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Research Article

TRANSFEROSOMES: AN ADVANCED APPROACH FOR ENHANCED TRANSDERMAL DRUG DELIVERY

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ABSTRACT

Transferosomes are an artificial vesicle that can be used for controlled and possibly targeted medication delivery. They are a patented drug delivery technique. Recently, transferosomes were developed, which can deliver both high- and low-molecular-weight medications. This has been used to increase the effectiveness of material transfer across intact skin. Some potential benefits include avoiding first pass metabolism, predicting and extending the duration of activity, minimising unfavourable side effects, using drugs with short half-lives, and improving physiological and pharmacological response. Edge activators and phospholipids are parts of the transferosome's composition. Transferosomes can enter cells directly or through other cells, creating an "osmotic gradient" in the process. Transferosomes are classified similarly to other vesicles including liposomes, niosomes, and micelles. Transferosomes are also find to have applications in animals in transdermal delivery, anti-inflammatory and anti-arthritic treatment, anti-cancer drug delivery etc. The current review discusses transferosome characteristics, preparation techniques, and mechanisms of action.

Keywords: Transferosomes, Targeted drug delivery, Controlled drug delivery, Osmotic gradient, Tran-cellular route.

INTRODUCTION

The majority of the time, it is impossible to establish an effective, successful therapeutic therapy. This is frequently because of a variety of factors, including the occurrence of hepatic first-pass metabolism, unfavourable side effects, the rejection of invasive treatments, and low patient compliance (Chaurasiya *et al*, 2019). To solve these issues, various medication delivery systems have been created and researched over the years. Transdermal delivery systems are a viable strategy because they are minimally invasive and have no first-pass effects. However, it is necessary to address the skin's barrier function, which hinders or prohibits the transdermal distribution of therapeutic substances (Modi *et al*., 2012). The aforementioned issue has been solved by nanoencapsulation employing a lipid-based vesicular system, such as liposomes. Conventional liposomes do not adequately permeate the living skin and blood circulation, which is an issue (Cevc Gregor et al,1996; Lymberopoulos, *et al*., 2017). So, rather than for transdermal delivery, liposomes have been used frequently as drug carriers for cutaneous distribution. Conventional liposomes have drawbacks as well, such as a poor ability to encapsulate hydrophilic medications, an unstable membrane that causes leaky behaviour, and a brief half-life (Xu *et al*,2012; Szoka Jr, Francis *et al*.,1978) Other unique vesicles including niosomes, sphingosomes, bilosomes, chitosomes, transfersomes, ethosomes, and invasomes have been discovered and developed as a result of these significant challenges. In the 1990s, Cevc *et al*. introduced transfersomes, a brand-new class of carrier system. The words transfero and soma are combined to form the word transferosome. The ultra-deformable quality of transfersomes is made possible by edge activator (EA), a membrane-softening substance found in Tween 80, Span 80, and sodium cholate. Transfersomes are made of phospholipids and EA. Transfersomes can alter the flexibility of their membranes and spontaneously flow through the skin pores when they get close to the skin pores. This so-called self-optimizing deformability is described in (Rai, Shubhra *et al*., 2017). Additionally, because transferomes are so easily malleable, they can easily pass through even the narrowest pores (Cevc *et al*.,

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2002). In depth preclinical studies, a wide range of phase I and phase II clinical trials, as well as the transcutaneous delivery of peptides and proteins and the sustained release of desired therapeutic agents were all successfully conducted using these self-optimizing, highly deformable lipid aggregates (Rajan *et al*, 2011; Cevc *et al.*, 2004). As a result, transfersomes are acknowledged as the most remarkable and inventive transdermal drug carrier available today.

Advantages

Due to the fact that they are produced from natural phospholipids, much like liposomes, they are biocompatible and biodegradable. They guard against metabolic breakdown of the medication that is encapsulated. Transferosomes demonstrate better medication penetration via the skin. These act as carriers for pharmaceuticals with both small and high molecular weights. The percentage of drug entrapment in transferosomes is higher for or drugs that are lipophilic, around 90%. Prevents atmospheric deterioration of the medication that is entrapped. Because of their great deformability and high entrapment efficiency, intact vesicles can be penetrated more effectively. They can serve as a vehicle for both low and high molecular weight medications, such as analgesics and anaesthetics. Insulin, gap junction protein, sex hormone, anticancer, corticosteroids, and albumin. Transferosomes can accommodate medicinal molecules with a wide spectrum of solubilities because their structural makeup combines hydrophobic and hydrophilic moieties. They can be utilized for both systemic and topical medication administration, acting as a depot that releases its contents gradually.

