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TRANSFEROSOMAL NASAL *IN SITU* GELS: A REVIEW

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ABSTRACT
The majority of medications are often administered orally or intravenously for quick effect, improved patient compliance and simplicity of medication administration. However, treating neurodegenerative and mental illnesses is extremely difficult due to the low bioavailability and restricted brain exposure of medications taken orally. As a result, the circumstance calls for drug delivery to the brain. Numerous parameters, including molecular weight, delivery method, drug's lipophilic nature and blood-brain barrier (BBB), are taken into account for brain targeting (BBB). These elements restrict the drug's ability to enter brain tissue via BBB. One of the potential solutions to these issues is intranasal medication administration, which avoids the BBB and reduces the dosage while improving drug exposure in the brain. For a targeted brain delivery system, transferosomes serves as the carrier. This drug delivery technique uses a precisely engineered artificial vesicle that resembles cell vesicles to distribute drugs in a regulated and possibly targeted manner. They share a similar phospholipid composition with liposomes, making them biocompatible and biodegradable. They are composed of edge activators such as sodium cholate, phospholipids like phosphatidyl choline and a little quantity of ethanol. This article provides a crucial overview of the characteristics, make-up, production methods, characterisation and uses of transfersomes as drug delivery vesicles.

KEYWORDS: Transferosomes, Nasal *in situ*, Brain targeting, Vesicular drug delivery.

INTRODUCTION
Drug delivery to the central nervous system (CNS) is still difficult to achieve in the creation of effective agents for central targets, primarily because the blood-brain barrier is impenetrable (BBB). The BBB restricts substrate penetration generally based on a number of factors, including lipophilicity, molecular size and selectivity for different ATP-dependent transport mechanisms. The BBB's endothelial cells express efflux transporters, such as P-glycoprotein (P-gp), which prevent numerous lipophilic substances, including potential therapeutic medicines, from reaching their intended target areas in the CNS. Numerous methods (such as using prodrugs, inhibiting efflux transporters, disrupting the endothelial tight junctions that, along with the cell membrane, form the physical barrier, and using nasal administration) have been investigated to lessen the effects of the BBB due to the critical importance of effective drug delivery to the brain. The purpose of this brief study is to highlight the nasal route's potential as a conduit for the preferred transport of therapeutic drugs to the brain.[1]

The Indian medical system of Ayurveda has long acknowledged intranasal therapy as a valid type of therapy. "NASYA KARMA" is another name for nasal therapy. One of the PANCHAKARMAS described in Ayurveda pertains to this one. It is the procedure used to give medication through the nostrils. When performed correctly and consistently, "Nasyakarma" can relieve conditions such as cervical spondylitis, headaches, facial paralysis, hemiplegia, disorders of the nose, frozen shoulder, mental illness, and skin issues. Nasyakarma will increase sense organ function and avoid head ailments (URDHWANGA).[2]

Early in the 1980s, the nasal route was introduced as a potential systemic delivery alternative to other traditional medication delivery systems. Because it is permeable to more compounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucous, and less dilution by gastrointestinal contents, nasal mucosa has been considered as a potential co-administration route to achieve faster and higher level of drug absorption. It is a helpful form of medication delivery for substances like proteins and peptides that are effective at low dosages and have no minimal oral bioavailability.[3]

Advantages of nasal drug delivery system[4]
- **Large surface area:** Nasal mucosa provides a sizable surface area for the accommodation of

medication or dose form, allowing for higher drug absorption.

- **High vascularity:** Nasal mucosa has a significant amount of blood vessels, which encourages massive medication absorption.
- **Rapid onset of action:** Due to the nasal mucosa's strong vasculation and huge surface area, medication is quickly absorbed and begins to work.
- **Non-invasive:** Nasal medication administration is a simple process. Individuals are able to self-administer medications.
- **Bypass BBB:** Medicines that are unable to cross the BBB can be administered via a nasal delivery device. The olfactory area of the nose, which avoids the BBB, is used to transport drugs from the nasal mucosa to the brain and CSF.
- **Better drug use with less side effects:** Drugs administered via the nasal route do not go through hepatic first pass metabolism; hence, fewer doses of the medicine are needed to cure a particular ailment, and adverse effects are also reduced.
- **Systemic effect and CNS drug delivery:** In addition to achieving targeted drug delivery to brain areas, medication administration by the nose has the potential to have systemic effects. Drug absorption through the respiratory region of the nose has systemic effects, and medication transport to the brain is correlated with this absorption.

