Review Article

Transferosomes For Effective Transdermal Drug Delivery

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Received: 11-01-2024; **Revised:** 20-03-2024; **Accepted:** 28-03-2024; **Published on:** 15-04-2024.

ABSTRACT

Recent advancements in drug delivery systems have sparked interest, particularly in vesicular drug delivery. Among these systems, Transdermal Drug Delivery Systems (TDDS) stand out due to skin permeability, allowing small, lipophilic drugs while being impermeable to macromolecules. Ethosomes and transferosomes, ultra-flexible lipid-based elastic vesicles, have been designed to enhance drug delivery. Transferosomes excel in transdermal delivery for both low and high molecular weight drugs, offering advantages like bypassing first-pass metabolism, extended duration of activity, and minimized side effects. Transferosome characterization involves assessing vesicle size, morphology, drug content, entrapment efficiency, penetration ability, occlusion effect, surface charge, *in vitro* drug release, and *in vitro* skin penetration. This comprehensive characterization ensures stability of labile drugs and controlled release. Transferosomes, with their unique ability to encapsulate hydrophilic, lipophilic, and amphiphilic pharmaceuticals, demonstrate higher penetration efficiency than traditional liposomes. This review endeavors to offer a comprehensive understanding of transferosomes, encompassing their concept, composition, mode of action, preparation methods, current applications, and potential pros and cons. It serves as a valuable reference for researchers in the drug delivery systems field.

Keywords: Transdermal drug delivery system, Transferosomes, Preparation of transferosomes, Evaluation of transferosomes, Application of transferosomes.

INTRODUCTION

The skin, a protective barrier, is harnessed for drug delivery. Transdermal methods shield against oral side effects, enhance drug effectiveness, and encourage patient compliance. Yet, not all drugs suit this route due to low skin permeability, largely governed by the stratum corneum—composed of lipophilic, dead corneocytes and lipids. Nano/micro technologies hold promise for transdermal delivery, especially nano/micro vesicles like liposomes, niosomes, and ethosomes. Though successful, these face hurdles penetrating the stratum corneum. However, deformable vesicles, transferosomes, show potential in overcoming these barriers, marking a promising future for transdermal therapeutic delivery[1].For a range of clinical conditions, transdermal distribution of medications via the skin to the systemic circulation offers a practical mode of administration. There are currently transdermal delivery systems available for the treatment of a number of diseases, including skin cancer, female sexual dysfunction, post-menopausal bone loss, urinary incontinence, depression, anxiety, and attention deficit hyperactivity disorder (ADHD), as well as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, and skin cancer. The substantial barrier to penetration across the skin, associated principally with the topmost stratum corneum layer of the epidermis, restricts the application of transdermal delivery to a wider spectrum of medications[2].Indeed, the development of Transdermal Drug Delivery Systems (TDDS) aims to overcome the skin barrier for effective drug delivery. The Stratum Corneum (SC) poses a challenge due to its compact nature, hindering drug penetration. Conventional liposomes have limitations in penetrating beyond the upper skin layers, acting as local drug reservoirs. Transferosomes, with added edge activators (EA), enhance penetration by increasing bilayer fluidity, allowing them to deform and penetrate deeper into the skin. The EA not only facilitates skin penetration but also acts as a permeation enhancer by disrupting the SC's organized intercellular lipids, aiding drug permeation. This makes transferosomes promising for delivering drugs into deeper skin layers at higher concentrations[3].

TRANSFEROSOMES

A transferosome is a synthetic vesicle that mimics a cell vesicle and is used to introduce genetic material or medications into a cell. A single naturally occurring amphipath, such as phosphatidylcholine, which has a propensity to self-aggregate into vesicles, makes up a transferosome. Then, at least one bilayer softener (such as a biocompatible surfactant) is added to the latter. Therefore, the vesicle-like transferosome often has an aqueous core encircled by a complex, extremely flexible, and adaptive lipid bilayer. A transferosome's basic structure is similar to that of a basic lipid vesicle, or liposome, but it is distinguished from the latter by having a more flexible and porous, or "softened," bilayer membrane[2].

Structure and composition of transferosomes

Transferosomes, comprising phospholipids like phosphatidylcholine, form lipid bilayers in aqueous settings, creating vesicles. Edge activators, often single-chain surfactants such as sodium cholate or Tween 20, boost bilayer elasticity and fluidity. These deformable lipid-

based droplets can navigate pores smaller than their size, passing through constrictions from 5-10 µm efficiently. Their adaptable, stress-responsive structure aids in maintaining integrity during transit. With components like Span 80 and dipotassium glycyrrhizinate, transferosomes excel in delivering drugs into or through the skin, owing to their biodegradability, biocompatibility, and resistance to metabolic degradation, ensuring high efficiency based on administration routes or applications[4,5].

