized batch for preparation of transdermal patch so it was selected for determination of drug content.

$$\textbf{\% Drug content} = \frac{\text{Actual amount of drug in patch}}{\text{Theoretical amount of drug in patch.}} \times 100$$

Table 4.73Observation for drug content:

Batch No	% Drug content	Average % drug content
H5	95.2	95.05
	94.3	
	95.7	

Result:

Here, Average drug content of three transdermal patch was determined spectroscopically and % drug content was found to be 95.05 %

Conclusion

In present study various formulations were evaluated using physicochemical evaluation parameters such as thickness, % elongation, tensile strength, % moisture content, moisture uptake. In-vitro drug release study was performed using Franz diffusion cell with cellophane membrane. Ex-vivo drug release study was also performed using Franz diffusion cell with guinea pig skin.Result revealed that patches showed good physical characteristics.The in vitro release study through dialysis membrane and ex-vivo study through guinea pig skin revealed that batch H5 containing 10 % w/v of HPC-EF and 10 % w/v of IPM and batch D5 containing 70% of Duro-Tak and 10 %w/v of IPM showed maximum drug release 92.41% and 89.14 % as compared to all other batches. Comparative study showed that batch H5 gave maximum release of Repaglinide (92.41%) at 24 hr. So it was selected for further studies.

4.7 FORMULATION OF ADHESIVE LAYER

4.7.1 Method of preparation

Adhesive layer was preparedusingDuro-Tak 87-9301 as pressure sensitive adhesive. Different concentration of Duro-Tak 87-9301 was accurately weighed and dissolved in 10 ml of ethanol and mixed until clear solution wasobtained. The uniform solution was casted on the scotch pack backing layer and dried at 80 ^{0}C temperature for 8 h. After 8 h, adhesive layer was taken out for further evaluation studies.

Table 4.74 Composition of Adhesive layer

Batch No	B1	B2	B3	B4	B5
Duro-Tak 87-9301 (PSA)	0.5	1	1.5	2	2.5

(%w/w)					
Ethanol (ml)	**10**	**10**	**10**	**10**	**10**

Evaluation parameters:

In this test, force required to remove an adhesive coating from a test substrate is referred aspeel adhesion. Molecular weight of adhesive polymer, the type and amount of additives are thevariables that determined the peel adhesion properties.

Peel adhesion study was carried out using Texture analyzer. In this test the adhesive solution was applied on guinea pig skin and then using probe single sided tape was applied to the skin and then probe was pulled from the skin at a 180° angle, and the force required to remove the probe was measured.

Table 4.75Evaluation parameters

Batch No	**Peel Adhesion (N/cm^2)**
B1	181.10
B2	263.03
B3	326.14
B4	421.79
B5	526.06

Discussion:

It was found that there was constant increase in adhesiveness as concentration of pressure sensitive adhesive was increased. Batches B1 to B3 (0.5 to 1.5 %w/w Duro-Tak 87-9301) showed insufficient adhesive property to skin and batch B5 (2.5%w/w Duro-Tak 87-9301) showed more stickiness. Batch B4 (2%w/w of Duro-Tak 87-9301) showed optimum adhesiveness as compared to all other batches.

4.8FORMULATION OF BACKING LAYER

Selection of the backing layer is very critical step in formulation of transdermal patch. Here, backing layer was formulated using different concentration of polyvinyl alcohol (PVA) and ethylcellulose (EC). Dibutylpthalate (DBP) was used as plasticizer.

4.8.1 PREPARATION OF POLYVINYL ALCOHOL BACKING LAYER

Backing layer was prepared using solvent casting method. Here, 4% w/v, 5% w/v, 6% w/v solution of polyvinyl alcohol was prepared in ethanol. Uniform solution was casted on the plastic petridish and dried at 40 ^{0}C for 24 hr, and then dried film was taken out for further evaluation studies.

