

Transferosomes as a Transdermal Drug Delivery- Formulation, Regulatory aspects and Future Outlook

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Abstract : Transferosomes are highly deformable lipid-based vesicular carriers developed to overcome the barrier properties of the stratum corneum and enhance topical and transdermal drug delivery. Unlike conventional rigid vesicles, transferosomes contain phospholipids and edge activators that provide membrane flexibility and enable the vesicles to deform and pass through narrow intercellular pathways within the skin. This unique property allows transferosomes to accommodate and transport a wide range of therapeutic agents, including hydrophilic, lipophilic, peptide, protein, and other macromolecular drugs. Various preparation approaches, including thin-film hydration, ethanol injection, reverse-phase evaporation, homogenization, freeze-thaw cycling, and extrusion, have been investigated for producing transferosome formulations with desirable vesicle characteristics. Important formulation and process variables include phospholipid and edge-activator concentration, solvent composition, hydration medium, vesicle size, and processing conditions. Characterization involves evaluation of vesicle size, morphology, entrapment efficiency, deformability, zeta potential, drug content, in-vitro release, skin permeation, and stability. Transferosomes have demonstrated potential applications in transdermal immunization, insulin and protein delivery, anticancer therapy, antifungal treatment, and corticosteroid delivery. Despite their advantages, challenges associated with formulation stability, manufacturing complexity, drug-loading capacity, excipient safety, and regulatory acceptance remain. Future development is expected to focus on optimized and scalable manufacturing, combination with physical penetration-enhancement techniques, targeted and stimuli-responsive systems, and application in biologic drug delivery. Overall, transferosomes represent a promising platform for improving the efficiency and versatility of topical and transdermal drug delivery.

Keywords: Transferosomes; Edge activators; Vesicular drug delivery; Iontophoresis based Transferosomes; Transcutaneous vaccination; Stimuli-responsive transferosomes.

INTRODUCTION

Novel Drug Delivery Systems (NDDS) are advanced approaches developed to overcome the drawbacks of conventional drug delivery methods. Conventional dosage forms usually release the drug immediately after administration, resulting in fluctuating plasma concentrations, reduced therapeutic efficacy, and increased side effects. In contrast, NDDS and Transdermal Drug Delivery Systems (TDDS) are designed to enhance the bioavailability, stability, and therapeutic performance of both new and existing drugs by modifying their release behavior, targeting specific sites of action, and maintaining consistent drug levels in the systemic circulation. Furthermore, encapsulating drugs within advanced

carrier systems protects the active ingredient from degradation, prolongs its residence time in the body, and reduces the required dose frequency, thereby improving patient compliance and overall treatment efficiency [1]. Marked commercially by the release of the scopolamine patch in 1979, transdermal administration offers significant benefits over traditional oral and parenteral routes. It allows for patient self-administration, enhances adherence, provides controlled drug release, and bypasses hepatic first-pass metabolism to maintain stable therapeutic levels [2,5]. Parenteral injection routes carry notable drawbacks such as pain, inability to reverse the drug, risks of embolism or hypersensitivity, and the generation of dangerous medical waste [2, 4]. In resource-limited settings,

improper needle disposal poses severe infection transmission risks, including bloodborne pathogens like HIV [2]. Additionally, oral delivery faces major challenges, such as drug breakdown within acidic gastrointestinal surroundings, variable pH, bitter taste, and limited bioavailability for delicate biomolecules [2, 4]. Consequently, TDDS serves as an ideal delivery platform for sensitive therapeutics like peptides, proteins, hormones, RNA-based therapies, and nonsteroidal anti-inflammatory drugs (NSAIDs) [2, 5]. Despite these clear clinical advantages, effective transdermal drug diffusion remains restricted by the skin's outermost barrier, the stratum corneum (SC) [2, 4, 5]. The SC operates via a "bricks and mortar" structural arrangement that maintains thermoregulation and prevents trans epidermal water loss [2]. The "bricks" consist of 18–20 layers of terminally differentiated, a nucleated corneocytes (0.2–0.4 μm thick and 40 μm wide) interconnected by Corne desmosomes, while the "mortar" is an intercellular lipid matrix composed of an equimolar mixture of ceramides, cholesterol, and free fatty acids [2]. Beneath this non-viable SC layer (50–100 μm thick) lies the viable epidermis and the vascularized dermis (1–2 mm thick), which contains capillary loops that absorb permeated drugs into systemic circulation [2]. Due to this tight barrier, passive skin permeation is strictly restricted to non-ionized, hydrophobic molecules with low molecular weights (typically below 500 Da), while larger or hydrophilic compounds exhibit severely reduced permeability [2, 4]. To bypass the stratum corneum barrier, various physical and vesicular technologies have been explored, including iontophoresis, electroporation, sonophoresis, microemulsions, microneedles, and lipid vesicles [2]. Early vesicular platforms, such as conventional liposomes (phospholipid bilayers) and noisome, were widely investigated as drug reservoirs and penetration enhancers [2, 3]. However, conventional liposomes and noisome are rigid vesicles that remain trapped in the superficial layers of the stratum corneum and cannot reach deeper epidermal tissues or deliver macromolecules effectively. Other modified carriers like ectosomes utilize high ethanol concentrations to fluidize lipid bilayers, whereas transethosome combine ethanol with edge activators for deeper penetration [2]. To solve the penetration limits of rigid carriers, ultra-deformable lipid vesicles known as transfersomes were conceptualized and named by Gregor Cevc in 1991 [2].

