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Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery System

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ABSTRACT: In recent years, transdermal drug delivery systems have gained considerable interest because they offer several advantages compared to traditional oral or injectable methods. These systems are non-invasive, user-friendly, and designed to improve patient compliance by enabling controlled drug release. However, one of their main challenges is the skin's natural barrier, which significantly restricts the absorption of molecules larger than 500 Da or those that are ionized. This limitation means that only a small number of drugs are suitable for skin-based delivery.

A promising way to overcome this hurdle is through the use of transfersomes. These are flexible, bilayer vesicles capable of carrying various types of drugs, whether they are lipophilic, hydrophilic, or amphiphilic. Unlike standard liposomes, transfersomes are exceptionally elastic, allowing them to squeeze through small skin pores without losing their structural integrity.

This review delves into the science of transfersomes, explaining how they work, how they are made, and the methods used to study their properties. It also examines the key factors that influence their effectiveness and highlights recent innovations in their application for delivering drugs through the skin.

Keywords:transfersomes; nano encapsulation; transdermal drug delivery.

I. INTRODUCTION

Achieving effective therapeutic treatment is often challenging due to factors such as hepatic first-pass metabolism, side effects, the invasiveness of some treatments, and low patient compliance. To address these challenges, researchers have developed various drug delivery systems over the years. One promising solution is transdermal drug delivery, which avoids the first-pass effect and is minimally invasive. However, the skin's natural barrier poses a significant obstacle, limiting the delivery of many therapeutic agents.

Nanoencapsulation using lipid-based vesicular systems like liposomes has been explored as a strategy to overcome this barrier. Liposomes help transport drugs across the skin through three primary mechanisms: adhesion to the skin surface, allowing direct transfer of the drug; fusion with the skin's lipid matrix to enhance drug partitioning; and lipid exchange between the liposome and cell membranes, aiding diffusion. Despite these advantages, conventional liposomes face challenges, such as shallow skin penetration, low encapsulation efficiency for hydrophilic drugs, membrane instability, and a short half-life. As a result, they are more suited for dermal delivery than for deeper, transdermal applications.

To address these limitations, researchers have developed novel vesicles, including niosomes, sphingosomes, bilosomes, chitosomes, transfersomes, ethosomes, and invasomes. Niosomes, introduced in the 1970s, are made of non-ionic surfactants, cholesterol (for rigidity), and sometimes ionic amphiphiles to enhance stability and entrapment efficiency. Compared to liposomes, niosomes are more chemically stable, cost-effective, and capable of improving drug residence time in the skin while reducing systemic absorption.

Chitosomes, described in the 1990s, are liposomes coated with chitosan, which improves stability and provides mucoadhesive properties. Chitosan enhances membrane structural integrity, reducing fluidity and increasing overall stability. Similarly, sphingosomes, developed in the late 1990s, use sphingolipids and cholesterol to achieve greater stability and resistance to hydrolysis compared to phospholipid-based liposomes.

Bilosomes are another innovation, combining non-ionic surfactants and bile salts, making them ideal for oral vaccine delivery due to their resistance to gastrointestinal enzymes and

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bile. Transfersomes, introduced in the 1990s, stand out for their ultra-deformable nature, allowing them to pass through narrow skin pores. These vesicles use phospholipids and edge activators like Tween 80 or Span 80 to achieve high flexibility and spontaneous pore penetration, making them highly effective for transdermal delivery of drugs, peptides, and proteins.

Transfersomes have undergone extensive preclinical testing and clinical trials. For instance, ketoprofen-loaded transfersomes (Diractin®) demonstrated superior efficacy in treating knee osteoarthritis with fewer side effects. Insulin-loaded transfersomes (Transfersulin®) showed potential in lowering blood glucose levels in diabetic rabbits. Similarly, transfersomes containing triamcinolone acetonide showed an improved risk-benefit profile compared to conventional formulations.

The success of transfersomes has inspired the development of related systems, such as ethosomes and invasomes. Ethosomes, containing high ethanol concentrations, enhance skin penetration by disrupting the stratum corneum's lipid structure. Invasomes, combining phospholipids, ethanol, and terpenes, similarly improve drug delivery by disrupting tight lipid packing in the skin.

This review focuses on transfersomes as the first-generation elastic vesicles, exploring their mechanisms, preparation methods, characterization techniques, and the factors influencing their properties. It also highlights recent advances in their application for transdermal drug delivery, offering valuable insights for researchers in this field.

II. TRANSFEROSOMES

Transferosomes are vesicular carrier systems that are specially designed to have at least one inneraqueous compartment that is enclosed by a lipid bilayer, together with an edge activator (Figure 1 & 2).

Transfersomes are specialized vesicles with an aqueous core surrounded by a lipid bilayer, giving them remarkable flexibility and self-regulating properties. Their elasticity allows them to deform and pass through extremely narrow skin pores or constrictions—much smaller than their own size—without losing integrity. Unlike traditional liposomes, which are made from natural or synthetic phospholipids like egg phosphatidylcholine (EPC) or dipalmitoyl phosphatidylcholine (DPPC), transfersomes include an additional component: edge activators (EAs). These single-chain surfactants enhance the flexibility of the vesicle membranes, making transfersomes highly deformable and improving their ability to permeate the skin.

