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DESIGN AND EVALUATION OF CONTROLLED RELEASE TRANSDERMAL DOSAGE FORM OF CARDIOVASCULAR DRUGS

A Thesis

Submitted for the degree of

DOCTOR OF PHILOSOPHY

In the

FACULTY OF PHARMACY

TO

SAURASHTRA UNIVERSITY, RAJKOT

Research guide:

Dr. H. M. TANK

M. Pharm, Ph. D

Submitted by:

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CERTIFICATE

This is to certify that the thesis entitled "**DESIGN AND EVALUATION OF CONTROLLED RELEASE TRANSDERMAL DOSAGE FORM OF CARDIOVASCULAR DRUGS**" submitted for the Ph.D. degree in pharmacy by **Mrs. Shital Dhiren Faldu** incorporate original research work carried out by her under my supervision and no part of the thesis has been submitted for any other degree.

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DECLARATION

I here by declare that the topic entitled "**DESIGN AND EVALUATION OF CONTROLLED RELEASE TRANSDERMAL DOSAGE FORM OF CARDIOVASCULAR DRUGS**" Which is submitted herewith to Saurashtra University, Rajkot for the award of Doctor of Philosophy in the Faculty of Pharmacy is the result of work done by me in S.S. Institute of Pharmaceutical Education and Research, Rajkot, under the guidance of Dr. H. M. Tank, Principal, S.S. Institute of Pharmaceutical Education and Research, Rajkot.

I further declare that the results of this work have not been previously submitted for any degree or fellowship.

Place: Saurashtra University, Rajkot — Mrs. Shital D. Faldu

Date: — M.Pharm.

DEDICATED

TO

MY BELOVED FAMILY

AND

FRIENDS...

ACKNOWLEDGEMENT

ACKNOWLEDGEMENT

No research is ever the outcome of single individual's talent or efforts. This project work would not have been possible without the hearted encouragement, support and co-operation of my parents, guide, teachers, friends and well wishers. This thesis is a fruit of infinite encouragement, infallible guidance and unstinted cooperation received from multidirectional aiming for a unidirectional goal. With heartiness I take opportunity to pen down few words of heartfelt expression for all involved directly or indirectly in making this thesis a success.

This work was carried out at the Department of Pharmaceutics and Pharmaceutical Technology, S.S. Institute of Pharmaceutical Education and Research, Rajkot during the year 2006-2009. I am indebted for the same.

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(Shital D. Faldu)

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Aim of present investigation

AIM OF PRESENT INVESTIGATION

A nation with a good social health is the happiest situation and the pharmacist's role is to contribute a lot towards healthcare management in the society through his community service and research apt to the country's needs. Today's pharmaceutical research is the mother of all the expertise including technology required to manufacture, simple as well as sophisticated remedies in the country.

The cost involved both in the terms of money and time in the development of a single new molecule has mandatory for pharmaceuticals companies to reconsider research focus. Great strides have been made in the management of disease through the invention of drugs over the past 50 years, clearly, unless a drug can be delivered to its target area at a rate and concentration that both minimize side effects and maximize therapeutic effect, the drug will not be maximally beneficial to the patient and in the extreme, an otherwise useful drug must be discarded.

Pharmaceutical field is the research and development intensive field. The search for safe and effective drugs continues to be major effort involving the pharmaceutical industries, universities and government. The complexities of discovering and testing new drugs have become enormous as a result of the many aspects of safety, efficacy and economics that determine acceptability of a drug. Indeed the situation as a whole has become almost a Gordian knot. The concept of controlled drug delivery has been embraced with great enthusiasm by many as the sword that will slice through Gordian knot.

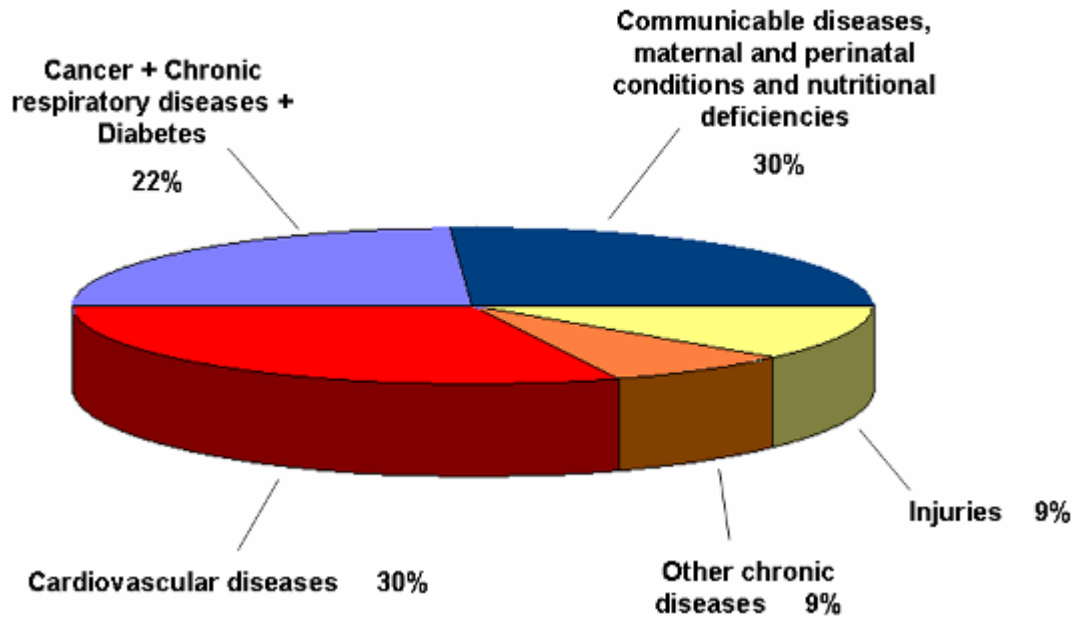
Current state of drug delivery approaches are humble steps in the direction of creating ZION on earth by attaining Zenith of health and cure for everyone. Last century had witnessed spectacular developments on the diverse kind of oral delivery systems. Nevertheless there is continuous need for developing delivery systems that can regulate drugs levels in a more efficacious, elegant and economic manner.

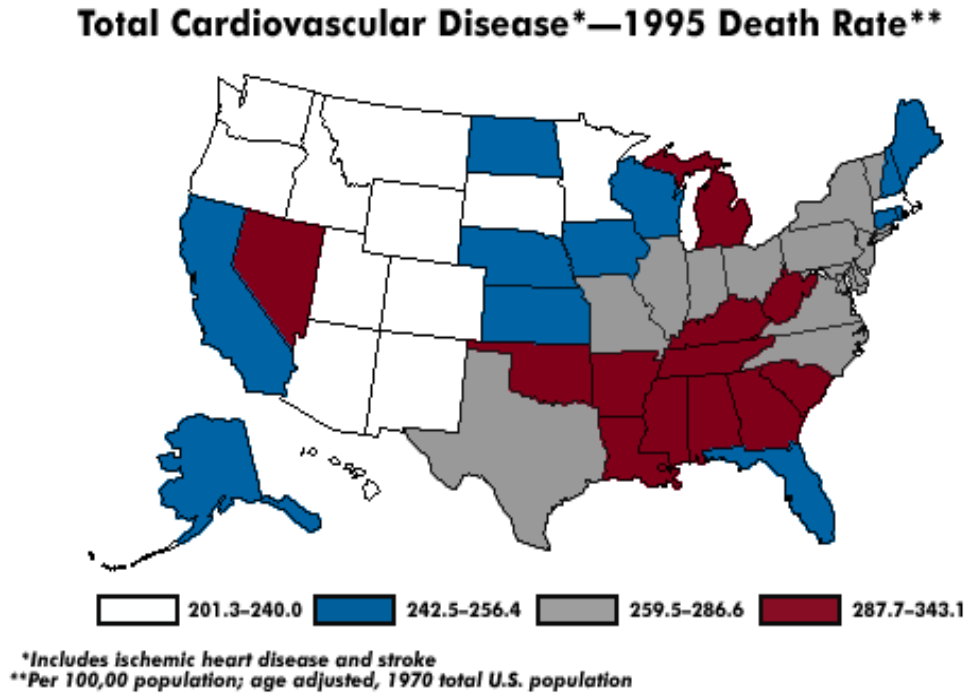
In an attempt to reduce the cost of drug development process and advantageously reap the benefits of patient's regime, companies are now investing strategically in the development of controlled drug delivery system [CDDS]. Evolution of an existing drug molecule from a conventional form to CDDS can significantly improves its performance in terms of patient compliance, safety and efficacy. CDDS that can precisely control the release rate or target drugs to a specific body site have had an enormous impact on the healthcare system. Drug delivery companies are engaged in

the development of multiple platform technologies to get competitive advantage, extended patent life and increase in market share of their products. The last two decade in the pharmaceutical industry have witnessed an avant-garde interaction among the field of polymer and material science, resulting in the development of CDDS.

There are high hopes in achieving satisfactory results in developing newer formulations and remedies for cardiovascular diseases, coronary heart diseases, bronchial asthma, diabetes, rheumatoid arthritis, hepatitis, cancer, AIDS etc. from indigenous and natural resources under WHO guidelines.

Cardiovascular diseases rank first amongst dreaded diseases, second is the cancer. Coronary cardiac problems and ischemic heart diseases (IHD) are major causes for death among other diseases.





As per WHO report for the cause of death, cardiovascular diseases contributing to about 30% of total deaths. In US about 23 to 30% deaths are due to IHD. Every year, out of 5 million patients presented to emergency room with chest discomfort approximately 1.5 million are hospitalized for acute coronary syndromes. Each year in US more then 1 million patient suffer an acute myocardial infraction. In India the situation is quite gloomy about 35% to 40% deaths are due to IHD, out of 5 millions are hospitalized for acute coronary syndrome.

Every CVD does not lead itself to deaths but it is responsible to economic loss to the nation also. The top ten therapeutic segments accounting nearly 30% of total world market, the leading out of 10% is CVD; which is growing at the rate of 20% per annum. The economic implication of preventive and therapeutic interventions directed against heart diseases along with medical care and medical interventions had become a subject if considerable and intensive research of CVD.

Globally the situation is very poor, as per WHO press release titled “Stop the global epidemic of chronic diseases” mentioned the following facts.

i) In 1999 CVD contributed to a third of global deaths.

ii) In 1999 low and middle income countries constituted to 78% of CVD deaths.

iii) By 2010 CVD is estimated to be leading cause of death in developing countries.

iv) Heart disease has no geographic, gender, socio-economic boundaries.

v) Majority of CVD are presentable and controllable.

vi) By 2015 almost 20 million people will die from CVD, every year.

WHO press release mentions an estimated economic loss of US $ 558 billion due to chronic diseases.

The drugs used in the treatment of cardiovascular disease like Diltiazem, Nifedipin, Verapamil, Atenolol etc. are not free from biopharmaceutics and pharmacokinetic problems of absorption, hepatic metabolism and poor bioavailability. Despite the tremendous advancement in drug delivery, the transdermal route remains the preferred route for the administration of therapeutic agents because the cost of therapy and ease of administration lead to high level of patient compliance.

Transdermal drug delivery system [TDDS] are the novel drug delivery system in which a constant, prolonged and therapeutically effective drug levels are maintained by using intact skin as a port of drug administration. Modern transdermal technology is entering its fourth decade after 2000. The 1970's was the era of experimentation. Since then this new mode of drug delivery not made journey to deliver simple bases clonidine, scopolamine, nicotine, to complicated molecules like insulin, acyclovir, nitroglycerin but it has also developed fabrication techniques, exploited pressure sensitive adhesive as matrix moderated delivery component, penetration enhancement concept, exploited ultrasonic waves sonophoresis, microelectronics, liposomes, microneedles, transfersomes etc. as strategic means for skin penetration to meet required steady state plasma concentration of drug.

Over the past 10 years there have been 8000 transdermal related presentation at the annual conventions of controlled release society and American Association of Pharmaceuticals Science.

Nitroglycerine, scopolamine, clonidine, fentanyl, estradiol are the drugs that have sizable transdermal market in US. The global drug delivery market of transdermal formulation is expected to increase from $ 6.7 billions in 2000 to $ 26.7 billion in 2010. Transdermal drug delivery system offers a solution to bioavailability problems. Also economically T.D.D.S. market is growing at the rate of 15 % per annum; offers a good economical gain along with some distinct advantages over other controlled drug

delivery system which are,

• No pricking with needle required.

• External mode of administration.

• Easy to discontinue therapy in case of side effect.

• No sterilization processing involved compared to parentaral manufacturing.

• Bioavailability problem are minimized.

Drugs that have a short biological half life need to be administered two or three times in a day. For the drugs which are susceptible to hepatic metabolism need to be administered in a higher doses to meet the requirement of steady state plasma concentration for therapeutic effect.

Diltiazem is classified as calcium channel blocker, is used in cardiovascular disease. It has a mean plasma half-life of 3.5 hrs and only 40% of the orally administered drug reaches the circulation due to hepatic metabolism.

Atenolol (TENORMIN) is a β1–selective antagonist, used in hypertention. Atenolol is very hydrophilic and appears to penetrate the brain only to a limited extent. Its half-life is somewhat about 5 to 8 hours. Only 50% orally absorbed dose reaches the systematic circulation.

To obviate the problems of bioavaibility and to enhance patient's compliance to the therapy transdermal drug delivery system of the above drugs shall be a proper solution.

The present search was directed to examine the biopharmaceutics aspect of selected drugs, prior to incorporate into delivery devices. The permeability of Diltiazem and Atenolol was evaluated using membrane, matrices and human skin.

In recent years the value of hydrophilic and hydrophobic polymers based drug delivery system as vehicles for controlled release delivery has been increasingly demonstrated as vehicle of numerous patents and research papers and their utilization n new product in the market place. The use of biopolymeric devices to control the release of a variety of drugs has become important in the development of modified release dosage form. In part the widespread and successful use of such polymeric systems can be attributed to their ease of manufacturing, relatively low cost, their favorable in vivo performance and their versatility in controlling the rate of drugs with a wide range of physiochemical properties.

The aim of present investigation was to develop transdermal drug delivery system of Diltiazem base and Atenolol using various hydrophilic and hydrophobic polymers.

Optimization techniques shall be applied in the present study to systematically study the influence of formulation variables on the development of dosage forms. Matrix diffusion and membrane moderated T.D.D.S., shall be deviced using various polymers. Specific strategies like use of drug penetration enhancers shall be employed to meet systemic requirement of drug. Different formulations variables shall be studied and optimized to achieve the desired release profile. Finally selected devices shall be evaluated for in vitro studies using human skin.

The present study was aimed at the development of membrane moderated and matrix moderated transdermal drug delivery system of Diltiazem and Atenolol using various polymers. Specific strategies like use of penetration enhancers shall be employed to meet systematic requirement of drug. Different formulation variable shall be studied and optimized to achieve the desired release profile.

Finally selected devices shall be evaluated for in vitro study using human live skin.

Chapter 1

Introduction

CHAPTER 1

1.1 CONTROLLED DRUG DELIVERY SYSTEM

1.1.1 Introduction

The administration of conventional oral dosage forms like tablets, capsules, liquids orals of drugs suffers a set back due to problem of gastro intestinal tract absorption, local irritation, dilution of drug strength , Liver first pass metabolism, degradation of drug by gastro intestinal tract enzymes, the protein binding of drug at an absorption surface and local toxicity. The bioavailability as well as duration of action is reduced which requires frequent administration, which in turn is associated with the problem of patients compliance to therapy and the economy of the treatment.

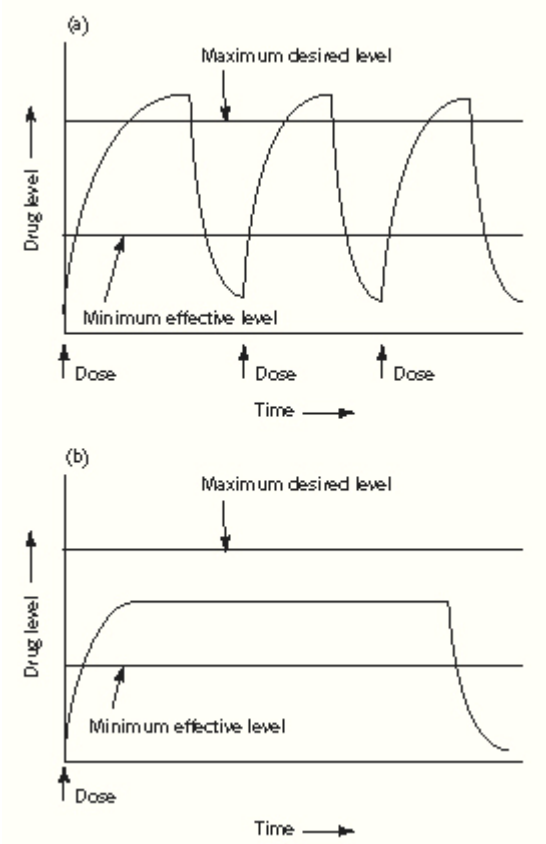
Parenteral route is the preferred route of administration for moderate to severe complication, even though patients compliance are rather low for this mode of drug delivery as it is invasive drug delivery technique, requiring frequent pricking with needle.

All conventional dosage form except intravenous infusion, follow second-order kinetic.[1] Dosage form releases drug initially at faster rate, leading to quick rise in blood level of drug and then falls exponentially until a further dose is administered. This results in peak and valleys pattern of drug concentration in blood and tissues. Thus, for most of the time the concentration of drugs either above the required therapeutic level or below it. The time course of various modes of administration[2] is represented in figure 1.1.1.

It is evident that the quality of the rate of absorption and the rates of metabolic elimination would result on the equilibrium distribution of the drug in tissues and blood, but however missing in the case of conventional dosage forms. This factor as well as some other factors such as repetitive dosing and unpredictable absorption, led to the concept of drug delivery system or the therapeutic system.

Drug delivery system may be a controlled release drug delivery system where there is predictive control over the release pattern, and subsequent tissue or blood levels can be achieved. From figure 1.1.1, it is observed that the equality of the rate of absorption and the rate of metabolism is only in the case of controlled drug delivery system.

Figure 1.1.1: Time course of various modes of administration



(1) Conventional single administration

(2) Multiple administrations

(3) Sustained release administration

(4) Controlled drug delivery administration

Administration of drug in conventional dosage form requires- large dose, frequent administration and lacks extended duration, with chances of toxicity. While in controlled drug delivery devices there is efficient utilization of drug, desired extended duration, with very low chances of toxicity, facilitating enhanced complication of patient, leading to better management of therapeutics. The efficacious use of drug influences cost factor and economy of therapy too.

It seems that the controlled delivery should be the goal for all products and now a day's drug firms have been allocating large resources on the reformulation of older, existing drugs, in sustained and controlled drug delivery often resulting in special economic gains.

1.1.2 Types of controlled release preparation [3-13]

On the basis of technical sophistication, controlled drug delivery system can be categorized into 3 major classes.

A. Rate programmed controlled drug delivery system

These drug delivery system are those from which the drug release has been programmed at specific rate profiles. They are further subdivided into following subclasses.

1. Dissolution controlled drug delivery system

Slow dissolution rate of the drug

Slow dissolution rate of the reservoir membrane or matrix

2. Diffusion controlled drug delivery system

Porous matrix controlled system

Porous membrane controlled systems

3. Erosion controlled drug delivery system

Surface erosion

Bulk erosion

4. Dissolution, Diffusion and/or Erosion controlled drug delivery system

Reservoir system (membrane controlled drug delivery system)

Matrix system (monolithic drug delivery system)

Hybrid systems (membrane cum matrix drug delivery system)

B. Stimuli activated drug delivery system

1. Activation by physical process

Osmotic pressure activated drug delivery system

Hydrodynamic pressure activated drug delivery system

Vapor pressure activated drug delivery system

Mechanical force activated drug delivery system

Magnetically activated drug delivery system

Thermally activated drug delivery system

Photo activated drug delivery system

Photo mechanically waves (laser) activated drug delivery system

Ultrasound activated drug delivery system

Electrically activated (Iontophoresis**)** drug delivery system

2. Activation by chemical process

pH activated drug delivery system

Ion activated drug delivery system

Hydrolysis activated drug delivery system

Chelation activated drug delivery system

3. Activation by biological system

Enzyme activated drug delivery system

Antibody interaction drug delivery system

Antigen activated drug delivery system

Inflammation activated drug delivery system

C. Site targeted drug delivery system

1. Polymeric carriers for drug targeting

2. Albumin as carrier for drug targeting

3. Lipoprotein as carrier for drug targeting

4. Liposomes as carrier for drug targeting

1.1.3 Drug release mechanisms for controlled drug delivery system

Most of the design of controlled release dosage form employs polymers for controlling the drug release. There are three fundamental mechanisms by which polymers release drugs.[14]

(1) Diffusion e.g. Reservoir type systems

- Microcapsules
- Matrix/laminates

(2) Chemical reaction Water or enzyme causes degradation of a polymer which is used to encapsulate a drug.

- Erodible
- Degradable systems
- Pendant chain systems

(3) Solvent activated external systems In this case drug is entrapped in the polymer until either solvent swells the polymer or water

imbibement creates osmotic pressure.

(4) Other general mechanism includes

Magnetic signal	Placement of drug into magnetic beads in matrix systems, Applying an external magnetic field, the beads can be made to Squeeze drug through the polymer.
Chemical signal	This can also be utilized to create self regulated systems. In such cases an external molecule such as glucose can diffuse into a polymer membrane and react with an enzyme that is immobilized. Enzymatic reaction causes pH sifts which alters membrane permeability or drug solubility and there by changes the release rate.

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1.2 TRANSDERMAL DRUG DELIVERY SYSTEM

1.2.1 Introduction

Conventional systems of medication that require multi dose therapy are having many problems. The controlled drug delivery is a newer approach is to deliver drug in to systemic circulation at a predetermined rate. Our system should duplicate continuous intravenous infusion, which not only by passes hepatic 'first pass' elimination but also maintains a constant, prolonged and therapeutically effective drug level in the body. This is made possible by using intact skin as a port of drug administration to provide continuous delivery of drug in to systemic circulation. Following skin permeation, the drugs first reach the systemic circulation. The drug molecules are then transported to the target site, which could be relatively remote from the site of administration, to produce therapeutic action.

Transdermal drug delivery offers the following potential advantages [1, 2, 3]

1. Avoid the risks and inconveniences of intravenous therapy and of varied conditions of absorption and metabolism associated with the oral therapy.
2. Continuity of drug administration in TDDS permits the use of a drug with short biological half-life.
3. Transdermal drug delivery improves the bioavailability that reduces the total daily dose.
4. Avoids first-pass hepatic metabolism.

5. Less chances of over or under dosing as the result of prolonged pre programmed delivery of drug at the required therapeutic rate.
6. Decrease gastrointestinal side effects.
7. Elimination drug food interactions.
8. Increased patient compliance in following manner
    - Provisions of simplified therapeutic regimen.
    - Painless delivery of drug.
    - Eliminates swallowing.
    - No chances of forgetting the dose once the device is applied on skin.
    - Easy to carry a patch in wallet or ladies purse.
9. Patches offer less friability problems of wear and tear than the tablets.
10. In a multi drug regimen TDDS avoids drug interaction in GIT.
11. It is easy to terminate the medication simply by removing the dug delivery device from the skin surface.
12. TDDS system can be taken without any aid, which makes it most suitable formulation; for instance, tablet and capsule need little water. Liquid oral preparation needs teaspoon and parentaral delivery needs specialized person whereas if a patient is told to apply TDDS patch, he/she can do it any where e.g. in office, in theatre, in club, in house without any aid.
13. Chance of toxicity due to additives e.g. preservatives, stabilizing agent antioxidants etc. are less as compared to other dosage forms.
14. Problem of dose dumping is least in TDDS, because stratum corneum is more resistant than the inner membranes (i.e. mucous membrane in case of oral controlled release delivery systems) and stratum corneum itself is a rate limiting factor.
15. Need not to be sterile, obviates processing problem.

Disadvantages of transdermal drug delivery system[4]

1. The limitation of transdermal drug delivery is principally associated with skins barrier function, which severely constrains the absolute amount of drug that can be absorbed across reasonable area of skin during the dosing period. Thus, the major disadvantage of the method is that it is limited to potent drug

molecule typically those requiring a daily dose on the order of 20 mg or less.

2. Even if the drug is sufficiently potent, it must yet satisfy other criteria to be considered a viable candidate for transdermal drug delivery. For example its physiochemical properties must allow to be absorbed percutaneously. This mean that its molecular weight should ideally be less than 500 Daltons and it should have adequate solubility in both lipophillic and aqueous environments since, to reach dermal micro circulation and gain access to systemic circulation, the molecule must cross that stratum corneum (a lipid barrier) and then transfer through the much-more-aqueous-in-nature viable epidermis and upper dermis. Absence of either oil or water solubility altogether, will preclude permeation at a useful rate.
3. The pharmacokinetic and pharmacodynamic characteristic of the drug must be such that relatively sustained and slow input provided by transdermal delivery makes sense. Tolerance inducing compounds are not intelligent choice for this mode of administration unless until an appropriate “wash out” period is programmed into the dosing regimen.
4. Drugs that can be given once a day orally, with reproducible bioavailability and which are well tolerated by patient do not really need a patch formulation.

Drugs must not be locally irritating or sensitizing since provocation of significant skin reactions beneath a transdermal delivery system will most likely prevent its regulatory approval.

1.2.2 Physiology of human skin[2]

The skin is the largest single organ in the body. An average human skin is known to contain, on an average 40-70 hair follicles and 200-250 sweat ducts per every square centimeter of the skin. These skin appendages, however actually occupy grossly only 0.1% of total stratum corneum surface henceforth the transappendageal route of percutaneous absorption has provided only a very limited contribution to the overall kinetic profile of transdermal permeation. Therefore, the transdermal permeation of most neutral molecule at steady can thus be considered as primarily diffusion through the intact stratum corneum in the interfollicular region. So, for fundamental understanding of TDD (Transdermal drug delivery), the structure should be

understood.[3] Figure 1.2.1 is transeverse section of the human skin. The skin can be divided in to two layers.

I Epidermis - It is superficial layer of stratified epithelium which is of ectodermal origin.

II Dermis or Corium - It is foundation of firm connective tissue upon which epidermis is laid and is of mesoderm origin.

The total thickness of skin has got considerable regional variation, ranging in human body from less than 1/10 of millimeter tip to 3 or even 4 millimeters.

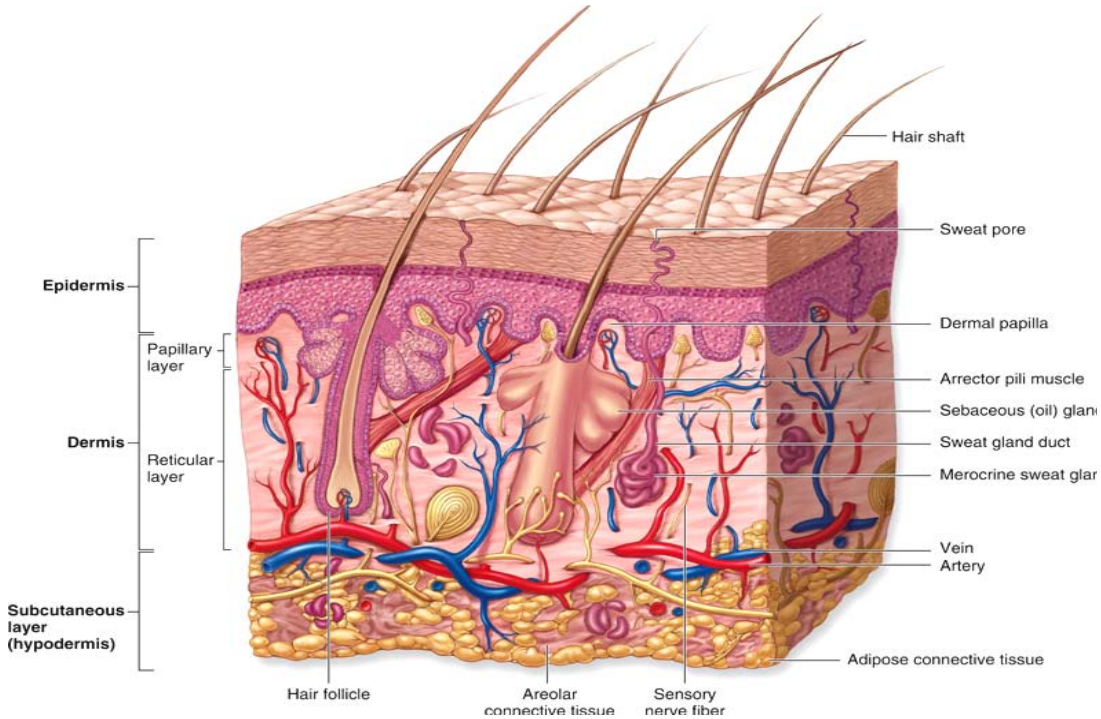
I. Epidermis [5,6]

In a typical part of the epidermis there are number of different strata in which the cells have distinct anatomical features. From below, the first stratum is the ***basal layer*** or *layer of Malpighi*. Its cells are mostly polygonal in shape, the deepest tending to a cylindrical columner form, and the most superficial becoming somewhat flattened.

Active mitotic proliferation takes place in the deeper layers, the development of new cells leading to a gradual displacement of the older cells towards the surface.

Hence, this stratum is also called ***stratum germinatum***. The epidermis is quiet avascular, and between the cells of stratum germinatum there are fine intercellular channels which probably allow the transmission of nutrient fluids derived from capillary blood vessels in the adjacent dermis. These channels are bridged across by delicate protoplasmic threads connecting one cell with another. The stratum germinatum, therefore, appears to be syncytium of cells.

Figure 1.2.1: Transverse section of human skin



Covering the ***stratum of Malpighi*** is a layer two or three cells deep called the stratum granulosam. These cells are flattened and contain abundance of granules in their cytoplasm.

Above stratum granulosam is ***stratum lucidam*** **-** it called so, because it has a clear translucent appearance in stained sections. At this level of their growth towards the surface, the cells have lost their clear-cut outline, the nuclei are becoming indistinct, and the granules of the subjacent stratum have become converted in to larger masses of an achromatic substance.

The surface stratum forms the greater part of' the whole thickness of the epidermis in many parts of the skin. Because of' horny character of the cellular elements that composes this layer; henceforth it is called the ***stratum corneum.*** In stratum corneum the cell structure has become completely obscured and nuclei are no longer evident.[6] The cells are now known as corneocytes. Corneocytes are dead, flattened and rich in keratin. Corneocytes are densely arranged and are surrounded by a complex mixture of intercellular lipids. The complex mixture of intercellular lipids comprises ceramides, free fatty aids, cholesterol and cholesterol sulphate. Their most important

feature is that they are structured into ordered bilayer arrays. The predominant diffusional path for a molecule crossing the stratum corneum appears to be intercellular.[7] The stratum corneum is traversed by the ducts of sweat glands and hair where these are present. Table 1.2.1 and Table 1.2.2 show the composition of the human stratum corneum and composition of stratum corneum lipids respectively.[8, 9, 10]

Table 1.2.1: Composition of human stratum corneum

Sr. No.	Components	Percentage	Gross Biochemical composition
1	Cell membrane	5	lipid and non-fibrous protein
2	Cell contents	85	Lipids (20 %) α-Protein (50 %) β- Protein (20 %) Non-fibrous protein (10 %)
3	Intercellular proteins	10	lipid and non fibrous proteins

Table 1.2.2: Composition of stratum corneum lipids

SR. NO.	COMPONENTS	PIG	HUMAN
1	Cholesteryl esters	1.7	10.0
2	Triglyceride	2.8	0.0
3	Fatty acids	13.1	9.1
4	Cholesterol	26.0	26.9
5	Ceramide 1	4.1	3.2
6	Ceramide 2	16.7	8.9
7	Ceramide 3	6.9	4.9
8	Ceramide 4	4.4	6.1
9	Ceramide 5	4.5	5.7
10	Ceramide 6	7.6	12.3
11	Glucosyl Ceramides	1.0	0.0
12	Cholesteryl Sulphate	3.9	1.9
13	Others	5.7	11.1

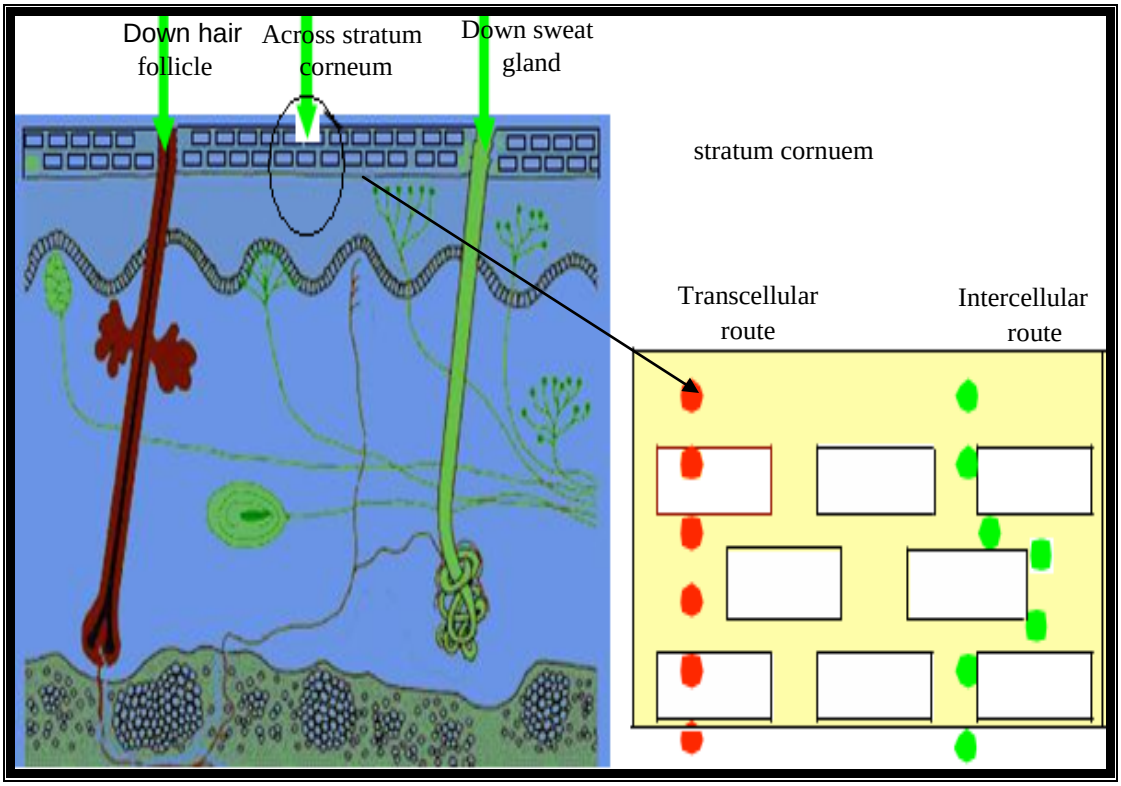
II. Dermis

The dermis or corium consists of a dense felt work of connective tissue in which bundles of collagenous fibres predominate, mingled with a certain proportion of elastic tissue in superficial levels. Dermis contains fine plexuses of blood vessels, lymphatics and nerves, hair follicles, sweat glands and sebaceous glands.[2,3] The thicker the epidermis, therefore, the more prominent are the papillae.[11]

1.2.3 Drug penetration pathways [12-24]

There are critically three ways in which a drug molecule can cross the intact stratum corneum: via skin appendages (shunt routes); through the intercellular lipid domains; or by a transcellular route (Figure 1.2.2) A particular drug is likely to permeate by a combination of these routes, with the relative contributions of these pathways to the gross flux governed by the physicochemical properties of the molecule.

Figure 1.2.2: Drug penetration pathways across skin.



THE APPENDGEAL ROUTE

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

TRANSCELLULAR ROUTE

Drugs entering the skin *via* the transcellular route pass through corneocytes. Corneocytes, containing highly hydrate keratin, provide an aqueous environment for which hydrophilic drugs can pass. The cells are surrounded by a lipid envelope which connects the cells to the interstitial lipids. Separating keratinised skin cells are multiple lipid bilayers; there are estimated to be up to 20 such lamellae between each corneocyte. Therefore, the diffusion pathway for a drug *via* the transcellular route requires a number of partitioning and diffusion steps.

INTERCELLULAR ROUTE

The intercellular pathway involves drug diffusing through the continuous lipid matrix. This route is a significant obstacle for 2 reasons. Firstly, recalling the 'bricks and mortar' model of the stratum corneum, the interdigitating nature of the corneocytes yields a tortuous pathway for intercellular drug permeation, which is in contrast to the relatively direct path of the transcellular route. It has been estimated that water has 50 times further to travel by the intercellular pathway, than the direct thickness of the horny layer. Secondly, the intercellular domain is a region of alternating structured bilayers. Consequently, a drug must sequentially partition into, and diffuse through repeated aqueous and lipid domains. This route is generally accepted as the most common path for small uncharged molecules penetrating the skin.

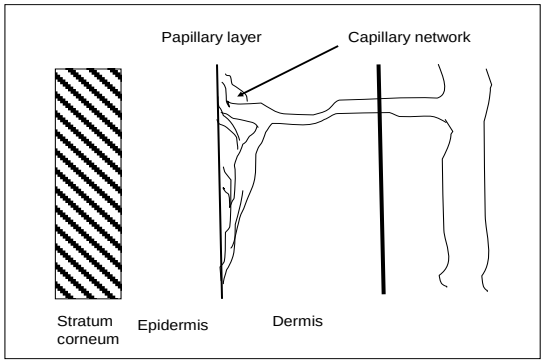
1.2.4 Stratum corneum the rate limiting barrier to percutaneous absorption

The skin contains a dead layer stratum corneum and viable layers, the viable tissue contains catechol-o-methyl transferase which metabolizes the drugs, the papillary

layer of Dermis contains so many capillaries and it is highly probable that most molecules enter the micro circulation soon after leaving the epidermis. Thus the average total resistance time of a drug species in the dermal aqueous phase may be only a fraction of minute.[25]

A simplified model of the human skin for mechanistic analysis[26] is shown in figure 1.2.3

Figure 1.2.3: Human skin model



For abdominal skin the thickness of Stratum corneum is 10µm, Viable epidermis 100 µm and for Upper portion of dermis is 100µm.

The diffusional resistant total.

$$\sum R_1 = \frac{hsc}{Dsc \cdot Ksc} + \frac{h_E}{D_E \cdot K_E} + \frac{h_D}{D_D \cdot K_D}$$

h = Thickness

D = Diffusion coefficient

K = Partition coefficient

Sc = Stratum corneum

E = Epidermis

The sum of tissue resistance Rs of skin calculated for water permeation [27] by using appropriate parameters for above equation.

$R_S = R_{SC} + R_E + R_D$

$= 9.1 \times 10^6 + 6.3 \times 10^3 + 6.3 \times 10^3$

$= 9.1 \text{ X } 10^6$ cm/sec.

The dermis and epidermis are extensively hydrated and diffusion coefficients are typical of liquid-state diffusion. In dermis molecules probably moves within water filled interstices. In contrast, the stratum corneum is semi fibrous structure which is characterized by semisolid diffusivity. According to these calculations the diffusional resistance of the stratum corneum to water is approximately 1000 times that of the either the viable epidermis or the superficial regions of dermis.

Polar Solutes (other than water) and larger molecules usually have smaller diffusion coefficients than that of the water, and stratum corneum becomes even more dominant in permeation process. In general conclusion, the stratum corneum (Horny layer) provides the rate limiting barrier for diffusion is true for the entire class of the water soluble substances.

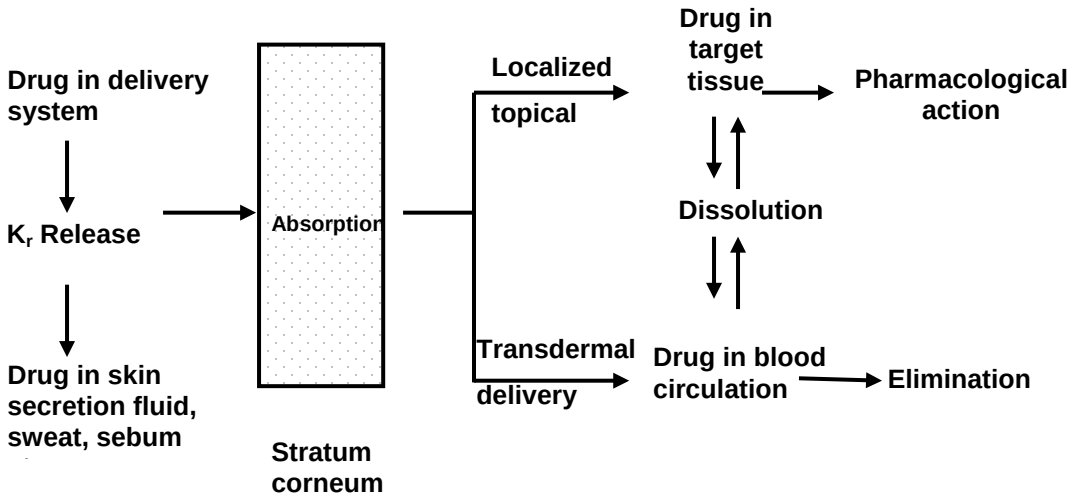
Lipid Soluble Molecules, Octanol applied to cadaver skin as an aqueous solution.[28] Comparing the result with that for water, it was observed that the resistance of the skin has decreased dramatically and that this reduction arose from the more favorable distribution of the octanol into the stratum corneum (as measured by K_{sc}). Thus for non polar molecules also stratum corneum provides rate limiting barrier to percutaneous absorption.

Probably the intercellular keratin structure, rather than cell wall of stratum corneum is the main site for membrane diffusional resistance,[27,28] although thickened plasma membrane has, been reported [29] to be the main diffusional barrier in stratum corneum.

The dependency of permeation on the partition coefficient it appears that the lipid-soluble molecules segregate and diffuse through lipid region in stratum corneum. A mathematical model for stratum corneum[30] does assume that there is strict separation of protein and lipid in stratum corneum.

Percutaneous absorption of drugs for localized therapeutic actions in the skin tissues or for systemic medication in the tissues remote from the site of topical drug application [26] is shown in figure 1.2.4.

Figure 1.2.4: Percutaneous absorption of drugs



1.2.5 Pharmacokinetic model for percutaneous absorption

In figure 1.2.5, it is shown that how a drug molecule is partitioned and diffused to various layers of skin and reaches to systemic circulation. In this model, the rate constants are assigned true physicochemical significance and may be predicted from basic physical properties.[31] In figure 1.2.6 events during percutaneous absorption are shown.[32]

The kinetic parameters (Figure 1.2.5) are associated with the following significance.[33]

$f(K_1)$ describes the input kinetics From the transdermal device for a "membrane controlled" system, $f(K_1)$ consists of both First-order (K^1) and zero-order (K^0) components. The former represents drug release from the contact adhesive; the latter signifies the membrane limited leaching of drug from reservoir.

K_r reflects the fact that there will be competition for the drug between the patch and the stratum corneum, if the system is well designed, then K_r will be small.

K_1 and K_2 are first order rate constants describing drug transport across the stratum corneum and viable tissue, respectively K_1 and K_2 are therefore proportional to the corresponding diffusion coefficients through these layers of skin and may be simplistically related to the penetrant molecular weight (M) by equation.

$$D=C.M^{1/3} \qquad (1)$$

Figure 1.2.5: Pharmacokinetic model for transdermal drug delivery system

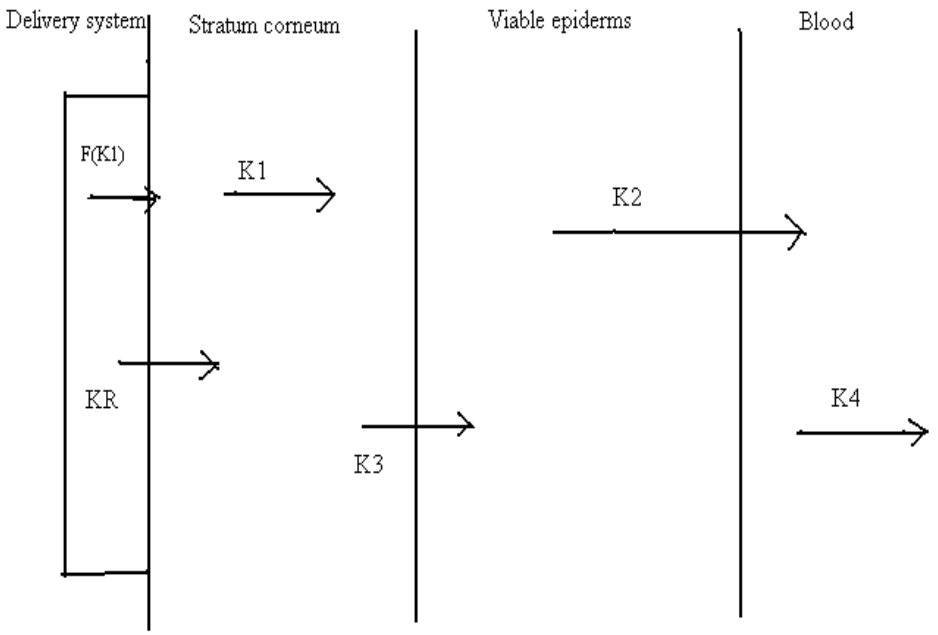
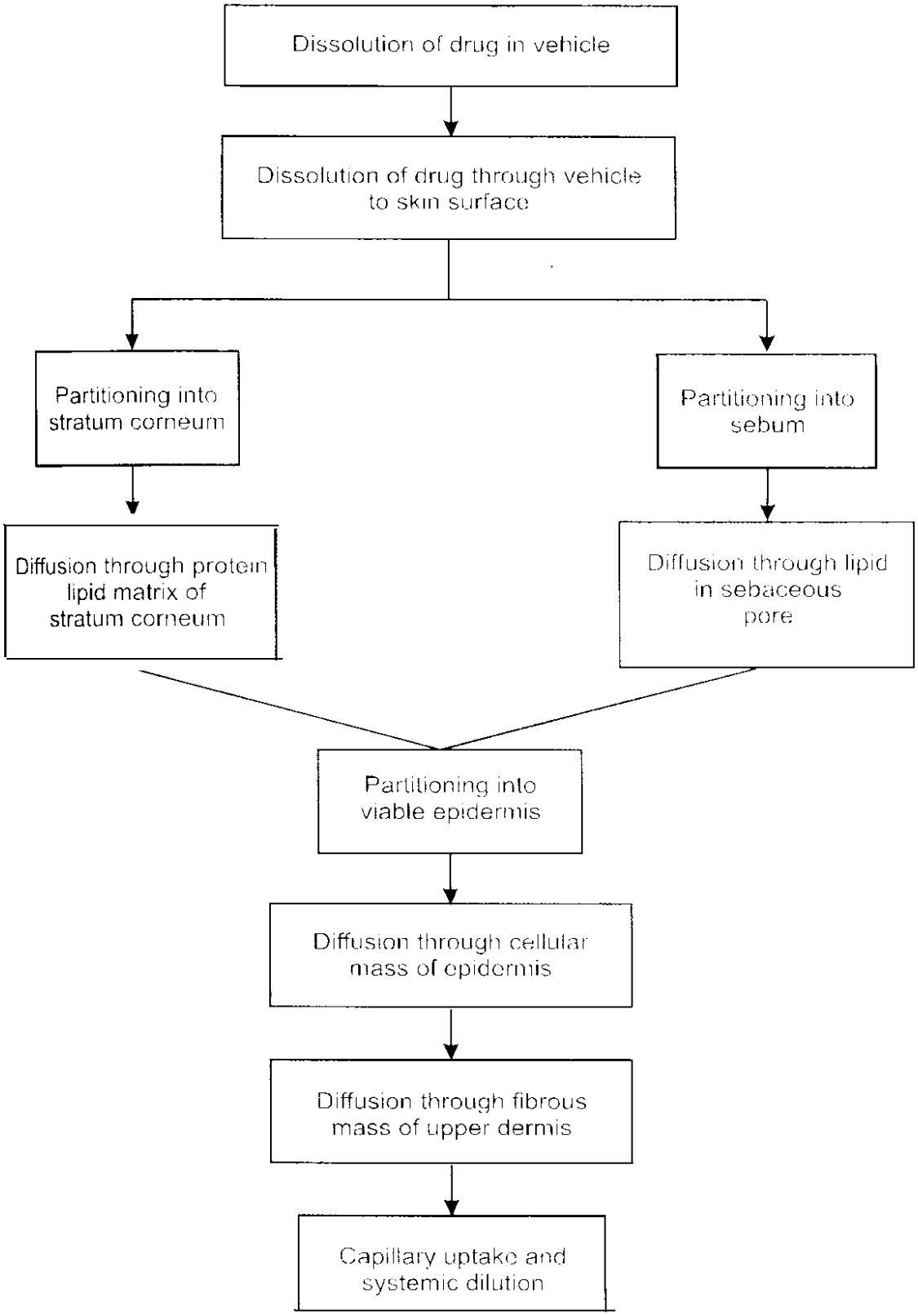


Figure1.2.6: Events governing percutaneous absorption



1.2.6 Pharmacokinetic Aspects for Transdermal Drug Delivery System

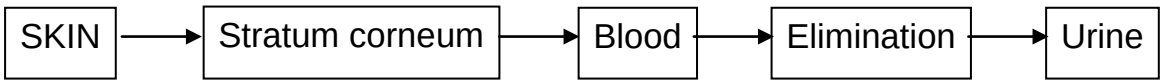
The development of drug delivery is extremely complex and requires the understanding of basic pharmacokinetic and bio-pharmaceutics.

For transdermal controlled drug delivery device the general diffusivity of drug across skin is the must. All the controlled release delivery devices are multiple dose devices designed to result in steady state concentration Css. The magnitude of Css depends on the dose rate R (amount of drug per unit of time), and total clearance CL (loss of drug from the volume of distribution per unit of time) of the drug.

Following are basic principles to understand design and evaluation.

(A) Compartment model

Single compartment model for transdermal delivery device is given below



(B) Terminal half life ($t_{1/2}$)

The terminal half life or biological half life is the time required to reduce the concentration in blood, plasma, or serum to one half, after equilibrium has been reached. It is an important parameter in selecting the drug candidate. The shorter the $t_{1/2}$, the greater will be the amount of drug to be incorporated into the device. Only the drugs whose $t_{1/2}$ can be correlated with the pharmacological response are candidates for controlled release drug delivery devices.

(C) The area under curve (AUC)

AUC is the measure of quantity of drug in the body. It is a "robust" pharmacokinetic parameter. It is the key parameter in determining absolute and relative bio-availability. It is determined by a compartment model- independent approach, using a trapezoidal rule.

It permits estimation of total clearance (CL) and apparent volume of distribution (V_d).

(D) Total clearance (CL)

The total clearance is that hypothetical volume of distribution of un-metabolized drug that is cleared per unit of time by any pathway of drug removal.

CL can be determined from the dose administered D, the absolute bio-availability F, and AUC

$$CL = \frac{DF}{AUC}$$

CL, V_d and $t_{1/2}$ interrelated as follow

$$CL = \frac{0.693vd}{t^{1/2}}$$

Upon multiple dosing, once steady state is reached CL is

$$CL = \frac{D}{AUC(T_n \rightarrow T_{n+1})}$$

Where, AUC ($T_n \rightarrow T_{n+1}$) is the AUC during any dosing interval

The total clearance is the key to estimate the dose rate R^0 (=D/T) for controlled release delivery device is related to the mean steady – state concentration $C_{ss.}$

(E) Apparent volume of Distribution (V_d)

It is a hypothetical volume, indicating the volume of fluid, which is required to dissolve the amount of drug at the concentration that is found in blood. It is a proportionality constant relating the amount of drug in the body to the measured concentration in blood.

$$V_d = \frac{DF}{AUC \cdot Kel}$$

(F) Mean Steady State Concentration (C_{SS})

The mean steady state concentration C_{ss} is not the numeric mean between peak (C_{ss} max) and through (C_{ss} min) at steady state but an integrated concentration. With constant rate infusion and in ideal controlled release device, no fluctuations occur at steady state hence,

$$C_{ss} = C_{ss}\ max = C_{ss}\ min$$

The value of C_{ss} can be estimated from the dose rate

$$Css = R^0/CL \quad or \quad Css = AUC\ (T_n \rightarrow T_{n+1})/T$$

The means steady-state concentration is usually the target concentration to be reached and maintained using controlled release delivery.

Incase of transdermal controlled delivery device the input rate R° is corrected for the surface area A of the delivery device.

$$R° = (C_{SS} CL)/ A$$

1.2.7 Mathematical model for transdermal diffusion and in vitro permeation study

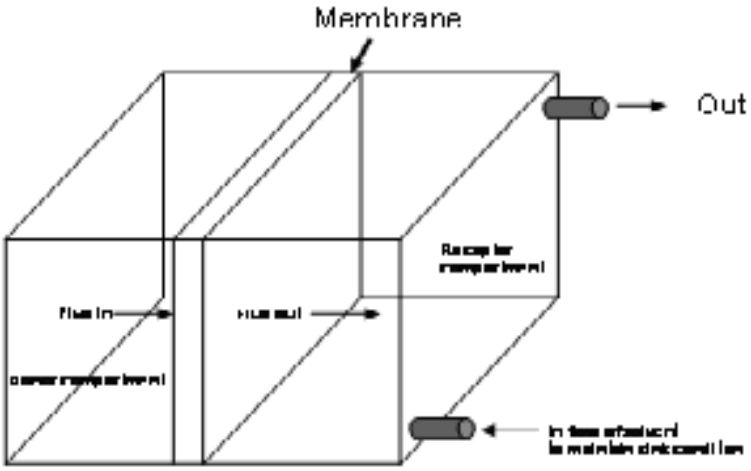


Figure 1.2.7: Diffusant flowing through into and out of region separated by a membrane

1.2.7.1 Diffusion equation[35]

For understanding drug permeation through skin let us revise Fick's laws regarding diffusion

Ficks First Law (Steady—state diffusion)

It states that amount M of material flowing through a unit cross section. s, of barrier in unit time t, is known as flux J.

$$J = \frac{dM}{dt} S \qquad \text{(I)}$$

This flux is in turn proportional to concentration gradient, dc/dx

$$J = -D \frac{dC}{dx} \qquad \text{(II)}$$

The negative sign of equation (II) signifies that the diffusion occurs in a direction opposite to that of increasing concentration. That is to say, diffusion occurs in the direction of decreasing concentration of diffusion, thus the flux is always a positive quantity. D is the diffusion coefficient of penetrant (diffusant) in cm^2/sec, C is concentration in g/cm^3, and x the distance in cm of movement perpendicular to the surface of barrier. In equation (I), mass M is usually given in grams, the barrier surface S in cm^2 and the time t, in seconds.

Fick's second law:

This law includes the study of mass transport with the rate of change diffusant concentration rather then the mass diffusing across a unit area of barrier in unit time. This equation is derived as follows:

In figure 1.2.7 it is shown that a diftusant is flowing into and out of region. A difference in concentration results from a difference in input and output. A barrier in a membrane form separates the donor and receptor compartments. The concentration of diffusant in volume element changes with time, i.e. $\Delta C/\Delta t$, as the flux or amount of diffusant changes with distance, $\Delta J/\Delta x$ in the x direction.

$$\frac{dC}{dx} = \frac{dJ}{dx} \qquad \text{(Ill)}$$

Differentiating the equation (II) of first law, with respect to x, one obtains

$$-\frac{dJ}{dx} = D\frac{d^2c}{dx^2} \qquad \text{(IV)}$$

Substituting dc/dt form equation (Ill) in to equation (IV) results in Fick's second law, namely,

$$-\frac{dc}{dx} = D\frac{d^2c}{dx^2} \qquad \text{(V)}$$

Equation (V) represents diffusion only in the x direction and this expression is sufficient to describe transdermal drug permeation.

Ficks adapted the two diffusion equation I and V to the transport of matter from the laws of heat conduction.[36]

1.2.7.2 Mechanisms and kinetics of transdermal drug delivery system

The rate of permeation, dq/dt, across various layers of skin tissues in the course of transdermal permeation can be expressed mathematically as:[37]

$$\frac{dQ}{dt} = P_s\left(C_s - C_r\right) \qquad (1)$$

Where C_d and C_r are, respectively, the concentration of pharmacologically active molecule in the donor phase. e.g. concentration on the stratum corneum surface, and in the receptor phase e.g. concentration in the systematic circulation; and Ps is the over all permeability coefficient of the skin tissues to the penetration pharmacologically active molecule, which is called the penetrant, and is defined by

$$P_s = K_s\, D_s/h_s \qquad (2)$$

Where K_s, is the partition coefficient for the interfacial partitioning of the penetrant molecule from the TDDS on to stratum corneum; D_s is the apparent diffusivity for the steady state diffusion of the penetrant molecule through the skin tissues; and h_s is the overall thickness of skin tissues. The overall permeability coefficient P for a skin penetrant can be considered as an invariant value if K_s**,** D_s and h_s in equation (2) are maintained at constant values under a given set of conditions.

Analysis of equation (1) suggest that, to achieve a constant rate of transdermal permeation, one needs to maintain a condition in which the drug concentration on the surface of' stratum corneum C_d is consistently and/or substantially greater than the dug concentration in the body

(C_r), i.e., $C_d > C_r$**;** under such condition equation 1 can be reduced to:

$$dQ/dt = P_s\, C_d \qquad (3)$$

The rate of transdermal permeation (dQ/dt) should be constant, as the magnitude of C_d remains fairly constant through out the course of skin permeation. To maintain the C_d at a constant value, it is necessary to deliver the drug in to the skin surface at a greater rate R_d, that is either a constant or always greater than the rate of skin absorption R_a, i.e., $R_d > R_a$.

By making R_d greater than R_a the drug concentration on the skin surface C_d will soon achieve a drug level which is equal to or greater than the equilibrium (or saturation) solubility of the drug in stratum corneum (C_s), i.e., $C_d \geq C_s$, and the maximum rate of transdermal permeation (Dq/dt)m is expressed by the following equation

$$\{Dq/dt\}_m = Ps\ C_s^{\ e} \qquad (4)$$

In such a case, the magnitude of (dq/dt)m is determined by inherent permeability coefficient P_s of the skin to the drug and the equilibrium solubility of the drug in the stratum corneum C_s^e. Analysis of urinary recovery data suggested that the rate of transdermal permeation dq/dt increased as the nitroglycerine dose C_d, applied on a unit surface area increases. It appeared that the maximum rate of transdermal permeation (1.585 mg/cm^2/day) was achieved for nitroglycerine in rhesus monkeys[38] when the applied dose reached the level of 4.786 m/cm^2 or higher.

1.2.8 Computation of desired release rate and maintenance dose

Controlled release drug delivery devices are in fact multiple - dose products and usually are comparable to intravenous constant rate infusion, Infusions delivers specific amounts of drug in extremely short dosing interval t , In drip infusion rate 20 drops per minute X mg of drug is delivered every 0.05 hour. Using an infusion pump of constant delivery t becomes infinitely small.

The delivery rate R° is

$$R^o = D/t \qquad (1)$$

If the drug elimination follows first order process and drug release from the delivery device follows a zero order process, the rate of drug release or drug input R^o must equal to the rate of elimination or rate of drug output

$$R^o = R_{output} \qquad (2)$$

$$R_{output} = DM * Kel \qquad (3)$$

The rate of drug output is the product of the maintenance dose DM and the elimination rate constant Kel.

$$K_{el} = \frac{0.693}{t_{1/2}} \qquad (4)$$

Equation (3) becomes

$$R_{output} = DM \frac{0.693}{t_{1/2}} \qquad (5)$$

Now, Amount = gms / CC = Concentration x Volume and DM i.e. maintenance dose is an amount of drug

DM = Concentration x Volume.

Now concentration is steady-state i.e. Css and Volume is V_d

$$DM = Css * V_d \qquad (6)$$

Substituting equation (6) in equation (3)

$$R_{output} = Css\ V_d\ Kel \qquad (7)$$

$$Kel\ V_d = CL \qquad (8)$$

$$R_{output} = C_{SS} * CL \qquad (9)$$

$$R^O = R_{output} = Css * CL$$

Input Rate for transdermal delivery device is dependant on area of device.

$$R° = R_{output} = (Css * CL) / A \qquad (10)$$

1.2.9 Factors affecting Transdermal Permeation

(A) Physicochemical properties of the diffusant

Partition Coefficient:

Partition coefficient plays an important role in establishing flux from a membrane and skin to receiver fluid. For drugs the passage through skin, startum corneum is rate limiting. The stratum corneum to vehicle partition coefficient is then crucially important in establishing a high initial concentration of diffusant in first layer of tissue. Drugs possessing both water and lipid solubility is favorably absorbed through skin. Transdermal permeability coefficient shows a linear dependency on partition coefficient.

A lipid water partition coefficient of 1 or greater is required for optimum transdermal permeability.[39-42] The partition coefficient of a drug molecule may be altered by chemical modification of its functional groups,[43] by varying the vehicle, or by incorporating lipophillic agent with drug e.g. pentanol, which effect skin vehicle partition coefficient.

Diffusant solubility:

Flux of a solute is proportional to the concentration gradient across the entire barrier phase. Therefore for maximal flux, solute should be in saturated solution in donor phase. The solubility of a solute can be controlled by controlling solvent composition of the vehicle.

Effective Concentrations:

The concentration differential is considered as driving force for diffusion, in actual it is the chemical potential gradient or activity gradient which is the fundamental parameter. The thermodynamic activity of penetrant in either the donor phase or the membrane may be radically altered by such phenomena as (1) pH changes (ii) complex formation (iii) co-solvents, (iv) presence of surfactants, micelle etc.

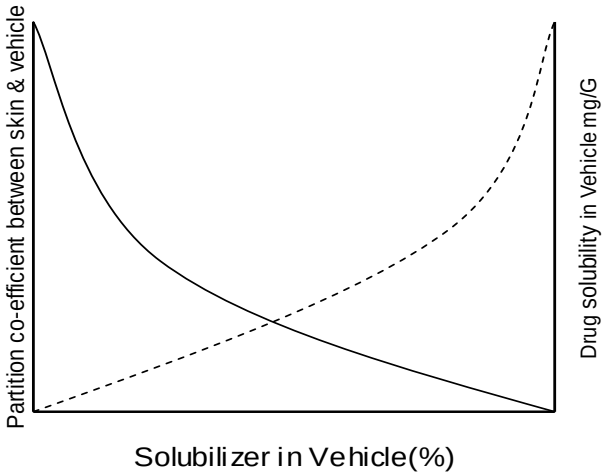
pH variation :

According to pH partition hypothesis, only unionized molecules pass across lipid membranes in significant amount.[45] Ionized species do not have favorable free energies for transfer to lipid phase. Weak acids and the weak basis dissociated to different degrees, depending on the pH and the pKa and pKb of diffusant. Thus the fraction of unionized drug in the applied phase determines the effective membrane gradients, and this fraction is function of pH.[46] Usually transdermal flux of drug increases with increasing pH up to approximately 1 to 2 pH units higher than the pKa value or decreasing 1 to 2 pH lower than pKb values, at which point the drug molecule exist totally as the non protonated form.

Co-solvents:

Co-solvents are used to increase solubility of solute in vehicle, so as to maximize the concentration gradient across stratum corneum, membrane. In general partition coefficient of a drug between a membrane and a solvent mixture falls as the drug solubility in the solvent system rises[47] as shown figure 1.2.8. Hence it is important not to over solubilize a drug in a vehicle if the aim is to promote penetration through stratum corneum.

Figure 1.2.8: Relationship between partition coefficient, Solubility and concentration of solubilizer in vehicle



Surface Activity and Micellization:

When surface active agent forms micelle, the total apparent solubility of agent in the aqueous phase increases dramatically, with a consequent decrease, in the apparent partition coefficient Figure 1.2.8. When drug and surfactant are different, the part played by surfactant is more complicated. Sometimes surfactant decreases permeation, reflecting reduced biological activity e.g. retarded rectal absorption of triiodophenol.[48]

Complexation:

Complex formation influences apparent solubility and apparent partition coefficient of a drug. When complex is formed in donor solution, flux of diffusant across skin or membrane decreases, because concentration of free drug falls. It is reported that complexes have lowered partition Coefficient than respective free drugs.[49]

Molecular Weight:

(a) Convection

Usually the lower the molecular weight the faster and more complete is the transport. Small molecules may pass through pores of the membrane by convective transport. Spherical compounds up to MW of 150 and thread like compounds up to MW 400 are considered of being permeable by convective transports.

(b) Diffusion:

Most drugs are transported across membrane by passive diffusion, since in passive diffusion the drug outside and inside the aqueous compartment is in true solution, but

dissolves in the lipid material of the membrane during the transport across the barrier. The compound must possess minimal lipid solubility.

The transport stream Q depends on the diffusion constant of drug in lipid material D, the surface area A, the partition coefficient K, the membrane thickness "h" and the concentration C_0 and C_i on both sides of the membrane.

$$Q = DAK (C_0 – C_i) / h$$

Under sink condition, where the drug is immediately carried away by the blood after crossing the membrane and diluted within the volume of distribution, according to Ficks first law. The flux and diffusion coefficient D decreases with increasing MW. The flux of some drugs and their molecular weights [50] are listed below.

The majority of drugs have low molecular weights (MW 200-250). In recent years increasing interest is being devoted to drugs of large molecular weights i.e. (MW 1000-30000) the peptides such as insulin (MW 6000) growth hormone (22600), oxytocin (1007), vasopressin (1200) and antibodies (> 150,000).

Table 1.2.3: Molecular weights (MW) and total flux across skin of some selected drugs

Drug	MW	Flux (µg/ cm^2 hr)
Ephedrine	165	300
Diethyl carbamazine	199	100
Octanol	130	23
Nitroglycerin	227	13
Scopolamine	303	3
Ethanol	48	3
Chlorpheniramine	275	3
Fentanyl	337	2
Estradiol	272	0.016
Testosterone	288	0.014
Progesterone	315	0.011
Quabain	585	0.008
Cortisone	402	0.0015
Hydrocortisone	362	0.00091
Digitoxin	765	0.00013

pKa: lonization at Physiological pH.

The nonionized moiety is usually lipid soluble hence may dissolve in the lipid material of a membrane and may be transported by passive diffusion, whereas the ionized moiety usually is not lipid soluble enough to permit permeation. The percent of ionization can be calculated from Henderson -Hasselbalch equation.

