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FORMULATION, DEVELOPMENT AND EVALUATION OF TOPICAL LIPOSOMAL GEL OF FLUCONAZOLE FOR THE TREATMENT OF FUNGAL INFECTION

Nikita Agrawal, Dr. Vimukta Sharma, Dr. Rahul Maheshwari

BM College of Pharmaceutical Education & Research, Indore M.P., India

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***Corresponding author:* Nikita Agrawal**

Email:

nikitacorporate@gmail.com

***Present address*:**

BM College of Pharmaceutical Education & Research, Indore M.P., India

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Abstract:

The present work on the preparation of topical liposomal gel containing fluconazole is an attempt to utilize the immense potential of liposomes as a carrier to increase the permeability. Liposomal encapsulation showed more drug retention compared with plain drug gel .

Further, the physicochemical modification in the drug by means of phospholipid membrane also promises to prolong the drug action. A number of problem associated with drug molecule such as bioavailability, degradation, stability and side effects can be overcome by incorporating it into liposomes. The liposomes of fluconazole was prepared by film hydration method and evaluated. The *In vitro* release of the formulation was studied and found to be more efficient than plain gel. Creation of reservoir effect for drug in skin due to deposition of other components of liposomes with drug into the skin and thereby increasing the drug retention capacity into the skin. Liposomal formulations were more spherical with stable zeta potential and mono-disperse with no clumping.

Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to control. Drug release of all batches was studied. Stability studies performed for Liposomal gel indicates the prepared liposomes have more stability at freezing temperature than that of room temperature.

Fluconazole molecules could be successfully entrapped in liposomes with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing fluconazole has potential application in topical delivery for the treatment of fungal skin ailments.

1. INTRODUCTION

Fungal infection of skin is now-a-days one of the common dermatological problem. The physicians have a wide choice for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among all the topical formulation clear transparent gels have been widely accepted in both cosmetics and pharmaceuticals[1]. In the past decade, liposomal formulations have been extensively used to enhance the drug delivery efficiency through various routes of administration. In a number of instances, liposomal drug formulations in any dosage form, have shown to be markedly superior to the conventional dosage forms, especially for intravenous and topical route of administration of drugs. The major reasons of topical liposomal drug formulations being better to other dosage forms are, ability:

- To reduce serious side effects and incompatibilities that may arise from undesirably high systemic absorption of drug;
- To enhance the accumulation of drug specifically at the site of administration because of the lipid nature of both liposomes and the biological membrane.
- To readily incorporate a wide variety of both hydrophilic and lipophillic drugs.

In recent years, increasing attention is being paid to the development of controlled drug delivery system (CDDS). The advantages of CDDS over conventional therapy are numerous, including better plasma level profile, increased patient compliance, lower dosage and toxicity, possibility of targeting and more efficient utilization of active agent. Liposomes are one of the most suitable drug delivery systems to deliver the drug to the target organ and minimize the distribution of the drug to non-target tissues.[2]

Novel drug delivery systems (NDDS), bear great potential for topical delivery. Among them lipidic and non-lipidic vesicular systems like liposome, niosome, transferosome and ethosome have been used since last decade to overcome the shortcomings with conventional topical formulations. These vesicular systems are found to be much more effective as they provide controlled release of drug. It is so as they form a depot in skin that keeps the therapeutic drug concentration for long period of time.[3,4]

In further chapters of this paper topically applied liposomal formulations is discussed with emphasis on their application for fungal infections using fluconazole as the drug of choice. The topical antifungal formulation would be formulated using drug fluconazole for the deeper fungal infections like candidiasis. Liposomal topical gel is formulated due to its enormous advantages

over conventional dosage forms. The mechanism by which liposomes facilitate deposition of drugs into the skin, route of permeation and its potential applications are discussed.[5]

Topical drug delivery is a very useful and attractive route for both local and systemic treatment. The delivery of drugs onto the skin is identified as an effective means of therapy for local dermatological diseases. Fluconazole, a synthetic antifungal agent, is a triazole derivative. It is used in the treatment of oropharyngeal, esophageal, or vulvovaginal candidiasis as well as other serious systemic candidal infections. It is also effective against superficial fungal infections and dermatophytoses.[6]

Topical liposome formulations could be more effective than conventional formulations. Liposomal carriers, well known for their potential in topical drug delivery and their drug release efficiency have been chosen to help fluconazole molecules to permeate in the skin layers. These vesicles are expected to provide lipid enriched hydrating conditions to retain the drug molecules within the skin layers. With this objective fluconazole loaded liposomal systems will be prepared and their topical performance will be compared with non liposomal systems containing fluconazole.[9]

INTRINSIC WORTH OF TOPICAL LIPOSOME

The major problem which lies with the efficacy of topical drugs is that they have to reach the site of action i.e skin and to stay there in an effective therapeutic concentration for a certain period of time. Although the skin is the organ which can be reached directly; drug application on the surface of skin does not mean the drug is getting to the right site of action.[10] This is the problem with the conventional dosage forms like creams and ointments etc. so in these type of dosage forms penetration enhancers, e.g. dimethylsulphoxide (DMSO) or propylene glycol is used which, on the one hand, improves transport rate through the epidermal barrier but, on the other hand, produce unwanted effects due to an increased systemic drug level. Moreover, irritative and even toxic side effects are reported sometimes leading to the conclusion that addition of penetration enhancers does not really improve topical dosage form.[11,12]

Topical drug administration is a localized drug delivery system which is applied anywhere in the body through ophthalmic, rectal, vaginal, and skin as the topical routes. As discussed topical application have many advantages over the conventional oral or other dosage forms. In general, they are deemed more effective, and less toxic due to the bilayer composition, properties and structure.[12]

QUICK VIEW OF TYPES OF TOPICAL PREPARATIONS

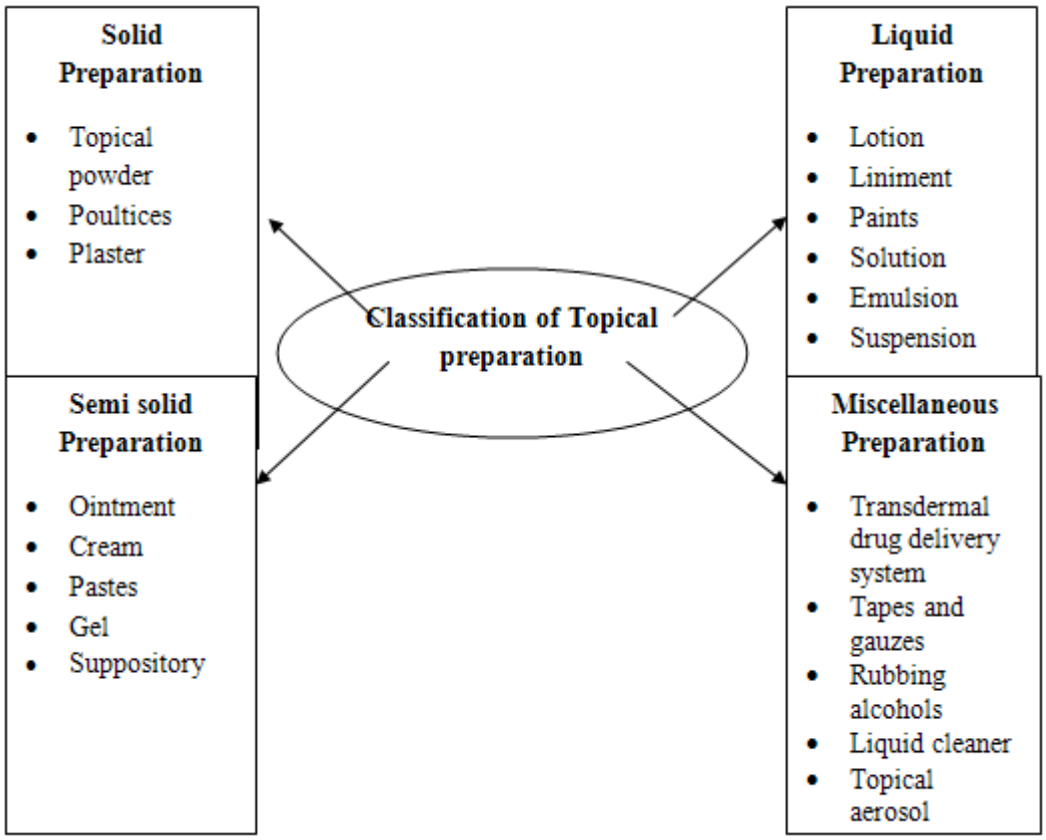

Figure 1: Types Of Topical Preparations

1.1 INTRODUCTION TO FUNGAL INFECTIONS

The fungal infections are incredibly familiar and can be current as well as systemic. The incidence of superficial fungal infections of skin, hair and nails has been increased in worldwide. It has been predictable that about 40 million people have suffered from fungal infections in developing and under de- veloped nations. The succession of fungal infections can be rapid and serious due to compromising with immune function. *Dermatophytes* are one of the the majority frequent causes of *tinea* and o*nchomycosis*. Candidal infec- tions are also among the most widespread superficial cu- taneous fungal infections. Even, *candida* can occupy deeper tissues as well as the blood which leads to life- threatening systemic candidiasis, when the immune system is weakened. The fungal infections can be treat by topically applied medicines as well as by oral administrations. However, oral use of medicine is not much significant in treating local fungal infections and also has systemic side effects.

Fungal infections of the skin are also known as 'mycoses'. They are universal and generally mild. However, in very sick or otherwise immune suppressed people, fungi can sometimes cause serious disease.[13]

1.1.1 CHARACTERISTICS OF FUNGI

Fungi are parasites or saprophytes i.e. they live off living or dead organic matter. Mycologists identify and classify fungi according to their exterior by microscopy and in culture, and by the process of reproduction, which may be sexual or asexual.

Growing fungi have divided filaments called hyphae, which make up the mycelium (like branches are part of a tree). Some fungi are compartmented by cross-walls (called septae).

Arthrospores are made up of fragments of the hyphae, breaking off at the septae. Asexual spores (conidia) form on conidiophores. The sexual reproductive phase of many fungi is unknown; these are 'fungi imperfecta' and include those which infect humans.

Yeasts form a subtype of fungus characterised by clusters of round or oval cells. These bud out comparable cells from their surface to divide and propagate. In some conditions they appearance a chain of cells called a pseudomycelium.

1.1.2 MAJOR FUNGAL INFECTIONS

The number of dissimilar kinds of fungi out there is vast, and, of course, some of them are pleasant to eat. Only a minute subset is capable of infecting humans.[14]

1.1.2.1 Superficial fungal infections

These influence the outer layers of the nails, the skin, and hair. The main groups of fungi causing superficial fungal infections are:

- Dermatophytes
- Yeasts i.e. candida, malassezia, piedra

1.1.2.2 Subcutaneous fungal infections

These occupy the deeper layers of the skin (the dermis, subcutaneous tissue and even bone). The causative organisms in general live in the soil or in rotting vegetation. They can get into the skin as a result of an injury but frequently stay localized at the site of injury. Deeper skin infections include:

- Mycetoma
- Chromoblastomycosis

1.1.2.3 Systemic fungal infections

Systemic mycoses may consequence from breathing in the spores of fungi, which usually live in the soil or rotting vegetation or as opportunistic disease in protected compromised individuals. Systemic fungal infections are more infectious as they are usually more difficult to diagnose, are

chronic in nature, and, in some cases, can become life-threatening. They occur more recurrently in individuals with compromised immune systems (cancer patients; AIDS patients; transplant patients). Prophylactic treatment is sometimes indicated in AIDS patients and bone marrow transplant patients, but risk of developing resistance is high. Life-threatening infections require the utilization of more potent but much more toxic antifungals.[15]

1.1.2.4 Inhaled fungal infection

Although uncommon, some may infect healthy individuals. The result is most often a mild infection and long lasting resistance to further attack, but occasionally these infections are more somber and chronic (especially in the immune suppressed).

