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Studies On Effect Of Formulation, Characterization And Evaluation Of Fluticasone Propionate Ethosomal Gel For The Treatment Of Skin Conditions

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ABSTRACT

Background and Objective:

Fluticasone Propionate is a glucocorticoid steroid with anti-inflammatory and immune-suppressive abilities. It is a synthetic glucocorticoid. It was well absorbed topically and shows improved bioavailability. Fluticasone propionate (FP) is the latest in a range of inhaled corticosteroids indicated for the treatment of asthma and other topical skin conditions.

Fluticasone propionate (FP) is a novel androstane glucocorticoid with potent anti-inflammatory activity which has been effectively used intranasally, as therapy for seasonal and allergic perennial rhinitis. Fluticasone propionate is a highly lipophilic molecule with good uptake, binding and retention characteristics in human lung tissue. It has high glucocorticoid receptor selectivity and amenity, demonstrating rapid receptor association and slow receptor dissociation. Fluticasone reduces swelling (inflammation), itching, and redness. This medication is a medium-strength corticosteroid and it is used to treat a variety of skin conditions (such as eczema, psoriasis, rash). It is available in several forms, including cream, ointment, and lotion.

The main objective of this research work was to develop a novel formulation and evaluation of transdermal drug delivery system by using a pure drug of Fluticasone Propionate in the form of ethosomal gel to provide a better anti-inflammatory activity and prolonged duration of action of drug to treat varying diseases associated with the skin.

Method and Results:

Preformulation study was carried out which includes description, solubility, melting point, specific optical rotation and moisture content determination. The organoleptic properties and solubility of the drug was evaluated and complies with standard. The melting point was found to be 273^0C and the specific optical rotation and moisture content determination was found to be 32.6^0 and 0.4% respectively.

The compatibility study of FT-IR spectrum of drug and drug-excipient was found to be satisfactory, which indicates that the drug and excipients were compatible. The study by DSC for studying the thermal properties and determination of λ MAX for maximum absorbance of the drug were found satisfactory with the obtained results.

Fluticasone propionate ethosomes were prepared by using cold method with different ratio of drug to excipients such as phospholipids (2% to 5%w/v), ethanol (20% to 40% v/v), propylene glycol (10 % v/v), cholesterol (0.005 g), drug (0.20 g) and distilled water (q.s) and were characterized for Surface morphology, Optical microscopy, Entrapment Efficiency, Vesicle size and Zeta Potential, Polydispersity Index and Release Kinetics. Among the eight formulations (F1 to F8), F1 was selected as optimized formulation. The average vesicle size, polydispersity index, Zeta potential, % Entrapment efficiency and % drug content of the F1 formulation were found in the range of 116.37±3.04 nm, 0.286±0.115, -31 mv, 89.45±0.51 respectively.

Fluticasone propionate ethosomes was formulated into topical gel formulation (EG1 to EG8) using different concentrations of Carbopol 934 (1%, 1.5%, 2%, 3% W/W), Triethanolamine (0.5%, 1%, 1.5% W/W) and buffer (pH 7.4) (q.s).

Various evaluation test also carried out with FP Ethosomal gel involving Physical examination, Washability, Viscosity, Spreadability, pH determination, drug content uniformity, extrudability, drug content study, Homogeneity, In Vitro studies and stability studies and all the evaluation test results was reported and found satisfactory results. The viscosity of the gel formulation was determined and was found that highest

viscosity of 17338 cps with the formulation (EG5). The pH of all formulations was found near to the skin pH value with the average of pH 6.3. The Spreadability and Extrudability results shows that in the range of 19.8 to 36.9 gm.cm/sec and excellent to satisfactory results respectively for all the formulations. The drug content uniformity of FP Ethosomal gel (EG1) showed 95.68% and the drug content study shows that the formulation (EG3) exhibiting 99.8% among the other formulations.

From the in-vitro drug release study, it was revealed that the topical ethosomal gel EG1 formulation lasts up to 8 hours and has a drug release of about 88.5%. From the stability study, it was revealed that the F8 formulation was stable at 4±1℃ and the results for up to 3 months at its storage founds satisfactory and it is clear that the formulation did not undergo any chemical changes found more stable at room temperature. Among the five formulations, EG1 was selected as the best formulation as it's showing higher drug content uniformity and better in-vitro drug release study amongst the other formulations.

Interpretation & Conclusion:

These results suggested that it is a potentially promising formulation for the efficient delivery of drugs by topical administration in the form of ethosomal gel. Fluticasone propionate ethosomal gel presents a promising approach for enhancing the transdermal delivery of this corticosteroid. By leveraging the unique properties of ethosomes, this formulation may improve therapeutic efficacy while minimizing systemic side effects and it is more potential for the topical drug delivery.

KEYWORDS: Ethosomes, Fluticasone propionate, Gel, skin conditions, Phospholipids, Triethanolamine and Carbopol 934.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

1.1 DRUG DELIVERY SYSTEM

Drug delivery system is outlined as a technological formulation or a tool that permits the introduction of a drug molecule within the body by controlling the rate, extent and the location of drug release. The development of a novel chemical entity is a very expensive and time-consuming process, and therefore designing of new delivery systems of an already existing drug molecule can significantly enhance the drug safety, efficacy, improve patient compliance, etc. Conventional drug delivery systems such as powders, tablets, capsules, etc., are associated with a variety of drawbacks viz. severe side effects of drugs, multidrug resistance issues, lack of target specificity etc.,

In this scenario, topical-transdermal-based drug delivery system could be better option to tackle such hurdles and offer many benefits over free drugs via increasing their solubility, preventing drug interactions, providing controlled release characteristics, could also increase the drug accumulation in diseased site, etc. Drug delivery systems control the rate at which a drug is released and the location in the body where it is released. Some systems can control both.

Types of Drug Delivery Systems

Different kinds of drug delivery systems exist to address varied medical needs. They may be classed into:

- **Oral delivery systems** - Tablets and capsules are typical forms, offering convenience and patient compliance.
- **Inhalation delivery systems** - Popular in treating respiratory conditions; examples include inhalers and nebulizers.
- **Injectable delivery systems** - Includes intramuscular, subcutaneous, and intravenous injections.
- **Topical delivery systems** - Involves application on the skin, like ointment, gel and creams.

1.2 TOPICAL DRUG DELIVERY SYSTEM

Topical drug delivery is a route of drug administration that allows the topical formulation to be delivered across the skin upon application, hence producing a localized effect to treat skin disorders like eczema. The formulation of topical drugs can be classified into corticosteroids, antibiotics, antiseptics, and anti-fungal. The mechanism of topical delivery includes the diffusion and metabolism of drugs in the skin.

The delivery of topical drugs needs to pass through multiple skin layers and undergo pharmacokinetics, hence factor like dermal diseases minimize the bioavailability of the topical drugs. The wide use of topical drugs leads to the advancement in topical drug delivery. These advancements are used to enhance the delivery of topical medications to the skin by using chemical and physical agents.

1.3 TRANSDERMAL DRUG DELIVERY SYSTEM (TDDS)

Innovations in the area of drug delivery are taking place at a much faster pace as compared with the last two decades. Improved patient compliance and effectiveness are inextricable aspects of new drug delivery systems A more radical approach has been to explore newer interfaces on the body for introducing therapeutics. One such approach, transdermal drug delivery, makes use of human skin as a port of entry for systemic delivery of drug molecules.

Transdermal drug delivery system (TDDS) is one of the systems lying under the category of controlled drug delivery, in which the aim is to deliver the drug through the skin in a predetermined and controlled rate. TDDS are adhesive drug-containing devices of defined surface area that deliver a predetermined amount of drug to the surface of intact skin at a programmed rate to reach the systemic circulation.[1]

Transdermal route has vied with oral treatment as the most successful innovative research area in drug delivery, as oral treatment involves attainment and maintenance of drug concentration in the body within a therapeutically effective range by introduction of a fixed dose at regular intervals, due to which the drug concentration in the body follows a peak and trough profile, leading to a greater chance of adverse effects or therapeutic failure; large amount of drug is lost in the vicinity of the target organ and close attention is required to monitor therapy to avoid overdosing.

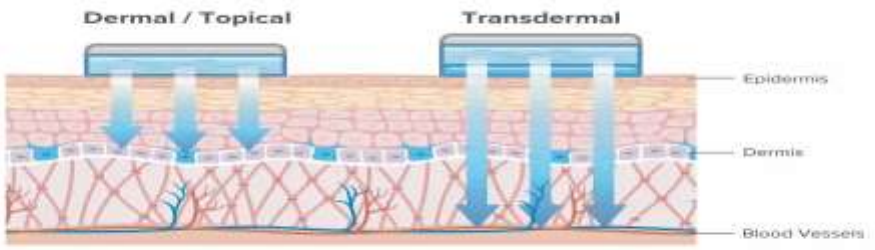

Figure No.1: Transdermal delivery of skin

Transdermal delivery provides a leading edge over injectables and oral routes by increasing patient compliance and avoiding first-pass metabolism, respectively.

Transdermal drug delivery systems are specifically designed for systemic treatments by delivering drugs through the patient's skin. Offering controlled release and maintaining continuous efficacy of the drug, they are considered an effective drug delivery method. The drug released from TDDS initially permeates the skin through the stratum corneum progressing through the epidermis and dermis without accumulating the active ingredient in the dermal layer. Once the drug reaches the dermal layer, it is released for systemic absorption via the dermal microcirculation.

The limitations of the oral route can be overcome and benefits of intravenous drug infusion such as to bypass hepatic "first pass" elimination (HEPE) to maintain constant prolonged and therapeutic effective drug levels in the body can be closely duplicated, without its potential hazards, by transdermal drug administration through intact skin.[2]

Overall, transdermally applied drugs are safely and conveniently delivered to children and elderly patients, treating a wide range of conditions and diseases. These include moderate to severe menopausal vasomotor symptoms, Alzheimer's disease, ADHD, major depressive disorder, chronic pain management, and various other conditions.

1.4 **SKIN:**

The skin acts as a formidable barrier to the penetration of drugs and other chemicals; it does have certain advantages which make it an alternative route for systemic delivery of drugs. The skin is the body's largest organ, made of water, protein, fats and minerals. Your skin protects your body from germs and regulates body temperature. Nerves in the skin help you feel sensations like hot and cold.

Transdermal drugs delivery system involves the route passage of substances from the skin surface through the skin layers, into the systemic circulation. The skin has been commonly used as a site for topical administration of drugs, when the skin serves as a port for administration of systemically active drugs.

The drug applied topically is distributed following absorption, first into the systemic circulation and then transport to the target tissue, which can be relatively remote from the site of drug application to achieve its therapeutic action.

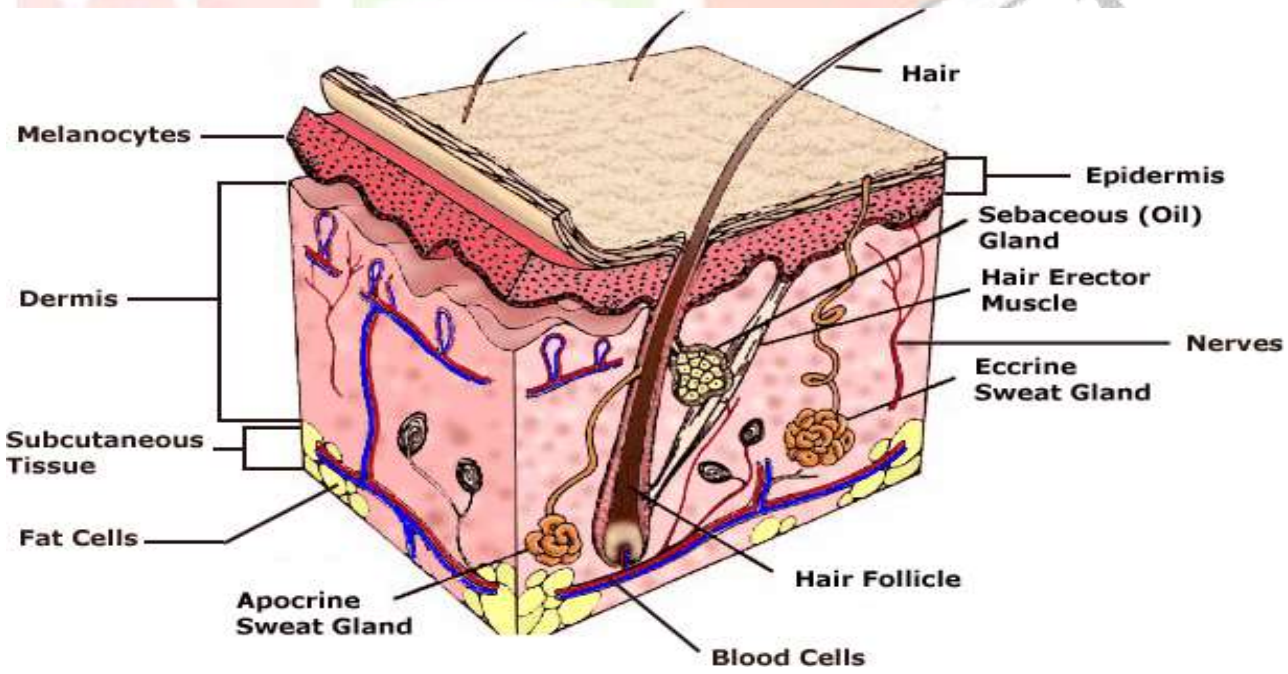

Figure No.2: Structure of skin

Table No 1: BIOCHEMISTRY OF SKIN

Layers	Epidermis	Dermis	Subcutaneous fat
Function	Barrier	Supportive	Insulation
Major components	Keratin	Collagen	Fat
Thickness	0.2mm	3.5mm	Variable
pH	4.2-6.5	7.1-7.3	-
Water content	10-25%	60-70%	-
Blood vessels	None	Many	Some

1. Adult human skin extends to approximately $2m^2$ in area and is around 2.5mm thick; its density is around 1.1 g/cm^3 and constitutes around 6% of the total body weight. The thick (10-20 mm) surface layer of epidermis termed as stratum corneum is highly hydrophobic and contains 10-15 layers of integrated corneocytes which are constantly shed and regenerated. The extra circular lipids account for 10% of the dry weight of this layer and remaining 90% are the intracellular proteins mainly keratin.
2. An average human skin surface is known to contain, on the average, 40 to 70 hair follicles and 200 to 250 sweat ducts on each square cm of skin area.
3. Presently there is an increasing recognition that skin also serves as the part of administration for systemically active drugs.

In this case the drug applied topically will be absorbed first into blood circulation and then we transported to target tissues, which would be rather remote from the site of drug application to achieve its therapeutic doses.

The skin is a multi–layer organ composed of many histological layers. It is generally described in terms of 3 major tissue organs.

1. The Epidermis
2. The Dermis
3. The Hypodermis

THE EPIDERMIS

Epidermis is the most superficial layer and is composed of stratified squamous type of epithelium. Epidermis is the top layer of the skin. Keratin, a protein inside skin cells, makes up the skin cells and, along with other proteins, sticks together to form this layer. From outside inward stratified epithelium may be divided in to 5 layers.

They are,

a. Stratum Corneum

b. Stratum Lucidum

c. Stratum Granulosum

d. Stratum Spinosum

e. Stratum Germinatum

a. Stratum Corneum: The Stratum Corneum is most superficially placed and consists of many layers of compacted, flattened, dehydrated, and keratinized cells. They are dead cells converted into proteins and are continuously shed. The cell outlines are indistinct and the nuclei are absent. The Stratum Corneum as a water content of only~20% as compared to normal 70 % in physiologically active Stratum Germinatum. This layer is thickest at the sole and the palm and thinnest at the lip. Hairs, loops, nails, feathers, scales, etc., are special outgrowth of this layer.[3]

b. Stratum Lucidum: This is a thin more or less transparent layer 3 to 5 cells deep placed below the Stratum Corneum. The cell outlines are indistinct and the nuclei are absent. The cells contain droplets of "eleidin" which is precursor of keratin.

c. Stratum Granulosum: The Stratum Granulosum is situated below the stratum lucidum and consists of 3 to 5 layers of flattened polyhedral cell filled with keratohyalin granules which takes a deep stain with hematoxylin.

d. Stratum Spinosum: This is a broad layer of variable thickness and is made up of polyhedral cells is apparently coved with minute spines, which interdigitate with similar spines of adjacent cells. There are consequently known as "prickle cells". As a microscopic study indicates that the prickle is not in fact cytoplasmic protrusions and the branches from two cells actually do not have cytoplasmic continuity, but attached by well-developed cytoplasmic nodes called as desmosomes.[4]

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e. Stratum Germination: This is growing layer is composed of a single layer of columnar epithelium which has got transverse, thin, short cytoplasmic processes on its basal lamina by means of which they anchor the epithelium to the underlying dermis. These cuboidal to columnar cells with oblong nuclei placed perpendicularly on the basement membrane produce new cells to replace those of the above layers by the process of mitosis.

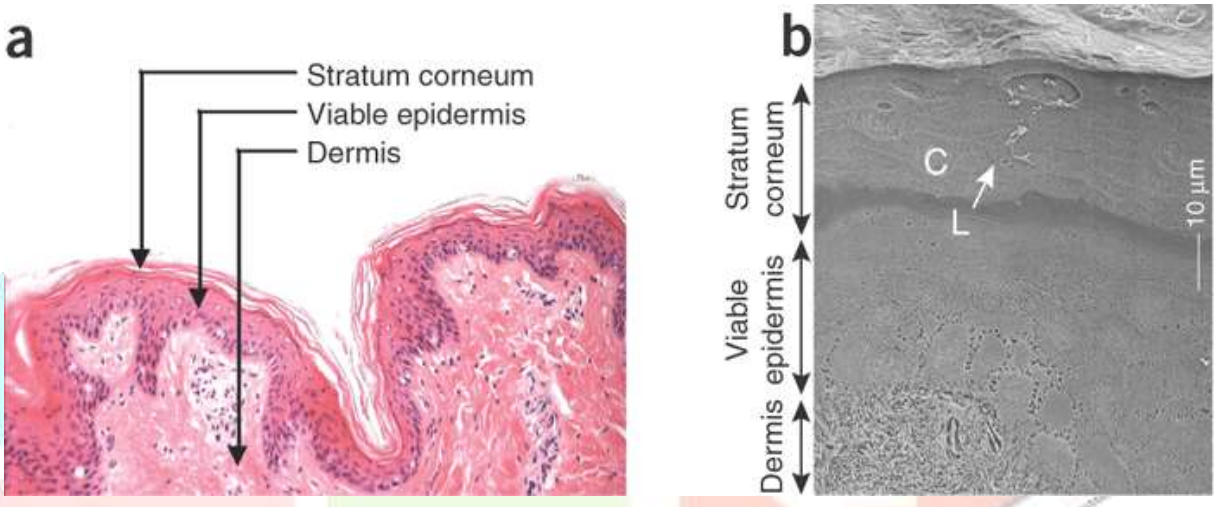

Figure No.3: Multi-layer tissues of skin

THE DERMIS

The true skin is made up of connective tissue and lies below the epidermal layer which binds to the underlying tissues. It is made up of chiefly of collagenous and elastic fibers which provide it with a tensile strength equal to that of a thin steel wire. This layer is utilized for the production of leather after chemical processing. The superficial part of the dermis is compact and forms the papillary layer because it sends innumerable finger like projections into the prickle cell layer of epidermis. The deeper part of the dermis is composed of rather loose connective tissue and is infiltrated with fat. The reticular layer of the dermis merges imperceptibly in to subcutaneous layer of fat.[5]

They are of 2 types,

a. Papillary Dermis

b. Reticular Dermis

a. **Papillary Dermis:**

The papillary dermis is the thin, upper layer that contains capillaries (tiny blood vessels) that help regulate skin temperature and provide nutrients to the epidermis.

This skin layer also contains the following:

- **Meissner corpuscles**, which are receptors that transmit sensations of delicate touch.
- **Lamellar corpuscles**, which are receptors that transmit sensations of vibration and pressure.

b. **Reticular Dermis:**

The reticular dermis is the thick, lower layer that contains connective tissues and dense collagen bundles. Collagen is the main protein that provides structure to skin and connective tissues. It gives skin elasticity and strength.

The thickness of the dermis varies by its location on the body. On the eyelids, it is roughly 0.6 millimeters thick. On the back, palms of hands, and soles of the feet, it's 3 millimeters thick.

THE HYPODERMIS

The hypodermis is also known as subcutaneous fat layer. Subcutaneous fat layer acts both as an insulator, a shock absorber, and reserve depot of calories and supplier of nutrients to the other two layers. This subcutaneous tissue or hypodermis is composed of loose, fibrous connective tissue which contains fat and elastic fibers. The base of the hair follicles is present in this layer, as are the secretary portion of the sweat glands, cutaneous nerves and blood and lymph networks. It is generally considered that the drug has entered the systemic circulation if it reaches this layer; however, the fat deposits may serve as a deep compartment for the drug and this can delay entry into the blood.

