TRANSFEROSOMES: NOVEL APPROACH FOR TRANSDERMAL DRUG DELIVERY

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ABSTRACT

Transdermal administration of drugs is generally limited by the barrier function of the skin. Vesicular systems are one of the most controversial methods for transdermal delivery of active substances. The interest in designing transdermal delivery systems was relaunched after the discovery of elastic vesicles like transferosomes, ethosomes, cubosomes, phytosomes, etc. For a drug to be absorbed and distributed into organs and tissues and eliminated from the body, it must pass through one or more biological membranes/barriers at various locations. Such a movement of drug across the membrane is called as drug transport. They are noninvasive and self-administered delivery systems that can improve patient compliance and provide a controlled release of the therapeutic agents. The greatest challenge of transdermal delivery systems is the barrier function of the skin’s outermost layer. Molecules with molecular weights greater than 500D and ionized compounds generally do not pass through the skin. Therefore, only a limited number of drugs are capable of being administered by this route. Encapsulating the drugs in transferosomes are one of the potential approaches to overcome this problem. They have a bilayered structure that facilitates the encapsulation of lipophilic and hydrophilic, as well as amphiphilic, drug with higher permeation efficiencies compared to conventional liposomes. Transferosomes are elastic in nature, which can deform and squeeze themselves as an intact vesicle through narrow pores that are significantly smaller than its size. This review aims to describe the concept of transferosomes, the mechanism of action, different methods of preparation and characterization and factors affecting the properties of transferosomes, along with their recent applications in the transdermal administration of drugs.
KEYWORDS: Transferosomes, nanoencapsulation, transdermal drug delivery, stratum corneum, surfactant, transferosomes, vesicles.

INTRODUCTION
An efficacious, successful therapeutic treatment cannot be achieved in most cases, often due to many reasons, such as the occurrence of hepatic first-pass metabolism, adverse side effects, the rejection of invasive treatments and poor patient compliance. Therefore, several drug delivery systems have been developed and studied over the past decades to overcome these problems. One promising approach is the use of transdermal delivery systems, as they are minimally invasive methods without first-pass effects. However, the barrier function of the skin that prevents or dampens the transdermal delivery of therapeutic agents has to be addressed. Nanoencapsulation using a lipid-based vesicular system such as liposomes has been used to overcome the aforesaid challenge. Liposomes facilitate drug transport through the skin by three possible mechanisms: adsorption to the skin surface with a subsequent transferring of the drug directly from vesicles to skin, fusion with the lipid matrix of the stratum corneum, thereby increasing drug partitioning into the skin, and lipid exchange between the liposomal membrane and cell membrane, facilitating the diffusion of the drug across the membrane. However, the problem with conventional liposomes is that they do not deeply penetrate into the viable skin and blood circulation. Therefore, liposomes have been widely used as drug carriers for dermal delivery and not for transdermal delivery. Conventional liposomes also have limitations, such as the poor encapsulation efficiency of hydrophilic drugs, an unstable membrane that results in leaky behavior and a short half-life. These major obstacles have led to the discovery and development of other novel vesicles such as niosomes, sphingosomes, bilosomes, chitosomes, transferosomes, ethosomes and invasomes. Niosomes were first reported in the early 1970s. They are composed of nonionic surfactants (i.e., of alkyl or di-alkyl polyglycerol ether class, alkyl ethers, alkyl esters or alkyl amides); cholesterol and, sometimes, ionic amphiphiles. The cholesterol provides rigidity to the vesicular bilayer, whereas nonionic surfactants increase the size and entrapment efficiency of niosomes. Furthermore, some ionic amphiphiles such as dicetyl phosphate (negatively charged molecule) and stearylamine (positively charged molecule) are used in the niosomes for enhancing the entrapment efficiency, efficacy and stability. Moreover, they are identified as a better drug carrier system over liposomes due to considering factors such as a high chemical stability, high bioavailability, high entrapment efficiency and being inexpensive. According to the literature, niosomes tend to enhance the residence time of therapeutic drugs...
in the stratum corneum and epidermis, meanwhile reducing the systemic drug absorption and thereby improving the trapped drug penetration across the skin. Chitosomes, liposomes-based vesicles coated with chitosan polymer, were first described in the early 1990s. Chitosan is used to improve the vesicular stability by modifying the surface properties of liposomes. Moreover, chitosan provided mucoadhesive properties to the liposomes. Chitosan increases the membrane structural integrity of liposomes, decreases the membrane fluidity and thereby enhances the physicochemical stability of liposomes. Sphingosomes were first introduced in the late 1990s. They can be identified as liposomes made from sphingolipids (such as lysoglycosphingolipids, hexadecasphinganine, n-acylsphingosines, phosphoglyco sphingolipids and gangliosides) and cholesterol. Sphingosomes are more stable than phospholipid liposomes, because sphingolipids consist of only amide and ether linkages that are more hydrolysis-resistant than that of lecithin ester linkages. They also contain fewer double bonds than lecithin and, therefore, are less subjected to rancidity. For bilosomes, they are vesicles composed of nonionic surfactants and bile salts (bile salts are incorporated into the niosome’s membrane), which are considered as non-lipoidal biocarriers. These novel vesicles were developed for the purpose of vaccine oral delivery due to its resistance towards the enzymes and bile salts in the gastrointestinal tract. Transferosomes are composed of phospholipids and edge activator (EA), which is a membrane-softening agent (such as Tween 80, Span 80 and sodium cholate) that facilitates the ultra-deformable property of the transferosomes. When transferosomes reach the skin pores, they are capable of changing their membrane flexibility and passing through the skin pores spontaneously. This is the so-called self-optimizing deformability. Moreover, transferomes are extremely deformable; therefore, they easily cross even the very narrow pores.\(^\text{[1-4]}\)