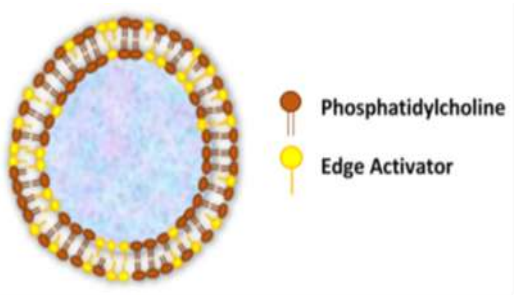

Figure 1. Structure of transfersomes

This unique composition enables transfersomes to address the limitations of conventional liposomes, such as limited permeability and structural fragility. Transfersomes retain their structure even after passing through smaller pores and deliver superior performance. The inclusion of EAs not only boosts vesicle flexibility but also helps dissolve hydrophobic drugs, increasing their entrapment within the vesicles. Additionally, EAs can fluidize and break down skin lipids, further enhancing drug penetration. The effectiveness of these enhancements depends on the type and concentration of EAs used.

Surfactants, commonly used as edge activators and penetration enhancers, are amphiphilic molecules with both hydrophilic and lipophilic properties. Non-ionic surfactants, which lack a charge, are often preferred due to their lower toxicity, reduced irritation, and better biocompatibility compared to cationic or anionic surfactants. These surfactants also maintain near-physiological pH levels and serve multiple roles, including acting as solubilizers, emulsifiers, and absorption enhancers, making them valuable for targeted drug delivery.

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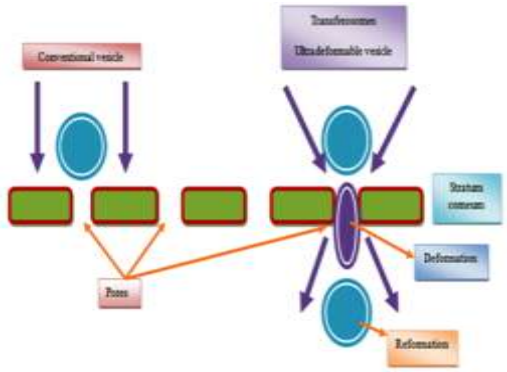

Figure 2. Schematic diagram describing interaction of the transferosomes with skin tissues

Transfersomes have proven effective in various applications, including peripheral drug targeting, transdermal immunization, and the delivery of a wide range of therapeutic agents. Studies show that transfersomes can transport both small and large molecules (200 Da to over 1,000,000 Da), as well as hydrophilic and lipophilic compounds, with an impressive transport efficiency exceeding 50%. The skin's natural barrier limits molecule penetration, but transfersomes significantly enhance the delivery of drugs, even outperforming liposomes. They have been shown to transfer lipophilic markers through murine skin with greater efficiency than unencapsulated drugs or conventional liposomes.

The size of the vesicles is critical for effective skin penetration. Larger vesicles (≥600 nm) struggle to reach deeper layers, while smaller ones (≤300 nm) penetrate further into the skin. Vesicles around 70 nm have demonstrated the highest deposition in the viable epidermis and dermis. Notably, transfersomes sized at 120 nm have exhibited enhanced penetration compared to larger vesicles.

The use of transfersomes in herbal formulations has also shown promising results, improving the bioavailability, stability, and delivery of active plant-derived compounds. This makes them particularly useful in skincare and therapeutic applications, offering sustained drug release and efficient delivery into deeper skin layers or systemic circulation.

With their remarkable ability to overcome the skin's natural barriers, transfersomes open new possibilities for drug delivery. They hold immense potential for creating innovative, efficient, and targeted therapeutic systems, paving the way for advancements in transdermal and systemic drug delivery methods.

2.1 Advantages of Transfersomes as Vesicle-based Transdermal Drug Delivery Systems:

- Transfersomes possess both hydrophilic and hydrophobic properties, making them a unique and versatile drug delivery system capable of transporting a wide range of therapeutic agents with varying solubility profiles.
- Their ultra-deformable and elastic nature enables them to pass through extremely narrow skin barrier constrictions—up to 5 to 10 times smaller than their own size—while maintaining their integrity.
- This high deformability allows drugs to penetrate the skin without significant loss of intact vesicles, making them suitable for both localized and systemic treatments.
- Transfersomes are highly adaptable and can accommodate various types of molecules, regardless of their size, structure, molecular weight, or polarity.
- Composed of natural phospholipids and edge activators (EAs), transfersomes are biocompatible and biodegradable, ensuring minimal adverse reactions.
- They are effective in delivering a diverse range of active compounds, such as proteins, peptides, insulin, corticosteroids, interferons, anesthetics, NSAIDs, anticancer drugs, and herbal formulations.
- Transfersomes are ideal for achieving sustained drug release, providing predictable and prolonged therapeutic effects.
- These vesicles enhance transdermal drug flux and improve the targeted delivery of bioactive agents.
- By bypassing first-pass metabolism—a common limitation in oral drug delivery—transfersomes optimize drug bioavailability.
- They help minimize side effects, protect drugs from metabolic degradation, and enhance the utility of drugs with short half-lives.
- Transfersomes often exhibit high entrapment efficiency, especially for lipophilic drugs, with rates reaching up to 90%. For instance, transferosome formulations of diclofenac diethylamine (DDEA) and curcumin (CRM) have achieved over 90% entrapment efficiency. However, this efficiency can vary based on factors like lipid and surfactant concentrations; higher lipid content improves encapsulation, while excessive surfactant can lead to mixed micelle formation and reduced efficiency. Using surfactants with low

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hydrophilic-lipophilic balance (HLB) values can enhance encapsulation efficiency in some cases.

