CHAPTER 1
INTRODUCTION
1. INTRODUCTION

High-throughput screening technologies in drug discovery present an efficient way to find new powerful substances. But in recent years it has become evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. Although, great success has been achieved in the management of diseases by the use of drugs in the last 5-6 decades. These achievements in drug development, however, have not been matched by a similar growth in the area of drug delivery. Unless a drug can be delivered to its target area at rate and concentration that both minimize side effects and maximize therapeutic effects, the drug will not be maximally beneficial to the patient and, in the extreme, an otherwise useful drug may be discarded.

Poor water solubility of drug molecules, insufficient bioavailability, fluctuating plasma levels or high food dependency are the main and common problems for designing the delivery system for drug. Hence, major efforts have been spent for the development of a drug delivery system through customized drug carriers to overcome these disappointing in vivo performance of the drug. For carriers non-toxicity (acute and chronic), sufficient drug loading capacity, possibility of drug targeting, controlled release characteristics, chemical and physical storage stability (for both drug and carrier) and feasibility of scaling up production with reasonable overall costs are requested [1-3].

Colloidal carriers have attracted the main interest because they are promising systems to fulfill the requirements mentioned above. A number of novel colloidal carriers have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery.

Based on the carrier material the conventional vehicles used as drug carriers can be divided into two main groups.
1. Microparticulate systems
   - Microspheres
   - Nanospheres
   - Solid lipid nanoparticles
   - Microcapsules

2. Vesicular systems
   - Liposomes
   - Niosomes
   - Ethosomes
   - Transferosomes
1.1 Need of Vesicular drug delivery systems

Encapsulation of drug in vesicular structure can be predicted to prolong the existence of the drug in the systemic circulation, enhance penetration into target tissue and reduce the toxicity, if selective uptake can be achieved [4]. Numbers of vesicular drug delivery systems have been developed such as liposomes, niosomes, transfersomes and ethosomes.

1.2 Niosomes

Niosomes the non-phospholipids vesicular alternative to liposomes, have been first reported by Vanlerberghe et al [5]. Niosomes are microscopic lamellar structures formed on admixture of cholesterol and single alkyl chain non-ionic surfactant with subsequent hydration in aqueous media. Niosomes as a drug carrier are capable of entrapping hydrophilic and hydrophobic drugs. Compared to phospholipids used in liposomes, the synthetic non-ionic surfactants used in the preparation of niosomes are chemically stable, precise in chemical composition and cheaper in cost [6].

Niosomes are reported to attain and retain better stability than liposomes and can prolong the circulation of the entrapped drugs. Because of the presence of non-ionic surfactant, they possess better intrinsic targeting potential and propensity towards the tumor, liver and brain. It may prove very useful for targeting the drugs in cancer, parasitic viral and other microbial diseases more effectively. Niosomes have been investigated for drug delivery through the most common routes of administration such as intravenous, intramuscular, subcutaneous, ocular, oral and transdermal. In recent years, niosomes have been extensively studied for their potential to serve as carriers for delivery of drugs, antigens, hormones and other bioactive agents.

Encapsulation of bioactive agent within niosomes prevents its premature biodegradation or inactivation, targets it to the ailing tissue and provides slow release thereby altering its distribution, metabolic stability and toxic manifestations.
1.2.1 Mechanism of niosomes formation

Non-ionic surfactant based vesicles are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in a closed bilayer structures as shown in Figure 1.1. The assembly into closed bilayers is rarely spontaneous (Lasic, 1990) and usually involves some input of energy such as physical agitation or heat [7]. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups enjoy maximum contact with same.

Fig. 1. Schematic representation of a niosome. ○ = hydrophilic head group, —— = hydrophobic tail.

Figure 1.1: Schematic representation of a niosome O = hydrophilic head group, —— = hydrophobic tail.
1.2.2 *Types of niosomes*

Niosomes can be classified as liposomes (Based on structural parameters) as shown in Table 1.1.

Table 1.1: Types of niosomes.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLV</td>
<td>Multilamellar large vesicles &gt; 0.5 (\mu)</td>
</tr>
<tr>
<td>OLV</td>
<td>Oligolamellar vesicles 0.1-1 (\mu)</td>
</tr>
<tr>
<td>UV</td>
<td>Unilamellar vesicles (all size range)</td>
</tr>
<tr>
<td>SUV</td>
<td>Small Unilamellar vesicles 20-100 nm</td>
</tr>
<tr>
<td>MUV</td>
<td>Medium sized unilamellar vesicles</td>
</tr>
<tr>
<td>LUV</td>
<td>Large unilamellar vesicles &gt; 100 nm</td>
</tr>
<tr>
<td>GUV</td>
<td>Giant unilamellar vesicles &gt; 1 (\mu)</td>
</tr>
<tr>
<td>MV</td>
<td>Multivesicular vesicles &gt; 1 (\mu)</td>
</tr>
</tbody>
</table>

1.2.3 *Salient Features of Niosomes* [8]

1. Niosomes entrap solute in a manner analogous to liposomes.
2. Niosomes are osmotically active and stable as well as they increase the stability of entrapped drug.
3. Surfactant does not require special handling and storage condition.
4. Niosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubility.
5. Niosomes exhibit flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation.
6. Niosomes improve oral Bioavailability of poorly absorbed drugs and enhance skin permeation of drugs.
7. They can be made to reach the site of action by oral, parenteral and topical route.

8. They offer their surface for the attachment of target oriented ligands such as hydrophilic groups and can incorporate hydrophilic moieties in bilayers to bring about changes in the in-vivo behavior of niosomes.

9. Niosomes made up from non-ionic surfactants are inexpensive, biodegradable, biocompatible and non-immunogenic.

10. Niosomal dispersion in aqueous phase can be emulsified in non-aqueous phase to regulate delivery rate of drug.

11. Niosomes improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

1.2.4 Materials Used for Niosomes Preparation [9]

Surfactants

Mainly, membrane components of niosomes are non-ionic surfactants. Principal among vesicle forming compounds are the alkyl ether lipids. These can be broadly divided into two classes based on the nature of their hydrophilic head group: alkyl ethers in which the hydrophilic head group essentially consists of repeat glycerol subunits, related isomers or larger sugar molecules, and those in which the hydrophilic head groups consists of a repeat ethylene oxide subunit. In addition, alkyl esters, amides and fatty acids and amino acid compounds also form vesicles.

Alkyl ethers

Alkyl glycerol ethers synthesized by L’oreal, France were the first compounds reported to form niosomal vesicular dispersions. A wide variety of drug delivery applications for the L’oreal non-ionic surfactants have been explored. Notable application of these compounds has been as vesicle dispersions in the field of experimental cancer
chemotherapy where both methotrexate and doxorubicin have been encapsulated and
the effect of delivery in niosomes studied. In the control of leishmaniasis, vesicles
formed from the alkyl glycerol ethers have been employed in the delivery of sodium
stibogluconate and direct comparisons made with liposomes. The encapsulation of
haemoglobin into these non-ionic surfactant vesicles as well as antipyrine has been
documented. Alkyl glycerol ethers have also been used to prepare non-ionic surfactant
vesicles for cosmetic application.

