"FORMULATION AND EVALUATION OF MATRIX TRANSDERMAL PATCH OF GINGER EXTRACT USING NATURAL PENETRATION ENHANCERS AND *EX-VIVO* PERMEATION STUDY THROUGH EXCISED GOAT SKIN"

Thesis submitted to the

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY,
Guwahati, Assam.

In partial fulfillment of the requirement for the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by:

Damanbhalang Rynjah
Reg. No.: 387305219 of 2019-2020
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Under the supervision of:

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2021

Dedication

Dedicated to
My Parents

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DECLARATION

I hereby declare that the thesis entitled **"FORMULATION AND EVALUATION OF MATRIX TRANSDERMAL PATCH OF GINGER EXTRACT USING NATURAL PENETRATION ENHANCERS AND *EX-VIVO* PERMEATION STUDY THROUGH EXCISED GOAT SKIN"** is a bonafide and genuine research work carried out by me under the supervision of **Dr. Tapash Chakraborty, Asst. Professor, and Asha Das, Asst. Professor, Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science,** Azara, Guwahati-17. The work embodied in this thesis is original and has not been submitted in part or full for the award of degree, diploma, associateship or fellowship of any other University or Institution.

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Certificate

GIPS/IAEC/M.PH/PRO/05/2021

This is to certify that the project proposal no.....entitled "***Formulation and evaluation of matrix transdermal patch of ginger extract using natural penetration enhancers and ex-vivo permeation study through excised rat skin***" submitted by ***Mr. Damanbhalang Rynjah*** has been approved/recommended by the IAEC of ***Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara***, Guwahati-781017, Assam in its meeting held on 25/02/2021 and .....(Number and Species of animals) have been sanctioned under this. 6 Nos zealand albino Rabbits &
16 Wistar albino rats.

Authorized by	Name	Signature	Date
Chairman:	Gouranga Das.		02.03.2021
Member Secretary:	Gouranga Das.		02.03.2021
Main Nominee of CPCSEA:	Dr. Smriti Rekha Dutta		02.03.21

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संख्या /No.: BSI/ERC/ Tech/2021/ 117 दिनांक /Dated:29.07.2021

सेवा में/To,

Damanbhalang Rynjah
M. Pharm. Student
Girijananda Chowdhury Institute of Pharmaceutical Sciences
Azara, Guwahati-17, Assam.

विषय /Sub.: Identification and authentication of plant specimen.

Dear Mr. Rynjah

With reference to your letter No.Nil dated 09-07-2021, regarding the subject cited above, I am to inform you that your plant specimen has been identified and confirmed as below:-

1. *Zingiber officinale* Roscoe (Zingiberaceae)

Thanking You/ धन्यवाद

भवदीय /Yours sincerely

29/7/2021

(Dr.N. Odyuo)
वैज्ञानिक-ई एवं कार्यालय प्रमुख/ Scientist-E,HoO

ABSTRACT:

The aim of the present study is to formulate and evaluate the matrix transdermal patch of ginger extract using natural penetration enhancers by *in vitro* and *ex-vivo* permeation studies. The ginger extract was extracted from ginger rhizome by simple maceration using ethanol as a solvent, and its characterization was done by TLC method, FT-IR, and from the absorption maxima. The transdermal patches of ginger extract was formulated by using solvent evaporation method and further required studies of different parameters were evaluated like the drug content and other related parameters which are required for evaluating the transdermal patch. In the *in vitro* and *ex vivo* permeation study, it was found that all the patches containing penetration enhancers show good release than the blank. Different parameters related to the permeation study was calculated and report like Flux value, cumulative drug permeated per square centimetre, Enhancement ratio, permeability coefficient and others. After comparing the cumulative drug permeated/cm^2 (Q_{24}) and the flux from all the patches, it was found that the EF3 is showing the highest drug permeated/cm^2 (Q_{24}) of 42.141±0.009 mg/cm^2 with the total flux of 3.308±0.001 mg/cm^2/hr. After running the One-way ANOVA, it was found that the cumulative drug release/cm^2 is not significant at $p<0.05$. The patch shows no irritation, after testing in rabbit and the mechanistic study report that there are changes in the proteins and lipids structure of the skin. Hence it can be concluded that the prepared patches can be formulated and further studies can be carried out like calculating the dose, checking its therapeutic effects, pharmacokinetics, and also animals and humans study before it can be used as an anti-emetic, anti-nausea transdermal patch.

Keywords: Ginger, transdermal patch, penetration enhancers, permeation study, anti-emetic.

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It is a great opportunity for me to acknowledge those who without them this thesis work would not be successful.

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DAMANBHALANG RYNJAH

M. Pharm 4th semester,

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LIST OF UNITS

Units	Description
cm	Centimetre
°C	Degree Celsius
Da	Dalton
hrs.	Hours
%	Percentage
°F	Degree Fahrenheit
g/mol	Gram/mole
kg	Kilogram
m	Metre
min	Minutes
mm	Millimetre
mg	Milligram
mg/ml	Milligram/milliliter
ml	Millimetre
$mg/cm^2/hr$	Milligram/ centimeter square/ hour
nm	Nanometre
v/v	Volume/volume
w/v	Weight/volume
λ_{max}	Wavelength of maximum absorbance
µg/ml	Microgram/millilitre

LIST OF ABBREVIATIONS

Abbreviation	Description
CAM	Complementary and Alternative medicine
CPCSEA	Committee for purpose of control and supervision of experiments on animals
DMAC	Dimethyl acetamide
DMF	Dimethyl formamide
DMSO	Dimethyl sulphoxide
ER	Enhancement ratio
EVAC	Ethylene Vinyl Acetate Copolymer
FTIR	Fourier Transform Infrared spectroscopy
GC/MS	Gas-chromatography/ mass spectroscopy
GIT	Gastro Intestinal Tract
HPMC	Hydroxy propyl methyl cellulose
HPLC	High Performance Liquid Chromatography
5-HT3	5-Hydroxytryptamine
IAEC	Institute Animals Ethics Committee
IPM	Isopropyl myristate
J	Flux
NPE	Natural permeability enhancers
PE	Polyethylene
PEG	Polyethylene glycol
Q_{24}	Cumulative release of drug per cm^2 for 24 hr.
R_f	Retention factor
TLC	Thin Layer Chromatography
TDDS	Transdermal drug delivery system
T_{lag}	Lag time
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The use of an herb or plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes is known as Herbal medicine, Botanical medicine, or Phytomedicine. An herb is a plant or plant part used for its scent, flavor, or therapeutic properties. Long before history has been recorded, plants had been used as medicines. Ayurveda, one of the oldest traditional medical systems had been primarily practiced in India for nearly over 5000 years which includes diet and herbal remedies while emphasizing the body, mind, and spirit in disease prevention and treatment by the use of plants and plant extracts. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Also, some of the indigenous cultures such as the African and Native Americans used herbs in their healing rituals. Herbal medicines are one type of dietary supplement being sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. Most people use herbal medicines to maintain or improve their health by believing that products labeled "natural" are always safe and good for them, though it is not true or partially true, as the herbal medicines do not have to go through the testing and quality control like that of a drug (Kar, 2008).

Advantages of herbal medicine over allopathic medicines:

- Allopathic medicines are very costly. In contrast, herbal medicines are very cheap. This cost-effectiveness makes them all the more alluring.
- Herbal medicines can be brought without prescription and they are available in almost all health stores. Some herbs can even be grown at home.
- For certain ailments, herbal medicines are considered to be more effective than allopathic medicines.

- Herbal medicines do not have any side effects, as they are free from chemicals. They are also milder than allopathic medicines.
- The natural detoxification process of the body is effectively enhanced by herbal medicines. They can be used to cleanse the colon, improve digestion and food absorption. Herbal medicines are also very good for boosting the immune system.
- Herbal medicines are very effective in curing various digestive disorders like colitis, indigestion, peptic ulcers, and irregular bowel movements.
- These types of medicines are best for people who are allergic to various types of drugs.
- Herbal medicines are also effective in boosting mental health; reducing the cholesterol level in the bloodstream and reducing weight by regulating appetite. They are also used to treat coronary artery diseases.

Disadvantages of herbal medicine over allopathic medicines:

- The main drawback is that herbal medicines take too much time to act. The entire process is very slow.
- There is also a remote chance that herbal medicine may not give the desired result.
- Herbal medicine is not good for serious trauma like broken bones.
- Lack of Dosage instruction.
- Poison risk associated with the wild herb.
- Some herbal medications can interact with medications like antidepressants.

- **Traditional medicine system:**

WHO defines traditional medicine as "medical knowledge systems that developed over generations within various societies before the era of modern medicine, including the health practices, approaches, knowledge, and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being". In developing countries like India, traditional medicine is often the only accessible and affordable treatment available and in India, it has a rich heritage of traditional medicine, and the traditional health care system has been flourishing for many centuries. The WHO regional office for the Americas (AMRO/PAHO) reported that 71% of the population in Chile and 40% of the population in Colombia has used traditional medicine. In many Asian countries traditional medicine is widely used, even though western medicine is often readily available just like in the case of Japan, 60-70% of allopathic doctors prescribe traditional medicines for their patients (Kar, 2008)

- **Ayurvedic medicine**

Ayurveda is derived from a Sanskrit word where ayus mean "longevity", and Veda means "related to knowledge" or "science". Ayurvedic medicine is a system of traditional medicine native to the Indian subcontinent right from before 2500 B.C, which involves curing, prevention of illness, and also in holistic view in man's health. It is also practiced in other parts of the world as a form of traditional medicine and Ayurveda remains an influential system of medicine in South Asia. Ayurveda is considered to be a form of complementary and alternative medicine (CAM) in the western world, where several of its methods, such as the use of herbs, massage, and yoga, are applied on their own as a form of CAM treatment. The medicinal preparation in this system is invariably complex mixtures, based mostly on plant products. Around

1,250 plants are cured by the use of various Ayurvedic preparations. Many Indian medicinal plants have come under scientific scrutiny since the middle of the nineteenth century, although in a sporadic fashion. The first significant contribution from Ayurvedic Materia medica came with the isolation of the alkaloids from the plant Sarpagandha (*Rauwolfia serpentina*), which is used in Ayurveda for the treatment of hypertension, insomnia, and insanity. This was the first important ancient-modern concordance in Ayurvedic plants. According to Ayurveda all objects in the universe including the human body are composed of five basic elements (Panchamahabhutas) namely earth, water, fire, air, and vacuum. There is a balanced condensation of these elements in different proportions to suit the needs and requirements of different structures and functions of the body matrix and its parts. The growth and development of the body matrix depend on its nutrition, i.e. on food. The food, in turn, is composed of the above five elements, which replenish or nourish the like elements of the body after the action of bio-fire (Agni). Treatment of the disease consists of avoiding causative factors responsible for disequilibrium of the body matrix or any of its constituent parts through the use of Panchkarma procedures, medicines, suitable diet, activity, and regimen for restoring the balance and strengthening the body mechanisms to prevent or minimize future occurrence of the disease. Normally treatment measures involve the use of medicines, a specific diet, and a prescribed activity routine. The use of these three measures is done in two ways. In one approach of treating the three measures antagonize the disease by counteracting the etiological factors and various manifestations of the disease and in the second approach the same three measures of medicine, diet, and activity are targeted to exert effects similar to the etiological factors and manifestations of the disease process. These two types of therapeutic approaches

are respectively known as Vipreeta and Vipreetarthkari treatments (Gokhale et al., 2009)

- **Siddha system**

Siddha system is one of the oldest systems of medicine in India. The term Siddha means achievements and Siddhars are the saint persons who achieved results in medicine. Eighteen Siddhars were said to have contributed towards the development of this medical system. Siddha literature is in Tamil and it is practiced largely in the Tamil-speaking part of India and abroad. The Siddha System is largely therapeutic. The principles and doctrines of this system, both fundamental and applied, have a close similarity to Ayurveda, with specialization in Iatro-chemistry. According to this system, the human body is the replica of the universe and so are the food and drugs irrespective of their origin. Like Ayurveda, this system believes that all objects in the universe including the human body are composed of five basic elements namely, earth, water, fire, air, and sky. The food, which the human body takes and the drugs it uses are all, made of these five elements. The proportion of the elements present in the drugs vary and their preponderance or otherwise is responsible for certain actions and therapeutic results. As in Ayurveda, This system also considers the human body as a conglomeration of three touches of humor, seven basic tissues, and the waste products of the body such as feces, urine, and sweat. Food is considered to be the basic building material of the human body which gets processed into humor, body tissues, and waste products. The equilibrium of humor is considered as health and its disturbance or imbalance leads to disease or sickness. This system also deals with the concept of salvation in life. The exponents of this system consider the achievement of this state is possible by medicines and meditation. The Siddha system is capable of treating all types of diseases other than emergency cases. In general, this system is effective in treating all

types of skin problems particularly Psoriasis, sexually transmitted diseases, urinary tract infections, diseases of the liver and gastrointestinal tract, general debility, postpartum anemia, diarrhea, and general fevers (Vasisht & Kumar, 2002)

- **Unani**:

Unani system of medicines originated in Greece and is based on the teachings of Hippocrates and Gallen and it developed into an elaborate medical system by Arabs, like Rhazes, Avicenna, Al-Zahravi, Ibne-Nafis, and others. Unani medicines got enriched by imbibing what was best in the contemporary systems of traditional medicines in Egypt, Syria, Iraq, Persia, India, China, and other Middle East countries. In India, the Unani system of medicine was introduced by Arabs and soon it took firm roots. The Delhi Sultans (rulers) provided patronage to the scholars of the Unani system and even enrolled some as state employees and court physicians. During the 13th and 17th century A.D. Unani medicine had its heyday in India. During the British rule, the Unani system suffered a setback due to the withdrawal of State Patronage but continued to be practiced as the masses reposed faith in the system. It was mainly the Sharifi family in Delhi, the Azizi family in Lucknow, and the Nizam of Hyderabad due to whose efforts Unani medicine survived during the British period. In India, the concept of research in the Unani system of medicine was originally perceived by Masihul-Mulk Hakim Ajmal Khan in the 1920s. A versatile genius of his time, Hakim Ajmal Khan spotted Dr. Salimuzzaman Siddiqui- a chemist- for undertaking chemical studies on some important medicinal plants used in Unani Medicine. Dr. Siddiqui undertook the task visualized by Masih-ul-Mulk and his discovery of medicinal properties of a plant, commonly known as Asrol (Pagal Booti), led to sustained research that established the unique efficacy of this plant known all over the world as *Rauwolfia serpentina*, in

neurovascular and nervous disorders, such as hypertension, insanity, schizophrenia, hysteria, insomnia and psychosomatic conditions, etc. (Gokhale et al., 2009)

- **Homeopathic:**

Homeopathy, founded by a German physician Samuel Hahnemann in 1790, is based on the idea that 'like cures like'; that is substances that cause certain symptoms in a healthy person can also cure those same symptoms in someone who is sick. This so-called law of similarity gives homeopathy its name 'homeo' for similar 'pathy' designating disease. In this experiment, Hahnemann developed a method of 'potentizing' homeopathic remedies by diluting them in a water-alcohol solution and then vigorously shaking the mixtures. The result convinced him that a high degree of dilution not only minimizes the side effects of the remedies but also simultaneously enhances their medical efficacy. Most Homeopathic remedies have undergone 'proving' or medical observation in which healthy individuals are given doses of undiluted homeopathic substances. Mental, emotional, psychic, and other details of the patients are most important. This leads the physician to a better understanding of which remedy will best suit a particular set of symptoms. Over the past 200 years, providing for almost 2,000 substances has been conducted (Yadav et al., 2012).

1.1. TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal is a route wherein active ingredients are administered in the form of a patch or ointment by delivering it across the skin for systemic distribution to show the desired effect. Transdermal patches, oils, creams, gels, etc. are some of the examples used for drug delivery. Transdermal medication conveyance offers an exceptionally worthwhile course for drug transport contrasted and different courses of medication organization. Nonetheless, the hindrance capacity of the skin peripheral layer, stratum

corneum (SC) is one of the primary impediments to it, and hence, skin penetration enhancers are acquiring the best advantage in drug research (Warner et al., 2003). It is realized that the largest organ in the body is the skin, and in this way, the transdermal drug delivery is a more specific and designated delivering technique since the large uncovered area of the tissue works with the absorption of active (therapeutic) substances especially for those that cannot be effectively taken orally (Amjadi et al., 2018). Along these lines, numerous methodologies have been refined to upgrade drug penetration through the skin which is a multifaceted structure, to be used in transdermal drug delivery (Takeuchi et al., 2017).

1.1.1. Anatomy and physiology of skin

Skin is known as the largest organ in the human body which covers an area of about $2m^2$ in an average human adult and approximately one-third of the blood supply, this multi-layered organ receives. With only 1 mm of its thickness, the skin divides the underlying blood flow network from the outside atmosphere. (Figure 1.1)

The human skin comprises of three distinct, mutually dependent tissues:

- Epidermis - Top most-outer layer and the only visible layer which is thick, composed of keratinized, stratified squamous epithelium made of five sub-layers namely; stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. The epidermis does not have any blood vessels within it.
- Dermis – It is thicker than the epidermis, placed in between the epidermis and hypodermis containing oil and sweat glands, connective tissues, lymph vessels, hair follicles, and nerve endings. It is the layer of skin that enables the function to protect from pathogens and helps in supporting the skin structure.

- Hypodermis – It is also known as subcutis or subcutaneous fat; the main function of this layer is to provide insulation to the body and keeps the body warm, a shock absorber that surrounds the vital organs. This layer gets attach to the muscles and tissues below it and it can thicker in some parts of the body and it depends on genetics.