**Mechanism of Action:** Vesicles are known as colloidal particles, which are an aqueous compartment enclosed by a concentric bilayer that are made-up of amphiphilic molecules. They are very useful as vesicular drug delivery systems, which transport hydrophilic drugs encapsulated in the inner aqueous compartment, whereas hydrophobic drugs are entrapped within the lipid bilayer. With regard to transferosomes, they are highly deformable (ultra-flexible) and self-optimizing novel drug carrier vesicles, in which their passage across the skin is mainly associated with the transferosomes’ membrane flexibility, hydrophilicity and the ability to maintain the vesicle’s integrity.\(^\text{[5]}\)
They efficiently penetrate through the intact skin if applied under nonocclusive conditions; this specific nonocclusive state of the skin is required mainly to initiate a transepidermal osmotic gradient across the skin. According to the study done by Cevc and Blume, hydrotaxis (xerophobia) is the permeation mechanism of transfersomes, which is further described as the transfersome’s moisture-seeking tendency towards deeper skin layers rather than dry outer background due to the state of moisture evaporation from the transfersomal formulation following its application on the skin (nonocclusive condition). The transdermal water activity difference, which originates due to the natural transdermal gradient, creates a significantly strong force that acts upon the skin through transfersomes vesicles, which enforce the widening of intercellular junctions with the lowest resistance and thereby generate transcutaneous channels 20–30 nm in width. These created channels allow the transfer of ultra-deformable, slimed transfersomes across the skin with respect to the hydration gradient. Moreover, the osmotic gradient develops as a result of evaporation of the skin surface water due to body heat, which exerts its action as the driving force to facilitate the flexible transport across the skin to deliver therapeutic agents from the site of application to the target area for local or systemic treatments in effective therapeutic concentrations and minimum systemic toxicity. Transfersomes demonstrate a higher permeation efficiency (through small skin channels) compared to conventional liposomes but have a similar bilayered structure that facilitates the encapsulation of lipophilic and hydrophilic, as well as amphiphilic, drugs. Transfersomes vary from liposomes, primarily due to their softer, better adjustable and ultra-deformable artificial membranes. Interdependency of the local

Fig. 1: Mechanism of action of transfersomes.
composition, as well as the shape of the lipid bilayer, makes the vesicles both self-optimizing and self-regulating. This property enables the transfersomes vesicles to cross numerous transport barriers efficiently. Therefore, transfersomes are supramolecular entities composed of at least one type of amphipathic agent and, by the addition of at least one type of bilayer-softening agent (edge activator), result in greatly increased lipid bilayer flexibility and permeability. Certain transfersomes have some amounts of alcohol (ethanol or propylene glycol) in their compositions as penetration enhancers and, also, used as cosolvents that have good solvating power. Ethanol has been proposed to induce modifications of the lipid bilayer polar head region. Following penetration, ethanol increases the fluidity of the intercellular lipid matrix and later on results in decreasing the density of the lipid lamellae. Transfersomes can penetrate through the stratum corneum and reach the target sites, including the dermis and blood circulation. Their penetration ability depends on the deformability of the transfersomal membrane, which can be attributed to the vesicle compositions. Therefore, the most suitable vesicle compositions must be identified through conducting individually designed experimental procedures for each therapeutic agent to obtain the most appropriate carriers with optimum deformability, drug carrying capacity and stability.[6-10]

