CHAPTER 1

Introduction & Literature
Introduction

Basically there are three routes of drug administration oral, topical & parenteral. Among that topical i.e. skin provide efficient route for API administration. Stratum corneum in epidermal layer found to be major barrier for penetration of API through or in to the skin. [Breimer D, 1985]

Topical drug delivery system means delivery of API through or in to the skin for direct treatment of cutaneous disorder or the cutaneous manifestation. [Atiyeh B et al. 2009] Topical drug delivery systems include a large variety of pharmaceutical dosage form like semisolids, liquid preparation, sprays and solid powders. Most widely used semisolid preparation for topical drug delivery includes gels, creams and ointments. [Gisby J et al., 2000]

Fungus infection (Mycoses) is a human infection is broadly of two types. Superficial & deep seated (systemic) superficial infection is by far common & comprises the various types of tinea or ringworm affecting the skin, hair & nails. Superficial mycoses having two types surface infection & cutaneous infection. [Theresa M, 1998] In cutaneous disorder mostly fungal infection occurs superficially. Novel topical drug delivery systems include noisomes, liposomes, and solid lipid nanoparticles etc. which are highly effective for topical route of drug administration. [Namdeo A, 1999]

Conventional preparation like Ointment, gel, cream, emulsion shows problem of drug penetration as well as API deposition. So to overcome this problem there is need to develop novel formulation act as carrier for better penetration through topical formulations. [Papps P et al., 2004]

Niosomal gels contain nanometric systems embedded in a gel. Nanometric systems have a greater surface area, which renders them highly satisfactory for the application of drug substances promoting a homogeneous drug release. Such structures have been investigated as alternatives to the classical formulations based on chemical skin permeation enhancers. [Wallace T & Rex J, 2002 & Wallace T, 1997] Additionally, the nanostructure systems have nano size so it is easy for application to the skin in dermatological product. [Don A, 1997]
1.1 Niosomes

Non ionic surfactant vesicle is a best alternative to phospholipids vesicle. [Moussaoui N et. al. 2002] Vesicles are closed bilayer structure prepared by self assembly of amphiphiles by means of aqueous media. Physical agitation and heat act as source of energy in vesicles preparation. Hydrophilic molecules trapped in aqueous core and lipophilic molecules entrapped in lipids bilayer. [Handjani-Vila R, 1979]

![Figure 1.1: Structure of Niosome](image)

**Advantages of niosome**

Non ionic surfactant vesicle is one of the best nano-carrier having lot of advantages like

- Niosome contain hydrophilic and hydrophobic domain so it can provide loadings of API with diverse solubility.
- Surfactant vesicles improve penetration of molecule through skin.
- Drug molecule entrapped in vesicle bilayer or aqueous core, so API diffuse through vesicle in sustained manner.[Xia W (2000]
- Vesicles are non toxic because of it degradable in natural way & compatible.[Papahadyopoulos D, 1980]
- Therapeutic efficiency of API is enhanced caused by vesicles targeted effect, tardy clearance from the circulation & protection of API from natural surroundings.
- Non phospholipids vesicle having better stability than liposome. [Baillie A, 1985]
Easy hydrolysis of phospholipids due to ester bonds leads to migration of phosphoryl group at stronger acidic area.

Peroxidation of unsaturated phospholipids is also a type of degradation observed in liposome.

Moreover, synthetic non-ionic surfactants are economical than phospholipids.

Non ionic surfactant vesicles enhance stability of trapped API.

Liposome and niosome having different properties since liposome is made up by phospholipids while niosome is prepared from non ionic surfactant & cholesterol. [Nasseri B, 2003]

1.2 Ideal properties of Non-ionic surfactant used in niosomes formation

Hydrophilic lipophilic balance & critical packing parameters of non ionic surfactant are important factors during vesicle formulation rather than micelles. [Rogerson A, 1988] The correlation among the surfactant structure containing size of polar head group plus length of non-polar region in vesicle preparation is illustrated in following figure.

![Critical packing parameter of surfactants](image)

Figure 1.2: Critical packing parameter of surfactants

Hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of any surfactant. HLB number should not be more than 8, if it is increased hydrophilicity will increases so stability of vesicle decreases. If Non-ionic surfactants like polysorbates (tween) are used in formation niosomes have HLB more than 10, thus large concentration of cholesterol requires for stability of niosomes and which in turn results decrease in entrapment efficiency. [Chandrashekhhar G, 1994]
1.3. Classification of non-ionic surfactants [Baillie A, 1986]

They are characterized as amphipathic (hydrophobic head and hydrophilic tail) molecules capable for forming vesicle after hydrated in water or solution.

a) Sorbitan esters
They are prepared by sorbitol esters along with oleic acid anhydrides. Sorbitan esters are water insoluble as reflected by their lower HLB values.

b) Polysorbates
They are poly-oxy -ethylene derivatives of sorbitan ester. They are prepared by condensation of sorbitol ester with ethylene oxide moles. Polysorbates are water miscible due to high hydrophilic lipophilic balance value greater than 10.

c) Poly-oxy ethylated glycol mono-ethers
Poly-oxy ethylated glycol mono-ethers are available as Brij series which includes Poly-oxy ethyl lauryl ethers (Brij 30, 35) and Poly-oxy ethyl cetyl ethers (Brij 52, 56)

General formula: Cx Ey

X: alkyl chain length and Y: ethylene oxide chain length

d) Poly-oxy ethylated alkyl phenols
Poly-oxy-ethylated t-alkyl phenols are available as Triton-X series which includes X-114 (E 7-8), X-100 (E 9-10) and X-102 (E 12-13).

e) Poloxamers
They are Poly-oxy-ethylene—Poly-oxy-propylene derivatives.
They are commercially available under trade name ‘Pluronics’. Pluronic F68 [Poly-oxy propylene mol.wt.(1501-1800)+140 mol. ethylene Oxide]

f) Bola- surfactant
These novel surfactants are made up of azacrown ether units connected with long alkyl chain group and capable to form colloidal structure after addition of cholesterol. [Robinson et. al. (1995).]
<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Commercial name</th>
<th>HLB Values</th>
</tr>
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<tbody>
<tr>
<td>Poly-oxyethylene Sorbitan monolaurate</td>
<td>Tween 20</td>
<td>16.7</td>
</tr>
<tr>
<td>Poly-oxyethylene Sorbitan monopalmitate</td>
<td>Tween 40</td>
<td>15.6</td>
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<tr>
<td>Poly-oxyethylene Sorbitan monostearate</td>
<td>Tween 60</td>
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<tr>
<td>Poly-oxyethylene Sorbitan tristearate</td>
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<td>10.5</td>
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<td>Poly-oxyethylene Sorbitan mono-oleate</td>
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<td>Sorbitan monolaurate</td>
<td>Span 20</td>
<td>8.6</td>
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<td>Sorbitan monopalmitate</td>
<td>Span 40</td>
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<td>Sorbitan monostearate</td>
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<tr>
<td>Polyoxyethylene 4 lauryl ether</td>
<td>Brij 30</td>
<td>9.7</td>
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</table>
1.4. Methods of niosomes preparation

a. Ether injection method [Khandare J, 1994]
In this method vesicles are made by injection or slowly introduction of lipids i.e. non ionic surfactants and cholesterol in ether into aqueous phase heated at 60-65°C. Mechanism of vesicle formation can be attributed to slow evaporation of solvent resulting in ether gradient between surfactant-cholesterol monolayer on interface of ether and water. Consequently may form vesicles from folding of bilayer sheets. Vaporisation of ether leads to formation of single layered vesicle with highest entrapment efficiency.