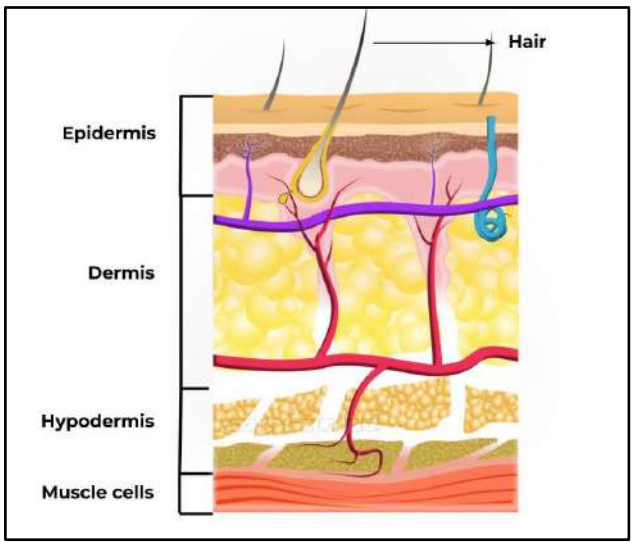


Figure 1.1: Structure of Skin.
[Structure Of Skin | Skin Structure and Function - LearnFatafat]

1.1.2. Skin as a barrier to drug permeation:

The outermost few microns of the skin the stratum corneum, aids to the barrier function of the skin. This layer of the skin is the most impermeable, forming a laminate of compressed keratin-filled corneocytes attached in a lipophilic matrix. The lipids of this matrix are distinguishing in many respects:

- From the skin surface to the base of the stratum corneum they provide the only continuous phase.
- Among the bio membranes, the absence of phospholipid is particular, and its composition (ceramides, free fatty acids, and cholesterol) is unique.

- The Stratum Corneum lipids exist as multi-lamellar sheets even though it is having a deficit of polar bilayer forming lipids.
- The essentially saturated, long-chain hydrocarbon tails aids a highly ordered, interlock configuration

However, the resistivity of the membrane cannot be entirely explained by the unusual lipid matrix and the architecture of the stratum corneum altogether has been proposed to play a role in the barrier property of the membrane. The corneocytes resembling a brick and mortar assembly are suggested to impart the membrane-impermeable to water concerning other bio membranes. And by the visualization studies localizing several permeants, in the intercellular channels by kinetic analysis of the in vivo skin penetration rates of the model compound and by evidence from thermo tropic biophysical studies of lipid domains the transport role of these pathways gets further more support (Naik et al., 2000).

1.1.3. Pathways for skin penetration:

The pathways for percutaneous absorption can happen through two different routes that are the transepidermal (intercellular and intracellular) and transappendageal which is also known as "Shunt pathway" (sebaceous glands, hair follicles, and sweat ducts).(Figure 1.2)

- **Transepidermal pathway**

  It includes intercellular and intracellular pathways. The intercellular pathways involve solute diffusion through the intercellular lipid domains through the tortuous pathway. This intercellular pathway shows a major resistance to skin permeation.

The intracellular or transcellular pathway involves permeation through the corneocytes then into the intercellular lipids. Permeation of compounds through this route uses the imperfections in the corneocytes that create openings comprised of water. This route is for hydrophilic drugs for delivery.

- **Transappendageal pathway**

  In the transappendageal pathway, the penetrant traverses the stratum corneum via a "shunt" pathway provided by the hair follicles or sweat glands. In particular, hair follicles play a major contributor to this pathway due to higher follicular distribution. Although the available surface area for the follicular route is assumed to be limited to approximately 0.1% of total skin surface area, it has recently been suggested that follicular number, opening diameter, and follicular volume are important considerations to define the extend of delivery. Also, the hair follicles extend deep into the dermis with a significant increase in the actual surface area available for penetration. Many studies have indicated the relevance of this pathway in skin permeation (Jain et al., 2017).

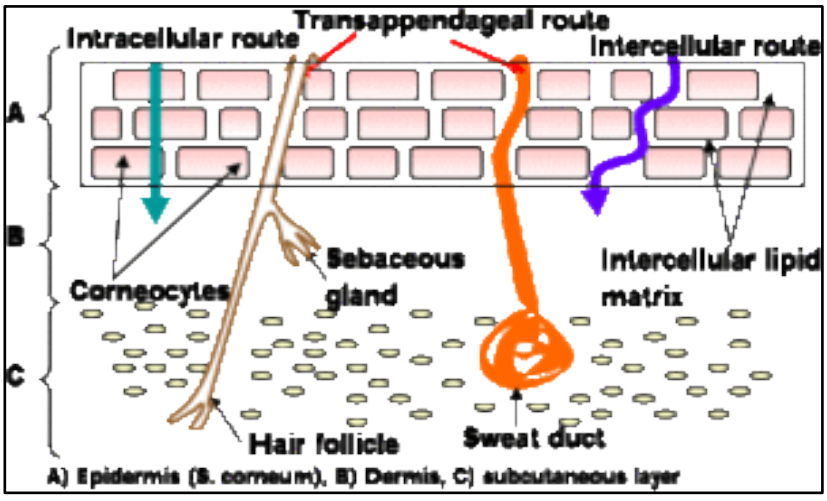


Figure 1.2: Pathways for skin penetration.
[Do cosmetic ingredients penetrate skin? (debraspence.com)]

1.1.4. Factors affecting transdermal permeation. (Yewale et al., 2021)

The factors that affect skin permeation are categorized into three types:

A. Physicochemical properties of the penetrant molecule.

- **Partition coefficient:** A partition coefficient of 1 (greater or equal to) is required for the optimal transdermal permeability and the partition coefficient can be modified by chemical modification without affecting the therapeutic property of the drug.
- **pH conditions:** Very high or very low pH values of the formulation can be destructive to the skin, but at an average pH value, the flow density of ionized drugs may be affected by a change in pH, the effect of which on transdermal permeability is the result of a change in the ratio between loaded and unloaded species.
- **Penetrant concentration:** The cause of a relative increase in the flux of the drug is due to a higher concentration of the penetrants. As the concentration of the penetrants is proportional to the concentration of the dissolved drug. If the concentration of the drug is greater than the solubility, then the excess solid drug will act as a reservoir system and helps to uphold a constant drug concentration for an extended period.

B. Physicochemical properties of the delivery system.

- **Release characteristics:** The amount of drug that gets solubilized in the vehicle is the release rate of the drug and the release mechanism of the drug depends on the factors-
  - If the drug molecules are dissolved or suspended in the formulation;

- The partition coefficient of the drug from the formulation to the skin; and
- The pH of the vehicle used in the formulation.

- **Composition of the drug delivery systems:** The composition of the drug delivery system, like thickness, boundary layers, vehicles, and polymers are not the only properties that affect the drug release rate but it also affects the drug permeability into the Stratum corneum.
- **Drug permeation enhancer:** Most of the drugs will not pass through the skin at the sufficient rate to show its therapeutic efficacy. So, permeation enhancers are added to the system to improve the transdermal permeation of drugs. Example, Organic solvents (Ethylene glycol, Polyethylene glycol, Dimethyl formamide, Dimethly sulfoxide, and Dimethyl acetamide), Surface active agents (Sodium dioctylsulfosuccinate, Sodium lauryl sulfate), azones (laurocapram), etc.

C. Physiological and pathological conditions of the skin.

The physiological and pathological conditions of the skin are discussed below as:

- **Reservoir effects of the horny layer:** The characteristics of some drugs for transdermal permeation totally depend on the horny layer of the skin due to the depot or reservoir action of the layer and hence it alters the drug permeation transdermally.
- **Lipid film:** The lipid nature of the skin layer and the epidermal cell lipid act as barrier function of the Stratum corneum and drug delivery.
- **Skin hydration:** Hydration of the stratum corneum enhances the skin permeability.

- **Skin temperature:** When the temperature of the skin was raised from 10 to 37 °C, the skin permeation was raised by 10 times its normal rate.

1.1.5. Basic components of transdermal drug delivery system (Saravanakumar et al., 2015).

- Polymer matrix or matrices
- The Drug
- Permeation enhancers
- Backing laminate
- Release liner
- Other excipients

Polymer matrix: Polymers are the foundation of transdermal system. The selection of polymer and design are of prime importance. And some of the considerations for polymer selection in transdermal delivery systems are:

- Should be stable and non-reactive with the drug moiety.
- Easily available, fabricated and manufactured in to desired formulations.
- The properties of polymer e.g. molecular weight glass transition temp. Melting point and chemical functionality etc. should be such that drug can easily diffused through it and with other components of system.
- Mechanical properties should not change if large amount of drug incorporate.
- Should provide consistent release of drug throughout the life of system

Table 1.1: Types of Polymers.

Natural polymers	Synthetic polymers	Synthetic elastomers
Cellulose derivatives	Polyethylene	Nitrile
Proteins	Polypropylene	Acrylonitrile
Gelatin	Poly vinyl alcohol	Hydrin rubber
Gum	Poly vinyl chloride	Silicone rubber
Starch	Polyamide	Polybutadiene
Chitosan	Polyurea	Neoprene
Waxes	Polystyrene	Polysiloxane
Shellac	Epoxy	Chloroprene
Natural rubber	Acetal copolymer	

Drug:

For successfully developing the transdermal drug delivery system, the drug should be chosen with great care. The following are some of the desirable properties of drug for transdermal drug delivery system.

Table 1.2: Parameters for an ideal transdermal patch.

Parameters	Properties
Dose	Less than 20mg/day
Molecular weight	Less than 1000 Dalton
Melting point	Less than 200 °C
Half life	Less than 10 hours
Shelf life	Up to 2 years
Partition coefficient	1 to 4
Aqueous solubility	Greater than 1 mg/ml

pH of the aqueous saturated solution	5-9
Drug affinity	Both lipophilic and hydrophilic
Skin permeability coefficient	Greater than 0.5x10-3 cm/hour
Skin reaction	Non-irritating and non-sensitizing
Oral bioavailability	Low

Release liners: The patch is covered by protective liner during storage until it is used .The release liner removed and discarded just before the application of patch over the skin since release liner is in intimate contact with the transdermal system hence it should be physically as well as chemically inert. The release coating layer is composed of Teflon and silicon.

Backing laminate: These are chosen for appearance, flexibility and need for occlusion. It protects the patch from outside environment .The backing layer serves the function of holding the entire system and protects the drug reservoir. Eg. Aluminium foil, Polyester films, Vinyl polyethylene, metallic plastic laminate, and foam pad.

Penetration Enhancer: Penetration enhancers are incorporated into a formulation to improve the diffusivity and solubility of drugs through the skin that would reversibly reduce the barrier resistance of the skin. These are the substances which stimulate the topically applied drugs to penetrate through the skin and are commonly known as penetration enhancer, accelerants, or absorption promoters.

1.1.6. Classification of transdermal patches

A) Single layer drug in adhesive type

In this type of transdermal patch the drug and excipient are inclusive with the skin adhesive which is serving as a single foundation as a breaking layer (Figure 1.3).

Figure 1.3: Single layer drug in adhesive

B) Multi-layer drug in adhesive type

In this system drug and excipients incorporated with adhesive but both layer of adhesive separated by single layer membrane. The released of drug occurred through diffusion phenomenon (Figure 1.4).

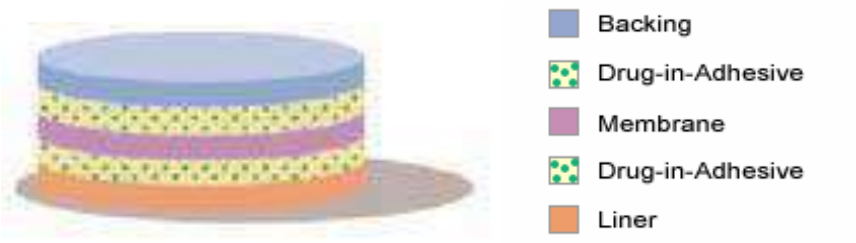


Figure 1.4: Multi-layer drug in adhesive

C) Drug matrix in adhesive

This system is designed by inclusion of semisolid matrix having drug in solution or suspension form which is in direct contact with the release liner (Figure 1.5).

Figure 1.5:Drug matrix in adhesive

D) Drug reserviour in adhesive

In the reservoir system, inclusion of liquid compartment containing drug solution/suspension between baking layer and semipermeable membrane followed by adhesive layer and release liner (Figure 1.6). (Sheth & Mistry, 2011)

Figure 1.6: Drug reservoir in adhesive

1.1.7. Approaches for developing transdermal drug delivery system

- **Membrane permeation controlled system.**

In this system, the medication repository is completely installed in a compartment shaped between a medication impermeable backing laminate and a rate-controlling polymeric layer. The drug particles are allowed to deliver across the rate-controlling layer basically by dispersion measure through the pores. In the reservoir compartments, the solid drugs are scattered homogenously in a strong polymeric framework (for example polyisobutylene) suspended in the unleachable thick fluid medium (for example silicon liquid) to shape a gel-like suspension, or to release in a dissolvable medium (for example alkyl alcohol) to shape a gel-like in the arrangement. The rate-controlling layer can be either a micro-porous or non-permeable polymeric film for example ethylene-vinyl acetate copolymer, having explicit medication penetrability. On the top surface of the polymeric film a slight layer of medication viable adhesive polymer, e.g., silicone adhesives can be applied, give personal contact of the transdermal framework with the skin surface. The delivery rate from this transdermal

framework can be custom fitted by fluctuating the polymer synthesis, the thickness of the rate-controlling membrane, adhesive, and permeability coefficient. (Shah et al., 2012**)**

- **Matrix diffusion-controlled system.**

The drug reservoirs in this approach are formulated by dispersing the drug particles homogeneously in a hydrophilic or lipophilic polymer matrix or by combining both. The resulting medicated polymer is then cast into a medicated disc with a definite surface area and controlled thickness. The drug particles in the polymer matrix can be dispersed by either homogenously mixing the finely pulverized drug particles (solid) with a liquid polymer or sometimes a highly viscid base polymer which then followed by cross-linking the polymer chains or by homogenously blending drug solids with a rubbery polymer at an elevated temperature and/or under vacuum. The polymer disc which comprises a drug reservoir is fixed onto an occlusive base plate in a compartment fabricated from a drug-impermeable backing and spread with an adhesive polymer to form a strip of the rim along with the medicated disc. Since the polymer cannot rapture, hence the absence of dose dumping is not possible and make the matrix dispersion transdermal system more beneficial. This type of transdermal system is best exemplified by the nitroglycerin releasing transdermal therapeutic system.

- **Adhesive dispersion type system.**

The drug reservoir is prepared by direct dispersing the drug in an adhesive polymer and then spreading the medicated adhesive by solvent casting method or by hot melting method onto a flat sheet containing drug-impermeable backing membrane to form a thin drug reservoir layer. A layer of rate-controlling non-medicated adhesive polymer of uniform thickness is spread on top of the medicated layer to produce an adhesive

diffusion-controlled drug delivery system which detachable release liner which in an ideal situation is removed and the patch is applied to the skin for some time. For this type of system, the transdermal therapeutic system used in angina pectoris and Valsartan (Angiotensin-II type I selective blocker) is developed and marketed.

- **Microreservoir type controlled system.**

It is a hybrid type of reservoir and matrix-dispersion type of drug delivery system; where the drug is suspended homogeneously in an aqueous system of liquid polymer to form a drug-reservoir and then suspending the dispersion homogeneously in a lipophilic polymer (e.g. Silicone elastomers) with shear mechanical force to form microscopic spheres of drug reservoirs by using high energy dispersion technique and this technology has been used in the development of Nitro-disc. The drug release pattern from a micro-reservoir type system can be either partition-controlled or matrix diffusion controlled depending on the drug relative magnitude of solubility in the liquid compartment and the polymer matrix. For this type of system, the Nitro-disc system for angina pectoris can be used.(Ghume et al., 2020)

1.1.8. Approaches in preparing transdermal patches

a) Circular Teflon mold method

Solutions containing polymers in various ratios are used in an organic solvent. Calculated amount of drug is dissolved in half the quantity of same organic solvent. Enhancer's indifferent concentrations are dissolved in the other half of the organic solvent and then added. Plasticizer (e.g., Di-N-butylphthalate) is added into the drug polymer solution. The total contents are to be stirred for 12 hrs. and then poured into a circular Teflon mold. The molds are to be placed on a leveled surface and covered with inverted funnel to control solvent

vaporization in a laminar flow hood model with an air speed of 0.5 m/s. The solvent is allowed to evaporate for 24 h. The dried films are to be stored for another 24 h at 25±0.5°C in desiccators.

b) Asymmetric TPX membrane method

A prototype patch can be fabricated for this a heat sealable polyester film (type 1009, 3m) with a concave of 1cm diameter will be used as the backing membrane. Drug sample is dispensed into the concave membrane, covered by a TPX [poly (4-methyl-1-pentene0] asymmetric membrane, and sealed by an adhesive. These are fabricated by using the dry/wet inversion process. TPX is dissolved in a mixture of solvent (cyclohexane) and non-solvent additives at 60°C to form a polymer solution. The polymer solution is kept at 40°C for 24 hrs and cast on a glass plate to a pre-determined thickness with a Gardner knife. After that the casting film is evaporated at 50°C for 30 sec, then the glass plate is to be immersed immediately in coagulation bath (maintained the temperature at 25°C). After 10 minutes of immersion, the membrane can be removed, air dried in a circulation oven at 50°C for 12 hr.

c) Mercury substrate method

The drug is dissolved in polymer solution along with plasticizer. It is followed by stirring for 10- 15 minutes to produce a homogenous dispersion and poured into a leveled mercury surface, covered with inverted funnel to control solvent evaporation.

d) "EVAC membranes" method

In order to prepare the target transdermal therapeutic system, 1% carbopol reservoir gel, polyethylene (PE), ethylene vinyl acetate copolymer (EVAC) membranes can be used as rate control membranes. If the drug is not soluble in

water, propylene glycol is used for the preparation of gel. Drug is dissolved in propylene glycol; carbopol resin will be added to the above solution and neutralized by using 5% w/w sodium hydroxide solution. The drug (in gel form) is placed on a sheet of backing layer covering the specified area. A rate controlling membrane will be placed over the gel and the edges will be sealed by heat to obtain a leak proof device.

e) Aluminum backed adhesive film method

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

f) Free film method

Free film of cellulose acetate is prepared by casting on custom made aluminum foil surface. A polymer solution (e.g., 2% w/w) is prepared using organic solvent (e.g., chloroform). The optimized concentration of plasticizer is incorporated to the polymer solution (e.g., 40% w/w of polymer weight). Small volume (e.g., 5 ml) of polymer solution is poured in a glass ring which is placed over the mercury surface in a glass petri dish. The rate of evaporation of the solvent is controlled by placing an inverted funnel over the petri dish. The film formation is noted by observing the mercury surface after complete evaporation of the solvent. The dried film is separated out and stored between the sheets of wax paper in desiccators until use. (Kesarwani et al., 2013)

1.1.9. Penetration enhancers

Permeation enhancers are the compounds which promote skin permeability (Raza et al., 2015). They are an important factor in transdermal drug delivery system which is used to improve the flux (J). Flux can be defined as the amount of material flowing through unit cross section area at time (t). (Dhamecha et al., 2009)

- **Ideal Properties of Penetration Enhancers:**

- Some of the ideal properties in a permeation enhancer are as follows:
- They must be pharmacologically inert, non-allergic, non-irritating and non-toxic.
- It must have compatibility with excipients and drugs.
- It must not have any pharmacological activity in the body.
- Cosmetically it must be acceptable.
- It must be odourless, tasteless and colourless. (Pfister & Hsieh, 1990)
- It should allow therapeutic agents into the body but should prevent the loss of endogenous material from the body i.e. they should work unidirectional.
- It must have chemical and physical stability.
- It must have reproducible and predictable duration of action.
- It must have a good solvent property. (Pathan & Setty, 2009)

Uses of Penetration Enhancers:

- It is used to increase the delivery of ionizable drugs. Example: timolol maleate etc.
- To deliver the impermeable drugs. Example: heparin etc.
- To maintain level in blood.
- To improve the efficacy of less potent drugs with higher dose. Example: oxymorphane.

