

LIPOSOMES AND ADVANCED VESICULAR SYSTEMS FOR TRANSDERMAL DELIVERY: OVERCOMING THE SKIN BARRIER

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Abstract

Transdermal drug delivery offers a non-invasive alternative to conventional routes of administration; however, its widespread application is limited by the stratum corneum's highly effective barrier function. Recently, liposomes and advanced vesicular systems have emerged as promising carriers to enhance drug permeation across the skin. This review critically evaluates liposomal systems and next-generation vesicular carriers, including niosomes, transfersomes, and ethosomes, with a particular focus on their roles in transdermal drug delivery. The structure, classification, advantages, and limitations of liposomes are discussed alongside approaches to formulation and characterization. The enhanced performance of deformable, ethanol-containing vesicular systems is evaluated relative to conventional liposomes. Overall, liposomal and advanced vesicular systems demonstrate significant potential in improving transdermal drug delivery; however, further studies focusing on long-term safety and scalable manufacturing are necessary for their successful clinical application.

Rezumat

Administrarea transdermică a medicamentelor reprezintă o alternativă non-invazivă la căile convenționale de administrare, însă utilizarea este limitată de funcția de barieră a stratum corneum. În ultimii ani, lipozomii și sistemele veziculare avansate au devenit soluții promițătoare pentru îmbunătățirea permeabilității cutanate a substanțelor active. Aceast studiu analizează sistemele lipozomale și transportorii veziculari de nouă generație, precum niozomii, transferozomii și etozomii, evidențiind rolul acestora în administrarea transdermică. Sunt prezentate structura, clasificarea, avantajele și limitările lipozomilor, precum și metodele de formulare și caracterizare a acestora. Totodată, este evaluată eficiența sistemelor veziculare deformabile și a celor care conțin etanol, comparativ cu lipozomii convenționali. Sistemele lipozomale și veziculare avansate prezintă un potențial important pentru optimizarea administrării transdermice a medicamentelor, însă pentru utilizarea clinică sunt necesare studii suplimentare privind siguranța pe termen lung și posibilitatea extinderii proceselor de fabricație la scară industrială.

Keywords: advanced vesicular system, liposome, skin barrier, *stratum corneum*, transdermal delivery

Introduction

Transdermal delivery systems are becoming increasingly popular, given their effective methods for delivering active ingredients across the skin barrier (1). Avoiding the first-pass effect, controlling the rate of drug release, improving patient compliance, and having the ability to stop treatment immediately are among the various advantages of transdermal delivery (2). However, because of its selective permeability, the stratum corneum-the skin's most substantial protective layer - functions as a semipermeable membrane, providing an effective barrier to most medications (3). Various approaches, such as iontophoresis, sonophoresis, electroporation, microneedles, ablation techniques, and penetration enhancers, have been developed to enhance the transdermal absorption of drugs because the stratum corneum is the primary barrier to transdermal delivery, which compromises therapeutic plasma levels of drugs (4, 5). Drug delivery systems based on nanotechnology have become effective means to overcome these restrictions,

providing improved drug penetration, prolonged release, and increased solubility of hydrophobic substances (6).

The generation of liposomal formulations is a potential strategy to improve transdermal delivery (7). Liposomes demonstrate great bioavailability and safety as nanocarriers. They are effective systems for transdermal delivery due to their high drug-loading capacity (8).

The goal of this review is to provide readers with information and highlight the advantages of transdermal drug delivery using liposomes and advanced vesicular systems, such as niosomes, transfersomes, and ethosomes.

Liposomes

Alec D. Bangham first described liposomes in the 1960s following the observation that dry phospholipid films spontaneously self-assembled into closed bilayer vesicles upon hydration. The resulting vesicles were subsequently visualized by negative-staining electron

microscopy (9, 10). Further studies indicated that liposomes could contain substances with different properties in their internal and membrane regions (11). Liposomes are spherical vesicles enclosed by one or more phospholipid bilayers that surround an aqueous core. Phospholipids spontaneously self-assemble into bilayer structures in aqueous media due to their amphiphilic nature, with hydrophilic head groups and hydrophobic hydrocarbon tails. This unique structural organization enables liposomes to encapsulate both hydrophilic and lipophilic compounds (12, 13).

