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A COMPREHENSIVE REVIEW OF TRANSFERSOMES IN DRUG DELIVERY

Roopa S.* and Fmith Celvia Miranda

Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka, India 574143.

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***Corresponding Author**
Roopa S.
Srinivas College of Pharmacy, Valachil, Farangipete Post, Mangalore, Karnataka, India 574143.

ABSTRACT

Transdermal drug delivery systems (TDDS), which are self-administrable and non-invasive, can improve bioavailability and patient compliance by bypassing first-pass metabolism. Transdermal novel drug delivery has various benefits over traditional delivery methods. However, physicochemical characteristics of numerous drugs make them less soluble when given topically. Enhancement of penetration of both low as well as high molecular weight drugs is the principle endeavour in designing these vesicles. Vesicular-based TDDS have attracted a lot of significant interest in recent years because they're designed for controlled, efficient, and targeted drug delivery. One of these delivery technologies, transferosomal-based formulations, has grown in popularity due to its ability to achieve all of the desired criteria and qualities. Transferosomes combine the characteristics of liposomes and niosomes because they contain both liposomes (phospholipids and cholesterols) and niosomes as components (nonionic surfactants; edge activators), as a result, they are referred to as the first generation of elastic liposomes. However transdermal drug delivery is difficult due to the presence of the skin's protective barrier. Transferosomal drug delivery overcomes all obstacles due to its unique characteristics, such as its ultra-deformable vesicular nature. The benefits, limitations, modes of penetration, formulations, production and assessment methodologies, and pharmaceutical uses of transferosomal drug delivery systems are discussed in this paper.

KEYWORDS: Transferosomes, Transdermal drug delivery system, Phospholipid, Permeation.

INTRODUCTION

In recent years, research scenario is moving towards the development of new type of drug delivery system with the goal of achieving both high therapeutic activity along with patient compliance. This creates an entirely new window of opportunity for spontaneous small-scale formulations in pharmacy settings. Novel vesicular drug delivery systems have made great progress in the field of nanotechnology, as these systems have a potential to carry a variety of drugs and have been widely used for various purposes such as drug targeting, controlled release and permeation enhancement of drug. Creating novel drug delivery systems to meet the requirements of specific patient populations is becoming more and more necessary. Due to the presence of first pass effect or medication interactions with other components of Gastro Intestinal tract (GIT) before absorption, an efficacious, successful therapy with no undesirable effects may not be possible in the majority of situations. Patient adherence to these kinds of treatments is low. Because of this, researchers have recently focused on investigating enhanced drug delivery techniques to reap the rewards of traditional therapy without having to deal with its disadvantages.[1,2]

Transdermal drug delivery systems are one of the more promising types of drug delivery systems due to their advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patient's convenience.[3]

Salient features of Transdermal Drug Delivery System[4]

1. It minimises the risk of harmful effects by providing a steady blood level in the plasma for medications with a narrow therapeutic index. A continuous infusion of a medication is provided over an extended period of time by a transdermal drug delivery device.
2. It offers less intra-patient variability and better patient compliance. It increases bioavailability by bypassing hepatic first pass metabolism.
3. It can be applied over an extended period of time and to medications with short half-lives, limited window of therapeutic application and poor oral absorption.

4. When compared to the oral route, it is preferable for unconscious individuals.
5. No disruption with gastric and intestinal fluids. Self-administration is possible and easy elimination of drug delivery in case of toxicity.

Pharmaceutical research has shown a significant deal of interest in the transdermal method of drug delivery because it avoids many of the issues that come with the oral route of drug administration. Several tactics have been employed recently to improve the transdermal distribution of bioactive compounds. These mostly consist of chemical permeation enhancers, microneedles, sonophoresis, iontophoresis, and vesicular system like liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes. Among these strategies, transfersomes appear promising.[5]

El Maghraby et al., suggested that ultradeformable vesicles improved skin disposition rather than penetration and, hence, are most useful for topical drug delivery.

Gregor Cevc et al., 1991 had introduced the concept of transfersomes. IDEA AG, a German business, has registered the trademark "Transfersome," which alludes to their exclusive medication delivery method. The Latin term "transferre," which means "to carry across," and the Greek word "soma," which means "a body," are the sources of the name, which means "carrying body." Transfersomes are ultradeformable vesicles for transdermal applications consisting of lipid bilayers with phospholipids and an edge activator and an ethanol/aqueous core. An artificial vesicle called a transfersome carrier is made to resemble a cell vesicle or a cell undergoing exocytosis, making it appropriate for targeted and controlled drug delivery. Transfersomes features membranes that are incredibly flexible and self-regulating, allowing the vesicle to be highly deformable. Microporous barriers can be effectively crossed by transfersome vesicles, even if the pores are significantly smaller than the vesicles themselves.[6,7]

Structure Of transfersome

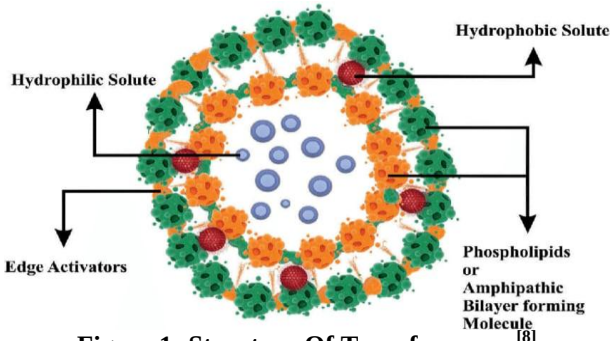


Figure 1: Structure Of Transfersomes.[8]

Advantages Of Transfersomes[9,10]

1. Since transfersomes have an architecture made up of both hydrophilic and hydrophobic moieties, they can hold a variety of medicinal compounds with varying solubilities. They do not suffer appreciable loss when they bend and pass through thin constriction (between 5 and 10 times smaller than their own diameter).
2. This system's high deformability allows intact vesicles to penetrate more effectively. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anaesthetic, corticosteroids, sex hormone, anticancer, insulin and albumin.
3. They are similar to liposomes such as being biocompatible and biodegradable because they are composed of natural phospholipids.
4. When it comes to lipophilic drugs, their entrapment efficiency is nearly 90%. They protect the drug from within, being broken down by metabolism, such as proteins and peptides.
5. They act as depot, releasing their contents slowly and gradually & can be used for both systemic as well as topical delivery of drug. They are easy to scale up, as procedure is simple and avoid unnecessary use or pharmaceutically unacceptable additives.
6. Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles through tight junctions.