To cross or to reach membrane or regions by passive diffusion within the body the percentage of drug nonionized at that site should be between at least 0.1 and 0.5%. Table 1.2.4 gives a serve of pH values of body fluids and anatomical units.[51]

Table 1.2.4: Body pH values

	pH	Range
Blood arterial	7.40	7.35-7.45
Blood venous	7.37	7.32-7.42
Plasma	7.40	7.38-7.42
Urine	5.70	4.80-7.50

Isoelectric Point

Isoelectric point is the pH at which the zwitterion concentration of protein or peptide is at a maximum and the net movement of molecule is negligible. The permeation across the membrane is at its minimum at the isoelectric point. The permeation across skin upon topical administration of vasopressin was minimum at isoelectric point. [52]

(B) Physicochemical properties of drug delivery system

Release characteristics:

The affinity of the vehicle for the drug molecule can influence the release of drug molecule from the vehicle.[53] The solubility in the vehicle will determine the release rate of drug. Generally more easily the drug is released from the drug delivery system; higher will be the rate of transdermal permeation. A linear relationship is observed between the median effective dose of a corticosteroid against product of partition coefficient and square-root of their solubility in vehicle,[54] pH of the vehicle can also influence the rate of release of drug from the drug delivery system.

Composition of drug delivery system:

The vehicle used in drug delivery system usually assumed to be "inert" but it is not so. The composition of the vehicle and drug delivery system influences greatly on percutaneous absorption of drug particles. It may affect not only the rate of drug release but also the permeability of stratum corneum by means of hydration, mixing with skin lipids, or other sorption promoting effects.

Enhancers / sorption promoters:

Sorption promoters or sorption enhancers are not drugs but they are molecules which reversibly decrease the barrier nature of the stratum corneum.[55,56,57,58] Sorption promoters allow the drugs to penetrate into skin and the permeate across more readily and thus increase systemic availability.[59,60]

Sorption promotes act by interaction with intracellular lipids leading to disrtruption of their organization and increasing their fluidity.[61] Some of them also interact with intercellular protein, keratin denaturation (azone and oleic acid) [62,63] while others act by both mechanism (DMSO and propylene glycol).[64] Another possible mechanism is by altering the skin hydration.[65,66]

The mechanism of these sorption promoters to some extent is also related to octanol/water partition co-efficient.[67] Recently, lipid-protein partition theory has been formulated to describe the potential mechanism of action of sorption promoters.[55,64,68]

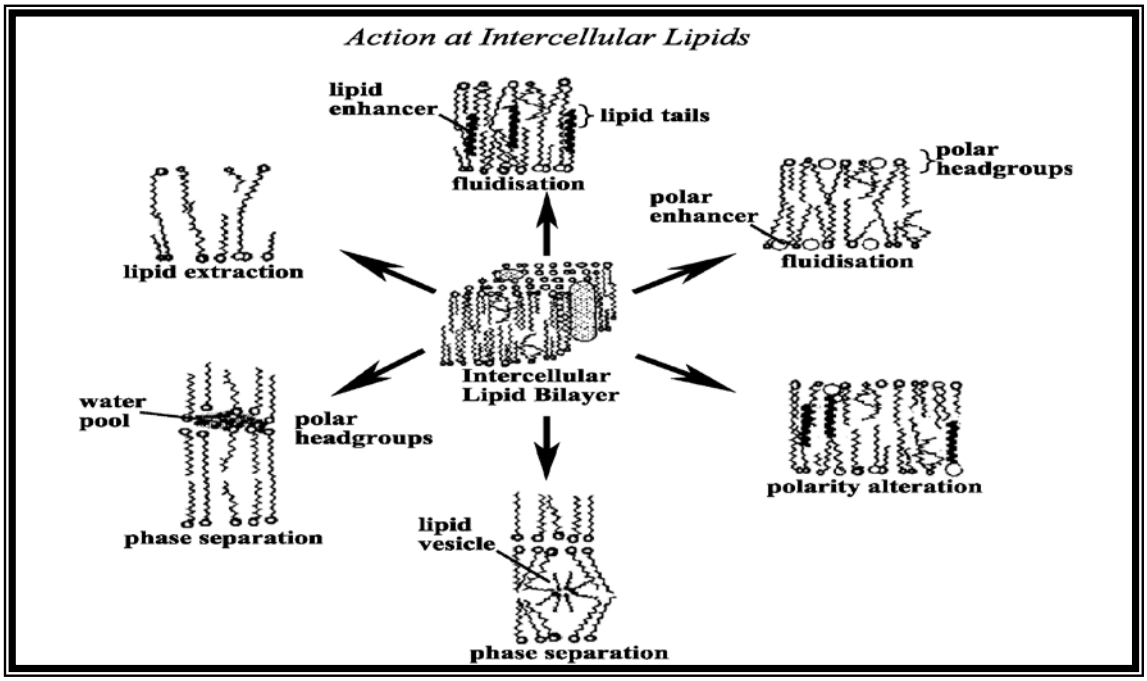
Mechanism of penetration enhancers on skin [69,70]

Most of the enhancer especially chemical enhancer works on the either lipophilic part or hydrophilic part of the bilayer of SC and alter its property and ultimately increases the delivery of the drug through the skin. Chemical enhancers may either work of lipid portion of the lipid bilayer (intracellular mechanism) or they may work on the protein part of the cell (cellular mechanism).

They may act as lipid enhancer and so here total lipidic portion increases and ultimately lipophilic drug can penetrate easily, some chemical enhancer are increases the total hydrophilic portion and so they increases flux of hydrophilic drug, some drug may able to change the polarity of bilayer and as rule of thumb like dissolve like drug of nearly similar polarity may penetrate through skin easily.

Some enhancer work of cellular part of SC. Enhancers (chemical or other) may increase the distance between the two cell and so large molecule can penetrate through skin or they may change the arrangement of cellular keratin fibers and generate vacuoles in it so it become more permeable to drug.

Figure 1.2.9: Action of chemical enhancer on Intercellular lipids



Some enhancer causes the phase separation of bilayer and so drug can penetrate through skin. Some enhancer develop pool of increases the flux of hydrophilic drug. Solvent like ethanol have tendency to dissolve lipid and it extract out lipid of SC and so obstruction for hydrophilic molecule decreases. Estraderm®, containing estradiol was the first commercial transdermal system to use ethanol as a permeation enhancer.

Figure 1.2.10: Action of penetration enhancer on the integrity of stratum corneum adhesion.

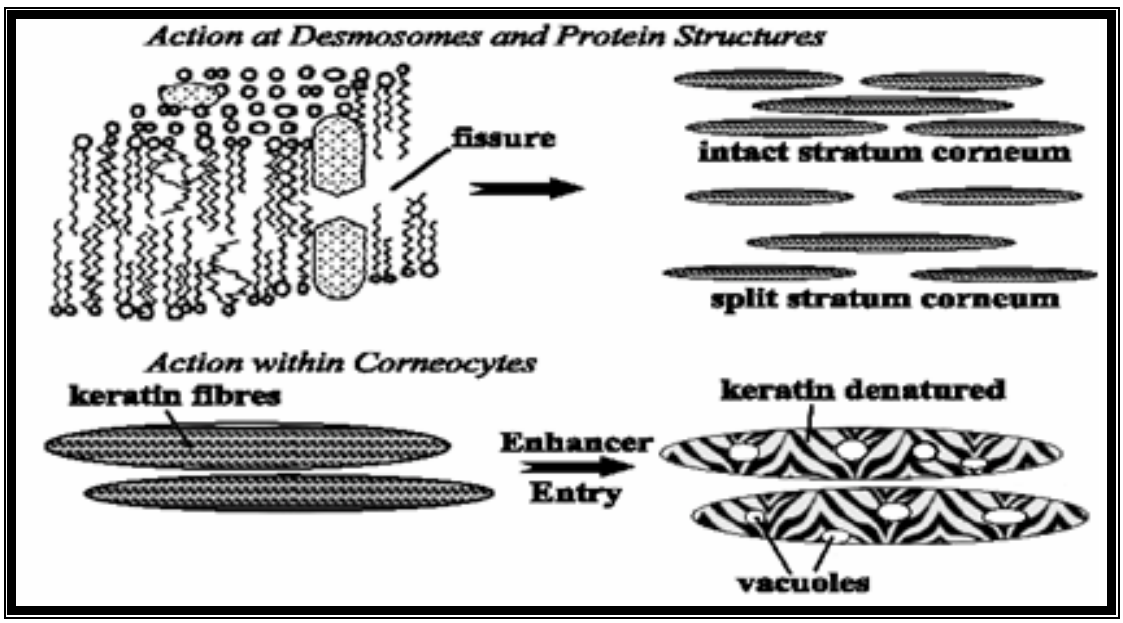


Table 1.2.5: Different classes of sorption promoters

Sr. No.	Sorption Promoters	Example	Refrences
1.	Sulfoxides and similar Compounds	Dimethyl sulfoxide Dimethyl acetamide Dimethyl formamide N- methyl formamide	57,60,71,72
2.	Pyrrolidone	2-pyrrolidone 1-methyl 2-pyrrolidone 5-methyl 2-pyrrolidone 1,5-methyl 2-pyrrolidone 1-ethyl 2-pyrrolidone`	57,60
3.	Fatty acids	Oleic acid, lauric acid, linolic acid, myristic acid	57,60,61,63,73
4.	Azone	Laurocapram and its derivatives	57,60,73,74,75,76
5.	Urea	Urea	57,58,60
6.	Surfactant	Sodium lauryl sulfate Triethyl ammonium bromide Synperonic NP series	57,60
7.	Alcohols	Ethanol Lauryl alcohol Linolenyl alcohol Octanol	57,60,77
8.	Glycols	Propylene glycol PEG 400	57,64,60,73
9.	Terpenes and terpenoids	Menthol Camphor	57,68,78,79
10.	N-pentyl N-acetyl prolinate	-	80
11.	Latam N-acetic acid esters	-	81

(C) Physiological and pathological conditions of the skin

Reservior Effect of Horny Layer:

A reservoir for potent fluorinated steroid was demonstrated to be associated with stratum corneum.[82] A depot for steroid resides in normal horny layer. Depot usually occurs for substances having small diffusivity and low solubility in stratum corneum.

Skin Hydration:

Hydration of stratum corneum facilitates the penetration of most materials through the skin. Hydration swells the cells of stratum corneum, wrinkles and its permeability dramatically increases.

Skin Temperature:

Thermal energy is necessary for the diffusivity and solubility of drug in skin tissues. Rise of temperature may increase vasodilation of skin vessels leading to an increased percutaneous absorption.

Regional Skin Sites:

Variation on cutaneous permeability will depend on the thickness of the stratum corneum, its nature and the density of skin appendages. Compounds like salicylic acid, hydrogen sulfide gas and lidocain base penetrates the different skin as follow; in decreasing order.

Scrotum > forehead > Scalp > back > Forearms > Palms > foot arch.

Injuries to skin:

Injury that disrupts the continuity of stratum corneum increases skin permeability. Chemical injury to the skin, erythema, hyperemia, etc increases transderrnal permeability.

Skin Metabolism:

Skin plays as a site of drug and chemical metabolism. Skin actively metabolizes steroids, hormones, chemical carcinogens, and drugs and that such metabolism may ultimately prove to be a critical determinant of therapeutic-efficacy of topically applied drugs and of the carcinogenic response in the skin.

Circulatory Effects:

Increases blood flow raises the concentration gradient across the skin. Vasoconstriction agent such as topical steroids could reduce their own rate of clearance from the skin.

Species difference:

Mammalian skin from different species display wide difference in anatomy, e.g. thickness of skin, numbers of sweat glands, and hair follicles per unit surface area, distribution of blood supply etc... And such factors affect both, the routes of penetration and the resistance to penetration.

(D) Biopharmaceutical aspect of drug.

Extent of Protein Binding:

Extent of drug binding the plasma protein and tissue proteins is not constant and may change due to physiological and pathological factors, or displacement. Proteins also work to carry drugs which may be utilized for drug transport.

Erythrocyte Uptake:

Erythrocyte may take up drugs. Uptake occur by several mechanisms, (a) Lipophillic drugs dissolves in erythrocyte membrane (b) Anions attracted by positively charged pores of RBC (c) Adsorption to erythrocyte membrane (d) Binding with erythrocytes to carbonic anhydrous drugs.

General absorbability:

The parameters molecular weight, PKa, solubility and partition coefficient gives reasonable information on the potential for absorption from a given site. To verify the absorbability weather by passive diffusion or active diffusion can be tested by guinea pig ileum.[83]

1.2.10 Types of Transdermal Delivery Systems

There are mainly four types of basic transdermal patches in the market.[84]

(1) Drug in adhesive type

In this type drug is loaded in adhesive itself and stratum corneum acts as rate controlling barrier. This is most old type of transdermal patch design. This type of transdermal drug delivery system is best illustrated by the development and marketing of a nitro glycerin releasing system named as deponit by PharmaSchwartz/Lohmann in Europe. Basic construction includes backing membrane, adhesive loaded with drug and release liner.

(2) Multi laminate type

This is most complicated type of design for transdermal patches. Basic construction includes backing membrane, drug in adhesive, rate controlling membrane, then again adhesive (loaded with drug) on to it. This shows that there are two adhesive layers. First layer that is in contact with the release liner is actually delivering drug and second layer of adhesive (after membrane) acts as depot of drug. The example is scopolamine releasing TDDS named as Transderm-scop by Ciba and clonidine releasing TDDS named as CataPress-TTS by Boehringer Ingelheim.

(3) Reservoir type

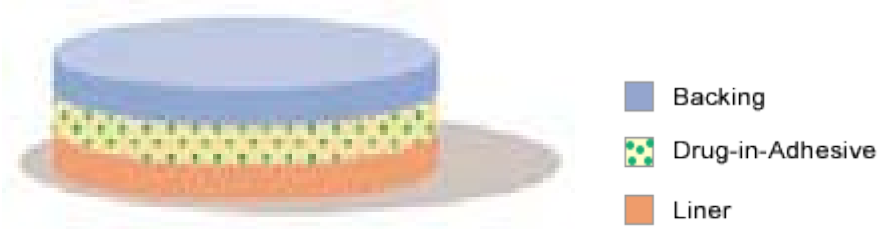
In this type the drug is incorporated in reservoir which is lined with membrane. The adhesive is coated on to this membrane. This membrane can be rate controlling. Basic construction includes backing membrane, drug in reservoir, membrane, adhesive and release liner. Example of this type of TDDS is nitro-glycerine releasing system named ass Nitrodisc by Searle.

(4) Matrix type

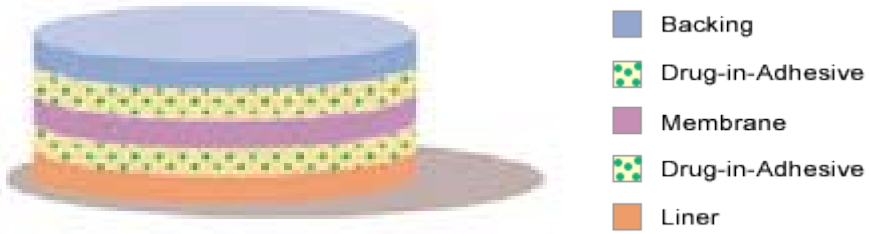
In this type, the drug is incorporated in the matrix of polymer which itself releases drug in zero order. The adhesive layer is just at the periphery and little inside the periphery of the patch. Basic construction includes backing membrane, adhesive, and drug in matrix and release liner. The example of matrix type transdermal patch in nitroglycerine releasing TDDS named as Nitro-dur by Key.

Figure 1.2.11 Basic patch construction

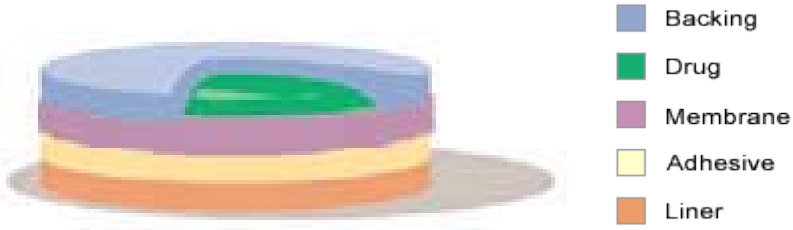
Single-layer Drug-in-Adhesive



Multi-layer Drug-in-Adhesive



Drug Reservoir-in-Adhesive



Drug Matrix-in-Adhesive

1.2.11 Drugs Studied For Transdemal Drug Delivery System

Table-1.2.6 indicates about the drugs studied for TDDS development. The information was collected after extensive literature survey from various journals.

Table- 1.2.6: Drugs studied for skin permeation and TDDS Development.

Drug	Therapeutic use	Skin model used	References
LNG- Estradiol	Contraceptive	In-vivo (Human)	85
Fentanyl	Post operative analgesic	In-vivo (Human)	86
Estradiol	To reduce hot flushes in post menopausal woman	In-vivo (Human)	87
Estrogen	Estrogen replacement therapy	In-vivo (Human)	88
Vipocetine	Cerebral diseases	In-vitro(male Wistar rats)	89
Acyclovir	Antiviral	Nude mouse skin	90
Physostigmine	Organophosphorus poisoning	Isolated human skin	91
Proparanol	Anthypertensive	Excised hair free rat skin and in vivo in male albino rabbits	92
Methotrexate	Recalcitrant Rheumatoid arthritis	Hairless mouse skin for In-vitro and Hairless mice for In-vivo	93
Diazepam	Anxiolytic	In-vivo mice	94
Zalcitabine	Anti-HIV	Hairless rat skin	95
Terbutaline	Antiasthmatic	Rat, guinea pig and human skin	96
Atenolol	Antihypertensive	Guinea pig and rat skin	97
Alizapride, Bromopride metoclopramide	Antiemetics	Rat skin	98
Nitroglycerine	Prophylaxis against angina-pectoris	In-vivo (human)	99
Glutathion	Endogenous		100

Peroxidase Catalase	Anti oxidant for photoprotection of skins	In-vivo (human)	
Tranilast	Keloids and hypertropic scars	In vivo (humans)	101
Elcatonin	Paget's disease	in-vivo (humans)	102
Nicotine	Adjunct to smoking cessation therapy	In-vivo (humans)	103
Flubriprofen Indomethacin	Anti-inflammatory	Full thickness rat skin	104
Clonidine	Antihypertensive	In-vivo (humans)	105
Testosterone	Hypogonadism	In-vivo (rhesus monkeys)	106
Nicardiapine	Antihypertensive	Hairless guinea pig	107
Tripolidine	----------	Hairless mouse skin	108
Insuline	Hypoglycemic	----------	109
Melatonine	----------	Porcine skin	110
Anti- estrogen	----------	Hair less mice skin	111
Tolbuterol	Antiasthmatic	In vivo, humans	112
Progesterone	Hormone replacement therapy	In-vitro porcine skin	113
Interlukin-2 and interferone alpha	For passive immunity	Murine RENCA cell line model	114
Haloperidol	Antipsychotic	In-vivo, Rat and Rabbit	115
Prednisolone	Antiinflammatory for eye	In-vivo rates	116
Pinacidil monohydrate	Vasodialator	In-vitro albino rat skin	117
Ketoprofen	Anti inflammatory	In-vitro rat	119
Gestodene	Prodrug of active progestine	In-vitro mice skin	120
7-Hydroxy coumarine	Anticoagulants	In-vitro human, snake	121
Chlorphenaramine maleate	Antiallergic	TESTSKIN® living skin equivalent	122

Diclofenac	Anti-inflammatory	In-vitro , cadaver skin	123
Nimesulide	Antiinflammatory	In-vitro porcine and human skin	124
Testosterone	Hypogonadism	In-vitro (rat skin)	125

1.2.12 **Commercially available Transdermal therapeutic system** [126,127]

Table 1.2.7: Commercially available transdermal therapeutic system

Drug/ Manufacturer	Trade Name	Duration	Type of system	Therapeutic Use
Scopolamine Alza/Ciba	Transderm® Scop	*2* days	Reservoir	Alleviate motion sickness
Nitroglycerine Alza/Ciba	Transderm ®-Nitro	1 day	Reservoir	Treatment and prophylaxis of angina pectoris
Hercom	NTS®		Matrix	
Searle	Nitrodisc®		Matrix	
Key	Nitro-dur ®		Matrix	
Wayth	Deponite ®		Sand witch	
Isosrbide dinitrate Nitro electric industrial	Frandol ® Tape ®	1 day	Matrix	Treatment and prophylaxis of angina pectoris
Clonidine Boehringer/Ingelneim	Catapress® TTS	7 days		Treatment of hypertension
Estradiol Ciba-Giegy	Estraderm ®	3 days	Reservoir	Relief from post menopausal symptoms
Nicotine Alza	Nicoderm ®	1 day	Reservoir	Aid in smoking cessation
Ciba-Giegy	Habitrol ®		Matrix	
Park-Davis	Prostep ®		Matrix	
Fentanyl Jenssens	Duragesic ®	5 days		Post operative analgesic

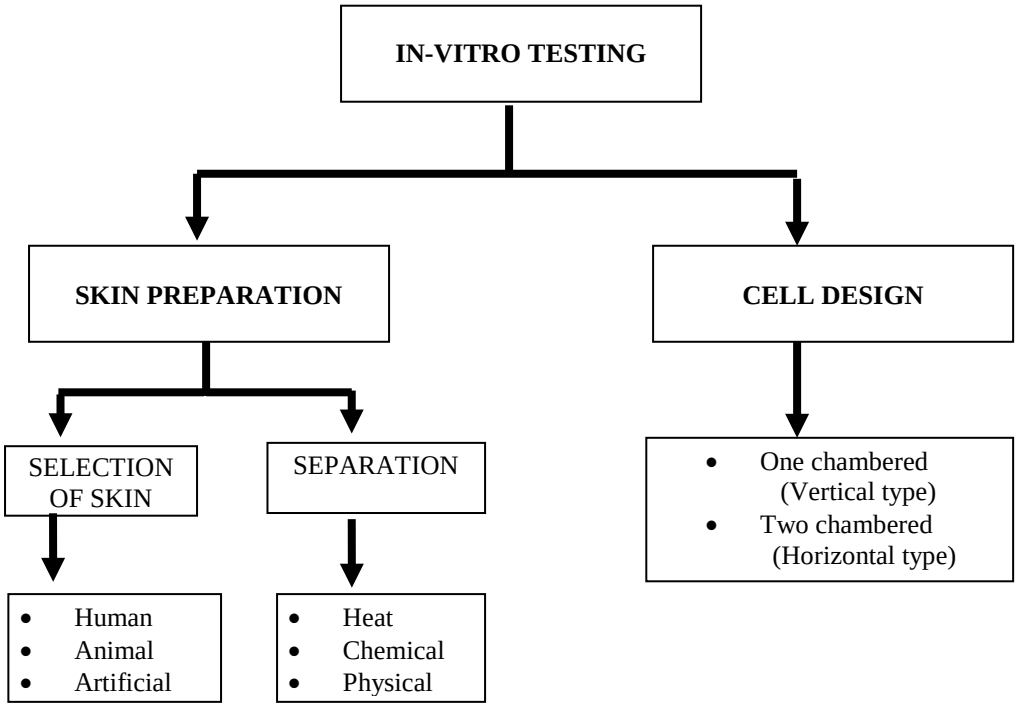
1.2.13 Evaluation of Transdermal Drug Delivery Device

Table 1.2.8: Testing of transdermal drug delivery system [128,129]

TYPE OF TEST ON FINAL PRODUCT	TESTS
Chemical test	• Content • Content uniformity • Purity • Residual Solvent
Physical test	**Release testing** • USP apparatus 5 (Paddle over disk) • USP apparatus 6 (Cylinder) • USP apparatus 7 (Reciprocating disk) **Test for adhesion** • **Peel property** • **Tack property** Thumbtack test Rolling ball tack test Quick stick test Probe tack test • **Shear strength**
Cutaneous toxicity	• Contact dermatitis • Growth of microorganisms • Cytotoxicity • Sensitization study
Percutaneous absorption model	• *In vitro* testing • *In vivo* testing

IN VITRO TESTING OF TRANSDERMAL DELIVERY SYSTEM

Figure 1.2.12: In Vitro testing procedure

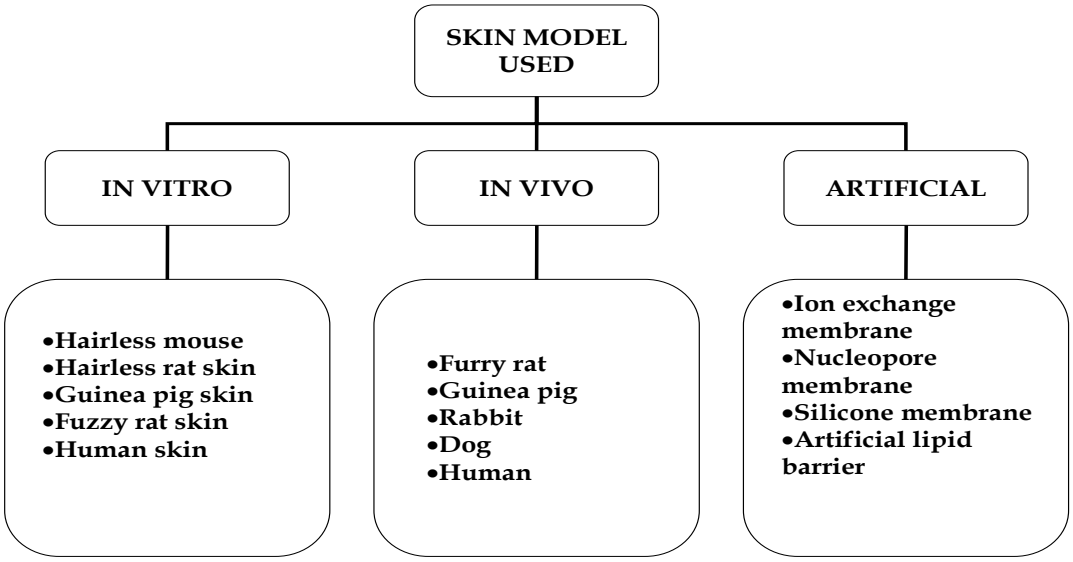


Proper skin preparation and appropriate cell design gives good *in vivo* results. Skin preparation includes selection of proper skin. One can choose human skin or can also go for animal or artificial membrane like Nylon, Cellulose acetate etc. Skin separation includes the treatments needed for separation of required part of skin from other unwanted parts. Then separated skin we mount on the diffusion cell which can be one chambered (one donor compartment) or two chambered (two donor compartment).

Selection of membrane for in vitro study of transdermal drug delivery system

Various skin models used by various researchers are given in Figure 1.2.13. Though there is no rule regarding to selection of the skin model. But generally researchers starts with artificial membrane, then *in vitro* animal skin, then *in vitro* human skin (cadaver skin), then *in vivo* animal skin, then finally *in vivo* human skin.

Figure 1.2.13: Selection of membrane.



Franz diffusion cell [130,131]

It is one chambered (vertical) type cell. Most widely used for in-vitro testing of TDDS. Many modifications have been made in the Franz diffusion cell design according to the requirement. Here skin is mounted on the plate above O ring. 20-70 ml phosphate buffer of pH 7.4 is filled in reservoir compartment. Transdermal patch is applied on upper layer of skin. Diffusion medium in reservoir is stirred at particular rpm. Sampling is done at particular interval from reservoir compartment i.e. specified volume of fluid is withdrawn and is replaced by equivalent amount of the same fluid.

In vitro drug release profile modeling

In-vitro drug release has been recognized as an important tool in drug development and as an important parameter in quality control. Under certain conditions, it can be used as a surrogate for the assessment of bio-equivalence or prediction of bioequivalence.[132]

A good understanding of the release mechanism of the dosage form as well as the physical chemical properties of the drug will enable development of accurate dissolution tests.[133]

An appropriate drug release test is required to characterize the drug product and ensure batch-to-batch re-producibility and consistent pharmacological/biological activity and to evaluate scale up and post approval changes such as manufacturing site changes, component and composition changes. The release of drug from a sustained release formulation is controlled by various factors through different mechanisms such as diffusion, erosion or osmosis. Several mathematical models are proposed by many researchers to describe the drug release profiles from various systems.[134,135]

In order to characterize the kinetics of drug release from dosage forms several model dependent methods are reported by various researchers.[136,137] The model dependent methods all rely upon a curve fitting procedure. Different mathematical functions have been used to model the observed data. Both the linear and non-linear models are being used in practice for dissolution modeling. Linear models include Zero order, Higuchi, Hixson-Crowell, quadratic and Polynomials, where as the nonlinear models include First order, Weibull, Korsmeyer- Peppas, Logistic etc.[138] There are several linear and non-linear kinetic models widely used to describe release mechanisms and to compare test and reference dissolution profiles in Table 1.2.9.

Table 1.2.9: Drug release profile model equations

Function	Equation
Zero-order	$\%diss = kt$
First-order	$\%diss = 100[1 - e^{kt}]$
Hixson-Crowell	$\%\sqrt{diss} = 100\left[\left[1 - \left(1 - \frac{kt}{4.6416}\right)^3\right]\right]$
Higuchi	$\%diss = kt^{0.5}$
Korsmeyer-Peppas	$\%diss = kt^{n}$
Weibull	$\%diss = 100\left[1 - e^{-(t/Td)^{\beta}}\right]$

Note: % diss = Percent dissolved at time t;

K = Dissolution rate constant

n = Release component which is indicative of drug release mechanism;

β = Shape parameter Td, time at which 63.2% of the drug is dissolved.

Zero order kinetics [132]

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly (assuming that area does not change and no equilibrium conditions are obtained) can be represented by the following equation:

$$W_0 - W_t = K_0 t$$

Where W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the amount of drug in the pharmaceutical dosage form at time t and K is proportionality constant. Dividing this equation by W_0 and simplifying:

$$f_t = K_0 t$$

Where $f_t = 1- (w_t/ w_0)$ and f_t represents the fraction of drug dissolved in time t and k_0 the apparent dissolution rate constant or zero order release constant. In this way, a graphic of the drug-dissolved fraction versus time will be linear if the previously established conditions were full filled.

First order kinetics [132,139]

This type of model to analyze drug dissolution study was first proposed by Gibaldi and Feldman and later by Wagner. The relation expressing this model:

$$\log Q_t = \log Q_0 + K_1 t / 2.303$$

Where, Q_t is the amount of drug released in time t, Q_0 is initial amount of drug in the solution and K_1 is the first order release rate constant. In this way a graphical relationship between f_t versus time to get the Zero order constant from the slope.

Korsmeyer Peppas model [132,140]

Korsmeyer et al., developed a simple semi empirical model, relating exponentially the drug release to the elapsed time (t).

$$\frac{Q_t}{Q_\infty} = K_k t^n$$

Where Q_t is the amount of drug released in time t, Q_0 is initial amount of drug in the solution; K_k is a constant incorporating structural and geometric characteristic of the drug dosage form and n is the release exponent, indicative of the drug release mechanism.

Table 1.2.10: Release exponent of Korsmeyer Peppas model and its corresponding drug transport mechanism.

Release exponent (*n*)	Drug transport Mechanism	Rate as a function of time
0.5	**Fickian diffusion**	**$t^{-0.5}$**
0.5<*n*<1.0	**Anomalous transport**	**t^{n-1}**
1.0	**Case-II transport**	**Zero-order release**
Higher than 1.0	**Super Case-II transport**	**t^{n-1}**

Higuchi Model [130,133]

The model purposed by the Higuchi [141] is described by following formula:

$$Qt = K_H t^{1/2}$$

Where Q_t is the amount of drug released at time t and K_H is the Higuchi release rate. This is the most widely used model to describe drug release from pharmaceutical matrices. A linear relationship between square root of time and concentration indicates that the drug release follows strict Fickian diffusion. For purpose of data treatment, the above equation is usually reduced to:

$$Q = Kt^{1/2}$$

Therefore a plot of amount of drug released versus the square root of time should be linear if drug release from the matrix is diffusion controlled. Alternatively, the drug release rate is proportional to the reciprocal of the square root of time. An important advantage of the above equations is its simplicity.

Hixson- Crowell model [132,138]

Hixson and Crowell recognizing that the particle regular area is proportional to the cubic root of its volume derived an equation that can be described in the following manner:

$$W_0^{1/3} - W_t^{1/3} = K_0 t$$

Where *W* is the initial amount of drug in the pharmaceutical dosage form, *W* is the remaining amount of drug in the pharmaceutical dosage form at time *t* and *K* is a constant incorporating the surface–volume relation.

Weibull model [142]

A general empirical equation described by Weibull was adapted to the dissolution / release process from pharmaceutical dosage forms, the Weibull equation expresses the accumulated fraction of the drug, *m*, in solution at time, *t*, by:

$$m = 1 - \exp\left[-\left(t - T_i\right)^{\beta} / a \right]$$

In this equation, the scale parameter, *a*, defines the time scale of the process. The location parameter, Ti, represents the lag time before the onset of the dissolution or release process and in most cases will be zero. The shape parameter, β, characterizes the curve as either exponential (β=1) (Case 1), sigmoid, S-shaped, with upward curvature followed by a turning point (β>1) (Case 2), or parabolic, with a higher initial slope and after that consistent with the exponential (β <1) (Case 3). This equation may be rearranged into:

$$\log\left[\ln\left(1 - m\right)\right] = b * \log\left(t - Ti\right) \log a$$

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1.3 SKIN IRRITATION AND SKIN SENSITIZATION STUDIES

1.3.1 Skin Irritation and Sensitization

The concept of skin irritation or inflammation has a very long history, Egyptian scrolls, dated perhaps as early as 2650 BC, have several references to a word indicating inflammation that is associated with wounds.

In 1889, Julius Cohnheim described inflammation as a series of changes to the affected area including redness, swelling, pain, warmth. Earlier to 1985 it was thought that chemical irritation to the skin occurs in phases like increased permeability and blood flow, infiltration of cells, leucocytosis etc. After 1985 Patrick et al., Agner and serup 1987 reported that irritation of chemical and resulting effects on the skin appeared to be related to the specific irritant applied. Further more, skin irritation appears to be produced by multiple mechanisms resulting invariable patterns of response.[1, 2]

We can broadly classify skin sensitization irritation and inflammation in to following categories.[3, 4]

1. Allergic Contact Dermatitis (Skin Sensitization)

Definition and Description [5,6]

Allergic contact dermatitis or dermatitis venenta, is the result of an interaction between the complicated pathophysiological mechanisms of type-IV cell mediated immunity and environmental sensitizer (allergens).

Acute contact dermatitis is characterized by papules and sharply demarcated erythema. Blisters are also produced following the release of cytotoxic compounds by white cells attracted to the affected site.

The key element in the formation of sub chronic and chronic allergic dermatitis is due to recurrent exposure to the causative agent.

2. Light induced cutaneous toxicity [4,7]

The wavelength of light found in UV-B spectrum is generally considered the primary source of toxic changes in the skin. The specific wavelength responsible for a particular biological response is termed as “action spectrum” for that effect. In some cases, xenobiotics play a role in these effects while in others, the interaction of light

with the normal compound of the skin is responsible in either case; adverse reaction of the skin to light (UV or Visible) is termed as photo sensitization.

2.1 Photo sensitization not related to xenobiotics

The exposure of the unprotected skin to UV light (from sunlight or artificial sources) can result toxic responses. These includes short term, generally reversible effects such as sunburn (erythema) and tanning (enhanced pigment darkening) as well to as long term, generally irreversible effects such as premature skin ageing (actinic elastosis) and development of the skin cancer.

2.2 Photo sensitisation related to xenobiotic exposure

Xenobiotics localized within the skin can interact with light and produces adverse reaction in the skin in many ways. These include phototoxicity, photo allergy depigmentation, induction of endogenous photosensitizes and induction of photosensitive disease states.[8]

3. Cutaneous Carcinogenesis [9]

The skin is the most common site of cancer in humans. Both benign and malignant tumors may be derived from viable keratinocytes and melanocytes of the epidermis, and rarely from skin appendages, blood vessels, peripheral nerves and lymphoid tissue of the dermis. Histologically, basal cell and squamous cell carcinomas that develop from keratinocytes account for 60% and 30% for all the skin cancers respectively.

4 Acne-like eruptions [4]

These reactions are initiated by the proliferation of the epithelium of sebaceous glands and formation of keratin cyst resulting in the development of a pustule filled with fatty compounds and other product of sebaceous origin.

Evaluating chemicals for the adverse effect on the skin

1 Sensitization testing in animals

Sensitizations test is done to evaluate the allergic potential of chemicals, to assess potential sensitization properties. Animals is treated with an intial or several doses of chemical (the induction phase) by intradermal or cutaneous application. Following an incubation or sensitization phases for approximately two weeks the

animal is treated with second dose or series of doses of the same test chemical (the challenge phase). Sensitization is evaluated by examining the skin reaction following the challenge phase compared with any skin reaction immediately following the induction phase.

Intradermal Techniques

Draize et, al.[10] were the first to describe standardized irritation and sensitization tests. The Draize test is the simplest and most predictive tests to perform. However the test have several draw backs including high incidence of negatives with weak sensitizers[11] further more, the test; recommends a consistent induction concentration of 0.1% injected intradermally without regard to use pattern or exposure potential of the chemicals.[12]

Freud's complete adjuvant test[13] is the test in which test substances is mixed with Freud's complete adjuvant (FCA a mixture of heat killed Mycobacterium tuberculosis, paraffin oil and mannide monooleate) prior to intradermal injection for induction. The use of FCA increases the immunological response and aids in the detection of weak sensitizers. This test utilizes three injections of this mixture at different sites during induction phase. In the challenge phase the test substance is generally applied on epidermally (non-occluded) in range of non-irritating concentrations. This test is considered as sensitive as the optimization test and is of low cost to perform. The primary drawback of this test is the use of intradermal induction doses which actually bypasses the effect of stratum corneum and stratum corneum is highly important to limit the absorption of potential sensitizers. Further more the use of FCA may cause the sensitizing potential of the test chemical to be over-estimated.[14]

Epicutaneous Techniques

The open epicutaneous test utilizes an induction phase of repeated applications of an undiluted test substance (which may be a formulation of a final product for consumer exposure) over several weeks. The challenge phase separated in to initial and rechallange phases. Klecak et al (1977) found this test to be sensitive, highly predictive test.[15]

The buckler test was designed to reproduce a human patch test in animals and therefore allows variation of conditions to optimize detection of moderate strong

sensitizers prior to testing in humans. This test utilizes induction and challenge phases of occluded epidermal doses of the test substances that may be allergic chemicals or final product formulations.[16]

2 Irritation testing in animals

The evaluation of compound for irritability in animals correlates well with the degree of skin response in humans.

Single Application Irritation Testing

The test described by Draize et al.[10] or slight modification of this test is the most widely used for predicting the potential skin irritation of chemicals and chemical mixtures. In this test hair are clipped from the back of a rabbit and four distinct areas for the application of test substances are identified. Two of four areas were abraded by making four epidermal incisions in the appropriate areas. All four areas were covered by gauze that is held in place with adhesive tape and the test substance is applied to the appropriate area under the gauze.

The entire trunk of the rabbit is wrapped in impervious cloth or plastic to hold the patches in place and decrease the evaporation of volatile test substances. The rabbits were generally remain wrapped for 24 hr after treatment and are evaluated for irritation at the time of unwrapping and 24 and 48 hr after being unwrapped. Generally four test substances are evaluated in a series of six rabbits.

Table-1.3.1: Division of various elements of irritation in to distinct categories of grading according to Draiz et al.

Sr. No.	Skin reaction	Score
1.	**Erythema and Escher Formation**	
	Very slight erythema (barely perceptible)	**1**
	Well defined erythema	**2**
	Moderate to severe erythema	**3**
	Severe erythema (beet redness) to slight Escher formation	**4**
	Total possible erythema score	**4**
2.	**Edema Formation**	
	Very slight Edema (Barely perceptible)	**1**
	Slight Edema edges of area well defined by definite raising)	**2**
	Moderate edema (area raised approximately 1mm)	**3**
	Severe edema (raised 1mm and extending beyond the area of exposure)	**4**
	Total possible edema score	**4**
	Total possible score for primary irritation	**8**

Repetitive Application Irritation Testing

The repetitive application over at least 7-14 days appears to be better able to predict the irritability of test substances than the single application. Additionally, the repetitive application test to assess irritability can be combined with an assessment for systemic toxicity by cutaneous route. This is highly relevant especially in the case of transdermal drug delivery. In repetitive irritant testing usually, three or more groups of five to ten animals per sex are used with each group receiving a different dose of test substance daily for 2,3,4 or 13 weeks. An additional group of animals is handled similarly but treated with the vehicle or when no vehicle is used with water to serve as control group is removed from the back of the animal. The animal is then wrapped with an occlusive dressing and returned to its home cage for the period of 6 hr. The

wrapping is removed and the back is gently wiped. The animals are observed daily for the signs of cutaneous irritation.

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1.4 CARDIOVASCULAR DISEASE

Cardiovascular diseases are the class of diseases that involve the heart or blood vessels (arteries and veins).

- Heart diseases which affect the heart in several different ways but they all cause the ultimate problem of disrupting the vital pumping action of the heart. e.g. Cardiac arrhythmias, Heart attack, Myocardial infraction, Valvular disorders, Coronary artery disease, Congenital heart disease; Heart muscle disease or Cardiomyopathy; Pericardial diseases.
- Vascular disease or Blood vessel disease which affects the blood vessels and ultimately causes heart dysfunctioning. e.g. Hypertension, Hypotension, Atherosclerosis, Embolism etc.

1.4.1 Cardiac arrhythmias[1-4]

The rhythm of the heart is normally generated and regulated by pacemaker cells within the sinoatrial (SA) node, which is located within the wall of the right atrium. SA nodal pacemaker activity normally governs the rhythm of the atria and ventricles.

Causes of Cardiac arrhythmias

A frequent cause of arrhythmia is coronary artery disease because this condition results in myocardial ischemia or infarction. When cardiac cells lack oxygen, they become depolarized, which lead to altered impulse formation and/or altered impulse conduction. The former concerns changes in rhythm that are caused by changes in the automaticity of pacemaker cells or by abnormal generation of action potentials at sites other than the SA node (termed ectopic foci). Altered impulse conduction is usually associated with complete or partial block of electrical conduction within the heart. Altered impulse conduction commonly results in reentry, which can lead to tachyarrhythmia. Finally, many different types of drugs (including antiarrhythmic drugs) as well as electrolyte disturbances (primarily K^+ and Ca^{++}) can precipitate arrhythmias.

Classification of Cardiac Arrhythmias

Bradycardia denotes a decrease in heart rate.

Tachycardia is the term denoting an increase in heart rate.

Extrasystoles: these are premature beats that occurs before the next expected beats. This may be artrial, ventricular or nodal, depending on its site of origin.

Paroxymal Supraventricular Tachycardia: It is the condition in which the heart rate suddenly increases to 150-200 beats/min and 1:1 AV conduction is maintained.

Atrial-flutter: It is a very rapid but regular beating of atria with a rate between 240-400 per min.

Atrial Fibrillation: It is a rapid, continuous chaotic and irregular beating of atria. It is of great danger.

Ventricular Fibrillation: It is a irregular and chaotic ventricular arrhythmia with rapid rate and disorganized spread of impulses throughout the ventricular myocardium. It is Very Dangerous.

Heart block: It is the term applied to a condition in which the nerve impulses are delayed or fail to get through from their source in the right atrium to the ventricles. It is obstruction of the impulse, in the heart thus suddenly, decreasing the heart rate and the pulse rate.

Cardiac arrhythmias treatment

When arrhythmias require treatment, they are treated with drugs that suppress the arrhythmia. These drugs are called antiarrhythmic drugs. There are many different types of antiarrhythmic drugs and many different mechanisms of action. Most of the drugs affect ion channels that are involved in the movement of sodium, calcium and potassium ions in and out of the cell. These drugs include mechanistic classes such as sodium-channel blockers, calcium-channel blockers and potassium-channel blockers. By altering the movement of these important ions, the electrical activity of the cardiac cells (both pacemaker and non-pacemaker cells) is altered, hopefully in a manner that suppresses arrhythmias.

Classification of Antiarrhythmic drugs

Class I*:* Na channel blockers (membrane-stabilizing drugs) block fast Na channels, slowing conduction in fast-channel tissues Class I drugs are subdivided based on the kinetics of the Na channel effects. e.g. Quinidine, Procainamide

Class Ia drugs have a intermediate kinetic

Class Ib drugs have fast kinetics

Class Ic drugs have slow kinetics.

Class II*:* Class II drugs are β-blockers, which affect predominantly slow-channel tissues (SA and AV nodes), where they decrease rate of automaticity, slow conduction velocity, and prolong refractoriness. Thus, heart rate is slowed. e.g. Propranolol

Class III: Class III drugs are primarily K channel blockers, which prolong action potential duration and refractoriness in slow- and fast-channel tissues. e.g. Amioderone, Sotalol

Class IV: Class IV drugs are the nondihydropyridine Ca channel blockers, which depress Ca-dependent action potentials in slow channel tissues and thus decrease rate of automaticity, slow conduction velocity, and prolong refractoriness. Heart rate is slowed. e.g. Verapamil, Diltiazem.

1.4.2 Hypertension[5-8]

Hypertension or high blood pressure is a condition in which the blood pressure in the arteries is chronically elevated. With every heart beat, the heart pumps blood through the arteries to the rest of the body. Blood pressure is the force of blood that is pushing up against the walls of the blood vessels. If the pressure is too high, the heart has to work harder to pump, and this could lead to organ damage and several illnesses such as heart attack, stroke, heart failure, aneurysm, or renal failure.

Classification of hypertension

Hypertension may be classified as essential or secondary. Essential hypertension is the term for high blood pressure with unknown cause. It accounts for about 95% of cases. Secondary hypertension is the term for high blood pressure with a known direct cause, such as kidney disease, tumors, or other diseases.

Causes of Hypertension

Though the exact causes of hypertension are usually unknown, there are several factors that have been highly associated with the condition. These include Smoking, Obesity or being overweight, Diabetes, Sedentary lifestyle, Lack of physical activity, High levels of salt intake (sodium sensitivity), Insufficient calcium, potassium, and magnesium consumption, High levels of alcohol consumption, Stress, Aging, Medicines such as birth control pills, Genetics and a family history of hypertension, Chronic kidney disease, Adrenal and thyroid problems or tumors.

Symptoms of Hypertension

There is no guarantee that a person with hypertension will present any symptoms of the condition. Extremely high blood pressure may lead to some symptoms, however, and these include severe headaches, Fatigue or confusion, Dizziness, Nausea,

Problems with vision, Chest pains, Breathing problems, Irregular heartbeat, Blood in the urine.

Treatment of Hypertension

Medications most often prescribed for high blood pressure include the following:

Water pills (diuretics)

Diuretics are used very widely to control mildly high blood pressure, and are often used in combination with other medications. They increase sodium excretion and urine output and decrease blood volume. The sensitivity to the effect of other hormones in your body is decreased. e.g. Hydrochlorothiazide.

Beta-blockers

Beta-blockers reduce heart rate and decrease the force of heart contraction, thereby reducing the pressure generated by the heart. They are preferred for people who have associated coronary heart disease, angina, or history of a heart attack, since they also prevent recurrent heart attacks and sudden death. e.g. Carvedilol, metoprolol, atenolol.

Side effects - Fatigue, depression, impotence, nightmares

Calcium channel blockers

Calcium channel blocking agents work by relaxing the muscle in the walls of the arteries.

They also reduce the force of contraction of the heart. e.g. Nifedipine, diltiazem, verapamil, nicardipine, amlodipine, felodipine.

Side effects - Ankle swelling, fatigue, headache, constipation, flushing

Angiotensin-converting enzyme (ACE) inhibitors

ACE inhibitors stop the production of a chemical called angiotensin II, a very potent chemical that causes blood vessels to contract, a cause of high blood pressure. Blockage of this chemical causes the blood vessels to relax. e.g. Captopril, enalapril, lisinopril.

Angiotensin receptor blockers

Angiotensin receptor blockers work on receptors in tissues all over the body to prevent uptake of angiotensin II, and therefore inhibit the vasoconstrictor effect of angiotensin II. e.g. Losartan, valsartan.

Alpha-blockers

Alpha-blockers relax blood vessels by blocking messages from the nervous system that cause muscular contraction.

Examples – Terazosin, doxazosin.

Blockers of central sympathetic (autonomic nervous) system

These agents block messages out of the brain from the autonomic nervous system that contract blood vessels. The autonomic nervous system is the part of the nervous system that is automatic and controls heart rate, breathing rate, and other basic functions. The effect of these drugs is to relax blood vessels, thus lowering blood pressure. e.g. Clonidine.

Direct vasodilators

Direct vasodilators relax (dilate) the blood vessels to allow blood to flow under lower pressure. These medications are often given through an IV line in an emergency (that is, in malignant hypertension). e.g. Nitroprussidediazoxide. Oral medications are hydralazine and minoxidil.

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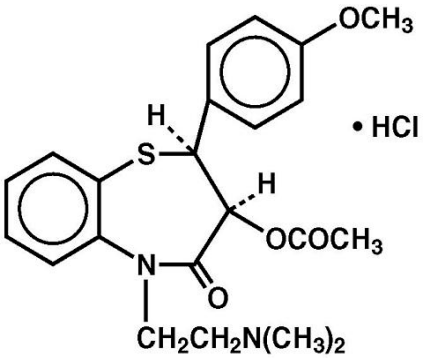
1.5 INTRODUCTION TO DRUG

1.5.1 Diltiazem HCI [1]

Diltiazem is a benzothiazepine calcium channel-blocking agent most similar to verapamil in its clinical effects. Diltiazem increases exercise capacity and improves multiple markers of myocardial ischemia, reduces heart rate, may improve cardiac output, improve myocardial perfusion, educes left ventricular work load and may prevent calcium induced perfusions injury that occurs after procedures such as angioplasty.

A. Physiochemical profile

Structure:



Empirical formula:	$C_{22}H_{26}N_2O_4S$ HCl
Molecular weight:	450.98
Chemical name:	1, 5-Benzothiazepin-4 (5H)-one, 3-(acetyloxy)--5- [2- (dimethylamino) ethyl]-2, 3-dihydro-2-(4-methoxyphenyl)-, monohydrochloride
Category:	Antianginal, Calcium Channel Blocker, Antihypertensive.
Description:	White crystalline powder or small crystals, odorless and bitter taste.
Melting point:	207.5-212°C

Solubility:

Table 1.5.1: Solubility of Diltiazem hydrochloride in different solvent	
Solvent	**Solubility**
Water	Freely soluble
Formic acid	Freely soluble
Methanol	Freely soluble
Chloroform	Freely soluble
Absolute alcohol	Sparingly soluble
Ether	Insoluble

B. Pharmacology [2,3,4]

Mechanism of action

Hypertension. Diltiazem produces its antihypertensive effect primarily by relaxation of vascular smooth muscle and the resultant decrease in peripheral vascular resistance. The magnitude of blood pressure reduction is related to the degree of hypertension; thus hypertensive individuals experience an antihypertensive effect, whereas there is only a modest fall in blood pressure in normotensives.

Angina. Diltiazem has been shown to produce increases in exercise tolerance, probably due to its ability to reduce myocardial oxygen demand. This is accomplished via reductions in heart rate and systemic blood pressure at submaximal and maximal workloads. Diltiazem has been shown to be a potent dilator of coronary arteries, both epicardial and subendocardial. Spontaneous and ergonovine-induced coronary artery spasms are inhibited by diltiazem.

Dosage: Adult: 30-60 mg 3 times in day.

C. Pharmacokinetic

Diltiazem is well absorbed from the gastrointestinal tract and is subject to an extensive first-pass effect, giving an absolute bioavailability (compared to intravenous administration) of about 40%. Diltiazem undergoes extensive metabolism in which only 2% to 4% of the unchanged drug appears in the urine. In vitro binding studies show Diltiazem is 70% to 80% bound to plasma proteins. The plasma elimination half-life following single or multiple drug administration is approximately 3.0 to 4.5 hr. Desacetyl Diltiazem is also present in the plasma at levels of 10% to 20% of the

parent drug and is 25% to 50% as potent as a coronary vasodilator as Diltiazem. Minimum therapeutic plasma Diltiazem concentrations appear to be in the range of 50 to 200ng/ml.

Table 1.5.2: Pharmacokinetic data of Diltiazem hydrochloride [3]	
Extend of absorption	80-90%
Bioavailability	40-67%
Onset of action	30-60 min
Peak plasma level	2-3 hr
Protein binding	70-80%
Therapeutic serum level	50-200 ng/ml
Metabolite	Desacethyl diltiazem
Excreted unchanged in urine	2-4%
Half life elimination	3.5-6 hrs

D. Indication

Angina, Atrial fibrillation, Hypertension, Cardiomyopathy, Diabetic neuropathy

E. Contraindications

Diltiazem is contraindicated in patients with sick sinus syndrome except in the presence of a functioning ventricular pacemaker, second- or third-degree AV block except in the presence of a functioning ventricular pacemaker, hypotension (less than 90 mm Hg systolic), hypersensitivity to the drug, acute myocardial infarction and pulmonary congestion documented by x-ray on admission.

F. Adverse effects

AV block, Confusion, Constipation, Depression, Heat failure, Hypotension, Stevens-Johnson syndrome

G. Market products of Diltiazem hydrochloride [5,6]

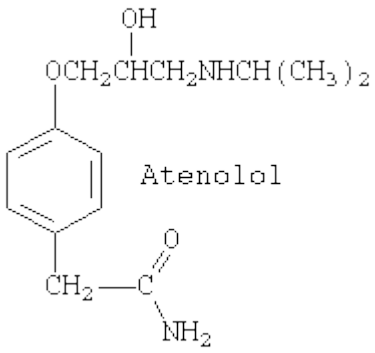
Table.1.5.3 Different formulations of Diltiazem hydrochlorides			
Conventional Dosage form			
Brand Name	**Formulation**	**Available Strength**	**Company**
Angizem	Tablet	30mg, 60mg	Sun Pharma
Dicard	Tablet	30mg, 60mg	Intas
Diltime	Tablet	30mg, 60mg, 90 mg	Zydus Cadila
Dilzem	Tablet	30mg, 60mg,	Torrent
Modified Release Dosage forms			
Angizem cd	Tablet	90mg, 120mg, 180mg	Sun pharma
Dilter CD	Capsule	60mg, 90mg, 120mg	Sun Pharma
Diltime	Tablet	120mg	Zydus Cadila
Dilzem	Tablet	90mg	Torrent
Injectable dosage form			
Dilzem	Injection	5mg/ml	Torrent

1.5.2 Atenolol [7,8,9]

Atenolol is a β-adrenolytic, cardioselective drug, having no intrinsic sympathomimetic activity.

A. Physicochemical profile

Structure



Emperical formula $C_{14}H_{22}N_2O_3$

Molecular weight	266.3
Chemical Name	(RS)-4-(2-hydroxy-3-isopropylaminopropoxy)phenylacetamide
Category	Antihypertensive
Description	Atenolol is a white and odorless powder.
Melting Point	152-155^{o}C
Dissociation Constant (pK_a)	9.6 @ 24^{o}C
Partition Co-efficient	0.23

Table 1.5.4: Solubility of atenolol in different solvents

Solvent	Solubility
Water	Sparingly Soluble
Ethanol	Soluble
Methanol	Soluble
Ether	Practically Insoluble

B. Pharmacology

Mechanism of action

Atenolol is a beta-adrenaoreceptor antagonist, or a more commonly known as a beta blocker. Atenolol slows down the strength of the heart's contractions and reduces its oxygen requirements and the volume of blood it has to pump. Hypertension (high blood pressure) may be treated with these drugs because of their ability to increase the diameter of the blood vessels thus allowing blood to flow under less pressure. Some of these medicines include a diuretic to help reduce blood pressure by increasing the body's excretion of excess fluid. Beta blockers are also used to treat Myocardial infarction (heart attack) and Arrhythmias (rhythm disorders), angina (chest pains), and disorders arising from decreased circulation and vascular constriction, including migraine.

Dosage

Adult 50-100mg daily

C. Pharmacokinetic

Bioavailability following oral administration is about 45%, but with individual variation this can triple or quadruple. Peak plasma concentrations occur 2-4 hours after oral administration and the duration of therapeutic effect is up to 24 hours. Plasma concentration increases comparatively with the patient's age. If taken with food, the absorption is reduced by approximately 20%. Protein binding is approximately 3% and the volume of distribution is 0,7 l/kg. Atenolol has low lipid solubility. Hepatic metabolism is negligible and the drug is eliminated, almost always unchanged, through the kidneys. Only 10% of Atenolol is eliminated as metabolite, none of which have any pharmaceutical activity in man. Plasma half-life is 6-9 hours. In case of impaired renal function half-life is prolonged, but impaired hepatic function has no effect on half-life.

D. Indication

Antihypertensive, Anti-Angina, Anti-Arrhythmic, Myocardial Infarctions, Alcohol Withdrawal, Anxiety States, Migraine Prophylaxis, Hyperthyroidism, Tremor

E. Contraindications

Atenolol is contraindicated in patient with Asthama, Congestive cardiac failure and Phaeochromocytoma.

F. Adverse effect

The most serious adverse effects are heart failure, heart block, and bronchospasm. Other more minor side-effects include fatigue and coldness of extremities. It causes CNS effect of depression, hallucination, confusion. It causes nausea, vomiting, constipation and abdominal pain. It causes skin rash, pruritis, decreased tear production, blurred vision and soreness.

G. Marketed product

Aloten (Core), Altol (Indoco), Angitol (Ind-Swift), Antipress (Saga Labs), Atcardil (Sun Pharma), AteCard (Dabur), Atecor (Win-Medicare), Atelol (Themis Pharma), Aten (Kopran), Atenex (Recon), Atenova (Lupin), Atormin (PCI), Atpark (Parke Davis), Beta (Stadmed), Betacard (Torrent), Betanol (Unisearch), beten (Sigma Labs), Biduten (Croydon), BP-NOL (Elder), Catenol (Alidac), Eucard (Malladi Drugs),

Hipres (Cipla), Lakten-50 (Shalaks), Lonol (Khandelwal), Normolol (Pace, SOL), Pertenol (Karnataka Antibiotics), Telol (Max), Tenase (Jenburkt), Tenolol (IPCA), Tensicard (Troikaa), Tensimin (Unique).

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1.6 INTRODUCTION TO POLYMERS

1.6.1 Ethyl Vinyl Acetate Copolymer [1-5]

1. Synonyms

Acetic acid, ethylene ester polymer with ethane, CoTran; ethylene vinyl acetate copolymer; EVA; EVA copolymer, EVM; poly(ethylene co-vinyl-acetate); VA/ethylene copolymer; vinyl acetate/ethylene copolymer

2. Chemical properties

Chemical name Ethylene vinyl acetate copolymer

CAS Registry Number (24937-78-8)

Empirical formula and molecular weight $(CH_2CH_2)x\ (CH_2CH(CO_2CH_3))y$

Structural formula Ethylene vinyl acetate copolymer is a Random copolymer of ethylene and Vinyl copolymer of ethylene and vinyl acetate

Functional category Membrane, Transdermal backing

3. Physical properties

Description

Ethylene vinyl acetate is available as white waxy solid in pellet or powder form. Films are translucent.

Density 0.92-0.94 gm/cm^3

Flash point 260^oC

Melting point 75-102^oC depending on polymer ratios

Table 1.6.1: Characteristics of different CoTran (3M Drug Delivery Systems) film grades

Grade	Vinyl acetate (%)	Thickness (µm)	Moisture vapour transmission rate (g/m^2/24h)
CoTran 9706	9	101.6	26.4
CoTran 9715	19	76.2	64.8
CoTran 9716	19	101.6	48.6

Stability and storage condition

Ethylene vinyl acetate copolymers are stable under normal conditions and should be stored in a cool, dry place. Films of ethylene vinyl acetate copolymers should be stored at 0-30^{o}C and less than 75% relative humidity.

Incompatibilities

Ethylene vinyl acetate is incompatible with strong oxidizing agents and bases.

4. Application in Pharmaceutical Formulation or Technology

Ethylene vinyl acetate copolymers are used as membranes and backings in laminated transdermal drug delivery system. They can also be incorporated as components in backings in transdermal systems. Ethylene vinyl acetate copolymers have been shown to be an effective matrix and membrane for the controlled delivery of atenolol[1,2], triprolidine[3,4] and furosemide.[5] The system for the controlled release of atenolol can be further developed using ethylene vinyl acetate copolmers and plasticizers.[1]

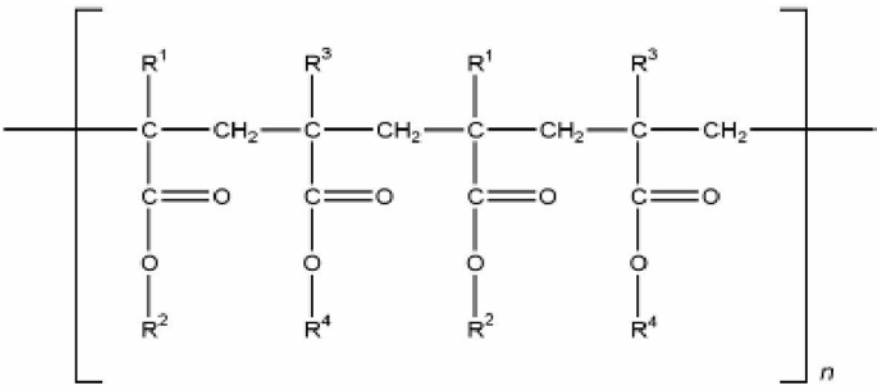
1.6.2 Polymethacrylates [6-9]

1. Synonyms

Acryl-EZE; Acryl-EZE MP; Eastacryl 30D; Eudragit; Kollicoat MAE 30 D; Kollicoat MAE 30 DP; polymeric methacrylates.

2. Chemical properties

Structural Formula



For Eudragit RL and Eudragit RS:

R^1 = H, CH_3

$R^2 = CH_3, C2H_5$

$R^3 = CH_3$

$R^4 = CH_2CH_2N(CH_3)_3\ ^+Cl^-$

Functional Category

Film former; tablet binder; tablet diluent.

Chemical Name and Cas Registry Number

Table 1.6.2: Chemical name, Trade name, Company name and CAS registry Number of Polymethacrylate

CHEMICAL NAME	TRADE NAME	COMPANY NAME	CAS NUMBER
Poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride)1 : 2 : 0.2	ERL 100	Röhm GmbH	[33434-24-1]
Poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride)1 : 2 : 0.1	ERS 100	Röhm GmbH	[33434-24-1]

3. Physical properties

Alkali Value: 23.9–32.3 for Eudragit RL 100

Density (Bulk): 0.390 g/cm^3

Density (Tapped): 0.424 g/cm^3

Density (True): 0.816–0.836 g/cm^3 for Eudragit RL and RS PO

Refractive Index: n^{20}D = 1.38–1.385 for Eudragit RL and RS

Viscosity (Dynamic): ≤15 mPa for Eudragit RL and RS

Stability and Storage Conditions

Dry powder polymer forms are stable at temperatures less than 30°C. Above this temperature, powders tend to form clumps, although this does not affect the quality of the substance and the clumps can readily be broken up. Dry powders are stable for at least 3 years if stored in a tightly closed container at less than 30°C.

Incompatibilities

Incompatibilities occur with certain polymethacrylate dispersions depending upon the ionic and physical properties of the polymer and solvent. For example, coagulation may be caused by soluble electrolytes, pH changes, some organic solvents, and extremes of temperature.

4. Application in pharmaceutical formulation or technology

Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents. Depending on the type of polymer used, films of different solubility characteristics can be produced.

Eudragit RL, RS are used to form water-insoluble film coats for sustained-release products. Eudragit RL films are more permeable than those of Eudragit RS, and films of varying permeability can be obtained by mixing the two types together.

1.6.3 Ethyl cellulose: [10-13]

1. Synonyms

Aquacoat EDC; Aqualon; Ethocel; Surelease

2. Chemical properties

Non proprietary name:

BP : Ethylcellulose

PhEur : Ethylcellulosum

USPNF : Ethylcellulose

Chemical name: Cellulose ethyl ether.

Empirical formula: $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$

Functional category:

Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity increasing agent.

Table1.6.3: Concentration of ethyl cellulose used

Use	Concentration (%)
Microencapsulation	10-20
Sustained release tablet coating	2-20
Tablet coating	1-3
Tablet granulation	1-3

3. Physical properties

Description:

Ethyl cellulose is a tasteless, free flowing, white to light tan-coloured powder.

Density 0.4 g/cm^3

Specific gravity 1.12-1.15g/cm^3

Glass transition temperature 129-133°C

Solubility

It is practically insoluble in glycerin, propylene glycol and water. EC that contains less than 46.5% ethoxyl groups if freely soluble in chloroform, methyl acetate, tetrahydrofuran. EC that contains not less than 46.5% ethoxyl groups if freely soluble in ethanol, ethyl acetate, methanol, and toluene.

Moisture content

Ethyl cellulose absorbs very little water from humid air or during immersion and that small amount evaporates readily.

Stability and storage conditions

Ethyl cellulose is stable, slightly hygroscopic material. It is chemically resistant to alkalis both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic material than are cellulose esters.

Table 1.6.4: Different grades of ethyl cellulose

Grade	Viscosity (mPa s)	Mean particle size (μm)
Ethocel Std 4 Premium	3-5.5	204
N-7	5.6-8	160
Ethocel Std 7FP Premium	6-8	9
Ethocel Std 7 Premium	6-8	210
N-10	8-11	225
Ethocel Std 10F Premium	9-11	5
Ethocel Std 10P Premium	9-11	5
N-14	12-16	212
Ethocel Std 20P Premium	18-22	-
N-22	18-24	243
Ethocel Std 45P Premium	41-49	-

N-50	40-50	305
N-100	80-105	-
Ethocel Std 100FP Premium	90-110	194
Ethocel Std 100P Premium	90-110	40

1.6.4 Carbopol 934[14-17]

Carbopol 934P is specially tailored for pharmaceutical industries. It can be useful for internal pharmaceutical dosage forms. Carbopol 934P is high purity grade and used for thickening, suspending and emulsifying. It is also useful in tablets for binding and sustained release formulations.

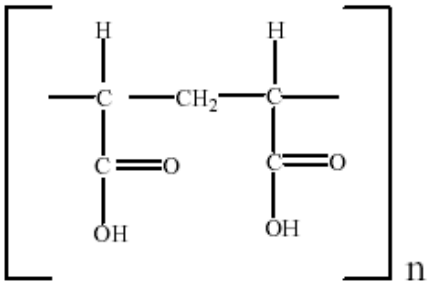
1. Synonyms

Carboxy polymethylene, carboxyvinyl polymer

2. Chemical property

Chemical name: carboxy polymethylene

Chemical structure:



Nonproprietary name:

carbopol 940 L R, carbomer, carbomera

Functional category:

Bioadhesive, suspending agent, viscosity increasing agent, release modifying agent, tablet binder.

3. Physical properties

Description:

A white, fluffy, acidic, hygroscopic powder with a slight characteristic odour.

Typical properties:

carbopol is soluble in water, alcohol, and glycerin. Agents that can neutralize carbopol include sodium hydroxide, potassium hydroxide, sodium bicarbonate, borax, amino acids, polar organic amines.

Specific gravity: 1.41

Density (bulk): 5 g/cm^3

Density (tapped): 1.4 g/cm^3

Viscosity (0.5%w/v): 40-60 poise

Acidity/ alkalinity: pH = 2.7-3.5 for a 0.5 % w/v aqueous dispersion,
pH = 2.5-3.0 for a 1 %w/v aqueous dispersion.