Composition of Liposomes

Liposomes are lipid-based vesicular systems formed by one or more lipid bilayers surrounding an aqueous core. Phospholipids constitute the main structural components, while cholesterol and other lipids may also be included (14). Phospholipids have both hydrophilic and hydrophobic parts. The polar head contains a phosphate group, while the fatty acid chains are hydrophobic. In aqueous environments, the polar heads interact with water, whereas the fatty acid chains orient toward each other. Glycerophospholipids are the most commonly used group, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol (15). Phospholipids used in liposomes are either natural or synthetic. Natural phospholipids, such as those derived from egg yolk or soy lecithin, resemble those found in biological membranes. Synthetic phospholipids are produced under controlled conditions and allow adjustment of parameters such as chain length and saturation (14, 16).

Neutral phospholipids tend to form more stable membranes, while charged ones introduce electrostatic interactions that alter lipid arrangement (16, 17). For fatty acid chains, saturated chains allow tighter, more ordered packing, whereas unsaturated chains, due to their double bonds, disrupt this arrangement and create a more fluid structure (18).

Cholesterol is commonly incorporated into liposomal membranes. Its effect varies with membrane composition but is more pronounced in systems containing saturated phospholipids (14, 19). In addition to these components, charged lipids may be included to modify surface properties, especially electrostatic interactions. Membrane structure is influenced by head group characteristics, fatty acid composition, and cholesterol content (11, 13).

Classification of Liposomes

Liposomes can be categorized by parameters such as size, bilayer number, structural modifications, and production methods. Liposomes are considered unilamellar and multilamellar structures. Unilamellar liposomes consist of an aqueous inner core

surrounded by a single phospholipid bilayer. There is a single aqueous compartment within the liposome. Unilamellar liposomes are also classified as small or large based on their vesicle diameter (15). Multilamellar liposomes are structures formed by the interlocking of multiple phospholipid bilayers. In these structures, the phospholipid bilayers exhibit a layered organization from the center outwards. Aqueous phases are present between each layer; multiple aqueous compartments are formed within the liposome (18).

Liposomes are classified into different size groups based on their vesicle diameters. This classification is based on the physical dimensions of liposomes in nanometers and micrometers. Small liposomes are defined as vesicles within a narrower diameter range. Liposomes in this group are generally on the nanometer scale and exhibit a more compact structure. Small liposomes have a higher surface area-to-volume ratio. Larger liposomes, on the other hand, are defined as vesicles that can reach hundreds of nanometers to micrometers in diameter. These liposomes have a larger internal volume and are physically larger structures (14).

Another classification is based on the structures found on the liposome surface. Unmodified liposomes have a classic membrane structure consisting of phospholipids and additional lipids. There is no polymer or other coating on the outer part of the membrane. Modified liposomes, on the other hand, are systems that contain additional components on the outer surface of the phospholipid membrane. An example of this group is stealth liposomes. Stealth liposomes are liposomal structures that carry hydrophilic polymer chains on their outer surface (15, 19). Polyethylene glycol (PEG) is one of the most commonly employed polymers for this purpose, and its surface attachment, known as PEGylation, reduces macrophage uptake and prolongs circulation time (20, 21, 22).

In addition to classifications based on lamellar structure, vesicle size, surface modification, and lipid composition, liposomes can be grouped according to the mechanical behavior of their membrane (14). Deformable or elastic liposomes are systems designed with a more flexible membrane structure, achieved by adding surfactants to the phospholipid bilayer (17).

Advantages and Disadvantages of Liposomes

One of the advantages of liposomes is their ability to carry active substances with different solubility properties within the same system. Hydrophilic and lipophilic compounds can be present within a single carrier platform (23). Active substances can be protected within the carrier system. Compounds sensitive to environmental factors such as light and

oxygen can be included in such systems. Liposomes are among the systems that exhibit controlled-release properties, are biocompatible, and can be used in various application routes (24). Another advantage of liposomes is that they allow for a homogeneous distribution of the active substance within the system. Liposomes are structures whose surface properties can be modified. This modifiability in surface properties expands the application area of the carrier system. They are evaluated in various fields besides pharmaceutical applications (15).