Transfersomes are specialized, highly deformable aggregates consisting of phospholipids (such as phosphatidylcholine) combined with edge activators (surfactants). The addition of edge activators weakens the lipid bilayer, conferring programmed elasticity and stress-responsive properties [2, 5]. Driven by the natural trans epidermal osmotic/hydration gradient between the dry outer skin surface (~15–20% water) and deeper hydrated tissue layers (~75–100% water), transfersomes dynamically deform and squeeze intact through narrow intercellular pores smaller than their own size [2]. This unique mechanism enables transfersomes to overcome the stratum corneum barrier, delivering both low- and high-molecular-weight hydrophilic and lipophilic therapeutics with superior bioavailability, reduced systemic toxicity, and sustained drug release [2,5].

2. TRANSFEROSOMES

The trademark Transfersome was registered by the German business IDEA AG and refers to a proprietary medicine delivery technology. The name translates to "carrying body" and is derived from the Latin word "transferer," which means "to carry across," and "some," which is a Greek word for "body." The transfersome is a synthetic vesicle that mimics the properties of exocytotic cell vesicles, making it ideal for controlled and potentially targeted medication delivery [6]. Transfersomes are specialized ultra deformable vesicular carriers developed primarily for topical and transdermal drug delivery. They consist of a phospholipid bilayer enclosing an aqueous compartment, together with an edge activator that modifies the organization of the lipid membrane and imparts enhanced flexibility and deformability [7,9]. This structural organization enables the incorporation of both hydrophilic and lipophilic therapeutic agents, with hydrophilic molecules being accommodated predominantly within the aqueous compartment and lipophilic molecules associated with the lipid bilayer. The characteristic ultra-deformability of transfersomes allows them to undergo substantial shape adaptation and traverse narrow pathways within the stratum corneum while maintaining vesicular integrity, thereby facilitating drug transport across the skin [7,8]. In contrast to conventional liposomes, the incorporation of single-chain surfactants as edge activators increases membrane flexibility and may also promote fluidization of skin lipids, contributing to enhanced skin

permeation. The performance of transfersomes is influenced by formulation variables such as the type and concentration of phospholipid and edge activator, as well as vesicle size, which can affect deformability, drug entrapment and skin deposition. [9] Consequently, transfersomes have been investigated for the delivery of a broad range of therapeutic agents, including hydrophilic, lipophilic and high-molecular-weight molecules, with applications in topical and transdermal delivery, peripheral drug targeting and sustained drug deposition within the skin [10].

3. COMPOSITION

3.1. Phospholipids

1. Primary structural component — phospholipids form the bilayer structure of transfersomes.
2. Phosphatidylcholine-rich lipids are commonly used because they readily form bilayers and support vesicle formation.
3. Their amphiphilic nature provides an aqueous compartment and hydrophobic bilayer domain, allowing incorporation of hydrophilic and lipophilic drugs.
4. Phospholipid characteristics such as acyl-chain composition, unsaturation and phase-transition behavior influence membrane fluidity, packing and permeability.
5. The phospholipid content must be appropriately balanced with the edge activator to maintain vesicle integrity, drug retention and deformability.

3.2. Edge Activators

1. Edge activators are surfactants or bile salts incorporated into the phospholipid bilayer to increase membrane flexibility.
2. They reduce lipid-packing resistance, allowing the vesicle membrane to deform under mechanical stress.
3. Common examples include Tween 80, Span 80, sodium cholate and sodium deoxycholate.
4. Their type and concentration strongly influence vesicle size, entrapment efficiency, deformability, stability and skin permeation.
5. An optimum edge-activator concentration is essential: insufficient amounts may limit

Ingredient	Examples	Function
Phospholipid	• Soya phosphatidylcholine • Egg phosphatidylcholine • Distearoyl phosphatidylcholine (DSPC)	Vesicle-forming component
Surfactant	• Sodium cholate • Sodium deoxycholate • Tween 80 • Span 80	Provides flexibility
Alcohol	• Ethanol • Methanol	As a solvent
Dye	• Rhodamine-123 • Rhodamine-DHPE • Fluorescein-DHPE • Nile red • 6-Carboxyfluorescein	For confocal scanning laser microscopy (CSLM) study
Buffering Agent	• Phosphate-buffered saline (PBS), pH 6.5 • 7% v/v ethanol • Tris buffer, pH 6.5	As a hydrating medium

Figure 1: Composition of transfersomes

4. ADVANTAGES OF TRANSFEROSOMES

4.1. Enhanced Penetration

Transfersomes possess high deformability and elasticity, allowing them to squeeze through narrow pores and penetrate deep into the skin or mucosal tissues. This property facilitates the efficient delivery of drugs to target sites that are otherwise difficult to reach, including deeper skin layers or systemic circulation.

