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Review Article

Transferosomes–Ultra Deformable Vesicles for Transdermal Drug Delivery System

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ABSTRACT

Transdermal administration systems have advantages over traditional oral and parenteral delivery methods, they have attracted a lot of attention recently. These self-administered, noninvasive delivery methods have the potential to enhance patient compliance while delivering a regulated release of medicinal substances. The barrier function of the skin's outermost layer presents the biggest hurdle for transdermal delivery methods. Ionized chemicals and molecules with molecular weights more than 500 Da typically cannot cross the skin. As a result, the number of medications that can be provided via this method is restricted. One potential solution to this issue is to encapsulate the medications in transferosomes. Their bilayered shape makes it easier to encapsulate drugs that are both lipophilic and hydrophilic as well as amphiphilic. The present work attempts to explain the idea of ultra deformable vesicular carriers (transferosomes), their mode of action, various synthesis and characterisation techniques, factors influencing their effective delivery of drug into deeper layers of the skin reaching the target site and thus improving its systemic bioavailability.

INTRODUCTION

Effective therapeutic therapies are rarely delivered due to first-pass hepatic metabolism, undesirable consequences, patient noncompliance, and refusal of aggressive treatments. In recent decades, many pharmaceutical administration strategies have been developed and studied to solve these concerns. Transdermal therapy delivery systems are non-intrusive with no first-pass effects, making them promising. The skin's barrier function reduces transdermal drug transfer; hence it must be addressed. Liposomes or similar lipid-based vesicular structures are used in nanoencapsulation

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to avoid the difficulty [1]. Liposomes help drugs pass through the skin through adsorption to the skin's surface, combining with the stratum corneum's lipid matrix, and lipid transmit between the liposomal and cell membranes. Conventional liposomes cannot enter living skin or vessels. Thus, liposomes are often used as cutaneous medication carriers instead of transdermal administration. Conventional liposomes have a limited half-life, fragile membranes that leak, and poor hydrophilic drug encapsulation. Due to these problems, ethosomes and noisome, bilosomes, chitosomes, transfersomes, sphingosomes, and invasomes were found and produced. Niosomes were initially reported in the early 1970s. The surfactants include cholesterol, ionic amphiphiles, and nonionic surfactants including alkyl or di-alkyl polyglycerol ethers, ethers, esters, and amides. Nonionic surfactants increase niosome diameter and entrapment efficiency, while cholesterol stiffens the vesicular bilayer. Stearylamine and dicetyl phosphate, two ionic amphiphiles, are also used in niosomes to improve stability, efficacy, and efficiency. Given their great chemical stability, bioavailability, entrapment effectiveness, and low cost, they are a better drug carrier technology than liposomes. The literature shows that niosomes increase therapeutic drug residency in the stratum corneum and epidermis, decrease systemic drug absorption, and increase trapped drug absorption through the skin. Because they resist bile salts and enzymes used for digestion, these vesicles were designed to administer vaccines orally. In the 1990s, Cevc et al. invented transfersomes, a novel carrier mechanism. Phospholipids plus edge activation (EA), a membrane-softening substance like Tween 80, Span 80, or sodium cholate, make transfersomes ultra-deformable. Transfersomes change membrane flexibility to spontaneously move through skin pores. Self-optimizing deformability. Transferomes flex easily, allowing them to flow through very tiny pores. These highly flexible, self-optimizing lipids aggregates have been successful in phase I and II clinical trials, preclinical testing, transcutaneous peptide and protein injection, and sustained drug release. Several transfersome-based formulations are in clinical studies. Researchers studying elastic vesicle-based transdermal drug delivery should understand the term, concept, preparation and characterization methods, and factors affecting the first development of elastic vesicles (transfersomes). This study covers transfersome modes of action, preparations, and characterization methods, with a focus on their latest transdermal drug administration usage. An edge stimulator and one or more inner aqueous compartments surrounded by a lipid bilayer make up transfersomes or transferosomes. Extremely malleable lipid bilayer-enclosed aqueous foci produce self-optimizing and self-regulating vesicles. Elastic transferosomes may squeeze and deform as whole vesicles via minuscule holes or skin constrictions without losing any material. Transfersomes are phospholipid-modified liposomal spheres with a single-chain detergent activating the edge.

2. Transfersome structure:

Lipid bilayer-enclosed aqueous cores create ultra-deformable, self-optimizing, and self-regulating vesicles. Elastic transferosomes may squeeze and deform as whole vesicles via minuscule holes or skin constrictions without losing any material. Single-chain surfactants activate edges and form transfersomes' phospholipids. [2]

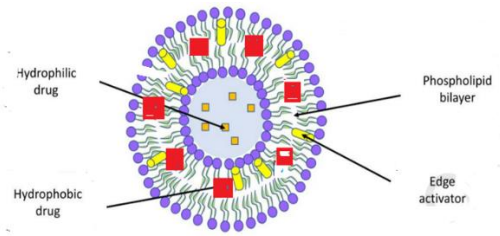

Figure 1- Structure of transfersome.

Edge activators (EAs) make transfersomes bendable and ultra-flexible, boosting their penetration, when mixed with the correct lipid. EAs are excellent membrane-destabilizing agents for vesicle membrane deformability. Transfersomes may enter pores smaller than their diameters, overcoming typical liposomes' fundamental drawbacks. Transfersomes with herbal components may provide skin care and therapeutic effects. Several studies have shown that transfersomes can penetrate deep into the skin to build skin drug depots for prolonged drug release, distribute therapeutic chemicals into deep skin layers, and transport medications into the bloodstream. Thus, transfersomes provide a promising new approach to drug. administration.[3]

3. Transdermal Delivery of Drugs System Benefits from Transfersomes

• Because of their hydrophilic and hydrophobic moieties, transfersomes can deliver therapeutic medicines with a wide range of solubility. • Transfersomes can pass through skin barrier constrictions 5-10 times smaller than their diameter due to their extreme deformability and elastic properties. • High vesicle deformability allows for medication passage through the skin without affecting vesicles. • Transfersomes are made up of naturally occurring phospholipids and extr They may be biocompatible and biodegradable.