However, liposomal systems also have some disadvantages. The physical stability of liposomes may not be maintained under all conditions. Vesicle aggregation, leakage, or structural degradation may occur during storage. Phospholipid-based structures are sensitive to certain reactions in terms of chemical stability. Processes such as oxidation and hydrolysis are among the problems encountered in liposomal systems. The production processes of liposomal systems can be technically complex. The fact that the preparation stages depend on numerous parameters can make controlling the process difficult. Especially in the scale-up phase, it may not always be possible to maintain the results obtained under laboratory conditions (13, 24).

Preparation Methods of Liposomes

Thin-film hydration method

Thin-film hydration is one of the longest-used and most frequently described methods in the literature for liposome preparation. The method is based on dissolving phospholipids in a suitable organic solvent. After the solvent is removed, the lipids are exposed to an aqueous environment. Volatile organic solvents, such as chloroform, chloroform:methanol mixtures, and ethanol, are generally preferred for this purpose (25). The lipid solution is prepared in a glass flask or similar container, and the solvent is evaporated. As a result of solvent removal, a homogeneous lipid film layer forms on the inner surface of the container. This formed dry lipid film is then hydrated with an aqueous phase (25). During hydration, the lipid layer swells, separates from the glass surface, and spontaneously forms vesicular structures. As hydration continues, multilayered liposome structures begin to form. The vesicles obtained at this stage usually show a multilamellar structure and vary in size. Prepared liposomes are sometimes subjected to additional processing such as sonication, membrane extrusion, and high-pressure homogenization (17, 26). As a result of these processes, single-layered liposomes or liposomes with a narrower size distribution can be obtained (27).

Reverse-phase evaporation method

In reverse-phase evaporation, phospholipids are typically dissolved in organic solvents such as chloroform or a chloroform-methanol mixture. After dissolving the lipids in the organic phase, the aqueous phase is added to the system. This results in a water-in-oil emulsion. At this point, the system is typically mechanically mixed or sonicated to improve stability. The resulting emulsion structure exhibits inverted micelle-like organization of lipid molecules. After preparation of the emulsion, the organic solvent is removed using a rotary evaporator or similar systems (28). During solvent removal, the system acquires a gel-like structure. Subsequent precipitation of this structure forms vesicular structures. This process ultimately yields liposome suspensions. If necessary, some post-hydration processes are applied. The system can be adjusted by mixing, filtration, or mechanical processes (13, 25, 26). *Microfluidics and other modern preparation techniques*

In microfluidic methods, the basic principle is based on the controlled combination of lipids and the aqueous phase within micrometer-scale channels. Microfluidic devices typically consist of micro-channels made of glass, silicon, or polymer. A lipid solution and an aqueous phase are mixed in these channels in specific ratios. In the microscale environment where mixing occurs, lipid molecules spontaneously organize into vesicular structures. Liposomes obtained by microfluidic preparation are usually observed to have a narrower size range (25, 28). In addition to microfluidic techniques, other techniques exist for liposome preparation. These include ethanol injection, detergent removal, and supercritical fluid-assisted techniques. In the ethanol injection method, lipids are dissolved in ethanol (13). This solution is then injected into the aqueous phase in a controlled manner. During injection, the lipids disperse in the aqueous environment, forming vesicular structures. In detergent removal methods, micelle structures are formed by combining lipids and detergents. Then, liposomes are formed by removing the detergent from the environment. Detergent removal is usually achieved through processes such as dialysis, adsorbent materials, or dilution (25, 28).

In supercritical fluid-assisted techniques, lipids are dissolved in fluids under supercritical conditions. Then, liposome formation is achieved by applying controlled pressure drops. These methods are reported in a smaller number of studies in the literature and are evaluated within the context of modern preparation techniques (29, 30).