Limitations Of Nasal Drug Delivery

- **Limited dose size:** The major drawback of nasal drug delivery is that only small doses may be given at once. This technique cannot be used to provide medications for which a significant dose is necessary to demonstrate their therapeutic impact. Per nostril, only 50–250µL of dosage can be administered.
- **Delivery of macromolecules:** Nasal mucosa cannot absorb molecules with a molecular weight of greater than 1000 Daltons.
- **Pathological conditions:** The pH and nasal secretions are altered by pathological disorders such as cold, flu, nasal allergy, and sinus infection. Effective medication administration through the nose is impossible under such circumstances.
- **Ciliary movement:** Ciliary movements cause mucus on the nasal surface to be expelled. It may shorten the duration a drug stays on the surface of the nasal mucosa, which may alter or lessen the drug's permeability.
- **Enzymatic degradation of proteins and peptides:** The inner nasal surface contains the enzymes exonuclease (mono and diamino peptidases) and endonuclease (serine and cysteine), which cleave peptide molecules at their N- and C-terminal residues as well as their centres.

When BBB is present, drug distribution into the brain tissue is poor, which prompts the development of other methods to deliver the drug to brain. Due to a special relationship between the nose and the central nervous system, medication molecules can be delivered to the brain tissues via the nasal route without passing through the BBB. The brain's olfactory nerve terminals offer a different route for medicines to enter the central nervous system. Based on the existing literature, it has been noted that when a medication is administered to rats via the nasal route, the resulting concentration is higher than usual.[5]

Pathways And Mechanisms Of Nasal Drug Delivery[6]

A growing body of evidence suggests that pathways involving nerves linking the nasal passages to the brain and spinal cord are significant, even though the precise processes behind intranasal medication transport to the CNS are still not fully known. Furthermore, routes via the lymphatic, vascular, and CSF have been used to transport chemicals from the nasal cavity to the brain. Depending on the qualities of neurotherapeutics, the features of formulations, and the administration method employed, a combination of these routes might be at play, though one pathway might prevail.

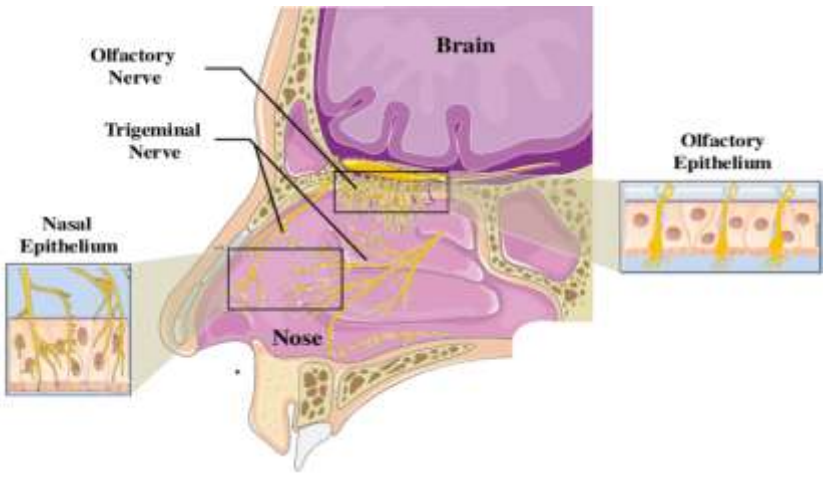

Fig. 1: Schematic diagram of Nasal drug delivery.

