Topical or Transdermal drug delivery system is challenging because of skin acting as limiting barrier. Thus, Vesicular systems such as liposomes, niosomes, transfersomes etc are used as effective methods for transdermal application of drug substances. Transfersomes are ultra deformable vesicles in nature, made up of phospholipid component along with a surfactant mixture which control flexibility of vesicles. They can squeeze themselves through pores of skin which is many times smaller than its size owing to its elasticity and flexibility and thus can act as carrier for low as well high molecular weight drugs eg. analgesics, anaesthetics, corticosteroids, sex hormone, anti-cancer, insulin, gap-junction proteins and albumins. Transfersomes can accommodate lipophilic, hydrophilic as well as amphiphilic drugs. Peripheral drug targeting, transdermal immunization can also be achieved with this type of drug delivery system.
INTRODUCTION

The skin covers a total surface area of approximately 1.8m² and provides the contact between human body and its external environment. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases. This has the advantage that the high concentrations of drugs can be localized at the site of action, reducing the systemic drug levels and therefore also reducing the systemic side effects (1). Transdermal drug delivery system has been gaining interest over conventional drug delivery systems so as to avoid hepatic first-pass effect to increase drug bio-availability and to decrease dosing frequency required for oral treatment (2).

Recently, many strategies have been used to augment transdermal delivery of bioactives. They include iontophoresis, electrophoresis, sonophoresis, chemical permeation enhancers, microneedles and vesicular systems (liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes). Among these, transfersomes appear to be promising approach (3). Introduced by Gregor Cevc, transfersomes are elastic or ultradeformable vesicles which consists of phospholipids and an edge activator. The system delivers drug reproducible either into or through skin by squeezing itself through a pore which is many times smaller than its size owing to its elasticity. The name means 'carrying body' derived from Latin word 'Transferre' meaning to carry across and greek word 'Soma' for a body (4).

Flexibility or elasticity of transfersome membrane is achieved by mixing suitable surface-active components (edge activator) in proper ratios. Thus, the resulting flexibility of transfersome membrane minimizes risk of complete vesicle rupture in skin and allows them to follow natural water gradient across epidermis, when applied under non-occlusive condition (5). They can penetrate the intact stratum corneum by either intracellular lipid or transcellular route. When applied on skin, carrier searches and exploits hydrophilic pathways or 'pores' between cells, where it opens wide enough to permit entire vesicle to pass through stratum corneum along with drug molecule, deforming itself extremely to accomplish this without losing its vesicular integrity. This enables them to cross various transport barriers efficiently.

Components Of Transfersomes:

<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactants</td>
<td>Sodiumcholate, sodium deoxycholate, span60, span-65, span-80, tween-20, tween-60, tween-80</td>
<td>Flexibility provider</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Dipalmitlyphosphatidylcholine, diestearylphosphatidylcholine, egg phosphatidylcholine, soya phosphatidy l choline, lecithin</td>
<td>Vesicle providing agents</td>
</tr>
<tr>
<td>Solvents</td>
<td>Ethanol, methanol, Chloroform</td>
<td>Solvent</td>
</tr>
<tr>
<td>Buffering agents</td>
<td>Saline phosphate buffer</td>
<td>Hydrating medium</td>
</tr>
<tr>
<td>Dye</td>
<td>Fluorescein-DHPE, Nile-red, Rhodamine-DHPE, rhodamine-123</td>
<td>For confocal laser microscopy study (7)</td>
</tr>
</tbody>
</table>
Salient Features Of Transfersome:

- Biocompatible and biodegradable as made from natural phospholipids and use of acceptable additives.
- Protect encapsulated drug from degradation, act as depot and release contents slowly and gradually.
- Used for both systemic as well as topical delivery of drugs.
- Used for both low as well as high molecular weight drugs eg. anaesthetics, sex hormones, corticosteroids etc.
- Highly flexible. Higher flux rate across skin and higher rate of skin penetration as compared to other vesicular systems.
- High deformability gives better penetration of intact vesicles.
- Possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result, can accommodate drug molecules with wide range of solubilities.
- Non-therapeutic delivery of therapeutic molecules across open biological barriers.
- Carrier-associated drug clearance through cutaneous blood vessels plexus (6)(7)(8)(9).