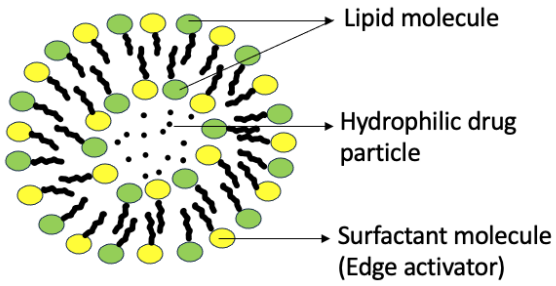

Figure 1: Structure of transferosomes referred from Solanki D, et al[16]

Transferosomes are a type of vesicular carrier that are composed of phospholipids and surfactants, which enable them to encapsulate medications that are both hydrophilic and hydrophobic. They have a structure that combines hydrophobic and hydrophilic moieties, enabling them to hold a variety of solubility-based medicinal compounds. Better penetration of intact vesicles is achieved by transferosomes because they may deform and pass through tiny constriction (between five and ten times smaller than their own diameter) without noticeable loss. They can be prepared by various methods, including thin-film hydration, reverse-phase evaporation, and ether injection methods[6].

Their makeup, transferosomes, enables them to get beyond the constraints of traditional drug delivery methods. They may encapsulate both hydrophilic and hydrophobic medications since they are made of phospholipids and surfactants. The high and self-optimizing deformability of transferosomes' membrane, which is adaptable to ambient stress, allows them to change their membrane composition locally and reversibly, enabling them to deform and pass through narrow constrictions without measurable loss. Since intact vesicles can penetrate more easily due to their great deformability, they function well as a transdermal drug delivery mechanism. Because of their infrastructure, which is made up of both hydrophobic and hydrophilic moieties, transferosomes may hold medicinal molecules with a variety of solubility levels. They have the capacity to transport both low and high molecular weight drugs. The original composition of transferosomes was soya phosphatidylcholine incorporating sodium cholate and a small concentration of ethanol. They have been used to deliver a range of drugs, including anti-inflammatory agents, antibiotics, antifungal agents, and anticancer drugs [6-9].

Table 1: Composition of transferosomes

S. No	Class	Example	Uses
1	Phospholipids	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmitoyl phosphatidyl choline	Vesicles forming compounds
2	Surfactant	Sodium cholate, sodium deoxycholate, tween 80, tween 20, span 20, span 80	For providing flexibility
3	Alcohol/Solvents	Ethanol, methanol, isopropyl alcohol, chloroform	As a solvent
4	Buffering agents	Saline phosphate buffer (p^H 6.4), phosphate buffer (p^H 7.4)	As a hydrating medium
5	Dye	Rhodamine-123, Rhodamine-DHPE, Fluorescein-DHPE, Nile-red	For confocal scanning laser microscopy (CSLM)

TRANSFEROSOMES V/S OTHER CARRIER SYSTEM

Transferosomes, niosomes, proniosomes, liposomes, ethosomes, and electrosomes are all types of vesicular drug delivery systems that have gained attention in the field of nanotechnology[10-13]. Among these, transferosomes are a promising option for transdermal drug delivery due to their ability to penetrate through the skin pores, encapsulate both hydrophilic and lipophilic molecules, prolong the drug's existence in the systemic circulation, target organs and tissues, and reduce drug toxicity while increasing bioavailability[11,12]. Transferosomes are elastomeric or deformable vesicles composed of phospholipid, surfactant, and water that improve transdermal drug delivery. They are evaluated for their entrapment efficiency, drug content, in-vitro drug release, degree of deformability, and other parameters[12]. Proniosomes, on the other hand, are dry formulations of water-soluble nonionic surfactant-coated carrier systems that immediately form niosomes upon hydration. They can solve the instability issues with liposomes and niosomes and can potentially increase the solubility, bioavailability, and absorption of certain medications. [10]. Niosomes are tiny lamellar structures that are created when cholesterol and non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class are mixed together and then hydrated in aqueous conditions. These structures can be utilized as drug carriers for both lipophilic and amphiphilic substances[13].

Advantages

- Transferosomes can enhance skin permeation better than conventional drug solutions, allowing for more efficient transdermal drug delivery[14].
- Transferosomes are ultra-deformable vesicles that can squeeze themselves through narrow pores smaller than their size, making them a useful drug delivery system for poorly soluble drugs[15].
- Transferosomes can be formulated with various lipids, surfactants, and edge activators, allowing for a wide range of solubilities and improved penetration[15].
- Transferosomes are made of lipids and surfactants that are biocompatible and biodegradable, making them suitable for drug delivery applications[16].
- They are composed of phospholipids and surfactants, which provide them with the ability to encapsulate both hydrophilic and hydrophobic drugs[16].
- Transferosomes can avoid first-pass metabolism, which is a common issue with conventional oral drug delivery systems, leading to improved patient compliance and reduced side effects[17].
- Transferosomes can improve the bioavailability of drugs by enhancing their penetration through the skin and avoiding the degradation caused by the gastrointestinal tract[17].
- Transferosomes can provide painless drug delivery and reduce the frequency of administration, improving patient acceptability[17].
- Act as a carrier for low and high molecular weight drugs, and penetrate the stratum corneum by generating a natural osmotic gradient [15,18,19].
- Transferosomes have been used for the potential ocular delivery of cyclosporine A and for the dermal delivery of minoxidil. They have also been designed for the selective delivery of active pharmaceutical ingredients (APIs) to inflamed cells in the respiratory tract [15,18,19].
- Self-administration is possible with these systems[18,19].

