REVIEW ARTICLE

Transfersome: A Novel Technique Which Improves Transdermal Permeability

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Abstract

Transdermal drug delivery offers various advantages on skin. However, its use is limited because the presence of stratum corneum, which serve as barrier to this route. Vesicular system has potential to evade these barriers include liposome, niosome, and transfersome. Transfersome is one of vesicular systems which has highly stress-adaptive, stress-responsive complex aggregate possessing an aqueous core surrounded by complex lipid bilayer. Transfersome has some potential advantages such as the ability to deform and pass through narrow pores, serve as a carrier for both low as well as high molecular drugs. There are some theories describe which affects the formation of transfersome. Transfersome composed of amphipathic ingredient such as phosphatidylcholine, other component is bilayer softening component such as surfactant, along with lipid and surfactant, preparation method for transfersome also alcohol as a solvent in various ratio and water or phosphate buffer solution for hydration of vesicles. The mechanism behind the transfersome penetration into skin is the development of osmotic gradient because of evaporation of surface water due to body heat. There are various methods exist for the preparation of transfersome such as film dispersion method, reverse phase evaporation method, high-pressure homogenization method, and ultrasonic dispersion method. Transfersome is pertinent in the field of insulin delivery, corticosteroid delivery, delivery of protein and peptide, also serve as carrier for anticancer drug, anesthetics, non-steroidal anti-inflammatory drugs, and herbal drug.

Key words: Edge activator, phosphatidylcholine, transfersomes, ultra-flexible

INTRODUCTION

In recent years, research scenario goes toward the development of new type of drug delivery system with the objective of high therapeutic activity along with patient compliance. Many drug delivery systems are developed with improved therapeutic activity, but some complications arise with some delivery systems are not as such overcomes.

Orally administered drugs experience a hostile environment in the gastrointestinal (GI) tract, where most drugs are degraded in variable pH conditions, or face solubility issues, and most importantly first-pass metabolism. In case of parenteral preparation, disadvantages are a lack of drug reversal, hypersensitivity reaction, risk of infection and emboli, and cost. Some drugs much bitter in taste, swallowing of such a bitter medication in oral delivery, and pain associated due to needle in parenteral delivery make them less patient compliance.[1]

From last few decades, considerable attention has been focused on the development of topical delivery of drugs because a number of advantages with this route. Skin in an average adult body covers a surface of approximately 2 m^2 and total weight of 3 kg; it receives about one-third of the blood circulating among the body. Topical drug delivery means the application of drug to skin for localized effect, and in transdermal drug delivery system (TDDS) skin is used as a potential route for the delivery of systemic action of drugs. TDDS is one of the systems with high patient compliance. Some potential advantages of transdermal route found over other conventional routes such as oral- and parenteral-like avoidance of first-pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter- and

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intra-patient variations, and most importantly, it provides patients convenience.[2,3] However, it also has some disadvantages such as possibility of local irritation effect, erythema, itching, and low permeability in the stratum corneum. A major obstacle to dermal and transdermal drug delivery is the permeation characteristics of the stratum corneum, which limits drug transport, making this route of administration frequently insufficient for medical use.[4] Stratum corneum is the top layer of the epidermis consists of keratinized, flattened remnants of once actively dividing epidermal cells, impermeable to water and behaves as a tough flexible membrane. Many technologies and systems have been investigated to evade this barrier including electrophoresis, iontophoresis, chemical permeation enhancers, microemulsions, sonophoresis, as well as utilizing vesicular systems such as liposome, niosomes, ethosomes, and transfersomes, and one of the most promising techniques is to formulate novel vesicular carriers for delivery through the skin as it delivered drug at sustained or controlled manner.[5-10] Various vesicular systems along with their advantages and disadvantages are given in Table 1.

Among all these transfersomes appear promising. A new type of vesicular drug carrier system called transfersome. The term transfersomes and the underlying concept were introduced in 1991 by Gregor Cevc. In broadest sense, a transfersomes is a highly adaptable and stress-responsive, complex aggregate possessing an aqueous core surrounded by a complex of lipid bilayer.[11,12] Transfersome is a term registered as a trademark by the German Company IDEA AG and used by it to refer to its proprietary drug delivery technology. The name means "carrying body" and is derived from the Latin word "transferred" meaning "to carry across," and the Greek word "soma," for a "body." A transfersome carrier is an artificial vesicle designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potentially targeted, drug delivery.[13] Most suitable form of transfersome is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. In terms of delivering of drugs through transdermal route, there are some problems encountered with some other vesicular systems such as poor skin permeability, breaking of vesicles, leakage of drug, and aggregation and fusion of vesicles. To overcome all the above problems, a new type of vesicular carrier has been developed called "transfersome" which is capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes are artificial vesicles, and they are more deformable than standard liposomes. Transfersomes have been reported to enhance the transdermal delivery of drugs when applied onto the skin nonocclusively.[12,14-17]

Advantages of transfersomes[12,17,18]

1. Transfersomes have ability to deform and pass through narrow pores (from 5 to 10 times less than their diameter) without measurable loss
2. This function gives better penetration of intact vesicles
3. They generally have high entrapment efficiency, as in case of lipophilic drug (approximately 90%)
4. They serve as a carrier for both low as well as high molecular weight drugs, e.g., sex hormone insulin analgesic, anesthetic, corticosteroids, anticancer, gap junction protein, and albumin
5. Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result, can accommodate drug molecules with wide range of solubility.

