1.1. Vesicular drug delivery systems

Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence.

Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes.

Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.

Currently, vesicles as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering. Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduce of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and liophilic drugs. Different novel approaches used for delivering the drugs by vesicular system include liposomes, niosomes, sphinosomes, transferosomes and pharmacosomes.

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphillic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphillic building blocks. Biologic origin of these vesicles was first reported in 1965 by Bingham, and was given the name Bingham bodies. Drug carrier can be engineered to slowly degrade, react to stimuli and be site-specific. The ultimate aim is to control degradation of drug
and loss, prevention of harmful side effects and increase the availability of the drug at the disease site\textsuperscript{9}. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved\textsuperscript{10}. Lipid vesicles are one type of many experimental models of biomembranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of vesicular drug delivery systems. Vesicular drug delivery system has some of the advantages like:

I. Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.

II. Improves the bioavailability especially in the case of poorly soluble drugs

III. Both hydrophilic and lipophilic drugs can be incorporated

IV. Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems\textsuperscript{8}.

These vesicular systems are accompanied with some problems like drug carriers and externally triggered (eg., temperature, pH, or magnetic sensitive) carriers load drugs passively, which may lead to low drug loading efficiency and drug leakage in preparation, preservation and transport in vivo\textsuperscript{11}.

\textbf{Liposomes}

Liposomes or lipid based vesicles are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures\textsuperscript{12}. The assembly into closed bilayered structures is a spontaneous process and usually needs some input of energy in the form of physical agitation, sonication, heat, etc\textsuperscript{13}. Since lipid bilayered membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes.

But limitations associated with liposomes are High production cost, Leakage and fusion of encapsulated drug / molecules, Sometimes phospholipid undergoes oxidation and hydrolysis, Short half-life, Low solubility and less stability\textsuperscript{14}.
Niosomes

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. Although, they are structurally similar to liposomes. Limitation associated with niosomes are Physical instability in niosomal dispersion during storage occurs due to vesicles aggregations, fusion and leaking. This may leads to hydrolysis of encapsulated drugs which affects the shelf life of the dispersion.

Sphingosomes

Sphingosome may be defined as “concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid. The hydrolysis in liposomes may be avoided altogether by use of lipid which contains ether or amide linkage instead of ester linkage (such are found in sphingolipid). Thus sphingolipid are been nowadays used for the preparation of stable liposomes known as sphingosomes. Limitation of spingosomes are Higher cost of sphingolipid hinders the preparation and use of these vesicular systems and Low entrapment efficacy.

Pharmacosomes

They are the colloidal dispersions of drugs covalently bound to lipids. Depending upon the chemical structure of the drug–lipid complex they may exist as ultrafine vesicular, micellar, or hexagonal aggregates. As the system is formed by linking a drug (pharmakon) to a carrier (soma), they are termed as pharmacosomes. They are an effective tool to achieve desired therapeutic goals such as drug targeting and controlled release.

Limitation are synthesis of a compound depends upon its amphiphilic nature, required surface and bulk interaction of lipids with drugs, required covalent bonding to protect the leakage of drugs, pharmacosomes, on storage, undergo fusion and aggregation, as well chemical hydrolysis.
Transferosomes

Transferosomes were introduced for the effective transdermal delivery of number of low and high molecular weight drugs. Transferosomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties\textsuperscript{21}. It consist of both hydrophilic and hydrophobic properties, high deformability gives better penetration of intact vesicles\textsuperscript{22}. These vesicular transfersomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration. Transferosomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. There is provision for this, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transferosomes membrane is achieved by mixing suitable surface-active components in the proper ratios.

Limitation are chemically unstable because of their predisposition to oxidative degradation, purity of natural phospholipids is another criteria militating against adoption of transferosomes as drug delivery vehicles and transferosomes formulations are expensive\textsuperscript{23}.

Future Perspective of vesicular system

Vesicular drug delivery systems are emerging with the diverse application in Pharmaceutical, Cosmetics and food industries. Their delivery of drug directly to the site of infection is leading to reduction of drug toxicity with no adverse effects. It also reduces the cost of therapy by imparting better biopharmaceutical properties to the drug, resulting in improved bioavailability, especially in case of poorly soluble drugs. Now a day’s various non-steroidal anti inflammatory drugs, proteins, cardiovascular, antineoplastic, antiglucoma, antidiabetic drugs that are incorporated with vesicular system are available in a commercial market that are playing a vital role to cure from a disease, hence improving the health of human kinds. Some of the emerging vesicular drug delivery systems are listed below (Table 1.1).
Table 1.1: Emerging vesicular drug delivery systems

<table>
<thead>
<tr>
<th>Vesicular system</th>
<th>Description</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquasomes</td>
<td>Three layered self assembly composition with ceramics carbon nanocrystalline particular core coated with glassy celllobiose</td>
<td>Specific targeting, molecular shielding.</td>
</tr>
<tr>
<td>Cryptosomes</td>
<td>Lipid vesicles with a surface coat composed of PC and of suitable polyoxyethylene derivative of phosphotidyl ethanolamine</td>
<td>Ligand mediated drug targeting.</td>
</tr>
<tr>
<td>Discomes</td>
<td>Niosomes solubilized with non ionic surfactant solutions (polyoxyethylene cetyl ether class)</td>
<td>Ligand mediated drug targeting.</td>
</tr>
<tr>
<td>Emulsomes</td>
<td>Nanosize lipid particles (bioadhesives nanoemulsion) consisted of microscopic lipid assembly with apolar core</td>
<td>Parenteral delivery of poorly water soluble drugs.</td>
</tr>
<tr>
<td>Enzymosomes</td>
<td>Liposomal constructs engineered to provide a mini bioenvironmental in which enzymes are covalently immobilized or coupled to the surface of liposomes.</td>
<td>Targeted delivery to tumor cells</td>
</tr>
<tr>
<td>Ethosomes</td>
<td>Ethosomes are lipid “soft malleable vesicles” embodying a permeation enhancer and composed of phospholipid, ethanol and water</td>
<td>Targeted delivery to deep skin layer cells</td>
</tr>
<tr>
<td>Genosomes</td>
<td>Artificial macromolecular complexes for functional gene transfer. Cationic lipids are most suitable because they possess high biodegradability and stability in the blood stream.</td>
<td>Cell specific gene transfer</td>
</tr>
<tr>
<td>Photosomes</td>
<td>Photolysase encapsulated in liposomes, which release the content photo-trigged charges in membrane permeability characteristics.</td>
<td>Photodynamic therapy</td>
</tr>
<tr>
<td>Virosomes</td>
<td>Liposomes spiked with virus glycoprotein, incorporated into the liposomal bilayers based on retro viruses derived lipids.</td>
<td>Immunological adjuvants</td>
</tr>
<tr>
<td>Vesosomes</td>
<td>Nested bilayer compartment in vitro via the interdigested bilayer phase formed by adding ethanol to a variety of saturated phospholipids.</td>
<td>Multiple compartment of the vesosomes give better protection to the interior contents in serum</td>
</tr>
<tr>
<td>Proteosomes</td>
<td>High molecular weight multi-submit enzyme complexes with catalytic activity, which is specifically due to the assembly pattern of enzymes</td>
<td>Better catalytic activity turnover than non associated enzymes.</td>
</tr>
</tbody>
</table>
1.2. **Transdermal drug delivery system**

Transdermal drug delivery systems (TDDS) are dosage forms designed to deliver a therapeutically effective amount of drug across a patient’s skin. In order to deliver therapeutic agents through the human skin for systemic effects, the comprehensive morphological, biophysical and physicochemical properties of the skin are to be considered. Transdermal delivery provides a leading edge over injectables and oral routes by increasing patient compliance and avoiding first pass metabolism especially. Transdermal delivery represents an attractive alternative to oral delivery of drugs and is poised to provide an alternative to hypodermic injection too.  

The first Transdermal system, Transderm-SCOP was approved by FDA in 1979 for the prevention of nausea and vomiting associated with ravel, particularly by sea. The evidence of percutaneous drug absorption may be found through measurable blood levels of the drug, detectable excretion of the drug and its metabolites in the urine and through the clinical response of the patient to the administered drug therapy.

TDDS offer pharmacological advantages over the oral route and improved patient acceptability and compliance. As such, they have been an important area of pharmaceutical research and development over the last few decades.

Today, there are 19 transdermal delivery systems for such drugs as estradiol, fentanyl, lidocaine and testosterone; combination patches containing more than one drug for contraception and hormone replacement; and iontophoretic and ultrasonic delivery systems for analgesia.

Molecules greater than 500 Da normally do not cross the skin. This prevents epicutaneous delivery of the high molecular weight therapeutics as well as non-invasive transcutaneous immunization. There is considerable interest in the skin as a site of drug application both for local and systemic effect. However, the skin, in particular the stratum corneum, poses a formidable barrier to drug penetration thereby limiting topical and transdermal bioavailability. In addition, transdermal systems are non-invasive and can be self-administered. They can provide release for long periods of time (up to one week). They also improve patient compliance and the systems are generally inexpensive. Perhaps the greatest challenge for transdermal delivery is that only a limited number of drugs are amenable to administration by this route.
current delivery methods, successful transdermal drugs have molecular masses that are only up to a few hundred Daltons, exhibit octanol-water partition coefficients that heavily favor lipids and require doses of milligrams per day or less\textsuperscript{28}.