b. Ethanol injection method [Tamhankar M, 1995]
In this method slow injection of surfactant: cholesterol: stearic acid and drug in ethanol through a needle into pre-heated aqueous phase. Mechanism of vesicle formation can be attributed to slow evaporation of ethanol solvent resulting in ethanol gradient across surfactant and cholesterol layer at ethanol-water interface and afterwards there is formation of vesicles.

c. Film hydration method [Maver L, 1985]
In this method non ionic surfactant & membrane stabilizer lipid are mixed in organic solvent like chloroform, methanol, and diethyl ether in round bottom flask. There is formation of thin film of solid mixture by evaporation of volatile solvent on the wall of round bottom flask. Afterwards rehydration of that film occurs by addition of solvent with gentle agitation. Film hydration method forms multi-lamellar niosome.

d. Sonication
Lipid mixture is added into the water or aqueous solvent and sonicated for specific time interval at definite temperature. Previously multi-lamellar vesicles are formed which undergo vibrations to produce uni-lamellar vesicles.

e. Reverse phase evaporation
Equimolar ratio of surfactant and lipid are added into organic solvent like chloroform, ether. Hydrophilic drug incorporated in to aqueous phase and sonication of lipid & aqueous phase at low temperature. Due to that gel was formed undergo sonication
after adding up phosphate buffer saline. Volatile solvent is removed by application of heat. Produced suspension is diluted with phosphate buffer saline & heated at 60-65°C for 15 min to give niosome.

f. Aqueous dispersion
It involves lipid dispersion in water having hydrophilic moiety for encapsulation with continuous agitation under controlled temperature conditions leads to homogenous vesiculation. Dispersion may be homogenized or ultra-centrifuged. Homogenization may be followed by bubbling of nitrogen till vesiculation is complete. Bubbles possibly provide spherical gas/air interface for niosome to get organize as per thermodynamic stability requirement. Nitrogen is subsequently release and allows hydration of amphiphiles to form vesicles.

g. Micro fluidization [Chauhan S, 1989]
In this method jet principle was used for niosome formation. Two phases i.e. lipid and aqueous phase interact with each other at high velocities. High velocity jet principle give rise to niosome having nano size, good uniformity and reproducibility.

h. Multiple membrane extrusion method [Blazek-Walsh A, 2001]
Extrude technique was used for niosome formation. In this technique thin film was made by evaporation of lipid mixture i.e. surfactant-membrane stabilizer. Lipid film hydrated with water or phosphate buffer saline having hydrophilic API and resulting mixture extruded via membrane filter made by polycarbonate. Extrusion takes place serially to obtain niosome of desired size.

i. Trans membrane pH gradient process [Uchegbu I., 1998]
Lipid mixture dispersed in organic solvent subsequently lipid film was obtained on wall of round bottom flask. Hydration of film takes place with addition of acid like citric acid by vigorous agitation. Produced multilamellar vesicles undergo freeze thaw cycle and later go through sonication. Later API aqueous solution is added and agitated. Mixture pH was raise to 7.0 – 7.2 by disodium phosphate 1M addition. Finally mixture is heated at 65°C to give niosome.
j. **Bubble method [Fernandez P, 2005]**

This is innovative technique used in niosome preparation excluding organic solvent. Round bottom flask contain three neck areas for reflux, thermometer and nitrogen supply inlet respectively. Lipid mixture was dispersed in phosphate buffer saline later on undergoes high pressure homogenizer. Instantly supply of nitrogen gas at 65-70 °C owing to bubble formation and obtained the vesicle.

k. **Niosomes from proniosomes [Hu C. 1999]**

In this method surfactant coating on the sorbitol or water soluble carrier, output of this method is dry formulation. Niosome was prepared form proniosome by the addition of water phase at elevated temperature which was greater than mean phase transition temperature.

![Figure 1.3: Mechanism of niosome formation](image)

1.5. **Methods of Size Reduction [Yoshioka T, 1994 & Venkatesh P, 2000]**

There are various techniques used for size diminution, is mentioned below

- Probe sonication produces nonionic surfactant vesicle in small size
- Extrusion method gives niosome of 140 nm size
- Combination of sonication and filtration produces non ionic surfactant vesicle of 150-200 nm size.
- Use of Microfluidiser can be used to achieve submicron niosomes of 50 nm sizes.
- Homogenization at elevated pressure give up the vesicle in nm but drug entrapment efficiency was low due to small vesicle size.
• **Different types of vesicles**

During formulation development different types of vesicles can be obtained depend on type and concentration of surfactant and stabilizer used, method of preparation and size reduction method. Following figure 1.4 illustrate different type of vesicles.

![Figure 1.4: Different types of vesicles](image)

**Figure 1.4: Different types of vesicles**

1.6 **Methods for removal** active pharmaceutical ingredient which is not trap

Determination of active pharmaceutical ingredient quantity incorporated in the niosome is of prime importance, since it influences release characteristics. Entrapment efficiency is defined as active pharmaceutical ingredient incorporated in niosome, total quantity drug incorporated is said to be 100%. So, to determine how much drug is unentrapped the following techniques are used.

* Extensive dialysis [Fernandez P, 2005]

In Extensive dialysis niosome suspension is removed using dialysis tubing by using phosphate buffer solution.
This method is economical and appropriate for thick dispersion having high vesicle diameter above 11 micrometer. But its limitation are method was tedious one require 10 -25 hrs, make thin niosome dispersion and not suitable for active pharmaceutical ingredient want specific removal system.

- Gel Filtration [Malhotra M, 1991]
The vesicle dispersion pass throughout stationary phase and phosphate buffer solution or normal saline were used as mobile phase.

Advantage: Speedy process require less time when Sephadex act as stationary phase

Disadvantage:
- Time consuming when stationary phase is Sepahrose
- Method is not economical if gel is not recycled. Not used for thick dispersion and dispersion having vesicle diameter greater than 15 micrometers.

- Centrifugation [Yoshida H, 1992]
The vesicle dispersion is centrifuged at high speed and supernatant is removed. The settled pellets were washed and re dispersed in saline or buffer solution.

Advantage: Quick (~30 min), economical methodology

Disadvantage: Small size vesicles unable to sediment properly and may cause breakage of delicate vesicle system.

1.7. Characterization of niosomes

- Micromeretic properties
  - Size [Yoshida H, 1992]
  
  Niosome is sphere in shaped and vesicle size could be find out by microscope technique, Beckmann’s particle size analyzer etc. This parameter is important because it has been observed that release rate, shelf life of preparation depends on vesicle size.

  - Shape and morphology [Sternberg B, 1992]
  
  It is determined by microscopic techniques under 400X magnification. Such studies are important because the composition and concentration of bilayer affects the shape. E.g. Vesicles prepared with C16G2 (Hexadecyl diglycerol ether) and Solulan C24
(Stearic stabilizer cholesteryl-poly-24-oxyethylene ether) alone formed polyhedral niosomes. Cholesterol, in addition, formed spherical-tubular niosomes.

Also, the method adopted for vesicle preparation is found to influence their final morphology. E.g. Hand shaking technique forms MLVs whereas ether injection method produces uni-lamellar vesicles.