Excipients Used in the Liposomes

Phospholipids are the basic structural unit in the preparation of liposomes (31). Phospholipid types such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol are commonly used in liposome formulations (32). Cholesterol is one of the most commonly used excipients in liposome formulations. Surfactants and charge-modifying components are among the excipients that affect the surface properties of liposomes. Cationic, anionic, or neutral components can be included in the formulation. Such substances can be located on the outer surface of the lipid bilayer or between lipids. Stearylamin, phosphatidic acid, and similar substances can be used in the liposome structure for this purpose (31). Organic solvents are used during liposome preparation. Solvents such as chloroform, methanol, ethanol, and diethyl ether are used to dissolve lipids and obtain a homogeneous lipid mixture. They are removed from the environment in the stages following the preparation process. Buffer systems and aqueous-phase components also play important roles in liposome formulations. The aqueous phase usually consists of pure water, phosphate buffers, or buffer solutions prepared within the physiological pH range. The pH and ionic strength of the aqueous phase can affect the properties of vesicles formed during lipid hydration (13). Components such as polymers, preservatives, or viscosity modifiers may be present in formulations developed for specific applications (32).

Characterization of Liposomes

To evaluate the formulation of liposomes, certain physicochemical properties must be determined. Particle size, surface charge, encapsulation efficiency, release properties, and stability are parameters that need to be characterized (33).

Particle size and polydispersity index (PDI)

Particle size and distribution are key parameters for the characterization of liposomes. The homogeneity of the size distribution is evaluated using the PDI. (28, 33). Dynamic light scattering (DLS) is a widely used technique for determining particle size. However, its accuracy may be limited for highly polydisperse samples or larger vesicles. In such cases, complementary techniques, including nanoparticle tracking analysis (NTA), laser diffraction, or microscopy, may provide more reliable particle size characterization (34, 35).

Zeta potential

Zeta potential provides information about the surface charge of a particle, whether it is cationic, anionic, or neutral. In this measurement, the electrophoretic mobility of the particles is determined by applying an electric field to the

environment and observing their movement, and the zeta potential value is calculated. Measurements are generally performed using light scattering-based techniques (28, 36). Low absolute zeta potential values (approaching zero) promote particle aggregation. In contrast, high absolute zeta potential values (commonly > 30 mV) provide greater electrostatic repulsion, decreasing the tendency to agglomerate and thereby improving colloidal stability and facilitating redispersion (37).

Encapsulation efficiency

Encapsulation efficiency indicates how much of the active substance is retained within the liposomal structure and is used to evaluate the formulation's delivery capacity. To determine this parameter, the active substance in its free form must first be separated from the liposomal structure. Methods such as centrifugation, ultrafiltration, dialysis, or gel filtration are used for this separation process. After the separation process, the liposome structure is broken down, releasing the retained substance, then quantified using high-performance liquid chromatography (HPLC), UV-Vis spectrophotometry, or fluorescence analysis (28, 33).

Drug release

The release behavior of liposomal systems is used to examine how the active substance is released over time. This can be used to determine whether the formulation provides controlled or extended release. Release studies are performed using dialysis with a semi-permeable membrane. The liposomal system is maintained in a specific medium, and samples are collected at specific time intervals to quantify the amount of active substance released. These analyses are performed using chromatographic or spectrophotometric methods (33, 38).

Stability

Stability assessments include examining both physical and chemical parameters to determine if the liposomal formulation maintains its structure and function over time. Changes in particle size, PDI, and zeta potential are monitored in physical stability analysis. In terms of chemical stability, lipid degradation and leakage of the encapsulated substance are examined. These changes are generally monitored using light scattering techniques and chromatographic analysis. Temperature and storage conditions play an important role in stability (28, 33).

Pharmacokinetics

Pharmacokinetic studies examine how liposomal systems act in the body by examining how substances are distributed, how long they remain in the bloodstream, and how long they are retained in the body. Liposomal carriers can alter the pharmacokinetic profile of the active substance, prolonging circulation time and consequently increasing accumulation in target tissues. These

properties can vary depending on formulation parameters such as particle size, surface charge, and lipid composition (33).

Effect of Liposomes on Skin

Mechanism of penetration

Liposomal systems do not enter the skin through a single pathway. Penetration pathways are divided into three: intercellular, transcellular, and transappendageal. Each pathway is considered in light of factors such as the organization of the stratum corneum, lipid-protein balance, and the distribution of epidermal appendages. In the stratum corneum, the intercellular pathway runs between corneocytes along the lipid lamellae. The movement therefore occurs around the cells rather than directly through them. These lipid regions contain a matrix rich in ceramides, cholesterol, and free fatty acids. After contact with these regions, small or flexible liposomes may undergo structural changes. The bilayer components can mix with surrounding lipids or form smaller aggregates (39, 40).