**Advantages of Transdermal Drug Delivery System (TDDS)**

The advantages of transdermal delivery over other delivery systems are as follows\textsuperscript{29}:

1. Avoidance of first pass metabolism of drugs.
2. Reduced plasma concentration levels of drugs, with decreased side effects.
3. Reduction of fluctuations in plasma levels of drugs, Utilization of drug candidates with short half-life and low therapeutic index.
4. Easy elimination of drug delivery in case of toxicity.
5. Reduction of dosing frequency an enhancement of patient compliance.
6. Transdermal medications deliver a steady infusion of a drug over and extended period of time. Adverse effects or therapeutic failure frequently associated with intermittent dosing can also be avoided.
7. Transdermal delivery can increase the therapeutic value of many drugs via avoiding specific problems associated with the drug. E.g. GI irritation, lower absorption, decomposition due to ‘hepatic first pass’ effect.
8. Due to above advantage, it is possible that an equivalent therapeutic effect can be elicited via transdermal drug input with a lower daily dose of the drug than is necessary, if e.g. the drug is given orally.
9. The simplified medication regimen leads to improved patient compliance and reduced inter and intra-patient variability.

**Limitation of TDDS**

The drug must have some desirable physicochemical properties for penetration through stratum corneum and if the drug dosage required for therapeutic value is more than 10 mg/day, the transdermal delivery will be very difficult if not impossible. Skin irritation or contact dermatitis due to the drug, excipients and enhancers of the drug used to increase percutaneous absorption is another limitation. Clinical need is another area that has to be examined carefully before a decision is made to develop a transdermal product. The barrier function of the skin changes from one site to another on the same person, from person to person and with age.
Limitations for a drug substance to be incorporated into a transdermal delivery system is:

- Heavy drugs molecules (>500 Da) usually difficult to penetrate the stratum cornea.
- Drugs with very low or high partition coefficient fail to reach blood circulation.
- Drugs that are highly melting can be given by this route due to their low solubility both in water and fat.
- Many approaches have been attempted to deliver medicament across skin barrier and enhance the efficacy.

Scientists previously believed that the skin was an effective barrier to inorganic particles. Damage from mechanical stressors was believed to be the only way to increase its permeability. Recently, however, simpler and more effective methods for increasing skin permeability have been developed. For example, ultraviolet radiation (UVR) has been used to slightly damage the surface of skin, causing a time-dependent defect allowing easier penetration of nanoparticles. Many approaches have been attempted to deliver medicament across skin barrier and enhance the efficacy. Other skin damaging methods used to increase nanoparticle penetration include tape stripping, skin abrasion, and chemical enhancement. Electroporation is the application of short pulses of electric fields on skin. Vesicles, particulate systems (liposome, niosome, transfersome, microemulsion, and solid lipid nanoparticle) has proven to increase skin permeability³⁰.

Transdermal drug absorption markedly alters drug kinetics and depends on a several parameters including the following-

- Medicament application site
- Thickness and integrity of the stratum corneum epidermis.
- Size of the molecule that is to be administered.
- Permeability of the membrane for the transdermal drug delivery.
- Hydration state of skin, Drug metabolism by skin flora.
- pH of the drug, Lipid solubility.
- Drug depot in skin
- Blood flow alteration in the skin by additives and body temperature
The toxic effect of the drug and problem in limiting drug uptake are major considerable potential for transdermal delivery systems, especially in children because skin thickness and blood flow in the skin usually vary with age. The increased blood supply in the skin along with thinner skin has significant effects on the pharmacokinetics of transdermal delivery for children. In some situations this may be an advantageous, while in others systemic toxicity may occur. This was observed after using scopolamine patches that are used to prevent motion sickness, a eutectic mixture of local anesthetics (EMLA) cream used to minimize the pain, corticosteroid cream applied for its local effect on skin maladies. Episodes of systemic toxic effects, including some fatalities in children have been documented with each of these, often secondary to accidental absorption through mucous membranes\(^3\)1.

From a global perspective, we propose that advances in transdermal delivery systems can be categorized as undergoing three generations of development from the first generation of systems that produced many of today’s patches by judicious selection of drugs that can cross the skin at therapeutic rates with little or no enhancement; through the second generation that has yielded additional advances for small molecule delivery by increasing skin permeability and driving forces for transdermal transport; to the third generation that will enable transdermal delivery of small molecule drugs, macromolecules (including proteins and DNA) and virus based/other vaccines through targeted permeabilization of the skin’s stratum corneum\(^3\)2.

**Skin structure**

The skin is the largest organ in the body; it protects against the influx of toxins and the efflux of water and is largely impermeable to the penetration of foreign molecules. Human skin consists of three main layers: the epidermis, dermis, and hypodermis (Figure 1.1).
The epidermis, in particular the stratum corneum, acts as the major barrier to drug absorption. The stratum corneum contains only 20% of water and is a highly lipophilic membrane; it is 10–20 mm thick depending on its state of hydration. The thickness of the epidermis varies from 0.06 mm on eyelids to 0.8 mm on the soles of the feet.

An applied drug must traverse these structural layers, encountering several lipophilic and hydrophilic domains on the way to the dermis where absorption into the systemic circulation is rapid due to the large capillary bed. Removing the stratum corneum speeds the diffusion of small water-soluble molecules into the systemic circulation by up to 1000 times\textsuperscript{33}. Alternatively, hydrophilic compounds can reach the dermis via shunt pathways such as hair follicles, sweat glands, nerve endings, and blood and lymph vessels. These routes contribute minimally to steady-state drug flux. The dermis is the thickest layer of the skin (3–5 mm) and possesses hair follicles, sweat glands, nerve endings, and blood and lymph vessels. It acts as the systemic absorption site for drugs.

There are variations between individuals in the rate at which drugs are absorbed via the skin due to factors such as thickness of the stratum corneum, skin hydration, underlying skin diseases or injuries, ethnic differences, and body temperature\textsuperscript{34}.
Routes of permeation through skin

The diffusant has two potential entry routes to the blood vasculature; through the epidermis itself or diffusion through shunt pathway, mainly hair follicles with their associated sebaceous glands and the sweat ducts. Therefore, there are two major routes of penetration.

- Transcorneal penetration
- Transappendegeal penetration

The viable tissue layer and the capillaries are relatively permeable and the peripheral circulation is sufficiently rapid. Hence diffusion through the stratum corneum is the rate-limiting step. The stratum corneum acts like a passive diffusion medium.

Overall, transdermal drug delivery offers compelling opportunities to address the low bioavailability of many oral drugs; the pain and inconvenience of injections; and the limited controlled release options of both. Building off the successes of first-generation transdermal patches, second-generation chemical enhancers and iontophoresis are expanding delivery capabilities for small molecules, whereas third-generation physical enhancers (including ultrasound, thermal ablation and microneedles) could enable transdermal delivery of macromolecules and vaccines.

These scientific and technological advances that enable targeted disruption of stratum corneum while protecting deeper tissues have brought the field to a new level of capabilities that position transdermal drug delivery for increasingly widespread impact on medicine.
1.3. **Transfersomes (Deformable liposomes)**

The term Transfersome and the underlying concept were introduced in 1991 by Gregor Cevc. In broadest sense, a Transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimising. This enables the Transfersome to cross various transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents\(^{39}\).

IDEA is a privately held biopharmaceutical company with headquarters in Munich, Germany. IDEA develops and commercializes noninvasive, targeted therapeutics, applied through the skin and/or nose. The proprietary carriers are typically applied on skin and can be engineered to achieve high drug concentration at or near the site of application, diminish local or systemic adverse side effects, and often increase drug potency. The Company's Transfersome ® carriers are topically applied on the skin and can be engineered to achieve high drug concentration at or near the site of application, increasing drug potency and diminishing side effects\(^{40}\).

Transfersomes were developed in order to take the advantage of phospholipids vesicles as transdermal drug carrier. These self-optimized aggregates, with the ultra flexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency. These vesicular transfersomes are several orders of magnitudes more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipid of the stratum corneum. There is provision for this, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transfersomes membrane is achieved by mixing suitable surface-active components in the proper ratios. The resulting flexibility of transfersome membrane minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under nonocclusive condition. Transfersomes can
penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayers properties\textsuperscript{41}.

**Mechanism of Penetration of Transfersomes**

Transfersomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm\textsuperscript{2} area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. The reason for this high flux rate is naturally occurring "transdermal osmotic gradients" i.e. another much more prominent gradient is available across the skin. This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75\% water content) and nearly completely dry stratum corneum, near to the skin surface (15\% water content). This gradient is very stable because ambient air is a perfect sink for the water molecule even when the transdermal water loss is unphysiologically high. All polar lipids attract some water this is due to the energetically favourable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration. Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration. So when lipid suspension (transfersomes) is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and try to escape complete drying by moving along this gradient. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes composed of surfactant have more suitable rheologic and hydration properties than that responsible for their greater deformability less deformable vesicles including standard liposomes are confined to the skin surface, where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility, so they can take full advantages of the transepidermal osmotic gradient (water concentration gradient).

The carrier aggregate is composed of at least one amphipathic (such as phosphatidylcholine), which in aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle. By addition of at-least one bilayer softening
component (such as a biocompatible surfactant or an amphiphile drug) lipid bilayer flexibility and permeability are greatly increased\textsuperscript{32-43}. The resulting, flexibility and permeability optimized, Transfersome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer (Figure 1.2).

