A Critical Assessment on Lipid Based Nanocarriers for Safe and Effective Delivery of Phytoconstituents

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Abstract

Despite the increasing popularity of herbal drugs for their ability to treat various ailments with minimal toxicity, side effects, or adverse reactions, these treatments face numerous challenges such as poor solubility in water or lipids, inadequate permeation, lack of specificity in targeting, instability in highly acidic pH, and susceptibility to liver metabolism. Transdermal drug delivery systems have captured significant attention in the realm of pharmaceutical technology and are among the most frequently produced pharmaceutical products worldwide. Nanovesicles increase drug bioavailability by protecting drugs from degradation, improving their solubility and stability, facilitating their transport across biological barriers, and enhancing their uptake by cells. This review provides a brief introduction to novel Nanovesicles delivery systems and covers Transferosomes, including their mechanism of action, preparation, characterization, and recent applications in transdermal drug delivery, with the aim of exploring and evaluating their potential for resolving the bioavailability issues associated with phytoconstituents. A comprehensive and methodical search from Science direct, PubMed and Google Scholar databases was conducted to gather and analyze relevant information for the review.

Keywords: *Herbal drugs, Transdermal Drug Delivery systems, Nanovesicles, Bioavailability, Phytoconstituents, Biological Barriers*

INTRODUCTION

Plants have been vital to human survival for thousands of years, providing herbal remedies that have been used in traditional medicine. With the advancement of science, researchers have explored the therapeutic potential of bioactive compounds found in plants to treat various diseases such as cancer, cardiovascular and neurodegenerative diseases. However, these bioactive compounds or phytoconstituents face significant delivery challenges due to their poor physicochemical, pharmacokinetic, and pharmacodynamic properties [1, 2]. These challenges include poor solubility, chemical instability in acidic pH, and inadequate permeation through the intestinal wall. Even when phytoconstituents are highly soluble in water, they are often unable to be absorbed due to their large molecular size or poor absorption through the lipid-rich membranes of the small intestine [3, 4].

Novel carrier systems, such as surfactant and polymer/lipid-based systems are being developed to improve the solubility and bioavailability of phytoconstituents, making them a promising area for future research in herbal medicine [5]. Novel Drug Delivery Systems (NDDS) such as nanoparticles, liposomes, microencapsulation, solid lipid nanoparticles, and cyclodextrin complexes can be used to increase the bioavailability of phytoconstituents and improve their therapeutic benefits [6]. These systems protect the phytoconstituents from degradation, increase their absorption and circulation in the body, and release them in a controlled manner for maximum efficacy. NDDS aim to strike a balance between hydrophilicity and lipophilicity to improve the solubility and bioavailability of phytoconstituents, making them a promising avenue for future research in herbal medicine [7].

Over the past few years, there has been increasing attention towards the development of novel nanomedicine techniques, which have shown remarkable benefits over traditional formulations [8]. These advanced drug delivery systems can enhance the solubility and stability of drugs, improve their bioavailability and reduce their toxicity, thereby enhancing the therapeutic efficacy of herbal formulations [9]. This review focuses on recent advancements in strategies for phyto-formulations, covering aspects such as their limitations, applications, and exploring the physical, chemical, kinetic, dynamic, and therapeutic properties of many bioactive compounds that remain largely unexplored.

VESICULAR NANO-CARRIER DRUG DELIVERY SYSTEM

In the current scenario, drug targeting and desired delivery with sustained or controlled release are critical factors for improving the effectiveness of therapy. Researchers have focused on formulating vesicular nanocarrier systems, such as Phytosomes, Liposomes, Niosomes, and Transferosomes, as these materials hold great promise in addressing the limitations associated with conventional medicines [10].

Phytosomes

It is an advanced herbal drug delivery system that improves stability, bioavailability and target specificity of active plant constituents. Phytosomes are vesicular drug delivery systems capable of delivering both water-soluble and lipid-soluble compounds along with phospholipid. The phospholipid and phytoconstituent exist in specific stoichiometric proportion of 2:1 or 1:2 [11]. Phytosomes offer improved stability and solubility, improved

membrane permeation ability, and improved bioavailability, making it an acceptable system for delivering herbal bioactives [12].

Phytosomes are consisting of therapeutic agents, carrier material, and solvent. The choice of standardized herbal extract or active phytoconstituent depends on its hydrophilicity or lipophilicity, while the carrier phospholipid is chosen based on its chemical stability. Phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine are commonly used phospholipids [13]. Solvent selection in the phospholipid complexation strategy depends on the solubility of the herbal bioactive and phospholipids. The phytosome preparation process involves mixing phospholipids and herbal extract/ phytoconstituent, dissolving them in an organic solvent, and then isolating the phytosomes through precipitation, lyophilization, spray drying, or vacuum drying [14].