The second group of alkyl ether amphiphiles, in which the hydrophilic region consists of
repeat oxyethylene units, has received considerable attention. These compounds form
niosomes. Okahata and associates [10] also report that the dioctadecyl compound
(compound 8F) produces small disc shaped structures. The amphiphilic neutral crown
ethers, in which the head group is composed of repeat oxyethylene units also forms
vesicles. Hydrophobic compounds may be solubilized in an oil phase and stabilized
surfactant oil droplets in turn encapsulated in CₙEOₘ alkyl ethers among other non-ionic
surfactants to give niosomes entrapping a largely hydrophobic interior.

**Alkyl Esters**

Alkyl esters, such as the sorbitan esters, widely used in foodstuffs, have been studied as
the basis of non-ionic surfactant vesicles. As drug delivery vehicles in the field of cancer
chemotherapy they have been employed in the encapsulation of methotrexate,
vincristine and doxorubicin. Recent reports on the preparation of vesicles with
polyoxyethylene sorbitan monolaurate (Polysorbate 20) have appeared even though this
is a relatively soluble surfactant. The encapsulation of diclofenac sodium into
polysorbate 60 (a mixture of polyoxyethylene sorbitan C₁₆ and C₁₈ esters) niosomes has
also been documented. Sucrose palmitate stearate, a dialkyl sucrose ester, has been
investigated as a vesicle forming compound and vesicles have been prepared from this
agent encapsulating the anticancer drug methylglyoxal-bis-guanyl-hydrazone. A non-
ionic surfactant vesicle formulation consisting of a mixture of alkyl ether and alkyl ester surfactants, namely polyoxyethylene-10-stearyl ether (C_{18}EO_{10}):glyceryl laurate (C_{12}G_{1}):cholesterol (27:15:57), has been used in the transdermal delivery of cyclosporin A.

Alkyl Amides

Alkyl galactosides and glucosides incorporating amino acid spacers have also been found to produce vesicles. While, as a general rule, the alkyl groups in all vesicle forming amphiphiles consist of fully or partially saturated C_{12} to C_{22} hydrocarbons, certain novel amide compounds bearing fluorocarbon chains also form vesicles and small disk shaped structures.

Fatty Acid and Amino Acid Compounds

In addition to alkyl glycosides, amino acid moieties, when made suitably amphiphilic by the addition of hydrophobic alkyl side chains, are vesicle formers. In the presence of a water-soluble carbodiimide condensing agent, these amino acid vesicles form peptide liposomes. Long chain fatty acids also form 'ufasomes', closed vesicles formed from fatty acid bilayers. The latter vesicles were prepared at pH 8 resulting in a degree of ionization and subsequent increase in the effective volume of the hydrophilic head group.

Nonionic surfactants are widely used in the preparation of niosomes are listed with their HLB value in the Table 1.2.
Table 1.2: Nonionic surfactants with their HLB value.

<table>
<thead>
<tr>
<th>Non ionic surfactants</th>
<th>HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitan monolaurate (Span 20)</td>
<td>8.6</td>
</tr>
<tr>
<td>Sorbitan monopalmitate (Span 40)</td>
<td>6.7</td>
</tr>
<tr>
<td>Sorbitan monostearate (Span 60)</td>
<td>4.7</td>
</tr>
<tr>
<td>Sorbitan mono-oleate (Span 80)</td>
<td>4.3</td>
</tr>
<tr>
<td>Sorbitan sesquioleate (Arlacel 83)</td>
<td>3.7</td>
</tr>
<tr>
<td>Sorbitan trioleate (Span 85)</td>
<td>1.8</td>
</tr>
<tr>
<td>POE 20 sorbitan monolaurate (Tween 20)</td>
<td>16.7</td>
</tr>
<tr>
<td>POE 4 sorbitan monolaurate (Tween 21)</td>
<td>13.3</td>
</tr>
<tr>
<td>POE 20 sorbitan mono-oleate (Tween 80)</td>
<td>15.0</td>
</tr>
<tr>
<td>POE 5 sorbitan mono-oleate (Tween 81)</td>
<td>10.0</td>
</tr>
<tr>
<td>POE 20 sorbitan trioleate (Tween 85)</td>
<td>11.0</td>
</tr>
<tr>
<td>POE 10 stearyl ether (Brij 76)</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Other membrane component

Cholesterol

Steroids are the important component of cell membrane and their presence in membrane brings about discernible changes in regard to bilayer fluidity and permeability. Cholesterol can be incorporated in vesicle bilayer in a significantly higher molar ratio, however by itself it does not form bilayers. Thus, it could be used to ameliorate and manipulate the bilayer characteristics.

Being amphiphilic in nature cholesterol aligns itself in such a way that its OH- group orients towards aqueous phase while aliphatic chain aligns parallel to the hydrocarbon chain of surfactant. Further, in a mixed molecular bilayer it occupies an alternate
position. The presence of steroidal skeleton along side the carbon chain of surfactant could possibly restrict the freedom of movements of the carbon of hydrocarbon segment due to the increased packing and decreased mobility of the hydrocarbon chains thus providing an absolute rigidization. Thus cholesterol could regulate the permeability of biological membrane by affecting the internal viscosity and molecular motion of lipids in the membrane.

The decrease in permeability is generally proportional to concentration of cholesterol. Varying the cholesterol content of the vesicle membrane may control the stability and drug release from the niosomes in vitro and in vivo. Cholesterol content has also been reported to increase the entrapment efficiency of niosomes prepared from Span surfactants. X-ray diffraction methods have demonstrated that cholesterol increase the width of phospholipids bilayers. The increased drug entrapment is most likely to be the result of increased vesicle size. Cholesterol also reduce leakage rate of entrapped drug.

**Charge imparting agents**

- Dicetyl phosphate has been reported to decrease the size of vesicle since the introduction of charges in the bilayer might increase the membrane curvature and alter the biodistribution of drug incorporated in vesicles [8,11].
- It imparts negative charge to niosomes.
- Charged particles also have different bio-distribution as compared to non charged ones, so it also helps in targeting of drug molecules.

**1.2.5 Methods of niosomes preparation**

Several methods have been reported for the preparation of niosomes [5,8,11], which includes:

I. Hand shaking method

II. Ether injection method

III. Sonication
IV. Micro-fluidization

V. Multiple membrane extrusion method

VI. Reverse phase evaporation technique (REV)

VII. Trans-membrane pH gradient (inside acidic) drug update process/ Remote loading

VIII. Aqueous dispersions method.

I. Hand shaking method (Thin film hydration technique)

The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether or chloroform) in a round bottom flask. The organic solvent is removed at room temperature (20° c) using a rotary evaporator, leaving a thin layer of solid mixture deposited on the wall of round bottom flask. The dried surfactant film can be rehydrated with aqueous phase at 50°C-60°C with gentle agitation. This process forms typical multilamellar niosomes. Hydrating the lipid above phase transition temperature of surfactant and vortexing during hydration helps to reduce the size of vesicles prepared by handshaking method. Hand shaking method produce vesicles with greater diameter (0.35 -13 μm) compared to those prepared by ether injection method ((50 - 100μm).