4.2. Increased Drug Bioavailability

The deformability of transfersomes enhances the bioavailability of drugs by promoting their absorption through the skin or mucosal membranes. This is particularly beneficial for drugs with poor oral bioavailability or those that require localized delivery.

4.3. Improved Stability

Transfersomes provide improved stability to encapsulated drugs by shielding them from enzymatic degradation, pH variations, and other environmental factors. This helps maintain the integrity and activity of the drug during storage and transportation.

4.4. Targeted Drug Delivery

Similar to invasomes, transfersomes can be surface-modified with targeting ligands to achieve targeted drug delivery. These ligands can recognize specific receptors or molecules on the target cells, allowing for precise and localized drug delivery while minimizing off-target effects.

4.5. Versatile Formulation

Transfersomes can encapsulate a wide range of drugs, including both hydrophilic and hydrophobic compounds. This versatility makes them suitable for various types of drugs, allowing for flexibility in drug formulation and delivery.

5. DISADVANTAGES OF TRANSFEROSOMES

5.1. Complex Manufacturing Process

The preparation of transfersomes requires specialized techniques and equipment, which can increase the complexity and cost of manufacturing. This can limit their widespread use and availability, especially for small-scale production or in resource-limited settings

MECHANISM OF TRANSFEROSOMES

- Transfersome Penetration: Hydration and lipid fluidization
- Deformability Driven Penetration: Squeezing Mechanism
- Osmotic Gradient
- Molecular Level Mechanism

5.2. Stability challenges

Transfersomes may face stability issues during storage, such as aggregation, leakage of encapsulated drugs, or changes in their physical properties. These challenges need to be addressed to ensure consistent and reliable performance of transfersomes.

5.3. Variable Performance

The performance of transfersomes can be influenced by various factors, including the physicochemical properties of the drug, formulation parameters, and the site of application. Achieving consistent and predictable performance across different drug molecules and conditions may require optimization and customization for each specific application.

5.4. Regulatory Considerations

Like other novel drug delivery systems, transfersomes may require specific regulatory considerations for approval and commercialization. Compliance with regulatory standards, safety assessments, and demonstrating the efficacy of transfersomes may add complexity and time to the development process.

5.5. Limited Drug Loading Capacity

Transfersomes may have a limited drug-loading capacity due to their vesicular structure. This can be a drawback when delivering drugs with high dosage requirements or large molecular sizes, potentially requiring higher doses or more frequent administration [14].

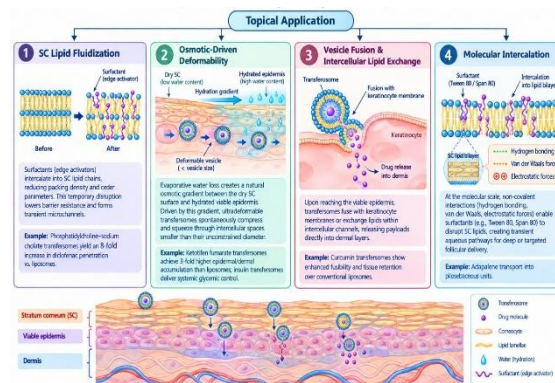


Figure 2: Penetration mechanism of transfersomes

6. METHOD OF PREPARATION

6.1 Suspension homogenization process

In this method transfersomes are prepared by mixing an ethanol soybean phosphatidylcholine solution with a suitable amount of edge activator molecule example: sodium cholate. This prepared triethanolamine –HCL buffer to yield a total lipid concentration 21. After all this at last the resulted suspension is sonicated, frozen and thawed for 2 to 3 times.

6.2 Vertexing sonicated method

In this technique mixed lipids (phosphatidylcholine, EA and the therapeutic agent) are put together in phosphate buffer and vortexed to attain a milky suspension. After this suspension is sonicated followed by extrusion through poly- carbonate membranes.

6.3 Thin film hydration method

In this method of preparation surfactants and phospholipids are dissolved in acceptable organic medium, such as methanol-chloroform and make a thin film using rotary evaporator. The organic solvent was evaporated at the above the lipid transition temperature at 50 °C is added to hydrate load of film and afterwards kept for rotation at 60 rpm for 1 hr. And kept at room temperature for 2 hrs. till the enlargement of vesicle is completed. Subsequently to achieve crave size and then the dispersion is sonicated. [5]

6.4 Modified handshaking process

In this method of preparation transfersomes are prepared by modified hand shaking lipid film hydration technique and this method is also known as lipid film hydration technique. The drug lecithin (Pc) and edge activator were dissolved or mixed in ethanol-chloroform in the ratio of 1:1. In this mixture drug, lipid and edge activators are the evaporator at the temperature

is (430c) hand shaking is achieved with a constant rotation 24. The thin lipid film is formed inside the flask. This film was kept for overnight for the complete evaporation of solvent. At the last the film was hydrated with the phosphate buffer (PH 7.4) with gentle shaking for 15 minutes at appropriately temperature.