1.1.3 A BRIEF DESCRIPTION OF SOME COMMON FUNGAL INFECTIONS

1.1.3.1 Candidiasis

Candidiasis is a fungal infection due to any types of *Candida* (a type of yeasts). When it affects the mouth, it is commonly called thrush. Symptoms embrace appearance of white patches on the tongue or other areas of the oral cavity i.e mouth and throat. Other symptoms may contain soreness in throat and problem in swallowing .If it affect the vagina, it is usually called as yeast infection. Symptoms include genital itching, burning sensation, and sometimes a white "cottage cheese-like" discharge from the vagina. Less commonly the penis may be affected, resulting in itchiness. Very rarely, the infection becomes invasive and spread throughout the body, consequential in fever sometimes.[16]

There are more than 20 types of *Candida* can cause infection, but *Candida albicans* is the most common. Infections of the mouth are the majority common among children less than one month old, the elderly, and those with weak immune systems. Conditions that result in a weaker immune systems include HIV/AIDS, after organ transplantation, diabetes, and the use corticosteroids. Other risks include dentures and following antibiotic therapy. Vaginal infections occur more commonly during pregnancy, in those with weak immune systems, and following antibiotic use. Risk for widespread infection includes being in an exhaustive care unit, , low birth weight infants, following surgery and those with weak immune systems.[10]

For infections of the mouth, treatment with topical clotrimazole or nystatin is usually effective. Oral or intravenous fluconazole, itraconazole, or amphotericin B may be used if these do not work. A number of topical antifungal medications are used for vaginal infections including clotrimazole. In certain groups at very high risk antifungal medications may be used preventatively.

Symptoms of candidiasis vary according to the area affected. Mainly candidial infections effect in minimal complications such as redness, itching, and discomfort, on the other offer the complications may be severe or yet fatal if left untreated. In immunocompetent persons, candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx or esophagus, the gastrointestinal tract, the rectum, anus, perianal/perirectal or ano-rectal area (in men as well as women), the perineum, urinary bladder, the finger and toe nails (onychomycosis), and the genitalia (vagina, penis, ectcetera).

Candidiasis is a very common cause of vaginal irritation, or vaginitis, and can also occur on the male genitals. In immunocompromised patients, *Candida* infections can affect the esophagus with the potential of becoming systemic, causing a much more serious condition, called candidiasis.

Oral candidiasis is the most familiar fungal infection of the mouth, and it also represent the mainly common opportunistic oral infection in humans. They are commonly treated with antimycotics; antifungal drugs; these contain topical nystatin, fluconazole, topical clotrimazole, and topical ketoconazole.

1.1.3.2 Mycosis

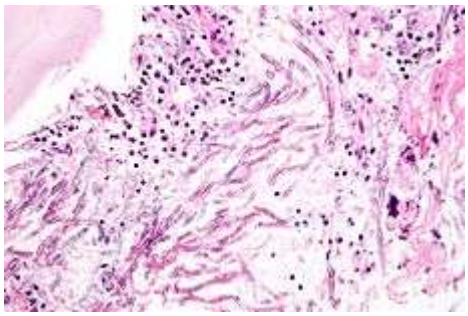

Figure 3: Micrograph showing a mycosis (aspergillosis).

Mycosis (plural: **mycoses**) is a fungal infection of animals, including humans. Mycoses are universal and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may instigate persistent infections; as a result, mycoses often start in the lungs or on the skin.

Individuals with weakened immune systems are also at risk of increasing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are groups at risk.[16]

1.1.3.2.1 Brief Classification

Mycoses are classified according to the tissue levels initially colonized.

- **Superficial mycoses**

External mycoses are limited to the outermost layers of the skin and hair.

An example of such a fungal infection is *Tinea versicolor*, a fungus infection that normally affects the skin of young people, especially the chest, back, and upper arms and legs. These fungus produce spots that are either lighter than the skin or a reddish-brown. This fungus exists in two forms, one of them causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities. However, almost all people with this very common condition are healthy.

1.1.3.3 ATHLETE'S FOOT

Athlete's foot is the term worn for a common disorder affecting the skin between the toes. The cleft between the fourth and fifth toes is the most frequently affected, with moist soft skin that peels off easily. Often the skin splits uncomfortably (a fissure). It may smell unpleasant. It is normally mild; very inflamed athlete's foot is generally due to resultant bacterial infection. The ordinary disease is the athlete's foot which most commonly affects children before puberty.

❖ **Causes**

- Athlete's foot can be due to one factor or a combination of factors.
- Bacterial infection (erythrasma), pseudomonas, Staphylococci and Streptococci).
- Mould infection.
- Soft corn (build-up of thick skin because the toes are pressing against each other).
- Injury e.g.vigorous removal of peeling skin.
- Skin conditions such as psoriasis, eczema or keratolysis exfoliativa.
- Fungal infection (tinea pedis).

❖ **Treatment**

Treatment for athlete’s foot begins with general measures:

- Dry carefully between the toes.
- Keep toes apart using a cotton or foam wedge.
- Should wear shoes that are loose around the toes or go bare foot.

- Apply a topical antifungal agent. These may also control many of the bacteria responsible for athlete's foot.
- Whitfield's ointment is particularly useful as it removes the surface layer of moist peeling skin (i.e. it is keratolytic) and eliminates bacteria and fungi.

1.1.3.4 CRADLE CAP (infantile seborrhoeic dermatitis)

Cradle cap is a patchy, greasy, scaly and crusty skin rash that occur on the scalp of newly born babies. Cradle cap is infantile seborrhoeic dermatitis that is restricted to the scalp. childish seborrhoeic dermatitis may also affect other areas of the body such as behind the ears, in the crease of the neck, armpits and diaper area.

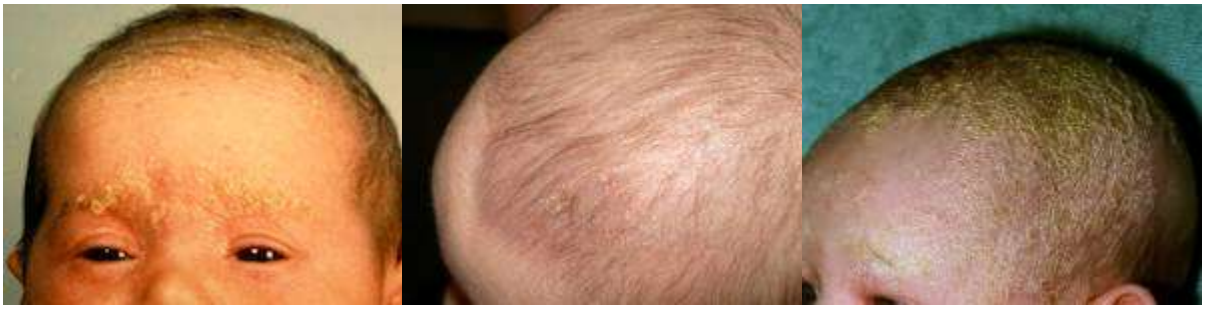

Figure 5: Cradle cap

1.3.3.6 ADULT SEBORRHOEIC ECZEMA

Seborrhoeic eczema affect scalp, face (creases around the nose, behind ears, within eyebrows) and upper trunk.

1.3.3.6.1 Diagnosis

Seborrhoeic eczema is diagnosed by its experimental appearance and behaviour. As *Malassezia* is a normal component of skin flora, its attendance on microscopy of skin scrapings is not diagnostic. Skin biopsy may be helpful but is rarely indicated.

1.3.3.6.2 Treatment

Treatment of seborrhoeic dermatitis often involve several of the following options.

- Keratolytics are used when essential, eg salicylic acid, lactic acid, urea, propylene glycol
- New antifungal agents are active against *Malassezia* eg ketoconazole, or ciclopirox shampoo or cream.
- Mild topical corticosteroids for 1-3 weeks to decrease inflammation in acute flare

- Topical calcineurin inhibitors (pimecrolimus cream, tacrolimus ointment) as required
- In resistant cases in adults, oral itraconazole, tetracycline antibiotics or phototherapy may be recommended.
- Ketoconazole or ciclopirox cream can be used once daily for 2 to 4 weeks, repeated as necessary.[18]

1.4 LIPOSOMES AS NOVEL DRUG DELIVERY SYSTEM

An ideal release system delivers drug at a specified rate in the body in a confident period of time for an effective treatment. Drugs used in the treatment of various fungal disease can be highly toxic to standard tissues. The toxicity of these drugs could be minimized by decreasing delivery to healthy cells and tissues.

Liposomes have been considered to be excellent models of cell membranes. They explain effective drug delivery which is frequently used in dermal applications. Liposomes were first described by British haematologist Alec D Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge. Liposomes are microscopic spherical vesicles together of one or more lipid bilayers with an aqueous core. Liposomes are lengthily used as carriers for numerous molecules in cosmetic and pharmaceutical industries. To grow delivery system that can entrap unstable compounds and to shield their functionality, food and undeveloped industries uses the liposome encapsulation.[22]

Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets. As a drug delivery system, liposomes, commercially, have increased rate of use because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues. Many studies have been conducted on liposomes with the aim of decreasing drug toxicity or targeting specific cells.[23]

One of the main aims of any behavior employing drug is to enlarge the therapeutic index of the drug and while minimizing its side effects. The clinical usefulness of most conservative chemotherapeutics is controlled either by the incapability to transport therapeutic drug concentrations to the target soft tissue and harmful toxic side effects on normal organs and tissues. Different approaches have been made to conquer these difficulty by providing the 'selective' delivery to the target area; the ideal answer would be to target the drug alone to those

cells, tissues, organs that are affected by the disease. The significance of liposomes lies in their composition, which makes them biodegradable and biocompatible.

The liposomes contain drugs can be administrated by many routes i.e. intravenous, oral, nasal, intramuscular, pulmonary, topical, and ocular. They can be delivered in many vesicles like creams, ointments, capsules, solutions, sprays, etc. Liposomes can be used for the treatment of many diseases:, endocrine, arthritis, cancer, bacterial, fungal, ocular, vaccines, fibrinolysis asthma, diabetes, diseases of immune system, drugs used to achieve relief from pain and topical anesthesia.

Liposomes are classified on the basis of structural parameters, and application in biology, biochemistry, cosmetics and medicine method of preparation, composition. Phospholipids which are capable of forming liposomes include both natural and synthetic phospholipids. The main sources of natural phospholipids are yolk egg and soya bean although they can be obtained from plant oils such as olive oil. Liposomes can be confidential as conventional, pH sensitive liposomes, cationic liposomes, immunoliposomes, temperature or heat sensitive liposomes, magnetic liposomes and sterically stabilized “stealth” liposomes.[23]

Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticuloendothelial system(RES). These liposomes are known as "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery instrument. Though, research currently seeks to investigate at what amount of PEG coating the PEG actually hinders binding of the liposome to the delivery site. In addition to a PEG coating, most stealth liposomes also have some sort of biological species attached as a ligand to the liposome in order to enable binding via a specific expression on the targeted drug delivery site.[24] These targeting ligands could be monoclonal antibodies (making an immunoliposome), vitamins, or specific antigens. Targeted liposomes can target nearly any cell type in the body and transport drugs that would naturally be systemically delivered.

The mainly common used phospholipids in preparation of liposomes are:

Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), dipalmitoylphosphatidylcholine (DPPC), dipalmitoyl-phosphatidylglycerol dioleoylphosphatidylethanolamine (DOPE). 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, distearoylphosphatidylcholine (DSPC), dipalmitoylphosphatidylserine.

The conservative liposomes are characteristically composed of phosphatidylcholine and cholesterol. On the other hand, "stealth" liposomes are lipid bilayers coated with polyethylene glycol(PEG). The "stealth" liposomes provide enhanced stabilization and additional protection of the entrapped substances. They allow longer circulation time for the drug delivery mechanism. The importance of a prolonged period of time can be achieved by continued release of the drug.

Liposomes are composed of accepted phospholipids that are biologically inert and feebly immunogenic, and they have low inherent toxicity. Furthermore, drugs with different lipophilicities can be encapsulated into liposomes: strongly lipophilic drugs are entrapped almost totally in the lipid bilayer, intensely hydrophilic drugs are situated entirely in the aqueous compartment.[25]

1.4.1 CLASSIFICATION OF LIPOSOMES BASED ON STRUCTURE PARAMETERS

1. Multilamellar Large vesicles > 0.5um
2. Oligolamellar vesicles (0.1-1um)
3. Unilamellar vesicles (All size range)
   a) Small unilamellar vesicles 20-100nm
   b) Medium sized unilamellar vesicles
   c) Large unilamellar vesicles >100nm
   d) Giant unilamellar vesicles >1um
4. Multivesicular vesicles >1um

1.4.2 STRUCTURE AND COMPOSITION OF LIPOSOME

Among the variety of novel drug delivery systems liposomes seem to have the best potential to accommodate both water and lipid soluble compounds to protect the liposome-encapsulated drug from metabolic degradation and to act as a delivery instrument, releasing active ingredients slowly and in a controlled manner.

Phospholipid, the component of the liposome lipid bilayer is usually extracted from egg yolk or soy bean oil consists of a hydrophilic head portion covalently attached to two hydrocarbon tails representing the lipophilic portion. Aggregation in a bilayer structure occurs by direction of the hydrophilic head groups towards the aqueous environment.[35] While keeping the lipophilic hydrocarbon chains sequestered inside. Formation of such a configuration provides the vesicle

with the lowest potential energy state during solvation of the polar head groups and hydrophobic interactions of the lipid chain.