- Transfersomes stand out for their ability to encapsulate lipophilic drugs effectively, making them highly advantageous for certain therapeutic applications.
- They are crafted from pharmaceutically accepted materials using standard methods, though their design and formulation require optimization tailored to specific drugs.
- The production process is straightforward and scalable, enabling easy large-scale manufacturing.

In summary, transfersomes offer a highly efficient, flexible, and innovative approach to transdermal drug delivery, making them an excellent choice for a variety of medical and therapeutic applications.

2.2 Limitations of transfersomes

- Transfersomes are prone to chemical instability due to their susceptibility to oxidative degradation. To mitigate this, the aqueous medium used in their formulation can be degassed and purged with inert gases like nitrogen or argon. Additionally, storing transfersomes at low temperatures and protecting them from light can further reduce the risk of oxidation. Post-preparation techniques such as freeze-drying or spray-drying can also enhance their storage stability.
- Achieving the required purity of natural phospholipids for transfersome preparation is another challenge. To address this, synthetic phospholipids can be used as a reliable alternative.
- The cost of transfersomal formulations is relatively high, primarily because of the expensive lipid excipients and specialized equipment required for production. However, phosphatidylcholine, a widely used lipid component, is often chosen for its affordability compared to other options.

While these limitations exist, careful formulation strategies and material choices can help address these challenges effectively.

III. MECHANISM OF ACTION

Understanding Vesicles and Transfersomes in Drug Delivery -

Vesicles are colloidal particles made up of an aqueous core enclosed by a bilayer of amphiphilic molecules. These structures serve as excellent carriers for drug delivery, encapsulating hydrophilic drugs within their aqueous core while trapping hydrophobic drugs in the lipid bilayer. Transfersomes, a novel type of vesicular carrier, stand out for their ultra-flexibility and self-optimizing properties, making them highly effective for transporting drugs across the skin.

The ability of transfersomes to penetrate intact skin relies heavily on their flexible membranes, hydrophilic nature, and capacity to maintain structural integrity. These vesicles are particularly effective under nonocclusive conditions, as this creates a transepidermal osmotic gradient that drives their movement through the skin. Research by Cevc and Blume highlights a mechanism called hydrotaxis, or xerophobia, which describes the transfersomes' natural tendency to move toward the moist deeper layers of the skin while avoiding the dry outer surface. This gradient-driven movement occurs due to water evaporation from the transfersomal formulation when applied to the skin.

The transdermal water activity gradient generates a strong force that widens intercellular junctions in the skin, forming channels approximately 20–30 nm in width. These channels allow the ultra-flexible transfersomes to pass through and deliver therapeutic agents to their target areas, either locally or systemically, with high efficiency and minimal systemic toxicity.

Transfersomes offer superior permeation capabilities compared to conventional liposomes. While they share a similar bilayer structure, transfersomes have softer, more adaptable membranes that enable their ultra-deformability. This flexibility, combined with the interdependent composition and shape of the lipid bilayer, makes transfersomes both self-regulating and self-optimizing, allowing them to overcome various transport barriers effectively.

Constructed from at least one amphiphilic agent and a bilayer-softening edge activator, transfersomes exhibit enhanced flexibility and permeability. Some formulations include alcohols like ethanol or propylene glycol, which act as penetration enhancers and co-solvents. These substances can modify the polar head region of the lipid bilayer, increasing its fluidity and reducing the density of the lipid lamellae.

The ability of transfersomes to reach the dermis and bloodstream depends largely on the deformability of their membranes, which is

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determined by their composition. Optimizing this composition requires carefully designed experiments tailored to each therapeutic agent to ensure the best combination of deformability, drug-carrying capacity, and stability.

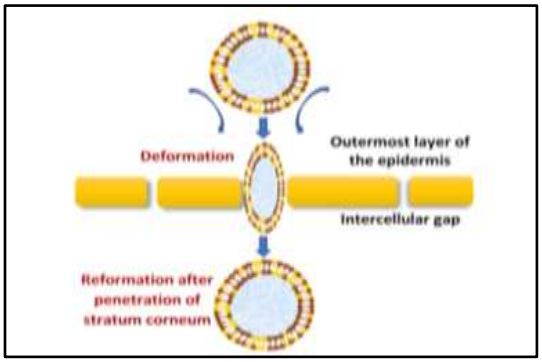

Figure 3. The mechanism of action of transfersomes

IV. PATHWAYS OF TRANSFERSOMES PENETRATION:

1. Through stratum corneum
2. Transfollicular
3. Through sweat glands

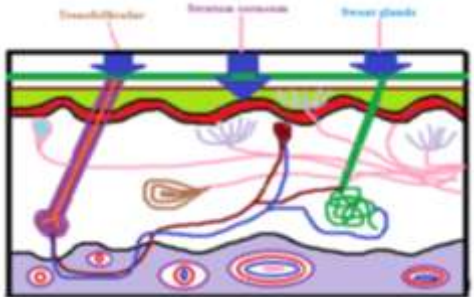

Figure 4. Pathways of transfersomes penetration

V. COMPOSITION OF TRANSFEROSOMES

Transfersomes are generally composed of:

• Firstly, the main ingredient, an amphipathic ingredient (e.g., soya phosphatidylcholine, egg phosphatidylcholine, etc.) that can be a mixture of lipids, which are the vesicle-forming components that create the lipid bilayer.