Limitations:

- Are chemically unstable because of their predisposition to oxidative degradation
- Purity of natural phospholipids is another criteria militating against adoption of transfersome as drug delivery vehicles.
- Transfersome formulations are expensive (7)(10).

Mechanism Of Penetration Of Transfersome :

Transfersomes when applied under suitable conditions can transfer 0.1mg of lipid per hour and cm² area across the intact skin. The high flux rate is due to 'transdermal osmotic gradient' i.e. another gradient available across skin. The gradient is developed due to skin penetration barrier that prevents water loss through skin and maintains a water activity difference in viable part of epidermis (75% water content) and nearly completely dry stratum corneum, near to skin surface (15% water content).

Systemic penetration is described in 3 proposed mechanism :-

1. Interaction between hydrophilic lipid residues and proximal water makes polar lipids to attract water molecules induce hydration, lipid vesicles move to site of higher water concentration. The difference in water content across skin stratum and epidermis develops transdermal osmotic gradient that leads to penetration of transfersome across skin.
2. Transfersome, by enforcing its own route induce hydration that widen hydrophilic pores of skin, through widen pores there is gradual release of drugs that binds to targeted organ.
3. Transfersome act as penetration enhancers, that disrupt the intercellular lipids from stratum which ultimately widens the pores of skin and facilitate the molecular interaction and penetration of system across skin (2).

Method Of Preparation:

Thin film hydration method is commonly used for preparation of transfersomes which is comprised of 2 steps:

First, a thin film is prepared, hydrated and then bought to desired size by sonication.
Secondly, sonicated vesicles are homogenized by extrusion through a polycarbonate membrane.
### Characteristics of Transfersomes

Characteristics of transfersome is similar to liposomes, niosomes and micelles. Following parameters have to be checked:

1. **Vesicle size distribution and zeta potential:**
   - Vesicle size, size distribution and zeta potential are determined by Dynamic Light Scattering Method (DLS) using a computerized inspection system by Malvern Zetasizer.

2. **Vesicle morphology:**
   - Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples are prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements. Transfersomes vesicles can be visualized by TEM, phase contrast microscopy, etc. The stability of vesicle can be determined by assessing the size and structure of vesicles over time. Mean size is measured by DLS and structural changes are observed by TEM.

3. **No. of vesicles per cubic mm:** This is an important parameter for optimizing the composition and other process variables. Non-sonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study. The Transfersomes in 80 small squares are counted and calculated using the following formula:

\[
\text{Total no. of transfersomes per cubic mm} = \left( \frac{\text{Total no. of Transfersomes counted} \times \text{dilution factor} \times 4000}{\text{Total no. of squares counted}} \right)
\]

### Method is summarised in tabular form:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mixture (Phospholipid+surfactant+organic solvent)</td>
</tr>
<tr>
<td>2.</td>
<td>Organic solvent evaporated above lipid transition temp. using rota evaporator</td>
</tr>
<tr>
<td>3.</td>
<td>Final traces of solvent removed under vacuum for overnight</td>
</tr>
<tr>
<td>4.</td>
<td>Deposited film hydrated with phosphate buffer by rotation at 60rpm for 1 hour</td>
</tr>
<tr>
<td>5.</td>
<td>Resulting vesicles allowed to swell for some time at room temp</td>
</tr>
<tr>
<td>6.</td>
<td>Above resulting vesicles sonicated at room temperature for 30 min using bath sonicator and at room temperature for 30 min using probe sonicator</td>
</tr>
<tr>
<td>7.</td>
<td>Sonicated vesicles homogenized by passing through sandwich of 200 and 100 nm polycarbonate membrane</td>
</tr>
</tbody>
</table>
4. **Entrapment efficiency:** The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency is determined by first separation of the un-entrapped drug by use of mini-column centrifugation method. After centrifugation, the vesicles are disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

\[
\text{Entrapment efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100
\]

5. **Drug content:** The drug content can be determined using one of the instrumental analytical methods such as modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program depending upon the analytical method of the pharmacopoeial drug.

