REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

A Recent Overview of Advanced Pharmaceutical Delivery Systems: Transfersome and Protransfersome as Promising Nanoencapsulation Techniques for Transdermal Drug Delivery

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Abstract

The study aims to characterize protransfersome gel as a transdermal drug delivery system for Repaglinide to ensure effective and sustained drug delivery. Repaglinide protransfersomal gel was created using the coacervation phase separation method and studied for vesicular morphology, dimensions, entrapment efficiency, and drug penetration through rat dermis. Numerous edge activator and formulation characteristics employed in the development of the optimal formula. All protransfersomal formulations were described, revealing that the created gel's color and physical state were discernible to the naked eye. The mean particle size of protransfersome ranged from 264 to 341 nm. TEM micrographs of the transfersome suspension revealed that the vesicles possess a homogeneous spherical morphology with a smooth surface. The formulation demonstrated excellent stability at 4 ± 1°C, and after three months of storage, there were no alterations in its liquid crystalline properties, drug content, or other distinctive criteria. The protransfersomal formulation is non-irritating. Consequently, the formulated preparation is deemed safe for topical use. The in vivo pharmacodynamic analysis of protransfersomal gel demonstrated a substantial enhancement in the bioavailability of repaglinide compared to oral drug administration.

Keywords: Protransfersome; Repaglinide; Edge Activator; Transdermal Drug Delivery.

1. Introduction

Transdermal delivery system TTD is a highly effective pathway for the distribution of a drug throughout the body's circulatory system. The reason for this is the extensive surface area of the skin, which allows for convenient access and several choices for the absorption of substances through the skin. Moreover, medications exhibit

more consistent pharmacokinetic profiles with less occurrence of peaks, hence reducing the likelihood of hazardous adverse reactions. This strategy not only enhances patient adherence by reducing the frequency of dosing, but it is also appropriate for patients who are unconscious or experiencing vomiting, as well as those who depend on self-administration ***(Ita, 2014)***.

TDD enhances bioavailability by circumventing pre-systemic metabolism. Nevertheless, the ability of medication molecules to pass through the skin is hindered by the protective layer of human skin epithelium, which acts as a barrier against external chemicals ***(Ramkanth et al., 2018)***. In recent times, a range of techniques have been employed to enhance the transdermal distribution of active medicinal components such as liposomes, niosomes, elastic liposomes (ethosomes), and transferosomes. Transferosomes, among other strategies, have the ability to easily change their shape under external force and can penetrate deep into the epidermal layers with high water content, ultimately reaching the subcutaneous tissues. Unlike traditional liposomes, transferosomes have the ability to transport large molecules, such as proteins, through the bloodstream ***(Rajan et al., 2011)***.

Transport of the drug through skin is best route of drug delivery because the skin is largest organ in human body. Drug carries which are used in transdermal drug delivery such as liposomes, niosomes, or microemulsions pose a problem that they remain mostly confined to the skin surface and therefore do not transport drugs efficiently through the skin. Because of the deformable nature of transfersomes, it penetrates through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form. Vesicle shape and size, entrapment efficiency, degree of deformability, number of vesicles per cubic mm can be characterized by in vitro studies. Thus, they act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin. The transdermal drug delivery system overtakes the pitfall associated with oral drug delivery system. It decreases the dosing frequency, diminish the GI based side effect by avoiding the first pass metabolism and enhances patient compatibility ***(Pandey et al., 2018)***.

The skin is the largest and most weighty organ in the human body. It encompasses the entire external surface, weighing around 3.6 kilograms, which accounts for 15% of the total body weight. Additionally, it has a surface area of approximately 2 square meters ***(Streckfus, 2022)***. The skin is the largest organ in the human body and is a component of the integumentary system. The skin envelops the external auditory meatus, encloses the tympanic membrane, and links with the conjunctiva of the eyelid ***(Streckfus, 2022)***. The skin is a complex system that acts as the body's main defense against viruses, UV radiation, toxins, and physical injury. It serves as a waterproof barrier, regulating water loss and controlling body temperature. Additionally, the skin plays a role in immune surveillance, sensory perception, fluid balance, and overall homeostasis. Its adaptability is evident through its varying thickness and specialized functions across different areas of the body ***(Oakley et al., 2020)***. In addition, it generates antimicrobial peptides that hinder infections, as well as hormones, neuropeptides, and cytokines that have biological impacts, not just in the immediate vicinity of the skin but also throughout the entire body ***(Neupane et al., 2020)***.

The skin consists of three distinct layers: the outermost layer is called the epidermis, the layer beneath it is known as the dermis or connective component of nutrition, and the deepest layer is called the subcutaneous tissue, which includes subcutaneous fat. These layers vary significantly in their structure and function **Figure (1).** The epidermis and dermis work together to create a unique matrix structure called the basement membrane. This membrane physically divides the two compartments of the skin and serves as a stable and dynamic interface ***(Breitkreutz et al., 2009)***. The epidermis plays a crucial role in protecting the body from environmental threats and preventing the loss of water and other bodily components to the outside environment. This is achieved through its function as both an inside-outside barrier and an outside-inside barrier, contributing to the overall defense mechanisms of the skin ***(Baroni et al., 2012)***.

Transfersomes (ultra-deformable vesicles) are the first generation of elastic, soft malleable vesicles which are tailor-made for enhanced delivery of active agents through the skin.