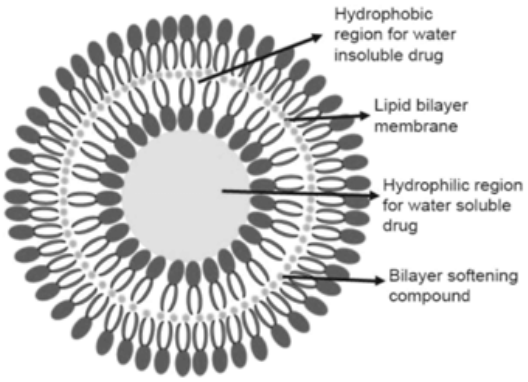

Figure 1. Schematic diagram of transferosome.

Disadvantages

Transferosomes are prone to oxidative destruction, which makes them chemically unstable. The natural phospholipids' purity is another factor that works against the use of transferosomes as drug delivery systems. These formulations cost a lot of money. Drugs' hydrophilic nature allows them to gently penetrate the skin and provide therapeutic benefits. Drugs with larger dosages are not appropriate for transdermal drug delivery systems. Skin sensitivity and skin inflammation are possible side effects of transdermal medication delivery. The drug molecule used for transferosomal delivery needs to be strong.

Factors affecting transdermal delivery

Mainly two factors affect transdermal delivery of drug which include: biological factors and physicochemical factors. Biological factors: Skin age: Younger skin is more permeable than older skin, making children more susceptible to the absorption of toxins through the skin. Skin condition: The patient's illness affects the patient's skin condition. In addition, skin cells are damaged by acids, alkalis, and solvents like methanol and chloroform, which increase penetration. Skin metabolism: The skin breaks down certain carcinogens, hormones, and medicines. Therefore, the effectiveness of a medicine that permeates the skin is predicted by the skin's metabolism. Skin site: Different sites have different keratins, skin thickness, and appendages. Physicochemical factors: Drug concentration: Flux is inversely proportional to the gradient of drug concentration across the barrier. Therefore, when the drug concentration is higher across the barrier, the concentration gradient will be bigger. Skin hydration: Hydration plays a key role in boosting skin permeability. In touch with water, the skin becomes more permeable. Humectants are therefore employed in the creation of transdermal delivery systems. Temperature and pH - As the temperature varies, the medicine permeates the body more readily. The diffusion coefficient falls as the temperature rises. The amount of unionised drug determines the amount of drug in the skin. Partition coefficient: The intercellular route is the pathway for highly lipophilic molecules (log K > 3) and for molecules with intermediate partition coefficients (log K 1

to 3), and additionally, the ability to partition out of the SC into an aqueous region via epidermal tissues, while hydrophilic molecules (log K 1) are likely to dominate the transcellular route.

COMPOSITION OF TRANSFEROSOMES

Transferosomes are mainly composed of 2 main aggregates like phospholipids and edge activators. Phospholipids: Phospholipids create the membrane and give vesicles stability. Consequently, both the destabilising agent and the phospholipids, which create the membrane, are necessary. The most widely utilised phospholipids are those derived from soy, such as soy phosphatidylcholine and hydrogenated soy phosphatidylcholine.

Edge activators

An edge activator typically consists of a single-chain, non-ionic surfactant that weakens the lipid bilayer and increases its fluidity and elasticity. Numerous edge activators, including sodium cholate, sodium deoxycholate, dicetylphosphate (DCP), KG (dipotassium glycrhizinate), and others, have been described for the formation of transferosomes. Vesicle characteristics like as size, entrapment effectiveness, and zeta potential are influenced by the kind and ratio of the various edge activators.

MECHANISM OF TRANSPORT

Transferosomes circumvent the obstacle of skin penetration by squeezing along the stratum corneum's internal sealing lipids. The process for improving the transport of active ingredients into and across the skin is currently not well understood. Two different action mechanisms have been suggested. Transferosomes serve as medication delivery systems because they stay intact after penetrating the skin. Transferosomes serve as penetration enhancers by disorganizing the stratum corneum's highly organised intercellular lipids, which thus makes it easier for drug molecules to enter and cross the stratum corneum. The use of transfersomes vesicles in medication administration therefore depends on the carrier's capacity to enlarge and circumvent the skin's hydrophilic pores. Similar to typical endocytosis, intracellular drug transport may entail vesicle lipid bilayer diffusion with the cell membrane. The process is very complicated and incorporates cutting-edge mechanical concepts together with material transport and hydration/osmotic force. Possible methods by which a penetrant could penetrate the skin barrier. Over the intact horny layer, Through the hair follicles and the sebaceous glands that are connected to them, or through the sweat glands.

