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Transferomes: Emerging Trends for Drug Delivery

Krishna moorthy S.B*[1], Sandeep. J[2], Jyothirmaie. K[3], Rajalakshmi.R[1]

[1]*Department of Pharmaceutics, Sree Vidyanikethan College of Pharmacy, A. Rangampet, Tirupathi, Andhra Pradesh, India-517102.*

[2]*Department of Pharmaceutics, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi, Andhra Pradesh, India-517503.*

[3]*Department of Pharmaceutics, Jagan's College of Pharmacy, Muthukur, Nellore, Andhra Pradesh, India- 524002.*

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Abstract

Transferosomes have become increasingly apparent that vesicular drug delivery elicits modest possessions in drug targeting. Transfersomes are a form of elastic or deformable vesicle, which were first introduced in the early 1990s. Elasticity can be achieved by using an edge activator in the lipid bilayer structure. 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.

Keywords: Transferosomes, stratum corneum, phospholipids, elasticity, transdermal.

Contents

***Corresponding author**
Krishna moorthy S.B
Department of Pharmaceutics,
Sree Vidyanikethan College of Pharmacy,
Tirupathi, Andhra Pradesh, India-517102.
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1. Introduction

In the last few years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs. These vesicles are preferred over other formulations because of their specific characteristics such as lack of toxicity, biodegradation, capacity of encapsulating both hydrophilic and lipophilic molecules, capacity of prolonging the existence of the drug in the systemic circulation by encapsulation in vesicular structures, capacity of targeting the organs and tissues, capacity of reducing the drug toxicity and increasing its bioavailability. Vesicular systems such as transferosomes formulations have been used as drug delivery vehicles for sustained release of proteins and peptides. These formulations have been used as carriers of cytotoxic drugs with the strategy based on reduction of toxicity and passive delivery to tumors [1-6].

Transfersome is a term registered as a trademark by the German company IDEA AG, and used by it to refer to its drug delivery technology. The name means "carrying body", and is derived from the Latin word 'transferre', 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 [7, 8].

Transfersomes (transfersomes) offer a versatile delivery concept for improving the stability as well as the potential to be used with a wide range of active compounds. They are (quasi) metastable, which makes the vesicle membrane ultra-flexible, and thus, the vesicles are highly deformable that squeeze through pores in the stratum corneum less than one-tenth of their own diameter when applied under non-occlusive conditions. Thus, even sizes up to 200–300 nm can penetrate intact skin. It is primarily due to the remarkable strong membrane adaptability that allows the Transfersomes vesicles to lodge in a confining pore, and thus permeate that pore [9-11].

2. Description

A. Mechanism of Penetration

Transferosomes when applied under suitable condition can transfer 0.1 mg of lipid per hour and cm^2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradients. The reason for this high flux rate is naturally occurring "transdermal osmotic gradients" i.e. another much more prominent gradient is available across the skin.[12] This osmotic gradient is developed due to the skin penetration barrier, prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content).[13] 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 Consequently all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration, So when lipid suspension (transfersomes) is placed on the skin surface, that is partly dehydrated by the water evaporation loss and then the lipid vesicles feel this "osmotic gradient" and try to escape complete drying by moving along this gradient [14, 15]. A Transfersomes 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.

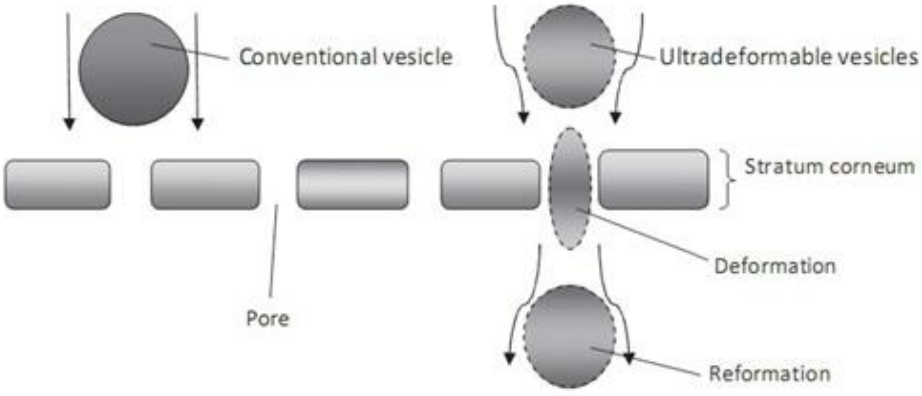

Figure 1. Schematic diagram of the two micro routes of penetration

Transferosomes when placed on skin surface
↓
Dehydrated by water evaporation loss
↓
Lipid vesicle feels osmotic gradient
↓
Move along this gradient, deform to pass to pass through pores in skin