1. Olfactory nerve pathway

A drug must pass through the nasal olfactory epithelium and, depending on the pathway used, the arachnoid membrane enclosing the subarachnoid space before it may reach the CSF or brain parenchyma from the olfactory area in the nasal cavity. In theory, there are three possible routes across the olfactory epithelium:

a. **Transcellular pathway;** particularly across sustentacular cells, most likely by passive diffusion, fluid phase endocytosis, or receptor-mediated endocytosis. The likelihood of passive diffusion is higher for more lipophilic medicines. It is mediated quickly and frequently. Drugs that are lipophilic are transported via this pathway, and it exhibits rate dependence on the lipophilicity of such medicines.

b. **Paracellular pathway;** through clefts between sustentacular cells and olfactory neurons or the tight junctions between sustentacular cells. Most likely, diffusion through aqueous channels causes hydrophilic medicines to be absorbed through the nasal passages (pores). This route is passive and slow. This pathway is in charge of transporting hydrophilic medicines, and it exhibits rate-dependent response to drug molecular weight. Medicines up to 1000 Da in molecular weight without an absorption enhancer exhibit good bioavailability, which can be increased to pharmaceuticals up to 6000 Da in molecular weight with an absorption enhancer.

c. **The olfactory nerve pathway;** where the medication is picked up by endocytosis or pinocytosis processes and delivered to the olfactory bulb by intracellular axonal transport. Hence, transcellular passive diffusion, paracellular passive diffusion, carrier-mediated transport, transcytosis, and efflux transport are the several methods of drug delivery across the nasal olfactory epithelium.

2. Trigeminal nerve pathway

The trigeminal nerve, the largest cranial nerve, which innervates the respiratory and olfactory epithelium of nose passages and enters the CNS in the pons, is a vital nerve that connects nasal passages to the CNS. The trigeminal nerve also has a short segment that ends in the olfactory bulbs. The trigeminal nerve's ophthalmic division (V1), maxillary division (V2), or mandibular division (V3) transmit sensory data from the nasal cavity, oral cavity, eyelids, and cornea to the central nervous system. The latter has both sensory and motor function while the first two simply have sensory function. As neurons from these branches travel through the nasal mucosa directly, the ophthalmic and maxillary branches of the trigeminal nerve are crucial for the transfer of drugs from the nose to the brain.

3. Pathways involving cerebrospinal fluid and nasal lymphatics

A route for intranasally administered medications to the CSF and other parts of the brain connects the perineurial regions including olfactory nerves, nasal lymphatics, and the subarachnoid space, which contains CSF. According to studies, radiolabeled tracers injected into the CSF of cerebral ventricles or the subarachnoid space drain to the underside of olfactory bulbs and into channels connected to olfactory nerves that cross the cribriform plate, where they eventually reach the nasal lymphatic system and cervical lymph nodes. After intranasal delivery, these pathways ensure that medications reach the CNS and are distributed throughout the brain by travelling from nasal passages to the CSF, brain interstitial spaces, and perivascular spaces.

To achieve a quicker and greater amount of medication absorption, the nasal mucosa has been examined as a potential delivery route. The high degree of vascularization and permeability of the nasal mucosa piqued researchers interest in using the nasal route for systemic drug distribution.

Systemic Medication Delivery To The Brain Via Colloidal Carriers[7]

In general, colloidal drug carriers consist of polymeric nanoparticles, lipid-based nanoparticles, polymeric micelles, nanoemulsions, liposomes, and other substances. The purpose of using colloidal drug carriers for nose to brain drug administration is to improve the selectivity towards cell or tissue, increase the bioavailability of medications by boosting their diffusion through biological membranes, and protect them from enzymatic destruction.

a. Nanoparticles

Nanoparticles, which are primarily made of biodegradable polymers, have been widely used in targeted drug delivery because they significantly improve nose to brain drug delivery by preventing encapsulated drugs from degrading biologically or chemically and by facilitating extracellular transport via the P-gp efflux system. This makes medicines more readily available for use in the CNS. The polymers that have been shown to be biodegradable, biocompatible, and non-toxic include poly lactic acid (PLA), poly glycolic acid (PGA), poly lactide-co-glycolic acid (PLGA), poly ε-caprolactone (PCL), and poly methyl methacrylate.