Natural phosphatidylcholine extracted from egg yolk or soy bean oil or its semisynthetic derivatives represents the main constituent in various liposomal formulations. The chemical structure of naturally occurring phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. Each may have between 10 to 24 carbon atoms together forming the hydrophobic (lipophilic) portion of the particle. The phosphate and choline moieties form the hydrophilic "head".

The fatty acid chains, depending on their length and degree of saturation, can exist in the gel phase in which the lipids are rigid, impermeable and easily aggregated upon storage or in the more fluid liquid crystalline phase. The temperature at which the gel phase converts to the liquid crystalline phase is known as the transition temperature. Cholesterol is normally added in minute quantities to most liposomal formulations to increase the fluidity of the liposomal gel phase enhance the retention of hydrophilic particles and to steady the bilayer membrane in a manner similar to that of biological membranes.[26]

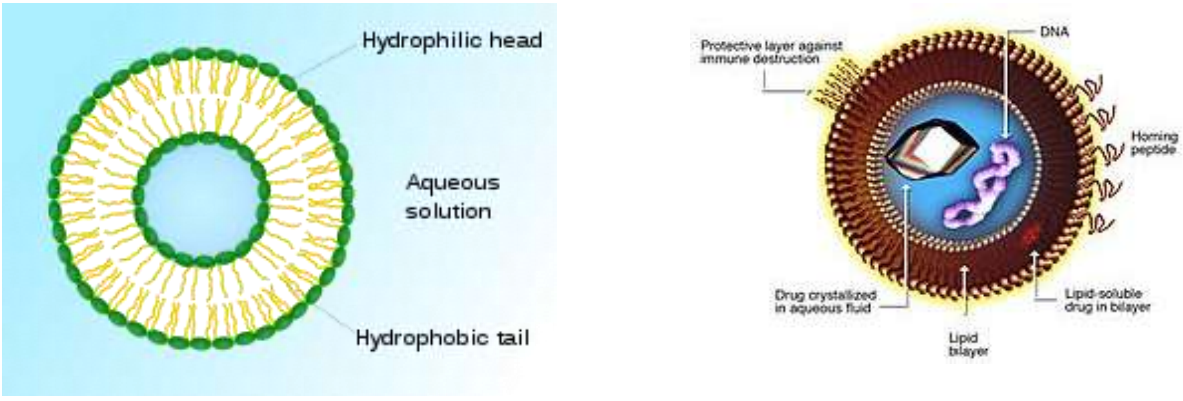

Figure 10: Structure of Liposomes.

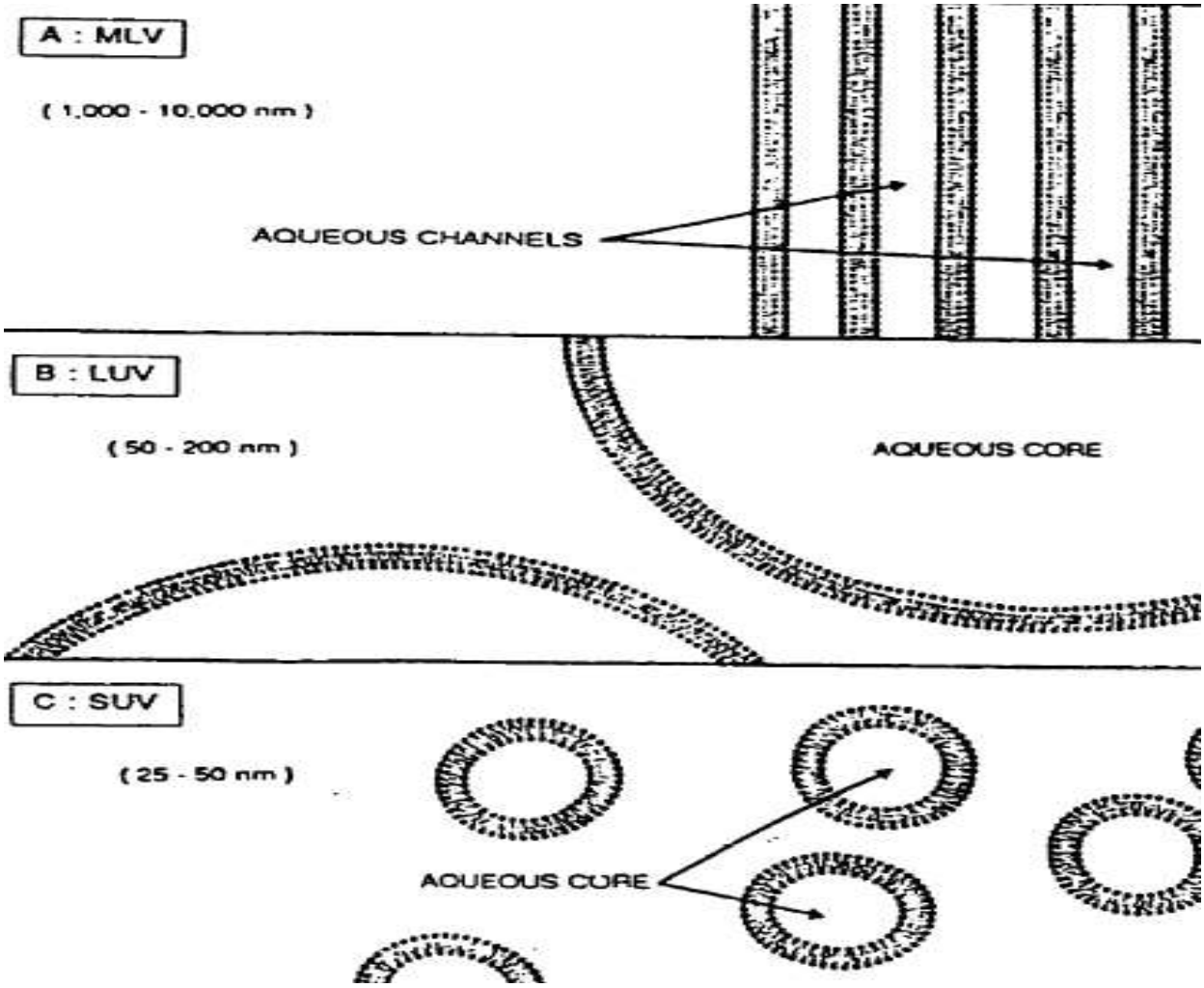

Figure 11: A diagrammatic representation of three main class of liposomes.(A) Multilamellar vesicles; (B) Large unilamellar vesicles; (C) Small unilamellar vesicles

1.4.3 METHODS OF PREPARATION OF LIPOSOMES

1.4.3.1 Multilamellar Liposomes (MLV)

1. Lipid hydration method:

This is the most widely used scheme for the research of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for a short time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the maximum melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities.MLV are simple to organize by this method and a variety of substances can been capsulated in these liposomes. The drawback of the process are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous.

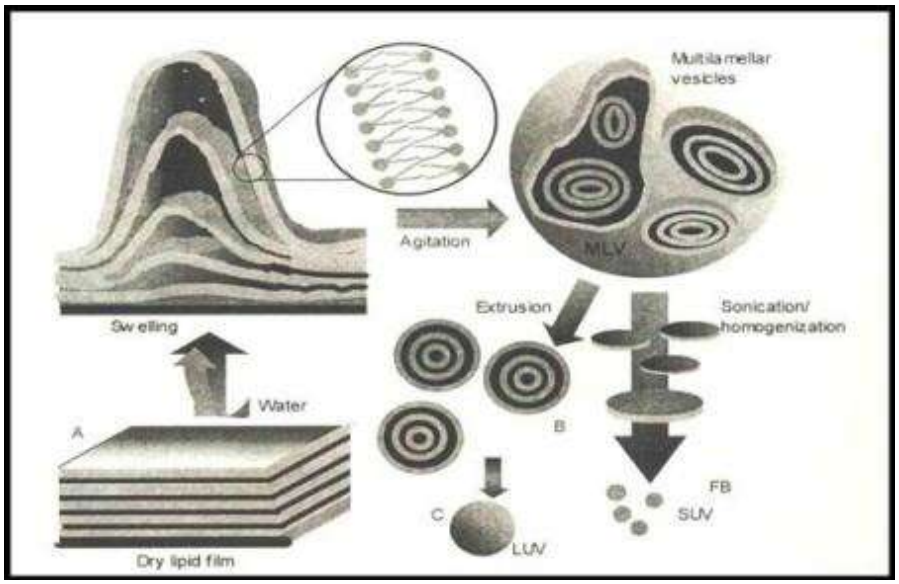

Figure 12: Multilamellar vesicles

2. Solvent spherule method:

A process for the preparation of MLVs of homogeneous size distribution .The procedure involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were created when controlled evaporation of organic solvent occurred in a water bath.

1.4.3.2 Small Unilamellar Liposomes (SUV):

1. Sanitation Method:

Here MLVs are solicited either with a bath type sonicator or a probe sonicator below an inert atmosphere. The main drawbacks of this method are very low interior volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulate, exclusion of large molecules, metal pollution from probe tip and existence of MLV along with SUV.

***2. French Pressure Cell Method**:*

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has a number of compensation over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs.

1.4.3.3 Large Unilamellar Liposomes (LUV)

They encompass high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

1. Solvent Injection Methods

a) Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70- 190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.

b) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the populace is heterogeneous (30-110 nm), liposomes are very dilute, it is complex to remove all ethanol because it forms azeotrope with water and the possibility of various biologically lively macromolecules to inactivation in the presence of even low amounts of ethanol.

c) Reverse Phase Evaporation Method

First water in oil emulsion is formed by sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure.

With this method high encapsulation competence up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication.

d) Calcium-Induced Fusion Method:

This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structure in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. The foremost advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, even though of a assorted size range. The chief drawback of this method is that LUVs can only be obtained from acidic phospholipids.

e) Freeze-Thaw Method:

SUVs are rapidly frozen and followed by slow thawing. The concise sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is unpaid to the fusion of SUV during the processes of freezing and or thawing. This type of fusion is strongly repressed by increasing the ionic force of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained.

1.4.3.4 Giant Liposomes

The procedure for the arrangement of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methylglucoside detergent alongside an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm.[26,27]

1.4.3.5 The advantages of liposomes as drug carrier systems

1. Similar to biological membranes they can accumulate water-soluble and **lipophilic** substances in their different phases i.e. it readily incorporate a wide diversity of hydrophilic and hydrophobic drugs.
2. They are similar to the epidermis with respect to their lipid composition which enables them to penetrate the epidermal barrier to a greater extent compared to other dosage forms.
3. According to studies performed so far liposomes are biodegradable and non-toxic which is important to avoid side effects.
4. The new aspect with liposomes is that they are thought to act as “drug localizers” - not only as “drug transporters”. i.e. to enhance significantly the accumulation of drug at the site of administration as a result of the high retentivity of liposomes with biological membranes.[42]

5. The uptake of intact liposomes by the reconstructed epidermis, these vesicles do not penetrate through healthy skin. Yet, this is to be expected in diseased skin with no intact epidermal barrier. This is particularly important as far as drugs like glucocorticosteroids or retinoids are concerned which are known to create severe systemic effects when absorbed percutaneously to a greater extent. Vehicles which can transfer these drugs to the wanted site of action within the skin would thus prevent systemic absorption and consecutively unwanted effects. This is the reason why liposomes as a promising form for current drug delivery.[27]
6. Liposome may serve as a local store for the sustained release of dermally active compounds including antibiotics, corticosteroids or retinoic acid.
7. By virtue of penetration of individual phospholipid molecules or nonionic ether surfactants into the lipid layers of the stratum corneum and epidermis they may serve as penetration enhancer and facilitate dermal delivery leading to higher restricted drug concentrations.

1.4.4 MECHANISM OF ACTION OF TOPICAL LIPOSOME

The mode by which liposome facilitate transfer of drug into living skin strata and beyond has always been a topic of much interest. They propose a simple hypothesis of liposomal action that accounts for a majority of the effects observed. For a liposomal formulation to be effective, especially for hydrophilic drugs, it is critical that the deferment experience significant dehydration. Since in most studies reported the lipid concentration scarcely exceeds 100 mg/ml, the bulk aqueous medium constitutes roughly 90% of the formulation. Thus, without a high degree of dehydration, no compensation over simple aqueous solution can be governed by employing liposomal systems, especially if the drug action is anticipated to occur within few hours after application. The dehydration of liposomal suspension can either be complete or reach an equilibrium stage wherein a certain amount of water is always held within the bilayers.[28]

Drug Criteria for Topical Liposomal Drug Delivery System which groups of substances are considered to be especially interesting for liposomal encapsulation in the field of dermatology?