Drawbacks Of Transfersomes[11,13]

1. Many drugs especially those drugs with hydrophilic properties penetrate the skin too slowly to be of therapeutic purpose.
2. The barrier function of the skin changes from one site to another on the same person, from person to person and also with age.
3. Drug molecule must be potent because patch size limits amount that can be delivered.
4. Not suitable for high drug doses.
5. Adhesion may vary with patch type and environmental conditions.
6. Skin irritation and hypersensitivity reactions may occur.
7. Drugs that require high blood levels cannot be administered.

Why Only Transfersomes For Skin[14]

Transfersomes are advantageous as phospholipids vesicles for transdermal drug delivery. They can deliver the medicine with great efficiency and repeatability through or into the skin, depending on the application or mode of administration, because to their ultra-flexible and self-optimized membrane features. The stratum corneum's internal sealing lipid allows transfersomes to squeeze past obstacles to skin penetration. These are typical of transfersomes because of the high vesicle deformability that allows entry in response to external mechanical force in a self-adapting manner. Transfersome membrane flexibility is controlled by combining appropriate surface-active ingredients with phospholipids in the right amounts. When applied under non-occlusive conditions, the ensuing flexibility of the transfersome membrane reduces the possibility of a full vesicle rupture in the skin and enables transfersomes to follow the natural water gradient across the epidermis. Transfersomes have the ability to spontaneously permeate the entire stratum corneum via two different intracellular lipid pathways, each with unique bi-layer characteristics. Since Bangham's 1963 discovery of liposomes, vesicular systems have been attracting a growing amount of interest. However, it has recently been clear that traditional liposomes have very little penetration value. According to research using confocal microscopy, undamaged liposomes stay on the top layer of the stratum corneum instead of penetrating into the granular layer of the epidermis. The rate of drug release and its deposition at the target site can be altered by altering the vesicular composition or surface characteristics.

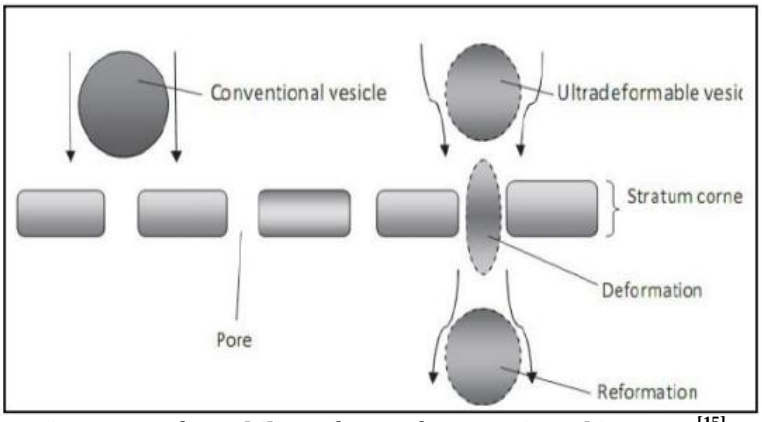


Figure 2: Deformability Of Transfersomes into skin pores.[15]

Mechanism Of Action[16]

Transfersomes enter the deeper skin layer after passing through the outermost skin layers. They are typically flushed out into the blood circulation thereafter. When the transfersomes are applied to the skin's surface, water evaporation creates "osmotic gradient," which serves as part of the penetration mechanism. Thus, concentration has no effect on the elastic vesicles' transit. The skin penetration barrier creates this osmotic gradient, which keeps the water activity differential in the viable portion of the epidermis constant and stops water loss through the skin. Due to their elastic nature, vesicles are able to pass through the stratum corneum's pores, despite the fact that these pores are only tenth of the vesicles' diameter. By forging its own pathway, transfersomes cause hydration, which widens the skin's hydrophobic pores. The drug then gradually releases via the expanded pores and binds to the intended organ. Transfersomes, function as penetration enhancers that disrupts the intercellular lipids from stratum which ultimately widens the pores of skin and promote the molecular interaction and penetration of system through the skin.

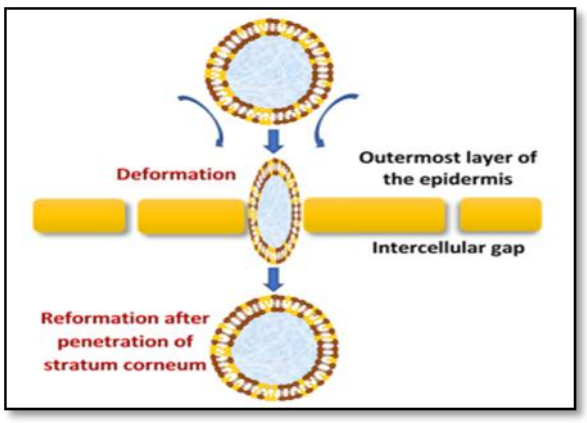
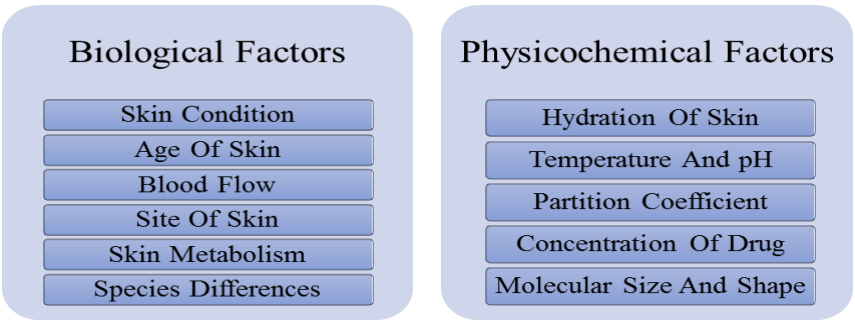


Figure 3: Mechanism Of Action Of Transfersomes.