The transcellular pathway allows molecules to pass directly through corneocytes and reach deeper layers. In liposomal systems, this route is often associated with components that interact strongly with cell membranes. In cell culture and reconstructed epidermis models, liposomes have been observed to bind to the cell surface and, in some cases, be taken up into the cell. After internalization, they may be traced within the cytoplasm and gradually degraded (41).

The transappendageal pathway refers to penetration through hair follicles, sebaceous glands, and sweat glands. These structures represent areas where the compact lipid organization of the stratum corneum is interrupted. Nano-sized carriers in particular can remain near follicular openings and gradually spread to nearby tissues (42).

The follicular canal has a different cellular and lipid organization compared with the surrounding epidermal surface. The canal also contains a sebum-rich environment that can influence the interaction between vesicles and surrounding lipids. In some imaging studies, signals from fluorescently labeled liposomes appear more intense around follicular openings, suggesting that carriers may accumulate in these areas and move deeper along the canal over time (43).

Improving skin hydration and barrier function

The main function of the skin barrier is to protect the tissue from the external environment and maintain epidermal water balance (44). The stratum corneum, the outermost epidermal layer, is made up of keratin-rich corneocytes. The main components of this lipid matrix are ceramides, free fatty acids, and cholesterol (45). The regular lamellar structure of the

intercellular lipid layers is the main factor limiting the uncontrolled loss of water from the epidermis (46). If this balance is disrupted, transepidermal water loss (TEWL) increases. Liposomes, with their phospholipid bilayer structure, can form a thin lipid layer on the skin surface. This semi-occlusive effect created by the vesicular structure on the stratum corneum creates a temporary moisture microenvironment on the epidermal surface (47). Liposomal systems support hydration in several ways. Formulations containing liposomes are often evaluated using parameters such as TEWL and stratum corneum hydration (47). In liposomal systems, phospholipids in the vesicle membrane exhibit a structure similar to the lipid matrix in the stratum corneum. In the stratum corneum, the lipid layers that fill the gaps between corneocytes are organized in a specific layered pattern. When phospholipids interact with stratum corneum lipids, a short-term change in the intercellular lipid matrix can occur (45).

In liposomal systems, the presence of cholesterol significantly affects the arrangement of the phospholipid bilayer. Cholesterol molecules are located between the phospholipid tails, balancing the membrane's fluidity and rigidity (48). In the stratum corneum, cholesterol is one of the main barrier lipids (45). The regular lamellar organization of barrier lipids plays a crucial role in maintaining epidermal water balance. Hydration from liposomal systems depends on vesicle stability and the time they remain on the epidermal surface. When the phospholipid membrane remains intact, the lipid layer formed on the skin surface can persist for a longer period (47). Liposomes that spread well and maintain their structure on the surface can create a more balanced moisture level in the epidermal microenvironment. Parameters such as phospholipid type, cholesterol content, and the ratio of additional lipids are key factors determining vesicle stability (48).

Future Perspectives

Targeted liposomal systems

Targeted liposomal systems aim to retain active substances in specific areas rather than their random distribution in the skin (49). The concept of targeting is explained by modifying certain fundamental properties of liposomes. In particular, adjustments are made to parameters such as surface structure, particle size, and lipid composition (50).

Approaches used in targeted liposomal systems are discussed in two main categories: passive and active targeting (49). Passive targeting relies on the interaction between the structural properties of liposomes and the skin barrier. Passive targeting does not involve a specific binding mechanism. The structural similarity of liposomes' phospholipid

bilayer to the epidermal lipid matrix allows these systems to interact more harmoniously with the skin surface. Components such as phospholipid type and cholesterol ratio are important. Particle size is an important parameter in passive targeting approaches. The surface charge of liposomes also affects their interactions with biological structures on the skin surface. Liposomes with cationic, anionic, or neutral surface properties have been reported (50).

