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FORMULATION AND EVALUATION OF LORAZEPAM LOADED TRANSFEROSOMES FOR THE MANAGE NEURODEGENERATIVE DISORDERS

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Abstract

Transferosomes are ultra-deformable vesicular carriers widely explored for enhanced transdermal drug delivery. These vesicles are composed mainly of phospholipids and edge activators, which impart high elasticity and deformability, enabling them to penetrate through the narrow pores of the stratum corneum. Transferosomes can encapsulate both hydrophilic and lipophilic drugs and deliver them effectively into deeper layers of the skin or systemic circulation. Due to their unique structure and flexibility, they provide improved drug permeation, prolonged release, enhanced bioavailability, and reduced systemic side effects compared to conventional dosage forms. Various preparation techniques such as rotary film evaporation, reverse phase evaporation, ethanol injection, vortex-sonication, and freeze-thaw methods are employed for their formulation. Characterization parameters including vesicle size, zeta potential, entrapment efficiency, morphology, drug content, and in vitro permeation studies are essential for evaluating their performance. Transferosomes have shown promising applications in the delivery of corticosteroids, NSAIDs, peptides, proteins, vaccines, herbal drugs, and anticancer agents. Despite limitations such as oxidative instability and difficulties in phospholipid purification, transferosomes remain a highly efficient and promising carrier system for controlled and targeted transdermal drug delivery. Their ability to overcome the barrier properties of the skin makes them an advanced and effective approach in modern pharmaceutical research.

Keywords: Transferosomes, Transdermal drug delivery, Ultra-deformable vesicles, Phospholipids, Edge activators.

Introduction

Transferosomes

Transferosomes, even called as ultradeformable vesicles for applying to skin holding a lipid bilayer with phospholipids and edge activator along with aqueous layer. Based on the lipophilicity the active substance is enclosed with in core or amongst the bilayer. In comparison to liposomes, transferosomes are having a great capacity to touch whole deeper areas of skin once applied topically (Fernández *et al*., 2020).

The trademark Transferosome was registered by the German business IDEA AG and refers to a proprietary medicine delivery technology. The name translates to "carrying body" and is derived from the Latin word "transferre," which means "to carry across," and "some," which is a Greek word for "body." The transfersome is a synthetic vesicle that mimics the properties of exocytotic cell vesicles, making it ideal for controlled and potentially targeted medication delivery (Solanki *et al*., 2016).

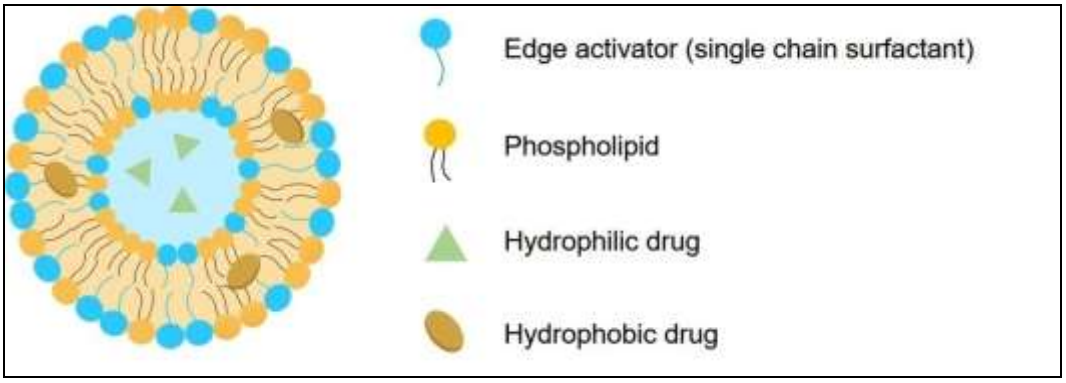

Figure: Structure of transferosomes

Composition of transferosomes

Transferosome, a lipidic vesicular carrier is composed of phospholipids which can either be of natural origin or can be synthetic in nature and edge activators30-33. The lipids after coming in contact with the aqueous environment, self-assemble to form a bilayer and in the process enclose a hydrophilic core in the centre. The edge activators or softening agents as they are also referred to as, added during the formulation steps, enhance the flexibility and deformability of the lipidic vesicle, by causing the lipid bilayer to destabilize. Their flexibility imparts them the ability to squeeze even through skin and membrane pores smaller than their own size without any rupture. Since transferosomes are made of lipids, both hydrophilic as well as lipophilic drugs can be

delivered by them. For imaging purposes, transferosomes can also be incorporated with dyes for example Nile red, rhodamone 123 etc (Solanki *et al*., 2016).