Factors Affecting Transdermal Drug Delivery[17]

Three aspects can be taken into consideration when formulating an effective transdermal drug delivery system: the drug, the skin, and the vehicles. There are primarily two components at play: Physicochemical and biological considerations.



1. Skin condition: These acids and alkalis are able to degrade the skin cells and dissolve through it, along with many less abrasive solvents such as chloroform, methanol etc. Patient in diseased state also suffers from changes in skin conditions.
2. Blood Flow: Modifications in peripheral blood flow may impact the drug's transdermal absorption.
3. Site of skin: Site-specific differences include skin thickness, stratum corneum type, and appendage density. These elements have a big impact on penetration.
4. Skin metabolism: Steroids, hormones, chemical carcinogens, and some medications are all metabolised by skin. Therefore, a medicine absorbed through the skin's metabolism impacts its effectiveness.
5. Hydration of Skin: Skin permeability greatly increases when it comes into contact with water. Increasing skin penetration is primarily dependent on hydration levels. Hence, humectant is generally used in transdermal delivery.
6. Concentration of Drug: The concentration gradient across the barrier determines the flow, and a higher concentration gradient indicates a higher drug concentration across the barrier.

Composition of transfersomes[18]

The transfersome is composed of two main aggregates namely,

- The first component is Amphipathic one (such as phosphatidylcholine) ingredient, which in aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle.
- The second one is bilayer softening agent significantly increases the flexibility and permeability of the lipid bilayer (e.g., biocompatible surfactant, amphiphile medication).

Because of its enhanced flexibility and permeability, the resulting Transfersome vesicle can quickly and readily change shape in response to external stimuli by modifying the local concentration of each bilayer component in response to the local stress that the bilayer is subjected to, as illustrated in Figure 4. Thus, the "softer," more flexible, and more easily modifiable artificial membrane of the Transfersome sets it apart from such more traditional vesicles.

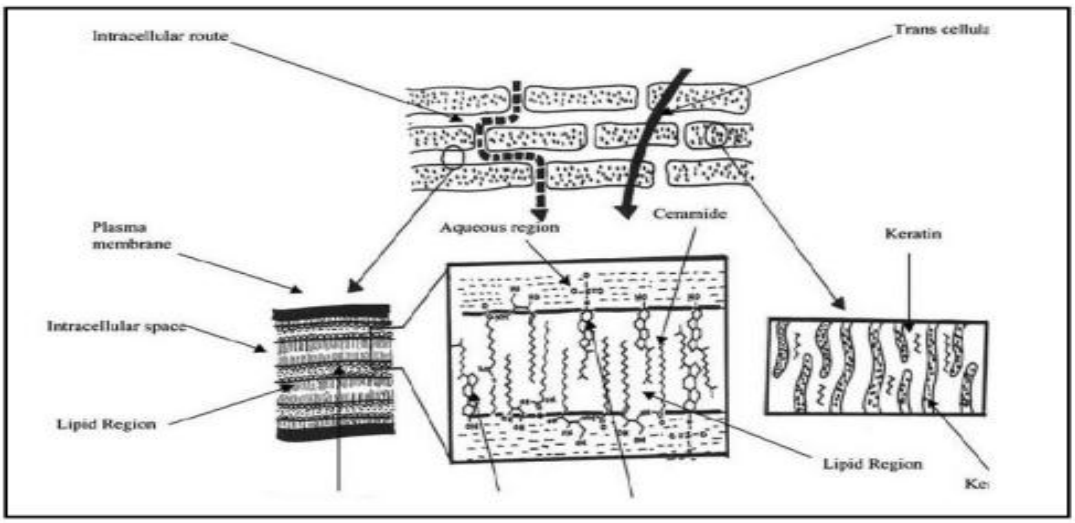


Figure 4: Micro routes for drug penetration across human skin.

MATERIALS AND METHOD

Many phospholipids, surfactants, alcohol, dyes, buffering agents and other materials are frequently employed in the formulation of transferosomes. The numerous additives that are utilised in the formulation of transferosomes are enumerated.

Various additives used in formulation of transfersomes[19,22]

1. Phospholipids

Phospholipids are crucial components in the preparation of transfersomes, typically used at concentrations ranging from 1% to 10%. These amphiphilic molecules possess a hydrophilic head and hydrophobic tail, which enables them to form lipid bilayers in an aqueous environment. In transfersome formulations, phospholipids serve as the primary structural component, forming the lipid vesicles that encapsulate drugs or active ingredients.

The concentration of phospholipids affects the stability, flexibility and deformability of transfersomes. Higher concentrations of phospholipids can lead to increased vesicle size and reduced membrane fluidity, impacting the ability of transfersomes to penetrate biological barriers effectively. Conversely, lower concentrations may result in decreased stability and structural integrity of the vesicles.

When phospholipids are used in excess, it can lead to several potential issues

1. Reduced deformability: Excessive phospholipid content can increase the rigidity of the transfersome membrane, limiting its ability to deform and squeeze through narrow pores, which is essential for efficient drug delivery.

2. Increased vesicle size: Higher concentrations of phospholipids can result in larger transfersome vesicles, which may hinder their penetration through the skin or other biological barriers.

3. Decreased drug loading capacity: The encapsulation efficiency of drugs within transfersomes may be compromised when phospholipid concentrations are too high, leading to lower drug loading capacities and reduced therapeutic efficacy.

4. Stability concerns: Excessive phospholipids can destabilize transfersome formulations, leading to aggregation, fusion or leakage of encapsulated drugs, thereby compromising the shelf-life and efficacy of the product.