- To deliver the drugs having high molecular weight like peptide and hormones.
- To decrease lag time of transdermal drug delivery system. (Bharkatiya & Nema, 2009)

Classification of penetration enhancer

- **Chemical enhancers**

Sulphoxides and similar chemicals dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), dimethyl acetamide (DMAC), Azones, Pyrrolidones, Fattyacids–Lauric acid, Myristic acid and capric acid, Oxizolidinones (4-decycloxazolidine-2-one) etc.

- **Physical Enhancers**

Iontophoresis,Sonophoresis,Phonophoresis,Magnetophoresis,Electroporation,Thermophoresi,Radiofrequency,Needless injection, hydration of stratum corneum, stripping of stratum corneum.

- **Natural Penetration enhancers**

Terpenes: Menthol, Linalool, Limonene, Carvacrol.

Essential oil: Basil oil, Neem oil, Eucalyptus, Chenopodium, Ylang-Ylang (Ita, 2015).

1.1.10. Natural penetration enhancer

Natural permeability enhancers (NPE) are comparatively new class of penetration enhancer to the pharmaceutical industry. Due to its advantages research in this field is keenly desired so that scale up is possible in the formulations suitable to be sold commercially.

Essential Oil as Natural Permeation Enhancer:

Essential oil are natural products which are extracted from aromatic plants having a concoction of a number of aromatic smelling volatile compound , primarily consisting compounds like terpenes , terpenoids, phenyl propanoids etc. (Chen et al., 2015). They can be accepted as natural alternative to synthetic skin penetration enhancer due to their promising penetration enhancing activity.

As penetration enhancer, Essential Oils helps in delivery of drug compounds into the skin by interacting with the intercellular lipids by different physical processes like increased disorder, phase separation, fluidization etc. As they are penetrated easily by the skin they are easily excreted also by the body with urine and feces. Hence, due to their better safety profile in comparison with other penetration enhancers their use is increasing.

Fate of essential oil molecules:

At the stratum corneum /viable epidermis junction essential oil molecule must partition into viable tissues before further diffusion to derma epidermal junction partitioning occurs once more at the site followed by diffusion through dermal tissues to the vascular capillaries in addition to this there are other potential fates for essential oil molecules entering the skin which include-

1) Irreversible binding to cutaneous proteins such as keratin.

2) Degradation or biotransformation by cutaneous enzymes.

3) Partitioning into and forming a reservoir in sub cuticles. (Aggarwal et al., 2013)

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE:

- Nachum Z *et.al.,* (2006) reviewed and reported that though the Scopolamine patch shows significant action towards motion sickness patients also complain about the adverse effects of the scopolamine transdermal patch where about 50-60% of the subjects report dry mouth, 20% report drowsiness, and 10% of the subjects report allergic contact dermatitis. (Nachum et al., 2006)
- Yewale C. *et al.,* (2021) that transdermal drug delivery is the most promising technology for drug application, with the enhancement of patient's compliance and reducing the adverse effects of drugs that occurred due to overdosing. Statistics predicted the market rise of the transdermal drug delivery system up to $49.37 billion by 2024. (Yewale et al., 2021)
- Hafeez A. *et al.,* (2013) carried out a study about transdermal patches and described the various generations of a transdermal patch, its advantages over other dosage forms, components of the transdermal patches, various methods of preparations were also stated and also the kinetics of transdermal permeation and its evaluation process details as a ready reference for the research scientist who is involved in TDDS. (Hafeez et al., 2013)
- Ali B. H. *et al.,* (2008) mention that why ginger is to be considered in novel formulations. Ginger is one of the oldest known spices that is used all around the world which has a wide array of benefits to treat many ailments and it is considered to be safe with very few and insignificant adverse effects which makes it a more suitable candidate for novel formulations. (Ali et al., 2008)
- Lete I *et al.,* (2016) concluded that ginger is having the best evidence to demonstrate that it is an effective and inexpensive treatment for nausea, vomiting in case of motion sickness, morning sickness, or chemotherapy-

induced, and with the given attainability of ginger preparations with known active ingredients, it would be good to perform the study to understand the efficacy of the ginger constituents (mainly gingerols and shogaols).

Ginger's constituents act peripherally within the GI tract by increasing the gastric tone, gastric emptying, and motility due to anticholinergic and anti-serotonergic actions. From this, it explains the possible mechanism of the ability of ginger to relieve symptoms of functional gastrointestinal disorders which include nausea and vomiting (Lete & Allué, 2016)

- Jin Z. *et al.,* (2014) investigated the action of ginger on serotonin (5-HT3 and 5-HT4) and cholinergic (M3) receptor activities and reported that the ginger main constituents (6-gingerol, 6-shogaol, and Zingerone) inhibit the 5-HT and M3 receptor response that followed a non-competitive, concentration-dependent manner of action which prevents the afferent inputs to the central nervous system that is stimulated by specific neurotransmitters. (Jin et al., 2014)
- Palatty P.L. *et al.,* (2013) concluded that in the preclinical studies ginger and its constituent's shows to be effective as an antiemetic agent against different emetogenic stimuli, the report also stated that the pharmacological activity of ginger is due to gingerols, paradols, and shogaols; however the clinical data shows some insufficient conclusion regarding the action against chemo-induced nausea vomiting which may be due to variations in the bioactive compounds as the studies were conducted in different countries.

  Because of its abundance, low cost, and safety in consumption, ginger remains a species with tremendous potential and countless possibilities for further investigation. Ginger has the potential to develop as a nontoxic broad-spectrum antiemetic agent when gaps existing in knowledge are bridged. The outcomes of

such studies may be useful for the applications of ginger in humans in various emesis and may open up a new therapeutic possibility. (Palatty et al., 2013)

- Zick S. M. *et al.,* (2008) found that in the i.v bolus studies performed in rats showed that free 6-gingerol is rapidly cleared from the plasma with a terminal half-life which ranges from 7.23 min to 8.5 min and also mentioned that 6-gingerol is not detectable after 30 min but gets transformed into its conjugate forms. (Zick et al., 2008)
- Ali B.H. *et al.,* (2008) reviewed that the total body clearance of 6-gingerol is 16.8 ml/min/kg, and serum protein binding of 6-gingerol was 92.4%; where the cause of elimination was studied and found that there was no significant difference in either plasma concentration-time curve or any pharmacokinetic parameters in the nephrectomised rats but there was a significant difference in the rats with hepatic damage which came into a conclusion that the renal excretion does not contribute to the disappearance of 6-gingerol from plasma in rats but the elimination of 6-gingerol is partly done by the liver which leads to the first-pass effect. (Ali et al., 2008)
- Minghetti P. *et al.,* (2007) mentioned that 6-gingerol and its derivatives are having a low molecular weight which is less than 300 Da, and with moderate solubility in water and oil and the log P (n-Octanol: water) is in the range of 2.5-3.8; while 8 and 10-gingerol derivatives are more lipophilic with the range of 3.5-4.9 with a slightly higher molecular weight more than 300 Da. (Minghetti et al., 2007)
- Govindarajan V.S. *et al.,* (2009) Ginger extract (mainly 6-gingerol) is showing to be soluble in methanol, acetonitrile, ether, chloroform, and benzene. In alcohol and benzyl benzoate it is soluble but with sedimentation, in fixed oil, it

is slightly soluble, in glycerine and in mineral oil it is insoluble. (Govindarajan & Connell, 2009)

- Shinde S.K. *et al.,* (2017) performed the isolation and standardization of ginger extract and the study was performed to extract and isolate gingerol from the ginger rhizome by using 95% ethanol by simple maceration process and the extract was then suspended in water to isolate gingerol and the characteristics studies and identification tests like TLC, HPLC, and FTIR was performed.

  The standard calibration curve of Ginger was performed by using methanol as a solvent and the maximum absorbance was shown at 281.4 nm which was taken as λ_{max} for ginger extract. (Sachin et al., 2017)
- Nair K. *et al.,* (2019) Ginger oleoresins were extracted by different methods like Hydro-distillation, solid-phase micro-extraction method, supercritical carbon dioxide method, and one of them is solvent extraction method; where acetone or ethanol was used to extract. (K. P. Nair, 2019)
- Kanadea R. *et al.,* (2016) in this study, concluded that acetone or ethanol is the better solvent, and soxhlet extraction is a better method for optimum extraction to produce a high yield of ginger oil. Soxhlet is a better method for extraction than other methods because of its high recovery, process simplicity, thermal stability, and low energy requirement. (Kanadea & Bhatkhandeb, 2016)
- Ral S. *et al.,* (2006) characterization of the ginger extract was carried out by using methanol extract of the dried ginger rhizome and the mobile phase used was n-hexane: diethyl ether with each ratio of 40:60 v/v. (Ral et al., 2006)
- Dhiman S. *et al.,* (2011) carried out a study on transdermal patches that describes the methods of preparation of different types of transdermal patches such as matrix patches, reservoir type, membrane matrix, drug-in-adhesive

patches, and micro reservoir patches. In addition, the various methods of evaluation of transdermal patches and methods to increase its transdermal permeation are also stated. (Dhiman et al., 2011)

- Chinchole P. *et al.,* (2016) studied transdermal drug delivery and described the various aspects such as skin and drug permeation, the kinetics of transdermal permeation various stages in transdermal drug delivery, components, approaches used in the development of transdermal drugs. In addition to this various methods of evaluation of transdermal patches are also stated. (Chinchole et al., 2016)
- Eisha G. *et al.,* (2016) prepared acetohexamide transdermal patches by solvent casting technique using the HPMC and PVP which were evaluated for various *in- vitro* parameters (Thickness, Folding endurance, Moisture content, Moisture uptake, Flatness, Water vapour transmission rate, etc.) and In- vitro permeation studies were also performed by using Franz diffusion cells. Variations in drug permeation profiles were observed among all the eight formulations prepared. (Eisha & Kuldeep, 2016)
- Nair R. S. *et al.,* (2013) performed a study by formulating a matrix type of transdermal patch of captopril by using different proportions of polymers such as Hydroxypropyl methylcellulose (HPMC), polyethylene glycol (PEG) 400, propylene glycol, and penetration enhancers such as menthol and aloe vera at different concentrations were used. In this study, the formulation with menthol as a penetration enhancer showed maximum drug permeation. (R. S. Nair et al., 2013)
- Lakshmi P.K. *et al***.,** (2017) studied essential oils as natural penetration enhancers and reported the role of Natural oils in enhancing the permeation of

drugs through the skin because of possessing different features like natural origin, favorable penetration enhancement, and partitioning action in the skin by the oils. Various natural oils, their classification, and influence as penetration enhancers were discussed in this study for example thyme oil, lemongrass oil, orange peel oil, etc. (P K et al., 2017)

- Karpanen T.J *et al.,* (2010) investigated the use of eucalyptus oil as a penetration enhancer and which was subjected to permeation studies on full-thickness human skin and it was found that the oil enhanced the permeation of chlorhexidine (2%(w/v)) into the dermis and the lower layer of the epidermis when it was combined with 70%(w/v) isopropyl alcohol and 10%(v/v) eucalyptus oil on comparison to the solution of chlorhexidine/isopropyl alcohol alone. (Karpanen et al., 2010)
- Akram M. *et al.,* (2018) prepared and evaluated the transdermal patches with optimized polymer concentration and five different enhancers namely Isopropyl-myristate (IPM), Span 80, Tween 20, eucalyptus oil, and limonene were used. The result of the in vitro permeation studies showed that these enhancers increase the drug release and ranked them in order of IPM> eucalyptus oil> Span® 80> Tween® 20> limonene. (Akram et al., 2018)
- Xie F. *et al.,* (2016) performed a study to understand the effects and possible enhancement mechanism of the enhancer by using ATR-FTIR. The skins were scanned before and after treatment of the penetration enhancers. There are different ranges of asymmetric and symmetric vibrations which determine the lipids and keratins which are present in the stratum corneum of the skin, and these absorption peaks are usually used as key parameters to investigate the

structural alterations of stratum corneum lipids and keratin induced by penetration enhancers. (Xie et al., 2016)

- Gupta V. *et al.*, (2015) mention the use of the goatskin model. The permeation of Cisplatin from novel topical formulations through the excised pig, goat, and mice skin was performed and reported that there was no significant difference among the pig and goat skin model for the permeation profile of Cisplatin, but there was a statistically significant difference between mice and pig, and mice and goatskin model for the permeation profile of Cisplatin. The conclusion was drawn that a positive correlation between the steady-state flux values of pig and goat skins was observed, however, mice skin showed significantly different permeation and accumulation profiles. It is also stated that pig and goat skin models are having similarities in structure and anatomical; and also the skin of pig and goatskin is composed of epidermis and dermis with characteristics like those of human skin. However, the skin of laboratory animals, for example, mice, rats, guinea pigs, rabbits, etc., shows marked anatomic differences from human skin. (Gupta & Trivedi, 2015).

CHAPTER 3

PLANT & EXCIPIENTS PROFILE

3. PLANT AND EXCIPIENTS PROFILE:

3.1. Ginger

Ginger or *Zingiber officinale*, Roscoe of the Zingiberaceae family is a rhizome that is possibly native to India and widely found, grown, and most consumed commercial crop in South, Southeast Asian countries, tropical Africa, Latin America, the Caribbean, and Australia.

Figure 3.1: Ginger (*Zingiber officinale*)

Vernacular names of *Zingiber officinale*:

English- Ginger

Hindi- Adrak

Assamese- Aada

Khasi- Sying bah

Taxonomical classification of *Zingiber officinale*:

Kingdom- Plantae

Order- Zingiberales

Family- Zingiberaceae

Genus- *Zingiber*

Species- *Z. officinale*

Underground parts: Branched and distinctive thickened rhizome (somewhat like a swollen hand). The rhizome has a brown corky outer layer and a pale yellow center with a spicy lemon-like scent.

Leaves: Shoots (pseudo-stems), up to 1.2 m tall, arise annually from buds on the rhizome. These pseudo-stems are formed from a series of leaf bases wrapped tightly around one another with the long (up to 7 cm), narrow (up to 1.9 cm wide), mid-green leaf blades arranged alternately.

Flowers: The flowering heads, borne on separate shorter stems, are cone-shaped spikes and composed of a series of greenish to yellow leaf-like bracts. Protruding just beyond the outer edge of the bracts, the flowers are pale yellow with a purplish lip that has yellowish dots and striations. Flowering stems are rarely if ever, producing in cultivated plants.

Cultivation: Ginger probably originated as part of the ground flora of tropical lowland forests, where many of its wild relatives can still be found. In cultivation, it requires hot, humid, shady conditions, and grows best in a fertile loam as it needs large quantities of nutrients.

Chemical constituents: Ginger being the most used spice worldwide is found to contain more than 400 different compounds which include sugar, protein, and fats. The major phenolic active constituents of ginger are 6-gingerol, 6-shogaol, and 6-paradol where gingerols and shogaols are accountable for the pungent taste and are bioactive compounds of ginger rhizomes. In addition, gingerols are thermolabile and get

converted to shogaols due to dehydration and these shogaols are more pungent than gingerols. Apart from the pungent compounds, ginger rhizome comprises compounds that are volatile and oily; detected by gas-chromatography/ mass spectroscopy (GC/MS). Three major classes of compounds found in the essential oils are sesquiterpenoids, monoterpenoids, and aldehydes and from these, the following constituents are identified, namely α-curcumene, α-farnesene, β-bisabolene, β-sequiphellandrene, and zingiberene. About 30% of Zingiberene contributes to the essential oil found in the ginger rhizome. Polysaccharides, organic acids, fibers, and lipids are also found to contain in the rhizome. Research in recent years suggested that the compounds present in ginger rhizome are very potent and useful in the cure and management of different diseases including cancer, tumors, allergic reaction, cough, and inflammation.