Table 4.76 Composition of polyvinyl alcohol backing layer

Ingredients	B1	B2	B3
Polyvinyl alcohol (PVA) (% w/v)	4	5	6
Water (ml)	20	20	20

Evaluation Parameters for batch B1 to B3

Table 4.77 Evaluation Parameters for batch B1 to B3

Concentration (% w/v)	Thickness (mm)	Tensile strength (N/cm^2)	%Elongation	Folding endurance
4	0.08	1.0371	63.6	> 100
5	0.09	2.009	78.12	>100
6	0.10	1.125	70.52	>100

Result:

Batch B2 (5 % w/v of PVA) showed better tensile strength and % elongation as compared to other batches.Film was flexible and suitable for preparation of backing layer.

4.8.2 PREPARATION OF ETHYLCELLULOSE BACKING LAYER

Here, different concentration of polymer of ethyl cellulose (2 %w/w, 4 %w/w, 6 %w/w) was dissolved in ethanol as solvent using ultrasonic sonicator with continous stirring. Dibutylpthalate (DBP) at 30% w/v concentration was used as plasticizer and was dissolved in above polymeric solution. This solution was casted on glass petridish and dried hot air oven at 45 ^{0}C temperature for 24 hr. and evaluated for various parameters.

Table 4.78 Composition ethylcellulose backing layer

Batch no	B4	B5	B6
Ethyl cellulose (EC) (%w/w)	2	4	6
Dibutylpthalate (DBP) (%w/v)	30	30	30
Ethanol (ml)	20	20	20

Evaluation Parameters for batch B4 to B6

Table 4.79 Evaluation Parameters for batch B4 to B6

Batch No	B4	B5	B6
Thickness (mm)	0.12	0.13	0.53
Tensile strength (N/cm^2)	0.922	0.963	0.906
% Elongation	12.5	21.87	21.42
Folding Endurance	> 100	> 100	> 100

Result

Batch B6 (6 % w/w of EC) and batch B4 (2% w/w of EC) did not give the satisfactory result for backing layer. In batch B6 precipitation was observed. Batch B5 had better tensile strength and % elongation than other batches. It was transparent and also showed suitable mechanical properties . So, 2% w/w of ethylcelluose was suitable for formulation of backing layer.

4.8.3 USE OF ACETONE AND ISOPROPYLALCOHOL (IPA) AS A SOLVENT IN BACKING LAYER

Table 4.80 Composition of EC backing layer

Ingredients	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**
Ethyl cellulose (EC) (% w/v)	2	4	6	2	4	6
Dibutylpthalate (DBP) (% w/v)	30	30	30	30	30	30
Acetone	-	-	-	20 ml	20 ml	20 ml
Acetone:IPA (65:35)	20 ml	20 ml	20 ml	-	-	-

Evaluation Parameters of batch B7 to B12

In batches B10 to B12 precipitation was observed. The patch could not be separated from casting surface. So acetone alone can not be used in preparation of ethylcellulose backing layer.

Table 4.81 Evaluation Parameters of batch B7 to B12

Batch no	**B7**	**B8**	**B9**
Thickness (mm)	0.18	0.42	1.73
Tensile Strength (N/cm^2)	0.303	0.574	0.231
% Elongation	12.1	37.5	11.9
Folding endurance	> 100	> 100	42

Result

Here, batch B8 gave higher tensile strength and percentage elongation than other batches of ethylcellulose. Batch B8 was also transparent, flexible and suitable for preparation of backing layer.

4.8.4 COMPARISON OF TENSILE STRENGTH AND % ELONGATION OF PVA AND EC BACKING LAYER

Table 4.82 Comparison of tensile strength and % elongation of batches B1 to B9 containing various polymers PVA and EC

Batch no	Tensile Strength (N/cm^2)	% Elongation
B1	1.037	63.6
B2	2.009	78.12
B3	1.125	70.52
B4	0.922	12.5
B5	0.963	21.42
B6	0.906	21.87
B7	0.303	12.1
B8	0.574	37.5
B9	0.231	11.9

Figure 4.29 Comparison of tensile strength of batches B1 to B9 containing various polymers PVA and EC