Table 1: Advantages and disadvantages of various vesicular approaches[6]

Methods	Advantages	Disadvantages
Penetration enhancers	Increase penetration through skin and give both local and systemic effect	Skin irritation immunogenicity, only for low molecular weight drugs
Physical methods, e.g., iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (<10%)
Liposomes	Phospholipids vesicle, biocompatible, biodegradable	Less skin penetration, less stable
Proliposome	Phospholipids vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of vesicles
Niosomes	Non-ionic surfactants vesicles, greater stability	Less skin penetration easy handling
Proniosomes	Will convert into niosome *in situ*, stable	But will not reach up to deeper skin layer
Transfersomes and protransfersomes	More stable, high penetration due to high deformability, biocompatible, and biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reach up to deeper skin layers	None, but for some limitations

Limitations of transfersomes[11,17,18]

1. Transfersomes are chemically unstable because of oxidative degradation make its predisposition
2. Purity of natural phospholipids is another criterion for achieve for adoption of transfersomes as drug delivery vehicles
3. Transfersomes are expensive to formulate.

THEORIES AND APPROXIMATIONS IN MICELLE AND VESICLE FORMATIONS

Micelle formation

Law of mass action

If we consider an aqueous solution containing neutral amphiphilic molecules (i.e., non-ionic surfactants), each molecule has a one alkyl chain as its hydrophobic tail. In general, amphiphiles have the ability to form various sizes and shapes aggregates. If we consider each micelle is spherical and there is no any effect of fluctuation on its size and shape, thus we assume that each of which surfactant molecule as monomer or part of *n*-mer. We denote the number densities of the monomers and *n*-mers by ρ_1 and ρn, respectively so that the total surfactant concentration is given by:

$$\rho = \rho_1 + n\,\rho n \quad (1)$$

The monomers and micelles concentration are given by the law of mass action (Chandler, 1986):[19]

$$\rho n\, a_3 = (\rho_1 a_3) \exp(-\beta \Delta G) \quad (2)$$

Where, β represents inverse temperature (i.e., β−1 = kBT), "*a*" is for a microscopic length that specifies the standard state convention and is the driving force for assembly, namely, the free energy of the *n*-mer, "*fn*" represents to that of n monomers, nf_1. We take "*a*" to be approximately the girth of a surfactant molecule.

$$\Delta G = fn - nf_1 \quad (3)$$

For large *n*, Equation (3) applies the existence of a threshold concentration of surfactant molecules ρcmc, at that concentration, the density of aggregates becomes significant. As long as it is physically sensible, due to this intersect is precipitous, its location is almost independent of the specific definition of the threshold. Specifically, to within corrections of order $n-1 \ln n$.

$$\ln \beta \text{cmc}\, a_3 = \beta\, \Delta G \ln^* \quad (4)$$

The driving force per surfactant, $\Delta G/n$, is a function of *n*, and it is to be evaluated at the most probable aggregation number, *n**. This number is the value of *n* that minimizes $\Delta G/n$.

Driving force

The contributions to Δ*G* can be found in three steps:

Creation of a cavity

A region which is vacated by water is filling by micelle. Suppose the extent of the surface is at least 1 nm^2, the free energy required to create this cavity is:

$$\Delta G_1 = \delta A \quad (5)$$

Where "*A*" denotes the surface area of the cavity, while δ represents the water-vapor surface tension. In general, cavity in liquid is formed due to some pressure-volume work. For water, if we consider standard condition, pressure application is too small so that it forms cavities with diameters <5 nm. We will limit our consideration to the vesicle size within this range.

Filling the hydrophobic core

Suppose disconnecting each hydrophobic tail in a surfactant of its relative hydrophilic head group and moving it to micelle core from water. Total of "*n*" tails must be moved to fill the cavity formed in Step 1. As such, one part of the free energy to fill the cavity is $-n\Delta\mu$, where $-\Delta\mu$ is the free energy change in transferring the hydrophobic tail (e.g., an alkane chain) from water into the oily hydrophobic core. An additional part of the free energy for filling the cavity is an interfacial contribution due to the presence of Van der Waals attractions between oil and water. These interactions cause the oil-water surface tension, γo/w, to be lower than the water-vapor surface tension, γ.

Thus, the free energy for filling the cavity is:[20]

$$\Delta G_2 = -n\Delta\mu - \Delta\gamma A \quad (6)$$

Where: $\Delta\gamma = \gamma - \gamma ow$.