4. Composition of Transfersomes

Transfersomes are generally composed of

1. Firstly, the main ingredient, an amphipathic ingredient (e.g., soy phosphatidylcholine, egg phosphatidylcholine, etc.) that can be a mixture of lipids, which are the vesicle-forming components that create the lipid bilayer.[11]

2. Secondly, 10–25% surfactants/edge activators; the most commonly used edge activators in transfersome preparations are surfactants as sodium cholates; sodium deoxycholate; Tweens and Spans (Tween 20, Tween 60, Tween 80, Span 60, Span 65 and Span 80) and dipotassium glycyrrhizinate, which are biocompatible bilayer-softening compounds that increase the vesicles’ bilayer flexibility and improve the permeability.[12]

3. About 3–10% alcohol (ethanol or methanol), as the solvent and, finally, hydrating medium consist with either water or a saline phosphate buffer (pH 6.5–7).

In an aqueous environment, the phospholipids self-assemble into flexible lipid bilayers and close to form vesicles. The biocompatible membrane softeners, which are also known as edge activators, are single-chain surfactants that incorporate into the transfersomes structure and
facilitate the destabilization of the vesicle’s lipid bilayer and enhance its fluidity and elasticity. The total amount of surfactants and the proper ratios of individual surfactants to phospholipids are responsible for the control of vesicles’ membrane flexibility and minimizing the risk towards vesicle ruptures in the skin. This result promotes transferosomes to follow the natural osmotic gradient across the epidermis following application under a nonocclusive manner. In summary, the penetration-enhancing effect of these vesicles depends on the concentrations and the types of surfactants, the types of lipids, the size shape and elasticity of the vesicles.\[13-15\]

**METHODS OF PREPARATION OF TRANSFEROSOMES**

**Rotary Film Evaporation Method:** The measured quantity of phospholipids and edge activators are utilized to produce a thin film in this approach. In an organic solvent, such as a combination of chloroform and methanol, a solution of phospholipids and edge activators is produced. The prepared solution is transferred to a round bottom flask that is rotated at a constant temperature (above the lipids' glass transition point) and low pressure. On the flask's walls, a coating of lipids and edge activator forms. The drug-infused aqueous medium is then used to hydrate the produced film. Lipids expand and produce bilayer vesicles as a result. Extrusion or sonication of bigger vesicles can produce vesicles of desired size.\[16,17\]

**Vortexing-sonication Method:** In a phosphate buffer, the phospholipids, edge activator, and medication are combined. After that, the mixture is vortexed till it forms a milky transferosomal suspension. It's then sonicated for a certain amount of time at room temperature in a bath sonicator before being extruded through polycarbonate membranes (example: 450 and 220 nm).\[18,19\]

**Modified Hand Shaking Method:** In an ethanol: chloroform (1:1) combination, the drug, lecithin (PC), and edge activator are dissolved. Above the lipid transition temperature (43°C), the organic solvent is evaporated with hand shaking. Rotation causes the formation of a thin lipid layer inside the flask wall. The thin layer is let to dry overnight to ensure complete evaporation of solvent. The film is then hydrated for 15 minutes at room temperature with phosphate buffer (pH 7.4) and moderate shaking. At 2-8°C, the Transferosomal suspension hydrated for a further hour.\[20,21\]

**Reverse Phase Evaporation Method:** In a glass beaker, the components such as cholesterol and phospholipids are added. The surfactant is then added to same beaker and dissolved in a
separate solvent solution. The beaker is left at room temperature for 24 hours to produce a thin layer. The drug solution is poured over the thin film and sonicated for 2 minutes at a frequency of 20 KHz using a probe sonicator. After that, the film is hydrated in phosphate buffer saline (pH 7.4) with edge activator before being sonicated for 2 minutes to get transferosomal suspension. After that, different transferosomal suspensions should be filtered using Whatman filter paper (No. 40).[22]

**Ethanol Injection Method:** The organic phase is made by dissolving the phospholipid, edge activator, and lipophilic drug in ethanol and stirring for the appropriate amount of time until a clear solution is obtained. The water-soluble compounds are dissolved in the phosphate buffer to form the aqueous phase. This is the time to incorporate the hydrophilic medication. Both solutions are heated to 45–50 °C. After that, the ethanolic phospholipid solution is injected dropwise into the aqueous solution while stirring continuously for the period specified. Transferring the resulting dispersion into a vacuum evaporator and then sonicating for particle size reduction is how ethanol is removed.[23]