Active targeting approaches involve modifying the liposome surface with specific molecules. Peptides, sugar derivatives, or vitamin-like molecules can be attached to the liposome surface, and interactions of these structures with cell-surface components can be targeted (50). Most of these systems have been evaluated at the experimental level, and data for clinical applications remain limited. Another approach to skin targeting is to subject the liposome surface to various chemical modifications. PEG-coated liposomes are frequently reported among liposomal systems with modified surface properties; in addition, formulations containing cationic or anionic lipids are also found in the literature (49).

However, there are some limitations in the development of targeted liposomal systems. The production process is relatively complex. Costs are higher than those of conventional systems. Standardizing these formulations can also be challenging. In addition, clinical data are still limited (50).

Advanced Vesicular Systems

Transfersomes

Transfersomes are elastic vesicular systems with a high degree of deformation capability. These systems contain a phospholipid bilayer and special surfactants called "edge activators." This structure allows them to stretch under mechanical pressure. This increases their ability to pass through narrow and irregular gaps in the skin barrier. With this feature, transfersomes serve as a carrier system that can pass through the existing stratum corneum rather than directly disrupt the skin barrier (51). Edge activators used in transfersomes increase the

elasticity of the phospholipid bilayer and allow the vesicle structure to deform without breaking down. This feature distinguishes transfersomes from classic liposomes. In the literature, transfersomes are classified as systems that utilize the carrier's physical properties in addition to passive diffusion for transdermal applications (52).

Niosomes

Niosomes are vesicular systems primarily composed of non-ionic surfactants. Cholesterol is frequently incorporated to improve membrane stability but is not an essential component of all formulations. In addition, charge-inducing agents, such as dicetyl phosphate or stearylamine, are often included to modify the surface charge and enhance vesicle stability (53, 54). In terms of chemical stability, niosomes are considered more durable compared to classical liposomal structures. They are particularly preferred for products that require long-term stability (55). The vesicular structure allows the active compound to remain associated with the carrier rather than being released immediately. Non-ionic surfactants are generally more stable than phospholipids. They are less sensitive to oxidative and hydrolytic degradation. Because of this, stability issues during storage tend to be lower (56).

Ethosomes

Ethosomes are elastic vesicular systems containing phospholipids, high levels of ethanol (20-45%), and water (57). The ethanol content increases the fluidity of the lipid bilayer of ethosomes and modifies their interaction with the skin barrier. Ethosomes interact particularly with the stratum corneum lipid structure, allowing the carrier to reach deeper layers within the skin (58). The presence of ethanol in ethosomal systems increases vesicle elasticity and supports transport by inducing transient changes in the skin lipid matrix (52). Transfersomes, niosomes, and ethosomes represent different nanocarrier approaches developed to overcome the limitations of classical liposomes (59). Figure 1 presents schematic representations of liposomes, niosomes, ethosomes, and transfersomes, while Table I summarizes recent studies on these vesicular systems.



Figure 1.

Schematic presentation of liposome, niosome, ethosome, and transfersome (created by Biorender.com)

Table I

Recent advances in vesicular systems for transdermal drug delivery

Drug	System	Key findings	Reference
Huperzine A	Ethosome	Compared with ordinary gel and cream, Huperzine A ethosome gel demonstrated noticeably higher permeation.	(60)
Curcumin	Ethosome	Ethosomes can facilitate curcumin's penetration into the skin.	(61)
Sinapic acid	Ethosome & transfersome	Compared to plain hydrogel and transfersome hydrogel, ethosome hydrogel showed greater permeability.	(62)
Diflunisal	Ethosome & transfersome	Both carriers showed better diflunisal absorption and movement through the skin than the liposomal hydrogel.	(63)
Methotrexate	Transfersome	Because transdermal flux is three times higher with transfersomal gel, it may be a viable alternative to conventional gel.	(64)
Diclofenac	Niosome	Compared with the commercial drug, the niosomal hydrogel enhanced the amount and rate of diclofenac transport across the skin.	(65)

Clinical Translation, Regulatory Status, and Marketed Products

Liposomes

Despite their significant advantages in transdermal delivery, liposomes still encounter several limitations in their clinical translation. Large-scale manufacturing process standardization remains a considerable challenge. Laboratory-scale preparation techniques often exhibit limited reproducibility, with batch-to-batch variations in particle size distribution and encapsulation efficiency that may adversely affect product quality and scalability (66).

