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TRANSFEROSOMES AS TRANSDERMAL DRUG DELIVERY CARRIERS

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ABSTRACT

Transdermal drug delivery is preferable over the oral and parenteral drug delivery systems due to absence of first pass metabolism and patient compliance. Dermal patches are becoming popular in drug delivery. Administration of drug across the skin is best route of drug delivery as the skin is largest human organ. Skin comprises of 1.5 -2.0 m^2 surface with total weight 3 kg. Stratum corneum, the outermost protecting envelop of the skin retard the entry of xenobiotics. A novel vesicular system, transferosomes have been developed to improve the permeability of the skin thereby enhancing the topical delivery of drugs. Transferosomes are composite vesicles consists of hydrophobic and hydrophilic moieties together enable them to accommodate drug molecules with wide range of solubility. Transferosomes has the ability to deform and pass through narrow passages which are 5 to 10 times less than their own diameter without measurable loss of drug. Transferosomes, a new age carrier vesicles have been explored extensively for topical application to enhance intact skin penetration as well as to improve skin retention of drugs for extended release of drugs.

KEY WORDS: Transdermal drug delivery, Transferosomes, Method of preparation, Applications.

INTRODUCTION

Transferosomes are the new vesicular carrier systems for the transdermal drug delivery. Administration of drug through the skin offer potential dominance over conventional methods such as oral and parenteral drug delivery. However, the major impediment of transdermal drug delivery system (TDDS) is the permeability of the drug across the skin because of the external stratum corneum consisting of corneocytes which restrict the entry of xenobiotics. The efforts have been done to increase the permeability of the drug molecules through different techniques like skin permeation enhancers(surfactants, sulfoxides etc), microneedles(vaccines), iontophoresis (peptides and oligonucleotides), electroporation(vaccines, oligonucleotides etc), sonophoresis(hormones, proteins, vaccines etc).Recent advances developed a vesicular composite carrier molecules that are capable of carrying drugs and macromolecules to deeper tissues. These inquisitions have resulted in the designing and development of novel vesicular carriers like ultra-flexible lipid-based elastic vesicles called transferosomes [1].In the year 1991, .Gregor Cevc. Developed a highly ductile, stress-responsive complex supra molecular aggregates and these were termed as transferosomes.

Transferosomes are ultra-deformable vesicles possessing an aqueous core surrounded by the complex lipid bilayer (similar to liposomes). The size and shape of these vesicles is self-coordinating and self-optimizing based on the composition. These are competent of carrying both high and low molecular weight drugs as well as hydrophilic and lipophilic drugs. Transferosomes are specifically optimized; ultra-flexible lipid molecular aggregates, enables them to cross various transport barriers efficiently and then act as a drug carrier for non-invasive targeted drug delivery and sustained release for most of the therapeutic agents.

The term "Transferosomes" was registered as a trademark by a German company IDEA AG, and used it to refer as its proprietary drug delivery technology. The name 'Transfero' is derived from the Latin word which means to carry across, and the Greek word 'soma' for a body. A transferosomes are artificial vesicular carriers which are

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designed to be like a cell vesicle or a cell engaged in exocytosis, and thus suitable for controlled and potentially targeted drug delivery [2].

Strong bilayer deformability is another valuable significance of transferosomes which include increased affinity of transferosomes to bind and retain water. An ultra-deformable and highly hydrophilic vesicle always tends to abstain dehydration, which may involve a transport process associated to forward osmosis, but not identical to it. For example, when Transferosomes are applied to an open biological surface, such as non-occluded skin, tends to penetrate its barrier and travel into the water-rich deeper strata to acquire adequate hydration. Reversible bilayer deformation can occur during barrier penetration, but either vesicle integrity or barrier properties must not get conceded for the underlying hydration affinity and gradient which has to remain unimpaired.