![Figure 1.2: Mechanism of Transfersomes](image)

Vesicle can act as drug carrier systems, whereby intact vesicles enter the stratum corneum, carrying vesicle-bound drug molecule into skin, under the influence of naturally occurring in vivo transcutaneous hydration gradient.

Vesicle can act as penetration enhancers, whereby vesicle bilayer enter the stratum corneum and subsequently modify its intercellular lipids, hence, raising its fluidity.

Phospholipids have a high affinity for biological membranes, thus, the mixing of vesicle–phospholipid bilayer with the intercellular lipid layer of skin may also contribute to permeability enhancement of deformable vesicles.

**Salient features of transfersomes**

Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility as shown in Figure 1.3. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anaesthetic,
corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes. They have high entrapment efficiency, in case of lipophilic drug near to 90%. They protect the encapsulated drug from metabolic degradation. They act as depot, releasing their contents slowly and gradually. They can be used for both systemic as well as topical delivery of drug. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.44

Figure 1.3: Ultradeformable vesicle (Transfersomes)45

**Limitations of transfersomes**45-47

- Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
- Purity of natural phospholipids is another criteria militating against adoption of transfersomes as drug delivery vehicles.
- Transfersomes formulations are expensive.

**Transfersomes v/s other carrier systems**

Liposomal as well as niosomal systems are not suitable for transdermal delivery because of their poor skin permeability, breaking of vesicles, leakage of drug, aggregation, and fusion of vesicles.48 To overcome these problems, a new type of
carrier system called "transfersome" which is capable of transdermal delivery of low as well as high molecular weight drugs has recently been introduced\textsuperscript{49}. Transfersomes are especially optimized by ultradeformable (ultraflexible) lipid supramolecular aggregates which are able to penetrate the mammalian skin intact. Each transfersome consists of at least one inner aqueous compartment which is surrounded by a lipid bilayer with especially tailored properties due to the incorporation of "edge activators" into the vesicular membrane\textsuperscript{50-51}.

Surfactants such as sodium cholate, sodium deoxycholate, span 80, and Tween 80, have been used as edge activators\textsuperscript{52-54}. It was suggested that transfersomes could respond to external stress by rapid shape transformations requiring low energy\textsuperscript{55}. These novel carriers are applied in the form of semidilute suspension without occlusion. Due to their deformability, transfersomes are good candidates for the non-invasive delivery of small, medium, and large sized drugs. Multiliter quantities of sterile, well-defined transfersomes containing drug can be and have been prepared relatively easily.

The presence of surface-active agents in the transfersomes enhances the rheological properties and sensitivity to the driving force which results from water concentration gradient across the skin. This enhances the propensity of sufficiently large but deformable penetrant, transfersomes to move across the skin barrier. Such capability combined with the inclination to deform into elongated shapes while maintaining the vehicle integrity can explain the usually high efficiency of transfersomes across the skin.

At first glance, transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes. However in functional terms, transfersomes differ vastly from commonly used liposomes in that they are much more flexible and adaptable. On the contrary, mixed micelles stay confined to the topmost part of the stratum corneum even they are applied non occlusive\textsuperscript{56}.

Transfersomes differ in at least two basic features from the mixed micelles, first a transfersomes is normally by one to two orders of magnitude (in size) greater than standard lipid micelles. Secondly and more importantly, each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. To differentiate the penetration ability of all these carrier systems proposed the
distribution profiles of fluorescently labelled mixed lipid micelles, liposomes and transfersomes as measured by the Confocal Scanning Laser Microscopy (CSLM) in the intact murine skin. In all these vesicles the highly deformable transfersomes transverse the stratum corneum and enter into the viable epidermis in significant quantity.

**Formulation and preparation of Transfersomes**

Materials which are widely used in the formulation of Transfersomes are various phospholipids, surfactants, alcohol, dye, buffering agent etc different additives used in the formulation of Transfersomes are summarized in Table 1.2.

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids</td>
<td>Soya phosphatidyl choline</td>
<td>Vesicles forming component</td>
</tr>
<tr>
<td>Surfactant</td>
<td>Sodium cholate</td>
<td>For providing flexibility</td>
</tr>
<tr>
<td></td>
<td>Sodium deoxycholate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Span-80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tween-80</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>Methanol</td>
<td>As a solvent</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isopropyl alcohol</td>
<td></td>
</tr>
<tr>
<td>Buffering agent</td>
<td>Saline phosphate buffer (pH 7.4)</td>
<td>As a hydrating medium</td>
</tr>
</tbody>
</table>

Transfersomes can be prepared by same method for liposome. Reported two methods are used for manufacturing of deformable vesicles. Firstly, thin film hydration technique and secondly modified hand shaking method. Conventional rotary evaporation sonication method is also widely used to get uniform sized vesicles and homogeneous dispersion. All the methods of preparation of transfersomes are comprised of two steps. First, a thin film is prepared hydrated and then brought to the desired size by sonication; and secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane.
Chapter 1

Introduction

Characterization of Transfersomes

The characterization parameters of Transfersomes are as follows: 44, 46, 56, 58-60.

- Entrapment Efficiency
- Vesicle Diameter
- Number of Vesicle per Cubic Mm
- Confocal Scanning Laser Microscopy (CSLM) Study
- Degree of Deformability or Permeability Measurement
- Turbidity Measurement
- Surface Charge and Charge Density
- Penetration Ability
- Drug content
- Occlusion effect
- In-vitro drug release
- In-vitro Skin permeation Studies (Ex-Vivo drug release study)
- Physical stability

In Vivo Fate of Transfersomes & Kinetics of Transfersomes Penetration

After penetration of Transfersome through the outermost skin layers, transfersomes reach the deeper skin layer, the dermis. From this latter skin region they are normally washed out, via the lymph, into the blood circulation and through the latter throughout the body, if applied under suitable conditions. Transfersomes can thus reach all such body tissues that are accessible to the subcutaneously injected liposomes. The kinetics of action of an epicutaneously applied agent depends on the velocity of carrier penetration as well as on the speed of drug (re) distribution and the action after this passage. The most important single factors in this process are Carrier in-flow, Carrier accumulation at the targets site and Carrier elimination.

Kinetics of the transfersomes penetration through the intact skin is best studied in the direct biological assays in which vesicle associated drugs exert their action directly under the skin surface. Local analgesics are useful for this purpose, for determining the kinetics of penetration, various lidocaine loaded vesicles were left to dry out on the intact skin. Corresponding subcutaneous injection is used as control. The animal's sensitivity to pain at the treated site after each application was then measured as a
function of time. Dermally applied standard drug carrying liposomes or simple lidocaine solution have never caused any analgesic effect. It was necessary to inject such agent preparations to achieve significant pain suppression. In contrast to this, the lidocaine-loaded transfersomes were analgesically active even when applied dermally. Maximum analgesic effect with the latter type of drug application was typically observed 15 minutes after the drug application. A marked analgesic effect was still noticeable after very long time. The precise reach as well as kinetics of transfersomes penetration through the skin are affected by: drug carrier interaction, application condition or form, skin characteristics, applied dose.

**Applications of Transfersomes as Drug Carrier Systems**

Transfersomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Transfersomes can be used for:

- Delivery of Insulin
- Carrier For Interferons & Interlukin
- Carrier for Other Proteins & Peptides
- Peripheral Drug Targeting
- Transdermal Immunization
- Delivery of NSAIDS
- Delivery of steroidal hormones and peptides
- Delivery of Anesthetics
- Delivery of Anticancer Drugs
- Delivery of Herbal Drugs

**Future direction**

No drug delivery system has been perfected in a single step. Likewise, the Transfersome® technology is expected to evolve further. This relates to potential use of self-regulating, ultradeformable carriers in devices (patches; electrically controlled epicutaneous reservoirs), and in design of formulation with additional special features, allowing, e.g., targeting of cellular subsets. The nearest term goal that remains to be reached is expansion of the positive experiences with NSAID targeting into peripheral tissues to other drugs with similar therapeutic demands.
1.4. Autoimmune disorders, RA & DMARDs

Autoimmune disorders

The immune system is the body's means of protection against microorganisms and other "foreign" substances. It is composed of two major parts. One component, B lymphocytes, produces antibodies, proteins that attack "foreign" substances and cause them to be removed from the body; the other component consists of special white blood cells called T lymphocytes, which can attack "foreign" substances directly.

An autoimmune disorder is a condition that occurs when the immune system mistakenly attacks and destroys healthy body tissue. An individual’s immune system protects one from disease and infection. If a person has an autoimmune disease, their immune system inaccurately attacks healthy cells in their body. There are more than 80 different types of autoimmune disorders. Autoimmune disease is a condition which is triggered by the immune system initiating an attack on self-molecules due to the deterioration of immunologic tolerance to auto-reactive immune cells. Smith and Germolec state that “autoimmune disorders affect approximately 3% of the North American and European populations, >75% of those affected being women.” The initiation of attacks against the body’s self-molecules in autoimmune diseases, in most cases is unknown, but a number of studies suggest that they are strongly associated with factors such as genetics, infections and/or environment.