Disadvantages

- They are sensitive to temperature and pH changes, which can affect their stability and drug release properties[15].
- Difficulty in achieving phospholipid purity and the high cost of formulation due to the use of expensive equipment and raw materials[16].
- Transferosomes may have limited drug loading capacity and may not be suitable for all types of drugs[20].
- It offers gradual therapeutic benefits for hydrophilic structures on the skin[21].
- It is not feasible or beneficial for lower-potent drugs and not appropriate for high drug doses[22].
- Transferosomes are chemically unstable because of oxidative degradation properties[22].

MECHANISM OF TRANSFEROSOMES

Transferosomes are a type of lipid-based vesicular system used for drug delivery, particularly for percutaneous administration. They are formed by hydrating a film of lipids, followed by high-speed homogenization techniques to create a stable dispersion of nanoparticles[23]. Drugs are transported into the stratum corneum via transferosomes, either intracellularly or transcellularly. The primary method for transferosome permeation through the skin has been described as the "osmotic gradient or transdermal gradient"[26]. where the colloidal particles that make up the vesicles create an amphiphilic bilayer. The hydrophilic medications are usually carried by the vesicular drug delivery systems in the internal aqueous compartment, while the hydrophobic drugs are trapped in the lipid bilayer[27]. On the other hand, because therapeutic agent carrier vesicles have a higher deformability and can associate with the tissue layer versatility and integrity of the transferosome, they facilitate passageway over skin, making transferosomes ultra-flexible and self-optimizing[28].

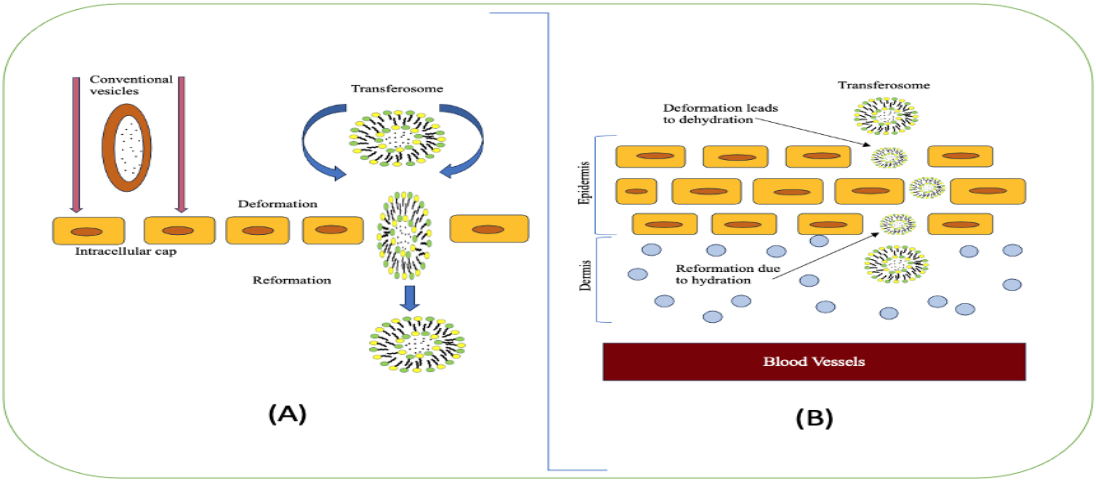

Figure 2: The mechanism of transferosomal penetration involves: (a) Deformation and subsequent reformation facilitating penetration, and (b) The tendency of transferosomes to seek moisture, aiding their permeation.[24]

The drug is incorporated into the lipid matrix of the transferosomes, which can improve drug solubility, stability, and encapsulation[24]. The release of the drug from the transferosomes is governed by the diffusion mechanism, which is influenced by factors such as the drug's concentration, the lipid matrix's properties, and the surrounding environment[23].Transferosomes can penetrate deeply into mucosal layers, enhancing drug bioavailability. This penetration is believed to be due to the elastic and deformable membrane of the transferosomes[24]. Transferosomes can be used for both transdermal and dermal drug delivery. The mechanism of skin permeation involves the interaction between the lipids in the transferosomes and the skin's lipid layer, which can facilitate the drug's penetration through the skin[25].