The hypodermis layer also does the following:

- Stores fat cells for energy reserves
- Gives the body its smooth, contoured appearance
- Regulates temperature through the contraction and dilation of blood vessels
- Serves as the attachment point for bones, muscles, and other organs to the skin
- Contains deep pressure sensors
- Produces a hormone called leptin that helps keep the body's metabolism in homeostasis (balance among all of your body's systems so they can function well)

Conditions that affect the subcutaneous layer of the skin include:

- Bedsores
- Hypothermia
- Panniculitis
- Sarcoidosis
- Third-degree burns
- Tumors

1.5 FUNCTIONS OF SKIN[6]

- ❖ Protection
- ❖ Regulation
- ❖ General sensation
- ❖ Gaseous exchange
- ❖ Absorption

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1.6 ROUTES OF PENETRATION

The following routes are observed in transportation of penetration through skin barrier:

1. Across the intact horny layer

2. Through the hair follicles with the associated sebaceous glands or

3. via the sweat glands

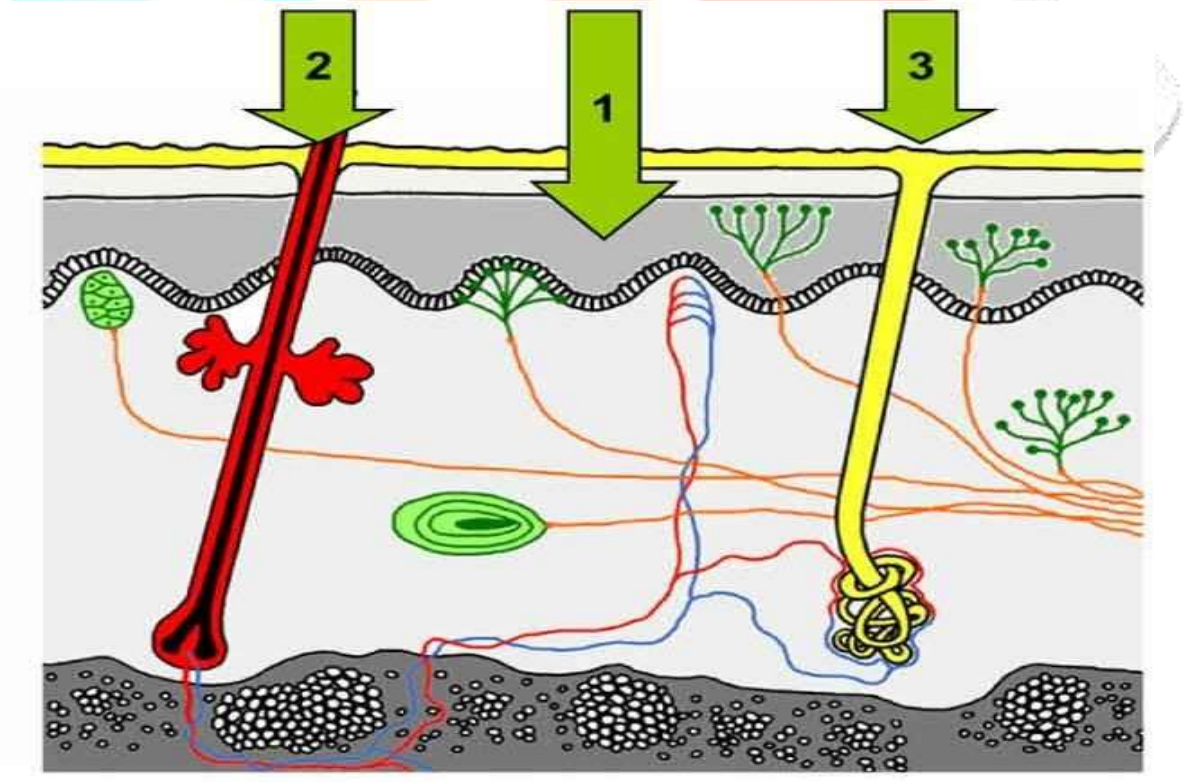


Figure No.4: Possible pathway for a penetrant to cross the skin barrier

Trans epidermal transport means that molecules cross the intact horny layer. Two potential micro-routes of entry exist, the Transcellular (or intracellular) and the intercellular pathways.

Figure No.5: Trans epidermal Transport

1.7 ADVANTAGES OF TRANSDERMAL DELIVERY[7]

1. Provide relatively steady and sustained drug concentration in plasma in contrast to conventional systems where peaks and troughs are a common feature.
2. Variability due to factors such as pH intestinal motility, food intake, etc., which make vast difference in the bioavailability of the drugs given through oral route, are not existent.
3. The hepatic first pass metabolism is avoided.
4. A constant rate of absorption is possible in a vast variety of adverse patient population.
5. Ease of administration and patient convenience.
6. Drug input terminable by mere removal of the transdermal patches.
7. Drugs that cause gastro intestinal upset can be good candidates for transdermal delivery this method avoids directs effects on stomach and intestine.
8. Increased therapeutic value due to avoidance of hepatic first pass effect, gastro intestinal irritation and low absorption problem.
9. Drugs that are having short biological half-life can be given by this

therapeutic system and it also reduces dosing frequency.

10. Transdermal patches are used for cessation of tobacco smoking.

11. Another advantage is convenience, especially notable in patches that require only once weekly application. Such a simple dosing regimen can aid in patient adherence to drug therapy.

1.8 DISADVANTAGES OF TRANSDERMAL DELIVERY

1. Can be used only for drugs, which require very small plasma concentrations for action.
2. Local irritation and arrhythmia are possible. Enzymes in epidermis or derived from microorganisms present on the skin may denature the drugs.
3. Another significant disadvantage of transdermal drug delivery is that skin is less permeable because it serves a protective barrier for the entry of foreign particles.
4. In order to maintain constant release states, transdermal patches must contain surplus of active drug.

In case of transdermal drug delivery systems, percutaneous absorption of drug molecule is important. The rate and extent of absorption of the drug should be adequate to achieve and to maintain uniform systematic and therapeutic levels throughout the duration of use. In general, once the drug molecules cross the stratum corneum barrier, passage into deeper dermal layers and into the systematic circulation occurs relatively quickly and easily[8]

1.9 FACTORS AFFECTING TRANSDERMAL PERMEABILITY

The factors controlling transdermal permeability can be broadly classified as:

1. Partition coefficient
2. pH conditions
3. Penetrant concentration
4. Physicochemical properties of transdermal drug delivery systems
5. Release characteristics

6.	Enhancement of transdermal permeation
7.	Physiological and pathological conditions of skin
8.	Reservoir effects of horny layer
9.	Lipid film
10.	Skin hydration
11.	Skin temperature
12.	Regional variation
13.	Pathological injuries to the skin
14.	Cutaneous self-metabolism

Table No 2: Factors affecting Transdermal Permeability[9]

S.NO	PARAMETERS	FACTORS
1.	FORMULATION	BOUNDARY LAYERS
		THICKNESS
		TEMPERATURE
		POLYMERS
		VEHICLES
		TORTUOSITY
		POROSITY OF MEMBRANES
		GEOMETRY OF SYSTEM
2.	SKIN	SPECIES (Causasian/Black)
		CONDITION OF SKIN (Healthy/Diseased, pre-treated, secretion)
		SITE TO SITE PERMEABILITY

3.	ADHESION	FLEXIBLE/ NON-FLEXIBLE AND BREATHABLE/ NON-BREATABLE BACKING MATERIAL
		ARRANGEMENT OF OTHER LAYERS
		CURING
		COHESIVENESS
		SIZE AND SHAPE
		TYPE AND CHARACTERISTICS OF POLYMER
4.	BIO-PHARMACEUTICAL	HALF-LIFE OF THE DRUG
		PHARMACOLOGICAL BLOOD LEVELS

1.10 STRATUM CORNEUM AND TWO MICROROUTES OF DRUG PENETRATION[10]

Optimizing Transdermal Drug Delivery Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, and most importantly, it provides patient convince. But one of the major problems in transdermal drug delivery is the low penetration rate through the outer most layer of skin. The non-invasive approaches for providing transdermal drug delivery of various therapeutics substances are

Drug and vehicle interactions:

1. Selection of correct drug or prodrug

2. Chemical potential adjustment

3. Ion pairs and complex coacervates

4. Eutectic systems

5. Stratum corneum modification

6. Selection of correct drug or prodrug

7. Chemical potential adjustment

8. Ion pairs and complex coacervates

9. Hydration

10.Stratum corneum bypassed or removed

11.Micro needle array

12.Stratum corneum ablated

13.Magnetophoresis

14.Photomechanical waves

15.Vesicles and particles

16.Liposomes and other vesicles

Vesicular systems are drug delivery system to deliver the drug dermally and transdermally. Liposomes have the potential of overcoming the skin barrier, as these are bilayered lipid vesicles, consisting primarily of phospholipids and cholesterols.

Liposomes were discovered in the early 1960's by Bangham and colleagues and subsequently became the most extensively explored drug delivery system. In early 1960's a great knowledge of vesicle derivatives has been tested for their abilities. Most experiments, however, have centered on liposomes, since derivations only add to their basic properties.

Vesicles are closed, spherical membrane that separates a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a

liposome would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells.

Generally, liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effect to a compound applied topically[11].

1.11 GENERATIONS OF TRANSDERMAL DELIVERY SYSTEMS[12]

a. First-generation transdermal delivery systems:

The first generation of transdermal delivery systems is responsible for most of the transdermal patches. First-generation delivery candidates must be low-molecular weight, lipophilic and efficacious at low doses. The first-generation approach to transdermal delivery is limited primarily by the barrier posed by skin's outermost layer called the stratum corneum, which is 10 to 20 μm thick. Underneath this layer is the viable epidermis, which measures 50 to 100 μm and is avascular. Deeper still is the dermis, which is 1–2 mm thick and contains a rich capillary bed for systemic drug absorption just below the dermal–epidermal junction.

b. Second-generation transdermal delivery systems:

The second generation of transdermal delivery systems recognizes that skin permeability enhancement is needed to expand the scope of transdermal drugs. The ideal enhancer should (i) increase skin permeability by reversibly disrupting stratum corneum structure, (ii) provide an added driving force for transport into the skin and (iii) avoid injury to deeper, living tissues. However, enhancement methods developed in this generation, such as conventional chemical enhancers, iontophoresis and noncavitational ultrasound, have struggled with the balance between achieving increased delivery across stratum corneum, while protecting deeper tissues from damage.

As a result, this second generation of delivery systems has advanced clinical practice primarily by improving small-molecule delivery for localized, dermatological, cosmetic and some systemic applications, but has made little clinically important effect on the delivery of macromolecules.

c. Third-generation transdermal delivery systems

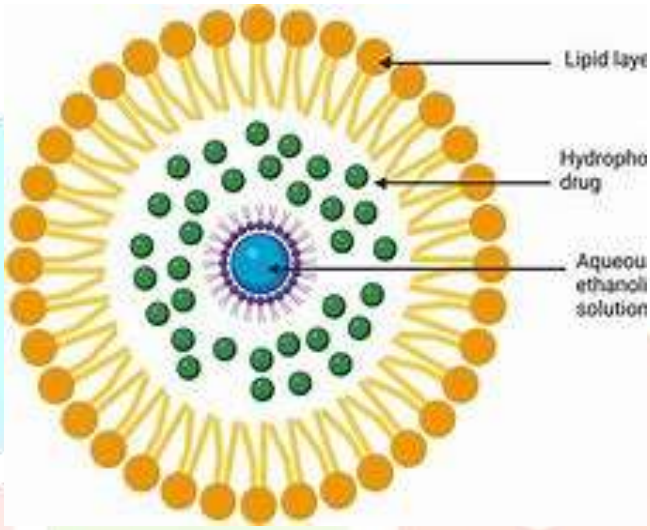
The third generation of transdermal delivery systems is poised to have a large impact on drug delivery because it targets its effects to the stratum corneum. This targeting enables stronger disruption of the stratum corneum barrier, and thereby more effective transdermal delivery, while still protecting deeper tissues. We have found that in this way, novel chemical enhancers, electroporation, cavitational ultrasound and more recently microneedles, thermal ablation and microdermabrasion. have been shown to deliver macromolecules, including therapeutic proteins and vaccines, across the skin in human clinical trials.

These advances were made possible in part by the emergence of technologies to localize effects to the stratum corneum combined with recognition that the safety afforded by localization should make these more aggressive approaches medically acceptable.

1.12 LIMITATIONS[13]

1. It cannot administer drug that requires high blood levels.
2. Drug or drug formulation may cause skin irritation and sensitization.
3. The barrier function of the skin changes from one site to another on the same person, from person to person and with age.
4. Not practical, when the drug is extensively metabolized in skin and when molecular size is great enough to prevent the molecules from diffusing through the skin.

CHAPTER 2



ETHOSOMES

2. ETHOSOMES

INTRODUCTION TO ETHOSOMES

Ethosomes are the modified form of liposomes that are high in ethanol content. Hence, they are called as “ethanolic liposomes”. The ethosomal system is composed of phospholipid,

ethanol and water. They can penetrate the skin and enhance compound delivery both to deep skin strata and systemically. This ethanol fluidizes both ethosomal lipids and bilayers of the stratum corneum intercellular lipid. The soft, malleable vesicles then penetrate the disorganized lipid bilayers[14].

Ethosomes can be defined as non-invasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents.

The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding of a vesicle derivative, known as an Ethosomes[15].

Ethosomes are the slight modification of well-established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water.

Ethosomes works as Vesicles, ethanol, and skin lipids interact synergistically in ethosomes function. Because ethosomes and skin lipids interact better than liposomes, they improve the distribution of active ingredients over liposomes. When ethanol interacts with the lipid molecules in the polar head group region, the transition temperature of the lipids in the stratum corneum is decreased. These cause the drug to be delivered into the deep layers of the skin by increasing fluidity and lowering lipid multilayer density. Furthermore, ethanol imparts smoothness and flexibility to vesicles, facilitating deeper penetration into the epidermal layer.

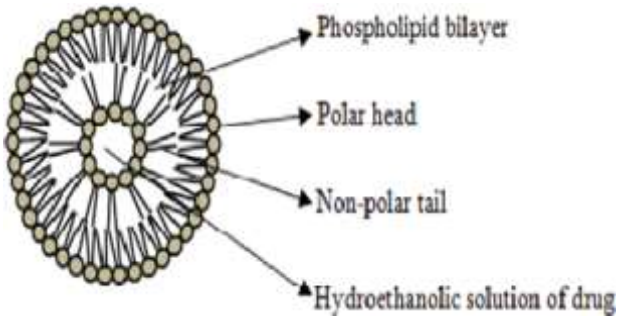


Figure No.6: Structure of Ethosome

The size range of Ethosomes may vary from tens of nanometers (nm) to microns (μ) Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux.

Different reports show a promising future of ethosomes in making transdermal delivery of various agents more effective. Ethosomes also offer a good opportunity for the non-invasive delivery of small, medium, and large sized drug molecules.

Preparation of ethosomes is easy with no complicated equipment involved and therefore can be scaled up to industrial level. These vesicular systems were found to be highly ancient carriers for the delivery of molecules with various lipophilicities into and through the skin, in in-vitro and in-vivo, in animal and clinical studies.

Ethosomes have undergone extensive research since they were invented in 1996, new compounds were added to their initial formula, which led to the production of new types of ethosomal systems. Different preparation techniques are used in the preparation of these novel carriers. For ease of application and stability, ethosomal dispersions are incorporated into gels, patches, and creams. Highly diverse in-vivo models are used to evaluate their efficacy in dermal/transdermal delivery, in addition to clinical trials[22].

2.1. Advantages of Ethosomal Drug Delivery

Ethosomal drug delivery systems contain several advantages. Few advantages are:

1. Ethosomes are platform for the delivery of large and diverse group of drugs across the skin (Peptides, protein molecules).
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The Ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. High market attractiveness for products. Relatively simple to manufacture with no complicated technical investments required for production of ethosomes[25].

2.2. Disadvantages of Ethosomal Drug Delivery

They required High blood levels cannot be administered limited only to potent molecules, those requiring a daily dose of 10mg or less.

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
2. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.

4. Adhesive may not adhere well to all types of skin.

5. May not be economical.

6. Poor yield

7. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.

8. In case if shell locking is ineffective then the Ethosomes may coalescence and fall apart on transfer into water.

9. Loss of product during transfer from organic to water media[26].

2.3 Ethosomal system types[27]:

Ethosomes can be subdivided based on their compositions (Fig.no:7).The major differences between various ethosomes for transdermal drug delivery is shown in Table 3.

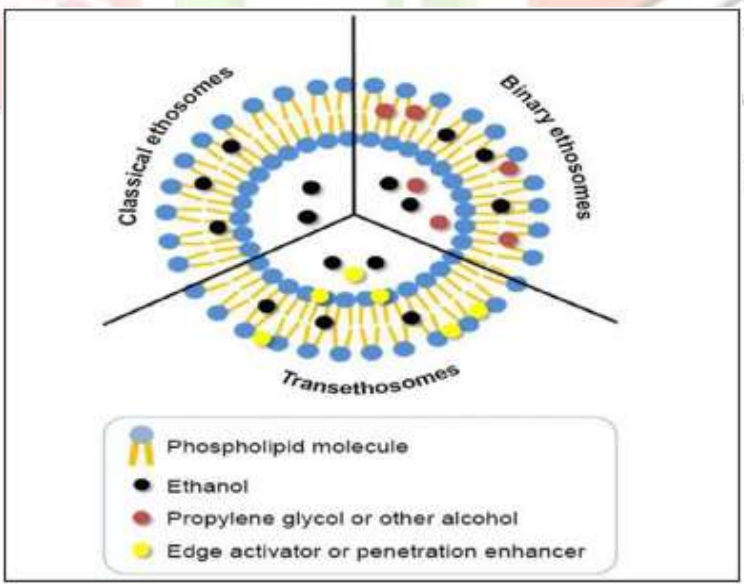


Figure No.7: Subtypes of Ethosomes

There are three types of ethosomal systems, classified on the basis of their compositions.

1. Classical ethosomes:

Classical ethosomes are a modification of classical liposomes and are composed of phospholipids, a high concentration of ethanol up to 45% w/w, and water. Classical ethosomes were reported to be superior over classical liposomes for transdermal drug delivery because they were smaller and had negative ζ-potential and higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profiles compared to classical liposomes.

2. Binary ethosomes:

Binary ethosomes were developed by adding another type of alcohol to the classical ethosomes. The most commonly used alcohols in binary ethosomes are propylene glycol (PG) and isopropyl alcohol (IPA).

3.Transethosomes:

Transethosomes are the new generation of ethosomal systems. This ethosomal system contains the basic components of classical ethosomes and an additional compound, such as a penetration enhancer or an edge activator (surfactant) in their formula. These novel vesicles were developed in an attempt to combine the advantages of classical ethosomes and deformable liposomes (transfersomes) in one formula to produce transethosomes. Many researchers have reported superior properties of transethosomes over classical ethosomes[16.]

Table 3. Difference between various ethosomes for transdermal drug delivery[28]

S. No.	Parameter	Classical ethosomes	Binary ethosomes	Transethosomes
1	Composition	1. Phospholipids	1. Phospholipids	1. Phospholipids
		2. Ethanol	2. Ethanol	2. Ethanol
		3. Stabilizer	3. Propylene glycol (PG) or other alcohol	3. Edge activator (surfactant) or penetration enhancer
		4. Charge inducer	4. Charge inducer	4. Charge inducer
		5. Water	5. Water	5. Water
		6. Drug/agent	6. Drug/agent	6. Drug/agent
2	Morphology	Spherical	Spherical	Regular or irregular spherical shapes
3	Size	Smaller than the classical liposomes	Equal to or smaller than classical ethosomes	Size based on type and concentration of penetration enhancer or edge activator used
4	Entrapment efficiency	Superior than traditional liposomes	Often higher than traditional ethosomes	Higher than the majority of typical ethosomes
5	Skin permeation	Usually greater than traditional liposomes	Usually on par with or superior to traditional ethosomes	Often higher than traditional ethosomes

S. No.	Parameter	Classical ethosomes	Binary ethosomes	Transethosomes
6	Stability	More robust than traditional liposomes	Stabler than classical ethosomes	There was no clear trend found

2.4 METHOD OF PREPARATION OF ETHOSOMES[35]

Cold method and hot method are the two conventional methods used for the preparation of Ethosomes. Classic mechanical dispersion method, ethanol-injection sonication method, thin-film hydration method, reverse-phase evaporation method was also reported in various literatures. Among this cold method is the most commonly used method.

a) Cold method

Dissolve phospholipid and other lipid material in ethanol in a covered vessel at room temperature by vigorous stirring. Add propylene glycol or another polyol during stirring. Heat the mixture up to 30^0C in a water bath. Heat the water up to 30^0C in a separate vessel and add to the above mixture slowly in a fine stream. The drug can be dissolved in water or ethanol depending on its hydrophilic/hydrophobic properties. Continue stirring for another 5 min and cool the resultant vesicle suspension at room temperature. The vesicle size of Ethosomal formulation can be modulated to desire extend using sonication or extrusion method. Finally, the formulation should be stored under refrigeration.

b) Hot method

In this method, disperse the phospholipid in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel mix ethanol and glycols and heat this mixture up to 40^0C. Once both mixtures reach 40^0C, add the organic phase to the aqueous one. Continue stirring for another 5 min and cool the resultant vesicle suspension at room

temperature. The drug can be dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. Modulation of Ethosomal vesicle size can be done by sonication or extrusion method.

c) Classic mechanical dispersion method

Dissolve phospholipid in an organic solvent or a mixture of organic solvents in a round-bottom flask (RBF). Remove the organic solvent using a rotary vacuum evaporator above lipid transition temperature to form a thin lipid film on the wall of the RBF. Traces of the solvent should be removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydrate the lipid film with hydroethanolic solution of drug by rotating the flask at suitable temperature with or without intermittent sonication.

d) Ethanol injection–sonication method

In this method, the organic phase containing the dissolved phospholipid in ethanol is injected to the aqueous phase, using a syringe system, at a flow rate of 200 µL/min, then homogenized with an ultrasonic probe for 5 minutes.

e) Thin-film hydration method

This represents the extension of the conventional liposome-preparation method, but in this method the lipid film is hydrated by a hydroethanolic solution. The phospholipid is first dissolved in chloroform only or a chloroform–methanol mixture at ratios of 3:110 or 2:186 in a clean, dry, round-bottom flask. Organic solvents are removed by a rotary vacuum evaporator at a temperature above the lipid-phase transition temperature. Then, the traces of the solvents are removed from the deposited lipid film under vacuum overnight. The lipid film is then hydrated with a water–ethanol solution or phosphate buffered saline–ethanol solution. During the hydration process, the lipid film is rotated and heated at the required temperature, which depends on the phospholipid property, for 30 minutes, 1 hour, or 6 hours.

f) The reverse-phase evaporation method

This is the least used method and specially designed to produce large unilamellar vesicles. The organic phase is prepared by dissolving the phospholipid in diethyl ether and then mixing it with the aqueous phase at a ratio of 3:1 v/v in an ultrasonic bath at 0℃ for 5 minutes to form a water-in-oil emulsion. The organic solvent is removed under reduced pressure to produce a gel, which turns into a colloidal dispersion upon vigorous mechanical agitation.