Liposomes

Liposomes are versatile drug delivery systems that can encapsulate both hydrophilic and lipophilic substances. They self-assemble using phospholipids and cholesterol to form a spherical shape, with sizes ranging from 50 nm to over 1 μm. Liposomes have limitations, such as leakage and short half-life, but new-generation liposomes have been developed to provide improved sustained/controlled release, stability, drug loading, target selectivity, prolonged action, and higher drug entrapment efficiency [15]. Synthesis methods include mechanical, organic solvent replacement, and fusion methods. PEGylation involves incorporating polyethylene glycol onto the surface, which improves stability and circulation half-lives. Liposomal formulations, including Doxil, DaunoXome, and Abraxane, are FDA-approved, and others are in clinical trials. Long circulating liposomes have PEG grafted on the exterior surface to prolong circulation time [16].

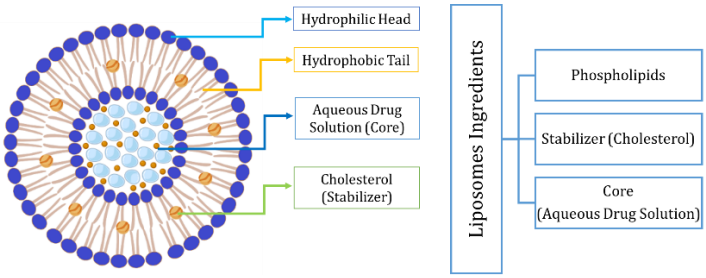

Figure 1: Detailed Compositional Structure of Liposomes

Niosomes

Niosomes are bi-layered lipid-based vesicular carriers that can encapsulate various types of molecules and exhibit longer circulation times than liposomes. They offer advantages over liposomes, including increased stability, reduced side effects, targeted drug delivery, increased efficacy, and reduced dosage requirements [17]. Niosomes are composed of nonionic surfactant, cholesterol, and di-ethyl ether, and can be produced using methods such as thin-film hydration and reverse phase evaporation. Niosomes are a promising drug delivery system that can overcome limitations associated with liposomes and have gained widespread acceptance [18].

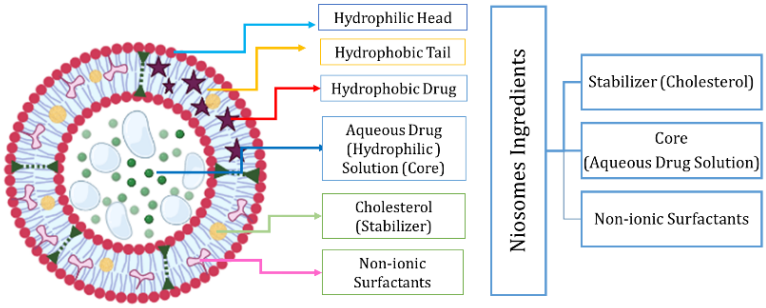

Figure 2: Detailed Compositional Structure of Niosomes

Ethosomes

Ethosomal systems, containing high concentrations of ethanol, phospholipids, and water, have been developed to enhance skin permeation for drug delivery. Classical ethosomes, with their negative ζ-potential, higher entrapment efficiency, and better stability profiles, are superior to liposomes for transdermal drug delivery. Binary ethosomes are created by adding another alcohol to classical ethosomes[19]. The latest generation, transethosomes, combine classical ethosomes and deformable liposomes with penetration enhancers or edge activators, providing better drug entrapment and entrapping larger molecular weight drugs. Researchers are exploring various edge activators and penetration enhancers to improve the characteristics of ethosomal systems [20].

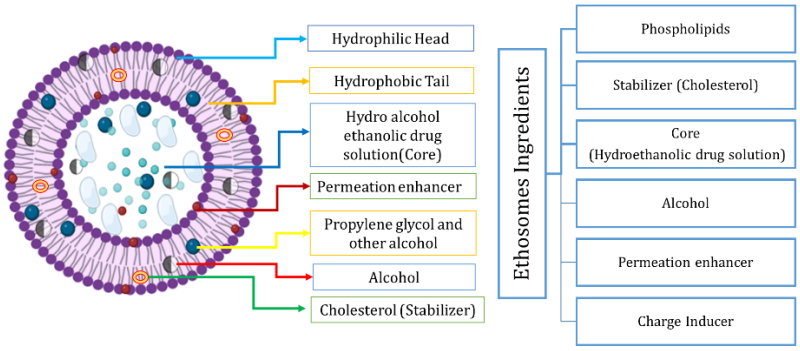

Figure 3: Detailed Compositional Structure of Ethosomes

Sphingosomes

These are liposomal preparations containing sphingolipids and cholesterol in varying ratios, forming one or more membranes. These lipids interact with neighboring molecules, with the polar headgroups orienting towards the exterior of the assembly. Sphingolipids tend to form ordered domains, resulting in the formation of ordered membranes [21] .The composition of naturally occurring sphingolipids varies greatly, but ceramide moieties with long-chain bases and saturated N-acyl chains promote partitioning into ordered membrane domains. The polar head group, ranging from a single hydroxyl to large carbohydrate assemblies, also influences the partitioning of these lipids [22].