TRANSFEROSOMES' INTERACTIONS WITH THE SKIN

Transferosomes are a type of deformable liposomes that can penetrate the stratum corneum, the outermost layer of the skin, more effectively than traditional liposomes, allowing for improved drug delivery[29,30].They are designed to enhance the delivery of drugs through the skin and have potential applications in various medical treatments[29].Transferosomes are elastomeric or deformable vesicles composed of phospholipid, surfactant, and water that improve transdermal drug delivery[12].They can accommodate drug molecules with a wide range of solubility and can act as a carrier for low as well as high molecular weight drugs[31].Transferosomes can deform and pass through narrow constrictions without measurable loss, penetrate through the pores of the stratum corneum, and get into the underlying viable skin in intact form due to their deformable nature[32].They have been used to deliver a variety of therapeutics, including hydrophilic actives, larger molecules, peptides, proteins, and nucleic acids[29].

PREPARATION OF TRANSFEROSOMES

Thin-film hydration method:

The phospholipids and edge activator, which are vesicle-forming ingredients, dissolve in a round-bottom flask using a volatile organic solvent mixture, such as a suitable (v/v) ratio of chloroform and methanol. This step allows for the incorporation of a lipophilic drug. Using a rotary vacuum evaporator, the organic solvent evaporates above the lipid transition temperature under reduced pressure to generate a thin layer. Vacuum conditions are maintained to eliminate any remaining traces of the solvent. After that, the thin film is hydrated by rotating it in a buffer solution (pH 7.4) for a predetermined amount of time at the right temperature. At this point, hydrophilic drug incorporation may take place. Smaller vesicles are produced by sonicating the resultant vesicles in a bath or probe sonicator after they have swelled at ambient temperature. Following sonication, these vesicles are homogenized by extrusion through a sandwich of 200–100 nm polycarbonate membranes [33-36].

Sonication-Vortexing Technique:

A mixture of medicine, phospholipids, and edge activators in a phosphate buffer is vortexed to form a milky transferosomal suspension. Sonication at room temperature follows, and the solution is extruded through 450 and 220 nm membranes to create transferosomes. RES, soya lecithin, and surfactant in a 2:1 chloroform/methanol ratio are mixed, dried, and hydrated with SNF at pH 5.5. Vesicles are then reduced in size using a probe sonicator, filtered through a 0.2-µm membrane, and cooled before storage at 4°C [22,37,38].

Adapted Handshaking Technique:

The modified handshaking method shares the core principle of the rotary evaporation-sonication technique. In this modified approach, a round-bottom flask contains the organic solvent, lipophilic drug, phospholipids, and edge activator. The goal is complete dissolution of all components to achieve a clear, transparent solution. Instead of utilizing a rotary vacuum evaporator, the solvent is evaporated via handshaking while the flask is partially immersed in a heated water bath (typically 40–60 °C). This process forms a thin lipid film along the inner wall of the flask overnight to allow complete solvent evaporation. Subsequently, the film is hydrated with an appropriate buffer solution, incorporating the hydrophilic drug with gentle shaking at a temperature above the lipid's phase transition temperature. This technique aligns closely with the thin film hydration method, combining lipophilic drug, organic solvent, edge activator, and phospholipids in a flask. The aim remains the same: achieving complete dissolution and a clear solution. The solvent is removed through handshaking evaporation, while the flask is partially submerged in a heated water bath, leading to the formation of a thin lipid coating on the flask's inner wall [22,37,38].

Suspension Homogenization Technique:

Transferosomes are prepared by mixing an ethanolic solution of soybean phosphatidylcholine with an appropriate amount of an edge-active molecule, such as sodium cholate. This prepared suspension is then mixed with Triethanolamine-HCl buffer to achieve the desired total lipid concentration. The resulting suspension undergoes sonication, followed by a process of freezing and thawing repeated two to three times to form the transferosome formulation [22,37,38].

Centrifugation method:

In the process, phospholipids, edge activator, and the lipophilic drug are initially dissolved in an organic solvent. The solvent is removed using a rotary evaporator under reduced pressure at the respective temperature, and any remaining traces are eliminated under vacuum. The deposited lipid film is then hydrated with an appropriate buffer solution through centrifugation at room temperature, allowing for hydrophilic drug incorporation. The resulting vesicles undergo swelling at room temperature. Simultaneously, in a parallel procedure,

phospholipids, surfactants, and the drug are dissolved in alcohol. The solvent is removed by rotary evaporation under reduced pressure at 40°C, with final traces eliminated under vacuum. The lipid film is hydrated with an appropriate buffer through centrifugation at 60 rpm for 1 hour at room temperature. The resulting vesicles from this stage undergo swelling for 2 hours at room temperature. The multilamellar lipid vesicles obtained from both processes are then further sonicated at room temperature[22,37,38].