II. Ether injection method

This method provides a means of making niosomes by slowly introducing solution of surfactants dissolved in diethyl ether into warm water maintained at 60°C. Typically the surfactant mixture is injected through a 14-gauge needle into an aqueous solution of the material to be encapsulated at 60°C. Vaporization of ether leads to formation of single layered vesicles. Depending on the conditions
used, the diameters of the resulting vesicles range from 50 to 1000 nm. This method produces unilamellar vesicles showing highest entrapment efficiency.

**III. Sonication**

The purpose of Sonication is to reduce the vesicle size. Increase in sonication time results in concomitant reduction in vesicle diameter. Multilamellar vesicles formed by thin film hydration method are sonicated either with probe sonicator or bath type sonicator. Probe sonication leads to more rapid size reduction. However, heat production, metal particle shedding from probe tip, and aerosol generation present problems. Temperature can be accurately regulated in bath type sonicator. Also for larger volume samples bath type sonicator is considered to be suitable. The finished product, i.e. vesicles are unilamellar in shape. Great care must be taken while working with a temperature sensitive solute. Also direct sonication of lipid mixture and aqueous phase can be carried out to obtain niosomes.

**IV. Micro-fluidization**

Micro-fluidization is the recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities (up to 1700 ft/sec) in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheets along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is greater uniformity, smaller size and better reproducibility in device scaleable to commercial production.

**V. Multiple membrane extrusion method**

The mixture of surfactant, cholesterol and dicetyl phosphate is formed into thin film by evaporation from chloroform. The film is hydrated with aqueous drug
solution and the resultant suspension extruded through two 0.1 μ polycarbonate membrane, in series, for up to 8 passages. Extrusion is a good method for controlling niosomes size and for reducing polydispersity. The study not only demonstrated effect of number of extrusion on vesicle size but also the effect of size on encapsulation of drug. It is found that using these method vesicles of mean size diameter 136 μm can be prepared.

**VI. Reverse phase evaporation technique (REV)**

In this method emulsion of aqueous phase in organic solvent containing lipid is prepared by sonication, followed by evaporation of organic solvent, resulting in a formation of vesicles. The lipid or surfactant forms a gel first and subsequently hydrates to form vesicles.

**VII. Trans-membrane pH gradient (inside acidic) drug update process/ Remote loading**

It is also called remote loading, in which multilamellar niosomes are prepared at acidic pH by hand shaking method then subjected to freeze-thaw cycle and later sonicated. Remote loading of drug is done by adding aqueous solution of drug, pH is adjusted to 7.0 - 7.2 and then mixture is heated. Niosomes so formed show greater entrapment efficiency and better retention of drug.

**VIII. Aqueous dispersions method**

The method is based on micro dispersion of surfactants in aqueous media containing solutes for encapsulation or entrapment. Continuous agitation under controlled condition leads to homogenous vesiculation. The dispersion may further be homogenized and ultra centrifuged.
1.2.6 Methods of separation of unentrapped drug [5,10]

It is essential to separate the drug, which has remained unentrapped after preparation of niosomes. This can be achieved following methods. Advantages and disadvantages of these methods are recorded in Table 1.3.

II. Separation by gel filtration (Sephadex G50) [6] (Uchegbu et al., 1994; Yoshioka et al., 1994).
III. Centrifugation (7000 x g for 30 min) [14]
IV. Ultracentrifugation (150000 x g for 1.5 h) [15]

Table 1.3: The advantages and disadvantages of the different methods for separation of the unentrapped drug.

<table>
<thead>
<tr>
<th>Method</th>
<th>Merits</th>
<th>Demerits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaustive dialysis</td>
<td>➢ Suitable for large vesicles &gt; 10 µm</td>
<td>➢ Extremely slow (5 – 24 h)</td>
</tr>
<tr>
<td></td>
<td>➢ Suitable for highly viscous systems</td>
<td>➢ Large volumes of dialysate required</td>
</tr>
<tr>
<td></td>
<td>➢ Inexpensive</td>
<td>➢ Dilutes the niosomes dispersion</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>➢ Quick</td>
<td>➢ Expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Dilutes the niosomes dispersion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Not suitable for highly viscous formulations.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>➢ Not suitable for formulations with a large</td>
</tr>
<tr>
<td></td>
<td></td>
<td>particle size (10 – 20 µm)</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>➢ Quick</td>
<td>➢ Fails to sediment the sub-micron niosomes.</td>
</tr>
<tr>
<td></td>
<td>➢ Inexpensive instrumentaion</td>
<td>➢ May lead to the destruction of fragile systems.</td>
</tr>
<tr>
<td></td>
<td>➢ Concentrates the niosomes dispersion</td>
<td></td>
</tr>
<tr>
<td>Ultracentrifugation</td>
<td>➢ Sediments all size</td>
<td>➢ Expensive instrumentation</td>
</tr>
<tr>
<td></td>
<td>➢ Concentrates niosome dispersion</td>
<td>➢ Long centrifugation times (1 – 1.5 h)</td>
</tr>
</tbody>
</table>
1.2.7 Characterization of niosomes [16]

Size shape and morphology

Vesicular structure of surfactant based vesicles has been visualized and established using freeze fracture electron microscopy while photon correlation spectroscopy could be successfully used to determine mean diameter of the vesicles. Electron microscopy can well be used for morphological studies of vesicles while master sizer based on laser beam is generally used to determine size distribution, mean surface diameter and size distribution of niosomes.

Entrapment efficiency

Niosomes with drug within the internal niosomal aqueous compartment are separated from the unentrapped drugs solution using the method of dialysis or by centrifugation.

- The difference between the amount of drug used for formulation and the amount dialyzed or obtained in supernatant gives the entrapment efficiency.

- Drug determination after complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 gives entrapment efficiency.

Release of drug from niosomes

A method of in-vitro release rate study reported recently, includes use of dialysis tubing. A dialysis sac is washed with distilled water and left soaking in distilled water. The vesicle suspension is pipetted in a bag made up of the tubing and sealed. The bag containing the vesicles is placed in 200 ml of buffer solution in a 250 ml of beaker with constant shaking at 25 °C or 37 °C. At various time intervals the buffer is analyzed for the drug content by appropriate assay method.
Stability/Leakage study

Stability of niosomes has been studied with respect to the amount of leakage/efflux of drug from the niosomes at:

a) 4 °C
b) Room Temperature (30 ± 2 °C)

The niosomes are stored in a suitable buffer and amount of drug effluxing is determined at various time intervals after Sephadex elution or dialysis.

