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Review Article

DRUG CARRIER TRANSFERSOMES: A NOVEL TOOL FOR TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

Transport of the drug through skin is best route of drug delivery because of the skin is largest organ human organ with total weight 3 kg and a surface of 1.5 -2.0 m^2. Drug carries used in transdermal drug delivery such as liposomes, noisomes, or microemulsions has problem that they remains mostly confined to the skin surface and therefore do not transport drugs efficiently through the skin. By using the concept of rational membrane design we have recently devised special composite bodies, so-called Transfersomes. Transfersomes penetrate through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form. This is because of its deformable nature. The system can be characterized by in vitro for vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm. 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.

Keywords: Transfersomes, Transdermal delivery, Liposomes, Phospholipids.

INTRODUCTION

Transdermal route offers several potential advantages over conventional routes 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 intra-patient variations, and most importantly, it provides patients convenience [**1**, **2**]. 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 [**3**].

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 transferosomes appear promising. A novel vesicular drug carrier system called transfersomes, which is composed of phospholipid, surfactant, and water for enhanced transdermal delivery. Transfersomes are a form of elastic or deformable vesicle, which were first introduced in the early 1990s [**4**, **5**]. 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 reproducibly 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 [**6**].

ADVANTAGES OF TRANSFERSOMES

Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss.

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.
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 [**2**, **3**, **7**].

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 [**1**, **3**, **7**].

TRANSFERSOMES V/S OTHER CARRIER SYSTEMS

At first glance, transfersomes appear to be remotely related to lipid bilayers vesicle, liposomes.

However in functional terms, transfersomes differ vastly from commonly used liposomes in that they are much more flexible and adaptable (**Table 1**). The extremely high flexibility of their membrane permits transfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersomes membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus bio surfactant) with sufficiently different packing characteristics into a single bilayer. The high resulting aggregate deformability permits transfersomes to penetrate the skin spontaneously. This tendency is supported by the high transfersomes surface hydro-philicity that enforces the search for surrounding of high water activity. It is almost certain that the high penetration potential of the transfersomes is not primarily a consequence of stratum corneum fluidization by the surfactant because micellar suspension contains much more surfactant than transfersomes (PC/Sodium cholate 65/35 w/w %, respectively). Thus, if the penetration enhancement via the solubilization of the skin lipids was the

reason for the superior penetration capability of transfersomes, one would expect an even better penetration performance of the micelles. In contrast to this postulate, the higher surfactant concentration in the mixed micelles does not improve the efficacy of material transport into the skin. On the contrary, mixed micelles stay confined to the topmost part of the stratum corneum even they are applied non occlusively. Transfersomes differ in at least two basic features from the mixed micelles, first a transfersomes is normally by one to two orders of magnitude (in size) greater than standard lipid micelles. Secondly and more importantly, each vesicular transfersomes contains a water filled core whereas a micelle is just a simple fatty droplet. Transfersomes thus carry water as well as fat-soluble agent in comparison to micelles that can only incorporate lipoidal substances. To differentiate the penetration ability of all these carrier systems proposed the distribution profiles of fluorescently labelled mixed lipid micelles, liposomes and transfersomes as measured by the Confocal Scanning Laser Microscopy (CSLM) in the intact murine skin. In all these vesicles the highly deformable transfersomes transverse the stratum corneum and enter into the viable epidermis in significant quantity [**1, 2, 7**].

Table 1: Comparison of different vesicles

Method	Advantage	Disadvantage
Liposomes	Phospholipid vesicle, biocompatible, biodegradable	Less skin penetration less stable
Proliposome	Phospholipid vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of vesicles
Physical methods e.g.iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
Niosomes	Non-ionic surfactants vesicles	Less skin penetration easy handling But will not reach up to deeper skin layer
Proniosomes	Greater stability, Will convert into noisome in situ, stable	Less skin penetration easy handling 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

PREPARATION OF TRANSFERSOMES

A. Thin film hydration technique is employed for the preparation of transfersomes which comprised of three steps:

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 [**1, 8, 9**].

B. Modified hand shaking, lipid film hydration technique is also founded for the preparation of transfersomes which comprised following steps

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 [**1, 10, 11**].