Salient Features of Transferosomes

- Transferosomes are vesicles which consist of both hydrophobic and hydrophilic moieties together and therefore these are suitable for drug molecules with wide range of solubility.
- Transferosomes have the ability to deform and can pass through narrow constriction which are 5 to 10 times less than their own diameter without any perceptible loss.
- These intact vesicles give prominent penetration because of its high deformability.
- These vesicular carriers are suitable for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, hormone, anticancer etc.
- These are made of natural phospholipids similar to liposomes which are biocompatible and biodegradable.
- Lipophilic drugs nearly have 90% entrapment efficiency.
- Metabolic degradation is prevented for encapsulated drugs.
- They also act as depot preparation as they release the contents slowly and gradually.
- They can be employed for both systemic as well as topical delivery of drug.
- Preparations of these transferosomes are simple and easy to scale up [3].

Advantages of Transferosomes

Transferosomes have the following advantages:

- First-pass metabolism of drugs are restraint.
- Fewer side effects can be observed.
- Lipophilic drugs have 90% entrapment efficiency.
- Transferosomes are carriers which are suitable for both low and high molecular weight drugs such as analgesic, protein, anesthetic, corticosteroids, hormone, anticancer etc.
- Transferosomes are convenient for drug molecules with wide range of solubility.
- They also act as depot preparation as they release the contents slowly and gradually.
- They are biocompatible and bio- degradable.
- They are suitable for both systemic and topical drug delivery.
- Metabolic degradation of encapsulated drugs can be prevented.
- Preparation of transferosomes is easy and they are easy to scale up.
- In case of toxicity, termination of drugs can be achieved easily.
- Decrease in dosing frequency and improvement in patient compliance can be achieved [4, 5].

Limitations of Transferosomes

- Transferosomes are chemically unstable as they are prone to oxidative degradation.
- Lack of purity of the natural phospholipids cause problems in choosing of transferosomes as drug delivery vehicles.
- Formulations of Transferosomes are costly. [3].

Comparison of Transferosomes with Other Carriers

When transferosomes are compared with normal micelles, transferosomes had shown higher penetration. In spite of having more concentration of surfactant in micelles does not improve the penetration across the skin because they were confined to upper layer of skin even when they were applied to non-occluded skin. The reason behind this due to micelles is less sensitive to transepidermal water activity gradient than transferosomes.

Transferosomes is greater in size than the standard lipid micelles. Vesicular transferosomes contains aqueous core surrounded by lipid bilayer whereas a micelle is a simple fatty droplet. As a result, transferosomes can carry hydrophilic as well as lipophilic agent in comparison to micelles that can only carry lipoidal substances.

Transferosomes are related to conventional liposomes. They differ from liposomes in terms of permeation across the skin; transferosomes are ultra-flexible and can pass through narrow constriction which is 5 to 10 times less than their own diameter without any perceptible loss, unlike conventional liposomes which are rigid in nature.

This high flexibility of transferosomes is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids + biosurfactant) with sufficiently different packing characteristics into a single bilayer. CSLM can be used to differentiate the penetration ability of all these carrier systems, Cervc et al., (1996) proposed the distribution profiles of fluorescently labeled mixed lipid micelles, liposomes, transferosomes as measured by the Confocal Scanning Laser Microscopy (CSLM) in the intact murine skin. In all these vesicles the

highly deformable and ultra-flexible transferosomes crosses the stratum corneum and enter into the viable epidermis in adequate quantity [6-8].

Mechanism of Penetration

Transferosomes when applied to the intact skin are able to transfer about 0.1mg to 0.5mg of lipid per hour across the intact skin. This value is considerably high when compare to the values driven by the transdermal concentration gradients. Skin penetration barrier leads to the development of osmotic gradient which restrict water loss from the skin and manage a water activity difference in the viable part of epidermis (75% water content) and close to the skin surface, around a dry stratum corneum (15% water content) and this gradient is very stable. There is an attraction of hydrophilic lipids towards water due to active interaction between hydrophilic lipid residues and their adjacent water. Thus induced dehydration is resisted by most of the lipid bilayers. Therefore all hydrophilic lipid vesicles move from dry location sites to the sites which have considerably high water content. When transferosomes are applied on the skin, they will get dehydrated to some extent due to water loss by evaporation and when lipid vesicles sense this osmotic gradient they try to avoid completely drying by migrating along the gradient. They can escape complete drying only if they are considerably deformable to pass through the narrow pores in the skin. As transferosomes are sufficiently flexible and have suitable rheologic and hydration properties and they can easily pass through the narrow pores in the skin. Conventional liposomes which are less flexible compare to transferosomes get confined to skin surface, where they get completely dehydrated and fused together and because of these conventional liposomes have less penetration power than transferosomes [9-11].