6. **Turbidity measurement:** Turbidity of drug in aqueous solution can be measured using nephelometer.

7. **Degree of deformability or permeability measurement:** In the case of transfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements.

8. **Surface charge and charge density:** Surface charge and charge density of Transfersomes can be determined using Zetasizer.

9. **In-vitro drug release:** In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from invitro studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32°C and samples are taken at different times and the free drug is separated by mini column centrifugation. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

10. **In-vitro Skin permeation Studies:** Modified Franz diffusion cell with a receptor compartment volume of 50ml and effective diffusion area of 2.50 cm² was used for this study. In vitro drug study was performed by using goat skin in phosphate buffer solution (pH 7.4). Fresh Abdominal skin of goat were collected from slaughterhouse and used in the permeation experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-40°C. To perform skin permeation study, treated skin was mounted horizontally on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50cm² and capacity of receptor compartment was 50ml. The receptor compartment was filled with 50ml of phosphate buffer (pH 7.4) saline maintained at 37 ± 0.5°C and stirred by a magnetic bar at 100RPM. Formulation (equivalent to 10mg drug) was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. The samples were analyzed by any instrumental analytical technique.

11. **Physical stability:** The initial percentage of the drug entrapped in the formulation is determined and are stored in sealed glass ampoules. The ampoules are placed at 4 ± 20°C (refrigeration), 25 ± 20°C (room temp), and 37 ± 20°C (body temp) for at least 3 months. Samples from each ampoule are analyzed after 30 days to determine drug leakage. Percent drug lose is calculated by keeping the initial entrapment of drug as 100% (10)(11)(12)(13).

**Applications Of Transfersomes:-**

1. **Controlled release and stability enhancement**
   Transfersomes are used for controlled release of administered drug and increase stability of labile drugs (6).

2. **Utility of high molecular weight drugs**
   Large molecules, incapable of diffusing into skin can be transported with help of Transfersomes. eg. (a)Encapsulation of insulin into transfersomes (transferulin) overcomes problem of inconvenience, large size along with showing 50% response as compared to subcutaneous injection. (b) also used as carrier for interferons like leukocyte derived interferons α (2)

3. **Transport of Proteins and Peptides**
   Because of degradation of proteins in g.i.t. through oral route and problem of large size through transdermal route, transfersomes are more suited as they provide somewhat similar bio-availability to subcutaneous injection (6).

4. **Transport of corticosteroids**
   Transfersomes is advantageous as less dose of corticosteroids can be encapsulated and it improves site specificity of overall safety margin of corticosteroids (10).
5. **Delivery of NSAIDS**
NSAIDS have greater GI side effects which can be overcome using transfersomes. Approach has been successfully used for drugs like Diclofenac, Ketoprofen etc (6).

6. **Delivery of anti-cancer drugs**
Favourable results appeared for anti-cancer drugs like methotrexate when tried for Transdermal delivery (10).

7. **Delivery of anaesthetics**
Transfersomal application of anaesthetics induces topical anaesthesia with less than 10 minutes. Maximum resulting pain insensitivity is nearly as strong as (80%) that of comparable subcutaneous bolus infection, but affects of transfersomal anaesthetics lasts longer (10).

8. **Delivery of herbal drugs**
Transfersomes can penetrate stratum corneum and supply nutrients for maintenance of skin. Technology has been used for Capsaicin.

**CONCLUSION:**
Transfersomes are specially optimized elastic vesicles, which can deform themselves, in response to an external stress to to penetrate the skin through pores and thus being able to carry large size molecules, a limitation possessed by transdermal drug delivery system. They are efficient and safer in composition and can carry unprecedented amount of drug per unit time across the skin ( upto 100 mg cm² h⁻¹). Drug release can also be controlled according to requirement. Thus, the approach can overcome the problems which can occur in other conventional techniques.

**REFERENCES:**

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