Solid lipid-based nanoparticles first appeared in the early 1990s and have since shown promise for the delivery of specific drugs. Lipid nanoparticles are divided into the following kinds based on the generations through which they have evolved:

- Solid lipid nanoparticles (SLN)
- Nanostructured lipid carriers (NLC)
- Polymer-lipid hybrid nanoparticles (PLN)
- Lipid-drug conjugate (LDC)

b. Nanoemulsions

Miniemulsions, nanoemulsions, ultrafine emulsions, multiple emulsions, etc. are terms that are frequently used to describe emulsions with droplet sizes on the

nanometric scale (usually in the range of 20–200 nm). These nanoemulsions are stable against sedimentation or creaming and seem transparent or translucent to unaided sight. These qualities make nanoemulsions as carriers of great interest for theoretical research and real-world applications in numerous industries, including chemical, cosmetic and medicinal.

c. Polymeric micelles

Block copolymer synthesis have improved, creating polymeric micelles that could be used as nanoscopic drug carriers. They are referred to as block copolymer self-assemblies and are promising nanocarriers for the transport of drugs and genes. Biodegradable and biocompatible block copolymers have been used to create polymeric micelles for medication delivery. Core-shell structure is a defining characteristic of polymeric micelles.

d. Liposomes

Liposomes are lyotropic liquid crystals that have an aqueous core enclosed by one or more bilayers of natural or synthetic lipids. They are made of materials that are comparatively biocompatible and biodegradable. Since the 1970s, liposomes have been the subject of much research as drug carriers for enhancing the delivery of medicinal substances to certain areas in the body. There are several liposome-based formulations that are offered for sale commercially, which is evidence of the effectiveness of liposomes as drug carriers. By conjugating or cross-linking the targeting moiety to the native liposome or by altering the liposomal formulation's surface, liposomes can be used to target and deliver therapeutic medicines to a specific region.

Colloidal carriers offer a wide range of applications in the field of medication delivery. Neurological conditions are currently difficult to treat because drugs must pass through the BBB to reach the CNS. Several techniques have been proposed to circumvent this problem. Among these, the use of intranasal nanocarrier delivery methods has shown promising results since it avoids the BBB, enables fast access to the brain, and does away with the limitations associated with oral and intravenous routes. Also, because liposomes, SLNs, nanoemulsions, microemulsions, nanosuspensions, polymeric nanoparticles, dendrimers and transferosomes significantly reduce the risk of medicine degradation and improve brain targeting, it looks to be advantageous to include medications into these nanocarrier systems.

Transferosomes

Transferosomes, also known as transfersomes, are ultradeformable vesicles for transdermal applications consisting of lipid bilayers with phospholipids and an edge activator and an ethanol/aqueous core. Depending on the lipophilicity of the active substance, it can be encapsulated within the core of the lipid bilayer.[8] The elasticity and deformability of transfersomes enable them to adjust their shape and squeeze through the nasal cavity to deliver proteins, vaccines, and other compounds more effectively.[9]

Transfersomes are categorized under novel vesicular drug carrier systems consisting primarily of phospholipids, surfactants as key excipients. They also come under a class of modified liposomes which are highly deformable, elastic or ultra-flexible.[10]

Advantages[11]

- Transfersomes can accommodate drug molecules with a wide range of solubilities due to their infrastructure, which combines hydrophobic and hydrophilic moieties.
- They can flex and squeeze through spaces that are five to ten times smaller than their own diameter without suffering serious damage.
- This system's high deformability allows intact vesicles to be penetrated more effectively. Drugs of both low and high molecular weights, such as analgesics, anaesthetics, corticosteroids, sex hormone, anticancer, insulin, and albumin, can be transported via them.
- Due to the fact that they are created from natural phospholipids, much like liposomes, they are biocompatible and biodegradable.
- They have a high entrapment efficiency, up to 90% in the case of lipophilic drugs.
- They guard the encapsulated medication against metabolic breakdown. e.g., protein and peptides.
- They serve as a depot and release their contents gradually. They can be utilised for both systemic and topical medication administration.
- As the process is straightforward and avoids needless use of pharmaceutically inappropriate ingredients, they are simple to scale up.
- At first look, transfersomes resemble liposomes, a type of lipid bilayered vesicle. Yet, in terms of functionality, transfersomes are far more versatile and flexible than routinely employed liposomes.
- Transfersomes are a clear choice for achieving predictable and prolonged activity as well as a sustained medication release.
- They have the ability to improve the site specificity of bioactive substances and increase transdermal flow.
- By avoiding the first-pass metabolism, which is a significant disadvantage of oral medication administration, the drug's bioavailability is maximised.
- Reduce the negative effects of the medication, safeguard it from metabolic breakdown, and increase the usefulness of medications with brief half-lives.