There are various symptoms and disorders which are encompassed in autoimmune diseases. They vary from organ specific to systemic, and include, insulin dependent diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, thyroiditis and multiple sclerosis to name but a few. The most common areas in the body which are targeted by autoimmune diseases are the thyroid gland, stomach, adrenal glands and pancreas. Systemic autoimmune diseases most commonly include the skin, joints and muscle tissue. It is widely accepted that the pathogenesis of autoimmune diseases is multifactorial, where the genetic, infectious and environmental factors play a role in determining the onset and progression of the disease. Despite this, the ability to quantify the environmental influences of autoimmune diseases is extremely difficult. Various evidences suggest that genetic factors are a major determinant of autoimmune disease susceptibility as well as progression. Different autoimmune diseases often co-exist within family members.
which points out, that common genes underlie multiple autoimmune diseases, and several diseases may share similar pathogenic pathways. This concept is additionally supported by various sets of evidence demonstrating variable prevalence degrees of autoimmune diseases in different geographical areas.\textsuperscript{69-70}

The development of autoimmune disease occurs as a result of an overactive immune response to body material and tissues present in the body. This means that the body attacks its own cells.\textsuperscript{71} The immune system confuses a specific part of the body as a pathogen and attacks it. Immunosuppression, which is a disease medication that decreases the immune response, is typically the treatment of an autoimmune disease.\textsuperscript{72}

There are various defense mechanisms that guard an individual from micro-organisms and potentially harmful material. Some of these mechanisms, such as physical barriers like the skin, phagocytic cells and certain chemical matter and enzymes, are active before contact with alien materials.\textsuperscript{73}

What causes the immune system to no longer tell the difference between healthy body tissues and antigens is unknown. One theory is that some microorganisms (such as bacteria) and drugs may trigger some of these changes, especially in people who have genes that make them more likely to get autoimmune disorders.\textsuperscript{74}

An autoimmune disorder may result in:

- The destruction of one or more types of body tissue
- Abnormal growth of an organ
- Changes in organ function

An autoimmune disorder may affect one or more organ or tissue types such as Red blood cells, Blood vessels, Connective tissues, Endocrine glands such as the thyroid or pancreas, Muscles, Joints, and Skin etc.

Some of the most common types of autoimmune disorders include Systemic Autoimmune Diseases & Localized Autoimmune Diseases. Rheumatoid arthritis is a systemic type autoimmune disease.
Symptoms of autoimmune disorders

Symptoms of an autoimmune disease vary widely and depend on the specific disease. A group of symptoms that occur with autoimmune diseases may include Dizziness, Fatigue, General ill-feeding and Low-grade fever.

Signs and tests for autoimmune disorders

The health care provider will perform a physical exam. Specific signs vary widely and depend on the specific disease. Tests that may be done to diagnose an autoimmune disorder are Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Treatment of autoimmune disorders

The goals of treatment are to reduce symptoms, control the autoimmune process, and maintain the body's ability to fight disease. Which treatments are used depends on the specific disease and your symptoms.

Some patients may need supplements to replace a hormone or vitamin that the body is lacking. Examples include thyroid supplements, vitamins, or insulin injections. If the autoimmune disorder affects the blood, you may need blood transfusions. People with autoimmune disorders that affect the bones, joints, or muscles may need help with movement or other functions.

Medicines are often prescribed to control or reduce the immune system's response. They are often called immunosuppressive medicines. Such medicines may include corticosteroids (such as prednisone) and nonsteroid drugs such as cyclophosphamide, azathioprine, or tacrolimus.

Rheumatoid Arthritis

Rheumatoid arthritis is a chronic, systemic inflammatory illness that may affect many tissues and organs, but primarily attacks synovial joints. The disease produces a surplus of synovial fluid. The pathology of the disease process often leads to the severe damage of articular cartilage and ankylosis of the joints. Rheumatoid arthritis can also generate diffuse inflammation in the lungs, pericardium, pleura, and sclera, and also nodular lesions, most frequent in subcutaneous tissue. The cause of rheumatoid arthritis is unknown, although autoimmunity plays a pivotal role in both its chronicity and progression.
Symptoms of RA

The main symptoms are pain and stiffness of affected joints (Figure 1.4). The immune system normally makes antibodies (small proteins) to attack bacteria, viruses, and other germs. It is not clear why this happens. In people with RA, antibodies are formed against the synovium (the tissue that surrounds joints). This causes inflammation in and around affected joints. Over time this can damage the joint, the cartilage, and parts of the bone near the joint. The most commonly affected joints are the small joints of the fingers, thumbs, wrists, feet, and ankles. RA can occur at any age, but is more common in middle age. Women get RA more often than men. Infection, genes, and hormone changes may be linked to the disease.

RA can occur at any age, but is more common in middle age. Women get RA more often than men. Infection, genes, and hormone changes may be linked to the disease.

Wrists, fingers, knees, feet, and ankles are the most commonly affected. The disease often begins slowly, usually with only minor joint pain, stiffness, and fatigue. Joint symptoms may include:

- Morning stiffness, which lasts more than 1 hour, is common. Joints may feel warm, tender, and stiff when not used for an hour.
- Joint pain is often felt on the same joint on both sides of the body.
- Over time, joints may lose their range of motion and may become deformed.

Other symptoms include Chest pain when taking a breath (pleurisy), Dry eyes and mouth (Sjogren syndrome), Eye burning, itching, and discharge, Nodules under the skin,
skin (usually a sign of more severe disease), Numbness, tingling, or burning in the hands and feet etc.

**Signs and tests of RA**

There is no test that can determine for sure whether you have RA. Most patients with RA will have some abnormal test results, although for some patients, all tests will be normal. Two lab tests that often help in the diagnosis are Rheumatoid factor test and Anti-CCP antibody test.

Other tests that may be done include Complete blood count, C-reactive protein, Erythrocyte sedimentation rate, Joint ultrasound or MRI, Joint x-rays, and Synovial fluid analysis.

**Treatment of RA**

RA usually requires lifelong treatment, including medications, physical therapy, exercise, education, and possibly surgery. Early, aggressive treatment for RA can delay joint destruction.

Medications for RA includes Disease modifying antirheumatic drugs (DMARDs), Anti-inflammatory medications, Antimalarial medications, Corticosteroids, Biologic agents, Surgery, and Physical therapy.

**Disease-Modifying AntiRheumatic Drugs (DMARDs)**

The drugs that work best for RA are called DMARDs. DMARD stands for Disease-Modifying AntiRheumatic Drug. These medicines don't just relieve pain. They slow or stop the changes in your joints. DMARDs drugs are the first drugs usually tried in patients with RA. They are prescribed in addition to rest, strengthening exercises, and anti-inflammatory drugs. Research shows that DMARDs work. They can slow down the disease and relieve pain. Table 1.3 shows list of DMARDs and its mechanism of action.
Table 1.3: Member drugs of DMARDs and its Mechanism

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adalimumab</td>
<td>TNF inhibitor</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Purine synthesis inhibitor</td>
</tr>
<tr>
<td>Chloroquine and hydroxychloroquine (antimalarials)</td>
<td>Suppression of IL-1 &amp; TNF-alpha, induce apoptosis of inflammatory cells and increase chemotactic factors</td>
</tr>
<tr>
<td>Ciclosporin (Cyclosporin A)</td>
<td>Calcineurin inhibitor</td>
</tr>
<tr>
<td>D-penicillamine</td>
<td>Reducing numbers of T-lymphocytes etc.</td>
</tr>
<tr>
<td>Etanercept</td>
<td>TNF inhibitor</td>
</tr>
<tr>
<td>Golimumab</td>
<td>TNF inhibitor</td>
</tr>
<tr>
<td>Gold salts (sodium aurothiomalate, auranofin)</td>
<td>Unknown - proposed mechanism: inhibits macrophage activation</td>
</tr>
<tr>
<td>Infliximab</td>
<td>TNF inhibitor</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Pyrimidine synthesis inhibitor</td>
</tr>
<tr>
<td>Methotrexide (MTX)</td>
<td>Antifolate</td>
</tr>
<tr>
<td>Minocycline</td>
<td>5-LO inhibitor</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Chimeric monoclonal antibody against CD20 on B-cell surface</td>
</tr>
<tr>
<td>Sulfasalazine (SSZ)</td>
<td>Suppression of IL-1 &amp; TNF-alpha, induce apoptosis of inflammatory cells and increase chemotactic factors</td>
</tr>
</tbody>
</table>

**Serious problems associated with DMARDs**

- Methotrexate (Rheumatrex®, Trexall®) can cause liver and kidney problems. It can also cause low red blood cell counts and painful mouth sores.
- Steroids like prednisone can weaken bones, raise blood sugar, and cause weight gain. That is why steroids are often prescribed in low doses and for a short time.
- DMARD shots can cause redness, itching, rash, and pain at the spot where the shot is given. More people taking anakinra (Kineret®) have these reactions than people taking other DMARD shots.
- About half of the people getting DMARDs by IV have a reaction. They get chills, dizzy, or sick to the stomach. But only about 2 out of 100 people stop their medicine because of reactions.
- It’s rare, but DMARDs given by IV can also cause a serious reaction, like a seizure.
Selection of regimen in RA

The choice of DMARD depends on a number of factors, including the stage and severity of the joint condition, the balance between possible side effects and expected benefits, and patient preference. Before treatment begins, the patient and clinician should discuss the benefits and risks of each type of therapy, including possible side effects and toxicities, dosing schedule, monitoring frequency, and expected results.

The most common DMARDs are methotrexate, sulfasalazine, hydroxychloroquine, and leflunomide. Less frequently used medications include gold salts, azathioprine, and cyclosporine.
1.5. Introduction of drugs

Two drugs from the class of DMARDs were used in this project work i.e. Methotrexate and Azathioprine. Physicochemical and biological properties of drugs are discussed here with.