MATERIAL FOR TRANSFEROSOMES

Phospholipids like phosphatidyl choline are included in transferosomes, a self-adaptable and well-optimized mixed lipid aggregate that spontaneously forms a lipid bilayer in an aqueous environment before closing to form a vesicle. To improve the flexibility and permeability of the lipid bilayer, a bilayer softening is applied. The name "edge activator" is given to the second component. An edge activator is often a single chain surfactant that causes the lipid bilayer to become less stable, increasing the fluidity and flexibility of the layer. In 1998, Van den Berg introduced the more recent elastic vesicles, which used non-ionic surfactant as the edge activator 30. By combining relevant surface-active chemicals in the right proportions, it is possible to change how flexible the tranferosome membrane is. By adapting the local concentration of each bi layer component to the local stress encountered by the bi layer, the resulting, flexibility and permeability optimised, transferosome vesicle can alter its shape to surrounding stress easily and quickly. When administered under non-occlusive conditions, this flexibility also reduces the chance of a complete vesicle rupture in the skin and enables transferosomes to follow the water gradient that naturally exists across the epidermis. Phospholipids (soya phosphatidylcholine, egg phosphatidylcholine, dipalmitylphosphatidylcholine, etc.) are the primary component of the vesicles, which also contain 10–25% surfactant to provide flexibility (ethanol, methanol), and a hydration medium made of saline phosphate buffer (pH 6.5-7). Nile red or a dye similar to Rhodamine 123 for Confocal Scanning Laser Microscopy. Materials commonly used for the preparation of transfersomes are summarized in Table below: a. Phospholipids: Soya phosphatidylcholine, egg phosphatidylcholine, Disteryl, Phosphatidylcholine. b. Surfactants: Sodium cholate, sodium deoxy cholate, Tween 80 and span-80. c. Alcohol: Ethanol, Methanol. d. Dye: Rhodamine- 123, Rhodamine-DHPE, Fluorescein-DHPE, Nil Red. e. Buffering agents: Saline phosphate buffer (P^H-6.5), 7% V/V ethanol, Tris buffer (P^H-6.5)

METHODS OF PREPARATION

Rotary Film Evaporation Method/ Modified Hand Shaking Method

This technique is often referred to as the modified hand shaking technique. Lecithin is dissolved in a 1:1 mixture of chloroform and ethanol along with the edge activator (surfactant) and medication. By hand shaking at a temperature above the lipid transition temperature, the mixture is exposed to evaporation to remove the organic solvent. To guarantee that the organic solvent is completely removed, the thin lipid film is left on all night. The thin film that has been created above is hydrated using a pH 6.5 buffer by rotating at 60 RPM for one hour at the appropriate temperature. The resultant vesicles swelled at room temperature for two hours. The resultant vesicles were sonicated at room temperature to create tiny vesicles.

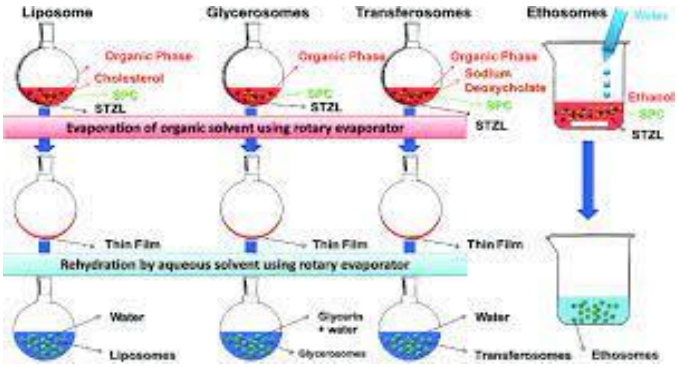

Figure 2. Rotary Film Evaporation Method.

Reverse Phase Evaporation Method

In this approach, lipids that have been dissolved in organic solvents are added to a flask with a flat bottom. Under nitrogen purging, aqueous media containing edge activators is introduced. Depending on the characteristics of the drug's solubility, it may be introduced to a lipid or aqueous media. The created system is then subjected to sonication until a homogeneous dispersion is achieved; this dispersion should not separate for at least 30 minutes. The organic solvent is then eliminated while operating at lower pressure. The system will now change into a thick gel, and vesicles will start to develop. Using centrifugation or dialysis, the non-encapsulated material and leftover solvents can be eliminated.