The interior of a micelle is densely packed and much likes a hydrocarbon liquid. Thus, Δμ is close to the transfer free energy for moving the associated alkenes chain from oil into water. However, it is slightly smaller than this value because the environment of an alkane chain in a micelle interior is more confining than that in bulk oil.[21] To the extent that the micelle is spherical, $A = 4\pi L2$, where *L* is the micelle radius. Since the interior is densely packed, *L* is given by $A = 4\pi L^{3/3} = n\delta a_2$, where ä is the mean length over which a polar head group is separated from an alkyl group within a surfactant molecule. From these considerations:

$$\Delta G_1 + \Delta G_2 = -n\Delta\mu + \mu g n^{2/3} \quad (7)$$

Where, $g = (36\pi)^{1/3}(\gamma ow a_2) \;\rangle\rangle\; 4.8 \times (\gamma owa2)(\gamma/a)^{2/3}$

The right-hand side of the equation is essentially the free energy for nucleating oil clusters in water.[22] It is the

hydrophobic driving force identified in the Lum–Chandler–Weeks theory.[23] The first term is proportional to the volume of hydrophobic units. The second term is proportional to the area of the interface. The first term is extensive in n and dominates at large *n*. Thus, if only ΔG_1 and ΔG_2 were significant, the strength of the driving force would grow without bound leading to macroscopic clusters.

Placing hydrophilic head groups on the micelle surface

In the last step, the hydrophilic head groups are again connected to the hydrophobic tails, which are at the water-oil interface so as to maintain positive solvation energy. Connectivity between heads and tails and maintaining the densely packed interior, simultaneously enforcing these positioning. These result in an entropic cost that increases super extensively with aggregate size. The form of this third contribution to the driving force is conveniently estimated from the electrostatic analogy of stoichiometric constraints.[24,25]

The result is:

$$\Delta G_3 = hn5/3/\beta \quad (8)$$

Where, $h = \frac{3}{4\pi^{2/3}}\left(\frac{46}{49}\right)\left(\frac{a}{\delta}\right)^{4/3} \approx 0.75\left(\frac{a}{\delta}\right)^{4/3}$

In assuming this analogy, it is important to note that the micelle volume is essentially that of the densely packed alkyl chains.

Micelle size and critical micelle concentration

Combining the three contributions discussed above gives the driving force in units of kBT.

$$\beta\Delta G \approx n\beta\Delta\mu + \beta g n^{2/3} + h n^{5/3} \quad (9)$$

Minimization of $\Delta G/n$ therefore gives:

$$n^* \approx \frac{\beta g}{2h} = \left(\frac{49\pi}{48}\right)\beta\gamma\delta^2 \quad (10)$$

With this aggregation number:

$$\ln \rho cmca3 = c(\beta\gamma owa2)^{2/3} - \beta\Delta\mu \quad (11)$$

Where,. $c = \left(\frac{5832}{49}\right)^{1/3} \approx 4.9$

The thermodynamic cycle of micelle formation can be observed in Figure 1.

Correlation between amphiphile structures with its phase behavior could be understood by a simple geometric model, which defines a dimensionless critical packing parameter (CPP) to describe the relative bulkiness of the hydrophobic part and the hydrophilic part in an amphiphile. With the CPP value increasing from a small to a high, the amphiphile changes from hydrophilic to hydrophobic, its preferred phase structure from direct structures through lamellar structure to reverse structures. This model provides a basis for the molecular design of amphiphiles.

When the size is small and molecules experience stress at that time molecules on the surface feels curvature.

The process of assembling *n* separated amphiphiles (a) to a micelle (d) can be performed in three steps: (1) creating a cavity in the solvent (light gray) (b), (2) transferring the hydrophobic chains (dark gray) from the aqueous solution into the cavity (c), (3) distributing the polar units (gray) over the surface of the cavity, and reconnecting them to the hydrophobic groups (d).

The stress plays a significant role when the size is below 100 nm. Generally, for bilayer formation, the molecules must be amphiphilic. Typical geometry for a vesicle formation is:

$$P = V/a_0\, Ic \quad (12)$$

Where:
P: Critical packing parameter,
Ic: Hydrophilic chain length,
V: Volume of hydrophilic part, and
a_0: Optimum surface area/molecule at interface.[26]

A pictorial representation of geometry and variants of packing arrangements are shown in Figures 2 and 3, respectively.[27]

Vesicle formation

Formation of large vesicles from small, preformed vesicles[28-32]

Small, uniform-sized vesicles were prepared as described. The vesicle solution contains solute to be entrapped was then warmed to 25°C, and a solution sodium deoxycholate was rapidly added and mixed to give a final mixture having a ratio of 1:2 of deoxycholate to phospholipids, respectively. The large vesicles start forming almost immediately, as indicated by the increase in the light scattering of the solution, a change from nearly clear for the small vesicles to a transparent opalescence for the large vesicles.