*Methotrexate (meth" oh trex' ate)*

**Brand Names:** Rheumatrex Dose Pack, Trexall, Amethopterin, Abitrexate, Mexate, Methylaminopterin, Ledertrexate, Antifolan.

**Categories:**

MTX is used in several diseases and so it can be categorized as Antineoplastic Agent, *Antirheumatic Agent*, Antimetabolite, Enzyme Inhibitor, Folic Acid Antagonist, Dermatologic Agent, *Immunosuppressive Agent*, Nucleic Acid Synthesis Inhibitor, and Abortifacient Agent.

**Mechanism of action:**

The mechanism involved in its activity against rheumatoid arthritis is not known. For the treatment of rheumatoid arthritis, inhibition of DHFR (dihydrofolate reductase) is not thought to be the main mechanism, but rather multiple mechanisms appear to be involved including:

- The inhibition of enzymes involved in purine metabolism,
- Leading to accumulation of adenosine;
- Inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells;
- Increasing CD95 sensitivity of activated T cells;
- Inhibition of methyltransferase activity,
- Leading to (de)-activation of enzyme activity relevant to immune system function.
Molecular structure:\[\text{Empirical Formula}: \quad C_{20}H_{22}N_8O_5\]
\[\text{Molecular weight}: \quad 454.44\]
\[\text{Chemical Name}: \quad (2S)-2-[[4-[(2, 4-diamino pteridin-6-yl) methyl methylamino] benzoyl] amino] pentanedioic acid.\]

**Dosing frequency (Usual Adult Dose for Rheumatoid Arthritis):**

Single dose of MTX for RA is 7.5 mg orally weekly and divided dose is 2.5 mg orally every 12 hours for 3 doses once a week. Maximum weekly dose is only 20 mg of MTX in RA.

**Description:** Orange-brown or Yellow, crystalline powder

**Solubility:** 2600 mg/L in water

**BCS class:** Class IV drug (low solubility, low permeability)

**Indications:**

Methotrexate is indicated in the management of selected adults with severe, active, rheumatoid arthritis (ACR criteria), or children with active polyarticular-course juvenile rheumatoid arthritis, who have had an insufficient therapeutic response to, or are intolerant of, an adequate trial of first-line therapy including full dose non-steroidal anti-inflammatory agents (NSAIDs).

It is used as a treatment for some autoimmune diseases, including rheumatoid arthritis, juvenile dermatomyositis, psoriasis, psoriatic arthritis, lupus, sarcoidosis, Crohn's disease (although a recent review has raised the point that it is fairly underused in Crohn's disease) eczema and many forms of vasculitis. Because of its effectiveness, low-dose methotrexate is now first-line therapy for the treatment of rheumatoid arthritis. Although methotrexate for autoimmune diseases is taken in
lower doses than it is for cancer, side effects such as hair loss, nausea, headaches, and skin pigmentation are still common. X-rays also showed that the progress of the disease slowed or stopped in many people receiving methotrexate, with the progression being completely halted in about 30% of those receiving the drug. Those individuals with rheumatoid arthritis treated with methotrexate have been found to have a lower risk of cardiovascular events such as myocardial infarctions (heart attacks) and strokes.

**Pharmacokinetics**:<sup>83-86</sup>: 

- **Bioavailability**: 60% at lower doses, less at higher doses.  
- **Half life**: Low doses (<30 mg/m<sup>2</sup>): 3 to 10 hours; High doses: 8 to 15 hours.  
- **Log P value**: -1.85  
- **pKa value**: 4.7  
- **Protein binding**: 35-50% (parent drug), 91-93% (7 hydroxy-methotrexate)  
- **Time to peak plasma concentration (hours)**: 0.67 to 4  
- **Peak plasma concentration (mcg/ml)**: 0.03 to 1.40  
- **Volume of distribution (L/kg)**: 0.4 to 0.8  
- **Metabolism**: Hepatic and intracellular (oral)  
- **Excretion**: Urine (80-100%), faeces (small amounts)

**Side effects**:<sup>84,87</sup>: 

Methotrexate can cause serious or life-threatening side effects on your liver, lungs, or kidneys. Most serious side effects includes Dry cough, shortness of breath; Diarrhea, vomiting, white patches or sores inside your mouth or on your lips; Blood in your urine or stools; Swelling, rapid weight gain, little or no urinating; Seizure (convulsions); Fever, chills, body aches, flu symptoms; Pale skin, easy bruising, unusual bleeding, weakness, feeling light-headed or short of breath; Nausea, upper stomach pain, itching, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes); or Severe skin reaction -- fever, sore throat, swelling in your face or tongue, burning in your eyes, skin pain, followed by a red or purple skin rash that spreads (especially in the face or upper body) and causes blistering and peeling.
Common methotrexate side effects may be Vomiting, upset stomach; Headache, dizziness, tired feeling; or Blurred vision.

**Adverse effects**

The most common adverse effects include: ulcerative stomatitis, low white blood cell count and thus predisposition to infection, nausea, abdominal pain, fatigue, fever, dizziness, acute pneumonitis, rarely pulmonary fibrosis and kidney failure.

Central nervous system reactions to methotrexate have been reported, especially when given via the intrathecal route, which include myelopathies and leucoencephalopathies. It has a variety of cutaneous side effects, particularly when administered in high doses.

**Drug interactions**

Penicillins may decrease the elimination of methotrexate and thus increase the risk of toxicity. The aminoglycosides, neomycin and paromomycin have been found to reduce GI absorption of methotrexate. Probenecid inhibits methotrexate excretion, which increases the risk of methotrexate toxicity. Likewise retinoids and trimethoprim have been known to interact with methotrexate to produce additive hepatotoxicity and haematotoxicity, respectively. Other immunosuppressants like ciclosporin may potentiate methotrexate's haematologic effects, hence potentially leading to toxicity. NSAIDs have also been found to fatally interact with methotrexate in numerous case reports. Nitrous oxide potentiating the haematological toxicity of methotrexate has also been documented. Proton-pump inhibitors like omeprazole and the anticonvulsant valproate have been found to increase the plasma concentrations of methotrexate, as have nephrotoxic agents such as cisplatin, the GI drug, colestyramine and dantrolene. Caffeine may antagonise the effects methotrexate on rheumatoid arthritis by antagonising the receptors for adenosine.

**Stability**

Stable, but light sensitive and hygroscopic, Incompatible with strong acids, strong oxidizing agents. Store at -15°C or below.

**Decomposition**

When heated to decomposition it emits toxic fumes including nitrogen oxides.
Contraindications: Methotrexate can cause fetal death or teratogenic effects when administered to a pregnant woman. Methotrexate is contraindicated in pregnant women with psoriasis or rheumatoid arthritis and should be used in the treatment of neoplastic diseases only when the potential benefit outweighs the risk to the fetus. Women of childbearing potential should not be started on Methotrexate until pregnancy is excluded and should be fully counseled on the serious risk to the fetus should they become pregnant while undergoing treatment. Pregnancy should be avoided if either partner is receiving Methotrexate; during and for a minimum of three months after therapy for male patients, and during and for at least one ovulatory cycle after therapy for female patients.

Marketed products:
- Rheumatrex ⇒ Tablet USP 2.5 mg for oral administration
- Otrexup ⇒ 25 mg/ml Injection (subcutaneous)
- Abitrexate® ⇒ 2.5 mg/ml Injection (PF)
- MTX Gel ⇒ 0.25% and 1% topical gel

Azathioprine (ay" za thye' oh preen):


Categories: Azathioprine is categorized as Immunosuppressive agent.

Mechanism of action:
Azathioprine is used to suppress the immune system. It is used to treat patients who have undergone kidney transplantation and for diseases in which activity of the immune system is important. Azathioprine is a prodrug (a precursor of a drug) which is converted in the body to its active form called mercaptopurine (Purinethol). The exact mechanism of action of azathioprine is not known. It suppresses the proliferation of T and B lymphocytes, types of white blood cells that are part of the immune system and defend the body against both infectious diseases and foreign materials.
**Chapter 1**

**Molecular structure**:

![Molecular Structure Image]

**Empirical Formula**:

C₉H₇N₇O₂S

**Molecular weight**:

277.263 g/mol

**Chemical Name**:

6-(3-methyl-5-nitroimidazol-4-yl)sulfanyl-7H-purine.

**Dosing frequency (Usual Adult Dose for Rheumatoid Arthritis)**:

The initial dose for rheumatoid arthritis is approximately 50 to 100 mg given as a single dose or twice daily. This can be increased every 1-2 months, up to a maximum dose of approximately 75 to 150 mg given twice a day.

**Description**:

Pale yellow solid with a slightly bitter taste

**Solubility**:

It is practically insoluble in water and only slightly soluble in lipophilic solvents such as chloroform, ethanol and diethylether. Soluble in Dichloromethane. It dissolves in alkaline aqueous solutions, where it hydrolyzes to 6-mercaptopurine.

**BCS class**:

Class IV drug (low solubility, low permeability)

**Melting point**:

238–245 °C (460–473 °F)

**Indications**:

It is indicated as an adjunct for the prevention of rejection in renal homo transplantation. It is also indicated for the management of active rheumatoid arthritis to reduce signs and symptoms.

Aspirin, non-steroidal anti-inflammatory drugs and/or low dose glucocorticoids may be continued during treatment with AZT. The combined use of AZT with disease modifying anti-rheumatic drugs (DMARDs) has not been studied for either added...
benefit or unexpected adverse effects. The use of AZT with these agents cannot be recommended.