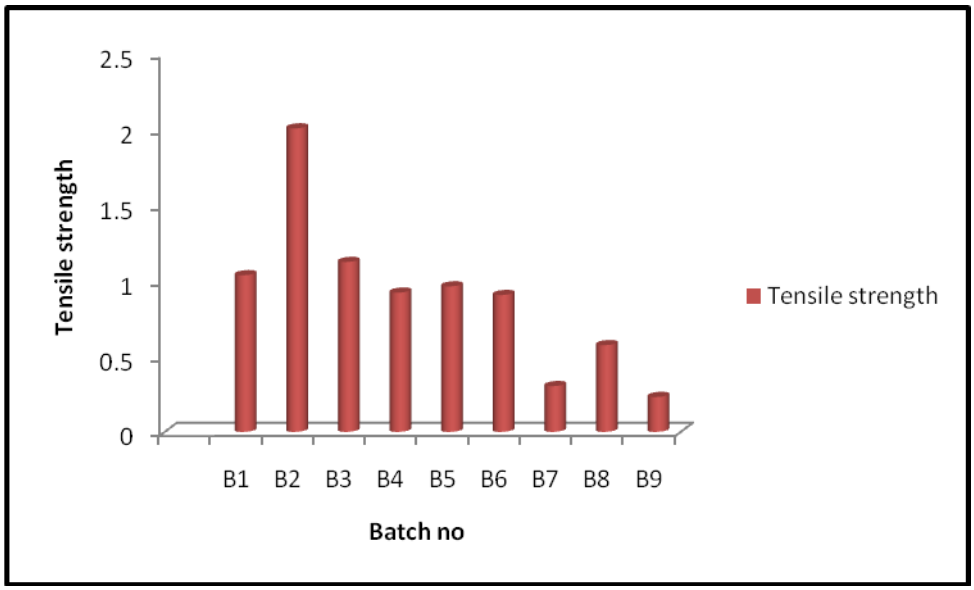
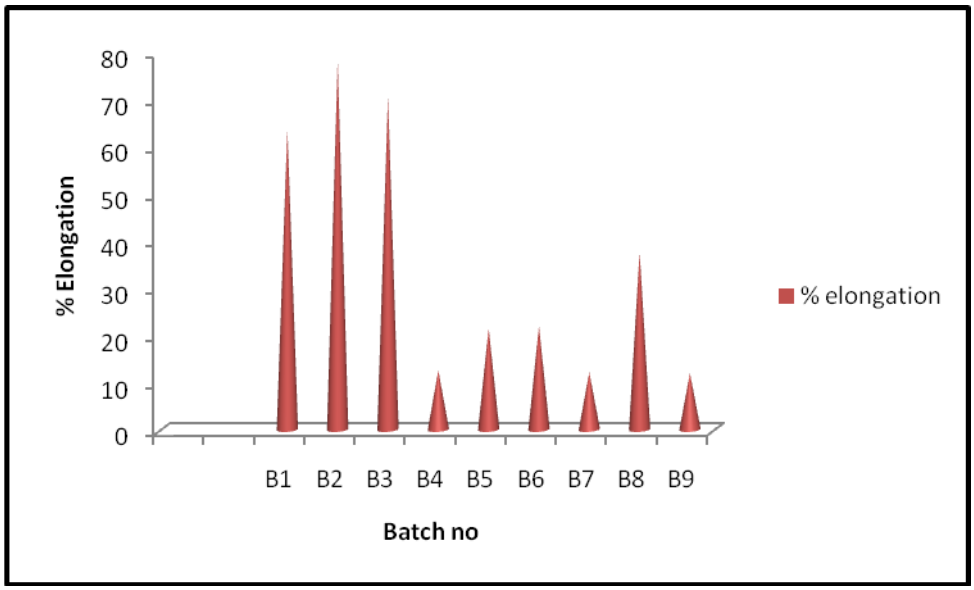


Figure 4.30 Comparison of % Elongation strength of batches B1 to B9 containing various polymers PVA and EC



Discussion

Backing layer was formulated by using different concentration of polyvinyl alcohol (PVA) and ethylcellulose (EC) using solvent casting method. All batches of backing layer were formulated and compared by mechanical properties and it was found that batch B2 (5 %w/v of PVA) showed maximum tensile strength (2.009 N/cm^2) and % Elongation (78.12) compared to all other batches. Backing layer containing 5 %w /v of PVA (Batch B2) had better flexibility than other formulations. It was transparent and suitable for use. So batch B2 (5% w/v of PVA) was selected as backing layer for transdermal