- **Entrapment efficiency** [Raja N, 1994]
  It was find out by removals of free drug as of the niosomal dispersions by dialysis, centrifugation or using sephadex columns. Direct estimation is carried out by use of markers and disruption of vesicles by surfactants like Solulan C-24, Triton X-100 or 50 % propanol. [Azmin M *et al.* 1985]

  In case of centrifugation method unentrapped drug can be determined from clear supernatant. Formula of entrapment efficiency (%) is given as bellow.

  \[
  \text{Entrapment Efficiency (\%) } = \frac{(A-B)}{A} \times 100 \quad \text{OR} \\
  \text{Entrapment efficiency (\%) } = \frac{(C)}{A} \times 100
  \]

  - \( A \) = Initial concentration drug
  - \( B \) = Drug in supernatant
  - \( C \) = Drug entrapped in niosomes

- **In-vitro release**
  API release rate was determined using dialysis method. Non ionic surfactant dispersion was filled in dialysis sac which is previously washed and sinks in phosphate buffer or water. Afterwards dialysis sac was placed in phosphate buffer solution maintained at 37°C. On specific duration buffer sample was withdrawn and find out drug concentration using suitable analytical method. [Malhotra M, 1991]

- **Vesicle surface charge**
  It is predicted with measure movement of vesicles, called as zeta potential.

  \[
  \zeta = \frac{\mu E 4 \pi \eta}{\Sigma}
  \]
Where, $\eta$ means Viscosity, $\zeta$ for Zeta potential, $\Sigma$ for Dielectric constant and $\mu E$ means electrophoretic mobility.

Charged species like dicetyl phosphate, cetylpyridinium chloride are added to niosomal formulations to improve physical stability.

### 1.8 Factors affecting characteristics of niosomes

- **Drug** [Farkas E, 2004 & Parthasarathi G, 1994]

  Blank niosome having small vesicle size when compare with API loaded niosome. It occurs due to contact of API with polar head region of surfactant vesicle. If vesicle was coated with PEG, in such case API trapped in long chain of PEG. Hence in such type of vesicles less probability to enlarge vesicle diameter.

- **Nature of surfactant**

  Hydrophilic lipophilic balance is important parameter to decide vesicle size. As HLB value goes on increasing vesicle size increases since lipophilicity of surfactant increases.

  Phase transition temperature of surfactant decided entrapment power of API in vesicle. Phase transition temperature means conversion of gel state to liquid state. In gel state the components in vesicle were closely controlled otherwise in liquid state constituents of vesicle were placed in disorder manner. Those surfactant having high phase transition temperature showed higher entrapment efficiency e.g. Sorbiton monosterate. [Arunothayanum P, 2000]

- **Cholesterol content and charge** [Gregoriadis G, 1981 & Weissman G, 1975]

  Cholesterol acts as stabilizer in niosome formulation. Cholesterol increase vesicle rigidity by accommodate between lipid bi-layer. It act as charge inducer so repulsion occur among the bi-layer and tend to increase in vesicle size. Cholesterol accommodate between lipid bi-layer so decrease entrapment of hydrophobic API. Vesicle stiffness increases so it retarded API diffusion form niosome.


  Preparation techniques give rise to niosome of different characteristics. There are various methods like Trans membrane pH gradient, sonication, rotary evaporation...
technique, ether injection. From that, sonication method produced large vesicle size with chances of aggregation. The pH gradient method can be produced niosome with better entrapment efficiency. Multi-lamellar vesicle is produced by rotary evaporation method on other hand ether injection method produced uni-lamellar vesicle.

1.9. Niosome Application

Non ionic surfactant vesicle is a novel delivery system appropriate to many disease conditions. Application of niosome are mentioned below

- Targeting of bioactive agents
  - Reticulo-endothelial system
    RES cell engulf the Non ionic surfactant vesicle. It is dependant on opsonins i.e. circulating serum part. RES niosomal drug delivery is used in the treatment of carcinoma. [Brewer J, 1992]

- Organs other than RES
  Organ excluding RES uses Niosome for targeting of API. [Moser P, 1989] It was found that antibody connect to the lipid surface vary easily so targeting to API carrier was easy. [Arvier M, 1990]

- Neoplasia
  Antracyclic derivative antibiotic having toxicity effects depend on dose. In such type of antibiotic niosome vesicle act as effective carrier, increased life span of patient suffer from tumor and reduce proliferation of cancerous cell. [Jayaraman C, 1996]

- Leishmaniasis
  It is a disease wherein organism exist in organ of the RES. Niosome act as alternative which reduce the side effect due to low dose of API & harmless to liver, kidney and spleen. [Hunter C, 1988]

- Peptide drug
  It was found that non ionic surfactant vesicle enhance stability of peptide. [Yoshida H, 1992]

- Immunology provoker
It was found that non ionic surfactant is a carrier which is used as adjuvant in immune system. [Elias P, 1983]

- Hemoglobin (Hb) carriers
Non ionic surfactant vesicles act as carrier for hemoglobin. It was found that spectrums of vesicle dispersion and free Hb were super imposable. [Katrin M, 2001 & Gopinathan M, 2002]

- TDDS by vesicle
The major drawback of TDDS is inefficient penetration of API through skin layers. Since from research work, it was seen that vesicles targeted pilosebaceous gland during topical or transdermal drug delivery system. Non ionic surfactant vesicles act as penetration enhancer in topical & transdermal API delivery. [Stanley J, 1982]

- Additional application
  - Controlled release: Active pharmaceutical ingredients having low minimum effective concentration to maximum safe concentration ratio & water solubility, such type of API were encapsulated in vesicle for sustained action. [Bell G, 1963]
  - Localized effect: Low permeability of Surfactant vesicles via epithelium and connective tissue maintained the active pharmaceutical ingredient restricted at the administration site. Due to localization effect surfactant vesicle delivery reduce dose as well as toxicity of active pharmaceutical ingredient. [Elias P, 1983]

1.10. Skin
Body surface is completely enclosed with the skin organ. Average surface area of skin is 2 m² & 1/3 rd blood circulation all over the body. Skin layer function as protective barrier due to defensive action to infective organism, remove waste by skin layer and regulate body temperature. Skin act as sensory organ thus play immense role in immune system by gathering any sensation from environment. Skin having three major layers like outermost epidermal layer then below epidermal, hypodermis and lastly endodermis.
Figure 1.5: Skin layers

Epidermal layer is further subdivided into five sub-layers. First is stratum corneum, outermost layer also known as horny layer. [Scheuplein R, 1965] Primary defensive layer i.e. stratum corneum is made up of corneocytes enclosed by lipid region. Keratinocytes or corneocytes suspended in lipid as well as enclosed by layer of lipid, appearance as brick and mortar which retain water but hinders penetration or permeation of API or any foreign particle. [Jacob S, 1970] Underneath the horny layer there was clear layer i.e. stratum lucidum afterwards granular layer known as stratum granulosum subsequently the stratum spinosum. Stratum spinosum or prickly cell layer is spiny appearance due to presence of desmosome. Afterwards, there was stratum germinativum consisting columnar basal cells linked to basal layer via hemi desmosomes. [Babiuk S, 2000] Stratum basal layer having regenerative properties composed of stem cell and keratinocytes. [Farmer E, 2000 & Jia-You F, 2004]

Stratum corneum acts as major barrier layer in topical or transdermal delivery of active pharmaceutical ingredient. [Suhonen, T et.al, 1999] Stratum corneum thickness relies on skin surface stimulation through abrasion and weight bearing. Hence palm and sole are thicker than other part of body. [Heather A, 2005] Horny layer, granular layer & clear layer are physiological important in skin functioning. All three upper layer of epidermis were removed leads to increase in permeability and water loss. [Schatzelin A, 1998] Cholesterol, fatty acid & ceramides lipids present in horny layer.