6.5 Centrifugation process

In this method phospholipids, surfactants and the drug are mixed in alcohol, then the solvent is removed by rotatory evaporation under reduced pressure at 400c. Detected solvent are removed under vacuum. After that set down or place lipid film is hydrated with appropriate (suitable) buffer by centrifuging at 60rpm for 1 hr. at room temperature. The resulting vesicles are swollen for 2 hrs. Then the multi-lamellar lipid vesicles obtained which are further sonicated at room temperature [12].

6.6 Reverse-Phase Evaporation Method

The phospholipids and edge activator are added to a round-bottom flask and dissolved in the organic solvent mixture (example: diethyl ether and chloroform). The lipophilic drug can be incorporated in this step. Then, the solvent is evaporated using rotary evaporator to obtain the lipid films. The lipid films are redissolved in the organic phase mostly composed of isopropyl ether and/or diethyl ether. Subsequently, the aqueous phase is added to the organic phase, leading to a two-phase system. The hydrophilic drug incorporation can be done in this stage. This system is then subjected to sonication using a bath solicitor until a homogeneous w/o (water in oil) emulsion is formed. The organic solvent is slowly evaporated using rotary evaporator to form a viscous gel, which then becomes 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 transfer Some are stored in appropriate conditions.

6.8 Ethanol Injection Method

The organic phase is produced by dissolving the phospholipid, edge activator and the lipophilic drug in ethanol with magnetic

stirring for the respective time, until a clear solution is obtained. The aqueous phase is produced by dissolving the water-soluble substances in the phosphate buffer. The hydrophilic drug incorporation can be done in this stage. Both solutions are heated up to 45–50 °C. Afterwards, the ethanolic phospholipid solution is injected dropwise into the aqueous solution with continuous stirring for the respective time. Ethanol removal is done by transferring the resultant dispersion into a vacuum evaporator and then sonicating for particle size reduction.

6.9 Freeze–Thaw Plus Extrusion Method

This combined approach utilizes the advantages of both freeze–thaw cycling and membrane extrusion for the preparation of highly stable and size-controlled transfersomes. Initially, hydrated vesicle dispersions are subjected to multiple cycles of freezing (typically at –80 °C) and thawing (at room temperature), which promotes lipid bilayer fusion, reduces drug leakage, and enhances entrapment efficiency. After this, the vesicle suspension is passed through polycarbonate membranes of defined pore sizes using an extruder to achieve uniform particle size and a narrow PDI. This technique is particularly beneficial when the goal is to obtain highly monodisperse vesicle populations suitable for intravenous or transdermal applications.

6.10 Supercritical CO₂ (SCF) and Flow-Reactor Based Continuous Techniques

Emerging technologies such as supercritical fluid-based methods and microfluidic flow-reactor systems are gaining prominence due to their scalability and environmentally friendly nature. In the SCF approach, lipids and drug substances are processed under supercritical CO₂ conditions, which act as a solvent and anti-solvent system depending on the setup. This method eliminates the need for harmful organic solvents and allows the production of solvent-free vesicles. Similarly, flow-reactor-based systems use microfluidic mixing under controlled flow rates and pressure to generate highly uniform and reproducible transfersomes. These advanced methods are especially promising for large-scale industrial production due to their continuous operation, precise control over process parameters, and potential for automation.[15]

6.11. Special Methods

6.11.1. Iontophoresis-Based Transfersomes

In this innovative approach, transfersomes are first prepared using a thin-film hydration method involving surfactants such as sodium cholate or sodium deoxycholate with soya phosphatidylcholine (SPC). The formulation is then optimized using Design of Experiments (DoE) to refine critical parameters such as surfactant concentration, drug loading, and vesicle size. The unique aspect of this method is the application of the prepared transfersomal formulation via iontophoresis—a technique that uses mild electrical currents to enhance skin penetration. Ex vivo models, such as porcine nipple skin, are often employed to evaluate the efficiency of this delivery system. This method is particularly effective for enhancing drug delivery through thicker skin regions or specific dermal layers.

6.11.2. Asiatic Acid Transfersomes Gel Method

This method is tailored for the topical delivery of bioactive such as Asiatic acid. The transfersomes are initially prepared using the thin-film hydration technique by combining various surfactants (e.g., Tween 80, Span 80, or sodium deoxycholate) with soybean lecithin to optimize vesicle properties. After hydration, the vesicles are further refined using high-pressure homogenization to reduce particle size and improve stability. The final transfersomal suspension is then incorporated into a hydrogel base to facilitate ease of application and prolonged retention on the skin. This combination results in a highly bioavailable topical formulation with enhanced therapeutic efficacy.[13]

7 FACTORS AFFECTING TRANSFEROSOME

Numerous process factors may have an impact on the transfersome qualities throughout the process of creating an optimal formulation. These factors mostly relate to the production of transfersomes formulations, which are denoted by the following,

7.1 Effect of Phospholipids: Edge Activator

The characteristics of vesicles, such as their size, charge and ability to entrap drugs are influenced by various factors. These include the concentration of surfactant, length and number of carbon chains in the surfactant molecules, hydrophilic nature of the head group and competition for space within the lipid bilayer and surfactant hydrophilic-lipophilic balance HLB value. Generally, higher surfactant concentration, longer and more numerous hydrocarbon chains, greater hydrophilicity of

the head group and higher HLB value led to smaller vesicle size being produced.