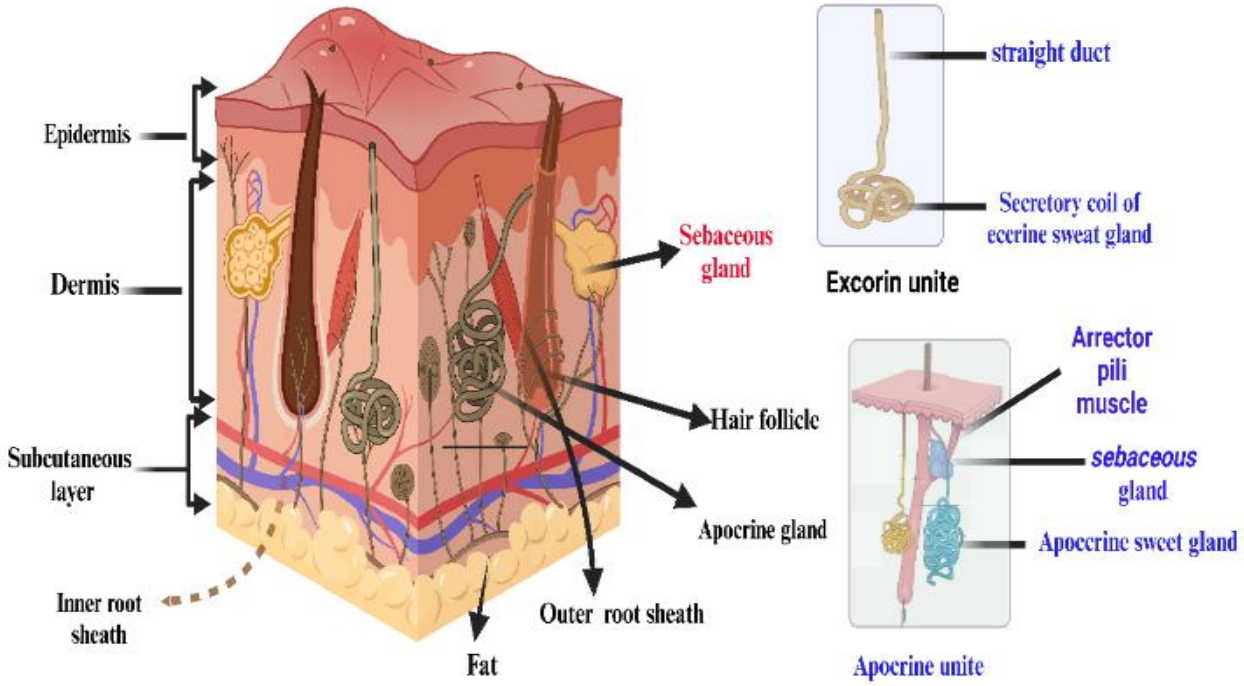

Figure (1):Cross-Section of Skin and Panniculus *(Akl et al., 2024)*

These are special type of liposomes composed of phospholipids and an edge activator, which destabilize lipid bilayers and increase the deformability of the vesicles making them more compliant to penetrate through intact skin. Along with its ability to accommodate hydrophilic drugs, transfersomes possess elasticity of several orders more than the standard liposomes enhancing skin penetration ***(Nirwan et al., 2021)***. Conventional liposomes have nominal penetration across stratum corneum (SC), that tends to accumulate over the surface delivery insignificant progress in the treatment of osteoporosis ***(Zheng et al., 2020)***. In contrast, transferosome are suitable for controlled and targeted drug delivery because of their ability to exhibit characteristics associated with cell vesicles ***(Patnaik et al., 2021)***. However, the problem of these ultra-flexible vesicles is self-stability. Therefore, to enhance the stability of these vesicles, use of liquid crystalline, pro-ultraflexible lipid vesicles (protransfersomes) has been proposed. Protransfersomes have the ability to get converted into ultra-flexible lipid vesicles (transfersomes) in-situ, upon absorbing water from the skin ***(Gamsjaeger et al., 2021)*** .

Protransfersomes exhibit superior entrapment efficiency and are suitable for incorporating both lipophilic and hydrophilic medicines, while also providing improved stability. Due to the characteristics of protransfersomes, such as their capacity to accommodate and improve the skin penetration of lipophilic substances, these molecules can serve as a good vehicle for delivering medications that have poor penetration abilities ***(Khatoon et al., 2019)***. The fluidic characteristic of protransfersomes restricts its application to the surface of the skin. Therefore, employing a vehicle for its transportation can yield advantageous outcomes. Khatoon et al. conducted a study on the transdermal application of clinidipine loaded transfersome gel. The study found that the gel had a higher entrapment efficiency and did not cause any hazardous effects. This conclusion is supported by previous studies conducted by ***(Srivastava et al., 2021)***.

Vesicular drug delivery systems

In recent years, there has been a concerted effort to design drug carrier systems aimed at enhancing drug safety, maximizing efficacy, and minimizing unfavorable or side effects. The utilization of lipid-based vesicular systems for drug delivery has attracted attention in the fields of topical and cutaneous drug administration

(Elsayed et al., 2007; Nasr-Eldin et al., 2024). Vesicles are very effective in penetrating the stratum corneum barrier in intact skin and efficiently delivering medication molecules. If selective uptake can be achieved, encapsulating a medication in a vesicular structure is expected to extend the drug's presence in the systemic circulation, improve its penetration into target tissue, and decrease its toxicity. Vesicles are composed of amphiphilic molecules arranged in a bilayer structure. The hydrophilic drug is contained within the inner aqueous compartment, while the vesicle bilayer consists of amphiphilic, lipophilic, and charged hydrophilic molecules. These molecules are held together by hydrophobic and/or electrostatic interactions ***(Shilakari et al., 2013)***.

The benefits of using lipid vesicles for topical and transdermal application are as follows: (i) the lipophilic nature of the vesicle wall allows for the incorporation of poorly soluble substances, enhancing their penetration; and (ii) the lipid vesicles provide a controlled release system, acting as a storage system for the incorporated molecule. Although the system has a benefit, it is hindered by inadequate drug permeability for molecules weighing more than 500 Da ***(Pathak and Raghuvanshi, 2015)***. The introduction of vesicular systems was a response to the issue of pharmaceuticals having low permeability. This led to a series of modifications in vesicles, including the creation of deformable vesicles. The deformable lipid vesicles are categorized as liposomes, niosomes, ethosomes, and transfersomes (distorted vesicles) **Figure (2)**.

Liposomes

Liposomes are vesicles composed of a bilayer of phospholipids. They have a particle size that can range from 20 nm to 10 μm. Liposomes can be categorized into three types based on their size: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and multi-lamellar vesicles (MLVs). Their circulation half-life is brief, but it can be extended by applying a layer of polyethylene glycol (PEG) units, known as stealth liposomes ***(Khatry et al., 2010)***. Liposomes are created by many techniques, such as lipid film hydration using either shaking or non-shaking methods, micro-emulsification, membrane extrusion, dried reconstituted vesicles, ether or ethanol injection method, and reverse phase evaporation method ***(Akbarzadeh et al., 2013)***.

Research on liposomal carriers for topical medication administration has been conducted since the 1980s and has generated significant attention. Liposomes enhance drug delivery through the skin through three potential mechanisms: attachment to the skin surface followed by direct transfer of the drug from the vesicles to the skin, merging with the lipid layer of the outermost layer of the skin, which increases the drug's distribution into the skin, and lipid exchange between the liposomal membrane and cell membrane, which aids in the drug's movement across the membrane ***(Yadav et al., 2011)***. Conventional liposomes have little ability to fully penetrate the living skin and enter the bloodstream. As a result, they tend to stay confined to the outer layer of the stratum corneum ***(Lymberopoulos et al., 2017)***. Consequently, liposomes have found extensive application as vehicles for delivering drugs to the skin, but not for delivering drugs through the skin. Conventional liposomes have some disadvantages, including low encapsulation efficiency (EE) of hydrophilic medicines, an unstable membrane leading to leakage, and a short half-life ***(Xu et al., 2012)***. The presence of significant barriers has prompted the exploration and advancement of alternative vesicles, such as niosomes, ethosomes, and transfersomes.