1.2.8 Stability of niosomes

Physical stability

This aspect on niosomes involves preservation of the niosomes structure and entrapment; i.e. the stability of niosome size and shape and the barrier properties of the bilayers for the encapsulated or membrane bound molecules. Changes in the particle size distribution in colloidal systems occur mostly via two mechanisms: one on the molecular level that can be an asymmetrical molecular exchange, whereas at the particle level, that is mostly aggregation, flocculation and/or fusion.

Aggregation is the formation of larger units composed of individual liposomes. It is a reversible process i.e. by applying mild shear forces, changing temperature, or by binding metal ions that initially induce aggregation.

Fusion is an irreversible process and it leads to formation of new niosomal structures. In contrast to aggregation fusion of niosomes may induce drug leakage, when encapsulated drug is water-soluble and does not interact with bilayer. Very small niosomes (< 100 nm) are more prone to fusion than larger niosomes due to stress coming from the high curvature of their membrane and also because they have a large surface to volume ratio. Bilayer defects are induced during the phase transition if niosomes are stored above or below phase transition temperature range.
Drug leakage depends on bilayer composition and physicochemical nature of drugs. Large polar or ionic, water-soluble drugs will be retained much more effectively than low molecular weight, amphiphilic compounds. Charged drugs may interact with oppositely charged bilayers, which will increase the niosome encapsulation efficiency compared to those that do not interact with bilayers. Bilayer in gel state or those containing substantial fractions of cholesterol tend to lose the associated drug only slowly; whereas liquid state bilayers are more prone to drug loss and are less stable during storage.
1.3 Proniosomes

Simple addition of aqueous phase to a dry powder of the nonionic surfactant is an inefficient and irreproducible method of making hydrated niosomes. The material tends to clump together and extensive agitation or other treatment is required to disperse the aggregates into a niosomes suspension (17).

The traditional method for producing niosomes or liposomes involves drying the lipid to a thin film from organic solvent, and then hydrating this film with the aqueous solvent of choice (5,13). The resulting multilamellar vesicles can be further processed by sonication, extrusion, or other treatments to optimize drug entrapment.

For many years, one of the most common methods for producing niosomes was hydration of a film shell-dried in a round bottom flask. The hydration of dried films is thought to involve a process of swelling of "blebs" from the surface and shearing of these protrusions to produce multilamellar niosomes.

The principal benefit of this method is its simplicity, but there remain some disadvantages. Once the dried film has been prepared, it is not possible to make up any less than the entire sample. Second, it is usually necessary to agitate the sample for a prolonged period, up to one hour at elevated temperature, to obtain a uniform preparation and even after long hydration period, there is sometimes surfactant residue remaining on the wall of the flasks, and the additional time is required.

Other methods, such as injection of lipids in water-miscible or water-immiscible solvents into an aqueous solution, detergent dialysis, or reverse-phase evaporation are complicated by the need to remove certain components following liposome formation (13). All of these methods are time consuming, and many involve specialized equipment.

The thin film approach allows only for a predetermined lot size so material is often wasted if smaller quantities are required for a particular application or dose.
However, even though exhibit good chemical stability during storage, there may be problems of physical instability in niosome dispersions. Like liposomes, aqueous suspension of niosomes may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion. Various approaches can be used to minimize the instability associated with aqueous niosome dispersions.

First, a dry product, which could be hydrated immediately before use would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability could also be minimized. The additional convenience of the transportation, distribution, storage, and dosing would make ‘dry niosomes’ a promising industrial product (18-19).

The objective in developing proniosomes was to devise a method of producing a nonionic surfactant based dosage at the point of use to avoid problems of physical and chemical stability found in storage of some surfactant-based dosage forms. First, by creating a dry formulation, issues related to hydrolysis of the active ingredient or surfactants are avoided; by forming the suspension as needed, precipitation and aggregation are avoided. Although the sorbitol-based proniosomes (19) accomplished these objectives, the effect of the carrier on entrapment efficiency remained problematic. Second, the provesicular approach can be extended to the niosomes that exhibit superior stability as compared to liposomes. Liquid crystalline proniosome may be converted into the niosomes in situ by absorbing water from the skin. Figure 1.2 shows the diagrammatic representation of the sequence/mechanism of niosomes formation from proniosomes upon hydration.
Figure 1.2: Mechanism of niosomes formation from proniosomes
1.3.1 Materials used for the proniosomes preparation

The major components used for proniosomes are listed below:

- Carrier
- Non-ionic surfactant
- Lecithin
- Cholesterol

**Carrier**

Carrier is the most important component of the proniosomes preparation

**Sorbitol**

Rhodes et al, 1999 first reported the preparation of proniosomes by using a sorbitol as a carrier. However sorbitol exhibited some limitations as a carrier. It was observed that certain solutes were affected by the presence of dissolved sorbitol. It was difficult to coat sorbitol particles because sorbitol is soluble in chloroform and other organic solvents. If the surfactant solution was applied too quickly, the sorbitol particles would degrade and the sample became viscous slurry. However, making proniosomes with a reduced amount of sorbitol was a tedious process and began to compromise the advantages of proniosomes related to minimizing film thickness.

**Maltodextrin**

Rhodes et al, 2001 first reported the maltodextrin as a carrier alternative to sorbitol for the proniosomes preparation.

**Advantages of Maltodextrin as a carrier**

The use of maltodextrin as a carrier in the proniosome preparation permitted flexibility in the amounts of surfactant and other components, which greatly enhances the potential application of proniosomes in a scaled-up production environment.

Although maltodextrin is a polysaccharide, it has minimal solubility in organic solvents.
It is possible to coat the maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent. An analogous process with sorbitol results in a solid, surfactant/sorbitol cake. Because the maltodextrin particle morphology is preserved during proniosomes preparation, maltodextrin particles can be used for a significant gain in surface area. The higher surface area results in a thinner surfactant coating, which makes the rehydration process more efficient.

For drugs where maltodextrin is found to affect encapsulation efficiency, the maltodextrin can be minimized by producing proniosomes with greater surfactant loading.

1.3.2 Methods of proniosomes preparation

Mainly two different types of proniosomal systems have been reported

1. Dry granular proniosomes
2. Proniosomal gel (Provesicular form)

1. Dry granular proniosomes

Spraying method

Proniosomes are made by spraying surfactant in organic solvent onto Sorbitol powder and then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant loading has been achieved.

The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves. The resulting niosomes are very similar to those produced by conventional methods and the size distribution is more uniform. It is suggested that this formulation could provide a suitable method for formulating hydrophobic drugs in a lipid suspension without concerns over instability of the suspension or susceptibility of the active ingredient to hydrolysis.
➢ **Slurry method**

A required quantity of surfactant and drug dissolved in organic solvent is transferred to a round bottom flask containing maltodextrin to form the slurry. The flask is attached to a rotary flask evaporator and the organic solvent is evaporated under reduced pressure until a dry free flowing product is obtained.