7.2 Effect of various solvents

There are several solvents utilized including methanol and ethanol. The compatibility of formulation ingredients with the solvent and their solubility in it determine which solvents are the best. For optimal film-forming capability and enhanced stability upon hydration, it is desirable for all components, including the drug and excipients, to fully dissolve in the solvent, resulting in a clear and transparent solution. The formulation's solvents may also serve as penetration enhancers, increasing the drug concentration flow across a membrane. William and Barry (2004) report that ethanol was employed in several trials to increase the flow of levonorgestrel, hydrocortisone, 5-fluorouracil and estradiol through rat skin.

7.3 Impact of different edge activators (surface active agents)

Transfersomes, being meticulously optimized ultra-flexible lipid vesicles, possess the unique ability to swiftly deform under external pressure. This characteristic facilitates their passage through skin pores, which are notably smaller than the vesicles themselves. Specific edge activators, along with their respective concentration are crucial for maximizing membrane deformability. This improvement is credited to the combined efficacy of transfersome in serving as both drug carriers and enhancers of permeation [15].

7.4 Effect of hydration medium

The choice between saline phosphate buffer with a pH range of 6.5-7 and water serves as crucial for achieving an optimal balance in the formulation characteristics, biological applicability and delivery method. Maintaining an appropriate pH level in the hydration medium is essential to ensure that medication remains in its unionized form, thereby enhancing its entrapment within transfersomes and facilitating penetration through the cellular membrane. This is particularly significant due to the similarity between the lipid bilayer of the transfersome and the phospholipid layer of the cell membrane, enabling intracellular transportation of the medications [16].

8.CHARACTERIZATION OF TRANSFEROSOME

8.1. Drug excipient interaction study

Fourier Transform Infrared spectrophotometer (FTIR) exhibits graphical representation of pure drug, a mixture of the soya

lecithin and drug, and a mixture of excipients (Span 80, Span 60, Span 20, Tween 20, etc.) and the drug. For the sample preparation potassium bromide is used and within a spectral range of 450–4000 cm⁻¹ data are collected. If the graphical representation shows no overlap between individual components, proves that components are not interacting with each other.

8.2. Preformulating studies of drug

Before incorporating the drug in formulation, Preformulating studies are a vital detection process for the physical and chemical properties of the drug.

8.2.1. Physical appearance

Organoleptic characteristics such as color, odor, and taste of the drug in powder form are examined.

8.2.2. Melting Point

The melting point of the drug is detected with the help of a capillary method or digital melting point apparatus. It is one of the important specifications for disclosing the purity of drugs. The drug sample becomes hot in a fuse capillary tube and the rate is 5°C/min.

8.2.3. Solubility study

Solubility studies are carried out with distilled water, 0.1N hydrochloric acid, methanol, ethanol, and Phosphate buffer pH 7.4 at room temperature (25±2° C). Solute or drug in excess amount is needed in solvent to produce supersaturation. Then it is shaken mechanically for around 48 hours at 25°C and equilibrium state is achieved. Membrane filter having porosity 0.45µ is used to filter the aliquots and solubility analysis is performed by UV spectrophotometer.

8.2.4. pH measurement

Digital pH meter is essential for pH detection of the formulation. In this method, a specific amount of drug in powder form is accurately weighed. Then it is dissolved in a certain amount of ethanol when the drug is insoluble in distilled water and made up the volume with distilled water by the sonication method using a sonicator. The solution is then filtered using filter paper and digital pH meter helps to determine the pH of the filtrate. [16]

8.3. Determination of Wavelength Maxima

Accurately 10 mg of the drug is weighed and dissolved in 100 ml of phosphate buffer having pH 7.4.

From this stock solution, 1 ml of is pipette out and by phosphate buffer having pH 7.4 made up the volume up to the final mark. The scan value of the solution is detected by using a UV spectrophotometer.

8.4. Morphology and Structure of transfersomes

The structure and morphology of the transfersomes consisting of drugs are detected by using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Transfersomes are diluted in distilled water and a drop of the diluted suspension is mounted on a clean slide. Staining agent like 1% phosphodiester acid (dissolved in distilled water), is used for better visualization or to increase the contrast of the image and observed after drying. An optical microscope is used for visualization of vesicles that are produced without sonication. Make a thin film by spreading the suspension on the slide and a coverslip is utilized to cover the sample. After drying it out, the sample is placed under optical microscope observation [17].

8.5. Entrapment Efficiency

Entrapment efficiency (%EE) represents the percentage of drug successfully entrapped within the transfersome vesicles. The untrapped drug is separated from the vesicular fraction by centrifugation or ultracentrifugation.

8.5.1. Direct method

In the direct method, the vesicles are separated and subsequently disrupted using a suitable solvent such as Triton X-100 or n-propanol to release the entrapped drug. The resulting solution is diluted, filtered through a 0.22–0.45 µm syringe filter and analyzed using a suitable analytical technique such as HPLC or UV-visible spectrophotometry.

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

8.5.2. Indirect method

In the indirect method, the free drug present in the separated supernatant is quantified, and the amount of entrapped drug is calculated by difference.