• Transfersomes can carry proteins, the hormone insulin, NSAIDs, or corticosteroids, interferons, anticancer drugs, peptides, anesthetics, and herbal treatments.

• Transfersomes are ideal for consistent and long-lasting action and medication release. • They improve site selectivity and transdermal bioactive material flow. • Eliminating first-pass metabolism, a major drawback of oral medicine, and increasing bioavailability.

• They require individualized optimization but may be made from pharmaceutically acceptable ingredients using ordinary procedures. Additionally, their simple production process simplifies scaling up.[4]

4. Transfersome limitations

Transfersomes are chemically unstable due to their oxidative degradation. Transfersome degradation can be decreased by adding nitrogen and argon to the aqueous media. Light protection and low storage temperature reduce oxidation risk. Post-preparation processes, such as freeze- and spray-drying, can enhance transfersome storage stability. The difficulty of purifying natural phospholipids hinders transfersome drug distribution. Therefore, synthesized phospholipids can be used as a substitute. Transfersomal formulations are expensive due to the raw materials and machinery needed to create lipid excipients. Due to its low cost, phosphatidylcholine is a popular lipid component. [5]

5. Mechanism of Action

Vesicles, or colloidal particles, are amphiphilic molecules with an aqueous chamber and a concentric bilayer. They are effective vesicular drug delivery devices because they can distribute

hydrophilic drugs in the inner water compartment and hydrophobic pharmaceuticals in the lipid bilayer. Transfersomes are flexible, self-optimizing drug carriers. They penetrate the skin due to their membrane flexibility and vesicle integrity.[6]

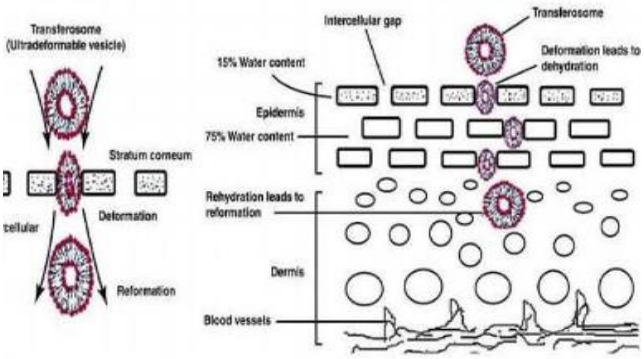

Figure 2- representing Mechanism of Action[24]

The Cevc and Blume study calls transfersome penetration hydrotaxis (xerophobia). The nonocclusive condition occurs when moisture loss from the transfersomal formulation occurs after skin application, causing the transferosome to look for moisture in deeper skin layers rather than the dry external backdrop. Transfersome vesicles push the skin with the natural transdermal gradient, which causes the epidermal water activity differential. Transcutaneous channels with a width of 20–30 nm result from these vesicles expanding connections between cells with the lowest resistance. These pathways enable ultra-deformable, slimed transfersomes to travel with a water gradient across the skin. Body heat evaporates skin surface water, creating the osmotic gradient. This gradient drives the adaptable transportation of therapeutic chemicals over the skin from the applied location to the target area for localized or systemic therapies in at least harmful systemic concentrations. Transfersomes feature a bilayered shape that encapsulates lipophilic, hydrophilic in nature and amphiphilic medicines and penetrate skin more efficiently than regular liposomes. Transfersomes have more flexible artificial membranes.[7] Vesicles are self-regulating and self-optimizing due to the lipid bilayer structure and local composition dependency. Transfersome vesicles can readily cross transport obstacles due to this trait. Transfersomes, supramolecular structures, increase lipid bilayer permeability and flexibility. They contain at least one edge activation and one amphipathic agent. Several transfersome formulations contain small concentrations of alcohol (ethanol or polypropylene glycol) as penetration-enhancing agents and cosolvents with high solvating capacity. Some believe ethanol can affect the fatty acid bilayer's polar head region. [8] After penetration, ethanol fluidizes the intercellular lipid matrix, lowering lipid lamellae density. Through the corneal layer, transfersomes can reach the dermis and circulate the blood. The vesicles' components distort the transfersomal membrane, allowing them to puncture. The optimal vesicle compositions for each medicinal product must be determined using specially designed experimental methods to offer the best carriers with the best deformation, drug capacity for transport, and stability. Vesicle composition affects transfersomal membrane deformability and penetration. The optimal vesicle composition for each medicine must be determined using specially devised experimental methods to give the best carriers with deformability, drug transport, and stability.

6.Transfersome structure

Transfersomes usually consist of an amphipathic material (e.g., phosphatidylcholine from eggs or soy) and a lipid bilayer, which can form vesicles.

Table-1: Examples of some lipids used in synthesis of nanovesicles.

Examples of some lipids used in synthesis of nanovesicles	
Common Name	**Example**
Polyoxymethylene polyoxypropylene block copolymer	Poloxamer 188, poloxamer 407
Saturated polyglycolized glycerides	Lauroyl macrogol glycerides, stearoyl macrogolglycerides
PEG-8 caprylic/capric glycerides	Caprylocaproyl macrogolglycerides
Vitamin E derivative	Tocopherol PEG succinate
Polyoxyethylene polyoxypropylene block copolymer	Poloxamer 188, poloxamer 407
Saturated polyglycolized glycerides	Lauroyl macrogolglycerides, stearoyl macrogolglycerides
PEG-8 caprylic/capric glycerides	Caprylocaproyl macrogolglycerides

•10–25% surfactants/edge activators: sodium cholates, sodium deoxycholate, Tweens and Spans (Tween 20, Tween 80, Tween 60, Span 60, Span 65, and Span 80), and dipotassium glycyrrhizinate are employed most often in transfersome preparations. These biocompatible bilayer-softening compounds increase vesicle permeability and flexibility. Surfactants for nanovesicle production

• Solvent: 3-10% ethanol or methanol; hydration medium: water or saline phosphate buffer (pH 6.5-7).[9] In water, phospholipids form flexible lipid bilayers and constrict to form vesicles. Biocompatible membrane softeners, also called edge activators, are single-chain surfactants that incorporate into transferosomes. They help destabilize the vesicle's lipid bilayer and increase its flexibility. Surfactants and their ratios to phospholipids regulate membrane flexibility and reduce the risk of ruptures in the skin. This allows transfersomes to move freely over the epidermis in line to the simple osmotic the gradient after application.