4.10 COMPOSITION OF FINAL TRANSDERMAL PATCH OF REPAGLINIDE

Table 4.82 Composition of final patch

SR NO.	INGREDIENTS	QUANTITY TAKEN
1	Repaglinide(mg)	81.67
2	HPC-EF(%w/v)	10
3	PEG-400(% w/v)	20
5	IPM(%w/v)	10
4	Ethanol(ml)	20

*** Each patch contain 4 mg Repaglinide in 2x2 cm^2 area**

Baking Layer: 5 % w/v PVA

Adhesive Layer: 2 % w/v Duro-Tak 87-9301

Table 4.83 Evaluation data for final patch

Sr No.	Evalution parameters	Observation
1	**Thickness (mm)**	0.37
2	**Tensile Strength (N/cm^2)**	1.169
3	**% elongation**	16.67
4	**Folding endurance**	>100
5	**Moisture Content (%)**	7.54
6	**Moisture Uptake (%)**	1.81
7	**In-vitro drug release (%)**	92.41
8	**Ex-vivo drug release(%)**	90.86

4.10 STABILITY STUDY OF TRANSDERMAL PATCH

The stability studies of the optimized batch H5 was carried out in in a sealed zip lock bag covered with aluminium foil carried out at 40°C/75% RH and 25°C/60% RH using stability chamber.

TABLE 4.84- RESULTS OF STABILITY STUDIES OF BATCH H5

Condition	Time	Tensile Strength (N/cm^2)	% Elongation	Folding endurance	Appearance
40°C/75%RH	Initial	1.169	16.67	>100	Acceptable
	1 month	Ongoing	Ongoing	Ongoing	Ongoing
	2 month	Ongoing	Ongoing	Ongoing	Ongoing
25°C/60%RH	Initial	1.169	16.67.	>100	Acceptable
	1month	Ongoing	Ongoing	Ongoing	Ongoing
	2 month	Ongoing	Ongoing	Ongoing	Ongoing

Condition	Time	Moisture content (%)	Moisture uptake (%)	In-vitro % drug release	Ex-vivo % drug release	Appearance
40°C/75%RH	Initial	7.54	1.81	92.41	90.86	Acceptable
	1 month	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
	2 month	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
25°C/60%RH	Initial	7.54	1.81	92.41	90.86	Acceptable
	1month	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
	2 month	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing

4.11 IN –VIVO STUDY FOR TRANSDERMAL PATCH

The studies were approved ny institutional Ethical Committee (IEC) with project number IEC/NU/III/IP/03 7th May 2013.

4.10.1 Skin irritation study

The transdermal patch of Repaglinide was applied on clean, dry area of forearm in human subject. After 24 hrs subject was examined for sign and symptoms of skin irritation. Primary outcome was the skin irritation score as measured by erythema. It was graded on 5 point scale:

Table 4.86 Point scale for skin irritation study

TEST	SKIN REACTION	SCORE
Erythema	None	0
	Very slight erythema	1
	Well defined erythema	2
	Moderate to severe erythema	3
	Severe erythema	4

Table 4.87Observation for skin irritation study:

TEST	SCORE	OBSERVATION
Erythema	0	No erythema observed

The skin irritation study was carried out on human skin and results showed that no skin irritation was observed on to the skin.

4.11.2 Patch adherence study

Patch adherence was evaluated at the end of wear period, immediately prior to removal of patch. Score corresponded to percentage of patch surface in contact with skin in according to 5 point scale.

The recommended scoring system for adhesion of transdermal patches is indicated as follows:

0 = ≥ 90% adhered (essentially no lift off the skin)

1 = ≥ 75% to < 90% adhered (some edges only lifting off the skin)

2 = ≥ 50% to < 75% adhered (less than half of the patch lifting off the skin)

3 = > 0% to < 50% adhered but not detached (more than half of the patch lifting off the skin

Without falling off)

4 = 0% adhered - patch detached (patch completely off the skin)

Observation:

Table 4.88 Observation for patch adherence study

TEST	SCORE	OBSERVATION
Patch adherence study	0	Patch adhered > 90%(completely on)

Discussion

After wear period it was observed that >90 % of patch adhered to the skin.