Vesicle forms within 5-10 min at 250°C (vesicles could also be formed at 40°C and required 15-30 min). The excess of the detergent was then removed by passage of the sample over 60 volume of Sephadex G-25 (medium porosity). The residual deoxycholate may represent detergent trapped within the vesicle

General procedures for vesicular preparations are:
- Direct hydration
- Hydration from organic solvent, and
- Detergent removal.

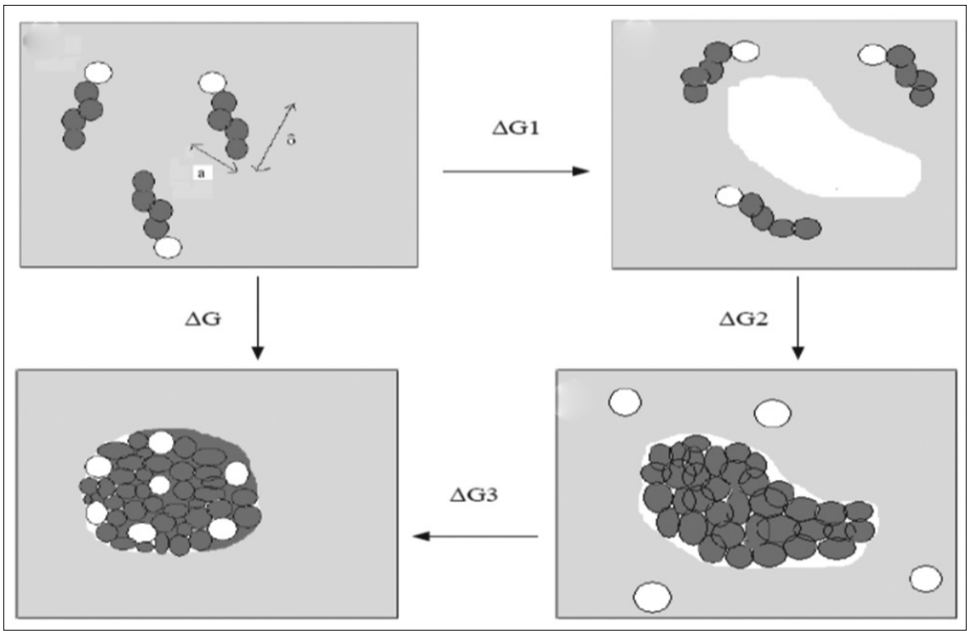

Figure 1: Thermodynamic cycle of micelle formation

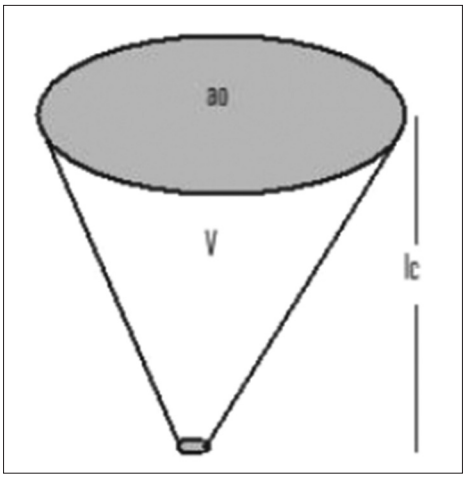

Figure 2: Representation of typical geometry for a vesicle formation

Hydration from organic solvent method is used for producing multilamellar vesicles (MLVs). MLVs extrusion through 0.1 μm polycarbonate filter and plus freeze-thaw technique, reverse phase evaporation, and detergent removal procedures are used for producing large unilamellar vesicles. Sonication is used for producing small unilamellar vesicles. Direct hydration procedure has certain advantages such as it is fast procedure. Its disadvantages include low trapped volume, low trapping efficiency, and unequal distribution of solute. The advantage of hydration from organic solvent procedure is high trapping efficiency, and its disadvantages include technically complex and limited by lipid solubility in organic phase. Detergent removal procedure has advantages such as reconstitution of proteins possible, high trapped volume, and its disadvantages are its difficult to remove detergents completely, and low trapping efficiency.

VESICLES AND ITS HYBRIDS

Liposomal formulations can be classified into two categories:

- Rigid vesicles – Liposomes and niosomes
- Elastic or ultradeformable vesicles – Transfersomes and ethosomes.