Therefore, optimizing the concentration of phospholipids is essential to ensure the desired characteristics and performance of transfersome formulations for effective transdermal drug delivery.

Examples: Soya phosphatidyl choline, Soya Lecithin Dipalmitoyl phosphatidyl choline, Distearoyl phosphatidyl choline.

Parveen S et al., developed topical transferosomal gel of bifonazole using varying percentages of soya lecithin (1%, 2%, 3%, and 4%). It was discovered that the phospholipid concentrations significantly impacted the vesicle nature, stability, drug content, entrapment efficiency, and size.[23]

2. Edge activators

Edge activators, also known as surfactants or penetration enhancers, play a crucial role in the preparation of transfersomes. These compounds are typically used at concentrations ranging from 0.5% to 5% in transfersome formulations.[24] Edge activators serve several important functions in transfersome preparations

1. Enhanced membrane fluidity: Edge activators disrupt the packing of phospholipids in the lipid bilayer, increasing membrane fluidity. This property is essential for improving the deformability and flexibility of transfersomes, allowing them to squeeze through pores and penetrate biological barriers more effectively.

2. Facilitated membrane fusion: Edge activators promote membrane fusion between transfersomes and biological membranes, such as the cell membrane or the lipid layers of the stratum corneum in the skin. This enhances the delivery of encapsulated drugs into target cells or tissues.

3. Stabilization of transfersomes: In addition to promoting membrane fluidity, certain edge activators can also help stabilize transfersome formulations by preventing aggregation, fusion, or leakage of encapsulated drugs.

4. Increased drug encapsulation efficiency: Edge activators can improve the encapsulation efficiency of drugs within transfersomes by facilitating their partitioning into the lipid bilayer or the aqueous core of the vesicles.

When edge activators are used in excess, several potential consequences may arise

1. Cytotoxicity: Some surfactants used as edge activators can exhibit cytotoxic effects at high concentrations, potentially causing irritation or damage to the skin or mucous membranes.

2. Destabilization of transfersomes: Excessive amounts of edge activators can destabilize transfersome formulations, leading to increased vesicle leakage, aggregation, or fusion. This can compromise the structural integrity and efficacy of the transfersomes.

3. Altered drug release kinetics: High concentrations of edge activators may affect the release kinetics of drugs from transfersomes, leading to either rapid or delayed

drug release profiles that are not optimal for therapeutic efficacy.

4. Reduced membrane fluidity: Contrary to their intended function, excessive edge activators may decrease membrane fluidity by disrupting the lipid bilayer structure excessively, which can hinder the deformability and penetration ability of transfersomes.

Therefore, it is essential to carefully optimize the concentration of edge activators in transfersome formulations to balance their beneficial effects on membrane properties with potential drawbacks such as cytotoxicity and destabilization.

Examples: Sodium Cholate, Sodium deoxycholate, tween 80, Span80.

Tawfeek HM et al., have formulated Lornoxicam in the form of topical transferosomal hydrogel using sodium deoxycholate as edge activator. Results revealed that optimum Lornoxicam transfersomes (LOR TRSs) had an encapsulation efficiency of 99.34 ± 0.2%, size of 233.5 ± 12.5 nm and zeta potential of −35.34 ± 0.78 mV. It is concluded that increment in the concentration of edge activator led to an increase in drug permeation along with increase in entrapment efficiency.[25]

3. Solvents

Solvents play a crucial role in the preparation of transfersomes by facilitating the dissolution of phospholipids, drugs and other components, as well as adjusting the vesicle size and properties. The concentration of solvents used in transfersome formulations varies depending on factors such as the solubility of the components, desired vesicle characteristics, and compatibility with biological systems. Typically, solvents are used in concentrations ranging from 10% to 50% in transfersome preparations.

The primary functions of solvents in transfersome formulations include

1. Phospholipid dissolution: Solvents are essential for dissolving phospholipids, which are the primary structural components of transfersomes. By dissolving phospholipids, solvents enable the formation of lipid bilayers and vesicles, which encapsulate drugs or active ingredients.

2. Drug solubilization: Solvents are used to solubilize drugs or active ingredients that may have limited solubility in aqueous or lipid phases. This facilitates the incorporation of drugs into transfersomes and ensures uniform distribution within the vesicles.

3. Vesicle size control: The choice of solvent and its concentration can influence the size, shape and stability of transfersomes. By adjusting the solvent concentration, it is possible to modulate the vesicle size and optimize the formulation for specific applications, such as transdermal drug delivery.

4. Enhanced membrane fluidity: Some solvents can interact with phospholipids and edge activators, increasing membrane fluidity and deformability of transfersomes. This property is crucial for improving the penetration ability of transfersomes through biological barriers.

If solvents are used in excess or if inappropriate solvents are chosen, several potential consequences may arise

1. Vesicle destabilization: Excessive solvent concentrations can destabilize transfersome formulations, leading to vesicle aggregation, fusion, or leakage of encapsulated drugs. This can compromise the structural integrity and efficacy of the transfersomes.

2. Drug precipitation: Incompatible solvents or high solvent concentrations may cause drugs or active ingredients to precipitate out of solution, reducing their encapsulation efficiency and therapeutic efficacy.

3. Cytotoxicity: Certain solvents used in transfersome preparations may exhibit cytotoxic effects at high concentrations, posing risks of irritation or damage to biological tissues.

4. Altered vesicle properties: The choice of solvent and its concentration can significantly impact the physical and chemical properties of transfersomes, including their size, shape, membrane fluidity and drug release kinetics. Excessive solvent concentrations may lead to undesirable changes in these properties, affecting the performance and stability of the transfersome formulation.

Therefore, it is essential to carefully select and optimize the concentration of solvents in transfersome formulations to ensure compatibility with the components and biological systems, as well as to achieve the desired vesicle characteristics and therapeutic outcomes.

Examples: Ethanol, methanol.