4. Applications of carbopol in pharmaceutical formulation or technology

Carbopol are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams; gels, and ointments for use in ophthalmic, rectal, and topical preparations. carbomer grades, even with low residual benzene content, such as Carbopol 934P, are no longer included in the PhEur 2002. Carbopol having low residuals only of ethyl acetate, such as Carbopol 971P or 974P, may be used in oral preparations, in suspensions, tablets, or sustained release tablet formulations. In tablet formulations, Carbopols are used as dry or wet binders and as a rate controlling Excipients. In wet granulation processes, water or an alcohol-water blend is used as the granulating fluid. Anhydrous organic solvents have also been used, with the inclusion of a polymeric binder. The tackiness of the wet mass can be reduced with the addition of certain cationic species to the granulating fluid or, in the case of water, with talc in the formulation.

Carbopol resins have also been investigated in the preparation of sustained release matrix beads, as enzyme inhibitors of intestinal proteases in peptide-containing dosage forms, as a bioadhesive for a cervical patch and for intranasally administered microspheres, and in magnetic granules for site-specific drug delivery to the esophagus.

Carbopols are also employed as emulsifying agents in the preparation of oil-in-water emulsions for external use. For this purpose, the Carbopol is neutralized partly with sodium hydroxide and partly have been investigated as a viscosity increasing aid in the preparation of multiple emulsions

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1.6.5 Hydroxypropyl methyl cellulose[18-21]

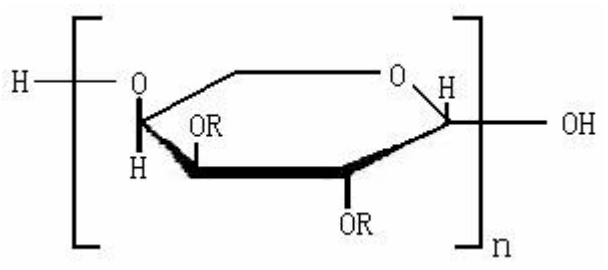
Hydroxypropyl methyl cellulose is mixed hydroxyl alkyl cellulose ether and may be regaded as thew propylene glycol ether of methyl cellulose. It is available in many grades of different viscosity range from 5 to 100000 cps.

1. Chemical properties

Chemical name and CAS registry number: cellulose, 2-hydroxypropyl methyl ether (9004-65-3)

Nonproprietary name: BP: Hypromellose, JP: Hydroxypropyl methyl cellulose, PhEurr: Hypromellosum, USP: Hypromellose

Chemical structure:



Where,

n is number of glucose units in cellulose molecule.

R is CH_3 or CH_2CH (0H)CH_3 .

Viscosity (2% aqueous solution)

HPMC K100 LV	100 cps
HPMC A15 C	2,000cps
HPMC K4M	4,000 cps
HPMC K15M	15,000 cps
HPMC K100M	1, 00,000 cps

Functional category

oating agent, film former, rate-controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity increasing agent.

2. Physical properties

Description: Appearance: white or off-white powder, odorless, tasteless

Particle size: about 98.5% through 100 mesh; 100% through 80 meshes.

Carbonation temperature: 280-300°C

Specific gravity:	1.26-1.31
Temperature to change color:	190-200°C
Surface tension:	42-56 dyne/cm (2% solution).
Acidity/alkalinity:	pH 5.5-8.0 for a 1%w/w aqueous solution
Ash:	1.5-3% depending upon the grade
Density (bulk):	0.341 g/cm^3
Density (tapped):	0.557 g/cm^3
Density (true):	1.326 g/cm^3
Glass transition temperature:	170-180^{o}C

Moisture content

Hypromellose absorbs moisture from the atmosphere, the amount of water absorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility

It is soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane and mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone solution, mixtures of dichloromethane and propan-2-ol and other organic solvents.

Stability and storage condition

Hypromellose powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3-11. Increasing temperature reduces the viscosity of solutions. Hypromellose undergoes a reversible sol-gel transformation upon heating and cooling, respectively. The gel point is 50-90^{o}C.

Incompatibilities

Hypromellose is incompatible with some oxidizing agents. Since it is nonionic, hypromellose will not complex with metallic salts or ionic organics to form insoluble precipitates.

Applications of hydroxypropyl methyl cellulose in pharmaceutical formulation or technology

It is suspending, viscosity enhancing and film forming agent. HPMC is most widely used in hydrophilic matrix sustaining release tables and other type of controlled release pharmaceutical dosage forms, because of its characteristic namely non-toxic

nature, its capacity to incorporate active pharmaceutical, manufacture of tablet by direct compression without previous granulation as well as pH independent nature.

1.6.6 Polyvinyl pyrolidone [22-27]

PVP (polyvinyl pyrrolidone, povidone, polyvidone) is a water-soluble polymer made from the monomer N-vinyl pyrrolidone:

1. Synonyms

Polvinylpolypyrrolidone; N-Vinylbutyrolactam polymer; poly[1-(2-oxo-1-pyrrolidynyl)ethylene];

2. Chemical properties

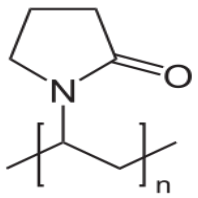
Chemical name

1-Ethenyl-2-pyrrolidoinone homopolymer (IUPAC); Poly[1-(2-oxo-1-pyrrolidinyl)ethylene]; polyvidone; polyvinylpyrrolidone; PVP; 1-vinyl-2-pyrrolidinone polymer

CAS Registry Number 9003-39-8

Empirical formula $(C_6H_9NO)_n$

Structural formula



Molar mass 2.500 - 2.5000.000 $g \cdot mol^{-1}$

Functional category Stabilizing agent, binding agent

4. Physical properties

Description White to light yellow, hygroscopic, amorphous powder

Density 1.2 g/cm^3

Melting point 110 - 180 °C (glass temperature)

Stability and storage condition

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from incompatible substances. Containers of this

material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

Incompatibilities:

It is incompatible with strong oxidizing agent and strong reducing agents.

4. Application in Pharmaceutical Formulation or Technology

It is used as a binder in tablet formulations. PVP added to Iodine forms a complex in solution which is known under the trade name Betadine. PVP also used as coating agent for photo-quality ink-jet papers and transparencies, as well as in inks for inkjet printers. PVP is used in shampoos and toothpastes, in paints, and adhesives. It is also used in contact lens solutions and in steel-quenching solutions. PVP is used in hair sprays, hair gels, as a food additive, as a stabilizer, as a blocking agent during Western blot analysis.

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Chapter 2

Review of work done

CHAPTER 2

2.1 LITERATURE REVIEWS ON TRANSDERMAL DRUG DELIVERY SYSTEM

Jamakandi and co-workers (2009)[1] used different polymeric grades of hydroxy propyl methyl cellulose (6cps, 15cps and K4M) for the development of transdermal drug delivery system of Nicorandil, an antiaginal drug. Matrix patch were evaluated for their physiochemical characterization followed by in vitro evaluation. Among the six different HPMC formulations, transdermal patch with 6 cps and 6 %w/v DMSO as permeability enhancer showed maximum release.

Janardhanan Bagyalakshmi and co-workers (2008)[2] developed membrane-moderated transdermal systems of Ampicillin sodium and to evaluate them with respect to various in vitro and in vivo parameters. The membrane type transdermal systems were prepared using a drug with various antinucleant polymers like hydroxyl propyl methylcellulose (HPMC), Methyl cellulose (MC), Cellulose acetate phthalate, chitosan, sodium alginate (SA) and sodium carboxy methyl cellulose in an ethanol: pH 4.7 buffers by the solvent evaporation technique with HPMC as the rate controlling membrane for all the system. The release and permeation of the drug from the SA patch was found to be the maximum. The in vivo study of SA patch exhibited a peak plasma concentration of 126µg/mL at t_{max} 4 hours.

Murthy T.E.G.K. and co-workers (2008)[3] studied the influence of hydrophilic polymers on the permeability of Carvidiol from cellulose acetate films. The dried films were evaluated for appearance, thickness and drug release characteristic. In vitro permeation studies were carried out with Franz- diffusion cell. The drug release followed zero order kinetics and controlled by diffusion mechanism.

Wahid and co-workers (2008)[4] chemically modified chitosan using acetaldehyde and propionaldehyde to form Schiff's bases. Drug free polymeric film of chitosan, chemically modified chitosan and chitosan/hydroxyl propyl methylcellulose blend were prepared and evaluated for various physiochemical characters. All the films were evaluated for bursting strength, swelling index, moisture uptake, thickness uniformity, drug content uniformity, tensile strength, percent elongation at break, flatness, water vapor transmission rate and in vitro drug permeation study.

Shaila Lewis and co–workers (2007)[5] characterized plasma concentration time profiles for nicotine after single application of nicotine transdermal system to the upper fore arm of healthy smokers. A 12cm^2 system was applied for 24h. Plasma nicotine concentration achieved a mean C_{max} value of 14.5ng/ml. the result indicated that the system has potential usefulness in smoking cessation. The pharmacokinetic profile of marketed patch system was compared with the developed system. Significant difference was observed in the pharmacokinetic parameters.

Shahi S. R. and co-workers (2007)[6] studied the effect of various penetration enhancers such as Isopropyl Myristate (IPM), Dimethyl sulphoxide (DMSO), Benzyl alcohol, Menthol oil, oleic acid, Eucalyptus oil to increase the permeability of Ketorolac Tromethamine. The efficiency of the enhancers to improve the topical delivery of Ketorolac Tromethamine was sequenced in the order of DMSO> Eucalyptus > isopropyl myristate > menthol > oleic acid > benzyl alcohol.

Vanja and co–workers (2006)[7] computed solubility of methotrexate using Fedor method. Permeability studied was carried out using artificial membranes such as cellophane and dialysis membrane and biological membrane such as rat skin and egg shell membrane. Flux and permeability coefficient determination showed that dialysis membrane and egg shell membrane exhibited similar barrier properties as that of rat skin when compared with cellophane.

Saxena and co-workers (2006)[8] prepared Transdermal patches of metaclopramide hydrochloride using polyvinylalcohol and polyvinylpyrolidone. The physiochemical parameters like thickness, drug content, weight variation, moisture content, moisture uptake and drug penetration studies were evaluated for the patch. The in vitro drug penetration studies showed that burst release of the drug in initial hours and thereafter the drug was released slowly up to 12 hr. The stability studies indicated that all the patches maintained good physical appearance and drug content for 6 month at 40^{o}C and 75% RH.

Bharkatiya and co-workers (2006)[9] prepared transdermal films of Nimesulide using four different polymers using solvent casting technique. Dibutylphthalate was used as

plasticizer. In vitro permeation profile of formulation containing drug reservoir with HPMC: PVP showed highest permeation. The release of drug from all formulations followed the diffusion controlled Higuchi model and zero order release kinetics

Sadhna Gupta and co-workers (2005)[10] formulated and evaluated metaprolol tartarate transdermal drug delivery system using Eudragit RL and Hydroxypropyl methylcellulose. This transdermal drug delivery system was characterized for their thickness, tensile strength and drug content. They were characterized for in vitro release kinetics and drug skin permeation studies. The system comprising of Eudragit RL: Hydroxypropylmethylcellulose in 40:60 ratio exhibited drug skin permeation 87.5µg/h/cm^2. The transdermal drug delivery system exhibited better and constant drug plasma profile for 24h as compared to oral administration.

Ramesh Panchangula and co–workers (2005)[11] developed transdermal reservoir patch of naloxone and evaluated for *in vivo* studies, stability studies and irritancy potential. Propylene glycol and oleic acid have been used as penetration enhancers and showed developed transdermal system of naloxone is efficacious, stable and safe upon single and multiple dose application.

Schurad B. and co-workers (2005)[12] investigated the transdermal in vitro permeation behavior of the Proterguride using hairless mouse skin as a model membrane. Drug in adhesive matrix formulations based on different types of pressure-sensitive adhesives (Eudragit® E 100 and Gelva®7883 as acrylates, Oppanol® B 15 SFN as polyisobutylene, and BioPSA® 7-4202 as silicone) with a drug load of 3% by weight were prepared. It was found that Gelva®-based patches show good physical stability, good skin adhesion, and moderate flux values and, thus, can be evaluated as a basis for a suitable formulation for the transdermal administration of Proterguride.

Kanikkannan N. and co-workers (2004)[13] prepared monolithic drug-in-adhesive type transdermal patches of melatonin containing penetration enhancers such as fatty alcohols, fatty acids, and terpenes. The addition of enhancers in the patch increased the permeation of melatonin through hairless rat skin. The flux values of patches containing octanol, nonanoic acid, and myristic acid were higher than the control patch (no enhancer), but the differences were not statistically significant ($P > 0.05$).

Decanol, myristyl alcohol, and undecanoic acid at 5% concentrations showed significantly higher flux values through hairless rat skin (enhancement ratios 1.7, 1.5, and 1.6 for decanol, myristyl alcohol, and undecanoic acid, respectively) ($P < 0.05$). Menthol and limonene at 5% w/w showed maximum permeation of melatonin among all enhancers studied (enhancement ratios was 2.1 and 2.0 for menthol and limonene, respectively) ($P < 0.001$).

Amir Mehdizadeh and co-workers (2004)[14] designed to evaluate different matrix, drug-in-adhesive and reservoir formulation of Fentanyl transdermal patches. He has designed drug-in-adhesive patches by designing full factorial design. The results showed that the release kinetics obeyed the square root of time or Higuchi model, indicating the diffusion controlled release mechanism. It was found that the amount of fentanyl needed for each $10cm^2$ three days drug-in-adhesive should be 3.3mg. The respective amount for reservoir and matrix patches were 2.5 and 5mg. it was concluded that acrylic pressure sensitive adhesive showed the best adhesion and release properties.

Murthy S N and co-workers (2004)[15] prepared formulation containing 5mg/patch salbutamol sulfate(SS), providing an input rate of 100µg/h of SS and subjected for pharmacokinetic and pharmacodynamic evaluation in moderately asthmatic patients. A linear correlation was observed between cumulative amount of drug diffused in vitro and cumulative AUC of serum concentration time curve. A steady state serum concentration of 2.87 ± 0.1 ng/ml was attained after an initial lag period of 4.67 ± 1.03 hr.

Y. S. R. Krishnaiah and co-workers (2004)[16] investigated the effect of limonene on the *in vitro* permeation of nimodipine across the excised rat abdominal skin from a 2% w/w hydroxypropyl methyl cellulose (HPMC) gel drug reservoir system. The HPMC gel formulations containing 1.5% w/w of nimodipine and selected concentrations of limonene (0% w/w to 8% w/w) were prepared, and subjected to *in vitro* permeation of the drug through excised rat abdominal epidermis. The flux of nimodipine across rat epidermis was markedly increased by the addition of limonene to the HPMC gels. A maximum flux of nimodipine was observed (203 ± 0.6 $\mu g/cm^2 \cdot h$) with an enhancement ratio of about 5.7 when limonene was incorporated in HPMC

gel at a concentration of 4% w/w. The results suggest that limonene is useful for enhancing the skin permeability of nimodipine from transdermal therapeutic systems containing HPMC gel as a reservoir.

Priyanka Arora and co-workers (2002)[17] deigned matrix type transdermal patches containing diclofenac diethylamine using different ratios of polyvinylpyrrolidone (PVP) and Eethylcellulose (EC) by solvent evaporation technique. All the prepared formulations were subjected to physical studies like moisture content, moisture uptake and flatness and in vitro release studies and in vivo skin permeation studies. In vitro permeation studies were performed across cadaver skin using a modified diffusion cell. They concluded that diclofenac diethylamine can be formulated into the transdermal matrices type patches to sustain its release characteristics and the polymer composition (PVP:EC, 1:2) was found to be the best choice for manufacturing transdemal patches of diclofenac diethylamine among the formulation studies.

M. Aquil and co-workers (2002)[18] developed matrix type transdermal drug delivery system of Pinacidil monohydrate by film casting technique or mercury substrate method and skin penetration studies using Keshary Chien diffusion cell on albino rat skin. The cumulative % of drug release in 48h was found to be highest (92.18%) from formulation Eudragit RL100: PVP K-30 (6:4).

Hong Zaho and co-workers (2002)[19] formulated matrix-type transdermal delivery systems of testosterone (TS) using three different pressure sensitive adhesives (PSA) The effect of PSA, skin permeation enhancers and solubilizers on the rat skin permeation rate of TS were systematically investigated. The highest skin permeation rate (4.14 μg/cm^2/hr) was achieved when 2% TS was loaded in DuroTak® 87-2516 together with 10% span 80 and 3% dodecylamine, the permeation enhancer. In vivo studied showed that the application of an experimental patch on rat abdominal skin resulted in a prompt and significantly higher plasma concentration of TS than that of a commercially product (Testoderm) designed to apply on scrotal skin.

Michael H. and co–workers (2002)[20] studied the release of permeation enhancers from transdermal drug delivery system of drug-in-adhesive type using known enhancers from eight types of adhesive polymers. They showed that, enhancers

released completely from the adhesive and the release rate depended on the types of adhesives. They also showed that acrylic adhesive and polyisobutylene adhesive showed slower drug release rate than silicon adhesive.

Kim J.H. and co-workers (2002)[21] investigated the effect of various pressure sensitive adhesives (PSA) on the percutaneous absorption of physostigmine across hairless mouse skin. Physostigmine showed the highest permeability from silicone adhesive matrix. Among, acrylic adhesive the permeability of physostigmine was the highest from grafted acrylic adhesive. This study also showed that several non-ionic surfactants, including PEG-20 evening primrose glycerides, enhanced the permeation of physostigmine across hairless mouse skin better than oleic acid.

Verma, P. R. and co-workers (2000)[22] developed matrix-dispersion-type transdermal drug delivery system of propranol using different ratios of mixed polymeric grades of Eudragit. In vivo evaluation was carried out on healthy human volunteers following a balanced incomplete block design (BIBD). In vitro dissolution rate constant k and pharmacokinetic parameters generated from plasma and urine were evaluated statistically. Statistically excellent correlation was found between percentages of drug absorbed from patch versus Cmax, $AUC^{0-24,}$ and $AUC^{0-\infty}$. A highly significant difference was observed when Cmax and AUC0- generated from plasma and urine data were compared, but when K_e, $t_{1/2}$e, ka, $t_{1/2}$ were compared, the difference was not significant. Urinary excretion data are suggested as a simpler alternative to blood-level data in studying the kinetics of absorption and deriving the absorption parameter.

Varshney and co-workers (1999)[23] used **A**rosol-OT (AOT), an ionic moiety of the surfactant docusate sodium, to enhance the permeation of nitroglycerine. The drug encapsulated in reservoirs with surfactant arranged in normal and reverse micelle pattern. They shown that 5% weight of AOT in water, arranged in normal micellar pattern, can increase the permeability of nitroglycerine 12.3 fold.

Kusum Devi and co–workers (1998)[24] developed and evaluated free films and transdermal patches of ketorolac tromethamine using polymers and pressure sensitive adhesives. PVP and PVA were used as polymer matrix materials and acrylic and

silicone based pressure sensitive adhesives were used as adhesive matrix materials. They observed that the permeation of drug with span 80 was more when compared to tween 80, oleic acid, propylene glycol as enhancers.

Narasimha Murthy and co–workers (1996)[25] carried out drug release studies from transdermal films of terbutaline sulphate using polymers such as hydroxypropyl methylcellulose and sodium carboxy methyl cellulose and reported that the hydroxypropyl methylcellulose films showed a greater rate of release compared to that of sodium carboxymethyl cellulose across all the barriers used.

Mishra A and co–workers (1996)[26] prepared transdermal formulations of testosterone designed for biphasic delivery, containing a blend of polymeric components polyvinyl alcohol and polyvinyl pyrrolidone in isopropyl alcohol. They reported that the film initially showed burst effect and then sustained release of testosterone.

McDaid and co–workers (1996)[27] prepared Amlodipine base from its besylate salt and various physiochemical properties relevant to transdermal delivery determined. Permeation of the drug from a range of hydrophilic and hydrophobic bases through hairless mouse skin was studied and the influence of the penetration enhancers in a sodium carboxymethyl cellulose 3% gel base was examined. The flux of drug could be further enhanced using variable percentage of ethanol in the donor phase. In vivo studied using rabbits were performed to assess the suitability of a reservoir type device. No adverse local effects in the animal model arising from the application of the transdermal device were observed.

Jain G K and co–workers (1996)[28] studied the effect of penetration enhancer (α-limonene) on poly vinyl alcohol and polyvinyl pyrrolidone films containing verapamil hydrochloride as drug. They reported that the drug permeation was enhanced by using α- limonene as permeation enhancer.

Thacharodi D and co–workers (1995)[29] studied different permeability of propranolol hydrochloride by controlled cross-linking with glutarldehyde to regulate

the drug release in the devices. Chitosan gel was used as the drug reservoir. The drug release profile through rabbit pinna skin showed that the drug delivery is completely controlled by the devices. The rate of drug released was found to be dependent on the type of membrane used.

Lee Y. L. and co-workers (1994)[30] developed transdermal delivery system for ketotifen. *In vitro* skin penetration results showed that ketotifen had optimal skin permeability at pH 7.5. In addition, ketotifen had higher rate of penetration through stripped skin. Ketotifen patch was fabricated in a stainless mold containing Eudragit S-100 and PEG 400. Other components, tween, span and fatty acids were also incorporated into the patch as penetration enhancers. For animal study, a patch with area of 30 cm^2 was applied on the dorsal skin of rabbit. The plasma level, after 10 hrs administrations were reached 60 ng/ml and maintained a constant level. The results proposed that ketotifen was successfully absorbed through the skin from the applied patch.

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2.2 LITERATURE REVIEW ON DRUGS

2.2.1 Diltiazem HCl

Ekapol Limpongsa and co-workers (2008)[1] prepared the suitable polymeric films for the development of diltiazem hydrochloride transdermal drug delivery systems. Hydroxypropyl methylcellulose (HPMC) and ethylcellulose (EC) were used as hydrophilic and hydrophobic film formers, respectively. Effects of HPMC/EC ratios and plasticizers on mechanical properties of free films were studied. Effects of HPMC/EC ratios on moisture uptake, in vitro release and permeation through pig ear skin of diltiazem HCl films were evaluated. The film composed of 8:2 HPMC/EC, 30% DBP and 10% IPM, IPP or Tween80 loaded with 25% diltiazem HCl should be selected for manufacturing transdermal patch by using a suitable adhesive layer and backing membrane. Further in vitro permeation and in vivo performance studies are required.

Prashant M. Satturwar and co-workers (2008)[2] conclude that Rosin in combination with PVP and with incorporation of Dibutyl phthalate (30% w/w) produces smooth flexible films with improved tensile strength and percentage elongation. The release rate of Diltiazem HCl from films and permeation across skin increases with increase in drug and PVP loading but is independent of film thickness. Patches containing Rosin: PVP (7:3) show promise for pharmacokinetic and pharmacodynamic performance evaluation in a suitable animal model.

Gopal Krishna Murthy T E and co-workers (2008)[3] prepared transdermal gels of Diltiazem hydrochloride using polymers like HPMC, NaCMC, MC, Carbopol, PEG6000 and PVP. The correlation coefficient values revealed that the diffusion profile follows zero order kinetic and the mechanism of drug release was governed by Peppas model. The diffusion exponent of release profile has a value indicate case II transport diffusion.

Lakshmana Prabhu and co-workers (2008)[4] developed matrix dispersion type transdermal drug delivery system of Diltiazem hydrochloride using different ratios of rosin with Eudragit RL and PVP. The patch prepared by the combination of rosin and

PVP was not transparent one and shows an uneven distribution of PVP, which may be due to the hydrophilic nature of PVP. The effects of polymers on the technological properties, i.e. drug release, water vapor transmission rate, % moisture loss, % moisture absorption and thickness were investigated. The patch containing Rosin: ERL PM (6:4) showed a release of 2651 µg in 24 hr in order to improve the release various proportion of camphor was included in the formulations. The patch containing rosin: ERL PM (6:4) with 5 %w/v of camphor showed a sustained release of the drug extending over a period of 24 hr. Further skin permeation and skin irritation was carried out on rat skin and rabbit respectively. Therefore it can be concluded that the patch containing Rosin : ERL PM (6:4) with 5%w/v of camphor achieved the desired objectives of transdermal drug delivery system, such as overcoming of first pass effect, extended release and reduced frequency of administration.

T E Gopal Krishna Murthy and co–workers (2007)[5] prepared and evaluated Eudragit RS 100 films as rate controlling membrane for transdermal drug delivery systems using Diltiazem HCl as a drug.. Acetone-methanol (8:2), chloroform-methanol (8:2), dichloromethane-methanol (8:2) and ethyl acetate-methanol (8:2) were used as solvents in the preparation of films. Dibutyl phthalate at a concentration of 15% w/w of the polymer was used as a plasticizer. The dry films were evaluated for Physical appearance, Thickness uniformity, Folding endurance, Water Vapor Transmission, Drug diffusion and Permeability Coefficient. Both Water vapor transmission and Drug diffusion rate followed zero order kinetics. The mechanism of drug release was governed by peppas model. The diffusion exponent of release profiles (slope) has a value of n>1, which indicates non-analmous transport diffusion. Eudragit RS 100 films employed with ethyl acetate: methanol in 8:2 ratios as casting solvent yielded low area of patch with desired release rate for both drugs.

Swamy P V and co-workers (2004)[6] designed mucoadhesive bilayered buccal devices comprising a drug containing mucoadhesive layer and a drug free backing membrane. Films composed of mixture of drug Diltiazem hydrochloride, chitosan, hydroxypropylmethylcellulose and ethyl cellulose. Films were fabricated by solvent casting technique and were evaluated for in vitro drug release, bioadhesion strength, and tensile strength, folding endurance, thickness and drug content uniformity. A combination of chitosan and hydroxypropyl methylcellulose (1:1) using propylene

glycol (50 % by weight of polymer) as a plasticizer gave promising result.

Ritu Gupta and co-workers (2003)[7] developed transdermal drug delivery system of Diltiazem HCl employing different ratios of polymers like ethyl cellulose and povidone and evaluated for the potential drug delivery using depilated freshly excised abdominal mouse skin. The cumulative amount of drug was found to be proportional to the square root of time, i.e. Higuchi kinetic. Film composed of povidone: EC (1:2) should be selected for the development of Diltiazem hydrochloride, using a suitable adhesive layer and backing membrane for potential therapeutic use.

S K Jain and co-workers (2003)[8] developed transdermal drug delivery system of Diltiazem to obtain a prolonged controlled drug delivery. Both the matrix diffusion controlled (MDC) and membrane permeation controlled (MPC) systems were developed. The matrix diffusion controlled systems used various combinations of hydrophilic and lipophillic polymers, whereas membrane permeation controlled systems were developed using the natural polymer chitosan. The MDC systems were prepared using the cast film method and the MPC systems by an adhesive sealing technique. Both the systems were characterized for in vitro and in vivo performance. The in vitro release studies showed that the release from the matrix diffusion controlled transdermal drug delivery systems follows a nonfickian pattern and that from the membrane permeation controlled transdermal drug delivery systems follow zero-order kinetics. The release from the matrix systems increased on increasing the hydrophilic polymer concentration, but the release from the membrane systems decrease on cross-linking of the rate controlling membrane and also on addition of citric acid to the chitosan drug reservoir gel. The in vivo studies of the selected systems showed that both systems are capable of achieving the effective plasma concentration for a prolonged period of time. The MPC system achieved effective plasma concentration a little more slowly than the MDC system, but it exhibited a more steady state plasma level for 24 hr.

Rao and co-workers (1998)[9] developed ethyl cellulose, polyvinyl pyrollidone (PVP) film containing Diltiazem HCl and Indomethacin. The influences of initial drug concentration, film composition and film thickness on the vitro drug release rate as well as drug permeation trough rat abdominal skin were studied. The in vitro skin

permeation profiles showed increased flux values with increased drug and PVP concentration in the film. They concluded that films composed of ethyl cellulose: PVP: Diltiazem (8:2:2) or indomethacin (8:2:3) with suitable adhesive layer and backing membrane could be developed for therapeutic purpose. The flux through rat skin was dependent on concentration of drug and PVP in matrix.

Tank H M and co-workers (1998)[10] prepared Verapamil free base and Diltiazem free base from corresponding salt forms and characterized prior to evaluate their in vitro diffusion kinetics from ethylene vinyl acetate- vinyl acetate 40% copolymer membrane. Base forms of original drug molecules exhibited enhanced transmembrane permeation as compared to corresponding salt form facilitating to deliver the drug in a controlled manner, across a convenient small surface area of delivery device.

P Rama Rao and co-workers (1997)[10] studied permeability of cellulose acetate(CA) free films casted from chloroform solution containing different plasticizer like Dibutyl phathalate(DBP), propylene glycol 600 (PEG 600) and propylene glycol (PG) with a view to developing a suitable rate controlling membrane for transdermal use. Permeability characteristics of free film were studied using the drug such as Diltiazem Hydrochloride and Indomethacin. The films plasticized with PEG600 showed higher permeability for both drug compared with other films.

Nitsch and co-workers (1991) [12] studied in vitro transcutaneous uptake of Diltiazem HCl from reservoir type formulation (ointment). Each formulation was evaluated for release of Diltiazem across skin employing Franz diffusion all over 12 hour period. The release of Diltiazem across skin increased with increase in concentration of Diltiazem.

2.2.2 Atenolol

S S Agrawal and co-workers (2007)[13] formulated matrix-type transdermal patches of Atenolol and metoprolol tartrate using polyvinylpyrrolidione, cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate and ethyl cellulose. The physiochemical evaluation of the polymer matrices was performed for suitability. In vitro permeation studied was performed using rat abdominal skin and result indicated

that maximum release was obtained at 48h and 85% of Atenolol and 44% metoprolol tartrate. The drug permeation studies across cadaver skin showed about 27% of reduction in the amount of drug release as that compared to rat abdominal skin.

Anroop B and co–workers (2005)[14] used prodrug approach to increase the permeability of Atenolol. They prepared Atenolol ester to increase its lipophillicity and permeation studied carried out in isolated porcine skin; promising result were obtained with caproate ester to increase lipophillicity of Atenolol with a view to enhance its permeation through lipophillic barrier of the skin. Atenolol caproate ester was found to be promising in improving its percutaneous absorption.

Cho C. W. and co-workers (2004)[15] have developed matrix transdermal film of Atenolol using ethyl vinyl acetate. The rate of drug release from EVA matrix increased with increasing temperature and drug loading dose. There was linear relationship between the flux of Atenolol and square root of loading dose. Among the plasticizer used, diethyl phthalate had the best enhancing effects on drug release.

S P Gupta and co–workers (2004)[16] developed polymer matrix system for transdermal delivery of Atenolol for its prolonged and controlled release systemic availability. To achieve the desired and controlled release rate, different combinations of Eudragit RL with polyvinyl pyrrolidone and polyethylene glycol 4000 were used in the preparations of polymeric matrix system. These preparations were evaluated for in vitro release and permeation of the drug across pig skin. The desired systems exhibited linear relationship between drug release (Q) versus time$^{0.8}$(hr$^{0.8}$). The product exhibiting required skin permeation 64 mcg/h/cm^2 to achieve an effective plasma concentration was selected for the in vivo performance evaluation. The study revealed that the designed polymeric matrix transdermal drug delivery system of Atenolol could be successful with improved performance.

Jin Kim and co-workers (2003)[17] prepared EVA matrix system for transdermal delivery of Atenolol. The effects of drug concentration, temperature, and plasticizers on drug release were studied from the Atenolol-EVA matrix. The release rate of drug from the EVA matrix increased with increased temperature and drug loading doses. The flux of Atenolol versus the reciprocal of the loading dose yielded a straight line. The release of Atenolol from the EVA matrix follows a diffusion-controlled model,

where the quantity released per unit area is proportional to the square root of time. Among the plasticizers used such as alkyl citrates and phthalates, tributyl citrate (TBC) showed the best enhancing effects.

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2.3 LITERATURE REVIEWS ON POLYMERS

Krishnaiah and co-workers (2009)[1] evaluated ethylene vinyl acetate (EVA) copolymer membranes with vinyl acetate content of 18% w/w (EVA1802) for transdermal delivery of ondansetron hydrochloride. The EVA1802 membranes containing selected concentrations (0, 5, 10 and 15 %w/w) of PEG6000 were prepared, and subjected to in vitro permeation studies from a nerodilol-based drug reservoir. Flux of ondansetron from EVA1802 membranes without PEG6000 was 64.1 ± 0.6 μg/cm^2.h, and with 10%w/w of PEG6000 (EVA1802-PEG6000-10) it increased to 194.9 ± 4.6 μg/cm^2.h. The EVA1802-PEG6000-10 membrane was coated with an adhesive emulsion, applied to rat epidermis and subjected to in vitro permeation studies against controls. Flux of ondansetron from transdermal patch across rat epidermis was 111.7 ± 1.3 μg/cm^2.h, which is about 1.3 times the required flux. It was concluded from the comparative pharmacokinetic study that TTS of ondansetron, prepared with EVA1802-PEG6000-10 membrane, provided average steady-state plasma concentration on par with multiple-dosed oral tablets, but with a low percent of peak-to-trough fluctuation.

R Sadashivivaih and co-workers (2008)[2] prepared matrix type transdermal drug delivery system of Haloperidol using different ratios of Ethyl cellulose(EC) and Polyvinyl Pyrrolidone(PVP) by solvent evaporation technique. In vitro studied was done using modified Franz diffusion cells through human cadaver skin utilizing 20% PEG-40 in normal saline. Higuchi and Peppas models wee used for optimizing the formulation.

Gannu R. and co-workers (2007)[3] developed and evaluated matrix type transdermal drug delivery systems (TDDS) of nitrendipine (NTDP). Ten formulations composed of Eudragit RL 100 and Hydroxypropyl methyl cellulose in the ratios of 5:0, 4:1, 3:2, 2:3, 1:4 in formulations A1, A2, A3, A4, A5 and Eudragit RS 100 and Hydroxypropyl methyl cellulose in the same ratios in formulation B1, B2, B3, B4, B5 respectively were prepared. All formulations carried 6 % w/w of carvone as penetration enhancer and 15% w/w of propylene glycol as plasticizer in dichloromethane and methanol as solvent system. Formulations A4 (flux 23.51 μg/hr/cm^2) and B5 (flux 22.98 μg/hr/cm^2) showed maximum skin permeation in the respective series. The flux

obtained with formulation A4 and B5 meets the required flux (19.10 μg/hr cm^2).

Shin S. C. and co-workers (2007)[4] studied the effect of different types of penetration enhancers on the permeability of quinupramine through the rat skin. The relative bioavailability of quinupramine in the matrix containing polyoxyethylene-2-oleyl ether as an enhancer was approximately 2.81 times higher than the group without an enhancer. These results showed that the quinupramine-EVA matrix containing a permeation enhancer could be a good transdermal delivery system for providing sustained plasma concentrations.

Ubaidulla U. and co-workers (2007)[5] developed a matrix-type transdermal therapeutic system containing carvedilol with different ratios of hydrophilic and hydrophobic polymeric combinations by the solvent evaporation technique. In vitro permeation results followed Higuchi kinetics (r = 0.9953- 0.9979), and the mechanism of release was diffusion mediated. Based on physicochemical and in vitro skin permeation studies, patch formulation containing ethyl cellulose: polyvinylpyrrolidone (7.5:2.5) and Eudragit RL: Eudragit RS (8:2) were chosen for further in vivo studies. The bioavailability studies in rats indicated that the carvedilol transdermal patches provided steady-state plasma concentrations with minimal fluctuations and improved bioavailability of 71% and 62% in comparison with oral administration.

Gattani S G and co-workers (2007)[6] prepared monolithic matrix type transdermal film of Lovastatin using two different polymer combinations Ethyl cellulose (EC) with Polyvinyl Pyrrolidone (PVP) and Eudragit RL100 (ERL) with Eudragit RS100 (ERS). All the formulation carried 10%w/w Lovastatin and 30%w/w Dibutyl Phthalate (DBP) as a plasticizer in chloroform as solvent system. On the basis of in vitro permeation studies formulation ERL: ERS, 4:1 having maxim rate of permeation and it was selected as optimized formulation. The correlation co-efficient obtained from Higuchi plot was found to be in the Range of 0.97-0.99 indicating the diffusion mechanism of drug release.

Singh Y. T. and co-workers (2007)[7] prepared transdermal patches of carvedilol using HPMC by the solvent evaporation technique. In this investigation, the

membranes of Eudragit RL100 and Eudragit RS100 were used to achieve controlled release of the drug. In vitro permeation studies were performed using hairless guinea pig skin and followed the super case II transport mechanism. The effects of non-ionic surfactants Tween 80 and Span 80 on drug permeation were studied. The non-ionic surfactants in the patches increased the permeation rate, Span 80 exhibiting better enhancement relative to Tween 80.

T E Gopal Krishna Murthy and co-workers (2006)[8] prepare rate controlling membrane of cellulose acetate and ethyl cellulose. The permeability characteristics were studied using propranolol hydrochloride as a drug. The dry films were evaluated for physical appearance, thickness, content uniformity, folding endurance, water vapor transmission, drug diffusion and permeability coefficient. The mechanism of drug release was governed by Peppas model. The diffusion exponent of release profile indicates non anomalous transport diffusion.

Das M. K. and co-workers (2006)[9] studied the effect of polymeric composition, drug content and plasticizer on the permeation of trazodone hydrochloride across the mouse epidermis for the development of transdermal therapeutic system. The polymers used were Eudragit RL100 and RS100. Triethylcitrate was used as plasticizer. The *in vitro* drug release increased with increasing amount of Eudragit RL100 in the film. It was observed that the maximum skin permeability was attained at a loading dose of 10% w/w in the film. The present study has demonstrated the potential of the fabricated pseudolatex transdermal films for sustained release of trazodone hydrochloride.

Tanwar Y. C. and co-workers (2005)[10] developed transdermal drug delivery systems of Salbutamol sulphate using Eudragit RL100 and PVP by solvent casting technique employing mercury as a substrate. Propylene glycol was used as a plasticizer. In vitro permeation profiles across the guinea-pig dorsal skin using K-C diffusion cell are reported. Incorporating PEG-400 and tween 60 into the films enhanced the permeation across guinea-pig skin. The permeation followed zero order kinetics and mechanism was found to be matrix diffusion

Mukherjee B. and co-workers (2005)[11] developed a suitable matrix type transdermal drug delivery system (TDDS) of Dexamethasone using blends of two different polymeric combinations, Povidone (PVP) and Ethyl cellulose (EC) and Eudragit RL100 with PVP. The formulations of PVP: EC provided slower and more sustained release of drug than the PVP : Eudragit formulations during skin permeation studies. They conclude that PVP–EC polymers are better suited than PVP– Eudragit polymers for the development of TDDS of Dexamethasone.

Khatun M. and co-workers (2004)[12] prepared polymeric films of Naproxane using Eudragit RS100 by solvent casting method. Naproxen was incorporated in two different percent loading (8.3 % w/w and 20.8 % w/w of films). Effects of two plasticizers (PEG 1500 and PEG 4000) and two release modifiers (PVA and HPMC) on in vitro drug release from naproxen loaded Eudragit RS films were assessed. Inclusion of PEG in Eudragit RS films caused the drug to be released by diffusion (Fickian) kinetics whereas PVA and HPMC containing formulations released drug by diffusion mechanism coupled with erosion.

N Udpa and co-workers (2004)[13] prepared matrix type transdermal patches containing glibenclamide using different ratios of ethyl cellulose (EC), polyvinyl pyrolidione(PVP) and Eudragit RL100(ERL) and Eudragit RS100 (ERS) by solvent evaporation technique. All the prepared formulations were subjected to physiochemical studies like thickness, weight variation, drug content, moisture content, moisture uptake, flatness in vitro release and in vitro permeation study through mouse skin. Based on in vitro skin permeation study ERL: ERS (1:4) and EC: PVP (3:2) were selected for in vitro experiment. The pharmacokinetic evaluation showed that the patches could maintain steady state concentration of drug within the pharmacologically effective range for prolonged period of time.

V Kusumdevi and co-workers (2003)[14] prepared transdermal patches of Verapamil HCl using four different polymers Eudragit RL100, Eudragit RS100, HPMC 15cps and Ehyl cellulose of varying degree of hydrophilicity and hydrophobicity. The effect of the polymers on the technological properties i.e. drug release, WVTR, % Moisture absorption , % moisture loss , folding endurance and thickness was investigated,.

Different formulation were prepared in accordance with the 2^3 factorial design with ERL100 being the patent polymer substitution with ERS 100, HPMC and EC decreased all the above values in accordance with their decreasing degree of hydrophilicity. In vitro release studies showed zero order release of the drug from all the patches. Percutaneous absorption studies were carried out in rabbits. The pharmacokinetic parameters calculated and it was concluded that the patch containing ERL 100 and HPMC in the ratio 8:2 has achieved the objectives of transdermal drug delivery system.

Paul W. S. and co-workers (2003)[15] investigated the influence of storage conditions and types of plasticizers on the properties and stability of Ethyl cellulose and polymethacrylate films. The effects of different plasticizers on the morphology, transparency, mechanical property and water vapor permeability of the prepared films were studied. The film samples were exposed to storage conditions of 30 ^oC and 50 or 75 %RH. The films prepared from aqueous Ethyl cellulose dispersions were relatively weaker and more brittle than acrylate films. Acrylate films did not show any significant change in mechanical property when stored at high humidity. They concluded that changes in mechanical property of Ethyl cellulose films on storage were mainly attributed to the loss of plasticizers during storage, causing further coalescence of Ethyl cellulose films and to a smaller extent, reduction in moisture content of the film.

V. Shankar and co-workers (2003)[16] prepared drug free polymeric films of ethyl cellulose using castor oil and glycerin at a concentration of 30%w/w and 40%w/w of dry polymer, as a plasticizer. The permeability characteristics of free film were studied using nifedipine as a drug in 4% HPMC gel. From in vitro in vivo study it was concluded that faster release was observed from Ethyl cellulose patch containing glycerol as plasticizer.

Dandagi P. M. and co-workers (2003)[17] prepared transdermal films of ketotifen fumarate using Eudragit L 100, Hydroxypropyl methyl cellulose and ethyl cellulose in combinations. Polyethylene glycol 400 was used as plasticizer. Permeation enhancers like Dimethyl sulfoxide and propylene glycol were used at different concentrations and *in vitro* drug release studies were carried out. They concluded that as the

concentration of permeation enhancers increased, the drug release increased.

Aqil Mohamed and co–workers (2003)[18] prepared and evaluated of monolithic matrix type transdermal drug delivery system of Metoprolol tartarate using different concentration ratios of Eudragit RL100 and Polyvinyl pyrrolidone K-30. They reported that, the release of drug from the matrix was controlled and the skin permeation rate followed zero order kinetics.

Kulkarni R. and co-workers (2002)[19] prepared and evaluated transdermal patch of Verapamil hydrochloride using Eudragit RS 100. They reported that films plasticized with polyethylene glycol showed higher permeability of verapamil hydrochloride as compared to Dibutyl phthalate. They also reported that permeability of drug decreased as the concentration of Dibutyl phthalate and polyethylene glycol increased.

Panigrahi L and co-workers (2002)[20] prepared pseudo-latex transdermal drug delivery system of Terbutaline sulphate using Eudragit RS 100. They reported that the drug release profiles from the patches followed apparent zero order patterns for a period of 12 hrs.

Kapoor and co-workers (2002)[21] formulated sodium alginate patches containing nicotine and used Ethyl cellulose or whatman filter paper as rate controlling membrane. Prepared patches were evaluated for thickness, drug content, content uniformity, weight variation, water uptake, stability and in vitro release characteristics using sigma membrane, excised rat skin and excised rabbit skin.

Arabi H and co-workers (2002)[22] studied the performance of a membrane controlled reservoir system (MCRS) for scopolamine hydrobromide (SH) using ethylene-vinyl acetate copolymer (EVA) and ethyl cellulose (EC) membranes. The results obtained showed that in the EVA membrane, the permeability of SH across membrane increased with the increase of vinyl acetate percentage in copolymer. Microscopic studies of EVA membrane surface showed that the size of surface porosity increases with the increase of vinyl acetate percentage in the co polymer. In the case of EC membrane, the results showed that the rate of release increases with the increase of porosity size of EC surface despite of having high molecular weight.

Rao P. R. and co-workers (2000)[23] formulated polymeric films containing propranolol hydrochloride using different ratios of ethyl cellulose (EC) and poly vinylpyrrolidone (PVP) using mercury substrate method. The release rate propranolol hydrochloride increased linearly with increasing drug concentration and PVP fraction in the film, but was found to be independent of film thickness. It was also observed that the release of drug from the films followed the diffusion-controlled model at low drug concentration. The in vitro skin permeation profiles displayed increased flux values with increase of initial drug concentration in the film, and also with the PVP content. From this study, it is concluded that the films composed of EC : PVP : Drug in 9:1:3, 8:2:2 and 8:2:3, should be selected for the development of transdermal drug delivery systems using a suitable adhesive layer and backing membrane for potential therapeutic applications.

Kulkarni Raghavendra and co–workers (2000)[24] prepared drug free polymeric films of polyvinyl pyrolidone (PVP), ethyl cellulose (EC), Eudragit RS 100 (ERS) and Ethylene vinyl acetate (EVA) using Verapamil HCl as model drug. The prepared films were evaluated for drug release study and they showed that, drug diffusion followed nearly zero order kinetics and it is in the order of PVP>EC>ERS>EVA.

S Sridevi and co-workers (2000)[25] developed acrylic based transdermal drug delivery system for Glibenclamide and evaluate its pharmaco-dynamic performance in male wistar rats. The drug embedded in a polymeric matrix of polymethyl methylacrylate and ethyl cellulose. Transdermal drug delivery system significantly sustained the hypoglycemic activity for 24 hr in normal rat when compared to oral administration where the effect declined after 8 hr.

Ocak and co–workers (1999)[26] developed a membrane controlled transdermal system of Isosorbide dinitrate using carbomer gel as the drug reservoir and ethylene vinyl acetate copolymers and polyethylene membranes were used as rate controlling membrane. The release rate achieved using the polyethylene rate controller mimicked the commercial product and was closed to the target flux and was thought to bee promising for commercial development.

Nagarajan M. and co–workers (1996)[27] prepared gelatin films of Salbutamol

sulphate transdermal drug delivery system using ethyl cellulose film as rate controlling membrane. They reported that controlled release of Salbutamol sulphate was observed for a period of 12-24 hrs and the drug release was found to depend on the proportion of gelatin used in drug reservoir layer. They showed that drug release from gelatin films can be controlled using ethyl cellulose film as rate controlling membrane.

Balasubramanian Iyer and co–workers (1979)[28] studied the release of suspended Trimcinolone acetonide from films of lanolin alcohol in combination with Ethyl cellulose and propylene glycol or cetyl alcohol followed the diffusion-controlled granular matrix model. The results suggested that the drug release follows a diffusion-controlled matrix model and a square root of time release profile. The release rate constants were proportional to drug concentration.

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2.4 LITERATURE REVIEWS ON SKIN IRRITATION AND SENSITIZATION STUDIES

Shin Irie and co-workers (2006)[1] assessed the skin reaction of PHK-301p, a newly developed nicotine patch. They conducted a phase I study that consisted of 2 parts: a skin irritation test (48-h closed patch test) and a photosensitivity test (24-h closed patch test + Ultraviolet A irradiation). Twenty healthy men were treated with PHK-301p and placebo. Both preparations were punched out to a circle of 6-mm diameter and were applied simultaneously to each participant. Skin irritation and photosensitivity were assessed by a physician who was kept unaware of the treatment. In the skin irritation test, moderate and mild erythemas were observed in each participant 72 h after application (24 h after removal) for PHK-301p. Mild erythema was observed in one participant 49 h after application (1 h after removal) for placebo. The skin irritation index, which was calculated based on the skin reactions of participants, was 7.5 for PHK-301p and 2.5 for placebo. In the photosensitivity test, one participant had mild erythema (±) approximately 25 and 72 h after application of PHK-301p. No solar urticaria was observed. From these results, it was concluded that PHK-301p is an acceptable product as a nicotine patch.

J. Bircher and co-workers (2006)[2] investigated adverse skin reaction for Nicotine transdermal therapeutic system (TTS). 14 volunteers (10 male, 4 female) with a History of former adverse skin reactions to this device were investigated. Skin tests for contact urticaria and patch tests for contact allergy were done with the individual components of the TTS. Irritant reactions due to occlusion were present in 9 subjects. The optimal test agent and concentration for elucidating the adverse skin reaction was on aqueous solution of 10% nicotine base. Nicotine should be added to the expanding list of transdermally delivered drugs which may elicit contact dermatitis.

Janet Tamada and co-workers (2004)[3] examined whether pre application of corticosteroid preparations could reduce skin irritation from iontophoresis used by the GlucoWatch G2® Biographer (Cygnus, Inc., Redwood City, CA) in monitoring interstitial glucose levels frequently and automatically. Numerous corticosteroid preparations were screened to identify formulations that did not interfere with adhesion of the Biographer to the skin or glucose sensing. Kenalog and Cortizone-10

sprays were selected and, in a double-masked, randomized, controlled trial, were applied to the forearms of 66 subjects with diabetes and allowed to dry. Biographers were applied and worn for 15 h, and home blood glucose measurements were taken every 30 min to assess accuracy. Irritation was assessed periodically by trained observers and study subjects. Skin irritation was reduced by both corticosteroid sprays, with the fraction of subjects who experienced moderate irritation reduced by 57% and 43% for the Kenalog and Cortizone-10. Pre application of these preparations did not affect the clinical utility of interstitial glucose readings. Pre application of Kenalog or Cortizone-10 Quick Shot sprays significantly reduced skin irritation due to iontophoresis, and did not interfere with glucose measurements.

Murthy N S and co-workers (1998)[4] developed transdermal films of terbutaline hydrochloride using hydroxypropyl methyl cellulose as monolithic matrix, for evaluation of pharmacokinetic and pharmacodynamic parameters. The skin irritation study revealed no signs of erythema or edema in rabbits. Transdermal drug formulation was more effective than oral dosage form as evident from the pharmacodynamic studies carried out on guinea pig, using histamine aerosol induced bronchospasm model.

Wilson and co-workers (1998)[5] determined whether topical pretreatment with triamcinolone acetonide 0.1% cream might be useful in reducing the incidence and/or severity of chronic skin irritation associated with the testosterone transdermal delivery (TTD) systems used to treat post-pubertal hypogonadism in males. Adult male volunteers wore three topical systems, which were applied to the upper back daily for 6 weeks: (1) TTD with no pretreatment of application site; (2) TTD with pretreatment of application site using triamcinolone acetonide 0.1% cream; and (3) an inactive occlusive dressing (control). Skin reactions were graded on a scale from 0 to 4 (0 = none, 4 = severe) and were assessed daily by research personnel. At assessment 1, pretreatment with triamcinolone acetonide 0.1% cream (compared with no pretreatment) was associated more often with scores of 0 (no erythema), with comparable occurrences of mild skin irritation, and with fewer occurrences of moderate erythema. Results of this study suggested that in patients using TTD systems, the incidence and severity of skin irritation at application sites may be reduced through pretreatment with triamcinolone acetonide 0.1% cream.

William P. Jordan and co-workers (1998)[6] compared the skin irritation of an investigational testosterone transdermal system (System I) with that of a marketed testosterone transdermal system (System II) in healthy men. In Part 1 of the study, System I was applied 10 times over 14 days to the same skin site on the backs of 26 healthy men. In Part 2, the skin irritation resulting from daily application of Systems I and II was assessed over 14 days in 17 men less than 65 years of age and 16 men 65 years of age or older. At the end of Part 1 of the study, 65.4% of the subjects experienced no erythema, 15.4% of subjects had faint erythema, and 19.2% had moderately intense erythema immediately after System I removal. At the completion of Part 2, none of the System I application sites were assessed as having moderately intense erythema, whereas one third (33.3%) of System II application sites demonstrated moderately intense erythema. There were no differences in erythema rates between younger and older subjects with either transdermal system. During this study, repeated application of System I to the same skin site resulted in acceptable non cumulative irritation, suggesting that application-site rotation may not be necessary. A comparison of the two systems demonstrates that System I results in significantly less application-site irritation than does System II and that older men do not have a higher rate of skin reactions.

William P. Jordan and co-workers (1997)[7] compared topical irritation rates for scrotal (Testoderm Testosterone Transdermal System; ALZA Corporation, Palo Alto, CA) and nonscrotal (Androderm Testosterone Transdermal System; SmithKline Beecham Pharmaceuticals, Philadelphia, PA) products. This open-label, crossover studied randomized 60 healthy, adult males to 14 days each of two treatments: one 40-cm^2 scrotal system delivering approximately 4 mg testosterone over 24 hours, or two 37-cm^2 nonscrotal systems worn on the back or upper outer arm, providing approximately 5 mg testosterone over 24 hours. Scrotal systems produced no confirmed contact allergy and less topical irritation than nonscrotal systems. The four subjects with contact allergy to nonscrotal systems used the scrotal system without a reaction, suggesting testosterone was not the allergen.

Krishna and co–workers (1996)[8] formulated a carboxy methylcellulose sodium based transdermal drug delivery system for propranolol and evaluated it for in vitro and in vivo performance. In vitro studies using excised hair free rat skin model

resulted in 66.54% permeation at the end of 24hr in a zero order permeation profile. Skin irritation studies in rats evaluated for flare and wheal with respect to a formalin control indicated that the drug containing patch invoked only a mild response over a 7 day period.

L. Speroff and co-workers (1996)[9] determined the efficacy and local tolerance of a new matrix transdermal drug-delivery system that delivers 0.02 mg of 17β-estradiol (E2) daily for 7 days for the relief of vasomotor symptoms. A total of 324 surgically or naturally menopausal women, all with prior hysterectomy and moderate to severe vasomotor symptoms participated in two independent, 12-week, randomized, double-blind, placebo-controlled studies. After a 4-week, treatment-free period, each woman received a continuous regimen of either one E2 transdermal system, two E2 transdermal systems, or placebo transdermal systems applied every week for 12 weeks. To measure local tolerance, skin irritation was objectively and systematically evaluated under blue light after removal of the transdermal systems. Few clinically significant skin reactions occurred, and only nine (3%) of the subjects withdrew because of a skin effect. After initial increase, serum E2 concentrations remained stable throughout the study, achieving values of approximately 20 and 40 pg/mL above baseline for one and two E2 transdermal systems, respectively.

Michael Robinson and co-workers (1991)[10] developed transdermal patch for the antihistamine triprolidine (TP) might provide benefits in terms of increased efficacy and reduced sedative side effects. They developed a binary vehicle $^{\text{delivery}}$ system comprised of TP in 0.5% oleic acid (OA) in propylene glycol (PG). Rabbit skin irritation and Buehler guinea pig skin sensitization testing indicated that this TP/OA/PG formula had both skin irritation and ACS potential. In clinical tests, skin irritation was due mainly to the OA–PG vehicle, but was enhanced in the presence of high TP concentrations. They suggested that the TP/OA/PG formula had a very high ACS potential. Subsequent predictive clinical patch testing was conducted with a buffered aqueous TP formula which provided in vitro skin penetration of the drug equivalent to the TP/OA/PG formula. These clinical studies demonstrated that TP itself had no significant irritation potential but still induced ACS reactions in a high proportion of test subjects.

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2.5 LITERATURE REVIEWS ON STUDY ON HUMAN SKIN

R Sadashivaiah and co-workers (2008)[1] prepared Matrix-type transdermal drug delivery systems of haloperidol lactate using different ratios of ethyl cellulose (EC):polyvinyl pyrrolidone (PVP) (3:2, 2:3, 4:1, 1:2, 2:1, and 1:4) by solvent-evaporation technique. Physicochemical parameters were characterized, and dissolution studies of the formulated films were performed. *In vitro* permeation studies were done using modified Franz diffusion cells through human cadaver skin utilizing 20% PEG 400 in normal saline. Permeation studies illustrated that 4% hyaluronidase enzyme was a good enhancer. The prepared films were subjected to scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FT-IR) spectral analysis. Higuchi and Peppas models were used for optimizing the formulation.

Petkar K. C. and co-workers (2007)[2] studied permeability of Losartan potassium (LP) across human cadaver skin. They also studied the influence of capsaicin, sex and site of application on permeation characteristics and determined an appropriate animal model for human skin permeability. Optimized controlled formulation (without capsaicin) released 42.17% (±1.85) of LP in 12 hr whereas treatment formulation (with capsaicin 0.028 % w/v) released 48.94% (±1.71) of LP with significant difference on null hypothesis. Influence of sex showed statistically significant difference for permeation of LP through male and female rats ($p<0.05$). In-*vitro* permeation of LP across human skin was compared with the permeation across rat and mice skins. It was suggested that the membranes are good models for human skin permeability. In conclusion simple transdermal adhesive patches formulations incorporating high molecular weight of LP can deliver a dose *in-vivo* and proposed model skin membranes can be utilized for future pharmacokinetics and toxic kinetic studies as well as metabolism studies of LP.

Ana Melero and co-workers (2007)[3] studied the influence of propylene glyol(PG), ethanol and oleic acid (OA) on nortryptyline hydrochloride (NTH) penetration through human epidermis at different Ph (5.5 and 7.4). A pH value of 5.5 in the donor solution decreases significantly the permeability co-efficient with respect to a pH value of 7.4. The vehicle showed an increasing enhancement effect in the order:

polysorbate 80 > ethanol/PG/OA > PG > ethanol > ethanol/lactic acid > lactic acid at pH 5.5 while they reduced the permeation of NTH at pH 7.4.

Panigrahi L. and co-workers (2005)[4] prepared a pseudolatex transdermal delivery of terbutalin sulphate and evaluate the effect of pH and organic ester penetration enhancers on permeation kinetics of terbutaline sulphate through mice abdominal and human cadaver skin. Increase in the permeation flux with increasing pH was observed. The permeation profile and related kinetic parameters of terbutaline sulphate was determined in presence of three ester type permeation enhancers incorporated in the films, viz. methyl laureate, isopropyl lanolate and isopropyl myristate. The more pronounced enhancing effect was obtained with isopropyl myristate amongst the three, regarding permeation flux, permeability coefficient and diffusion coefficient. This was attributed to its solubility parameter being nearer to the solubility parameter of human skin, and probably due to its passage across the skin barrier through the lipid pathway.

Dimitrios A and co-workers (2004)[5] performed in vitro permeation of ondansetron through human cadaver epidermis. In vitro release studies were carried out using modified Franz diffusion cells and human epidermis, taken from cadaver skin by heat separation technique. To estimate the effect of the type and concentration of the penetration enhancers and the skin from different donors, an 3^2 asymmetrical factorial design was used. Formulations containing lauric acid and oleic acid as penetration enhancers, showed the largest *Q* values [amounts of ondansetron permeated per unit area of epidermal membrane ($\mu g/cm^2$)] at 24, 48, and 72 hr, as well as steady state flux values, among all formulations tested. The other enhancers increased the flux in the following order: lauryl alcohol > glycerol monooleate > Azone® > cineole > oleyl alcohol > 1-methyl-2-pyrrolidinone. Moreover, the concentration of the penetration enhancer and the type of the skin were proved to significantly affect the permeation rate of ondansetron through human epidermis. From the results obtained, it was shown that the formulations containing lauric acid or oleic acid at 5% or 10% could increase sufficiently the permeation of ondansetron. Therefore, the transdermal administration of ondansetron seems feasible.

G.D. Gupta and co-workers (2004)[6] prepared matrices of Repaglinide and evaluated for their physico-chemical characteristics: weight variation, thickness uniformity, tensile strength and percent elongation at break, drug content uniformity, in-vitro permeation study, interaction studies and stability studies. In-vitro permeation studied was carried out across human cadaver skins using a modified Keshery- Chien diffusion cell. Different models were applied to evaluate release mechanism and kinetics. Based on the best fit of permeation data to different mathematical models it was concluded that all the formulations followed zero order kinetics with matrix diffusion pattern. The permeation rate was enhanced which was confirmed by skin flux, enhancement factor and permeability coefficient.

Deepak Gondaliya and co-workers (2003)[7] designed and evaluated unilaminate transdermal adhesive matrix system of bupropion base using Eudragit E. The in vitro release and epidermal flux through human cadaver skin were studied. The delivery rate of bupropion ranged from 10.5mg to 31.4mg/day/3.14cm^2 and release of drug from the matrices obeyed zero order kinetics. Triethylcitrate and dibutylphtalate have no influence on the diffusion of bupropion through human cadaver skin. Succinic acid in the adhesive matrix retarded the diffusion while propylene glycol and myristic acid alone and combination enhanced the flux of bupropion.

Pongjanyakul and co–workers (2003)[8] fabricated nicotine transdermal patches using an acrylic adhesive emulsion to form a transparent matrix film. The in vitro release behavior and permeation of nicotine across abdominal human epidermis was studied using United States Pharmacopeias dissolution apparatus 5 (paddle over disk) and modified Franz-diffusion cell, respectively. The release of nicotine from transdermal patches showed a good linear co relation with the square root of time. This indicated a matrix diffusion controlled release mechanism. This study also showed that the transdermal system provides a good delivery system with more than 65% of the nicotine delivery being controlled by the device.

Sanjay K. Iain and co-workers (2003)[9] prepared transdermal drug delivery systems based on polymeric pseudolatex and matrix diffusion controlled systems for salbutamol and compared for in vitro skin permeation profile and in vivo performances. Poly (isobutylene) was used as release controlling polymer in both the

systems. In vitro skin permeation was studied using the human cadaver skin in franz diffusion cell. Permeation rate constants for matrix diffusion controlled system and pseudolatices were 10.625 and 13.750 mcg/hr/cm^2 respectively. The prepared transdermal systems were tested on human volunteers having chronic reversible airways obstruction and compared with oral treatments (Asthaline).

H M Tank and co-workers (1998)[10] prepared verapmil free base from corresponding salt form and characterized prior to evaluate its in vitro diffusion kinetic from isolated epidermis of human cadaver skin. The flux of verpamil using donor phase from pure verapmil base and saturated alcoholic solution of base across isolated epidermis were 88.2± 3.95 and 304.2 ± 14.15 mg/cm^2 h 10^3 respectively.

Narasimha Murthy S and co-workers (1997)[11] prepared Terbutaline sulphate transdermal films using various cellulose like sodium carboxy methyl cellulose, cellulose acetate and ethyl cellulose. They studied the drug release from human cadaver skin and reported that, the films made from hydrophilic polymers showed a greater rate of release than that of hydrophobic polymers.

Murthy N S and co–workers (1997)[12] designed transdermal films of terbutalin sulphate as monolithic matrices using cellulose polymers like hydroxypropyl methyl cellulose (HPMC), sodium carboxymethyl cellulose (CMC), PEG 400 and propyleneglycol were used as enhancer in various ratios. In vitro diffusion studies were carried out across isolated stratum corneum of fresh human cadaver skin using a polycarbonate feeding bottle modified as a diffusion cell. The release of drug from formulation followed zero order kinetics. The transdermal permeability across human skin was enhanced with the increasing plasticizer concentration.

Bret Berner and co–workers (1989)[13] studied the skin permeation of nitroglycerine and ethanol as a function of the volume fraction of ethanol. An optimal concentration range of aqueous ethanol produces 5-10 fold increases in nitroglycerine flux across skin. For aqueous ethanol solution saturated with nitroglycerine with an ethanol volume fraction less than or equal to 0.7, the flux of nitroglycerine across is liner with the ethanol flux and is traced to a liner solubility relationship and a constant diffusion coefficient.

Pramod Sarpotdar and co-workers (1986)[14] studied the effect of polyetylene glycol 400 on the penetration of drugs through human cadaver skin. Polyethylene glycol 400 was used in various concentrations in the donor and the receptor compartment. It was observed that polyethylene glycol 400 had significant effects on the penetration rates of compounds, both when used in the donor as well as in the receptor compartment. These effects were barrier specific and are related to the alteration of the skin structure and the mass flow of water.

Chandrasekaran and co-workers (1977)[15] developed a mathematical model for estimating the temporal pattern of scopolamine delivery from a transdermal therapeutic system through human skin in vivo. The model was useful in optimizing the design of the therapeutic system. The transdermal therapeutic system in 2.5 cm^2 in area and the strength is specified by its temporal pattern of drug release, 200 µg promising dose, and 10µg/hr for 72 hr.

James Ostrenga and co–workers (1971)[16] investigated the penetration of two topical steroids, fluocinolone acetonide and fluocinonide, through human abdominal skin for various propylene glycol-water gels. The similarity between the in vivo and in vitro composition profiles for both steroids suggested that clinical efficacy can be predicted from in vitro data and from the physical properties of the steroids. The correlations indicated that the in vivo results were directly dependent upon penetrability.

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Chapter 3

Experimental Setup

3.1 PLAN OF WORK

3.1.1 Objective of the study

1. To screen the cardiovascular drugs which is able to penetrate the skin.
2. To find out skin permeation flux for the cardiovascular drugs which is successful to permeate skin.
3. To perform drug additive interaction studies.
4. To prepare matrix diffusional and membrane moderated transdermal drug delivery system.
5. To carry out permeability study of device through human live skin.
6. To develop protocol for cutaneous toxicity studies and perform cutaneous toxicity study for transdermal drug delivery system.
7. To perform stability study of final selected formulation.