2. **Proniosomal gel (Provesicular form)**

The surfactant, drug and alcohol are taken in a clean, dry wide mouth small glass tube and mixed. The open end of the glass tube and mixed. The open end of the glass tube is covered with a lid to prevent the loss of solvent from it and the tube is warmed on a water bath at 60-70 °C for 5 min, until the surfactants dissolve completely. The aqueous phase is added and warmed on a water bath till clear solution is formed which upon cooling converts to proniosomal gel. In case of formulations in which drug is not properly dissolved, the drug and surfactants are dissolved in chloroform or ether followed by vacuum evaporation of the solvent.

Principle: When the surfactant molecules are kept in contact with water, there are three ways the lipophilic chains of surfactant can be transformed into a disordered, liquid state called lyotropic liquid crystalline state (neat Phase). These are: increasing temperature to Kraft point, addition of solvent, which can dissolve lipids, use of both, temperature and solvent. Neat phase or lamellar phase contains bilayers arranged as sheets over one another within intervening aqueous layer. These types of structures give typical X-ray diffraction pattern and thread like birefringent structures are seen under polarized light microscope. For ternary lecithins, non-ionic surfactants as monoglycerides and alcohol systems, lamellar liquid crystals are formed at Kraft temperature in presence of alcohols. The lamellar liquid crystalline phase is converted to dispersion of niosomes at higher water concentrations. The organization of lipid / ethanol /water mixture into lamellar structures can be
conveniently utilized for transdermal delivery of drugs. This method avoids the use of pharmaceutically unacceptable solvents and is easy to scale-up.

1.4 Transdermal drug delivery systems

To maximize drug utilization, it is necessary to deliver the drug to the target tissue in the correct amount and at the proper time to elicit a desired response. Drug delivery to or via the skin is one of the drug delivery systems, which presents both unique opportunities and obstacles due to the skin structure, physiology and barrier properties.

The skin covers a total surface area of approximately 1.8 m² and provides the contact between the human body and its external environment [20]. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs. Transdermal drug delivery presents a number of advantages over classical drug delivery systems administered via other routes [6]:

1. Ease of self-administration.
2. Good patient compliance.
3. Avoidance of variations in gastro-intestinal absorption.
4. Bypass of the hepatic first pass metabolism.
5. Production of sustained and constant plasma concentration of drugs
6. Reduction in repeat dosing intervals
7. Reduction of potential adverse side effects
8. Removal of TDSDS provokes an immediate decrease of drug plasma level.
9. Substitute for oral or parenteral administration in certain clinical situations.
10. Adaptability to drug with a short half-life.

An understanding of transport behaviour of drugs is vital for designing an effective topical or transdermal product, as well as reasonably predicting and comparing drug behaviour in various formulations.
Transdermal drug delivery makes use of the skin as portal of entry of pharmacologically active molecules.

1.4.1 Routes of penetration

At the skin surface, molecules contact cellular debris, microorganisms, sebum and other materials, which negligibly affect permeation. The penetrant has three potential pathways to the viable tissue — through hair follicles associated with sebaceous glands, via sweat ducts, or across continuous stratum corneum between these appendages. Fractional appendageal area available for transport is only about 0.1%; this route usually contributes negligibly to steady state drug flux. The pathway may be important for ions and large polar molecules that struggle to cross intact stratum corneum. Appendages may also provide shunts, important at short times prior to steady state diffusion. Additionally, polymers and colloidal particles can target the follicle.

The intact stratum corneum thus provides the main barrier; its ‘brick and mortar’ structure is analogous to a wall. The corneocytes of hydrated keratin comprise the ‘bricks’, embedded in a ‘mortar’, composed of multiple lipid bilayers of ceramides, fatty acids, cholesterol and cholesterol esters. These bilayers form regions of semicrystalline, gel and liquid crystals domains. Most molecules penetrate through skin via this intercellular micro-route and therefore many enhancing techniques aim to disrupt or bypass its elegant molecular architecture. Viable layers may metabolise a drug, or activate a prodrug. The dermal papillary layer is so rich in capillaries that most penetrants clear within minutes. Usually, deeper dermal regions do not significantly influence absorption, although they may bind e.g. testosterone, inhibiting its systemic removal. Three main pathways postulated for the diffusion of solutes through the stratum corneum: transcellular, intercellular and transappendageal are shown in Figure 1.3.
Figure 1.3: Permeability of solute through the skin to the bloodstream or underlying tissues.

Some ways for circumventing the stratum corneum barrier are [21]:

- Drug/Vehicle interaction
- Stratum corneum modified (Chemical Enhancer/Hydration)
- Stratum corneum bypassed or removed (Microneedled array, Ablation, Follicular delivery)
- Electrically assisted method (Ultrasound, Iontophoresis, Electroporation, Magnetophoresis)
- Vesicles (Liposomes, Niosomes, Ethosomes, Transfersomes)

1.4.2 Vesicles as a transdermal and dermal delivery

Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more (multilamellar vesicles) concentric
bileayers. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions [22]. A wide variety of lipids and surfactants can be used to prepare vesicles. Most commonly, the vesicles are composed of phospholipids or non-ionic surfactants. These are referred to as liposomes and niosomes or nonionic surfactant vesicles, respectively. The composition of the vesicles influences their physicochemical characteristics such as, size, charge, thermodynamic phase, lamellarity and bilayer elasticity. These physicochemical characteristics have a significant effect on the behaviour of the vesicles and hence on their effectiveness as a drug delivery system. Figure 1.4 shows the routes of penetration of different types of vesicles entrapped with drug.

![Figure 1.4: Routes of penetration of vesicles as a carrier entrapped with drug](image)

The effectiveness of vesicles as transdermal drug delivery vehicles

The first papers to report on the effectiveness of vesicles for skin delivery were published in the early 1980s. The reported data, however, were conflicting. Mezei and Gulasekharom reported that liposomal encapsulation of triamcinolone acetonide
increased drug disposition in the epidermis and dermis [23-24]. Several other groups suggested a slower skin transport of highly polar compounds in vesicle formulations than in buffer solutions. The transport rate of lipophilic compounds was reported to be very similar to that of free drug solutions. Most groups concluded that liposomes did not act as transport system [25-28]. Conflicting results continued to be published concerning the effectiveness of vesicles, enhancing the controversy of vesicles as transdermal delivery vehicles. Several transport studies have reported that vesicles only enhanced the drug disposal in the skin, suggesting that vesicles are only useful for topical dermal delivery [29-33]. Others, however, have suggested that application of drugs in vesicles could lead to therapeutic drug concentrations in the systemic circulation and are suitable candidates for transdermal delivery [34-37].

Physico-chemical properties of vesicles

The following physicochemical properties of vesicles influence their performance:

Size: The size of the vesicles i.e. liposomes is shown to affect the skin penetration behavior of drug caffeine. Small vesicles exhibit lower skin permeation; higher accumulation in the skin, and a longer lag time before steady-state permeation is reached. Large vesicles show low skin permeation with low accumulation in the skin and a shorter lag time than from small vesicles [38].