Gupta A et al., have formulated Econazole topical transferosomal gel by using methanol as a solvent. The examined transfersome (TF-4) has vesicular shape with large internal aqueous core, with 200–150 nm in diameter. When the level of methanol increases research reveals a consequential impact on vesicular size. Their study demonstrates that elevated methanol concentrations lead to a significant enlargement of vesicles within the cellular environment. This phenomenon not only highlights the sensitivity of vesicular dynamics to methanol exposure but also underscores the potential implications for cellular function and integrity. Understanding the relationship between methanol concentration and vesicular size enlargement is crucial for assessing the toxicological

effects of methanol and for designing strategies to mitigate its adverse consequences in pharmaceutical and biomedical applications.[26]

4. Buffering agent

Buffering agents are substances used to maintain the pH of a solution within a specific range, thereby stabilizing its acidity or alkalinity. In the preparation of transfersomes, buffering agents are employed to maintain the pH of the formulation, typically in the range of 5.5 to 7.4, which is compatible with the physiological pH of the skin and other biological tissues. The concentration of buffering agents used in transfersome formulations varies depending on factors such as the desired pH range, the buffering capacity of the agent, and the compatibility with other components. Generally, buffering agents are used in concentrations ranging from 0.1% to 2%.

The primary functions of buffering agents in transfersome preparations include

1. pH stabilization: Buffering agents help maintain the pH of transfersome formulations within a narrow range, which is critical for preserving the stability and activity of encapsulated drugs or active ingredients. Fluctuations in pH can degrade drugs or alter their chemical properties, leading to reduced efficacy or safety concerns.

2. Enhanced skin compatibility: By maintaining a pH similar to that of the skin (around pH 5.5 to 6.5), buffering agents improve the compatibility of transfersome formulations with the skin, minimizing irritation or adverse reactions upon application. This is particularly important for transdermal drug delivery applications, where transfersomes come into direct contact with the skin.

3. Stabilization of vesicle structure: Buffering agents can help stabilize the structure and integrity of transfersomes by minimizing pH-induced changes in the lipid bilayer or vesicle morphology. This is crucial for preserving the physical properties and drug delivery capabilities of transfersomes during storage and application.

4. Enhanced drug stability: Some drugs or active ingredients may exhibit pH-dependent stability, with degradation or precipitation occurring outside specific pH ranges. Buffering agents help maintain the pH of transfersome formulations within the optimal range for drug stability, thereby preserving their therapeutic efficacy and shelf-life.

If buffering agents are used in excess or if inappropriate buffering agents are chosen, several potential consequences may arise

1. Altered drug release kinetics: Excessive buffering agent concentrations can affect the release kinetics of drugs from transfersomes by influencing the pH microenvironment within the vesicles. This may lead to either accelerated or delayed drug release profiles, which may not be optimal for therapeutic efficacy.

2. Skin irritation: Some buffering agents may exhibit irritant properties at high concentrations or if the pH of the formulation deviates significantly from the physiological range. This can lead to skin irritation, redness, or discomfort upon application of transfersome formulations.

3. Formulation instability: Incompatible buffering agents or excessive concentrations may destabilize transfersome formulations, leading to vesicle aggregation, fusion, or leakage of encapsulated drugs. This can compromise the structural integrity and efficacy of the transfersomes.

4. pH-induced drug degradation: Inappropriate pH conditions resulting from excessive buffering agent concentrations may accelerate the degradation of pH-sensitive drugs or active ingredients, leading to reduced therapeutic efficacy or safety concerns.

Therefore, it is essential to carefully select and optimize the concentration of buffering agents in transfersome formulations to ensure compatibility with the desired pH range, the stability of encapsulated drugs, and the skin compatibility of the formulation.

Examples: Saline phosphate buffer (pH 7.4).

Khan MI et al., have developed an *in vitro/ex vivo* evaluated lecithin-based deformable transfersomes and transfersome-based gels for combined dermal delivery of meloxicam and dexamethasone. Through his findings, it's evident that an increase in phosphate buffer concentration leads to enhanced pH stability, thereby prolonging drug shelf life and ensuring therapeutic efficacy. Moreover, Khan's work demonstrates how this buffer system facilitates controlled drug release, promoting optimal bioavailability and patient compliance. By understanding the effects of phosphate buffer 7.4, pharmaceutical scientists can refine drug delivery systems for improved patient outcomes and pave the way for the development of more effective therapeutic interventions.[27]

5. Dyes

Dyes, also known as colorants or pigments, are substances used to impart color to transfersome formulations for various purposes. The concentration of dyes used in transfersome preparations varies depending on factors such as the desired color intensity, compatibility with other components, and regulatory considerations. Typically, dyes are used in concentrations ranging from 0.01% to 1% in transfersome formulations.

The primary functions of dyes in transfersome preparations include

1. Visual identification: Dyes are often used to visually identify transfersome formulations, especially in topical or transdermal drug delivery applications where multiple formulations may be used concurrently. By imparting distinct colors to transfersome formulations, dyes facilitate easy identification and differentiation between products.

2. Product aesthetics: Dyes can enhance the aesthetic appeal of transfersome formulations by imparting attractive colors or shades, which may improve consumer acceptance and compliance with therapy. Aesthetic considerations are particularly relevant for cosmetic or skincare products where appearance plays a significant role in consumer preference.

3. Labeling and branding: Dyes can be used to align transfersome formulations with specific brands or product lines by incorporating signature colors or branding elements. This helps reinforce brand recognition and differentiation in the marketplace, contributing to brand loyalty and consumer trust.

4. Quality control: Dyes can serve as indicators of formulation homogeneity and consistency during manufacturing and quality control processes. Uniform dispersion and distribution of dyes throughout transfersome formulations indicate proper mixing and formulation integrity, ensuring product quality and performance.

If dyes are used in excess or if inappropriate dyes are chosen, several potential consequences may arise
1. Skin irritation or sensitization: Some dyes may exhibit irritant or sensitizing properties, particularly at high concentrations or in individuals with sensitive skin. Excessive use of dyes in transfersome formulations may increase the risk of skin irritation, allergic reactions, or other adverse effects.