Different chemical constituents of ginger rhizome (*Zingiber officinale*) are described in Figure 3.2. with different chemical structures of

a) Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone)
b) Gingerol (5-hydroxydecan-3-one)
c) β-phellandrene ((5*S*)-2-methyl-5-propan-2-ylcyclohexa-1,3-diene)
d) Zingiberene ((5R)-2-methyl-5-[(2S)-6-methylhept-5-en-2-yl] -cyclohexa-1,3-diene)
e) β-sesquiphellandrene (3-(6-methylhept-5-en-2-yl)-6-methylidenecyclohexene)
f) 1,4-cineol (1-methyl-4-propan-2-yl-7-oxabicyclo[2.2.1]heptane)
g) Shogaol ((*E*)-1-(4-hydroxy-3-methoxyphenyl)dec-4-en-3-one)
h) Farnesene ((3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene)
i) Limonene (1-methyl-4-prop-1-en-2-ylcyclohexene)

j) Geraniol ((2*E*)-3,7-dimethylocta-2,6-dien-1-ol)

k) Citral ((2*E*)-3,7-dimethylocta-2,6-dienal)

l) Camphene (2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane)

m) α-terpineol (2-[(1*S*)-4-methylcyclohex-3-en-1-yl]propan-2-ol)

n) 6-paradol (1-(4-hydroxy-3-methoxyphenyl)decan-3-one)

o) Curcumene (1-methyl-4-(6-methylhept-5-en-2-yl)benzene)

p) Borneol (1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol)

q) β-elemene ((1*S*,2*S*,4*R*)-1-ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl)cyclohexane)

r) Zingiberenol (1-methyl-4-(6-methylhept-5-en-2-yl)cyclohex-2-en-1-ol)

s) Linalool (3,7-dimethylocta-1,6-dien-3-ol).

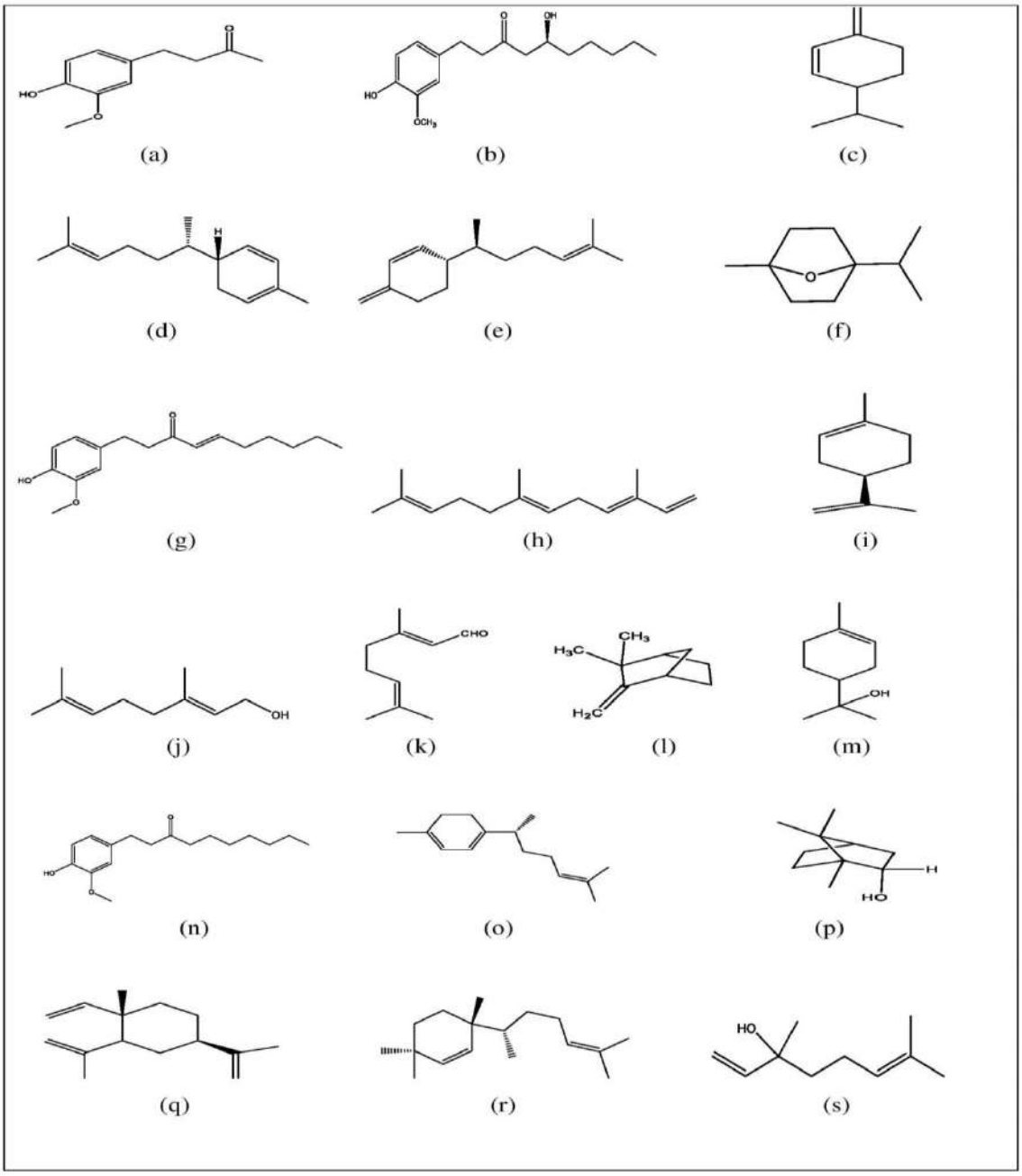


Figure 3.2: Different chemical structures of chemical constituents found in ginger (*Zingiber officinale*). (a) Zingerone, (b) Gingerol, (c) β-phellandrene, (d) Zingiberene, (e) β-sesquiphellandrene, (f) 1,4-cineol, (g) Shogaol, (h) Farnesene, (i) Limonene, (j) Geraniol, (k) Citral, (l) Camphene, (m) α-terpineol, (n) 6-paradol, (o) Curcumene, (p) Borneol, (q) β-elemene, (r) Zingiberenol, (s) Linalool.

Uses: It has been used in cooking as a spice for a very long time and also as a traditional remedy to treat various ailments. Such as nausea, vomiting, pain, arthritis, indigestion, gastro reflux, etc. In addition to these, several uses of ginger are useful in treating, managing, or preventing diseases like cardiovascular disease, diabetes, respiratory disease, obesity, nausea, and vomiting induced by chemotherapy and in neurodegenerative diseases; it also in-holds some of the biological properties like antimicrobial, anti-inflammation, antioxidant, anticancer, and wound healing properties. Apart from these many therapeutic activities, ginger may also be considered to be the plant that can treat or help the patient with SARS-CoV-2 and also as an adjuvant in SARS-CoV-2 management (Rynjah et al., 2021).

Concerning its antiemetic properties, ginger and its constituents act peripherally, within the gastrointestinal tract, by increasing the gastric tone and motility due to anti-cholinergic and anti-serotonergic actions. It is also reported to increase gastric emptying. This combination of functions explains the widely accepted ability of ginger to relieve symptoms of functional gastrointestinal disorders, such as dyspepsia, abdominal pain, and nausea, which are often associated with decreased gastric motility. Although the exact mode of action of ginger concerning its antiemetic properties is still being unraveled, three recent studies have investigated the action of ginger on serotonin (5-hydroxytryptamine, 5-HT3, and 5-HT4) and cholinergic (M3) receptor activities. Working on the evidence that emetogenic chemotherapeutic drugs increase 5-HT concentration and activate visceral vagal afferent nerve activity, Jin et al used patch-clamp methods to study the effects of ginger and its pungent constituents on 5-HT-evoked inward currents in rat no-dose ganglia neurons. Results showed that 6-shogaol, 6-gingerol, and Zingerone could inhibit the 5-HT response in a concentration-dependent manner, with 6-shogaol exhibiting the greatest potency (Lete & Allué, 2016).

3.2. Menthol

IUPAC name: 5-Methyl-2-(propan-2-yl) cyclohexan-1-ol.

Molecular formula: $C_{10}H_{20}O$

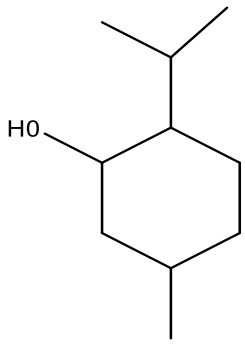


Figure 3.3: Chemical structure of Menthol.

Molecular mass: 156.269 g/mol

Melting point: 36-38 °C

Boiling point: 214.6 °C

Flashpoint: 196°F

Log P: 3.4

Solubility: Slightly soluble in water.

Appearance: White crystal, Peppermint/cooling odor, Peppermint/cooling taste

Stability/Shelf life: Stable under recommended storage conditions

Decomposition: When heated to decomposition it emits acrid smoke and irritating fumes

Uses: Menthol is having multiple applications in various ways like an Antipruritic, topical analgesic, penetration enhancer in transdermal drug delivery, as a decongestant for sinuses and chest, in oral hygiene (mouthwash, oral sprays, toothpaste, chewing gum, and candy), in perfumery, as an antispasmodic, and relaxant of smooth muscle in upper gastrointestinal endoscopy (Harris, 2006). Various extracts from peppermint contain menthol as a major active constituent and have been used for centuries as

traditional medicines for several ailments including infections, insomnia, and irritable bowel syndrome as well as an insect repellent.

Side effects: Menthol can produce sharp, irritating, and disagreeable perceptions (Graf, 1983). It can also cause glucose-6-phosphate dehydrogenase deficiency in newborn babies which may be developed into severe jaundice after menthol administration due to the inability of the neonates to conjugate menthol and can cause isolated cases of spasm of the larynx with a few cases of the nervous or digestive system disturbance. Menthol may cause allergic reactions and applying a menthol-containing ointment to the nostrils of infants may cause instant collapse (Leung, 2010).

3.3. Eucalyptus oil

IUPAC name: 1,3,3-trimethyl-2-oxabicyclo[2.2.2] octane

Molecular formula: $C_{10}H_{18}O$

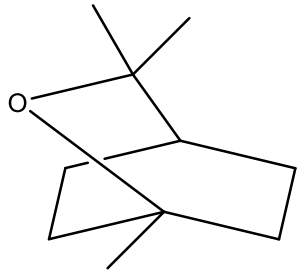


Figure 3.4: Chemical structure of Eucalyptus oil.

Molecular weight: 154.25

Melting point: 34.7 °C

Boiling point: 176.4 °C

Log P: 2.74

Solubility: Miscible with ether, alcohol, chloroform, glacial acetic acid, and fixed or volatile oils. Soluble in alcohols, most fixed oils, glycerin, and propylene glycol.

Appearance: Colourless liquid or oil, Camphor-like odor, bitter-sweet flavor

Stability/Shelf life: Stability is good.

Uses: Eucalyptus oil is having many therapeutic properties like analgesic, antibacterial, antifungal, anti-inflammatory, antioxidant, anti-rheumatic, and many others. Eucalyptus oil can be used as a topical application, ingestion, and aromatherapy. In cosmetics, it is used as a denaturant, masking agent, and as tonic. In Pharmaceutical it is used as a flavor, cough drops, perfumery, and reduce plaque-related gingivitis. Apart from therapeutic use, eucalyptus oil is used in household and commercial or industrial products (Dhakad et al., 2018). It has been reported that eucalyptus oil is also used as a penetration enhancer for the transdermal delivery of drugs. A recent review has reported that eucalyptus essential oil can be used as an off-label to protect from COVID-19 by reducing the patient's symptoms and morbidity risk factors (Abbass, 2020).

Side effects: Cineol being the main component in eucalyptus oil is responsible for its therapeutic actions as an expectorant and an antiseptic. It is reported that eucalyptus oil was considered not to be an ocular corrosive or severe irritant. Irritation may be shown but it is safe when used in minute amounts to the skin.

3.4. Hydroxypropyl methylcellulose

Chemical formula: $C_{56}H_{108}O_{30}$

IUPAC name: (2*R*,3*R*,4*S*,5*R*,6*R*)-2,3,4-trimethoxy-6-(methoxymethyl)-5-[(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trimethoxy-6-(methoxymethyl)oxan-2-yl]oxyoxane;1-[[(2*R*,3*R*,4*S*,5*R*,6*S*)-3,4,5-tris(2-hydroxypropoxy)-6-[(2*R*,3*R*,4*S*,5*R*,6*R*)-4,5,6-tris(2-

hydroxypropoxy)-2-(2-hydroxypropoxymethyl)oxan-3-yl]oxyoxan-2-yl]methoxy]propan-2-ol.

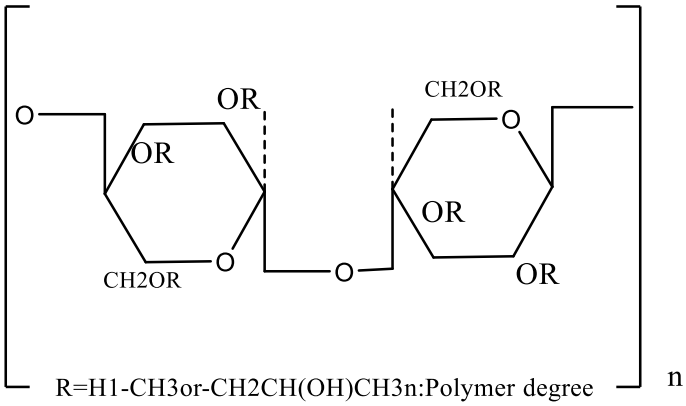


Figure 3.5: Chemical structure of HPMC.

Molecular weight: 1261.45 g/mol

Synonym: Hypromellose

Density: 0.50 — 0.70 g/cm3

Melting point: Browns at 190-200°C; chars at 225-230°C

Appearance: odorless and tasteless, white or creamy-white colored fibrous or granular powder.

Chemistry: Hypromellose has the polymeric backbone of cellulose, a natural carbohydrate that contains a basic repeating structure of anhydroglucose units. During the manufacture of cellulose ethers, cellulose fibers are heated with a caustic solution which in turn is treated with propylene oxide, yielding hydroxy-propyl substitution on the anhydroglucose units of methyl ether of cellulose. The fibrous reaction product is purified and ground to a fine, uniform powder. Hypromellose possesses varying ratios of Hydroxypropyl and Methyl substitution which in turn determine the organic solubility as well as thermal gelation temperature of the aqueous solution.

Solubility: Soluble in cold water, forming a viscous solution; practically insoluble in hot water, chloroform, ethanol (95%), and ether but soluble in mixtures of ethanol and

dichloromethane, the mixture of water and alcohol, mixture of methanol and dichloromethane.

Uses: Hypromellose is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations. Hypromellose is also used in liquid oral dosage forms as a suspending and/or thickening agent at concentrations ranging from 0.25–5.0%. Hypromellose is also used as a suspending and thickening agent in topical formulations. Compared with methylcellulose, hypromellose produces aqueous solutions of greater clarity, with fewer un-dissolved fibers present, and is therefore preferred in formulations for ophthalmic use. Hypromellose at concentrations between 0.45–1.0% w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solutions. Hypromellose is used as an emulsifier, suspending agent, and stabilizing agent in topical gels and ointments. As a protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. Recent study reported that hypromellose along with plasticizers and anesthetic drugs were formulated into a buccal film which will act as a needleless anesthetics administration, and its effect was studied. These results are a step forward in the rational development of formulations for the replacement of needles in dentistry (Ferreira, L. et al., 2021).

3.5. Polyethylene glycol 400

Chemical formula: $C_2H_6O_6$

IUPAC name: α-Hydro-ω-hydroxypoly(oxy-1,2-ethanedyl)

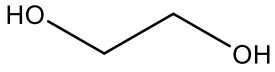


Figure 3.6: Chemical structure of Polyethylene glycol 400.

Molecular weight: 62.07

Density: 1.1135 g/cu at 20 °C

Melting point: 4-8 °C

Boiling point: 197 °C

Log P: -1.36

Appearance: Clear, colorless syrupy liquid, sweet in taste, and odorless.

Solubility: Miscible with water, very soluble in acetone, in alcohol, and methylene chloride, practically insoluble in fatty oils and mineral oils.

Decomposition: When heated to decomposition it emits acrid smoke and irritating fumes.

Uses: Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations, including parenteral, topical, ophthalmic, oral, and rectal preparations. Polyethylene glycol has been used experimentally in biodegradable polymeric matrices used in controlled-release systems. Polyethylene glycols are stable, hydrophilic substances that are essentially nonirritant to the skin and they do not readily penetrate the skin, although the polyethylene glycols are water-soluble and are easily removed from the skin by washing, making them useful as ointment bases (Hunyadvari-Ugri et al., 1989).

3.6. Propylene glycol:

Chemical formula: $C_3H_8O_2$

IUPAC name: 1,2-Propanediol

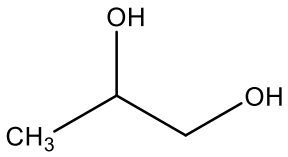


Figure 3.7: Chemical structure of Propylene glycol

Molecular weight: 76.09

Density: 1.0361 g/cu cm at 20 °C

Melting point: -60 °C

Boiling point: 187.6 °C

Log P: -0.92

Appearance: Thick odorless, colorless liquid.