Keratin contains free amino acids functioning as buffer and defends the skin from acids or alkali. Below the horny layer the true skin present recognized as dermal layer.
or corium. Dermis is composed of collagen fibers & 2-3 mm thick. Collagen, elastic tissue, and reticular fibers are three tissues present in dermal region. Dermal layer consist of two layers reticular and papillary sheet. In this papillary layer is thinner than reticular layer which is made up by thick collagen fibers while in papillary layer thin arrangement of fibers. The hair follicles are located with this the erector pili muscle that connect to every follicle. Hair follicles have oil gland and scent gland also eccrine sweet gland though not linked with hair follicle. This layer having blood vessel and nerves regulate the skin as well as body temperature. Meissner's and Vater-Pacini corpuscles are specialized nerve sensor of touch and pressure. [Naik A, 2000]

Superficial fastratum corneumia is a last layer of subcutaneous tissue regulates the body temperature. The thickness varies of this layer varies from person to person.

Papillary and reticular are the two layers of dermis. In that upper, papillary layer is thinner compare to reticular layer. Dermal layer have numerous specialized cells and structures.

- API release pathway through skin layers

There are numerous diffusional barriers in topical or transdermal drug delivery system. Diffusion of active pharmaceutical ingredient takes place through out various layers of skin. Initially release of API occurs from formulation then via epidermal and dermal layer respectively. Each layer having distinct barrier properties depend on composition of layers. Horny layer include major two routes for API delivery like lipid channel and hydrophilic keratinized cell. Hydrophobic molecules lipid channel is route of entry. It was found that hydration enhance diffusion of polar drug compare to nonpolar drug. [Behl C, 1980]
In topical or Trans dermal drug delivery partitioning of API among vehicle and stratum corneum is a rate limiting step and during partitioning API was stored in horny layer. [Jia-You F, 2004]

So, stratum corneum acts as a major barrier in topical drug delivery. Though epidermal and dermal drug’s diffusion are rate controlling steps for hydrophobic molecules once stratum corneum is damaged. [Spiclin P, 2003]

**Factors affecting API skin diffusion**

- **Physiological**
  1. **Age**
     Skin structure changes take place due to aging. As discussed earlier dermal blood flow is important parameter in drug distribution but skin aging reduce API flux through dermal layer. Permeation enhance upon hydration of the skin, on aging ability of API to diffuse is varied.

  2. **Skin hydration**
     In drug permeation skin hydration is one of the important factors. As skin hydration goes on increasing there is increase in pore size due to enlargement and softening of
tissue present in the skin. Skin hydration increases the flux of respective API through the skin layers so it increases drug diffusion. [Kirjavainen M, 1999]

3. Application site of body
Effective delivery of drug through topical route is depending on site of application. Skin is thicker in palms and soles side compared to eyelids and lips region. In palm region skin thickness is in the range of 400 – 600 micrometer while in other site it is 10 – 20 micrometer in range.

4. Skin integrity
Stratum corneum and other skin layer integrity alter in disease condition. At the same time diffusion capacity also varied as there alterations in skin barrier function.

5. Metabolism
Metabolizing enzymes are present in epidermis, hair follicles and sebaceous glands. In topical drug delivery, it was found that enzyme available in skin metabolizes 5% of API available during therapy. Steroidal hormone is example of metabolizing enzyme present in the skin.

6. Additional parameters
It includes temperature and pH of skin surface. In diffusion of API through the skin layers enhances as skin layer temperature increases.
Unionized molecules pass through the lipophilic layer of skin. So skin pH change ionization of weakly basic and weakly acidic drugs accordingly diffusion of API alters.

➢ Preparation parameters

1. Medium materials
Topical delivery contains active pharmaceutical ingredient or drug for giving desired therapeutic action. It also includes other substance which helps API during delivery. Substance other than active pharmaceutical ingredient identified as excipients. These excipients include solvent, penetration enhancer, preservative and stabilizer etc. known as vehicle. Drug diffusion is smooth when drug in the vehicle, which form layer during application mix with sebum followed by tissue cell contact. Drug
efficiency during topical delivery relies on drug ability to diffuse out of the formulation or vehicle and pass into the skin. Diffusion coefficient of drug molecules depends on solubility of API in vehicle as well as vehicle viscosity. As vehicle viscosity increases the value of diffusion coefficient increases leads to poor drug penetration. Partitioning of drug from vehicle to the skin depend on solubility in vehicle. If drug was soluble in vehicle then it becomes difficult to diffuse from vehicle to the skin. Highly soluble drug having more partitioning in vehicle so penetration decreases. Thus it is necessary to have proper balance of drug solubility which gives aesthetic appeal as well as support diffusion from vehicle through the skin layers. Oleaginous vehicles increase skin hydration by the way of preventing moisture evaporation. It leads to increase skin water content and percutaneous diffusion of API through skin barrier layers. [Cevec G, 1996]

2. Permeation enhancers

It enhances solubility of API in the intercellular lipid of stratum corneum. Other increases hydration by denaturation of keratin in the horny layer. [Lagerquist C, 1992] Example of penetration enhancers are animal and vegetable oils which having good penetration ability than minerals oils. Acetone, benzene and propylene glycol also act as penetration enhancers. [Lopez-Pinto J, 2005]

3. API concentration

Drug molecule flux increases as there is increasing concentration gradient of API through the skin diffusion layer as stated in Fick’s first law of diffusion. Drug absorption ability per unit area is directly proportional to the concentration. If formulation is saturated with API it gives maximum flux as well as better penetration through skin layers. Formulation having saturated drug concentration indicates release pattern near to zero order kinetics.

> API physiochemical characteristics

1. API Solubility

Drug which show better solubility in oil give efficient percutaneous absorption. At the same time it is also important drug molecule is soluble in aqueous phase in some extent to penetrate hydrophilic skin layers present below the horny layer. Active
pharmaceutical ingredient having proper balance among hydrophobic and lipophobic solubility leads to greater concentration in dermal layer.

2. Diffusion Coefficient (D)

The diffusion coefficient is used as an indicator of the rate of penetration and degree of resistance to penetration of a molecule through the skin. It encompasses factors such as protein binding of the drug, the tortuosity of the diffusion pathway, some properties of the drug molecule and interactions between the vehicle and the skin. In the skin, the value of D decreases as the penetrant reaches deeper more compact layers of the stratum corneum. [Verma D, 2003]

Drug penetration rate and degree of resistance of drug depend on respective diffusion coefficient (D). Various factors affects alter D like protein binding, chemical interaction between drug and vehicle, interaction among skin layer and drug. Drug penetrates at deeper layer as D value decreases.

D is expressed in Fick’s first law of diffusion

\[ J = - D \frac{dc}{dx} \]

Where;

J = Rate of transfer per unit area of surface (flux)
C = Concentration of diffusing substance
x = Space coordinate measured normal to the section
D = Diffusion coefficient

Rate of transfer per unit area of surface (flux) is inversely proportional to drug concentration. Skin permeation of drug molecules enhances as there is increasing in D value. In skin membrane it is hard to distinguish partition coefficient value (K) from diffusion coefficient value (D).