Niosomes

The discovery of niosomes dates to the early 1970s. Niosome is a vesicular system composed of a solitary nonionic surfactant with an alkyl chain, and occasionally cholesterol, which forms a closed bilayer structure in an aqueous environment. The utilized non-ionic surfactants consist of sorbitan esters and polysorbates. Cholesterol contributes to the stiffness of the vesicular bilayer, while nonionic surfactants enhance the size and encapsulation efficiency of niosomes ***(Ge et al., 2019)*** . In addition, some ionic amphiphiles, such as diacetyl phosphate (a negatively charged molecule) and stearyl amine (a positively charged molecule), are employed in niosomes to improve the encapsulation efficiency, effectiveness, and stability ***(Marianecci et al., 2014)***.

In addition, niosomes are recognized as a superior method for delivering drugs compared to liposomes. This is because they possess several

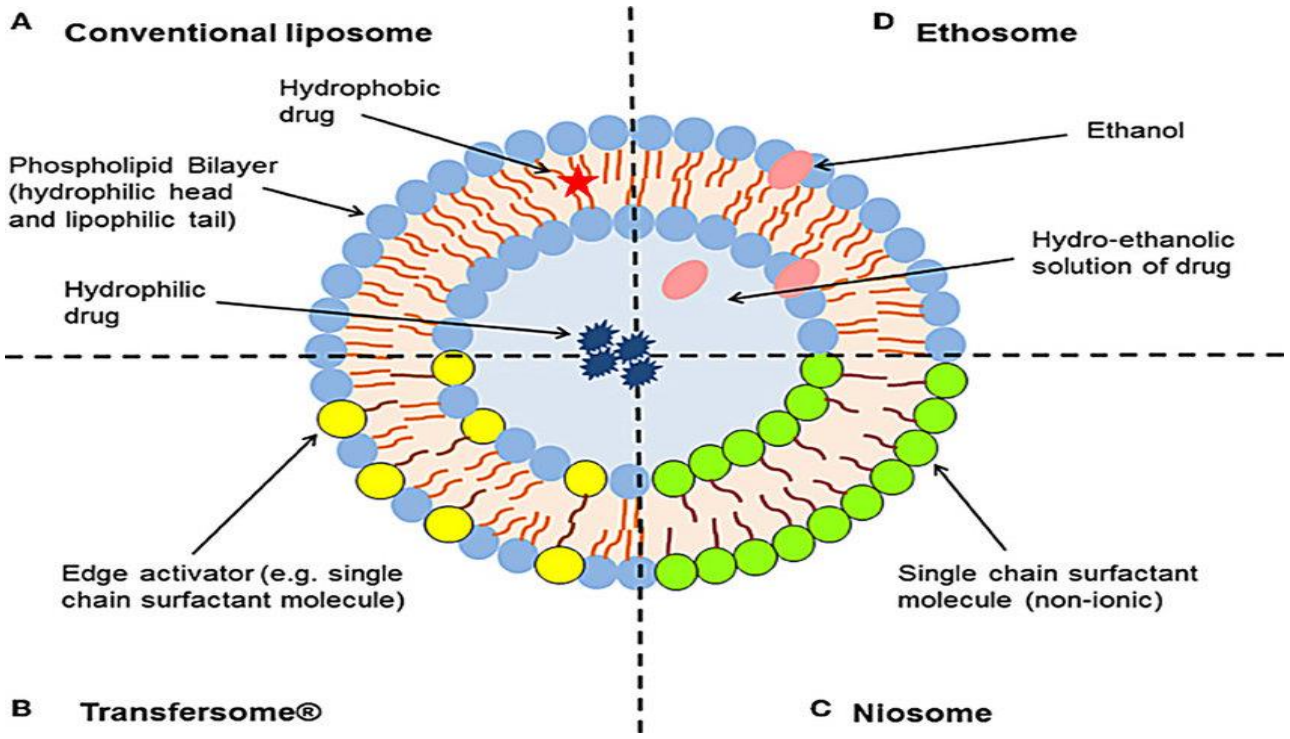

Figure (2): Schematic representation of the different types of lipid-based vesicular delivery systems

advantageous characteristics, including exceptional chemical stability, high encapsulation efficiency (EE), enhanced skin penetration resulting from a high concentration of surfactants, higher bioavailability, and cost-effectiveness. According to the literature, niosomes have been found to increase the amount of time that therapeutic medications stay in the outermost layer of the skin (stratum corneum) and the layer beneath it (epidermis). At the same time, they decrease the absorption of the drug into the bloodstream, resulting in better penetration of the drug into the skin ***(Xu et al., 2012)***. They are produced by techniques such as lipid film hydration, transmembrane pH gradient, and bubble procedures. Over the past decade, new classes of lipid vesicles such as deformable liposomes (transfersomes) and ethosomes have been developed as an enhanced type of liposomes ***(Maghraby et al., 2001)*** .

Ethosomes

Ethosomes are liposomes containing phospholipids, a high concentration of ethanol, and water. They serve as a non-invasive carrier system for delivering physiologically active substances, both hydrophilic and lipophilic, to the deeper layers of the epidermis and systemic circulation. Because ethosomes have high concentrations of ethanol, they are highly stable and have a flexible vesicle structure. This leads to changes in the lipid bilayer of the skin, which in turn improves the capacity of the vesicles to penetrate the stratum corneum. Research has demonstrated that ethosomes are more effective than liposomes in enhancing the transport of drugs through the skin, primarily because of their superior ability to penetrate the skin ***(Mehanna and Mneimneh, 2021)***.

Deformed vesicles (Transfersomes) and protransfersome

The primary purpose of formulating transfersomes and protransfersomes is to facilitate the delivery of drugs into or through the skin, resulting in a specified therapeutic response rate. Transfersomes and protransfersomes are highly flexible vesicles that utilize phospholipids as their main constituent to transport drugs. These vesicle systems, such as liposomes are less elastic compared to other systems. Therefore, they are well-suited for delivering drugs through the skin. Drug penetration through the skin is a challenging task, but it can be

facilitated by using transfersomes and protransfersomes. These substances can squeeze themselves into the deeper layers of the stratum corneum. These objects possess inherent adaptability and exhibit exceptional deformability, enabling them to penetrate the skin by altering their shape and size in response to mechanical force from the surrounding environment. The flexibility of transfersomes and protransfersomes membrane is attained through the combination of appropriate surface-active components in the correct proportions ***(Rane and Gujarathi, 2017)***. The flexible structure of vesicles minimizes the likelihood of full rupture in the skin. When applied without blocking, it enables them to track the natural water gradient across the skin.

