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# Transferosomes: An emerging tool for effective transdermal drug delivery

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## ABSTRACT

Transdermal drug delivery is proving to be superior to the conventional oral delivery of drugs owing to its distinct virtues. But it has got its own limitations as well- inability to transport large molecules, inability to overcome the barrier properties of stratum corneum and many more. Using this route of drug delivery along with novel approaches can help to solve these problems. Formulating the drug in a transfersome is one such approach. Transfersome, which was first introduced in the early 1990s, is an ultradeformable vesicle, elastic in nature which can squeeze itself through a pore which is many times smaller than its size owing to its elasticity. Transfersomes are applied in a non-occluded method to the skin and have been shown to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. Transfersomes are made up of a phospholipids component along with a surfactant mixture. The ratio of individual surfactants and total amount of surfactants control the flexibility of the vesicle. The uniqueness of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic drugs. These drugs find place in different places in the elastic vesicle before they get delivered beneath the skin. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. Peripheral drug targeting, transdermal immunization can also be achieved with this type of drug delivery system.

**Key words:** Transfersome, Vesicle, Ultradeformable, Flexible, Skin delivery.

## INTRODUCTION

The transdermal route of drug delivery has gained great interest of pharmaceutical research, as it circumvents number of problems associated with oral route of drug administration. Recently, various strategies have been used to augment the transdermal delivery of bioactives. Mainly, they include electrophoresis, iontophoresis, chemical permeation enhancers, microneedles, sonophoresis, and vesicular system like liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes. Among these strategies transfersomes appear promising. A novel vesicular drug carrier system called transfersomes, which is composed of phospholipid, surfactant, and water for enhanced transdermal delivery. Transfersomes, a novel class of modified liposomes, are variously described as deformable, highly deformable, elastic or ultra-flexible liposomes or vesicles, which were first introduced in the early 1990s. Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. Because of their self-optimized and ultra flexible membrane properties, they are able to deliver the drug reproducible either into or through the skin, depending on the choice of administration or application, with high efficiency. The vesicular transfersomes are 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 (Paul, 1995; Patel, 2009; Roger, 2006; Ashok, 2007)

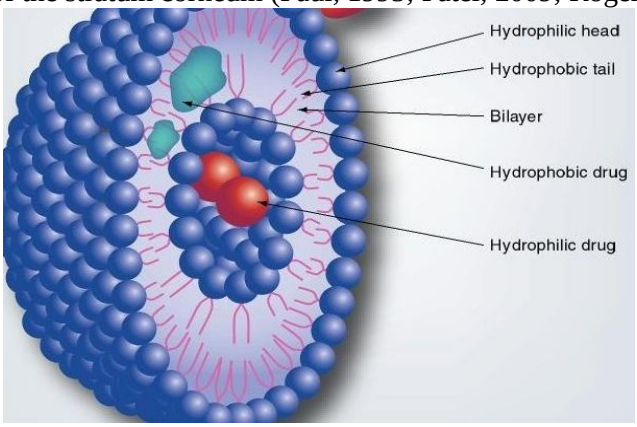


**Figure 1: Structure of Transfersomes**

Also they possess a unique ability to get accommodated with a wide range of solubility and act as efficient carriers for both low as well as high molecular weight drugs, e.g. analgesic, corticosteroids, hormones, anticancer drugs, insulin, proteins etc with high entrapment efficiency & a unique advantage of protection of the encapsulated drug, from metabolic degradation. However, their major disadvantage arises due to their chemical instability and high susceptibility to oxidative degradation. Transfersomes could be easily prepared using various processes - suspension homogenization process, aqueous lipid suspension process, modified handshaking process and centrifugation process. Transfersomes' inherent potential advantages are highly utilized in 'Transdermal Immunization', 'Peripheral Drug Targeting' & for 'Transdermal Delivery 'of Insulin, NSAIDs, Heparin, Anti Cancer drugs, etc. Various patent applications have been admitted, using Transfersomes for various applications, e.g., 'Transcutaneous Administration' of Antigens, Delivery of larger molecules across nasal mucosa, Intra-Epithelial delivery of chemical agents, Delivery of biologically active drugs, delivery of DNA using lipid vesicles, etc. Recently, marketing approval of Drug 'diractin' using ketoprofen has gained its significance for Transfersomal delivery of the drug.