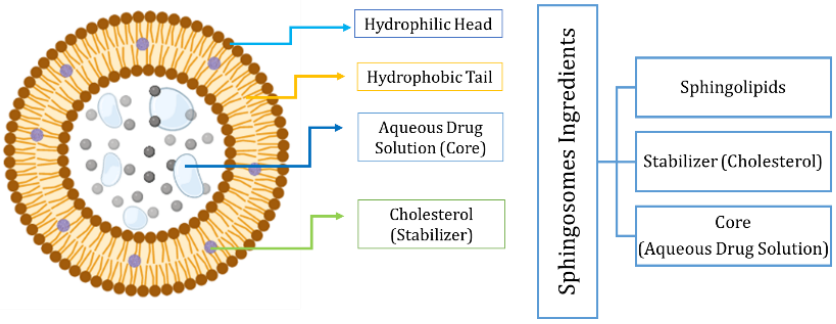

Figure 4: Detailed Compositional Structure of Sphingosomes

Penetration Enhancer-Containing Vesicles

Modified vesicles, developed by Cevc and Blume in 1992, have paved the way for the emergence of Penetration Enhancer Containing Vesicles (PEVs) as versatile systems for transdermal drug delivery [23]. PEVs can be prepared with different lipid components and permeation promoters (PE), with glycerol being a commonly used PE in Glycerosomes. In a study conducted, it was reported that Glycerosomes containing sodium diclofenac showed greater fluidity than conventional liposomes and optimized drug deposition on the skin. Additionally, studies with minoxidil demonstrated that PEVs increased vesicle deformability and improved drug retention, making them an effective option for transdermal drug delivery [24].

Menthosomes

Menthosomes are vesicular carrier systems containing phospholipids, menthol, and surfactants that increase the elasticity of lipid bilayers. They enhance the skin permeation of drugs by improving drug partitioning and diffusion. Menthosome alters the lipid packing arrangement in the stratum corneum, reducing the hexagonal/orthorhombic hydrocarbon packing ratio [25]. Duanjgit et al. developed menthosomes to improve the transdermal delivery of meloxicam. They prepared ten formulations and optimized them using the response surface method. The selected formulation had higher meloxicam penetration and followed predicted values. The results showed the potential of transdermal delivery of meloxicam using menthosomes [26].

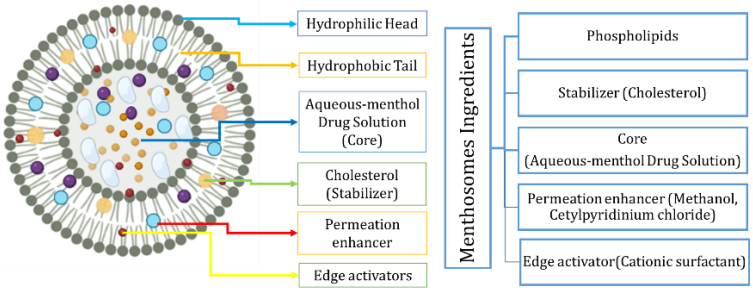

Figure 5: Detailed Compositional Structure of Menthosome

Cubosomes

Cubosomes are a type of nanoparticle that form through self-assembly of surfactant-like molecules in liquid crystalline phases with cubic symmetry. They have a solid lipid crystalline structure similar to honeycomb, and range in size from 100 to 500 nm. Cubosomes are optically isotropic and viscous, with a clear, gel-like appearance. Bi-continuous water and

oil channels are formed by amphiphilic molecules, with two distinct hydrophilic regions separated by a bilayer [25]. Cubosomes have advantages over hexosomes due to their tunability, controlled by changes in temperature, ionic strength, or pH. They are also biodegradable, non-toxic, and can solubilize hydrophobic, hydrophilic, and amphiphilic compounds. Cubosomes are physically stable in water, and have the potential for controlled delivery of drugs and other actives, as their narrow pore sizes facilitate diffusion through the regular channel structure of the cubic phase. They are useful in maintaining stability and efficacy of biologically active compounds. Colloidal dispersions of Cubosomes can be stabilized with polymer [26,27].