IMPORTANCE OF TRANSFERSOMES FOR SKIN DELIVERY

Transfersomes have an advantage as phospholipids vesicles used for transdermal drug delivery. Due to their self-optimized and ultra-flexible membrane properties, they successively deliver drug either into or through the skin, depends on the choice of administration or application, along with high efficiency. Transfersomes are superior to the standard liposomes in elastic property and thus more suitable for the skin penetration. Transfersomes squeezing themselves along the intracellular sealing lipid of the stratum corneum and overcome the skin penetration difficulty. Mechanism described in Figure 4. Transfersomes have high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-assembling manner. Flexible nature of transfersomes membrane is achieved by addition of suitable surface-active components in phospholipids in proper ratio.[33] Flexibility in transfersomes membrane reduces the risk of vesicle rupture in the skin and permits transfersomes to follow the natural water gradient across the epidermis when applied under non-occlusive condition. Transfersomes spontaneously penetrate the intact stratum corneum along with two routes in the intracellular lipid that differ in their bilayers properties.[34] Figure 4 shows available microroutes for drug penetration across human

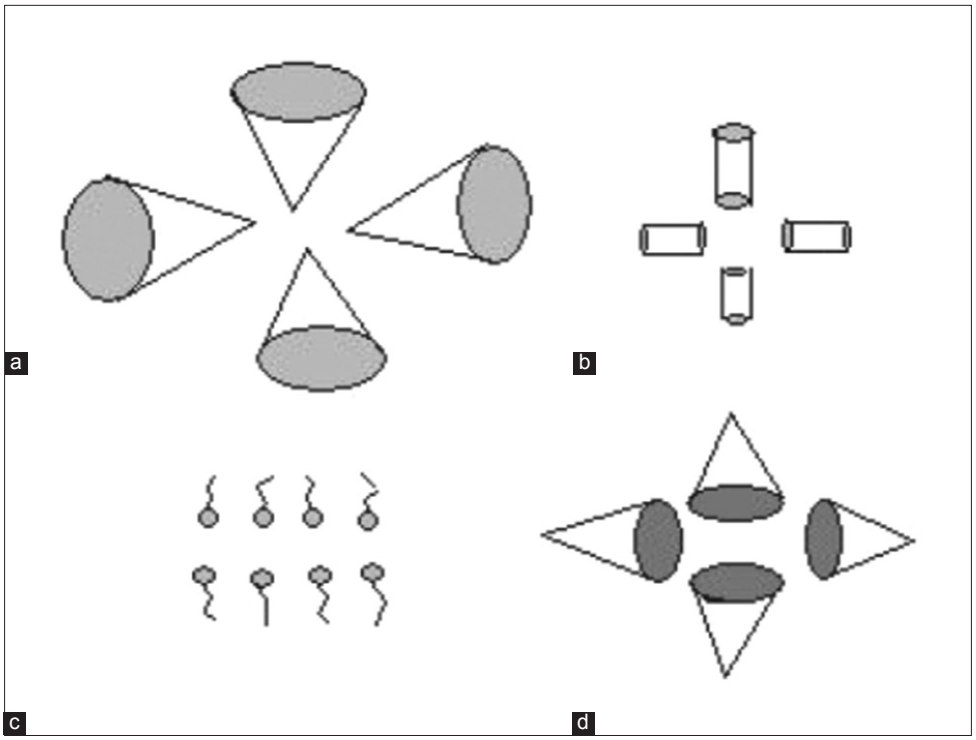

Figure 3: (a) $P = 1/3$, micelle formation, (b) $P = 1/2$, cylindrical micelle, (c) $P = V/a_0 lc$ $P = 1$, planar bilayer, (d) $P > 1$, inverted micelle

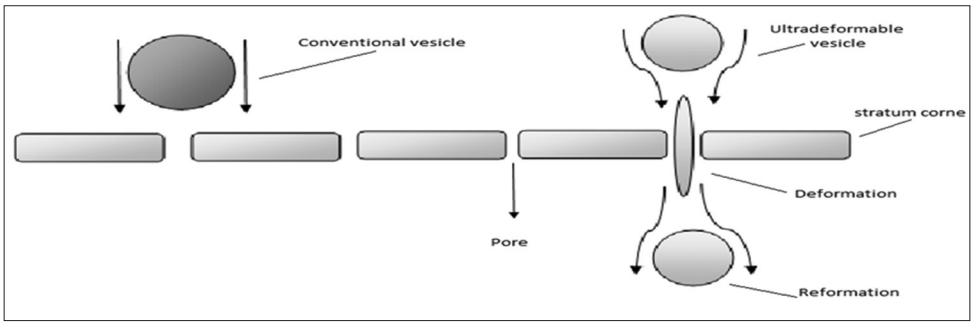

Figure 4: Deformability of transfersomes into skin pores[11]

skin intracellular and transcellular. Liposome discovered in 1963 by Bingham since from then attraction toward vesicular system is increased.[35] However, recently, it observed that liposomes have low penetration capacity. According to confocal microscopic studies observed that intact liposomes are unable to penetrate into a granular layer of epidermis, they remain on the upper surface layer of stratum corneum. Drug release rate and the deposition to the target site can be adjusted by modification of vesicular composition or surface property of transfersome membrane.[36]

COMPOSITION OF TRANSFERSOMES[18]

The transfersome has two main components:

- First one is amphipathic ingredient (such as phosphatidylcholine), which is in aqueous solvent self-assembled into lipid bilayer that forms a simple vesicle
- Second is bilayer softening component (such as a biocompatible surfactant or an amphiphilic drug), so lipid bilayer membrane flexibility and permeability are increased.