It is used to treat many inflammatory conditions, including rheumatoid arthritis, lupus, inflammatory muscle diseases (dermatomyositis and polymyositis), vasculitis, multiple sclerosis, myasthenia gravis, autoimmune hepatitis and inflammatory bowel disease. It also is used to prevent rejection of transplanted organs.

**Pharmacokinetics**

Bioavailability ⇒ 60±31%

Half life ⇒ 26–80 minutes (azathioprine), 3–5 hours (drug plus metabolites)

Log P value ⇒ 0.10

pKa value ⇒ 7.87 (at 25°C), 8.2

Protein binding ⇒ 20–30%

Time to peak plasma concentration (hours) ⇒ 1 to 2

Peak plasma concentration (mcg/ml) ⇒ 0.6

Volume of distribution (L/kg) ⇒ 1.54

Metabolism ⇒ Activated non-enzymatically, deactivated mainly by xanthine oxidase (oral)

Excretion ⇒ Renal, 98% as metabolites

**Side effects**

The most common serious side effects of azathioprine involve the cells of the blood and gastrointestinal system. Azathioprine can cause serious lowering of the white blood cell count, resulting in an increased risk of infections. This effect is reversed when the dose of azathioprine is reduced or temporarily discontinued.

Azathioprine can cause nausea, vomiting, and loss of appetite, which may resolve when the daily dose is reduced or divided and taken more than once a day.

Azathioprine can cause liver toxicity (for example, in less than 1% of rheumatoid arthritis patients). Less often, azathioprine may cause hepatitis (liver swelling or damage), pancreatitis (swelling or damage to the pancreas gland behind the stomach, which can cause abdominal pain) or an allergic reaction that may include a flu-like illness or a rash. All patients taking azathioprine require regular testing of blood for blood cell counts and liver tests to monitor for side effects of azathioprine.
Other side effects encountered less frequently include fatigue, hair loss, joint pains, abdominal pain and diarrhea.

**Adverse effects**\(^7\):

Nausea and vomiting are common adverse effects, especially at the beginning of a treatment. Such cases are met with taking azathioprine after meals or transient intravenous administration. Side effects that are probably hypersensitivity reactions include dizziness, diarrhea, fatigue, and skin rashes. Hair loss is often seen in transplant patients receiving the drug, but rarely occurs under other indications.

Because azathioprine suppresses the bone marrow, patients can develop anaemia and will be more susceptible to infection; regular monitoring of the blood count is recommended during treatment. Acute pancreatitis can also occur, especially in patients with Crohn's disease.

**Drug interactions**\(^5\):

Allopurinol (Zyloprim) that is used for treating increased blood levels of uric acid and preventing gout increases azathioprine levels in the blood which may increase the risk of side effects from azathioprine. Therefore, it is important to reduce the dose of azathioprine by approximately 1/3 to 1/4 in patients taking allopurinol.

The use of angiotensin-converting enzyme (ACE) inhibitors to control high blood pressure in patients taking azathioprine has been reported to induce anemia (low levels of red blood cells) and severe leukopenia (low levels of white blood cells).

Azathioprine reduces blood levels of the blood thinner, warfarin (Coumadin), and thus may reduce the blood thinning effect of warfarin.

**Storage temperature**\(^5\): 15-25\(^\circ\)C in a dry place and protected from light.

**Contraindications**\(^5\):

Azathioprine can cause birth defects. A 2003 population-based study in Denmark showed that the use of azathioprine and related mercaptopurine resulted in a seven-fold incidence of fetal abnormalities as well as a 20-fold increase in miscarriage. Birth defects in a child whose father was taking azathioprine have also been reported. Although no adequate and well-controlled studies have taken place in humans, when given to animals in doses equivalent to human dosages, teratogenesis was observed.
Transplant patients already on this drug should not discontinue on becoming pregnant. This contrasts with the later-developed drugs tacrolimus and mycophenolate, which are contraindicated during pregnancy.

Traditionally, as for all cytotoxic drugs, the manufacturer advises not to breastfeed whilst taking azathioprine. However, the "Lactation Risk Category" reported by Thomas Hale in his book "Medications and Mothers' Milk" lists azathioprine as "L3", termed "moderately safe".

Azathioprine should be used with caution in patients with medical history especially, liver disease, kidney disease, blood disorders, any infection or any allergy. Azathioprine is not recommended during pregnancy or lactation. Hepatic function should be carefully assessed in patients receiving azathioprine especially in patients with pre-existing hepatic disease. Determination of serum alkaline phosphatase, bilirubin and aminotransferase concentration should be performed periodically.

**Marketed products**[^1][^2][^3]:

- **Imuran** ⇒ Tablets 50 mg
- **Azasan** ⇒ 100 mg, 50 mg tablet
- **Apo-Azathioprine** ⇒ 50 mg Tablet
- **Azathioprine sod** ⇒ 100 mg vial
- **Mylan-Azathioprine** ⇒ 50 mg Tablet
- **Novo-Azathioprine** ⇒ 50 mg Tablet

[^1]: [Imuran](#) | [Tablets 50 mg](#)
[^2]: [Azasan](#) | [100 mg, 50 mg tablet](#)
[^3]: [Apo-Azathioprine](#) | [50 mg Tablet](#)
1.6. **Introduction of lipid, edge activators and polymers**

Commonly used excipients in the formulation of Transfersomes and transfersomal gel are lipid, edge activators and polymers respectively. These excipients will influence properties of vesicles. They are discussed here with. Lipid used in this project is PHOSPHOLIPON 90G. SPANS and TWEENS are used as edge activators. Carbopol 934 is used to develop gel formulation of transfersomal vesicles.

**Lipid**

The lipids are a large and diverse group of naturally occurring organic compounds that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology. They are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water\textsuperscript{103}.

**PHOSPHOLIPON 90 G\textsuperscript{104-109}**

**Description:** Purified phosphatidylcholine from soybean lecithin, well established Lipoid brand characterizes natural and hydrogenated lecithin fractions and phospholipids from soybean, eggs, milk, marine sources, cottonseed, rapeseed (canola) or sunflower.

**Molecular Structure:**

![Molecular Structure of PHOSPHOLIPON 90G](image)

**Molecular Formula:** $C_{42}H_{80}NO_{8}P$

**Molecular Weight:** 758.07

**Composition:** Phosphatidylcholine: [g/100 g] 94.0 – 102.0
Chapter 1

Purity:

Lysophosphatidylcholine ⇒ [g/100 g] n.m.t. 4.0
Peroxide value ⇒ n.m.t. 5.0
Acid value ⇒ n.m.t. 0.5
Toluene insoluble ⇒ [g/100 g] n.m.t. 0.05
Water ⇒ [g/100 g] n.m.t. 1.5
Non-polar lipids ⇒ [g/100 g] n.m.t. 3.0
Tocopherol ⇒ [g/100 g] n.m.t. 0.3
Heavy metals ⇒ [mg/kg] n.m.t. 10
Residual solvents Ethanol ⇒ [g/100 g] n.m.t. 0.2

n.m.t. = not more than
n.l.t. = not lower than

Physical Properties:

Colour ⇒ Yellowish
Consistency ⇒ Waxy solid

Bacteriological Data:

Total aerobic microbial count (TAMC) ⇒ [/g] n.m.t. 102
Total combined yeasts & moulds count (TYMC) ⇒ [/g] n.m.t. 101
Bile-tolerant gram-negative bacteria ⇒ [/g] absent
Pseudomonas aeruginosa ⇒ [/g] absent
Staphylococcus aureus ⇒ [/g] absent
Salmonella ⇒ [/10g] absent

Ingredients: Purified phosphatidylcholine from soybean lecithin, DL-α-tocopherol, ascorbyl palmitate

Packaging: welded in PE and PE-coated aluminium foil

Regulatory status: Lecithin (Phospholipid) is approved by the United States Food and Drug Administration for human consumption with the status "generally recognized as safe" (GRAS). Lecithin is admitted by the EU as a food additive, designated as E322.

Maximum permissible limit: 425-550 mg/day (orally)
Storage:

Recommended storage: Under dry condition, at max. $8^0\text{C}$, sealed under inert gas. Storage at $-20^0\text{C}$ further improves the shelf life. To avoid the negative impact on product quality by humidity, a frozen product unit must not be opened without conditioning of the package to ambient temperatures.

Possible Application:

- Phosphatidylcholine source for drugs and dietetics
- Solubility enhancement of actives, syrups, tonics,
- Solubilizer for parenteral administration forms,
- For pharmaceutical oral or topical applications
- Emulsifier for pharmacy, dermatology and cosmetics

Phospholipids in Pharmaceutical Applications:

Phospholipids (PL) are amphiphilic molecules and are an integral part of the membrane of any living cell. They are indispensable to life and are involved in the metabolism and respiration of the cells. Due to their functional properties, phospholipids are ideal additives in pharmaceutical applications that offer excellent opportunities to the developers of pharmaceutical formulations.

Drug delivery systems are one important application field. The application of following systems makes it possible to impact on the release, absorption, bioavailability and efficacy of drug and vital substances. Typical drug delivery systems are:

- Liposomes
- Emulsions
- Solid Lipid Nanoparticles (SLN)
- Mixed Micelles
- Suspensions
- Actives coated with phospholipids

pH Level: 5 to 8.
**Side effects:** The most important side effects with phospholipid are as below:

1. Gastrointestinal problems like diarrhea
2. Changes in weight (loss and gain)
3. Loss of appetite
4. Skin rashes
5. Nausea, dizziness, vomiting and confusion
6. Low blood pressure (which is just as dangerous as high blood pressure)
7. Blurred vision and occasional fainting

**Edge activators**

Edge activators are bilayer softening component, such as biocompatible surfactant in to which an amphiphilic drug is added to increase lipid bilayer flexibility and permeability. Surfactant proportionally increases the fluidity of the membrane and enhances the penetration through the skin\textsuperscript{110}.