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3.1.2 Selection of cardiovascular drugs suitable for transdermal drug delivery system

After extensive literature survey and contemplating, we selected following drugs on the basis of prerequisite physio-chemical and pharmacokinetic properties for transdermal drug delivery system. These drugs are

1. Diltiazem hydtrochloride
2. Atenolol

The pharmacokinetic data of these drugs is collected from best available sources and is presented in Table 3.1.1. These drugs have been selected on the basis of

· Shorter half-life
· Small dose
· Small molecular weight
· Moderate lipid solubility
· Extensive first pass metabolism
· Peak plasma level at nano gram level

Table 3.1.1: Pharmacokinetic data of drugs

Pharmacokinetic data	Diltiazem	Atenolol
Extent of absorption	80-90 %	50%
Bioavailability	40-67 %	45%
Time for Peak plasma level	2-3 hr	2-4 hr
Protein binding	70-80 %	6-16 %
Therapeutic serum level	40-200 ng/ml	280 ng/ml
Volume of distribution	1.9-4.3 liter/ kg	0.7 liter/ kg
Half life of elimination	3.5-6 hrs	6-7 hr

All the above properties make these drugs as potential candidate to be formulated in to transdermal drug delivery system

3.1.3 Study of design aspects and other biological consideration of transdermal patches.

Apart from studying permeability studies of selected drugs other aspects like design, manufacturing and biological consideration are also important. We have listed these as follows:

1. Drug additive interaction study
2. Preparation of matrix and membrane moderated transdermal drug delivery system.
3. Design proper diffusion cell for in vitro permeability study
4. Permeability study through human live skin.
5. Skin irritation tests for selected drugs and other additives used in transdermal patch

3.2 MATERIALS USED IN PRESENT INVESTIGATION

Ingredients	Suppliers
Diltiazem Hydrochloride BP Atenolol	Sun Pharmaceutical Ind. Ltd., India
Eudragit RL 100 Eudragit RS 100 Ethyl cellulose 22 CPS USP/EP	Signet Chemical Corporation, India
Ethylene Vinyl Acetate Copolymer (Vinyl Acetate 40%)	Aldrich Chemicals, U.K.
Polyvinyl Pyrrolidone Triethanol amine Propylene glycol	Loba Chemical Pvt. Ltd., Mumbai, India
Hydroxy propyl methyl cellulose 400 CPS, USP 2208	Signet Chemical Corporation, India
Carbopol 934	Corel Pharma Chem., Ahmedabad, India
Ethanol	Baroda Chemical Industries Ltd., Vadodara, India
Silica	Department of Pharmaceutical Science, Rajkot, India
Sodium Chloride Acetone	Finar Chemicals Ltd., Ahmedabad, India
Dibutyl Phthalate Hydrochloric acid	E-Merck Specialities Pvt Ltd., Mumbai, India
Ammonia solution	S D Fine Chem. Ltd., Mumbai, India
Solvent ether I.P. Mercury Toluene	Ranbaxy Fine Chemical Ltd., New Delhi, India
Natural Rubber solution	Beta Surgical Ltd., Rajkot, India

3.3 INSTRUMENTS USED IN PRESENT INVESTIGATION

Instruments	Suppliers
Sartorius electronic balance (Model CP-224 S)	Shimadzu, Kyoto, Japan
Digital pH meter	Systronic, Ahmedabad, India
UV/VIS Double beam spectrophotometer 2204	Shimadzu, UV-1700, Kyoto, Japan
Differential scanning calorimeter	DSC – Shimadzu 60 with TDA trend line software, Shimadzu, Tokyo, Japan
FTIR Spectrophotometer 8400	Shimadzu Corporation, Japan
Magnetic Stirrer Mechanical water bath shaker NSW-133 Centrifuger REMI R-23	Remi equipment, Mumbai, India
Micrometer (0.001mm)	Mitutoyo, Japan
Hot air oven	Tempo Instrument Pvt Ltd., Mumbai, India
Thermostatic water bath	Istrument manufacturing corp Pvt. Ltd., Ambala, India
Speed regulator	Remi equipment, Mumbai, India
Stability chamber	Thermo Lab., Mumbai, India
Rabbit holder	Self prepared

3.4 SOFTWARE USED IN PRESENT INVESTIGATION

Instrument	Supplier
Diffusion profile calculator Microsoft office	Self developed Microsoft Pvt. Ltd.

Chapter 4

Preparation of Diltiazem base from official salt form and its characterization

CHAPTER 4

4. PREPARATION OF DILTIAZEM BASE FROM OFFICIAL SALT FORM AND ITS CHARACTERIZATION

4.1 AIM OF PRESENT INVESTIGATION

The goal of present investigation was to design and evaluate transdermal drug delivery system of Diltiazem for the treatment of cardiovascular diseases. Extensive efforts have recently been focused on placing a drug delivery system in a particular region of body for maximizing drug availability and minimizing dose dependent side effects. Diltiazem hydrochloride (DTZ), a drug used in the treatment of angina pectoris has recently become very popular for treatment of old age hypertension.[1, 2] The drug is well absorbed form gastrointestinal tract, but its bioavailability is low due to extensive first pass metabolism.[3]

Diffusion of drug molecule across a membrane is influenced by a physicochemical nature of drug and membrane polymer. In general human skin is considered as hydrophobic and it is reported [4, 5] that the salt form of original drug molecule has poor permeability across skin as compared to its base form. Commonly used membranes for controlled release systems are hydrophobic in nature. Here an attempt is being made to test the scope of the above information to get enhanced release of base form of Diltiazem. The aim of present work is to prepare and characterize base forms of Diltiazem molecule from corresponding salt forms and evaluate their in vitro permeation kinetics.

4.2 Experimental

4.2.1 Preparation of Diltiazem free base from official salt form

Diltiazem base was prepared from hydrochloric salt. 1 gm of Diltiazem hydrochloride was dissolved in 20 ml of distilled water. Strong ammonia solution was added and pH was adjusted upto 9.5. Diltiazem free base was precipitated out. The base was extracted and purified using solvent ether. Extraction process was carried out four times using 20 ml ether. Ethereal phase was collected and evaporated at 40^{o}C.White amorphous powder of Diltiazem base was obtained.

4.2.2 Characterization of Diltiazem free base

The physiochemical properties of Diltiazem free base was determined using following

parameters.

Determination of melting point

Melting point of drug was determined by taking small amount of drug in a capillary tube closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed in triplicates and average value was noted.

Determination of partition co-efficient [6,7,8,11]

The partition co-efficient study was performed using n-octanol as oily phase and phosphate buffer, pH 7.4, as aqueous phase. The two phases were mixed in an equal quantity and were saturated with each other on a mechanical water bath shaker NSW-133 at 32^{o}C for 24 hr. The saturated phases were separated by centrifugation at 2000 rpm on a REMI R-23 centrifuge. Standard plots of drug were prepared for both, the phosphate buffer and octanol. Equal volumes (10ml each) of the two phases were taken in conical flasks and, to each; 100mg of weighed amount of drug was added. The flasks were shaken at 32^{o}C for 6h to achieve a complete partitioning at 100rpm. The two phases were separated by centrifugation at 1000 rpm for 5min and they were then analyzed for respective drug contents by UV/VIS spectroscopy method. The partition co- efficient of drug $K_{o/w}$ was calculated using the following formula:

$K_{o/w}$ = (Concentration in octanol/ Concentration in phosphate buffer pH 7.4)

Solubility studies [8]

The solubility study of Diltiazem base was performed in phosphate buffer solution, pH 7.4, in distilled water, methanol, chloroform, ether, alcohol (95%), acetone, toluene, glycerol, liquid paraffin, triethanol amine and silicone oil separately by adding excess amounts of drug in each case and keeping the excess drug containing flasks on a water bath shaker NSW-133 for 24hr at 32^{o}C.

UV /VIS Spectroscopic Analysis

UV spectrum of Diltiazem base was recorded on UV/VIS Spectrophotometer by scanning 5 μg/ml solution of Diltiazem base in 0.01N hydrochloric acid and scanned between 200-400nm using UV/VIS Spectophotometer.

Infrared (IR) Spectroscopic Analysis

The Fourier Infrared (FTIR) spectrums of moisture free samples of Diltiazem base was recorded on IR spectrophotometer by potassium bromide (KBr) pellet method. The scanning range was 4000 – 400 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC) Analysis

DSC scans of the powered samples were recorded using DSC- Shimadzu 60 with TDA trend line software. Drug was weighed (7-10 mg) and heated at a scanning rate of 10°c/min under dry nitrogen flow (100 ml/min) between 50-350°c. Aluminium pans and lids were used for drug sample. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

4.3 Result and discussion

Diltiazem base prepared form official hydrochloric salt was white amorphous powder, which showed following characteristics.

4.3.1 Melting point

Melting point of Diltiazem base was determined by capillary tube method and it was found to be 102° C±1.4337 (average of three readings). This value is same as that of the literature citation. [9]

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4.3.2 Partition co-efficient[10]

Octanol and in vitro study fluid (here phosphate buffer, pH 7.4) are considered to be the standard system to determine drug partition coefficient between skin and in vitro study fluid.[10] The logarithamic value of partition coefficient (log P) value was experimentally found to be 2.198. The result's obtained also indicate that the drug possess sufficient lipophillicity, which fulfills the requirements of formulating it into a transdermal patch. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin. As per literature survey for successful transdermal drug delivery system partition coefficient should be in the range of 1 to 4.

4.3.3 Solubility study

Solubility of Diltiazem base was evaluated in different solvent. The results are mentioned in Table 4.3.1.

Table 4.3.1 Solubility of Diltiazem base in different solvents

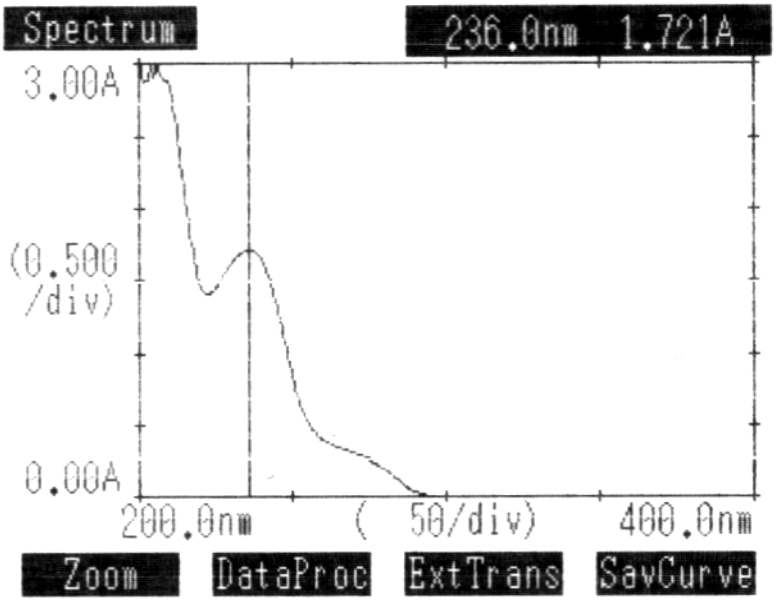
Solvent	Solubility
Phosphate buffer (pH 7.4)	Insoluble
Distilled water	Insoluble
Methanol	Freely soluble
Chloroform	Freely soluble
Ether	Freely soluble
Alcohol (95%),	Freely soluble
Acetone	Freely soluble
Toluene	Freely soluble
Glycerol	Insoluble
Liquid paraffin	Soluble
Triethanolamine	Soluble
Silicone oil	Insoluble
0.01N Hydrochloric acid	Soluble

An attempt was made at this point to learn whether the media phosphate buffer, pH 7.4, was able to maintain sink condition in diffusion as well as in permeation studies. Here form solubility studied data it was found that solubility of drug was poor in phosphate buffer, pH 7.4. Therefore it becomes difficult to maintain sink condition during diffusion study. Diltiazem base was soluble in 0.01 N HCl and it was selected as a diffusion medium.[9]

4.3.4 UV/VIS Spectroscopic analysis

The UV maxima of resultant solution were measured with Shimadzu, Japan UV/VIS Spectophotometer. The UV maxima of Diltiazem base in the solution was found to be 236.0 nm, which was suitable for the preparation of standard curve and estimation of Diltiazem base from various formulations. Figure 4.3.1 shows the UV spectrograph of Diltiazem base in 0.01N HCl.

Figure 4.3.1: UV spectrograph of Diltiazem in 0.01N HCl



Point pick

Abscis.	Ordinat.		Abscis.	Ordinat.	
1	238.0	1.707	11	236.0	1.721
2	237.8	1.709	12	235.8	1.720
3	237.6	1.711	13	235.6	1.719
4	237.4	1.713	14	235.4	1.717
5	237.2	1.715	15	235.2	1.715
6	237.0	1.717	16	235.0	1.714
7	236.8	1.718	17	234.8	1.712
8	236.6	1.719	18	234.6	1.710
9	236.4	1.719	19	234.4	1.708
10	236.2	1.720	20	234.2	1.706

4.3.5 Infrared (IR) Spectroscopic Analysis

Diltiazem was subjected for FTIR spectroscopic analysis, to characterize drug. The FT-IR spectra obtained for pure drug is given in Figure 4.3.2. The characteristic peak of the pure drug and group was mentioned in Table 4.3.2. FT-IR Spectra for base was compared with that given for FT-IR spectra of official salt form.[13] Diagnostic peaks and finger print regions were identical. These characteristics peaks are useful in drug – excipients compatibility study.

Figure 4.3.2 FT-IR Spectra of pure drug (Diltiazem)

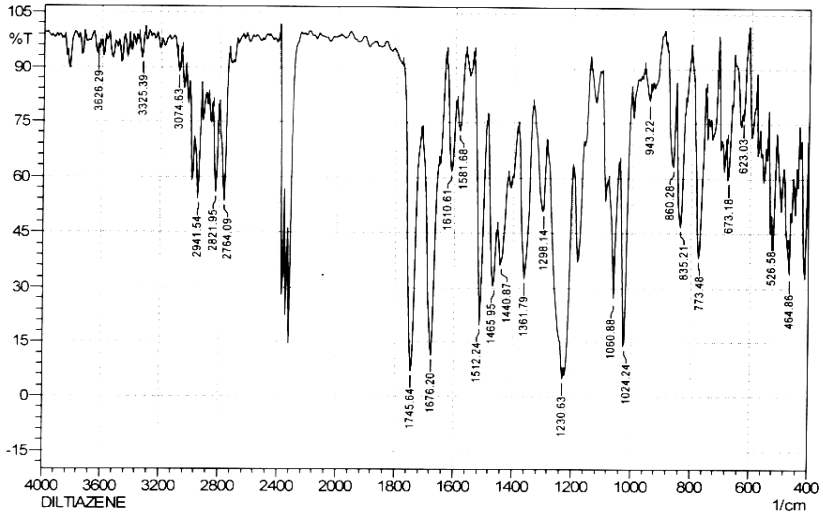


Table 4.3.2: FT-IR Spectral data of Diltiazem[12]

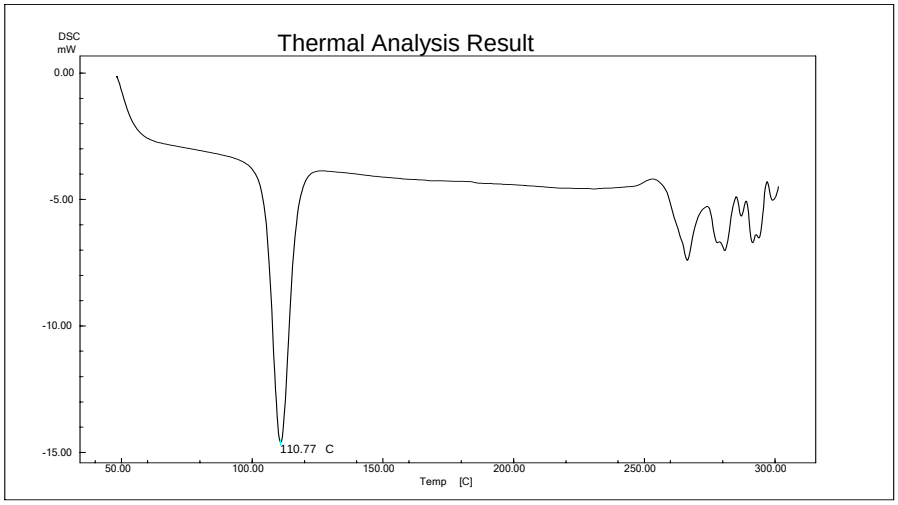
Frequency (cm^{-1})	Assignment
3074.63	Aromatic C-H stretch
2941.54	Aliphatic C-H stretch
2821.95	O-CH_3 C-H stretch
1745.64	Acetate C = O stretch
1676.20	Lactam C = O stretch
835.21	o-substituted aromatic C-H out-of-plane deformation
773.48	p-substituted aromatic C-H out-of-plane deformation

4.3.6 Differential Scanning calorimetry (DSC) analysis

Differential Scanning Calorimetry enables the quantitative detection of all processes in which energy is required or produced (endothermic or exothermic phase transformation). DSC curves obtained for pure drug is shown in figure 4.3.3. Pure powered Diltiazem showed a melting endotherm at 110.77 ^{0}C.

DSC study is useful for further drug excipients interaction study to check suitability of polymers.

Figure 4.3.3: DSC thermogram of Diltiazem



4.4 CONCLUSION

In the present study, Diltiazem base was prepared from its official hydrochloride salt and characterized using different parameters. Melting point, Refractive index were determined to check purity of drug. From solubility study it was found that 0.01N HCl was able to maintain sink condition, so it was suitable as a diffusion medium. The results obtained from Partition co-efficient study revealed that the drug possessed sufficient lipophillicity, which fulfills the requirements of formulating it into a transdermal patch. Differential scanning calorimetry and Fourier transform infrared spectroscopy gave idea regarding chemical structure of pure drug. UV/VIS Spectroscopic data are useful for the preparation of standard curve and estimation of Diltiazem base released from various formulations.

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Chapter 5

Preparation and characterization of polymeric matrix diffusional transdermal drug delivery device of Diltiazem

CHAPTER 5

5. PREPARATION AND CHARACTERIZATION OF POLYMERIC MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF DILTIAZEM

AIM OF PRESENT INVESTIGATION

Controlled release dosage forms have been extensively used to improve therapy with several important drugs. However, the development processes are faced with several difficulties. In an attempt to reduce the cost of drug development process and advantageously reap the benefits of patient regime, health care firms are now investing strategically in the development of new drug delivery system.[1]

Globally, cardiovascular diseases are the number one cause of death and are projected to remain so. An estimated 17.5 million people died from cardiovascular disease in 2005, representing 30 % of all global deaths. Of these deaths, 7.6 million were due to heart attacks and 5.7 million due to stroke. About 80% of these deaths occurred in low- and middle-income countries. If current trends are allowed to continue, by 2015 an estimated 20 million people will die from cardiovascular disease (mainly from heart attacks and strokes). [2]

Diltiazem, a calcium channel blocker, has been shown to be safe and effective in the treatment of patients in atrial fibrillation and/or atrial flutter. It has a mean plasma half-life of 3.5 hrs and only 40-67% of the orally administered drug reaches the circulation due to hepatic metabolism. The model predicts that mean plasma Diltiazem concentration of 79, 172, and 294 ng/ml are required to produce a 20%, 30%, and 40% reduction in heart rate, respectively.[3] This concentration can be achieved by preparing matrix diffusion controlled transdermal drug delivery system.

The transdermal controlled drug delivery is a newer approach to deliver drug in to systemic circulation at a predetermined rate. This system should duplicate continuous intravenous infusion, which not only bypasses hepatic ‘first pass’ elimination but also maintains a constant, prolonged and therapeutically effective drug level in the body. This is made possible by using intact skin as a port of drug administration to provide continuous delivery of drug in to systemic circulation. Following skin permeation, the drugs first reach the systemic circulation. The drug molecules are then transported to the target site, which could be relatively remote from the site of administration, to produce therapeutic action.

Matrix diffusion controlled release transdermal drug delivery systems are monolithic systems which are the simplest and least expensive means of controlling the release of an active agent. Here the active agent is physically blended with the polymer agent. The release rate is governed by Higuchi equation. Parameter influencing the release characteristics of monolithic devices can be classified as solute dependent factors like solubility, partition co-efficient and diffusion coefficient of drug in the polymer matrix. The solute independent parameters are system variables like geometry, tortuosity, pores, concentration, volume fraction and diffusion layer etc. in the present investigation solute related factors were considered to fabricate the devices using different polymer matrix. Polymer matrices employed were of non polar and hydrophobic nature.

With perception to above objective, it is necessary to modify current solid dosage forms in to controlled transdermal drug delivery system. A first step in this process is to illustrate how formulation and process variables could give drug release through skin. The aim of present investigation is to formulate and optimize the Diltiazem matrix diffusion controlled transdermal drug delivery system. In the present investigation, the influence of various grades and concentration of polymers were studied. Study was carried out to formulate an elegant product exhibiting desired therapeutic performance, from a small and cute dosage form.

In order to achieve this goal, following criteria were set

- The dosage form should remain intact for a period of 24 hr.
- Drug should be delivered in a controlled manner.
- The size of dosage form should be small with a view to enhance convenience of patient as well as compliance to therapy.
- Plasma concentration should be achieved within short period of time.

5.1 ANALYTICAL METHOD FOR ESTIMATION OF DILTIAZEM BASE

Diltiazem base can be estimated by various methods such as UV Spectroscopy, HPLC, HPTLC etc. In the present investigation Diltiazem was estimated by UV Spectrophotometry.

5.1.1 Determination of λmax

A solution of Diltiazem was prepared in 0.01N HCl and UV spectrum was taken using Systronic 2201 UV/VIS double beam spectrophotometer. UV spectrum of

Diltiazem base was recorded by scanning 5 μg/ml solution of Diltiazem base in 0.01N Hydrochloric acid and scanned between 200-400 nm.

5.1.2 Preparation of calibration curve

The calibration curve of Diltazem base in 0.01N HCl was prepared by measuring the absorbance of the solution in the range of 4-20 μg/ml. the absorbance of the solution was measured at the wavelength 236.0nm.

Dilitazem (50mg) was dissolved in 40ml 0.01N HCl and volume was made up to 50ml using 0.01N HCl in 50 ml volumetric flask. This stock solution (1mg/ml) was further diluted with 0.01N HCl to obtained solution of 4 to 20μg/ml. Absorbance of each solution was measured at 236.0 nm using Shimadzu, Japan UV/VIS Spectrophotometer with 0.01N HCl as a reference standard. The standard curve was generated for entire range of 4 to 20 μg/ml. The experiment was performed in triplicate and based on average absorbance; the equation for the best line fit was generated.

5.1.3 Result and discussion

Determination of λmax

The UV maxima of resultant solution were measured with Systronic UV- 2201 UV/VIS Spectophotometer. Figure 4.3.1 shows the UV spectrograph of Diltiazem base in 0.01N HCl

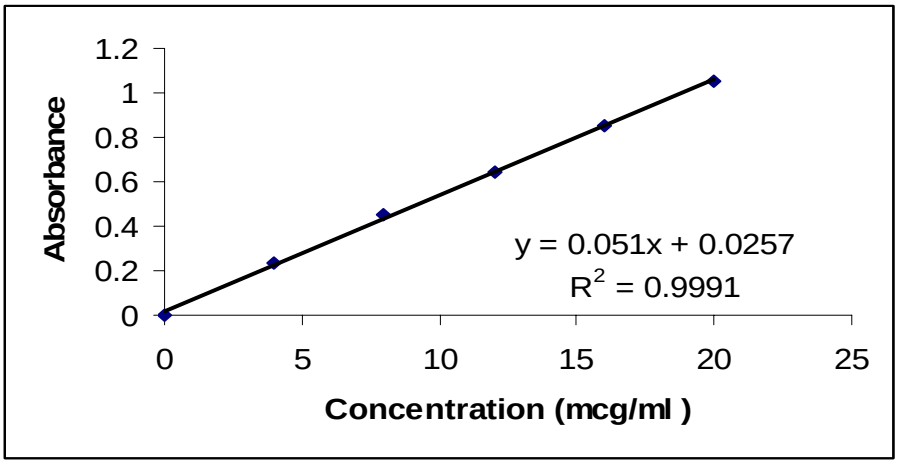
Calibration curve

The calibration curve of Diltuiazem base in 0.01N HCl was prepared by measuring the absorbance of the solution in the range of 4-20 μg/ml. the absorbance of the solution measured at the wavelength 236.0 nm. The results of standard curve are shown in Table 5.1.1 and Figure 5.1.2

Table 5.1.1: Calibration curve of Diltiazem in 0.01N HCl at 236.0 nm

Concentration (µg/ml)	Absorbance
0	0.000 (0.000)
4	0.237 (0.004)
8	0.450 (0.002)
12	0.647 (0.001)
16	0.849 (0.006)
20	1.055 (0.005)
Correlation coefficient = 0.9991	
Absorbance = 0.051 x concentration + 0.0257	
Values in parenthesis indicates standard deviation (n=3)	

5.1.1: Calibration curve of Diltiazem in 0.01N HCl at 236.0nm



5.2 PREFORMULATION STUDIES

The following pre formulation studies were performed for Diltiazem

5.2.1 Infrared (IR) Spectroscopic analysis [4]

In the preparation of film formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. Pre formulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between Diltiazem and the selected polymers. The individual drug and drug with excipients were scanned separately.

The Fourier Infrared (FTIR) spectrums of moisture free samples were recorded on IR spectrophotometer by potassium bromide (KBr) pellet method. The scanning range was 4000 400 cm^{-1} and the resolution was 1 cm^{-1}. Pottasium bromide was mixed with drug and polymer and the spectra were taken. FT-IR spectrum of Diltiazem was compared with FR-IR spectra of Diltiazem with polymers. Disappearance of Diltiazem peaks or shifting of peak in any of the spectra was studied.

5.2.2 Differential scanning calorimetry analysis

Differntial scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e., endothermic or exothermic phase transformation). DSC curves for pure drug. Diltiazem, Ethylene vinyl acetate copolymer (vinyl acetate 40%) and their composites were recorded using DSC-Shimadzu 60 with TDA trend line software. Drug and polymer was weighed (7-10 mg) and heated at a scanning rate of 10^{o}c/min under dry nitrogen flow (100 ml/min) between 50-350^{o}C. Aluminum pans and lids were used for drug sample. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

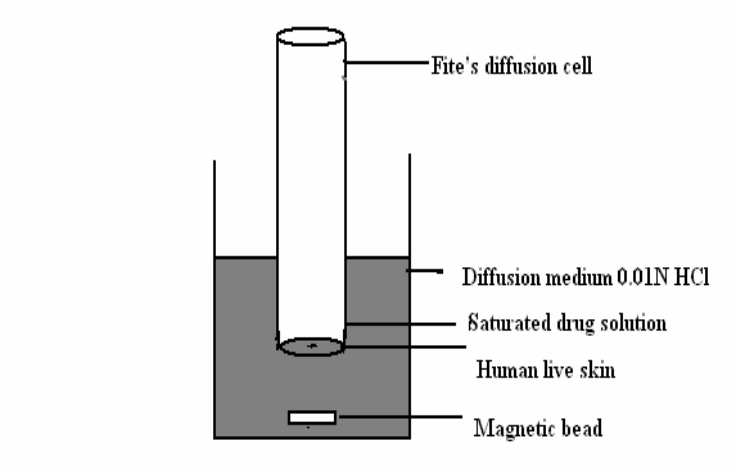
5.2.3 Drug permeability study using human live skin [5-10]

The permeation studies were performed in a Fite's diffusion cell (cell capacity of 10 ml, cross sectional area was 1.32 cm^2). The drug permeation study was performed using human live skin. The skin was used after fulfilling all the ethical requirements. The skin was store at 4 to 5^{o} C in saline solution until usage.

The dermatomed skin (thickness 140 μm) was washed with soap solution, followed by washing with distilled water. Skin was further washed with distilled water until it showed zero absorbance at 236.0 nm. Skin (thickness 140 μm) was cut, measured and mounted between the donor and receptor compartment of the diffusion cell. The dermal side of skin was facing receptor compartment. The receptor compartment of the diffusion cell was filled with 200 ml of 0.01 N HCl. The donor compartment contained 1 ml solution of Diltiazem base in 0.01N HCl having concentration 6.38 mg/ml. The Fite's diffusion cell was kept in side the beaker containing receptor fluid. The temperature of diffusion medium was maintained at 37 ± 0.5^{o}C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead. The diagrammatic representation of in vitro

diffusion study through human live skin is given in Figure 5.2.1.

Figure 5.2.1: Drug permeability study using Fite's diffusion cell



The samples were withdrawn (2 ml, each time) at different time interval and an equal amount of 0.01N HCl, was replaced each time. Absorbance of the sample was read spectrophotometrically at 236.0 nm taking 0.01N HCl solution, as a blank. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. The regression analysis of steady state data was done and release rate was computed. The experiment was triplicated and mean result recorded.

5.2.4 Improvement of skin permeability using enhancers

The first transdermal drug delivery system was developed for scopolamine for motion sickness in 1981. In spite of the therapeutic success achieved in last 15 years by using transdermal drug delivery system, the numbers of transdermal drug delivery system available in the market are very few. This is mainly dew to limited permeation behavior of drug across skin.

The limitation of transdermal drug delivery due to ionic characteristic of drugs and large molecular weight of drugs can be overcome to some extent by use of permeability enhancers. Sorption promoters or sorption enhancers are not drugs but they are molecules which reversibly alter the barrier nature of the stratum corneum. [11,12,13,14] Sorption promoters allow the drugs to penetrate into skin and the drug permeate across skin more readily and thus increase systemic availability. [15,16]

Azone, propylene glycol and alcohol was evaluated for Diltiazem permeation enhancement activity across human live skin. A solution of Diltiazem base (6.38 mg/ml) was prepared in 0.01N HCl and it was incorporated with enhancer to 10%

concentration level. The permeation study was carried out as described in section 5.2.3.

To improve the permeability of Diltiazem across human live skin, alcohol in gradually increasing concentration in donor phase was added and evaluated for influence on permeation kinetic of Diltiazem.

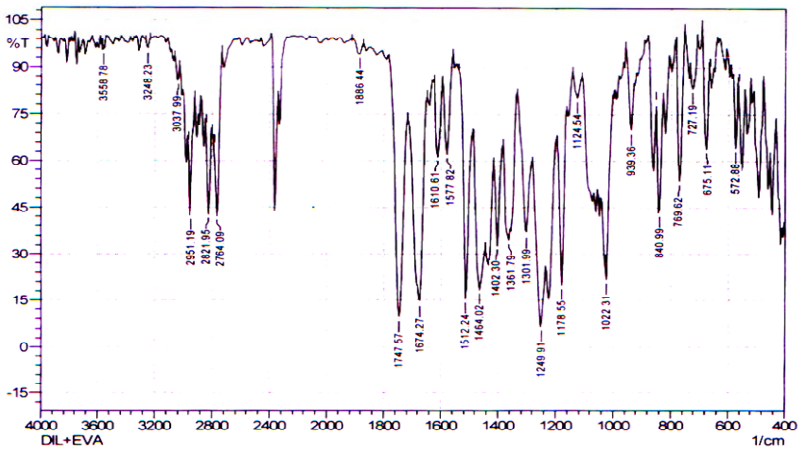
5.2.5 Result and discussion

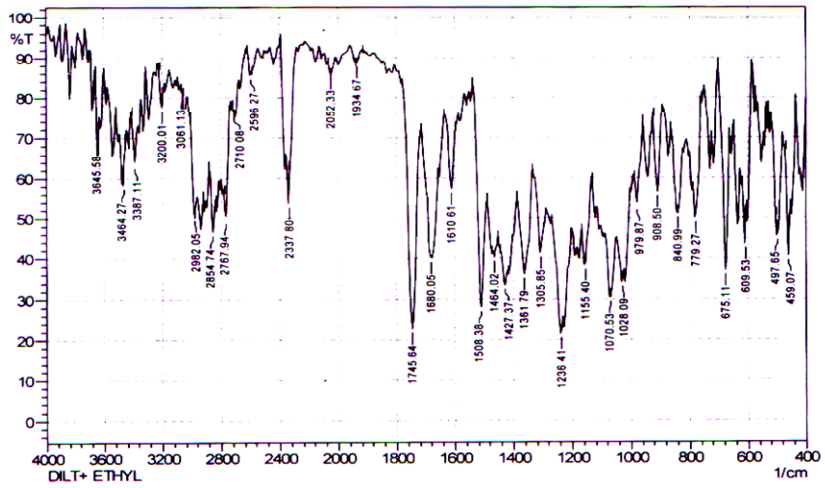
FT-IR spectroscopic and Differential scanning calorimetric studied was carried out to assess any interaction between the drug and the excipients. Simultaneously permeability study of drug was carried out through live human skin and also assesses effect of permeability enhancers on permeability of drug through human live skin.

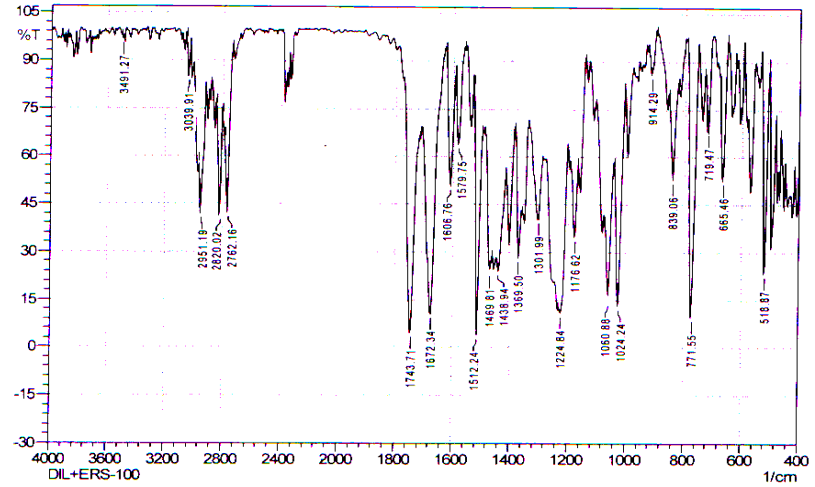
Infrared (IR) Spectroscopic analysis

The chemical interaction between the drug and the polymer often leads to identifiable changes in the Infrared (IR) profile of complexes. The FT-IR spectrum of pure Diltiazem was shown in figure 4.3.2. The FT-IR spectra of Diltiazem with EVA (VA 40%)copolymer, Ethyl cellulose, Eudragit RL100, Eudragit RS 100 are shown in Figure 5.2.2. the presence or absence of characteristics peaks associated with specific structural groups of the drug molecule was noted. From the FTIR spectra it was revealed that no interaction occurred between Diltiazem and different polymers.

Figure 5.2.2: IR specta of Diltiazem composites




%T
3645.58
3464.27
3387.11
3200.01
3061.13
2982.05
2854.74
2767.94
2710.08
2596.27
2337.80
2052.33
1934.67
1745.64
1680.05
1610.61
1508.38
1464.02
1427.37
1361.79
1305.85
1236.41
1155.40
1070.53
1028.09
979.87
908.50
840.99
779.27
675.11
609.53
497.65
459.07
DILT+ ETHYL
1/cm


%T
3491.27
3039.91
2951.19
2820.02
2762.16
1743.71
1672.34
1606.76
1579.75
1512.24
1469.81
1438.94
1369.50
1301.99
1224.64
1176.62
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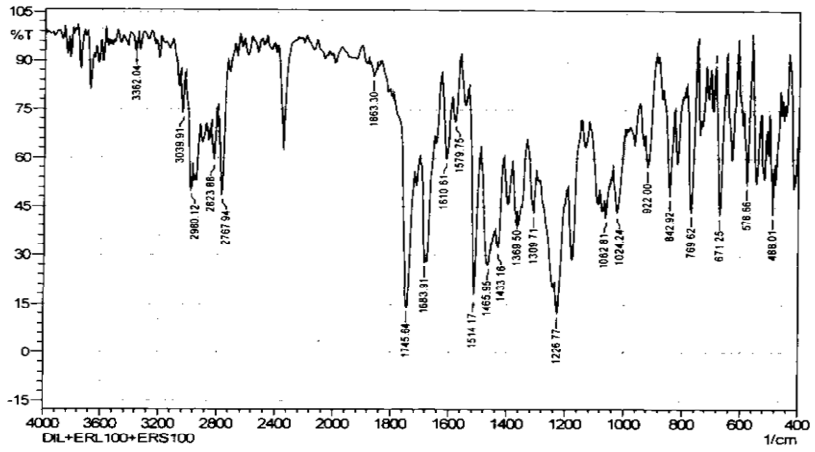
1024.24
914.29
839.06
771.55
719.47
665.46
518.87
DIL+ERS-100
1/cm


%T
3362.04
3039.91
2980.12
2823.86
2767.94
1863.30
1745.64
1683.91
1610.61
1579.75
1514.17
1465.55
1433.18
1369.50
1330.71
1226.77
1082.81
1024.24
922.00
842.92
769.62
671.25
578.66
486.01
DIL+ERL100+ERS100
1/cm

Differential scanning calorimetric analysis

Differntial scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced, during phase transformation (i.e., endothermic or exothermic phase transformation). DSC curves for pure drug, Diltiazem is shown in Figure 4.3.3. DSC curve of EVA and Diltiazem composite are shown in Figure 5.2.3. Pure powdered Diltiazem showed a melting endotherm at 110.77^{0}C temperature. DSC scan of EVA showed a broad endotherm due to the presence of residual moisture in polymers. DSC thermogram of Diltiazem base with EVA exhibits endothermic peaks at near to 111.07 ^{0}C temperature. This reveals the absence of any interaction occurring between drug and polymers.

Figure 5.2.3(a): DSC thermogram of EVA (VA 40%) copolymer

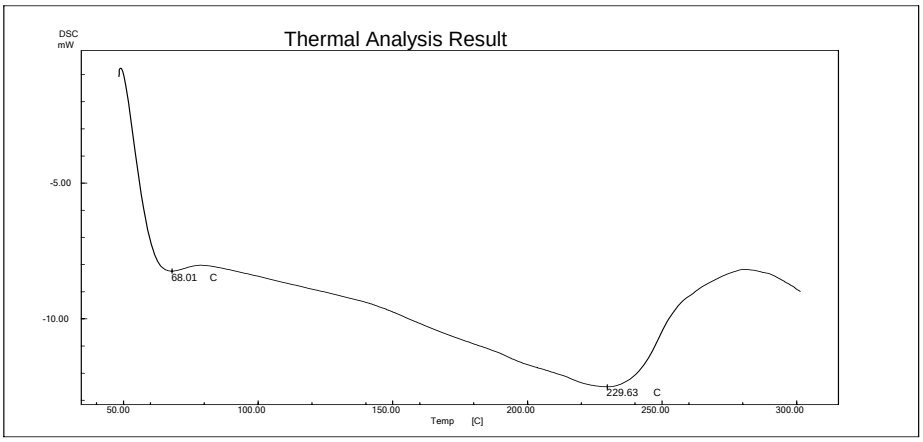
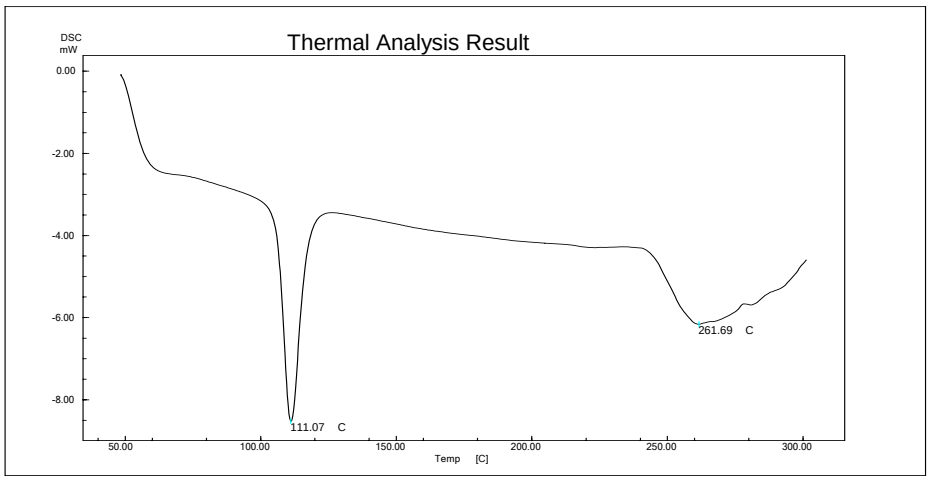


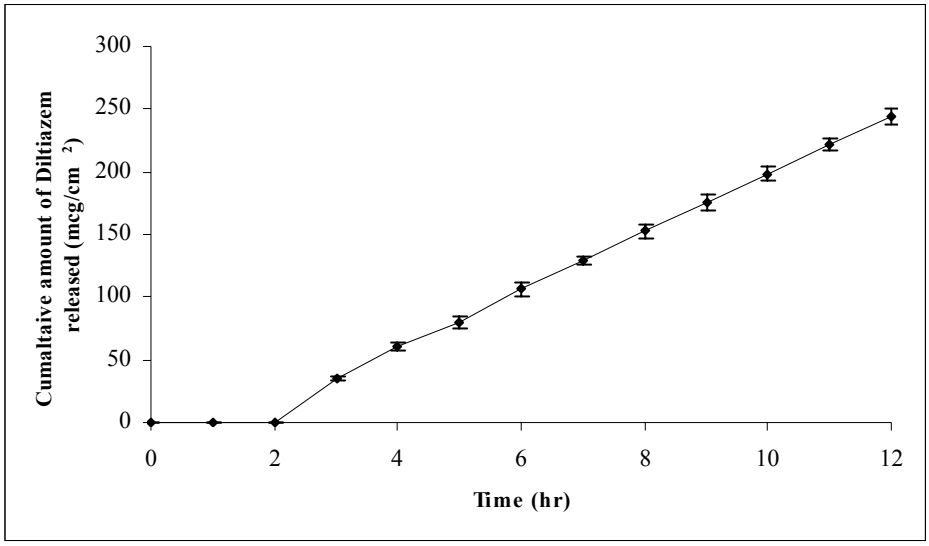
Figure 5.2.3(b): DSC thermogram of Diltiazem and its composites



Drug permeability study using human live skin

In vitro permeation study is predictive of in vivo performance of a drug. Permeation studies were performed for saturated solution of Diltiazem base in 0.01N HCl across human live skin using 0.01N HCl as a diffusion medium in the receptor compartment of a modified diffusion cell at 37 ± 0.5 ^{o}C. The amount of drug permeated per square centimeter at each time interval was calculated and plotted against time (Figure 5.2.4).

Figure 5.2.4: Permeation study of Diltiazem in 0.01N HCl through human live Skin



*** Standard deviation, n=3**

The permeation of Diltiazem hydrochloride across isolated human skin has been reported **9.38 µg/cm^2 hr** and it was very poor.[10] The conversion of salt from Diltiazem hydrochloride to base form, exhibited improved diffusivity across skin preparation. The flux of Diltiazem through human live skin was **23.84 µg/cm^2 hr** with time lag **1.5 hr.** The diffusion co-efficient was **2.178 x 10^{-5} cm^2/ hr,** permeability was **3.51 x 10^{-3} cm/hr** and permeability co-efficient **33.38 X 10^{-2} µg/cm hr.** The high intrinsic diffusion coefficient of lipophillic drug seems to have played role in improvement of skin flux. The results of permeation of pure drug across live human skin indicated that the base form of Diltiazem has quite good permeation characteristics.

The magnitude of flux was just sufficient to meet pharmacokinetic requirement of steady state plasma concentration. The improvement in percutaneous permeation of Diltiazem base was due to its hydrophobic nature which partitions easily from solution to skin. The further improvement in flux was done using different skin

penetration enhancer, so as to have a small size of device meeting the requirement of steady state plasma concentration of drug.

Improvement of permeability using enhancers

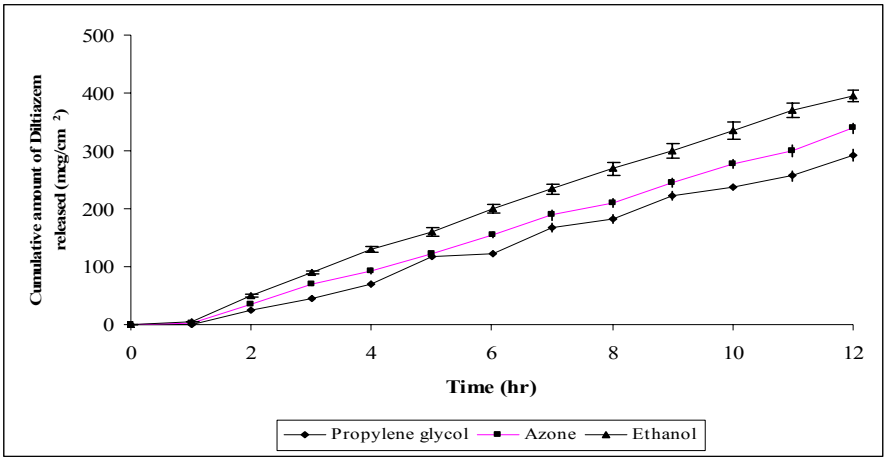
The permeation behavior of drug across the skin is controlled by stratum corneum. It is composed largely of fatty material along with keratin and other bioorganic compounds. Usually hydrophobic drug penetrate more as compare to hydrophilic and ionic compounds. To design a convenient transdermal controlled delivery for patient, the size of drug delivery play an important role and usually small size of delivery device meeting pharmacokinetic requirement of drug, is preferred for better compliance to therapy by a patient.

The skin flux of Diltiazem from hydrochloric acid solution was found to be **23.84 µg/cm^2 hr.** To improve skin flux, three chemical enhancers propylene glycol, Azone and ethanol were evaluated. The permeation behavior of Diltiazem in presence of skin penetration enhancer is presented in Figure 5.2.5.

The flux was improved and rank order of flux was propylene glycol < Azone < Alcohol. It was found to be **26.05, 30.01** and **34.95 µg/cm^2 hr** for **propylene glycol, azone** and **ethyl alcohol** respectively. Propylene glycol has a skin hydration property and by this way it improves the permeation of molecule. However it is hydrophilic as compare to azone and alcohol but Diltiazem is hydrophobic in nature so due to this reason the flux is less as compare to azone and alcohol. Azone is used to improve permeation of both types of drugs i.e. hydrophilic as well as hydrophobic. As such azone is water immiscible and soluble in organic solvent. Due to its hydrophobicity the flux is improved but it is less than that observed with alcohol. The permeation behavior of azone is still remaining to be explored, but it seems that it temporarily alters the keratin structure of stratum corneum, and this behavior seems to have improved the permeability of Diltiazem.

Permeation of Diltiazem in presence of alcohol was greatly improved and the highest flux **(34.95 µg/cm^2 hr)** was found with alcohol as compare to propylene glycol and azone at same concentration (10%) level.

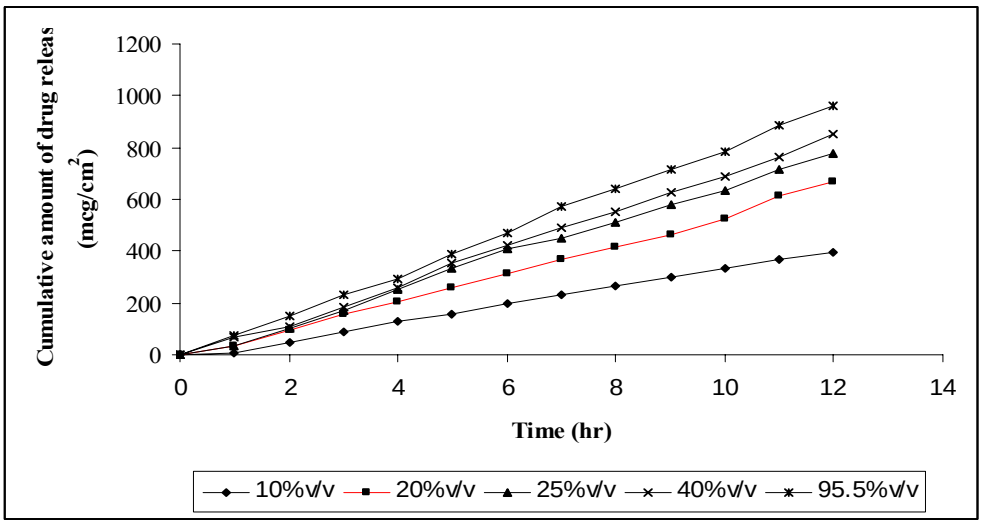
Figure 5.2.5: Permeation study of Diltiazem through human live skin using penetration enhancers



*** Standard deviation, n=3**

Permeation of Diltiazem in presence of alcohol was substantially improved. To optimize the suitable concentration of alcohol in order to get desired permeation rate meeting pharmacokinetic requirement of steady state plasma concentration of drug. Alcohol in gradually increasing concentration was further evaluated for Diltiazem permeation enhancement activity across human live skin.

Figure 5.2.6: Permeation study of Diltiazem in presence of ethanol through human live skin



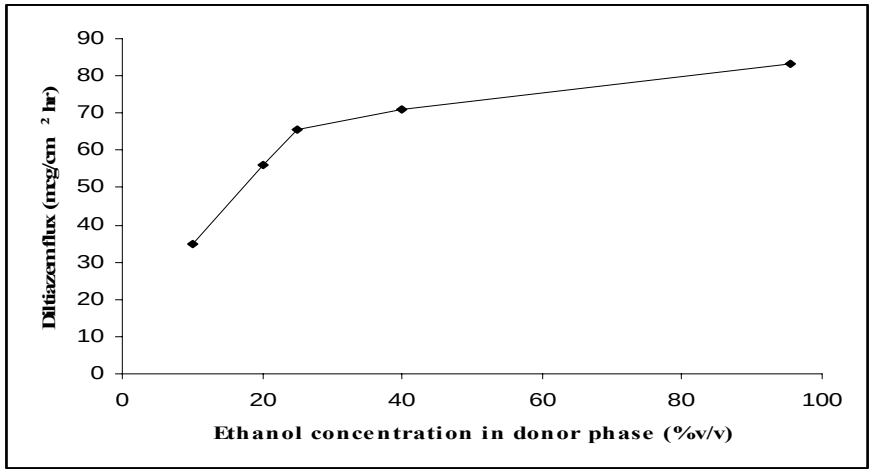
Alcohol concentration in donor phase was maintained **10%, 20%, 25%, 40%** and **95.5% alcohol.** The permeation profiles are shown in Figure 5.2.6. The Diltiazem flux was **34.95, 56.05, 65.58, 71.00** and **83.14 µg/cm^2 hr** for 10%, 20%, 25%, 40%

and 95.5% alcohol. Table 5.2.1 and Figure 5.2.7 present the relationship between concentration of alcohol in donor phase and solubility of Diltiazem in skin.

Table 5.2.1: Permeation kinetic of Diltiazem with increasing concentration of alcohol

Concentration of ethanol (% v/v)	Skin flux ($\mu g/cm^2$ hr)	Lag time (hr)	Diffusion coefficient (cm^2/hr)	Permeability coefficient ($\mu g/cm$ hr)
10%	34.95	0.51	6.410×10^{-5}	0.4893
20%	56.05	0.42	7.780×10^{-5}	0.7847
25%	65.58	0.33	9.890×10^{-5}	0.9181
40%	71.00	0.26	1.256×10^{-4}	0.9940
95.5%	83.14	0.20	1.633×10^{-4}	1.1639

Figure 5.2.7: Effect of ethanol concentration on skin flux of Diltiazem



Here after 25% Alcohol the improvement in Cs becomes steady. The possible explanation for the improvement in permeation of Diltiazem across skin is given below. The lipid content of stratum corneum as reported [29] was 44.5±1.17 %w/w. The principle barrier for skin penetration rests in stratum corneum. A random brick model for drug transport across stratum corneum is reported.[29] Stratum corneum consists of protein rich thin plates (cells) separated from one another by thin layer intercellular lipids. Following parallel pathways for drug diffusing across stratum corneum have been proposed.

1. through the cell/intercellular in series
2. through the lipid intercellular routes
3. through the flattened protein cells.
4. through the lipid intercellular route which is lipophillic

It is reported that the lipid layers play a significant role in skin permeation for lipophillic drugs.[29] The factor γ for lipophillic drug, $\gamma = KD_L/Dp >>> 10^4$ indicating a high intrinsic diffusion coefficient across lipid layer (D_L) as compared to intrinsic diffusion coefficient across protein rich layer (Dp) of sratum corneum K, was lipid-protein partition coefficient. Diltiazem base is hydrophobic, freely soluble in lipid solvents. It is the lipophillic nature of this drug rendered the drug to travel freely (as compared to Dlitiazem hydrochloride) from the lipid layer. Alcohol being lipid solvent seems to have fluidized the lipid barrier of skin facilitating solution diffusion membrane mechanism in stratum corneum for Diltiazem penetration.

Ethanol being a good solvent for Diltiazem base seems to have improved the thermodynamic activity of Diltiazem base. It is reported that higher the concentration of drug in cells of stratum corneum more will be the flux[29]. Alcohol improves the thermodynamic activity of Diltiazem in stratum corneum by maintaining high concentration in the stratum corneum. As concentration in donor phase increased the skin flux is also increased. The solubility of Diltiazem in stratum corneum (Cs) is improved and this improvement has lead to overall improvement in permeation behavior of Diltiazem across skin. At low alcohol concentration level permeation improvement from protenous phase seems to be responsible and at higher concentration level changes in fatty barrier seems to be responsible. At very high concentration i.e. above 40 to 95.5 %v/v dehydrating effect of alcohol seems to play role showing very small change in Cs and Cs becomes steady.

From above explanation 25 %v/v alcohol in donor phase is just sufficient to have desired skin flux to meet pharmacokinetic requirement of steady state Diltiazem concentration in blood. The flux was **65.58 µg/cm^2 hr** so, 25 %v/v alcohol was selected for preparation of reservoir patch of Diltiazem.

5.3 PREPARTION AND CHARACTERIZATION OF POLYMERIC MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICES OF DILTIAZEM

5.3.1 Preparation of polymeric matrix device

Matrix – type transdermal patches containing Diltiazem were prepared using different ratios of drug to polymers (Table 5.3.1).The polymers were weighed in requisites ratios keeping the total polymer weight 800mg, and dissolved in a given solvent. Diethyl Phathalate (2% w/w of polymer composition), Di-n-butyl Phalate (30% w/w of polymer composition) and glycerin (40% w/w of polymer composition) were used as a plasticizer for EVA, ERL100, ERS100 and EC respectively. Diltiazem (533.33mg) was added and mixed using a mechanical stirrer. The uniform dispersion of polymeric solution of drug (10 ml) was poured on the mercury surface (73.86 cm^2), and dried at room temperature. After 24h, the films were cut into a 3.14 cm^2 area and backing membrane (biaxial oriented polyethylene film) was then glued. A glossy paper having a smooth surface was used as a release liner. The devices were stored in desiccators until used.

Table 5.3.1: Composition of polymeric matrix diffusional patches of Diltiazem

Formulation code	Polymers	Plasticizers (%w/w of polymer composition)	Solvent
F1	EVA (VA 40%) copolymer	DEP (2 %)	Toluene
F2	EC	Glycerin (40 %)	Chloroform
F3	ERS100	DBP (30%)	Chloroform
F4	ERL100: ERS100 (1:4)	DBP (30 %)	Chloroform

Figure 5.3.1: Matrix diffusional transdermal patch of Diltiazem

5.3.2 Physiochemical evaluation of polymeric matrix device

Thickness

The thickness of the laminate was assessed at six different points of the prepared medicated film using thickness gauge micrometer (0.001mm, Mitutoyo, Japan). For each formulation, three randomly selected laminated were used.

Weight variation

The weight variation for each batch was determined using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan. Six patch from each batch (3.14 cm^2), were weighed individually and the average weight was calculated.

Drug content

The Diltiazem content of each prepared film was measured in triplicate and analyzed by UV-VIS spectrophotometer and expressed as the percentage of nominal lode**.** Patches (n=3) of specified area (3.14 cm^2), were cut and weighed accurately. The pieces were taken into 100 ml volumetric flask and dissolved in respective solvent. The solution was filtered through whatman filter paper (Nyulge Nune, UK). This stock solution was diluted 100 times using respective solvent and the absorbance of the resulting solution was measured at specific wavelength. The content of Diltiazem was calculated at 281.5 nm for toluene and 259 nm for chloroform using calibration curve prepared using respective solvent system.[17, 18]

Flatness

The flatness was measured manually for the prepared films. Longitudinal strips were cut out from each film, one from the center and two from either side. The length of each strip was measured and the variation in the length because of non uniformity in

flatness was measured by determining percentage constriction, considering 0% constriction is equivalent to 100 % flatness.[5] Flatness was determined using below given formula:

% Constriction = $[(l_1 – l_2)/ l_2] * 100$

Where,

l_1 = Initial length of each strip

l_2 = Final length of each strip

The flatness for Diltiazem matrices was measured in triplicate and average reading was considered.

Folding endurance

The folding endurance was measured manually for the prepared films. The folding endurance of the films was determined by repeatedly folding a strip measuring 2x2 cm size at same place till it break.[19] The number of times the film could be folded at the same place without breaking gave the value of folding endurance.

Moisture content (Loss on drying) [20]

The inherent moisture presents in material may influence the stability of dosage forms, especially if it contains a drug that is sensitive to water. The absolute method is employed to determine the moisture content which gives a weight loss registered during process.

Three patch from each batch (3.14 cm^2), were weighed individually and the average weight was calculated. This weight was considered as an Initial weight. Then all the patches were kept in a desiccators containing activated Silica at normal room temperature for 24hr. The final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption was calculated as a difference between initial and final weight with respect to final weight.

% Moisture content = [(Initial weight – Final weight)/ Final weight]* 100

Moisture absorption [21]

Moisture uptake also influences the stability of dosage form. Low moisture uptake protects the material from microbial contamination. So for transdermal drug delivery system it is necessary to determine % Moisture absorption of matrices.

Three patch from each batch (3.14 cm^2), were weighed individually and the average weight was calculated. This weight was considered as an Initial weight. Then all the patches were kept in a desiccators containing 200 ml saturated solution of Sodium chloride (Relative humidity of 75%) at normal room temperature for 72h. The final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The % Moisture absorption was determined using below formula:

% Moisture absorption = [(Final weight – Initial weight)/ Initial weight]* 100

Water vapor transmission rate (%WVTR) [22,23]

For this study vials of equal diameters were used as transmission cells. These cells were washed thoroughly and dried in oven, about 1 gm of activated silica was taken in cells and the polymeric films measuring 3.14cm^2 were fixed over the brim with the help of an adhesive. The cells were weighed accurately and initial weight was recorded, and then kept in a closed desiccators containing 200 ml saturated solution of potassium chloride. The cells were taken out and weighed after 6, 12, 24, 36, 48 and 72 hr of storage. The amount and rate of water vapor transmitted was calculated by the difference in weight using below given formula:

% Water vapor transmission rate = (Final weight- Initial weight)/ time * Area

5.3.3 Result and discussion

The present investigation deals with the development of Diltiazem base loaded polymeric matrix using different polymers. The preliminary screening was carried out for the selection of best polymer.

A diffusion mediated matrix controlled transdermal drug delivery system for Diltiazem base was successfully prepared using different polymers using mercury subtract method and all matrices were evaluated using different physiochemical parameters.

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of film was measured at six different points and the average thickness was noted. The thickness results are given in Table 5.3.2. The results indicate that there was no much difference

in the thickness with in the formulations. Thickness in the different formulations was in the range of **173.33 ± 1.443 µm** to **85 ± 2.5 µm.** Maximum thickness was found in formulation F1, while minimum found in formulation F4. These results revealed that thickness was found to increase as hydrophobic portion of polymer increases. The results of thickness also indicate uniform distribution of the drug and polymer over the mercury surface. The rank order of thickness of Diltiazem loaded polymeric matrices was

EVA (40% vinyl acetate)> EC> ERS 100> ERL100:ERS100 (1:4)

Table 5.3.2: Results of thickness uniformity of F1 to F4 matrix formulations

Sr. No.	Formulation code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	172.5	175.0	172.5	173.33 ± 1.443
2	F2	115.0	117.5	117.5	116.66 ± 1.443
3	F3	150.0	152.5	150.0	150.83 ± 1.443
4	F4	85.0	87.5	82.5	85.00 ± 2.500

***Standard deviation, n=3**

Weight variation

Drug loaded films (3.14cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 5.3.3. The weight of 3.14 cm^2 film ranged from **50.30 ± 0.100 mg** to **58 ± 0.500 mg**. The weight of the patches was found to be uniform among different batches.

Table 5.3.3: Results of weight variations of F1 to F4 matrix formulations

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	53.0	53.1	52.9	53.00 ± 0.100
2	F2	52.5	52.6	52.3	52.46 ± 0.152
3	F3	58.0	57.5	58.5	58.00 ± 0.500
4	F4	50.3	50.2	50.4	50.30 ± 0.100

***Standard deviation, n=3**

In a weight variation test, the pharmacopoeial limit for the percentage deviation of all the films of less than mg is ± 10%. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for weight variation as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Diltiazem in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation, with acceptable deviation.

Drug content

Drug content of the matrices was carried out to ascertain that the loading of drug is uniform in the formulation. The results obtained are represented in Table 5.3.4.

The films were found to contain **97.87% - 101.23%** of the labeled amount of Diltiazem indicating uniformity of drug content. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for content uniformity as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Diltiazem in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation, with acceptable deviation.

Table 5.3.4: Results of % drug content of F1 to F4 matrix formulations

Sr. No.	Formulation code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	98.57	96.52	98.53	97.87 ± 1.172
2	F2	101.20	100.05	101.23	100.82 ± 0.672
3	F3	97.80	98.90	99.02	98.57 ± 0.672
4	F4	101.50	101.20	101.00	101.23 ± 0.251

***Standard deviation, n=3**

The drug content analyses of prepared formulation showed that the process employed to prepared patches was capable of giving uniform drug content, with minimum batch variability.

Flatness

The flatness was measured manually for the prepared films. An ideal patch should be formulated in such a way that it possesses a smooth surface and it should not constrict with time. Flatness studies were performed to assess the same. The results of the flatness study showed that none of the formulations had the differences in the strip length before and after their cuts. It indicates 100% flatness observed in the formulated patches. Thus, no amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the patches and thus they could maintain a smooth surface when applied on to the skin.

Folding endurance

Folding endurance was determined manually for drug loaded polymeric matrices. The folding endurance of the films was determined by repeatedly folding a strip measuring 2x2 cm size at same place till it break. The number of times the film could be folded at the same place without breaking gave the value of folding endurance. The results of folding endurance are given in Table 5.3.5.

Table 5.3.5: Results of folding endurance of F1 to F4 matrix formulations

Sr. No.	Formulation Code	Folding endurance			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	248	250	247	248.33 ± 1.527
2	F2	245	244	247	245.33 ± 1.527
3	F3	15	17	18	16.66 ± 1.527
4	F4	19	17	18	18.00 ± 1.000

***Standard deviation, n=3**

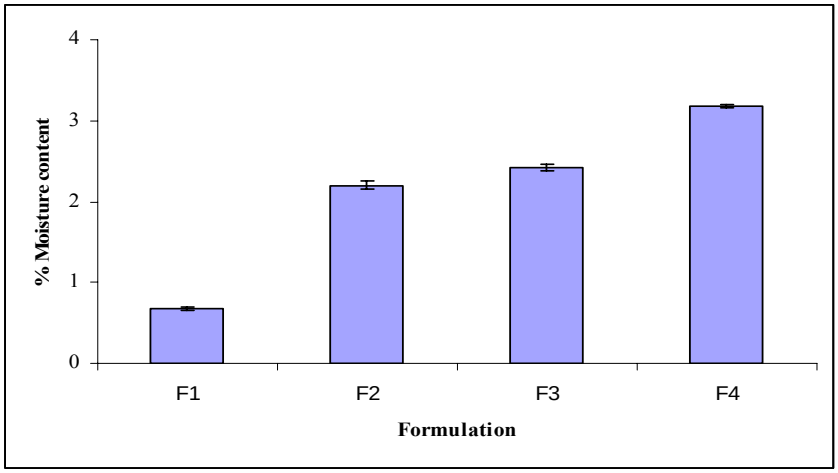
Here formulation F1 and formulation F2 shows good folding endurance as compare to formulation F3 and F4.

Moisture content (Loss on drying)

The moisture content was determined by keeping the drug loaded polymeric matrix patches in desiccator containing activated silica for 24hr. The percentage moisture content was calculated from the weight differences relative to the final weight. The

results of the moisture content studies for different formulations are shown in Figure 5.3.2.

Figure 5.3.2: % moisture content of F1 to F4 matrix formulations



*** Standard deviation, n=3**

The moisture content in all the formulations was found to be low and ranged from **0.681 ± 0.019** to **3.181 ± 0.024%.** The result revealed that the moisture content was found to increase with increasing concentration of hydrophilic polymers. The small moisture content in the formulations helps them to remain stable and from being a completely dried and brittle film. The rank order of % moisture content of Diltiazem loaded polymeric matrices was

EVA (40% vinyl acetate) < EC < ERS 100 < ERL100:ERS100 (1:4)

Moisture absorption

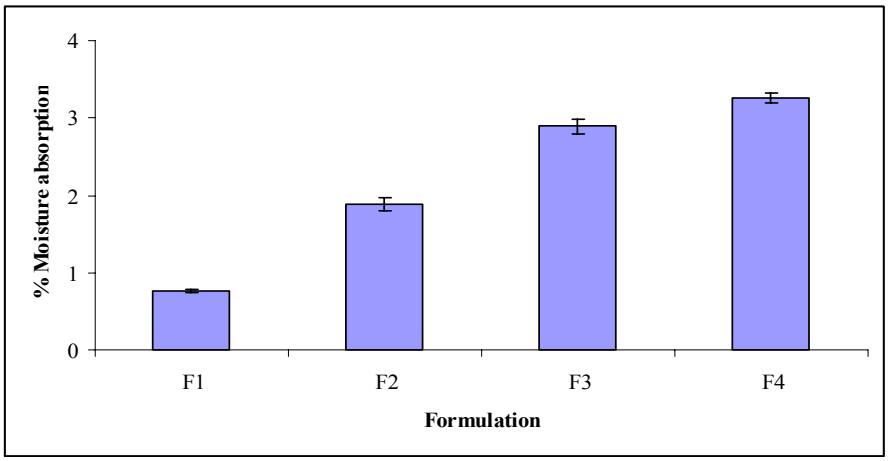
Moisture uptake also influences the stability of dosage form. Low moisture uptake protects the material from microbial contamination. So for transdermal drug delivery system it was necessary to determine % Moisture absorption of matrices.

The results of the moisture content studies for different formulations are shown in Figure 5.3.3.