7. Methods of Preparation

No formula or technique exists for this procedure, despite various patented transfersome preparation methods. Conduct individually designed tests for each therapy to identify, design, and optimise the optimal setup conditions and vesicle compositions to obtain the best transporters with the best deformation, drug capacity for transport, and stability. Thin film hydration, also known as rotary evaporation-sonication, is the conventional transfersome preparation method. Modified preparation methods include vortexing-sonication, modified handshaking, centrifugation, suspension uniformity, reverse-phase evaporation, high-pressure homogenizing, and alcohol injection.

7.1 Rotating Evaporation-Sonication Method/Thin Film Hydration Technique

The phospholipids (phospholipids) and edge activator, which are responsible for vesicle formation, are dissolved in a round-bottom flask using a mixture of flammable organic solvents, such as methanol and chloroform, in a suitable volume-to-volume ratio. This step may involve the integration of the lipophilic drug. A rotating vacuum evaporator is employed to volatilize the organic solvent at a temperature higher than the lipid temperature of transition while operating at lower pressure, resulting in the formation of a thin layer. Apply suction using a vacuum cleaner to eliminate any lingering solvent residue. Once the thin film is created,

it is moisturized by spinning it at the correct temperature for the required duration using a solution of buffer with the suitable pH level, such as pH 7.4. At this juncture, the incorporation of the hydrophilic medication can be finalized. In order to generate minuscule vesicles, the resulting vesicles are subjected to sonication using either a bath or probe sonicator, following their expansion at room temperature. The process of extrusion, which involves passing through a sandwich of polycarbonate membranes with thicknesses ranging from 200 nm to 100 nm, helps to evenly distribute the sonicated vesicles.

7.2 Sonication-Vortexing Technique: The medicine, phospholipids, and edge activator are mixed together in a phosphate buffer. Subsequently, the mixture is vigorously agitated to generate a cloudy transfersomal suspension. Following sonication for the specified duration at the surrounding temperature utilizing a bath sonicator, the substance is forced through polycarbonate membranes with pore sizes of 450 and 220 nm, as an illustration.

7.3 Revised Protocol for Handshaking: Both the modified handshaking method and the rotating evaporation-sonication approach have a common fundamental principle. In the modified handshaking protocol, a round-bottom flask is used to combine phospholipids, an edge activator, an organic solvent, and a lipophilic medicament. In order to obtain a clean and transparent solution, it is necessary for every excipient to be fully dissolved by the solvent. The organic solvent is subsequently eliminated through evaporation while agitating, rather than utilizing a centrifugal vacuum evaporator. Simultaneously, a water bath, which is kept at an elevated temperature (such as 40-60 ◦C), is used to partially immerse the round-bottom flask. A slender lipid layer subsequently forms along the inner surface of the flask. During the night, the solvent is allowed to completely evaporate in the flask. Afterwards, the film that is produced is moisturized at a temperature higher than its phase transition temperature and gently agitated with the suitable buffer solution. The process of incorporating the hydrophilic medication is now complete[10].

7.4. The suspension homogenization method used is To create transfersomes, mix an appropriate amount of edge activator with an ethanolic phospholipid solution. Once the suspension is formed, it is mixed with a buffer solution to achieve the overall lipid content. The ultimate concoction is thereafter subjected to freezing, thawing, and sonication, in that specific sequence, for a total of two to three repetitions.

7.5 Centrifugation Procedure

The lipophilic medicine, edge activator, and phospholipids are dissolved by the organic solvent. Subsequently, the solvent is extracted at the suitable temperature by use of a low-pressure rotating evaporator. The residual solvent is completely removed under a vacuum. By subjecting the lipid film to centrifugation at room temperature, it is effectively hydrated with the appropriate buffer solution. At this juncture, the incorporation of the hydrophilic medication can be finalized. When the vesicles are exposed to room temperature, they undergo swelling. The multilamellar lipid vesicles undergo further sonication at room temperature.

7.6. The method of reverse-phase evaporation The phospholipids are dissolved in an organic solvent mixture (such as diethyl ether and chloroform) in a round-bottom flask using an edge activator. This step may involve the integration of the lipophilic drug. The lipid coatings are subsequently acquired by employing a rotary evaporator to volatilize the solvent. The organic phase, composed mainly of isopropyl ether and/or diethyl ether, acts as where the lipid coatings are dissolved again. The organic component is subsequently merged with the phase of water to generate a biphasic system. At this juncture, the incorporation of the hydrophilic medication can be finalized. Subsequently, the system is subjected to sonication using a bath sonicator until a homogeneous water-in-oil emulsion is achieved. The rotary evaporator is employed to progressively evaporate the organic solvent, resulting in the formation of a thick gel that ultimately transforms into a vesicular suspension.

7.7 Homogenization using a High-Pressure Method The phospholipids, an edge activator, and medicine are uniformly dispersed in either PBS or distilled water including alcohol. Subsequently, they are simultaneously stirred and exposed to ultrasonic vibration. Subsequently, the mixture is periodically agitated using ultrasonic technology. Afterwards, a homogenizer with high pressure is employed to

homogenize the resulting mixture. Subsequently, the transfersomes are placed in appropriate storage.