• secondly, 10–25% surfactants/edge activators; the most commonly used edge activators in transfersome preparations are surfactants as sodium cholates; sodium deoxycholate; Tweens and Spans (Tween 20, Tween 60, Tween 80, Span 60, Span 65 and Span 80) and dipotassium glycyrrhizinate, which are biocompatible bilayer-softening compounds that increase the vesicles' bilayer flexibility and improve the permeability.

• about 3–10% alcohol (ethanol or methanol), as the solvent and, finally, hydrating medium consist with either water or a phosphate buffer (pH 6.5–7).

Table 1. Composition of Transfersomes

Sr. No.	Class	Example	Uses
1	Phospholipids	Soya lecithin Soya phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearoyl phosphatidyl choline	Vesicles forming components
2	Surfactant	Sod. Cholate Sod. Deoxycholate Span-80 Span-60 Tween-80 Tween-20	Edge activator
3	Alcohol	Ethanol Methanol Chloroform	As a solvent
4.	Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein-DHPE Nile-Red	For CSLM study
5	Buffering Agent	Phosphate Buffer	As a hydrating Medium

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In an aqueous environment, the phospholipids self-assemble into flexible lipid bilayers and close to form vesicles. The biocompatible membrane softeners, which are also known as edge activators, are single-chain surfactants that incorporate into the transfersomes structure and facilitate the destabilization of the vesicle's lipid bilayer and enhance its fluidity and elasticity. The total amount of surfactants and the proper ratios of individual surfactants to phospholipids are responsible for the control of vesicles membrane flexibility and minimizing the risk towards vesicle ruptures in the skin. This result promotes transfersomes to follow the natural osmotic gradient across the epidermis following application under a nonocclusive manner. In summary, the penetration-enhancing effect of these vesicles depends on the concentrations and the types of surfactants, the types of lipids, the size shape and elasticity of the vesicles.

VI. PREPARATION METHODS (MANUFACTURING PROCESS)

While several patented methods exist for preparing transfersomes, there is no universal protocol or fixed formula for their production. This means the optimal preparation conditions and vesicle compositions must be identified and tailored through carefully designed experiments specific to each therapeutic agent. These experiments aim to create carriers with the best possible deformability, drug-loading capacity, and stability.

The most commonly used method for making transfersomes is the **thin film hydration technique**, often referred to as the rotary evaporation-sonication method. Beyond this, several modified techniques are available, including vortexing-sonication, the modified handshaking process, suspension homogenization, centrifugation, the reverse-phase evaporation method, high-pressure homogenization, and the ethanol injection method. Each of these methods has unique steps and applications that suit specific requirements for drug delivery systems.

6.1Thin Film Hydration Technique/Rotary Evaporation-Sonication Method

To prepare transfersomes, phospholipids and an edge activator (the vesicle-forming components) are dissolved in a volatile organic solvent mixture, such as a blend of chloroform and methanol in an appropriate volume ratio. If the drug being delivered is lipophilic, it can be added during this step. A thin lipid film is created by evaporating the organic solvent above the lipid's transition temperature under reduced pressure using a rotary vacuum evaporator. To ensure all traces of solvent are removed, the setup is kept under vacuum.

The thin lipid film is then hydrated with a buffer solution of suitable pH (for example, pH 7.4) by rotating it for a specified time at the required temperature. If the drug is hydrophilic, it can be incorporated during this hydration step. The resulting vesicles are allowed to swell at room temperature, then sonicated using either a bath or probe sonicator to produce smaller vesicles. Finally, the sonicated vesicles are homogenized by extruding them through polycarbonate membranes with pore sizes ranging from 200 nm to 100 nm.

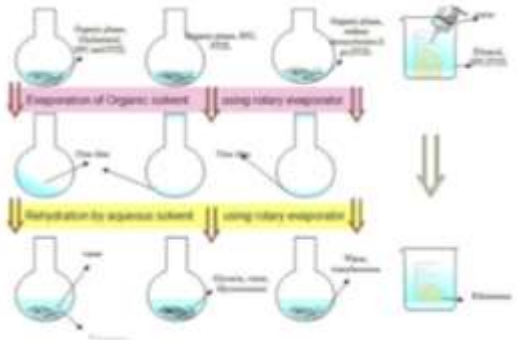

Figure 5. Rotary film evaporation method

6.2Vortexing-Sonication Method

The phospholipids, edge activator and the drug are mixed in a phosphate buffer. The mixture is then vortexed until a milky transfersomal suspension is obtained. It is then sonicated, using a bath sonicator, for a respective time at room temperature and then extruded through polycarbonate membranes (example: 450 and 220 nm).