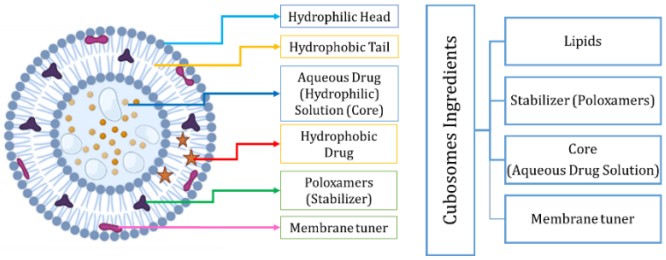

Figure 6: Detailed Compositional Structure of Cubosomes

Archaeosomes

Archaeosomes are vesicles with bilayers of Total Polar Lipids (TPL) extracted from microorganisms in the Archaea domain. These microorganisms, unlike those in the Eukarya and Bacteria domains, require harsh conditions for optimal growth and include hyperthermophiles, thermoacidophiles, obligate anaerobes, and extreme halophiles. Archaea consists of single-celled organisms that differ from eukaryotic cells and have specific cell and cell-membrane modifications to survive in harsh environments [28]. Archaeosomal drug vesicular carrier systems have been developed using the physicochemical stability and encapsulation properties of archaeosomes. These systems can overcome biological and physical barriers in drug delivery and induce long-lasting immune responses when used as adjuvants in vaccine delivery. Attar et al. formulated archaeosomes extracted from the Haloarcula 2TK2 strain, encapsulating rifampicin and isoniazid for the treatment of tuberculosis [29]. They compared the effectiveness of archaeosomes and liposomes as drug vesicular carrier systems and found that archaeosomes showed potential for the treatment of tuberculosis when used with known antitubercular agents. Optimization of archaeosomal membrane composition is crucial in improving drug-loading capacity and efficacy [30].

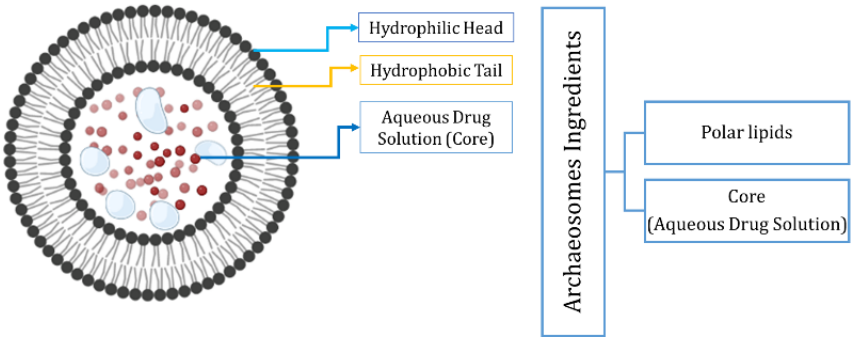

Figure 7: Detailed Compositional Structure of Archaeosomes

Invasosomes

Invasomes are lipid-based, deformable vesicles used for non-invasive drug delivery that penetrate deeper into the skin or systemic circulation. These vesicles are soft, elastic, nanosize, and composed of various phospholipids, low concentrations of ethanol, terpene(s), and water. The penetration of invasomes occurs via ethanol, terpene, and invasome effects, where ethanol fluidizes the intercellular lipoidal layer of the skin and reduces the density of the multilayered lipid cell membrane [31]. Terpene enables percutaneous drug absorption and continuous systemic absorption without causing skin irritancy. Invasomes improve the membrane elasticity and volume of deformed vesicles. Invasomal formulations for various therapeutic applications have been reported, including transdermal delivery of Dapsone [32]. The study by El-Nabarawi et al. showed that the invasomes could enhance the skin drug deposition of Dapsone, with a 2.5-fold increment in in vivo skin deposition and nearly a 2-fold higher AUC compared to the drug solution. Therefore, invasomes have the potential to be a carrier to improve the skin deposition characteristics of drugs [33].