Transfersome vesicle can, therefore, gain its shape easily and rapidly, by adjusting available concentration of each bilayer component to the local stress experienced by the bilayer as shown in Figure 5. Therefore, the transfersome differs from conventional vesicle mainly by its physical properties such as softer, more deformable, and better adjustable artificial membrane.

Different additives used for preparation of transfersome[14,37-39]

Widely used materials for preparation of transfersomes are phospholipids, surfactant as edge activator, alcohol, dye

buffering agent, etc., example of above material given in Table 2.

MECHANISM OF ACTION[37,40]

Mechanism behind the penetration of transfersome is the development of osmotic gradient because while lipid suspension applies on skin surface water gets evaporated. Transfersomes have strong bilayer deformability and therefore they have increased affinity to bind and retain water. Dehydration is not happened in case an ultradeformable and highly hydrophilic vesicle; it is not identical to forward osmosis but may involve in transport process related to forward osmosis. Upon application on skin surface (non-occluded), it penetrates skin barrier and reaches at the deeper strata (water rich portion), where they get hydrated. Then, reach at deeper epidermal layer through dehydration of lipid vesicles within the stratum corneum by natural transepidermal activity. Therefore, transfersome uptake is a function of hydration gradient that exists across the epidermis, stratum corneum, and ambient atmosphere.

PREPARATION OF TRANSFERSOME[41,42]

The preparation methods of transfersomes are classified into the following four types: Film dispersion method, reverse evaporation method, high-pressure homogenization method, and ultrasonic dispersion method. Film dispersion method is the one of the common preparation methods of lipophilic drug transfersomes.

A. Thin film hydration technique is employed for the preparation of transfersomes which comprised three steps:[12,18,43]
   1. A thin film is prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in a volatile organic solvent (chloroform-methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50°C for dipalmitoylphosphatidylcholine) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight.
   2. A prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 h at the corresponding temperature. The resulting vesicles were swollen for 2 h at room temperature.
   3. To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min using a bath sonicator or probe sonicated at 4°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

B. Modified handshaking method, lipid film hydration technique is also founded for the preparation of transfersomes which comprised following steps:[44-47]
   1. Drug, lecithin (PC), and edge activator were dissolved in ethanol:chloroform (1:1) mixture.

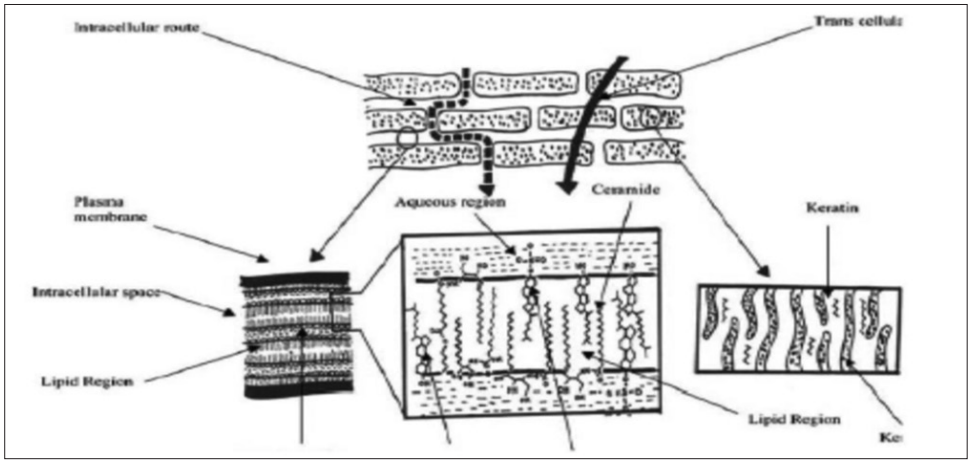

Figure 5: Micro routes for drug penetration across human skin[11]

Table 2: Different material with example and use

Class	Example	Uses
Phospholipids	Soya phosphatidylcholine, dipalmitoylphosphatidylcholine, distearoyl phoshatidylcholine	Vesicles forming component
Surfactant	Sodium cholate, sodium deoxycholate, tween-80, Span-80	For providing flexibility
Alcohol	Ethanol, methanol, diethyl ether	As a solvent
Dye	Rhodamine-123, rhodamine DHPE, fluorescein-DHPE Nile-red	For CSLM study
Buffering agent	Saline phosphate buffer (pH 6.4) phosphate buffer pH 7.4	As a hydrating medium

Organic solvent was removed by evaporation while handshaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent.

2. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 min at corresponding temperature. The transfersome suspension further hydrated up to 1 h at 2-8°C.

OPTIMIZATION OF FORMULATION CONTAINING TRANSFERSOMES[46]

There are some process variables such as lecithin, surfactant ratio, effect of various solvents, effect of various surfactants, and hydration medium. This could affect the preparation and properties of the transfersomes. Procedure for preparation of transfersomes will accordingly optimize and validate. The process variables depend on the procedure for manufacturing of formulation.