1. There are drugs which are known to have severe side-effects by the predictable way of topical administration, e.g. topical glucocorticosteroids.
2. There are substances which normally are helpful by systemic application but not by topical application, e.g. interferon.
3. There are drugs which only show inadequate effects when applied topically. e.g. hamamelis distillate.

1.4.5 Marketed Formulation Of Topical Liposome

❖ **Celadrin®:**

Celadrin® Topical Liposome Lotion- 4 oz. (a proprietary blend of esterified fatty acid carbons), MSM (Methylsulphonylmethane), *Arnica spp*. Flower Tincture and Natural Menthol (1.25%).

Celadrin® is a registered trademark of Imagenetix, Inc.

1.5 INTRODUCTION TO GEL DOSAGE FORMS

Gels are semisolid systems in which a liquid phase is inhibited surrounded by a three dimensional polymeric matrix, in which a high degree of corporal cross linking has been introduce. It consists of either natural or synthetic gums.[24] The structural material that is worn to form the gel network is composed of inorganic particles or organic macromolecules, primarily called as polymers. Cross link network can be shaped via chemical or physical interactions. This had lead to classification of gel into chemical and physical gel systems, respectively. Chemical gels are connected with permanent chemical covalent bonding between atoms while physical gels are coupled with relatively weaker and reversible inter molecular interactions such as hydrogen bonding, dipole dipole interactions, Vander Waals forces electrostatic interactions and hydrophobic interactions.[29]

DEFINITION - GEL (U.S.P.):

A semisolid system consisting of dispersion complete up of either small inorganic particles or large organic molecules enclosing and interpenetrated by continues phase liquid. Gels consist of two phase system in which inert particles are not dissolve but merely dispersed throughout the continuous phase and large organic molecules are dissolved in the continuous phase, arbitrarily coiled in the flexible chains. Gels are used dermatologically and have so many favorable properties such as thixotropy, easily removed, emollient, non-staining, greaseless, easily spreadable, and compatible with several excipients and water soluble or miscille, non grittiness. These all characteristics make them valuable in topical drug delivery system.[26,29]

Gels are defined as “the semisolid system in which a liquid phase is constrained within a polymeric matrix containing high degree of physical and chemical cross-linking”.

1.5.1 STRUCTURE OF GEL

The gel is inflexible due to the attendance of a network formed by the interlinking of particle gelling agent. The structure of network depends on nature of the particles and the type of force that determine the properties of gel.[28] The entity particles of hydrophilic colloid may consist of either spherical or an isometric aggregates of small molecules, or single macromolecules. In linear macromolecules the network is comprise of entangled molecules, the point of contact between which may either be relatively small or consist of several molecules aligned in a crystalline order.[31] The force of appeal responsible for the linkage between gelling agent particles may range from strong most important valencies, as in silicylic acid gels, to weaker hydrogen bonds and vander waals forces. The weaker nature of these latter forces is indicate by the fact that a slight increase in temperature frequently cause liquefaction of gel.

5.2 PROPERTIES OF GELS

1. Ideally, the gelling agent for pharmaceutical or cosmetic use should be inert, safe, and should not react with other formulation components.
2. The gelling agent incorporated in the preparation should create a reasonable solid-like nature during storage that can be easily broken when subjected to shear forces generated by shaking the bottle, squeezing the tube, or through topical application.
3. It ought to possess suitable anti-microbial to avert from microbial attack.
4. The topical gel be supposed to not be tacky.
5. The ophthalmic gel be supposed to be sterile.

1.5.3 CHARACTERISTICS OF GELS

a) Swelling

When a gelling agent is kept in make contact with with liquid that solvates it, then an appreciable amount of liquid is taken up by the agent and the volume increases. This method is referred to as swelling. This phenomenon occurs as the in the money penetrates the matrix. Gel-gel relations are replaced by gel solvent interactions.[29,30] The quantity of swelling depends on the number of linkages between entity molecules of gelling agent and on the strength of these linkages.

b) Syneresis

Many gels frequently contract spontaneously on standing and exude some fluid medium. This consequence is known as syneresis. The quantity to which syneresis occurs, increases as the concentration of gelling agent decreases. The incidence of syneresis indicates that the original gel

was thermodynamically unstable. The mechanism of contraction has been related to the reduction of elastic stress developed during the setting of the gels. As these stresses are relieved, the interstitial space available for the solvent is summary, forcing the liquid out.[31]

c) Ageing

Colloidal systems usually exhibit slow unprompted aggregation. This process is referred to as ageing. In gels, ageing results in measured formation of a denser network of the gelling agent.

d) Structure

The rigidity of a gel network is formed by the interlinking of particles of the gelling agents. The nature of the particle and the stress, straightening them out and lessening the resistance to flow.

e) Rheology

Solutions of the gelling agents and dispersion of flocculated solid are pseudo artificial i.e. exhibiting Non Newtonian flow behaviour, characterized by a decrease in viscosity with increase in shear rate. The tenuous arrangement of inorganic particles discrete in water is disrupted by functional shear stress due to breaking down of inter particulate association, exhibit a greater tendency to flow. Similarly, for macromolecules the applied shear stress aligns the molecules in the course of Organic phase (single phase system).

1.5.4 USES

- To deliver drug directly to the skin, mucous membrane or the eye.
- As long acting forms of drug injected intramuscularly.
- As binders in tablet granulation, protective colloids in suspensions, thickeners in oral liquid and suppository bases[32,33]

1.5.5 CLASSIFICATION OF GELS

Gels can be confidential based on colloidal phases, physical nature, nature of solvent used and rheological properties.

Table 3: Types of gel based on colloidal phases

Class	Description	Examples
Inorganic	Usually two phases system	Bentonite Magma
Organic	Usually single phases system	Carbopol, tragacanth

Hydrogel	Organic hydrogel natural and synthetic gums inorganic	Methyl cellulose, sodium carboxymethyl cellulose, pluronic,
Organogel	Hydrocarbon type animal, vegetable fats soap base greases hydrophilic organogel polar	Petrolatum, mineral oil/ polyethylene gel, cocoa butter aluminium stearate with heavy

1.5.5.1 Based on colloidal phases

They are classified into:-

- Inorganic (two phase system)
- Organic (single phase system)

1.5.5.1.1 Two phase system

If fractional size of the dispersed phase is relatively large and structure, in this organization is not always stable. They ought to be thixotropic-forming semisolids on standing and develop into liquid on agitation.

1.5.5.1.2 Single-phase system

These consist of large organic molecules existing on the twisted strands dissolved in a continuous phase. This larger organic molecule either accepted or synthetic polymers are referred as gel formers, they tend to tangle with each other their accidental motion or bound together by Vander walls forces.[31]

1.5.5.2 Based on nature of solvent

- Hydro gels (water based):

  Here they include water as their incessant liquid phase.

  E.g.: bentonite magma, Gelatin, , carpooler, cellulose derivatives and poloxamer gel.

- Organic Gels (with a non-aqueous solvent):

  These enclose a non-aqueous solvent on their continuous phase.

  E.g. plastibase (low molecular wt polyethylene dissolved in mineral oil & short Cooled) Olag (aerosol) gel and dispersion of metallic stearate in oils.

- Xerogels:

Solid gels with low in the chips concentration are known as xerogels. These are created by evaporation of solvent or freeze drying, leaving the gel structure behind on write to with fresh fluid, they swells and can be reconstituted.

E.g. Tragacanth ribbons, acacia tear β-cyclodextrin, dry cellulose and polystyrene.

1.5.5.3 Based on rheological properties

Usually gels display non-Newtonian flow properties.

They are classified into,

a) Plastic gels

b) Pseudo plastic gels

c) Thixotropic gels.

(a) Plastic gels

E.g. - Bingham bodies, flocculated suspensions of Aluminum hydroxide exhibit a artificial flow and the plot of rheogram gives the yield worth of the gels above which the elastic gel distorts and begins to flow.

(b) Pseudo-plastic gels

E.g.: - Liquid dispersion of tragacanth, sodium alginate, Na CMC etc. exhibits pseudo-plastic flow. The viscosity of these gels decreases with escalating rate of shear, with no yield value. The rheogram results from a shearing action on the long chain molecules of the linear polymers. As the shear stress is increased the disordered molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.[32]

(c) Thixotropic gels

The bonds between particle in these gels are very weak and can be broken down by shaking. The resultant solution will revert back to gel due to the particles colliding and linking together again (the reversible isothermal gel-sol-gel transformation). This occurs in colloidal system with non-spherical particles to build up a scaffold like structure.

E.g.: Kaolin, bentonite and agar.

1.5.5.4 Based on physical nature

(a) Elastic gels:

Gels of agar, pectin, Guar gum and alginates exhibit an elastic behaviour. The rubbery molecules being linked at the point of junction by quite weak bonds such as hydrogen bonds and dipole attraction. If the molecule possesses free –COOH group then supplementary bonding takes place by salt overpass of type –COO-X-COO between two adjacent strand networks.

E.g.: Alginate and Carbapol.

(b) Rigid gels:

This can be twisted from macromolecule in which the structure linked by primary valance

bond.

E.g.: In silica gel, silic acid molecules are held by Si-O-Si-O bond to give a polymer structure

possessing a network of pores.

1.5.6 PREPARATION OF GELS

Gels are in general in the industrial scale equipped under room temperature. Though a small number of polymer need special treatment before processing. Gels can be organized by following methods.

1. Thermal changes

2. Flocculation

3. Chemical reaction

1) Thermal changes

Solvated polymers (lipophilic colloids) when subjected to thermal changes causes gelatin. A lot of hydrogen formers are more soluble in hot than cold water. If the temperature is reducing, the degree of hydration is reduced and gelatin occurs. (Cooling of a concentrated hot explanation will produce a gel.

E.g.: - Gelatin, agar sodium oleate, guargummed and cellulose derivatives etc. In dissimilarity to this, some materials like cellulose ether have their water solubility to hydrogen bonding with the water. Raising the temperature of these solutions will interrupt the hydrogen bonding and reduced solubility, which will cause gelation. Hence this method cannot be adopted to prepare gels as a all-purpose method.[34][35]

2) Flocculation

Here gelation is produced by adding just adequate quantity of salt to precipitate to produce age state but insufficient to bring about complete precipitation. It is essential to ensure rapid mixing to circumvent local high concentration of precipitant.

E.g.: Solution of ethyl cellulose, polystyrene in benzene can be gelled by rapid mixing with suitable amounts of a non-solvent such as petroleum ether. The calculation of salts to hydrophobic solution brings about coagulation and relation is hardly ever observed. The gels formed by flocculation process are Thixotropic in behaviour. Hydrophilic colloids such as gelatin, proteins and acacia are only affected by high concentration of electrolytes, when the effect is to "salt out", the colloidal and gelation doesn't occur.

3) Chemical reaction

In this process gel is created by chemical interaction between the solute and solvent. E.g.: aluminium hydroxide gel can be equipped by interface in aqueous solution of an aluminium salt and sodium carbonate an augmented concentration of reactants will produce a gel structure.

PREPARATION OF CARBOPOL GEL AND INCORPORATION OF LIPOSOMES INTO CARBOPOL GEL

7.2.1 PREPARATION OF LIPOSOMES

Different weight ratio of phospholipids(soyalecithin): choleseterol and stearic acid was weighed in different ratios and dissolved in chloroform:methanol mixture (2: 1 v/v) in 250 ml round bottom flask. A thin film was formed on the inner side of round bottom flask by evaporating organic solvent under vacuum in rotary evaporator at 45-50 °C. Subsequently, the flask kept overnight under vacuum to ensure the complete removal of residual solvent. The dry lipid film was hydrated with 20 ml phosphate buffer solution (pH 7.4) containing fluconazole at a temperature of 60±2 °C. The dispersion will be left undisturbed at room temperature for 2-3 h to allow complete swelling of the lipid film and hence obtain vesicular dispersion.

7.2.2 PREPARATION OF CARBOPOL GEL

Aqueous liposomal formulations were prepared by conventional lipid film hydration method. As a vehicle for incorporation of liposomes for topical delivery, a carbopol gel was made.