7.8 Intravenous Ethanol Administration Technique

The phospholipid, edge activator, and lipophilic medicine are dissolved in ethanol using magnetic stirring for the necessary duration to produce a clear solution, resulting in the formation of the organic phase. The water-soluble substances are fully dissolved in the phosphate buffer solution to form the aqueous phase. At this stage, the incorporation of the hydrophilic medication can be finalized. Both solutions attain temperatures of 45–50 °C. Afterwards, the water-based solution is consistently agitated for the specified duration while the ethanol-based phospholipid solution is introduced gradually, one drop at a time. To remove ethanol, the dispersion is placed in a vacuum evaporator and subjected to sonication to decrease the particle size.

8. Factors Affecting the Properties of Transfersomes

Various process variables can impact the characteristics of transfersomes during the formulation development process. The elements mostly pertain to the production of transfersomal formulations, as illustrated by the following:

8.1 Phospholipids' Impact: Ratio of Edge Activator: To get the best possible outcomes, it is crucial to adjust the proportion of phospholipid to edges activator (lecithin:surfactant) as it significantly affects the size of the vesicles, the efficiency of trapping substances within them, and their ability to penetrate. In general, it has been observed that higher concentrations of surfactants can decrease EE. Reduced surfactant levels can cause vesicles to become larger, while a greater concentration of the border promoter can lead to the formation of openings in the bilayer and a decline in the vesicles' ability to pass through. The enhanced porosity of the vesicles membrane, leading to leakage, can be ascribed to the organization of molecules of surfactant inside the bilayer of lipids structure, which creates pores. [11].

8.2 The Impact of Different Solvents

Multiple solvents, including ethanol and methanol, are used. The selection of a solvent is contingent upon the suitability of the constituents of the formulation with its solvent and their ability to dissolve in it. For optimal outcomes, it is advisable for both the medicine and any other ingredients to be completely dissolved in the solvent. This will lead to a solution that is transparent and clear, enhancing the ability to produce films and ensuring stability after hydration. The solvents in the formulation can also act as penetration enhancers, increasing the drug's ability to pass through the membrane. Williams and Barry (2004) conducted experiments in which ethanol was used to increase the penetration of 5-fluorouracil, estradiol, hydrocortisone, and levonorgestrel through the skin of rats. For example, ethanol enhances penetration through many ways. It improves the solubility of drugs in vesicles by acting as a solvent. Furthermore, it penetrates the outermost layer of the skin, called the stratum corneum, by altering the tissue's ability to breakdown things and improving the spread of medication within the membrane. The addition of more ethanol to the formulation may cause a decrease in %EE, which can be explained by the deeper penetration of the vesicle phospholipid bi layer. This can enhance the following discharge of the enclosed drug.

8.3 Effects of Various Edge Activators (Surfactants)

The flexibility and ability to capture substances of transfersome s are determined by the specific edge activators included in their compositions. The unique chemical composition of the EA could be the underlying factor responsible for this phenomenon. The size of vesicles can be decreased by raising the concentration of surfactant, enhancing the hydrophilic nature of the surfactant's head group, lengthening the carbon chain, and adjusting the hydrophilic a lipophilic balance (HLB). The appropriate quantity of surfactant to incorporate into the formulation is contingent upon the packing density of the phospholipid and the nature of the interaction between the surfactant and phospholipid. Surfactants can influence the permeability of materials by transfersomes. The release of the medication ciprofloxacin was observed to be affected by the type and amount of surfactant employed, with the inclusion of Tween 80 notably enhancing the release. Cipolla et al. conducted a study in 2014. [13].

Table-2 : Various Surfactants used in synthesis of nanovesicles.

Surfactants used in synthesis of nanovesicles	
Name	**Formula**
Span 80	$C_{24}H_{44}O_6$
Span 60	$C_{24}H_{46}O_6$
Span 40	$C_{22}H_{42}O_6$
Span 65	$C_{60}H_{114}O_8$
Tween 80	$C_{32}H_{60}O_{10}$
Tween 60	$C_{35}H_{68}O_{10}$
Tween 20	$C_{26}H_{50}O_{10}$
Sodium deoxycholate	$C_{24}H_{39}NaO_4$

8.4. The influence of the hydration medium on the system

Two possible options for a hydrating medium are water or saline phosphate buffer with a pH range of 6.5-7. The pH level of the combination must be modified in order to achieve a harmonious balance between the qualities of the formulation, its biological applications, and the route of administration. Transfersomes, due to their similarity to the phospholipid layer of the cell membrane, only allow unionised drugs to pass through their intracellular channel and bind to the phospholipid bilayer of the membrane. Using the correct pH of the hydration medium is crucial as it helps maintain the drug's unionization, hence enhancing its ability to penetrate and be trapped.

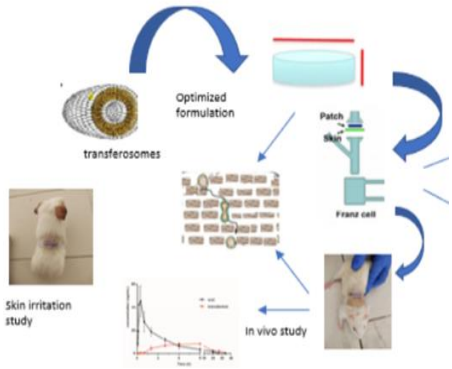

Figure 3– Representing the invitro/ invivo evaluation of transfersemal transdermal delivery.