**Characterization of the Transferosomes**

1. **Vesicle Size, Zeta Potential and Morphology:** The vesicle size is one of the important parameters during transfersome preparation, batch-to-batch comparison and scale-up processes. During storage, the changing of the vesicle size is an important variable in terms of the physical stability of the formulation. Vesicles smaller than 40 nm are prone to fusion processes because of the high curvature state of their bilayer membranes, whereas much larger and electroneutral transfersomes are aggregated through van der Waals interactions due to relatively greater membrane contact areas. Vesicle size is a factor that influences the ability to encapsulate the drug compounds in transfersomes. For lipophilic and amphiphilic agents, a high lipid-to-core ratio is favored, while a larger aqueous core volume is preferred for the encapsulation of hydrophilic compounds. Generally, the dynamic light scattering (DLS) method or photon correlation spectroscopy (PCS) can be used to determine the vesicle diameter. The vesicle’s suspension can be mixed with an appropriate medium, and the vesicular size measurements can be obtained in triplicate. Moreover, as another approach, the sample can be prepared in distilled water and filtered through a 0.2 mm membrane filter. The filtered sample is then diluted with filtered saline to measure the size of the vesicles by DLS or PCS. Moreover, the DLS method-associated computerized inspection system by Malvern Zetasizer can be used for
the determination of the vesicle size and size distribution, whereas the structural changes are observed by transmission electron microscopy (TEM). The zeta potentialis measured by the electrophoretic mobility technique using Malvern Zetasizer. The visualization of transfersome vesicles can be done by using the phase contrast microscopy or TEM.[24,25]

2. **Number of Vesicles Per Cubic mm**: This parameter is important for the optimization of the composition of the transfersomes and other process variables. Unsonicated transfersomal formulations are diluted five times using 0.9% sodium chloride. A hemocytometer with an optical microscope is used to study this sample. The transfersomes with a vesicle size of more than 100 nm can be observed by optical microscope. The number of transfersomes in small squares are counted and calculated using the following formula[26]:

   \[
   \text{Total number of transfersomes per cubic} = \frac{\text{(Total number of transfersomes counted} \times \text{df} \times 4000)}{\text{Total number of squares counted}}.
   \]

3. **Entrapment Efficiency (%EE)**: The entrapment efficiency (%EE) is the amount of drug entrapped in the formulation. The EE is determined by separating the unentrapped drug from the vesicles using various techniques, such as mini-column centrifugation. In this process, direct or indirect methods can be used to determine the %EE. After ultracentrifugation, the direct approach would be removing the supernatant followed by disrupting the sedimented vesicles using a suitable solvent that is capable of lysing the sediment. Subsequently, the resulting solution can be diluted and filtered using a syringe filter (0.22–0.45 um) to remove the impurities. The drug content is determined by employing analytical methods, such as modified high performance liquid chromatography (HPLC) or spectrophotometrically, which depends on the analytical method of the active pharmaceutical ingredient (API). The percentage drug entrapment (the entrapment efficiency) is expressed as[27,28]:

   \[
   \%\text{Entrapment efficiency} = \frac{\text{(Amount of the drug entrapped} \times 100)}{\text{Total amount of the drug added}}
   \]

4. **In Vitro Drug Release**: The in vitro drug release profile can provide fundamental information on the formulation design and details on the release mechanism and kinetics, enabling a scientific approach to optimize the transfersomal formulation. The in vitro drug release of transfersomes is typically evaluated in comparison to the free drug or the reference product. Various research studies have evidently provided successful data
related to the drug release profiles of developed transfersome formulations. Celecoxib transfersomal gel for the rheumatoid arthritis treatment showed the release of 75% of the entrapped drug within 6 h, which is more than a 30% increment relative to the commercial gel. Ketoconazole-loaded transfersomal gel showed an initial burst of the drug release of 40.67%, which was higher than that of ketoconazole suspension (27.35%) after 6 h. The in vitro release profile of lidocaine from the transfersomal vesicles showed more than 80% of the drug released after 6 h. In brief, Franz diffusion cells are employed in the in vitro drug release study. The donor chamber is fixed to the receptor chamber by means of adhesive tape. The fluid in the receptor chamber is constantly stirred by a magnetic bar. As normal skin surface temperature is approximately 32°C, therefore, in the release study, the temperature of the receptor fluid should be kept at the in vivo skin surface temperature of 32°C. A mixed cellulose ester membrane of an average pore size of 0.45 um is used. The membranes are soaked in the release media (phosphate buffer) at room temperature overnight in order to allow the membrane pores to swell. The aliquots of 1 mL of the receptor medium are withdrawn at appropriate time intervals (such as 0, 0.5, 1, 2, 3, 4, 5 and 6 h), and simultaneously, the receptor medium is replaced by an equal volume of the fresh PBS to maintain the sink conditions. The obtained samples can be analyzed by using appropriate methods such as UV, HPLC and high performance thin layer chromatography (HPTLC).[29,30]