The moisture absorption in all the formulations was found to be low and ranged from **0.7584 ± 0.0276** to **3.2617 ± 0.05696%.** The result revealed that the moisture absorption was found to increase with increasing concentration of hydrophilic polymers. The small moisture absorption in the formulations helps them to remain stable and protects the material from microbial contamination and bulkiness of the patches. The rank order of % moisture absorption for Diltiazem loaded matrices was

EVA (40% vinyl acetate) < EC < ERS 100 < ERL100:ERS100 (1:4)

Figure 5.3.3: % Moisture absorption of F1 to F4 matrix formulations

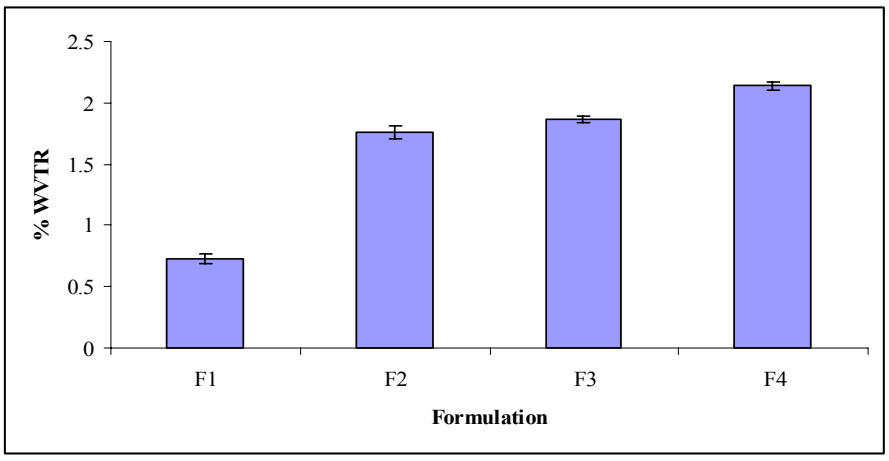


*** Standard deviation, n=3**

Water vapor transmission rate (%WVTR)

The water vapor transmission rates of different formulation were evaluated, the results are shown in Figure 5.3.4. Diltiazem films containing ERL100 showed higher % WVTR as compared to other polymers. This may be due to the hydrophilic nature of ERL 100. Formulation F1 and F2 showed less % WVTR as compared to F3 and F4. The rank order of % water vapor transmission rate for Diltiazem loaded polymeric matrices was **EVA (40% vinyl acetate) < EC < ERS 100 < ERL100:ERS100 (1:4)**

Figure 5.3.4: % water vapor transmission rate of F1 to F4 matrix formulations



*** Standard deviation, n=3**

5.4 IN VITRO DIFFUSION STUDY OF MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF DILTIAZEM

The release rate determination is one of the most important study to be conducted for all controlled release delivery systems. The diffusion studies of patches are very crucial, because one needs to maintain the drug concentration on the surface of stratum corneum consistently and substantially greater than the drug concentration in the body to achieve a constant rate of drug permeation.[24]

5.4.1 Experimental

In vitro diffusion studies of Diltiazem from various transdermal patches was studied using modified Keshary-Chien diffusion cell (Figure 5.4.1). The diffusion cell consists of two parts; the upper parts i.e. The donor compartment and contains the active ingredients and the carrier adhesive/patch; the bottom part contains the receptor solution, the water jacket for temperature control, and the sampling port.

The effective permeation area of the diffusion cell and receptor cell volume was 3.14cm^2 and 40 ml, respectively. The temperature was maintained at 37±0.5^oC. The receptor compartment contained 40 ml of 0.01N HCl stirred by magnetic stirrer.

Samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at different time intervals. The absorbance of the withdrawn samples were measured using UV VIS spectrophotometer at 237.8 nm using 0.01N HCl as a blank. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of square root of time for different formulations. The release rate $Q/\sqrt{T}$ was determined by simple regression analysis of steady state data.

Figure 5.4.1: Modified Keshary-Chien diffusion cell

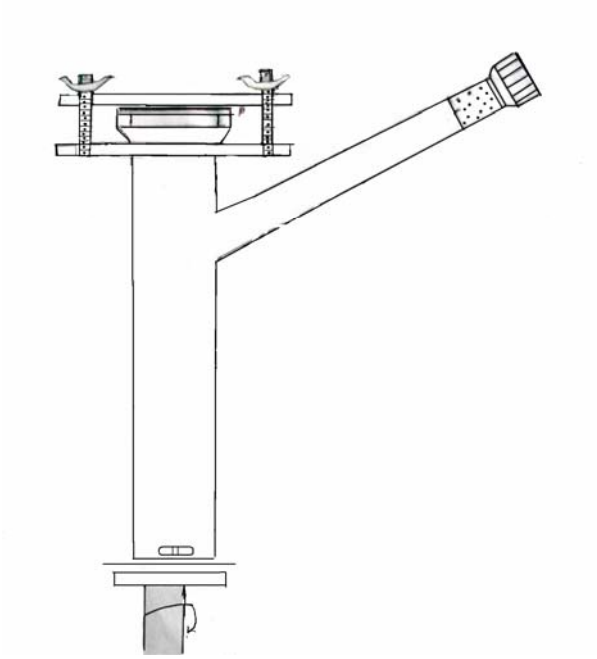


Figure 5.4.2: Experimental setup

5.4.2 Result and discussion

Diffusion studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. In vitro release profile is an important tool that predicts in advance how the drug will behave in vivo [25]. The results of in vitro drug diffusion studies of transdermal patches are depicted in Table 5.4.1 and Figure 5.4.3.

Table 5.4.1: In vitro diffusion profiles of Diltiazem from F1 to F4 formulations

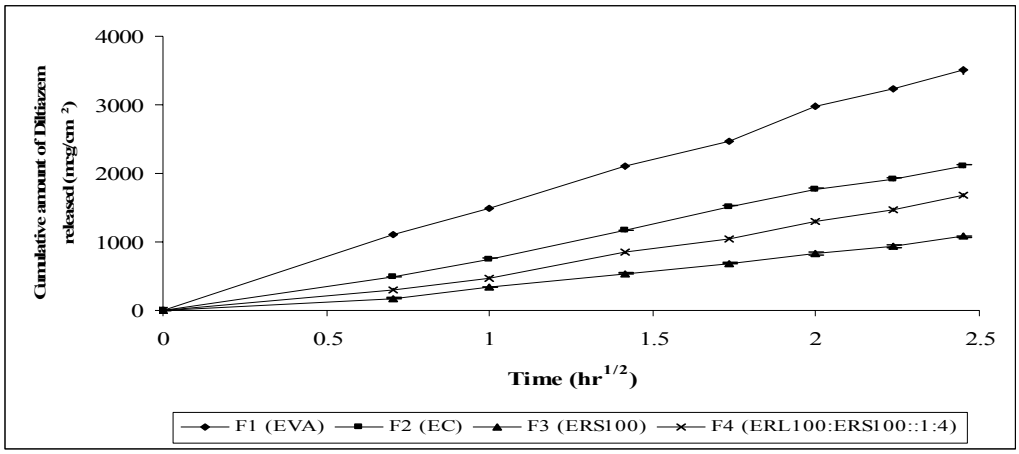
Time ($hr^{½}$)	Cumulative amount of drug release from device ($\mu g/cm^2$)			
	Formulation code			
	F1	F2	F3	F4
0.707	1105.51 ± 15.20	486.25 ± 5.39	180.16 ± 5.34	305.87 ± 6.32
1.000	1499.85 ± 20.33	750.17 ± 10.34	339.65 ± 6.40	475.53 ± 8.44
1.414	2099.63 ± 25.46	1164.70 ± 15.34	540.96 ± 7.35	845.80 ± 10.32
1.732	2470.25 ± 26.59	1520.00 ± 18.45	688.56 ± 10.49	1050.56 ± 12.53
2.000	2985.46 ± 32.46	1775.24 ± 20.58	830.25 ± 11.40	1295.23 ± 15.42
2.236	3231.56 ± 30.29	1920.56 ± 22.59	940.56 ± 17.39	1475.47 ± 18.48
2.449	3500.23 ± 45.38	2100.36 ± 25.79	1075.82 ± 19.84	1675.69 ± 19.32
$Q/\sqrt{T}$ ($\mu g/cm^2\ \sqrt{hr}$)	**1488.10**	**946.30**	**503.29**	**794.08**
Correlation coefficient	**0.9976**	**0.9959**	**0.9990**	**0.9987**

***Standard deviation, n=3**

The results of diffusion study of Diltiazem loaded polymeric matrix formulated using various polymers are presented in Table 5.4.1 and profiles are shown in Figure 5.4.2. The release rate $Q/\sqrt{T}$ ($\mu g/cm^2\ \sqrt{hr}$) was determined by simple regression analysis of steady state data. The release of Diltiazem from all the matrices followed square root law. The rank order of release was

EVA (40% vinyl acetate) >EC > ERL100:ERS100 (1:4) > ERS 100

Figure 5.4.3: In vitro diffusion profiles of Diltiazem from F1 to F4 formulations



*** Standard deviation, n=3**

Formulation **F1 EVA (VA 40%) copolymer** exhibited maximum Q/√T release rate **1488.10 µg/cm^2√h** while Formulation **F3** exhibited minimum Q/√T release rate **(503.29 µg/cm^2 √h**). The physiochemical property of polymer plays important role in drug release characteristics, from the polymeric matrix. EVA (VA 40%) copolymer is more hydrophobic as compare to other polymers and enhanced permeation from the matrix. The solubility characteristic of Diltiazem base in EVA matrix seems to have played, the significant role in the release characteristics. The higher polymer solubility has played significant improvement in release of drug from EVA (VA 40%) copolymer matrix. EVA (VA 40%) copolymer matrix provides a good release for Diltiazem base. Based on physiochemical and in vitro release experiments, formulation **F1** may be chosen for further in vitro permeability study through human live skin.

In vitro release kinetic

The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 5.4.2. Here R is regression coefficient.

Table 5.4.2.: Data of various parameters of model fitting of formulation F1 to F4

Formulation code	Zero order equation	First order equation	Higuchi's equation
F1	**0.9774**	**0.9326**	**0.9960**
F2	**0.9604**	**0.8915**	**0.9930**
F3	**0.9907**	**0.9309**	**0.9984**
F4	**0.9851**	**0.9074**	**0.9981**

The process of drug release in most controlled release devices including transdermal patches is governed by diffusion [26] and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three – dimensional network of polymers chain. The in vitro release profile could be best expressed by Higuchi's equation for the permeation of drug from the matrix.

In our experiment, the in vitro permeation profiles of all formulations could be best expressed by **Higuchi's equation** (R^2 = 0.9930 to 0.9984) for the permeation of drug

from a homogeneous- polymer matrix type delivery system that depends mostly on diffusion characteristics.[27]

5.5 TO OPTIMIZE DRUG LOADING IN MATRIX FOR ELEGANT FORMULATION

5.5.1 Experimental

Drug loading (i.e. concentration) in matrix influences parameter of patch like folding endurance, thickness, weight variation, flatness, texture appearance, brittleness etc. EVA (VA 40%) copolymer was selected to device final matrix diffusional therapeutic system. Inclusion of Diltiazem in gradually increased concentration changes its above characteristics. The drug is compatible with polymer as seen in DSC profile.Higher concentration of drug can be loaded in matrix to achieve enhanced Q/√T release rate. Simultaneous changes that occur in the matrix are the texture, appearance, folding endurance etc which influences elegance of medicated laminate. A compromise is necessary between the loading of drug to have a maximum available Q/√T release rate and elegance of formulation. Most of the investigations, employing diffusion study are directed towards determining flux, permeability coefficient and diffusion coefficient of drug across the polymeric vehicle components like membrane and matrices. Many unexplored points still exist whose study might prove profitable. For medicated laminates of monolithic characteristics, containing the dispersed drug, release rates are proportional to square root of concentration. Release of drug from laminated containing dissolved drug is expected to be linearly related to the initial concentration. This aspect of physical pharmaceutics has been less extensively studied.

The release of drug from matrices is described by the well known Higuchi model. But when the drug has significant solubility in the polymer the release has been described by the following model equation. [28]

$$Q = q/A = 2C (Dt/ \pi)^{1/2}$$

Where, Q is the amount of drug (q) release to the sink at time t per unit area A of contact. D is diffusion coefficient of drug in the polymer and C is the initial concentration of drug in polymer mg/cm^3. This equation showed the relationship

existing between the release rate Q/√T and C, the overall concentration of drug dissolved in polymer. The main purpose of present investigation was to test the scope and usefulness of above model in a series of Diltiazem loaded homogeneous EVA (VA 40% copolymer) matrices.

Preparation of polymeric matrix device

Matrix – type transdermal patches containing Diltiazem were prepared using different ratios of polymers and drugs (**Table 5.5.1**).The polymers were weighed in requisite ratios and dissolved in toluene. Diltiazem was added and mixed slowly with a mechanical stirrer. The uniform dispersion of polymeric solution of drug (10 ml) was poured on the mercury surface (73.86 cm^2), and dried at room temperature. After 24h, the films were cut into a 3.14 cm^2 area and backing membrane (biaxial oriented polyethylene film) was then glued. A glossy paper having a smooth surface was used as a release liner. They were kept in desiccators until used.

Table 5.5.1: Composition of matrix diffusional transdermal patches of Diltiazem

Formulation code	Name of polymer	Ratio of drug/polymer	Amount of drugs (mg)
C1	EVA (VA 40%) copolymer	10:90	160.00
C2	EVA (VA 40%) copolymer	20:80	200.00
C3	EVA (VA 40%) copolymer	30:70	342.85
C4	EVA (VA 40%) copolymer	40:60	533.33
C5	EVA (VA 40%) copolymer	50:50	800.00

Physiochemical evaluation of polymeric matrix device

Prepared Diltiazem containing matrices were evaluated for various parameters like thickness , weight variation, drug content, flatness, folding endurance , moisture content, moisture absorption % WVTR etc, as per procedure given in the chapter 5, section 5.3.2.

In vitro diffusion profile

In vitro diffusion study of different matrices containing Diltiazem was carried out as per the procedure given in chapter 5, section 5.4.1.

Data Analysis

Q, cumulative amount of Diltiazem released into infinite per unit surface area of device was plotted as a function of square root of time for each disc. Release rate Q/√T was computed for each disc from simple regression analysis of steady state data**.** The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles.

5.5.2 Result and Discussion

The present investigation deals with the development of Diltiazem base polymeric matrices using different concentration of drug and polymer. This preliminary screening was carried out to for the selection of best matrices. A diffusion mediated matrix controlled transdermal drug delivery system for Diltiazem base was successfully prepared using different drug and polymers ratio using mercury subtract method and all matrices were evaluated using different physiochemical parameters.

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of films was measured and the average thickness was noted. The thickness results were given in **Table 5.5.2**.

The results indicate that there was no much difference in the thickness with in the formulations. Thickness in the different formulations was in the range of **216.66 ± 2.88 µm** to **87.5 ± 2.5 µm**. Maximum thickness was found in formulation F6, while minimum found in formulation F1.

These results revealed that thickness was found to increase as drug concentration increases. The thickness results also indicate uniform distribution of the drug and polymer over the mercury surface. The thickness of each film was determined and decrease in thickness of various transdermal drug delivery system were found to be of the order of

C5 > C4 > C3 > C2 > C1

Table 5.5.2: Results of thickness uniformity of C1 to C5 matrix formulations

Sr. No.	Formulation Code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	85.0	90.0	87.5	87.50 ± 2.500
2	C2	117.5	117.5	120.0	118.33 ± 1.443
3	C3	145.0	150.0	147.5	147.50 ± 2.500
4	C4	170.0	170.0	172.5	171.66 ± 1.443
5	C5	215.0	215.0	220.0	216.66 ± 2.886

***Standard deviation, n=3**

Weight variation

Drug loaded films (3.14cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 5.5.3 The weight of 3.14 cm^2 film ranged from **32.46 ± 0.152 mg** to **53 ± 0.100 mg**. The weight of the patches was found to be uniform among different batches.

Table 5.5.3: Results of weight variations of C1 to C5 matrix formulations

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	40.2	40.0	40.4	40.20 ± 0.200
2	C2	44.5	44.6	44.4	44.50 ± 0.100
3	C3	47.0	47.2	47.0	47.06 ± 0.115
4	C4	53.0	52.0	51.5	52.16 ± 0.763
5	C5	54.0	55.0	54.0	54.33 ± 0.577

***Standard deviation, n=3**

In a weight variation test, the pharmacopoeial limit for the percentage deviation of all the films of less than mg is ± 10%. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for weight variation as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Diltiazem in the film formulations. Hence it was

concluded that drug was uniformly distributed in all the formulation, with small deviation. The results also showed that as the concentration of Diltiazem increases weight of matrices also increases. The weight of each film was determined and decrease in weight of various transdermal drug delivery system were found to of the order of **C5 > C4 > C3 > C2 > C1**

Drug content

Drug content of the matrices was carried out to ascertain that the drug is uniformly distributed in the formulation. The results obtained are represented in Table 5.5.4.

Table 5.5.4: Results of % Drug content of C1 to C5 matrix formulations

Sr. No.	Formulation Code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	97.80	98.90	99.02	98.57 ± 0.672
2	C2	101.50	101.20	101.00	101.23 ± 0.251
3	C3	99.02	97.80	98.90	98.57 ± 0.672
4	C4	98.57	96.52	98.53	97.87 ± 1.172
5	C5	101.20	100.05	101.23	100.82 ± 0.672

The films were found to contain 97.57%-101.23% of the labeled amount of Diltiazem indicating uniformity of drug content. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for content uniformity as per official requirements. From the results obtained, it was clear that there was proper distribution of Diltiazem in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation, with acceptable deviation.

Flatness

The flatness was measured manually for the prepared films. The results of the flatness study showed that none of the formulations had the differences in the strip length before and after their cuts. It indicates 100% flatness observed in the formulated patches. Thus, no amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the patches and thus they could maintain a

smooth surface when applied on to the skin.

Folding endurance

Folding endurance was determined manually for drug loaded polymeric matrices. The results of folding endurance are given in Table 5.5.5.

Table 5.5.5: Results of folding endurance of C1 to C5 film formulations

Sr. No.	Formulation code	Folding endurance			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	506	504	502	504.00 ± 2.000
2	C2	446	445	448	446.33 ± 1.527
3	C3	381	378	380	379.66 ± 1.527
4	C4	248	246	250	248.00 ± 2.000
5	C5	184	182	185	183.66 ± 1.527

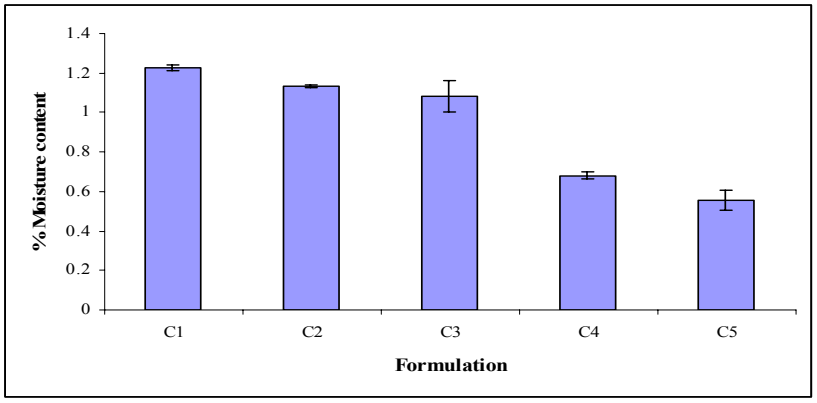
***Standard deviation, n=3**

Here formulation C1 and formulation C2 showed good folding endurance as compare to formulation C4 and C5. As the concentration of drug increases and polymer concentration decreases the folding endurance decreases and patch becomes more friable.

Moisture content (Loss on drying)

The moisture content was determined by keeping the drug matrices patches in dessicator containing activated silica for 24h. The percentage moisture content was calculated from the weight differences relative to the final weight. The results of the moisture content studies for different formulations are shown in Figure 5.5.1.

Figure 5.5.1: Percentage moisture content of C1 to C5 matrix formulations



*** Standard deviation, n=3**

The moisture content in all the formulations was found to be low and ranged from **0.556 ± 0.052** to **1.225 ± 0.013%.** The result revealed that the moisture content was found to decrease with increasing concentration of Diltiazem base. The small moisture content in the formulations helps them to remain stable and from being a completely dried and becoming brittle and friable. The rank order of decrease moisture content of various transdermal drug delivery systems are given below.

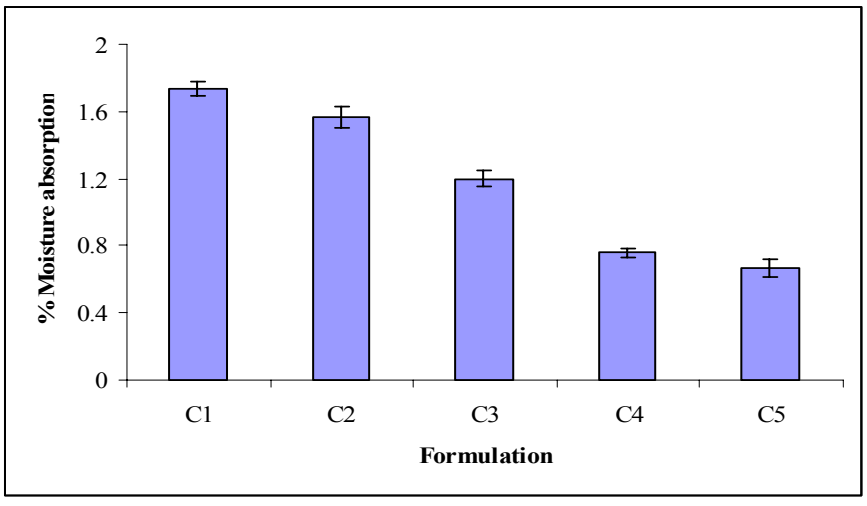
C1 > C2 > C3 > C4 > C5

Moisture absorption

The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The results of the moisture absorption studies for different formulations are shown in Figure 5.5.2.

The moisture absorption in all the formulations was found to be low and ranged from **0.6551 ± 0.0548** to **1.7361 ± 0.0453%.** The result revealed that the moisture absorption was found to decrease with increasing concentration of hydrophobic Diltiazem base. The % Moisture absorption of each film was determined and decrease in % moisture absorption of various transdermal drug delivery system were found to of the order of **C1 > C2 > C3 > C4 > C5**

Figure 5.5.2: Percentage moisture absorption for C1 to C5 matrix formulations



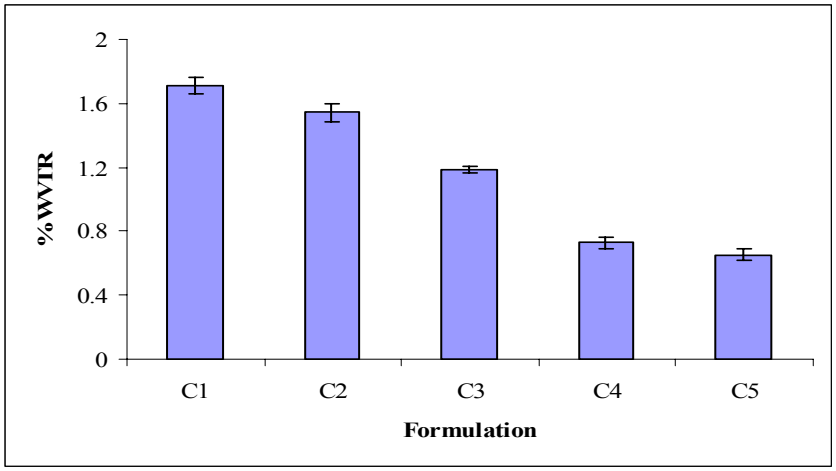
*** Standard deviation, n=3**

Water vapor transmission rate (%WVTR)

The water vapor transmission rates of different formulation were evaluated and the

results are shown in Figure 5.5.3.

Figure 5.5.3: % WVTR of C1 to C5 matrix formulations



*** Standard deviation, n=3**

The % water vapor transmission rate of each film was determined and decreases % water vapor transmission rate of various transdermal drug delivery system were found to be in the order of **C1 > C2 > C3 > C4 > C5.**

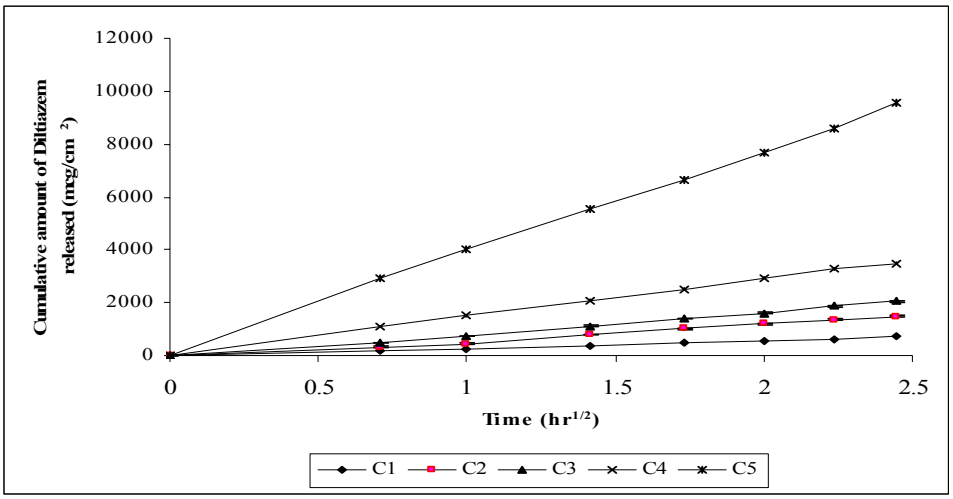
In vitro diffusion profile

Diffusion studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. In vitro release profile is an important tool that predicts in advance how the drug will behave in vivo [25]. Diffusion studies for different formulations were performed using modified Keshary-Chien diffusion cell using 0.01N HCl as a diffusion medium at 37 ± 0.5 ^{0}C. Cumulative amount of Diltiazem released from the device into the diffusion medium was plotted against square root of time for each disc subjected under diffusion study. Release rates Q/√T were derived from simple regression analysis of steady state diffusion data. The results of in vitro drug diffusion study for transdermal patches are depicted in Table 5.5.6 and Figure 5.5.4

Table 5.5.6: In vitro diffusion profiles of Diltiazem from EVA (VA 40%) copolymer polymeric matrices

Time (hr ^{½})	Cumulative amount of drug release from device (µg/cm^2)				
	Formulation code				
	C1	C2	C3	C4	C5
0.707	155.40 ± 5.75	333.70 ± 10.44	497.60 ± 9.43	1105.51 ± 8.54	2900.60 ± 30.54
1	250.10 ± 8.33	452.70 ± 11.53	743.49 ± 12.32	1499.85 ± 15.43	4007.65 ± 49.45
1.414	367.50 ± 10.43	779.20 ± 15.33	1126.83 ± 14.53	2099.60 ± 20.43	5567.66 ± 59.23
1.732	465.23 ± 15.34	1020.23 ± 20.54	1389.56 ± 18.30	2525.36 ± 25.32	6656.47 ± 78.34
2	560.23 ± 20.43	1195.23 ± 21.43	1598.63 ± 25.32	2940.36 ± 26.32	7645.12 ± 85.34
2.236	625.36 ± 22.54	1365.89 ± 22.75	1870.56 ± 26.30	3265.48 ± 30.53	8575.15 ± 99.23
2.449	701.45 ± 25.66	1490.23 ± 31.43	2055.45 ± 30.35	3500.23 ± 40.43	9540.23 ± 99.34
Q/√T µg/cm^2√hr	**310.95**	**690.61**	**891.44**	**1397.10**	**3749.10**
Correlation coefficient	**0.9992**	**0.9966**	**0.9986**	**0.9993**	**0.9991**

Figure 5.5.4.: In vitro diffusion profiles of Diltiazem from EVA (VA 40%) copolymer polymeric matrices



*** Standard deviation, n=3**

Release rate Q/√T increased with increasing concentration of Diltiazem base in EVA (VA 40%) matrix. In our experiments, variable release profiles of Diltiazem from the different experimental patches composed of various proportion of drug and polymers were observed. Cumulative amount of drug diffused per square centimeter of patches,

into the diffusion medium when plotted against square root of time, showed linear relationship. Regression analysis was done to calculate Q/√T release rate. The rank order of release rate observed was

C1 < C2 < C3 < C4 < C5

The formulation **C5** exhibited the maximum Q/√T (**3749.10 µg/cm^{2}h$^{1/2}$**) release rate, which were significantly different, compared to the lowest values in the formulation C**1** (**310.95 µg/cm^{2}h$^{1/2}$**). Based on physiochemical characteristics and in vitro release experiments, formulation **C4** may be chosen for further in vitro permeability study through human live skin.

In vitro release kinetic

The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 5.5.7. Here R is regression coefficient.

Table 5.5.7: Data of various parameters of model fitting of C1 to C5 formulations

Formulation	Zero order equation	First order equation	Higuchi's equation
C1	**0.9897**	**0.9400**	**0.9987**
C2	**0.9720**	**0.8904**	**0.9979**
C3	**0.9877**	**0.9334**	**0.9979**
C4	**0.9785**	**0.9308**	**0.9988**
C5	**0.9904**	**0.9486**	**0.9985**

In our experiment, the in vitro permeation profiles of all formulations could be best expressed by **Higuchi's equation** (R^2 = 0.9979 to 0.9988) for the permeation of drug from a homogeneous- polymer matrix type delivery system that depends mostly on diffusion characteristics.

5.6 IN VITRO PERMEATION STUDY OF MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF DILTIAZEM ACROSS HUMAN LIVE SKIN

5.6.1 Treatment and preparation of skin for permeation study

Skin covers the entire external surface of the human body, representing the largest single organ. The integument acts as a protective barrier from environmental insults including trauma, radiation, harsh environmental conditions and infection.

Human live skin was collected from unused portion of human male patients from private hospital with consent of plastic surgeon and patient, who have no problem regarding reactions to medicines or problems of breathing. Healthy skin is taken from a place on patient's body called the donor site. Common sites for the collection of skin graft include the upper anterior and lateral thighs. Most people having a skin graft have a split-thickness skin graft. If the entire thickness of the dermis is included, the appropriate term is full-thickness skin graft (FTSG). If less than the entire thickness of the dermis is included, this graft is referred to as a split-thickness skin graft (STSG). STSGs are categorized further as thin (0.005-0.012 in), intermediate (0.012-0.018 in), or thick (0.018-0.030 in), based on the thickness of the harvested graft. For study of controlled transdermal drug delivery system skin site is required.

Regardless of technique, adequate anesthesia must be established because harvesting of skin grafts is a painful procedure. Lidocaine with epinephrine injected at the donor site may reduce blood loss and provide greater tissue turgor that assists in harvesting. Here surgery will probably be done while volunteers are under general anesthesia (unconscious and will not feel pain). In order to remove the thin and well preserved skin slices and stripes from the donor, surgeons use a special surgical instrument called a dermatome. This usually produces a split-thickness skin graft, which contains the skin with only a portion of the dermis.

Dermatomes are typically air-powered or electric, although manually operated devices exist. Commonly used dermatomes include the Padgett and Zimmer, among others. All of these dermatomes harvest with a rapidly oscillating blade. The thickness is easily adjusted on the instrument. The width is typically adjusted in 1- to 2-inch

increments by applying blade guards of different widths.

A surgeon was familiar with the installation of the blade and depth settings and must check these before operating the device. Donor site was washed off with Betadine or other agent to prepare the donor site to allow the device to easily slide over the skin. Then skin was lubricated using mineral oil so dermatome allows easy gliding of the dermatome. The dermatome was held in the dominant hand of the operator at a 30-45° angle from the donor skin surface. With the non operating hand providing traction behind the dermatome, the assistant provides traction in front of the dermatome to help stretch and flatten the skin. The dermatome was runned when it engages the skin surface and then advanced in a smooth continuous motion over the skin with gentle downward pressure. After an appropriate length has been harvested, the dermatome is tilted away from the skin and lifted off of the skin to cut the distal edge of the graft and completed the harvesting.

The graft may then be gently washed of lubricant. The donor-site area is covered with a sterile dressing for 3 to 5 days. Collected skin graft was washed with saline to remove blood and other material and then kept in saline aseptically. This skin graft stored in a refrigerator at 4 - 5 ^{0}C until further used.

Figure 5.6.1: Dermatome

Figure 5.6.2: Dermatome blade guards

Figure 5.6.3: Split-thickness skin graft

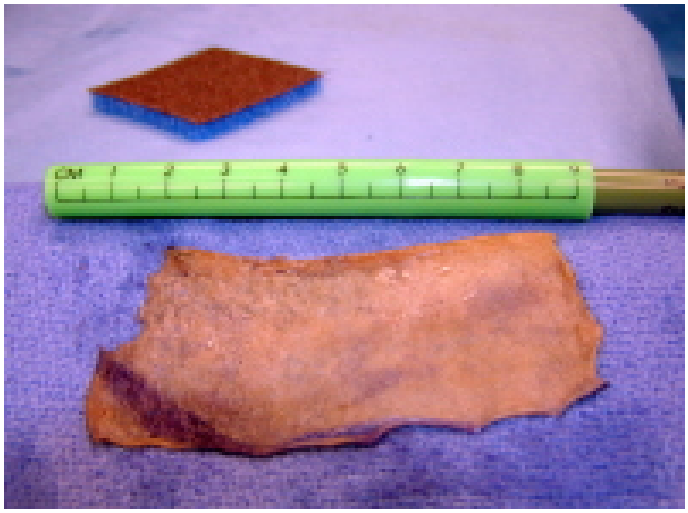


Figure 5.6.4: Skin graft donor site 8 days after the skin was taken

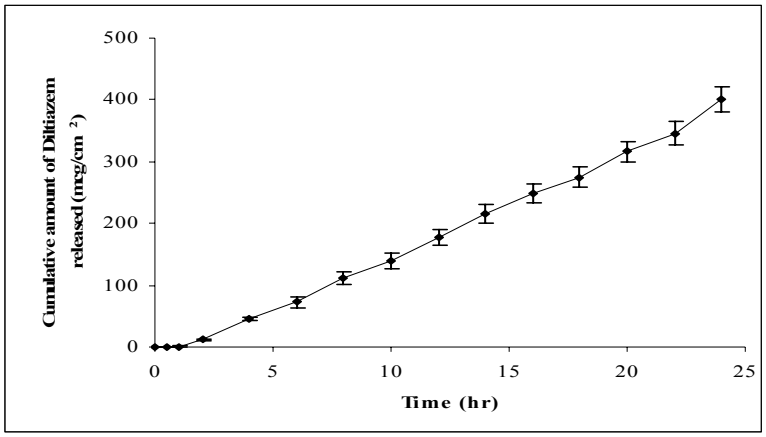
5.6.2 In vitro permeation study through human live skin

Permeation study was performed in a modified Keshary-Chien diffusion cell. The permeation study was performed using human live skin. The skin was used after fulfilling all the ethical requirements. Skin was kept at room temperature in saline and then washed with soap solution to remove adhering matter. A section of skin was cut having thickness 140µm and placed on the brim of diffusion cell in such a way that the dermal side of the skin was in the donor compartment and patch was affixed on the skin so that aluminum backing was upward. The receiver compartment was filled with 40 ml 0.01 N HCl. The transdermal patch containing Diltiazem: EVA (40:60) with backing membrane was firmly pressed on the human skin. To perfectly fix the release face of patch on the skin, the release face was covered with a very thin layer (10µm) of natural rubber solution. Once the adhesion to the skin surface was confirmed, flange of the diffusion cell mounted in such a way that the patch was situated precisely over the flange aperture. The whole assembly was kept on a magnetic stirrer and diffusion medium in the receiver compartment was constantly and continuously stirred using a magnetic bead. Samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at different time intervals till 24 hrs. The absorbance of the withdrawn samples were measured using UV VIS spectrophotometer at 236.0 nm using 0.01N HCl as a blank. Cumulative amount of drug released per square centimeter of patch were plotted as function of time. The release rate was determined by simple regression analysis of steady state data. The experiments were triplicated and mean release rate was recorded.

5.6.3 Results and discussion

In vitro permeation study is predictive of in vivo performance of a drug. The result of in vitro skin permeation of Diltiazem from selected formulation C4 are shown in Figure 5.6.5

Figure 5.6.5: In vitro skin permeation profile of Diltiazem from Diltiazem:EVA (40:60) matrix patch through human live skin



*** Standard deviation, n=3**

Various parameters of diffusion kinetics of Diltiazem released from device across human live skin are presented in Table 5.6.1. The parameters listed in this table are useful for biopharmaceutics and pharmacokinetics of the matrix diffusional system evaluated.

Table 5.6.1: Parameters of diffusion kinetics of Diltiazem from Diltiazem:EVA (VA 40%) co-polymer(40:60) matrix patch through human live skin

Sr. No.	Parameters	Value
1	Skin flux (Jss)	16.26 µg/ cm^2 hr
2	Time lag (t_L)	1.63 hr
3	Skin thickness (µm)	140 µm
4	Diffusion coefficient	2.004 x 10^{-5} cm^2/sec
5	Solubility of drug in skin (Cs)	11.36 mg/cm^3

The time lag (t_L) for devices is presented in Table 5.6.2. The average diffusion coefficient, D of Diltiazem was determined using $D = h^2/6\ t_L$ relationship, where Skin thickness h was 140 x 10^{-4} cm. The amount of Diltiazem retained by skin area used for permeation was calculated by dividing steady state flux with gradient of diffusivity and it was found to be **11.36 mg/cm^3** of skin. The amount of Diltiazem retained in skin need to be incorporated in contact adhesive to serve as a prompt dose (priming dose) before steady state is established. The release of Diltiazem from

matrix diffusional system into infinite sink can be approximated by equation

Release rate = $Q/\sqrt{T} + H_e^{-Kt}$

The first term on right hand side of the equation represents the time dependent delivery rate i.e Q vs. √T pattern. The second term represents the temporal pattern of drug release during priming dose period.

Comparison of permeation kinetics: Patch without priming Dose: Patch with priming Dose

The release rate (µg/hr) has linear relationship with area of release face of a transdermal rug delivery system. The final patch needs to be provided with adhesive system and priming dose. To assess release kinetic of a complete patch, a patch of 3.14 cm^2 area was provided with a peripheral adhesive system (4 mm rim).from the data of solubility of drug in skin, a 0.216 % w/v Diltiazem in natural rubber solution was used as an adhesive system. This solution was uniformly layered in 4 mm rim surrounding 3.14 cm^2 area of patch, to obtain 10 µm thick adhesive systems containing priming dose of 159 µg/cm^2 area of delivery device. The device was firmly secured on human live skin and was subjected to diffusion experiment using 0.01 N HCl as diffusion medium. The release profile is shown in **Table 5.6.2** and **Figure 5.6.6.**

Figure 5.6.6: In vitro skin permeation profile of Diltiazem from matrix patch through human live skin with priming dose and without priming dose

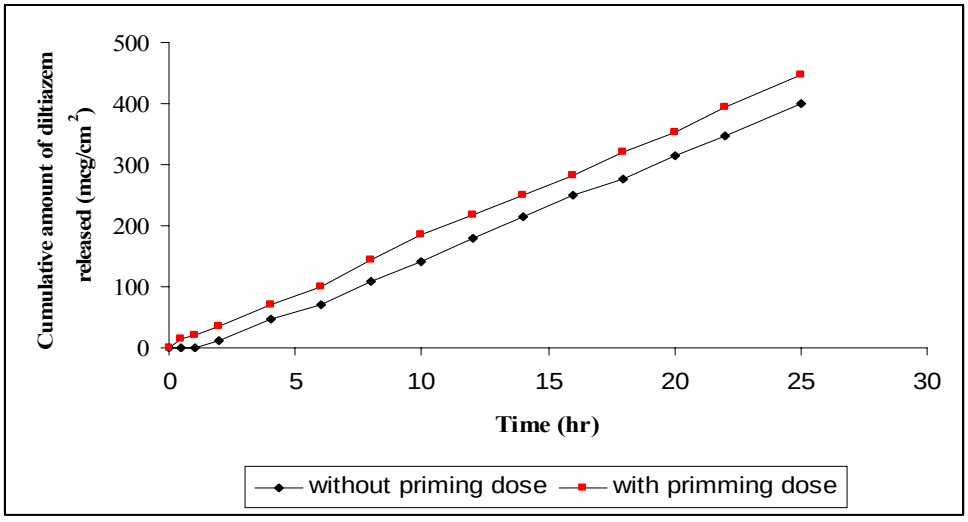


Table 5.6.2: Parameter of in vitro skin permeation kinetics of Diltiazem from matrix patch through human live skin with priming dose and without priming dose

Parameters	Cumulative amount of drug release (µg/cm^2) Without priming dose	Cumulative amount of drug release (µg/cm^2) With priming dose
Release rate (µg/cm^2 hr)	16.26	18.24
Correlation coefficient	0.9985	0.9986
Time lag (t_L)	1.63 hr	1.03 hr

Computation of desired release rate (in vivo input) for target steady state plasma concentration of drug

For Diltiazem $t_{1/2}$ = 7 hrs, V_d = 2.0 litres/Kg and targetted steady state plasma concentration (Css) = 40 ng/ml and therefore the desired drug release can be calculated as follows.

In vivo input = in vivo output

$= C_{ss} * V_d * K_e * 70$

= 40 ng/ml * $2x10^3$ ml/kg * 0.693/7 * 70 kg

= 554.4 µg/hr

Desired release rate = 554.4 µg/hr

Computation of area of patch required for target steady state plasma concentration (Css)

In vivo input = in vivo output = 554.4 µg/hr

$J_{ss(skin)}$ x A = $C_{ss} * V_d * K_e * 70$ = 554.4 µg/hr

Area of patch = (554.4 µg/hr)/ $J_{ss(skin)}$

= 554.4/18.24 cm^2

= 30.4 cm^2

Formulation of final patch

Final formulation was selected as 24 hour once a day device having area is 30.4 cm^2. This formulation was provided with priming dose incorporated in natural rubber adhesive as follow.

The amount of Diltiazem retained in skin need to be incorporated in contact adhesive to serve as prompt dose (priming dose). For preparation of final matrix diffusion drug delivery device containing peripheral adhesive system **0.216 % w/v** Diltiazem in natural rubber solution is to be used as an adhesive system **0.62 ml** of this solution was layered over **8.29 cm^2** (4 mm) rim area of delivery device giving a 10µm thick layer, providing a priming dose of **159 µg/cm^2** area of delivery device. The release of Diltiazem from adhesive formulated for final device into infinite sink followed first order kinetics with rate constant 0.114 hr^{-1}.

5.7 STABILITY STUDY

5.7.1. Stability study of matrix diffusional transdermal drug delivery device

Stability is defined as the ability of particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic and toxicological specification. Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to the product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. The objective of accelerated stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature**.** Finally selected and optimized matrix diffusional transdermal drug delivery system of Diltiazem was subjected to stability study.

The accelerated stability study was carried out according to ICH guideline by storing the samples at 25 ^{0}C / 60% RH, 30 ^{0}C/ 65% RH and 40 ^{0}C/ 75% RH for 90 days in a stability chamber (Thermo Lab., Mumbai, India). These samples were analyzed UV Spectrophotometerically and checked for changes in physical appearance and drug content at an interval of 15 days**.**

5.7.2 Results and Discussion

Formulation **F4** was selected for stability study and observed for change in color,

appearance, flexibility and drug content. Temperature and humidity values selected were as per the ICH guidelines and the test was carried out in a stability chamber. The stability study was carried out at 25 ^{0}C / 60% RH, 30 ^{0}C/ 65% RH and 40 ^{0}C/ 75% RH for 90 days. Diffusion study was carried out and it was observed that formulation stored at 40 ^{0}C exhibited higher Q/√T release rate as compared to those stored at 25 ^{0}C and 30 ^{0}C. The release rate at 30 ^{0}C was altered but it was in order. The product stored at 25 ^{0}C exhibited no change in release rate.

Drug degradation study was carried out as per ICH guideline at above mentioned physical condition of temperature and humidity. Periodic samples were subjected to drug content analysis. The % retained in device was worked out and from the plot of log % retained versus time degradation rate constant was computed for 25 ^{0}C, 30 ^{0}C and 40 ^{0}C temperatures. The degradation was higher at an elevated temperature. The first order rate constant of degradation for room temperature was **1.105 x 10^{-3} $week^{-1}$**. The self life calculated was **95 week**.

Results of stability study indicated a good stability for laminate (matrix) containing drug delivery device. The results of stability study indicated that the products should not be stored at an elevated temperature and also should not be refrigerated as at lower temperature transdermal patches lost overall flexibility and turned rigid loosing elegancy. The product should be stored at room temperature.

5.8 SKIN IRRITATION AND SKIN SENSITIZATION STUDY

5.8.1 Experimental

Selection criteria for indication for skin irritation

Skin irritation and skin sensitization though are different types of physiological responses yet they have several common indications. Skin sensitization is systematic response and skin irritation is primarily is local response.A protocol was devised for evaluation of skin irritation and/or sensitization in such a manner that the signs at the sight of application would be assessed in common for the both and further, to distinguish sensitization from irritation; additional observation has been made. The assessment of signs and selected criteria was such that it itself indicated differences in irritation and sensitization.

The signs and selected criteria as indication for skin irritation and sensitization are as follows:

1. Erythema	**Score**
• No redness or barely perceptible	0
• Slight redness, spotty or diffuse	1
• Moderate uniform redness, well defined erythema	2
• Intense redness (moderate to severe erythema)	3
• Severe erythema (fiery red)	4

2. Scaling	**Score**
• Absent totally	0
• Fine	1
• Moderate	2
• Severe with large flakes	3

3. Fissures	**Score**
• No fissure seen	0
• Fine cracks	1
• Single or multiple boarder fissures	2
• Fine cracks with hemorrhage or exudation	3

4. Oedema formation	**Score**
• Oedema absent totally	0
• Very slightly oedema (barely perceptible)	1
• Slight oedema(edges of area well defined by definite raising)	2
• Moderate oedema (area raised approx 1 mm)	3
• Severe oedema (area raised more than 1 mm and extending beyond the area of exposure)	4

5. Ecchymosis (Hemorrhage in to skin or bruising)	**Score**
• Absent	0
• Very slight hemorrhage mainly aggravated by shaving	1
• Clear sign of hemorrhage	2
• Spreaded hemorrhage and clearly perceptible bruising	3

- Severe bruising and hemorrhage 4

6. Necrosis (Areas of dead skin) **Score**

- Absent 0
- Little exudates but no part of dead skin seen 1
- Exudates and clear regions containing layers /flaked dead skin 2

Study plan

The study plan for evaluating skin sensitization and irritation was made using common where critical assessment indicated whether chemical sensitizing or irritating or safe. The sensitization included induction period following a short sensitization period giving allergic reaction at the application site; this has been discussed under literature review. Once this sensitivity was established, the subsequent exposure to the chemical will lead to higher response both locally (at the site of application) and systematically due to secondary immune reaction (hypersensitivity). A 28 days protocol had been made which is divided in to the following phases.

1. Pre-exposure period (2 days)
2. Induction phase (5 days)
3. Rest (12 days)
4. Challenge phase (till 28 days)

The protocol is presented in Table 5.8.1

Table 5.8.1: A typical protocol of irritation and sensitization study.

SR. NO.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																					
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE									
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
2	Scaling																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
3	Fissures																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
4	Oedama formation																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
5	Ecchymosis																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
6	Necrosis																										
		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg		Avg	
Total of all score																											
Total average per animal																											

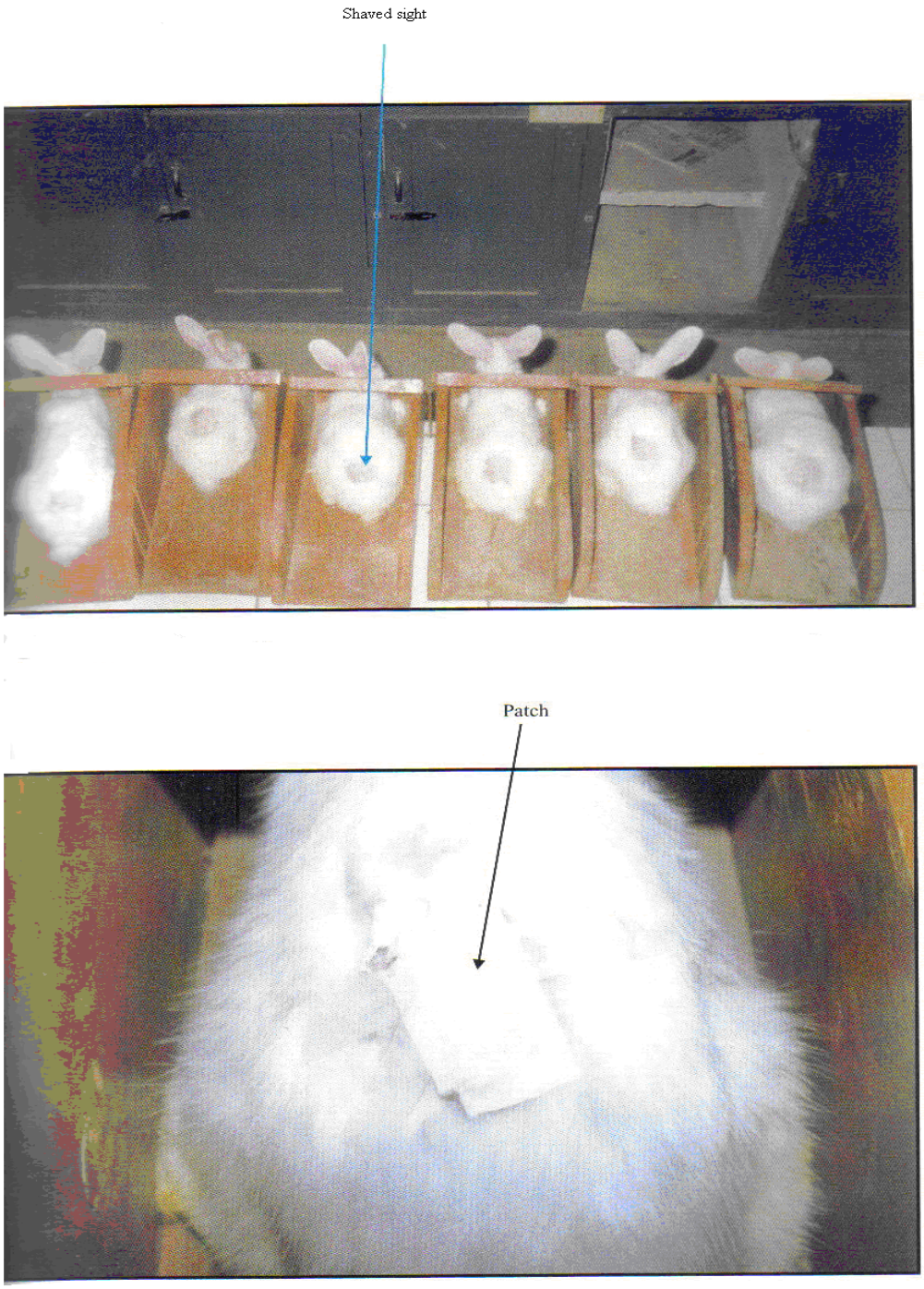


Figure 5.8.1(a): Cutaneous toxicity studies on albino white rabbits

Figure 5.8.2(b): Demonstration of patch application during cutaneous toxicity study

Shaved site for patch application

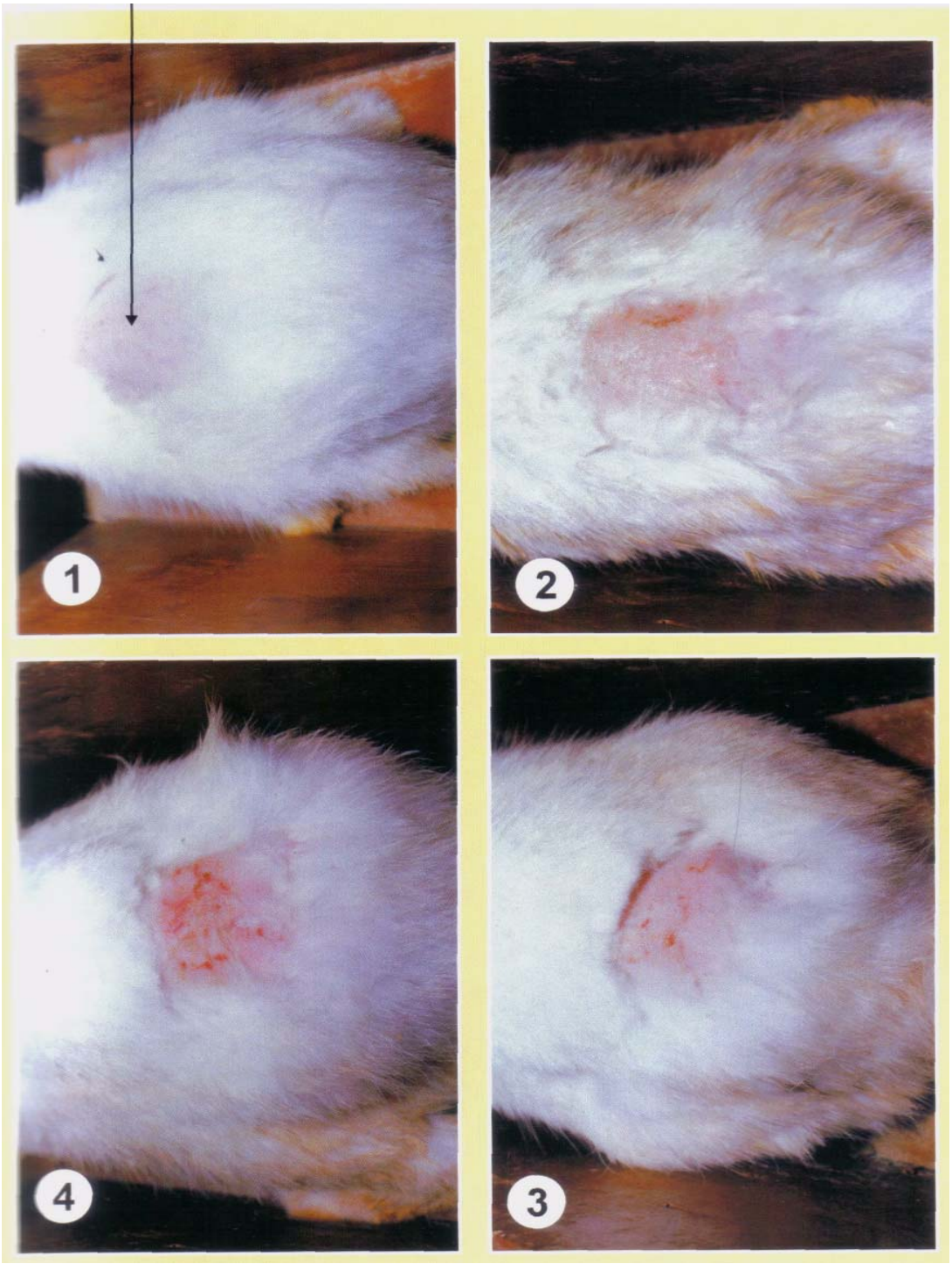


Figure 5.8.2: Various scores of erythema

Application time was specified for each day and observations were taken at specified days score of each sign was entered in the respective cells of the table.

Selection of animal species

New Zealand white rabbit were selected due to following reasons.

1. Availability of large surface area of skin compared to guinea pig and mouse.
2. Ease in hair removal at the back
3. It was observed that the rabbits were less interested to remove the patches applied on to them comparing to rats.
4. Comparative ease in handling the species than the rat and the guinea pig.
5. Albino rabbit is species of choice in the skin irritation testing. [31,32]

Methodology involved in cutaneous toxicity study

Cutaneous toxicity comprised critical steps involving skills like invasion free shaving, selecting a site which is comfortable and beyond the reach of the limbs of animal and time management on account of large numbers of samples to be investigated with six parameters for almost a month.

1. Shaving and selection of site of drug application

According to protocol one set of animal contained, four New Zealand white rabbits. The back of six rabbits was shaved with fresh Gillete® 7 O' Clock super platinum blade is quite satisfactory non-invasive technique. Finally, four rabbits having best shaved sites were selected out of six, to carry out study. This was done to omit any chance of interference of invasion and irritation signs.

2. Toxicity application patch

Normal saline was used for the preparation of control patch. A 0.5 ml sample of the control article was then applied to site by introduction under a double gauze layer to an area of skin approximately 2.0 cm^2. The concentration of NaCl was 0.9 %w/v in distilled water. This was applied to the skin surface (shaved) and made adhered with the help of medical adhesive tape (entirely covered) from 3 M company product named "3 M Micropore". Then animals were returned to their cages. After 24 hr exposure, the article was removed. The test sites were wiped with tap water to remove

any remaining test article residue.

The final Diltiazem matrix patch (2.2 cm^2) was supported with aluminum foil. This was applied to the skin surface (shaved) and made adhered with the help of "3 M Micropore"- medical adhesive tap (3M, Corporation, U.K.).

5.8.2 Results and discussion

The sensitization included induction period following a short sensitization period giving allergic reaction at the application site. Once this sensitivity was established, the subsequent exposure to the chemical would lead to higher responses both locally (at the site of application) and systematically due to secondary immune reaction (hypersensitivity).

Sensitization testing

The protocol gave provisions for refractory period as induction phase, secondary a exposure as challenge phase. Following observations would indicate the agent as a sensitizer.

1. If score of respective sign has increased during the days when the patch is not applied.
2. Any exaggerated response during challenge phase.
3. Any systemic response in terms of decreased activity, fever tremor or any other unusual symptoms. (giving indication of systemic reactions which is characteristics of sensitivity)

Irritation testing

The same protocol employed for sensitization studies performed irritation evaluation. The limitation was of assessing sensitization testing first, because if chemical or agent was recognized as sensitizer and then separate study for irritation assignment was needed. In such case, separate study is not recommended since the chemical would be discarded earlier if it was found to be sensitizer. Hence forth, the question was of assessing the agent whether a local irritant after establishing it non-sensitizing. Therefore, one common protocol can judge whether a non-sensitizing agent was producing irritation or not following observations would indicate the agent as an irritant

1. If the score of each sign decreased slightly or not increases during zero application days, but the same increases with repeated application.
2. Elevated scores showed decrease in rest phase.
3. Scores started decreasing after 1st day of induction phase.
4. Scores elevated at day 19, but decreased till day 28.

Control patch (Normal saline)

Normal saline is not irritant or sensitizing in nature. However, the study for normal saline was incorporated so that any response due to animal variation or irritation due to shaving (non-chemical method) should be investigated. Table 5.8.2 shows 28 days study of irritation and sensitization for normal saline. Except scaling no other scores for signs of irritation were zero. At the end of 28 days score for each sign was zero except necrosis and oedema formation. Therefore, protocol indicated control as a safe (non-sensitizing and non-irritating) which (n-saline) otherwise is also safe. Inversely this was also proved that our protocol was systematic and designed in proper manner. This result was useful during study of other drugs. Score 1 for any individual sign for individual animal was not irritating and its symptoms have been explained earlier in experimental section. Though for scaling, score 1 (fine) for individual animal has not come in study of control, otherwise also it has been considering irritating.

Table 5.8.3: The average score for each sign after day 20 and day 28 during irritation and sensitization study of normal saline

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.5	0.0
2	Scaling	0.0	0.0
3	Fissures	0.5	0.0
4	Oedema formation	1.0	0.5
5	Ecchymosis	0.0	0.0
6	Necrosis	0.5	0.25

Table 5.8.2: Skin irritation and sensitization study using 0.9 % NaCl as a control

SR.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE							
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0
		0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0	Avg	0.5	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0	Avg	0
2	Scaling	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0
3	Fissures	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	0	Avg	0
4	Oedama formation	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0
		0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	1	Avg	.75	Avg	.5	Avg	.25
5	Ecchymosis	1	0	1	1	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
		1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0.5	Avg	1	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0
6	Necrosis	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	1
		0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	.5	Avg	.25
Total of all score		2		4		2		9		9		6		6		2		0		9		8		4		2	
Total average per animal		0.5		1		0.5		2.25		2.25		1.5		1.5		0.5		0		2.25		2		1		0.5	

Study of Diltiazem matrix patch

The results of skin irritation and skin sensitization study for Diltiazem matrix patch is presented in Table 5.8.4. Following inferences has been drawn from irritation and sensitization studies of Diltiazem matrix diffusional transdermal drug delivery system.

From Table 5.8.5, it was clear that the Diltiazem was non irritating since the maximum score for each sign for given concentration of drug was not more than 1, though the number of animals giving score 1 for the various sign was increased. However, score 1 is not considered under irritating category therefore Diltiazem patch was considered as safe. Though it does not show average score more than 1 at day 20 but this indication solely cannot be taken as a sign of non-irritating. The reason for this was that individual animal show score more than 1, but the average was less than 1 (which means that some animals showed score 0, out of four animals).

Diltiazem is non- sensitizing in nature. The reason for this was reduction in various scores even after repeated application. All the tables' indication and total score irritating scores were reduced from day 20 to day 28. Therefore, the primary and secondary sensitization was absent.

Table 5.8.5: The average score for each sign after day 20 and day 28 during irritation and sensitization study of Diltiazem matrix patch

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.75	0.25
2	Scaling	0.5	0.25
3	Fissures	0.5	0.25
4	Oedema formation	0.5	0.0
5	Ecchymosis	0.25	0.0
6	Necrosis	0.5	0.25

Table 5.8.4: Skin irritation and sensitization study of Diltiazem matrix patch

SR. NO.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE									
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
		Avg	0	Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	.75	Avg	.75	Avg	0.5	Avg	0.5
2	Scaling	0	0	0	1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
		Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0.5	Avg	.75	Avg	.75	Avg	.25
3	Fissures	0	0	0	0	0	0	1	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25
4	Oedama formation	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
		1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	.25	Avg	0
5	Ecchymosis	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
		1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	0	1	1	1	1	0	0
		Avg	0.5	Avg	.75	Avg	1	Avg	1	Avg	1	Avg	.75	Avg	.75	Avg	.25	Avg	.25	Avg	.25	Avg	0.5	Avg	0.5	Avg	0
6	Necrosis	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
		0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
		Avg	.25	Avg	.25	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25
Total of all score		5		7		11		12		13		10		8		4		2		12		14		12		5	
Total average per animal		1.25		1.75		2.75		3		3.25		2.5		2		1		0.5		3		3.5		3		1.25	

Table 5.8.6: Maximum sensitization and irritation score of various signs at last day for Diltiazem matrix patch

Sr. No.	Sign	Score	N
1	Erythema	**1**	**1**
2	Scaling	**1**	**1**
3	Fissures	**10**	**1**
4	Oedema formation	**0**	**4**
5	Ecchymosis	**0**	**4**
6	Necrosis	**1**	**1**

5.9 CONCLUSION

EVA (VA 40%) copolymer matrix modulated transdermal drug delivery system of Diltiazem has been prepared successfully. Among different polymers evaluated EVA (VA 40%) copolymer gave a medicated matrix, which was stable, non irritant and non sensitizing to skin and was safe. It also complied with official and non official pharmaco-technical specification. The matrix device evaluated for Diltiazem release in vitro into infinite sink and across human live skin, enabled to provide adequated rate of Diltiazem, meeting requisite pharmacokinetic requirement of steady state plasma concentration for 24 hours, giving once a day drug delivery system.

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Chapter 6

Preparation and characterization of memebrane moderated reservoir type transdermal drug delivery device of Diltiazem

CHAPTER 6

6. PREPARATION AND CHARACTERIZATION OF MEMBRANE MODERATED RESERVOIR TYPE TRANSDERMAL DRUG DELIVERY DEVICE OF DILTIAZEM

AIM OF PRESENT INVESTIGATION

Polymers have officially entered the field of pharmacy for design of novel drug delivery systems. Polymers are the key ingredients in drug delivery systems where they are mainly used to deliver the drug in predetermined fashions and in controlled manner. They are used either as carriers of medicament i.e. matrix or as a barrier to moving drug molecules i.e. membrane. In either case the rate of release from dosage form can be controlled and in this aspect the delivery systems differs from the conventional dosage forms.

For conventional administration the size of the dosage form is usually influenced by the dose of the drug while in case of novel, sustained and controlled drug delivery systems size of the dosage form and hence the elegancy of dosage form is influenced by the pharmacokinetic aspect i.e., steady state blood concentration or target concentration desired which in turn will be influenced by the amount of drug delivered per unit area per unit of time by the drug delivery system. The drug flux from a delivery device will depend on the physicochemical characteristics of rate controlling polymer as well as drug molecule and its formulation also.

It is fact that as the size of any dosage forms when increases beyond certain limit; the convenience of therapy is reduced. For membrane or matrix moderated novel drug delivery system intended for percutaneous administration of drug an elegant and cute formulation is preferable. The most commonly used membranes for controlled release systems are nonporous, homogeneous, polymeric films. Diffusion of a drug molecule from a membrane moderated delivery device is governed by steady state Fick's law equation.

$Jss = D K Cd / h$

Where, Jss is a steady state flux ($\mu g/cm^2.h$), K is drug partition coefficient, D is diffusion coefficient cm^2/hr, Cd is concentration difference on either side of membrane which is usually donor phase concentration mg/ml, h is thickness of membrane in cm. So far membrane remaining saturated with drug, a constant amount of drug will diffuse across a unit surface area of membrane. There is linear

relationship between magnitude of drug flux and area of drug delivery device. For many purposes a constant release rate is desirable for extended period of time. The transdermal entry of a drug into systemic circulation at desired rate can be achieved by using a suitable rate controlling membrane and drug reservoir.[1] The permeability of drug through polymeric free films was dependent on characteristics of the polymer [2, 3], casting solvent [4] and plasticizer used.[5]

Here an attempt is being made to evaluate the diffusion characteristics of base forms of originally official salts form, across various rate controlling polymeric membrane. The aim was to determine flux and permeation rate of Diltiazem base across various polymeric rate controlling membranes with a target to receive a membrane exhibiting promising rate, so that a dosage form releasing requisite amount of drug from a small surface area can be formulated to have elegant and acceptable dosage form.

6.1 Preparation of polymeric rate controlling membrane using different polymers

6.1.1 Preparation of polymeric rate controlling membrane

Polymeric membranes were prepared by solution casting technique on a substrate. Glass substrate was used where the components of membrane facilitated easy lifting of the membrane. In the cases where difficulty of lifting the membrane arose, mercury pool technique was used to cast the film. The composition along with different conditions employed is listed in a Table 6.1.1.

Table 6.1.1: Composition of rate controlling membrane

Polymer	Solvent	Plasticizer	% w/w of plasticizer	Substrate	Temperature (^{0}C)
EVA (VA 40%) copolymer	Toluene	DEP	2.00	Glass	85
EC	Chloroform	DEP	2.00	Glass	85
PVAC	Benzene	DEP	5.00	Mercury	90
ERS 100	Chloroform	DEP	15.00	Mercury	60

Specified weight of membrane polymer and additives were dissolved in respective solvents (10ml) by heating the mixture at controlled temperature until a clear solution was formed. The solution cooled to room temperature. The solution was poured in leveled glass Petri dish (Glass substrate) or on the pool of mercury contained in leveled glass Petri dish (Mercury pool substrate). The solvent was allowed to evaporate overnight. The membrane were carefully lifted and dried at room temperature at least for 24 hours. The membranes were stored in air tight container until used.

6.1.2 Physiochemical evaluation of polymeric rate controlling membrane

Physical appearance

All prepared membrane was evaluated for its physical appearance like color, texture, distribution of polymers and uniformity.

Thickness

The thicknesses of the membrane were assessed using micrometer (0.001mm, Mitutoyo, Japan). Thickness at five different places was measured and average thickness was recorded.

Density

Density was determined by accurately weighing a piece of 2 x 2 cm. the volume of pieces was calculated using its thickness and density was calculated as

Density = Mass of membrane / volume of membrane

Water sorption characteristics

Accurately weighed piece of membrane was kept and equilibrated in distilled water for 24 hrs and percentage of water sorbed was found out from weight difference after 24 hr.

6.1.3 Results and discussion

The present investigation deals with the development of polymeric membrane using different plasticizer and solvent. This preliminary screening was carried out to for the selection of best polymeric membrane. It was possible to cast the membrane of different polymers. The physical parameters were evaluated as follows.