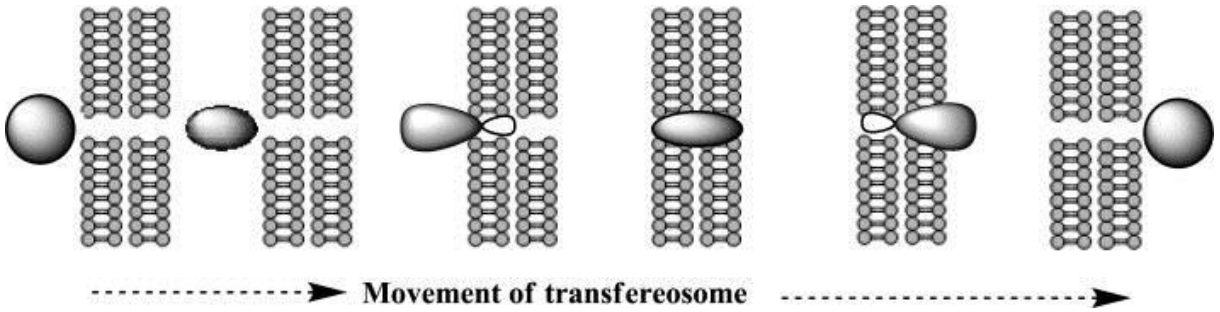


**Figure.2.Transfersomes are ultra deformable (up to 105 times that of conventional Liposomes) squeezing through small pores in the SC.**

**Advantages of transfersomes:** Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss (Chein, 1987; Kumar, 2004; Misra, 1997).

1. They have high entrapment efficiency, in case of lipophilic drug near to 90%.
2. This high deformability gives better penetration of intact vesicles.
3. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
4. Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility.
5. They act as depot, releasing their contents slowly and gradually.
6. They can be used for both systemic as well as topical delivery of drug.
7. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes. The carrier aggregate is composed of at least one amphiphat somes.
8. They protect the encapsulated drug from metabolic degradation.
9. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.
10. Non therapeutic delivery of therapeutic molecules across open biological barriers.
11. Transport of small molecule drugs having specific physico-chemical probe.
12. Carrier-associated drug clearance through cutaneous blood vessels plexus.

**Limitations of Transfersomes:**

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

**Table 1: Composition for Transfersome Formulations**

| Class | Example | Uses |
|---|---|---|
| **Surfactants** | Sodiumcholate, sodium desoxycholate,span60, span-65, span-80,tween-20,tween-60,tween-80 | Flexibility provider |
| **Phospholipids** | Dipalmitylphosphatidylcholine, distearylphosphatidylcholine, egg phosphatidylcholine, soya phosphatidyl choline, lecithin | Vesicle providing agents |
| **Solvents** | Ethanol, methanol, Chloroform | Solvent |
| **Buffering agents** | Saline phosphate buffer (pH 6.4) | Hydrating medium |
| **Dye** | Fluorescein-DHPE, Nile-red,Rhodamine-DHPE,rhodamine-123, | For conofocal laser microscopy study |

**Mechanism of action:** The mechanism for penetration is the generation of "osmotic gradient" due to evaporation of water while applying the lipid suspension (Transfersomes) on the skin surface. The transport of these elastic vesicles is thus independent of concentration. The trans-epidermal hydration provides the driving force for the transport of the vesicles. As the vesicles are elastic, they can squeeze through the pores in stratum corneum (though these pores are less than one-tenth of the diameter of vesicles).

A Transfersome vesicle applied on an open biological surface, such as non occluded skin, Tends to penetrate its barrier and migrate into the water-rich deeper strata to secure its adequate hydration. During penetration through the stratum corneum, reversible deformation of the bilayer occurs. But it should be noted that while this deformation is occurring, vesicle integrity, gradient and barrier properties for the underlying hydration affinity should not be compromised. Since it is too large to diffuse through the skin, the Transfersome needs to find and enforce its own route through the organ. The Transfersome vesicles usage in drug delivery consequently relies on the carrier's ability to widen and overcome the hydrophilic pores in the skin. Intracellular drug transportation may involve diffusion of vesicle lipid bilayer with the cell membrane like normal endocytosis. The mechanism is thus complex and involves advanced principles of elasto-mechanics combined with material transport and hydration/osmotic force (Heather, 2005; Boinpally, 2002).