Transfersomes

Transfersomes, which are distorted or elastic vesicles, were initially introduced by ***(Cevc, 1996)*** . Transfersomes are vesicular carriers that consist of a lipid bilayer enclosing at least one inner aqueous compartment. They also contain an edge activator, such as Tween 80, Span 80, or sodium cholate, which is a substance that softens the membrane and allows the transfersomes to be highly deformable. See **Figure (3)**. The edge activators disrupt the stability of the lipid bilayer in liposomes and enhance their flexibility ***(El Maghraby et al., 2004)***. The presence of an aqueous core enclosed by a lipid bilayer enables the formation of highly flexible vesicles that possess the ability to optimize and regulate themselves **(Walve et al., 2011)**. Transferosomes are characterized by their elasticity. When they come into contact with skin pores, they have the ability to adjust their membrane flexibility, deform, and compress themselves as intact vesicles without any noticeable loss. As a result, they can easily pass through narrow pores or constrictions in the skin that are considerably smaller than the size of the vesicles, ***(Sachan et al., 2013)***.

A transfersome typically consists of soybean phosphatidyl choline, sodium cholate, and a little amount of ethanol. Malformed vesicles are employed for the transportation of minuscule compounds, proteins, and peptides. They possess the capacity to pass through narrow passages that are one-tenth the size of their own diameter, enabling them to spontaneously penetrate through the outermost layer of the skin known as the stratum corneum ***(Foldvari and Kumar, 2017).***

Multiple studies have demonstrated that transfersomes effectively address the main limitations of traditional liposomes. The distribution of drugs through the skin using transfersomes has proven to be more efficient than rigid liposomes, mostly because to the presence of EA. Edge activators act as membrane destabilizing agents, enhancing the flexibility of vesicle membranes. When combined with the right lipid in the correct proportion, they create an optimal mixture that allows transfersomes to become highly deformable and extremely flexible. This leads to an increased permeability capability ***(Pandey et al., 2014)***. In addition, the transfersomes retained their widths without fragmentation, even after passing through the smaller pores. In addition, the encapsulation agents (EAs) employed in transfersomal formulations can enhance the solubility of hydrophobic medications, hence augmenting the efficacy of drug entrapment ***(Opatha et al., 2020)***.

The impact of EAs on skin permeability is contingent upon their specific types and concentrations. EAs Surfactants are a class of chemicals that function as edge activators and penetration enhancers, as described by ***(Kim et al., 2020)***. These compounds are recognized for their amphiphilic nature, including of a lipophilic alkyl chain linked to a hydrophilic head group ***(Kumar & Rajeshwarrao, 2011)***. In general, anionic surfactants are more effective than cationic surfactants in enhancing skin penetration. Anionic surfactants also have a lower critical micelle concentration. On the other hand, nonionic surfactants with an uncharged polar head group are better tolerated than both cationic and anionic surfactants ***(Kim et al., 2020)***. Nonionic surfactants are regarded as having lower toxicity and hemolytic properties, as well as being less irritating to cellular surfaces. Additionally, they have a tendency to maintain a pH level close to that of the body in a solution. Furthermore, they possess diverse capabilities such as solubilizing, emulsifying, and strongly inhibiting P-glycoprotein. These characteristics are valuable for improving drug absorption and targeting specific tissues ***(Kumar and Rajeshwarrao, 2011)***.