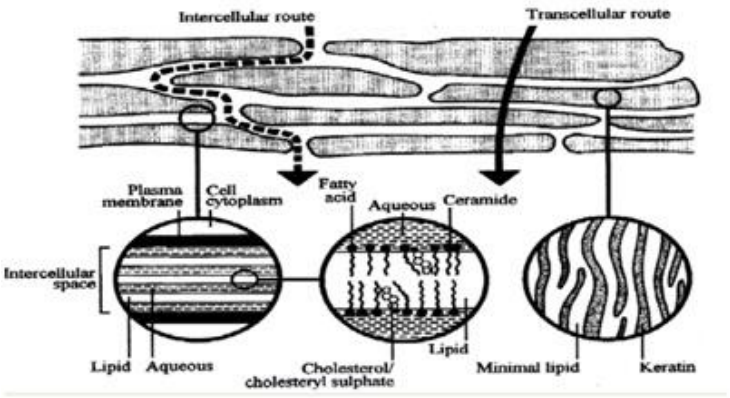

Figure 2. Schematic diagram of the penetration mechanism.

A. Transcellular mechanism: In this pathway hair follicles and sweat ducts offers pores that by pass the stratum corneum.
B. Intercellular mechanism**:** In this type of route drug directly goes to the systemic circulation via lipid matrix between keratocytes.
C. Intracellular mechanism: In this route drug molecule penetrates directly across the stratum corneum by diffusion method. Stratum corneum is the main barrier for drug molecules from transdermal drug delivery system. These are three main pathways for drug molecules to penetrate stratum corneum. Hydrophilic drugs permeate by Intercellular pathway and lipophilic drugs permeate by Intracellular (Transcellular) mechanism [16].

Pathways of drug penetration [17,18]

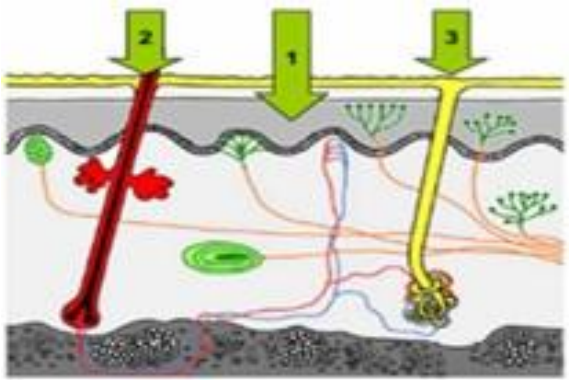

Figure 3. Drug penetration pathways.

1. Through stratum corneum , 2. Transfollicular, 3. Through sweat gland

B. Structure of A Transferosome

Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility Vesicles are water-filled colloidal particles. The walls of these capsules consist of amphiphilic molecules (lipids and surfactants) in a bilayer conformation. In an excess of water these amphiphilic molecules can form one (unilamellar vesicles) or more (multilamellar vesicles) concentric bilayers. Hydrophilic drugs can be entrapped into the internal aqueous compartment, whereas amphiphilic, lipophilic and charged hydrophilic drugs can be associated with the vesicle bilayer by hydrophobic and/or electrostatic interactions [19].

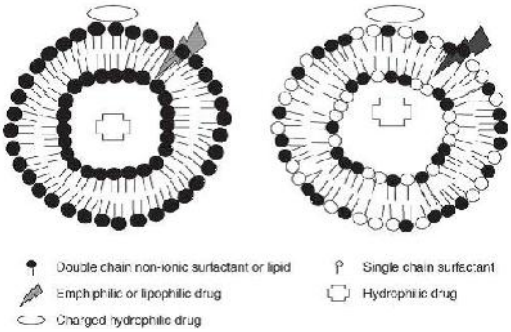

Figure 4. Structure of trasferosomes

C. Preparation of Transferosomes

Thin film hydration technique

1. A thin film is prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in 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 dipalmitoyl, phosphatidyl choline) 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 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr 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.

D. Modified hand shaking, lipid film hydration technique

1. Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking 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 minute at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8°C [20-23]

E. Optimization of Formulation Containing Transfersomes

There are various process variables which could affect the preparation and properties of the Transfersomes. The preparation procedure was accordingly optimized and validated. The process variables are depending upon the procedure involved for manufacturing of formulation. The preparation of transfersomes involves various process variables such as,

a. Lecithin: surfactant ratio
b. Effect of various solvents
c. Effect of various surfactants
d. Hydration medium

Optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant.[24, 25].