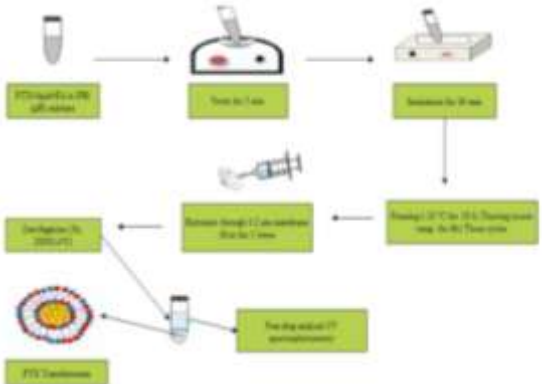

Figure 6. Vortex or Sonication method

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6.3Modified Handshaking Process

The modified handshaking method follows the same foundational principles as the rotary evaporation-sonication technique, with a slight variation in the process. In this method, the organic solvent, lipophilic drug, phospholipids, and edge activator are added to a round-bottom flask. These components are thoroughly mixed until fully dissolved, forming a clear, transparent solution. Instead of using a rotary vacuum evaporator, the organic solvent is manually evaporated while gently handshaking the flask. During this process, the flask is partially immersed in a water bath maintained at an elevated temperature (typically between 40–60°C).

As the solvent evaporates, a thin lipid film forms along the inner wall of the flask. To ensure complete removal of the solvent, the flask is left overnight. The next day, the lipid film is hydrated using a suitable buffer solution with gentle shaking at a temperature above the phase transition temperature of the lipids. If the drug is hydrophilic, it can be incorporated during this hydration step.

6.4Suspension Homogenization Method

Transfersomes are prepared by mixing an ethanolic phospholipid solution with an appropriate amount of edge activator. The prepared suspension is subsequently mixed with buffer to yield a total lipid concentration. The resulting formulation is then sonicated, frozen and thawed respectively two to three times.

6.5 Centrifugation Process

Phospholipids, an edge activator, and a lipophilic drug are first dissolved in an organic solvent. The solvent is then carefully removed using a rotary evaporator under reduced pressure at a controlled temperature. To eliminate any residual solvent, the mixture is subjected to vacuum treatment.

The resulting thin lipid film is hydrated with a suitable buffer solution, and the hydration process is carried out by centrifuging at room temperature. At this stage, hydrophilic drugs can also be incorporated into the formulation. The hydrated vesicles are allowed to swell at room temperature, forming multilamellar lipid vesicles. These vesicles are then further processed by sonication at room temperature to refine their structure.

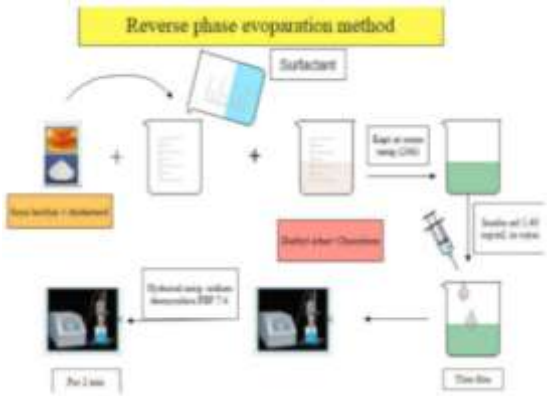

Figure 7. Reverse-Phase Evaporation Method

6.6 Reverse-Phase Evaporation Method

Phospholipids and an edge activator are combined in a round-bottom flask and dissolved in an organic solvent mixture, such as diethyl ether and chloroform. At this stage, a lipophilic drug can also be incorporated into the solution. The solvent is then evaporated using a rotary evaporator to create lipid films.

These lipid films are redissolved in an organic solvent, typically made up of isopropyl ether, diethyl ether, or a combination of both. Next, an aqueous phase is added to the organic phase, resulting in a two-phase system. Hydrophilic drugs can be introduced during this step. The mixture is then sonicated in a bath sonicator until a uniform water-in-oil (w/o) emulsion forms.

Finally, the organic solvent is gradually evaporated using a rotary evaporator, transforming the emulsion into a viscous gel, which subsequently forms a vesicular suspension.

6.7 High-Pressure Homogenization Technique

The phospholipids, edge activator and the drug are uniformly dispersed in PBS or distilled water containing alcohol and followed by ultrasonic shaking and stirred simultaneously. The mixture is then subjected to intermittent ultrasonic shaking. The resulting mixture is then homogenized using a high-pressure homogenizer. Finally, the transfersomes are stored in appropriate conditions.

6.8 Ethanol Injection Method

To prepare the organic phase, the phospholipid, edge activator, and lipophilic drug are dissolved in ethanol while being stirred magnetically until a clear solution forms. For the aqueous phase, water-soluble substances are dissolved in phosphate buffer. At this stage, hydrophilic drugs can be added.

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Both the organic and aqueous solutions are heated to a temperature of 45–50°C. Following this, the ethanolic phospholipid solution is slowly added dropwise to the aqueous solution with continuous stirring for a specified duration. To remove ethanol, the resulting dispersion is transferred to a vacuum evaporator. Finally, the mixture is sonicated to reduce particle size.