3. Partition Coefficient (K)

K value means capability of API to partition out of the vehicle to the horny layer. Horny layer is major barrier for drug diffusion at that time K value is important factor. Partition coefficient value depends upon solubility in vehicle, ionization capacity and
drug loading in formulation. [Valenta C, 2003] High K value associated with drug binding to the stratum corneum on other hand low K value indicates low partitioning capacity of API.

4. API binding with protein
Drug molecule having more numbers of atoms then there is greater chances of protein binding through the formation of hydrogen bond. So, for effective penetration drug molecule comprise fewer atoms. Ideal log P value is 2.6 for most favorable API penetration. Increase protein binding leads to higher log P value.

5. Drug particle Morphology
Molecular weight is important determinant in drug penetration through the skin. An ideal molecular weight is less than or nears to 500 dalton. Molecular weight is inversely proportional to flux, so small molecule diffuses through skin layer easily compared to the high molecular weight compound.

1.11 Gel

Gels are transparent to opaque semisolids including high proportion of vehicle to polymer i.e. gelling mediator. Polymer or viscosity modifier adds into suitable vehicle colloidal network was developed. Developed colloidal network restrict flow of fluid by immobilization and trapping of the solvent molecules. [Brown M, 2005]

Topical drug delivery system of skin infections uses various dosage forms like solid, semisolid and liquid formulation. Non ionic surfactant dispersion gives release pattern in sustained way but developed gel formulation improve topical applicability. Various dosage forms are available in semisolid form, containing distinctive properties. From various semisolid preparations the gel was widely used in topical delivery system. Gel is sufficiently viscous form so it stays on the skin for extensive duration of time prior to washing. This characteristic gives release pattern in sustained manner. [Kasapis S, 1999]

Developed niogel preparation having constructive characteristics like greaseless, thixotropic, spreadable, nonstaining and it is also compatible with added ingredients in formulation.
Classification

Gels are classified based on nature of solvent used for hydration of the polymer

i) Aqueous gels (Hydrogels): The term has evolved to refer specially to aqueous gels containing an insoluble polymer.

ii) Non aqueous gels (Organogels): Organogels contain a nonaqueous solvent as the continuous phase e.g. Plastibase.

iii) Xerogels: These are solid gels containing low concentration of solvents. Xerogels are often produced by evaporation of the solvent, leaving the gel framework behind. They can return to the gel state by introduction of an agent that, on imbibition, swells the gel matrix. Examples of xerogels include dry gelatin, tragacanth, dry cellulose and polystyrene respectively.

In some literature review mentioned two classification scheme of gel.

First scheme devides gel into inorganic and organic. Inorganic are hydrogel which are two phase system means the gel mass consisting of flocules of small distinct particles. Organic gels are single phase system means macromolecules distributed throughout the boundaries between them.

Second scheme hydrogel and organogel, hydrogel include ingredients that are dispersible as colloids or soluble in water like organic hydrogel, sodium CMC, bentonite gum, hydrophilic colloid. Organo-gel includes the hydrocarbon, animal & vegetable fat, soap base greases & the hydrophilic organo-gel.

Polymer used in gel formulation:

Basically three types of polymers were used in gel preparation. First is natural like polysaccharides, proteins. Second one is semi synthetic for e.g. cellulose derivative. Finally synthetic polymers like poly acrylamide, poloxamer, polyethylene copolymer.

Numbers of terms are commonly used in discussing some of the characteristics of gel including imbibitions, swelling, syneresis, thixotropy & xerogel. In that imbibitions is the taking up of certain amount of liquid without measurable increase in volume otherwise swelling is the taking up of certain amount of liquid with measurable increase in volume. Syneresis interaction between particles of the disperse phase
become so great on standing, the disperse medium is squeezed out in droplet and the gel shrink. While, thixotropy is a reversible gel-sol formation & no change in volume and temperature and it gives type of non-newtonian flow. And lastly in xerogel liquid is removed from gel & only framework remains like gelatin sheets, tragacanth ribbon & acacia tears.

**Carbopol polymer**

The extensive use of carbopol for gel preparation has been recognized to its properties like good aqueous solubility, gel formation at low concentration, good bioadhesivity, and compatibility with many active components and stability. [Tamburic S, 1995] Carbomer (carbopol) resin first described in 1955, are ingredient in variety of dosage form controlled release tablet, oral suspension, topical gel. Carbomer resins are high molecular weight allyl pentarerythrotol-cross-linked-acrylic acid based polymers modified with C 10 – C 30 allyl acrylate. [Rowe R, 2006] They are floppy white dry powder with large bulk density. The .5 % & 1 % aqueous dispersion are pH 2.7 to 3.5 & 2.5 to 3 respectively. More carbomers resin with viscosity 0- 80, 000 cps. Carbomers 910, 934, 934P, 940 & 1342 are official in the UAP 32- NF 27

In solid state, the carbopol molecule often exists as a strongly coiled spiral form. The unwinding of this spiral structure upon hydration leads to increase in viscosity. The complete unwinding of the molecule frequently ensures the highest viscosity.

The unwinding of the carbopol resin may be explained by one of the mechanisms as described below. The most common mechanism is based on use of appropriate base for neutralization of the polymer. [Bremecker D, 1984] This neutralization imposes ionization of polymer leading to generation of negative charge on the polymer chains. [Unlu N, 1992] Unfolding of the structure thus occurs through repulsion between these charges that on intertwining forms a three-dimensional matrix resulting in delopment of viscous gel. [Hernandez M, 1998]

The second mechanism consists of hydrogen bond formation induced by addition of a hydroxyl donor structure to the resin. This process leads to formation of gel even at acidic pH, however, maximum thickening may be achieved after several hours.
Polyols such as polyethylene glycol, glycerin and propylene glycol or non-ionic surfactants containing five or more ethoxy groups can be used as hydroxyl donors. [Tabernera T, 2002]

To manufacture clear, uniform, air free gels, certain key processing characteristics must be provided. The nature of the carbomer requires initial high shear mixing to form a uniform smooth dispersion, followed by low shear mixing during the neutralization step. In addition, mixing under vacuum, will withdraw entrapped air from the dispersion during manufacture and prevent further air entrapment by incidental surface breaks. Minimization of air entrapment is necessary from the aesthetic standpoint and most importantly, controlling fill weights during packaging operations. [Tamburic S (1995]

1.12. Fungal Infection

Dermatophyte is a type of fungus that infect top layer of skin. Fungi had been recognized as causitaive agent of human disease earlier than bacteria. Fungi causing favus (*Trichophyton schonleinii*) & thrush (*Candida albicans*) had been described as early as in 1839.

Fungus infections however are extremely common & some of them are serious and even fatal. With the control of most bacterial infections in the developed countries, fungus infection assumed greater importance. Fungi are eukaryotic protista that differ from bacteria & other prokaryotes in many ways. They possess rigid cell walls containing chitin, mannan & other polysaccharides. [Skalko N, 1992]

**Resons behind fungal infection**

Ringworm is type of fungal infection grows rapidly in moist part of the body like skin between fingers and toes and other parts of the body where skin is folded. Towels, puppies, hats, kitten and brushes are act as mediators of fungal infection. Warm and moist environment are suitable for fungal growth.
Classification of fungal infection

Depending on the cell morphology, fungi can be divided into four classes: yeast, yeast-like fungi, moulds, dimorphic fungi. Yeast is unicellular fungi which occur as spherical or ellipsoidal cells & reproduce by simple budding. On culture they form smooth, creamy colonies. The only pathogenic yeast is Cryptococcus neoformans.