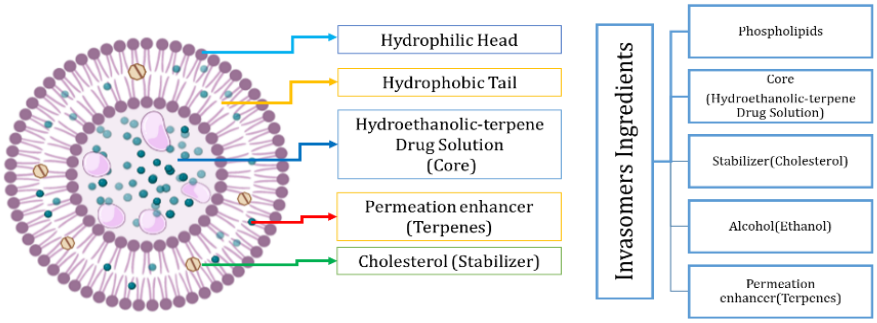

Figure 8: Detailed Compositional Structure of Invasomes

Ufasomes

Ufasomes, which are self-assembled nano-sized bilayer vesicles with oleic acid as a penetration enhancer, have been reported to improve the skin permeation of drugs. Oleic acid enhances the action of ufasomes by fluidizing the subcutaneous lipid and promoting phase separation. The formation of ufasomes occurs at a narrow pH range (7–9) because fully ionized and unionized fatty acid carboxylic acid groups form bilayer vesicles at this pH range [34]. Ufasomes penetrate the skin by lipid exchange between the stratum corneum layer and lipid carrier, and then fuse with the fatty acid in sebum. Sharma and Arora developed ufasomes of methotrexate for topical delivery using oleic acid as the penetration enhancer. In vitro skin delivery of methotrexate was better with ufasomes compared to standard liposomes, and ufasomes deposited about 50% of the initially administered dose in the skin. These results suggest that ufasomes could potentially be used as a carrier for topical treatment of rheumatoid arthritis[35,36].

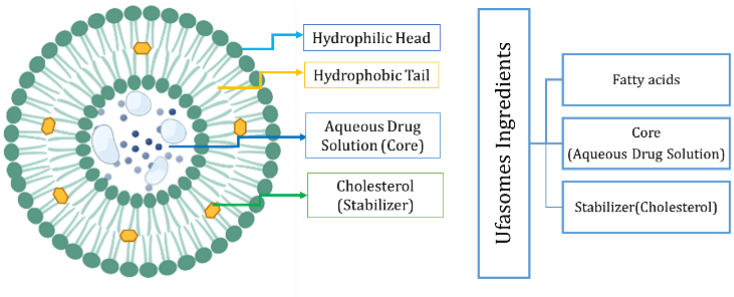

Figure 9: Detailed Compositional Structure of Ufasomes

Transferosomes

Transferosomes are defined as specially designed highly deformable vesicles, consisting of at least one inner aqueous compartment enclosed by lipid vesicles. They are like liposomes in morphology, but, functionally, they are suitably deformable to go through membrane pores. Transferosome is a trademarked technology of the German company IDEA AG [37]. It consists of two words “transferred” and “some” where “transferred” means carrying and “some” means body. Transferosomes consist of phospholipid, surfactant, and water. They are elastic and extremely adaptable aggregates and this property assist in their quick penetration through the intercellular lipid pathway of the subcutaneous tissue. They have a diameter of approx. 100 nm. Tranferosomes can encapsulate both hydrophilic and hydrophobic moieties (herbal/synthetic) in their structural framework are highly biocompatible, sustained release and effectively used in topical application [38, 39].

Composition

Transfersomes typically comprise three main components. The first is an amphipathic ingredient, like a combination of soy or egg phosphatidylcholine, which forms the vesicles' lipid bilayer. The second component comprises 10-25% surfactants or edge activators, including sodium cholates, sodium deoxycholate, Tweens, Spans, and dipotassium glycyrrhizinate, which are biocompatible and enhance the vesicle's flexibility and permeability [40]. The third component includes 3-10% alcohol, such as ethanol or methanol, serving as a solvent and a hydrating medium, with water or a saline phosphate buffer having a pH range of 6.5-7.

Transfersomes' flexible lipid bilayers form through self-assembly of phospholipids in water, aided by edge activators that increase their fluidity and elasticity [41]. Controlling the ratio of surfactants to lipids and the overall amount of surfactants regulates the membrane flexibility and minimizes rupture risk, promoting natural osmotic gradient for non-occlusive application. Penetration-enhancing effect depends on surfactants and lipids types, vesicle size, shape, and elasticity. Overall, transfersomes' efficacy depends on surfactants and lipids concentrations, vesicle characteristics, and their ability to follow natural osmotic gradient [42].

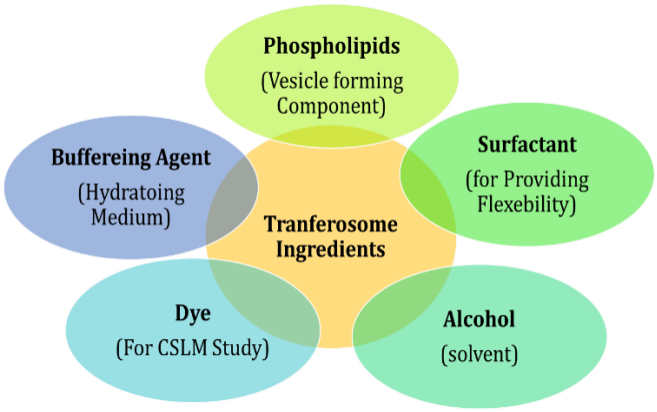

Figure 10: Detailed Compositional Structure of transfersomes

Method of Preparation

It is worth noting that although there are various patented methods for transfersome preparation, there is no standardized protocol or specific formula. Optimum conditions must be identified and optimized for each therapeutic agent to achieve appropriate carriers with ideal characteristics such as deformability, drug carrying capacity, and stability [43].