Carbopol 934 (1 g) was dispersed in distilled water (88 g) by stirring at 800 rpm for 60 minutes. Then, propylene glycol (10 g) was added and the mixture was neutralised by

dropwise addition of tri-ethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 5.5.[5,69]

Table 6: Table showing composition of Fluconazole loaded liposomal gel

Formulation	Fluconazole	Phospholipids	Cholesetrol	Stearic acid	Carbopol 934	Ratio P:Ch:S:C
F1	100mg	100mg	500mg	500mg	500mg	1:1:5:5:5
F2	100mg	200mg	500mg	500mg	500mg	1:2:5:5:5
F3	100mg	100mg	1000mg	1000mg	700mg	1:1:10:5:5
F4	100mg	200mg	1000mg	1000mg	700mg	1:2:10:10:7
F5	100mg	300mg	500mg	500mg	500mg	1:3:5:5:5
F6	100mg	300mg	1000mg	1000mg	700mg	1:1:10:10:7
F7	100mg	400mg	500mg	500mg	500mg	1:4:5:5:5
F8	100mg	400mg	1000mg	1000mg	1000mg	1:4:5:5:7

7.2.3 INCORPORATION OF LIPOSOMES OF OPTIMIZED BATCH INTO CARBOPOL GEL

Fluconazole loaded liposomes were prepared by thin film hydration technique using soya lecithin, cholesterol and drug in different weight ratios.[70] Liposomes containing fluconazole (separated from the unentrapped drug) were mixed into the 1% (w/w) Carbopol gel with an electrical mixer (25 rpm, 2 min), the amount of liposomes of optimized batch added into the gel, such that the prepared gel have 2% w/w fluconazole concentration (20 mg drug per 1gm of gel). Plain drug gels (2% w/w) were made under the same conditions. Instead of liposomes, those samples contained free fluconazole were incorporated.

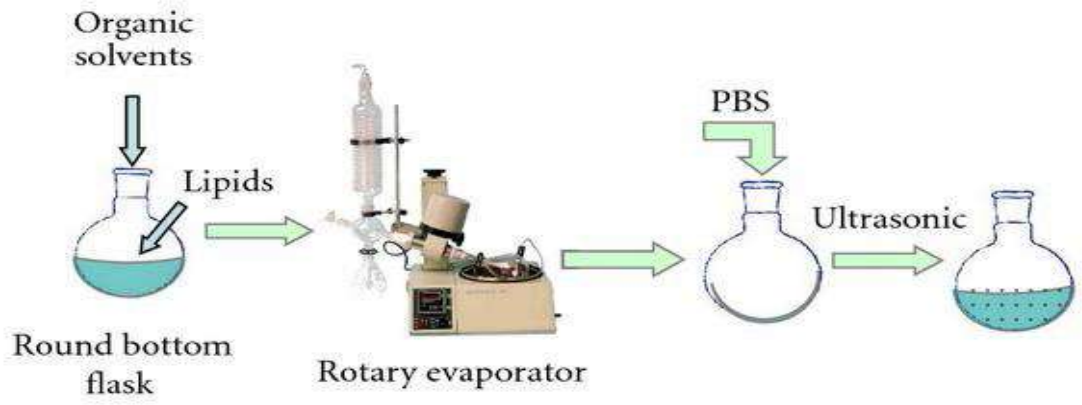

Figure 17: Assembly for thin film hydration method

7.3 EVALUATION OF LIPOSOMAL GEL

The characterization parameters for the purpose of evaluation was carried out in following categories, which include physical, chemical and biological parameters.[17]

Physical classification evaluates various parameters, including size, shape, surface features, lamellarity, phase behaviour and drug release profile.

Chemical characterization includes those studies which establish the purity and potency of various liposomal constituents.

Biological characterization parameters are helpful in establishing the safety and suitability of the formulations for the in vivo use or the therapeutic application.

Physical and chemical characterizations are very important for meaningful comparison of dissimilar liposomes arrangements for different batches. Biological consideration helps to ensure safety of use in humans.

A. Physicochemical Evaluation of liposomal gel

1) Physical examination: The prepared Liposomal gel was evaluated for colour, and transparency.

2) pH: The pH values of 1% aqueous solutions of the prepared gels were measured by digital pH meter.(Chameline model)

3) Drug Content uniformity:

Preparation of standard graph

Stock solution of Fluconazole: Stock solution of 100µg/ml was prepared by dissolving 10 mg of fluconazole in 100 ml of methanol. Dilution in the range of 10 to 40 µg/ml were scanned for determining λmax from 200-400 through UV spectrophotometer(Systronic model) and λmax was found to be at 260 nm for fluconazole.

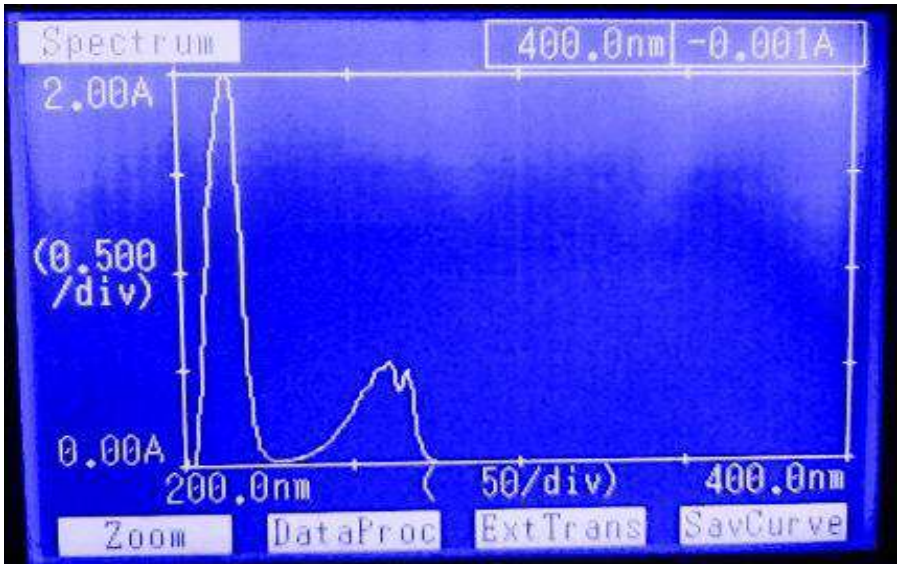

Figure 18: λmax of Fluconazole in methanol

Table 7: Absorbance of different dilutions of drug at 260 nm in methanol

S.No.	Concentration (µg/ml)	Absorbance (mean ± SD)(n=3)
1.	0	0
2.	10	0.131 ± 0.001
3.	20	0.262 ± 0.004
4.	30	0.355 ± 0.002
5.	40	0.448 ± 0.003

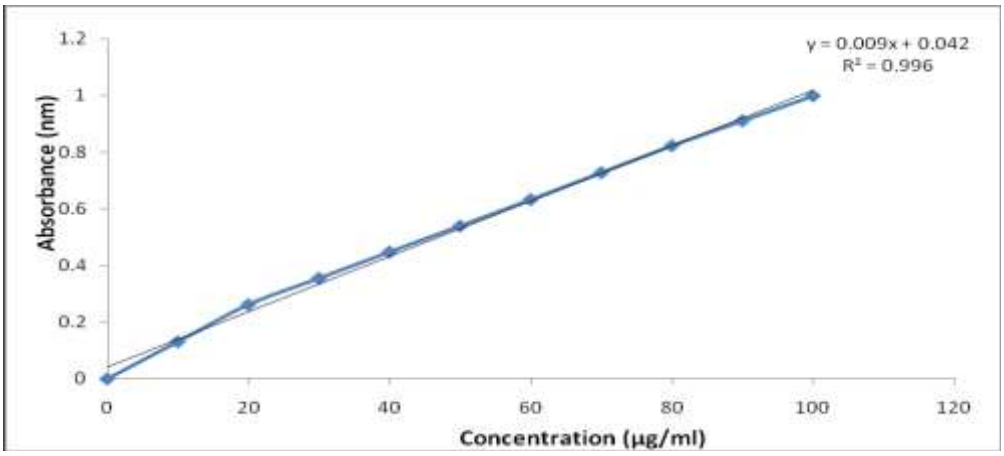

Figure. 19: Standard calibration curve of fluconazole at 260nm in methanol

The gel sample (100 mg) withdrawn and drug content was determined using a UV spectrophotometer at 260 nm. Similarly, the content uniformity determined by analyzing the drug concentration in gel taking from 3 to 4 different points from the container. In case of liposomal gel, it will be shaken with adequate quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer at 260 nm.

4) Spreadability: It was determined by wooden block and glass slide apparatus. Two glass slide of 20×20 cm were selected. The gel formulations were placed over one of the slides. The other slide was placed upon the top of the gel such that the gel was sandwiched between the two slides in an area occupied by a distance of 60. cm along 100g weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gels adhering to the slide was scrapped off. The two slides in positioned were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20 g weight was tied to upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm and separate away from the lower slide under the direction of weight was noted. Weights were added to the pan and the time noted for upper slide (movable) to separate completely from the fixed slides. Spreadability intended by using the formula:

$S = M.L / T$

Where, S = Spreadability M = Weight tide to upper slide L = Length of glass slide

T = Time taken to separate the slide completely from each other

5) Homogenicity: Developed gel was experienced for homogenicity by visual inspection after the gel has been set in the container. This tested for their appearance and presence of any aggregates.

6) Viscosity Studies: Viscosity measurements were done on Brookfield viscometer by selecting suitable spindle number and rpm. 50 g of preparation was kept in 50 ml beaker which was set till spindle groove was dipped and rpm was set and dial reading was measured after three minutes. From the reading obtained, viscosity was calculated by using factor.

7) Rheological study*:* The semisolid preparations should flow or deform after applying the force and regain its elasticity as the force is removed. Thus, to understand the rheological properties of liposomal gels rheological study has been performed.

8) *In-vitro* drug diffusion study

Rat abdominal skin was used for diffusion studies as an *in-vitro* experimentation. Subcutaneous fatty tissue will be unconcerned from the skin using a scalpel and surgical scissors. After the fatty tissue was removed, the surface of the skin will be cleaned with saline solution. [80]

Experiment

A Franz diffusion cell was used to perform the experiment. *In vitro* absorption studies are generally carried out in vertical franz diffusion cell. According to Food and Drug Administration (FDA) regulations, it is an ideal tool for quality control of topical preparations. It has a receptor and a donor chamber, which is filled with phosphate buffer medium solution from the receptor chamber. The jacketed cell embodied is stirred throughout the study at 500 rpm employing a magnetic stirrer.. The dialysis membrane is sandwiched between the two chambers and clamped in place tightly. Full shaved abdominal skin was mounted between the cells and the receptor compartment was 10 ml. The donor medium consisted of 1 gm liposomal gel. To maintain the sink condition Phosphate buffer pH (6.5): Ethanol (3:1) was used as receptor medium. Stirring rate and temperature were kept 400 rpm and 37°C respectively. At different intervals (1, 2, 4, 8, 12 and 24 h), the receptor samples were removed and replaced with fresh receptor medium. Receptor samples were then analyzed for drug content spectrophotometrically at 260 nm wavelengh. Cumulative amount of drug release was determined as a function of time and the release rate was calculated.

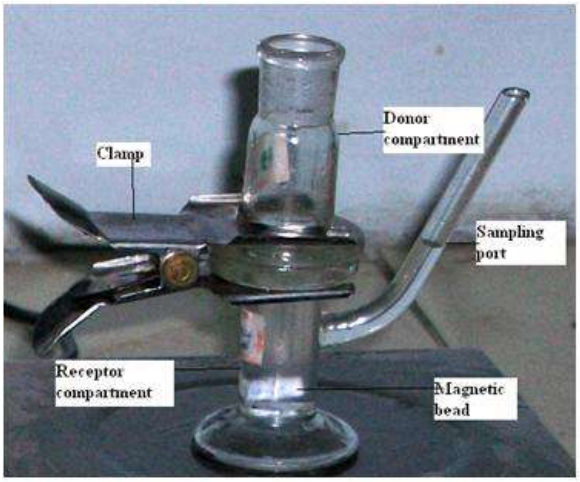

Figure 20: Franz diffusion cell

7.4 Physicochemical Evaluation Of Liposomes

1) Entrapment efficiency

Drug associated with liposome were divided from unentrapped drug using centrifugation method. Liposomes were centrifuged[70]. Supernatant contain unentrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome determined as follow:

EE (%) = [(Cd–Cf)/Cd] 100

Where Cd is concentration detected of total drug and Cf is concentration of free drug.EE is entrapment efficiency.