- **Composition of Ethosomes[42]**

Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The non-aqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. The high concentration of ethanol makes the Ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

- **Advantages of high alcohol content**

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in Ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol. Touitou discovered and investigated lipid vesicular systems

embodying ethanol in relatively high concentration and named them Ethosomes. The basic difference between liposomes and Ethosomes lies in their composition. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in Ethosomes was suggested to be the main reason for their better skin permeation ability[46].

The high concentration of ethanol (20-50%) in Ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the Ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature of Ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of Ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like Acyclovir, Minoxidil, Trihexyphenidyl, Testosterone, Cannabidiol and Zidovudine[47].

2.5 Mechanism of ethosomal system skin permeation[22,36,51]

Ethanol and phospholipids are reported to act synergistically to enhance the skin permeation of drugs in ethosomal formulations. Ethanol fluidizes the lipid bilayers of the ethosomal vesicles and the stratum corneum simultaneously, changing the arrangement and decreasing the density of skin lipids. Therefore, the highly malleable and soft vesicles of an ethosomal system will penetrate the altered structure of the stratum corneum and create a pathway through the skin. The release of the therapeutic agent occurs by the fusion of these vesicles into cell membranes in the deeper layers of the skin.

The permeation of molecules from an ethosomal system through the skin involves several key mechanisms such as,Stratum Corneum Disruption, Vesicle Flexibility, Enhanced Solubility, Diffusion Gradient, Molecular Interaction and Sustained Release Mechanism.

By leveraging these mechanisms, ethosomal systems effectively enhance the transdermal delivery of a variety of therapeutic agents. A proposed mechanism is illustrated as,

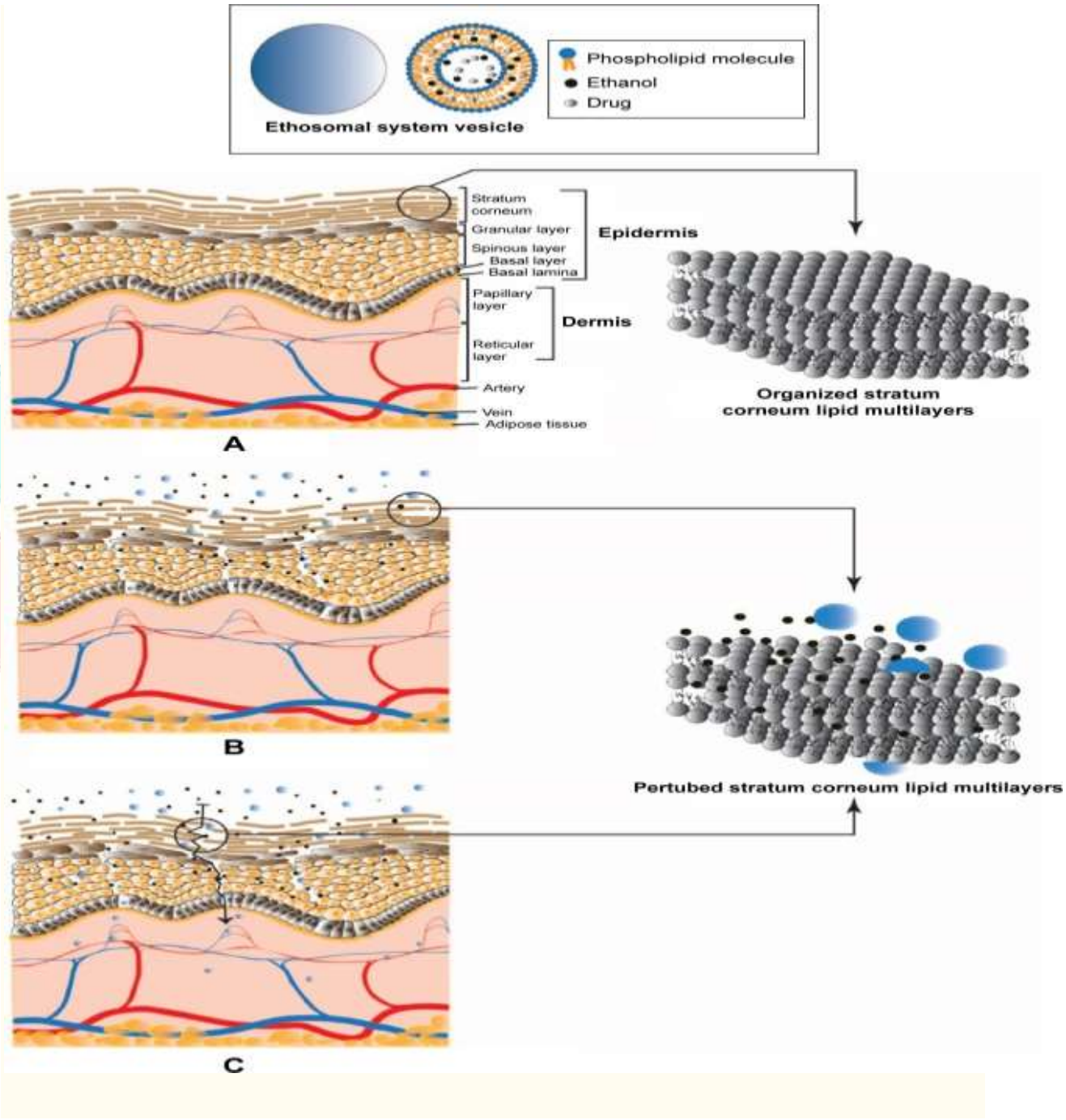


Figure No.9: Proposed mechanism for permeation of molecules from ethosomal system through the skin.

Notes: (A) Normal skin; (B) Skin-lipid perturbation by ethanol effects; (C) Penetration of the soft malleable ethosomal system vesicles.

2.6 SKIN RASHES AND IRRITATION[37,43]

A rash, also called dermatitis, is swelling (puffiness) or irritation of the skin. It might be red, itchy, dry, bumpy, scaly, crusty or blistered. Rashes are a symptom of many different medical conditions. Things that can cause a rash include other diseases, irritating substances, allergies and your genetic makeup. Some rashes develop immediately while others form over several days. If you scratch your rash, it might take longer to heal. 'Contact dermatitis' and 'eczema' are terms that describe common rashes.

Causes

Dry skin, exposure to poison oak and poison ivy, and pets, food and consumer products all cause rashes. Ingredients in soaps, cosmetics, detergents, dyes, latex, deodorants, fragrances and rubber products can produce skin irritation and rashes. Rashes can also signal a fungal or bacterial infection, or a minor symptom of a more serious medical problem.

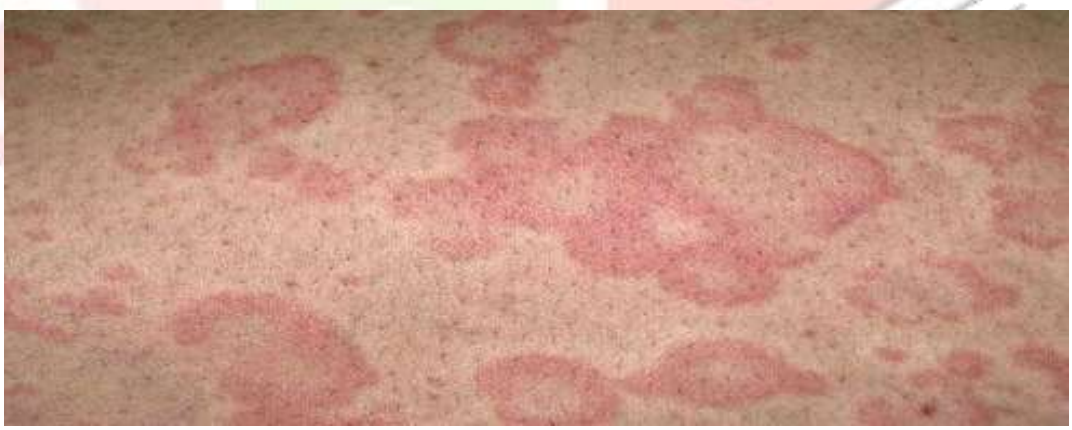


Fig No.10: Common skin rashes

Common types of rashes

Common types of rashes are:

1.) Eczema, also called atopic dermatitis. Eczema can cause dry, chapped, bumpy areas around the elbows and knees or more serious cases of red, scaly, and swollen skin.

2.) Irritant contact dermatitis is caused by contact with something irritating, such as chemicals, soaps, detergents and the sun. Irritant contact dermatitis can be red, swollen and itchy.

3.) Allergic contact dermatitis is a rash caused by contact with something you are allergic to, such as rubber, hair dye, or nickel, a metal found in some jewelry. Allergic contact dermatitis can produce a red, scaly, crusty rash. Contact with poison ivy, oak and sumac can cause this rash.

Treatments

Rash treatments include moisturizers, lotions, baths, cortisone creams that relieve swelling, and antihistamines, which relieve itching. For eczema, the physician may suggest moisturizers called emollients that retain water in skin, keeping it smooth while soothing any itch. For poison ivy, physicians may recommend cool showers and calamine lotion, and an antihistamine for severe cases to decrease itching and redness. For rashes caused by an allergen, including hives, the doctor will need to determine which food, substance, medicine, or insect caused the problem. A medical test can determine which allergens are causing trouble.

There are many over-the-counter products for treating simple rashes and skin irritation. Antihistamine creams, anti-itch lotions and moisturizers commonly treat rashes. If a rash persists for more than two or three days, visit a physician who can prescribe an effective treatment.

ECZEMA

Fig No.11: Eczema and it's types

Eczema is a condition that causes your skin to become dry, itchy and bumpy. This condition weakens your skin's barrier function, which is responsible for helping your skin retain moisture and protecting your body from outside elements.

Eczema is a type of dermatitis.

Dermatitis is a general term for conditions that cause inflammation of the skin. Examples include atopic dermatitis (eczema), contact dermatitis and seborrheic dermatitis (dandruff). These conditions cause red rashes, dry skin and itchiness among other symptoms. Severe cases can include crusty scales, painful cracks in your skin or blisters that ooze fluid.

The main causes of dermatitis vary depending on the type. However, environmental triggers can often be the reason for specific flare-ups.

The trigger is what causes your skin to have a reaction. It could be a substance, your environment, or something happening in your body.

"Dermatitis" is a word used to describe a number of skin irritations and rashes caused by genetics, an overactive immune system, infections, allergies, irritating substances and more. Common symptoms include dry skin, redness and itchiness. In the word "dermatitis," "derm" means "skin" and "itis" means "inflammation." The word as a whole means "inflammation of the skin." The rashes range from mild to severe and can cause a variety of problems, depending on their cause.

The types of dermatitis include, but not limited to:

- Atopic dermatitis (eczema)
- Contact dermatitis
- Diaper dermatitis (rash)
- Dyshidrotic dermatitis
- Seborheic dermatitis
- Deboresic dermatitis

Common triggers that cause dermatitis to flare include stress, hormonal changes, the environment and irritating substances

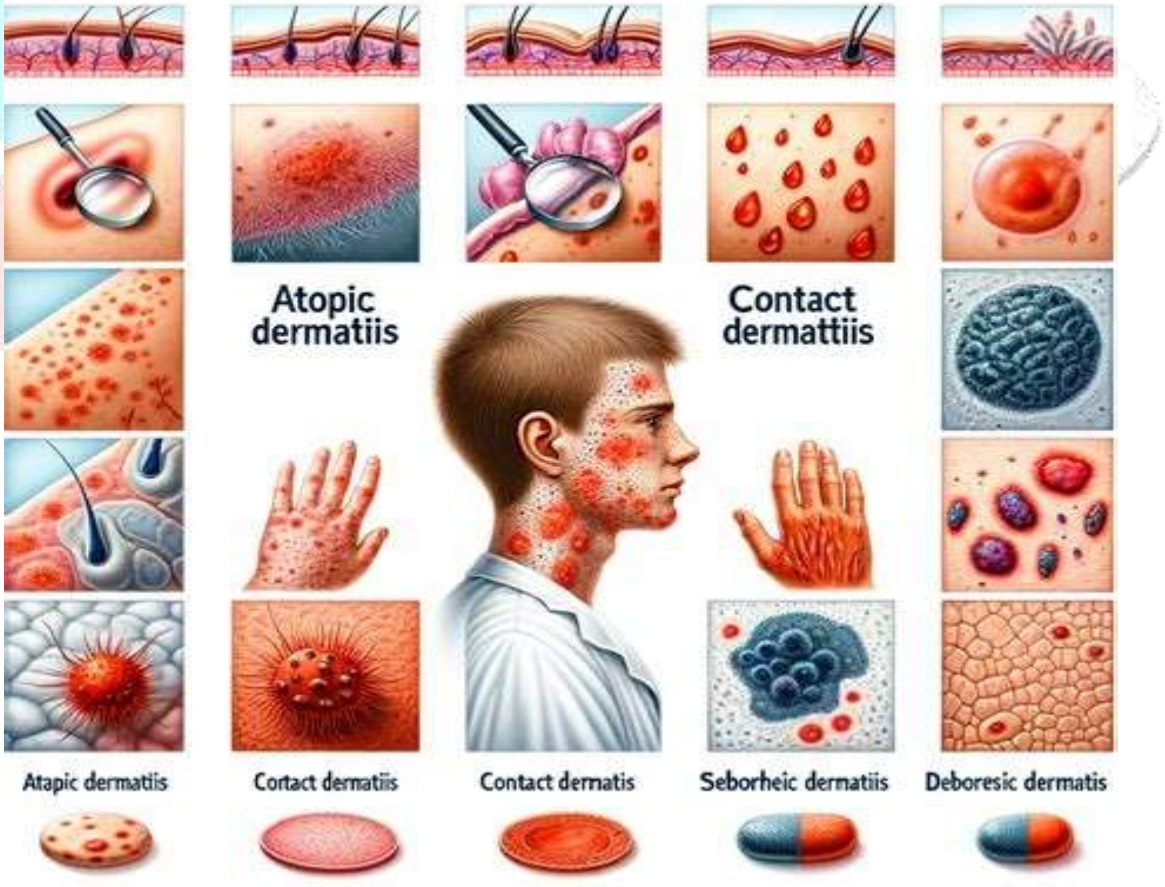


Fig No.12: Types of Dermatitis

PSORIASIS

It is a chronic inflammatory skin disease-with increased epidermal proliferation related to dysregulation of immune system estimated to affect around 2-3% of the world population. This disease has different types: psoriasis vulgaris, guttate psoriasis, erythrodermic psoriasis, pustular psoriasis and nail psoriasis.

The first type is the most common form of psoriasis, which is characterized by red, scaly and raised plaques. Classic psoriasis vulgaris mainly infects specific areas such as elbows, knees and the scalp. It can also remain localized or become generalized over time and the plaques may differ in size.

Treatment of psoriasis depends on many factors such as the extent of the disease, its influence on patient's life, and the life perception of patient's illness. Different types of treatment can be used such as ultra violet B (UVB), psoralen plus ultraviolet A (PUVA), methotrexate (MTX), cyclosporine, vitamin D3 analogues, topical retinoids and topical corticosteroids such as Fluticasone Propionate (FP).

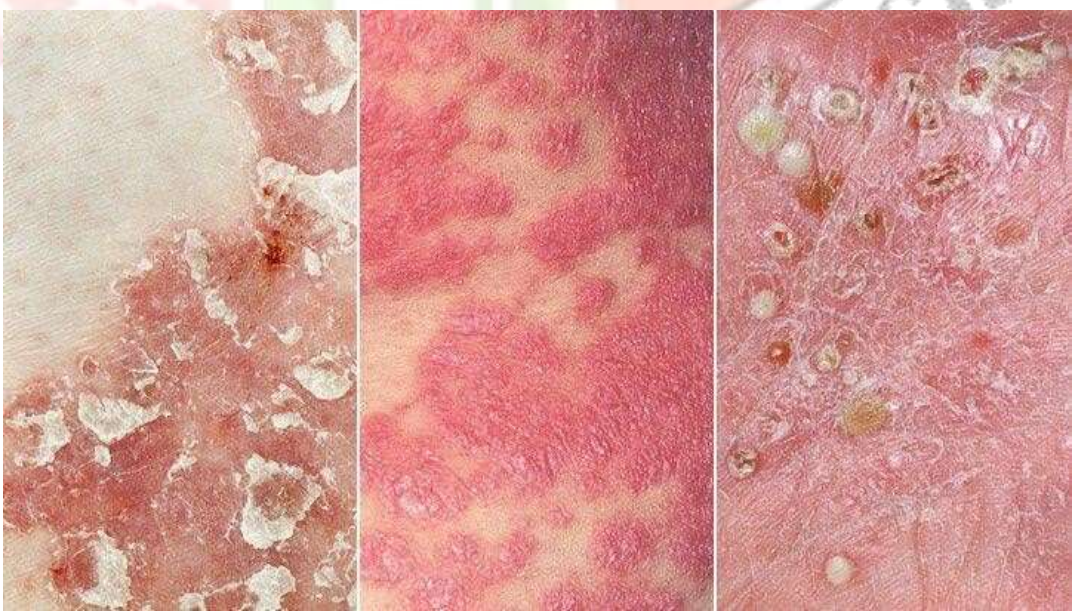


Fig No.13: Types of psoriasis

FP is a highly potent glucocorticoid receptor agonist which possesses immunosuppressive, anti-inflammatory and anti-proliferative effects. FP has been used in topical therapy for the treatment of mild to moderate psoriasis.

Targeting of topically applied drugs is becoming a major Centre of interest for many pharmaceutical groups working in dermatology to improve drug penetration into different skin layers.

Thus, several vesicular systems were used in the treatment of psoriasis such as Fluticasone Propionate (FP) loaded liposomes hydrogel, FP loaded transfersomes, FP loaded niosomes and corticosteroid Nano capsule suspension.

Transfersomes have been utilized for dermal and transdermal drug delivery, also have several advantages over other Nano systems such as biocompatibility, biodegradability and transportation of therapeutic agents through narrow constriction without any significant loss. The high deformability of transfersomes gives better penetration of intact vesicles.

Therefore, the aim of this work is to design and evaluate Fluticasone Propionate (FP) loaded ethosomes as a topical formulation for the treatment of localized plaque psoriasis.

Gels are biphasic swollen networks occupying both the cohesive characteristics of solids, and the diffusive transport properties of liquids. In contrast to ointments and creams, gels often grant immediate release of active pharmaceutical ingredient, regardless of the water solubility of the drug. They have limited risk of inflammation and unwanted reaction and are remarkably biocompatible.

Gels are easy to apply on the skin and there is no need to remove it. Gels for skin application possess various agreeable characters. They are thixotropic and non-greasy having emollient action. Gels are readily spreadable that can be conveniently wiped out upon washing. Since the gels are washable with water.

Carbopol is a synthetic polymer made of carbomers. Carbomer polymers are cross linked together and form a microgel structure that is useful in dermatological applications. As these polymers are anionic in nature so neutralization in necessary for microgel structure that's why

organic amines like tri ethanolamine are used for such purpose.

CHAPTER 3

AIM & OBJECTIVE

3. AIM AND OBJECTIVES

AIM

To formulate design, characterize and evaluate Fluticasone Propionate Ethosomal gel for the treatment of skin conditions.