Rotary Evaporation-Sonication Method or Thin Film Hydration Technique

The method involves dissolving phospholipids and edge activators in a volatile organic solvent mixture, such as chloroform and methanol. The lipophilic drug can also be incorporated at this stage. The organic solvent is then evaporated under reduced pressure using a rotary vacuum evaporator to form a thin lipid film. The deposited thin film is hydrated using a buffer solution with the appropriate pH and rotation for a specific time and temperature. The hydrophilic drug can be incorporated at this stage [44]. The resulting vesicles are then sonicated in a bath or probe sonicator to obtain small vesicles, which are then homogenized by extrusion through a sandwich of polycarbonate membranes. This method allows for the incorporation of both hydrophilic and hydrophobic drugs and can be easily scaled up for large-scale production. The size and properties of the vesicles can be controlled by adjusting the lipid composition, sonication parameters, and extrusion process. However, it is important to note that this method can be time-consuming and requires a significant amount of expertise to optimize the preparation conditions for each therapeutic agent [45].

Modified Handshaking Method

In this method, lipophilic drug, phospholipids, edge activator, and organic solvent are mixed in a round-bottom flask until a clear, transparent solution is formed. The solvent is then removed by evaporation while shaking the flask by hand, and a thin lipid film is formed inside the flask wall. The flask is left overnight to allow complete evaporation of the solvent. The film is then hydrated with a buffer solution by gently shaking the flask at a temperature above its phase transition temperature. Hydrophilic drug incorporation can be done during this stage [46, 47].

Vortexing-Sonication Method

In the vortexing-sonication method, a mixture of phospholipids, edge activator, and drug is stirred in a phosphate buffer until a milky suspension of transfersomes is formed. The suspension is then sonicated in a bath sonicator at room temperature for a specific time [48]. The transfersomes are subsequently extruded through polycarbonate membranes to obtain uniform size and shape. This approach is essential for ensuring optimal performance of the transfersomes as drug carriers [49].

Suspension Homogenization Method

To create transfersomes, an ethanolic phospholipid solution is mixed with an edge activator, followed by the addition of buffer to achieve the desired lipid concentration. The mixture is then sonicated to form transfersomes and subjected to two to three cycles of freezing and thawing to ensure uniform size and shape of the vesicles, which is critical for their effectiveness as drug carriers[50].

Centrifugation Method

In this method, a lipid film is first formed on the walls of a round-bottom flask by evaporating a mixture of phospholipids, edge activator, and a lipophilic drug in an organic solvent. The lipid film is then hydrated with a buffer solution and the resulting suspension is centrifuged at a high speed to separate the large lipid aggregates from the small transfersomes. The supernatant containing the transfersomes is then collected and subjected to further processing such as extrusion through polycarbonate membranes to obtain uniform-sized vesicle [51,52].

Reverse-Phase Evaporation Method

To prepare Transferosomes using the reverse-phase evaporation method, phospholipids and edge activator are dissolved in an organic solvent mixture, such as diethyl ether and chloroform, in a round-bottom flask. A lipophilic drug may also be incorporated in this step. The solvent is then evaporated using a rotary evaporator to obtain a lipid film, which is subsequently re-dissolved in the organic phase consisting mostly of isopropyl ether and/or diethyl ether. Next, the aqueous phase containing the hydrophilic drug is added to the organic phase, resulting in the formation of a two-phase system[53]. The system is then sonicated using a bath sonicator until a homogeneous water-in-oil emulsion is formed. The organic solvent is slowly evaporated using the rotary evaporator to create a highly viscous gel-like substance, which then forms a vesicular suspension. This method allows for the incorporation of both hydrophilic and lipophilic drugs and provides a high encapsulation efficiency [54].

High-Pressure Homogenization Technique

In this method, a phospholipid solution is mixed with an edge activator, and the resulting mixture is passed through a high-pressure homogenizer to break up the phospholipid aggregates and form transfersomes. The homogenization process is repeated several times to achieve uniform size and shape of the transfersomes [55]. The resulting suspension is then subjected to further processing steps such as centrifugation and filtration to remove any

debris or aggregates. The high-pressure homogenization technique is a widely used method for the preparation of transfersomes due to its ability to produce stable and homogeneous vesicles with high entrapment efficiency [56].

Ethanol Injection Method

The ethanol injection method for preparing transfersomes involves two phases: organic and aqueous. The organic phase is produced by dissolving phospholipids, an edge activator, and a lipophilic drug in ethanol with magnetic stirring until a clear solution is obtained. The aqueous phase is created by dissolving water-soluble substances in a phosphate buffer. Both solutions are heated to 45-50°C**[57].** The ethanolic phospholipid solution is then injected dropwise into the aqueous solution while continuously stirring. Ethanol removal is achieved by transferring the resulting dispersion into a vacuum evaporator and sonicating for particle size reduction[58].