9. Characterisation Transfersomes

Characterization parameters of transfersomes, such as vesicle shape and size, variation in size, diversity index, zeta potential, then the amount of vesicles per a cubic mm, capture efficiency, extent of deformability, and skin permeability, are commonly determined using published methods. These techniques are beneficial for optimizing the transfersomal formulation. Below, you will find a comprehensive explanation of each characterization technique. [14]

9.1 Size, Zeta Potential, and Morphology of Vesicles

The vesicle size is a crucial factor in the production of transfersomes, conducting batch-to-batch comparisons, and scaling up methods. The variability in vesicle size during preservation is a crucial element that impacts the physical stability of the formulation. Due to their highly curved form, bilayer membranes of vesicles smaller than 40 nm are more prone to undergo fusion processes. On the other hand, larger and electroneutral transfersomes assemble by van der Waals contacts, mostly due to their relatively larger

barrier contact surfaces. The size of the vesicles is a determining factor in the ability of medicinal compounds to be enclosed in transfersomes. For the packing of hydrophilic compounds, it is better to have a larger volume of the aqueous core. On the other hand, for lipophilic and an amphiphilic agents, a higher ratio of lipids to the core is preferred. The size of the vesicle may often be determined using either the photon correlation spectroscopy (PCS) or the dynamic light scattering (DLS) method. One can mix the vesicle suspension with an appropriate medium and then measure the size of the vesicles three times. In addition, the sample can be made in water that is distilled and filtered using a 0.2 mm membranes filter as an alternative approach. To determine the dimensions of the vesicle using DLS or PCS, the filtrated specimen is diluted with filtering saline solution. In addition, although the technique of transmission electron microscopy (TEM) is utilized for the examination of structural alterations, the computerized inspection system connected with the DLS approach by Malvern Zetasizer can be employed to precisely determine the size and distribution of vesicles. The Malvern Zetasizer is utilized in the electrophoretic mobility technique for the purpose of detecting the zeta potential. Transfersome vesicles can be visualized using either phase contrast microscopy or transmission electron microscopy.

Vesicle density per cubic millimeter: This parameter is essential for optimizing both other process factors and the composition of transfersomes. The somal components have been diluted with 0.9% sodium chloride five times, using inappropriate transfer methods. The sample is analyzed using an optical microscope and a hemocytometer. Transfersomes having vesicles larger than 100 nm are visible under an optical microscope. The formula shown below is utilized to calculate and determine the quantity of transfersomes within small squares: The total number of squares counted multiplied by the dilution factor and then multiplied by 4000 is the total amount of transfersomes per cubic millimeter.

9.2 Entrapment Efficiency, expressed as a percentage (%EE)

The entrapment efficiency (%EE) refers to the amount of medication that is trapped within the formulation. The determination of the encapsulation efficiency (EE) is achieved by extracting the non-encapsulated drug from the vesicles using various techniques, such as mini-column centrifugation. The percentage of EE in this operation can be determined using either direct or indirect methods. An effective approach involves extracting the liquid portion from ultracentrifugation and subsequently employing an appropriate solvent capable of breaking down the settled vesicles in the sediment. The pollutants can be removed by diluted and filtering the resulting solution using a syringe filter with a pore size of 0.22–0.45 µm. The drug content of the active pharmaceutical ingredient (API) can be determined using several analytical methods, such as spectrophotometric analysis or modified high-performance liquid chromatography (HPLC).

The entrapment efficiency, or percentage of drugs entrapped, is given as follows:

%Entrapment efficiency = Total amount of drug added times 100 amount of drug entrapped

Using a suitable solvent to dilute the supernatant and filter it to remove contaminants is the indirect method of determining the %EE. Using the proper analytical technique, the concentration of the drug in the supernatant is identified as the free drug.

As a result, the proportion of drug entrapment is stated as follows:

%Entrapment efficiency is equal to the total amount of added drug minus the total amount of free drug multiplied by 100.

9.3 Deformability Degree

This parameter is significant since it influences the transfersomal formulation's penetration. Pure water is used as the benchmark in this investigation. The mixture is run through many microporous filters with known pore diameters ranging from 50 to 400 nm. DLS measurements are used to record the particle size and size distribution following each pass . The expression for the degree of deformability is:

J (rv/rp) = D

where J is the amount of suspension extruded over a 5-minute period, rv is the vesicle's size, and rp is the barrier's pore size. D is the degree of deformability.

9.4 Drug Release in Vitro : The optimization of transfersomal composition can be achieved through the analysis of the in vitro medication release profile. This analysis provides crucial information about the formulation design, as well as details about how it releases and kinetics. The evaluation of Transfersomes' drug release in vitro is often conducted in comparison to the unbound drug or the standard product. Several experiments have yielded valuable information regarding the drug release properties of developed transfersome formulations. [16] Franz diffusion cells are employed for in vitro drug release studies, in short. The donor cylinder is affixed to the receiver chamber using adhesive tape. A magnetic bar continuously agitates the water in the target receptor chamber. To ensure accurate results in release studies, it is crucial to keep the receptor fluid temperature at 32 ± 1 ◦C, which is consistent with the median skin surface temperature in vivo. The membrane utilized is a mixed cellulosic ester barrier with a standard pore diameter of 0.45 µm. In order to facilitate the expansion of the membrane pores, the membranes are submerged in the release medium (phosphate buffer) at the ambient temperature for a duration of one night. In order to maintain the sink conditions, 1 mL portions of the receptor medium are extracted at specific time intervals (0, 0.5, 1, 2, 3, 4, 5, and 6 hours). Simultaneously, the receptor media is substituted with an equal volume of new PBS. The obtained samples can undergo appropriate analysis methods, such as high-performance thin-layer chromatography (HPTLC), UV, and HPLC.

9.5 Studies on In Vitro Skin Permeation

This study aims to assess the efficiency of transdermal delivery methods in transporting medications and identify the factors that enhance the transdermal flux of drugs, typically quantified in µg/cm2/h. Before conducting expensive in-vivo experiments, the information obtained from this work can be used to improve the composition and anticipate the in vivo performance of different transdermal delivery systems. [17]. It is necessary to evaluate the ability of proposed formulations to penetrate human skin. Nevertheless, the use of human skin for permeation studies is hindered by its limited availability, ethical concerns, and religious restrictions. Several animal models, such as ape, porcine, rat, mouse, guinea pig, and snake skins, have been suggested as more easily obtainable substitutes for human skin. This model offers the advantage of possessing a higher degree of consistent permeability and reactivity compared to animal and human skins. Essentially, the skin permeation inquiry employs Franz diffusion cells. The membranes are positioned horizontally on the receptor compartments, with the stratum corneum facing upward towards the donor compartments. The receptor compartments of the Franz diffusion cells are filled with a solution of phosphate buffer saline, which is then stirred using a magnetic bar. The receptor fluid temperature must be kept at 37 ± 0.5 ◦C in order to accurately replicate blood circulation beneath the skin [40,93]. Once the donor compartment is placed on the membrane, an appropriate amount of the testing formulation is poured, and the top of the diffusion cell is opened to mimic nonoccluded conditions.