OBJECTIVE

Fluticasone Propionate is a glucocorticoid steroid with anti-inflammatory and immune-suppressive abilities. It is a synthetic glucocorticoid. It was well absorbed topically and shows improved bioavailability. Fluticasone propionate was first approved in 1990.

This medication is used to treat a variety of skin conditions (such as eczema, psoriasis, rash). Fluticasone reduces swelling (inflammation), itching, and redness. This medication is a medium-strength corticosteroid. It is available in several forms, including cream, ointment, and lotion.

The main objective of this research work was to develop a novel formulation and evaluation of transdermal drug delivery system by using a pure drug of Fluticasone Propionate in the form of ethosomal gel to provide a better anti-inflammatory activity and prolonged duration of action of drug to treat varying diseases associated with the skin.

PLAN OF WORK

- ✓ **LITERATURE REVIEW**
- ✓ **SELECTION AND PROCUREMENT OF DRUG AND EXCIPIENTS**
- ✓ **PRE-FORMULATION STUDIES**
- ✓ **To carry out the preformulation studies of drug as follows,**
  - Organoleptic properties
  - Solubility studies
  - Melting point
  - Specific optical rotation
  - Moisture content determination
- ✓ **COMPATIBILITY STUDIES**
  - Fourier Transform Infra-Red Spectroscopy studies
  - Differential scanning calorimetry (DSC)
  - **Determination of λ max**
- ✓ **Preparation of Ethosomes**

✓ **Physicochemical characterization**

• Surface morphology

• Optical microscopy

• Entrapment Efficiency

• Vesicle size and Zeta Potential

• Polydispersity Index

• Release Kinetics

✓ **Preparation of Ethosomal gel**

✓ **POST FORMULATION STUDIES**

✓ **The Ethosomal gel will be evaluated for the following parameters:**

• Physical examination

• Washability

• Viscosity

• Spreadability

• pH measurement

• Drug content uniformity

• Extrudability

• Drug content study

• Homogeneity

• In vitro release studies for gel

• Stability studies

- ✓ **RESULTS AND DISCUSSION**
- ✓ **SUMMARY AND CONCLUSION**
- ✓ **FUTURE PROSPECTUS**
- ✓ **BIBLIOGRAPHY**

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS USED IN FORMULATION

For the formulation of Fluticasone Propionate ethosomal gel, the following Raw materials are procured from the mentioned vendor sources.

Table No 6: List of raw materials used

MATERIALS	SOURCE
Fluticasone Propionate **(API)**	J K Chemicals, Vapi, Gujarat, India
Phospholipids (soya lecithin)	A M Nutratech Private Limited, Chennai
Ethanol	Central drugs & pharmaceuticals, Chennai
Propylene glycol	Reliable Petro Chems, Chennai
Cholesterol	SD fine chemicals, Mumbai
Carbopol 934 powder	Dr. Milton laboratories, Chennai

Triethanolamine	A B Enterprises, Mumbai

4.2 EQUIPMENT / INSTRUMENTS USED IN FORMULATION

For the formulation of Fluticasone Propionate ethosomal gel, the following equipment/instruments are procured from the following manufacturers/ suppliers.

Table No 7: List of Equipment / Instruments used

EQUIPMENT / INSTRUMENTS	MANUFACTURERS / SUPPLIERS
Electronic weighing balance	Mc Dalal, Chennai
Magnetic stirrer	Remi Instruments, Mumbai
Digital pH-meter	Labman scientific instrument (LMPH9), Chennai
Ultra Sonicator	Lark, Chennai
Melting point apparatus	Guna Enterprises, Chennai
Glass Wares	Borosil, Mumbai
Brookfield viscometer	BRK Instruments, India
Optical microscopy	Leica, Germany
Dialysis membrane	Hi media laboratory, Mumbai
Scanning Electron Microscopy	Tescan, China
UV-Visible Spectrophotometer	Shimadzu UV, Japan
FT-IR Spectrophotometer (4000/6000Series)	Bruker Optics, Germany

Stability Chamber	Newtronics, Mumbai

CHAPTER 5

EXPERIMENTAL WORK

EXPERIMENTAL METHODS IN PREFORMULATION STUDIES

Preformulation testing is the first step in rational development of dosage forms of a drug substance. It is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug combination with pharmaceutical excipients in the dosage form. Hence, preformulation studies were performed for the obtained sample for identification and compatibility studies. It includes the physicochemical properties of drug and an assessment of their relevance to the final formulation.

Preformulation studies of ethosomal gels are critical for developing an effective transdermal drug delivery system. These studies evaluate various factors that influence the formulation's stability, efficacy and compatibility with the active pharmaceutical ingredient (API). It includes the following, but not limited to Selection of Drug, Lipid Selection, Formulation Composition, Characterization of Ethosomes, Stability Studies, Release Studies, Skin Penetration Studies, Compatibility Studies, Formulation Optimization and Regulatory Considerations.

Preformulation studies are essential for the successful development of ethosomal gels. Each step, from drug selection to formulation optimization, contributes to creating a stable and effective product for transdermal drug delivery. By carefully analyzing each component, to develop ethosomal formulations that significantly enhance drug permeation and therapeutic efficacy[52].

5.1 ORGANOLEPTIC PROPERTIES

Organoleptic properties refer to the characteristics of a substance as experienced by the senses including appearance, color, odor, taste, and texture. For ethosomal gels, these properties are important as they can influence patient acceptance and overall usability. Here's a detailed look at the organoleptic properties of ethosomal gels:

The general appearance like nature, color, odor, texture etc. were performed by visual observations and compared with the standard of drug given in pharmacopoeia for identification of drug.

Color: To a small quantity of drug were taken on the white butter paper and it was viewed in a well illuminated place.

Odor: A very less quantity of drug was smelled to get the odor.

Texture: A small quantity of drug was taken and view for its texture properties.

The organoleptic properties of pure drug was evaluated and the obtained results were given in the **Table no.10.**

5.2 SOLUBILITY STUDY

To know the solubility of Fluticasone Propionate, solubility study is performed in water, Dimethyl Sulfoxide, Dimethyl Formamide, Acetone, Dichloromethane, Ethyl acetate, Chloroform, Methanol and 95% Ethanol.

i) Solubility in water:

Known amount of Fluticasone Propionate is dissolved in 5ml of distilled water and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using distilled water as blank.

ii) Solubility in Dimethyl Sulfoxide:

Known amount of Fluticasone Propionate is dissolved in 5ml of Dimethyl Sulfoxide and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Dimethyl Sulfoxide as blank.

iii) Solubility in Dimethyl Formamide:

Known amount of Fluticasone Propionate is dissolved in 5ml of Dimethyl Formamide and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Dimethyl Formamide as blank.

iv) Solubility in Acetone:

Known amount of Fluticasone Propionate is dissolved in 5ml of Acetone and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Acetone as blank.

v) Solubility in Dichloromethane:

Known amount of FP is dissolved in 5ml of Dichloromethane and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Dichloromethane as blank.

vi) Solubility in Ethyl acetate:

Known amount of FP is dissolved in 5 ml of Ethyl acetate and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Ethyl acetate as blank.

vii) Solubility in Chloroform:

Known amount of Fluticasone Propionate is dissolved in 5 ml of Chloroform and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Chloroform as blank.

viii) Solubility in Methanol:

Known amount of Fluticasone Propionate is dissolved in 5 ml of Methanol and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using Methanol as blank.

ix) Solubility in 95% Ethanol:

Known amount of Fluticasone Propionate is dissolved in 5 ml of 95% Ethanol and the solution is filtered by using whatmann filter paper. The absorbance of filtrate is measured spectrophotometrically by using 95% Ethanol as blank.

The Solubility test results of Fluticasone propionate with varying solvents were performed and the results shown in the **Table no.11**

5.3 MELTING POINT

For determination of melting point, USP method was followed. Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which the drug started to melts and the temperature, when the entire drug gets melted was noted. It is one of the parameters to determine the purity of drugs. The accurate determination of the melting point can be essential for confirming the identity and quality of the compound in the formulation.

5.4 SPECIFIC OPTICAL ROTATION

The specific optical rotation of fluticasone propionate is a measure of its optical activity, which can be used to assess the purity and stereochemistry of the compound.

Reagent used: Ammonium hydroxide

Preparation of 6N ammonium hydroxide:

Dilute 400 ml of Ammonia water, Stronger Ammonium hydroxide with water to make 1000 ml.

Procedure:

Heat 80 ml of water to 50^0C. Allow to cool and add 0.2 mL of 6N ammonium hydroxide. Allow to stand for 30 mins and dilute with water to 100 ml. Measure the specific

rotation of the sample solution at 589 nm at 20^0C (Sodium lamp) by five replicates using water as blank.

5.5 MOISTURE CONTENT DETERMINATION

The titrimetric determination of water is based upon the quantitative reaction of water with an anhydrous solution of Sulphur dioxide and iodine in the presence of a buffer that reacts with hydrogen ions. In the original titrimetric solution, known as Karl Fisher Reagents, the sulfur dioxide and iodine were dissolved in pyridine and methanol. The test specimen may be titrated with the reagent directly, or the analysis may be carried out by a residual titration procedure.

Equipment and Reagents used:

- **Karl Fischer titrator**
- **Karl Fischer reagent** (a solution containing iodine and sulfur dioxide)
- **Solvent** (e.g. pyridine and methanol)
- **Sample-** Fluticasone propionate

Procedure:

Sample Preparation:

A quantity of about 5g fluticasone propionate was taken for the sample preparation.

Solubilization:

Dissolve the sample in a suitable solvent and ensure that the solvent is compatible with the Karl Fischer reagent.

Titration Setup:

Prepare the Karl Fischer titrator and ensure that the titrator is calibrated and that the reagent is fresh.

Performing the Titration:

Introduce the sample solution into the titration vessel.

Start the titration. The Karl Fischer reagent will react with the water in the sample, and the titrator will monitor the amount of reagent consumed.

End Point Detection:

The titrator will indicate the end point when the water has been completely reacted, typically observed through a change in the potential measured by the electrode.

Calculating Moisture Content:

The amount of Karl Fischer reagent used at the end point can be used to calculate the moisture content. This is typically expressed as a percentage (% w/w).

The formula to calculate moisture content is:

$$\%\,Water = \frac{B.R\,X\,F}{Wt} x100$$

Where,

B.R = volume of Karl Fischer reagent consumed in ml

F= Karl Fischer reagent factor in mg/ml and

Wt= weight of sample taken in g

Sample Stability:

Ensure that the sample is stable in the solvent and does not react with the Karl Fischer reagent.

Environmental Conditions:

Conduct the titration in a controlled environment to prevent external moisture interference.

The Karl Fischer method is a reliable technique for determining the moisture content in fluticasone propionate. Following the standard procedures and maintaining proper calibration will yield accurate and reproducible results.

5.6 COMPATIBILITY STUDIES:

5.6.1 Fourier Transform Infra-Red Spectroscopy studies:

Fourier Transform Infrared Spectroscopy (FTIR) is a valuable technique for analyzing the functional groups and molecular structure of fluticasone propionate API. Here's an overview of the FTIR analysis process and expected spectral characteristics:

The Fourier Transform Infra-Red analysis were conducted for the structure characterization. These studies were carried out to determine authenticity of the drug and drug-excipient compatibility using Fourier Transform Infrared Spectrophotometer. KBr pellets were prepared for the active ingredient Fluticasone propionate. The drug sample was scanned on IR spectrophotometer between 400-4000 cm^{-1} using KBr disc. Analyze the resulting spectrum, identifying peaks corresponding to different functional groups. The obtained IR spectrum was interpretated with the structure of Fluticasone Propionate.

The primary objective of this investigation was to identify a stable storage condition for drug in solid state and identification of compatible excipients for formulation. The FTIR spectra of pure drug Fluticasone propionate, drug with excipients and the gel formulation was done and given in the **Table no. 12** and **13** with the **Fig.15** and **16** respectively.

FTIR is an effective method for confirming the identity and purity of fluticasone propionate API by analyzing its functional groups. Comparing the obtained spectrum with reference spectra can help verify the presence of specific chemical bonds and functional groups, ensuring the integrity of the API.

5.6.2 Differential scanning calorimetry (DSC):

Differential Scanning Calorimetry (DSC) is an analytical technique used to study the thermal properties of materials, including fluticasone propionate. It is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a reference and sample are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the study. Mainly, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. A DSC study of fluticasone propionate is essential for understanding its thermal properties, stability, and potential interactions in formulations. The DSC analysis of Fluticasone propionate was given in **Fig. 17**.

5.6.3 Determination of λ max:

The primary aim in determination of λ max is to identify the wavelength at which fluticasone propionate exhibits maximum absorbance in a UV-Vis spectrophotometer. This information is crucial for developing methods for quantitative analysis. It is a fundamental step in developing analytical methods for its quantification and understanding its behavior in different formulations and also ensuring its quality and efficacy. The absorption maxima of Fluticasone Propionate were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer as follows,

REAGENT PREPARATION:

Preparation of standard drug solution

An accurately weighed quantity of 100 mg of Fluticasone Propionate were taken in the 100 ml capacity volumetric flask and 100ml of phosphate buffer pH 7.4 were added. This is labelled as stock solution. It contains 1mg/ml of drug.

Preparation of standard graph of FP

From the stock solution,10 ml was taken in a 100ml of volumetric flask and the solution were made up to 100ml with ethanolic phosphate buffer pH 7.4. This solution containing 100µg/ml. From that series of dilution containing 10, 20, 30, 40 and 50µg/ml of FP solution

was prepared. The absorbance of the above dilution was measured in UV spectrophotometer at 239 nm using the buffer of pH 7.4 buffer solution as blank. The concentration of FP and corresponding absorbance was given in the **Table no. 14** and **Fig No.18**. The absorbance was plotted against the concentration of FP and this calibration curve were used for estimating the Fluticasone Propionate in the samples.

5.7 PREPARATION OF ETHOSOMES:

Cold Method:

- Ethosomal formulations were prepared by using the cold method.
- The ethanolic vesicular system were composed of phospholipids (2% to 5%w/v), ethanol (20% to 40% v/v), propylene glycol (10 % v/v), drug and distilled water.
- Phospholipids were dissolved along with the drug in ethanol.
- This mixture was heated to 40^0C and a fine stream of distilled water were added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container.
- Mixing were continued for an additional 5min, while maintaining the systemat 40^0C.
- The preparation was left to cool at room temperature for 30min and then it was sonicated at 40^0C for five cycles of 3min each with a minute rest between cycles using ultra solicitor.

- Eight formulation were prepared using different concentrations of phospholipid and ethanol, among them optimized formulation was selected for characterization and evaluation studies.

The various compositions employed in the Formulation of Fluticasone Propionate Ethosomes were represented in the **Table No. 8** as follows,

Table No 8: Compositions of Fluticasone Propionate Ethosomal formulations

S.NO	Ethosomal Formulations (F)	Fluticasone propionate (g)	Phospholipids (soya lecithin) (%)	Cholesterol (g)	Propylene glycol (%)	Ethanol (%)
1	F1	0.20	2	0.005	10	20
2	F2	0.20	4	0.005	10	30
3	F3	0.20	5	0.005	10	20
4	F4	0.20	3	0.005	10	20
5	F5	0.20	2	0.005	10	40
6	F6	0.20	3	0.005	10	20

7	F7	0.20	4	0.005	10	30
8	F8	0.20	3	0.005	10	40

5.8 PHYSICOCHEMICAL CHARACTERIZATION:

The optimized Ethosomes containing drug were characterized by studying various physico-chemical properties as follows,

5.8.1 Surface morphology

Analyzing the surface morphology of fluticasone propionate using Scanning Electron Microscopy (SEM) provides valuable insights into the physical characteristics of the API, such as particle shape, size and surface features.

The size and shape of the vesicles were observed in the scanning electron microscopy (SEM). One drop of ethosomal suspension (F1 to F8) were mounted on a clear glass stub.

It was then air dried and gold coated using sodium aurothiomalate to visualize under scanning electron microscope (SEM) at 10,000 magnifications. The Scanning electron microscopic images of F1 formulation was given in **Fig no.19**.

5.8.2 Optical microscopy

Optical microscopy can be useful technique for visualizing and studying the morphology and structural characteristics of fluticasone propionate ethosomes.

Reflected light microscopy is used to study the surface of a sample. Formulation was placed under the microscope lens and polished into a flat surface to allow compositional and structural data to be collected by using the analysis software with a suitable objective lens. The microscopic image of Ethosomes F1 Formulation by using optical microscopy was represented in **Fig no.20.**

5.8.3 Determination of entrapment efficiency

10 ml of Ethosomal solution were taken in Centrifuge tube. Ethosomal formulations should be subjected to 5000 rpm for 1 hr. Using laboratory centrifuge, the unentrapped drug concentration was determined spectrophotometrically at 239nm. The drug entrapment percentage were calculated using the given equation,

Entrapment efficiency = DE/DT x 100

Where,

DE =Amount of drug in the Ethosomal sediment,

DT=Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment

The results for entrapment efficiency was shown in **Fig no. 21** and **Table no. 15** and **16.**

5.8.4 Vesicle size and Zeta Potential

The vesicle size and zeta potential of fluticasone propionate ethosomes are the key parameters in the ethosomal formulation.

Vesicle Size:

Typical Size Range: Ethosomes for fluticasone propionate usually have a size range of 100 nm to 200 nm.

Factors Affecting Size: The size can be influenced by the lipid composition, concentration of the drug, and the method of preparation (e.g., sonication, extrusion).

Zeta Potential:

Typical Values: The zeta potential of fluticasone propionate ethosomes typically ranges from -30 mV to -60 mV.

Importance of Zeta Potential: A zeta potential within this range indicates good stability, as

higher absolute values suggest strong electrostatic repulsion between vesicles, reducing the likelihood of aggregation.

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba scientific, nanoparticle analyzer instrument and the results shown in **Table no. 16** and **17** respectively.

5.8.5 Polydispersity Index

The polydispersity index (PDI) of fluticasone propionate ethosomes can vary based on the formulation and preparation method used. Generally, PDI is a measure of the distribution of molecular mass in a given polymer sample, with values typically ranging from 0 (monodisperse) to >1 (polydisperse).

For ethosomes, which are lipid-based carriers for drug delivery, a lower PDI (close to 1) indicates a more uniform size distribution, which is often desirable for consistent drug delivery and therapeutic efficacy. Values can range widely based on factors like the concentration of the drug, the type of lipids used, and the method of preparation.

The polydispersity index (PDI) is a measure of the distribution of molecular mass in a given sample and is often used in the characterization of colloidal systems like ethosomal gels.

The PDI is calculated using the formula:

$$\text{PDI} = \frac{\text{Weight Average Molecular Weight}}{\text{Number Average Molecular Weight}}$$

A PDI value of 1 indicates a uniform size distribution, while values above 1 indicate a broader distribution.

Interpretation:

A lower PDI (close to 1) indicates a more homogeneous population of ethosomes, which can be beneficial for drug delivery applications, as uniform size can improve skin penetration and drug release profiles.

In general, a PDI value below 0.3 is considered ideal for ethosomal systems, indicating a narrow size distribution and consistent performance in drug delivery and the result of PDI for optimized formulation was given in the **Table no. 17**.

5.8.6 Release kinetics of selected formulation

The release kinetics of fluticasone propionate from ethosomes can vary based on several factors, including the formulation, the lipid composition, and the method of preparation.

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing Zero order (cumulative % drug release v/s. time), First order (log cumulative % drug retained v/s. time), Higuchi model (cumulative % drug retained v/s. Square root of time) and Peppa's model (log cumulative % drug release v/s. log time). The results of optimized formulation (F1) was given in the **Table no. 18** and the respective **Fig**. were shown in **26, 27** and **28** respectively.

5.9 PREPARATION OF ETHOSOMAL GEL:

- The best achieved Ethosomal vesicles suspension, it was incorporated into carbopol gel (1%, 1.5%, 2%, 3% W/W).
- The specified amount of Carbopol 934 powder is to be slowly added to ultrapure water and kept at 100 for 20min.
- Triethanolamine (0.5%, 1%, 1.5% W/W) were added to it dropwise.

Appropriate amount of formulation containing drug were incorporated into gel base.

- Water q.s were added with other formulation ingredients should be achieved.
- Gel containing free drug were prepared by similar method using 1.5% Carbopol.

Table No 9: Composition of Fluticasone Propionate Ethosomal gel

Gel formulation	Fluticasone Propionate Ethosomal suspension (ml)	Carbopol (%)	Triethanolamine (%)	Buffer (pH 7.4)
EG1	20	1	0.5	q.s
EG2	20	1.5	1	q.s
EG3	20	2	1.5	q.s
EG4	20	1	1.5	q.s

EG5	20	3	1	q.s
EG6	20	1.5	0.5	q.s
EG7	20	2	1.5	q.s
EG8	20	1	0.5	q.s

5.10 EVALUATION TESTS FOR ETHOSOMAL GEL:

5.10.1 Physical Examination

The Prepared emulgel formulations were inspected visually for their color, homogeneity, consistency and phase separation.

Homogeneity:

The homogeneity of fluticasone propionate ethosomal gel is crucial for ensuring consistent drug delivery and therapeutic efficacy.