Reverse-Phase Evaporation Technique:

In a round-bottom flask, phospholipids and edge activator are dissolved in an organic solvent mixture (e.g., diethyl ether and chloroform), allowing for the incorporation of a lipophilic drug. After rotary evaporation to obtain lipid films, they are redissolved in an organic phase primarily composed of isopropyl ether and/or diethyl ether. The aqueous phase, containing edge activators (surfactants), is introduced to create a two-phase system, facilitating hydrophilic drug incorporation. Sonication in a bath sonicator produces a homogeneous water-in-oil (w/o) emulsion. Slow evaporation of the organic solvent using a rotary evaporator result in a viscous gel, eventually forming a vesicular suspension. Alternatively, in another method, lipids are dissolved in an organic solvent in a round-bottom flask, followed by the addition of an aqueous medium containing edge activators under nitrogen purging. The drug can be added based on its solubility. Sonication creates a homogeneous dispersion, and after removing the organic solvent under reduced pressure, a viscous gel transforms into vesicles. Non-encapsulated material and residual solvents are removed through dialysis or centrifugation [39,40].

High-Pressure Homogenization Technique:

Phospholipids, along with an edge activator and the drug, are uniformly dispersed in a solution of PBS or distilled water, incorporating alcohol. The mixture undergoes simultaneous ultrasonic shaking and stirring. Subsequently, intermittent ultrasonic shaking is applied. The resulting blend is homogenized using a high-pressure homogenizer. The transferosomes, formed through this process, are then stored under specific conditions to ensure stability and efficacy [41,42].

Ethanol Injection Method:

The organic phase is prepared by dissolving phospholipids, edge activators, and a lipophilic drug in ethanol with magnetic stirring, creating a clear solution. Simultaneously, the aqueous phase forms by dissolving water-soluble substances in a phosphate buffer, where hydrophilic drug incorporation can occur. Both solutions are heated to 45–50 °C. The ethanolic phospholipid solution is then injected dropwise into the aqueous solution under continuous stirring. The resulting dispersion undergoes ethanol removal through vacuum evaporation and sonication for particle size reduction, forming bilayered structures as lipid molecules precipitate upon contact with aqueous media[43,44].

FACTORS AFFECTING THE PROPERTIES OF TRANSFEROSOMES

Transferosomes are a promising nanoencapsulation technique for transdermal drug delivery due to their advantages over conventional oral and parenteral delivery systems, such as noninvasive and self-administered delivery, improved patient compliance, and controlled release of therapeutic agents. They have a bilayered structure that facilitates the encapsulation of lipophilic, hydrophilic, and amphiphilic drugs with higher permeation efficiencies compared to conventional liposomes[22]. Transferosomes are elastic in nature, allowing them to deform and squeeze themselves through narrow pores that are significantly smaller than their size.

The optimization of transferosome formulations involves manipulating various process variables during manufacturing to influence the properties of the transferosomes[22]. These variables encompass factors associated with the production of transferosomal formulations, and they are outlined as follows:

1. **Bilayered structure:** The bilayered structure of transferosomes helps in the encapsulation of various types of drugs, improving their permeation efficiency.
2. **Elasticity:** The elastic nature of transferosomes allows them to deform and squeeze themselves through narrow pores, facilitating drug delivery.
3. **Molecular weight:** Molecules with molecular weights greater than 500 Da and ionized compounds generally do not pass through the skin, which is a challenge for transdermal delivery systems. Encapsulating drugs in transferosomes can help overcome this barrier.
4. **Preparation methods:** Different methods of preparation and characterization can influence the properties of transferosomes, such as the choice of lipids, surfactants, and other excipients used in their formulation.
5. **Drug type:** The type of drug being administered can also affect the properties of transferosomes, as different drugs may have different requirements for encapsulation and delivery.

Overall, the properties of transferosomes can be tailored by optimizing the formulation and choice of components, which can lead to improved drug delivery and efficacy in transdermal applications.

CHARACTERIZATION AND EVALUATION OF THE TRANSFEROSOMES

Transferosomes share similar characterization aspects with liposomes, niosomes, and micelles. Various published methods exist for assessing their characteristics, including vesicle shape and size, size distribution, polydispersity index, zeta potential, number of vesicles per cubic millimeter, entrapment efficiency, deformability degree, and measurements of skin permeability [22,45,46]. Below, you'll find a detailed explanation for each of the mentioned characterization methods.

Vesicle size, Morphology and zeta potential:

Dynamic Light Scattering (DLS) via Malvern Zetasizer assesses vesicle size, distribution, and zeta potential crucial for transferosome characterization. Size impacts preparation, batch consistency, and stability. DLS monitors mean size, while Transmission Electron Microscopy (TEM) reveals structural changes. Zeta potential is measured for stability, and visualization is achieved through microscopy. This comprehensive approach ensures transferosome suitability for drug encapsulation [47,48,49].

Number of vesicles per cubic mm Studies:

The vesicle size parameter holds significant importance in optimizing transferosome composition and other process variables. Non-sonicated transferosomal formulations are diluted five times with a 0.9% sodium chloride solution. Further study involves the use of a hemocytometer and an optical microscope. This analysis allows for the observation of transferosomes with a vesicle size exceeding 100 nm, providing valuable insights for the refinement of composition and process parameters [46,50].