Solubility: Soluble in water, ethanol, acetone, and benzene. It is miscible with acetone and chloroform.

Decomposition: When heated to decomposition it emits acrid smoke and irritating fumes.

Uses: Propylene glycol has become widely used as a solvent, extractant, and preservative in a variety of parenteral and non-parenteral pharmaceutical formulations. It is a better general solvent than glycerin and dissolves a wide variety of materials, such as corticosteroids, phenols, sulfa drugs, barbiturates, vitamins (A and D), most alkaloids, and many local anesthetics. Propylene glycol is commonly used as a plasticizer in aqueous film-coating formulations (Madan & Madan, 2012).

CHAPTER 4

AIM & OBJECTIVES

4. Aim and Objectives

4.1. Aim:

The aim of the present study is to formulate and evaluate the matrix transdermal patch of ginger extract using natural penetration enhancers by *in vitro* and *ex-vivo* studies.

4.2. Objectives:

- Collection and authentication of Ginger rhizome.
- Extraction of the dried ginger rhizomes (*Zingiber officinale)* by simple maceration using Ethanol as a solvent.
- Identification and characterisation of the plant extract for the presence of ginger constituents (Gingerol & Shogaol) and determine its physico-chemicals properties of Ginger extract.
- Preformulation studies of ginger extract with the excipients.
- Formulation and evaluation of the physico-chemical properties of the transdermal patch using different natural penetration enhancers.
- *In-vitro* permeation test of drug through cellulose dialysis membrane using a Franz-diffusion cell.
- *Ex-vivo* permeation test of drug through excised-goat skin using a Franz-diffusion cell.
- To perform a skin irritation study on rabbit skin.
- To determine the permeation mechanistic pathway.

4.3. Rationale of the study:

The transdermal drug delivery is a promising route of drug delivery system because of its potential advantages over conventional system like bypassing the hepatic first pass metabolism, gastrointestinal effects and improved patience compliance.

From the literature it was found that in the i.v bolus studies performed in rats showed that free 6-gingerol is rapidly cleared from the plasma with a terminal half-life which ranges from 7.23 min to 8.5 min and also mentioned that 6-gingerol is not detectable after 30 min but gets transformed into its conjugate forms and it has been seen that ginger and its components are suitable for formulating into a transdermal system to bypass the first pass metabolism. But the main hindrance to its use is the outermost layer of the skin the stratum corneum which acts as a protective barrier and provides the greatest resistance to skin penetration becoming the major rate limiting step of percutaneous absorption.

In order to overcome this limitation the use of penetration enhancer emerged which can be of physical type, chemical type and natural type. Focusing on the natural penetration enhancer, a number of literature review supported that naturally occurring essential oil can be employed as permeation enhancer owing to their advantages property like less toxicity, less allergic reaction and easy availability as they are found in abundance in nature. A number of researches has also been reported on the oils like peppermint oil, eucalyptus oil, orange oil, niaouli oil, menthol, etc. in order to investigate there penetration enhancing property in transdermal drug delivery.

In this quest of studying the potency of natural permeation enhancers, in the present work eucalyptus oil and menthol were used and the release studies were carried-out in cellulose dialysis membrane and excised goat skin to see the permeation release of the ginger extract through the membranes by considering the permeation parameters.

CHAPTER 5

MATERIALS & METHODOLOGY

5. MATERIALS AND METHODOLOGY:

5.1. MATERIALS

- **Instruments used in the study**

Table 5.1: List of instruments used in the study.

INSTRUMENTS	COMPANY NAME
Digital weighing balance	Denver instruments
UV-visible spectrophotometer	Shimadzu UV 1800
Fourier Transform Infrared Spectroscopy	Bruker FT-IR spectrophotometer (model 220, Germany)
Magnetic stirrer with hot plate	Rolex, India.
pH meter	Indosati Scientific Lab Equipments
Modified Franz diffusion cell	Local made
Soxhlet apparatus	Local made
Heating mantle	Indosati 1000 ml
Water bath	Rectangular Water bath
Refrigerator	Gofrej
Ultrasonic bath	PCi^{TM} Analytics
Digital screw gauge	Aero Space (0-20 mm, 0.001 mm)

- **Animals use in the study**

Adult male albino rabbits (1.5-2 kg) were supplied by Animal House of Girijananda Chowdhury Institute of Pharmaceutical Science which was inhabited under standard laboratory condition with proper diet. The animals were received after the proposed study was approved by the Institute Animals Ethics Committee (IAEC), and Committee for purpose of control and supervision of experiments on animals (CPCSEA), Government of India bearing the number GIPS/IAEC/M.PH/PRO/05/2021. The goat ears were purchased from a slaughter house, clean, prepared and stored till further study.

5.2. METHODOLOGY

- **Plant material**

The plant (Zingiber officinale) is selected after a detailed literature survey based on medicinally important plants.

- **Collection of plant:** The ginger rhizome was purchased from a local farmer of Shillong, Meghalaya and it was authenticated by Botanical Survey of India, Shillong bearing authentication number BSI/ERC/Tech/2021/117.
- **Drying of Plant parts (shade drying):** Ginger was cut into small pieces, spread and dried it by shade drying at a temperature of 20-40 °C. The dried pieces of rhizome were then weighed and stored in tightly closed container in a desiccator till further use.
- **Grinding of the dried materials and sieving through sieve no. 60:** The dried ginger was then ground into coarse particle with the use of a mechanical grinder then passed through a sieve no. 60 which yields a good particle size.

- **Extraction:** The dried powdered ginger was extracted with 95% ethanol by simple maceration process and allowed to stand overnight. The mixture was then filtered and the solvent was evaporated by distillation (maintaining at a temperature below 40 °C) to obtain thick pasty mass known as oleo-resin. This ginger oleo-resin was then stored in a refrigerator at a cool temperature.

➢ **Determination of absorption maxima of ginger extract**

100 µg/ml standard solution of ginger extract was prepared by dissolving the ginger extract in small amount of methanol and then diluted with phosphate buffer (pH 7.4) and the solution was scanned at the range from 200-400 nm using UV-visible spectrophotometer.

➢ **Standard curve calibration of Ginger extract**

1mg/ml stock solution of ginger extract was prepared in phosphate buffer (pH 7.4), 1ml from the stock solution was pipetted out in 100 ml of phosphate buffer (pH 7.4) and in the series of 10 ml volumetric flasks different serial dilutions were done to get the final concentration of (20, 40, 60, 80, 100) µg/ml. The solutions were analyzed at the resultant absorption maxima by using UV-visible spectrophotometer.

5.2.1. Physicochemical characterization of ginger extract

- **Organoleptic properties**

  The organoleptic properties of ginger extract such as colour, odour, taste, pH, and physical state were examined visually.

- **Solubility**

  The solubility of the extract was determined by shake flask method. Excess amount of the ginger extract was taken in three different 10 ml

tubes containing various solvents like water, methanol, and phosphate buffer of pH 7.4. They were then continuously shaken for couple of hours and allowed to stand overnight. The samples were then centrifuged and the supernatant was collected, followed by dilution and then run for UV-visible spectrophotometric analysis of the sample. (Glomme et al., 2005)

- **Partition coefficient**

  The partition coefficient of ginger extract was determined by shake flask method. In 10 ml of water, 10 mg of ginger extract was mixed and to it 10 ml of n-Octanol was added and shake to mix for maximum time in a separating funnel and it was then allowed to stand for 24 hrs. The water part was separated and from there 0.5 ml of water-drug mixture was withdrawn and followed by dilution and analyzed by UV-visible spectrophotometer. (Schönsee & Bucheli, 2020)

- **TLC Method**

  1 g of the coarsely powdered ginger rhizome was refluxed under with 25 ml of methanol for 15 minutes, then cooled and filtered. The residue was washed with 10 ml of methanol. All of the filtrates were combined and concentrated to 10 ml. The sample was loaded in the plate and then kept in a chamber containing mobile phase of Hexane: Diethyl ether (40:60) and allow to run upto 80 % of the plate and was then taken out and allowed to dry. The plate was then sprayed with Vanillin sulphuric acid reagent (1% Ethanolic vanillin and 10% Ethanolic sulphuric acid) which is used for detection of higher alcohols, phenols, steroids, essential oils,

and esters and followed by heating for 10 min at 100 °C in the hot air oven (Wagner et al., 2001).

- **FT-IR study**

  Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. A small quantity of the extract was spread on the sample platform and the extract was scanned from 4000 - 400 cm^{-1}. The_FTIR spectra were also taken to check the extract-excipients compatibility of ginger extract, blank film (containing 50% HPMC and 50% PEG 400), and films loaded with extract and penetration enhancers.

5.2.2. Patch preparation:

Matrix type transdermal patches of ginger extract were prepared by solvent casting method. The polymeric solutions were prepared by dissolving the polymers (HPMC-K100 and PEG 400) in ethanol. A weighted amount of ginger extract was dissolved in the polymeric solution; then 10% w/w propylene glycol (plasticizer) was added to the solution and followed by natural penetration enhancer. The solutions were stirred thoroughly with a mechanical stirrer to obtain a homogeneous mixture. The contents were then poured into a petri dish and allowed to air-dry at room temperature. After ensuring the complete evaporation of the solvent, patches of desired dimensions were cut and the dried patches were packed in aluminum foil and stored in desiccators containing silica gel till further studies. The formulated ginger extract patches were evaluated within one week of preparation (Nair et al., 2013). The compositions of the patches were prepared as shown in Table 5.2.

Table 5.2: Formulation compositions of Ginger extract transdermal patches.

Formulation code	Drug (%) Ginger extract	Polymer (%)		Plasticizer (%)	Penetration enhancer (%)	
		HPMC K100	PEG 400	Propylene glycol	Eucalyptus oil	Menthol
EF1	20	100	-	10	2	-
MF1	20	100	-	10	-	2
EF2	20	75	25	10	2	-
MF2	20	75	25	10	-	2
EF3	20	50	50	10	2	-
MF3	20	50	50	10	-	2
EF4	20	25	75	10	2	-
MF4	20	25	75	10	-	2

5.2.3. **Physico-chemical characterization of formulated patches:**

The formulated ginger extract patches were evaluated for their physical appearance, average thickness, weight variation, drug content uniformity, moisture content, and folding endurance.

- Physical appearance: - All the patches were visually inspected for colour, flexibility, homogeneity, and smoothness.
- Film thickness: - The thicknesses of the prepared patches were measured at three different places using a digital screw gauge. The mean values and standard deviations were calculated.

- Weight variation test: - 1 cm^2 of prepared patches were cut and weighed in a digital balance and the average weights and standard deviation of each patch were calculated.
- Drug content uniformity: - For estimating the drug content, 1 cm^2 area of the patch was cut and was put into a 100 ml phosphate buffer (pH 7.4) and shake for 24 hrs. The solution is then subjected to ultra-sonication for 15 min and after which it is filtered and the drug content is analyzed by UV spectrophotometer at 281.2 nm. The drug content of each patch was estimated from the standard graph.
- Folding endurance: - A small strip of the prepared patch of about 2 cm x 2 cm was subjected to this test by folding the patch at the same place repeatedly for several times until a visible crack was observed.
- Moisture content: - The percentage of moisture content was measured by keeping the patches in a desiccator till the patch weight reaches to a constant. The initial weight and final weight of the patches were taken. Percentage moisture content was calculated using the formula:

$$\%Moisture\ content = \frac{(Initial\ weight - Final\ weight)}{Initial\ weight} \times 100\%$$

- Tack properties: - A transdermal patch with the best permeation release was selected, cut to a required size, and adhered on to the sample holder. Prior to the running of the test samples, the instrument was calibrated and experimental parameters, such as target force (5.0 g), approach speed (0.5 mm/s), return speed (5.0 mm/s), return distance (10.0 mm), and hold time (1 s) were set. Parameters, such as absolute +Force, positive area (FT), and separation distance was recorded (Banga et al., 2019).

5.2.4. *In vitro* and *ex-vivo* permeation study:

- *In vitro* release study: - The in vitro study was carried out using modified Franz diffusion cell. The dialysis membrane with a molecular weight of 12000 - 14000 Da and was placed between compartments of the diffusion cell. The dialysis membrane had been soaked for 24hrs in 40% v/v PEG 400 in phosphate buffer of pH 7.4 prior to the study. The donor compartment was open at the top and exposed to the atmosphere. The receptor compartments contained 30 ml of phosphate buffer of pH 7.4 and the contents were stirred at a speed of 400 rpm. The whole assembly was kept on a magnetic stirrer and the study was conducted at a temperature of 37±0.5 °C. The samples of 1ml were collected at preset time points up to 24 hrs. and replenished with a fresh phosphate buffer of pH 7.4 to maintain the sink condition and the drug content in the samples was estimated using UV/visible spectrophotometer at 281.2 nm. The cumulative release per cm^2 of the released drug was calculated and plotted against time. The results of the patch containing ginger extract were compared with the blank patch (without penetration enhancers).
- Preparation of goat skin: - The goat skin hair was removed, then the epidermal layer was surgically removed and the adhering fat was removed by wiping it with isopropyl alcohol, it was then soaked in phosphate buffer of pH 7.4, and then wrapped in aluminum foil and store in the deep freezer till further studies. The prepared skin was used within one week.
- *Ex vivo* permeation study: - Excised goat skin was mounted between the compartments of the diffusion cell with the stratum corneum facing the

donor compartment. The stratum corneum side of the skin was kept in intimate contact with the transdermal patch under the test. The receiver compartment contained 30ml of phosphate buffer of pH 7.4, and stirred with a magnetic stirrer at a speed of 400 rpm. The whole assembly was kept on a magnetic stirrer and the study was conducted at 37±0.5 °C. The amount of the permeated drug was determined by removing 1 ml at preset time points up to 24 hrs. and replenished with an equal volume of fresh medium and the absorbance were measured at 281.2 nm spectrophotometrically. The cumulative release of drug permeated per cm^2 was calculated and plotted against time. The results of the patch containing ginger extract were compared with the blank patch (without penetration enhancers).

5.2.5. **Skin irritation study:**

The skin irritation study was carried out by using healthy rabbits. The evaluation was based on a scoring method described by Draize et al, where the scores are assigned from 0 to 4 based on the severity of erythema or edema.

5.2.6. Permeation enhancement mechanistic study:

- **FT-IR**

  The rat epidermis treated with the transdermal patch containing patchouli oil as a permeation enhancer (which serves as the test) and untreated rat epidermis (which serves as the control) were subjected to FT-IR studies and was compared to analyse the mechanistic study.

5.2.7. Data analysis:

For estimating the *in-vitro* release parameters the flux of indomethacin across rat epidermis was calculated from the slope of cumulative release curve per cm^2 of the patch cross the skin versus time using linear regression analysis. The lag time (T_{lag}) was calculated from the X-intercept from the linear portion of the plot, the cumulative amount of drug permeated Q_{24} (mg/hr) was the amount of drug penetrating through the skin after 24 hrs. The enhancer ratio was also estimated by using the equation: $E_{SS}=J_{SS}$ (enhancer treated) / J_{ss} (control). One-way ANOVA was carried out for the cumulative amount of drug permeated/cm^2 and reported as given in the report. All the data hence found was expressed as a mean ± standard deviation (SD) (Patel et al., 2011).

CHAPTER 6

RESULTS & DISCUSSIONS

6. RESULTS AND DISCUSSIONS

6.1. Determination of absorption maxima of Ginger extract:

After diluting the ginger extract solution to an appropriate concentration, the absorption maxima of ginger extract was scanned in the range 200-400 nm and the λ_{max} was found to be 281.2 nm (Figure 6.1)

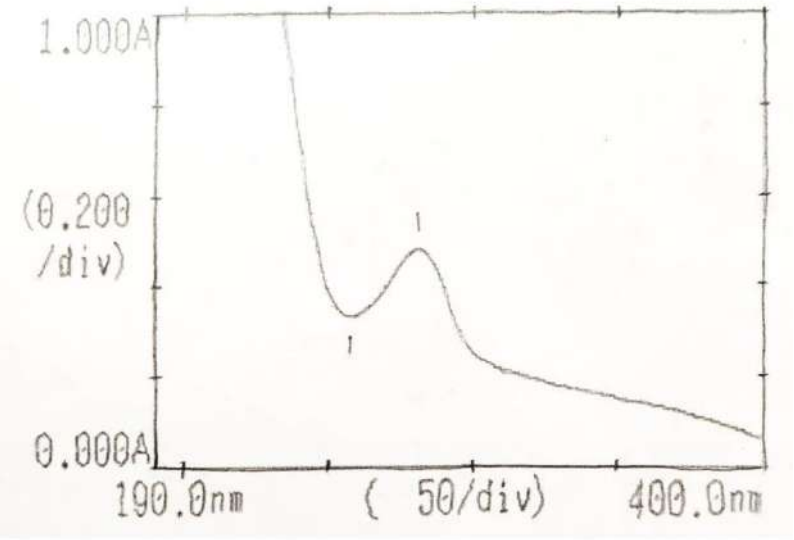


Figure 6.1: Absorption maxima of Ginger extract.

6.2. Standard curve calibration of Ginger extract:

After the serial dilutions of Ginger extract with the concentration from (20, 40, 60, 80, 100) µg/ml and after scanning the solutions in UV-visible spectrophotometer, the calibration curve was found to show linearity with the equation, y=0.0048x+0.0062 and the regression coefficient was found to be 0.9956 (Table 6.1 and Figure 6.2).

Table 6.1: Standard calibration curve absorbance of Ginger extract by UV-visible spectrophotometer.