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

Where % EE = Percentage entrapment efficiency.

8.6. Vesicle Size, Size Distribution and Morphology

Vesicle size is an important characteristic of transfersomes because it can influence their stability, drug loading and skin penetration. Dynamic light scattering (DLS) is commonly employed to determine the vesicle diameter and size distribution. The diluted vesicular suspension may be filtered before measurement, and the analysis is generally performed using a particle-size analyzer such as a Malvern Zetasizer. Morphological characteristics and structural integrity of the vesicles can be further investigated using microscopic techniques such as transmission electron microscopy (TEM).

8.7. Number of Vesicles

The number of vesicles per cubic millimeter can be determined using a hemocytometer in combination with an optical microscope. The unsonicated transfersosomal formulation is appropriately diluted, for example with 0.9% sodium chloride solution, and the vesicles are counted within the designated squares of the hemocytometer. This parameter provides an estimate of vesicle concentration within the formulation. Total no. of vesicles per cubic mm = (Total no. of vesicles counted $\times$ dilution factor $\times$ 4000)/Total no. of squares counted

8.8. Drug Content

Drug content indicates the total amount of drug present in the transfersosomal formulation and can be determined using an appropriate validated analytical method. High-performance liquid chromatography

(HPLC) equipped with a UV detector is one of the instrumental techniques used for quantitative determination of drug content. The analysis provides information regarding the actual drug concentration present in the prepared formulation.

8.9. Turbidity

Turbidity measurement can be used to characterize the optical properties of transfersosomal dispersions. The turbidity of a drug-containing aqueous system may be determined using a nephelometer. Changes in turbidity can provide information regarding the dispersed vesicular system and its physical characteristics.

8.10. Deformability/Permeability

Deformability is a critical characteristic that distinguishes transfersomes from conventional vesicular systems. It evaluates the ability of vesicles to pass through pores smaller than their original diameter while retaining their structural integrity. In the deformability test, the transfersomal

suspension is extruded through microporous membranes having defined pore diameters, commonly ranging from approximately 50 to 400 nm. The amount of suspension passing through the membrane and the vesicle size before and after extrusion are determined. The degree of deformability can be expressed using the relationship:

$$D = J \times (r_v/r_p)$$

where D is the degree of deformability, J is the amount of suspension extruded during a specified period, r_v is the vesicle radius and r_p is the pore radius of the membrane.

8.11. Occlusion Effect

The occlusion effect is associated with the influence of skin hydration on transfersosomal penetration. The skin possesses a hydration gradient, with the deeper layers being more hydrated than the relatively dry surface. This hydration gradient can act as a driving force for the movement of elastic vesicles through the skin. Occlusion reduces water evaporation from the skin and consequently alters skin hydration and the hydration forces involved in vesicular penetration. Therefore, the effect of occlusion may be considered when evaluating the transdermal performance of transfersosomal formulations.

8.12. Surface Charge and Zeta Potential

The surface charge of transfersomes can be characterized by measuring their zeta potential using a Zetasizer. Zeta potential provides information about the electrical properties of the vesicle surface and can be useful for assessing the physical stability of the vesicular dispersion. Surface charge may also influence interactions between vesicles and biological membranes.

8.13. Penetration Ability

The skin penetration ability of transfersomes can be investigated using fluorescence-based techniques. Fluorescent markers incorporated into transfersome vesicles can be visualized using fluorescence microscopy to assess the distribution and penetration of vesicles or their incorporated components within different skin layers. This approach is useful for investigating the ability of transfersomes to enhance dermal drug delivery.

8.14. In-vitro Drug Release

The transfersome formulation can be performed using a Franz diffusion cell equipped with a suitable semi-permeable membrane. The diffusion cell consists of donor and receptor

compartments separated by the membrane, with an effective diffusion area of approximately 2.303 cm². The receptor compartment is filled with phosphate-buffered saline (PBS, pH 7.4) and maintained at 37 ± 0.5 °C under continuous agitation at 100 rpm to simulate physiological conditions and maintain uniform distribution of the released drug. An accurately measured quantity of the transfersome formulation is applied to the donor compartment over the semi-permeable membrane. At predetermined time intervals, aliquots are withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh diffusion medium to maintain sink conditions and a constant receptor volume. The withdrawn samples are appropriately diluted, when necessary, and analyzed using a suitable analytical method such as UV-visible spectrophotometry or HPLC to determine the drug concentration. The cumulative amount of drug permeated through the membrane is calculated and plotted against time to obtain the in-vitro release/permeation profile of the transfersomal gel formulation. The study provides information regarding the release behavior and permeation characteristics of the formulation and can be used to compare different formulations and assess the ability of the transfersomal system to enhance drug transport [18].

8.15. In-vitro Skin Permeation

In-vitro skin permeation studies are performed to evaluate the transport efficiency of the drug through biological membranes and to determine factors influencing drug permeation flux. Franz diffusion cells are commonly used for this purpose. Suitable biological skin models, including human or animal skin, may be employed depending on the study design. The skin is positioned between the donor and receptor compartments, and the transfersomal formulation is applied to the donor side. The receptor compartment is filled with an appropriate medium, maintained under controlled temperature and stirring conditions. Samples are withdrawn at predetermined intervals and replaced with fresh medium to maintain sink conditions. Drug concentration in the collected samples is determined by spectrophotometric or chromatographic methods. The resulting data can be used to determine permeation parameters such as cumulative drug permeation and permeation flux and to optimize the formulation before further in-vivo studies.