Yeast like fungi grow partly as yeast and partly as elongated cells resembling hyphae. Candida albicans is pathogenic yeast like fungus. Moulds from true mycelia & reproduce by the formation of different types of spores. Dermatophytes are example of pathogenic moulds.

Dimorphic fungi can occur as filament or as yeast depending on the condition of growth. In host tissue or culture at 37°C. They occur as yeasts, while in the soil & in culture at 22°C they occur as moulds. Most fungi causing systemic infection are dimorphic fungi.

The systematic classification of fungi based on their sexual spore formation, recognizes & classes like phycomycetes, ascomycetes, basidomycetes & dueteromycetes (fungi imperfecti). The commones culture media used in mycology are saboured’s glucose agar (pH 5.4), cornmeal agar.

Fungus infection (Mycoses) is a human infection is broadly of two types. Superficial & deep seated (systemic) superficial infection is by far common & comprises the various types of tinea or ringworm affecting the skin, hair & nails.

Superficial mycoses having two types surface infection & cutaneous infection. In the former, the fungi live exclusively on the dead layers of skin & its appendages. They have no contact with living tissue & hence elicit no inflammatory response. The only changes produced are cosmetic effects. Tinea (pityriasis) versicolor, Tinea nigra, & Piedra fall in to this group. The most important cutaneous infection is dermatophytosis caused by dermatophytes. Candida albicans caused cutaneous infection confined to the skin & mucosa. Candida infection represents bridge connecting superficial & deep mycoses.
Photographs of Fungal infection

Rash caused by candida

Candida infection

Classification of antifungal drug

a. Polyene antifungal
   The polyene have high affinity for ergosterol present in fungi cell membrane: combine with it, get inserted into the and several molecules together orient themselves in such a way as to form a ‘micropore’. The hydrophilic side forms the interior of the pore through which ions, aminoacids and other water soluble substances move out. The micropore is stabilized by membrane sterols which fill up the spaces between the polyene molecules on the lipophilic side – constituting outer surface of the pore thus cell permeability is markedly increased. Examples: Nystatin, Amphotericin-B and Natamycin. [KD Tripathi, 1991]

b. Azole antifungal
   Mechanism of action of azole antifungal hamper cytochrome P450 demethylase synthesis. Function of this enzyme to transform lanosterol to ergosterol which is essential in synthesis of cell membrane. Azole antifungals are efficient like miconazole, clotrimazole and triazole.

c. Allylamines
   Allylamines reduce the synthesis of squalene epoxidase enzyme essential for ergosterol synthesis. E.g. are Naftifine, Amorolfine and Terbinafine.
d. Antimetabolite
All susceptible fungi are capable of deaminating flucytosine to 5-fluorouracil may be converted to fluorodeoxyuridylic acid which inhibits thymidylate synthetase and ultimately interferes with DNA synthesis. Example: Flucytosine.

e. Other topical- Tolnaftate, undecyclic acid, benzoic acid.
Most of the yeast and molds infection like candida are sensitive towards polyene antifungal like nystatin. It is broad spectrum antibiotic active against *candida albicans* which is obtained from *streptomyces naursei*. Furthermore fungal infection is associated with inflammation hence combined therapy of antifungal and anti-inflammatory such as Triamcinolone acetonide is prescribed for the effective treatment. The molecular weight of Nystatin is 926.13 and it is highly lipophilic. As per BCS classification it is Class-IV drug which has low permeability and low solubility. Due to its toxicity profile it cannot be formulated into injectable formulations.

So drug passage or permeation through skin layers is a challenge, by considering these problems we have encapsulated the drug into the non-ionic surfactant vesicles (niosomes) for effective delivery of drug to the site of action. Thus Nystatin was selected for the development of topical niosome formulations.

1.13 Literature Review

- **David et al. (1997)** developed organogel having poly(acrylic acid) act as bioactive implant. The developed organogel characterized for physical and chemical parameters. In rheological characterization, adhesion index, release study and analytical methods gave information regarding preclinical and clinical parameter evaluation of bioactive implant. Formulated bioactive agent showed effective therapy against oral cavity infectious disease.

- **Muhannad Jumaa et al. (2002)** studied chitosan activity in aqueous solutions and in lipid emulsion. To check chitosan antimicrobial characteristics two types of chitosan were used. It was found that for parenteral application and mucosal delivery, chitosan act as antimicrobial preservative.
• **Bowler PG et al. (2004)** described silver dressing preparation in which Ag used as broad spectrum antimicrobial properties. In development of dressing integrating silver with hydrofiber technology mix with gelling properties containing silver and hydrofiber techniques. Developed dressing was act as primary dressing effective against wound healing either chronic or acute microbial infection.

• **Skinner, M. C et al. (2010)** stated for *C. trachomatis* mixture of WLBU2 and 3-OG showed effective therapy. In combined microbicidal formulation have less tendency to induce resistant for *Chlamydia* strains when drug in dosage form showed varied mechanism of action. Combined dosage was preferred over single dosage because in combined form active ingredients gave synergistic effect which reduces the dose of the final product.

• **EP Guenin (1995)** described liposome or non ionic surfactant vesicle interaction with hairless skin of mouse. From the study it was found that water permeation rate was more in phospholipid vesicle at pH 2 while at pH 5 no change in water permeation rate. Non ionic surfactant vesicle not showed marked effect in water permeation rate.

• **Dufes et al. (2000)** stated use of transferring and ligands as drug targeting in polymeric chitosan based vesicle and non ionic surfactant vesicle. It was found that chitosan based vesicles engulfed via A431 cells and glucose bearing vesicles bound concavalavin A Gold to their surface.

• **MP Kamath (2000)** developed non ionic surfactant vesicle and phospholipid vesicle formulation containing rifampicin. Developed formulation was evaluated for *in vitro* & *in vivo* release pattern. It was observed that entrapment of rifampicin in vesicle delay the drug release by reason of slow API release in systematic circulation. Plasma drug concentration time profile showed higher area under curve (AUC) means better bioavailability compared to free rifampicin.

• **JY Fang et al. (2001)** developed enoxacin phospholipid vesicle and non ionic surfactant vesicle. It was found that enoxacin vesicle delivery through skin layer was enhanced. It was stated that inclusion of cholesterol increase the stability but addition of negative charge inducer decreases the niosome stability.
• **Ribier et al. (2001)** and formulated cosmetic which anhydrous in nature containing surfactants, oils, waxes, fatty bodies and vesicular lipidic composition means having amphiphilic lipid of non ionic or ionic in nature. Obtained patent on cosmetic composition of anhydrous in nature containing fatty composition.

• **Dhoot et al. (2003)** stated microencapsulated liposomes diminish the burst effect and showed release in controlled manner. It was due to cross linking ions could affect encapsulated protein release. It was found that release pattern of microencapsulated liposome was higher than free liposome.

• **S Agarwal et al. (2004)** determined paremeters effect like cholesterol content and hydrophilic lipophilic balance of surfactant on properties of Primaquine phosphate niosome. It was found that lower the HLB value gives small initial vesicle size. Surfactant HLB value was found to be directly proportional to mean size of the vesicle. It was observed that due to increase in cholesterol content there is higher entrapment efficiency of drug in vesicle.