2) Zeta potential (z) determination

Charge on empty and drug loaded vesicles surface will be determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined. Zeta potential is the measure of the magnitude of the electrostatic or charge repulsion or attraction between particles and, known to affect stability. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation, and can be applied to improve the formulation. Almost all particulate or macroscopic materials in contact with a liquid acquire an electronic charge on their surfaces [71]

3) Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) will be assessed by keeping the liposomal suspensions and liposomal gel at two dissimilar temperature situation for particular period of time. Samples will be reserved periodically and analyzed for the drug content

and particle size for liposomal suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies.[72]

4) Vesicular morphology

The morphology of vesicles was studied by scanning electron microscope. For SEM one drop of each sample from liposome were mounted on a stub covered with clean glass respectively. The drop was spread out on the glass homogenously. A sputter coater was used to sputter coat the samples with platinium and samples were examined under Jeol 6480 LVJSM at an accelerating voltage 20kv.

RESULTS AND DISCUSSION

8.1 Preformulation Studies

8.1.1 FTIR studies

Fourier Transform Infrared (FTIR) studies of Fluconazole and polymer Carbopol were performed at Pinnacle Biomedical Research Institute Bhopal, India, in order to carry out drug polymer compatibility study (Figure 8.1 and 8.2) using UNIVERSAL Q 200 V 23.5 instrument.

The principle peaks of fluconazole were observed in the spectra of the drug / polymer physical mixtures indicating no interactions had been occurred.

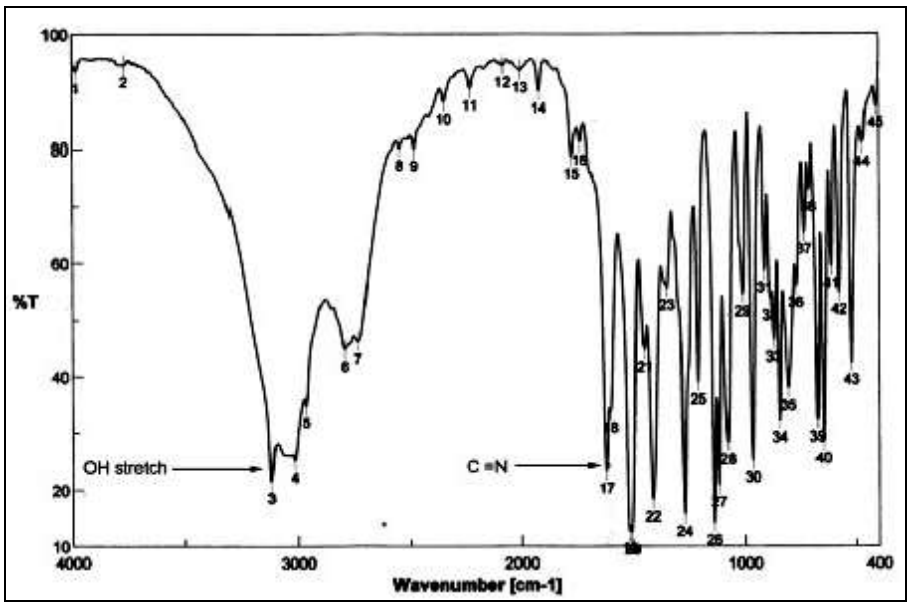

Figure 21: FT-IR of drug fluconazole

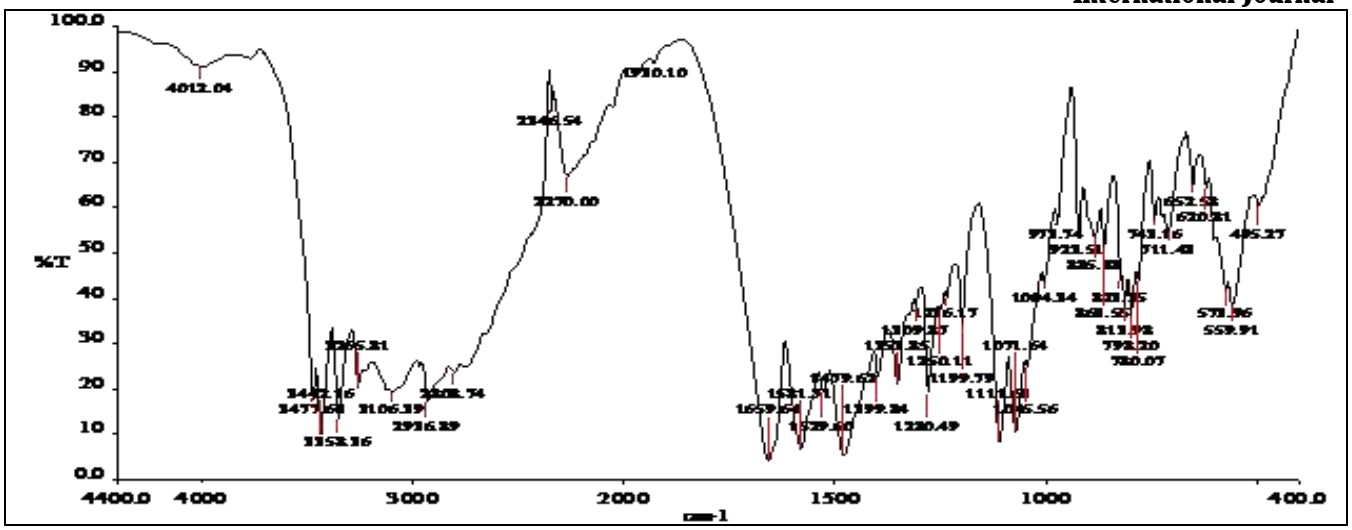

Figure22: FT-IR of polymer: Carbopol 934

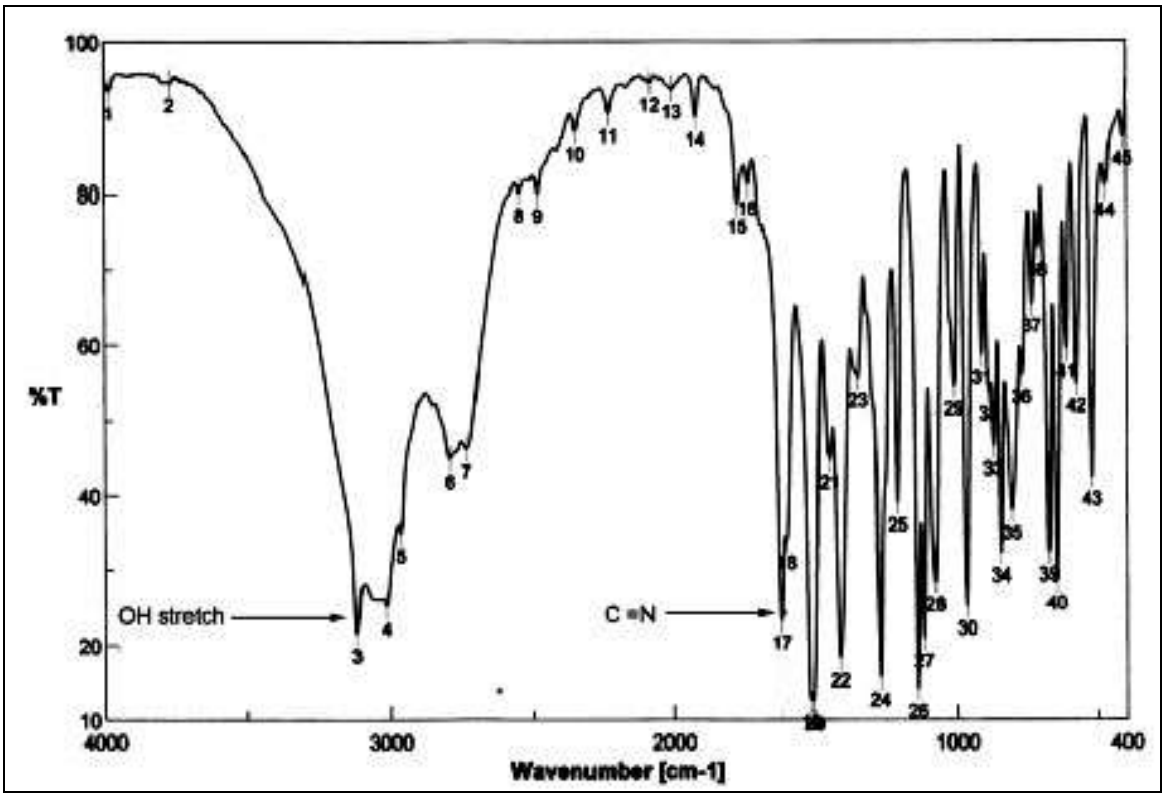

Figure 23: FT-IR of drug fluconazole and Carbopol 934

Table .8 FTIR interpretation in vibration mode for mixture of fluconazole and carbopol 934

Peak obtained in drug (frequency cm-1)	**Description**	**Peak obtained in mixture (frequency cm-1)**

3176.76	OH Stretching	3192.19
2817.36	CH2 Stretching	2880.41
2966.52	CH (Aromatic Stretching)	2956.87
1560.41	C = N Stretch	1562.34
1456.80	CH (Aromatic bending)	1456.20
1133.93	C - F Stretch	1139.39

8.1.2 Differential scanning calorimetry(DSC)

Methodology:

Differential Scanning Calorimetry (DSC) studies of drug Fluconazole and polymer Carbopol was carried out by heating the samples from 40°C to 240°C at the rate of 20°C/min, using UNIVERSAL Q 200 V 23.5 instrument.

Table. 9 Summary of DSC for decomposition of Fluconazole, carbopol 934 and mixture.

Parameters	**Pure Drug Fluconazole**	**Carbopol 934**	**Fluconazole in Mixture of Fluconazole & Carbopol**	**Carbopol 934 in Mixture of Fluconazole & Carbopol**
DSC Onset Temp. ^{0}C (Initial Temp.)	135.11 ^{0}C	90.8 °C	138.60 ^{0}C	100.55^{0}C
Final Temperature ^{0}C (Peak)	136.36 ^{0}C	93.5 °C	140.6 °C	102.3 ^{0}C
Enthalpy in KJ/Mole H	57	34	59	36
Characteristic of	Endotherm	Endotherm	Endotherm	Endotherm

Peak				

DSC was carried out for the drug fluconazole, for polymer carbopol and mixture. Pure fluconazole gave melting endotherms at 136.36 ^{0}C indicating that the drug is in crystalline form.

In the DSC thermogram of Carbopol 934, glass transition temperature (Tg) was observed near the range of 90-110 ^{0}C. The ***Carbopol 934*** sample gave an ***endothermic melting*** transition at 93.5 °C. Decomposition of Carbopol 934 was observed near 110 ^{0}C where Carbopol melted and decomposed sequentially.

DSC thermograms of physical mixtures showed the broad endotherms due to water removal at about 100 to 140.80C. The physical mixture of Fluconazole and polymer carried for DSC the melting of fluconazole observed between 100^{0}C and 102.3^{0}C and Carbopol showed onset temperature 100.5^{0}C and the peak transition temperature is 102.3^{0}C. It proves no interaction between drug and carbopol 934.