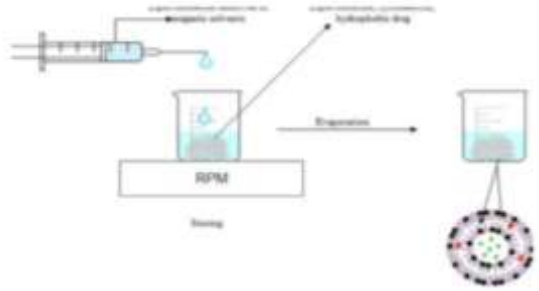

Figure 8. Ethanol Injection Method

6.9 Freeze thaw method

This process involves freezing the created multi lamellar vesicles suspension and then transferring it to a tube and dipping it in a nitrogen bath at -300 degrees Celsius for 30 seconds. After the suspension has frozen, it is treated to a high temperature in a water bath for 8-9 rounds. Diagrammatic view of this method is given Fig. 9

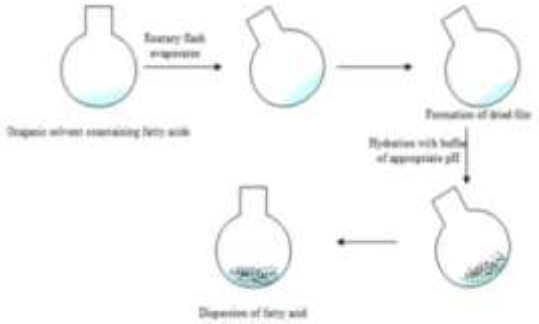

Figure 9. Freeze thaw method

VII. FACTORS AFFECTING PROPERTIES OF TRANSFEROSOSMES

In the process of obtaining an optimized formulation of transfersomes, there are number of process variables that could affect the properties of the transfersomes. These variables basically involve the manufacturing of transfersomal formulations, which are identified as follows:

7.1. Effect of Phospholipids

The ratio of phospholipid to edge activator (e.g., lecithin to surfactant) is a critical factor that significantly influences entrapment efficiency, vesicle size, and permeation capability. Studies indicate that a higher concentration of surfactant can reduce entrapment efficiency. This reduction is likely due to increased membrane permeability caused by the arrangement of surfactant molecules within the lipid bilayer, which can create pores in the vesicle membrane. These pores increase fluidity, potentially leading to the leakage of encapsulated drugs.

If the edge activator concentration continues to rise, the formation of additional pores in the bilayer may occur, further reducing the vesicle's ability to permeate effectively. Conversely, using lower concentrations of surfactants can result in larger vesicle sizes. Additionally, studies have shown that higher phospholipid concentrations tend to decrease vesicle size, highlighting the importance of optimizing the phospholipid-to-surfactant ratio to achieve the desired vesicle properties.

7.2. Effect of Various Solvents

Solvents like ethanol or methanol are commonly used in formulations, with the choice depending on the solubility of all ingredients and their compatibility with the solvent. Ideally, all excipients, including the drug, should dissolve completely in the solvent to create a clear, transparent solution. This ensures optimal film-forming capability and stability after hydration. Additionally, these solvents can act as penetration enhancers, aiding in the drug's ability to permeate through membranes.

Research by Williams and Barry (2004) demonstrated that ethanol has been utilized in several studies to improve the skin flux of drugs like hydrocortisone, 5-fluorouracil, estradiol, and levonorgestrel in rats. Ethanol enhances permeation through multiple mechanisms, including increasing drug solubility within vesicles and penetrating the stratum corneum. It alters the solubility characteristics of this tissue, thereby improving drug partitioning into the membrane. However, increasing ethanol concentration in a formulation can lower the entrapment efficiency (%EE), likely due to heightened permeability of the vesicular phospholipid bilayer, which can lead to drug leakage.

7.3. Effect of Various Edge Activators (Surfactants)

The deformability and entrapment efficiency of transferosome vesicles are

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significantly influenced by the type of edge activators (EAs) used in their formulation. This variation can be attributed to differences in the chemical structure of the EAs. Generally, increasing the surfactant concentration, the hydrophilicity of its head group, carbon chain length, or its hydrophilic-lipophilic balance (HLB) tends to reduce the vesicle size. For instance, surfactants such as Tween 80, Span 80, and sodium deoxycholate have been used to prepare transfersomes, with higher surfactant concentrations resulting in smaller vesicles. This is likely because concentrations exceeding 15% may promote micelle formation rather than vesicle formation.

A higher surfactant concentration is also associated with a smaller polydispersity index (PDI), indicating a uniform size distribution of vesicles. This uniformity plays a crucial role in reducing interfacial tension and producing a homogenous formulation. Additionally, increased surfactant concentrations may enhance the charge on vesicles, reducing aggregation and improving system stability.

Surfactants also impact the entrapment efficiency of vesicles. For example, using a surfactant with a low HLB value can improve the encapsulation of lipophilic drugs. Higher surfactant concentrations can increase the number of vesicles, thereby providing a greater hydrophobic bilayer volume for drug entrapment. However, exceeding the vesicular loading capacity with too much lipophilic drug can destabilize the membrane, causing drug leakage, reducing entrapment efficiency, and impairing skin permeation.

Membrane permeability of vesicles is further influenced by the surfactant's carbon chain length and transition temperature. The optimal surfactant amount depends on the phospholipid's packing density and its interaction with the surfactant. Surfactants also affect the permeation properties of transfersomes. For instance, a study by Cipolla et al. (2014) found that the release of ciprofloxacin depended on the surfactant type and concentration, with Tween 80 significantly enhancing drug release.

7.4. Effect of the Hydration Medium

The hydration medium for transfersomes can consist of either water or a saline phosphate buffer with a pH range of 5.5 to 7. It's essential to adjust the pH level of the formulation to strike a balance between its physical properties, biological compatibility, and intended route of administration. The lipid bilayer of transfersomes closely resembles the phospholipid structure of cell membranes, allowing only unionized drugs to bind to the bilayer and penetrate through the intracellular route. Therefore, selecting an appropriate pH for the hydration medium is crucial. This ensures the drug remains in its unionized form, enhancing both its entrapment within the vesicles and its ability to permeate biological barriers effectively.