Count and calculate the transferosomes in 80 small squares using the following formula:

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

Entrapment Efficiency (%EE) Studies:

Entrapment efficiency (%EE), expressed as % drug entrapment, is assessed by separating unentrapped drug from vesicles through methods like mini-column centrifugation. Following ultracentrifugation, the direct approach involves removing supernatant, disrupting sedimented vesicles with a suitable solvent, and then diluting and filtering the solution. Analytical methods like modified HPLC or spectrophotometry are employed to determine drug content, dependent on the API's analytical method. The entire process ensures accurate quantification of drug entrapment in the formulation [22,51,52].

The percentage entrapment efficiency can be calculated using the following formula:

$$Percentage\ Entrapment\ Efficiency\ (\%EE) = \frac{Amount\ of\ the\ drug\ entrapped}{\text{Total amount of the drug added}} \times 100$$

To ascertain the %EE indirectly, one dilutes the supernatant with a suitable solvent, followed by filtration to eliminate impurities. The concentration of the drug in the supernatant is then assessed as the free drug using a pertinent analytical method.

The drug entrapment percentage can be expressed as follows:

$$Percentage\ Entrapment\ Efficiency\ (\%EE) = \frac{Total\ amount\ of\ the\ drug\ added - Amount\ of\ the\ free\ drug}{\text{Total amount of the drug added}} \times 100$$

Degree of Deformability:

The drug entrapment percentage is crucial, influencing the permeation of transferosomal formulation. In this study, conducted with pure water as the standard, transferosomes preparation undergoes successive passes through microporous filters (50 to 400 nm). DLS measurements track particle size and distribution after each pass, with permeability and deformability assessed for transferosome characterization [22,49,53].

The degree of deformability or permeability measurement is expressed as:

$$D = \frac{rv}{rp} \times J$$

Where;

D = Degree of deformability

J = Amount of suspension extruded during 5 min

rv = Size of the vesicle and rp = Pore size of the barrier

***In vitro* drug release:**

The in vitro drug release profile is crucial for optimizing transferosomal formulations. Studies compare releases to free drugs or reference products. Notable examples include a Celecoxib transferosomal gel releasing 75% within 6 h, surpassing commercial gel by 30%, and Ketoconazole-loaded transferosomal gel with an initial 40.67% burst. Lidocaine transferosomes show over 80% release in 6 h. Using Franz diffusion cells at 32 °C, a 0.45 µm membrane, and phosphate buffer, in vitro studies involve withdrawing 1 mL aliquots at intervals for analysis via UV, HPLC, or HPTLC. Permeation rate determination is performed by incubating transferosomes at 32°C, separating free drug through minicolumn centrifugation, and calculating release from initial entrapped amounts. These studies inform formulation optimization before costly in vivo assessments[54-56].

***In vitro* skin permeation:**

The study aims to determine transport efficiencies of transdermal delivery systems and identify factors influencing drug flux (expressed as µg/cm2/h). Using Franz diffusion cells, pig skin, synthetic membranes like Strat M®, or modified cells with goat skin, experiments simulate in vivo conditions. Synthetic membranes show close correlations to human skin. For pig skin, fluxes and concentrations closely align with human skin. The Franz diffusion cells, filled with phosphate buffer, maintain 37 ± 0.5 °C, simulating blood circulation. Testing formulations are added to the donor compartment; samples withdrawn at intervals for analysis using HPLC or spectroscopy. The data aids in predicting in vivo behavior, optimizing formulations before costly in-vivo studies[57-59].

Stability of Transferosomes:

Transferosome vesicle stability is assessed through DLS and TEM for size and structural changes over time. Optimized

formulations are stored in sealed amber vials under ICH guidelines: long-term at 25 ± 2°C/60% RH ± 5% or 30 ± 2°C/65% RH ± 5%, and accelerated at 40 ± 2°C/75% RH ± 5%. Refrigerated drug products undergo long-term storage at 5 ± 3°C and accelerated at 25 ± 2°C/60% RH ± 5%. A significant change is failure to meet specifications. Initial drug entrapped percentage is determined, stored at 4 ± 2°C, 25 ± 2°C, and 37 ± 2°C for 3 months, with analysis after 30 days for percent drug loss calculation[26].

Drug content:

The drug content is analyzed utilizing an instrumental method, often a modified high-performance liquid chromatography approach. This involves equipment like an ultraviolet detector, column oven, auto sampler, pump, and a computerized analysis program tailored to the pharmacopoeial method[60].

Turbidity measurement:

The nephelometer is commonly employed to measure turbidity in aqueous solutions[61].

Surface charge and charge density:

The Zetasizer is employed to determine the surface charge and charge density of transferosomes[62].

Penetration ability:

The penetration ability of transferosomes is typically evaluated using fluorescence microscopy. This method is commonly employed for assessing the penetration ability of transferosomes[63].

Occlusion effect:

Skin occlusion, beneficial for traditional topical preparations in aiding drug permeation, poses a challenge for elastic vesicles. In the context of vesicle permeation through the skin, hydrotaxis, the movement of water from the dry surface to water-rich deeper regions, is a major driving force. Hydrotaxis plays a crucial role by influencing hydration forces and preventing water evaporation from the skin[62].