Propensity of Penetration

Epicutaneous lipid application leads to transepidermal concentration gradient and this result in movement of lipids from application site in to body. The movement of lipid depends on the mobility of molecule administered and the permeability of skin. The magnitude of the transport driving force also plays an important role:

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

Composition of Transferosome

Materials commonly used in the preparation of transferosomes are summarized in Table No. 2

Preparation of Transferosome

In this method, the first step includes dissolving phospholipids (vesicle forming component) and surfactant (provide flexibility) in volatile organic solvent mixture (chloroform: methanol). Lipophilic drug is incorporated at this stage in above mixture. A thin film is prepared by evaporating organic solvent (room temperature for pure phospholipid vesicles and 50^0C for dipalmitoyl phosphatidyl choline). It can be done by using rotary evaporator. Keep under vacuum for 12hr. The accumulated lipid films were hydrated using phosphate buffer (pH 6.5) by rotating at 60 rpm for 1 hr. The formed vesicles were kept at room temperature for swelling for 2 hr. At this stage hydrophilic drug can be incorporated. For preparing small vesicles, the resulted large multi vesicles are sonicated using bath or probe sonicator for 30 minutes at 4^0c. Homogenization of sonicated vesicles is done by manual extrusion by using a sandwich of 200 and 100nm polycarbonate membrane [12].

Modified Hand Shaking, Lipid Film Hydration Technique

Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture at 60^0c and stirred for some time. A thin film was prepared by evaporating organic solvent by hand shaking the mixture above lipid transition temperature of 43°C. For complete evaporation of organic solvent lipid film was kept overnight. Phosphate buffer (pH 7.4) was used to hydrate the thin film with gentle shaking for 15 minute at equivalent temperature. The transferosome suspension was further hydrated up to 1 hour at 2-8°C.

Characterization of Transferosomes

The following are characterization parameters of transferosomes:

Vesicle size distribution of transferosomes and zeta potential

Dynamic Light Scattering method by Malvern Zetasizer can be used to determine the vesicle size, size distribution and zeta potential [13].

Vesicle diameter

Photon correlation spectroscopy or dynamic light scattering (DLS) method is used to determine the vesicle diameter. Distilled water is use to prepare samples and filtration is done using a 0.2 mm membrane filter and then diluted with filtered saline and photon correlation spectroscopy or dynamic light scattering (DLS) method is use to measure the diameter of vesicles [13].

Number of vesicles per cubic mm

It is a critical parameter for optimizing the composition of transferosomes and other process variables.0.9% sodium chloride solution is used to dilute transferosomal formulations which are not sonicated at least for five times and then by using Haemocytometer and optical microscope number of vesicles are counted [14]. The Transferosomes vesicles are counted in 80 and calculated using the following formula: Total number of Transferosomes per cubic mm= (Total number of Transferosomes counted × dilution factor × 4000) / Total number of square counted.

Entrapment efficiency

Percentage of the drug entrapped in vesicles can be determined by initially separating the un-entrapped drug by using mini column centrifugation method. Then 0.1% Triton X-100 or 50% n-propanol was used to disarray the vesicles [15]. The entrapment efficiency can be calculated by using following formula:

Entrapment efficiency = (Amount entrapped / Total amount added) ×100

Drug content

Determination of drug content can be done by using one of the analytical method high performance liquid chromatography (HPLC) method using a UV detector [16, 17].

Turbidity measurement

Nephelometer can be used to determine the turbidity of drug in aqueous solution [13].

Degree of deformability or permeability measurement

The permeability study is the critical parameter for characterization of transferosomes. Pure water is used as a standard to study degree of deformability. Transferosomes preparation is made to pass through between known size of different microporous filters, having a pore diameter of 50 nm and 400 nm. Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) method is used to measure particle size and size distributions [13, 19].