Mode of action

Transfersomes are a type of drug delivery system that uses ultra-flexible phospholipid vesicles to deliver drugs transdermally with high efficiency. They have self-optimized membranes that can pass through narrow constrictions with minimal loss, resulting in better penetration of intact vesicles. Compared to standard liposomes, transfersomes are significantly more elastic, making them ideal for skin penetration [59]. The flexibility of transfersome membranes is achieved by mixing suitable surface-active components in the right ratios, which minimizes the risk of complete vesicle rupture in the skin. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayer properties. To increase the vesicle's flexibility and permeability, at least one amphipathic component is required, which self-assembles into a lipid bilayer vesicle [60]. Additionally, the addition of a bilayer softening component such as a biocompatible surfactant or amphiphilic drug enhances the transfersomes' affinity for water, enabling it to penetrate barriers such as non-occluded skin and migrate to deeper strata for hydration. To deliver drugs, Transfersomes widen and overcome hydrophilic pores in the skin or other barriers, gradually releasing the drug molecules to diffuse and bind to their targets. Drug transport may involve the Transfersomes' lipid bilayer fusing with the cell membrane or being actively taken up by the cell in a process called endocytosis. The figure below illustrates the potential micro pathways for drug penetration through human skin, including intracellular and transcellular routes [61].

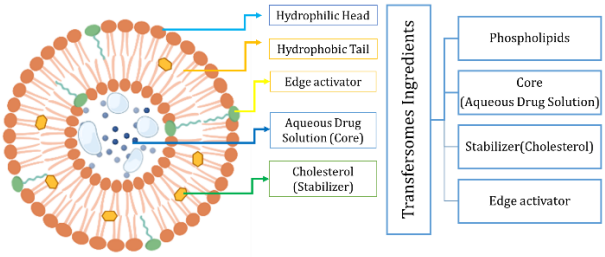

Figure 11: Detailed Compositional Structure of Transfersomes

CHARACTERIZATION

The characterization of transfersomes is important for evaluating their physicochemical and biological properties. The physical characterization methods such as size and size distribution analysis, zeta potential analysis, and FTIR provide information on the stability and composition of transfersomes [62]. The biological characterization methods such as in vitro drug release, cell viability and cytotoxicity assays, and skin permeation studies provide information on the efficacy and safety of transfersomes in Transdermal drug delivery [63].

Physical Characterization Methods

Size and size distribution analysis

The size and size distribution of transfersomes are important parameters that determine their stability, drug loading capacity, and in vivo performance. Various techniques can be used to measure the size and size distribution of transfersomes, including dynamic light scattering (DLS), static light scattering (SLS), nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) [64].

Zeta potential analysis

Zeta potential is the potential difference between the surface of a particle and the surrounding liquid medium. It provides information on the stability and surface charge of particles. Zeta potential can be measured using techniques such as Laser Doppler Anemometry (LDA) and electrophoretic light scattering (ELS)[65]. A high zeta potential indicates that particles are highly charged and are likely to repel each other, resulting in increased stability. A low zeta potential indicates that particles are less charged and are likely to aggregate, resulting in decreased stability [66].

Fourier transform infrared spectroscopy (FTIR)

FTIR measures the absorption of infrared radiation by chemical bonds in a sample. The FTIR spectrum of transfersomes can be used to identify the presence of different functional groups and confirm the composition of the lipid bilayer [67].

Biological Characterization Methods

In vitro drug release

In vitro drug release studies are performed to provide information on the release kinetics and release rate of the drugs from transfersomes, which is an important parameter that determines their efficacy and therapeutic potential. In vitro drug release studies can be performed using various techniques such as dialysis, ultrafiltration, and Franz diffusion cell method [68].

Cell viability and cytotoxicity assay

Various cell-based assays can be used to evaluate the biocompatibility and cytotoxicity of transfersomes, including the MTT assay, lactate dehydrogenase (LDH) assay, and live/dead staining assay. The MTT assay measures the viability of cells by assessing their ability to convert the yellow MTT dye to purple formazan crystals [69]. The LDH assay measures the release of LDH, which is an indicator of cell membrane damage. Live/dead staining assay is a

fluorescence-based assay that distinguishes live cells from dead cells using different fluorescent dyes [70].

Skin permeation studies

Skin permeation studies are performed to evaluate the ability of transfersomes to penetrate the skin and deliver drugs to the underlying tissues. Various skin permeation models can be used to evaluate the permeation of transfersomes, including excised skin, in vitro skin models, and in vivo animal models. In excised skin models, the skin is removed from a donor animal and mounted on a Franz diffusion cell [71].