Transfersomal formulations are extensively

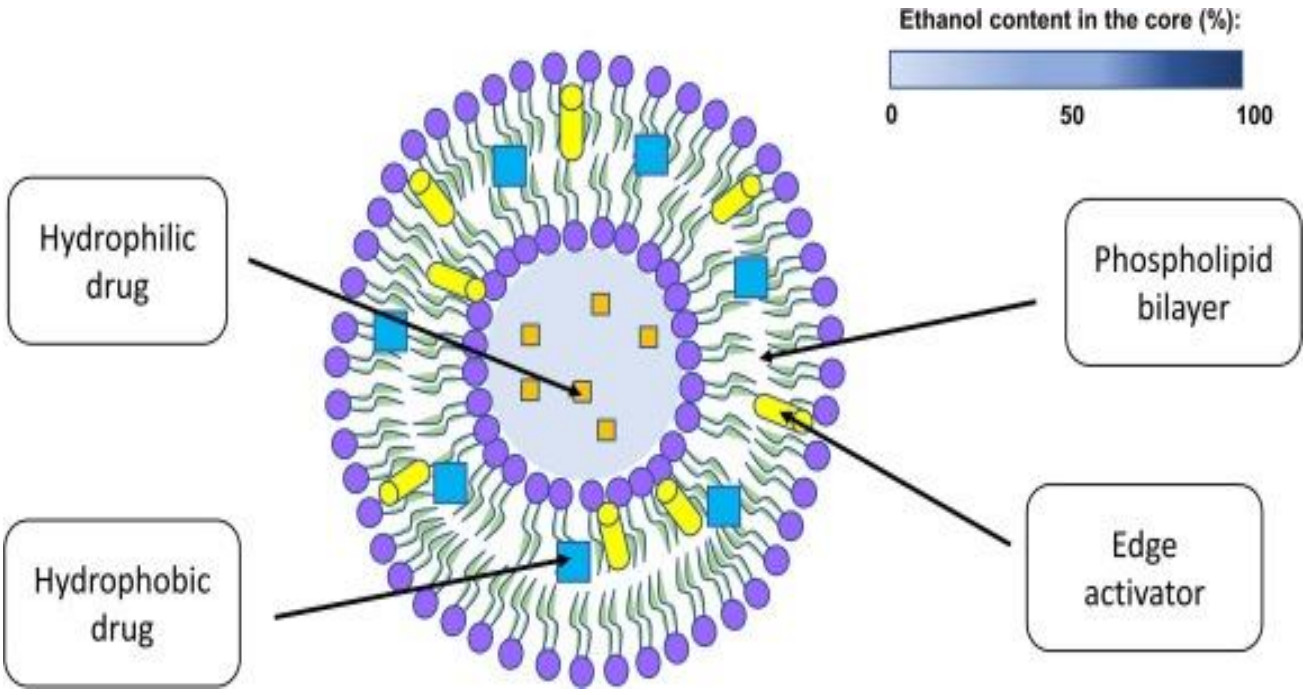

Figure (3): Structure of transfersomes, *(Fernández-García et al., 2020)*

employed in the targeting of drugs to peripheral areas, transdermal immunization, and are well acknowledged as a significant approach for the transdermal delivery of various medicinal agents. Multiple research publications have demonstrated that transfersomes have the ability to transport bioactive molecules of both low and high molecular weight ($200 \leq MW \leq 106$), as well as hydrophilic and lipophilic molecules, through the skin with a transport efficiency exceeding 50% ***(Opatha et al., 2020)***. The specific configuration of cells in the skin effectively inhibits significant molecular exchanges between the surface and deeper layers of the skin. Water can only permeate the epidermal barrier at a rate of 0.4 mg/cm2/h. The study revealed that the molecules with molecular weights ranging from 18 Da (water) to 750 Da (drugs) exhibited transcutaneous permeation rates ranging from 0.1 g/h/cm2 to a maximum of 1 µg/h/cm2. Furthermore, the transfersomes demonstrated the ability to transport these drug molecules through the skin by more than 50% compared to the unentrapped drug ***(Cevc, 1996)***.

Furthermore, studies have shown that transfersomes can transfer lipophilic fluorescent markers through the skin of mice at a rate above 50%, which is higher than that achieved by liposomes. The inclusion of both lipophilic and hydrophilic components in the vesicular structure leads to a broad spectrum of solubility for transfersomes ***(Pawar, 2016)***. Research has determined that vesicles measuring 600 nm or larger are incapable of penetrating the deeper layers of the skin, whereas those measuring 300 nm or less are able to go deeper into the skin ***(Vinod et al., 2012)***. Nevertheless, vesicles measuring 70 nm or less have demonstrated the highest concentration of their contents in both the viable epidermal and dermal layers of the skin. In addition, a study by ***(Jiang et al., 2018)***, found that transfersomes with a size of 120 nm demonstrated statistically significant improvements in skin penetration compared to bigger transfersomes.

Protransfersome

Protransfersome, a type of vesicular nanocarrier known for its exceptional skin penetration and excellent stability, is extensively utilized in transdermal drug delivery ***(Rahmi and Pangesti, 2018)***. The flattened liquid crystal structure of the

substance is transformed into an ultraflexible vesicle called transfersome when it absorbs water from the skin during in situ hydration ***(Gupta and Trivedi, 2012) (Sayali et al., 2015)***. Transfersome is an ultra-deformable vesicle that exhibits exceptional flexibility and deformability, enabling it to traverse three paths for penetrating the skin ***(Chaurasiya et al., 2019)***. Protransfersome technology enables quick penetration through the stratum corneum and entry into the deeper layers of the skin by utilizing the intercellular lipid pathway of the stratum corneum. The substance can merge with the cell membrane, allowing it to enter the transcellular pathway. It may also permeate through the intact hair follicle pathway to reach the deeper layers of the skin ***(Sala et al., 2018)***. Protransfersome consists of amphipathic lipid components, such as phosphatidylcholine, which create a double-layer membrane of vesicles. Additionally, it contains a surfactant that acts as an edge activator, enhancing the flexibility and deformability of the vesicles ***(Chen et al., 2019)***. Protransfersome typically has a higher quantity of phospholipids compared to transfersomes. The protransfersome does not undergo extrusion throughout the production process to generate unilamellar vesicles, as seen in the transfersome. The protransfersome is a carrier system that undergoes conversion into transfersome upon interaction with water in situ ***(Gupta et al., 2014)***. Thus, when observed under a light microscope, the protransfersome exhibits a palisade crystalline liquid structure, while transfersomes appear vesicular in liquid media ***(Miatmoko et al., 2015)***.

Protransfersome, a gel-based product, offers a potential solution to the issues commonly seen with aqueous dispersions of transfersome, such as problems with physical stability (aggregation, fusing, leaking). By utilizing a gel form, Protransfersome aims to eliminate these problems. The enhanced convenience of transportation, distribution, storage, and dosing renders 'Protransfersome gel' a highly promising industrial product. Apply a layer of Protransfersome gel onto a multilamellar transfersome suspension for hydration. in gel form may avoid many of the problems associated with aqueous dispersions of transfersome and minimize problems of physical stability (aggregation, fusion, leaking).

Advantages of Transfersomes and Protransferosomes

- Transfersomes and protransferosomes carriers are composed of hydrophilic and hydrophobic moieties, which result in becoming a unique drug carrier system that can deliver therapeutic agents with wide range of solubility. Transfersomes and protransferosomes can squeeze themselves through constrictions of the skin barrier that are very narrow, such as 5 to 10 times less than the vesicle diameter, owing to their ultra-deformability and elastic properties.

- High vesicle deformability facilitates the transport of drugs across the skin without any measurable loss in intact vesicles and can be used for both topical, as well as systemic, treatments. They are made up of natural phospholipids and EAs.

- Transfersomes and protransferosomes can be used for the delivery of various active compounds, including proteins and peptides, insulin, corticosteroids, interferons, anesthetics, NSAIDs, anticancer drugs and herbal drugs. Transfersomes and protransferosomes are an obvious choice for achieving a sustained drug release, as well as a predictable and extended duration of activity. They can increase the transdermal flux and improving the site specificity of bioactive agents. Avoiding the first-pass metabolism, which is a major drawback in oral drug administration, and results in optimized bioavailability of the drug.

- Minimize the undesirable side effects of the drug, as well as protect the drug from metabolic degradation; moreover, the utility of short half-life drugs. In most cases, a relatively high entrapment efficiency (EE) of nearly 90% of the lipophilic drug can be achieved by protransfersomes.

Mechanism of Action of protransferosomes

Vesicles are colloidal particles with a flattened liquid crystal structure. When water is absorbed from the skin during in situ hydration, they transform into ultra flexible vesicles called transfersomes. Vesicular drug delivery methods are

highly beneficial for transporting hydrophilic medications enclosed in the inner aqueous compartment, while hydrophobic pharmaceuticals are contained within the lipid bilayer. Transfersomes are advanced drug carrier vesicles that are highly deformable and self-optimizing. Their ability to pass through the skin is primarily attributed to the transfersomes' membrane flexibility, hydrophilicity, and the preservation of the vesicle's integrity, **Figure (4)** ***(Fernández-García et al., 2020)*.**