Surfactants are amphipathic molecule that consist of a nonpolar hydrophobic portion usually a straight or branched hydro carbon or fluorocarbon chain containing 8-18 carbon atoms, which is attached to hydrophilic portion. The hydrophilic portion can be ionic, non-ionic, or zwitter-ionic\textsuperscript{110}.

The main component of the vesicular system is the phospholipid bilayer, having the similar lipid composition as that of the skin. Edge activators are mainly incorporated in the lipid bilayer. An edge activator is often a single chain surfactant that destabilizes the lipid bilayer of the vesicles and increases the deformability of the bilayer by lowering its interfacial tension\textsuperscript{110}.

**Interaction of edge activators with lipid bilayer:**

HLB gives the surfactant affinity for water and lipid. HLB of tween 80, span 80 are 4.3, 15 respectively. The molar ratio of surfactant to lipid should be determined for considering the distribution of surfactant between lipid and aqueous components of vesicle\textsuperscript{110}.

Surfactant concentrates and interacts with skin by reducing the interfacial tension. They can bind with the cell / cell membrane and can change the porosity/permeability characteristic of cell membrane. Surfactant can penetrate to the deeper corneal regions
of the skin by diffusion. After diffusion the surfactant starts denaturation of the protein and finally the stratum corneum swells. The surfactant concentration can be increased up to CMC. The surfactants, anionic/cationic can interact with the protein by ionic bonds, while nonionic surfactants bind via hydrophobic interaction.  

**Criteria for selecting surfactants as permeation enhancer:**

Surfactants use their unique property of reducing the interfacial tension, for enhancing the skin permeation. The permeation capacity of surfactant depends solely on its affinity to bind with the polar or non-polar portion of the lipid bilayer.  

- The surfactant with relatively shorter alkyl chain forms vesicle.
- As the carbon chain length of surfactant is lowered, entrapment efficiency may become higher.
- Enhancers containing the ethylene oxide chain length of 2-5, HLB value of 7-9 and an alkyl chain length of C16-C18 were effective flux promoters.
- An increase in ethylene oxide chain can contribute to its enhanced flux.
- HLB of sodium oleate, tween 80, span 80 were 18, 15, and 4.3 respectively. HLB of surfactant relates its polarity in order to bind with the skin. The HLB value 4.3 indicates more lipophilic nature than surfactants having higher HLB value 15, thus can encapsulate lipophilic drug more efficiently.

**Impact of edge activators in vesicular drug delivery:**

These systems have several other advantages:

- They can encapsulate both hydrophilic and lipophilic moieties,
- Prolong half-life of drug by increasing duration of systemic circulation due to encapsulation,
- Ability to target organs for drug delivery,
- Increases the
- Deformability of bilayer by lowering the interfacial tension,
- Biodegradability,
- Better permeability and
- Lack of toxicity.
SPANS

Sorbitan is a mixture of isomeric organic compounds derived from the dehydration of sorbitol. Sorbitan is primarily used in the production of surfactants such as polysorbates. Sorbitan is an intermediate in the conversion of sorbitol to isosorbide\(^{116}\).

Sorbitan esters (also known as \textbf{Spans}) are lipophilic nonionic surfactants that are used as emulsifying agents in the preparation of emulsions, creams, and ointments for pharmaceutical and cosmetic use. Sorbitan esters are also used as emulsifiers and stabilisers in food\(^{117}\).

\textit{Sorbitan monoesters}\(^{118-119}\)

\textbf{Chemical structure:}

![Chemical structure of sorbitan monoester]

\textbf{Description:} It is an ester of sorbitan (a sorbitol derivative) and stearic acid and is sometimes referred to as a synthetic wax.

\textbf{IUPAC name:} Octadecanoic acid \([2-\{(2R,3S,4R)-3,4\text{-dihydroxy-2-tetrahydrofuranyl}\}-2\text{-hydroxyethyl}]\) ester

\textbf{Molecular formula:} C\(_{24}\)H\(_{46}\)O\(_6\)

\textbf{Molar mass:} 430.62 g/mol

\textit{Sorbitan Esters of Fatty Acids}

Sorbitan monoesters of palmitic, stearic, oleic, lauric acids and triesters of stearic acid are as follows:

- Sorbitan monolaurate ⇒ Span 20
- Sorbitan monopalmitate ⇒ Span 40
- Sorbitan monostearate ⇒ Span 60
- Sorbitan monooleate ⇒ Span 80
- Sorbitan tristearate ⇒ Span 85
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**Span 20 (Sorbitan monolaurate)** \(^{120-124}\)

**Category:** Non-ionic surface active agent

**Chemical structure:**

![Chemical structure of Span 20](image)

**IUPAC name:** Dodecanoic acid \([2-[(2R,3R,4S)-3,4-dihydroxy-2-tetrahydrofuranyl] 2-hydroxyethyl] ester\)

**Molecular formula:** \(C_{18}H_{34}O_6\)

**Molar mass:** 346.46 g/mol

**Description:** A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible lauric acid. Amber-coloured oily viscous liquid, light cream to tan beads or flakes or a hard, waxy solid with a slight odour

**Density:** 1.032 g/cm\(^3\)

**Solubility:** insoluble in water, but dispersible in hot and cold water.

**HLB value:** 8.6

**Chemical Properties:** Liquid

**Acute toxicity:** LD50 Oral - rat - 33.600 mg/kg

**Usage:** used as a lubricant, stabilizer and as an emulsifier.

**Storage:** Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

**Span 80 (Sorbitan monooleate)** \(^{119,124-126}\)

**Category:** Non-ionic surface active agent
Chemical structure:

Molecular formula: $C_{24}H_{44}O_6$

Molar mass: 428.60

Description: A mixture of the partial esters of sorbitol and its mono- and dianhydrides with edible oleic acid. Amber-coloured oily viscous liquid, light cream to tan beads or flakes or a hard, waxy solid with a slight odour.

Density: 0.986 g/mL at 25 °C (lit.)

Solubility: Soluble at temperatures above its melting point in ethanol, ether, ethylacetate, aniline, toluene, dioxane, petroleum ether and carbon tetrachloride; insoluble in cold water, dispersible in warm water.

Flash point: >113 °C

HLB value: 4.3

Usage: Emulsifier, stabilizer, used in food products and oral pharmaceuticals.

Storage: Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

TWEENS

Tweens are polyethoxylated sorbitan esters. In simple terms, they are ethoxylated spans. Tweens are hydrophilic in nature.

Polyoxyethylene Sorbitan Esters Fatty Acids

Polyoxyethylene (20) sorbitan monoesters of lauric, oleic, palmitic and stearic acid and triester of stearic acid are as below:

- polyoxyethylene (20) sorbitan monolaurate $\Rightarrow$ Tween 20
- polyoxyethylene (20) sorbitan monopalmitate $\Rightarrow$ Tween 40
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on

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tyethylene (20) sorbitan monostearate ⇒ Tween 60
polyoxyethylene (20) sorbitan tristearate ⇒ Tween 65
polyoxyethylene (20) sorbitan monooleate ⇒ Tween 80

**Tween 20 (polyoxyethylene (20) sorbitan monolaurate)** \(^{119,127-128}\)

Polysorbate 20 (common commercial brand names include Alkst TW 20 and Tween 20) is a polysorbate surfactant whose stability and relative non-toxicity allows it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. It is a polyoxyethylene derivative of sorbitan monolaurate, and is distinguished from the other members in the polysorbate range by the length of the polyoxyethylene chain and the fatty acid ester moiety. The commercial product contains a range of chemical species\(^{128}\).

**Category:** Non-ionic surface active agent

**Chemical structure:**

![Chemical structure of Polyoxyethylene (20) sorbitan monolaurate](image)

**IUPAC name:** Polyoxyethylene (20) sorbitan monolaurate

**Molecular formula:** \(C_{58}H_{114}O_{26}\)

**Molar mass:** 1227.54 g/mol

**Description:** Clear, yellow to yellow-green viscous liquid.

**Density:** 1.1 g/mL (approximate)

**Boiling point:** > 100 °C

**Flash point:** 110 °C (230 °F; 383 K)

**pH:** 7

**HLB value:** 16.7. A high HLB number like 16.7 indicates that the surfactant will travel into the water phase.
Use: as an excipient in pharmaceutical applications to stabilize emulsions and suspensions.

Storage: Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

**Tween 80 (polyoxyethylene (20) sorbitan monooleate)**\(^{119,129-130}\)

Polysorbate 80 (Tween 80) is a nonionic surfactant and emulsifier often used in foods and cosmetics. This synthetic compound is a viscous, water-soluble yellow liquid\(^{130}\). Polysorbate 80 is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups, which are polymers of ethylene oxide.

**Chemical structure:**

![Chemical structure of Tween 80](image)

**IUPAC name:** Polyoxyethylene (20) sorbitan monooleate

**Molecular formula:** \(C_{64}H_{124}O_{26}\)

**Molar mass:** 1310 g/mol

**Description:** Amber colored viscous liquid

**Density:** 1.06–1.09 g/mL, oily liquid

**Boiling point:** > 100 °C

**pH:** 7

**Solubility:** Very soluble in water, soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

**Viscosity:** 300–500 centistokes (@25°C)

**Flash point:** 113 °C (235 °F; 386 K)
HLB value: 15.0. A high HLB number like 15.0 indicates that the surfactant will travel into the water phase.