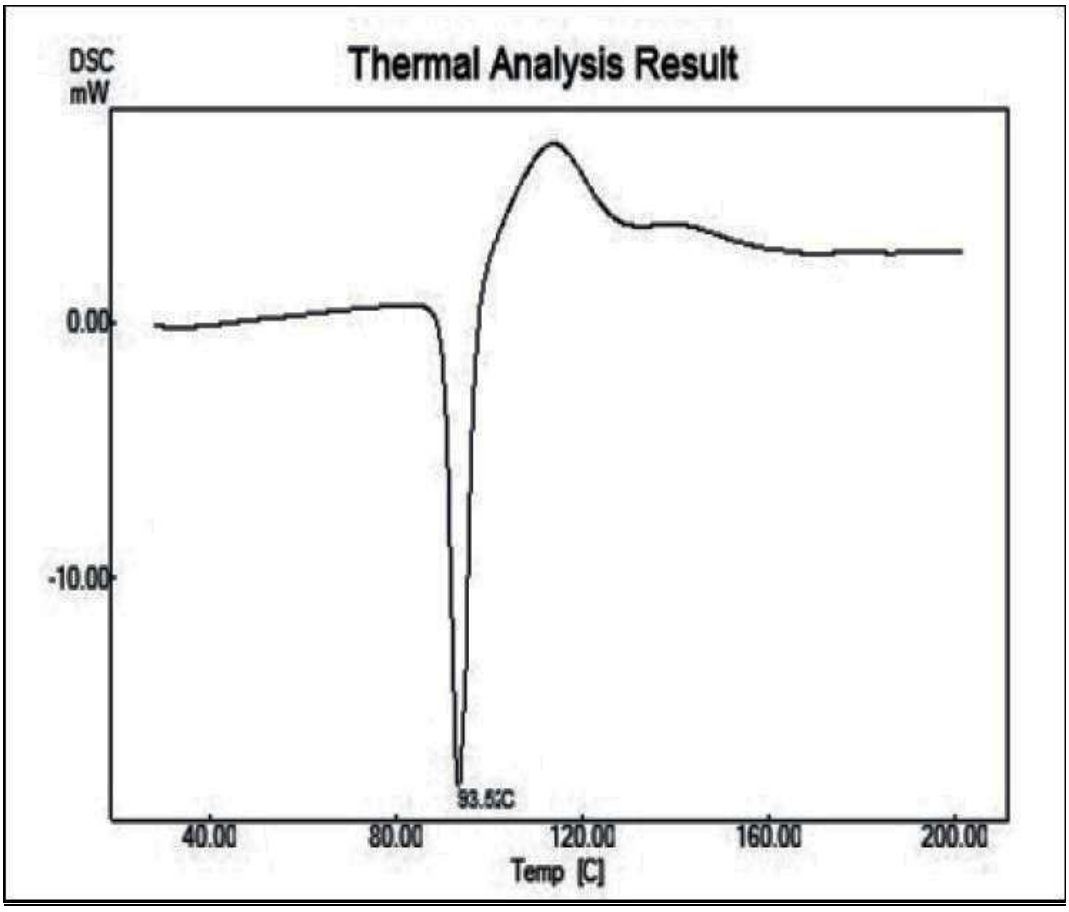

Figure 24: DSC of Polymer Carbopol 934

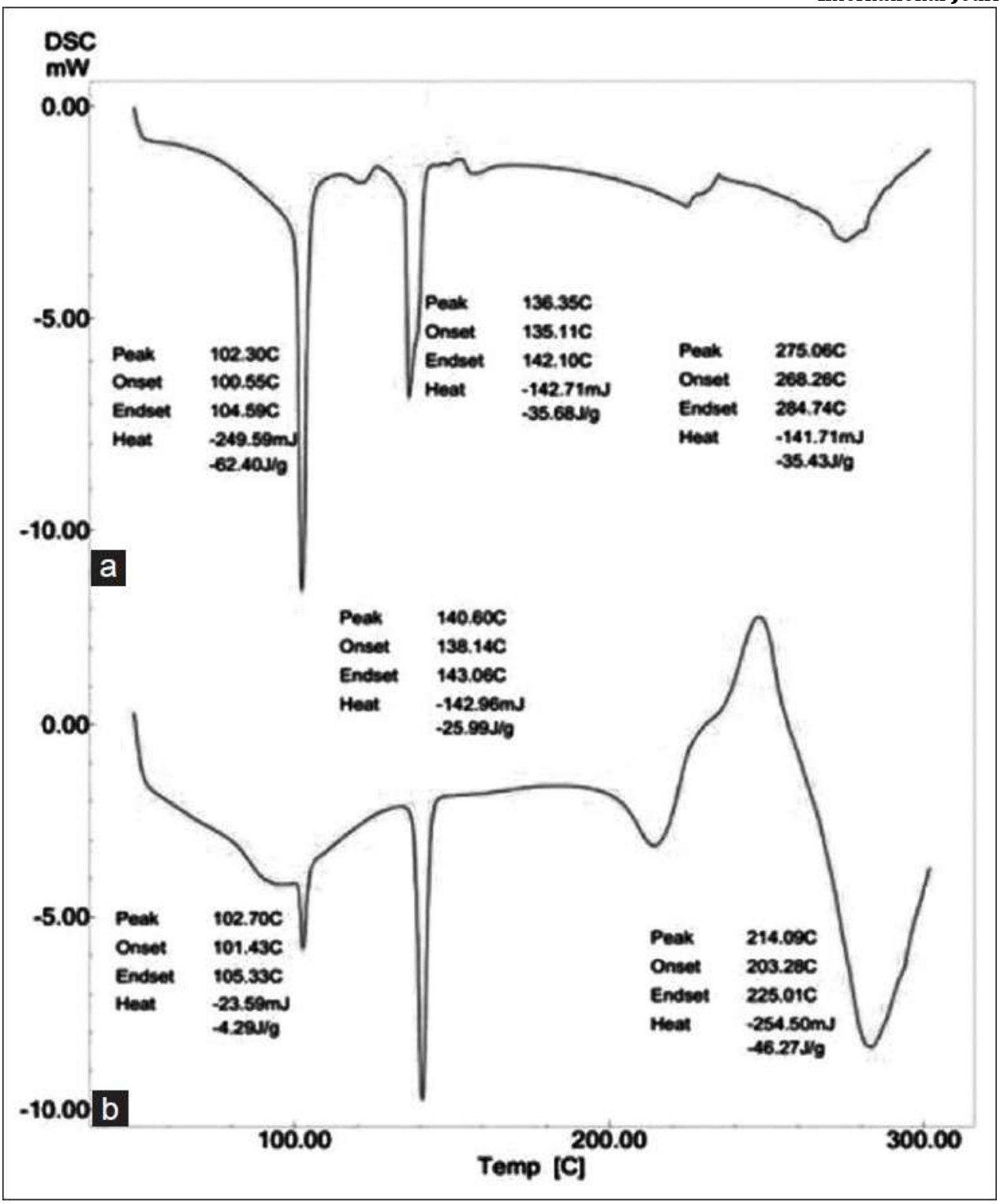

Figure 25: DSC of drug fluconazole and Polymer Carbopol 934 with drug fluconazole

8.2 Physicochemical Evaluation of liposomal gel

8.2.1.1 Physical examination: The prepared Liposomal gel was evaluated for colour, and transparency; the prepared gel was in acceptable limits.

8.2.1.2 pH: The pH values of 1% aqueous solutions of the prepared gels were measured by pH meter, the average pH of formulations were comes to 7.3 pH.

8.2.1.3 Drug Content uniformity: The gel sample (100 mg) withdrawn and drug content was determined using a UV spectrophotometer at 260 nm. Similarly, the content uniformity determined by analyzing the drug concentration in gel taking from 3 to 4 different points from the container. In case of liposomal gel, it will be shaken with adequate quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer at 260 nm.

There was no significant difference observed in the % drug at various locations, indicating that the method used to disperse the liposomal dispersion in the gel base is satisfactory.

8.2.1.4 Spreadability: It was determined by wooden block and glass slide apparatus. Weights were added to the pan and the time noted for upper slide (movable) to separate completely from the fixed slides. Spreadability intended by using the formula:

S = M.L / T

Where, S = Spreadability M = Weight tide to upper slide L = Length of glass slide

T = Time taken to separate the slide completely from each other

8.2.1.6 Homogenicity: Developed gel was experienced for homogenicity by visual inspection after the gel has been set in the container. This tested for their appearance and presence of any aggregates that comes in acceptable range.

8.2.1.7 Viscosity Studies: Viscosity of prepared gel was carried out by using Brookfield Viscometer. The rotation was varied from 30 to 50 rpm at 25±1 ◦C.

8.2.1.8 Rheological study*:* The semisolid preparations should flow or deform after applying the force and regain its elasticity as the force is removed. Thus, to understand the rheological properties of liposomal gels and for selection of optimum concentration of carbopol having desired rheological properties, different concentrations (1, 1.5, 2% w/w) of carbopol 934 were used to prepare liposomal gels at 25 °C with neutralization method. The rheologies of all samples were determined to identify the minimum concentration of carbopol required for the formation of gel with good visco-elastic properties.

8.2.2 *In-vitro* studies

Rat abdominal skin was used for diffusion studies as an ex-in-vivo experimentation. Subcutaneous fatty tissue will be unconcerned from the skin using a scalpel and surgical scissors.

After the fatty tissue was removed, the surface of the skin will be cleaned with saline solution.

Skin Permeation Test

Rat was sacrificed by exposing to excess chloroform. To the abdominal skin, depilatory was applied and kept for 10 m to remove the hair from the skin. After 10 m of application, skin was washed with water. Skin was excised from rat with scalpel and fatty layer was removed by keeping the skin in warm water at 0 60 C. After 2 m, fatty layer was peeled off gently and skin was washed with water and kept for saturation in phosphate buffer saline pH 7.4 for about 30 m before it was used for permeation studies. Fresh skin was used every time. Skin permeation studies with fluconazole containing liposome formulations were carried out using abdominal rat skin, employing modified Franz-diffusion 12 cells. The results obtained were compared with that of non-liposomal formulations of fluconazole. The skin was prepared by mounting on the receptor chamber with 2 cross-sectional area of 3.91 cm exposed to the receptor compartment. The receptor compartment was filled with phosphate buffer pH 7.4. It was jacketed to 0 maintain the temperature 37 + 0.5 C and was kept stirring at 50 rpm. Prior to application of formulations, the skin was allowed to equilibrate at this condition for 1 h. Liposomal or non-liposomal fluconazole formulation (amount equivalent to 5 mg of drug) was applied uniformly on the dorsal side of skin. Aliquots of 2 ml were withdrawn periodically and replaced with same amount of saline solution to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically at a wavelength of 260 nm. Max For determination of drug deposited in skin, cell was dismantled after a period of 8 h and skin was carefully removed from the cell. The formulation applied on skin surface was swabbed first with phosphate buffer pH 7.4 and then with methanol. The procedure was repeated twice to ensure no traces of formulation are left onto skin surface. The skin was then cut into small pieces and drug present in skin was extracted in phosphate buffer pH 7.4 using bath sonicator and determined spectrophotometrically after suitable dilution and filtration.[81]

Table 10(a): Drug release profile of fluconazole loaded liposome Formulation F1 to F4 and plain gel

Time(hr)	**F1**	**F2**	**F3**	**F4**	Plain Gel
1	17.3±1.98	23.4±0.98	14.6±1.42	14.0±1.24	14.8±2.00
2	17.5±1.31	23.5±1.08	14.9±1.55	14.2±1.22	15.0±2.12

3	19.9±1.33	24.2±1.21	16.0±1.32	16.3±1.32	16.7±1.98
4	22.4±1.42	26.7±1.24	19.8±1.57	19.0±1.36	20±1.22
5	29.3±2.01	29.9±0.99	22.6±1.67	23.0±1.52	26.7±1.35
6	32.5±1.98	30.6±1.35	23.8±1.28	25.0±1.35	33.0±1.87
8	40.0±2.22	57.3±1.75	41.3±1.98	32.7±1.87	39.7±1.98

Table 10(b): Drug release profile of fluconazole loaded liposome Formulation F5 to F8

Time(hr)	F5	F6	F7	F8
1	19.6±1.02	14.5±1.82	17.3±2.01	19.6±1.82
2	19.8±1.32	14.7±1.38	17.4±2.22	19.6±1.67
3	22.0±1.54	16.6±1.37	19.9±2.70	22.7±1.37
4	25.6±1.65	18.8±1.65	21.2±1.00	27.4±1.46
5	26.6±1.11	20.3±1.29	24.3±1.28	30.7±1.66
6	19.9±2.01	27.3±1.58	23.8±1.28	26.6±1.65
8	30.0±1.98	31.0±1.62	28.0±1.65	28.2±1.73

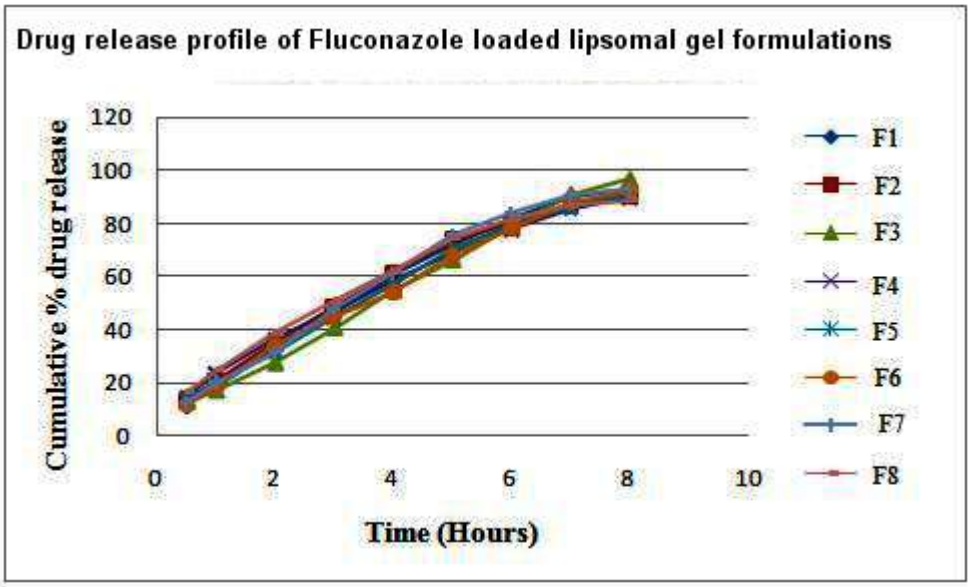

Figure 26: Drug release profile of fluconazole loaded liposomes

8.3 Physicochemical evaluation of liposomes

Vesicle Morphology

The various drug loaded formulations of liposomes (including the blank particles) appeared more or less spherical when observed by Scanning Electron Microscopy SEM.