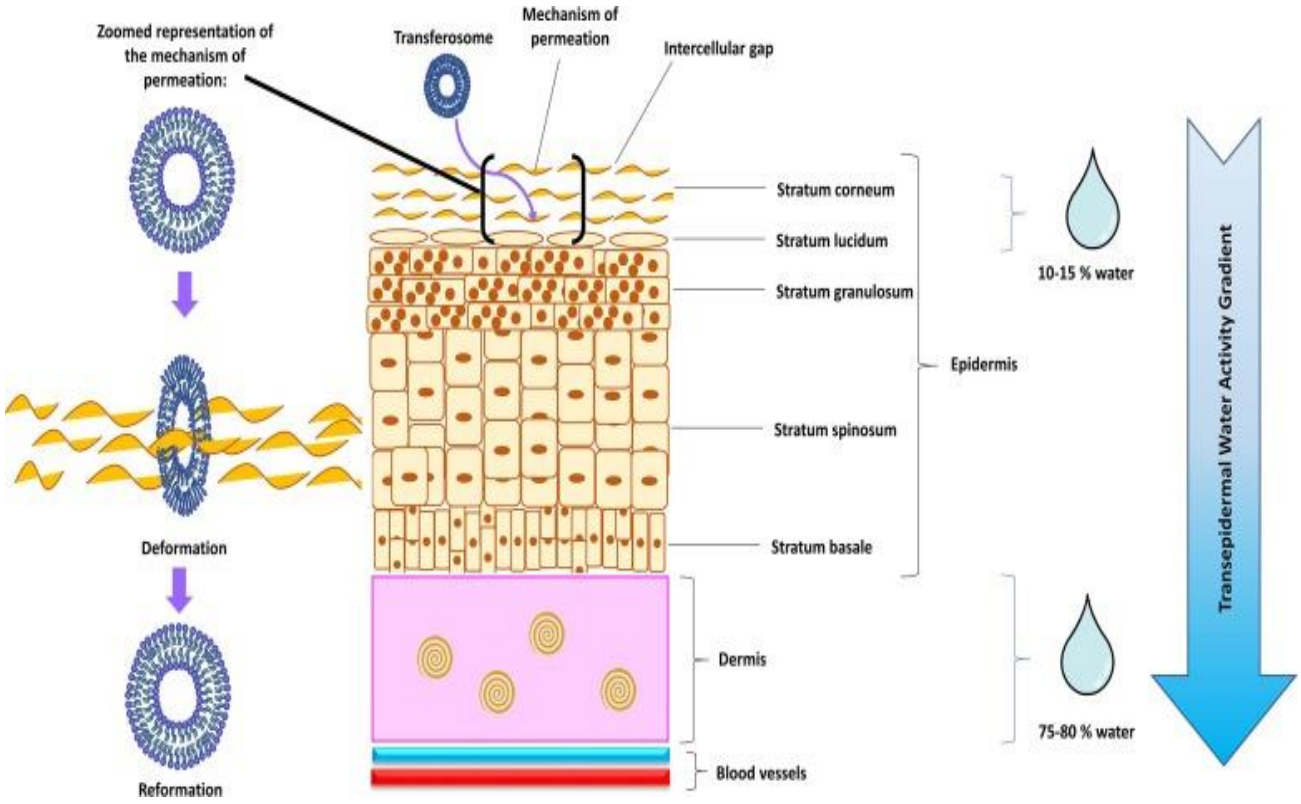

Figure (4): Schematic representation of the mechanism of penetration of transferosomes across the skin

Composition of protransfersomes

Protransfersomes are generally composed by four key elements:

- *Phospholipids:* is the main ingredient (e.g., soy phosphatidylcholine, egg phosphatidylcholine, etc.) that can be a mixture of lipids, which are the vesicle-forming components that create the lipid bilayer ***(Jiang et al., 2018)***.
- Edge activators such as a surfactant or bile salt ranging from 10–25%; the most commonly used edge activators in transfersome preparations are surfactants as sodium cholates; sodium deoxycholate; Tweens and Spans (Tween 20, Tween 60, Tween 80, Span 60, Span 65 and Span 80) and dipotassium glycyrrhizinate, which are

- biocompatible bilayer-softening compounds that increase the vesicles' bilayer flexibility and improve the permeability ***(Jiang et al., 2018; (Kotla et al., 2017)***

- Alcohol (ethanol or methanol, ~ 3–10%) as the solvent and, finally, hydrating medium consist with either water or a saline phosphate buffer (pH 6.5–7) ***(Garg et al., 2017)***.

Preparation Methods

While there exist multiple patented methods for transfersome preparation, there is currently no universally accepted protocol or exact formula for this process ***(Cevc et al., 1998)***. The traditional approach for transfersome preparation involves the utilization of the thin film hydration process, which is sometimes referred to as the rotary evaporation-sonication method. Additional altered techniques for preparation include vertexing-sonication, the modified handshaking procedure, suspension homogenization, centrifugation, reverse-phase evaporation, high-pressure homogenization, and ethanol injection ***(Rathbone et al., 2002)***. The traditional approach for protransfersome preparation involves the utilization of the thin film hydration process, which is sometimes referred to as the rotary evaporation-sonication method. Additional altered techniques for preparation include vertexing-sonication, the modified handshaking procedure, suspension homogenization, centrifugation, reverse-phase evaporation, high-pressure homogenization, and ethanol injection.

Thin Film Hydration Technique / Rotary Evaporation-Sonication Method

The phospholipids and edge activator, which are substances that form vesicles, are dissolved in a round-bottom flask using a volatile organic solvent mixture, such as chloroform and methanol, in an appropriate volume-to-volume ratio. Hydrophobic medication can be included in this stage. To create a thin layer, the organic solvent is evaporated at a temperature above the lipid transition point while applying reduced pressure with a rotating vacuum evaporator. Maintain a vacuum environment to eliminate any remaining amounts of the solvent. The thin film that has been deposited is subsequently hydrated by immersing it in a buffer solution of the desired pH (e.g., pH 7.4) and rotating it for a specific duration at the corresponding temperature. The process of incorporating hydrophilic medication can be carried out during this stage. The resultant vesicles exhibit swelling at ambient temperature and are subjected to sonication in either a bath or probe sonicator to achieve diminutive vesicles. The sonicated vesicles are made uniform by forcing them through a stack of polycarbonate membranes with pore sizes ranging from 200 nm to 100 nm ***(Modi and Bharadia, 2012)***.

Modified Handshaking Process

The modified handshaking method shares the fundamental idea of the rotary evaporation-sonication method. The modified handshaking technique involves the addition of the organic solvent, lipophilic drug, phospholipids, and edge activator into a round-bottom flask. It is necessary for all the excipients to fully dissolve in the solvent and form a solution that is clear and transparent. Subsequently, the organic solvent is eliminated through evaporation by manually shaking the mixture, as opposed to employing a rotary vacuum evaporator. Meanwhile, the round-bottom flask is partially submerged in a water bath that is kept at a high temperature, such as 40-60 degrees Celsius. A slender lipid layer is subsequently created along the inner surface of the flask. The flask is left overnight to ensure the solvent evaporates completely. The film is subsequently hydrated by gently shaking it with the suitable buffer solution at a temperature higher than its phase transition temperature. The process of incorporating the hydrophilic medication can be carried out during this stage ***(Dhopavkar and Kadu, 2017)***.