Entrapment efficiency of drug is the tool used for optimization. Other variables were kept constant at the time of preparation of particular system.

CHARACTERIZATION OF TRANSFERSOMES

The characterization of transfersomes is generally similar to liposomes, niosomes, and micelles.[48] The following characterization parameters have to be checked for transfersomes.

1. Vesicle size distribution and zeta potential:[11,12]
   Dynamic light scattering method (DLS) using a computerized inspection system by Malvern Zetasizer used for determination of vesicle size, size distribution, and zeta potential.
2. Vesicle morphology:[11,12]
   Photon correlation spectroscopy or DLS method generally used for vesicle diameter determination. Prepared sample in distilled water was filtered through 0.2 mm membrane filter and diluted with filtered saline and then size measurement done using photon correlation spectroscopy or DLS measurements. Transmission electron microscopy (TEM) and phase contrast microscopy can be commonly used for visualization of transfersomes vesicles. The stability of vesicle can be determined by assessing the size and structure of vesicles with respect to time. DLS and TEM used for mean size and structural changes, respectively.
3. Number of vesicles per cubic mm:[18]
   This parameter is very important for optimization of composition and other process variables. Transfersome formulations which are unsonicated are diluted 5 times with 0.9% sodium chloride solution. Hemocytometer and optical microscope are used for further study.
   The transfersomes in 80 small squares are counted and calculated using the following formula:
   Total number of transfersomes per cubic mm = (Total number of transfersomes counted × dilution factor × 4000)/total number of squares counted
4. Entrapment efficiency:[12]
   Generally, expressed in terms of % drug entrapment. In this method, unentrapped drug first separated using minicolumn centrifugation method. After that, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as:
   Entrapment efficiency = (Amount entrapped/Total amount added) × 100
5. Drug content:[49]
   The drug content is determined using one of the instrumental analytical methods such as a modified high-performance liquid chromatography method using an ultraviolet detector, column oven, auto sample, pump, and computerized analysis program depending on the analytical method of the pharmacopoeial drug.
6. Turbidity measurement:[12]
   Nephelometer is one of the methods which generally used for turbidity measurement in aqueous solution.
7. Degree of deformability or permeability measurement:[12-43]
   Permeability study is one of the important and unique parameters for characterization in case of transfersomes. The deformability study is done by taking pure water as standard. Transfersomes preparation is passed through a number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by DLS measurements.
8. Penetration ability:[18-43]
   Fluorescence microscopy can generally use for evaluation of penetration ability of transfersomes.
9. Occlusion effect:[12]
   Occlusion of skin is considered to be useful for permeation of drug in case of traditional topical preparations. However, the occlusion also proves to be harmful for elastic vesicles. Hydrotaxis is the major driving force for permeation of vesicles through the skin is hydrotaxis (movement in the direction) of water, from its relatively dry surface to water rich deeper regions. It affects hydration forces as it prevents evaporation of water from skin.
10. Surface charge and charge density:[12,18]
    Surface charge and charge density of transfersomes can be determined using zetasizer.
11. *In-vitro* drug release:[12,18]
    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 *in vitro* 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 minicolumn 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).

12. *In vitro* skin permeation studies:[46-50]
    Modified Franz diffusion cell with a receiver compartment volume of 50 ml and effective diffusion area of 2.50 cm^2 was used for this study. *In vitro* drug study was performed using goat skin in phosphate buffer solution (pH 7.4). Fresh abdominal skin of goat was 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 upward toward the donor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2.50 cm^2 and capacity of receptor compartment was 50 ml. The receptor compartment was filled with 50 ml of phosphate buffer (pH 7.4) saline maintained at 37 ± 0.5°C and stirred by a magnetic bar at 100 rpm. Formulation (equivalent to 10 mg 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 the calculation of release profile. The samples were analyzed by any instrumental analytical technique.
13. Physical stability:[47-49]
    The initial drug entrapped (percent) in the formulation was determined and was stored in sealed glass ampoules. The ampoules were placed at 4 ± 2°C (refrigeration), 25 ± 2°C (room temperature), and 37 ± 2°C (body temperature) for at least 3 months. Samples from each ampoule were analyzed after 30 days to determine drug leakage. Percent drug loss was calculated by keeping the initial entrapment of drug as 100%.