Penetration ability

Fluorescence microscopy is used to study penetration ability of transferosomes [14, 19].

Occlusion effect

Occlusion of skin is considered to be useful for permeation of drug. Hydrotaxis (movement in the direction of moisture) is the major driving force for penetration of vesicles through the skin approximately from dry surface of skin to water abundant deeper regions of skin. Evaporation of water from skin is prevented by hydration forces which are affected by occlusion effect [13].

Surface charge and charge density

Zetasizer is used to study Surface charge and charge density of Transferosomes [13, 14].

Confocal scanning laser microscopy

Conventional light microscopy and electron microscopy had drawbacks of fixation, section and staining of skin samples. Misinterpretation arising due to incompatibilities with analogous processing technique can be reduced by the use of CSLM. This technique uses lipo leakage. Percentage of drug lost was calculated by considering the initial entrapment of drug as 100% [22, 23].

fluorescence markers which are incorporated in Transfersomes and these markers emit light which can be used for following purpose: 1. Determination of mechanism of penetration of transferosomes across the skin. 2. To study histological organization of skin. 3. To differentiate mechanism of penetration of transferosomes with liposomes, niosomes and micelles [15]. CSLM uses different fluorescence markers. They are: Fluorescein- DHP, Rhoda mine- DHPE, NBD-PE, Nile red.

In-vitro drug release

Permeation rate can be determined by studying in vitro drug release of drug. Drug release can be determined for transferosomal suspension by incubating the suspension at 32 ^{0}C and samples are withdrawn at different times and the mini column centrifugation is used to separate the free drug. The amount of drug released is calculated obliquely from the amount of drug entrapped at zero times as the initial amount i.e., 100% entrapped and 0% released [13, 14].

In-vitro Skin permeation Studies

Modified Franz diffusion cell contain receiver compartment, donor compartment having volume of 50ml capacity and effective diffusion area of 2.50 cm^2 which is used for In vitro study. Goat skin dipped in phosphate buffer solution (pH 7.4) is used to study In vitro drug release. Hair from abdominal skin was removed and then the skin was hydrated using saline solution. The adipose tissue layer was removed from the skin by using a cotton swab. Isopropyl alcohol solution is used to store skin at 0-40˚C. For skin permeation study, treated skin was kept horizontally on the receptor compartment to the stratum corneum side facing upwards towards the donor compartment of modified Franz diffusion cell. 2.50cm^2 effective permeation area of donor compartment was exposed to receptor compartment. The receptor compartment having 50ml of phosphate buffer (pH 7.4) saline was maintained at 37 ± 0.5˚C and stirred by a magnetic bar at 100rpm. Formulation of Transferosomes corresponding to 10mg drug was kept on the skin and the top of the diffusion cell was covered. At applicable time intervals 1 ml sample from the receptor medium were withdrawn and instantaneously replaced by an equal volume of fresh phosphate buffers (pH 7.4) to maintain sink conditions. Correction factors for each sample were considered in calculation of release profile. The samples were examined by any analytical technique [21].

Physical stability

The ampoules containing transferosomal formulation were kept at 4 ± 2 ^{0}C (refrigerated temperature), 25 ± 2 ^{0}C (room temperature), and 37 ± 2 ^{0}C (body temperature) for about 3 months. Samples from each ampoule were examined after 30 days to determine drug

APPLICATIONS

Transfersomes as drug carrier

For an effective transdermal drug delivery, Drug carrier must accomplish two basic criteria. 1. Drug carrier should compose a gradient that commute the drug carrier complex from skin surface in to the deeper regions of skin. 2. Drug vesicles must be able to penetrate through the skin barrier without significant loss of therapeutic material.
Various applications can be summarized as follows:

Transferosomes as a carrier for protein

The delivery of proteins and peptides into the body is extremely difficult even with the use of chemical penetration enhancers and physical methods such as iontophoresis, sonophoresis etc have some limitations. But if these macromolecules are combined with optimized and ultra-deformable agents as carriers, then the transdermal or systemic delivery of therapeutic agent will be effective. For example, Transfersomes loaded serum albumin showed relatively similar bioavailability such as that resulting from subcutaneous injection [24].