• **Vyas et al (2005)** formulated topical DNA delivery system encapsulated in niosome vesicle. Hepatitis B surface antigen (HBsAg) act as encoding agent for DNA encapsulated in non ionic surfactant vesicle. Developed topical niosome gives a comparable serum antibody and endogenous cytokines level compare to i.m. recombinant hepatitis B surface antigen and topical liposome. It was found that developed niosomal DNA delivery system is effective topical immunization approach. Developed formulation is economical, simple than phopholipid vesicle.

• **A Girigoswami (2006)** used span 80, tween 80, span 20 and tween 20 for formulation of non ionic surfactant vesicle. To check donor-receptor distances study like fluorescence resonance energy transfer was carried out. It was observed that efficiency of fluorescence resonance energy is enhanced in non ionic surfactant vesicle compared to micelles. It was found that niosome formation is based on hydrophilic lipophilic balance of non ionic surfactant.

• **Wei Hua et al. (2007)** formulated non ionic surfactant vesicle made up by polyethylene glycol, water and span 80. It was observed that prepared niosome was stable up to one year. The ingredients used have an effect on preparation and
characteristics of the non ionic surfactant vesicle. However ionic concentration and temperature do not alter the stability radius.

- **Paolino et al. (2008)** formulated novel niosomal system using cholesterol, span 80 and \(\alpha,\omega\)-hexadecyl-bis-(1-aza- 18-crown-6) (Bola) in molar ration 2:5:2 was used as a topical 5-fluorouracil therapy. Developed formulation was used in skin cancer therapy. It was found that Bola-niosome enhances penetration rate up to 4-8 folds compared to drug aqueous solution.

- **Manosroi et al. (2008)** prepared niosomes by a novel supercritical carbon dioxide fluid (scCO2) technique. Niosomes by the scCO2 method with 10 % (w/w) ethanol gave higher trapping efficiency (12.22 ± 0.26%) than those by the conventional chloroform film method with sonication (10.85 ± 0.24%) and the scCO2 method without ethanol (8.40 ± 1.60%). This present study has demonstrated the trapping efficiency enhancement of water-soluble compounds in niosomes by the scCO2 method with 10 % (w/w) of ethanol.

- **Patel et al. (2009)** prepared phospholipid vesicle of ketoconazole by method of thin film hydration. Application improved by formation of gel using carbopol polymer having concentration 1%. Prepared liposomal gel was evaluated for deposition study, drug diffusion study and antifungal activity. Ketoconazole phospholipid vesicle showed sustained release compared with non liposomal formulation. Liposomal gel showed good antifungal activity than simple ketoconazole cream or gel formulations.

- **Bhaskaran et al. (2009)** used sorbiton monostearate as nonionic surfactant in salbutamol sulphate vesicle formulation. The various methods were used during niosome preparation like hand shaking, thin film hydration, transmembrane pH gradient and ether injection technique. Entrapment efficiency of niosome varied in the range of 62 to 87 %. *In vitro* diffusion study showed delayed release up to 24 hrs. From various techniques transmembrane pH gradient diffuse 78.4 % salbutamol sulphate in one day. Optimized formulation undergoes lyophilization and evaluated for functional group using IR Spectroscopy. Bioavailability study and tissue distribution studies were carried out using rabbits and albino rats.
• **Srinivas et al. (2010)** formulated aceclofenac non ionic surfactant vesicle for bioavailability improvement. Prepared non ionic surfactant vesicles were evaluated for drug diffusion study, entrapment efficiency and vesicle diameter. Drug diffusion study was carried out using dialysis membrane to clarify drug diffusion mechanism. From various model Peppas model was fit to drug diffusion release.

• **Choi MJ et al. (2005)** described topical and transdermal drug delivery system application. Though in many research work mentioned the advantages of TDDS. But skin horny layer acts as permeability barrier in topical delivery system. Presented review article describe liposome and niosome effect on enhancing drug penetration also gives information of impact of vesicle size, vesicle type and composition on topical or transdermal drug delivery.

• **Majid Tabbakhian et al. (2006)** Observe finasteride encapsulated surfactant vesicle enhance its concentration in Pilosebaceous unit compared to finasteride solution during topical applicability. Film hydration technique was used in vesicle preparation, and evaluated for transition temperature, vesicle size and encapsulated efficiency. From drug diffusion study and permeation studies, it was found potentials of phospholipid vesicle and surfactant vesicle dispersion in finasteride delivery to the Pilosebaceous unit.

• **Mahesh N. (2006)** described UV exposure causes skin degenerative effect. To overcome this problem antioxidant formulation were used. Factorial design approach ($3^2$) was used during vesicle formulation, in that cholesterol and phospholipid act as independent variables used in three various levels. Etanol injection method was used in vesicle formulation. Stability study of liposomal dispersion and liposomal gel were carried out for three months at 65 % humidity. Developed phospholipid vesicle showed greater deposition in skin for dermal delivery.

• **Nicolas Atrux (2010)** review described potential of nanocarrier in topical drug delivery system. Various nanocarriers were used in topical application like solid lipid nanoparticle, phospholipid vesicle, surfactant vesicle and nanocrystal etc. The active pharmaceutical ingredient having high molecular weight and low
lipophilicity was poor molecules in topical delivery system. This problem is overcome by entrapment of API in nanocarrier which enhance penetration and deposition of active pharmaceutical ingredient.

• **L. Zhang et al. (2010)** review emphasis on nanoparticle formulation for antimicrobial therapy. Antimicrobial agents have good therapeutic efficiency but inadequate therapeutic index and side effect which mab the gut flora reduction, dermal irritation and peeling. Recently antimicrobial entrappemnt in nanoparticle can overcome above mentioned disadvantages of antimicrobial drug and increase therapeutic efficiency. For better growth in nanotechnology field for the antimicrobial therapy combined efforts of nanoengineers and microbiologists is needed.

• **Elka Touitou et al. (1998)** explained various techniques for quantitative evaluation of active pharmaceutical ingredient. For topical and transdermal drug delivery quantification of drug is important parameters. A traditional investigation technique contains parallel slicing through deeper skin tissue layer and tape stripping. While qualitative autoradiography, heat separation, use of induced follicle free skin and heat separation techniques were used recently. From recent technology skin quantitative autoradiography is innovative techniques used in visualization and quantification of penetrant throughout skin section.

• **Manivannan Rangasamy et al. (2008)** used ether injection method and hand shaking process for preparation of acyclovir nonionic surfactant vesicle. The ingredients used in surfactant vesicle preparation are span 80 and stability enhancer cholesterol in the ration of 1:1, 1:2 and 1:3. From this two methods ether injection method gives smaller vesicle size 0.5-2.5 microns compared to vesicle 0.5-5 microns which was obtained from hand shaking techniques. It was found that surfactant concentration i.e. span 80 is directly proportional to entrapment efficiency. *In vitro* diffusion study show sustained release of Acyclovir.

• **Shimon Ben-Shabat (2005)** explained conjugation techniques is best alternative to enhance skin penetration. Conjugation occurs between linolenic acid – linolenic acid which are polyunsaturated fatty acids and calcipotriol – vitamin D3
extensively used in topical therapy in psoriasis. Developed conjugates prohibit keratinocytes proliferation in culture and hydrolysis causes antiproliferative activity. Penetration study revealed that conjugates penetrated skin efficiently compared calcipotriol alone. Conjugate act as prodrug so after penetration through skin conjugate is capable to undergo bioprocessed mechanism and converted into active therapeutic agent.