- Measure the viscosity of the gel at various shear rates.
- Compare the viscosity values of different samples taken from various locations.

Observation: Consistent viscosity readings across samples indicate good homogeneity.

Consistency:

Evaluating the consistency of ethosomal gel is essential to ensure its performance, stability, and effectiveness.

- Store gel samples at different temperatures (e.g., room temperature, refrigerator, and freezer).

- **Monitoring:** Observe for any changes in consistency over time (e.g., phase separation, viscosity changes).

Observation: Consistent performance across different temperatures indicates good stability.

Phase Separation:

Evaluating phase separation in ethosomal gel is important for ensuring stability and uniformity of the formulation.

- Freeze the gel samples at -20°C for 24 hours, then thaw at room temperature.
- Repeat the freeze-thaw cycle 2-3 times.

Observation: To monitor the changes in phase separation after each cycle.

All the observations for formulations (F1 to F8) were recorded and given in the **Table.no.19**.

5.10.2 Washability

A small quantity of gel was as applied on the skin and washed with water in order to check the Washability of gel.

5.10.3 Viscosity

The viscosity of the formulated batches was determined using a Brookfield Viscometer BRK Instrument, India with spindle 64. The formulation whose viscosity was to be determined was added to the beaker and was allowed to settle down for 30 min at the assay temperature (25±1ºC) before the measurement was taken. Spindle was lowered perpendicular in to the centre of emulgel taking care that spindle does not touch bottom of the jar and rotated at a speed of 50 rpm for 10 min and the angular velocity was found to be increased from 5,10,50,100 rpm and the values were noted. The results of viscosity observed were recorded and plotted in the **Fig.29**.

5.10.4 Spreadability

To determine Spreadability of the gel formulations, two glass slides of standard dimensions were selected. Formulation whose Spreadability was to be determined was placed over one slide and the other slide was placed over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower

slide is held firm by the opposite fangs of the clamp allowing the upper slide to slip off freely by the force of weight tied to it. 20 gm weight was tied to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted and given in **Fig.30**. The Spreadability was calculated by using the following formula,

$$S = ML/T$$

Where,

S is the Spreadability in g/s,

L is length of glass slide,

M is the mass in grams and

T is the time in seconds.

5.10.5 pH measurement

1gm Fluticasone Propionate Ethosomal gel were mixed in 100ml distilled water with homogenizer. Then the pH meter electrode was immersed in prepared gel solution and the readings were recorded from digital pH meter (**Fig.31**) in triplicate and the average value were calculated and shown in **Fig.32.**

5.10.6 Drug content uniformity

A measured amount of formulated gel was taken and dissolved in 100ml of buffer pH 7.4. Mechanical shaker were used to shake the gel solution continuously for 2hrs. The solution thus prepared were to be filtered and analyzed spectrophotometrically at 239nm using suitable phosphate buffer (pH 7.4) as blank and the results obtained during this study was shown in the **Table no. 20**.

5.10.7 Extrudability

The prepared ethosomal gel formulations were filled in clean, lacquered aluminum

collapsible tubes with a 5 mm opening nasal tip. Extrudability was then determined by measuring the amount of gel extruded through the tip when a constant load of 1 kg was placed over the pan. The extrudability of prepared ethosomal gel formulations was calculated by using following formula.

$$\textbf{Extrudability} = \frac{\textbf{Amount of gel extruded from the tube x 100}}{\textbf{Total amount of gel filled in the tube}}$$

The Extrudability results of the gel formulation was given in the **Table no. 21**.

5.10.8 Drug content study

Drug content study was done to determine the amount of drug present in the certain quantity of the formulation. Took 1 g of the formulation into 10 ml volumetric flask, add 1 ml methanol in it and shake well and make up the volume with PBS pH 7.4. The Volumetric flask was kept for 2 hrs and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered the mixer, then measure the absorbance by using spectrophotometer at 239 nm.

Drug Content = (Conc. × Dilution Factor × Vol. taken) × Conversion Factor

The drug content study was determined and plotted against the respective formulation codes in the **Fig no. 33**.

5.10.9 In vitro release studies

In-vitro release of Fluticasone Propionate Ethosomes

The in-vitro release of Ethosomal formulation were carried out by using simple diffusion cell apparatus. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5cm, open at both end of the tube is tied with sigma dialysis, which serves as a donor compartment.

Ethosomes equivalent to 5mg of Fluticasone Propionate were taken in a dialysis tube and placed in 200ml phosphate buffer (pH 7.4). the medium was stirred by using magnetic stirrer and the temperature were maintained at 37 ± 2^0c.

The samples (5ml aliquots) were collected at suitable time interval sample were analyzed for drug content by UV visible spectrophotometer at 239 nm after appropriate dilutions by using phosphate buffer as blank.

Cumulative corrections were made to obtain the total amount of drug release at each time interval.

The cumulative amount of drug release across the diffusion cell was determined as a function of time. The cumulative % drug release was calculated using standard calibration curve.

Details of dissolution testing:

Dissolution media: Phosphate buffer pH 7.4

Speed: 50 rpm

Aliquots taken at each time interval: 1 ml

Temperature: 37 ± 2^0c

Wavelength: 239 nm

The results of *In-vitro* drug release profile for Fluticasone propionate Ethosomal formulation was given in the **Table no. 22** and **23** and the results of *In-vitro* cumulative % drug release versus Time curve of FP Ethosomal formulation **Fig. no. 34** and **35** respectively.

In addition to the *In-vitro* release studies, the Comparison of FP Ethosomal formulation **(F1)**

and ethosomal gel **(EG1)** was carried out and the Cumulative % drug release for these formulations was carried out and given in the **Table no. 24** and **Fig. 36** respectively.

5.10.10 Stability Studies

Introduction:

In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection. Stability of a drug can be defined as the time from the date of manufacture and the packaging of the formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously.

Objective of the study:

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled "Stability testing of New Drug Substances and Products (QIA) describes the stability test requirements for drug registration application in the European Union, Japan and the States of America.

ICH specifies the length of study and storage conditions.

- **Long-Term Testing**: 25^0 C $\pm 2^0$ C at 60% RH ±5% for 12 Months

- **Accelerated Testing:** 40^0 C $\pm 2^0$ C at 75% RH ±5% for 6 Months

The stability studies were performed at 40^0C$\pm 2^0$C/75%RH as per ICH guidelines. Samples

were analyzed at periodic time intervals for 3 months for the estimation of pH and drug content. It was observed that there were no change in the physical appearance of the formulation.

The drug content were analyzed and there were a marginal difference between the formulations stored at different temperatures as shown in the **Table. 25** in the **Fig, 37, 38** and **39.**

Well closed container was used for the storage of optimized gel formulation. The gel formulations will be stored at 40˚C and 75% relative humidity for 90 days.

Samples were drawn at a forethought time interval of 30 days, 60 days and 90 days. The gel formulation can be evaluated for their physical properties including appearance, color, and presence of clogs, consistency and phase separation. Gel can also be evaluated for chemical parameters like change in pH and drug content.

CHAPTER 6

RESULTS

RESULTS

EVALUATION TEST

ORGANOLEPTIC PROPERTIES:

Pure Fluticasone Propionate was examined for color, odor, texture and appearance.

Table No 10: Organoleptic properties of Fluticasone Propionate

DRUG	TESTS	SPECIFICATIONS	OBSERVATIONS
Fluticasone Propionate	Appearance	White to off-white powder	**White powder**
Fluticasone Propionate	Color	White crystalline powder	**White powder**
Fluticasone Propionate	Odour	Odorless or faint odor	**Odorless**
Fluticasone Propionate	Texture	Fine and smooth Texture	**Clear smooth texture**

The observations noted were compared to the specifications given in the pharmacopoeia to confirm the identity of the drug and it were found that observations noted complies with the specifications.

SOLUBILITY STUDY:

Solubility studies are performed to determine the solubility of drug in different solvents.

Table No11: Solubility of Fluticasone Propionate in various solvents.

S.NO.	Quantity of drug	Solvent	Quantity of solvent	Inference
1.	50 mg	Distilled Water	5 ml	**Insoluble**
2.	50 mg	Dimethyl Sulfoxide	5 ml	**Freely Soluble**
3.	50 mg	Dimethyl Formamide	5 ml	**Freely Soluble**

4.	50 mg	Acetone	5 ml	**Sparingly soluble**
5.	50 mg	Dichloromethane	5 ml	**Sparingly soluble**
6.	50 mg	Ethyl acetate	5 ml	**Sparingly soluble**
7.	50 mg	Chloroform	5 ml	**Sparingly soluble**
8.	50 mg	Methanol	5 ml	**Slightly soluble**
9.	50 mg	95% Ethanol	5 ml	**Slightly soluble**

MELTING POINT:

Melting point of Fluticasone Propionate measured by using melting point apparatus, Guna Enterprises, Chennai were found to be **273^{0}C**. Melting point were measured three times and the mean were noted. A sharp transition took place from solid to liquid at 273^{0}C, indicating that the sample were pure and free from impurities.

SPECIFIC OPTICAL ROTATION:

The Specific optical rotation of Fluticasone propionate drug was found to be **32.6^0** against the specification limits of +31.0^0 to +36.0^0 and founds satisfactory.

MOISTURE CONTENT DETERMINATION:

The moisture content determination of Fluticasone Propionate by Karl Fisher titration method was found to be **0.4%** against the specification limit of ≤0.5% and founds satisfactory.

i) COMPATIBILITY STUDIES BY FTIR:

These studies were carried out to determine authenticity of the drug and the compatibility using Fourier Transform Infrared Spectrophotometer. KBr pellets were prepared of the active ingredient. The drug sample was scanned on IR spectrophotometer between 400-4000 cm^{-1} using KBr disc. The obtained IR spectrum was interpretated with the structure of FP.

The FTIR results for pure drug Fluticasone Propionate and FTIR Fluticasone Propionate+ Excipient was given in the **Fig. 15, 16** and **Table No. 12** and **13** respectively.

Fig No. 15: FTIR profile of pure Fluticasone Propionate

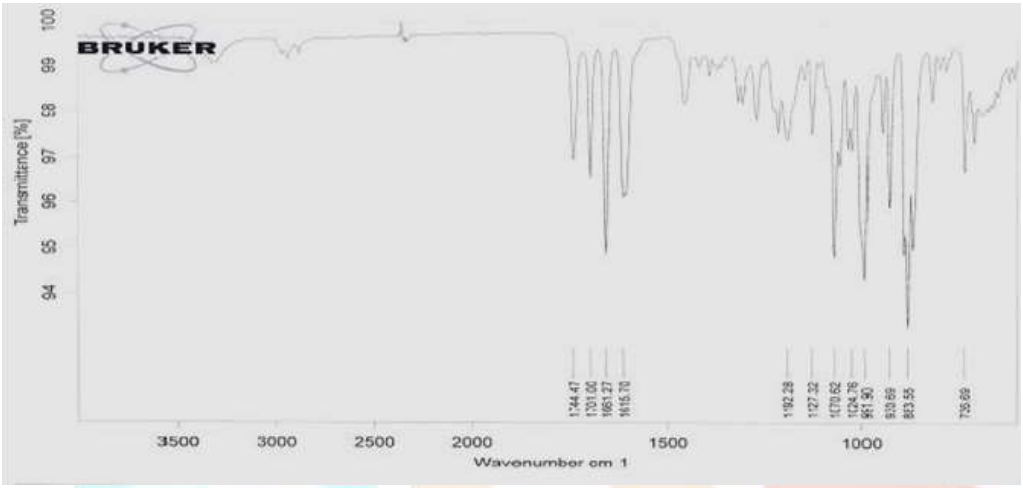


Table No 12: FTIR spectra interpretation of Fluticasone Propionate

S.No	TYPE OF VIBRATIONS	WAVE NUMBER (cm^{-1})
1	C=C stretching	1661.27
2	OH Stretching	1192.28
3	S-H thiol Stretching	991.90
4	C=O ketone Stretching	1744.47
5	C-F Stretching	1024.76
6	C-O Stretching	930.69
7	C-H Stretching	883.55

Fig No.16: FTIR of Fluticasone Propionate+ Excipient

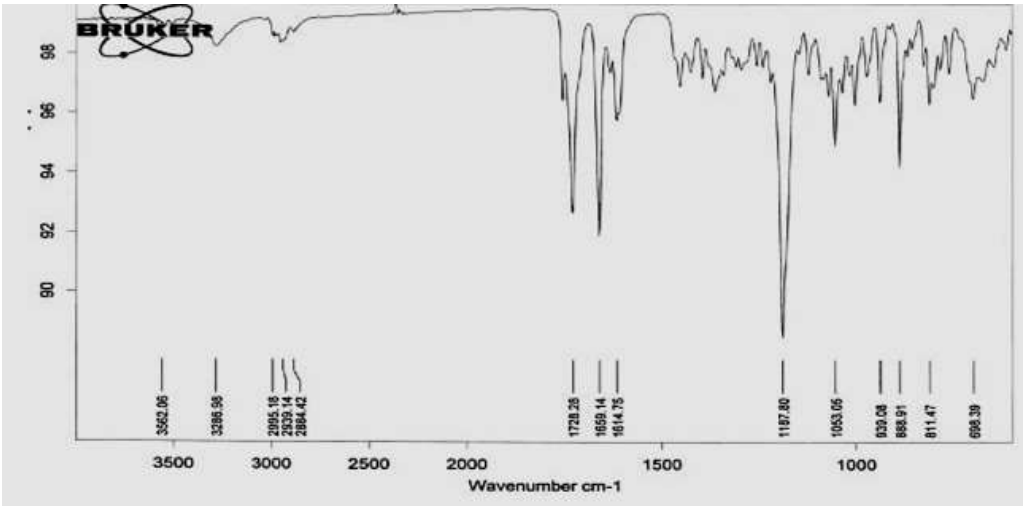


Table No 13: FTIR spectra interpretation of FP + Excipient

ii) BY

S.No	TYPE OF VIBRATIONS	WAVE NUMBER (cm^{-1})
1	C=C stretching	1659.14
2	OH Stretching	1187.80
3	S-H thiol Stretching	939.08
4	C=O ketone Stretching	1728.28
5	C-F Stretching	1053.06
6	C-O Stretching	888.91
7	C-H Stretching	811.47

DIFFERENTIAL SCANNING CALORIMETRY:

A small quantity of about 5mg Fluticasone propionate was taken for the analysis and place it in a DSC aluminium pan.

Set the temperature range appropriate for fluticasone propionate, typically from 25℃ to 300°C. Heat the sample at a constant rate (usually 5-20°C/min).

Record the heat flow as a function of temperature and it was found to be **293.51°C**. These values are essential for ensuring the stability and efficacy of fluticasone propionate in pharmaceutical formulations.

The DSC analysis of Fluticasone propionate was given in the below figure.

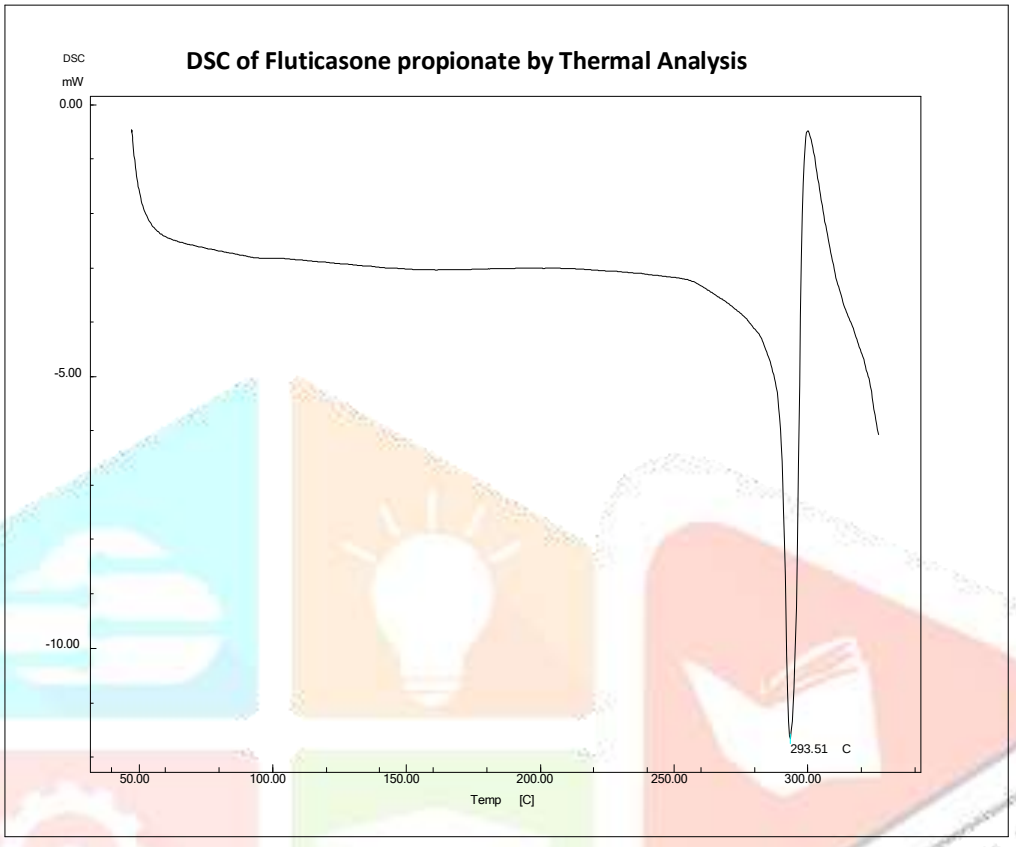


Fig No.17: Differential scanning calorimetry of Fluticasone Propionate

iii) BY DETERMINATION OF λ MAX:

CALIBRATION CURVE OF FLUTICASONE PROPIONATE:

Table No 14: Calibration Curve of Fluticasone Propionate in pH 7.4 at 239nm

C c Concentration (µg/ml)	**Ab Absorbance (nm)**
0	0
10	0.13
20	0.20

30	0.25
40	0.29
50	0.35

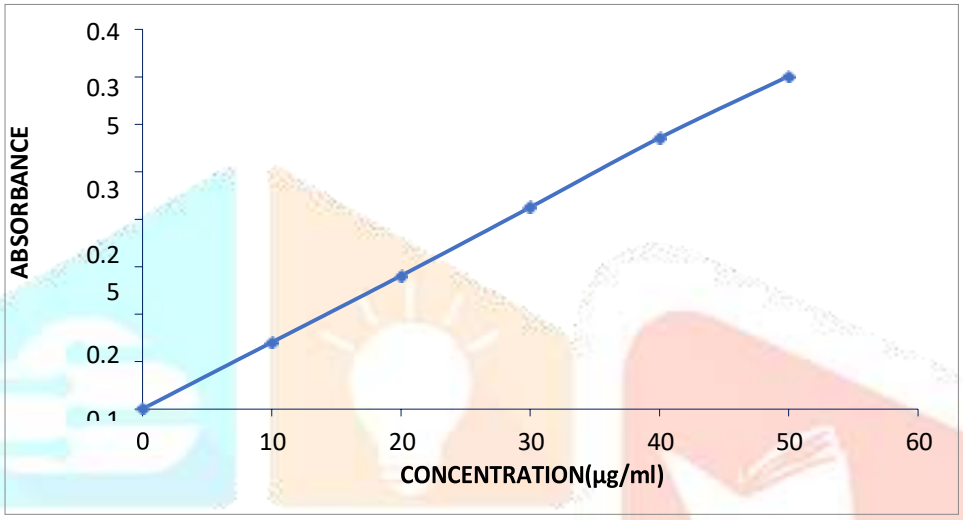


Fig No.18: Calibration curve of Fluticasone Propionate in phosphate buffer pH 7.4 at 239nm.

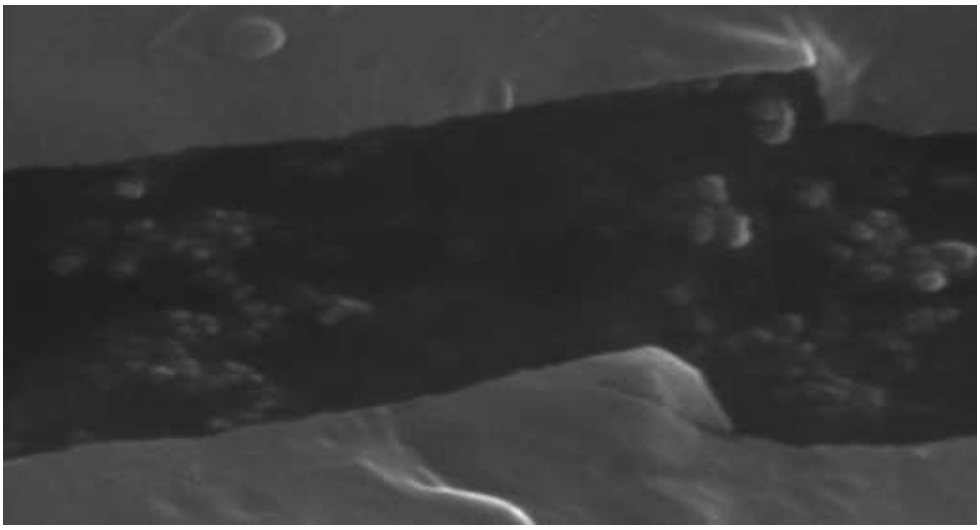
The method that were developed for the estimation of Fluticasone Propionate have showed a maximum absorption at a wavelength of 239nm in pH 7.4.