Surface charge: Reports show that application of positively charged liposomes results in almost twice the amount of lipids deposited in deeper layers of the skin compared to application of the negatively charged liposomes. Net positive charge formulations may result in marked irritation [39].

Fluidity: The composition of the lipid bilayer is critically important in determining the pharmaceutical properties of liposomes through influences on membrane fluidity, permeability, surface properties, and stability. Membrane fluidity refers to the existence of thermal phase transitions in phospholipids aggregates. As temperature increases
These lipids move from a relatively ordered gel state to a more disordered fluid-like liquid crystalline state. In the gel state, vesicle membranes are more stable, less permeable to solutes and less likely to interact with destabilizing macromolecules than in the liquid crystalline state. Loading and leakage of drug from liposomes are affected at phase transition temperature (Tm). The maximum bilayer permeability occurs at the Tm [40]. In contrary to this finding, Ykomizo and Sagitani [41] suggest that phospholipids containing unsaturated fatty acids as lipophilic group (decrease in Tm value) are strong penetration enhancers for the percutaneous delivery of indomethacin.

**Percutaneous absorption promoters:** Percutaneous absorption promoters such as limonene and other hydrocarbon terpenes may distribute, in the presence of ethanol in the intercellular double-layered lipid membrane in the stratum corneum where cholesterol is present. It influences the membrane structure in the gel state on the basis of its affinity for cholesterol, enhances the fluidity of the membrane, and accordingly results in good absorption enhancement [42-45].

**The mode of action of vesicles**

Several mechanisms mediating the vesicle-skin interactions have been described in the literature. It has been suggested that vesicle-skin interactions can occur either at the skin surface or in the deeper layers of the stratum corneum. Hofland et al. and Abraham et al. have demonstrated adsorption and fusion of vesicles onto the skin surface, resulting in the formation of lamellae and rough structures on top of the outermost corneocytes [46-47]. Changes in the deeper layers of the stratum corneum were observed only after treatment of the skin with liquid-state liposomes and non-ionic surfactant vesicles. No ultrastructural changes in the skin were found when gel-state non-ionic surfactant vesicles were applied [47-48]. They explained their results by a molecularly dispersed penetration of lipid or surfactant into the intercellular matrix. Studies with thermal analysis that enable to detect lipid phase transitions confirmed this
mechanism. This suggests that components of liquid state vesicles can enter the deeper layers of the stratum corneum where they can modify the intercellular lipid lamellae, whereas the components of gel state vesicles remain on the skin surface. The superior mode of action of liquid-state vesicles for skin interactions is the most probable explanation for the fact that they are more effective in enhancing drug transport into and across the skin. This is in accordance with a study that found a correlation between the skin penetration and the fluidity of the vesicle bilayers determined by electron spin resonance [49]. However, it is still debatable whether vesicles can enter the stratum corneum as intact entities. In general, rigid liquid- and gel-state vesicles do not enter the stratum corneum as intact entities.

1.4.3 Niosomes as tools for transdermal drug delivery

Since 1987, the topical liposome products have been exploited by the cosmetic industry, with more than 100 liposome and non-ionic surfactant vesicle products in cosmetic market. Recently niosomes are gaining popularity in the field of topical and transdermal drug delivery because of their following characteristic features.

- Niosomes increase skin penetration of drugs.
- Niosomes can act as local depot for sustained release of dermally active compounds.
- It can serve as a solubilizing matrix for both hydrophilic and lipophilic types of drugs.
- When non-ionic surfactants are incorporated into niosomes they are much better tolerated by the skin than when they are present in an emulsion.

Niosomes appear to have application in topical and transdermal products both containing hydrophobic and hydrophilic drugs. Besides, niosomes have also been used
Many studies performed in the last decade showed significantly higher absorption rates as well as a greater local pharmacological effect for drugs applied to the skin entrapped in carrier system (liposomes, niosomes) as compared to conventional topical formulations.

Several mechanisms can be used to explain the ability of niosomes to modulate drug transfer across skin [5,51]

- Adsorption and fusion of niosomes onto the surface of skin leading to a high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drugs.

- The effect of vesicles as the penetration enhancer reduces barrier properties of stratum corneum. (As the surfactants are the component of niosomes, they increase transdermal permeation and percutaneous absorption by decreasing surface tension, improving, and wetting of skin and enhance the distribution of the drugs.)

- The lipid bi-layers of niosomes act as rat-limiting membrane barrier for the drugs.
1.5 Inflammation

Inflammation is a host defense mechanism in response to various infections or metabolic stimuli. The complexity and dynamics of the inflammatory process may be analyzed in several ways: acute versus chronic, immunogenic versus non-immunogenic and cellular versus humoral aspects. At a macroscopic level, the response usually is accompanied by the familiar clinical signs of erythema, edema, tenderness, and pain. Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms. [55]

i. An acute transient phase, characterized by local vasodilation and increased capillary permeability.

ii. A delayed, subacute phase, most prominently characterized by infiltration of leukocytes and phagocytic cells

iii. A chronic proliferative phase, in which tissue degeneration and fibrosis occur.

Many different mechanisms are involved in the inflammatory process.

An operational scheme of the generation and function of various humoral factors involved in inflammation is outlined in Figure 1.5 Four principal and interacting cascades involved are:

i. The complement system

ii. The plasmin system

iii. The clotting system (kinins)

iv. The arachidonic acid cascade (e.g. prostaglandins)

Various metabolites or factors derived from these cascades serve to amplify or regulated vascular permeability, cellular infiltration, and tissue injury. Vasoactive amines (histamine and serotonin), lysosomal hydrolases, and mediators elaborated from the activated lymphocytes (lymphokines) and other monocytes are also involved.
Figure 1.5: Four principal and interacting cascades (Humoral factors in acute inflammation)

The principal cellular events involved in both immune and non-immune inflammation are shown in Figure 1.6. The three-way interaction of macrophages, subsets of T-lymphocytes (e.g. T-suppressors, helpers, killers), and B-lymphocytes constitutes another dynamic system, which is mainly responsible for the pathological consequences in rheumatoid arthritis and other immunologic inflammatory diseases.

The superposition of these cellular and humoral events produces inflammation, pain and tissue destruction in a variety of diseases. Among them, a family of arthritic disorders is by far the most important area of drug application [56].
Acute Inflammation

Release of Mediators
- Kinins
- Histamine
- Serotonin
- SRS
- Clotting Factors

Local Vascular Response
- Increased Blood Flow
- Increased Vascular Permeability - Edema
- Cellular Infiltration - PMN, Macrophage

Immune Inflammation

Antigen

Antibodies
- Mast cell
- Histamine, Serotonin, Heparin

Immune complexes

Complement system

Monocyte

Macrophage

Kinins, Prostaglandins, etc.