Niosomes

Although niosomes have demonstrated promising therapeutic potential in numerous preclinical studies, only a limited number of formulations have been evaluated in clinical studies. Most niosomal systems remain at the preclinical stage, with research primarily confined to laboratory investigations and animal studies. Consequently, the lack of robust human clinical data continues to hinder their regulatory approval and commercial translation, as the safety, efficacy, and long-term tolerability of niosomal drug products have not yet been comprehensively established. Another important challenge is the lack of harmonized regulatory guidelines that specifically address the development and characterization of niosomal drug delivery systems. Consequently, regulatory agencies generally evaluate niosomal formulations using existing frameworks for liposomal products or nanomedicines. However, these guidelines do not fully account for the unique physicochemical characteristics and biological behavior of niosomes. Large-scale manufacturing also remains a significant obstacle. Scaling up laboratory formulations may alter vesicle size, drug loading, encapsulation efficiency, and physicochemical stability, thereby affecting batch-to-batch reproducibility and overall product quality (67).

Ethosomes

Ethosomal systems have been extensively investigated as promising nanocarriers for transdermal drug delivery because of their ability to

enhance skin permeation and improve drug bioavailability. Despite encouraging preclinical outcomes, only a limited number of ethosomal formulations have been evaluated in clinical studies. In contrast, the majority remain confined to *in vitro*, *ex vivo*, and preclinical *in vivo* investigations. Several early pharmacokinetic and clinical studies have reported improved skin permeation and therapeutic performance following ethosomal drug delivery; however, large-scale randomized controlled trials remain scarce. The clinical translation of ethosomal formulations remains limited by several challenges, including formulation instability, difficulties in large-scale manufacturing, batch-to-batch variability, and the lack of dedicated regulatory guidelines. Moreover, differences in vesicle composition, ethanol concentration, and interindividual variations in skin physiology may contribute to inconsistent therapeutic outcomes. Therefore, additional well-designed clinical studies are required to establish the long-term safety, efficacy, and tolerability of ethosomal drug delivery systems. Currently, no harmonized regulatory guidelines specifically address ethosomal drug delivery systems. Consequently, regulatory agencies generally evaluate ethosomal formulations within broader regulatory frameworks established for nanotechnology-based medicinal products (68).

Transfersomes

Transfersomes are promising vesicular carriers for transdermal drug delivery because of their ability to improve drug bioavailability and reduce systemic adverse effects. Despite these advantages, their successful clinical translation into commercially available pharmaceutical products remains limited due to several challenges, including physicochemical instability, formulation complexity, variability in skin physiology, the need for comprehensive safety evaluation, and difficulties in large-scale manufacturing. Although functional transfersosomal formulations have been successfully developed at the laboratory scale, industrial-scale production remains a major challenge because of production

variability and sensitivity to processing conditions. Furthermore, scalability is hindered by the lack of standardized procedures for demonstrating batch-to-batch reproducibility in accordance with regulatory requirements. Currently, no harmonized regulatory framework specifically addresses the development, characterization, and regulatory approval of transferosomal drug delivery systems. Consequently, transferosomal formulations are generally evaluated within broader regulatory frameworks established for lipid-based nanocarriers or liposomal products (69, 70).

Safety, Skin Tolerability, and Toxicological Considerations

In liposomes, the safety of penetration enhancers has not yet been fully evaluated. Risks, including irritant dermatitis and other skin-associated side effects, could arise from long-term use of penetration enhancers (66).

In ethosomes, ethanol is present at concentrations between 20 and 45%, which poses potential risks. Erythema, dryness, or skin irritation can result from high ethanol content, especially when applied repeatedly or over an extended period. According to *in vitro* studies, ethanol disrupts the stratum corneum lipid layer, increasing permeability but potentially impairing the skin barrier's function, which may result in localized toxicity (71).

Edge activators in transfersomes, such as sodium cholate and Tween 80, can trigger irritation or hypersensitivity reactions that appear as swelling, redness, itching, and, rarely, respiratory distress. High concentrations of edge activators may disrupt cell membrane integrity and cellular health. Preclinical safety studies, the selection of biocompatible surfactants, and the titration of edge activators to the lowest effective dose can all help lower these potential risks. Developing a prodrug or encapsulating surfactants can further reduce local irritation (69).