Physical appearance

The physical characteristics of each membrane was evaluated visually and noted. The results are given in Table 6.1.2. From results obtained it was found that as compare to other polymeric membrane, the membrane containing EVA is more elastic and flexible.

Table 6.1.2: Physical characteristics of plain membrane

Polymer	Appearance	Texture	Distribution of polymers
EVA (VA 40%) copolymer	Opaque	Flexible, Elastic	Uniform
EC	Transparent	Flexible	Uniform
PVAC	Transparent	Flexible	Uniform
ERS 100	Transparent	Flexible	Uniform

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of films was measured at three different points and the average thickness was noted. The results of thickness are given in Table 6.1.3. The results indicate that there was no much difference in the thickness with in the membrane. Thickness in the different membrane was in the range of **61.66 ± 2.88 µm** to **34.16 ± 1.433 µm**. Maximum thickness was found in ERS100, while minimum found in EVA.

Table 6.1.3: Results of thickness of polymeric rate controlling membrane

Sr. No.	Polymers	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	EVA (VA40%) copolymer	35.0	32.5	35.0	34.16 ± 1.433
2	EC	45.0	42.5	47.5	45.00 ± 2.500
3	PVAC	50.0	52.5	50.0	50.83 ± 1.433
4	ERS 100	60.0	60.0	65.0	61.66 ± 2.886

***Standard deviation, n=3**

Density and water sorption

The density and water sorption characteristics of each membrane were determined. The results are given in Table 6.1.4. The results indicate that there was no much difference in the density with in the membrane. Density of the different membrane were in the range of **1.1002** to **0.9260 mg/ml**. water sorption characteristics was found almost zero for all the polymers. Results indicates that the membrane were hydrophobic.

Table 6.1.4: Physical characteristics of membrane

Sr. No.	Name of Polymer	Density (mg/ml)	Water sorption (wt/24hr)
1	EVA (VA 40%) copolymer	1.0220	NIL
2	EC	0.9260	NIL
3	PVAC	1.0660	NIL
4	ERS 100	1.1002	NIL

6.2 In vitro permeability study of Diltiazem through different polymeric membranes

6.2.1 Experimental

The permeation studies were performed in a Fite's diffusion cell (cell capacity of 10 ml, cross sectional are 1.32 cm^2).

The permeation studies were performed using polymeric membrane. A section of membrane was cut, measured and placed on the Fite's diffusion cell. A saturated drug solution (1 ml solution of Diltiazem base in ethanol having concentration 300 mg/ml) was kept in the donor compartment. The receiver compartment was filled with 200 ml of 0.01N HCl. The Fite's diffusion cell was kept in side the beaker containing receptor compartment. The temperature of diffusion cell was maintained at 37 ± 0.5 ^oC by circulating water jacket. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead.

The samples were withdrawn (2 ml, each time) at different time interval and an equal amount of 0.01N HCl was replaced each time. Absorbance of the samples was read spectrophotometrically at 236.0 nm taking 0.01N HCl solution, as a blank. The cumulative amount of drug permeated per square centimeter at each time interval was

calculated and plotted against time. The release rate µg/cm^2 hr was determined by simple regression analysis of steady state data.

6.2.2 Results and Discussion

Drug permeability studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. In vitro release profile is an important tool that predicts in advance how the drug will behave in reservoir type transdermal drug delivery system. Drug permeability studies for different polymeric membranes were performed in a modified Fite's diffusion cell using 0.01N HCl, as a diffusion media at 37 ± 0.5 ^{o}C. The results of in vitro drug diffusion studies from polymeric membrane are depicted in Figure 6.2.1. The release flux "Jss" was determined from the regression analysis of steady state data.

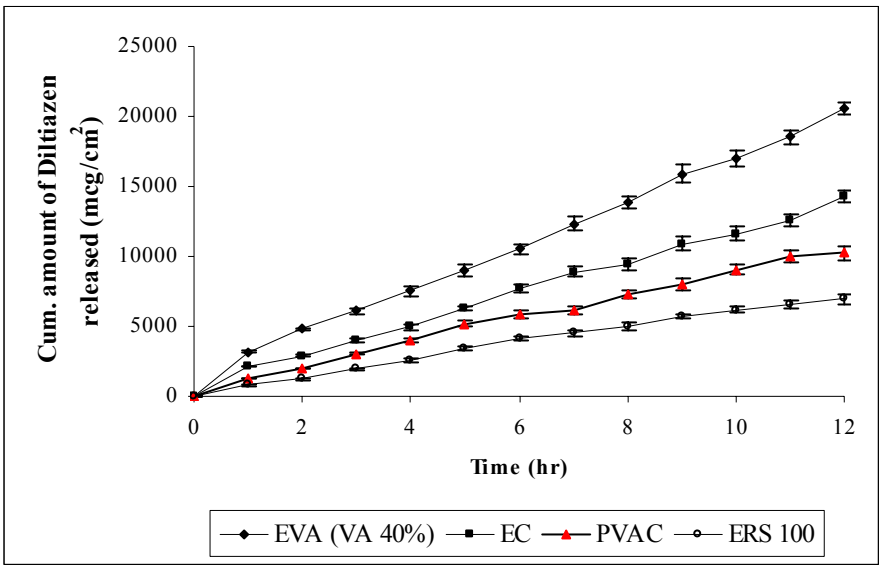
Table 6.2.1: Parameters of permeation kinetic of Diltiazem across polymeric membrane

Parameter	EVA (VA 40%) copolymer	EC	PVAC	ERS 100
Flux (µg/cm^2 hr)	1633.40	1099.8	836.34	581.29
Correlation coefficient	0.9969	0.9958	0.9946	0.9910
Permeability coefficient (µg/cm hr)	5.579	4.949	4.251	3.584

All the membrane was sufficiently hydrophobic and Diltiazem base was also hydrophobic exhibited permeation across all the membrane. Membrane thickness independent permeation rate is an important tool to rate the membranes for permeation study. However, the permeation coefficient for EVA (VA 40%) copolymer was the highest; this is due to good partitioning of Diltiazem base from EVA (VA 40%) copolymer than other polymer used to prepare membrane. EVA (VA 40%) copolymer was selected as rate controlling membrane, to device reservoir type transdermal drug delivery system. This membrane was further characterized for

various diffusion parameters.

Figure 6.2.1: Release profiles of Diltiazem through various polymeric membrane



*** Standard deviation, n=3**

6.3 Effect of thickness of membrane on release profile

6.3.1 Experimental

The release of drug from membrane is described by the well known steady state Fick's law equation.

$$Jss = D K Cd / h$$

Where, Jss is a steady state flux (µg/cm^2.h), K is drug partition coefficient, D is diffusion coefficient cm^2/hr, Cd is concentration difference on either side of membrane which is usually donor phase concentration mg/ml, h is thickness of membrane in cm. So far membrane remaining saturated with drug, a constant amount of drug will diffuses across a unit surface area of membrane. There is linear relationship between magnitude of drug flux and area of drug delivery device.

Thickness of membrane is an important parameter to formulate a robust device. A very thin membrane should give a product prone to rupture by minor mechanical injury to patch. Thickness influence release characteristics of drug and for to select a membrane giving substantial flux, still giving a robust formulation the diffusion study of EVA (VA 40%) copolymer rate controlling membrane of different thickness was carried out using Fite's diffusion cell. The Diltiazem release flux was computed by regression analysis of steady state data.

The main purpose of present investigation was to prepare EVA (VA 40%) copolymer rate controlling membrane of different thickness and to evaluate diffusion characteristics of Diltiazem using these membranes.

Preparation of EVA (VA 40%) copolymer membrane

Rate controlling polymeric membranes of EVA (VA 40%) copolymer were prepared as per method described in Chapter 6.1.1. To prepare polymeric membrane having different thickness, different quantity of polymeric solution was casted over mercury pool.

Drug permeability study through polymeric membrane

The permeation studies were performed in a Fite's diffusion cell, as per the procedure described in chapter 6.2.1. The cumulative amount of drug permeated per square centimeter at each time interval was calculated and plotted against time. The release rate $\mu g/cm^2$ hr was determined by simple regression analysis of steady state data.

6.3.2 Results and Discussion

Drug permeability studies for different polymeric membranes were performed in a Fite's diffusion cell using 0.01N HCl, as a diffusion media at 37 ± 0.5 ^oC. The results of in vitro drug diffusion studies from polymeric membrane are depicted in Table 6.3.1 and Figure 6.3.1.

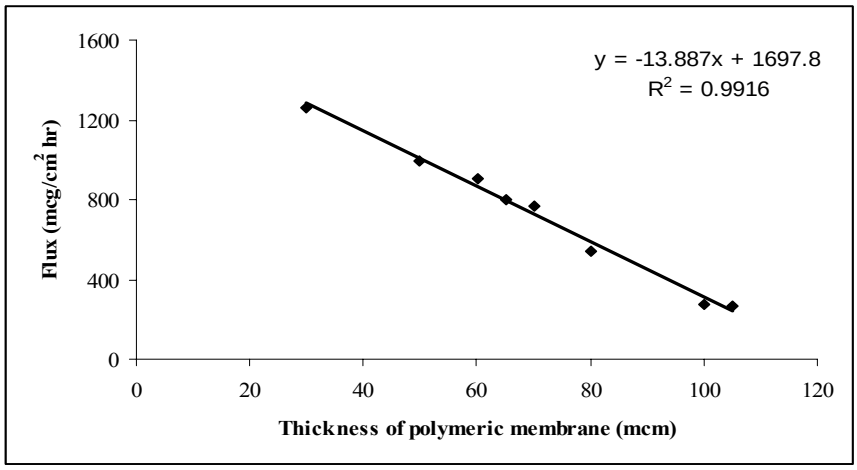
Table 6.3.1: Release flux of Diltiazem from different thickness EVA (VA 40%) copolymer membrane

Sr. No.	Thickness (µm)	Flux ($\mu g/cm^2h$)
1	30	1378.12
2	50	996.25
3	60	901.27
4	65	801.95
5	70	763.87
6	80	539.84
7	100	277.68
8	105	264.18

EVA (VA 40%) copolymer membrane having thickness 30 μm exhibited maximum flux for Diltiazem. The flux of Diltiazem, across membrane covering reservoir with saturated solution of Diltiazem, followed near to zero order release, exhibiting a high release rate. Substantial high flux of Dilitazem was due to "Burst effect phenomena" was due to high diffusivity or solubility of drug in polymer, the drug molecules appearing fast at the receptor side of barrier membrane.

Usually for an ideal membrane, for a permeant passing through it, a linear relationship without intercept on either axis is expected, when release rate is plotted as function of thickness. The negative intercept on X-axis indicates burst effect phenomena, showing high diffusivity of drug in the membrane.

Figure 6.3.1: Correlation between thickness of polymeric membrane and release rate



Various parameters of kinetics of Diltiazem released from polymeric membrane are presented in Table 6.3.2. The parameters listed in this table are useful for biopharmaceutical aspect of the membrane moderated system evaluated. The most commonly used membrane for controlled release systems are nonporous, homogeneous polymeric films. Diffusion of a drug molecule from a membrane moderate delivery device is governed by steady state Fick's law

$$Jss = D. K. Cd/ h$$

Where, Jss is steady state flux μg/cm^2 hr, K is drug partition co-efficient, D is diffusion coefficient cm^2/hr and Cd is concentration difference on either side of membrane which is usually donor phase concentration mg/ml, h is thickness of

polymeric membrane in cm. so far membrane remaining saturated with drug, a constant amount of drug will diffuse across an unit surface area of membrane.

Table 6.3.2: Various parameters of kinetics of Diltiazem released from polymeric membrane

Thickness ($\mu m \times 10^{-4}$)	Flux ($\mu g/cm^2h$)	Membrane permeability $P = (Jss/Cd) \times 10^{-3}$ (cm/hr)	Permeability coefficient $P' = Jss \times h$ ($\mu g/cm\ hr$)
30	1300.12	4.3337	3.9003
50	996.25	3.3208	4.9812
60	901.27	3.0000	5.4076
65	801.95	2.6730	5.2126
70	763.87	2.5460	5.3470
80	539.84	1.7990	4.3187
100	277.68	0.9256	2.7768
105	264.18	0.8206	2.5484

Membrane permeability P (cm/hr) and membrane thickness independent permeation rate P' (mg/cm.hr) were calculated using below equation

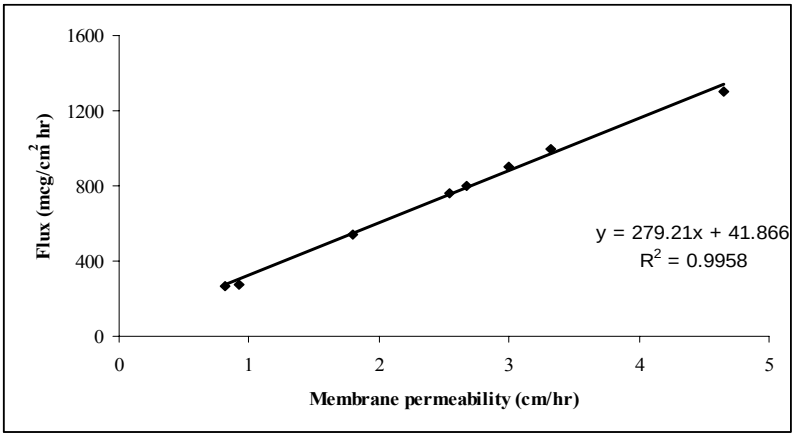
$P = Jss/Cd$

$P' = Jss.\ h$

A linear relationship was observed between release flux and membrane permeability. The correlation coefficient was 0.9958. As thickness increased the release rate decreased. The thickness independent permeation rates remained in order and limit.

At thickness higher than 100 µm and above the permeation rate decreases as thickness goes on increasing above certain level, the barrier properties seems to be turning to matrix characteristics showing enhanced path length for moving drug molecule to partition into diffusion medium. Due to this permeation rate seems to be decreasing.

Figure 6.3.2: Correlation between membrane permeability and release rate through polymeric membrane



6.4 Formulation and optimization of reservoir device of Diltiazem

Here an attempt is being made to evaluate the diffusion characteristics of base forms of originally official salts forms, across EVA (VA 40%) copolymer hydrophobic membrane. The aim was to determine flux and permeation rates of Diltiazem base through polymeric membrane and to formulate and optimize the Diltiazem membrane moderated reservoir type controlled transdermal drug delivery system of Diltiazem.

6.4.1 Experimental

Component of Device

The following components were used to fabricate the controlled drug delivery system

Preparation of Diltiazem base

Diltiazem free base was prepared as per method describe in chapter 3.

Backing membrane

Polyethylene coated aluminum foil was used as protective backing.

Drug reservoir

Low density polyethylene sheet 0.220 mm to 0.400 mm thick was drilled to a circular hole of desired size and glued on to the polyethylene coated aluminum foil, forming a reservoir well. The drug formulation was filled in the reservoir.

EVA (VA 40%) copolymer rate controlling membrane

The method of preparation is described in chapter 6.1.1. 4% w/v solution of EVA (VA 40 %) copolymer containing Dibutyl phalate (2% w/w of polymer weight) was used as a plasticizer. The films were cut into a 3.14 cm^2 area and covered with glossy

paper having a smooth surface. The film was kept in desiccators until used.

Adhesive system

Natural rubber solution prepared by masticating natural rubber with fixatives, tackifiers, preservatives in volatile solvents (hexane) was received readymade and was used as an adhesive.

Peel off release liner

Silicon sheet was used as peel off protective liner.

Fabrication of reservoir device

Membrane moderated reservoir type device was fabricate to evaluate the permeation kinetic of Diltiazem base. Low density PVC valley was taken as a reservoir. 0.3 ml Alcoholic solution of Diltiazem base having concentration 300 mg/ml was filled in the reservoir. The reservoir was covered with EVA membrane having thickness 30μm using adhesive. Reservoir patch was covered with liner and placed in desiccators until used.

Drug content analysis of reservoir patch

The plain patch without Diltiazem and a medicated patch were used for drug content analysis. The reservoir patch was taken into 100 ml volumetric flask and dissolved in 10 ml toluene. The solution was filtered through whatman filter paper (Nyulge Nune, UK). This stock solution was diluted 100 times using toluene. The amount of drug present was determined UV-VIS spectrophotometerically at 281.5 nm. The blank solution was prepared using reservoir patch free from drug.

6.4.2 Results and discussion

A membrane moderated transdermal controlled drug delivery system for Diltiazem base was successfully prepared using EVA (VA 40%) copolymer and reservoir patch was evaluated by drug content analysis.

Drug content

Drug content of the reservoir patch was carried out to ascertain that the drug is properly added in the formulation. The prepared reservoir patch found to contain **95.34 ± 0.345** of the labeled amount of Diltiazem. The average percentage deviation of formulation was found to be with in the limit, and hence the formulation passed the test for content uniformity as per official requirements. The drug content analysis of

prepared formulation showed that the process employed to prepared patches was capable of giving uniform drug content, with minimum batch variability.

6.5 In vitro diffusion study of reservoir device containing Diltiazem

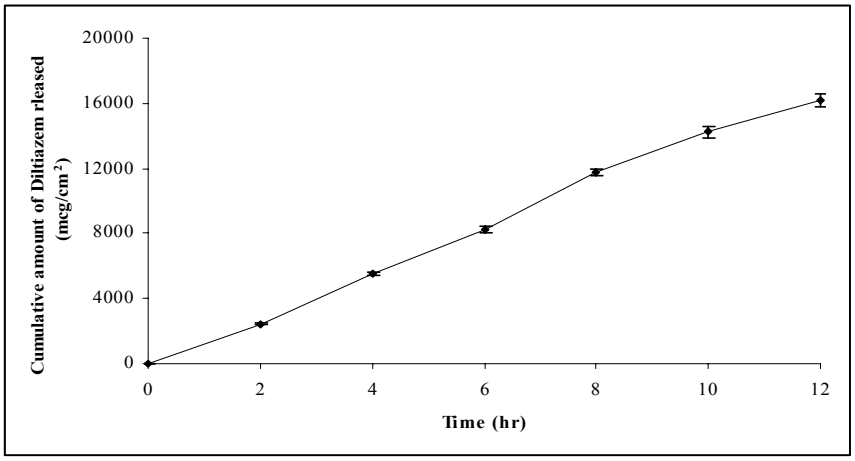
6.5.1 Experimental

Modified Keshary-Chien diffusion cell was used for diffusion study. Device was clamped on the brim of the cell. Membrane was kept facing the diffusion medium. Diffusion medium was stirred with bar type magnetic stirrer and maintained at 37 ± 0.5^{o}C. The effective permeation area of the diffusion cell and receptor cell volume was 3.14 cm^2 and 40 ml, respectively. The receptor compartment contained 40 ml of 0.01N HCl. Periodic samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at different time intervals for 6 hr. The absorbance of the withdrawn samples were measured using UV VIS spectrophotometer at 236.0 nm using 0.01N HCl as a blank. The experiments were done in triplicate. Cumulative amount of drug released per square centimeter of patch were plotted against function of time. The release rate was determined by simple regression analysis of steady state data.

6.5.2 Result and discussion

Diffusion studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. The results of in vitro drug diffusion profile for transdermal patch is depicted in Figure 6.5.1.

Figure 6.5.1: In vitro diffusion profiles of Diltiazem from reservoir device



*** Standard deviation, n=3**

Cumulative amount of drug diffused per square centimeter of patches, into the in vitro fluid when plotted against time, showed near to zero order release. The release flux **(1397.50 µg/cm^2 hr)** was calculated from the regression analysis of steady state data. The correlation coefficient was **0.9929** and exhibited zero order kinetic.

6.6 In vitro human live skin permeation study of Reservoir device of Diltiazem

6.6.1 Experimental

Here an attempt is being made to evaluate the diffusion characteristics of base forms of the drug molecule, across human live skin. The aim was to determine flux and permeation rate of Diltiazem base through human live skin. In general human skin is considered hydrophobic. The survey of permeability of hydrophobic molecule indicates that majority of lipophillic drug crosses the skin barrier at a faster rate. The objective was to test scope of this information so as to get cute and elegant drug delivery system.

Treatment and preparation of skin for permeation study

Human live skin was received from six healthy human male patients of an age 25 to 30 years. The skin was treated and stored as per method mentioned in Chapter 3.

In vitro permeation study through human live skin

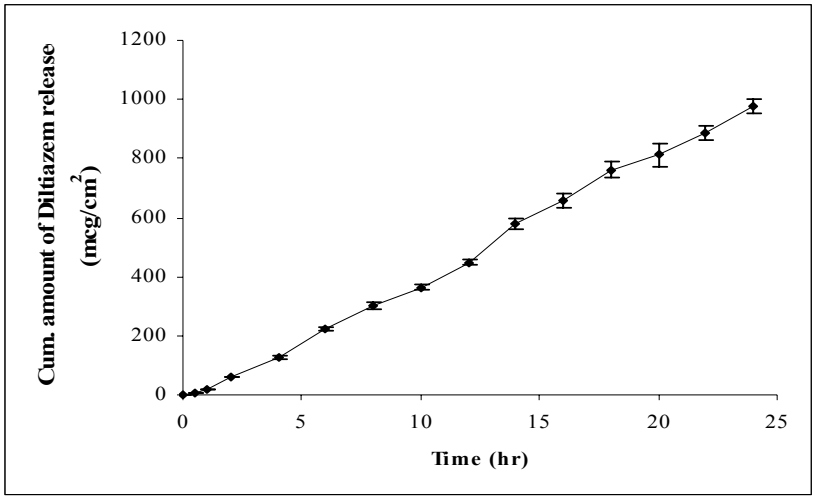
The permeation study was performed in a modified Keshary-Chien diffusion cell. The permeation study was performed using human live skin. The skin was used after fulfilling all the ethical requirements. Skin was kept at room temperature in saline and then washed with soap solution followed by washing with distilled water. A section of skin was cut and clamped on the brim of diffusion cell in such a way that the dermal side of the skin was facing the receiver compartment. The receiver compartment was filled with 40 ml 0.01 N HCl. The transdermal patch containing Diltiazem was firmly pressed onto the center of human skin. Once the adhesion to the skin surface had been confirmed, flange of the diffusion cell mounted in such a way that the patch was situated precisely over the flange aperture. The whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred using a magnetic bead. Samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port

of the diffusion cell at different time intervals till 24 hrs. The absorbance of the withdrawn samples were measured using UV VIS spectrophotometer at 236.0 nm using 0.01N HCl as a blank. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time. The release rate was determined by simple regression analysis of steady state data.

6.6.2 Results and discussion

In vitro permeation studied is predictive of in vivo performance of transdermal drug delivery system. The results of in vitro skin permeation of Dltiazem from reservoir device across human live skin are shown in Figure 6.6.1

Figure 6.6.1: In vitro skin permeation profile of Diltiazem from reservoir device through human live skin



*** Standard deviation, n=3**

Various parameters of diffusion kinetics of Diltiazem released from device across isolated skin are presented in Table 6.6.1. The parameters listed in Table 6.6.1 are useful for biopharmaceutics and pharmacokinetics of reservoir device evaluated.

Reservior device containing Diltiazem provided **40.77 ± 0.9584 µg/ cm^2 hr** skin flux. The time lag (t_L) for devices is presented in Table 6.6.1. The average diffusion coefficient, D of Diltiazem was determined using $D = h^2/6\ t_L$ relationship, where skin thickness h was 140 x 10^{-4} cm. The amount of Diltiazem retained by skin area used for permeation was calculated by dividing steady state flux with gradient of diffusivity and it was found to be **17.298 mg/cm^3** of skin.

Table 6.6.1: Parameters of diffusion kinetic of Diltiazem from reservoir device through human live skin

Sr. No.	Parameters	Value
1	Skin flux (Jss)	**40.77 µg/ cm^2 hr**
2	Time lag (t_L)	**0.908 hr**
3	Skin thickness (µm)	**140 µm**
4	Diffusion coefficient	**3.2996 x 10^{-5} cm^2/sec**
5	Solubility of drug in skin (Cs)	**17.298 mg/cm^3**

The amount of Diltiazem retained in skin need to be incorporated in contact adhesive to serve as a prompt dose (priming dose) before steady state is established. The release of Diltiazem from reservoir system into infinite sink can be estimated by equation.

Release rate = Jss (in sink) + H_e^{-Kt}

The first term on right hand side of the equation represents the steady state delivery rate i.e. Q Vs T pattern. The second term represents the temporal pattern of drug release during priming dose period.

Comparison of permeation kinetic: Patch without priming Dose: Patch with priming Dose

The release rate **(**µg/hr) has linear relationship with area of release face of a transdermal drug delivery system. The final patch needs to be provided with adhesive system and priming dose. The quantity of Diltiazem to be loaded in adhesive as priming dose was derived from separate diffusion experiment, on trial and error basis, where EVA (VA 40%) copolymer rate controlling membrane was layered with 0.075 ml adhesive solution contains 2.19 mg/ml Diltiazem over 1cm^2 area (gives a smear of approximate 10µm thick) and was affixed over human skin and subjected to in vitro diffusion study. The steady state release rate of Diltiazem was **29.78 µg/cm^2 hr** with diffusion coefficient of **2.41x10^{-5} cm^2/hr**, for skin thickness of 140 µm. Amount of Diltiazem absorbed per cm^3 of skin was approximated by dividing steady state flux with gradient of diffusivity, which was found to be **17.298 mg per cm^3** of skin.

To assess release kinetic of a complete patch, a patch of 3.14 cm^2 area of delivery device was covered with adhesive system. From the data of solubility of drug in skin, **0.323 % w/v Diltiazem** in rubber solution was used as an adhesive system. **0.236 ml** of this solution was uniformly layered over **3.14 cm^2** area of patch, to obtain 10 µm thick adhesive systems containing priming dose of **242 µg/cm^2** area of delivery device. The device was firmly secured on skin of human live skin and was subjected to diffusion study using 0.01 N HCl as diffusion medium.

Figure 6.6.2: In vitro skin permeation profile of Diltiazem from reservoir patch through human live skin with priming dose and without priming dose

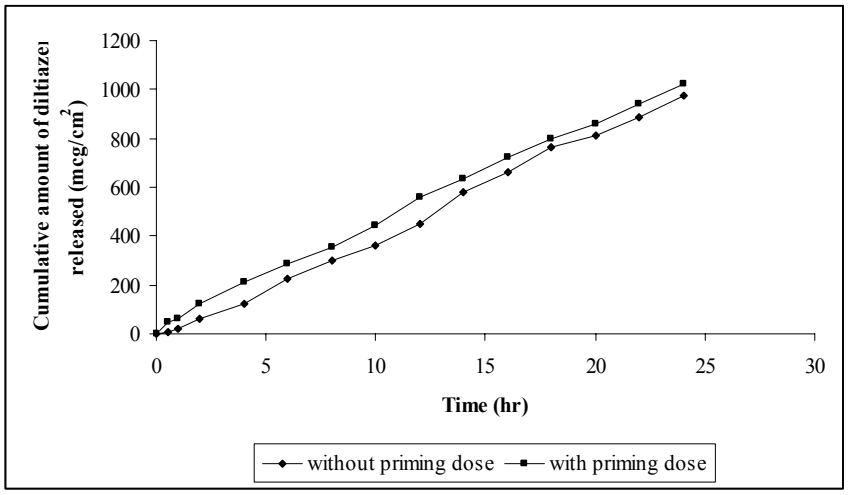


Table 6.6.2: Parameters of in vitro permeation kinetics of Diltiazem from reservoir device through human live skin with priming dose and without priming dose

Parameters	Cumulative amount of drug release (µg/cm^2) Without priming dose	Cumulative amount of drug release (µg/cm^2) With priming dose
Release rate (µg/cm^2 hr)	40.77	43.95
Correlation coefficient	0.9953	0.9921
Time lag (t_L)	0.908 hr	0.463 hr

Computation of area of patch required for target steady state plasma concentration of Diltiazem

As mentioned in section 5.6.3 desired release rate of Diltiazem was 554.4 µg/ hr.

In vivo input = in vivo output = 554.4 µg/hr

$J_{ss(skin)}$ x A = $C_{ss} * V_d * K_e * 70$ = 554.4 µg/hr

Area of patch = (554.4 µg/hr)/ $J_{ss(skin)}$ = 12.61 cm^2

Formulation of final reservoir type transdermal drug delivery system of Diltiazem meeting targeted steady state plasma concentration

The effective area for desired steady state release of Diltiazem computed is 12.61 cm^2. An adhesive system covering the membrane is to be provided. Priming dose needs to be included in the adhesive layer.

The adhesive solution (readymade received- Beta Surgical, Rajkot) was prepared using natural rubber masticated with fixative, tackifiers, preservatives and solvent hexane.

For preparation of final patch 0.323 % w/v Diltiazem base in natural rubber solution is to be used as an adhesive system. 0.95 ml of this solution is to be uniformly layered over release face of device to obtained 10 µm thick adhesive system containing 242 mg Diltiazem/cm^2 of delivery device as a priming dose.

The release phase is to be provided with a protective liner. The device to be packed in brown paper covering and to be stored at 20 to 25 ^{0}C.

6.7 Modification in formulation (Gel in reservoir)

Incorporation of Diltiazem base in reservoir formulation of a device containing EVA (VA 40%) copolymer as a rate controlling membrane material would provide an elegant , efficient and economic membrane moderated transdermal controlled drug delivery which would be convenient for patient but here attempt was carried out to incorporate drug in carbopol gel, to facilitate easy fabrication. As enclosing of semisolid is preferred to solution in the reservoir so far processing is concerned.[6]

Here an attempt is being made to evaluate the diffusion characteristics of Diltiazem base, from a gel formulation across EVA (VA 40%) copolymer hydrophobic membrane. The aim was to formulate a gel containing Diltiazem base. The medicated gel is to be evaluated for permeation kinetics through EVA (VA 40%) copolymer membrane (30 µm) containing reservoir system. Further permeation study was carried

out using human live skin.

6.7.1 Experimental

6.7.1.1 Component of Device

The following components were used to fabricate the membrane moderated reservoir type controlled drug delivery system of Diltiazem base.

Backing membrane

Polyethylene coated aluminum foil was used as protective backing.

Drug reservoir

A low density flexible polymer sheet of 2 to 5 mm thick was drilled to a circular hole of 2 cm diameter and it was affixed on polyethylene coated aluminum foil to form a reservoir well. The drug formulation was filled in the reservoir.

EVA rate controlling membrane

EVA (VA 40%) copolymer membrane (30 μm) was used as a rate controlling membrane.

Preparation of transdermal gel[7]

Diltiazem base was dissolved in alcohol and this solution was mixed with propylene glycol and distilled water. Required weight of carbopol was dispersed in aqueous phase and soaked overnight. The dispersion was then mixed with triethanolamine to neutralize carbopol. The gel formed was stirred with bar type stirrer at slow speed to get homogeneous gel. The pH of gel was 6.9.1. Compositions of gel formulation are given in Table 6.7.1.

Table 6.7.1: Composition of Diltiazem gel

Ingredients	Amount of ingredients		
	F1	F2	F3
Diltiazem base	0.5 gm	0.5 gm	0.5 gm
Carbopol	0.1 gm	0.1 gm	0.1 gm
Ethanol	3.0 ml	4.0 ml	5.0 ml
Propylene glycol	2.0 ml	1.0 ml	0.5 ml
Triethanolamine	0.35 ml	0.35 ml	0.35 ml
Distilled Water	Up to 10 gm	Up to 10 gm	Up to 10 gm

Adhesive system

Natural rubber solution prepared by masticating natural rubber with fixatives, tackifiers, preservatives in volatile solvents (hexane) was received readymade and was used as an adhesive.

Peel off release liner

Silicon sheet was used as peel off protective liner.

6.7.1.2 Fabrication of reservoir device containing gel

Membrane moderated reservoir type device were fabricate to evaluated the permeation kinetic of Diltiazem base where the reservoir well was filled with 1.5 gm of Diltiazem gel. The reservoir was covered with EVA (VA 40%) copolymer membrane having thickness 30µm. The reservoir filled with donor phase gel contained 75mg of Diltiazem base. A 10µm thick adhesive layer was provided on release face. The patch was covered with silicone liner, packed in brown paper and stored at room temperature in desiccators until used.

6.7.1.3 Physiochemical evaluation of reservoir patch containing gel

Drug content

The Diltiazem content of prepared reservoir patch was measured in triplicate and analyzed by UV-VIS spectrophotometer at 236nm. Patches (n=3) of specified area (3.14 cm^2), were taken into 100 ml volumetric flask and dissolved in 10 ml methanol. Membrane was removed and washed with methanol. The solution was filtered through whatman filter paper (Nyulge Nune, UK). This stock solution was diluted 100 times using methanol. The absorbance of the resulting solution was measured at about 236nm using corresponding blank solution. The blank solution was prepared using reservoir patch free from drug.

6.7.1.4 In vitro diffusion study of reservoir device containing Diltiazem gel

Modified Keshary-Chien diffusion cell was used for diffusion study. Silicone liner was peeled off and the device was clamped on the brim of the cell. Membrane was kept in such a way that it was facing the diffusion medium. Diffusion medium 0.01N HCl was stirred with bar type magnetic stirrer and maintained at 37 ± 0.5^oC. The effective permeation area was 3.14cm^2. Periodic samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution. The absorbance of the

withdrawn samples were measured using UV VIS spectrophotometer at 236.0nm using 0.01N HCl as a blank. The experiments were done in triplicate. Cumulative amount of drug released per square centimeter of patch were plotted against function of time for formulation. The release rate was determined by simple regression analysis of steady state data.

6.7.1.5 In vitro human live skin permeation study of Reservoir device of Diltiazem gel

Diffusion study of Diltiazem from selected reservoir device across human live skin was performed using modified Keshary-Chien diffusion cell. A section of skin was cut and placed on the brim of diffusion cell in such a way that the dermal side of the skin faced donor compartment. The patch of Diltiazem was affixed on the skin in such a way that backing membrane was facing upward. Diffusion study was carried as mentioned in section 6.6.1.

The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time. The release rate was determined by simple regression analysis of steady state data.

6.7.2 Result and discussion

A membrane moderated reservoir type transdermal controlled drug delivery system for Diltiazem base was successfully prepared using carbopol gel and patch was evaluated for drug content.

Drug content

Drug content of the reservoir patch was carried out to ascertain that the drug is uniformly distributed in the formulation. The prepared film formulations (3.14cm^2) were dissolved in 10 ml toluene in a 100 ml volumetric flask and the volume was adjusted up to 100 ml using toluene. The amount of drug present was determined by measuring the absorbance spectrophotometrically at 281.5 nm using toluene as a blank. The results obtained are represented in Table 6.7.2.

Table 6.7.2: Results of % Drug content of F1 to F3 reservoir patch contains Diltiazem gel

Sr. No.	Formulation Code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	99.02	97.80	98.90	98.57 ± 0.672
2	F2	98.57	96.52	98.53	97.87 ± 1.172
3	F3	101.20	100.05	101.23	100.82 ± 0.672

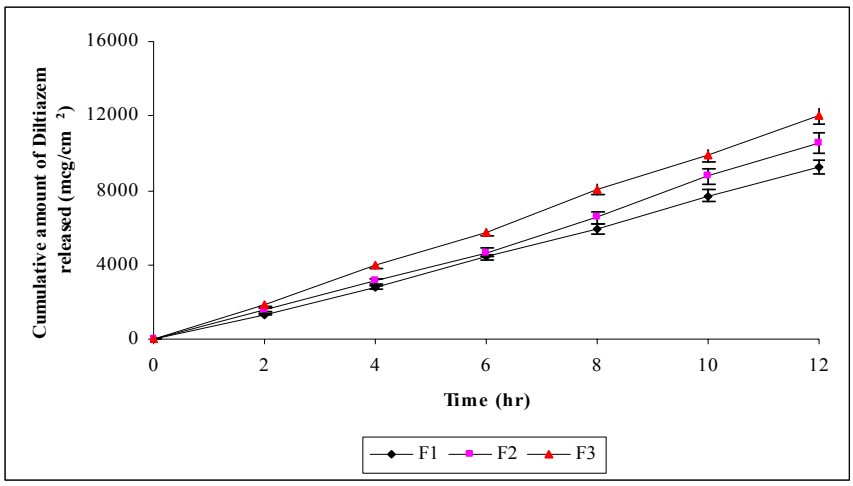
***Standard deviation, n=3**

The patches were found to contain **97.87% - 100.82%** of the labeled amount of Diltiazem indicating uniformity of drug content. The average percentage deviation of all formulations was found to be with in the limit, and hence all the formulation passed the test for content uniformity as per official requirements. The drug content analyses of prepared formulation showed that the process employed to prepare patches was capable of giving uniform drug content, with minimum batch variability.

In vitro drug diffusion study of reservoir device containing gel

Diffusion study for membrane moderated reservoir type transdermal controlled drug delivery system was performed in a modified Keshary-Chien diffusion cell using 0.01N HCl, as a diffusion medium at 37 ± 0.5 ^{0}C. The results of in vitro drug diffusion studies from transdermal patches are depicted in Figure 6.7.1.

Figure 6.7.1: In vitro diffusion profiles of Diltiazem from reservoir containing Diltiazem gel



***Standard deviation, n=3**

Table 6.7.3: Parameters of in vitro permeation kinetic of Diltiazem from Reservior device containing Diltiazem gel

Parameters	F1	F2	F3
Flux	779.36	883.46	1003.6
Correlation coefficient	0.9984	0.9955	0.9993

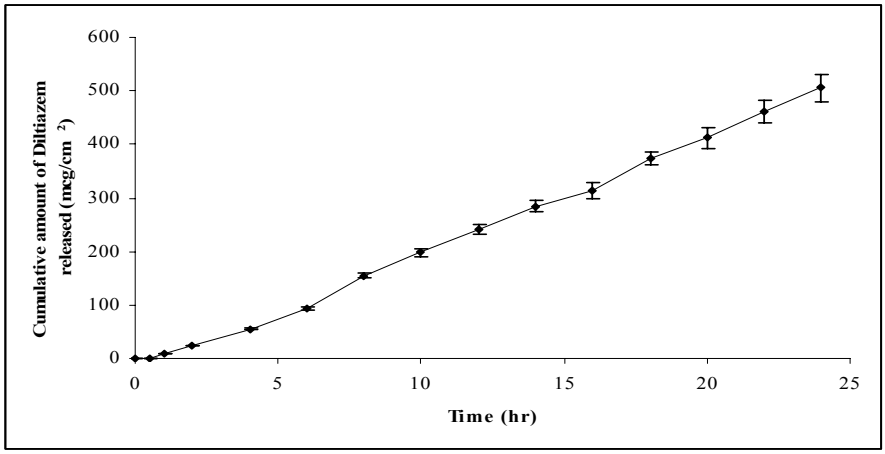
A device containing reservoir filled with a gel containing Diltiazem was covered with EVA membrane of 30μm thickness. In vitro diffusion study of Diltiazem released into infinite sink was carried out using the modified Keshary-Chien diffusion cell. Release of drug from the devices was diffusion under membrane controlled. The rank order of release of flux was F1< F2 < F3.

The release flux was calculated from the regression analysis of steady state data. Formulation **F1** exhibited minimum release rate **(779.36 μg/cm^2 h)** while Formulation **F3** exhibited maximum release rate (**1003.6 μg/cm^2 h**). Based on in vitro release experiments, formulation **F3** was chosen for further in vitro permeability study through human live skin.

In vitro human live skin permeation study of selected Reservoir device of Diltiazem containing Diltiazem gel

Device F3 exhibited maximum in vitro release flux and it was selected for fabrication of final device. Diffusion study of Diltiazem from selected reservoir device across human live skin was performed using modified Keshary-Chien diffusion cell. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time. The release rate was determined by simple regression analysis of steady state data. The results of in vitro skin permeation of Dltiazem from reservoir device are shown in figure 6.7.2.

Figure 6.7.2: In vitro skin permeation profile of Diltiazem from reservoir device containing gel through human live skin



***Standard deviation, n=3**

Various parameters of diffusion kinetics of Diltiazem released from device across human live skin are presented in Table 6.7.4.

Table 6.7.4: Parameters of diffusion kinetics of Diltiazem from reservoir device containing gel through human live skin

Sr. No.	Parameters	Value
1	Skin flux (Jss)	**22.209 µg/ cm^2 hr**
2	Time lag (t_L)	**1.3237 hr**
3	Skin thickness (µm)	**140 µm**
4	Diffusion coefficient	**2.4496 x 10^{-5} cm^2/sec**
5	Solubility of drug in skin (Cs)	**12.5877 mg/cm^3**

The time lag (t_L) for devices is presented in Table 6.7.4. The average diffusion coefficient, D of Diltiazem was determined using $D = h^2/6\ t_L$ relationship, where skin thickness h was 140 x 10^{-4} cm. The amount of Diltiazem retained by skin area used for permeation was calculated by dividing steady state flux with gradient of diffusivity and it was found to be **12.5877 mg/cm^3** of skin.

Comparison of permeation kinetics: Patch without priming Dose: Patch with priming Dose

The release rate (μg/hr) has linear relationship with area of release face of a transdermal rug delivery system. The final patch needs to be provided with adhesive system and priming dose. The quantity of Diltiazem to be loaded in adhesive as priming dose was derived from separate diffusion experiment, on trial and error basis where EVA (VA 40%) copolymer rate controlling membrane was layered with 0.075 ml adhesive solution contains 1.52 mg/ml Diltiazem over 1cm^2 area (gives a smear of approximate 10μm thick) and was affixed over an human skin skin and subjected to in vitro diffusion study. The steady state release rate of Diltiazem was **20.80 μg/cm^2 hr** with diffusion coefficient of **1.68 x 10^{-5} cm^2/hr**, for an skin thickness of 140 μm. Amount of Diltiazem sorbed per cm^3 of skin was approximated by dividing steady state flux with gradient of diffusivity, which was found to be **12.380 mg/cm^3** of skin.

To assess release kinetic of a complete patch, a patch of 3.14 cm^2 area was provided with adhesive system. From the data of solubility of drug in skin, **0.23 % w/v** Diltiazem in rubber solution was used as an adhesive system. This solution was uniformly layered in 4 mm rim surrounding **3.14 cm^2** area of patch, to obtain 10 μm thick adhesive systems containing priming dose of **173 μg/cm^2** area of delivery device. The device was firmly secured on skin of human live skin and was subjected to diffusion experiment using 0.01 N HCl as diffusion medium. The release profile is shown in Figure **6.7.3.**

Figure 6.7.3: In vitro skin permeation profile of Diltiazem from Reservior patch containing gel through human live skin with priming dose and without priming dose

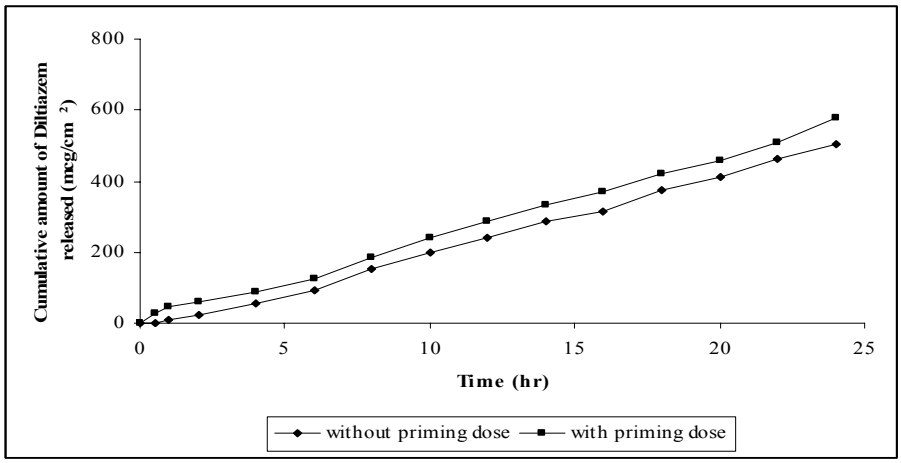


Table 6.7.5: Parameters of in vitro permeation kinetics of Diltiazem from reservoir device containing gel through human live skin with priming dose and without priming dose

Parameters	Cumulative amount of drug release ($\mu g/cm^2/hr$) Without priming dose	Cumulative amount of drug release ($\mu g/cm^2/hr$) With priming dose
Release rate ($\mu g/cm^2$ hr)	22.209	23.78
Correlation coefficient	0.9979	0.9965
Time lag (t_L)	1.3237 hr	0.735 hr

Computation of area of patch required for target steady state plasma concentration (Css) of Diltiazem

As per mentioned in section 5.4.3 desired release rate for target steady state plasma concentration of Diltiazem is 554.4 µg/ hr

Area of patch = (554.4 µg/hr)/ $J_{ss(skin)}$

= 554.4/23.78 cm^2

= 23.31 cm^2

Formulation of final reservoir type transdermal drug delivery system containing Diltiazem gel meeting targeted steady state plasma concentration

The effective area for steady state release of Diltiazem was 23.31cm^2. Adhesive system is to cover the release phase. The adhesive solution is to be prepared using natural rubber masticated with fixative, tackifiers and preservatives dissolved in n-hexane.

For preparation of final reservoir type transdermal drug delivery device of Diltiazem containing release phase covering adhesive, 0.231 %w/v solution of Diltiazem in rubber solution is to be used. This solution is to be layered over 23.31 cm^2 area of delivery device giving a 10µm thick layer, providing a priming dose of 173 $\mu g/cm^2$ area of delivery device.

The release face of the device is to be provided with a protective silicone liner. The device is to be packed in a brown paper covering and stored at 20 to 25 ^{0}C

6.8 STABILITY STUDY

6.8.1 Stability study of reservoir device containing Diltiazem

Membrane moderated reservoir device containing Diltiazem in form of solution and gel was subjected to accelerated thermal stability study. The accelerated stability studies were carried out according to ICH guideline by storing the samples at 25 ^{0}C / 60% RH, 30 ^{0}C/ 65% RH and 40 ^{0}C/ 75% RH for 90 days in a stability chamber (Thermo Lab., Mumbai, India). These samples were analyzed by UV Spectrophotometer method and checked for changes in physical appearance and drug content at an interval of 15 days**.**

6.8.2 Results and Discussion

Reservoir device containing alcoholic solution of Diltiazem and Diltiazem in carbopol gel was selected for stability study and observed for change in color, appearance, flexibility and drug content. Temperature and humidity values selected were as per the ICH guidelines and the test was carried out in a stability chamber. The stability studied was carried out at 25 ^{0}C / 60% RH, 30 ^{0}C/ 65% RH and 40 ^{0}C/ 75% RH for 90 days.

In case of reservoir device contains ethanolic solution of Diltiazem, it was observed that formulation stored at 40^{0}C there is drastic change in physical characteristic of product. Crystals of drug was observed in the reservoir stored at higher temperature. The release rate for product stored at 30 ^{0}C was also changed. The content of reservoir exhibited change in color from colourless to slight yellowish brown. However the product stored at 25 ^{0}C exhibited a little change in the flux, but the flux was in order. There was no change in color and appearance for the product stored at room temperature. The first order rate constant of degradation for room temperature was **1.981 x 10^{-3}**. The self life calculated was **53 week**. The results of stability study indicated that the products should be stored at a temperature not exceeding 25 ^{0}C.

Diffusion study of reservoir patch containing carbopol gel was carried out and it was observed that formulation stored at 40^{0}C, exhibited burst effect phenomena. The release pattern and drug flux was altered. Due to decrease in viscosity of gel at an elevated temperature the content of reservoir turned quite soft. The drug flux for the product stored at room temperature was in order. There was no change in appearance, color for the product stored at room temperature. The first order rate constant of

degradation for room temperature was **1.721 x 10^{-3}**. The self life calculated was **61 week.** Results of stability study indicates that product should be stored at temperature not exceeding 25 ^{0}C.

6.9 SKIN IRRITATION AND SKIN SENSITIZATION STUDY

6.9.1 Experimental

Skin irritation and skin sensitization though are different types of physiological responses yet they have several common indications. Skin sensitization is systematic response and skin irritation is primarily is local response.

A protocol was devised for evaluation of skin irritation and/or sensitization in such a manner that the signs at the sight of application would be assessed in common for the both and further, to distinguish sensitization from irritation. The skin irritation and skin sensitization study was carried out as per procedure mentioned in chapter 5.8.1. The final Diltiazem reservoir patch containing solution (1.0 cm^2) and reservoir patch containing Diltiazem gel (1.5 cm^2) was supported with aluminum foil. This was applied to the skin surface (shaved) and made adhered with the help of "3 M Micropore"- medical adhesive tap (3M, corporation, U.K.)

6.9.2 Results and discussion

The sensitization included induction period following a short sensitization period giving allergic reaction at the application site. Once this sensitivity was established, the subsequent exposure to the chemical would lead to higher responses both locally (at the site of application) and systematically due to secondary immune reaction (hypersensitivity).

Study of reservoir patch containing Diltiazem solution

The results of skin irritation and skin sensitization study for Diltiazem reservoir patch containing alcohol are presented in Table 6.9.1. Following inferences has been drawn from irritation and sensitization studies of Diltiazem reservoir transdermal drug delivery system.

From Table 6.9.2, it is clear that the Diltiazem is non irritating since the maximum score for each sign for given concentration of drug was not more than 1, though the number of animals giving score 1 for the various sign was increased. However, score

1 is not considered under irritating category therefore Diltiazem reservoir patch was considered as safe. Though it does not show average score more than 1 at day 20 but this indication solely cannot be taken as a sign of non-irritating. The reason for this was that individual animal show score more than 1, but the average was less than 1 (which means that some animals showed score 0, out of four animals).

Diltiazem is non- sensitizing in nature. The reason for this was reduction in various scores even after repeated application. Results shows that total irritating scores were reduced from day 20 to day 28. Therefore, the primary and secondary sensitization was absent.

Table 6.9.2: The average score for each sign after day 20 and day 28 during irritation and sensitization study of reservoir patch containing Diltaizem solution

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.5	0.25
2	Scaling	0.0	0.0
3	Fissures	0.25	0.0
4	Oedema formation	0.5	0.0
5	Ecchymosis	0.0	0.0
6	Necrosis	0.25	0.0

Table 6.9.1: Skin irritation and sensitization study of reservoir patch containing Diltiazem solution

SR.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE									
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25
2	Scaling	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0
3	Fissures	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0
4	Oedama formation	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0.5	Avg	.25	Avg	.25	Avg	0
5	Ecchymosis	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	.25	Avg	.25	Avg	0	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0
6	Necrosis	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0
Total of all score		**2**		**2**		**1**		**4**		**5**		**5**		**4**		**0**		**0**		**6**		**5**		**5**		**1**	
Total average per animal		**0.5**		**0.5**		**0.25**		**1**		**1.25**		**1.25**		**1**		**0**		**0**		**1.5**		**1.25**		**1.25**		**0.25**	

day for reservoir patch containing Diltiazem solution

Sr. No.	Sign	Score	N
1	Erythema	**1**	**1**
2	Scaling	**0**	**4**
3	Fissures	**0**	**4**
4	Oedema formation	**0**	**4**
5	Ecchymosis	**0**	**4**
6	Necrosis	**0**	**4**

Study of reservior patch containing Diltiazem gel

The results of skin irritation and skin sensitization study for reservoir patch containing Diltiazem gel are presented in Table 6.9.4. Following inferences has been drawn from irritation and sensitization studies of reservoir containing Diltiazem gel.

Table 6.9.5: The average score for each sign after day 20 and day 28 during irritation and sensitization study of reservoir patch containing Diltiazem gel

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.75	0.25
2	Scaling	0.25	0.25
3	Fissures	0.0	0.0
4	Oedema formation	0.5	0.5
5	Ecchymosis	0.25	0.0
6	Necrosis	0.25	0.25

Table 6.9.4: Skin irritation and sensitization study of reservoir patch containing Diltiazem gel

SR.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE							
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22	
1	Erythema	1	0	1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
		Avg	.25	Avg	.25	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	.25	Avg	.75	Avg	.75	Avg	0.5	Avg	.25
2	Scaling	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0.25
3	Fissures	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0
4	Oedama formation	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5
5	Ecchymosis	1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0
		1	0	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0
6	Necrosis	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1	0	1	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	0.5	Avg	.25	Avg	.25
Total of all score		4		4		4		6		6		5		3		1		2		8		9		7		5	
Total average per animal		1		1		1		1.5		1.5		1.25		0.75		0.25		0.5		2		2.25		1.75		1.25	

score for each sign for given concentration of drug was not more than 1, though the number of animals giving score 1 for the various sign was increased. However, score 1 is not considered under irritating category therefore Diltiazem patch is considered as safe. Results shows that total irritating scores were reduced from day 20 to day 28. Therefore, the primary and secondary sensitization was absent.

Table 6.9.6: Maximum sensitization and irritation score of various signs at last day for reservoir patch containing Diltiazem gel

Sr. No.	Sign	Score	N
1	Erythema	**1**	**1**
2	Scaling	**1**	**1**
3	Fissures	**0**	**4**
4	Oedema formation	**1**	**2**
5	Ecchymosis	**0**	**4**
6	Necrosis	**1**	**1**

6.10 CONCLUSION

EVA (VA 40%) Copolymer membrane moderated transdermal drug delivery system of Diltiazem was successfully prepared. Among different polymers used to prepared rate controlling membrane, EVA (VA 40%) copolymer, 30 μm thick membrane provided promising Diltiazem flux. Two different formulations of Diltiazem (I) alcoholic solution (II) Diltiazem gel were used to design two membrane moderated devices. On evaluation devices were found to be stable, non-irritant, non-sensitizing and safe. Devices complied to official and non-official pharmacokinetic specifications. The in vitro evaluation for drug release from device (I) and (II) across human live skin provided 43.95 and 23.78 μg/cm^{2}hr, Diltiazem flux respectively. The fluxes were adequate to meet pharmacokinetic requirements of steady state plasma concentration of Diltiazem for 24 hrs from 12.61 cm^2 reservoir device containing alohoolic solution of Diltiazem and 23.31 cm^2 reservoir device containing gel, giving once a day drug delivery system of Diltiazem.

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Chapter 7

Preparation and characterization of adhesive matrix diffusional transdermal drug delivery device of Diltiazem

CHAPTER 7

7 PREPARATION AND CHARACTERIZATION OF ADHESIVE MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF DILTIAZEM

AIM OF PRESENT INVESTIGATION

The adhesive matrix diffusional system tends itself to simple and easy processing of delivery device. The matrix (laminate, disc) being static in nature offers less problems of drug and formulation stability and results in robust product with near to no problems of handling and administration to patients.[1] Laminated form of delivery device offers easy transfer of technique from laboratory operations to a large scale manufacturing. As preparation of sheets, laminates etc from polymer beads is the major activity of plastic industry. Screw extrusion or injection molding can facilitate the formation of drug laminates from the polymer beads mixed with additives and active agents. For the preparation of adhesive drug matrix diffusion controlled transdermal device laminates are prepared using drug loaded adhesive and this layer is covered by protective liner and fixed on backing membrane, followed by punching of laminates in desired size.[2]

This type of transdermal drug delivery system can be easily prepared on large scale as a continuous manufacturing process as compare to polymeric matrix diffusion controlled transdermal drug delivery device and membrane moderate reservoir type controlled transdermal drug delivery device.[3]

Adhesive matrix diffusion controlled release transdermal drug deliver systems are monolithic systems which are the simplest and least expensive means of controlling the release of an active agent. Here the active agent is physically blended with the adhesive agent. The release rate is governed by Higuchi equation. The release in such system is proportional to square root of time and release is available until approximately 60 % of the drug is released. Thereafter release is related exponentially to time, exhibiting first order release.[4]

Parameter influencing the release characteristics of monolithic devices can be classified as solute dependent factors like solubility, partition co-efficient and diffusion coefficient of drug in the matrix. The solute independent parameters are system variables like geometry, tortuosity, pores, concentration, volume fraction and diffusion layer etc. In the present investigation solute related factors were considered

to fabricate the devices using adhesive.[5]

With perception to above objective, it is necessary to modify current solid dosage forms in to controlled transdermal drug delivery system. A first step in this process is to illustrate how formulation and process variables could give drug release through skin. The aim of present investigation is to formulate and optimize the Diltiazem adhesive matrix diffusion controlled transdermal drug delivery system.

7.1 Formulation and evaluation of adhesive drug matrix device of Diltiazem

7.1.1 Experimental

Preparation of adhesive matrix device of Diltiazem[6]

The transdermal therapeutic system comprised a backing membrane, an adhesive layer containing drug and a release liner. Adhesive matrix – type transdermal patches containing Diltiazem were prepared using different ratios of drug to adhesive (Table 7.1.1). Diltiazem was accurately weighed and dissolved in very small quantity of alcohol. This solution was mixed with natural rubber adhesive (Readymade received – Beta surgical, Rajkot). The uniform dispersion of drug and adhesive was spreaded on a drug impermeable polyethylene coated aluminum foil (5 x 3.5 cm^2) with the help of TLC kit spreader to form a thin drug adhesive layer and dried at room temperature. After 24h, the films were cut into a 1.13 cm^2 area and covered with glossy paper which is used as a protective liner. Dosage forms were kept in desiccators until further used.

Table 7.1.1: Composition of prepared adhesive matrix films

Formulation code	Adhesive (mg/cm^2)	Diltiazem (mg/cm^2)
F1	10	4
F2	10	6
F3	10	8
F4	10	10

Physiochemical evaluation of adhesive matrix device

Thickness

The thickness of the patches was assessed at six different points using thickness gauge

micrometer (0.001mm, Mitutoyo, Japan).

Weight variation

The weight variation for patch was determined using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan. Six patch from single batch (1.13 cm^2), were weighed individually and the average weight was calculated.

Drug content

The Diltiazem content of drug adhesive matrix was estimated in triplicate and analyzed by UV-VIS spectrophotometer at 236.0 nm. Patches (n=3) of specified area (1.13 cm^2), were cut and weighed accurately. The pieces were extracted using 10ml 0.01 N HCl in a 100 ml volumetric flask. It was shaken thoroughly and solution was transferred to a 100 ml volumetric flask. Similar procedure was repeated twice and collected fractions were mixed and filtered using whatman filter paper (Nyulge Nune, UK). This solution was diluted 100 times using 0.01 N HCl and UV absorbance of the resulting solution was measured at 236.0 nm using 0.01 N HCl as a blank.

Moisture content (Loss on drying)

The inherent moisture presents in material may influence the stability of dosage forms, especially if it contains a drug that is sensitive to water. The absolute method is employed to determine the moisture content which gives a weight loss registered during storage.

Three patch from each batch (1.13 cm^2), were weighed individually and the average weight was calculated. This weight was considered as an Initial weight. Then all the patches were kept in a desiccators containing activated Silica at normal room temperature for 24 hr. Then, the final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption was calculated as a difference between initial and final weight with respect to final weight.

% Moisture content = [(Initial weight – Final weight)/ Final weight] * 100

Moisture absorption

Moisture uptake influences the stability of dosage form. Low moisture uptake protects the material from microbial contamination. So for transdermal drug delivery system it was necessary to determine % Moisture absorption by matrices.

Three patch from each batch (1.13 cm^2), were weighed individually and the average weight was calculated. This weight was considered as an Initial weight. Then all the patches were kept in a desiccators containing 200 ml saturated solution of Sodium chloride (Relative humidity of 75%) at room temperature for 72h. The final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The % Moisture absorption was determined using below formula:

% Moisture absorption = [(Final weight – Initial weight)/ Initial weight] * 100

7.1.2 Results and discussion

The present investigation deals with the development of Diltiazem base adhesive matrix using different concentration of drug and adhesive. A diffusion mediated matrix controlled transdermal drug delivery system for Diltiazem base was successfully prepared using adhesive and it was evaluated using different physiochemical parameters.

Thickness

The results of thickness measurements are given in Table 7.1.2. The results indicate that there was no much difference in the thickness within the formulations. Thickness difference among formulations ranged from **110 ± 0.00 µm** to **83.33 ± 1.443 µm.** The results also indicated uniform distribution of the adhesive. The results indicated that as the concentration of Diltiazem increased thickness also increased.

Table 7.1.2: Results of thickness uniformity of F1 to F4 adhesive matrix formulations

Sr. No.	Formulation Code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	85.0	82.5	82.5	83.333 ± 1.443
2	F2	85.0	82.5	85.0	84.166 ± 1.443
3	F3	90.0	95.0	92.5	92.500 ± 2.500
4	F4	110.0	110.0	110.0	110.00 ± 0.000

***Standard deviation, n=3**

Weight variation

Drug loaded films (1.13 cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 7.1.3.

Table 7.1.3: Weight variations of F1 to F4 adhesive matrix formulations

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	12.34	12.56	12.43	12.44 ± 0.1106
2	F2	14.08	14.20	14.34	14.20 ± 0.1301
3	F3	15.84	15.50	15.00	15.44 ± 0.4225
4	F4	17.30	17.69	17.60	17.53 ± 0.2042

***Standard deviation, n=3**

The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for weight variation as per official requirements. All formulations showed acceptable pharmaco-technincal properties.

Drug content

Drug content of the drug adhesive matrix patch was carried out to ascertain that the drug loading is proper in the formulation. The prepared adhesive matrix patches (1.13 cm^2) were extracted using 0.01 N HCl. The amount of drug present was determined by measuring the absorbance spectrophotometrically at 236.0 nm using 0.01 N HCl as a blank. The results are represented in Table 7.1.4.

Table 7.1.4: Results of % Drug content of F1 to F4 adhesive matrix formulations

Sr. No.	Formulation Code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	101.50	101.20	101.00	101.23 ± 0.251
2	F2	98.57	96.52	98.53	97.87 ± 1.172
3	F3	101.20	100.05	101.23	100.82 ± 0.672
4	F4	97.80	98.90	99.02	98.57 ± 0.672

***Standard deviation, n=3**

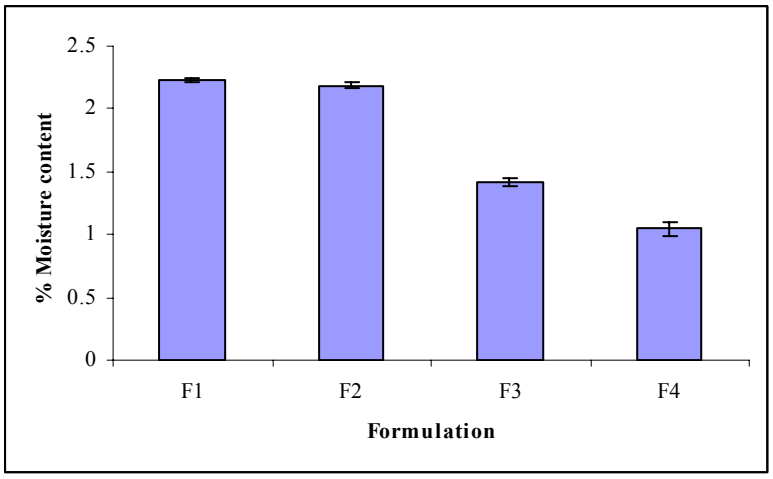
The medicated adhesive matrices were found to contain **97.87 %-101.23 %** of the labeled amount of Diltiazem indicating uniformity of drug content. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for content uniformity as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Diltiazem in the adhesive matrix formulations. Hence it was concluded that drug was uniformly distributed in all the formulations.

The drug content analyses of prepared formulation showed that the process employed to prepared patches was capable of giving uniform drug content, with minimum batch variability.

Moisture content (Loss on drying)

The moisture content was determined by keeping the patches in desiccators containing activated silica for 24h. The percentage moisture content was calculated from the weight differences relative to the final weight. The results of the moisture content studies for different formulations are shown in Figure 7.1.1.

Figure 7.1.1: Percentage moisture content from Diltiazem containing different adhesive matrix device



*** Standard deviation, n=3**

The moisture content in all the formulations was found to be low and ranged from **1.049 ± 0.054** to **2.225 ± 0.013%.** The % Moisture content of each device was determined and rank order of moisture content of various transdermal drug delivery

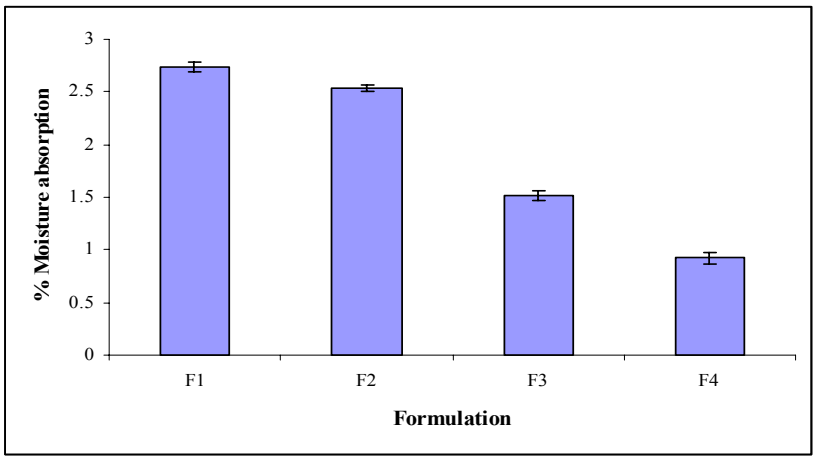
system were **F1 > F2 > F3 > F4.**

The result revealed that the moisture was present in the formulation due to presence of adhesive material but small moisture content in the formulations helps device to maintain its texture, flexibility etc.

Moisture absorption

The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The results of the moisture absorption studies for different Diltiazem adhesive matrix formulations are shown in Figure 7.1.2.

Figure 7.1.2: Percentage moisture absorption from Diltiazem containing different adhesive matrix device



*** Standard deviation, n=3**

The moisture absorption in all the formulations was found to be low and ranged from **0.9203 ± 0.0570** to **2.7361 ± 0.0453%.** The result revealed that the moisture absorption was found to decrease with increasing concentration of hydrophobic Diltiazem base. The % Moisture absorption of each film was determined and rank order of moisture absorption of formulations was **F1 > F2 > F3 > F4.**

7.2 In vitro drug diffusion study of adhesive matrix diffusional transdermal drug delivery device of Diltiazem

7.2.1 Experimental

In vitro diffusion study of Diltiazem from adhesive matrix transdermal patch was

carried out using modified Keshary-Chien diffusion cell. The transdermal therapeutic system with 3.14 cm^2 of surface area was placed on the brim of diffusion cell facing receptor compartment. The receptor compartment contained 40 ml of 0.01N HCl as a diffusion medium. The diffusion medium was stirred with bar type magnetic stirrer. The temperature was maintained at 37 ± 0.5^oC. Samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at different time intervals till 6 hrs. The absorbance of the withdrawn samples were measured UV VIS spectrophotometrically at 236.0 nm using 0.01N HCl as a blank. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of square root of time for different formulations. The release rate Q/√T was determined by simple regression analysis of steady state data.