The standard calibration curve obeys beer Lambert's law at the given concentration range of 10µg/ml-50µg/ml and shows λ MAX of 0.35 nm absorbance at 50µg/ml concentration.

PHYSICOCHEMICAL CHARACTERIZATION

A) SURFACE MORPHOLOGY BY SEM ANALYSIS:

The Surface morphology study of prepared ethosomes by SEM analysis indicates a powerful tool for evaluating and providing valuable insights into the formulation's structural characteristics.



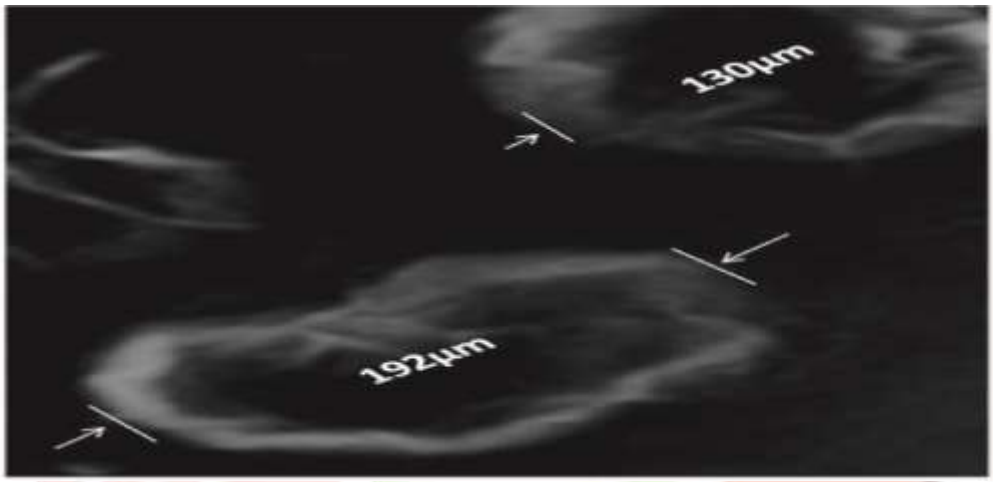


Fig No.19: Scanning electron microscope images of F1 formulation

The SEM image indicated that the Ethosomes are found to be abundant, irregular in shape and smooth.

SEM is a valuable technique for examining the surface morphology of fluticasone propionate ethosomal gel. The insights gained from this analysis can inform formulation development, stability studies, and optimize drug delivery performance. Proper sample preparation and SEM settings are critical to obtaining high-quality images that accurately reflect the structure of the ethosomal gel.

B) **OPTICAL MICROSCOPY:**

The Optical microscopic image of ethosomes F1 formulation was obtained and shown in the below figure. An optical microscope (Leica, Germany) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomal formulation.

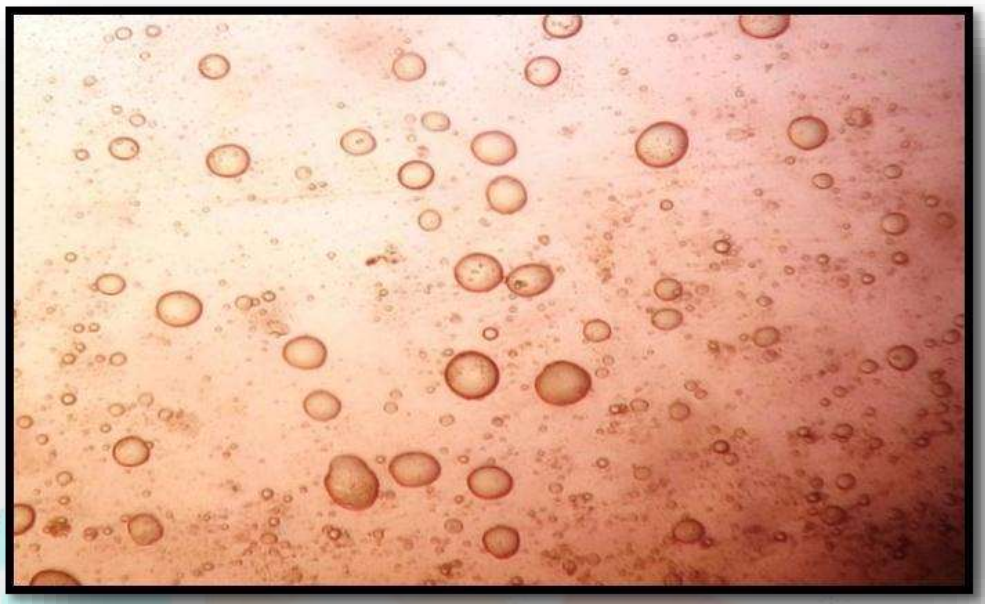


Fig No.20: Microscopic image of Ethosomes F1 Formulation

C) ENTRAPMENT EFFICIENY:

The Drug entrapment efficiency was calculated by using the following formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100$$

Table No 15: Evaluation parameters for formulation (F1 to F8)

Formulation code (F)	Entrapment efficiency (%)
F1	88.9

F2	79.5
F3	83.5
F4	82.3
F5	79.2
F6	69
F7	73.4
F8	75.7

Percentage entrapment of FP Ethosomes were found to be in the range of **68-90%** as shown in the Fig no.21. The entrapment efficiency were found to be higher for **F1** formulation **(88.9%)** and the evaluation parameters for formulations (F1 to F8) given in Table no.15. Amounts of Soya lecithin, ethanol and cholesterol used for ethosomes preparation seemed to influence the entrapment efficiency. Of all the factors examined, the concentration of ethanol were found to influence the entrapment efficiency to a significant extent. Increase in the amount of ethanol, increases the entrapment efficiency and the reason could be due to the formation of thinner membrane.

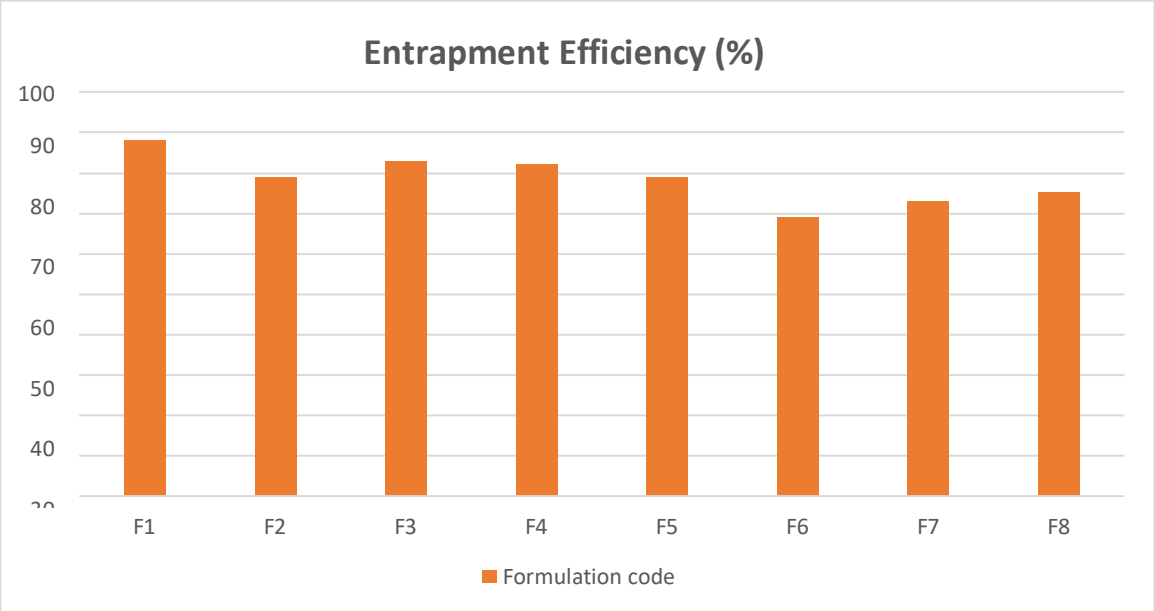


Fig No.21: The entrapment efficiency of ethosomes

D) AVERAGE VESICLE SIZE AND ZETA POTENTIAL:

The Prepared ethosomal formulations were optimized on basis of vesicle size, shape, surface charge and entrapment efficiency. Vesicle size of ethosomes were examined under trinocular microscopic (magnification 400X) and also determined by light scattering method and found that **average vesicle size** of optimized formulation **F-1** was **116.37±3.04 nm** and the **Zeta potential** was **-33±1.8**. It was observed that the vesicles size of ethosomes was increase with increasing the concentration of phospholipids and similarly vesicle size was decrease with increasing the concentration of ethanol due to its surfactant action. There was no significant difference in average vesicle size was observed with increasing the drug concentration. But in increasing the stirring time the vesicle size was decrease from 176.54±5.06 to 105.32±6.95 after 15 min. of stirring time (**Fig. 22** and **Fig. 23**).

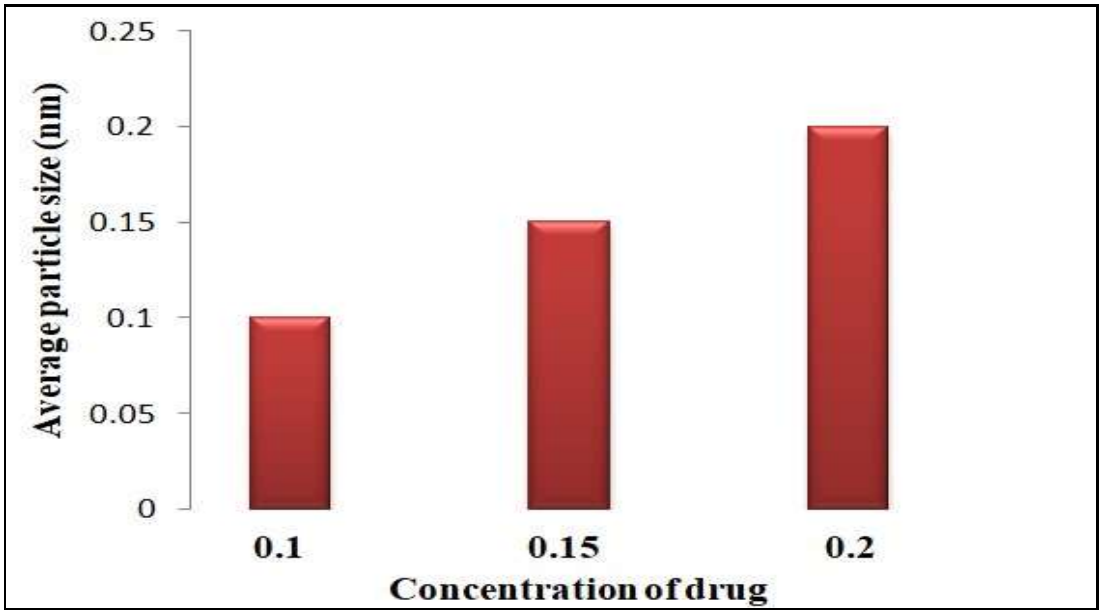


Fig. 22: Effect of drug concentration on vesicle size

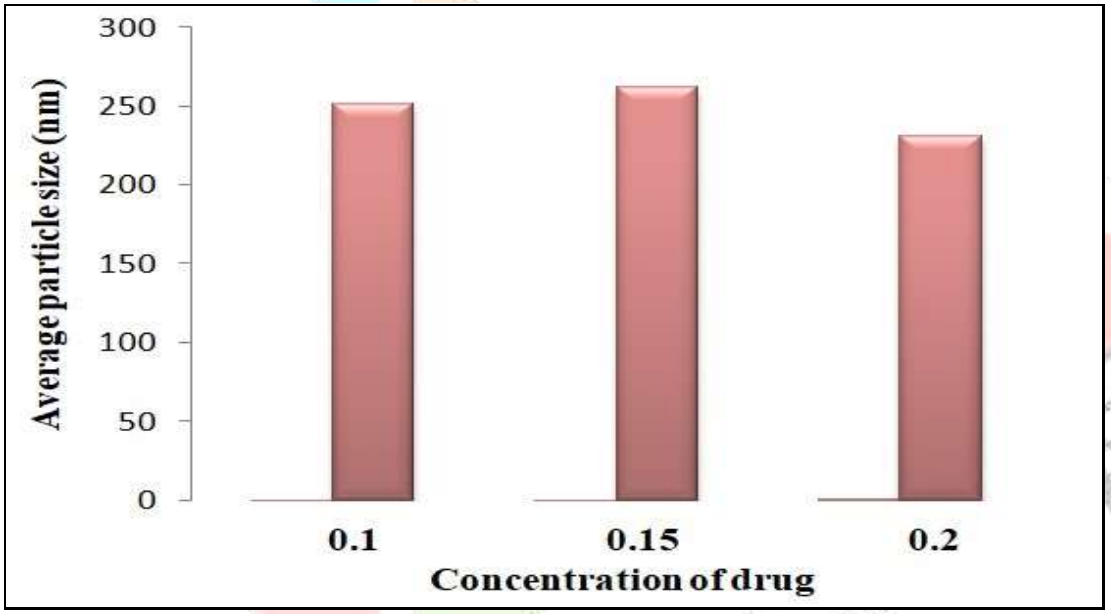


Fig. 23: Effect of stirring time on vesicle size

Table 16: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size	Entrapment Efficiency
F1	116.37±3.04	89.45±0.51
F2	125.56±4.56	78.67±0.84
F3	136.40±2.48	83.31±0.23

F4	105.50±4.58	82.10±0.21
F5	132.12±5.62	79.84±0.45
F6	113.56±4.10	69.03±0.12
F7	128.56±3.56	73.42±0.62
F8	125.37±3.16	75.35±0.38

E) **POLYDISPERSITY INDEX (PDI)**

The low PDI value represents the uniformity of formulation in which there is no major difference in size of vesicles. The **PDI** of optimized formulation **(F-1)** was found I in the range of **0.286±0.115**. PDI of formulation was varied with increasing or decreasing the concentration ratio of lipid and surfactant and stirring time. It was observed that when lipid ratio in formulations was decreased and surfactant concentration was increased then the PDI was found decrease. When stirring time increase from 5 to 15 min then the PDI value was 0.274, 0.163 and 0.452 respectively for F-5, F-6 and F-7. It was observed that on 10 min of stirring time the PDI was increased and it was due to the high mechanical forced of stirring time which was resultant in heat generation which leads to agglomerates or denaturing the lipid molecules after breaking the vesicles **(Fig. 24** and **Fig. 25)**.

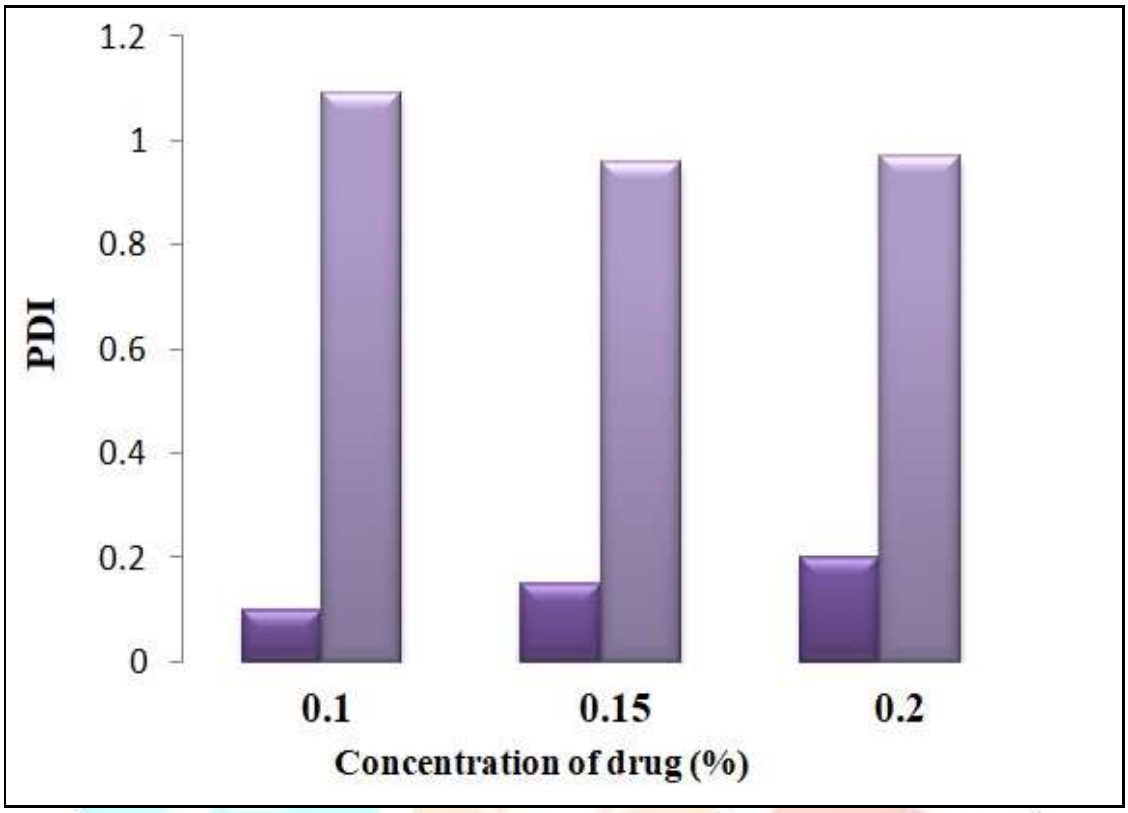


Figure 24: Effect of drug concentration of drug on PDI.

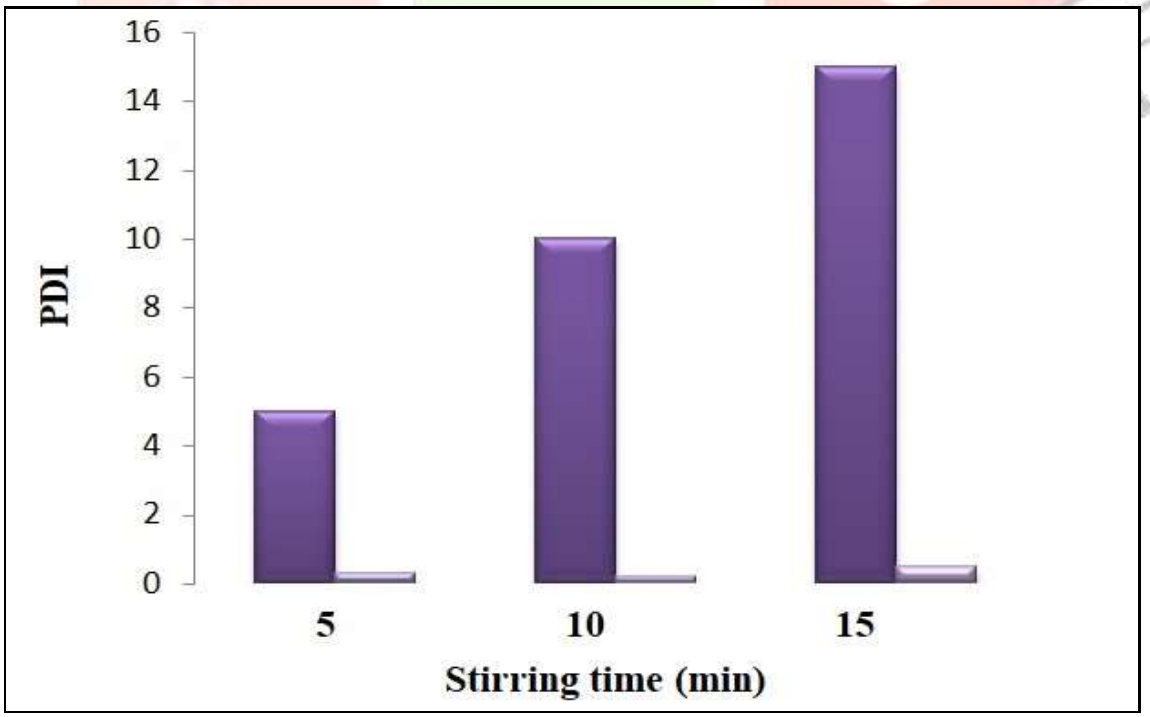


Figure 25: Effect of stirring time on PDI.

Table 17: Vesicle size and entrapment efficiency of optimized ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Polydispersity Index	Zeta potential
F1	116.37±3.04	89.45±0.51	0.286±0.115	-31mv

F) RELEASE KINETICS OF SELECTED FORMULATION:

Formulation Code	Zero order		First order		Higuchi		Korsmeyer peppas	
	R^2	K_0(-) (1/S)	R^2	K_1(-) M/L. S	R^2	K_H	R^2	n
F1	0.899	11.01	0.906	10.00	0.962	30.30	0.978	0.67

Table 18: Data of release kinetics

The data were treated according to zero order, first order, higuchi model and korsmeyer peppas pattern for kinetics of drug release during dissolution process. The regression equation of optimized formulation F1 were find out according to zero order equation 0.899, first order equation 0.906, higuchi model 0.962 and peppas model 0.978 and was given in the **Table no. 18** and **Fig.no. 27,28 and 29** respectively. These values clearly indicate that the formulation showed to be best expressed by Higuchi model for release kinetics. This model is based on the hypotheses that,

(i) Initial drug concentration in the matrix is much higher than drug solubility.