Table 2: Components used in the formulation of transferosomes

Components	Examples	Purpose
Edge Activator	Span 80, Tween 80, Sodium deoxy Cholate, Sodium Cholate	To impart flexibility to formed vesicles
Phospholipid	Phosphati dylcholine, Soya Phosphati dylcholine, Dipalmitoyl phosphate dylcholine	To form self-assembled vesicles
Solvents	Chloroform, Methanol, Ethanol	Solvent system to dissolve different components
Hydrating Agent	Distilled Water or Saline phosphate buffer Distilled Water	To hydrate the lipid film formed after evaporation of the solvent
Active Pharmaceutical ingredient	Miconazole Nitrate, Itraconazole, Ketoprofen, Diclofenac Sodium	For providing pharmacological effect

Mechanism of Action of Transferosomes

According to current research, transfersomesare drug delivery systems that can pass through undamaged skin. The lipid's interaction with water causes the lipid to attract water molecules, causing hydration, and the lipid vesicles to migrate to the water-rich concentration part. This change in watercontent across the stratum and epidermis of the skin increases the trans dermal osmotic gradient, allowing transferosomes to penetrate the skin. As a result of its self-optimizing deformability, once a transfersome reaches a pore depicts the mechanism in visual form for easier comprehension. If transferosomes are applied to the skin under non-occlusive conditions, they can easily permeate the skin. To establish the trans-epidermal osmotic gradient across the skin, the skin must be non-occlusive.

According to the literature, the Transferosomes' penetration mechanism is its moisture-seeking proclivity for deeper skin layers, also known as xerophobia

(hydrotaxis). The moisture loss from the transferosomal formulation upon application to the skin causes this moisture seeking behaviour (non-occlusive state).

The natural transdermal water activity variation across the skin layers creates a powerful force that activates the Transferosomes, causing the widening ofintercellular connections and the formation of transcutaneous channels with a diameter of 20-30nm. These pathways are created to allow ultra-deformable microscopic Transferosomes to pass through the epidermal layers furthermore, the osmotic gradient created by body heat evaporating moisture from the skin's superficial layers is used as a driving force to facilitate the flexible passage of therapeutic agents from the site of application to the specific area for local or system therapies in effective therapeutic concentrations with minimal systemic toxicity.

Transferosomes have a better penetration efficiency (through small skin pores) than traditional liposomes, but they have a similar bilayer structure that allows for the encapsulation of hydrophobic, hydrophilic, and amphiphilic medicines. Transferosomes differ from liposomes in that their non-natural membranes are softer, more flexible, and ultra-deformable (Rai *et al*., 2017).

Transferosomes are supramolecular units made up of a bilayer of amphipathic agent (phospholipid), and adding a bilayer unstiffening agent improves the elasticity and penetrability of the bilayer (edge activator). Alcohol is present in high or low concentrations in the formulations of several transferosomes as penetration enhancers and as solvating cosolvents. Vesicles are self-regulating and self-optimizing due to the shape of the lipid bilayer and the interdependency of local composition. Transferosomes can effectively and easily bypass many transport barriers in the body due to the existence of this feature.

Ethanol has been suggested as a way to modify the polar head area of the lipid bilayer. Following penetration, ethanol increases the fluidity of the intercellular lipid matrix and, as a result, the density of the lipid lamellae decreases. Transferosomes can pass through the stratum corneum and reach the dermis and blood flow, among other places.

The deformability of the transfersomal membrane, which can be linked to the vesicle compositions, determines their ibility to penetrate. As a result, thebest vesicle

compositions must be found by executing specially tailored experimental procedures for each medicinal drug in order to acquire the best carriers (Ascenso *et al*., 2015).