7.2.2 Result and discussion

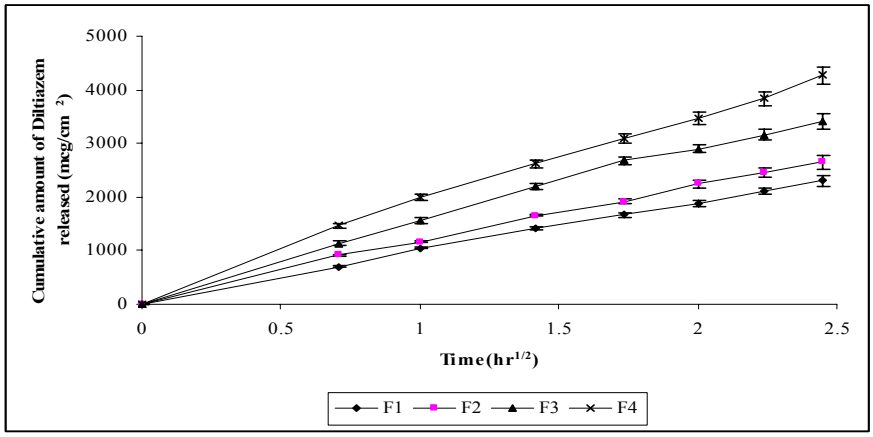
Diffusion study is important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. The results of in vitro diffusion studies are depicted in Table 7.2.1 and Figure 7.2.1.

Table7.2.1: Parameters of in vitro permeation kinetic of Diltiazem from adhesive matrix patch

Parameters	Formulation code			
	F1	F2	F3	F4
Q/√T (µg/cm^2√hr)	897.13	1012.50	1308.3	1556.70
Correlation coefficient	0.9975	0.9980	0.9928	0.9981

Release of Diltiazem from the drug loaded adhesive matrix followed square root law. The release rate Q/√T was computed by regression analysis of steady state data and it was the slope of the linear relationship obtained between cumulative amounts of Diltiazem released versus square root of time.

Figure 7.2.1: In vitro diffusion profiles of Diltiazem from drug adhesive matrix patch



*** Standard deviation, n=3**

Release rate Q/√T increased with increasing concentration of Diltiazem base in adhesive matrix. In our experiments, variable release profiles of Diltiazem from the different experimental patches composed of different proportion of drug and adhesive were observed. The rank order of release rate observed was **F1 < F2 < F3 < F4.**

The formulations **F4** exhibited the maximum Q/√T (**1556.70 µg/cm^{2}h$^{1/2}$**) release rate, which was significantly different, compared to the lowest value in the formulation **F1** (**897.13µg/cm^{2}h$^{1/2}$**). Based on in vitro release experiments, formulation **F4** was chosen for further in vitro permeability study through human live skin.

In vitro release kinetic

In vitro diffusion study of Diltiazem released into infinite sink was carried out using the modified Keshary-Chien diffusion cell. The release data were fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 7.2.2. Here R is regression coefficient.

Table 7.2.2.: Data of various parameters of model fitting in formulations

Formulation	Zero order equation	First order equation	Higuchi's equation
F1	**0.9888**	**0.9505**	**0.9993**
F2	**0.9736**	**0.9218**	**0.9970**
F3	**0.9529**	**0.8948**	**0.9896**
F4	**0.9903**	**0.9570**	**0.9975**

In our experiment, the in vitro permeation profile of adhesive matrix patch could be best expressed by Higuchi's equation for the permeation of drug from an adhesive matrix device.

7.3 In vitro human live skin permeation study of drug adhesive matrix device of Diltiazem

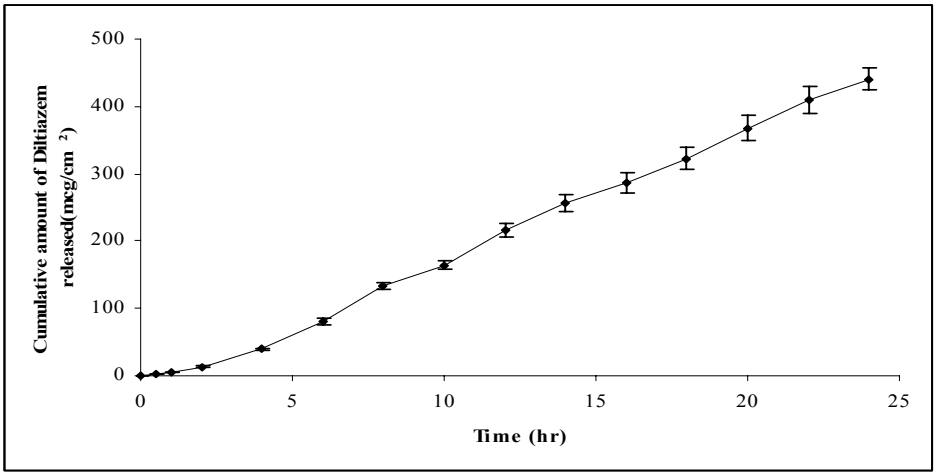
7.3.1 Experimental

The permeation study was performed in a modified Keshary-Chien diffusion cell. The experiment was performed as mention in section 5.6.2. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time for adhesive matrix formulation. The release rate was determined by simple regression analysis of steady state data.

7.3.2 Results and discussion

The result of in vitro skin permeation of Diltiazem from adhesive matrix formulation is shown in figure 7.3.1 and Table 7.3.1.

Figure 7.3.1: In vitro skin permeation profile of Diltiazem from drug adhesive matrix patch through human live skin



*** Standard deviation, n=3**

Parameters of diffusion kinetics of Diltiazem released, from device across human live skin are presented in Table 7.3.1.

Table 7.3.1: Parameters of diffusion kinetic of Diltiazem from drug adhesive matrix patch through human live skin

Sr. No.	Parameters	Value
1	Skin flux (Jss)	19.64 µg/ cm^2 hr
2	Time lag (t_L)	1.47 hr
3	Human live skin thickness (µm)	140 µm
4	Diffusion coefficient	2.222 x 10^{-5} cm^2/sec
5	Solubility of drug in skin (Cs)	12.37 mg/cm^3

Adhesive matrix device containing Diltiazem provided **19.64 µg/ cm^2 hr** skin flux. The time lag (t_L) for devices is presented in Table 7.3.2. The diffusion coefficient, D of Diltiazem was determined using $D = h^2/6\ t_L$ relationship, where skin thickness h was 140 x 10^{-4} cm. The amount of Diltiazem retained by skin area used for permeation was calculated by dividing flux with gradient of diffusivity and it was found to be **12.37 mg/cm^3** of skin.

Computation of area of patch required for target steady state plasma concentration (Css)

As mentioned in section 5.4.3, desired input rate through skin for target steady state plasma concentration of Diltiazem is 554.4 µg/ hr

Area of patch = (554.4 µg/hr)/ $J_{ss(skin)}$

= 554.4/19.64cm^2

= 28.22 cm^2

Formulation of final patch

Final formulation was selected as 24 hour once a day device having area is 28.22 cm^2. For the preparation of final adhesive matrix diffusion drug delivery device, 0.58 ml of natural adhesive solution was layered over 4 mm rim of delivery device giving 10 µm thick layers. This layer assists the perfect adhesion of device on the skin.

7.4 STABILITY STUDY

7.4.1. Stability study of adhesive matrix diffusional transdermal drug delivery device

The objective of accelerated stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature**.** The optimized formulation of Diltiazem adhesive matrix patch was selected for the stability study.

The accelerated stability study were carried out according to ICH guideline by storing the samples at 25 ^{0}C / 60 % RH, 30 ^{0}C/ 65 % RH and 40 ^{0}C/ 75 % RH for 90 days in a stability chamber (Thermo Lab., Mumbai, India). These samples were analyzed by UV Spectrophotometer method and checked for changes in physical appearance and drug content at an interval of 15 days**.**

7.4.2 Results and Discussion

Final formulation selected was subjected to stability study and observed for change in color, appearance, flexibility and drug content. Temperature and humidity values selected were as per the ICH guidelines and the test was carried out in a stability chamber. The stability study was carried out at 25 ^{0}C / 60 % RH, 30 ^{0}C/ 65 % RH and 40 ^{0}C/ 75 % RH for 90 days. Diffusion study was carried out and it was observed that formulation stored at 40 ^{0}C exhibited higher Q/√T release rate as compared to those stored at 25 ^{0}C and 30 ^{0}C. The initial release of drug was higher at an elevated temperature due to decreased viscosity of adhesive matrix. Release rate at 30 ^{0}C was altered but it was in order. The product stored at 25 ^{0}C exhibited no change in release rate. The degradation was higher at an elevated temperature. The first order rate constant of degradation for room temperature was **1.329×10^{-3} week^{-1}**. The self life calculated was **79 week.**

Results of stability study indicated a good stability for adhesive matrix loaded drug delivery device. The results of stability study indicated that the products should be stored at a temperature not exceeding 40 ^{0}C and also should not be refrigerated as at lower temperature it lost overall flexibility and turned rigid loosing elegancy. The product should be stored at room temperature.

7.5 SKIN IRRITATION AND SKIN SENSITIZATION STUDY

7.5.1 Experimental

Skin irritation and skin sensitization though are different types of physiological responses yet they have several common indications. Skin sensitization is systematic response and skin irritation is primarily is local response. A protocol was devised for evaluation of skin irritation and/or sensitization in such a manner that the signs at the sight of application would be assessed in common for the both and further, to distinguish sensitization from irritation. The skin irritation and skin sensitization study was carried out as per procedure mentioned in chapter 5.8.1.

The final Diltiazem adhesive matrix patch (2.0 cm^2) was supported with aluminum foil. This was applied to the skin surface (shaved) and made adhered with the help of "3 M Micropore"- medicated adhesive tap (3M Corporation, U.K.).

7.5.2 Results and discussion

The results of skin irritation and skin sensitization study for Diltiazem adhesive matrix patch is presented in Table 7.5.1. From this table total average score at day 20 and total average score at day 28 were obtained respectively.

From Table 7.5.2, it was clear that the Diltiazem adhesive matrix patch was non-irritating, since the maximum score for each sign did not exceed than 1. Further Table 7.5.3 shows that average score at day 20 and day 28 was not exceeding more than 1 for any sign. Average score not exceeding more than 1 at any day for any sign was clearly indicative for non-irritation nature of the agent.

Diltiazem is non-sensitizing in nature. The reason for this was reduction in scores even after repeated applications. All the tables show that maximum individual scores, average scores, total irritation scores were reduced at day 28^{th} in comparison to all these scores at day 20^{th}. Reduction in scores even after repeated application of test patch indicated absence of primary and secondary sensitization reaction.

Table 7.5.1: Skin irritation and sensitization study of Diltiazem adhesive matrix patch

SR.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE							
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	.75	Avg	0.5	Avg	0.5	Avg	0
2	Scaling	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25
3	Fissures	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	.75	Avg	.75	Avg	.75	Avg	0.5
4	Oedama formation	1	0	0	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	2	0	2	0	2	0	1
		0	0	0	0	0	1	1	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	0	1	0	1
		Avg	.25	Avg	.25	Avg	0.5	Avg	1	Avg	.75	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	1.25	Avg	1.25	Avg	.75	Avg	0.5
5	Ecchymosis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	2	2	2	2	2	2	1	1
		0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	2	2	1	1	1	1	1	1
		Avg	0.5	Avg	0.5	Avg	0.5	Avg	1	Avg	1	Avg	1	Avg	0.5	Avg	0	Avg	0	Avg	2	Avg	1.5	Avg	1.5	Avg	1
6	Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	2	1	1	0	0
		0	0	0	0	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	0	1	0	0
		Avg	0	Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	.25	Avg	1.25	Avg	1.25	Avg	.75	Avg	0
Total of all score		3		4		7		16		15		13		10		3		1		24		20		17		9	
Total average per animal		0.75		1		1.75		4		3.75		3.25		2.5		0.75		0.25		6		5		4.25		2.25	

Table 7.5.2: The average score for each sign after day 20 and day 28 during skin irritation and sensitization study of Diltiazem adhesive matrix patch

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.75	0.0
2	Scaling	0.0	0.25
3	Fissures	0.75	0.5
4	Oedema formation	1.25	0.5
5	Ecchymosis	2.0	1.0
6	Necrosis	1.25	0.0

Table 7.5.3: Maximum sensitization and irritation score of various signs at last day for Diltiazem adhesive matrix patch

Sr. No.	Sign	Score	N
1	Erythema	**0**	**4**
2	Scaling	**1**	**1**
3	Fissures	**1**	**2**
4	Oedema formation	**1**	**2**
5	Ecchymosis	**1**	**4**
6	Necrosis	**0**	**4**

7.6 CONCLUSION

An adhesive matrix diffusional transdermal controlled drug delivery device of Diltiazem was successfully prepared. The processing was non complicated, very simple and economic still meeting requirements of systemic administration of drug. Diltiazem loaded natural rubber based adhesive matrix were prepared and devices were fabricated containing varied drug loading. Device containing 10 mg/cm^2 of

Diltiazem was found to meet the biopharmaceutical and pharmacokinetic requirement for the therapeutic use of device. On evaluation the device was found to be stable, non-irritant, non-sensitizing and safe. It complied with official and non- official pharmacotechnical specifications. In vitro evaluation for Diltiazem release from device across human live skin provided zero order release rate **19.64 µg/ cm^2 hr.** The flux was adequate to meet pharmacokinetic requirement of steady state plasma concentration for 24 hours, giving once a day drug delivery system of Diltiazem.

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Chapter 8

Preparation and characterization of polymeric matrix diffusional transdermal drug delivery device of Atenolol

CHAPTER 8

8 PREPARATIONS AND CHARACTERIZATION OF POLYMERIC MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF ATENOLOL

AIM OF PRESENT INVESTIGATION

The age-old theory that imparted the status of "dead, impermeable barrier devoid of biological activity" to skin had already been challenged by the development of pioneering transdermal products[1,] but a less than impressive commercial growth in this sector had raised some doubts about the feasibility of this route as an efficient device of drug delivery. The journey of transdermal research had commenced with a lots of enthusiasm, as it heralded the promise of noninvasive cutaneous application. The projected advantages were publicized so much that the target consumers were prepared to accept the product even if they were costlier alternatives to the conventional therapy. This acceptability factor had encouraged researchers and industries alike to take up challenging project in this particular area. For the last two decades, it remained an area of vital research interest, and data was generated for almost every available drugs.[2]

Transdermal dosage forms, though a costly alternative to the conventional formulations, are becoming popular because of some unique advantages. Controlled zero order absorption, simple administration mode and the option of easy removal in case of adverse manifestations make them particularly desirable in cardiovascular therapy. Nitroglycerine and Isosorbide dinitrate, the two antiischaemic drugs; and the Clonidine, an antihypertensive molecule, are being extensively used in the transdermal form. Studies that compared with the established dosage forms had shown that though patches were costlier than conventional prescription products they reduced the occurrence of hospitalization and diagnostic cost. Currently numbers of antihypertensive drugs are under investigation for transdermal administration.

Antihypertensive is group of cardiovascular agents that had generated excitement amongst the transdermal scientists, specifically the β-adrenergic receptor antagonist. These drugs have a multiple utilities and are administered in hypertension, ischaemic heart disease including certain types of arrhythmias. Since antihypertensive suffer from the disadvantages of extensive first pass metabolism and variable bioavailability, they were considered ideal transdermal candidate.[3]

Atenolol, a β-adrenergic receptor antagonist, has been shown to be safe and effective in the treatment of patients with hypertension. It has a mean plasma half-life of 6 hrs and only 45% of the orally administered drug reaches the circulation due to hepatic metabolism. A model reported predicts that mean plasma Atenolol concentration of 43, 99 and 175 ng/ml are required to produce a 20%, 30%, and 40% reduction in blood pressure respectively. [4] This concentration can be achieved by preparing matrix diffusion controlled transdermal drug delivery system. Atenolol, a hydrophilic drug, with a relatively high effective concentration was less investigated compare to other β- blockers. Limited data available on the permeation studies shows that permeation increases in presence of chemical enhancers.[5]

Matrix diffusion controlled release transdermal drug deliver systems are monolithic systems which are the simplest and least expensive means of controlling the release of an active agent. Here the active agent is physically blended with the polymer agent. The release rate is governed by Higuchi equation. Parameter influencing the release characteristics of monolithic devices can be classified as solute dependent factors like solubility, partition co-efficient and diffusion coefficient of drug in the polymer matrix. The solute independent parameters are systems variables like geometry, tortuosity, pores, concentration, volume fraction and diffusion layer etc. in the present investigation solute related factors were considered to fabricate the devices using different polymer matrix. Polymer matrices employed were of non polar and hydrophobic nature.

With perception to above objective, it is necessary to modify current solid dosage forms in to controlled transdermal drug delivery system. A first step in this process is to illustrate how formulation and process variables could give drug release through skin. The aim of present investigation is to formulate and optimize the Atenolol matrix diffusion controlled transdermal drug delivery system. In the present investigation, the influence of various grades and concentration of polymers were studied. Study was carried out to formulate an elegant product exhibiting desired therapeutic performance, from a small and cute dosage form.

In order to achieve this goal, following criteria were set

- The dosage form should remain intact for a period of 24hr.
- Drug is delivered in a controlled manner.
- The size of dosage form should be small with a view to enhance convenience of patient as well as compliance to therapy.

- Plasma concentration should achieve within short period of time.

8.1 ANALYTICAL METHOD FOR ESTIMATION OF ATENOLOL

Atenolol can be estimated by various methods such as UV Spectroscopy, HPLC, HPTLC etc. In the present investigation Atenolol was estimated by UV Spectrophotometry.

8.1.1 Determination of λmax

A solution of Atenolol was prepared in 0.01N HCl and UV spectrum was taken using Shimadzu UV/VIS double beam spectrophotometer. UV spectrum of was recorded by scanning 5 µg/ml solution of Atenolol in 0.01N Hydrochloric acid and scanned between 200-400 nm.

8.1.2 Preparation of calibration curve

The calibration curve of Atenolol in 0.01N HCl was prepared by measuring the absorbance of the solution in the range of 20-100 µg/ml. the absorbance of the solution was measured at 273.5 nm.

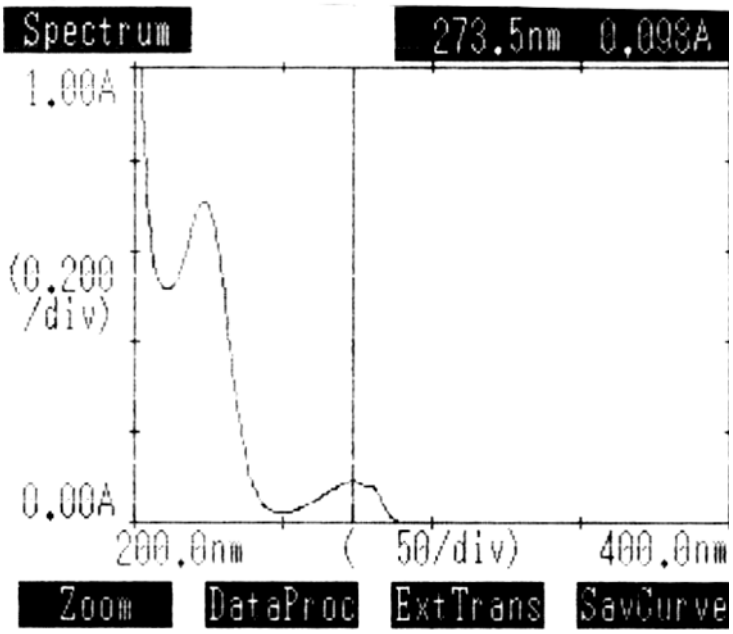
Atenolol (100mg) was dissolved in 40ml 0.01N HCl and volume was made up to 100ml using 0.01N HCl in 50 ml volumetric flask. This stock solution (1mg/ml) was further diluted with 0.01N HCl to obtained solution of 20 to 100 µg/ml. Absorbance of each solution was measured at 273.5 nm using Shimadzu UV/VIS Spectrophotometer with 0.01N HCl as a reference standard. The standard curve was generated for entire range of 20 to 100 µg/ml. The experiment was performed in triplicate and based on average absorbance; the equation for the best line fit was generated.

8.1.3 Result and discussion

Determination of λmax

The UV maxima of resultant solution were measured with Shimadzu UV- 2201 UV/VIS Spectophotometer. Figure 8.1.1 shows the UV spectrograph of Atenolol in 0.01N HCl.

Figure 8.1.1: UV spectrograph of Atenolol in 0.01N HCl



Point pick

Abscis.	Ordinat.		Abscis.	Ordinat.
1	275.0	0.097	11	273.0	0.097
2	274.8	0.097	12	272.8	0.096
3	274.6	0.098	13	272.6	0.096
4	274.4	0.098	14	272.4	0.095
5	274.2	0.098	15	272.2	0.094
6	274.0	0.098	16	272.0	0.094
7	273.8	0.098	17	271.8	0.093
8	273.6	0.098	18	271.6	0.093
9	273.4	0.097	19	271.4	0.092
10	273.2	0.097	20	271.2	0.091

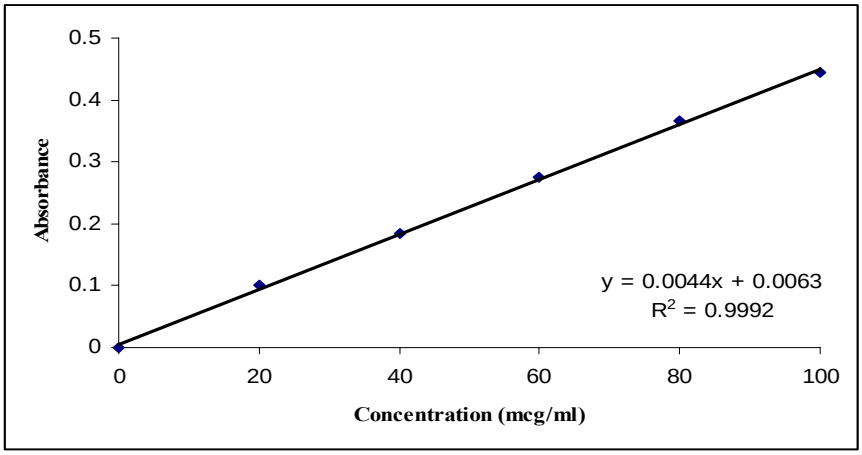
Calibration curve

The calibration curve of Atenolol in 0.01N HCl was prepared by measuring the absorbance of the solution in the range of 20-100 µg/ml. the absorbance of the solution measured at the wavelength 273.5 nm. The results of standard curve are shown in Table 8.1.1 and Figure 8.1.2

Table 8.1.1: Calibration curve of Atenolol in 0.01N HCl at 273.5nm

Concentration (µg/ml)	Absorbance
0	0.000 (0.000)
20	0.101 (0.005)
40	0.184 (0.002)
60	0.275 (0.001)
80	0.365 (0.006)
100	0.445 (0.007)
Correlation coefficient = 0.9992	
Absorbance = 0.0044 x concentration + 0.0063	
Values in parenthesis indicates standard deviation (n=3)	

Figure 8.1.2: Calibration curve of Atenolol in 0.01N HCl at 273.5nm



8.2 PREFORMULATION STUDIES

The physiochemical properties of Atenolol were determined using following parameters.

8.2.1 Determination of melting point

Melting point of drug was determined using capillary tube closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded. This was performed in triplicates and average value was noted.

8.2.2 Solubility studies

The solubility studies were performed in phosphate buffer solution, pH 7.4, in distilled water, methanol, chloroform, ether, alcohol (95%), acetone, acetic acid, Isopropanol, Dioxane, acetonitril and ethyl acetate separately by adding excess amounts of drug in each case and keeping the excess drug containing flasks on a water bath shaker NSW-133 for 24hr at 32^{o}C.

8.2.3 Infrared (IR) Spectroscopic analysis

In the preparation of film formulation, drug and polymer may interact as they are in close contact with each other, which could lead to the instability of drug. Pre formulation studies regarding the drug-polymer interaction are therefore very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility between Atenolol and the selected polymers. The individual drug and drug with excipients were scanned separately.

The Fourier Infrared (FTIR) spectrums of moisture free samples were recorded on IR spectrophotometer by potassium bromide (KBr) pellet method. The scanning range was 4000 to 400 cm^{-1} and the resolution was 1 cm^{-1}.Pottasium bromide was mixed with drug and polymer and the spectra were taken. FT-IR spectrum of Atenolol was compared with FR-IR spectra of Atenolol with polymers. Disappearance of Atenolol peaks or shifting of peak in any of the spectra was studied.

8.2.4 Differential scanning calorimetry analysis

Differntial scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced during transformation of state(i.e., endothermic or exothermic phase transformation). DSC curves for pure drug Atenolol, Eudragit RL100 and Eudragit RS100 mixture and their composites were recorded using DSC-Shimadzu 60 with TDA trend line software. Drug and polymer was weighed (7-10 mg) and heated at a scanning rate of 10^{o}C min under dry nitrogen flow (100 ml/min) between 50-350^{o}c. Aluminum pans and lids were used for drug sample. Pure water and indium were used to calibrate the DSC temperature scale and enthalpy response.

8.2.5 Result and discussion

Melting point

Melting point of Atenolol was found to be 154 ± 2.5 ^{o}C (average of three readings).

This value is same as that of the literature citation**.**

Solubility study

Solubility of Atenolol was evaluated in different solvent. The results are mentioned in Table 8.2.1.

Table 8.2.1 Solubility of Atenolol in different solvents

Solvent	Solubility
Methnol	Soluble
Acetic acid	Soluble
Dimethyl sulfoxide	Soluble
Methanol:Acetone (3:7)	Soluble
Ethanol (96%)	Sparingly soluble
Water	Slightly soluble
Isopropanol	Slightly soluble
Acetone	Very Slightly soluble
Dioxane	Very Slightly soluble
Acetonitril	Insoluble
Ethyl acetate	Insoluble
Chloroform	Insoluble

Infrared (IR) Spectroscopic analysis

FT-IR spectroscopic studied was carried out to assess any interaction between the drug and the excipients. The chemical interaction between the drug and the polymer often leads to identifiable changes in the Infrared (IR) profile of complexes. The FT-IR spectrum of pure Atenolol is shown in figure 8.2.1(a). The FT-IR spectra of atenolol with Ethylene vinyl acetate copolymer (Vinyl acetate 40%), Ethyl cellulose, Eudragit RL100, Eudragit RS 100 are shown in Figure 8.2.1(b), the presence or absence of characteristics peaks associated with specific structural groups of the drug molecule was noted. From the FTIR spectra it was revealed that no interaction occurred between Atenolol and different polymers.

Figure 8.2.1(a): FT-IR Spectra of pure drug (Atenolol)

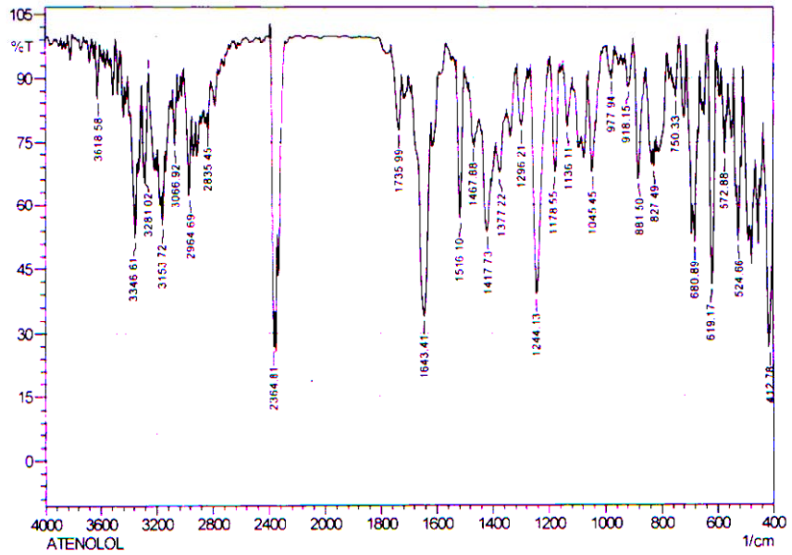
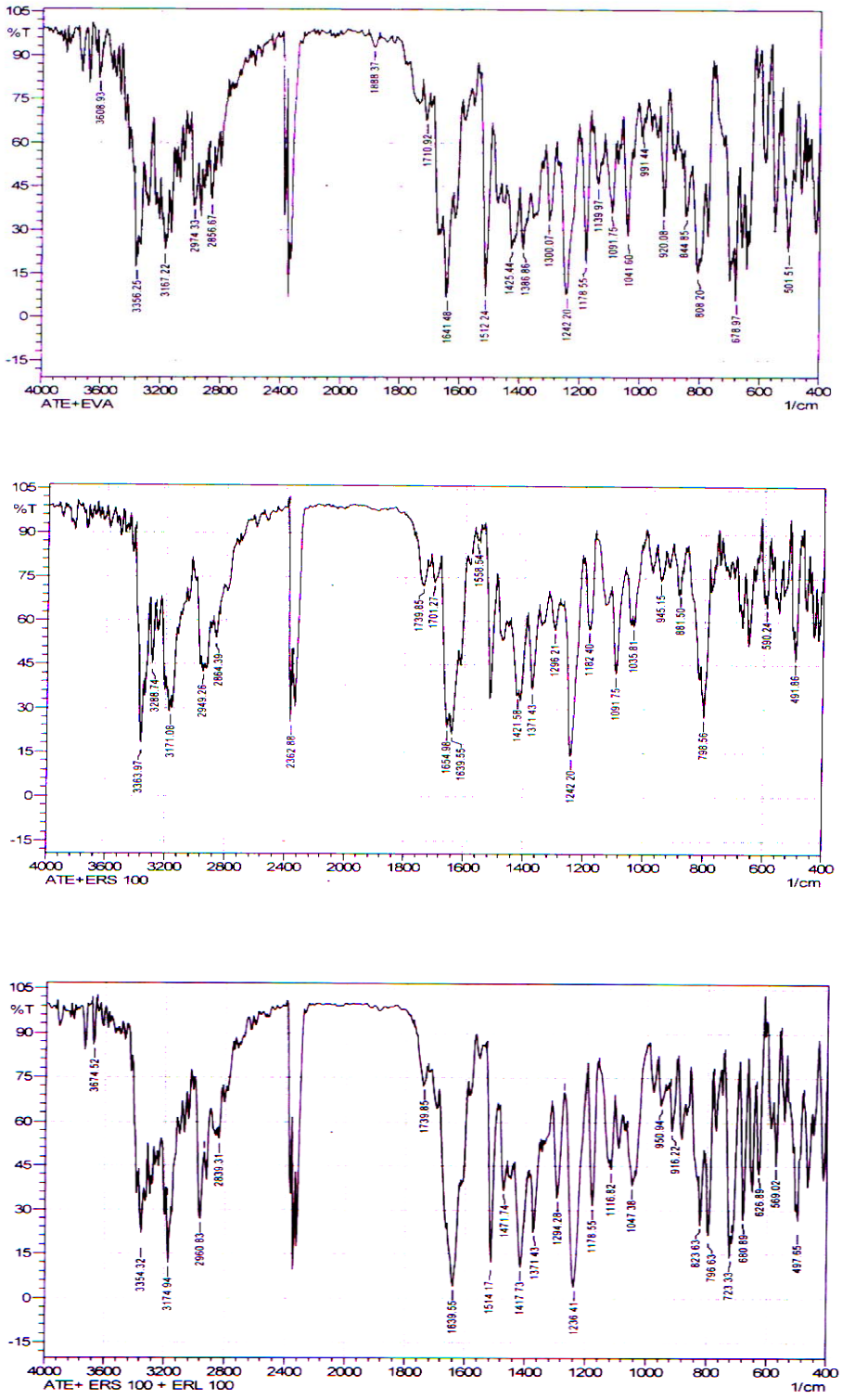
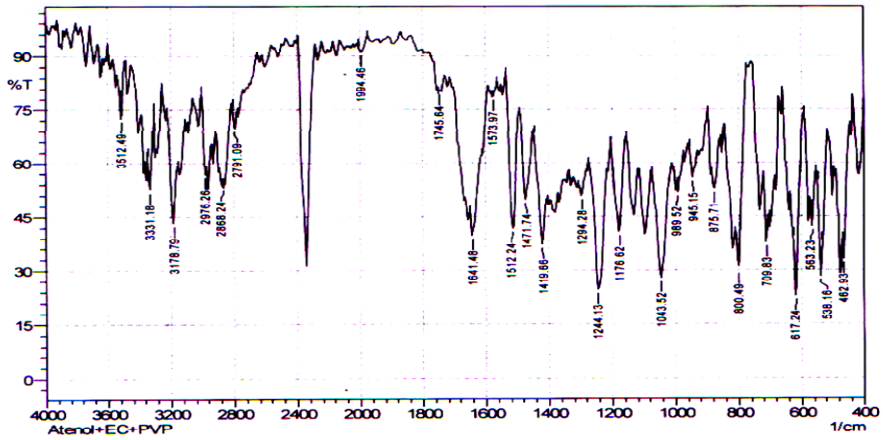


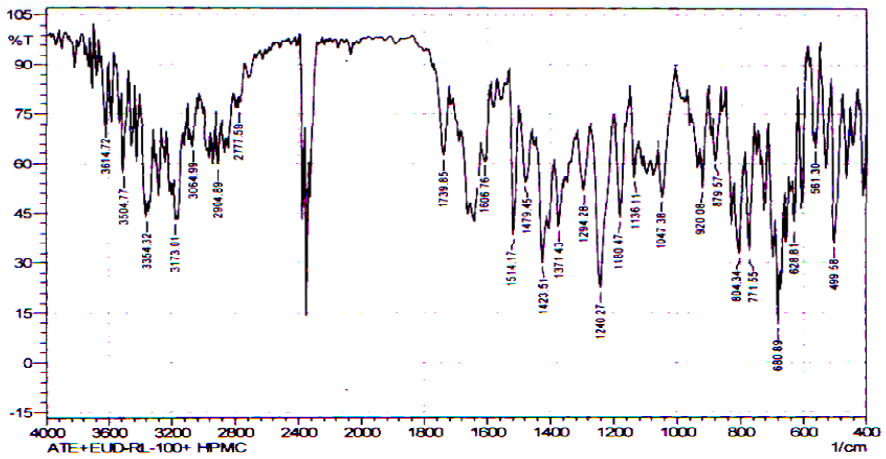
Table 8.2.2: FT-IR Spectral data of Atenolol [6]

Frequency (cm^{-1})	Assignment
3346.61	-CO-NH-
3153.72	-CO-NH-
2964.69	C-H stretching (alkane)
1643.41	C=O stretching (amide)
1516.10	-N-C=O, amide
1417.73	H_2N-CO-
1377.22	C-O stretching (alcohols)
1244.13	Arylether
1178.55	i-pr

Figure 8.2.1(b): IR specta of Atenolol composites







Differential scanning calorimetric analysis

Differential scanning calorimetry enables the quantitative detection of all processes in which energy is required or produced during transformation of state (i.e., endothermic or exothermic phase transformation). DSC curves for pure drug Atenolol was shown in figure 8.2.2(a). DSC curve of ERS 100, ERL 100 and Atenolol composite are shown in Figure8.2.2 (c). Pure powdered Atenolol showed a melting endotherm at 158.95 ^{o}C temperature. DSC scan of ERL100 and ERS100 showed a broad endotherm due to the presence of residual moisture in polymers.

DSC thermogram of Atenolol with ERL100 and ERS100 exhibits endothermic peaks at near to 161.80 ^{o}C temperature. Results reveal absence of any interaction occurring between drug and polymers.

Figure 8.2.2(a): DSC thermogram of Atenolol

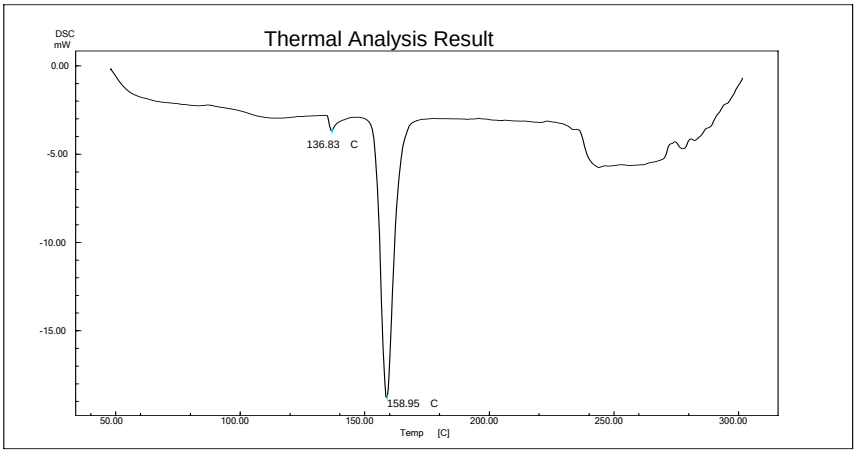


Figure 8.2.2(b): DSC thermogram of ERL100 and ERS 100

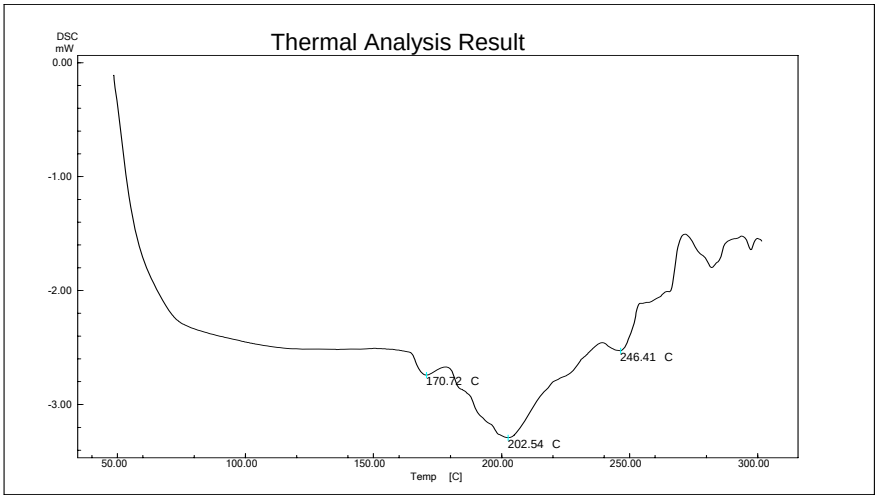
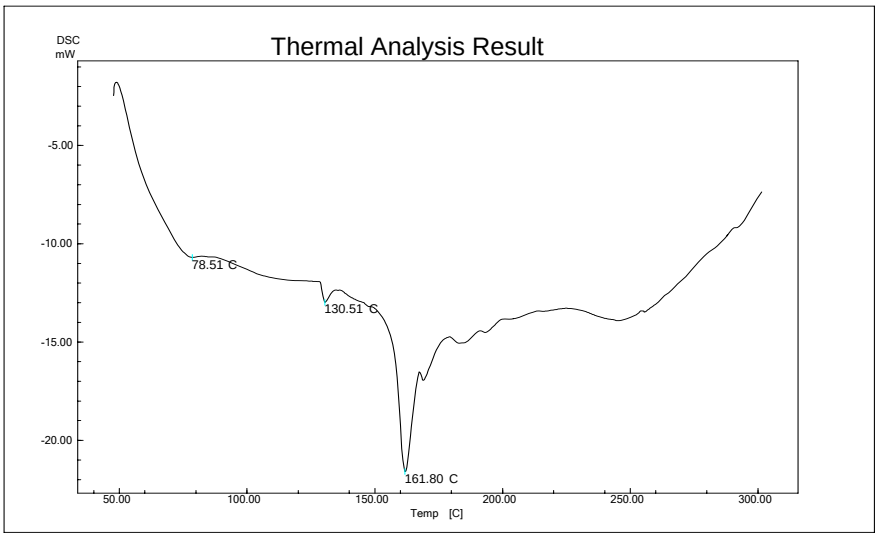


Figure 8.2.2 (c): DSC thermogram of Atenolol composite



8.3 PREPARTION AND CHARACTERIZATION OF POLYMERIC MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF ATENOLOL

8.3.1 Preparation of polymeric matrix device

Matrix – type transdermal patches containing Atenolol were prepared using different polymers (Table 8.3.1).The polymers were weighed in requisites ratios keeping the total polymer weight 800 mg, and dissolved in a given solvent. Di-n-butyl Phalate (30% w/w of polymer composition) were used as a plasticizer. Atenolol (533.33mg) was added and mixed using a mechanical stirrer. The uniform dispersion of polymeric solution of drug (10 ml) was poured on the mercury pool. Solvent was evaporated at room temperature, and matrices were carefully lifted. Laminates were punched out to have a 3.14 cm^2 area. Laminates were affixed on polyethylene coated aluminum foil backing. The release face of medicated laminate was covered with peelable silicone liner. The devices were stored in desiccators, at room temperature until further used.[7]

Table 8.3.1: Composition of prepared films

Formulation code	Polymers	Plasticizers (30% w/w of polymer composition)	Solvent
F1	EVA (VA 40%) copolymer	----------	2ml acetic acid and 10 ml toluene
F2	ERS100	DBP	Methanol
F3	ERL100: ERS100(1:1)	DBP	Methanol
F4	EC:PVP (2:3)	DBP	Methanol
F5	ERL 100:HPMC (2:3)	DBP	Methanol

Figure 8.3.1: Matrix diffusional transdermal patch of Atenolol

8.3.2 Physiochemical evaluation of polymeric matrix device

Prepared Atenolol containing matrices were evaluated for various parameters like thickness , weight variation, drug content, flatness, folding endurance , moisture content, moisture absorption, % WVTR etc, as per procedure given in the chapter 5, section 5.3.2.

8.3.3 Result and discussion

The present investigation deals with the development of Atenolol loaded polymeric matrix using different polymers. The preliminary screening was carried out for the selection of best polymer. A diffusion mediated matrix controlled transdermal drug delivery system for Atenolol was successfully prepared using different polymers using mercury subtract method and all matrices were evaluated using different physiochemical parameters.

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of film was measured at six different points and the average thickness was noted. The results of thickness measurements are given in Table 8.3.2. The results indicate that there was no much difference in the thickness with in the formulations. Thickness in the different formulations was in the range of **182.5 ± 2.5 µm** to **85.0 ± 2.5 µm**. Maximum thickness was found in formulation F1, while minimum found in formulation F4. These results revealed that thickness was found to increase as hydrophobic portion of polymer increases. The results of thickness measurements also indicate uniform distribution of the drug and polymer over the mercury surface. The rank order of thickness of Atenolol loaded polymeric matrices was **EVA (40% VA)**

copolymer > ERS 100 > ERL100:ERS100 (1:1)> EC: PVP (2:3) >ERL 100: HPMC (2:3)

Table 8.3.2: Results of thickness uniformity of F1 to F5 film formulations

Sr. No.	Formulation Code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	180.0	185.0	182.5	182.50 ± 2.500
2	F2	165.0	165.0	162.5	164.16 ± 1.443
3	F3	122.5	125.0	120.0	122.50 ± 2.500
4	F4	115.0	112.5	115.0	112.50± 1.443
5	F5	85.0	87.5	82.5	85.00 ± 2.500

***Standard deviation, n=3**

Weight variation

Drug loaded films (3.14cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 8.3.3. The weight of 3.14 cm^2 film ranged from **50.30 ± 0.100 mg** to **58 ± 0.500 mg.** The weight of the patches was found to be uniform among different batches.

Table 8.3.3: Results of weight variations of F1 to F5 film formulations

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	52.4	52.5	52.1	52.33 ± 0.208
2	F2	55.3	55.8	55.6	55.56 ± 0.251
3	F3	58.3	58.7	58.5	58.50 ± 0.200
4	F4	50.6	50.1	50.9	50.53 ± 0.404
5	F5	53.5	53.1	53.7	53.43 ± 0.305

***Standard deviation, n=3**

In a weight variation test, the pharmacopoeial limit for the percentage deviation of all the films of less than mg is ± 10%. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed

the test for weight variation as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Atenolol in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation, with small deviation.

Flatness

The flatness was measured manually for the prepared films. An ideal patch should be formulated in such a way that it possesses a smooth surface and it should not constrict with time. Flatness studies were performed to assess the same. The results of the flatness study showed that none of the formulations had the differences in the strip length before and after their cuts. It indicates **100%** flatness observed in the formulated patches. Thus, no amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the patches and thus they could maintain a smooth surface when applied on to the skin.

Folding endurance

Folding endurance was determined manually for drug loaded polymeric matrices. The folding endurance of the films was determined by repeatedly folding a strip measuring 2x2 cm size at same place till it break. The number of times the film could be folded at the same place without breaking gave the value of folding endurance. The results of folding endurance are given in Table 8.3.4.

Table 8.3.4: Results of folding endurance of F1 to F5 film formulations

Sr. No.	Formulation Code	Folding endurance			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	248	250	247	248.33 ± 1.527
2	F2	108	110	112	110.00 ± 2.000
3	F3	55	50	57	54.00 ± 1.000
4	F4	19	17	18	18.00 ± 1.000
5	F5	35	38	45	39.33 ± 5.131

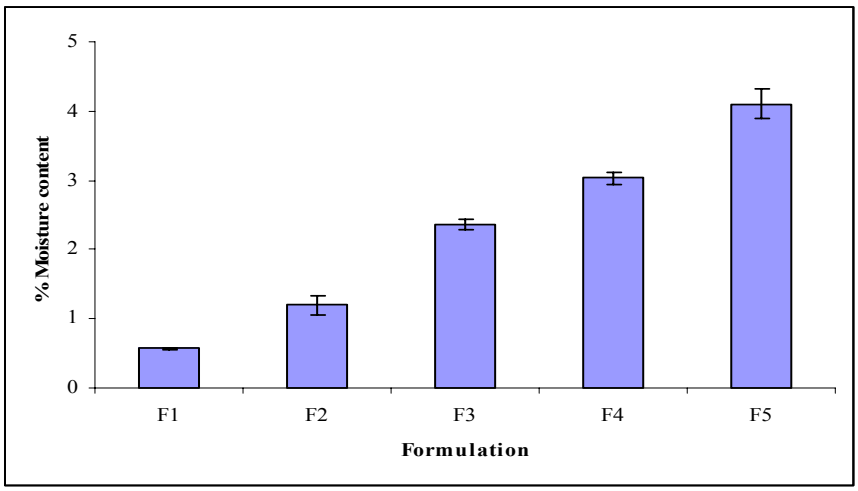
***Standard deviation, n=3**

Here formulation F1, F2 and F3 shows good folding endurance as compare to formulation F4 and F5.

Moisture content (Loss on drying)

The moisture content was determined by keeping the drug loaded polymeric matrices patches in dessicator containing activated silica for 24h. The percentage moisture content was calculated from the weight differences relative to the final weight. The results of the moisture content studies for different formulations are shown in Figure 8.3.2.

Figure 8.3.2: % moisture content in drug loaded polymeric matrices of Atenolol



*** Standard deviation, n=3**

The moisture content in all the formulations was found to be low and ranged from **0.571 ± 0.013** to **4.103 ± 0.210 %.** The result revealed that the moisture content was found to increase with increasing concentration of hydrophilic polymers. The small moisture content in the formulations helps them to maintain texture.

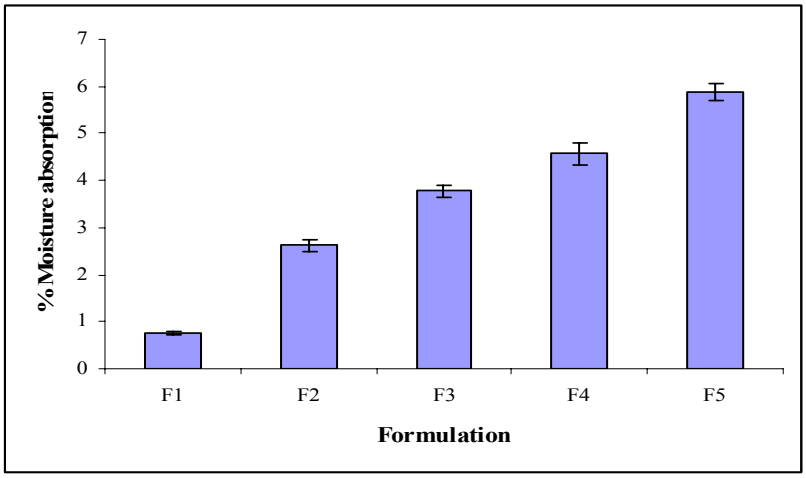
The rank order of % moisture content of Atenolol loaded polymeric matrices was **EVA (VA 40%) copolymer < ERS 100 < ERL100:ERS100 (1:1) < EC: PVP (2:3)>ERL 100: HPMC (2:3)**

Moisture absorption

% Moisture absorption was determined by keeping the drug matrices in a desiccators containing 200 ml saturated solution of Sodium chloride (Relative humidity of 75%) at normal room temperature for 72hr. The final weight was noted when there was no further change in the weight of individual patch. The percentage moisture absorption

was calculated as a difference between final and initial weight with respect to initial weight. The results of the moisture content studies for different formulations are shown in Figure 8.3.3.

Figure 8.3.3: % Moisture absorption in polymeric matrices of Atenolol



*** Standard deviation, n=3**

The moisture absorption in all the formulations was found to be low and ranged from **0.7400 ± 0.0360 to 5.8734 ± 0.1706.** The results revealed that the moisture absorption was found to increase with increasing concentration of hydrophilic polymers. The rank order of % moisture absorption for Atenolol loaded matrices was

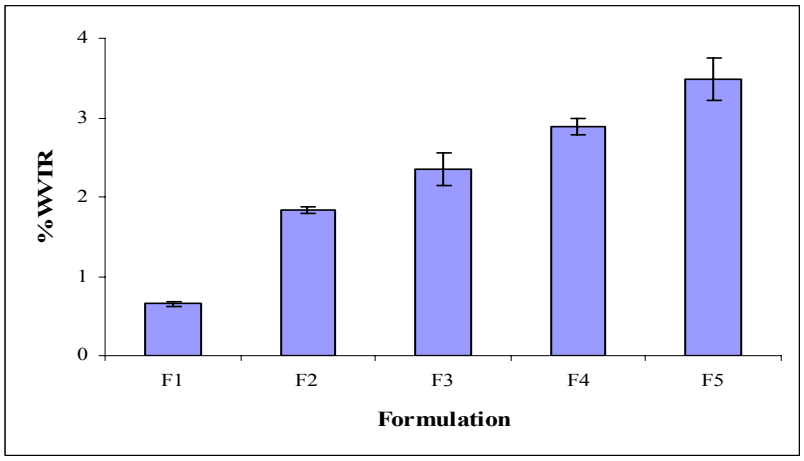
EVA (VA 40%) copolymer < ERS 100 < ERL100:ERS100 (1:1) < EC: PVP (2:3) <ERL100: HPMC (2:3)

Water vapor transmission rate (%WVTR)

The water vapor transmission rates of different formulation were evaluated, the results are shown in Figure 8.3.5. Atenolol films containing ERL100: HPMC showed higher % WVTR as compared to other polymers. This may be due to the hydrophilic nature of ERL 100 and HPMC. Formulation F1 and F2 showed less % WVTR as compared to F4 and F5. The rank order of % water vapor transmission rate for Atenolol loaded polymeric matrices was

EVA (VA 40%)copolymer < ERS 100 < ERL100:ERS100 (1:1) < EC: PVP (2:3) <ERL100: HPMC (2:3)

Figure 8.3.5: % water vapor transmission rate from drug loaded polymeric matrices of Atenolol



*** Standard deviation, n=3**

8.4 IN VITRO DIFFUSION STUDY OF ATENOLOL LOADED MATRIX DIFFUSIONAL FILMS

The release rate determination is one of the most important studies to be conducted for all controlled release delivery systems. The diffusion study of patches is very crucial, because one needs to maintain the drug concentration on the surface of stratum corneum consistently and substantially greater than the drug concentration in the body to achieve a constant rate of drug permeation.

8.4.1 Experimental

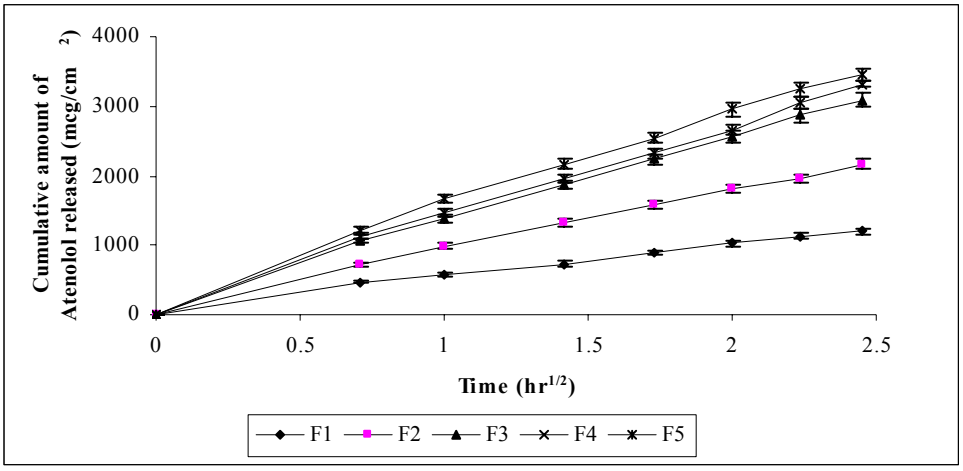
An in vitro diffusion study of Atenolol from various polymeric matrices was studied using modified Keshary-Chien diffusion cell.[8] The effective permeation area of the diffusion cell and receptor cell volume was 3.14cm^2 and 40 ml, respectively. The temperature was maintained at 37 ±0.5^{o}C. The receptor compartment contained 40 ml of 0.01N HCl stirred by magnetic stirrer.

Samples (2 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at different time intervals. The absorbance of the withdrawn samples were measured using UV VIS spectrophotometer at 237.5 nm using 0.01N HCl as a blank. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of square root of time for different formulations. The release rate Q/√T was determined by simple regression analysis of steady state data.

8.4.2 Result and discussion

Diffusion studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release. In vitro release profile is an important tool that predicts in advance, the extent of concentration builds up in vivo. The results of in vitro drug diffusion studies from transdermal patches are depicted in Table 8.4.1 and Figure 8.4.1.

Figure 8.4.1: In vitro diffusion release profiles of Atenolol from different matrix



*** Standard deviation, n=3**

Table 8.4.1: In vitro diffusion profiles of Atenolol from different polymer matrix

Time (hr ½)	Cumulative amount of drug release from device (µg/cm²)				
	Formulation code				
	F1	F2	F3	F4	F5
0.707	473.34 ± 25.67	726.48 ± 29.40	1060.46 ± 35.78	1120.23 ± 39.56	1210.39 ± 42.56
1	571.56 ± 25.46	990.49 ± 35.78	1370.89 ± 55.39	1459.23 ± 55.29	1678.34 ± 60.43
1.414	731.22 ± 31.56	1320.40 ± 51.34	1870.45 ± 60.99	1953.34 ± 61.64	2169.90 ± 71.34
1.732	890.56 ± 35.67	1570.30 ± 55.78	2241.36 ± 71.64	2321.90 ± 71.56	2541.20 ± 80.43
2	1025.89± 40.79	1810.37 ± 59.88	2564.90 ± 79.45	2659.86 ± 79.40	2958.68 ± 100.50
2.236	1136.30± 64.99	1950.34 ± 64.99	2863.94 ± 90.57	3045.75 ± 80.38	3245.46 ± 106.34
2.449	1200.13 ± 51.34	2160.80 ± 70.01	3090.34 ± 101.45	3320.00 ± 48.67	3458.95 ± 78.46
Q/√T (µg/cm² √hr)	**434.45**	**810.72**	**1220.90**	**1265.00**	**1337.80**
R	**0.9958**	**0.9989**	**0.9917**	**0.9963**	**0.9936**

The results of diffusion study of Atenolol released from polymeric matrix, formulated using various polymers are presented in Table 8.4.1 and profiles are shown in Figure 8.4.1. The release rate Q/√T **(**µg/cm^2 √hr) was determined by simple regression analysis of steady state data. The release of Atenolol from all the matrices followed square root law. The rank order of release was **EVA (VA 40%)copolymer < ERS 100 < ERL100:ERS100 (1:1) < EC: PVP (2:3) < ERL100: HPMC (2:3)**

The in vitro permeation experiment indicated that when the hydrophilic polymer concentration increased, the amount of drug permeation increased. As described by Rao and Diwan,[9] initial rapid dissolution of the hydrophilic polymers occurs when the patch is in contact with the hydrated skin, resulting in the accumulation of high amount of drug on the skin surface and thus leading to the saturation of the skin with drug molecule at all the time.[6]

Formulation **F1 EVA (VA 40%) copolymer** exhibited minimum Q/√T release rate **(434.45 µg/cm^2√h)** while Formulation **F5** exhibited maximum Q/√T release rate (**1337.80 µg/cm^2 √h**). The physiochemical property of polymer plays important role in drug release characteristics, from the polymeric matrix. EVA (VA 40%) copolymer is more hydrophobic as compare to other polymers and exhibited reduced permeation from the matrix. It was observed that as the concentration of hydrophilic polymer increased in the formulation the rate of diffusion increased subsequently. "Burst effect" was observed in the formulation F4 and F5 and this may be due to sufficient solubility of drug in the polymer.

In vitro release kinetic

The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 8.4.2.

The process of drug release in most controlled release devices including transdermal patches is governed by diffusion and the polymer matrix has a strong influence on the diffusivity as the motion of a small molecule is restricted by the three-dimensional network of polymers chain. The in vitro release profile could be best expressed by Higuchi's equation for the permeation of drug from the matrix.

Table 8.4.2.: Data of various parameters of model fitting of formulations

Formulation	Zero order equation	First order equation	Higuchi's equation
F1	**0.9804**	**0.9470**	**0.9962**
F2	**0.9836**	**0.9440**	**0.9985**
F3	**0.9836**	**0.9404**	**0.9998**
F4	**0.9935**	**0.9606**	**0.9968**
F5	**0.9835**	**0.9503**	**0.9977**

The importance of polymer dissolution on drug release from matrices has been known for ensuring the controlled release performance and the reproducibility of rate and duration of drug release. Initial "burst release" was observed in patches F4 and F5. This may be because of the much higher % of hydrophilic polymer. This hydrophilic components allow faster release of drug exhibiting small "time lag" to establish a concentration profile in the patches resulting in a "burst effect" in diffusion studies.[10,11] When burst release as well as higher release rate was considered, formulation F4 and F5 may be avoided from the preparation of a physiochemically stable and controlled release patch type formulation. Formulation F1 and F2 gave the slowest release. Thus it can be reasonably be suggested that the formulation **F3** is best suited for further in vitro permeability study through human live skin.[12,13]

8.5 TO OPTIMIZE FORMULATION USING BLENDS OF POLYMERS

8.5.1 Experimental

Different blend of polymer in matrix influences parameter of patch like folding endurance, thickness, weight variation, flatness, texture appearance, brittleness etc. ERL100 and ERS100 were selected to device final matrix diffusional therapeutic system. The drug is compatible with polymer as seen in DSC profile. A matrix laminate with different concentration of polymer was prepared with an aim to achieve improved Q/√T release rate. As the composition of laminate varies, it changes the texture, appearance, folding endurance etc which influences elegance of medicated laminate. A compromise is necessary between the loading of drug to have a maximum available Q/√T release rate and elegance of formulation.

In this study, various matrix type transdermal patches containing Atenolol of variable combination of ERL100 and ERS 100 were prepared. It was desired to design a

polymer matrix that allows one to control the release of Atenolol via the most appropriate choice of polymeric blend of ERL 100 and ERS 100 among the formulation studied, using the different diffusion pathway of the individual polymeric composition to produce the desired overall prolong drug release.

Preparation of matrix moderated Transdermal drug delivery device of Atenolol

Matrix – type transdermal patches containing Atenolol were prepared using different ratios of polymer (Table 8.5.1).The polymer were weighed in requisite ratios and dissolved in 10 ml mixture of acetone: methanol (7:3). Atenolol (15 mg/cm^2) and plasticizer Di-butyl phalate (30 %w/w of polymer weight) was added and mixed slowly with a mechanical stirrer. The uniform dispersion of polymeric solution of drug (10 ml) was poured on the mercury surface (73.86 cm^2), and solvent evaporated at room temperature. The dried film was carefully lifted. Films were punched out to have 3.14 cm^2 areas. Films were affixed on polyethylene coated aluminum foil as backing membrane. It was covered with peelable silicone liner and stored in desiccators at room temperature until further used.

Table 8.5.1: Composition of various formulations of Atenolol

Formulation code	Polymeric blend (10%w/v)	Ratio of polymer	Amount of drugs (mg/cm^2)
C1	ERL 100 : ERS 100	1:4	15
C2	ERL 100 : ERS 100	2:3	15
C3	ERL 100 : ERS 100	1:1	15
C4	ERL 100 : ERS 100	3:2	15
C5	ERL 100 : ERS 100	4:1	15

Physiochemical evaluation of polymeric matrix device

Prepared Atenolol containing matrices were evaluated for various parameters like thickness, weight variation, drug content, flatness, folding endurance, moisture content, moisture absorption, % WVTR etc, as per procedure given in the chapter 8, section 8.3.2.

In vitro diffusion profile

In vitro diffusion study of different matrices containing Atenolol was carried out as

per the procedure given in chapter 8, section 8.4.1.

Data Analysis

Cumulative amount of Atenolol released into infinite sink, per unit surface area of device was plotted as a function of square root of time for each disc. Release rate Q/√T was computed for each disc from simple regression analysis of steady state data**.** The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles.

8.5.2 Result and Discussion

The present investigation deals with the development of Atenolol polymeric matrix using different concentration of polymer. This preliminary screening was carried out for the selection of best matrix. A diffusion mediated matrix moderated transdermal controlled drug delivery system for Atenolol was successfully prepared using different polymer ratio using mercury subtract method and all matrices were evaluated using different physiochemical parameters.

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of films was measured and the average thickness was noted. The results of thickness measurements are given in Table 8.5.2.

Table 8.5.2: Results of thickness uniformity of C1 to C5 film formulations

Sr. No.	Formulation code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	465	460	462.5	462.5 ± 2.500
2	C2	470	470.5	472.5	471.0 ± 1.322
3	C3	480	487.5	482.5	483.33 ± 3.810
4	C4	510	510	510.5	510.16 ± 0.288
5	C5	525	530	527.5	527.50 ± 2.500

***Standard deviation, n=3**

The results indicate that there was no much difference in the thickness within the

formulations. Thickness in the different formulations was in the range of **462.5 ± 2.500 µm** to **527.50 ± 2.500 µm.** Maximum thickness was found in formulation C5, while minimum found in formulation C1. These results revealed that thickness was found to increase as hydrophilic portion of polymer increased. The results of thickness measurement also indicated uniform distribution of the drug and polymer over the mercury surface.

Weight variation

Drug loaded films (3.14cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 8.5.3. The weight of 3.14 cm^2 film ranged from **0.187 ± 0.002 gm** to **0.215 ± 0.01gm**. The weight of the patches was found to be uniform among different batches.

Table 8.5.3: Results of weight variations of C1 to C5 film formulations

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	0.225	0.215	0.205	0.215 ± 0.01
2	C2	0.190	0.185	0.195	0.190 ± 0.005
3	C3	0.189	0.185	0.188	0.187 ± 0.002
4	C4	0.194	0.193	0.208	0.198 ± 0.008
5	C5	0.209	0.207	0.210	0.208 ± 0.001

***Standard deviation, n=3**

In a weight variation test, the pharmacopoeial limit for the percentage deviation of all the films of less than mg is ± 10%. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for weight variation as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Atenolol in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation.

Drug content

Drug content of the matrices was carried out to ascertain that the requisite amount of drug is loaded in the formulation. The prepared film formulations (3.14cm^2) were

dissolved in 10 ml mixture of Acetone: Methanol (7:3) in a 100 ml volumetric flask and the volume was adjusted up to 100 ml using Acetone: Methanol (7:3). The amount of drug present was determined by measuring the absorbance spectrophotometrically at 282.0 nm using mixture of Acetone: Methanol (7:3) as a blank. The results obtained are presented in Table 8.5.4.

Table 8.5.4: Results of % Drug content of C1 to C5 film formulations

Sr. No.	Formulation Code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	99.45	98.40	98.63	98.82 ± 0.551
2	C2	101.45	100.60	100.32	100.79 ± 0.588
3	C3	100.35	102.37	99.60	100.77 ± 1.432
4	C4	97.46	98.70	98.50	98.22 ± 0.665
5	C5	99.90	102.38	101.36	101.21 ± 1.246

***Standard deviation, n=3**

The films were found to contain **98.22% - 101.21 %** of the labeled amount of Atenolol indicating uniformity of drug content. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for content uniformity as per official requirements.

Flatness

The flatness was measured manually for the prepared films. The results of the flatness study showed that none of the formulations had the differences in the strip length before and after their cuts. It indicates 100% flatness observed in the formulated patches. Thus, no amount of constriction was observed in the film of any formulation and it indicates smooth flat surface of the patches and thus they could maintain a smooth surface when applied on to the skin.

Folding endurance

Folding endurance was determined manually for drug loaded polymeric matrices. The folding endurance of the films was determined by repeatedly folding a strip measuring 2x2 cm size at same place till it break. The number of times the film could be folded

at the same place without breaking gave the value of folding endurance. The results of folding endurance are given in Table 8.5.5.

Table 8.5.5: Results of folding endurance of C1 to C5 film formulations

Sr. No.	Formulation Code	Folding endurance			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	C1	20	25	22	22.33 ± 2.516
2	C2	20	24	23	22.33 ± 2.081
3	C3	19	18	20	19.00 ± 1.000
4	C4	17	18	18	17.66 ± 0.577
5	C5	20	22	23	21.66 ± 1.527

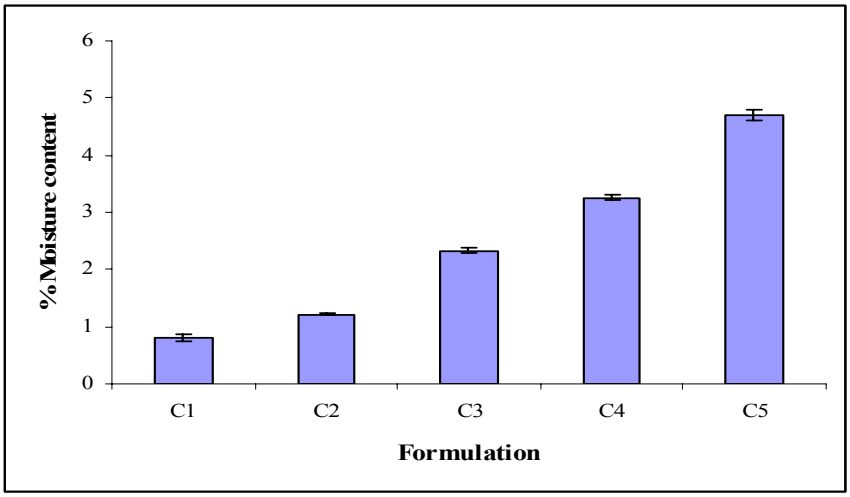
***Standard deviation, n=3**

Here formulation C1 and formulation C2 showed good folding endurance as compare to formulation C3 and C4.