(ii) Drug diffusion takes place only in one dimension.

(iii) Drug particles are much smaller than system thickness.

(iv) Matrix swelling and dissolution are negligible.

(v) Drug diffusivity is constant

(vi) Perfect sink conditions are always attained in the release environment.

The dissolution data was also plotted to the well-known exponential equation (Korsmeyer-peppas equation), which is often used to describe the drug release behaviour from polymeric system. According to this model, a value of n<0.45 indicates fickian release, between n>0.45 to n<0.89 for non-fickian (anomalous) release and n>0.89 indicate super case II type of release. Case II generally referred to the erosion of the polymeric chain and anomalous transport (non-fickian) refers to a combination of both diffusion and erosion control drug release. The n-value describe in table 19. On the basis of n-value the optimized formulation (F1) exhibit non-fickian type drug release.

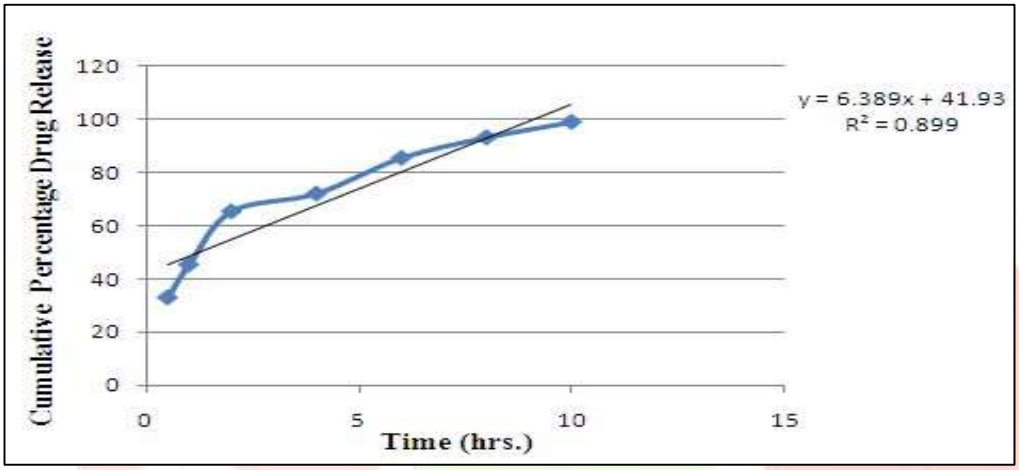


Figure 26: Cumulative Percent Drug Released Vs Time (Zero Order Plots)

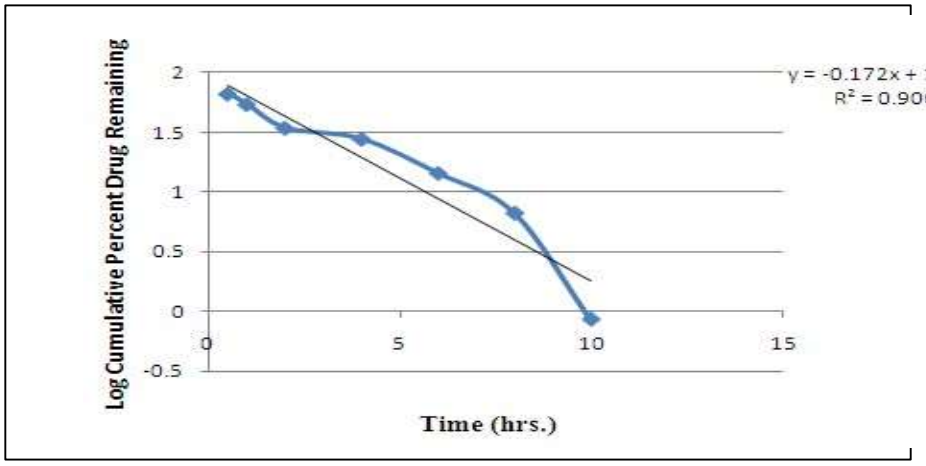


Figure 27: Log Cumulative Percent Drug Remaining Vs Time (First Order Plots)

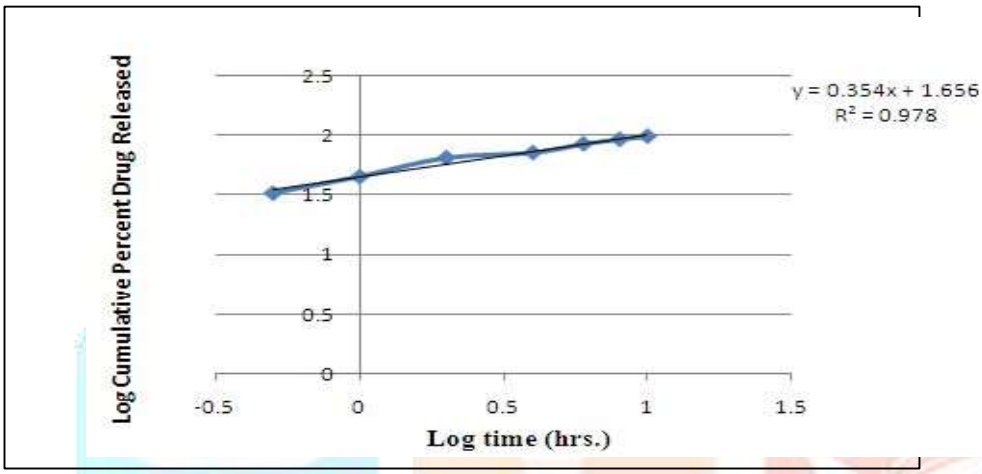


Figure 28: Log Cumulative Percent Drug Released Vs Log Time (Peppas Plots)

EVALUATION OF ETHOSOMAL TOPICAL GEL:

i) Physical Examination

The ethosomal gel formulations were white, viscous, creamy preparation with a smooth homogeneous texture and glossy appearance. The Physical Examination results of Fluticasone propionate ethosomal gel formulation **(EG1 to EG8)** was given in the below table.

Table 19: Results of Color, Homogeneity, Consistency and Phase Separation

S. No.	Formulation Code (EG)	Color	Homogeneity	Consistency	Phase Separation
1	EG1	White	Fair	+	None
2	EG2	White	Excellent	+++	None
3	EG3	White	Excellent	+++	None
4	EG4	White	Excellent	+++	None
5	EG5	White	Fair	+++	None
6	EG6	White	Good	++	None
7	EG7	White	Excellent	+++	None
8	EG8	White	Excellent	+++	None

ii) Washability

All the prepared formulations were subjected to Washability and found that all of them are easily washable without leaving any residue on the surface of the skin.

iii)Viscosity

The ethosomal gel was rotated at 50 rpm for 10 min with spindle 64. The corresponding reading was noted. The viscosity of the ethosomal gel was obtained. The viscosity of the formulations increases as concentration of polymer increases. The data for formulation (EG1 to EG8) was shown in below Fig. 4. The formulation **(EG5)** shows maximum viscosity of about **17338 cps** among the other formulations.

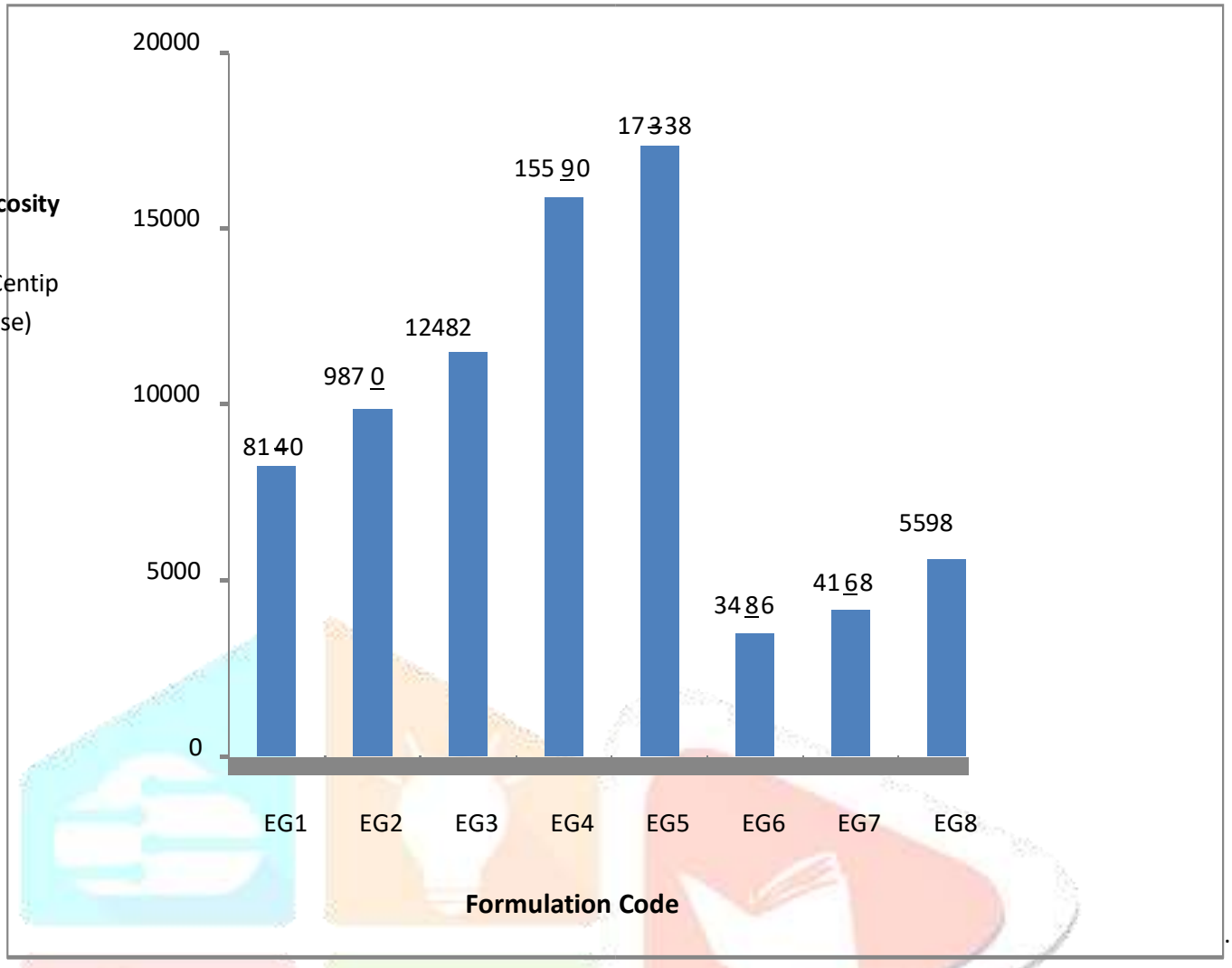


Fig. 29: Viscosities of Different Formulations EG1-EG8

iv)Spreadability

The Spreadability of FP Ethosomal gel was considered high by having a low spread of time. The therapeutic efficacy of gels depends on their spread. The gel spreading helps in the uniform application of the gel to the skin, so the prepared gels must have a good Spreadability and satisfy the ideal quality in topical application. Spreadability was in the range of **19.8 to 36.9 gm.cm/sec**.

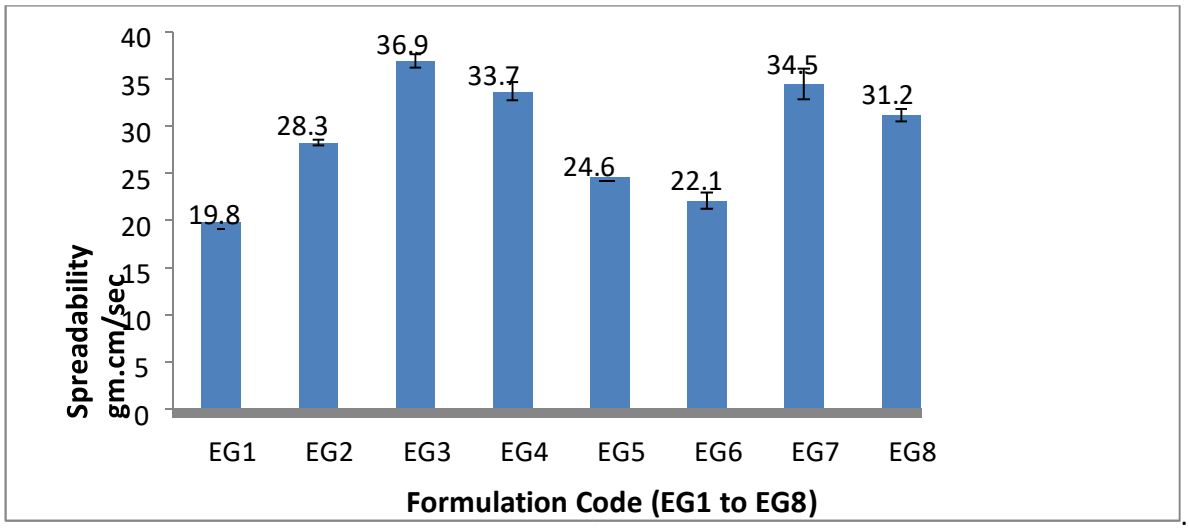


Fig. 30: Spreadability results of Formulations EG1-EG8

v)pH determination

The pH were measured three times and mean were noted. Hence, pH of Fluticasone Propionate were found to be **6.3**.

Fig no.31: pH determination apparatus image

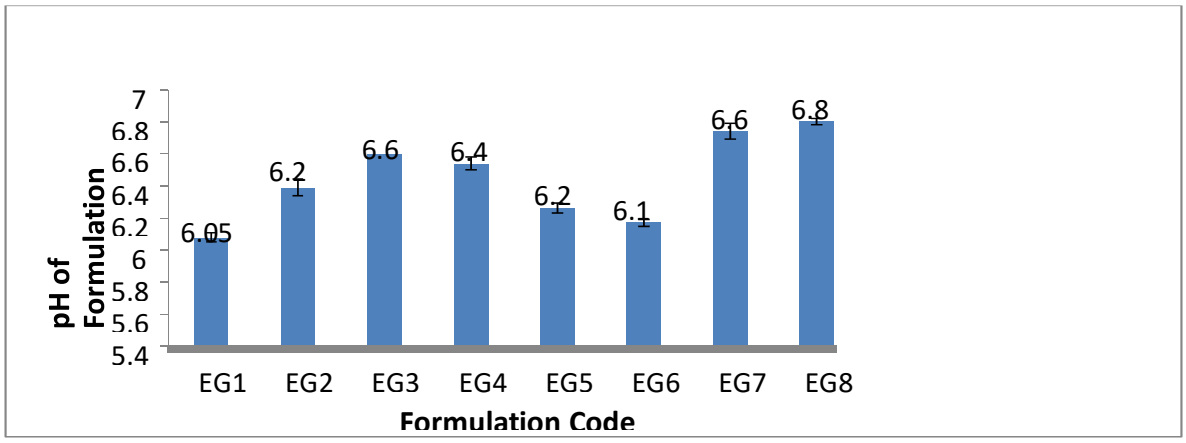


Fig. 32: pH values of Different Formulations EG1-EG8

vi)Drug content uniformity

The drug content were estimated spectrophotometrically 50mg equivalent of gel were taken and dissolved in methanol and filtered. The volume were made up to 10ml with methanol. The resultant solution were suitably diluted with methanol and absorbance were measured at 239nm and found that the drug content uniformity of about **95.68%** found for the formulation **(EG1)**.

Table No 20: Drug content of Ethosomal gel

S.NO	FORMULATION CODE (EG)	DRUG CONTENT (%)
1	EG1	95.68
2	EG2	91.50
3	EG3	93.26
4	EG4	88.53
5	EG5	92.88
6	EG6	94.62
7	EG7	90.46
8	EG8	89.75

vii) EXTRUDABILITY

Table No.21: Extrudability results of Formulations EG1-EG8

S.no.	Formulation Code	Extrudability
1	EG1	+++
2	EG2	+++
3	EG3	++
4	EG4	+++
5	EG5	++
6	EG6	+
7	EG7	+++
8	EG8	++

Excellent +++, Good++ and Satisfactory+

viii)DRUG CONTENT STUDY

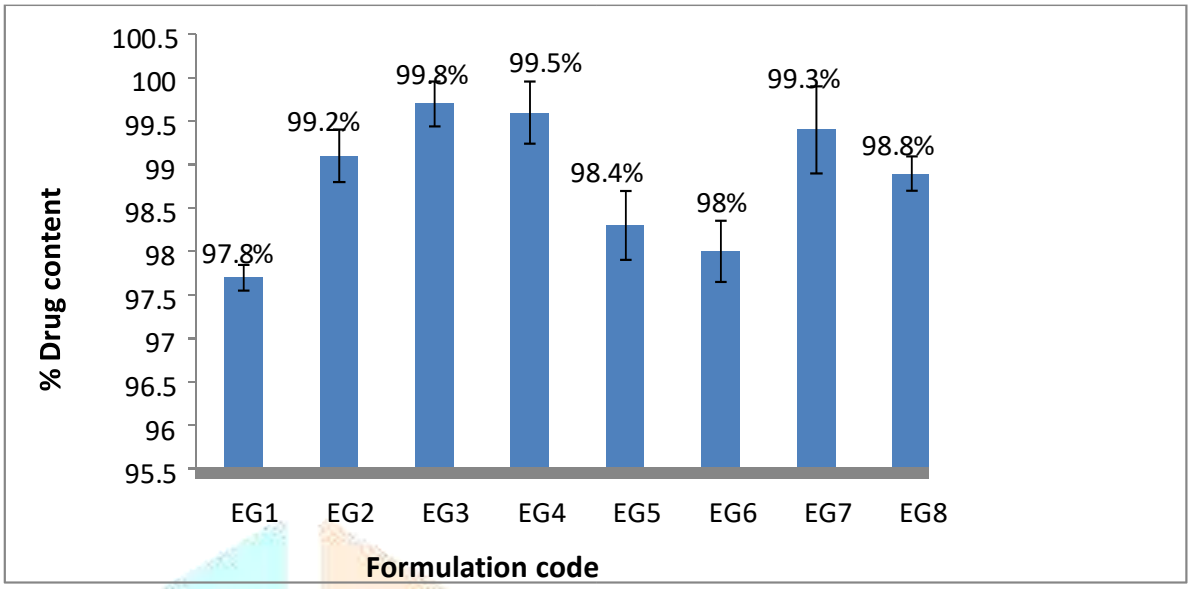


Fig. 33: Drug content study of Formulations EG1-EG8

***ix)In vitro* release studies of Fluticasone Propionate ethosomal gel**

The cumulative percentage drug release from various Ethosomal gel formulations (EG1-EG8) is shown in the below given table. Formulation **(EG1)** shows higher cumulative drug release of **88.5 %** at its **8th hr**. It shows higher entrapment efficiency and drug release than other formulations.