9.6 Stability of Transferosomes
By assessing the dimensions and composition of the vesicles over time, one can determine the stability of transferosomes. The average dimensions and alterations in structure can be determined using Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), respectively. The optimized transfersomal formulations can be stored at different temperatures in tightly sealed amber vials. The recommendations set by the International Conference on Harmonisation (ICH) specify that the general storage conditions for stability testing of novel medicinal substances and products are either 25 ± 2 ◦C/60% relative humidity (RH) ± 5% RH or 30 ± 2 ◦C/65% RH ± 5% for a duration of 12 months. The suggested storage condition for accelerated testing is a temperature of 40 ± 2 ◦C and a relative humidity of 75% ± 5% for a duration of six months. Drug products intended for refrigeration should be stored at a temperature of 5 ± 3 ◦C for 12 months, followed by a shorter research period of six months at a temperature of 25 ± 2 ◦C and a relative humidity of 60% ± 5% RH. Noncompliance with the drug product's standards is deemed a significant alteration.

10. The use of transferosomes in transdermal drug administration has been extensively studied in recent decades. [18]

10.1 Delivery of Antioxidants
In order to enhance their ability to protect against UV radiation, as well as their antioxidant and anti-aging properties, nanotransfersomes containing EGCG and hyaluronic acid were created using improved thin-film hydration and high-pressure homogenization techniques. The high-pressure homogenization approach was used in 2019 to create transfersomes containing resveratrol. It was found that the generated transfersomes could improve the stability, bioavailability, solubility, and safety of resveratrol.
10.2 Anticancer Drug Delivery: A study was conducted to treat melanoma by applying transfersome-embedded oligopeptide hydrogels containing paclitaxel topically. These hydrogels were created utilizing the thin-film dispersion process. Studies have shown that transfersomes composed of sodium deoxycholate, tween80, and phosphatidylcholine have the ability to effectively penetrate tumor tissues. In 2003 and 2004, Cevc and Blume conducted a study on the biological activity and characteristics of transfersomes loaded with the halogenated corticosteroid triamcinolone acetonide. These transfersomes were created using the standard thin-film hydration process. The results showed that transfersomes had a reduced therapeutic dose, increased biological potency, and prolonged effect.
10.3 Anti-Inflammatory Drug Delivery: Various research teams developed and studied transfersomes loaded with diclofenac sodium, celecoxib, mefenamic acid, and curcumin with the purpose of delivering these drugs topically. Furthermore, study findings have shown that transferomes have the ability to improve the stability and efficacy of anti-inflammatory drugs. The text contains concise explanations of several elements related to formulation and preparation.

11. Several methods of transferring substances via the skin in transdermal administration have been identified [19].

- Insulin is treated to either ultrasonication or intermediate-pressure homogenization using thin-film hydration (10 Pa), triethanolamine–HCl buffer (pH 6.5), ethanolic SPC, and SC (8.7% SPC by weight, 1.3% SC by weight, and 8.5% ethanol by volume). Both humans and animals were intentionally induced with therapeutically significant hypoglycemia, and the outcomes were positive.
- The technique used for high-pressure homogenization involved 5 cycles at a pressure of 1500 bar. The ingredients used were

Resveratrol, Plantacare® 1200 UP, TW80, PC, and TW20. The use of a coating did not impact the antioxidant activity, but it did improve the instability, bioavailability, solubility, and safety.

- Anabolic steroids Corticoid Glucocorticosone was subjected to a traditional film process for 12 hours at a pressure of 10 Pa. The procedure involved using a buffer with a pH of 6.5, and a mixture of methanol and chloroform in a 1:1 volume ratio. The resulting mixture was homogenized with SPC and TW20 in a 1:1 molar ratio relative to SPC. Extended duration of action, increased biological efficacy, and reduced therapeutic dose.
- Diclofenac sodium: The rotary evaporation–sonication procedure was recommended instead of the vortexing method since it is simpler and requires less time. Chloroform and methanol at a ratio of 2:1 (volume/volume), EPC, SC, SDC, TW80, SP80, SP85, and PBS with a pH of 7.4 were all subjected to sonication using a bath sonicator. The drug's distribution through the skin in a laboratory setting was significantly improved by vesicles containing tween 80. These vesicles also shown the highest level of ability to change shape and were more efficient than bile salts and spans.
- The process involved in preparing celecoxib includes using a modified handshaking technique, SPC, SDC, hydrating it with PBS (pH 7.4), sonication with a bath sonicator, and using a 3:1 v/v ratio of chloroform to methanol. Proven to be an effective therapeutic approach for administering medication to treat rheumatoid arthritis. Meloxicam is formed into a bilayer structure by combining EPC and CH in a molar ratio of 10:2, together with either SC, sodium oleate, or dicetylphosphate. Significantly increased skin permeation.
- The formulation of Felodipine consists of a mixture of lipid and edge activator in a ratio of 95:5. The preparation process involves vortexing and sonication techniques. The components used in the formulation include SPC, EPC, TW80, and SP80. Transfersomes efficiently and non-invasively penetrate the skin, resulting in fast and effective delivery of therapeutic drugs into the bloodstream at a lower dosage.
- Telmisartan was dissolved in a mixture of chloroform and methanol (in a 1:1 ratio). The resulting solution was then diluted in ethanol and subjected to sonication utilizing a probe sonicator in a standard rotating evaporation sonication method, employing SC, PBS (pH 6.4) as the solvent. Enhanced transdermal permeation and exhibited prolonged drug delivery.
- Sertraline: The traditional rotary evaporation sonication method is used to apply sonication to ethanol, soy lecithin, and SP80 that have been hydrated with 7% v/v ethanol. This medication has a higher effectiveness in treating depression and also has significantly better ability to pass through barriers.
- Libocaine: The thin-film hydration method was employed to dissolve SPC, CH, SC, SP80, and brij 35 in different molar ratios using sonication. The solvents utilized were a combination of methanol, chloroform, and ethanol in a 2:1:1 volume-to-volume-to-volume ratio, along with an isotonic phosphate buffer at pH 5.8. Enhanced skin penetration coupled with a more potent local anesthetic action.
- Repaglinide was sonicated using a bath sonicator in a mixture of methanol and chloroform (in a ratio of 3:1) together with soy lecithin, SP80, SP60, SP40, TW80, and PBS (with a pH of 6.8) using the handshaking thin-film hydration method. Improved

antihyperglycemic medication delivery with topical application, targeted to specific sites, and with prolonged release.