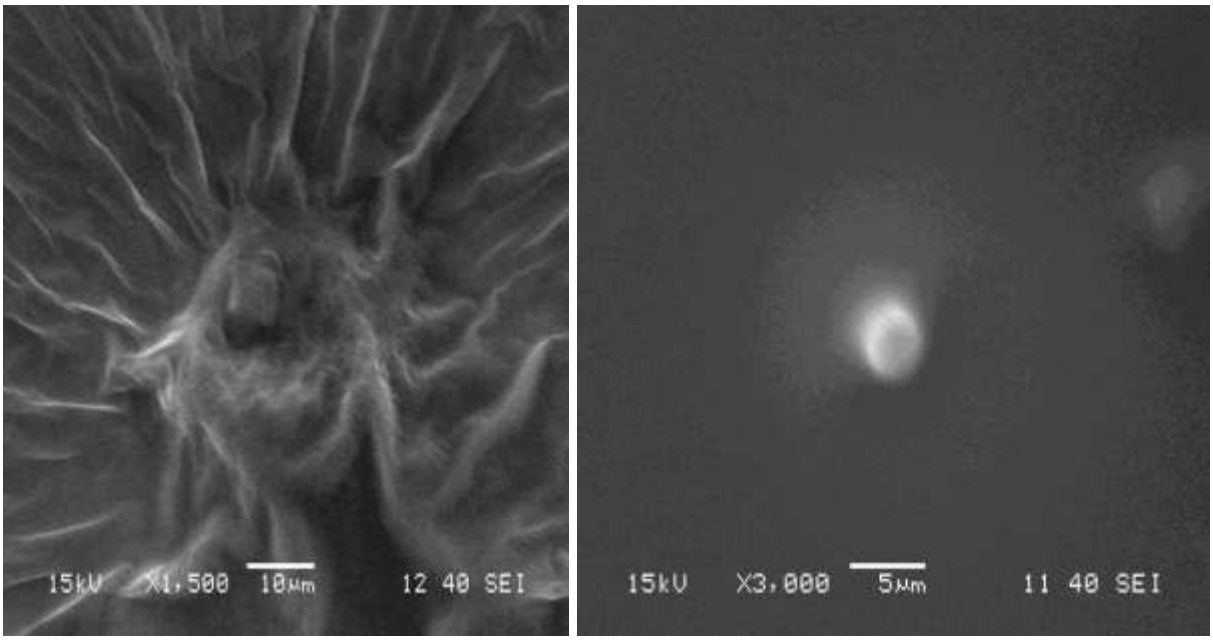

Figure 27: Scanning Electron Microscopy image of Liposome

8.3.1 Entrapment efficiency:

Drug associated with liposome were divided from unentrapped drug using centrifugation method. Liposomes were centrifuged. Supernatant contain unentrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome determined as follow:

EE (%) = [(Cd–Cf)/Cd] 100

Where Cd is concentration detected of total drug and Cf is concentration of free drug.EE is entrapment efficiency.

8.3.2 Zeta potential ( ) determination:

Charge on empty and drug loaded vesicles surface will be determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined.[33]

In the present study the obtained for liposomes are shown in Table …. The values of potential (-54.1 63.6 mV for vesicles) showed prepared liposome have sufficient charge to avoid aggregation of vesicles.

The zeta potential of the liposomes ranged between -12.2 mV to -29.0 mV indicating incipient stability.

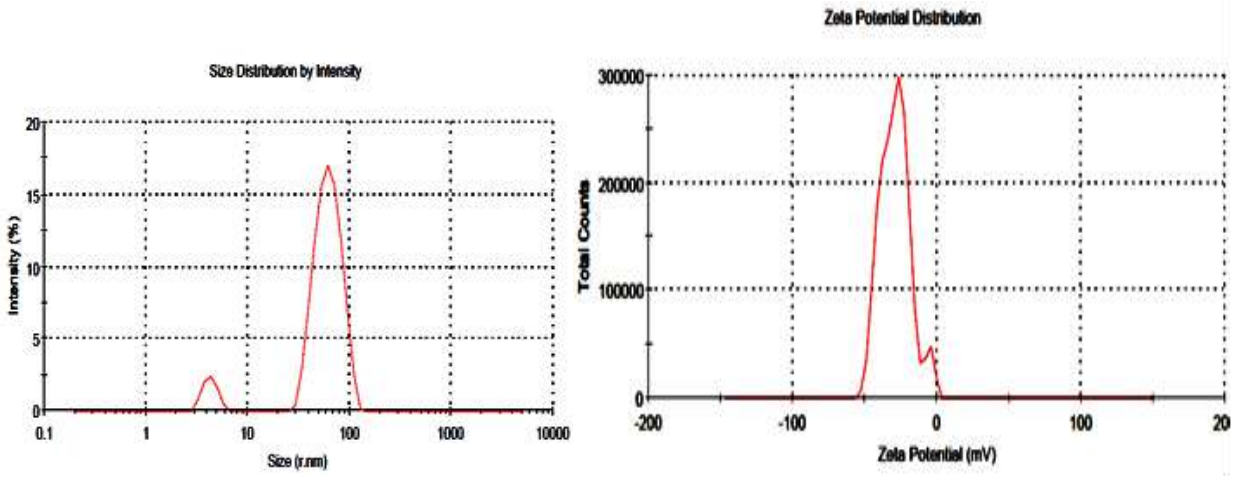

Figure 28: Size and zeta potential of liposome

8.3.3 Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) will be assessed by keeping the liposomal suspensions and liposomal gel at two dissimilar temperature situation for particular period of time. Samples was withdrawn periodically and analyzed for the spreadibility,

viscosity, pH, and drug content particle size for liposomal suspension in the manner described under entrapment efficiency and particle size distribution studies.[33,76]

The liposomal gel were packed and kept for one month at 0^0C- 2^0C, 40^0C/ 75% RH in a stability chamber, 60^0C/80% in incubator . At the interval of 15 days gel were withdrawn and evaluated for physical properties like Spreadibility, pH, viscosity and content uniformity carried out.

Table 11: Stability studies of Liposomal Gel

				Storage Condition			
S. No	**Parameters**	**0°C**		**25°C±2°C, 60% RH**		**40°C±2°C, 75% RH**	
		15days	**30days**	**15days**	**30days**	**15days**	**30days**
1.	**Spreadability (gcm/sec) ±SD**	54.46±0.79	53.92± 0.78	55.65± 0.81	54.79± 0.47	53.55± 0.78	51.62 ± 0.74
2.	**Viscosity (Centipoise)**	15174	157678	16695	16687	13546	13959
3.	**pH**	5.47	5.49	5.48	5.48	5.53	5.58
4.	**% Drug Contents**	95.05± 0.44	95.10± 0.51	95.49± 0.45	95.55± 0.43	93.61± 0.65	93.08 ± 0.67

Table 12: Entrapment efficiency, Vesicle Size and Zeta Potential of various formulations

Batch No.	**Entrapment Efficiency**	**Vesicle Size**	**Zeta Potential**

	(EE ± SD,%)	**(Size ± SD, µm)**	**(z ±SD,mV)**
1	55.6±1.23	2.88±0..22	- 54.5 ± 0.77
2	57.3±0.54	3.46±0.83	- 55.9 ± 1.43
3	59.8±0.87	3.98±0.99	- 57.5 ± 1.73
4	61.02±1.23	4.06 ± 0.40	- 58.6 ± 2.41
5	62.88±0.71	3.50 ± 0.92	- 54.5 ± 1.57
6	63.92±1.67	4.28 ± 1.62	- 58.9 ± 1.43
7	64.07±0.93	5.83 ± 1.49	- 55.09 ± 0.87
8	65.22±0.66	7.15 ± 1.14	- 53.4 ± 2.97

Table 13: *In-vitro* skin permeation and skin retention of fluconazole from different formulations

Fluconazole formulations	**Mean cumulative % drug permeated**	**Permeation flux mg/ cm2 / h**	**% Drug retained in skin**
Liposomal gel	30.46	0.1754	8.21
Liposomal dispersion	32.47	0.1871	10.03
Marketed gel	23.41	0.1341	5.21
Carbopol plain gel	24.68	0.1531	3.64
Aqueous solution	27.2	0.1570	5.79

Table 14: Effect on entrapment efficiency, vesicle size for liposomal dispersion and drug deposition from liposomal gel during stability

	Entrapment efficiency %		Vesicle size (□ m)		Drug deposition %	
No.of days	4-8 ^{0}C	Room Temp.	4-8 0 C	Room Temp.	4-8 ^{0}C	Room Temp.
0	64.88	64.88	3.5	3.5	0.41074	0.41074
30	63.92	61.73	4.28	4.3	0.3981	0.3174
60	61.39	58.32	4.13	5.2	0.3127	0.2349

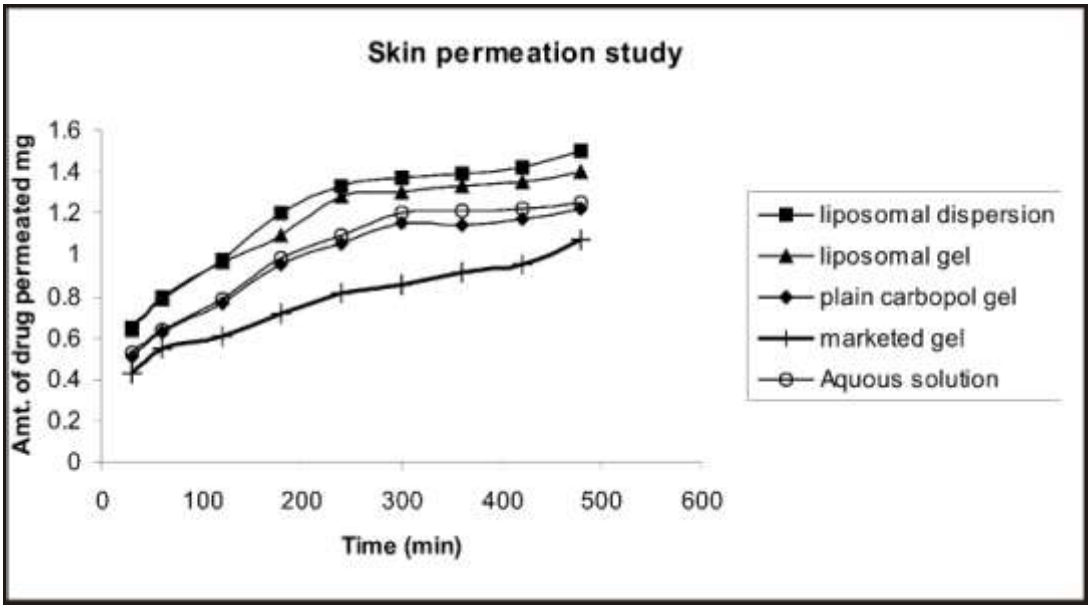

Figure 29: Permeation profile of different fluconazole containing systems

9. SUMMARY AND CONCLUSION

The present work on the preparation of topical liposomal gel containing fluconazole is an attempt to utilize the immense potential of liposomes as a carrier to increase the permeability. Liposomal encapsulation showed more drug retention compared with plain drug gel .

Further, the physicochemical modification in the drug by means of phospholipid membrane also promises to prolong the drug action. A number of problem associated with drug molecule such as bioavailability, degradation, stability and side effects can be overcome by incorporating it into liposomes. The liposomes of fluconazole was prepared by film hydration method and evaluated. The *In vitro* release of the formulation was studied and found to be more efficient than plain gel. Creation of reservoir effect for drug in skin due to deposition of other components of liposomes

with drug into the skin and thereby increasing the drug retention capacity into the skin. Liposomal formulations were more spherical with stable zeta potential and mono-disperse with no clumping.

Variables such as amount of phospholipid, amount of stabilizer have a profound effect on the vesicle size and entrapment efficiency. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to control. Drug release of all batches was studied. Stability studies performed for Liposomal gel indicates the prepared liposomes have more stability at freezing temperature than that of room temperature.

Fluconazole molecules could be successfully entrapped in liposomes with reasonable drug loading. Hence from results obtained it can be concluded that liposomal gel containing fluconazole has potential application in topical delivery for the treatment of fungal skin ailments.

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