Ethanol Injection Method

The organic phase is formed by dissolving the phospholipid, edge activator, and lipophilic drug in ethanol using magnetic stirring for a specific duration, until a transparent solution is achieved. The aqueous phase is formed through the dissolution of water-soluble compounds in the phosphate buffer. The process of incorporating hydrophilic medication can be carried out during this stage. Both solutions are heated to a temperature range of 45–50 º C. Subsequently, the solution containing phospholipids dissolved in ethanol is added slowly in small drops to the water-based solution while stirring continuously for the

specified duration. The process of removing ethanol involves passing the resulting dispersion into a vacuum evaporator and then subjecting it to sonication to reduce particle size ***(Balata et al., 2020)***.

Reverse-Phase Evaporation Method

The phospholipids and edge activator are introduced into a round-bottom flask and dissolved in a mixture of organic solvents, such as diethyl ether and chloroform. Hydrophobic medication can be included in this stage. Next, the solvent is removed through evaporation using a rotary evaporator to obtain the lipid films. The lipid films are dissolved again in the organic phase, which is primarily made of isopropyl ether and/or diethyl ether. Afterwards, the water-based phase is introduced into the organic phase, resulting in a system with two distinct phases. The process of incorporating hydrophilic medication can be performed at this point. Subsequently, the system undergoes sonication using a bath sonicator until a uniform water in oil (w/o) emulsion is achieved. The organic solvent is evaporated gradually using a rotary evaporator to produce a thick gel, which then transforms into a vesicular suspension ***(Chen et al., 2014)***.

High-Pressure Homogenization Technique

The phospholipids, edge activator, and the medication are evenly distributed in a solution of PBS or distilled water with alcohol, and then subjected to ultrasonic agitation and simultaneous stirring. Subsequently, the mixture undergoes periodic ultrasonic agitation. Subsequently, the mixture is homogenized by means of a high-pressure homogenizer. Ultimately, the transfersomes are kept under suitable circumstances ***(Wu et al., 2019)***.

Suspension Homogenization Method

Transfersomes are created by combining an ethanol-based solution of phospholipids with a suitable quantity of an edge activator. The suspension that has been created is then combined with buffer to produce a final concentration of lipids. The final formulation is subjected to sonication, followed by freezing and thawing cycles, repeated two to three times ***(Ghai et al., 2012)***.

Vertexing-Sonication Method

The phospholipids, edge activator, and the medication are combined in a phosphate buffer solution. The mixture is agitated vigorously until a milky transfersomal suspension is produced. Subsequently, the sample is subjected to sonication using a bath sonicator for a specific duration at room temperature, and then passed through polycarbonate membranes with pore sizes of 450 and 220 nm, respectively ***(Yusuf et al., 2014)***

Physicochemical characterization of the Transfersomes and protransfersomes

The physicochemical characterization of PTFs is essential to confirming quality control and stability. Both physical and chemical properties can be determined for PTFs. Microscopic and macroscopic techniques are used in the development of colloidal systems. Various techniques like particle size analysis, zeta potential, transmission electron microscopy, differential scanning calorimetry (DSC), X-ray scattering, and laser diffraction (LD), were performed to investigate the structure, mobility, and molecular environment of the compounds. These techniques also reveal the physical and chemical stability of the formulation; surface charge tends to determine whether the particles will flocculate or not ***(Khosa et al., 2018)***.

Measurement of particle size

The particle size is an important parameter in process control and quality assurance because the physical stability of vesicle dispersion depends on particle size, and as particle size decreases, surface area characteristics increase as a function of total volume. Photon correlation spectroscopy (PCS) based on laser light diffraction provides an appropriate method for investigation and can be applied to particles ranging from 200 nm to 1 μm. For particles below 200 nm, Rayleigh's theory holds that the scattering intensity is proportional to the sixth potency of the particle diameter. Both Fraunhofer's and Rayleigh's theories are only approximations of Mie's theory, which claims that the scattering intensity depends on the scattering angle, the absorption, and the size of the particles, as well as the refractive indices of both the particles and the dispersion medium ***(Nasr-Eldin et al., 2024; Poonia et al., 2016)***.

Measurement of zeta potential

Zeta potential is the electric potential of a particle

in a suspension. It is a parameter that is very useful for the assessment of the physical stability of colloidal dispersions. In suspensions, the surfaces of particles develop a charge due to the ionization of surface groups or the adsorption of ions. This charge depends on both the surface chemistry of the particles and the media around them. The surface charge generates a potential around the particle, which is at its highest near the surface and decays with distance into the medium. The zeta potential can be measured by determining the velocity of the particles in an electrical field (electrophoresis measurement) ***(Nasr-Eldin et al., 2024; Shah et al., 2014)***.

Encapsulation efficiency (EE) and drug loading (DL)

The encapsulation efficiency (EE) is defined as the ratio of the encapsulated drug in nanoparticles to the whole drug first incorporated into the PTFs multiplied by 100 ***(Kumbhar and Pokharkar, 2013)***. But it should be kept in mind that the damaged portion of the bioactive compound during the production and storage of PTFs must be subtracted. The EE can be calculated after quantifying the free portion or encapsulated portion of the drug. The unencapsulated free drug may crystallize and/or dissolve in the aqueous phase. The crystallized portion usually has a micron size, precipitates in the PTFs system, and can conveniently be removed by microfiltration or mild centrifugation and then quantified ***(Nguyen et al., 2012)***. The continuous phase can be separated from the nanoparticles by techniques such as ultrafiltration or ultra- centrifugation and the amount of dissolved or encapsulated drug can be quantified ***(Huang et al., 2013)***. Moreover, the percentage of dissolved drug in the aqueous phase may be determined by extraction with organic solvents. Several researchers reported the EE as the percentage of non-crystallized drugs within the PTFs system rather than as the percentage of drugs entrapped within phospholipid nanoparticles ***(Nguyen et al., 2012)***.