Immediately following removal of patch, the amount of adhesive remaining on patch site was examined and graded on 4 point scale:

Table 4.89 Point scale for remaining amount of adhesive at patch site study

TEST	SKIN REACTION	SCORE
Amount of adhesive remaining on patch site	None	0
	Light	1
	Medium	2
	Heavy	3

Observation

Table 4.90 Observation for amount of adhesive remaining on patch site

TEST	SCORE	OBSERVATION
Amount of adhesive remaining on patch site	1	Light

Discussion:

Amount of adhesive remaining on patch site was observed. Table 4.66 showed that light amount of adhesive was remained on patch site.

SUMMARY

Transdermal route is one of the potential alternative route that can improve undesirable characteristics of oral and parenteral therapy. Repaglinide is an anti-diabetic, oral hypoglycemic agent and is used in management of type-2 diabetes mellitus. Its oral therapy is often associated with several problems like poor bioavailability, frequent dosing leading to patient incompliance and other systemic and oral adverse effects, the most important being hypoglycemia. Transdermal drug delivery system provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy.

Therefore Repaglinide was chosen as a model drug and attempt was made to deliver Repaglinide in transdermal dosage form. Transdermal patches of Repaglinide were prepared by solvent casting method using HPC-EF and Duro-Tak 87-9301 as polymer.

In present study various formulations were evaluated using physicochemical evaluation parameters such as thickness, % elongation, tensile strength, % moisture content, moisture uptake. In-vitro drug release study was performed using Franz diffusion cell with cellophane membrane. Ex-vivo drug release study was also performed using Franz diffusion cell with guinea pig skin.

Result revealed that patches showed good physical characteristics. Drug purity was confirmed by using FTIR and melting point determination studies. Teflon petridish was selected as casting surface and 20% w/v of PEG-400 was suitable plasticizer for preparation of transdermal patches containing HPC-EF polymer. Batch H5 containing 10% w/v of HPC-EF and 10% w/v of IPM showed maximum in-vitro (92.41%) and ex-vivo (90.86%) drug release as compared to all other batches. Higuichi model showed maximum r^2 value (0.979) so drug release can be best expressed by Higuchi's equation diffusion characteristic.

Scotch-pack backing layer was selected for the preparation of transdermal patches containing Duro-Tak 87-9301. Batch D5 containing 80% w/w of Duro-Tak 87-9301 and 10% w/v of IPM showed maximum in-vitro (89.15%) and ex-vivo (90.74%) drug release as compared to all other batches. Higuichi model showed maximum r^2 value (0.954) so drug

release can be best expressed by Higuchi's equation diffusion characteristic.Comparative study for HPC-EF and Duro-Tak 87-9301 was done, results shown that batch H5 (10 % of HPC-EF and 10% of IPM) gave maximum drug release compared to all other batches and it was selected as best batch for transdermal patch. .

In vitro and ex-vivo correlation study was also performed. Results indicate in-vitro diffusion profile is similar to ex-vivo diffusion profile. Average drug content of three transdermal patch of batch H5 was determined spectroscopically and % drug content was found to be 95.05 %

Different concentration of PVA and EC were used for preparation of backing layer. Batch B2 (5 % of PVA) showed maximum tensile strength and % percentage elongation as compared to all other batches. It showed acceptable mechanical properties and it was transparent, flexible and suitable for preparation of backing layer.

Adhesive layer was prepared using 0.5, 1, 1.5, 2.0, 2.5 % w/w of Duro-Tak 87-9301 as pressure sensitive adhesive. Peel adhesion study was performed by using Texture analyser and results showed that 2% w/w of Duro-Tak 87-9301 gave optimum adhesiveness as compared to other batches.In vivo study (skin irritation study and patch adherence study) was carried out on human skin and results showed that no skin irritation was observed on to the skin.

Thus, Repaglinide can be successfully incorporated in TDDDS using HPC-EF as polymer.

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