5. In Vitro Skin Permeation Studies: This study is performed to determine the transport efficiencies of the transdermal delivery systems and identify the factors that increase the transdermal flux of the drugs, which is typically expressed in units of ug/cm²/h. The information obtained from this study can also be used to predict in vivo behaviors from different transdermal delivery systems and used for the optimization of the formulation prior to performing more expensive in-vivo studies. Ideally, the human skin should be used for the evaluation of permeation properties of candidate formulations. However, the limited availability, ethical problems and religious restrictions of the human skin make it less attractive for the permeation study. Various animal models, such as primate, porcine, rat, mouse, guinea pig and snake skins, have been suggested as more accessible substitutes for human skin. However, it should be noted that percutaneous absorption through various animal skins may differ significantly from the results obtained with human skin models. According to the published data, it is evidently suggested that pig skin is the most suitable animal model for human skin due to the fact that the fluxes
through the, as well as the concentrations in the skin, were exhibited to be of the same order of magnitude for both of those tissues, with minor differences of, at most, two or four-fold, respectively. Moreover, as another option, synthetic membranes (example: Strat M®) have been employed in transdermal permeation studies. It has been reported that synthetic membranes show very close correlations to human skin. This model has the advantage as being more consistent in permeability, as well as responsiveness, in comparison with human and animal skins. In brief, Franz diffusion cells are employed in the skin permeation study. The selected membranes are horizontally mounted on the receptor compartments as the side, indicating the stratum corneum facing upwards toward the donor compartments. The receptor compartments of the Franz diffusion cells are filled with phosphate buffer saline solution, which is stirred by a magnetic bar. As the receptor fluid is used to mimic blood circulation beneath the skin, the temperature of the receptor fluid should be kept at 37°C. An appropriate amount of the testing formulation is added into each donor compartment as it is placed on the membrane, and the top of the diffusion cell is opened to mimic nonoccluded conditions. Specific volumes of aliquots of the receptor medium are withdrawn at appropriate time intervals, and simultaneously, the receptor medium is replaced by an equal volume of the fresh receptor medium to maintain the sink conditions. The obtained samples can be analyzed by HPLC or the spectroscopic method.[31,32]

6. Stability of Transfersomes: The stability of transfersome vesicles can be determined by assessing the structure and the size of vesicles with respect to time. DLS and TEM can be used for the determination of the mean size and structural changes, respectively. The optimized transfersomal formulations can be stored in tightly sealed amber vials at different temperature conditions. According to ICH (International Conference on Harmonization) guidelines, under the stability testing of new drug substances and products, the general case for the storage condition is described as, for the long term, 25±2°C/60% relative humidity (RH) ±5% RH or 30±2°C/65% RH ±5% for 12 months and, for accelerated testing, 40±2°C/75% RH ±5% for six months. Drug products intended for refrigeration should be subjected to long-term storage at a condition of 5±3°C for 12 months and accelerated study for 25±2°C/60% RH ±5% RH for six months. A significant change for the drug product is defined as the failure to meet its specifications.[33-35]
CONCLUSION

Transfersomes are ultra-deformable carriers that allow for more effective distribution of a wide range of drug compounds through the skin barrier than traditional vesicular systems. The vesicles are flexible and deformable and thus easily passes through the skin pores for the effective delivery of drugs. They are custom-designed vesicular systems that must be adjusted for specific cases of medicines of interest in order to produce the most effective formulations and pharmacological reactions. Tranferosomes are said to have a variety of applications, including the delivery of vaccines, proteins, anti-cancer drugs, cortico-steroids, anaesthetics, and herbal drugs, and also has improved patient compliance, bio-availability, low toxicity and site specific delivery, and can serve as an emerging tool for transdermal delivery of nearly all drugs and bio-actives.

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