Sl. No.	Concentration (µg/ml)	Absorbance (λ_{max}=281.2)
1	0	0
2	20	0.0963
3	40	0.2201
4	60	0.2939
5	80	0.3777
6	100	0.4877

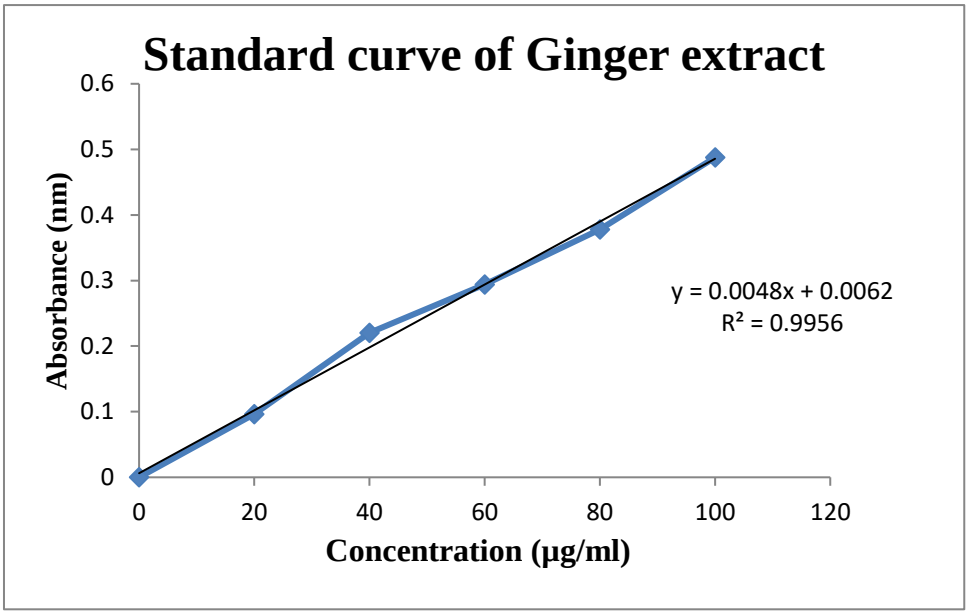


Figure 6.2: Standard calibration curve of Ginger extract.

6.3. Physicochemical characterization of Ginger extract:

- **Organoleptic properties**

The results of organoleptic properties of Ginger extract such as colour, odour, taste, pH, and physical state are presented in the Table 6.2.

Table 6.2: Organoleptic properties of Ginger extract.

Parameters	Results
Colour	Reddish-brown
Odour	Aromatic and pleasant
Taste	Characteristic pungent, and hot
pH	4.5
Physical state	Thick oily-pasty mass (oleo-resin)

- **Solubility**

Ginger extract (mainly gingerols) are highly lipophilic in nature as reported in many literatures (Bakht et al., 2014) and from the study it was found that after obtaining the absorbance by UV-visible spectrophotometer, the concentrations of different solvents (water, methanol, and phosphate buffer 7.4) were determined by considering from the standard curve and the results were obtained, showing the highest solubility in methanol (0.83204±0.003 mg/ml), slightly soluble in phosphate buffer 7.4 (0.17592±0.008), and lowest in water (0.12994±0.004). We can draw into conclusion that ginger extract is soluble in non-polar solvent. (Table 6.3 and Figure 6.3)

- **Partition co-efficient**

The lipophilicity of ginger extract can be explained by seeing the partition co-efficient and this partition co-efficient are considered as an important factor for predicting the skin permeability through the lipophilic stratum corneum layer. The ideal or required partition coefficient range for transdermal absorption is from 1-4. The obtained partition coefficient from the study was 2.35±0.16, which is within the range and hence the ginger extract can be formulated into a transdermal system Table 6.3. While considering the partition coefficient of any drug, the value should not exceed 3; as retardation in the drug absorption can be seen above this value which makes the drug difficult to pass through other skin layers which are hydrophilic (Schneider et al., 2009).

Table 6.3: Solubility and Partition coefficient data.

Solubility (mg/ml)			**Partition co-efficient (Log P)**
Water	**Methanol**	**Phosphate buffer**	**n-Octanol/water**
0.129±0.004	0.832±0.003	0.175±0.008	2.35±0.16

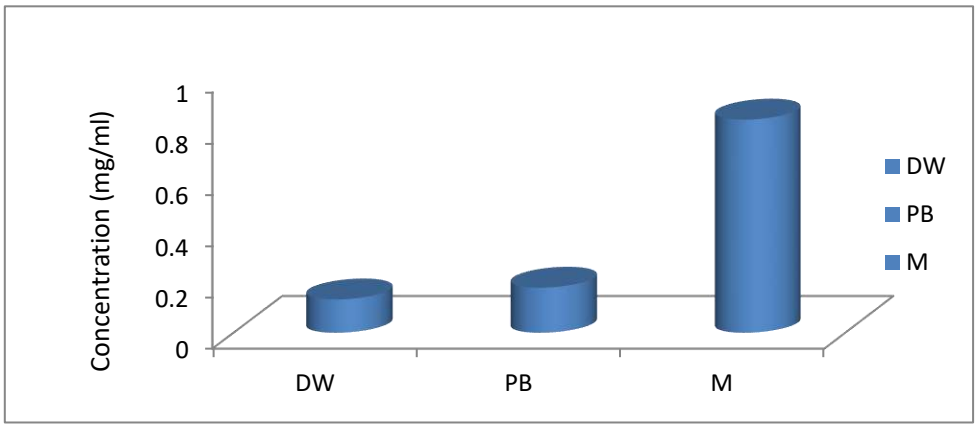


Figure 6.3: Solubility profile of Ginger extract.

DW- distilled water, PB- phosphate buffer pH 7.4, and M- methanol.

- **TLC method**

TLC method was used to characterise the Ginger extract and to identify the presence of different constituents. The main focus of this study was to identify the presence of gingerols (mainly 6-gingerol) which are the compounds of interest responsible for the anti-emetic/anti-nausea action (Lete & Allué, 2016). Clear spots of different compounds were observed in the TLC plate and the spots were compared with the reference and the retention factor (R_f) values were compared. A blue spot was seen in the plate with an R_f value of 0.17, indicates and confirm the presence of 6-gingerol (Wagner et al., 2001) given in Table 6.4 and Figure 6.4

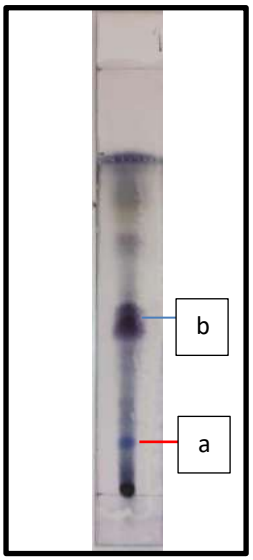


Figure 6.4: TLC of Ginger extract (a) Gingerol, (b) Terpenes.

Table 6.4: R_f values for Ethanolic extract of Ginger extract by TLC.

Solution	Solvent system	Ratio	Rf value
Test solution	Hexane: Diethyl ether	40:60	0.17

- **FT-IR study**

FT-IR study was performed mainly to see the drug (ginger extract) - excipients interactions with ginger extract and ginger mixture containing polymers and penetration

enhancers in it. After interpreting the FT-IR spectra of ginger extract several characteristics peaks were found like 1207.26 cm^{-1} indicating OH-bending (Phenols), 2854.68 cm^{-1}, 2924.43 cm^{-1} indicating the presence of C-H stretching (alkane), 1708.40 cm^{-1} indicating for C=O stretching (ketone), 1375.79 cm^{-1} for C-H bending (alkane), 1452.54, 1603.26 cm^{-1} for C=C stretching (aromatic), 1079.78 cm^{-1}, 1152.95 cm^{-1} indicating O-H bending (aromatic), and 1268.29 for C-O-C bond (ether). These spectra were compared with the mixture and detect for any interactions on the shifting or disappearing of the spectra. The report shows that there were slightly shift in the spectra but there were no disappearing of spectra, and these were given in Table 6.5 and in Figure 6.5-6.7.

Table 6.5: FT-IR interpretation of ginger extract and excipients.

Functional group	**Wave number (cm^{-1})**			
	Range	**Ginger extract**	**Ginger extract + polymers + eucalyptus oil**	**Ginger extract + polymers+ menthol**
OH-bending (Phenols)	3200	3398.82	3427.82	3387.28
C-H stretching (alkane)	2960-2850	2854	2852.47	2872.18
C=O stretching (ketone)	1680-1760	1708.40	1738.04	1648.29
C-H bending (alkane)	1340	1375.79	1348.59	1348.87
C=C stretching (aromatic)	1450-1600	1452.54	1453.59	1454.11
O-H bending (aromatic)	1050-1150	1079.78	1058.48	1081.71
C-O-C bond (ether)	1040-1250	1235.21	1248.99	1249.15

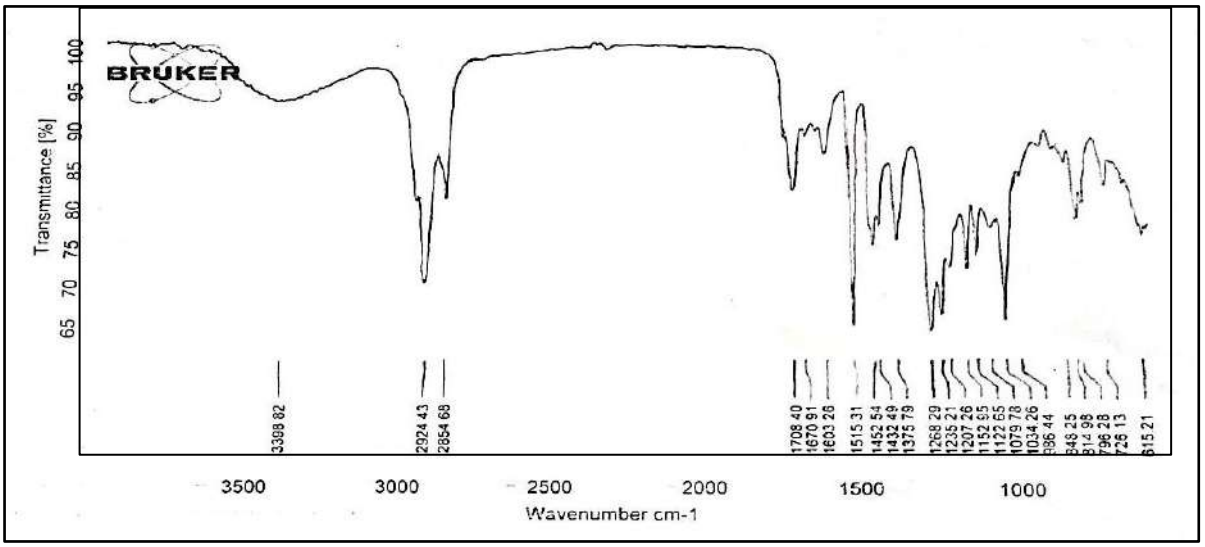


Figure 6.5: FT-IR spectrum of Ginger extract.

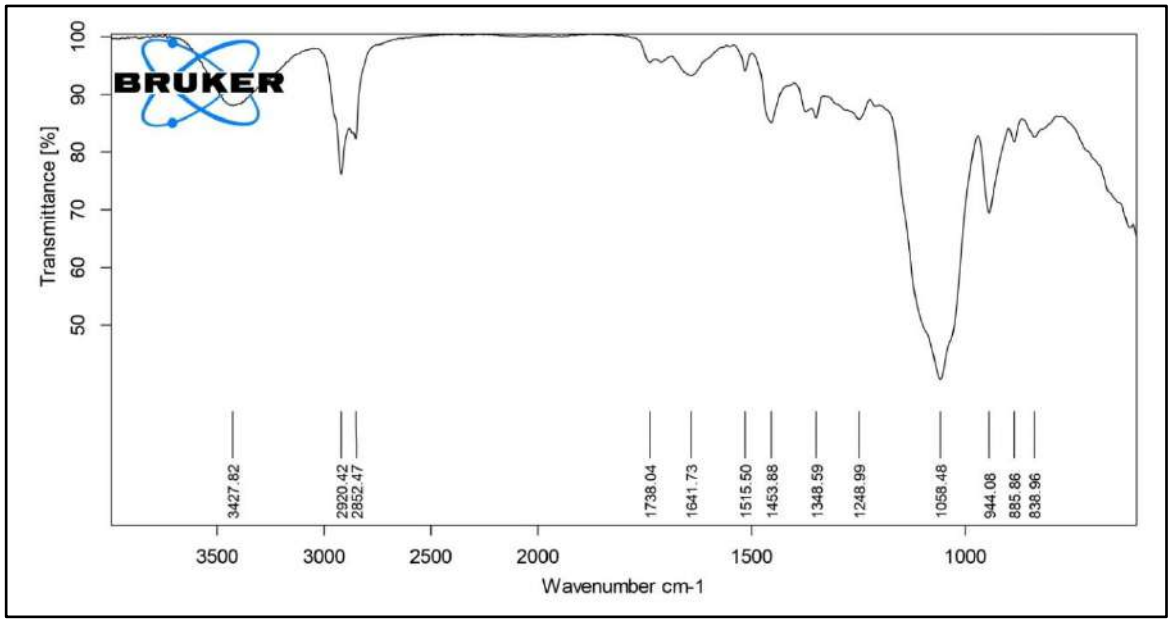


Figure 6.6: FT-IR spectrum of Ginger extract, polymers, and eucalyptus oil.

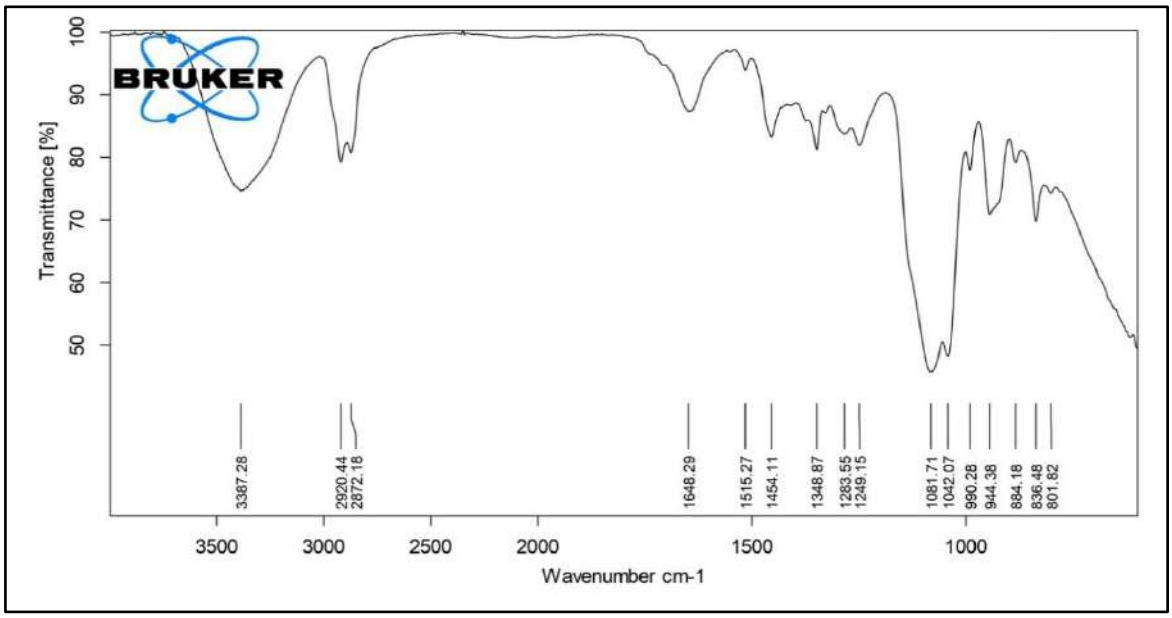


Figure 6.7: FT-IR spectrum of Ginger extract, polymers, and Menthol.

6.4. Physico-chemical characterization of formulated patches:

The physical appearance of the prepared patches like colour, flexibility, homogeneity, and smoothness were given in Table 6.6. The results of evaluations of various physiochemical parameters of ginger extract transdermal patches were presented in Table 6.7. The values of film thickness, weight variations, drug content uniformity, folding endurance, moisture content, and tack properties were given in Table 6.7. The results show that the patches were of an appropriate thickness and there were very less differences in the weight of each prepared patches. The percent of drug content of each patch were above 90% and the patch which shows the maximum drug content were EF3 with 97.742 ±0.27 % and MF3 with 97.032±0.54 %. The folding endurance was found to be >50 folds in all the formulations, and this indicates that the prepared patches have good tensile strength, flexibility, capability to withstand the mechanical pressure and able to maintain the integrity with general skin folding when applied. The percent moisture content of the prepared patches were determined for the present of moisture in

the prepared patches for which if excess moisture is present it can lead to fungus growth or degradation of the patches. The tack property test was performed to know the adhesive property of the patch on the skin layer. As the probe was pulled off, it resulted in debonding between the two surfaces Table 6.8 and Figure 6.8.

Table 6.6: Physical appearance.

Factors	Appearance
Colour	Yellow
Flexibility	Very flexible
Homogeneity	Homogenous
Smoothness	Smooth

Table 6.7: Film thickness, weight variations, drug content, folding endurance, moisture content.

Formulation code	*Film thickness (mm)	*Weight variations (g)	*% Drug content (mg)	*Folding endurance	*% Moisture content (%)
EF1	0.084±0.0005	0.0083±0.00017	91.620±0.05	>50	0.548±0.012
EF2	0.063±0.0005	0.0082±0.00015	94.211±0.04	>50	0.920±0.316
EF3	0.072±0.002	0.0093±0.0001	97.742±0.27	>50	0.980±0.008
EF4	0.070±0.001	0.0094±0.00011	93.735±0.42	>50	0.803±0.281
MF1	0.081±0.004	0.0083±0.00015	91.969±0.16	>50	0.721±0.301
MF2	0.063±0.0005	0.0085±0.00015	94.073±0.24	>50	0.887±0.306
MF3	0.069±0.003	0.0093±0.00017	97.032±0.54	>50	1.468±0.495
MF4	0.070±0.002	0.0090±0.00015	96.421±0.08	>50	1.330±0.749

*Values were expressed in terms of mean±SD (n=3).