2. Formulation instability: Incompatible dyes or excessive concentrations may destabilize transfersome formulations, leading to changes in vesicle morphology, aggregation, or leakage of encapsulated drugs. This can compromise the structural integrity and efficacy of the transfersomes, affecting their performance and shelf-life.

3. Regulatory compliance: Regulatory authorities impose limitations on the use of certain dyes in cosmetic, pharmaceutical, and skincare products due to safety concerns or regulatory restrictions. Exceeding permissible concentrations or using banned dyes in transfersome formulations can lead to non-compliance with regulatory standards and legal implications.

4. Undesirable color effects: Excessive concentrations of dyes may result in overly intense or unnatural colors in transfersome formulations, which may be aesthetically unappealing or inconsistent with consumer expectations. This can negatively impact consumer acceptance and perception of the product.

Therefore, it is essential to carefully select and optimize the concentration of dyes in transfersome formulations to achieve the desired color intensity, product aesthetics, and regulatory compliance while minimizing the risk of adverse effects or formulation instability. Conducting appropriate compatibility tests and adhering to regulatory guidelines are crucial steps in the formulation process to ensure the safety, efficacy, and acceptance of transfersome products.

Examples: Rhodamine-123, Rhodamine-DHPE, Fluorescein-DHPE Nile red.
Khatoon K et al., have developed cilnidipine loaded transfersomes for transdermal application used Rhodamine B dye for confocal laser scanning microscopy and he discovered that Rhodamine B loaded transfersomal formulation showed significantly deeper penetration compared to plain gel.[28]

Active Pharmaceutical Ingredients[29]
The most crucial characteristics of a drug candidate to be used in a transferosomal gel are that it belongs to BCS class 2, which means it has low solubility and high permeability, low molecular weight, and low dose administrability. It is possible to increase the formulation's solubility in a number of ways.

Table 1: List of drugs used for transfersomes.

Drug	Drug class	BCS class	Method
Ibuprofen	NSAIDs	2	Rotary evaporation method
Naproxen	NSAIDs	2	Thin film hydration technique
Oestradiol	Steroids	1	Coacervation phase separation method
Insulin	Steroids	1	Thin film hydration technique
Fluconazole	Antifungal	2	Thin film hydration technique
Itraconazole	Antifungal	2	Film dispersion method
Nystatin	Antifungal	2	Rotary evaporation sonication method
Valsartan	Antihypertensive	2	Conventional rotary evaporation method

Methods For Preparation Of Transfersomes[30,33]

1. Vortex-sonication method

Using this technique, a phosphate buffer is combined with mixed lipids (phosphatidylcholine, EA, and the medicinal drug) and vortexed to create a milky suspension. After the suspension is sonicated, it is extruded via membranes made of polycarbonate.

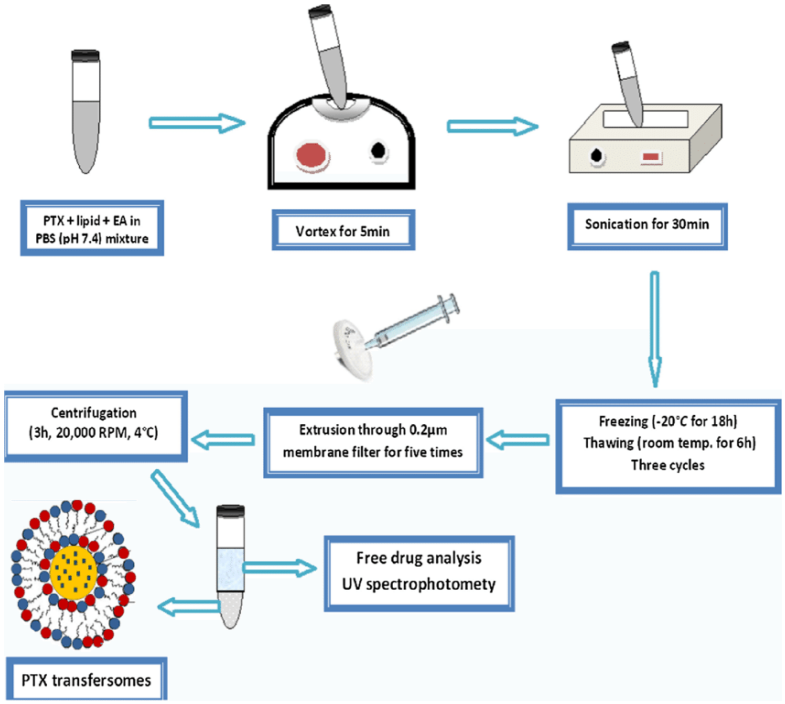


Figure 5: Vortex or Sonication Method

2. Suspension homogenization process

In this procedure, a suitable concentration of an edge-active chemical, such as sodium cholate, is combined with an ethanolic soybean phosphatidylcholine solution to create transfersomes. To get a total lipid concentration, this prepared solution is then combined with Triethanolamine-HCl buffer. The resultant suspension is frozen, thawed, and then sonicated two or three times.