Methods to Prepare transfersomes

- **Rotary Film evaporation method**

  Modified hand shaking method is another name for this approach. In this approach, API, lecithin, and edge activator are solubilized in a 1:1 mixture of chloroform and ethanol by manual shaking at a temperature higher than the lipid's transition temperature, and the resulting liquid is maintained for evaporation to remove the organic solvent. The thin lipid coating is left overnight to allow complete removal of the organic solvent. The film is then hydrated by rotating it at 60 RPM for 1 hour at room temperature with a pH 6.5 buffer. The leftover vesicles swell for 2 hours at room temperature. Small vesicles were made from leftover vesicles that had been sonicated at room temperature (Marwah *et al*., 2016).

- **Reverse phase evaporation method**

  This method is carried out 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 (Malakar *et al*., 2012).

- **Vortex or sonication method**

  Edge activators and phospholipids are assorted by continuous swirling in order to disperse in phosphate buffer in this procedure. After forming a milky suspension, it is sonicated in a bath sonicator before being extruded through polycarbonate membranes (Sunitha and Anusha, 2020).

- **Ethanol Injection Method**

  This method is more beneficial than others. The medication and water

solution are warmed up at a consistent temperature with continuous agitation in this procedure. Phospholipids and edge activators are combined with an ethanolic solution in aqueous media and then reacted (Chaurasiya *et al*., 2019).

- **Freeze Thaw Method**

  Ethanol Injection MethodThis method is more beneficial than others. The medication and water solution are warmed up at a consistent temperature with continuous agitation in this procedure. Phospholipids and edge activators are combined with an ethanolic solution in aqueous media and then nitrogen bath at -300 degrees Celsius for 30 seconds. After the suspension has frozen, it is treated to a high temperature 8-9 rounds (Gupta *et al*., 2012).

Characterization of transfersomes

Drug excipient interaction study:

Fourier Transform Infrared spectrophotometer (FTIR) exhibits graphical representation of pure drug, a mixture of the soya lecithin and drug, and a mixture of excipients (Span 80, Span 60, Span 20, Tween 20, etc) and the drug. For the sample preparation potassium bromide is used and within a spectral range of 450−4000 cm^{-1} data are collected. If the graphical representation shows no overlap between individual components, proves that components are not interacting with each other (Lachman *et al*., 1986).

Preformulation studies of drug:

Before incorporating the drug in formulation, Preformulation studies are a vital detection process for the physical and chemical properties of the drug (Kaur and Saraf, 2011).

Physical appearance:

Organoleptic characteristics such as color, odor, and taste of the drug in powder form are examined (Sazoka and Papahadjopoulos, 1978).

Melting Point:

The melting point of the drug is detected with the help of a capillary method or digital melting point apparatus[26-28]. It is one of the important specifications for disclosing the purity of drugs. The drug sample becomes hot in a fuse capillary tube and the rate is 5ºC/min (Williams *et al*., 2001).

Solubility study:

Solubility studies are carried out with distilled water, 0.1N hydrochloric acid, methanol, ethanol, and Phosphate buffer pH 7.4 at room temperature (25±2º C). Solute or drug in excess amount is needed in solvent to produce supersaturation.

Then it is shaken mechanically for around 48 hours at 25ºC and equilibrium state is achieved. Membrane filter having porosity 0.45µ is used to filter the aliquots and solubility analysis is performed by UV spectrophotometer (Ravi *et al*., 2012).

pH measurement:

Digital pH meter is essential for pH detection of the formulation. In this method, a specific amount of drug in powder form is accurately weighed. Then it is dissolved in a certain amount of ethanol when the drug is insoluble in distilled water and made up the volume with distilled water by the sonication method using a sonicator. The solution is then filtered using filter paper and digital pH meter helps to determine the pH of the filtrate (Gupta *et al*., 2012).

Determination of Wavelength Maxima:

Accurately 10 mg of the drug is weighed and dissolved in 100 ml of phosphate buffer having pH 7.4. From this stock solution, 1 ml of is pipette out and by phosphate buffer having pH 7.4made up the volume up to the final mark. The scan value of the solution is detected by using a UV spectrophotometer.

Morphology and Structure of transfersomes

The structure and morphology of the transfersomes consisting of drugs are detected by using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Transfersomes are diluted in distilled water and a drop of the diluted suspension is mounted on a clean slide. Staining agent like 1% phosphotungestic acid (dissolved in distilled water), is used for better visualization or to increase the contrast of the image and observed after drying. An optical microscope is used for visualization of vesicles that are produced without sonication. Make a thin film by spreading the suspension on the slide and a coverslip is utilized to cover the sample. After drying it out, the sample is placed under optical microscope observation (Rathore and Duggal, 2012).