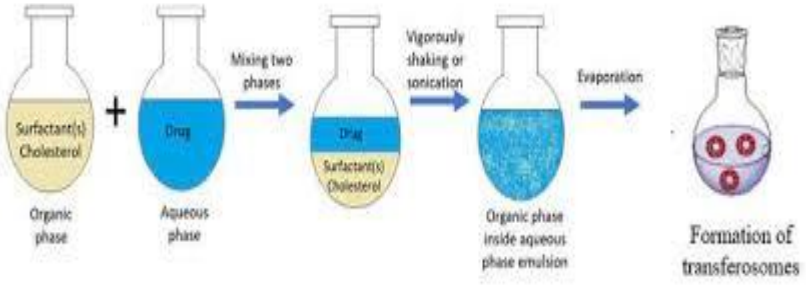

Figure 3. Reverse Phase Evaporation Method.

Vortex/Sonication Method

In this procedure, phospholipids and edge activators are thoroughly combined before being suspended in phosphate buffer. The created milky suspension is then extruded through polycarbonate membranes after being sonicated with a vortex or bath sonicator.

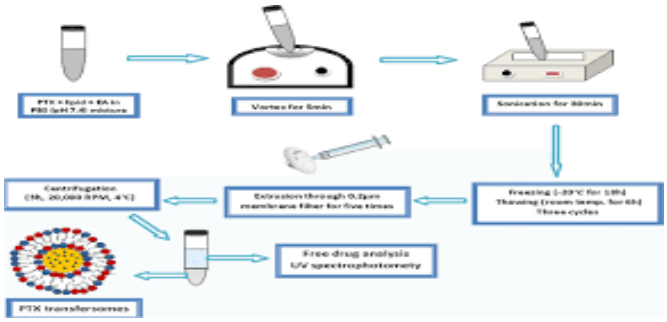

Figure 4. Vortex/Sonication Method.

Ethanol Injection Method

This approach involves continuously swirling at a steady temperature while heating the drug and water solution. Drop by drop, phospholipid- and edge-activating-containing ethanolic solution is added to an aqueous solution. Lipid molecules precipitate out of the solution and create bilayered structures when it comes into touch with watery medium. Compared to other ways, this one is better.

Figure 5. Ethanol Injection Method.

Freeze Thaw Method

With this technique, multi-lamellar vesicles are suspended and subjected to alternating cycles of freezing at very low temperatures and then being heated to extremely high temperatures. The prepared suspension is put in a tube and submerged for 30 seconds in a nitrogen bath (-30° C). It is heated to a high temperature in a water bath after it has frozen. This procedure is carried out 8 to 9 times.

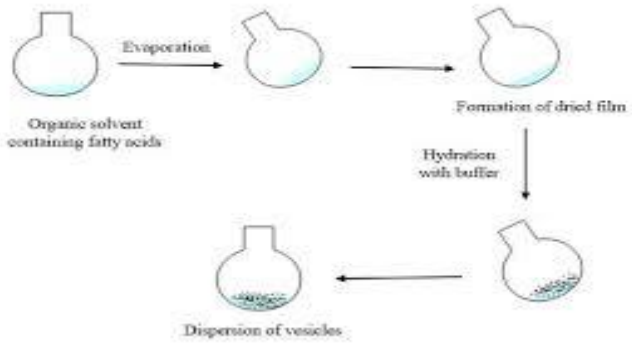

Figure 6. Freeze Thaw Method.

CHARACTERIZATION OF TRANSFEROSOMES

Entrapment Efficacy

The percentage entrapment efficiency (%EE) measures the amount of drug entrapped in the formulation. Using both direct and indirect approaches, minicolumn centrifugation is utilised to separate the un entrapped medication from the vesicles in order to calculate the entrapment efficacy (EE). After ultracentrifugation, the supernatant is removed using the direct method by breaking the vesicles with 0.1 trion x 100 or n-propanol. The resultant solution is diluted and filtered using a syringe filter (0.22 - 0.45 m) to eliminate the contaminants. The drug content is ascertained by spectrophotometric analysis or high-performance liquid chromatography (HPLC). The %EE is computed as follows:

$$\%\text{Entrapment efficiency} = \frac{\textit{Amount of drug entrapped}}{\textit{Total amount of drug added}} \times 100$$

Vesicle size, morphology and zeta potential

The vesicle diameter can be calculated using the Dynamic Light Scattering (DLS) method. In this, a suitable medium is mixed with a vesicle suspension, and measurements of the vesicular size can be taken either in triplicate or by preparing the sample in distilled water and filtering it through a 0.2mm membrane filter. The filtered sample is diluted with saline before being used to estimate the size of

the vesicles by DLS. The Malvern Zeta Sizer is then used to determine the size and size distribution of the vesicles, while transmission electron microscopy (TEM) is used to observe the structural changes.