Inflammation

Anaphylaxis

Destruction of antibody carrying cells

Immediate hypersensitivity

Delayed hypersensitivity

Lymphocyte

Activation

Macrophage

Multiplication

Lymphokines

Figure 1.6: Cellular events in acute and immune inflammation
1.5.1 Inflammatory diseases

Inflammatory diseases can be classified as

a) Rheumatic diseases related to arthritic disorders.
   - Rheumatoid arthritis
   - Gout
   - Systemic lupus erythematosus
   - Osteoarthritis
   - Ankylosing spondylitis (Bechterew's syndrome)

b) Dermatologic lesions

c) Ocular inflammation

d) Periodontal disease

e) Other prostaglandin-mediated disorders
   - Primary dysmenorrhoea
   - Ductus arteriosus
   - Miscellaneous

Among the inflammatory disease arthritis is the leading cause of physical disability in the world, afflicting about 50 million people and annually sending more than half to seek professional help in alleviating arthritic aches and pains. Of this number, arthritis forces about 10 million to restrict their normal activities in some way [57]. Together with the rheumatic diseases that affect soft tissues, arthritis encompasses more than 100 different conditions. Of these, the most widespread is degenerative joint disease (Osteoarthritis), which is most common among older people.

With increasing life expectancy, the number of people living long enough to suffer degenerative joint disease keeps growing. Rheumatoid arthritis, which may occur at any age, is one of the most destructive of the joint diseases. Of the rheumatic diseases, treatment has been most successfully developed for gout.
Rheumatic diseases are comprised of a large family of clinical syndromes with a common involvement of the joints, chiefly the synovial joints, and/or para-articular structures.

> Rheumatoid arthritis

Rheumatoid arthritis is a chronic multisystem disease of unknown cause. Rheumatoid arthritis is a common disease having peak incidence in 3rd and 4th decades of life, with 3-5 times higher preponderance in females. The condition has high association with HLA-DR4 and HLA-DR1 and familial aggregation. The onset of disease is insidious, beginning with prodrome of fatigue, weakness, joint stiffness, vague arthralgias and myalgias. This is followed pain and swelling of joints usually in symmetrical fashion, involving joints of hands, wrists and feet. Approximately 20% of patients develop rheumatoid nodules located over the extensor surfaces of the elbows and fingers. About 80% of cases are seropositive for rheumatoid factor (RF). Immunologic reactions appear to play a major role in the perpetuation of rheumatoid inflammations. Clinical evidence supporting this concept is as follows:

1. Extensive infiltration and proliferation of lymphocytes in the synovium.
2. Active local synthesis of IgG and RF
3. Presence of antigen-antibody complexes in synovial fluids and leukocytes

"RA Cells"

Etiopathogenesis

Present concept on etiology and pathogenesis proposes that RA occurs in an immunogenetically predisposed individual to the effect of microbial agents acting as trigger antigen. More recently, the role of superantigens which are produced by several microorganisms with capacity to bind to HLA-DR molecules (MHC-II region) has been proposed Figure 1.7 [58].
Genetic susceptibility
(MHC class II)

Antigenic stimulation

CD4+ T-cells

Cytokines
(TNF-A, INF-Y, IL-1)

Activate

B-cells

Anti-IgG antibody
(Rheumatoid factor)

Formation of immune complex, inflammatory cells, pannus

Inflammatory damage to synovium, small vessels, collagen

Destruction of cartilage, bone, fibrosis, ankylosis

Joint deformities

Endothelial cells

Release of
Adhesion molecules

Macrophages

Cytokines,
Proteases

Figure 1.7: Pathogenesis of rheumatoid arthritis.
Gout

Gout is a disorder of purine metabolism manifested by the following features, occurring singly or in combination:

i. Increased serum uric acid concentration

ii. Recurrent attacks of characteristic type of acute arthritis in which crystals of monosodium urate monohydrate may be demonstrable in the leucocytes present in the synovial fluid.

iii. Aggregated deposits of monosodium urate monohydrate in and around the joints of the extremities

iv. Renal disease involving interstitial tissue and blood vessels.

v. Uric acid nephrolithiasis.

Figure 1.8 shows the pathogenesis of gout. Clinically, the natural history of gout comprises four stages: asymptomatic hyperuricaemia, acute gouty arthritis, asymptomatic intervals of intercritical periods, and chronic tophaceous stage.
Figure 1.8: Pathogenesis of gout

- Systemic lupus erythematosus (SLE)
  It is a chronic inflammatory disease of unknown origin. It may affect many different organs with diverse manifestations, including fever, erythematous, rash, renal, neurological and cardiac abnormalities. The pathogenesis of SLE probably starts with an immunologic abnormality, possibly of viral origin. The
production of anti-DNA and anti-ribosomal antibodies leads to formation of immune complexes and complement fixation. The deposition of immune complexes in vessel walls and the basement membranes of glomeruli initiates a sequence of local inflammatory reactions analogous to those seen in the rheumatoid synovium: neutrophil infiltration, phagocytosis of immune complexes, release of Lysosomal enzymes, and damage of basement membrane. Amilial factors have also been implicated in pathogenesis [59].

> Osteoarthritis

Osteoarthritis is an extremely common degenerative joints diseases with a low degree of inflammation. It is characterized by progressive deterioration of articular cartilage and abnormal bone formation in the joint. Earlier histological studies and recent biochemical analysis have identified several possible pathogenic factors. Microcrystalline hydroxy apatite from faulty bone metabolism is an inflammatory substance. Lysosomal hydrolases are discharged by leukocytes and chondrocytes at the base of articular cartilage. Altered metabolic activities of chondrocytes e.g. DNA replication and proteoglycan biosynthesis, are indicated by an initial increase in the repair phase of cartilage, followed by a marked decline. Finally the defective repair mechanism is overwhelmed by the degradative process leading to joint destruction. An imbalance of nutritional factors and bone metabolism also contributes to the chronic degenerative disease. Much progress has been made in elucidating the structure and biochemical properties of connective tissue in recent years [60].
1.5.2 Nonsteroidal anti-inflammatory drugs

Treatment for arthritis has several goals: pain relief, reduction of stiffness, control of inflammation, maintenance of joint mobility, and prevention of deformity.

Very often, these goals require a combination of therapies, including drug therapy, a regimen of rest and exercise, physical therapy, the use of heat and cold, and, if indicated, surgical correction of deformed joints or their replacement with artificial ones.

Most patients can be treated non-surgically. There are medications that can relieve symptoms and slow disease progression. The medication recommended by the physician is based on the patient's medical condition, age, other drugs the patient currently takes, safety, cost and patient preference. Primary goal in the treatment of these diseases is relief from the pain and inflammation. Figure 1.9 showing the generation of arachidonic acid metabolites and their roles in inflammation [61]. Nonsteroidal anti-inflammatory drugs (NSAIDs) can relieve pain and inflammation.