Table 2: Parameters and characterization method for transferosomes

Parameter	Method/equipment
Zeta potential	Electrophoretic mobility technique
Vesicle size, size distribution	Dynamic light scattering method (DLS Method)
Vesicle morphology	DLS method, Photon correlation spectroscopy and Transmission electron microscopy
Number of vesicles for cubic mm	Hemocytometer and optical microscope
Entrapment efficiency	$\%\text{Entrapment efficiency} = \frac{Amount\ of\ the\ drug\ entrapped}{Total\ amount\ of\ the\ drug\ added} \times 100$ $\%\text{Entrapment efficiency} = \frac{\text{Total amount of the drug added} - \text{Amount of the free drug}}{Total\ amount\ of\ the\ drug\ added} \times 100$
Drug content	The HPLC method has been adapted by incorporating an ultraviolet detector, auto-sampler, column oven, pump, and computerized analysis program, tailored to the specific analytical requirements of the active pharmaceutical agent.
Degree of deformability	Microporous filter with DLS and Transmission electron microscopy
Surface charge and charge density	DLS method by Malvern Zetasizer and Thin-layer chromatography
In vitro drug release	Franz diffusion cell with cellulose membrane and Extrusion method
In vivo skin permeation studies	Franz diffusion cell
In vivo permeation ability	Histological study used to determine the bioadhesive potential and retention of transferosomes in the skin, Confocal scanning laser microscopy (CSLM), Fluorescence microscopy
Stability studies	DLS method and transmission electron microscopy

APPLICATION OF TRANSFEROSOMES

Transferosomes are lipid-based nano systems used for topical drug delivery, offering advantages such as increased solubility and permeability of drugs with low bioavailability [64,65]. They have been applied in various studies for delivering different drugs, including antifungal agents and ferulic acid[65,66]. Some notable applications of transferosomes include:

Carvedilol Nano lipid Transferosomes:

In a study, carvedilol, a low bioavailable drug (25-35%), was encapsulated in nanostructured lipid carrier (NLC) loaded transferosomes using a Box-Behnken design. The optimized formulation showed improved dermato pharmacokinetic and pharmacodynamic parameters when compared to a conventional formulation [64,67].

Antifungal Agent Transferosomes:

Transferosomes containing an antifungal agent were prepared using a Rotary Flask Evaporation-Sonication method. The Plackett-Burman design was employed to identify significant formulation and process parameters affecting vesicle size, such as the amount of lipid and surfactant, volume of ethanol and hydration medium, and hydration time[66].

Ferulic Acid Transferosomes:

In a comparative study, ferulic acid, an antioxidant molecule derived from natural sources, was incorporated into transferosomes and monoolein aqueous dispersions (MADs). The study found that transferosomes with poloxamer 188 in their composition created a multilamellar system, effectively controlling the release of the drug. The type of non-ionic surfactant impacted the drug release rate, and patch tests revealed that all transferosome formulations tested were safe when applied under occlusive conditions for 48 hours[65].

Rotigotine HCL and Rasagiline Mesylate Transferosomes:

In another study, transferosomes were optimized for the delivery of Rotigotine HCL and Rasagiline mesylate. The Plackett-Burman and Box-Behnken designs were used for screening and optimization of the formulation. The study found that transferosomes were spherical particles with a uniform distribution, suitable for drug delivery. The vesicle size, entrapment efficiency, and in vitro drug permeation investigation showed promising results for the delivery of these drugs[68].

Anti-cancer drugs transferosomes:

Transferosome technology was applied to explore transdermal distribution of anti-cancer drugs like methotrexate, yielding positive results and offering a promising therapeutic approach, particularly for treating skin cancer [71,72]. Research has highlighted the potential of transferosomes in delivering phytoconstituents with anti-cancer properties, among other therapeutic applications. These findings suggest that transferosomes hold promise for the targeted delivery of anti-cancer agents and warrant further investigation in the field of cancer therapy[70]. Jiang et al. (2018) demonstrated successful topical chemotherapy for melanoma using transferosome-embedded paclitaxel hydrogels, revealing effective tumor tissue penetration with phosphatidylcholine, tween 80, and sodium deoxycholate components[69].

Insulin transferosomes:

Transferosomes can improve the bioavailability of insulin by protecting it from degradation in the gastrointestinal tract and increasing its absorption in the intestinal mucosa. Transferosomes can reduce the side effects of insulin, such as hypoglycemia, by controlling the rate of insulin absorption and maintaining a stable blood glucose level. Transferosomes can be used for oral delivery of insulin, which is a non-invasive and patient-friendly method. Clinical trials have been conducted to evaluate the efficacy and safety of transferosomal insulin in type 2 diabetes patients. The results showed that transferosomal insulin was effective in controlling blood glucose levels and had a good safety profile. Transferosomal insulin is available in the market under the brand name "Insulin Transfer"[73].