Table 2: 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	For providing flexibility
Alcohol	Ethanol, methanol	As a solvent
Buffering agent	Saline phosphate buffer (pH 6.4)	As a hydrating medium
Dye	Rhodamine-123, Rhodamine-DHPE, Fluorescein-DHPE Nilered	For CSLM study

Table 3: List of drugs used for transfersomes

Drug	Inference
Oestradiol	Improved transdermal flux
Norgesterol	Improved transdermal flux
Hydrocortosone	Biologically active at dose several times lower than currently used formulation.
Human serum albumin	Antibody titer is similar or even slightly higher than subcutaneous injection.
Interferon-α	Controlled release, Overcome stability problem.
Insulin	High encapsulation efficiency. Transfer across the skin with an efficiency of >50%. Provide noninvasive means of therapeutic use.

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,

1. Lecithin : surfactant ratio
2. Effect of various solvents
3. Effect of various surfactants
4. Hydration medium

Optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant [**12, 13**].

CHARACTERIZATION OF TRANSFERSOMES

The characterization of transfersomes is generally similar to liposomes, niosomes and micelles [**14**]. 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 system by Malvern Zetasizer [**1, 2**].

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 [**1, 2**].

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 [**7**]. 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**

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 [**2**]. The entrapment efficiency is expressed as:

Entrapment efficiency = (Amount entrapped / Total amount added) ×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 [**15**].

6. **Turbidity measurement**

Turbidity of drug in aqueous solution can be measured using nephelometer [**2**].

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 [**2, 16**].

8. **Penetration ability**

Penetration ability of Transfersomes can be evaluated using fluorescence microscopy [**7, 16**].

9. **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. Occlusion affects hydration forces as it prevents evaporation of water from skin [**2**].

10. **Surface charge and charge density**

Surface charge and charge density of Transfersomes can be determined using zetasizer [**2, 7**].

11. **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 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 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) [**2, 7**].

12. **In-vitro Skin permeation Studies**

Modified Franz diffusion cell with a receiver compartment volume of 50ml and effective diffusion area of 2.50 cm^2 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 [**12, 17**].

13. **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% [**13, 15**].

APPLICATION OF TRANSFERSOMES

1. **Delivery of insulin:**

By transferosomes 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 transferosomes (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 [**18**].

2. **Delivery of corticosteroids:**

Transferosomes have also used for the delivery of corticosteroids. Transferosomes improves the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transferosomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases [**6**].

3. **Delivery of proteins and peptides:**

Transfersomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract. These are the reasons why these peptides and proteins still have to be introduced into the body through injections. Various approaches have been developed to improve these situations. The bioavaibility obtained from transferosomes is somewhat similar to that resulting from subcutaneous injection of the same protein suspension. The transferosomal preparations of this protein also induced strong immune response after the repeated epicutaneous application, for example the adjuvant immunogenic serum albumin in transferosomes, after several dermal challenges is as active immunologically as is the corresponding injected proteo-transferosomes preparations [**19**, **20**].

4. **Delivery of interferons:**

Transferosomes have also been used as a carrier for interferons, for example leukocytic derived interferone-α (INF-α) is a naturally occurring protein having antiviral, antiproliferive and some immunomodulatory effects. Transferosomes 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 interleukin-2 and interferone-α containing transferosmes for potential transdermal application .they reported delivery of

IL-2 and INF- α trapped by transferosomes in sufficient concentration for immunotherapy [**21**].

5. **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 [**1, 22**].

6. **Delivery of anesthetics:**

   Application of anesthetics in the suspension of highly deformable vesicles, transferosomes, induces a topical anesthesia, under appropriate conditions, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transferosomal anesthetics last longer [**1**].

7. **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 [**23**].

8. **Delivery of Herbal Drugs:**

   Transfersomes can penetrate stratum corneum and supply the nutrients locally to maintain its functions resulting maintenance of skin in this connection the Transfersomes of Capsaicin has been prepared by Xiao-Ying et al. which shows the better topical absorption in comparison to pure capsaicin [**22**].

DISCUSSION AND 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 effi ciently as water, which is 1500 times smaller.

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