Non-steroidal anti-inflammatory drug can be classified as:

**I Non-selective Cox Inhibitors:**

a. Salicylates and their congeners

b. Para-aminophenol derivatives: Phenacetin, Paracetamol

c. Pyrazolone derivatives: Phenyl butazone and related compound

d. Indoles and related drugs: Indomethacin, Sulindac

e. Heterocyclic arylacetic acid derivatives: Diclofenac, Ketorolac

f. Propionic acid derivatives: Ibuprofen, Fenoprofen, Ketoprofen

g. Fenamates: Flufenamic acid and mefenamic acid

h. Oxicams: Piroxicam

**II Selective Cox-2 Inhibitors:**

Nimesulide, Babumetone, Etodolac, Meloxicam, Celecoxib,
Figure 1.9: Generation of arachidonic acid metabolites and their roles in inflammation. 

Cell membrane phospholipids 

Steroids inhibit $\rightarrow X \rightarrow$ Phospholipases 

HETEs $\leftarrow$ HPETEs $\rightarrow$ lipooxygenases 

Arachidonic Acid $\rightarrow$ Cyclooxygenase $\leftarrow X$ COX-1 and COX-2 inhibitors 

5-Lipoxygenase 

5-HETE $\rightarrow$ 5-HPETE $\rightarrow$ 12-Lipoxygenase 

Leukotriene B4 $\rightarrow$ Leukotriene A4 (LTA4) $\rightarrow$ Leukotriene C4 (LTC4) $\rightarrow$ Leukotriene D4 (LTD4) $\rightarrow$ Leukotriene E4 (LTE4) 

Vasoconstriction, Bronchospasm, increased permeability 

Prostaglandin G2 (PGG2) $\rightarrow$ Prostaglandin H2 (PGH2) $\rightarrow$ Prostacyclin (PGI2) $\rightarrow$ Thromboxane A2 (TXA2) 

Vasodilation, inhibit platelet aggregation 

Lipoxin A4 (LXA4) $\rightarrow$ Lipoxin B4 (LXB4) $\rightarrow$ PGD2 $\rightarrow$ PGE2 $\rightarrow$ PGF2 

Vasodilation, inhibit neutrophil chemotaxis, stimulate monocytes adhesion 

Vasodilation, potentiate edema 

Figure 1.9: Generation of arachidonic acid metabolites and their roles in inflammation. COX = cyclooxygenase, HETE = Hydroxyeicosatetraenoic acid.
Mechanism of non-steroidal anti-inflammatory drugs. [55]

Though these drugs have different chemical structures, they produce qualitatively similar analgesic, antipyretic and anti-inflammatory effects. According to the current unifying concept of NSAID action, during inflammation, pain and fever, arachidonic acid is liberated from phospholipid fraction of the cell membrane. Arachidonic acid is then converted via cyclooxygenase (Cox-1 and -2) pathways to prostaglandins. The steps are (1) oxidation of arachidonic acid to the endoperoxide PGG$_2$ and (2) its subsequent reduction to the hydroxyendoperoxide PGH$_2$; the latter is then transformed into the primary prostanoids PGE$_2$, PGF$_2\alpha$, PGD$_2$, PGI$_2$ and TXA$_2$. Cox-1 and Cox-2 both use the same endogenous substrate arachidonic acid and form the same products by the same catalytic mechanism. Their major difference lies in the pathophysiological functions. COX-1 activity is constitutively present in nearly all cell types at a constant level and is involved in tissue homeostasis; whereas COX-2 activity is normally absent from cells (except those of kidneys and brain) but is inducible by bacterial liposaccharides IL2 and TNF in activated leucocytes and other inflammatory cells. Thus, COX-1 is physiological while COX-2 is usually pathological. Prostaglandins sensitize blood vessels to the effects of other inflammatory mediators that increase permeability. Pgs particularly PGE and PGI produce hyperalgesia associated with inflammation. They sensitize the chemical receptors of the afferent pain endings to other mediators such as bradykinin and histamine. Further, release of prostaglandins in the CNS may lower the threshold of the central pain circuits. Prostaglandins cause headache and vascular pain when infused IV and are also involved in the pyretic response in man.

The quantitative differences in the actions of different prostaglandins inhibitors and their propensity to cause adverse reactions may be explained by the differences in the sensitivities of COX in different tissues to the various NSAID. For example, the cyclooxygenase in the brain is much more sensitive to paracetamol than the...
cyclooxygenase in the blood vessels. Many NSAID non-selectively inhibit COX-1 and COX-2, while others act more selectively on COX-2. Thus, piroxicam and indomethacin are 10-40 fold selective for COX-1 whereas nabumetone is 15 fold selective for COX-2. Propionic acid derivatives like ibuprofen, fenamates, and aspirin inhibit COX-1 and COX-2 equally. Further, the anti-inflammatory drugs, which are weak prostaglandins inhibitors, appear to act by inhibiting the activation of T-lymphocytes, which are abundant in the inflamed tissues and release cytokines. The cytokines play an important role in mediating inflammation. Aspirin has both properties: prostaglandins synthesis inhibition and inhibition of T-lymphocyte activation and of their ability to release cytokines. NSAID may also unmask T cell suppressing activity, thereby suppressing the production of rheumatoid factors.

1.5.3 Experimental evaluation of anti-inflammatory activity

The three important aspects of inflammation that render themselves readily to measurement are erythema (local vasodilation), edema (increased capillary permeability) and formation of granulation tissue. Compounds claimed to possess anti-inflammatory activity can be evaluated either by their ability to reduce or more of these phenomena in experimentally induced inflammation or by testing their anti-inflammatory activity in experimental arthritis produced in animals.

The commonly employed methods are:

**Erythema assays:** irradiation of the shaven back skin of a guinea pig with ultra violet light causes erythema. Erythema can also be produced in human beings with certain specific irritants like tetrahydrofurylnicotinate. The anti-inflammatory property of new agent is assays by comparing its ability to reduce the erythema with that of a known anti-inflammatory drug.

**Edema assays:** Anti-inflammatory activity of a drug can also be measured by noting the reduction in edema produced by the local injection of substances like formaldehyde,
carrageenin, histamine, or dextran. A modification involves the measurement of leakage of a protein bound marker (Evans blue, $^{131}I$) from the circulation into the tissues; the results obtained however, vary considerably from laboratory to laboratory.

**Granuloma assays**: the 'cotton wool pellet' and 'granuloma pouch' are the most commonly used methods. The former involves SC implantation of weighed cotton wool pellets, impregnated with a foreign material like carrageenan, in rat. This causes localized inflammation. The animals are sacrificed after the drug treatment; the cottons pellets, now encapsulated and heavily infiltrated with connective tissue are removed, dried, weighed and compared with those in control animals not given the drug.

In the granuloma pouch assay, an irritant like croton oil diluted with cotton seed oil or air is injected SC in the rat, usually on its back. After drug treatment the animal is sacrificed, the pouch is dissected, its exudates content is measured and compared with that in control animals.
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