3. Thin film hydration technique

It is employed for the preparation of Transfersomes which comprised of three steps.

a. Phospholipids and surfactant, the elements that create vesicles, are dissolved in a volatile organic solvent (methanol, chloroform) to produce a thin film. The organic solvent is subsequently evaporated using a rotary evaporator above the lipid transition temperature, which is room temperature for pure phosphatidyl choline vesicles and 50°C for dipalmitoyl phosphatidyl choline. Under hoover, the last remnants of the solvent were eliminated overnight.

b. A produced thin film is rotated at 60 rpm for one hour at the appropriate temperature to hydrate it with buffer (pH 6.5). At room temperature, the resultant vesicles swelled for two hours.

c. The resultant vesicles were sonicated for 30 minutes at room temperature or 50°C to prepare small vesicles using a vortex shaker, bath sonicator, or probe that has been sonicated for 30 minutes at 4°C. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

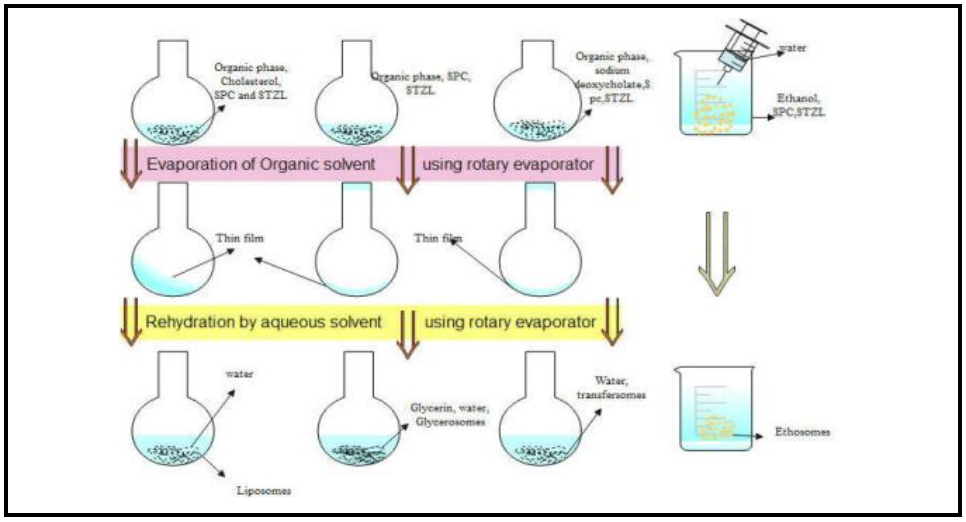


Figure 6: Thin Film Hydration Method.

Dudhipala N et al., have developed aceclofenac (AF) loaded transfersomes gel for transdermal application. AF-loaded transfersomes (AF-TS) were prepared by using the film hydration method and further it was evaluated for *ex vivo* skin permeation studies compared with marketed Hifenac 30 g gel. Optimized AF-TS showed vesicle size, PDI, and zeta potential of 111.1 ± 3.2 nm, 0.19 ± 0.02, and -29.6 ± 1.2 mV, respectively. Entrapment efficiency of 74.1 ± 1.8% with pH 5.8 phosphate buffer as a hydration medium and 17.1 ± 0.9 elasticity at 0.15%w/v EA and 1%w/v lipid concentration were observed.[34]

4. Modified hand shaking, lipid film hydration technique

It is also considered for the preparation of Transfersomes which comprised following steps

- A 1:1 mixture of ethanol and chloroform was used to dissolve the drug, soya lecithin (PC) and edge activator. Evaporation with hand shaking was used to remove the organic solvent above the lipid transition temperature (43°C). With rotation, a thin lipid coating developed inside the flask wall. The thin coating was left overnight to allow the solvent to completely evaporate.

- After that, the film was hydrated for 15 minutes at the appropriate temperature using phosphate buffer (pH 7.4) and moderate shaking. At 2–8°C, the transfersome suspension continued to hydrate for an hour.

5. Reverse phase evaporation method

The process for this method is as follows: lipids and organic solvents were combined together in a round bottomed flask under nitrogen purging aqueous media containing edge activators. Depending on the drug's solubility, it's mixed with either a lipophilic or a lipophobic media. After sonication, the prepared material is left for 30 minutes until it appears to be a homogeneous combination. Organic phase is eliminated when pressure is kept to a minimum. The substance transforms into a viscous gel that creates vesicles.

Patel P et al., have developed and evaluated Diacerin loaded transferosomal gel for arthritis by using reverse phase evaporation method. The statistical model indicated that as the concentration of lipid increased then entrapment efficiency also improved. The *in-vitro* release of diacerein from optimized transferosomal gel followed Higuchi kinetic model (R2 =0.9815). The developed formulation was stable, non-irritating, and showed a maximum amount of % swelling inhibition using carrageenan inducing hind rat paw oedema at a different time interval. Thus, it can be concluded that the developed diacerein-loaded transferosomal gel has a better potential to treat arthritis. The developed formulation showed sustained drug release, increased skin permeation, and efficient anti-inflammatory activity.[35]

Patent Reports on Transfersomes as carriers for the delivery of therapeutic agents

S.No.	Application no. of patent	Applicant	Results
1.	US 20020048596A1(2002)26	Gregor Cevc.	The patent claims the use of NSAID in transfersomes for transport through natural barriers and constriction of skin.
2.	US 7175850 B2 (2007)	IDEA AG, Munich (DE)	Described the administration of corticosteroids via transfersomes on mice skin for oedema suppression activity. They were tested against commercial reference cream
3.	US 20070042030 A1 (2007)	IDEA AG,	It is non-invasive and painless therapy,

		Munchen (DE)	resulted in >90% of the applied drug dose reaching the destined organ of the body.
4.	US 7591949 B2 (2009)	IDEA AG, Munich (DE)	Claimed the penetrant capability of transfersomes because these deformable complex droplets adapt the pore of the skin. They deform and penetrate the skin along the natural moisture gradient rather than coalescing locally.

Characterization Of Transfersomes[36,38]

In general, transfersomes are characterised similarly to liposomes, niosomes, and micelles. For transfersomes, the following characterisation parameters need to be evaluated.

1. Vesicle size & size distribution

Transmission electron microscopy (TEM) can be used to visualise transfersomes, and a computerised inspection system from Malvern Zetasizer can be used to determine the size and size distribution of vesicles using the dynamic light scattering (DLS) approach.

2. Vesicle diameter

The dynamic light scattering (DLS) method or photon correlation spectroscopy can be used to measure the diameter of vesicles. Samples were prepared in distilled water, passed through a 0.2 mm membrane filter, and then diluted with filtered saline. Dynamic light scattering measurements or photon correlation spectroscopy were used to determine the samples' sizes.