Proteins and peptides transferosomes:

Transferosomes have emerged as a successful tool for transdermal delivery of a variety of therapeutics including hydrophilic actives, larger molecules, peptides, and proteins. Transferosomes possess a dynamic structure and increased surface hydrophilicity, crucial for facilitating the transport of drugs and other solutes. They leverage hydration gradients as a driving force to efficiently deliver molecules through the skin. They provide increased stability by preventing degradation of the actives by oxidation, light, and temperature. Therefore, transferosomes can be applied as a delivery system for proteins and peptides[29,74,75]. Additionally, soybean proteins and peptides have been studied for their potential use as hypotensive agents [76,77].

Interferon transferosomes:

Transferosomes have been employed for transporting interferons like leukocytic-derived interferon (INF), a naturally occurring protein with antiviral, antiproliferative, and immunomodulatory properties. The use of transferosomes in transdermal drug delivery systems (TDDS) holds promise for delivering medication in a controlled manner while also improving the stability of sensitive medications [37,71].

Anesthetic transferosomes:

Transferosomes, a type of lipid-based drug delivery system, have been studied for their potential application in delivering anesthetics. Research has shown that transferosomes can enhance the penetration of local anesthetics into the skin, resulting in a longer duration of anesthesia compared to traditional creams. For example, transferosomes containing lidocaine and tetracaine have been found to be effective in providing prolonged anesthesia in animal models. This suggests that transferosomes may offer a promising approach for the topical delivery of anesthetics, potentially improving their efficacy and duration of action. Transferosome suspension of anesthetics yields a topical anesthetic in under 10 minutes, providing comparable pain insensitivity (80%) to a subcutaneous bolus dosage. Notably, transferosomal anesthetics exhibit prolonged duration of action. The inclusion of PAMAM G3 in the transferosomal formulation enhances local anesthetic effectiveness by 1.62 times compared to prior formulations [78,79].

Corticosteroid transferosomes:

Transferosomes are a type of vesicular system that have been extensively studied for delivering various drugs, including corticosteroids, through the skin[7]. They possess a high deformability, allowing them to pass through narrow constrictions without measurable loss, and can

accommodate drug molecules with a wide range of solubility [80,81]. This makes them a promising approach for transdermal drug delivery, overcoming the barrier posed by the skin's impermeability to large molecules and hydrophilic drugs. Research has shown the efficacy of transferosomes in enhancing site-specific corticosteroid delivery and increasing the permeation and therapeutic effects of corticosteroids [82,83].

Non-steroidal anti-inflammatory drugs [NSAIDs] transferosomes:

Many NSAIDs encounter significant gastrointestinal side effects. Transdermal delivery through transferosomes offers a solution to these issues. Studies conducted on diclofenac and ketoprofen reveal promising results. Notably, a transferosome formulation of ketoprofen received approval from the Swiss regulatory agency (Swissmedic) in 2007, set to be marketed as "Diractin." IDEAAG reports ongoing clinical development for additional therapeutic products utilizing transferosome technology. On the other hand, NSAIDs are non-steroidal anti-inflammatory drugs that are used for the treatment of inflammation, pain, and fever. They have been evaluated for their potential use in the pharmacotherapy of neurodegenerative, cardiovascular, diabetes, and cancer diseases [15,84,85].

Herbal transferosomes:

Transferosomes, a type of vesicular carrier, have been increasingly studied for the delivery of herbal medicines through the skin. These vesicular systems, including transferosomes, phytosomes, niosomes, ethosomes, and cubosomes, have been reported to enhance the skin transport of phytoconstituents, thereby improving their bioavailability and therapeutic efficacy. The use of nano vesicular systems based on phospholipids for the topical application of phytoconstituents has gained attention due to the ease of administration, local drug targeting, patient compliance, controlled drug delivery, and bypassing first-pass metabolism. This approach holds potential for overcoming the limitations associated with the oral administration of herbal extracts and phytoconstituents, such as poor lipid solubility, high molecular size, and first-pass metabolism, ultimately improving patient adequacy and therapeutic outcomes [86,87].

CONCLUSION

The transdermal drug delivery route faces limitations due to the barrier properties of the skin's stratum corneum layer, preventing the transport of high molecular weight therapeutic agents. Transferosomes, uniquely designed vesicles, respond to external stress by squeezing through narrow skin pores, enhancing transdermal flux of therapeutic agents. They offer advantages such as high penetration power, increased stability, systemic drug release capability, and greater deformability compared to vesicular systems like niosomes and ethosomes. With a structure comprising both hydrophobic and hydrophilic moieties, transferosomes accommodate a wide range of drug solubilities. These ultra-deformable carriers demonstrate superior efficacy, driven by osmotic gradients, making them promising for diverse drug delivery. Further optimization and scientific exploration of transferosomes could lead to innovative therapeutic approaches against various diseases, positioning them as a key player in future drug delivery applications.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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