Table 6.8: Tack property test of ginger patch containing eucalyptus oil (EF3):

Formulation code	Parameters		
	Absolute +Force (g)	Positive area (FT) (g.s)	Separation Distance (mm)
EF3	6.5	0.6	0.8

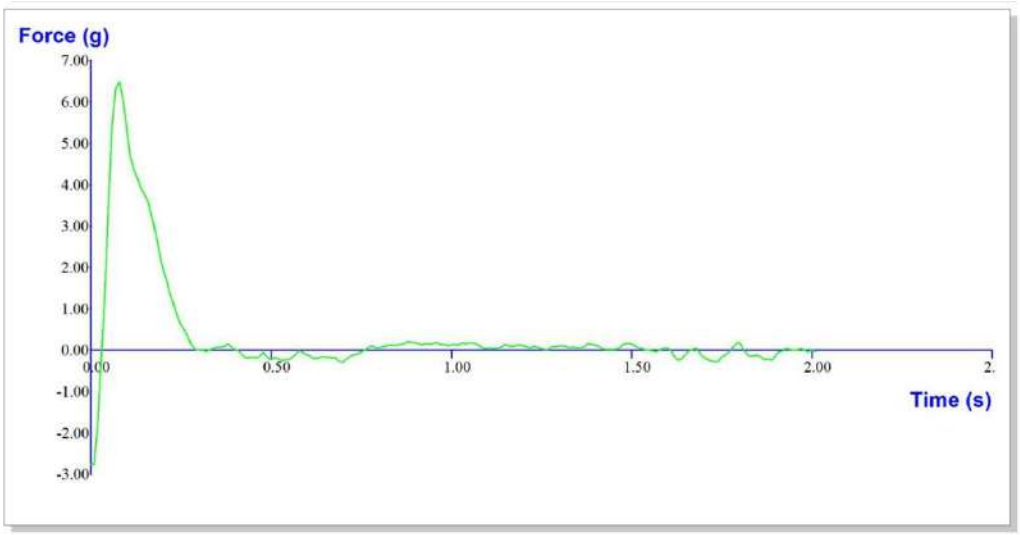


Figure 6.8: Tack property test of ginger patch containing eucalyptus oil (EF3).

6.5. *In vitro* and *ex vivo* permeation study:

All the eight formulations were subjected to *in vitro* and *ex vivo* permeation studies across cellulose dialysis membrane (molecular weight cut off 12000-14000 Da) and excised goat skin respectively for establishing the different permeation parameters. The results of permeation studies were shown in Table 6.9 and Table 6.10 and Figure 6.9-6.12. From the data of the study, it was revealed that the transdermal flux of the blank formulation (absent of penetration enhancers) was 0.76±0.06 mg/cm^2/hr in *in vitro* permeation study and 1.12±0.001 mg/cm^2/hr in *ex vivo* permeation study, which is very

less in comparison to the flux value of formulated patches containing penetration enhancers.

The formulations containing the different concentrations of polymers with Eucalyptus oil as penetration enhancers are given with formulation code EF1 (100% HPMC K100; 2% eucalyptus oil), EF2 (75% HPMC K100: 25% PEG 400; 2% eucalyptus oil), EF3 (50% HPMC K100: 50% PEG 400; 2% eucalyptus oil), and EF4 (25% HPMC K100: 75% PEG 400; 2% eucalyptus oil) and MF1 (100% HPMC K100; 2% menthol), MF2 (75% HPMC K100: 25% PEG 400; 2% menthol), MF3 (50% HPMC K100: 50% PEG 400; 2% menthol), and MF4 (25% HPMC K100: 75% PEG 400; 2% menthol).

In the *in vitro* permeation study, all of these formulations show increase in the flux ranging from 1.300 ±0.002 mg/cm^2/hr to 3.308±0.001 mg/cm^2/hr for EF1 to EF4 and 1.122±0.002 mg/cm^2/hr to 2.893±0.003 mg/cm^2/hr for MF1 to MF4 and with the cumulative drug permeated/ cm^2 (Q_{24}), the ginger extract permeated over 24 hr was found ranging from 21.135±0.016 mg/cm^2 to 42.141±0.009 mg/cm^2 for EF1 to EF4 and 19.369±0.037 mg/cm^2 to 37.048±0.04 mg/cm^2 for MF1 to MF4. After comparing the cumulative drug permeated/cm^2 (Q_{24}) and the flux from all the patches, it was found that the EF3 is showing the highest drug permeated/cm^2 (Q_{24}) 42.141±0.009 mg/cm^2 with the total flux of 3.308±0.001 mg/cm^2/hr as given in the Table 6.9 represented by Figure 6.8. The other release parameters like lag time (T_{lag}), enhancement ratio of steady state (E_{ss}), and permeability coefficient were given in the Table 6.9. After performing the One-way ANOVA it was found that the cumulative drug release/cm^2 results were not significant at $p<0.05$ for both formulations containing eucalyptus and menthol.

In the *ex vivo* permeation study, like that of *in vitro* permeation study there was an increase in the flux and cumulative drug release/ cm^2. The flux range from 1.449±0.438 mg/cm^2/hr to 3.007±0.006 mg/cm^2/hr for EF1 to EF4 and 1.427±0.006 mg/cm^2/hr to 2.162±0.001 mg/cm^2/hr for MF1 to MF4 and with the cumulative drug permeated/cm^2 (Q_{24}) for 24 hr was found ranging from 21.482±0.011 to 35.411 mg/cm^2 for EF1 to EF4 and 16.444±0.051 mg/cm^2 to 25.617±0.015 mg/cm^2 for MF1 to MF4. After comparing the cumulative drug permeated/ cm^2 (Q_{24}) and the flux from all the patches, it was found that the EF3 is showing the highest drug permeated/cm^2 (Q_{24}) 35.411±0.005 mg/cm^2 with the total flux of 3.007±0.006 mg/cm^2/hr as given in Table 6.10 represented by Figure 6.10. The other release parameters like lag time (T_{lag}), enhancement ratio of steady state (E_{ss}), and permeability coefficient were given in the Table 6.10. After performing the One-way ANOVA it was found that the cumulative drug release/cm^2 results were not significant at $p<0.05$ for both formulations containing eucalyptus and menthol.

Hence, the result evidenced that EF3 (50% HPMC K100: 50% PEG 400; 2% eucalyptus oil) shows best release in both *in vitro* and *ex vivo* permeation study and also came to know that increase in the amount of propylene glycol (known to be used as penetration enhancer) also aids to the increase in the overall permeation of the ginger extract.

Table 6.9: Permeation parameters of the different formulations of transdermal patches of ginger extract studied across cellulose dialysis membrane.

Formulation code	Q_{24} (mg/cm^2)[a]	J (mg/cm^2/hr)[b]	T_{lag} (hr)[c]	ER[d]	Permeability coefficient
EF1	21.135±0.016	1.300±0.0008	6.23±0.208	1.706±0.145	0.026±0.00001
EF2	24.890±0.063	1.492±0.002	5.3±0.173	1.957±0.164	0.029±0.00005
EF3	42.141±0.009	3.308±0.001	3.23±0.251	4.341±0.367	0.066±0.00002
EF4	34.512±0.06	2.741±0.007	4.5±0.2	3.597±0.312	0.054±0.0001
MF1	19.369±0.037	1.122±0.002	11.63±0.152	1.473±0.126	0.022±0.00004
MF2	24.055±0.050	1.536±0.004	13.13±0.152	2.015±0.168	0.0307±0.00008
MF3	37.048±0.044	2.893±0.003	3.63±0.23	3.797±0.321	0.057±0.00006
MF4	34.238±0.004	2.782±0.0006	7.23±0.152	3.651±0.308	0.055±0.00001

[a]Q_{24}- cumulative drug release/cm^2 at 24 hr; [b]J- flux of the different formulations; [c]T_{lag}- lag time of the different formulations; [d]ER- enhancement ratio. Values were expressed in terms of mean±SD (n=3).

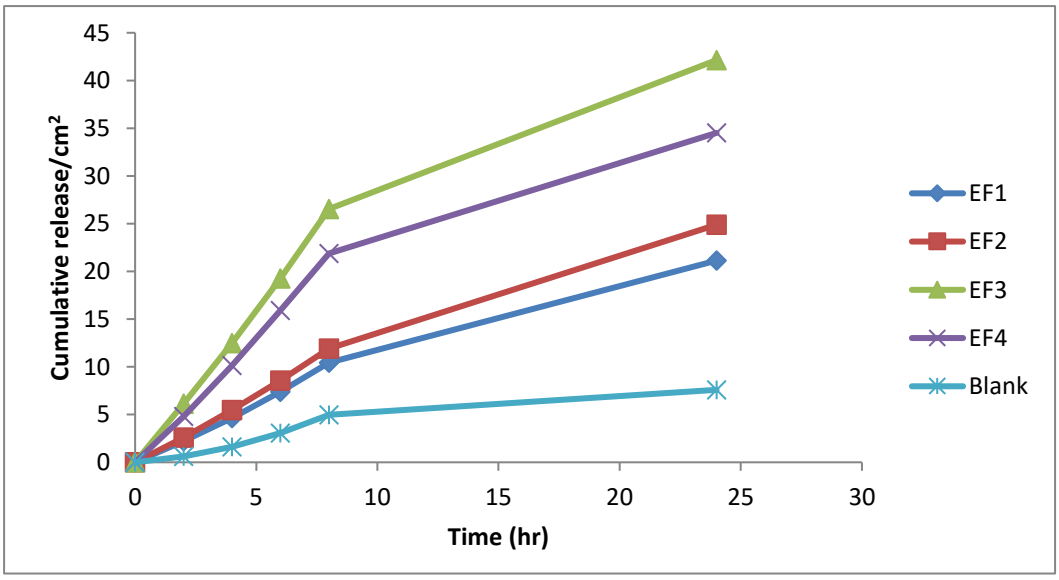


Figure 6.9: *In vitro* drug permeation of Ginger patches containing eucalyptus oil.

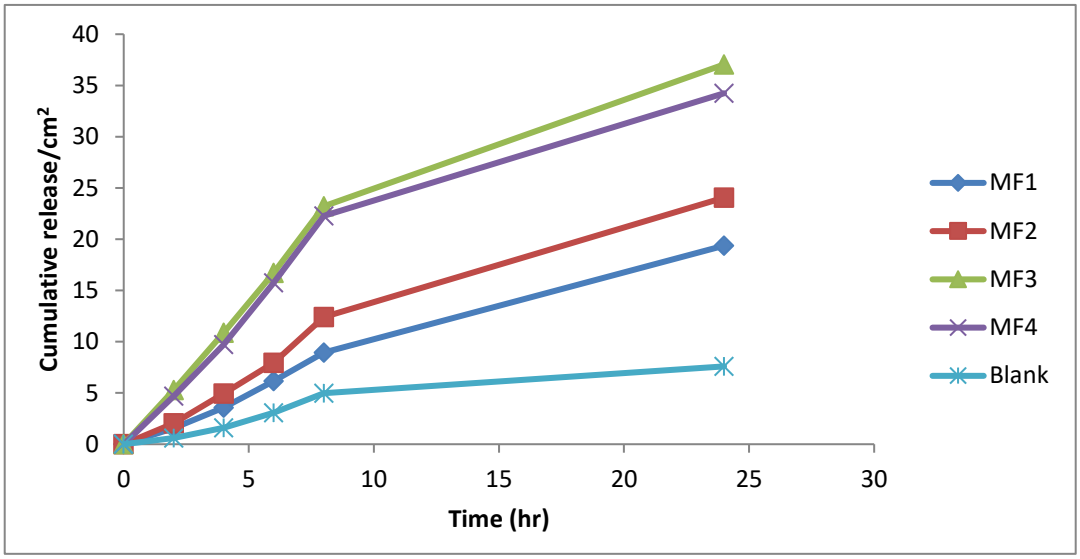


Figure 6.10: *In vitro* drug permeation of Ginger patches containing menthol.

Table 6.10: Permeation parameters of the different formulations of transdermal patches of ginger extract studied across goat skin.

Formulation code	Q_{24} (mg/cm^2)[a]	J (mg/cm^2/hr)[b]	T_{lag} (hr)[c]	ER[d]	Permeability coefficient
EF1	21.482±0.011	1.528±0.309	12.3±0.17	1.362±0.275	0.03±0.006
EF2	27.776±0.017	1.449±0.438	12.26±0.25	1.291±0.388	0.028±0.008
EF3	35.411±0.005	3.007±0.006	9.93±0.25	2.680±0.003	0.06±0.0001
EF4	26.104±0.052	2.359±0.006	7.53±0.25	2.103±0.008	0.047±0.0001
MF1	16.444±0.051	1.427±0.006	8.7±0.17	1.272±0.004	0.028±0.0001
MF2	20.097±0.050	1.605±0.004	8.4±0.1	1.430±0.002	0.032±0.00009
MF3	25.617±0.015	2.162±0.001	8.23±0.25	1.927±0.002	0.043±0.00003
MF4	22.472±0.092	1.824±0.015	10.4±0.17	1.625±0.001	0.036±0.0003

[a]Q_{24}- cumulative drug release/cm^2 at 24 hr; [b]J- flux of the different formulations; [c]T_{lag}- lag time of the different formulations; [d]ER- enhancement ratio. Values were expressed in terms of mean±SD (n=3).

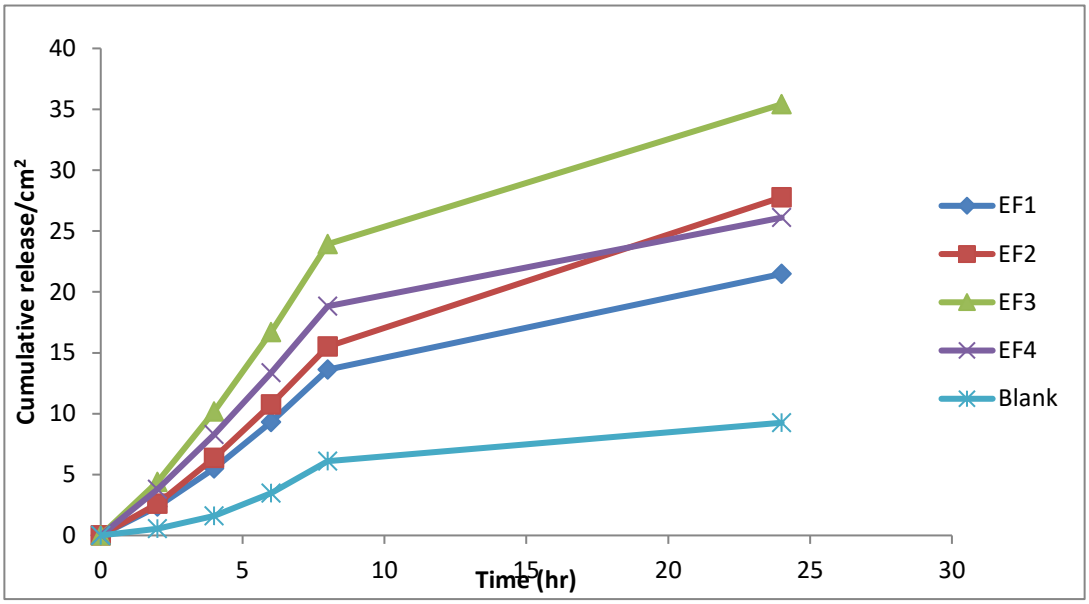


Figure 6.11: *Ex vivo* drug permeation of Ginger patches containing eucalyptus oil.