VIII. CHARACTERIZATION OF THE TRANSFEROSOMES

There are several published methods used to determine the characterization parameters of the transfersomes, such as the vesicle shape and size, size distribution, polydispersity index, zeta potential, number of vesicles for cubic mm, entrapment efficiency, degree of deformability and skin permeability measurements, which are beneficial for the optimization of the transfersomal formulation. Each characterization method mentioned above is explained in detail below.

8.1 Vesicle Size, Zeta Potential and Morphology

The size of vesicles plays a critical role in the preparation of transfersomes, batch-to-batch consistency, and scale-up processes. During storage, changes in vesicle size are a key factor influencing the physical stability of the formulation. Vesicles smaller than 40 nm tend to undergo fusion due to the high curvature of their bilayer membranes, while larger, electrically neutral transfersomes may aggregate through van der Waals forces because of their larger membrane contact areas. Vesicle size also impacts the drug-encapsulation efficiency in transfersomes. A high lipid-to-core ratio is ideal for lipophilic and amphiphilic compounds, whereas a larger aqueous core is better suited for hydrophilic drugs.

The size of vesicles can be measured using dynamic light scattering (DLS) or photon correlation spectroscopy (PCS). For this, the vesicle suspension is mixed with an appropriate medium, and measurements are taken in triplicate. Alternatively, the sample can be prepared in distilled water, filtered through a 0.2 mm membrane filter, and diluted with filtered saline before size determination using DLS or PCS.

Advanced techniques, such as a Malvern Zetasizer, allow for precise measurement of vesicle size and distribution, while structural changes can be visualized using transmission electron microscopy (TEM). The zeta potential, which provides insights into the stability of the vesicles,

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can also be measured using electrophoretic mobility with the Malvern Zetasizer. Visualization of transfersome vesicles can be achieved with phase-contrast microscopy or TEM.

8.2. Number of Vesicles Per Cubic mm

This parameter is important for the optimization of the composition of the transfersomes and other process variables. Unsonicated transfersomal formulations are diluted five times using 0.9% sodium chloride. A hemocytometer with an optical microscope is used to study this sample. The transfersomes with a vesicle size of more than 100 nm can be observed by optical microscope [88,89]. The number of transfersomes in small squares are counted and calculated using the following formula

$$\text{Total Number of transfersomes per cubic mm} = \frac{\text{Total Number of transfersomes counted} \times \text{dilution factor} \times 4000}{\text{Total Number of squares counted}}$$

8.3. Entrapment Efficiency (%EE)

The entrapment efficiency (%EE) is the amount of drug entrapped in the formulation. The EE is determined by separating the unentrapped drug from the vesicles using various techniques, such as mini-column centrifugation. In this process, direct or indirect methods can be used to determine the %EE. After ultracentrifugation, the direct approach would be removing the supernatant followed by disrupting the sedimented vesicles using a suitable solvent that is capable of lysing the sediment. Subsequently, the resulting solution can be diluted and filtered using a syringe filter (0.22–0.45 µm) to remove the impurities. The drug content is determined by employing analytical methods, such as modified high-performance liquid chromatography (HPLC) or spectrophotometrically, which depends on the analytical method of the active pharmaceutical ingredient (API). The percentage drug entrapment (the entrapment efficiency) is expressed as:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of the drug added}} \times 100$$

The indirect approach to determine the %EE is diluting the supernatant using a suitable solvent and filtering it to remove the impurities. The concentration of the drug in the supernatant is determined as the free drug by an appropriate analytical method. Thereby, the percentage drug entrapment is expressed as:

$$\%\ \text{Entrapment efficiency} = \frac{\text{Total amount of the drug added} - \text{Amount of the free drug}}{\text{Total amount of the drug added}} \times 100$$

8.4. Degree of Deformability

This parameter is important, as it affects the permeation of the transfersomal formulation. This study is done using pure water as the standard. The preparation is passed through many microporous filters of known pore sizes between 50 to 400 nm. The particle size, as well as the size distribution, are noted after each pass using DLS measurements. The degree of deformability is expressed as:

$$D = J\left(\frac{rv}{rp}\right)$$

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

8.5. In Vitro Drug Release

The in vitro drug release profile is crucial for understanding how a transfersomal formulation behaves, including insights into its release mechanism and kinetics. This helps guide the optimization of the formulation. To assess the drug release of transfersomes, it's often compared to the free drug or a reference product. Numerous studies have shown promising results with various transfersomal formulations. For example, a transfersomal gel of celecoxib for rheumatoid

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arthritis demonstrated a 75% release of the drug within 6 hours, which was over 30% more than the commercial gel. In another study, ketoconazole-loaded transfersomal gel showed an initial burst release of 40.67%, compared to 27.35% from a ketoconazole suspension after 6 hours. Lidocaine-loaded transfersomes released more than 80% of the drug after 6 hours.