Use: used as a surfactant in soaps and cosmetics, or a solubilizer such as in a mouthwash.

Storage: Store in cool place. Keep container tightly closed in a dry and well-ventilated place.

Span & Tween

Both are considered a non-ionic surface active agent. They are listed in the USP/NF, BP, EP, etc., as an approved pharmaceutical excipient for use in oral preparations.

Tween 80 and Span 80 are approved for use in specific food products and are generally recognized as safe (GRAS). They are well tolerated upon oral administration and are practically non irritating, possessing very low toxicity potential. They are generally regarded as non-toxic and non-irritating.

Tween 20 is 20 mole ethoxylate of sorbitan monolaurate and Tween 80 is 20 mole ethoxylate of sorbitan mono oleate. Only difference is in hydrophobe. Tween 20 has lauric acid and Tween 80 has oleic acid in hydrophobe. Both are nonionic surfactant. They are used as solubliser, O/W emulsifier and detergent for shampoos.

Polymers

CARBOPOL

Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are produced from primary polymer particles of about 0.2 to 6.0 micron average diameter. The flocculated agglomerates cannot be broken into the ultimate particles when produced. Each particle can be viewed as a network structure of polymer chains interconnected via cross-linking.

Carbomers readily absorb water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and it’s essentially insolubility in water makes Carbopol a potential candidate for use in controlled release drug delivery system.

Carbopol polymers are offered as fluffy, white, dry powders (100% effective). The carboxyl groups provided by the acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol polymers have an average
equivalent weight of 76 per carboxyl group. The general structure can be illustrated below:\(^{133}\):

![Carbopol Structure Diagram]

Carbopol polymers are manufactured by cross-linking process. Depending upon the degree of cross-linking and manufacturing conditions, various grades of Carbopol are available. Each grade is having its significance for its usefulness in pharmaceutical dosage forms.

Carbopol 934 P is cross-linked with allyl sucrose and is polymerized in solvent benzene. Carbopol 71G, 971 P, 974 P are cross-linked with allyl penta erythritol and polymerized in ethyl acetate. Polycarbophil is cross-linked polymer in divinyl glycol and polymerized in solvent benzene. All the polymers fabricated in ethyl acetate are neutralized by 1-3\% potassium hydroxide. Though Carbopol 971 P and Carbopol 974 P are manufactured by same process under similar conditions, the difference in them is that Carbopol 971 P has slightly lower level of cross-linking agent than Carbopol 974 P. Carbopol 71 G is the granular form Carbopol grade.

**Physical Properties\(^{133}\)**

The three dimensional nature of these polymers confers some unique characteristics, such as biological inertness, not found in similar linear polymers. The Carbopol resins are hydrophilic substances that are not soluble in water. Rather, these polymers swell when dispersed in water forming a colloidal, mucilage-like dispersion.

Carbopol polymers are bearing very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Table 1.4 describes physical and chemical Properties of Carbopol.
Table 1.4: Physical and Chemical Properties of Carbopol

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Fluffy, white, mildly acidic polymer</td>
</tr>
<tr>
<td>Bulk density</td>
<td>Approximately 176-208 kg/m³ (13 lbs/Ft³) *</td>
</tr>
<tr>
<td>Chemical description</td>
<td>High molecular weight, non-linear polyacrylic acid cross linked with polyalkenyl polyether</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.41</td>
</tr>
<tr>
<td>Particle size</td>
<td>2-7 microns</td>
</tr>
<tr>
<td>Moisture content</td>
<td>2.0% maximum</td>
</tr>
<tr>
<td>Equilibrium moisture content</td>
<td>8-10% (at 50% relative humidity)</td>
</tr>
<tr>
<td>Pka</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>pH of 1.0% water dispersion</td>
<td>2.5 - 3.0</td>
</tr>
<tr>
<td>pH of 0.5% water dispersion</td>
<td>2.7 - 3.5</td>
</tr>
<tr>
<td>Equivalent weight</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>Ash content</td>
<td>0.009 ppm (average) **</td>
</tr>
<tr>
<td>Glass transition temperature</td>
<td>100-1050°C</td>
</tr>
</tbody>
</table>

* Polymers produced in cosolvent (a cyclohexane / ethyl acetate mixture) have a bulk density of 176 kg/m³ (11 lbs/ft³).

** Polymers produced in ethyl acetate have an ash content (as potassium sulfate) of 1-3% on average.

**Rheological properties**

Different grades of Carbopol polymers exhibit different rheological properties, a reflection of the particle size, molecular weight between crosslinks (Mc), distributions of the Mc, and the fraction of the total units, which occur as terminal, i.e. free chain ends. Table 1.5 contains viscosity range of carbopol polymers.

Table 1.5: Viscosity range of different Carbopol Polymers

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Viscosity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol 934 NF</td>
<td>30500 – 39400</td>
</tr>
<tr>
<td>Carbopol 934 P NF</td>
<td>29400 – 39400</td>
</tr>
<tr>
<td>Carbopol 71 G NF</td>
<td>4000 – 11000</td>
</tr>
</tbody>
</table>

*Brookfield RVT Viscosity, cP 0.5 wt % mucilage at pH 7.5, 20 rpm at 25°C.

**How Carbopol® Polymers Thicken**

State I: Powder- Before contact with water, cross-linked polyacrylic acid is tightly coiled.

State II (Polymer Dispersion): Hydrated- When dispersed in water, cross-linked polyacrylic acid begins uncoiling.
State III (Polymer Mucilage): Neutralized- Neutralization with a base creates negative charges along backbone. These repulsive forces uncoil polymer into an extended structure.

Different neutralizers are sodium hydroxide, ammonium hydroxide, triethanolamine, arginine, potassium hydroxide, diisopropanolamine, etc.

Carbopol® polymer will precipitate out of solution if neutralizer is not chosen correctly.

**Applications of carbopol polymers**\(^\text{133}\)

The readily water-swellable Carbopol polymers are used in a diverse range of pharmaceutical applications to provide:

- Controlled release in tablets, Carbopol polymers offer consistent performance over a wide range of desired parameters (from pH-derived semi-enteric release to near zero-order drug dissolution kinetics) at lower concentrations than competitive systems.
- Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications. Noveon AA-1 USP polycarbophil is the recognized industry standard for bioadhesion.
- Thickening at very low concentrations to produce a wide range of viscosities and flow properties in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs.
- Permanent suspensions of insoluble ingredients in oral suspensions and topical.
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants.

**Topical Applications**\(^\text{133}\)

Carbomers are very well suited to aqueous formulations of the topical dosage forms. Many commercial topical products available today have been formulated with these polymers, as they provide the following numerous benefits to topical formulations:
Safe & Effective — Carbopol polymers have a long history of safe and effective use in topical gels, creams, lotions, and ointments. They are also supported by extensive toxicology studies.

Non-Sensitizing — Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage.

No Effect on the Biological Activity of the Drug — Carbopol polymers provide an excellent vehicle for drug delivery. Due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug.

Excellent Thickening, Suspending, & Emulsification Properties for Topical Formulations

**Flow Characteristics of Carbopol Systems**

Carbopol polymers can be used to provide a wide range of flow properties. High molecular weight, highly crosslinked polymers such as Carbopol 940 and 934 provide mucilages with a very short flow rheology. Short flow can be characterized as a gelled consistency similar to mayonnaise. In contrast, Carbopol 941, a lower molecular weight, more highly crosslinked polymer, provides a fairly long flow rheology. Materials with long flow rheology are "stringy" and flow like honey.

**Carbopol 934**

Poly (acrylic acid) (PAA or Carbomer) is generic name for synthetic high molecular weight polymers of acrylic acid. They may be homopolymers of acrylic acid, crosslinked with an allyl ether pentaerythritol, allyl ether of sucrose or allyl ether of propylene. Carbomer codes (910, 934, 940, 941 and 934P) are an indication of molecular weight and the specific components of the polymer.

Carbopol® 934 polymer is a cross-linked polyacrylate polymer. It offers excellent stability at high viscosity and produces thick formulations for opaque gels, emulsions, creams and suspensions.

Carbopol 934 can be used in thick formulation such as viscous gel, thick emulsions and heavy suspensions. It gives permanent stability at high viscosity. In aqueous system, carbopol 934 exhibits short flow properties, which are of interest in application such as cosmetics and spray on.
Molecular structure:

![Chemical structure of Carbomer 934]

**Appearance:** White, fluffy powder

Carbomer 934 is a high molecular weight polymer of acrylic acid cross-linked with allyl ethers of sucrose. Carbomer 934, previously dried in vacuum at 80°C for 1 hour, contains not less than 56.0 percent and not more than 68.0 percent of carboxylic acid (–COOH) groups.

**Packaging and storage:** Preserve in tight containers.

**Labeling:** Label to indicate “it is not intended for internal use.”

**Odor:** Slightly acetic

**Viscosity:** cp, 25°C by Brookfield RVT, 20 rpm, neutralized to pH 7.3 - 7.8. 2,050 - 5,450 - 0.2 wt% mucilage, 30,500 - 39,400 - 0.5 wt% mucilage
1.7. References


Chapter 1

Introduction


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