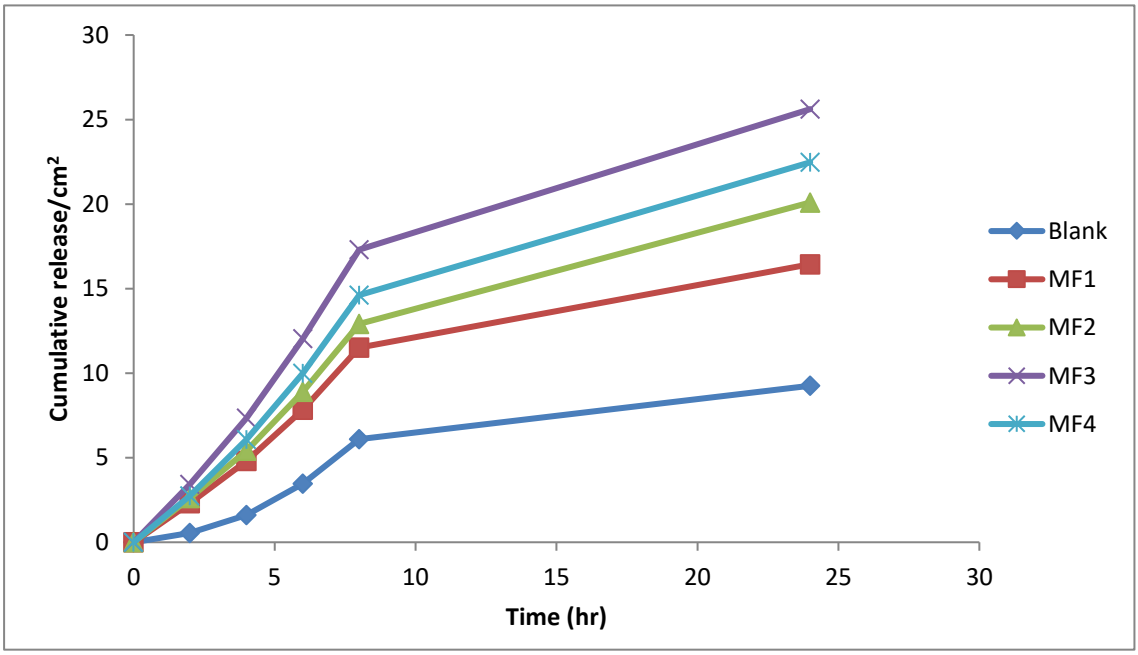
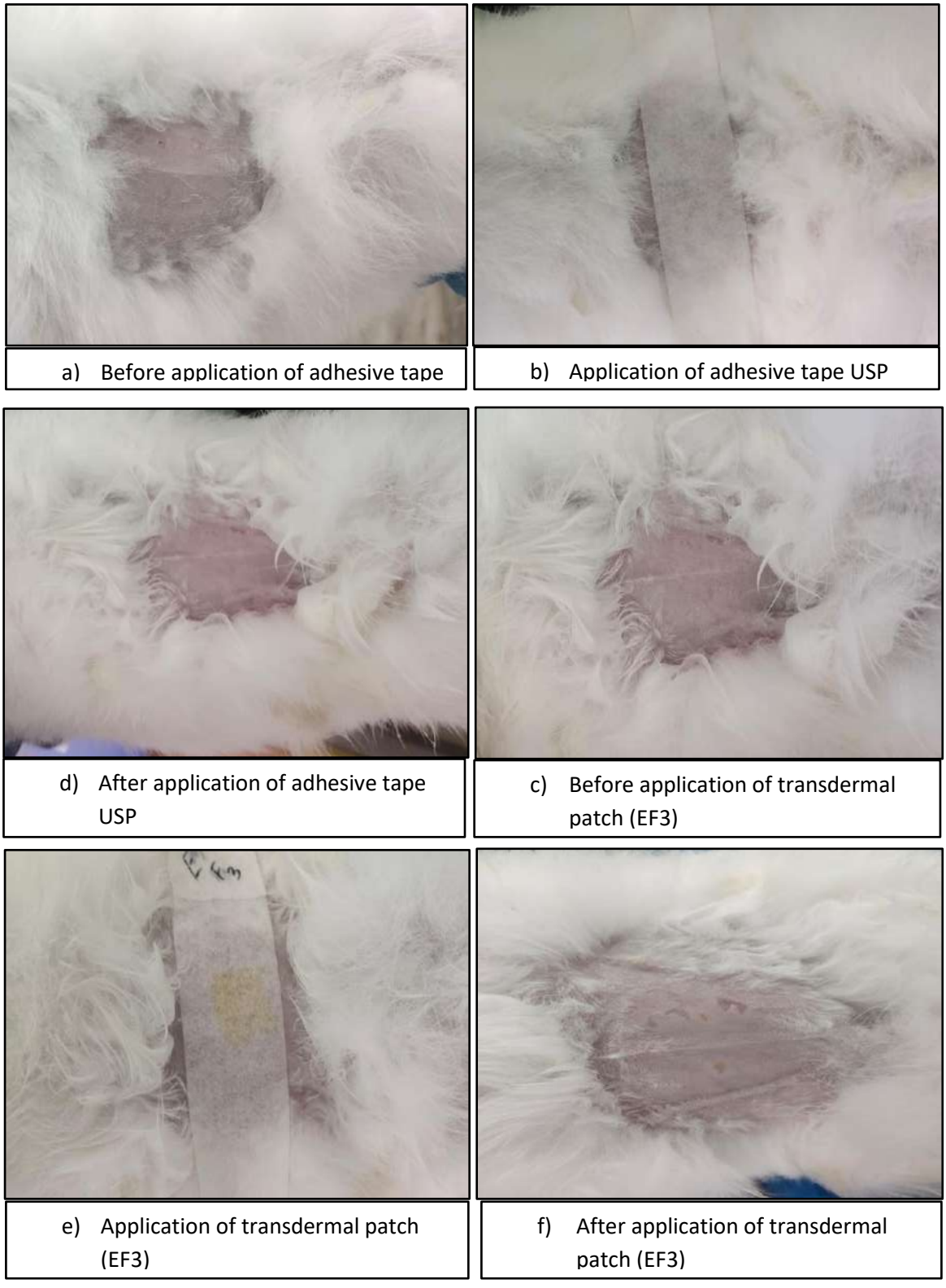


Figure 6.12: *Ex vivo* drug permeation of Ginger patches containing menthol.

- **Skin irritation study:**

On performing skin irritation study test for formulation EF3 on healthy male rabbits for 24 hours and comparing it to the control (adhesive tape USP); no edema or erythema was observed on the site of application of the patch indicating that they are non-irritable

to the skin hence can be considered safe for transdermal patches. The observed results are depicted in Figure 6.13.



a) Before application of adhesive tape

b) Application of adhesive tape USP

d) After application of adhesive tape USP

c) Before application of transdermal patch (EF3)

e) Application of transdermal patch (EF3)

f) After application of transdermal patch (EF3)

Figure 6.13: Photographs of skin irritation study

6.6. Permeation enhancement mechanistic study:

- **FT-IR study:**

To understand more further how the drug or ginger extract permeate through the skin layers and to elucidate the effect of eucalyptus oil and menthol alteration on the intercellular lipids in the stratum corneum keratin and protein. So FT-IR studies were conducted in goat skins, first with the control (blank) which contain no penetration enhancer and second with the test (epidermis treated with penetration enhancers that is eucalyptus oil and menthol).

It was seen that in the control or untreated patch numbers of peaks due to molecular vibrations of the proteins of the stratum corneum and the spectra showed absorption at 3200-2700 cm^{-1} are due to C-H stretching motions of the alkyl groups present in both proteins and lipids. The spectra which are linked to proteins is a broad band which is weak when compared to the lipids absorption which exhibit peaks at 2920 cm^{-1} and 2851cm^{-1}; due to the asymmetric and symmetric CH_2 vibrations of long chain hydrocarbons of lipids. The narrow bands are characteristics to the long alkyl chains of ceramides, cholesterol and fatty acids which are the major components of the stratum corneum lipids.

Strong bands at the range of 1700-1550 cm^{-1} (1740, 1642, and 1542 cm^{-1}) are due to the amide I and amide II stretching vibrations of stratum corneum proteins. The amide bands arise from C=O stretching vibration and the amide II bands are from C-N bending vibration. The amide I band, consisting of components bands, representing various secondary structures of keratin as shown in Figure 6.14.

After comparing the bands of the control and that of treated skin (in both eucalyptus and menthol containing patches), there was shifting, decrease in height, and area of the

peaks which indicate that the lipids were extracted from the stratum corneum by the penetration enhancers (eucalyptus oil and menthol) and it was seen that there was change in the amide I stretching peak on treatment with penetration enhancers which is due to the extraction of proteins and as amide I bands represents various secondary structures of keratin; it is proposed that the penetration enhancers used in the study enhanced the permeation of ginger extract by extraction of the stratum corneum lipids as well as changes in the proteins conformations (Vashisth et al., 2014) Figure 6.15 and 6.16.

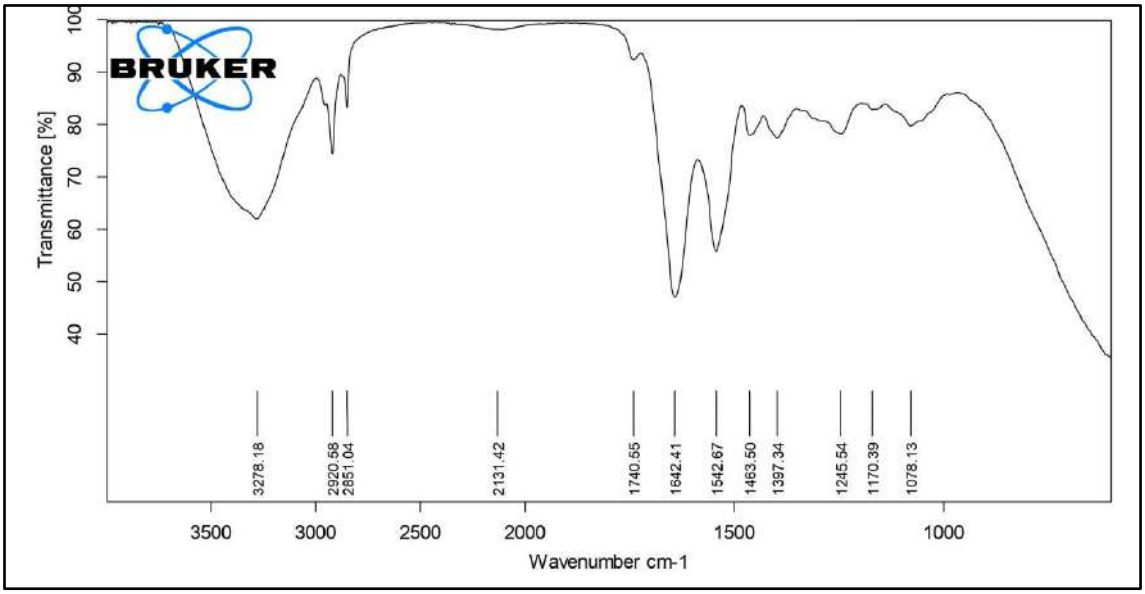


Figure 6.14: FT-IR spectra of goat epidermis untreated with transdermal patch.

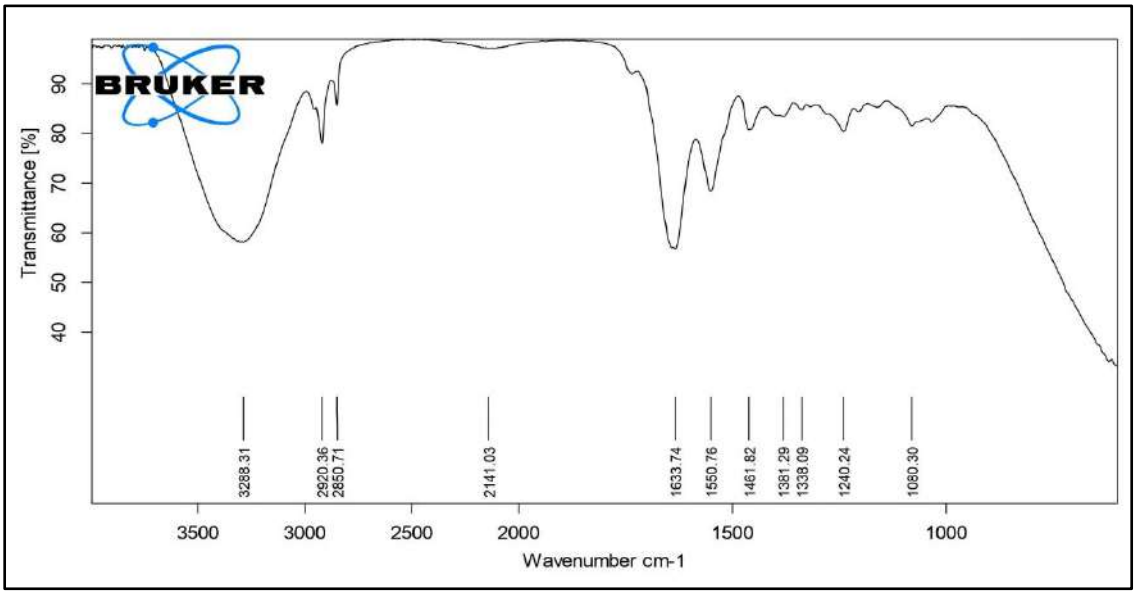


Figure 6.15: FT-IR spectra of goat epidermis treated with transdermal patch containing eucalyptus oil.

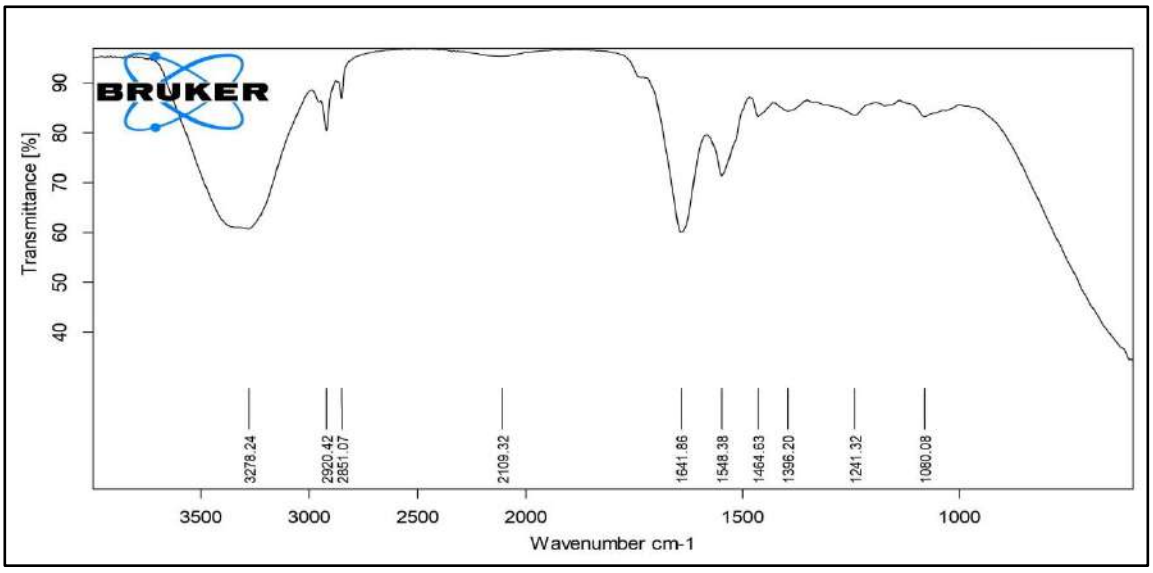


Figure 6.16: FT-IR spectra of goat epidermis treated with transdermal patch containing menthol.

CHAPTER 7

CONCLUSION

7. Conclusion:

In this study, the effects of natural penetration enhancers (Eucalyptus oil and menthol) on the transdermal permeation of Ginger extract across cellulose dialysis membrane and excised goat-skin was evaluated successfully using a modified Franz-diffusion cell. The transdermal matrix patch formulations of ginger extract were prepared in order to by-pass the first pass effect that is seen in ginger constituents (6-gingerol) by direct delivering the drug into the systemic circulation and the formulation was carried out by solvent evaporation method and various parameters related to the fulfilment of transdermal patch were evaluated. On account of the results, it was found that all the prepared formulations showed good uniformity concerning drug content and other physicochemical parameters. The skin irritation study performed indicated no irritation (no sign of skin rashes or edema) which concluded that the patch is safe for the skin. The *in vitro* and *ex vivo* permeation study showed that there was an increase in the drug permeation in the patches containing eucalyptus and menthol as penetration enhancers and from all the formulations, EF3 showed the best release. After running the one-way ANOVA it was found that they are not significant at $p<0.05$. Further study was carried out, where the permeation mechanistic pathway was determined by FT-IR studies and came to a conclusion that the penetration enhancers (eucalyptus oil and menthol) bring changes in the stratum corneum proteins and lipids structure which possibly results in the alteration of skin permeability. Hence it can be concluded that the prepared patches can be formulated and further studies can be carried out like calculating the dose, pharmacokinetics, checking its therapeutic effects, and also animals and humans study before it can be used as an anti-emetic, anti-nausea transdermal patch.

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RECENT DEVELOPMENT IN THE FORMULATIONS OF GINGER FOR THERAPEUTIC APPLICATIONS AND AN OVERVIEW TOWARDS THE ACTION ON SARS-COV-2.

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ABSTRACT: Ginger or *Zingiber officinale*, Roscoe of the *Zingiberaceae* family is a rhizome that is widely found and most consumed in South east Asian countries; also used as a traditional remedy to treat various ailments like nausea, vomiting, pain, arthritis, indigestion, gastro reflux, cardiovascular disease, diabetes, obesity, microbes, cancer, inflammation, oxidation, and wounds to name some of its activity which is based on the various chemical constituents present in ginger as it is found to contain more than 400 different compounds which include sugar, protein, and fats. The major phenolic active constituents of ginger are 6-gingerol, 6-shogaol and 6-paradol; which are safe and showing only a few insignificant adversarial effects. Here, this review aims to summarize and discuss the ideas on how ginger is formulated and improve from conventional to novel formulations using novel techniques; to improve the pharmacological, biopharmaceutical and chemical properties of ginger extract and its compounds. Different novel formulations of ginger like a tablet, capsule, powder, cream, gel, transdermal patch, nanoparticles, liposomes and phytosomes along with its therapeutic actions that were developed in recent years. Future aspects in research are suggested for the advances in the novel formulation of ginger using each isolated compound and improving bioavailability, therapeutic effect, and delivery. Also, this article discusses the *in silico* studies that have been carried out for ginger and its phytochemicals that may be considered as potential agents in the treatment of SARS-CoV-2.

INTRODUCTION: Natural medicinal products or herbal medicines have been taken as supplements or as medicines, and they are considered to be an alternative medicine in treating or preventing diseases. The uses of natural therapies have been most preferred by consumers. Though it is partially true, most people fallaciously believe that herbal products are superior, safe (no side effects) and much effective than allopathic medicines.

The secondary metabolites present in medicinal plants like phenolics, alkaloids, saponins, terpenes, lipids, and carbohydrates are substantially important in preventing the onset of many degenerative diseases like cancer, tumors [1] high-cholesterol, aging activities [2] and many others. Ginger or *Zingiber officinale*, Roscoe of the *Zingiberaceae* family, is a rhizome that is widely found and most consumed in Southeast Asian

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