12. Transferosomes in market

The transferosomal formulation, named Diractin®, containing ketoprofen, was approved by SwissMedic in 2007 and introduced to the market. This combination was suggested as an analgesic for knee osteoarthritis. Transferosomes shown superior ability to deliver ketoprofen to deeper tissues, such as muscle, in comparison to conventional anti-inflammatory gels. However, the EMA revoked the approval of the medicine six months after it was granted, as it was believed to have only marginally superior effectiveness compared to ketoprofen freevehicle. The higher production cost of transferosomes compared to regular gels is a key issue leading to the company's withdrawal from the market. There is a direct correlation between increasing drug prices and elevated production expenses. However, the minimal enhancement in permeability offered by the transferosomal formulation does not warrant the increased treatment cost. Transferosomes exhibit higher permeability compared to other topical dosage forms and drug delivery systems, provided they are prepared correctly. The higher expense of this product can be rationalized when synthesizing complex chemicals with exceptionally low permeability characteristics, or in special conditions that require greater permeability into deeper tissues, including the bloodstream. Under such conditions, the utilization of transferosomes would yield greater therapeutic advantages, hence justifying the cost expenditure. The text is referenced by the number [20].

13. Prospects for the future: Lipid nano-carriers are a widely researched and utilized technology for carrying compounds across or into the outermost layer of the skin, called the stratum corneum. Transferosomes possess a notable superiority compared to liposomes and niosomes due to their exceptional flexibility, which is obtained through the utilization of an edge activator. This enables them to infiltrate deeper levels of the skin by utilizing both intercellular and paracellular pathways, traversing the corneocytes.

14. Recent progress

Investigation of nanovesicles in phytochemical compounds

Tansferosomes possess the capacity to mitigate the issue of limited bioavailability associated with these medicines. Transferosomes have proven to be quite successful in encapsulating various phytochemical compounds to date. Abdallah et al. successfully developed a durable nano-transferosome for Silymarin gel, with a focus on addressing hyperglycemia. The stability of the nano-transferosome remained for a period of three months. In vivo studies demonstrated that the formulated mixture significantly reduced blood glucose levels. An ex vivo experiment revealed a significant transdermal flow, measuring 92.41 µg/cm2 h. Abdallah et al. (2022) suggest that the use of Span 80, a nonionic surfactant that enhances the flexibility of the membrane, could be responsible for the enhancement of skin permeability. [21]

14.1 Integrating nanovesicles with Artificial Intelligence

The technique has the capability to generate accurate prediction models from a dataset of significant size, which may then be utilized to develop an improved nanoformulation. Artificial neural networks (ANN) can be utilized even in the absence of a large data set. Li et al. (Y. Li et al., 2015) used ANN poly lactic-co-glycolic acid (PLGA) nanoparticles. The researchers discovered that using ANN resulted in a significant decrease in prediction errors for drug release and particle size. Specifically, the errors decreased from 19.4% to 2.99% and from 28.0% to 2.93% correspondingly (Y. Li et al., 2015).

Transferosome formulations can be generated utilizing the identical procedure. A separate cohort of researchers examined the impact of formulation variables on the efficacy of nanoparticle entrapment and the size of the particles. They employed artificial neural networks (ANN) in conjunction with continuous genetic algorithms to conduct their investigation. The researchers successfully identified the optimal verapamil-loaded nanoparticle by doing the minimum number of required experiments (Y. Li et al., 2015). Reker et al. conducted a recent study where they employed a random forest machine learning model to determine the most effective combination of medication and excipient. A total of 100 drug nanoparticles, which have the ability to assemble themselves, were obtained from a pool of 2.1 million pairs.
[22, 23]

CONCLUSION:

Transfersomes, in comparison to conventional vesicular systems, are extremely flexible carriers that facilitate the transportation of many therapeutic substances across the epidermal barrier with enhanced effectiveness. The osmotic gradient is the fundamental mechanism that drives the movement of transfersomes are as into the deeper parts of the skin. Transferosomes are vesicular systems that are specifically designed and optimized for each pharmaceutical to provide the most effective formulations and desired pharmacological effects. Further investigation into transfersomes has the potential to yield novel therapeutic approaches for a range of ailments.