The EE influences the release characteristics of PTFs and depends on the PTFs ingredients which influence factors such as the solubility of the drug in the lipid phase, nanoparticle crystallinity index, viscosity of the continuous phase, and thereby the diffusion coefficient of the drug, production method, and conditions used. The solid state of lipidic nanoparticles in PTFs immobilizes the incorporated drug and leads to higher EE in comparison to nanoemulsion. If the drug, which should be encapsulated, has poor water solubility and is sensitive to degradation under physicochemical stresses during the processing and storage of food and GI stresses (e.g., carotenoids), having a high EE is appropriate. But if the drug is more stable to physicochemical stresses and only has low water solubility and, thereupon, low bioavailability, having a low EE is appropriate. In the latter case, the nanoemulsion is preferred over PTFs, provided that the drug doesn't crystallize in the aqueous phase and the nanoemulsion has acceptable physicochemical stability. The drug loading (DL) of PTFs is defined as the ratio of the encapsulated drug to the amount of lipid phase or lipid nanoparticles in the PTFs formulation multiplied by 100 ***(Das et al., 2012)***. DL is predominantly influenced by the polymorphic state of the lipid, the solubility of the drug in the melted form of lipid, the types of lipids, and the chemical and physical structure of the solid lipid matrix.

In-Vitro release of active compounds from PTFs

The in-vitro release profile of an active compound from PTFs depends on the production temperature, the amount of surfactant used in the formulation, and the solubility of the active compound in the surfactant. The high temperatures used during the hot homogenization process often led to a biphasic release profile from PTFs, with an initial burst release followed by a prolonged release. The reduction in production temperature reduces the extent of the burst release, with almost no burst release reported for prednisolone nanoparticles manufactured using the cold homogenization technique ***(Muller and Runge, 1998; Nasr-Eldin et al., 2024)***. During the heating step of the hot homogenization process, the solubility of the active compound in the aqueous phase increases, leading to the partitioning of the active compound from the melted lipid droplet to the water phase. During the subsequent cooling step, the lipid matrix starts crystallizing with a relatively high amount of active material still concentrated in the aqueous phase. Further cooling leads to supersaturation of the active compound in the aqueous phase, which then tries to partition back into the lipid phase. This leads to a high concentration of active compounds in the outer shell, resulting in a high amount of burst release. The burst release is directly

proportional to the solubility of the active in the aqueous phase during PTFs production, the extent of burst release can also be controlled by the amount of surfactant used in the formulation. When the active compound is solubilized by the surfactant, higher concentrations of surfactants in the formulation lead to more burst release.

Scanning electron microscopy (SEM)

This technique can be used to investigate the shape of the particles prepared and to assess the particle size of these particles. Aqueous PTFs dispersions can be applied and spread on a sample holder (thin carbon film). The samples will be placed inside the vacuum column of the microscope, and the air will be pumped out of the chamber. An electron gun placed at the top of the column emits a beam of high-energy primary electrons. The beam of electrons passes through the lenses, which concentrate the electrons in a fine spot and scan across the specimen row by row. As the focused electron beam hits a spot on the sample, secondary electrons are emitted by the specimen through ionization. A detector counts these secondary electrons. The electrons are collected by a laterally placed collector, and these signals are sent to an amplifier ***(Beloqui et al., 2016)***.

Transmission electron microscopy

TEM provides a way to directly observe nanoparticles and the physical characterization of nanoparticles, with the former method being better for morphological examination. TEM has a smaller size limit of detection, is a good validation for other methods, and affords structural requirements. It is important to be aware of the limited number of observations and the impact of a vacuum on the particles ***(Kanwar et al., 2019; Nasr-Eldin et al., 2024)***.

Atomic force microscopy (AFM)

AFM is optimal for measuring morphological and surface features that are extremely small. In this technique, a probe tip with atomic-scale sharpness is rostered across a sample to produce a topological map based on the forces at play between the tip and the surface. The probe can be dragged across the sample (contact mode) or allowed to hover just above (noncontact mode), with the exact nature of the particular force employed serving to distinguish among the sub-techniques ***(Müller et al., 2000)***.

Differential scanning calorimetry (DSC)

DSC is usually used to get information about both the physical and energetic properties of a compound or formulation. DSC measures heat loss or gain because of physical or chemical changes within a sample as a function of temperature. DSC and powder are performed for the determination of the degree of crystallinity of the particle dispersion ***(Khosa et al., 2018; Nasr-Eldin et al., 2024)***. The rate of crystallinity using DSC is estimated by comparing the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion.

X-ray diffraction (powder X-ray diffraction)

The geometric scattering of radiation from crystal planes within a solid allows the presence or absence of the former to be determined, thus permitting the degree of crystallinity to be assessed ***(Mehnert and Mäder, 2012)***.Various established techniques are employed to assess the properties of Transfersomes and protransfersomes, including their vesicle shape and size, size distribution, polydispersity index, zeta potential, number of vesicles per cubic mm, entrapment efficiency, degree of deformability, and skin permeability measurements ***(Ascenso et al., 2015; Modi and Bharadia, 2012)***, These methods are advantageous for optimizing the formulation of protransfersomes.

Difference between transferosome and protransferosome

Nevertheless, the issue with these very flexible vesicles lies in their inherent self-stability. Thus, to improve the stability of these vesicles, the utilization of liquid crystalline, highly flexible lipid vesicles (protransfersomes) has been suggested. Protransfersomes can undergo in-situ conversion into highly flexible lipid vesicles, known as transfersomes, when they absorb water from the skin ***(Gamsjaeger et al., 2021; Gupta and Trivedi, 2012)***. In addition to their ability to introduce active molecules through a regulated, transdermal delivery method, protransfersomes are recognized for their superior entrapment efficiency and their suitability for incorporating both lipophilic and hydrophilic medicines with improved stability. Due to the characteristics of protransfersomes, which

include the capacity to accommodate and improve the skin penetration of lipophilic substances, these molecules can serve as a good vehicle for delivering medications that have poor penetration abilities ***(Madheswaran et al., 2017)***.

CONCLUSION

Protransfersomes are specially optimized particles or vesicles, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can thus be used to bring drugs across the biological permeability barriers, such as skin. When tested in artificial systems. Transfersomes can pass through even tiny pores (100 mm) nearly as efficiently as water, which is 1500 times smaller. Drug laden transfersomes can carry unprecedented amount of drug per unit time across the skin (up to 100mg cm2h-1). Transdermal drug delivery system is frequently used due to its several advantages over other routes drug delivery but the penetration of drug via the stratum corneum is a rate limiting step, its major limitations like, it cannot be able to transport the larger size molecule. That is why vesicular system like Transfersomes are developed to overcome these limitations. The elastic vesicles deform themselves to penetrate the skin through pores. It is more efficient & safer in composition then others. In this type of delivery, Drug release can also be controlled according to the requirement. Thus, this approach can overcome the problems which occur in conventional techniques.

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