Table 1. Different Additives Used in Formulation of Transfersome.

Class	Example	Uses
Phospholipids	Soya phosphatidyl Choline, Dipalmitoyl Phosphatidyl choline, Distearoyl phoshatidyl choline.	Vesicles forming component
Surfactant	Sod. Cholate, Sod.deoxycholate tween-80 Span-80	Providing flexibility
Alcohol	Ethanol	solvent
Dye	Rhodamine-123, Rhodamine-DHPE, Fluorescein-DHPE Nilered	CSLM study
Buffering Agent	Saline phosphate buffer (pH 6.4)	hydrating medium

F. Characterisation of Transferosomes

I. Vesicle size, size distribution and vesicle Diameter [26]

Transfersomes can be visualized by transmission electron microscopy (TEM) and vesicle size and size distribution can be determined by dynamic light scattering (DLS) technique. 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 measurements.

II. Vesicle shape and type

Transfersomes vesicles can be visualized by using TEM, with an accelerating voltage of 100 kv. These vesicles can be visualized without sonication by phase contrast microscopy by using an optical microscope. Dynamic light scattering is also used for determining vesicle shape.

III. Number of vesicle per cubic mm [27]

This is an essential parameter for optimizing the composition and other process variables. Transfersome formulations (without sonication) can be diluted five times with 0.9% of sodium chloride solution and studied with optical microscopy by using haemocytometer.

$$\text{Total number of vesicles per cubic mm} = \frac{\text{total number of transferosomes counted} \times \text{dilution factor}}{\text{total number of squares counted}} \times 4000$$

IV. Degree of Deformability or Permeability Measurement

Degree of deformability is an important and unique parameter of transfersomal formulations because it differentiates transfersomes from other vesicular carriers like liposomes that are unable to cross the stratum corneum intact. 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. The degree of deformability was calculated by using the following formula, as reported by Berge vanden et al. [28]

$$D=J\times(rv/rp)2$$

where,

D = deformability of vesicle where,

D = deformability of vesicle membrane,

J = amount of suspension, which was extruded during 5 min

rv = size of vesicles (after passes) and

rp = pore size of the barrier.

V. Propensity of penetration

The magnitude of the transport driving force plays an important role:

Flow = Area x (Barrier) Permeability x (Trans-barrier) force

Therefore, the chemically driven lipid flow across the skin always decreases dramatically when lipid solution is replaced by the same amount of lipids in a suspension.

VI. Confocal Scanning Laser Microscopy (CSLM) study

Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for following purpose:

1. For investigating the mechanism of penetration of transfersomes across the skin,
2. For determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways. For comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles. Different fluorescence markers used in CSLM study are

a. Fluorescein-DHPE(1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine-N-(5-fluores denthiocarbamoyl), triethylammonium salt)
b. Rhodamine-DHPE (1,2- dihexadecanoyl-sn-glycero- 3ogisogietgabikanube-N-LissamineTmrhodamine B sulfonyl), triethanolaminesalt)
c. NBD-PE (1, 2-dihexadecanoylsn- glycero-3- phosphoethanolamine-N-(7-nitro-Benz-2- oxa-1, 3-diazol- 4-yl) triethanolamine salt)
d. Nile red

VII. Drug Content

The drug content can be determined using a modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program.

VIII. Entrapment efficiency

Entrapment efficiency was determined by first separation of un-entrapped drug by the use of minicolumn centrifugation method. After centrifugation, the vesicle was disrupted using 0.1% Triton X-100 or 50% n-propanol and then followed by suitable analytical technique to determine the entrapped drugs.

IX. In vitro Drug Release [29, 30]

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 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).

X. Surface charge and charge density, Turbidity measurement:

Surface charge and charge density of transferosomes can be determined using zetasizer. Turbidity of drug in aqueous solution can be measured using nephelometer.

XI. *IN-VIVO*

1. Tape stripping method

Tape stripping technique is a widely accepted and it is used to examine the localization and distribution of substances within the stratum corneum. It is the simplest method for reducing the barrier imposed by the stratum corneum is to remove it. In this technique, an adhesive tape removes a layer of corneocytes. *In vivo*, removal of the stratum corneum by tape stripping is performed by the repeated application of adhesive tapes to the skin's surface. This can be used to investigate stratum corneum cohesion *in vivo* by quantifying the amount of stratum corneum removed. Tape stripping method can be studied in combination with electron microscopy and FT-IR. There are different parameters that can affect the quantity of stratum corneum removed by a piece of tape, and these include tape stripping mode, skin hydration, cohesion between cells, the body site and inter-individual differences.