In these studies, Franz diffusion cells are typically used to evaluate the drug release. The donor chamber is connected to the receptor chamber with adhesive tape, and the receptor chamber is stirred with a magnetic bar. The temperature of the receptor fluid is maintained at 32 ± 1°C, which reflects the typical temperature of human skin. A mixed cellulose ester membrane with a pore size of 0.45 µm is used for the study. Before the test, the membrane is soaked in the release medium (usually phosphate buffer) overnight at room temperature to allow the pores to swell. At set time intervals, usually 0, 0.5, 1, 2, 3, 4, 5, and 6 hours, 1 mL of the receptor medium is withdrawn, and the same volume of fresh PBS is added to maintain proper conditions. The collected samples are then analysed using methods like UV, HPLC, or high-performance thin layer chromatography (HPTLC).

8.6. In Vitro Skin Permeation Studies

This study aims to evaluate the efficiency of transdermal delivery systems and identify factors that enhance the transdermal flux of drugs, typically measured in units like µg/cm²/h. The insights gained from this research can help predict how these systems will behave in vivo, allowing for the optimization of formulations before more costly in-vivo studies are conducted. Ideally, human skin would be used to test the permeation properties of formulations. However, due to ethical concerns, limited availability, and religious restrictions, human skin is not always a practical option. As a result, various animal models—such as primate, porcine, rat, mouse, guinea pig, and even snake skin—are often used as alternatives. However, it's important to note that the absorption rates through animal skin may differ from those observed with human skin. Published studies suggest that pig skin is the most comparable to human skin, as the flux and concentration in pig skin are similar to those in human skin, with only minor differences.

Another alternative is the use of synthetic membranes, such as Strat M®, which have been shown to closely mimic human skin in terms of permeability and responsiveness. This model offers more consistency compared to both human and animal skin models. In these permeation studies, Franz diffusion cells are typically used. The selected membranes are mounted horizontally in the receptor compartments, with the stratum corneum side facing upwards toward the donor compartments. The receptor chambers are filled with a phosphate buffer saline solution and stirred with a magnetic bar to simulate blood circulation beneath the skin. The temperature of the receptor fluid is maintained at 37 ± 0.5°C. The test formulation is placed in the donor compartment, and the top of the diffusion cell is left open to simulate nonocclusive conditions. Samples of the receptor medium are withdrawn at specific time intervals, with fresh receptor medium added to replace the withdrawn volume to maintain sink conditions. These samples are then analysed using techniques like HPLC or spectroscopic methods.

8.7. Stability of Transfersomes

The stability of transfersome vesicles can be determined by assessing the structure and the size of vesicles with respect to time. DLS and TEM can be used for the determination of the mean size and structural changes, respectively. The optimized transfersomal formulations can be stored in tightly sealed amber vials at different temperature conditions. According to ICH (International Conference on Harmonization) guidelines, under the stability testing of new drug substances and products, the general case for the storage condition is described as, for the long term, 25 ± 2 ◦C/60% relative humidity (RH) ± 5% RH or 30 ± 2 ◦C/65% RH ± 5% for 12 months and, for accelerated testing, 40 ± 2 ◦C/75% RH ± 5% for six months. Drug products intended for refrigeration should be subjected to long-term storage at a condition of 5 ± 3 ◦C for 12 months and accelerated study for 25 ± 2 ◦C/60% RH ± 5% RH for six months. A significant change for the drug product is defined as the failure to meet its specifications.

IX. APPLICATIONS OF TRANSFEROSOMES

As the Transdermal Delivery System Over the past decades, the applications of the transfersomes in the field of transdermal drug administration have been extensively studied. Some of these applications are described in the section below.

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9.1. Delivery of Antioxidants

In 2017, Avadhani and colleagues developed nanotransfersomes containing epigallocatechin-3-gallate (EGCG) and hyaluronic acid using a modified thin-film hydration method followed by high-pressure homogenization. This approach was aimed at improving their effectiveness as UV protectants, antioxidants, and anti-aging agents. In 2019, Wu and his team prepared transfersomes containing resveratrol using the high-pressure homogenization technique. Their study found that the resulting transfersomes enhanced the stability, bioavailability, solubility, and safety of resveratrol.

9.2. Delivery of Anticancer Drugs

Research conducted by Jiang et al. in 2018 was associated with the topical chemotherapy of melanoma by transfersome-embedded oligopeptide hydrogels containing paclitaxel prepared by the thin-film dispersion method. Transfersomes composed of phosphatidylcholine, tween80 and sodium deoxycholate were shown to effectively penetrate into tumour tissues [52]

9.3. Delivery of Corticosteroids

In 2003 and 2004, Cevc and Blume conducted studies on halogenated corticosteroid triamcinolone-acetonide-loaded transfersomes, which were prepared using the traditional thin-film hydration method. Their findings revealed that transfersomes enhanced the biological activity, extended the duration of effect, and reduced the required therapeutic dose.

9.4. Delivery of Anti-Inflammatory

Drugs Diclofenac sodium, celecoxib, mefenamic acid and curcumin-loaded transfersomes were developed and studied for the purpose of topical administration by several research groups. Research findings suggested that transfersomes could improve the stability and efficacy of the anti-inflammatory drugs [76,103,104].

X. CONCLUSIONS

Transferosomes are highly flexible carriers that enable more effective delivery of various drug molecules through the skin, outperforming traditional vesicular systems. The key factor driving the movement of transfersomes into the deeper layers of the skin is the osmotic gradient. These vesicular systems are carefully designed and need to be optimized based on the specific drug in question to achieve the best formulation and desired therapeutic outcomes. Ongoing research on transfersomes could open up new and promising treatments for a wide range of diseases.

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