3. No. of vesicles per cubic mm

For the purpose of optimising the composition and other process factors, this parameter is essential. Transfersome formulations that are not sonicated are diluted five times using a 0.9% common salt solution. After that, an optical microscope and hemocytometer can be employed for more research. The Transfersomes in 80 small squares are counted and calculated using the subsequent formula

Total number of Transfersomes per cubic mm = (Total number of Transfersomes counted × dilution factor × 4000) / Total number of squares counted.

4. Entrapment efficiency

Generally, expressed in terms of % drug entrapment. Using a minicolumn centrifugation technique, the unentrapped medication was first separated in this manner. Following that, 50% n-propanol or 0.1% Triton X-100 were used to break apart the vesicles.

The entrapment efficiency is expressed as

Entrapment efficiency = (Amount entrapped/Total amount added) × 100.

5. Drug content

Depending on the analytical method of the pharmacopoeial drug, the drug content is determined using one of the instrumental analytical methods, such as a modified high performance liquid chromatography method using an ultraviolet detector, column oven, auto sample, pump, and computerised analysis programme.

6. Turbidity measurement

One tool that's frequently used to assess turbidity in aqueous solutions is the nephelometer.

7. Surface Charge and Charge Density

To calculate the surface charge and charge density, a zeta sizer is used.

8. Penetration Ability

The standard method for evaluating this is microscopy.

9. Degree of deformability or permeability measurement

Permeability analysis is one of the most important and unique characterisation for transfersomes. The analysis of deformability is finished using pure water as the reference. The process of preparing transfersomes involves creating an excessive amount of pores with known sizes (by sandwiching different microporous filters with pore diameters ranging from 50 nm to 400 nm, depending on the transfersome suspension used initially). Dynamic light scattering (DLS) measurements are used to record particle sizes and size distributions following each journey.

10. *In-vitro* drug release

A drug release study conducted *in-vitro* is used to calculate the penetration rate. Prior to more costly *in vivo* tests, the formulation is optimised using data from *in vitro* research, the time required to reach steady state permeation, and the permeation flow at steady state. Transfersomes suspension is incubated at 32°C, samples are taken at various periods, and minicolumn centrifugation is used to separate the free drug in order to determine drug release. The amount of drug released is then computed indirectly using the initial amount (100% entrapped and 0% released) and the amount of drug entrapped at zero times.

11. Physical stability

The initial drug entrapped (percent) in the formulation was determined and was stored in sealed glass ampoules with a tight seal. The ampoules were placed at 4 ± 2°C (refrigeration), 25 ± 2°C (room temperature), and 37 ± 2°C (body temperature) for at least 3 months. After 30 days, samples from each ampoule were analyzed to

determine drug leakage. Percent drug loss was calculated by keeping the initial entrapment of drug as 100%.

APPLICATIONS

Targeted and controlled drug distribution to different human tissues is made possible by the successful loading of different medicines onto transfersomes.

1. Delivery of insulin: Insulin is typically administered subcutaneously, which is inconvenient. Encapsulating insulin into transferosomes (transfersulin) overcomes all of these issues. After transferring insulin application on the intact skin, the first signs of systemic hypoglycemia are observed after 90 to 180 min, depending on the specific carrier composition. Delivery of insulin by transferosomes is the successful means of non-invasive therapeutic use of such large molecular weight drugs on the skin.
2. Delivery of corticosteroids: Transferosomes have also been used to deliver corticosteroids; by optimising the dose of medication applied topically, transferosome-based corticosteroids are biologically active at doses several times lower than the formulations currently used to treat skin conditions. Transferosomes also improve the site specificity and overall drug safety of corticosteroid delivery into skin.
3. Delivery of anaesthetics: Under the right circumstances, applying anaesthetics to a suspension of transferosomes—very deformable vesicles—induces a topical anaesthesia in less than ten minutes. Transferosomal anaesthetics have a longer-lasting impact than equivalent subcutaneous bolus injections, with a maximum resultant pain insensitivity that is almost as high (80%).
4. Delivery of Anticancer Drugs: Transfersome technology has been used to try transdermal delivery of anti-cancer medications like methotrexate. The outcomes were positive. This offered a fresh method of treating cancer, particularly skin cancer.
5. Delivery of Herbal Drugs: Transfersomes are able to enter the stratum corneum and provide the necessary nutrients locally to sustain the skin's functions. In light of this, Xiao-Ying *et al.*, have developed capsaicin transfersomes, which exhibit superior topical absorption when compared to pure capsaicin.
6. There are several GI adverse effects linked to NSAIDS. These can be avoided by employing highly deformable vesicles for transdermal administration.

Table 3: List of drugs that are administered using transferosomes.

S.No.	Drugs	BCS class	Category	Inferences
1.	Oestradiol	1	Steroids	Improves transdermal flux
2.	Norgestrel	2	Steroids	Improves transdermal flux
3.	Tacrolimus	2	Immunosuppressants	Better skin permeations compared to liposomes result in better antipsoriatic activities
4.	Tetanus toxoid	1	Vaccine	For transdermal immunization
5.	Curcumin	4	Antibiotics	Better permeation for anti-inflammatory activity
6.	Stavudine	1	Antiretroviral drugs	Improved the *in-vitro* skin delivery of Stavudine for antiretroviral activity
7.	Mefenamic acid	2	NSAID	Good spread ability and drug release profile produced 12 h better outcomes

CONCLUSION

Transfersomes are a significant advancement in drug delivery, offering enhanced bioavailability and controlled release through their ultra-flexible, lipid-based vesicles. They excel in delivering small molecules, peptides, proteins, and nucleic acids by traversing biological barriers more effectively than traditional systems. Clinically, transfersomes are promising for conditions like rheumatoid arthritis, cancer, and skin disorders due to their customizable and biocompatible nature. Despite challenges in production and regulation, ongoing research aims to optimize their formulation and validate their efficacy. In conclusion, transfersomes have the potential to revolutionize drug delivery, improving treatment effectiveness and patient outcomes in pharmaceutical sciences.

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