Table No 22: *In vitro* drug release profile for Fluticasone propionate Ethosomal formulation (EG1-EG4)

Time (hrs)	EG1	EG2	EG3	EG4
0	0	0	0	0
1	13.65	19.68	22.9	15.5
2	25.72	28.48	30.01	22.6
3	42.36	33.02	35.6	31.86
4	49.8	42.6	47.12	45.5

5	56.86	49.7	53.5	50.63
6	63.59	54.55	62.20	65.6
7	78.09	60.1	70.6	72.41
8	88.5	68.44	75.5	79.8

Table No 23: *In vitro* drug release profile for Fluticasone propionate Ethosomal formulation (EG5-EG8)

Time (hrs)	EG5	EG6	EG7	EG8
0	0	0	0	0
1	12.88	14.65	25.8	14.66
2	22.76	26.22	33.6	28.5
3	38.51	31.88	35.6	37.43
4	52.63	44.5	48.19	49.96
5	58.16	51.8	53.5	54.78
6	61.4	59.66	65.16	69.5
7	73.15	63.8	71.83	78.26
8	82.55	74.56	78.28	84.36

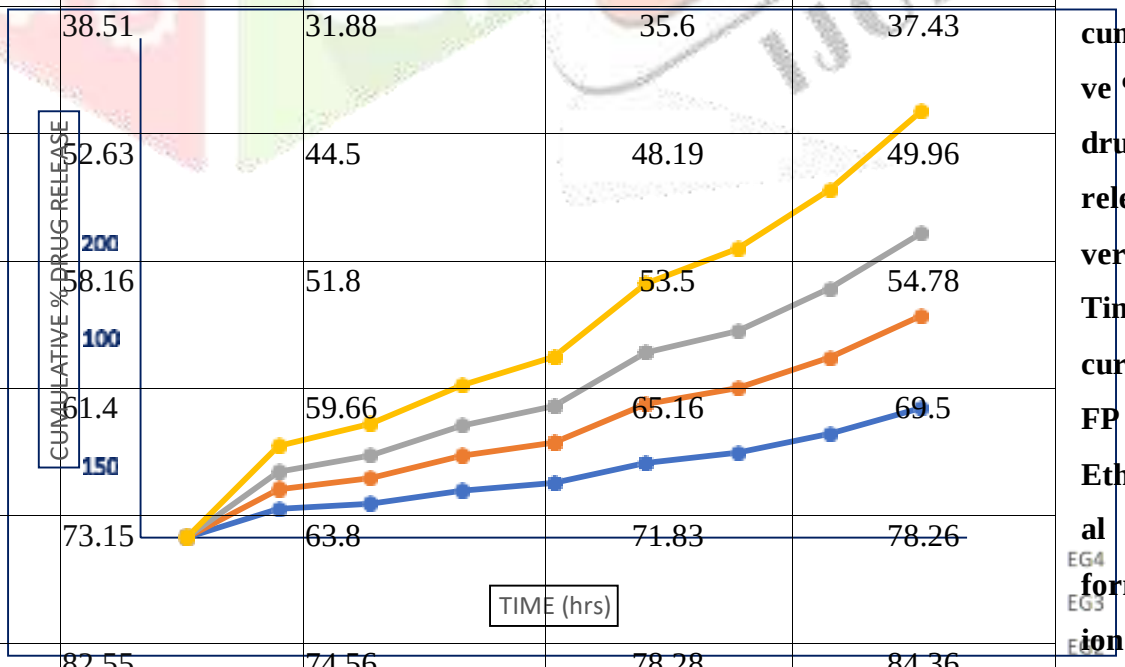


Fig No.34: *In-vitro* cumulative % drug release versus Time curve of FP Ethosomal formulation (EG1-EG4)

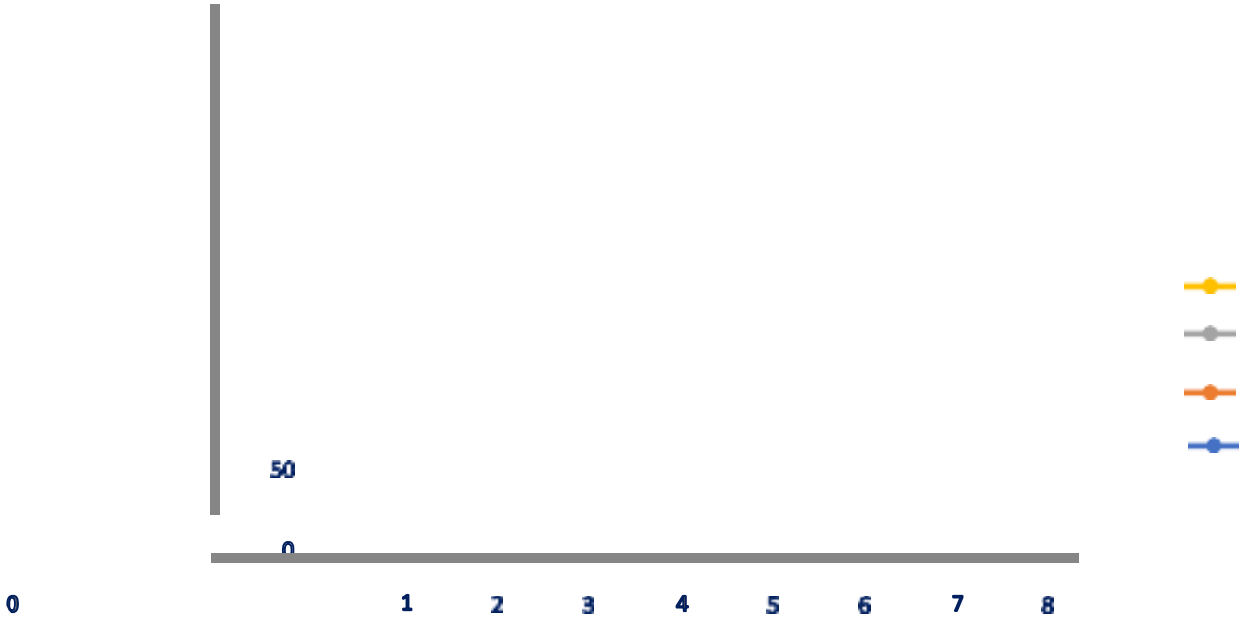
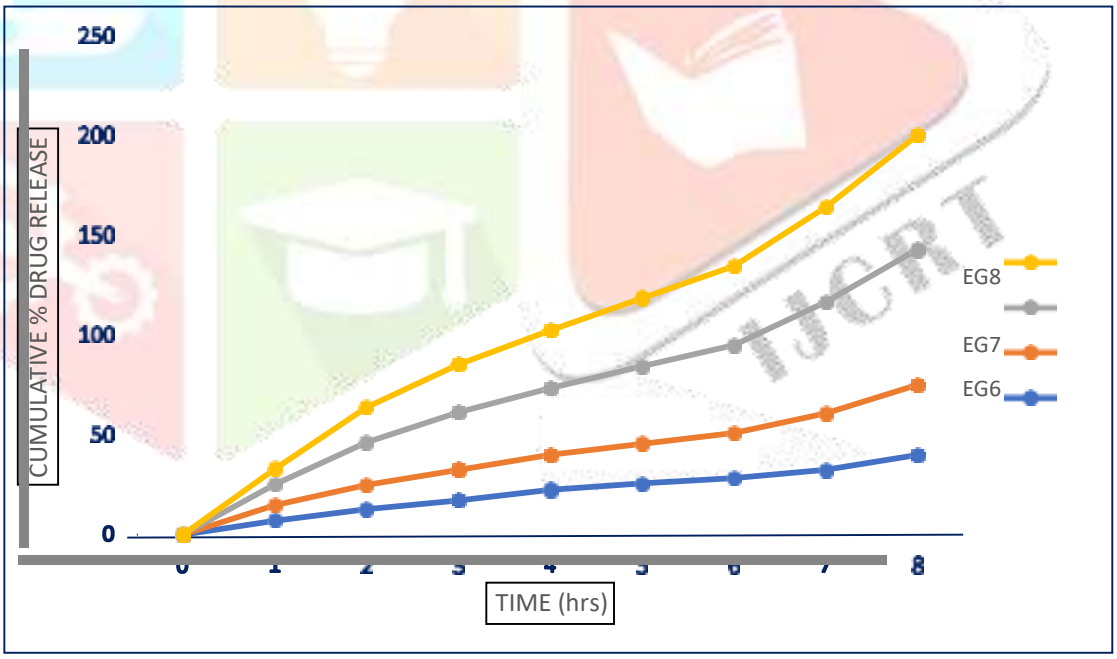


Fig No.35: *In-vitro* cumulative % drug release versus Time Curve of FP Ethosomal formulation (EG5-EG8)



Comparison of FP Ethosomal formulation and gel

The Cumulative % drug release was studied for the optimized formulation F1 and optimized Ethosomal gel EG1 and the comparisons shown below,

Table No 24: Comparison of *In vitro* drug release for FP ethosomal formulation F1 and Ethosomal gel EG1

Time(hrs)	Ethosomal formulation (F1)	Ethosomal gel (EG1)
0	0	0
1	10.5	13.65
2	21.3	25.72
3	28.8	42.36
4	33.4	49.8
5	38.9	56.86
6	43.9	63.59
7	55.8	78.09
8	67.8	88.5

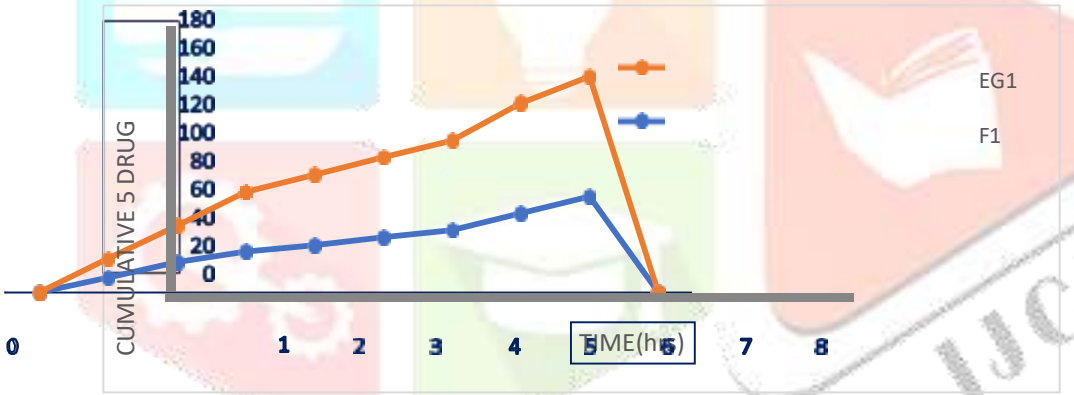


Fig No.36: Cumulative % drug release Vs time profile of optimized formulation F1 and optimized Ethosomal gel EG1

X) Stability Study:

Stability study data was revealed that the optimized formulation (F-8) was stable after 3 months of storage at 5ºC while at 24-27±2°C, the formulation was found unstable. Stability of formulation was observed on the basis of % drug remain, average vesicles size and physical appearance. The average vesicle size of ethosomes was found 178.37±5.07, 179.32±2.49 and 186.84±5.84 nm and after 1, 2 and 3 months of storage at 4.0 ±0. 2°C while at 25-28±2°C the average vesicle size was found 192.56±5.22, 215.54±3.65 and 386.54±7.82nm after 1, 2 and 3 months of storage. Drug remaining in ethosomal formulation was 48.62±1.39, 35.29±1.08 and 23.83±2.11 % after 1, 2 and 3 months of storage at 25-28±2°C while there

were no significant changes in % drug remain and physical appearance in ethosomal formulation was observed after 3 months of storage at 4ºC which is shown in the Table no. 25 and Fig. 37, 38 and Fig. 39 respectively.

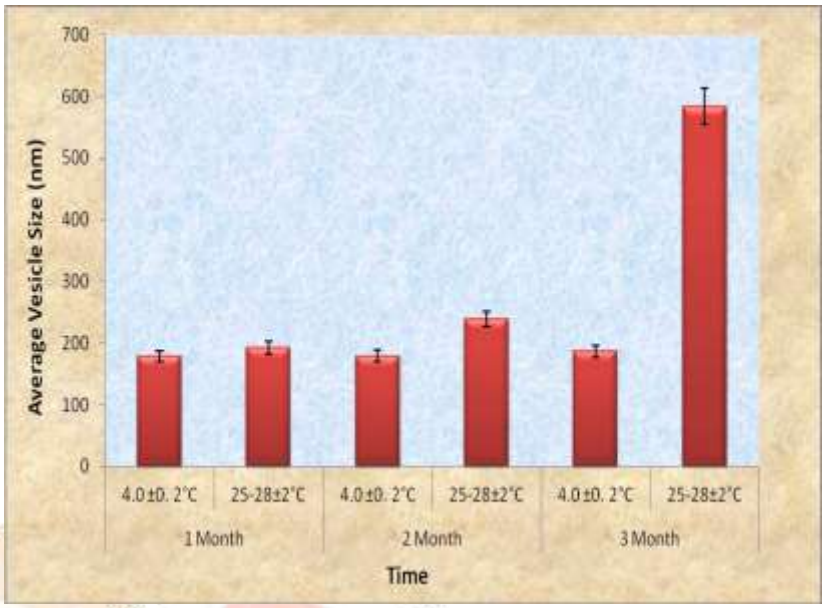


Figure 37: Average vesicle size after storage at different temperature for 3 months.

Figure 38: % Drug remain after storage at different temperature for 3 months

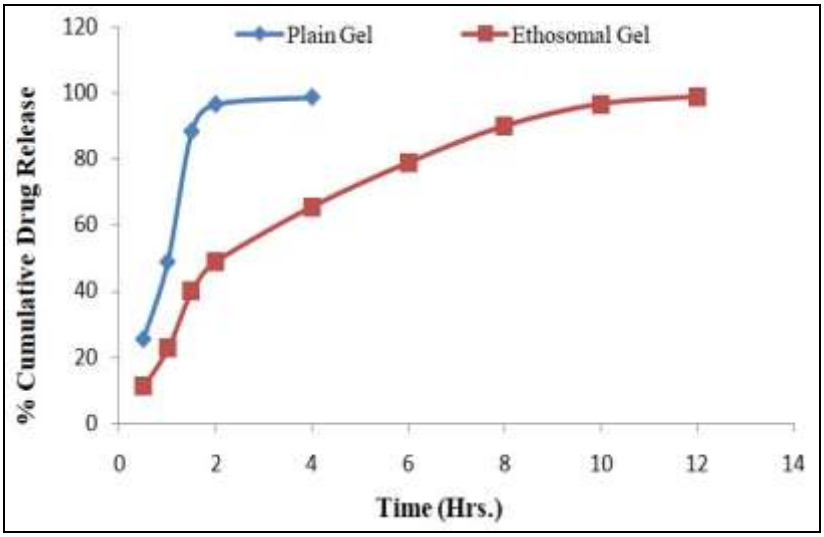


Figure 39: *In vitro* drug release of plain gel and ethosomes based gel

Table No 25: Stability studies of FP Ethosomal gel

PARAMETERS	INITIAL	1 MONTH	2 MONTHS	3MONTHS
pH value	6.6	6.7	6.6	6.8
Drug content	92.75	93.5	93.68	94.73
Viscosity at 50rpm	14956	19264.5	17042.8	18335.6

Ethosomal topical gel formulations retained good stability throughout the study.

CHAPTER 7

DISCUSSION

DISCUSSION:

Transdermal route offers several potential advantages over conventional routes. These advantages include avoidance of first pass metabolism, predictable and extended duration of action, minimizing undesirable side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in the blood level and most importantly it provides patient convenience. But, one of the major problems for efficient drug delivery is low penetration rate. While optimizing the topical drug delivery, vesicular system appears as upcoming development.

"Ethosomal system" which shows topical delivery with higher transdermal flux and higher skin deposition as it is attractive and has desirable advantages.

Fluticasone Propionate Ethosomes were prepared using the cold method with little modification. Studies were performed on Ethosomes containing 20%, 30%, and 40%w/w ethanol with sonication.

The preformulation studies involving description, solubility, melting point, specific optical rotation and moisture content determination of the drug were found to be comparable with the standard. Based on all the above preformulation studies, the drug was suitable for making the transdermal formulation of gel.

Then, it involves for the compatibility studies such as FT-IR, differential scanning calorimetry study and the determination of λ max.

The ethosomes were prepared and employed for physicochemical characterization studies such as Surface morphology, Optical microscopy, Entrapment Efficiency, Vesicle size and Zeta Potential, Polydispersity Index and Release Kinetics and the respective results were posted and founds satisfactory.

After confirm existence of vesicles and their size, drug entrapped by vesicular system was evaluated. The entrapment efficiency of Ethosomal formulation (F1) were found to be 88.9%. *In-vitro* release for Ethosomal formulation was carried out using dialysis membrane showed higher value i.e. 88.5 % at its 8^{th} hour.

In order to investigate the possible interaction between the drug and selected polymers, FTIR spectroscopy studies were carried out. IR spectrum of pure drug and physical mixture of drug-excipient were obtained and characterized.

It was observed that there were no changes in these main peaks in IR spectra of mixture of drug and excipient, which shows there were no physical interaction because of some band formation between drug and excipient.

Characterization of gel-based FP loaded ethosomes:

Prepared gel was evaluated for viscosity, % entrapment, extrudability, Spreadability and drug release study. It was found that viscosity of prepared gel was 11300±6000 cps, Extrudability was Excellent as well as good and Spreadability (g.cm/sec) was found that 20.5±15.2 respectively. In vitro drug release from ethosomes was carried out using diffusion cell method and found 88.5±0.65% in 8^{th} hr. In first 30 mins, it was 11.28±0.58% drug release which slightly high. It was due to the release of free drug present inside after leaching from ethosomes. Drug release from ethosomes formulation was found in very sustained and controlled manner.

The SEM analysis of Ethosomal formulation indicated that the vesicles are abundant, irregular in shape and smooth.

Various evaluation test also carried out with FP Ethosomal gel involving Physical examination, Washability, Viscosity, Spreadability, pH determination, drug content uniformity, extrudability, drug content study, Homogeneity, *In Vitro* studies and stability studies.

The gel spreading helps in the uniform application of the gel to the skin, so the prepared gel has good Spreadability and satisfy the ideal quality in topical application.

Drug content uniformity were estimated and showed higher value i.e., 95.68% of FP ethosomal gel (EG1).

The stability studies were carried at for 3month as per ICH guidelines. It was observed that there were no changes in the physical appearance and parameters such as pH value, viscosity and drug content of the formulation.

CHAPTER 8

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Fluticasone propionate is a potent corticosteroid used primarily for its anti-inflammatory properties in treating respiratory conditions and skin disorders. Ethosomal gels, which incorporate ethosomes-liposomes enriched with ethanol-enhance the transdermal delivery of hydrophilic drugs. This formulation combines the benefits of fluticasone propionate with the improved skin penetration and bioavailability provided by ethosomes.

It is well known that if drug molecules presenting any difficulties in its solubility and bioavailability along the GI tract are candidates for other routes of administration and if the site of action for drug candidate is superior, effective penetration enhancers are required to provide the drug molecule deeper into skin tissue for optimized therapeutic delivery of drug.

It is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not penetrate the skin. Recently derived ethosomal system can deliver drug molecules into and through the skin. An attempt was made to formulate the highly efficient ethosomal drug delivery system using Fluticasone propionate as a model drug. Since, many formulations are available commercially with this drug in topical form. It is the novel formulation by using Fluticasone propionate into ethosomal gel formulation.

The techniques used were simple and reproducible. The prepared ethosomes were spherical and discrete in shape. However, ethosomes prepared by sonication method were more uniform and smaller in size which is essential for skin penetration.

While comparing the entrapment efficiency, ethosomes containing 30% w/w ethanol and prepared by sonication showed highest value respect to all other formulation. So, it is concluded ethosomal prepared by sonication and containing 30% w/w ethanol as the best formulation considering all other aspects. The highest value of transdermal flux for sonicated ethosomes containing 30% w/w ethanol is the indication of complete and rapid penetration through the skin may be because of tiny vesicular size.

This is an encouraging observation for drugs which are poorly absorbed from skin, when effect of sonication was compared on ethosomal formulation, sonicated formulation possessed better or suitable characteristics (smaller size, uniform size, distribution, highest entrapment efficiency).\

From the above observations, it can be concluded that sonication is an essential tool for the preparation of ethosomes. Thus, the specific objectives listed in the introduction were achieved namely design, characterization and release studies of FP ethosomes.

Certainly, these finding can be applied for transdermal drug delivery of Fluticasone Propionate for treatment of skin irritation, rashes.

The present study was concluded that the Ethosomes offer advantages of rapid onset and maximum release of drug with reduced side effects. These findings may help the industry for development and scaling up a new formulation.

Fluticasone propionate ethosomal gel presents a promising approach for enhancing the transdermal delivery of this corticosteroid. By leveraging the unique properties of ethosomes, this formulation may improve therapeutic efficacy while minimizing systemic side effects.

Further studies, including in vivo evaluations, are essential to confirm the clinical benefits and safety of this delivery system. Overall, fluticasone propionate ethosomal gel was a novel formulation and it could represent a valuable addition to topical corticosteroid therapies, offering better patient adherence and therapeutic outcomes.

FUTURE OF ETHOSOMES

The use of ethosomes in skin cosmetic delivery is still in its early stages, and there are several challenges that need to be addressed such as long-term stability study, shelf-life and skin irritation. However, the potential benefits of using ethosomes as a cosmetic delivery system are significant, and further research is needed to fully explore their potential in this field.

The potential use of ethosomes in skin care delivery have also been investigated for the delivery of other cosmetic actives, such as hyaluronic acid, collagen, and peptides-94. These ingredients have shown great promise in improving skin hydration, firmness, and elasticity and their delivery using ethosomes may enhance their efficacy.

Ethosomes have shown promising results as drug delivery systems in the pharmaceutical industry, and their potential for skin care applications is increasingly being explored. The unique structure and composition of ethosomes make them suitable for the delivery of active cosmetic ingredients for the treatment of hair loss, acne, and skin whitening such as vitamins, antioxidants, and skin-lightening agents, through the skin. Their ability to enhance the penetration of drugs through the skin makes them a versatile and effective alternative to traditional transdermal drug delivery systems. The use of ethanol in their composition allows for increased stability, enhanced drug-loading capacity, and targeted drug delivery. However, the preparation of ethosomes can be complex and time-consuming and their stability can be affected by temperature and humidity. However, further research is needed to fully understand the potential benefits and limitations of using ethosomes in these indications and to optimize the formulations and methods of administration.

FUTURE SCOPE:

In future I would like to extent my present studies by including remaining characterization and evaluation. It may include,

- Vesicle shape (Transmission Electron Microscopy)

- HPA Axis Suppression test
- Carcinogenity tests
- Mutagenicity and Clastogenicity tests
- HPLC Assay
- Skin permeation studies by Confocal Laser Scanning Microscopy (CLSM)
- *In-Vivo* Skin permeation studies
- Statistical analysis

Additionally, FP gel has been evaluated in clinical trials for its efficacy in treating various skin conditions. These trials typically assess outcomes such as symptom improvement, quality of life and patient satisfaction.

CHAPTER 9

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