Research Reports Mentioning Delivery of Phytoconstituent(s) through Transfersomes

Capsaicin transfersomes were prepared and evaluated for entrapment efficiency, drug release rate, and in vitro skin permeation. The study found that capsaicin transfersomes had a high entrapment efficiency of 96.7%, showed enhanced skin penetration compared to cream and suspension, and were affected by the different levels of skin [72]. Another study involves the development of transfersomes containing three drugs and measured their encapsulation efficiency (EE) and drug release *in vitro.* The results showed that lipophilic or hydrophilic drugs with high molecular weight and opposite charges to the membrane have higher EE. DHAD, which can insert into the membrane, had slower drug release compared to VCR [73]. One more study developed transfersomes for delivering Berberine and Curcumin to the brain to treat Alzheimer disease. The formulations showed sustained release, safety, and good cellular penetration, improving spatial memory and reducing oxidative stress markers. The study concluded that the transfersomes were promising carriers for co-delivering drugs to the brain [74]. A topical dosage form was also developed for Silymarin due to its low absorption and poor solubility. A Box Behnken design was used to develop 15 formulations, and the optimized formula was added to HPMC gel to create a transfersomal gel. The Silymarin loaded transfersomal gel had optimal characteristics, showed the greatest transdermal flux, and significantly reduced blood glucose levels in vivo [75]. Another study aimed to enhance the transdermal delivery of berberine chloride (BBR) by using transfersomal emulgel. The study found that the transfersomal emulgel formulation, specifically F2, provided a 3.8-fold increase in BBR permeation through the skin compared to non-transfersomal emulgel [76]. Additional research investigated the use of quercetin-loaded deformable transfersomes in chitosan film for the treatment of osteoporosis. The transfersomes had good characteristics, and when administered to a glucocorticoid-induced osteoporosis rat model, they led to significant improvements in femur thickness, density, serum biochemical parameters, and histomicrographic analysis [77].

The another set of research reports showing the development of transfersomal carrier to encapsulate resveratrol, improving its stability and enhancing permeability, with optimal production conditions of 5% PC/EA (3:1) and 5% ethanol in distilled water, resulting in a particle size of 40.13 ± 0.51 nm and entrapment efficiency of 59.93 ± 0.99%, with comparable antioxidant activity to unencapsulated RSV and reduced cytotoxicity [78]. Additionally, Genistein-loaded transfersomes, lipid-based nanovesicles, were developed for intranasal delivery to the brain as a potential therapy for neurodegenerative diseases. The transfersomes containing Span 80, GEN-TF2, were found to attenuate ROS formation and

reduce apoptosis in the PC12 cell line, suggesting a potential role in adjuvant therapy for oxidative stress-related neurodegenerative diseases [79]. A group of researchers have developed ginsenoside Rhl, found in red ginseng, and has potential therapeutic effects. Ethosomes and transfersomes, new vesicular carriers, were used to enhance its topical delivery. Characterization showed a size range of 108.5 to 322.9 nm, negative charge from -20.95 to -31.37 mV, and %EE between 45.0 to 65.0%. Transfersomes had significantly higher skin permeation compared to ethosomes and conventional liposomes, making them a promising carrier for ginsenoside Rhl [80]. Baicalin vesicular systems were compared to enhance its therapeutic potential for ocular diseases, with liposomes, PEVs, and transfersomes being studied. Liposomes had the highest extent of absorption, and transfersomes showed the fastest onset of action, increasing bioavailability 4-5 times compared to baicalin solution [81]. A cream containing curcuma longa extract-loaded vesicular systems (liposomes, ethosomes, and transfersomes) for photoprotection and evaluate their effects on skin hydration and sebum content. Results showed that the transfersomal creams had the highest efficacy, followed by ethosomal and liposomal creams, improving skin hydration and sebum content, and suggesting potential use as photoprotective formulations [82]. The study aimed to prepare tanshinone transfersomes (TTs) and evaluate their deformability. The TTs were prepared by film dispersion method followed by sonication and were found to be spherical vesicles with good entrapment efficiency, stability, and high deformability in relation to the molar ratio of sodium cholate to lecithin and external pressure [83].

CONCLUSION

Owing to the flexible benefits of lipid based nanocarriers, the discussed vesicular systems are in high demand for the formulation scientists and research scholars too. The high bioavailability, greater stability and easy manufacturing are the reasons of their acceptability. Due to their unique compositional structure they allow both; water soluble and water insoluble drug to get loaded with the sufficient concentration to satisfy the dose and the need of the patient. The near future of vesicular systems is of great success with more and more macromolecules to be developed, delivered and commercialized with wide safety and efficacy protocol.

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