Zeta potential

Zeta potential was measured using a Horiba SZ100 spectrophotometer. The formulations were diluted with Millipore water before being subjected to measurements, and each measurement was performed in triplicate (Zaafarany *et al*., 2010).

Entrapment efficiency:

Centrifugation helps to calculate percentage entrapment efficiency. Phosphate buffer saline (PBS) with pH7.4 is needed to disperse different transfersomal formulations. A centrifuge is applied to centrifuge the formulations for 40 min at 10000 rpm. The supernatant (clear solution) is used for the determination of free drug after centrifugation. UV spectrophotometer is essential to detect the absorbance of that clear solution at the maximum wavelength of the drug.

Vesicle size and size distribution:

Previously, the vesicle size of tranfersome is determined without sonication by optical microscopy (a stage eyepiece micrometer) which is calibrated with a micrometer scale. After sonication, the Polydispersity Index (PDI) measurement is done by dynamic light scattering with the Zetasizer after sonication (Verma *et al*., 2003).

Drug content determination:

Transfersomes consist drug is mixed with gel, is transfersomal gel. The evaluation of drug content in transfersomal gel is performed by dissolving a certain amount of the formulation in a specific amount of ethanol for lysis of vesicles. Mix the solution properly by sonicator. The analysis is done by measuring the absorbance of the solution and the formulation against ethanol as a blank with a UV-Visible spectrophotometer at the maximum wavelength of the drug (Maurya, 2010).

Applications

- These are worthy transporter option to deliver the drug in to skin layers for treatment of dermal cancer.
- Delivery of drugs with high molecular weight through mucosal layers is possible
- Delivery of biologically active drugs and DNA using lipid vesicles.

- The ultra-deformable vesicles can be employed to overcome the drugs which are predicted to have GI side effects example, NSAIDs.
- These are employed in delivering the corticoseriods.
- Transfersomes can be used as carriers in delivery of interferons.
- To transport the peptides and as well as proteins transferosomes are good choice.
- Transcutaneous vaccines were shown better results in hepatitis B.
- These are applicable to deliver the herbal drugs, anticancer drugs (Solanki *et al*., 2016).

Advantages

- Transferosomalcarriers are made up of both hydrophilic and hydrophobic units, making them the only drug delivery system capable of delivering therapeutic compounds in a wide range of solubility.
- Because of their ultra-deformability and elastic qualities, transferosomescan squeeze through skin barrier constrictions that are thin, such as 5-10 times smaller than vesicle size.
- High vesicle deformability allows medications to flow through the skin without causing significant vesicle loss, and can be employed for both topical and systemic therapy.
- Regardless of size, shape, molecular weight, or polarity, these carriers are highly adaptable and efficient in accommodating a range of agents.
- As these units are prepared with natural phospholipids and edge activators. These are biodegradable and biocompatible.
- Transfersomes are used to transfer proteins and peptides, insulin, corticosteroids, inferferons, anaesthetics, NSAIDS, anti-tumor medications, and herbal remedies, among other active chemicals.Transferosomes are prime choice for obtaining a sustained drug release, as well as expectable and extended period of activity.
- These have the ability to boost transdermal flow while also improving bioactive agent site selectivity.

- Scaling up is simple due to the tiny and simple production method.
- Bypassing first-pass metabolism, which is a major flaw in oral medication administration, resulting in improved drug bioavailability.
- The entrapment rate of transferosomes is quite high in the case of hydrophilic medicines, reaching nearly 90% in some cases (Rajan *et al*., 2011).

Limitations of Transferosomes

- Because of their proclivity for oxidative stress, transferosome formulations are chemically unstable. When aqueous media is degassed and purged with inert gases such as nitrogen and argon, the oxidation of transferosmes is dramatically reduced.
- Another challenge of using transferosomes as drug delivery vehicles is obtaining the purity of natural phospholipids, which is difficult to produce. As a result, synthetic phospholipids could be used as a substitute (Bhasin and Londhe, 2018; Opatha *et al*., 2020).

Conclusion

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

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