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Conflict of Interest

The authors declare no conflict of interest

REFERENCES

1. Rother, M.; Lavins, B.J.; Kneer, W.; Lehnhardt, K.; Seidel, E.J.; Mazgareanu, S. Efficacy and safety of epicutaneous ketoprofen in Transfersome (IDEA-033) versus oral celecoxib and placebo in osteoarthritis of the knee: Multicentre randomised controlled trial. Ann. Rheum. Dis. 2007, 66, 1178–1183. [CrossRef]
2. Kumar, A.; Pathak, K.; Bali, V. Ultra-adaptable nanovesicular systems: A carrier for systemic delivery of therapeutic agents. Drug Discov. Today 2012, 17, 1233–1241. [CrossRef]
3. Fesq, H.; Lehmann, J.; Kontny, A.; Erdmann, I.; Theiling, K.; Rother, M.; Ring, J.; Cevc, G.; Abeck, D. Improved risk-benefit ratio for topical triamcinolone acetonide in Transfersome in comparison with equipotent cream and ointment: A randomized controlled trial. Brit. J. Dermatol. 2003, 149, 611–619. [CrossRef]
4. Cevc, G. Transfersomes: Innovative transdermal drug carriers. In Modified-Release Drug Delivery Technology, 1st ed.; Rathbone, M.J., Hadgraft, J., Roberts, M.S., Eds.; Marcel Dekker, Inc.: New York, NY, USA, 2002; Volume 126, pp. 533–546. ISBN 0-8247-0869-5.
5. Touitou, E. Composition of Applying Active Substance to or Through the Skin. U.S. Patent 5540934A, 30 July 1996.
6. Touitou, E.; Dayan, N.; Bergelson, L.; Godin, B.; Eliaz, M. Ethosomes novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J. Control. Release 2000, 65, 403–418. [CrossRef]
7. Shah, S.M.; Ashtikar, M.; Jain, A.S.; Makhija, D.T.; Nikam, Y.; Gude, R.P.; Steiniger, F.; Jagtap, A.A.; Nagarsenker, M.S.; Fahr, A. LeciPlex, invasomes, and liposomes: A skin

penetration study. Int. J. Pharm. 2015, 490, 391–403.
8. Qadri, G.R.; Ahad, A.; Aqil, M.; Imam, S.S.; Ali, A. Invasomes of isradipine for enhanced transdermal delivery against hypertension: Formulation, characterization, and in vivo pharmacodynamic study. Artif. Cells Nanomed. Biotechnol. 2016, 45, 139–145.
9. Walve, J.R.; Bakliwal, S.R.; Rane, B.R.; Pawar, S.P. Transfersomes: A surrogated carrier for transdermal drug delivery system. Int. J. Appl. Biol. Pharm. Technol. 2011, 2, 204–213.
10. Sivannarayana, P.; Rani, A.P.; Saikishore, V.; VenuBabu, C.; SriRekha, V. Transfersomes: Ultra deformable vesicular carrier systems in transdermal drug delivery system. Res. J. Pharm. Dos. Forms Technol. 2012, 4, 243–255.
11. Sachan, R.; Parashar, T.; Soniya, S.V.; Singh, G.; Tyagi, S.; Patel, C.; Gupta, A. Drug carrier transfersomes: A novel tool for transdermal drug delivery system. Int. J. Res. Dev. Pharm. Life Sci. 2013, 2, 309–316.
12. Li, J.; Wang, X.; Zhang, T.; Wang, C.; Huang, Z.; Luo, X.; Deng, Y. A review on phospholipids and their main applications in drug delivery systems. Asian J. Pharm. Sci. 2015, 10, 81–98.
13. Bhasin, B.; Londhe, V.Y. An overview of transfersomal drug delivery. Int. J. Pharm. Sci. Res. 2018, 9, 2175–2184.
14. Lei, W.; Yu, C.; Lin, H.; Zhou, X. Development of tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement in vivo. Asian J. Pharm. Sci. 2013, 8, 336–345. [CrossRef]
15. Pandey, A. Role of surfactants as penetration enhancer in transdermal drug delivery system. J. Mol. Pharm. Org. Process. Res. 2014, 2, 1–10. [CrossRef]
16. Duangjit, S.; Opanasopit, P.; Rojanarata, T.; Ngawhirunpat, T. Characterization and in vitro skin permeation of meloxicam-loaded Liposomes versus Transfersomes. J. Drug Deliv. 2010, 2011, 1–9. [CrossRef]
17. Aggarwal, N.; Goindi, S. Preparation and evaluation of antifungal efficacy of griseofulvin loaded deformable membrane vesicles in optimized guinea pig model of Microsporum canis—Dermatophytosis. Int. J. Pharm. 2012, 437, 277–287. [CrossRef]
18. Chen, J.; Lu, W.-L.; Gu, W.; Lu, S.-S.; Chen, Z.-P.; Cai, B.-C. Skin permeation behavior of elastic liposomes: Role of formulation ingredients. Expert Opin. Drug Deliv. 2013, 10, 845–856. [CrossRef]
19. Duangjit, S.; Opanasopit, P.; Rojanarata, T.; Ngawhirunpat, T. Effect of edge activator on characteristic and in vitro skin permeation of meloxicam loaded in elastic liposomes. Adv. Mater. Res. 2011, 194, 537–540.
20. Kneer, W., Rother, M., Mazgareanu, S., Seidel, E.J., European, I.-s.g., 2013. A 12-week randomized study of topical therapy with three dosages of ketoprofen in Transfersome(R) gel (IDEA-033) compared with the ketoprofen-free vehicle (TDT 064), in patients with osteoarthritis of the knee. Journal of pain research 6, 743-753. doi: 10.2147/JPR.S51054.
21. Abdallah, M. H., Abu Lila, A. S., Shawky, S. M., Almansour, K., Alshammari, F., Khafagy, E. S., & Makram, T. S. (2022). Experimental design and optimization of nano-transfersomal gel to enhance the hypoglycemic activity of silymarin. Polymers (Basel), 14(3), 508. https://doi.org/10.3390/polym14030508.
22. Damoiseaux, R., George, S., Li, M., Pokhrel, S., Ji, Z., France, B., Xia, T., Suarez, E., Rallo, R., Madler, L., Cohen, Y., Hoek, E. M. V., & Nel, A. (2011). No time to lose-high throughput screening to assess nanomaterial

safety. Nanoscale, 3(4), 1345-1360. https://doi.org/10.1039/c0nr00618a.

23. Liu, X., Jia, Y., Han, Z., Hou, Q., Zhang, W., Zheng, W., & Jiang, X. (2021). Integrating a concentration gradient generator and a single-cell trapper array for high-throughput screening the bioeffects of nanomaterials. Angewandte Chemie International Edition England, 60(22), 12319-12322. https://doi.org/10.1002/anie.202101293.
24. Kumar A., Pathak K. and Bali V; Ultra adaptable nanovesicular systems: a carrier for systemic delivery therapeutic agents; Drug Discovery Today; 2012; 17; Numbers 21/ 22; 1233-1241.

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