- **Mahmoud Mokhtar (2008)** developed flurbiprofen proniosomal gel or solution using various non ionic surfactants like sorbiton monostearate, sorbiton monolaurate, sorbiton palmitate and sorbiton maleate by addition of cholesterol. Centrifugation techniques and exhaustive dialysis techniques were used in determination of entrapment efficiency. From entrapment efficiency evaluation it was found that cholesterol concentration was inversely proportional to entrapment efficiency. From various non ionic surfactants used in vesicle preparations sorbiton monostearate showed high entrapment efficiency compared to other surfactants due to high glass transition temperature.

- **Hong et al, (2001)** developed topical preparation of enoxacin encapsulated in phospholipid vesicle and surfactant vesicle respectively. Franz diffusion cell was used to determine *in vitro* percutaneous absorption. Developed prepearration showed high concentration of enoxacin in the skin. From stability study, it was observed that surfactant vesicles are more stable compared to phospholipid vesicles. Cholesterol addition during vesicle preparation enhances stability of vesicles. Optimized vesicles formulation is best carrier in topical delivery of antibacterial drug due to low toxicity profile.

- **Barolli (1999)** described synthesized Hexakis [butohytris (ethoxy)] cyclophosphazene (3a), hexakis [dodecyloxytetakis (ethoxy)] cyclophosphazene (3b) and hexakis [hexadecyloxyicosaneakis (ethoxy)] cyclophosphazene and used niosome preparation. Cholesterol and synthesized compounds formulated niosome but aggregate strongly. So to minimize this problem stabilizer like dicetylphosphate was used. Carboxyfluorescein was used to determine entrapment efficiency of drug.
• **Arora et al. (2010)** developed topical dosage form of ketoprofen encapsulated in non ionic surfactant vesicle. Film hydration technique was used in niosome preparation using surfactant, stabilizer cholesterol, active pharmaceutical ingredient and deaggregating agent dicetyl phosphate. Developed surfactant vesicle was evaluated for *in vitro* release study and various physical parameters. UV spectroscopic method was used in chemical parameters evaluation.

• **Aditya K et al. (1994)** presented review article described novel techniques used in topical antifungal therapy. To clarify the concept of various novel approaches used in antifungal therapy, it is essential to understand traditional dosage form in fungal disease containing azole, poleyne, griseofulvin and other derivatives.

• **Aranya M et al. (2003)** developed niosome using surfactant and cholesterol mixture. Concentration of surfactant and cholesterol have impact on entrapment efficiency of the niosome. From various surfactants sorbiton monosterate showed higher entrapment efficiency. Cholesterol enhances stability of prepared vesicle due to increase rigidity of bilayer membrane.

• **P. Loan et al (2002)** supports the hypothesis that elastic vesicles act as best vehicle for transdermal drug delivery. Proposed vesicles establish good balance among solubility, elasticity of vesicle, vesicle stability and good API transport through the skin layers.

• **El Maghraby (2008)** present review article describe importance of vesicles in permeation improvement techniques as a model for human skin. From various vesicles, liposome in prime importance as a model in skin permeation study. Validation parameters like precision and accuracy are studies in both liposome vesicular system and traditional skin model.

• **Mahesh N (2006)** formulated phospholipid vesicle using ethanol injection method. To reduce number of batches during formulation development factorial design was applied. In that two indpendant variables like phospholipid and cholesterol were selected at higher, middle and lower level. Vesicle size and drug deposition were considered as dependant variables. Prepared phospholipid vesicles
were evaluated for vesicle size, entrapment efficiency, drug diffusion and deposition study and zeta potential determination.

- **Elka Touitou et al. (1998)** took overview of analytical methods used for quantification of active pharmaceutical ingredient in skin layer. From that, spectroscopic method was considered as economical, noninvasive, rapid assay technique. Among all spectroscopic techniques fluorescence spectroscopy was found to be most sensitive. Fluorescence method is divided into two types’ indirect and direct fluorescence spectroscopy. Direct method is applicable to self fluorescent molecule and indirect technique contains determination of UV absorbing penetrants.

- **U. S. Pat. No. 20080269184, Yuasa et al. (2008)** discloses non ionic surfactant vesicle is a best anticancer agent in cancer therapy. It contains metalloporphyrin complex implant in that demonstrates effectivity on cancer treatment by decreasing oxygen level in cancer cell. Non ionic surfactants remain in blood having good antioxidant capacity.

U. S. Pat. No. 20080050445, Alcantar et al.(2008) discloses novel technology like nanovesicle and further developed as a gel form. Present invention gives some clue in other technological in need of controlled release rate of active pharmaceutical ingredient and chemical moiety. In nanoparticle development phase drug encapsulated in vesicle so gives controlled release that release pattern further reduce by incorporation it in to gel network. So it may be advantageous to that chemical moiety showed immediate release pattern.

- **U. S. Pat. No. 6051250, Ribier et al. (2000)** discloses use of stabilizing agent in vesicle stabilization process. Vesicle prepared from ionic or non ionic amphiphilic lipid show stability problem. To reduce stability problem stabilizing agents was added like propylene glycol alginate, gellan gum and glycerol alginate. Present investigation gives idea regarding importance of stabilizing agent in vesicle stability used in topical application.
1.14 Need & objective

Dermatophyte causes the fungal infection, in which dermatophyte infect top layer of skin. Fungal infection is a critical problem throughout the world; to overcome this problem various oral and topical antifungal therapies were used. At primary stage of fungal infection it is easy to treatment. But in immunodeficiency and malnourished patient it becomes critical to cure fungal infection may be it becomes life threatening problem. Antifungal dosage form sale extensively throughout the world. The mainstay of management of fungal infection and dermatophytes associated with skin and nail injuries has been oral and topical antifungal drug delivery systems. [Rex J et al., 2004]

Patient suffering from cutaneous candidiasis cure with combined formulation of nystatin and triamcinolone acetonide reveal prominent healing of erythema & pruritis than therapy of nystatin and triamcinolone acetonide alone. Horny layer act as major barrier layer in topical drug delivery, it hinders drug deposition through the skin. To overcome shortcoming of conventional preparation nanocarrier was selected by enhancing drug penetration through skin layers and also increase deposition of drug. [Robinson J et al. 1990, Jia-You et al., 2004]

Polyene antifungal moiety like nystatin is effective over yeast and mold infections containing candida. It is broad spectrum antibiotic active against *candida albicans* which is obtained from *streptomyses naursei*. Furthermore fungal infection is associated with inflammation hence combined therapy of antifungal and anti-inflammatory such as Triamcinolone acetonide is prescribed for the effective treatment. The molecular weight of Nystatin is 926.13 and it is highly lipophilic. As per BCS classification it is Class-IV drug which has low permeability and low solubility. Due to its toxicity profile it cannot be formulated into injectable formulations. [Bosch, William H et. al., 2003]

Non ionic surfactant vesicle is best alternative in topical and transdermal delivery system. Vesicle enhances penetration of API through the skin layer and also showed sustained release pattern. Advantages of surfactant vesicle are biodegradable in nature, low side effect; enhance skin penetration and deposition through skin. Driving force essential for permeation of hydrophobic moiety is high thermodynamic gradient
of the active pharmaceutical ingredient at the interface. Vesicle acts as penetration enhancer by diminishing horny layer barrier properties. [Brown M & Jones S, 2005]

Therefore aim of research work is to formulate and optimize niosomal system containing antifungal agent. Furthermore topical applicability of developed niosomal system was enhanced by incorporating it in to gel framework. [Gisby J & Bryant J., 2000]