Number of vesicles per cubic mm

Using 0.9% Nacl, unsonicated transferosomal formulations are multiplied by five. This sample is examined using a hemocytometer and an optical microscope, which allows for the observation of transferosomes with vesicle diameters larger than 100 nm. Small squares are used to count the transferosomes, and the result is calculated as (Opatha, Shakthi Apsara Thejani,et al,2020).

Total number of transferosomes per cubic mm =

$$\frac{\text{Total number of transferosomes counted X dilution factor X 4000}}{\text{Total number of squares counted}}$$

Drug content

One of the instrumental analytical techniques, such as modified high performance liquid chromatography (HPLC) using a UV detector, column oven, auto sample, pump, and computerised analysis program(Sheo DM, Shweta A, et al,2010) can be used to determine the drug content.

Turbidity measurement

Turbidity of a drug in an aqueous solution is determined by using a nephelometer (Sheo DM, Shweta A, et al,2010).

Degree of deformability / permeability measurement

In order to determine deformability, pure water is used as the control. The preparation is filtered through a large number of microporous filters with pore sizes ranging from 50 to 400 nm. After each pass, DLS measurements are used to determine the particle size and size distribution, which are then calculated using the formula as

$$D = J\left(\frac{r_v}{r_p}\right)$$

Where D = degree of deformability, J = amount of suspension extruded during 5 min, rv = size of the vesicle and rp = pore size of barrier

Occlusion effect

It is believed that occluding the skin helps topical medications penetrate the skin more easily. On the other side, the issue also affects elastic vesicles. Vesicle penetration through the skin from its comparatively dry surface to its water-rich deeper layers is primarily fueled by the hydrotaxis of water. Because occlusion prevents water from escaping from the skin, it affects hydration forces.

Penetration ability, Surface charge and charge density

Fluorescence microscopy is used to assess the capacity of Transferosomes to penetrate and the surface charge and charge density of Transferosomes is determined by Zetasizer (Pawar, Ashish Y et al, 2016)(Kulkarni, P. R., et al, 2011).

In vitro drug release

The Franz diffusion cell determines in vitro drug release. Using adhesive tape, the donor chamber is attached to the receptor chamber. A magnetic bar stirs the fluid in the receptor chamber continually. Aliquots of 1 ml of the receptor media are removed at the appropriate times (such as 0, 0.5, 1, 2, 3, 4, and 6 h), and the withdrawn medium is simultaneously replaced with an equivalent volume of fresh phosphate buffer to maintain the sink conditions. UV or HPLC analysis may be used to analyse the samples that were collected (Sheo *et al.*, 2010).

In vitro skin permeation studies

The goal of this study is to determine the transport efficiencies and pinpoint the elements that contribute to a rise in the transport flux, which is commonly measured in units of g/cm2/h. In order to foresee in vivo behaviours from various transdermal delivery methods and to improve the formulation before more expensive in vivo tests are conducted, the findings from this study can be used. It has been asserted that skins from monkeys, pigs, rats, mice, guinea pigs, and snakes are suitable substitutes for human skin. Franz diffusion cell apparatus is used in the examination into skin permeability. The effective permeability area between the donor and receptor compartments was 2.50 cm2, and the receptor compartment had a capacity of 50 ml. In the receptor compartment, 50 ml of phosphate-buffered saline (pH 7.4) was stirred at 100 RPM using a magnetic bar. The formulation (equal to 10 mg of medication) was applied to the skin through the top of the diffusion cell. Aliquots of the receptor media are removed at the appropriate intervals and replaced at the same time with an equivalent volume of fresh phosphate buffer medium (pH-7.4) to maintain the sink conditions. HPLC or spectroscopic analysis can be used to assess the samples that have been collected.

CONCLUSION

The transdermal route has a long history of use, and because of its many benefits, new transdermal administration techniques are always being developed. Thus, the development of transferosomes, which are ultradeformable vesicles, will undoubtedly be a key step in resuming research on the use of vesicles as transdermal

drug delivery systems. The usage of elastic vesicles provides a few benefits over other transdermal administration methods, including: They allow for increased drug penetration through the skin; their composition is safe, and their ingredients are accepted for use in pharmaceutical and cosmetic products; they can increase transdermal flux, prolong the release of bioactive molecules, and enhance site specificity; and they can accommodate drug molecules with a wide range of solubility.As a result, increased distribution of bioactive compounds through the skin using an ultradeformable vesicular carrier creates new difficulties and potential for the creation of newer, improved therapeutics. Since transferosomes are specially designed, optimised vesicles with the ability to respond to an external stress by quick, energy-efficient shape changes, it can be inferred that the new, ultra-flexible drug carrier (transferosome) can resolve all the issues related to transdermal delivery.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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