Transferosomes as a carrier for insulin

Transferosomes loaded insulin carried across the skin shows more than 80% efficacy if properly optimized. Approximately 35±10% decrease in blood glucose level can be seen with Transfersomes loaded insulin which is relatively similar to subcutaneously injected insulin dose.

Transferosomes as a carrier for interferon

Leukocytic derived interferon-a have antiviral, antiproliferative and immunomodulatory effects. Interferon loaded Transfersomes were reported to have shown better transdermal delivery [25].

Transferosomes as means of transdermal immunization

Transdermal immunization is possible with the use of transferosomes as a carrier for water soluble protein such as integral membrane protein, human serum albumin, and gap junction protein [26, 27].

Transferosomes as a carrier for corticosteroids

Corticosteroids have anti-inflammatory, immunosuppressive, vasoconstrictor and antiproliferative actions and can be used in various dermatological conditions like atopic eczema, allergic contact dermatitis, arthritis etc. Uses of transferosomes as carrier in delivery of corticosteroids across the skin can be effective in terms of improved site specificity and drug safety [28].

Transferosomes as carrier for topical analgesics and anaesthetic agent

Aqueous solution of analgesics has skin permeability problems and do not reduce the pain when applied locally over skin. Lipophilic analgesics have few chances of desired therapeutic effect when applied under appropriate conditions. With trasferosomes as a carrier above problems can be overcome. It can carry sufficient amount of drug in to the skin and produces the anesthetic effect within 10 min of application, similar to subcutaneous bolus injection.

Transferosomes as a carrier for anticancer agents

Transferosome can be used as a carrier for anticancer drugs to reduce the side effects like depression and thrombosis. When Tamoxifen loaded transferosomes was applied on shaved murine back, most of it penetrated across the skin and integrated dose of Tamoxifen loaded transferosome in uterus was found to be higher when applied transdermally than given by oral administration.

Transferosomes as a carrier for non- steroidal anti-inflammatory agents

Lipid suspensions were used for NSAIDs from long time but the results with the use of transferosomes were drastically higher. It improved penetration of diclofenac and ibuprofen across the skin and the reason behind this the captivity of drug to the carrier that restrain the disappearance of drug in the blood capillaries once penetrated across the skin.

Table 1. Comparison of different approaches for transdermal drug delivery

S.No.	Method	Advantage	Disadvantage
1.	Penetration enhancers	Increase permeation of drugs across the skin and accord both local and systemic effect.	Skin irritation, immunogenicity, only for low molecular weight drugs.
2.	Physical methods Eg.1. Intophoresis 2. sonophoresis	Iontophoresis- Increment in the permeation of intermediate size charged molecule. Enhanced therapeutic efficacy	Only for charged drugs Produce skin irritation
3.	Liposomes	Phospholipid vesicle, biocompatible, biodegradable	Permeation is low, less stable
4.	Niosomes	Nonionic surfactant vesicle, greater stability	Less skin penetration
5.	Ethosomes	Increase skin penetration, low risk of toxicity	Poor yield, coalescence of ethosomes may occur

6.	Transferosomes	Ultra-flexible, biocompatible, biodegradable, suitable for both low and high molecular weight.	Formulation is quite expensive with no other disadvantages

Table 2. Different additives used in formulation of transferosomes

S.no.	Class	Example	Use
1.	Phospholipids	Soya phosphatidyl choline, egg phosphatidylcholine, dipalmitoylphosphatidyl choline	Vesicles forming Component
2.	Surfactant	Sod.cholate, Sod.deoxycholate, Tween-80, Span-80, Tween 20	Providing flexibility
3.	Solvents	Ethanol, methanol, isopropyl alcohol	As a solvent
4.	Buffering agent	Saline phosphate buffer (pH 6.4), phosphate buffer pH 7.4	As a hydrating medium
5.	Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-red	Used for confocal laser Scanning microscopy study