8.16. Stability Study

Stability studies are conducted to evaluate the ability of transfersomal formulations to maintain their physical and chemical characteristics during storage. The formulations are stored in suitable, tightly closed containers under controlled temperature and relative-humidity conditions. Long-term and accelerated stability studies can be performed according to applicable ICH conditions. During the study period, samples are periodically examined for changes in drug content, entrapment efficiency, drug leakage and other relevant formulation characteristics. Stability assessment is important for determining the suitability of the optimized transfersomal formulation for storage and subsequent development.

8.17. Release kinetics

A graph is plotted for different kinetic models following the report of in vitro permeation study. Zero-order kinetics display the Cumulative percent of drug permeated versus Time and first-order kinetics display the log cumulative percentage of drug remaining versus Time in graphical representation. Higuchi's model displays a graphical representation of the cumulative percent drug permeated versus square root of time. The drug release mechanism can be calculated by putting data to Korsmeyer- Peppas equation which displays a graphical representation of the log cumulative percentage of drug released versus log time. The value coming from the slope of the straight line is applied in exponent n detection. The diffusion mechanism is known as fickian when $n=0.5$ and the mechanism is non-fickian when it is $0.5 < n < 1.0$. If $n = 1$ it is Case II (relaxational) transport and if $n > 1$ it is super case II transport it is called as straggling [17,18].

9. APPLICATION OF TRANSFEROSOMES

9.1. Transdermal Immunization

Transfersomes facilitate the transfer delivery of proteins and antigens, including human serum albumin and membrane proteins, across the skin. Their elastic and highly deformable structure enhance penetration through the skin barrier, making them promising carriers for non-invasive immunization and vaccine delivery.

9.2. Anti-Cancer Drug Delivery

Transfersomes have been investigated for topical delivery of anticancer agents. Jiang et al. reported paclitaxel-loaded transfersome embedded oligopeptide hydrogels for topical

melanoma therapy. The formulation, composed of phosphatidylcholine, Tween 80 and sodium deoxycholate, demonstrated enhanced penetration into tumor tissues.

9.3. Insulin delivery

Transfersomal systems have been explored for non-invasive insulin delivery. Their ability to enhance drug absorption and provide controlled delivery may improve insulin bioavailability and help maintain stable blood glucose levels, offering a potential alternative to conventional administration routes.

9.4. Protein and Peptide Delivery

Transfersomes can facilitate the transdermal delivery of large and complex biomolecules such as proteins and peptides. Their deformability enables these macromolecules to cross the skin barrier, supporting their potential application in non-invasive protein and vaccine delivery.

9.5. Antifungal Drug Delivery

Transfersomes have been investigated as carriers for antifungal agents. Formulation and process variables such as lipid concentration, surfactant concentration, ethanol volume, hydration-medium volume and hydration time can influence vesicle characteristics and are therefore important during formulation optimization.

9.6. Corticosteroid Delivery

Transfersomes have been investigated for the topical delivery of corticosteroids such as triamcinolone acetonide. Studies by Cevc and Blume reported enhanced and prolonged biological activity compared with conventional drug formulations, with therapeutic effects achieved at lower doses.[19]

10. Regulatory Aspects of Transfersomes

The development of transfersome-based drug delivery systems requires careful consideration of the safety, toxicity, quality, and regulatory acceptability of the excipients used in the formulation. Transfersomes generally contain phospholipids, surfactants or edge activators, solvents, and other formulation components. Since these materials directly influence vesicle formation, deformability, stability, and skin permeation, their selection should be based on established safety profiles and suitability for topical or transdermal administration. Regulatory and pharmaceutical organizations maintain information on excipients considered safe for pharmaceutical use. The US FDA Inactive Ingredient Guide provides information regarding approved inactive ingredients

according to dosage form and route of administration. Therefore, the use of well-characterized and established excipients can help minimize potential toxicity and irritation concerns during transfersome development.[25].

Phospholipids are essential structural components of transfersomes because they form the flexible lipid bilayer surrounding the vesicle. Unsaturated phosphatidylcholine, including soybean phosphatidylcholine (SPC) and egg phosphatidylcholine, is commonly employed to obtain deformable vesicles. Edge activators are incorporated to increase membrane flexibility and facilitate penetration through the skin. Commonly reported edge activators include Tween 80, Span 80, Tween 20, sodium cholate, and sodium deoxycholate. However, their concentration should be carefully controlled because excessive surfactant levels may produce irritation or other undesirable effects. The choice of edge activator can also influence vesicle size, elasticity, stability, drug entrapment, and skin permeation. Ethanol is another important component that may enhance transdermal delivery. It can interact with the polar head groups of lipid molecules and increase lipid fluidity, thereby facilitating penetration through the stratum corneum. Thus, the regulatory development of transfersomes involves balancing formulation performance with excipient safety and tolerability. Appropriate selection and concentration of phospholipids, edge activators, and solvents are essential for developing a safe, effective, and reproducible transfersomal drug-delivery system [20].