Stability, Storage, and Scalable Manufacturing

Vesicular drug delivery systems must maintain their physicochemical stability throughout storage to ensure consistent therapeutic performance and product quality. However, vesicular formulations are inherently susceptible to both physical and chemical instability. Physical instability may result in aggregation, fusion, sedimentation, and changes in vesicle size distribution, whereas chemical instability mainly involves phospholipid oxidation, hydrolysis, and drug leakage. These changes may adversely affect encapsulation efficiency, drug release behavior, and overall formulation stability (72).

Several strategies have been proposed to improve the storage stability of vesicular systems. Among these, freeze-drying (lyophilization) is one of the most widely employed approaches because it significantly improves long-term preservation by removing the aqueous phase. The incorporation of cryoprotectants or lyoprotectants, including sucrose, trehalose, glucose, lactose, and mannitol, helps preserve vesicle integrity during freezing and drying while minimizing vesicle fusion, aggregation, and drug leakage after reconstitution. Appropriate storage conditions and formulation optimization are also important for maintaining vesicle stability during storage (72).

Despite considerable advances in vesicle preparation methods, large-scale manufacturing remains a major challenge for the successful commercialization of vesicular drug delivery systems. Many conventional laboratory-scale preparation methods are difficult to translate into industrial production because they often exhibit limited scalability, process reproducibility, and batch-to-batch consistency. Therefore, manufacturing processes that produce vesicles with reproducible physicochemical characteristics are essential for successful clinical translation. Recent advances in industrial production technologies have focused on improving process control, reproducibility, and scalability while meeting regulatory expectations for pharmaceutical manufacturing (73).

Convenience, improved patient compliance, rapid dose termination, and prevention of the first-pass effect are key advantages of transdermal delivery. Furthermore, this method of administration eliminates common issues with oral medications, such as gastrointestinal discomfort, inconsistent absorption, and enzymatic and acid-mediated breakdown. Transdermal delivery of drugs does not cause the pain, unintentional needle sticks, or temporarily elevated blood concentrations that are frequently associated with injections. Unfortunately, only a few medications can be delivered at therapeutic concentrations due to poor skin permeability (74).

In addition to being the largest organ in humans, the skin serves as a superior biological barrier (75). The skin, including the underlying hypodermis, is the largest organ of the human body, covering a surface area of approximately 1.5–2.0 m² and accounting for approximately 15% of total body weight (76). The skin's enormous surface area makes it an attractive site for drug absorption. However, because the stratum corneum is the primary barrier to be overcome, drug absorption through the skin is extremely difficult (39). The stratum corneum serves as the skin's fundamental barrier, allowing only certain compounds to pass through, like lipophilic and low-molecular-weight medications. This layer is

comparatively impermeable and extremely cohesive (41). Therefore, overcoming the stratum corneum barrier, delivering the medication to the skin, and transporting it through cellular and vascular tissue to the target tissue are the main challenges facing transdermal drug delivery systems. The issue is that the medication can only be delivered in small quantities through the skin (77).

Owing to their superior biocompatibility and structural resemblance to biological membranes, liposomes have been extensively investigated as efficient drug delivery systems for transdermal administration (78). However, rather than penetrating deeper into living tissues, conventional phospholipid liposomes typically show poor penetration through intact skin, accumulating in the stratum corneum or within appendages such as hair follicles. Their comparatively rigid bilayers, which may merge with or break upon touching stratum corneum lipids, are thought to be the reason for this restricted penetration. Such interactions could result in early drug release at the surface (79). Niosomes, transfersomes, and ethosomes are examples of new types of lipid vesicles with flexible, ultradeformable characteristics that have emerged by altering the composition and structure of conventional liposomes to further improve the skin penetration of encapsulated compounds (80).

Conclusion

Liposomes represent a promising strategy for overcoming the stratum corneum barrier; however, owing to the inherent limitations of conventional liposomes, more advanced vesicular systems, such as niosomes, transfersomes, and ethosomes, have been increasingly explored. Further research in this field is expected to expand in the coming years.

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