Fig 1. Mechanism of penetration of Transfersomes

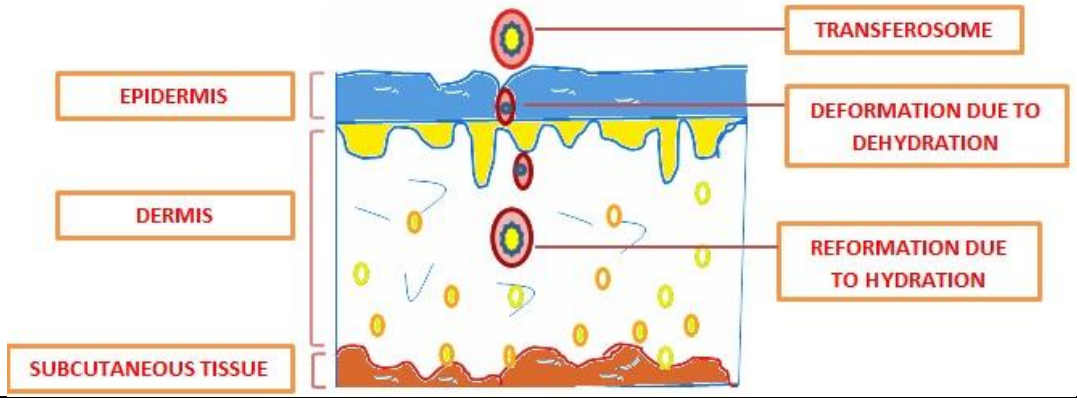
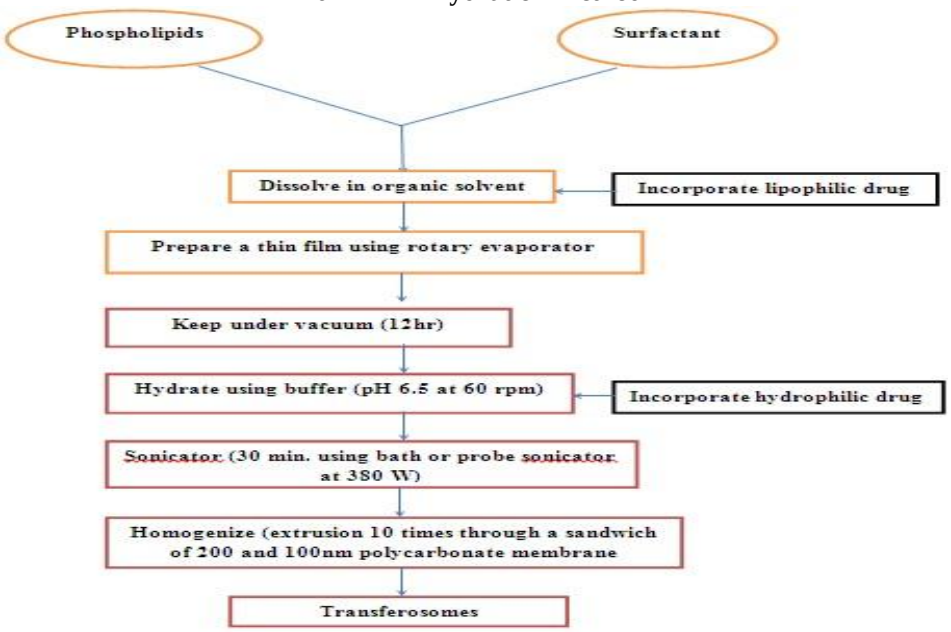


Fig 2. Preparation method of Transferosomes
1thin Film Hydration Method



CONCLUSION

A novel vesicular system called Transferosome overcomes the permeability problems of drug across the skin. These have the capability to accommodate both hydrophilic and lipophilic components and are flexible in nature which can transport both high and low molecular weight compounds such as analgesic, protein, anesthetic, corticosteroids, hormone, anticancer etc. Transferosomes loaded insulin carried across the skin shows more than 80% efficacy if properly optimized. Transferosomes are ultra-flexible and can pass through narrow constriction which is 5 to 10 times less than their own diameter without any perceptible loss, unlike conventional liposomes which are rigid in nature. This novel carrier system will be a great help in transdermal drug delivery system.

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Nil

CONFLICT OF INTEREST
No interest

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