APPLICATION OF TRANSFERSOMES

1. Delivery of insulin:[51]
   Transfersome is one of the successive ways to deliver such large molecular weight drugs on the skin. Insulin is generally administered by subcutaneous route that is inconvenient for patient. Encapsulation of insulin in transfersome (transfersulin) overcomes all problems arises with conventional insulin delivery. After application of transfersulin on the intact skin, therapeutic effect observed after 90-180 min, depending on the carrier composition.
2. Delivery of corticosteroids:[33]
   Problems arise with corticosteroids delivery is mask by incorporation it into transfersomes. Site specificity and overall drug of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose safety is achieved by transfersome encapsulation. Dose required for biological activity of corticosteroid is less by use of transfersomes technology.
3. Delivery of proteins and peptides:[52,53]
   Transfersomes have been widely used as a carrier for the transport of proteins and peptides also safely given by means of transfersome technology. Proteins and peptide has problem is it is difficult to transfer into the body, are large biogenic molecules, GI tract degradation is problem arise when given orally. That's reasons why these peptides and proteins still given by means of injectables. A number of approaches have been developed to improve this condition. Transfersome is somewhat identical to that resulting from subcutaneous injection of protein suspension in terms of bioavailability. On repeated epicutaneous application, transfersome preparation of protein also induced a strong immune response. For example, the adjuvant immunogenic serum albumin in transfersomes, after several dermal challenges, is as active immunologically as is the corresponding injected proteo-transfersomes preparations.
4. Delivery of interferon (INF):[54]
   INF also delivered using transfersome as a carrier, for example, leukocyte-derived INF-α is a naturally occurring protein having antiviral, antiproliferative, and some immunomodulatory effects. Transfersomes as drug delivery systems have the potential for providing controlled release of the administered drug and increasing the stability of labile drugs. Hafer *et al*. studied the formulation of transfersome containing interleukin-2 (IL-2) and INF-α for potential transdermal application. They reported delivery of IL-2 and INF-α promising by transfersomes insufficient concentration for immunotherapy.
5. Delivery of anticancer drugs:[11,55]
   Transfersome technology provides a new approach for cancer treatment, especially skin cancer. Result found to be favorable when methotrexate was tried for transdermal delivery using transfersome technology.
6. Delivery of anesthetics:[11]
   Application of transfersome containing anesthetics induces a topical anesthesia, under suitable conditions, within 10 min. Effect when we said in case of pain in sensitivity is nearly as strong (80%) as of a comparable subcutaneous bolus injection, but transfersomal anesthetics preparation has last longer effect.
7. Delivery of non-steroidal anti-inflammatory drugs (NSAIDs):[56]
   Problems arise with most of NSAIDs are a number of GI side effects. This can be overcome by transdermal delivery using transfersome. Studies have been carried out on diclofenac and ketoprofen. Ketoprofen in a

Table 3: Different studies related to transfersomes

Drug	Category	Result	References
Insulin	Antidiabetic	The *in vivo* study of optimized in alloxan- induced diabetic rat demonstrated prolonged hypoglycemic effect in diabetic rats over 24 h after transdermal administration	[57]
Itraconazole	Anti-fungal	The study on itraconazole-loading transfersomes provided the key technical support for the preparation development of lipophilic itraconazole with transdermal administration. The obtained transfersomes can further be made into transdermal gel	[42]
Carvedilol	-blocker	The nanotransfersomal vesicles were significantly more efficient in nasal delivery of carvedilol with absolute bioavailability of 63.4%	[58]
Piroxicam	COX-2 inhibitor	The study substantiated that the transfersomal gel can be used as a feasible alternative to the conventional formulations of piroxicam with advanced permeation characteristics for transdermal application	[59]
Terbinafine hydrochloride	Anti-fungal	The terbinafine hydrochloride transfersomes was highly absorbed by the skin. The absorption rate was significantly higher than that of the commercial cream either in the transdermal test *in vitro* or in the pharmacokinetic studies *in vivo*	[60]
Diclofenac	NSAIDs	Diclofenac lotion comprising ultradeformable vesicles, transfersomes, combines the safety of the conventional diclofenac formulations for the topical administration with a high or even improved efficacy. The latter matches that of the best available oral preparations of such drug. This suggests that the simple to use, topical transfenac formulations have the potential to replace oral therapy with diclofenac. We believe that such novel formulations will compete successfully with the combined oral/topical treatment of rheumatoid disease	[61]
Repaglinide	Antidiabetic	Tranfersome improves the transdermal delivery, side specificity, prolongs the release of drug repaglinide	[62]

NSAIDs: Non-steroidal anti-inflammatory drugs, COX-2: Cyclooxygenase-2

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.

8. Delivery of herbal drugs:[55]
   Herbal drug also delivered by transfersome approach. Xiao-Ying *et al*. who shows the better topical absorption of transfersomes of capsaicin in comparison to pure capsaicin.

RELATED RESEARCH ARTICLES

Related to transfersome various research articles published in various reputed journals using various drugs. Some of them discuss in Table 3.

CONCLUSION

From above discussion and research work carried out by various researcher, it can be concluded that transfersome is one of the promising novel drug delivery systems with reference to its stability, biocompatibility, reduce toxicity, and extended controlled delivery of drug which are generally plays an important role in case of topical or transdermal delivery. By topical or transdermal route vesicular system is more promising as compared to conventional system. Transfersome is one of the vesicular systems which are highly effective than other vesicular lipid systems such as liposome and noisome. Its highly deformable ultra-flexible structure makes it as a suitable carrier which carries a high amount of drug alternately improves bioavailability, improves controlled release pattern of drug.

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