2. Occlusion Effect

Occlusion of skin is considered to be helpful for permeation of drug in case of traditional topical preparations. But the same proves to be detrimental for elastic vesicles. Hydrotaxis (movement in the direction) of water is the major driving force for permeation of vesicles through the skin, from its relatively dry surface to water rich deeper regions. [28] Occlusion affects hydration forces as it prevents evaporation of water from skin.

XII. Stability Studies

Transfersomes stability was determined at 4°C and 37°C by TEM visualization and DLS size Measurement at different time intervals (30, 45, and 60 days), following vesicles preparation.

G. Limitations of Transfersomes

a. Like liposomes, transfersomes have certain limitations:
b. Transfersomes are chemically unstable because of their predisposition to oxidative degradation.
c. Lack of purity of the natural phospholipids comes in the way of adoption of transfersomes as drug delivery vehicles.
d. Transfersomes formulations are expensive to prepare.

H. Application of Transfersomes

1. Delivery of insulin [31]

Transfersomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin. Transfersulin application on the intact skin, the first sign of systemic hypoglycaemia are observed after 90 to 180 min, depending on the specific carrier composition.

2. Delivery of proteins and peptides [32, 33]

The transfersomal preparations of protein induced strong immune response after the repeated epicutaneous application, for example the adjuvant immunogenic bovine serum albumin in transfersomes, after several dermal challenges is as active immunologically as is the corresponding injected proteo-transfersomes preparations.

3. Delivery of steroidal hormones and peptides [34]

Transfersomes based cortiosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases. Flexible vesicles of ethinylestradiol showed significant anti-ovulatory effects as compared to plain drug given orally and traditional liposomes given topically**.** Extensive work has been done on other drugs like hormones and peptides viz Estradiol, low molecular-weight Heparin, Retinol, Melatonin.

4. Delivery of Anticancer Drugs [35]

Anti cancer drugs like methotrexate were tried for transdermal delivery using transfersome technology for treatment of skin cancer.

5. Delivery of Herbal Drugs [36]

Transfersomes of Capsaicin has been prepared by Xiao-Ying et al. which shows the better topical absorption in comparison to pure capsaicin.

6. Delivery of NSAIDS [37]

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.

7. Delivery of Anesthetics

Transfersome based formulations of local anesthetics- lidocaine and tetracaine showed permeation equivalent to subcutaneous injections.

I. Future Aspects [38]

This relates to potential use of self-regulating, ultradeformable carriers in devices (patches; electrically controlled epicutaneous reserviors), and in design of formulation with additional special features, allowing, e.g., targeting of cellular subsets. The nearest term goal that remains to be reached is expansion of the positive experiences with NSAID targeting into peripheral tissues to other drugs with similar therapeutic demands.

Table 2. List of drugs used for transfersomes

S.No	Drug	Inference
1	Oestradiol	Improved transdermal flux
2	Norgesterol	Improved transdermal flux
3	Hydrocortosone	Biologically active at dose several times lower than currently used formulation.
4	Human serum albumin	Antibody titter is similar or even slightly higher than subcutaneous injection.
5	Interferon-α	Controlled release, Overcome stability problem. High encapsulation efficiency.
6	Insulin	Transfer across the skin with an efficiency of >50%. Provide non-invasive means of therapeutic use

3. Conclusion

The transdermal route of drug administration has been a route of choice since ancient times because of its merits. But it itself limits its use as it is unable to transport larger molecules, penetration through the stratum corneum is the rate limiting step, physicochemical properties of drugs hinder their own transport through skin. The development of novel approaches like transferosomes have immensely contributed in overcoming these problems. These elastic vesicles can squeeze themselves through skin pores many times smaller than their own size and can transport larger molecules. Since a properly designed ultradeformable may even claim the transport of drug equivalent to the subcutaneous injection, this technology can provide effective tool for non-invasive therapy When tested in artificial systems transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller This carrier system does not depend upon the concentration gradient and mainly works on the principle of hydrotaxis and elasto-mechanics.

Ultra deformable vesicles hold great prospective in delivery of huge range of drug substances which includes large molecules like peptides, hormones and antibiotics, drugs with poor penetration due to unfavorable physicochemical characters, drugs for quicker and targeted action, etc.. The bio-distribution of radioactively labelled phospholipids applied in the form of transfersomes after 24 h is essentially the same after an epicutaneous application or subcutaneous injection of the preparations. The use of transfersomes carrier result in delivery of high concentration of active agents to/through the skin, regulated by system composition and their physical characteristics.

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