**Preparation of Transfersomes:**

**Reverse Phase Evaporation method:** Soya lecithin, cholesterol and other lipids should be taken in a clean beaker. Then, surfactant is poured in the same beaker and dissolved in a different solvent mixture. The beaker is kept at the room temperature for 24 h until the thin film is formed. Drug solution is poured onto the thin film and sonicated using probe sonicator at a frequency of 20 KHz for 2 min. After that, the film was hydrated using edge activator in phosphate buffer saline (pH 7.4) and then further sonicated for 2 min to obtain transfersomal suspension. Then various formulated transfersomal suspensions should be passed through Whitman filter paper (No. 40). Then, these transfersomal suspensions will be transferred to 5% w/v methylcellulose gel and stored in cool and dark place.

**Modified Hand Shaking, Lipid Film Hydration Technique:**

1. Drug, lecithin (PC) and edge activator are dissolved in ethanol: chloroform (1:1) mixture. Organic solvent can be removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film will be formed inside the flask wall while rotation. The thin film will be kept overnight for complete evaporation of solvent.

2. The film is then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersomal suspension is further hydrated up to 1 hour at 2-8 $^{0}$ C.

**Thin Film Hydration Technique:**

1. A thin film can be prepared from the mixture of vesicle forming ingredients that is phospholipids and surfactant by dissolving in volatile organic solvent (chloroform or methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50$^{0}$C for (dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent will be removed under vacuum for overnight.

2. The prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles will be swollen for 2 hrs at room temperature.

3. To prepare small vesicles, the resulting vesicles can be sonicated at room temperature $50^0$C for 30 minutes using a bath sonicator or probe sonicated at $40^0$C for 30 minutes. The sonicated vesicles will be homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

**Characterization of transfersomes:** The characterization of transfersomes is generally similar to liposomes, niosomes and micelles. Following characterization parameters have to be checked for transfersomes.

**1. Vesicle size distribution and zeta potential:** Vesicle size, size distribution and zeta potential were 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 were 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:

$$\textbf{Total number of Transfersomes per cubic mm} = \left(\frac{\textbf{Total number of Transfersomes counted} \times \textbf{dilution factor} \times \textbf{4000}}{\textbf{Total number of squares counted}}\right)$$

**4. Entrapment efficiency:** The entrapment efficiency is expressed as the percentage entrapment of the drug added. Entrapment efficiency was determined by first separation of the un-entrapped drug by use of mini-column centrifugation method. After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:

$$\textbf{Entrapment efficiency} = \left(\frac{\textbf{Amount entrapped}}{\textbf{Total amount added}}\right) \times \textbf{100}$$

**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 320C 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 receiver 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 was determined and were stored in sealed glass ampoules. The ampoules were placed at 4 ± 20C (refrigeration), 25 ± 20C (room temp), and 37 ± 20C (body temp) for at least 3 months. Samples from each ampoule were analyzed after 30 days to determine drug leakage. Percent drug lose was calculated by keeping the initial entrapment of drug as 100%.

**Application of transfersomes:**

**1. Delivery of insulin:** By transfersomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient. Encapsulation of insulin into transfersomes (transfersulin) overcomes these entire problems. After transfersulin application on the intact skin, the first sign of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition.

**2. Delivery of corticosteroids:**Transfersomes have also used for the delivery of corticosteroids. Transfersomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transfersomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.

**3. Delivery of Anticancer Drugs:** Anti cancer drugs like methotrexate were tried for transdermal delivery using transfersome technology. The results were favorable. This provided a new approach for treatment especially of skin cancer.

**4. Delivery of NSAIDS:** NSAIDS are associated with number of GI side effects. These can be overcome by transdermal delivery using ultra-deformable vesicles. Studies have been carried out on Diclofenac and Ketoprofen. Ketoprofen in a Transfersome formulation gained marketing approval by the Swiss regulatory agency (SwissMedic) in 2007; the product is expected to be marketed under the trademark Diractin. Further therapeutic products based on the transfersome technology, according to IDEA AG, are in clinical development (Schatzlein, 1998; Gamal, 1999; Wearner, 1988).

## CONCLUSION

Transfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems. Transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller. Drug laden transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm2h-1). The systemic drug availability thus mediated is frequently higher than, or at least approaches 80-90%. The bio-distribution of radioactively labeled phospholipids applied in the form of transfersomes after 24 h is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. When used under different application conditions, transfersomes can also positioned nearly exclusively and essentially quantitatively into the viable skin region.

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