11. Recent Advancements

11.1. Smarter and hybrid vesicle

Polymer-coated and hybrid transfersome systems are being explored to improve vesicle stability, regulate drug release and modify skin penetration. Transfersome-in-gel systems and other hybrid platforms may provide additional control over drug deposition and release compared with conventional vesicular formulations. Recent work also highlights the development of multifunctional transfersomes with modified membrane properties for improved delivery performance [19].

11.2. Stimuli-responsive transfersomes

Smart transfersomal systems responsive to environmental stimuli such as pH, temperature or enzymes are being investigated to provide more controlled and site-specific drug

release, particularly for applications involving cancer, wound healing and inflammatory skin conditions [2,22].

11.3. Broader therapeutic cargo

Recent studies have expanded transfersosomal applications beyond conventional small-molecule drugs to include NSAIDs, anticancer agents, phytochemicals, peptides, proteins, vaccines and nucleic acids. Their flexible membrane and hydration-driven transport mechanisms make them attractive for delivering compounds that are difficult to transport across the stratum corneum using conventional topical systems.

11.4. Transcutaneous vaccination

Transfersomes have also been investigated as carriers for antigens and vaccines, with the potential to enhance antigen transport across the skin and support non-invasive immunization strategies. Their ability to accommodate larger biological molecules has increased interest in their use for transcutaneous delivery of biologics. [21]

11.5. Design optimization and computational approaches

Transfersome performance is strongly influenced by phospholipid composition, edge-activator type and concentration, vesicle size, deformability and processing conditions. Increasingly systematic formulation approaches are therefore being used to establish relationships between formulation variables and drug-loading, stability and permeation characteristics. Computational and data-driven approaches, including artificial intelligence, represent emerging tools for reducing experimental workload and supporting formulation optimization [21,23].

11.6. Pro-transfersomes and related platforms

Pro-transfersomal systems are being investigated as alternative approaches in which a relatively dry or low-water precursor generates transfersomes following hydration. Such systems may offer advantages in storage, handling and formulation stability, although their scalability and long-term pharmaceutical performance require further investigation [23].

12. Future Outlook

12.1. Clinical translation and regulatory harmonization

Despite extensive experimental research, the clinical translation of transfersomes remains constrained by issues related to **stability, reproducibility, scale-up, manufacturing cost and regulatory standardization**. The development of standardized characterization methods and clearly defined critical quality

attributes will be important for advancing transfersomes formulations toward broader pharmaceutical use [24]

12.2. Next-generation therapeutics

Future development is expected to increasingly address the transdermal delivery of **peptides, proteins, vaccines and nucleic-acid-based therapeutics**, together with combination approaches involving microneedles, iontophoresis, other physical enhancement technologies and targeted ligands. These strategies may broaden the range of therapeutics that can be administered through the skin [23].

12.3. Standardized characterization and safety profiling

Future studies should emphasize standardized **in-vitro and in-vivo models**, particularly for correlating deformability and vesicle characteristics with actual skin permeation and therapeutic outcomes. Greater attention is also required for long-term stability, excipient safety, edge-activator effects and reproducibility between laboratory-scale and larger-scale manufacturing processes [26].

CONCLUSION

Transfersomes have emerged as a promising vesicular platform for topical and transdermal drug delivery because of their distinctive ultra-deformability and skin-penetrating ability. The incorporation of edge activators into phospholipid bilayers provides the flexibility required for vesicles to deform and pass through the restrictive stratum corneum, thereby enhancing drug transport and deposition within the skin. Their ability to incorporate both hydrophilic and lipophilic drugs further increases their formulation versatility. A range of preparation techniques and characterization methods can be employed to obtain transfersomes with suitable particle size, entrapment efficiency, deformability, stability, and drug-release characteristics. Their potential applications extend from conventional small-molecule drugs to proteins, peptides, vaccines, insulin, corticosteroids, antifungal agents, and anticancer therapeutics. However, issues related to physical stability, drug leakage, manufacturing reproducibility, limited drug loading, excipient tolerability, scalability, and regulatory requirements must be addressed before broader clinical and commercial implementation. Future research should therefore emphasize quality- by -design based formulation optimization, scalable manufacturing, standardized evaluation methods, mechanistic understanding of skin penetration, and

integration with technologies such as microneedles and iontophoresis. Development of targeted and stimuli-responsive transfersomal systems may further expand their therapeutic applications. Overall, continued optimization and rigorous safety and efficacy evaluation could support the advancement of transfersomes as versatile carriers for next-generation topical and transdermal therapies.

Supplementary Materials

No supplementary materials are associated with this article.

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Author Contributions

S.P.R. and S.R. conceived and designed the review; S.R. and R.S.N. conducted the literature search and collected the relevant information; S.P.R., S.R., and R.S.N. analyzed and organized the literature; S.R. prepared the initial draft of the manuscript; S.P.R. and R.S.N. critically reviewed and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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