Moisture content (Loss on drying)

The moisture content was determined by keeping the drug matrices patches in desiccator's containing activated silica for 24hr. The percentage moisture content was calculated from the weight differences relative to the final weight. The results of the moisture content studies for different formulations are shown in Figure 8.5.1.

Figure 8.5.1: % moisture content of C1 to C5 film formulations containing Atenolol



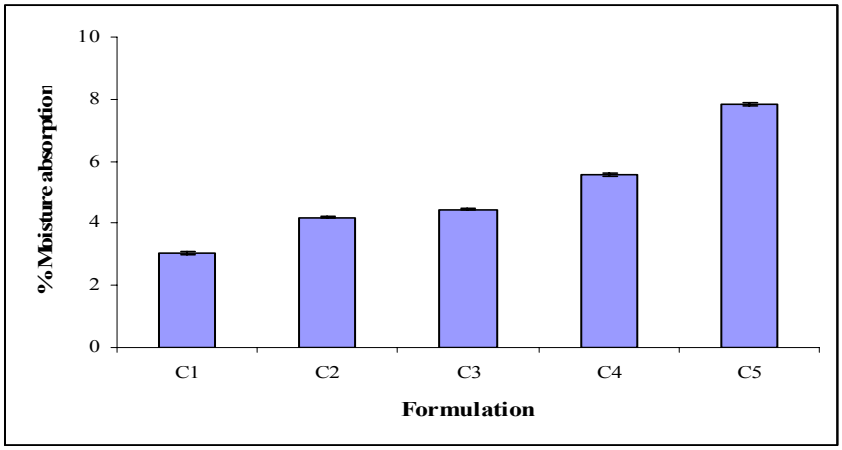
*** Standard deviation, n=3**

The moisture content in all the formulations was found to be low and ranged from **0.8023 ± 0.0525** to **4.6970 ± 0.1006 %.** The result revealed that the moisture content was found to decrease with increasing concentration of hydrophilic polymer ERL 100.

Moisture absorption

The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The results of the moisture absorption study for different formulations are shown in Figure 8.5.2.

Figure 8.5.2: % Moisture Absorption of C1 to C5 film formulations



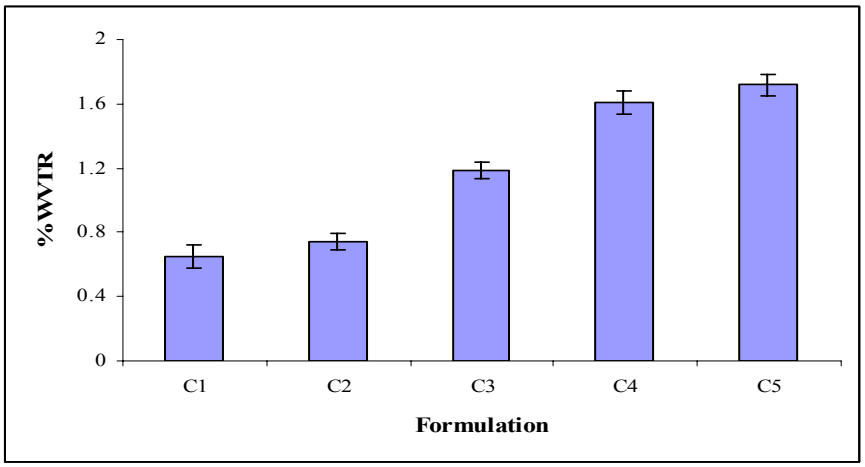
*** Standard deviation, n=3**

The moisture absorption in all the formulations was found to be low and ranged from **3.049 ± 0.0669** to **7.833 ± 0.0550 %.** The result revealed that the moisture absorption was found to increases with increasing concentration of hydrophilic portion of polymer. Rank order of moisture absorption for the formulation was **C5 > C4 > C3 > C2 > C1.**

Water vapor transmission rate (%WVTR)

The water vapor transmission rates of different formulation were evaluated and the results are shown in Figure 8.5.3. The % water vapor transmission rate of each film was determined and rank order of water vapor transmission for Atenolol containing matrices was C**1 > C2 > C3 > C4 > C5.** The water vapor transmission influences the permeation of the drug from the barriers.

Figure 8.5.3: Results of % WVTR of C1 to C5 film formulations

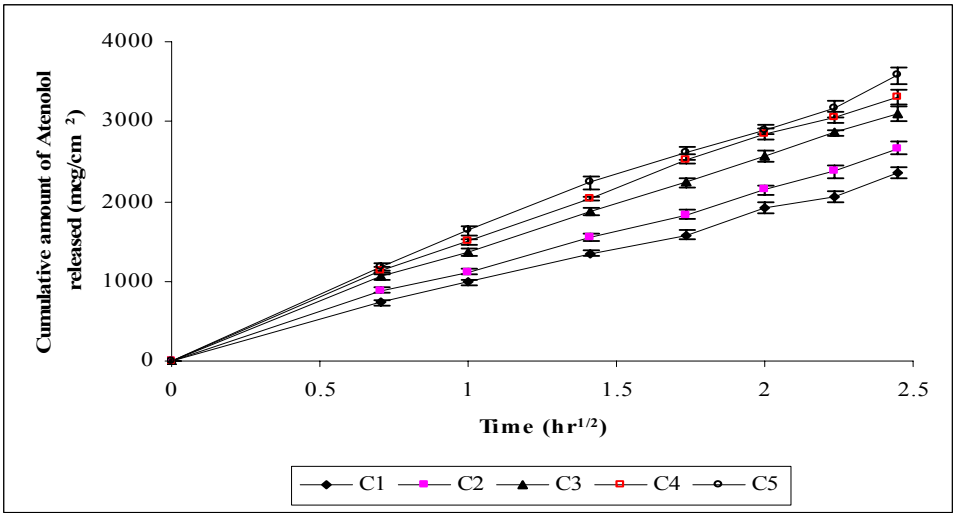


*** Standard deviation, n=3**

In vitro diffusion profile

Diffusion studies for different formulations were performed in a modified Keshary-Chien diffusion cell using 0.01N HCl, as a diffusion medium at 37 ± 0.5^0C. Experiments were triplicated. Cumulative amount of Atenolol released from the device into receptor fluid was plotted as a function of square root of time for each medicated disc subjected under diffusion study. Release rates Q/√T were derived from simple regression analysis of steady state diffusion data. The results of in vitro drug diffusion study for transdermal patches are depicted in Table 8.5.6 and Figure 8.5.4

Figure 8.5.4.: In vitro diffusion profiles of Atenolol from blend of polymer Matrices



*** Standard deviation, n=3**

Table 8.5.6: In vitro diffusion profiles of Atenolol from blend of polymer matrices

Time ($hr^{½}$)	Cumulative amount of drug release from device ($\mu g/cm^2$)				
	Formulation code				
	C1	C2	C3	C4	C5
0.707	731.22 ± 29.47	884.20 ± 35.39	1060.46 ± 39.67	1141.34 ± 45.32	1180.11 ± 50.29
1	990.49 ± 35.34	1121.24 ± 41.56	1370.89 ± 45.37	1492.39 ± 42.56	1631.15 ± 62.56
1.414	1350.92 ± 41.23	1543.98 ± 46.89	1870.45 ± 52.34	2035.30 ± 28.46	2232.24 ± 72.42
1.732	1579.46 ± 55.45	1833.50 ± 55.67	2241.36 ± 58.39	2531.59 ± 65.34	2604.56 ± 78.56
2	1920.59 ± 67.89	2142.30 ± 60.93	2564.90 ± 70.30	2842.50 ± 69.80	2901.32 ± 67.38
2.236	2064.68 ± 69.50	2371.10 ± 71.45	2863.94 ± 35.67	3051.49 ± 75.29	3160.45 ± 97.40
2.449	2353.90 ± 71.85	2664.20 ± 78.56	3090.34 ± 89.40	3301.11 ± 90.40	3581.54 ± 105.37
Q/√T ($\mu g/cm^2\sqrt{hr}$)	**909.56**	**1014.1**	**1220.9**	**1258.5**	**1309.1**
Correlation coefficient	**0.9949**	**0.9969**	**0.9917**	**0.9962**	**0.9940**

Release rate Q/√T increased with increasing concentration of Atenolol in ERL100: ERS100 matrix. In our experiments, variable release profiles of Atenolol from the different experimental patches, composed of various blend of polymers were observed. Cumulative amount of drug diffused per square centimeter of patches, when plotted against square root of time, showed linear graphic of the data obtained from different formulations. The rank order of Q/√T release rate for formulation was **C1 < C2 < C3 < C4 < C5.** The formulations **C5** exhibited the greatest **(1309.1µg/cm^{2}h**) release values, which were significantly different, compared to the lowest values in the formulation **C1** (**909.56 µg/cm^2 h**). Based on physiochemical data and in vitro release experiments, formulation **C5** was chosen for further in vitro permeability study through human live skin.

In vitro release kinetic

The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 8.5.7.

Table 8.5.7.: Data of various parameters of model fitting of formulations Containing Atenolol

Formulation	Zero order equation	First order equation	Higuchi's equation
C1	**0.9896**	**0.9564**	**0.9922**
C2	**0.9917**	**0.9533**	**0.9973**
C3	**0.9836**	**0.9404**	**0.9998**
C4	**0.9618**	**0.9113**	**0.9934**
C5	**0.9809**	**0.9386**	**0.9937**

In our experiment, the in vitro permeation profiles of all formulations could be best expressed by Higuchi's equation (**R^2 = 0.9922 to 0.9998**) for the permeation of drug from a polymeric matrix.

8.6 In vitro permeation study of matrix diffusional transdermal drug delivery device of Atenolol across human live skin

8.6.1 Treatment and preparation of skin for permeation study

Human live skin was collected from unused portion of human male patients from private hospital with consent of plastic surgeon and patient, who have no problem regarding reactions to medicines or problems of breathing. The skin graft was collected, treated and stored as mentioned in section 5.6.1.

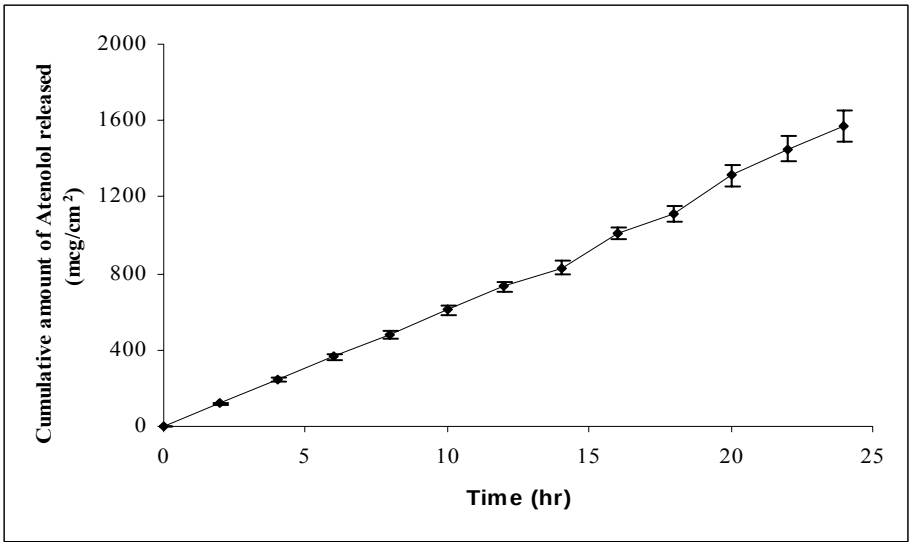
8.6.2 In vitro permeation study through human live skin

Diffusion study of Atenolol from selected matrix device across human live skin was performed using modified Keshary-Chien diffusion cell. A section of skin was cut and placed on the brim of diffusion cell in such a way that the dermal side of the skin faced donor compartment. The patch of Atenolol was affixed on the skin in such a way that backing membrane was facing upward. Diffusion study was carried as mentioned in section 6.6.1. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time. The release rate was determined by simple regression analysis of steady state data.

8.6.3 Result and discussion

In vitro permeation studied is predictive of in vivo performance of a drug. The results of in vitro skin permeation of Atenolol from formulation **C5** are shown in figure 8.6.1

Figure 8.6.1: In vitro skin permeation profile of Atenolol from matrix patch through human live skin



*** Standard deviation, n=3**

Various parameters of diffusion kinetics of Diltiazem released from device across excised human live skin are presented in Table 8.6.1. The parameters listed in this table are useful for biopharmaceutical and pharmacokinetic of the matrix diffusional system evaluated.

Table 8.6.1: Parameters of diffusion kinetic of Atenolol from Atenolol matrix diffusion controlled transdermal drug delivery system

Sr. No.	Parameters	Value
1	Skin flux Jss	**68.73 µg/cm^2 hr**
2	Time lag (t_L)	**1.06 hr**
3	Skin thickness (µm)	**140 µm**
4	Diffusion coefficient	**3.081 x 10^{-5} cm^2/sec**
5	Solubility of drug in skin (Cs)	**31.230 mg/cm^3**

From the time lag (t_L) for device the average diffusion coefficient, D of Atenolol was determined using $D = h^2/6\ t_L$ relationship where thickness of human skin was 140 x 10^{-4} cm. The amount of Atenolol retained by skin area used for permeation was computed by dividing skin flux with gradient of diffusivity.

$$Cs = Jss/ (D/h)$$

The value of Cs for device is presented in Table 8.6.1. The amount of Atenolol retained in skin need to be incorporated in contact adhesive to serve to saturate the skin area with permeate before steady state is established. The amount of permeate so incorporated in the adhesive system of device serves as priming dose. The release of Atenolol from matrix diffusional system into infinite sink can be approximated by equation.

$$\text{Release rate} = Q/\sqrt{T} + He^{-Kt}$$

The first term on right hand side of the equation represents the time dependent delivery rate. The second term represents the temporal pattern of drug release during priming dose period.

Comparison of permeation kinetics: Patch without priming Dose: Patch with priming Dose

The release rate (μg/hr) has linear relationship with area of release face of a transdermal drug delivery system. The final patch needs to be provided with adhesive system and priming dose. To assess release kinetic of a complete patch, a patch of 3.14 cm^2 area was provided with a peripheral adhesive system (4 mm rim). From the data of solubility of drug in skin, a 0.583 % w/v Atenolol in natural rubber solution was used as an adhesive system. This solution was uniformly layered in 4 mm rim surrounding 3.14 cm^2 area of patch, to obtain 10 μm thick adhesive systems containing priming dose of 437 $\mu g/cm^2$ area of delivery device. The device was firmly secured on skin of human live skin and was subjected to diffusion experiment using 0.01 N HCl as diffusion medium. The release profile is shown in **Table 8.6.2** and **Figure 8.6.2.**

Figure 8.6.2: In vitro skin permeation profile of Atenolol from matrix patch through human live skin with priming dose and without priming dose

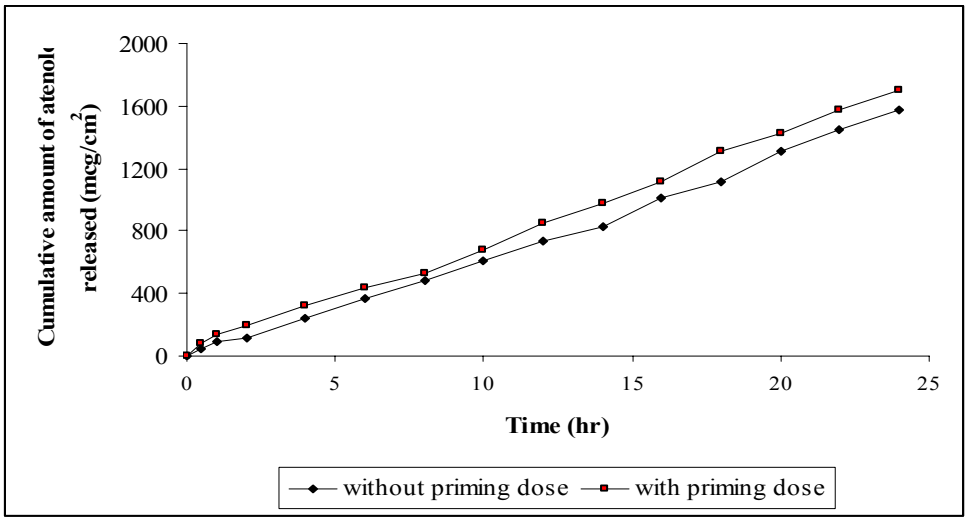


Table 8.6.2: Parameters of in vitro skin permeation kinetic of Atenolol from matrix patch through human live skin with priming dose and without priming dose

Parameters	Cumulative amount of drug release (µg/cm^2) Without priming dose	Cumulative amount of drug release (µg/cm^2) With priming dose
Release rate (µg/cm^2 hr)	68.73	70.84
Correlation coefficient	0.9954	0.9983
Time lag (t_L)	1.06 hr	0.86 hr

Computation of desired release rate (in vivo input) for target steady state plasma concentration of Atenolol

For Atenolol $t_{1/2}$ = 6 hrs, V_d = 0.7 liters /Kg and targeted steady state plasma concentration (Css) = 120 ng/ml the desired drug release can be calculated as follows.

In vivo input = in vivo output

$= C_{ss} * V_d * K_e * 70$

$= 120$ ng/ml * $0.7x10^3$ ml/kg * 0.693/6 * 70 kg

$= 679.14$ µg/hr

In vivo input = in vivo output = 679.14 µg/hr

Desired release rate = 679.14 µg/hr

Computation of area of patch required for target steady state plasma concentration (Css)

In vivo input = in vivo output = 679.14 µg/hr

$J_{ss(skin)} \times A = C_{ss} * V_d * K_e * 70 = 679.14$ µg/hr

Area of patch = (679.14 µg/hr)/ $J_{ss(skin)}$

$= 679.14/ 70.84$ cm^2

$=$ **9.68 cm^2**

Formulation of final patch

As per British J. clinical Pharm.[14] steady state plasma concentration built up after administration of 25mg, 50mg and 100 mg Atenolol orally in human volunteers are recommended to develop peak plasma concentration of 126 ng/ml, 219 ng/ml and 390 ng/ml respectively to control varied dose dependent physiological cardiac complains. Transdermal controlled drug delivery system is recommended as an alternative safe therapy with better compliance to therapy by the patient to reduced frequency of administration leading to better disease management. Following three patches of different configuration are formulated as an alternative to above oral administration, to meet requirement of desired steady state plasma concentration for varied cardiac conditions. Table 8.6.3 shows the composition of the formulations.

Table 8.6.3: composition of final Atenolol matrix moderated transdermal devices

Components	Patch 1	Patch 2	Patch 3
Oral dose of Atenolol	**25 mg**	**50 mg**	**100 mg**
Css, mean steady state plasma concentration (ng/ml)	**126**	**219**	**390**
Area of transdermal patch (cm^2)	**9.7**	**16**	**30**
Predicted steady state plasma concentration	**120**	**200**	**375**
Steady state flux, Jss, through human live skin (µg/cm^2 hr)	**70.84**	**70.84**	**70.84**
Priming dose in adhesive system (µg/cm^2)	**437**	**437**	**437**
Protective liner	**Silicon sheet**	**Silicon sheet**	**Silicon sheet**

Area of transdermal drug delivery device is directly related to mean steady state plasma concentration that builds up after administration of device. The above formulations are 24 hour (once a day) devices. The specific size matrix formulation having area 9.7, 16 and 30 cm^2 are affixed over polyethylene coated aluminum foil backing. The formulations are to be provided with priming dose incorporated in natural rubber adhesive as follow. The amount of Atenolol retained in skin need to be incorporated in contact adhesive to serve as prompt dose. For preparation of above devices containing peripheral adhesive system a 0.583 % w/v Atenolol in natural rubber adhesive solution is to be layered surrounding 4 mm rim of the delivery device, providing 437 µg Atenolol per cm^2 area of the adhesive system giving a 10 µm thick adhesive layer. The finally fabricated devices are to be provided with silicone sheet as a protective liner and to be stored at room temperature.

8.7 STABILITY STUDY

8.7.1. Stability study of matrix diffusion controlled device

The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. The objective of accelerated stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing

the temperature**.** The optimized formulation of Atenolol matrix patch was subjected for the stability study.

The accelerated stability study was carried out according to ICH guideline by storing the samples at 25 ^{0}C / 60% RH, 30 ^{0}C/ 65% RH and 40 ^{0}C/ 75% RH for 90 days in a stability chamber (Thermo Lab., Mumbai, India). These samples were analyzed by UV Spectrophotometer method and checked for changes in physical appearance and drug content at an interval of 15 days**.**

5.7.2 Results and Discussion

Final formulation was subjected to stability study and observed for change in color, appearance, flexibility and drug content. Temperature and humidity values selected were as per the ICH guidelines.

Diffusion study was carried out and it was observed that formulation stored at 40 ^{0}C exhibited higher Q/√T release rate as compared to those stored at 25 ^{0}C and 30 ^{0}C. The release rate at 30 ^{0}C was altered but it was in order. The product stored at 25 ^{0}C exhibited no change in release rate.

Drug degradation study was carried out as per ICH guideline at above mentioned physical condition of temperature and humidity. Periodic samples were subjected to drug content analysis. The % retained in device was worked out and from the plot of log % retained versus time degradation rate constant was computed for 25 ^{0}C, 30 ^{0}C and 40 ^{0}C temperatures.

The degradation was higher at an elevated temperature. The first order rate constant of degradation for room temperature was **1.280×10^{-3} week^{-1}**. The self life calculated was **82 week.** Results of stability study indicated storage of product at room temperature.

8.8 SKIN IRRITATION AND SKIN SENSITIZATION STUDY

8.8.1 Experimental

Skin irritation and skin sensitization though are different types of physiological responses yet they have several common indications. Skin sensitization is systematic response and skin irritation is primarily is local response. A protocol was devised for evaluation of skin irritation and/or sensitization in such a manner that the signs at the sight of application would be assessed in common for the both and further, to distinguish sensitization from irritation. The skin irritation and skin sensitization study

was carried out as per mentioned in chapter 5.8.1.

The final Atenolol matrix patch (2.25 cm^2) was supported with aluminum foil. This was applied to the skin surface (shaved) and made adhered with the help of "3 M Micropore" – medicated adhesive tap (3 M Corporation, U.K.).

8.8.2 Results and discussion

The results of skin irritation and skin sensitization study for Atenolol matrix patch are presented in Table 8.8.1. From this table total average score at day 20 and total average score at day 28 were obtained. From the average score it was clear that the Atenolol matrix patch was non- irritating, since the maximum score for each sign did not exceed than 1 except in one case i.e. score 2, for necrosis in one animal but this score was reduced to 1 at 28^{th} day. This indicates that it had happened due to bruising during shaving. Therefore these scores can be ignored. Further Table 8.8.3 shows that average score at day 20 and day 28 was not exceeding more than 1 for any sign. Average score not exceeding more than 1 at any day for any sign was clearly indicative for non-irritation nature of the formulation.

Atenolol is non-sensitizing in nature. The reason for this was reduction in scores even after repeated applications. All the tables show that maximum individual scores, average scores, total irritation scores were reduced at day 28^{th} in comparison to all these scores at day 20^{th}. Reduction in scores even after repeated application of test patch indicated absence of primary and secondary sensitization reaction.

Table 8.8.2: The average score for each sign after day 20 and day 28 during irritation and sensitization study of Atenolol matrix patch

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	1.0	0.25
2	Scaling	0.5	0.25
3	Fissures	0.25	0.0
4	Oedema formation	0.75	0.0
5	Ecchymosis	0.25	0.25
6	Necrosis	0.5	0.25

Table 8.8.1: Skin irritation and sensitization study of Atenolol matrix patch

SR. NO.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE									
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0
		0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0
		Avg	0	Avg	0	Avg	.25	Avg	.75	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	1	Avg	0.5	Avg	.25	Avg	.25
2	Scaling	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	1	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1	1	0	1	0	1
		Avg	0	Avg	0	Avg	.25	Avg	0.5	Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25
3	Fissures	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
		0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	0	Avg	0	Avg	0
4	Oedama formation	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0
		0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	1	0	1	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	.25	Avg	.75	Avg	0.5	Avg	0.5	Avg	0
5	Ecchymosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	.25
6	Necrosis	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	0	1	0	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	.25	Avg	.25	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25
Total of all score		**0**		**0**		**2**		**9**		**5**		**5**		**4**		**1**		**1**		**13**		**9**		**6**		**4**	
Total average per animal		**0**		**0**		**0.5**		**2.25**		**1.25**		**1.25**		**1**		**0.25**		**0.25**		**3.25**		**2.25**		**1.5**		**1**	

Table 8.8.3: Maximum sensitization and irritation score of various signs at last day for Atenolol matrix patch

Sr. No.	Sign	Score	N
1	Erythema	**1**	**1**
2	Scaling	**1**	**1**
3	Fissures	**0**	**4**
4	Oedema formation	**0**	**4**
5	Ecchymosis	**1**	**1**
6	Necrosis	**1**	**1**

8.9 CONCLUSION

ERL100:ERS100 matrix moderated transdermal drug delivery system of Atenolol has been prepared successfully. Among different polymers evaluated ERL100:ERS100 in ratio of 1:1 containing 15 mg/cm^2 of Atenolol provided a medicated matrix, which was stable, non-irritant and non-sensitizing to skin and was safe. It complied with official and non-official pharmacotechnical specification. The matrix device evaluated for Atenolol release in vitro into infinite sink and across human live skin, enabled to provide adequate rate of Atenolol, meeting requisite pharmacokinetic requirement of steady state plasma concentration for 24 hours, giving once a day drug delivery system. Alternative to three oral conventional doses (25mg, 50mg and 100mg) devices were provided by fabricating 9.7 cm^2, 16 cm^2 and 30 cm^2 matrix diffusional controlled transdermal drug delivery system of Atenolol respectively, as once a day drug delivery systems.

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Chapter 9

Preparation and characterization of adhesive matrix diffusional transdermal drug delivery device of Atenolol

CHAPTER 9

9 PREPARATION AND CHARACTERIZATION OF ADHESIVE MATRIX DIFFUSIONAL TRANSDERMAL DRUG DELIVERY DEVICE OF ATENOLOL

AIM OF PRESENT INVESTIGATION

The adhesive matrix diffusional system tends itself to simple and easy processing of delivery device. The matrix (laminate, disc) being static in nature offers less problems of drug and formulation stability and results in robust product with near to no problems of handling and administration to patients. Laminated form of delivery device offers easy transfer of technique from laboratory operations to a large scale manufacturing. As preparation of sheets, laminates etc from polymer beads is the major activity of plastic industry. Screw extrusion or injection molding can facilitate the formation of drug laminates from the polymer beads mixed with additives and active agents. For the preparation of adhesive drug matrix diffusion controlled transdermal device laminates are prepared using drug loaded adhesive and this layer is covered by protective liner and fixed on backing membrane, followed by punching of laminates in desired size.[1]

This type of transdermal drug delivery system can be easily prepared on large scale as a continuous manufacturing process as compare to polymeric matrix diffusion controlled transdermal drug delivery device and membrane moderate reservoir type controlled transdermal drug delivery device.

Adhesive matrix diffusion controlled release transdermal drug deliver systems are monolithic systems which are the simplest and least expensive means of controlling the release of an active agent. Here the active agent is physically blended with the adhesive agent.[2] The release rate is governed by Higuchi equation.[3] The release in such system is proportional to square root of time and release is available until approximately 60% of the loaded drug is released from the matrix.[4] Thereafter release is related exponentially to time, exhibiting first order release.

Parameter influencing the release characteristics of monolithic devices can be classified as solute dependent factors like solubility, partition co-efficient and diffusion coefficient of drug in the matrix. The solute independent parameters are system variables like geometry, tortuosity, pores, concentration, volume fraction and diffusion layer etc. In the present investigation solute related factors were considered

to fabricate the devices using adhesive. With perception to above objective, it is necessary to modify current solid dosage forms in to transdermal controlled drug delivery system. A first step in this process is to illustrate how formulation and process variables could give drug release through skin. The aim of present investigation is to formulate and optimize the adhesive matrix diffusion controlled transdermal drug delivery system of Atenolol.

9.1 Formulation and evaluation of adhesive drug matrix device of Atenolol

9.1.1 Experimental

Preparation of adhesive matrix device of Atenolol

The transdermal therapeutic system comprised a backing membrane, an adhesive layer containing drug and a release liner. Adhesive matrix – type transdermal patches containing Atenolol were prepared using different ratios of drug to adhesive (Table 9.1.1). Atenolol was accurately weighed and dissolved in very small quantity of alcohol. This solution was mixed with natural rubber adhesive (Readymade received – Beta surgical, Rajkot). The uniform dispersion of drug and adhesive was spreaded on a drug impermeable polyethylene coated aluminum foil (5x3.5 cm^2) with the help of TLC kit spreader to form a thin medicated adhesive layer and dried at room temperature. After 24h, the films were cut into a 3.14 cm^2 area and covered with pellabel silicone protective liner. Dosage forms were kept in desiccators until further used.

Table 9.1.1: Composition of prepared adhesive matrix films of Atenolol

Formulation code	Adhesive (mg/cm^2)	Atenolol (mg/cm^2)
F1	15	5
F2	15	10
F3	15	15

Physiochemical evaluation of adhesive matrix device

Prepared Atenolol containing adhesive matrices were evaluated for various parameters like thickness , weight variation, drug content, moisture content, moisture absorption etc, as per procedure given in the chapter 7, section 7.1.1.

9.1.2 Results and discussion

The present investigation deals with the development of Atenolol adhesive matrix using different concentration of drug and adhesive. A diffusion mediated matrix controlled transdermal drug delivery system for Atenolol was successfully prepared using adhesive and it was evaluated using different physiochemical parameters.

Thickness

With the help of micrometer (0.001mm), Mitutoyo, Japan, the thickness of drug adhesive matrix was measured and the average thickness was noted. The results of thickness measurements are given in Table 9.1.2. The results indicate that there was no much difference in the thickness with in the formulations. Thickness in the different formulations was in the range of **122.5 ± 2.5 µm** to **141.6 ± 2.886 µm.** The results indicated uniform distribution of the adhesive.

Table 9.1.2: Thickness uniformity of F1 to F3 adhesive matrix Atenolol formulations

Sr. No.	Formulation code	Average thickness (µm)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	120.0	122.5	125.0	122.5 ± 2.500
2	F2	130.0	135.0	132.5	132.5 ± 2.500
3	F3	145.0	140.0	140.0	141.6 ± 2.886

***Standard deviation, n=3**

Weight variation

Drug loaded films (3.14 cm^2) were weighed using Sartorius electronic balance (Model CP-224 S), Shimadzu, Japan and the results of weight variation are given in Table 9.1.3.

In a weight variation test, the pharmacopoeial limit for the percentage deviation of all the films of less than mg is ± 10%. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for weight variation as per official requirements. All the formulations showed acceptable pharmaco-technincal properties.

Table 9.1.3: Weight variations of F1 to F3 adhesive matrix Formulations of Atenolol

Sr. No.	Formulation Code	Average weight (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	45.23	46.53	45.89	45.88 ± 0.650
2	F2	60.00	58.96	60.13	59.69 ± 0.641
3	F3	75.20	74.63	76.43	75.42 ± 0.919

***Standard deviation, n=3**

Drug content

Drug content of the drug adhesive matrix patch was carried out to ascertain that the drug is properly added in the formulation. The results of drug content analysis are represented in Table 9.1.4.

Table 9.1.4: % Drug content of F1 to F3 adhesive matrix formulations of Atenolol

Sr. No.	Formulation code	Drug content (mg)			
		Trial 1	Trial 2	Trial 3	Mean ± S.D.*
1	F1	99.46	98.46	98.42	98.78 ± 0.589
2	F2	97.23	96.23	96.13	96.56 ± 0.587
3	F3	96.89	95.45	95.74	96.02 ± 0.761

***Standard deviation, n=3**

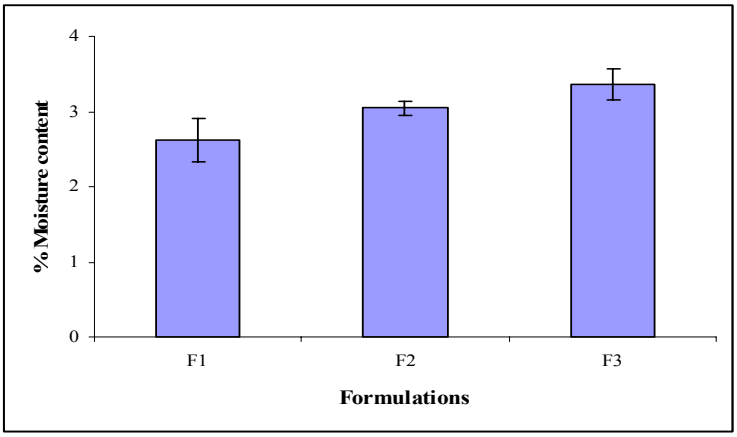
The films were found to contain 96.02 % - 98.78 % of the labeled amount of Atenolol indicating uniformity of drug content. The average percentage deviation of all formulations was found to be within the limit, and hence all the formulation passed the test for content uniformity as per official requirements. All the formulations showed acceptable pharmaco-technincal properties. From the results obtained, it was clear that there was proper distribution of Atenolol in the film formulations. Hence it was concluded that drug was uniformly distributed in all the formulation.

Moisture content (Loss on drying)

The results of the moisture content study for different formulations are shown in Figure 9.1.1. The moisture content in all the formulations was found to be low**.** The result revealed that the moisture content was found to increases with increasing concentration of Atenolol. The % Moisture content of each device was determined, the rank order of moisture absorption was **F1 < F2< F3.**

The result revealed that the moisture was present in the formulation due to presence of adhesive material. Small moisture content in the formulations helps to maintain the texture of the formulation also prevents the dosage form being brittle.

Figure 9.1.1: % moisture content from containing different adhesive matrix device of Atenolol



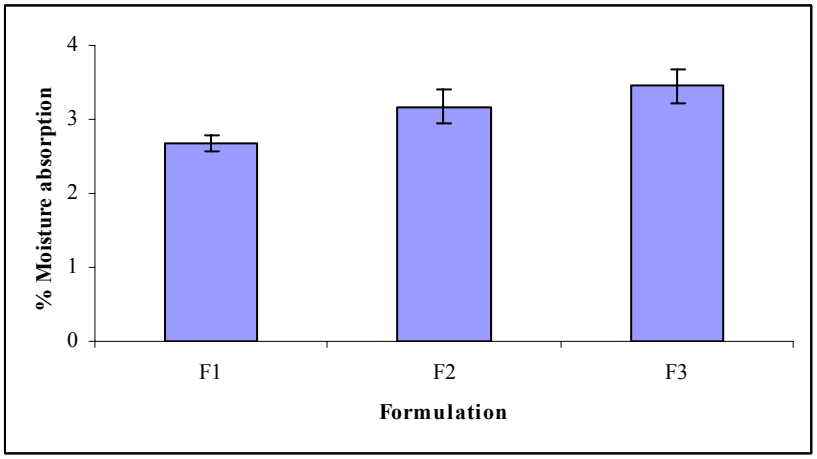
*** Standard deviation, n=3**

Moisture absorption

The percentage moisture absorption was calculated as a difference between final and initial weight with respect to initial weight. The results of the moisture absorption studies for different Atenolol adhesive matrix formulations are shown in Figure 9.1.2.

The result revealed that the moisture absorption was found to increase with increasing concentration of Atenolol. The % Moisture absorption of each film was determined, the rank order of moisture absorption was **F3 > F2 > F1.**

Figure 9.1.2: % moisture absorption from Atenolol containing different adhesive matrix device



*** Standard deviation, n=3**

9.2 In vitro drug diffusion study of adhesive matrix diffusional controlled transdermal drug delivery device of Atenolol

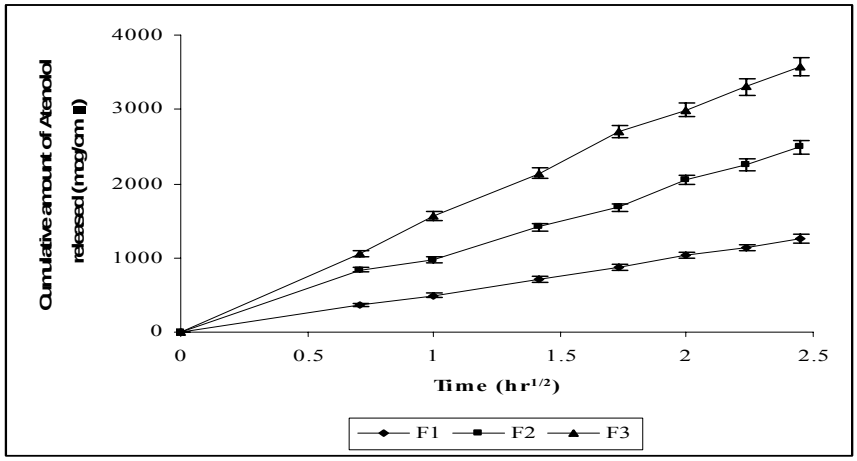
9.2.1 Experimental

In vitro diffusion study of Atenolol from adhesive matrix moderated transdermal patch was carried out using modified Keshary-Chien diffusion cell as per procedure given in the chapter 7, section 7.2.1. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of square root of time for different formulations. The release rate Q/√T was determined by simple regression analysis of steady state data.

9.2.2 Result and discussion

Diffusion study is important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release.[5,6] The results of in vitro drug diffusion studies from transdermal patches are depicted in Figure 9.2.1.

Figure 9.2.1: In vitro diffusion profiles of Atenolol released from drug adhesive matrix patch



*** Standard deviation, n=3**

Release of Atenolol from the drug loaded adhesive matrix followed square root law. The release rate Q/√T was determined by simple regression analysis of steady state data.

Release rate Q/√T increased with increasing concentration of Atenolol in adhesive matrix. In our experiments, variable release profiles of Atenolol from the different experimental patches composed of different proportion of drug and adhesive were observed. The rank order of release rate observed was **F1 < F2 < F3.**

The formulations **F3** exhibited the maximum Q/√T (**1469.90µg/cm^2√h**) release rate, which was significantly different, compared to the lowest value in the formulation **F1** (**512.65 µg/cm^2√h**). The formulation **F2** exhibited **995.25 µg/cm^2√h** release rate.

Based on in vitro release experiments, formulation **F3** was chosen for further in vitro permeability study through human live skin.

In vitro release kinetic

In vitro diffusion study of Atenolol released into infinite sink was carried out using the modified Keshary-Chien diffusion cell. Release of drug from the corresponding devices was diffusion under membrane controlled. The release data was fitted into various mathematical models using software to know which mathematical model will best fit to obtained release profiles. The obtained R values for various models are given in Table 9.2.1.

Table 9.2.1.: Data of various parameters of model fitting for Atenolol adhesive matrix formulations

Formulation	Zero order equation	First order equation	Higuchi's equation
F1	0.9785	0.9250	0.9987
F2	0.9850	0.9377	0.9969
F3	0.9698	0.9195	0.9959

The in vitro release profile could be best expressed by Highuchi's equation for the permeation of drug from the matrix.

9.3 In vitro human live skin permeation study of adhesive drug matrix device of Atenolol

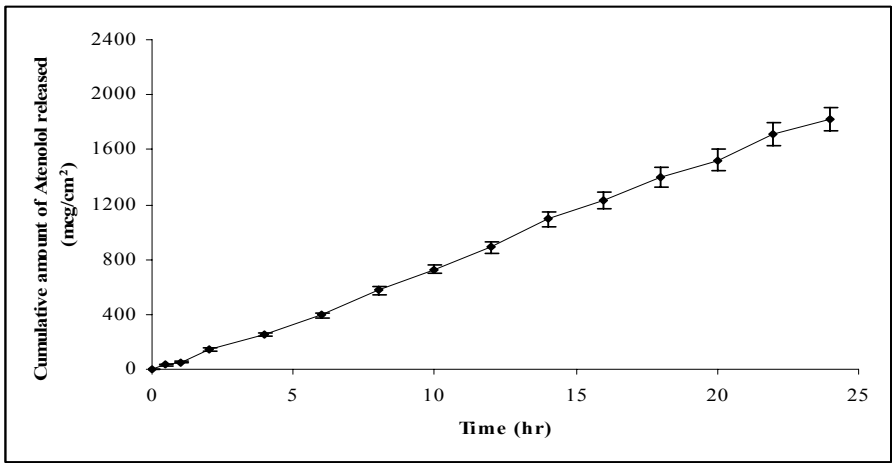
9.3.1 Experimental

The permeation study was performed in a modified Keshary-Chien diffusion cell. The experiment was performed as mention in section 5.6.2. The experiments were done in triplicate. Amount of drug released per square centimeter of patch were plotted against function of time for adhesive matrix formulation. The release rate was determined by simple regression analysis of steady state data.

9.3.2 Results and discussion

Permeation study was performed for drug adhesive matrix patch across human live skin using 0.01 N HCl, as a diffusion medium. The result of in vitro skin permeation of Atenolol from adhesive matrix formulation is shown in Figure 9.3.1

Figure 9.3.1: In vitro skin permeation profile of Atenolol from drug adhesive matrix patch through human live skin



*** Standard deviation, n=3**

Various parameters of diffusion kinetics of Atenolol released from device across human live skin are presented in Table 9.3.1.

Table 9.3.1: Parameters of diffusion kinetics of Atenolol from drug adhesive matrix patch through human live skin

Sr. No.	Parameters	Value
1	Skin flux (Jss)	79.83 µg/ cm^2 hr
2	Time lag (t_L)	0.82 hr
3	Human live skin thickness (µm)	140 µm
4	Diffusion coefficient	2.845 x 10^{-5} cm^2/sec
5	Solubility of drug in skin (Cs)	39.35 mg/cm^3

Adhesive matrix device containing Diltiazem provided **79.83 µg/ cm^2 hr** skin flux. The time lag (t_L) for devices is presented in Table 9.3.2. The diffusion coefficient, D of Diltiazem was determined using $D = h^2/6\ t_L$ relationship, where skin thickness h was 140 x 10^{-4} cm. The amount of Diltiazem retained by skin area used for permeation was calculated by dividing flux with gradient of diffusivity and it was found to be **39.35 mg/cm^3** of skin.

Computation of area of patch required for target steady state plasma concentration (Css)

As mentioned in section 8.6.3, desired input rate through skin for target steady state plasma concentration of Atenolol is 679.14 µg/ hr

Area of patch = (679.14 µg/hr)/ $J_{ss(skin)}$

= 679.14/79.83cm^2

= 8.5 cm^2

Formulation of final patch

As per British J. of clinical Pharm.[7] steady state plasma concentration built up after administration of 25mg, 50mg and 100 mg Atenolol orally in human volunteers are recommended to develop peak plasma concentration of 126 ng/ml, 219 ng/ml and 390 ng/ml respectively to control varied dose dependent physiological cardiac complains in the human. Transdermal controlled drug delivery system compared to conventional

administration is recommended as a safe alternative with better compliance to therapy by the patient, with reduced frequency of administration, leading to better disease management. Following three patches of different configuration were formulated as an alternative to above oral administration, to meet requirement of desired steady state plasma concentration for varied cardiac condition. Table 9.3.3 shows the parameters of the formulations.

Table 9.3.2: Parameters of final Atenolol adhesive matrix transdermal device

Components	Patch 1	Patch 2	Patch 3
Oral dose of Atenolol	**25 mg**	**50 mg**	**100 mg**
Css, mean steady state plasma concentration (ng/ml)	**126**	**219**	**390**
Area of transdermal patch(cm^2)	**8.5**	**14.2**	**26.6**
Predicted steady state plasma concentration	**120**	**200**	**375**
Steady state flux, Jss, through human live skin (µg/cm^2 hr)	**79.83**	**79.83**	**79.83**
Protective liner	**Silicon sheet**	**Silicon sheet**	**Silicon sheet**

The above formulations are 24 hour (once a day) devices. The drug loaded adhesive matrix was layered on specific dimension of polyethylene coated aluminum foil backing. For the preparation of final adhesive matrix diffusion controlled drug delivery device peripheral plain adhesive was used for good adherence of device on the skin. The release face was covered with peelable protective liner.

9.4 STABILITY STUDY

9.4.1. Stability study of adhesive matrix diffusional transdermal drug delivery device

The objective of accelerated stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature**.** The optimized formulation of Atenolol adhesive matrix patch was subjected for the stability study. The accelerated stability study was carried out according to ICH

guideline by storing the samples at 25 ^{0}C / 60 % RH, 30 ^{0}C/ 65 % RH and 40 ^{0}C/ 75 % RH for 90 days in a stability chamber (Thermo Lab., Mumbai, India). These samples were analyzed by UV Spectrophotometer method. Dosage forms were evaluated for changes in physical appearance and drug content at an interval of 15 days**.**

9.4.2 Results and Discussion

Final formulation was subjected for stability study and observed for change in color, appearance, flexibility and drug content. Temperature and humidity values selected were as per the ICH guidelines and the test was carried out in a stability chamber. The stability study was carried out at 25 ^{0}C / 60 % RH, 30 ^{0}C/ 65 % RH and 40 ^{0}C/ 75 % RH for 90 days.

Diffusion study was carried out and it was observed that formulation stored at 40 ^{0}C exhibited higher Q/√T release rate as compared to those stored at 25 ^{0}C and 30 ^{0}C. The initial release of drug was higher due to decreased viscosity at an elevated temperature. Release rate at 30 ^{0}C was altered but it was in order. The product stored at 25 ^{0}C exhibited no change in release rate. The degradation was higher at an elevated temperature. The first order rate constant of degradation for room temperature was **1.4×10^{-3} week^{-1}**. The self life calculated was **75 week**.

9.5 SKIN IRRIITATION AND SKIN SENSITIZATION STUDY

9.5.1 Experimental

Skin irritation and skin sensitization though are different types of physiological responses yet they have several common indications. Skin sensitization is systematic response and skin irritation is primarily is local response.

A protocol was devised for evaluation of skin irritation and/or sensitization in such a manner that the signs at the sight of application would be assessed in common for the both and further, to distinguish sensitization from irritation. The skin irritation and skin sensitization study was carried out as per mentioned in chapter 5.8.1.

The final Atenolol adhesive matrix patch (2.0 cm^2) was supported with aluminum foil. This was applied to the skin surface (shaved) and made adhered with the help of "3 M Micropore" – medical adhesive tap (3 M, Corporation, U.K.).

9.5.2 Results and discussion

The results of skin irritation and skin sensitization study for Atenolol adhesive matrix

patch is presented in Table 9.5.1. From this table total average score at day 20 and total average score at day 28 were obtained respectively.

From Table 9.5.2, it was clear that the Atenolol adhesive matrix patch was non-irritating, since the maximum score for each sign did not exceed than 1. Further Table 9.5.3 shows that average score at day 20 and day 28 was not exceeding more than 1 for any sign. Average score not exceeding more than 1 at any day for any sign was clearly indicative for non-irritation nature of the agent.

Atenolol is non-sensitizing in nature. The reason for this was reduction in scores even after repeated applications. All the tables show that maximum individual scores, average scores, total irritation scores were reduced at day 28th in comparison to all these scores at day 20th. Reduction in scores even after repeated application of test patch indicated absence of primary and secondary sensitization reaction.

Table 9.5.2: The average score for each sign after day 20 and day 28 during skin irritation and sensitization study of Atenolol adhesive matrix patch

Sr. No.	Sign	Average score at day 20	Average score at day 28
1	Erythema	0.75	0.0
2	Scaling	0.0	0.25
3	Fissures	0.75	0.5
4	Oedema formation	1.25	0.5
5	Ecchymosis	2.0	1.0
6	Necrosis	1.25	0.0

Table 9.5.1: Skin irritation and sensitization study of Atenolol adhesive matrix patch

SR.	Name And Concentration	SENSITIZATION / IRRITATION TESTING																							
		PRE-EXPOSURE PERIOD				INDUCTION PHASE										REST		CHALLENGE PHASE							
		Φ = 30M		Φ = 30M		Φ=24H		Φ=0		Φ=0		Φ=0		Φ=0		After 12		Φ=24H		Φ=0		Φ=0		Φ=0	
		D1		D2		D3		D4		D5		D6		D7		Days		D19		D20		D21		D22		D28	
1	Erythema	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	0	Avg	0	Avg	.75	Avg	0.5	Avg	0.5	Avg	0
2	Scaling	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0	Avg	0	Avg	0	Avg	0	Avg	0	Avg	.25
3	Fissures	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	1	1	1	1
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
		Avg	0	Avg	0	Avg	0	Avg	0.5	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	.75	Avg	.75	Avg	.75	Avg	0.5
4	Oedama formation	1	0	0	1	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	2	0	2	0	2	0	1
		0	0	0	0	0	1	1	1	1	1	0	1	0	1	0	0	0	0	1	1	1	1	0	1	0	1
		Avg	.25	Avg	.25	Avg	0.5	Avg	1	Avg	.75	Avg	0.5	Avg	0.5	Avg	.25	Avg	0	Avg	1.25	Avg	1.25	Avg	.75	Avg	0.5
5	Ecchymosis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	2	2	2	2	2	2	1	1
		0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	2	2	1	1	1	1	1	1
		Avg	0.5	Avg	0.5	Avg	0.5	Avg	1	Avg	1	Avg	1	Avg	0.5	Avg	0	Avg	0	Avg	2	Avg	1.5	Avg	1.5	Avg	1
6	Necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	2	1	1	0	0
		0	0	0	0	1	0	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	0	1	0	0
		Avg	0	Avg	0	Avg	.25	Avg	0.5	Avg	0.5	Avg	0.5	Avg	.25	Avg	.25	Avg	.25	Avg	1.25	Avg	1.25	Avg	.75	Avg	0
Total of all score		3		4		7		16		15		13		10		3		1		24		20		17		9	
Total average per animal		0.75		1		1.75		4		3.75		3.25		2.5		0.75		0.25		6		5		4.25		2.25	

Table 9.5.3: Maximum sensitization and irritation score of various signs at last day for Atenolol adhesive matrix patch

Sr. No.	Sign	Score	N
1	Erythema	**0**	**4**
2	Scaling	**1**	**1**
3	Fissures	**1**	**2**
4	Oedema formation	**1**	**2**
5	Ecchymosis	**1**	**4**
6	Necrosis	**0**	**4**

9.6 CONCLUSION

An adhesive matrix diffusional transdermal controlled drug delivery device of Atenolol was successfully prepared. The processing was non-complicated, very simple and economic, still meeting requirements of systemic administration of drug. Atenolol loaded natural rubber based adhesive matrix were prepared and devices were fabricated containing varied drug loading. Device containing 15mg/cm^2 of Atenolol was found to meet the biopharmaceutical and pharmacokinetic requirement for the therapeutic use of device. On evaluation, the device was found to be stable, non-irritant, non-sensitizing and safe. It complied with official and non- official pharmaco-technical specifications. In vitro evaluation for Atenolol release from device across human live skin provided zero order release rate **79.83 µg/cm^2 hr.** The flux was adequate to meet pharmacokinetic requirement of steady state plasma concentration for 24 hours, giving once a day drug delivery system of Atenolol. Alternatives to three oral conventional doses (25mg, 50mg and 100mg) devices were provided, by fabricating 8.5cm^2, 14.2cm^2 and 26.6cm^2 adhesive matrix diffusional transdermal drug delivery system of Atenolol, respectively as once a day drug delivery system.

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Chapter 10

Summary

CHAPTER 10

10. SUMMARY

Administration of drugs in conventional manner is not free from toxicity and G.I. tract related problems. Also hepatic metabolism reduces bioavailability of drug and to initiate and sustained therapeutic action higher doses of drug is needed. Only I/V dreeps can solve the above problem, however continuous presence of needle in the circulatory portal is inconvenient to patient. Ultimately patient compliance is reduced, and management of disease suffers. T.D.D.S is an answer and also a safe and convenient alternative to I/V dreeps. It obviates hepatic metabolism also, the problems concerned with G.I. tract route are absent. Processing of T.D.D.S is also simple as compared to parentral and solid dosage forms.

10.1 Preparation of Diltiazem base from official salt form and its characterization

- Hydrophobic drug penetrate more as compared to ionics and hydrophilic drug and with this information Diltiazem base was prepared from its official salt form and it was characterized prior to incorporate into drug delivery device
- In the present study, Diltiazem base was prepared from its official hydrochloride salt and characterized using different parameters. Melting point, Refractive index was determined to check the purity of drug.
- From solubility study it was found that 0.01N HCl was suitable to maintain sink condition so, it was selected as a diffusion medium.
- The results obtained from Partition co-efficient study revealed that the drug possessed sufficient lipophillicity, which fulfills the requirements of formulating it into a transdermal patch.
- Differential scanning calorimetry and Fourier transform infrared spectroscopy gave idea regarding chemical structure of pure drug.
- UV/VIS Spectroscopic data are useful for the preparation of standard curve and estimation of Diltiazem base from various formulations.

10.2 Preparation and characterization of polymeric matrix diffusional transdermal drug delivery device of Diltiazem

- Present investigation was aimed to formulate a robust, stable and simple matrix diffusional transdermal drug delivery system of Diltiazem.

- Different matrix forming polymers like EVA (VA 40 %) copolymer, Ethyl cellulose, ERS 100 and ERL 100 were employed to form medicated matrices. Casting on glass substrate as well as mercury substrate can give medicated matrices.
- Matrix laminates were characterized for its physical characteristics like thickness, weight variation, drug content, % moisture absorption, % moisture loss and % water vapor transmission rate etc.
- Drug polymer interaction study was done with differential scanning calorimetry and FTIR and it was observed that there was no interaction occurring between drug and polymer.
- Diltiazem containing medicated matrices of polymer EVA (VA 40 %) copolymer, EC, ERS 100 and ERL 100: ERS 100 (1:1) was subjected to diffusion study using modified Kesharay- Chien's diffusion cell. EVA (VA 40%) copolymer exhibited maximum Q/√T release rate. The diffusion kinetic from all the polymers obeyed square root law and Higuchi model was best fit for all the medicated matrices.
- Selected medicated matrices were further subjected to diffusion study to get desired Q/√T release rate, by incorporating gradually increasing concentration of Diltiazem. EVA (VA 40 %) copolymer: Diltiazem in 40:60 ratio exhibited maximum (desired) release rate. Also laminates complied with desired physical characteristics of drug distribution, elegance, thickness, content uniformity of drug etc.
- Marix diffusional T.D.D.S of Diltiazem was fabricated using polyethylene coated aluminum foil as backing membrane, Diltiazem loaded EVA (VA 40%) copolymer laminate as drug supplying layer, a adhesive system to keep the dosage form remaining attached throughout the duration of administration to the patient. A peelable silicone liner was used to protect device.
- Matrix diffusional T.D.D.S. of Diltiazem (3.14 cm^2) was evaluated for diffusion characteristic using human live skin (thickness – 140 μm). The release followed zero order kinetic, after a time lag. The skin flux was 18.24 $\mu g/cm^2$ hr.
- Using pharmacokinetic data of Diltiazem, Cmax, $t_{1/2}$, k_e, CL_{total} a targeted steady state plasma concentration that can be build up from the delivery device was computed. From the computation data, it was planned out too finally

fabricate once a day matrix diffusional Transdermal drug delivery device of Diltiazem. It was found that 30.4 cm^2 delivery device could meet the requirement of steady state plasma concentration for 24 hr.

- The delivery device was subjected to accelerated stability study as per ICH guideline. Results of stability study suggested a self life of 95 week for the device stored at room temperature.
- The dosage form can be used as once a day transdermal drug delivery device of Diltiazem, for cardiac problems.

10.3 Preparation and characterization of membrane moderated Reservoir type transdermal drug delivery device of Diltiazem

- Polymeric membranes were prepared using solution casting method.
- Membranes were prepared using EVA (VA 40%) copolymer, Ethyl cellulose, Polyvinyl acetate, ERS 100 as a polymer.
- Membranes were characterized physiochemically for appearance, transparency, thickness, density and water sorption etc.
- Membranes were evaluated for Diltiazem permeability. EVA (VA 40%) copolymer membrane exhibited maximum flux compared to other membrane. Therefore it was selected as rate controlling membrane to device reservoir type transdermal drug delivery system of Diltiazem.
- EVA (VA 40%) copolymer membrane of different thickness were prepared and evaluated for Diltiazem permeation kinetic with a view to optimize and select requisite thickness of membrane for devicing final drug delivery device. 30 μm thick EVA (VA 40%) copolymer rate controlling membrane gave Diltiazem flux of 1378.12 $\mu g/cm^2$ hr and it was selected to device final patch.
- A reservoir patch (3.14 cm^2 areas) was fabricated using selected membrane containing alcoholic solution of Diltiazem and evaluated for permeation kinetics using human live skin. The skin flux of Diltiazem 40.77 $\mu g/cm^2$ hr was quite promising to device a cute and elegant reservoir type device.
- The priming dose of Diltiazem was worked out from the data of diffusion kinetic of Diltiazem from device across human live skin. Incorporation of priming dose in release face covering adhesive system gave skin flux, 43.95 $\mu g/cm^2$ hr of Diltiazem.

- From the pharmacokinetic data of Diltiazem the requisite area required to fabricate final reservoir device was computed. 12.61 cm^2 size of reservoir device was sufficient to meet targeted steady state Diltiazem concentration for 24 hr.
- Accelerated stability study as per ICH guideline indicated self life of 53 weeks for the product stored at room temperature.
- Results of skin irritation and skin sensitization study on rabbit showed that patch was non irritating and free from skin irritation effects.
- A patch was deviced using Diltiazm gel filled in the Reservoir compartment of (3.14 cm^2) patch and evaluated for diffusion study using human live skin. Skin flux of Diltiazem was found to be 22.209 μg/cm^2 hr.
- The priming dose of Diltiazem was computed using parameters of diffusion kinetics of Diltiazem released from device across human live skin. Incorporation of priming dose in release face covering adhesive system gave skin flux of 23.78 μg/cm^2 hr of Diltiazem.
- Area of final reservoir device containing Diltiazem gel was computed from pharmacokinetic data of Diltiazem. A 23.31 cm^2 reservoir type transdermal drug delivery device containing Diltiazem gel was sufficient to meet targeted steady state Diltiazem concentration for 24 hr.
- Accelerated stability study as per ICH guideline indicated self life of 61 weeks for product stored at room temperature.
- Results of skin irritation and skin sensitization study on rabbit showed that the patch was non-irritating and free from skin sensitization and was safe for use.

10.4 Preparation and characterization of adhesive matrix diffusional transdermal drug delivery device of Diltiazem

The processing of Reservoir patch is complicated as compared to matrix type devices. Further among matrix devices, the processing of medicated film is little more complicated as compared to simple layering of a medicated mass on a backing surface. The very simple process for product still meets the requirement of systemic administration of drug; inspires one to prepare such devices. Attempt was made to formulate adhesive matrix type transdermal drug delivery system of Diltiazem.

- Natural rubber base adhesive was received ready made (Beta surgical, Rajkot)
- Drug loaded matrices of different concentration were prepared using TLC kit.

- Adhesive matrix type devices were evaluated for physiochemical test like thickness, weight variation, drug content, % moisture content and % moisture absorption etc.
- In vitro diffusion study of all the formulation followed square root law and Higuchi equation could be best fit for the drug release from the devices. Formulation F4 exhibited maximum Q/√T release rate, which was 1556.70 $\mu g/cm^2$ hr.
- In vitro human live skin permeation kinetic showed zero order release rate 19 64 $\mu g/cm^2$ hr.
- From the pharmacokinetic data and results of human live skin diffusion study, requisite area required to fabricate final device was worked out. A 28.22 cm^2 size Diltiazem loaded adhesive matrix device was found to meet targeted steady state Diltiazem concentration for 24 hours.
- Accelerated stability study as per ICH guideline indicated self life of 79 weeks for the product stored at room temperature.
- Results of skin irritation and skin sensitization study on rabbit showed that patch was non irritant, free from skin sensitization and safe for use.

10.5 Preparation and characterization of polymeric matrix diffusional transdermal drug delivery device of Atenolol

The aim of present investigation is to formulate and optimize the Atenolol matrix diffusion controlled transdermal drug delivery system. In the present investigation, the influence of various grades and concentration of polymers were studied. Study was carried out to formulate an elegant product exhibiting desired therapeutic performance, from a small and cute dosage form.

- Different matrix forming polymers like EVA (VA 40 %) copolymer, Ethyl cellulose, Polyvinly pyrolidone, HPMC, ERS 100 and ERL 100 were employed to form medicated matrices. Casting on glass substrate as well as mercury substrate can give medicated matrices.
- Matrix laminates were characterized for its physical characteristics like thickness, weight variation, drug content, % moisture absorption, % moisture loss and % water vapor transmission rate etc.

- Drug polymer interaction study was done with differential scanning calorimetry FTIR and it was observed that there was no interaction occurring between drug and polymer.
- Atenolol containing medicated matrices of polymer EVA (VA 40 %) copolymer, EC: PVP, ERS 100, ERL100: ERS100 (1:1) and ERL 100: HPMC were subjected to diffusion study using modified Kesharay- Chien's diffusion cell. EVA (VA 40%) copolymer exhibited maximum Q/√T release rate. The diffusion kinetic from all the polymers obeyed square root law and Higuchi model was best fit for all the medicated matrices.
- ERL100 and ERS100 were selected to device final matrix diffusional therapeutic system. In this study various matrix type transdermal patches containing Atenolol of variable combination of ERL100 and ERS100 were prepared. Also laminates complied with desired physical characteristics of drug distribution, elegance, thickness, content uniformity of drug etc.
- Marix diffusional T.D.D.S of Atenolol was fabricated using polyethylene coated aluminum foil as backing membrane, laminate as drug supplying layer, an adhesive system to keep the dosage form remaining attached throughout the duration of administration to the patient. A peelable silicone liner was used to cover the laminate to protect it.
- Matrix diffusional T.D.D.S. of Atenolol (3.14 cm^2) was evaluated for diffusion characteristic using human live skin (thickness – 140 μm). The release followed zero order kinetic, after a time lag. The skin flux was 70.84 $\mu g/cm^2$ hr.
- Using pharmacokinetic data of Atenolol, Cmax, $t_{1/2}$, k_e, CL_{total} a targeted steady state plasma concentration that can be built up from the delivery device was computed. As per British J. Clinical Pharma., three different oral conventional doses of Atenolol are recommended (25 mg, 50 mg, and 100 mg) for different cardiac complaints in human beings. Three polymeric matrix moderated transdermal drug delivery devices of Atenolol of different sizes i.e. 9.7 cm^2, 16 cm^2 and 30 cm^2 were fabricated to serve as once a day devices, as an alternative to above mentioned three conventional doses.
- The delivery device was subjected to accelerated stability study as per ICH guideline. Results of stability study suggested a self life of 82 week for the device stored at room temperature.

- Skin sensitization and skin irritation study was carried out and it was found that the dosage form was nonirritating and safe for use.
- The device can be used as once a day transdermal drug delivery device of Atenolol, for cardiac problems.

10.6 Preparation and characterization of adhesive matrix diffusional transdermal drug delivery device of Atenolol

The processing of Reservoir patch is complicated as compared to matrix type devices. Further among matrix devices, the processing of medicated film is little more complicated as compared to simple layering of a medicated mass on a backing surface. The very simple process for product still meets the requirement of systemic administration of drug; inspire one to prepare such devices. Attempt was made to formulate adhesive matrix type transdermal drug delivery system of Atenolol.

- Natural rubber base adhesive was received ready made (Beta surgical, Rajkot)
- Drug loaded matrices of different concentration were prepared using TLC kit.
- Adhesive matrix type devices were evaluated for physiochemical test like thickness, weight variation, drug content, % moisture content and % moisture absorption etc.
- In vitro diffusion study of all the formulation followed square root law and Higuchi equation could be best fit for the drug release from the devices. Formulation F3 exhibited maximum Q/√T release rate, which was 1469.9 $\mu g/cm^2$ hr.
- In vitro human live skin permeation kinetic showed zero order release rate 79.83 $\mu g/cm^2$ hr.
- Using pharmacokinetic data of Atenolol Cmax, $t_{1/2}$, $k_{e,}$ CL_{total} a targeted steady state plasma concentration that can be build up from the delivery device was computed. As per British J of Clinical Pharma., three different oral conventional doses of Atenolol are recommended (25 mg, 50 mg, and 100 mg) for different cardiac complaints in human beings. Three polymeric matrix moderated transdermal drug delivery devices of Atenolol of different sizes i.e. 8.5 cm^2, 14.2 cm^2 and 26.6 cm^2 were fabricated to serve as once a day devices, as an alternative to above mentioned three conventional doses.

- The delivery device was subjected to accelerated stability study as per ICH guideline. Results of stability study suggested a self life of 75 week for the device stored at room temperature.
- Skin sensitization and skin irritation study was carried out and it was found that the dosage form was nonirritating and safe for use.

Poster selected

Poster selected

1. Formulation, Characterization and evaluation of transdermal patches of Diltiazem, 12th February, 2009, Pittcon conference, Chicago, U.S.A.

Formulation, Characterization and evaluation of transdermal patches of Diltiazem

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Abstract:

Dilitiazem free base was prepared from corresponding official salt & characterized prior to incorporation in various polymeric matrix diffusional transdermal therapeutic system.

Medicated matrices were prepared using different polymers like ethylene vinyl acetate (VA 40%) copolymer, Ethyl cellulose, Eudragit RS 100, Eudragit RL 100 : Eudragit RS 100 (1 : 4), were used as polymers. Solution casting on mercury substrate technique was used to prepare medicated film. The films were investigated for water vapour transmission rate, % Moisture loss, % Moisture absorption, folding endurance and thickness.

Transdermal therapeutic systems were fabricated using 120 – 140 μm thick medicated film laminated on polyethylene sheet, which in turn was laminated over polyethylene coated aluminum foil backing. Adhesive system was peripheral & devices were covered with peelable protective liner.

Therapeutic systems were evaluated using modified version of keshary – chien type diffusion cell, where 0.01M HCl was used as diffusion medium. Diltiazem released from the device was estimated using UV-visible spectrophotometer at 237.8 nm. The release of diltiazem from all the patches was diffusion controlled & followed linear Q versus √T relationship. In vitro release rates of Diltiazem for EVA (VA 40%), Ethyl cellulose, Eudragit RS 100, Eudragit RL 100: Eudragit RS 100 (1: 4), patches were 1488.10, 946.30, 503.29 & 794.08 $\mu g/cm^2/\sqrt{h}$ respectively EVA (VA 40%) copolymer: Diltiazem (60: 40) therapeutic system exhibited maximum release rate. The Diltiazem flux from EVA (40%) copolymer system across dermatomed human live skin (thickness-140 μm) was investigated. The Epidermal flux was 16.26

μg / cm^2 / hr & was maintained for 24 hours.

Prediction of in vivo performance using a reported model indicated a small size of therapeutic system to be sufficient to meet the pharmacokinetic requirement of steady state plasma concentration of diltiazem.