Structure and Composition[12]

Transferosomes are mixed lipid aggregates that create the vesicles. They are made of phospholipids like

phosphatidyl choline-an amphipathic component, and an edge activator, a substance that softens lipid bilayers.

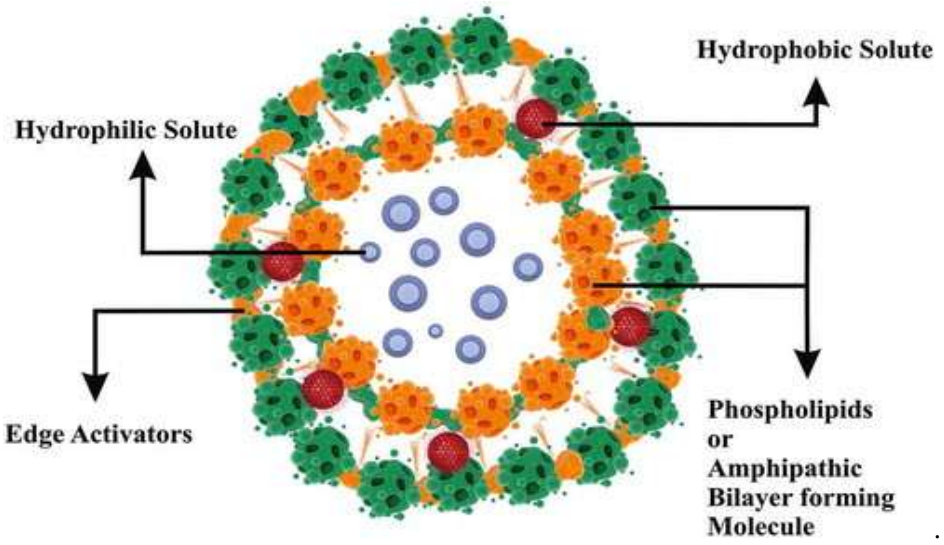

Fig. 2: Structure of Transferosome.

Phospholipids: Phospholipids, such as soya phosphatidylcholine, egg phosphatidylcholine, and dipalmitoyl phosphatidylcholine, are the primary constituents of the vesicles. A 10–25% surfactant is added for flexibility, along with different solvents like ethanol and methanol and a hydrating medium made of saline phosphate buffer (pH 6.5-7). Dyes like Rhodamine 123, Nile red etc., can be used.

Phosphatidylcholine is a fatty substance that can come from either animal or plant sources and is primarily an unsaturated fatty acid. Up to 70% of the total fatty acids in these unsaturated fatty acids are linoleic acid.

Soy phosphatidylcholine, which has a very low phase-transition temperature of below 0°C in water-containing systems, is primarily responsible for the ability of phospholipids to fluidize the lipid bilayer. This property can be observed by measuring the increase in trans epidermal water loss (TEWL) after application for a brief period of time.

Edge Activators: The edge activator, also known as a "bilayer softening component," is introduced to increase the permeability and flexibility of lipid bilayers. It may be a biocompatible surfactant or an amphiphilic medication.

An edge activator primarily consists of a single chain non-ionic surfactant that causes the lipid bilayer to become less stable, enhancing the fluidity and flexibility of the layer. The transferosome membrane's flexibility can be altered by properly combining the right surface-active chemicals.

The most common edge activators used in transferosome preparations are sodium cholates, sodium deoxycholate, Tweens and Spans (Tween 20, Tween 60, Tween 80; Span 60, Span 65, and Span 80), as well as dipotassium glycyrrhizinate. These surfactants are biocompatible, increase the vesicle's bilayer flexibility, as well as improve permeability.

Method of Preparation

- **Thin film hydration technique:**[13] A flask with a round bottom and a 2:1 solvent mixture of methanol and chloroform is used initially. The interior layers of the flask are then coated with the produced lipid layer after known quantities of Phospholipid, Drug, and the Edge Activator (EA) of choice have been dissolved in a solvent mixture and allowed to evaporate at 45^0C at 60 rpm (rotary evaporator). Vacuum is used to remove any extra solvent. Once a thin coating has developed, it is reconditioned with phosphate buffered saline (PBS) with a pH of 6.4 and left at room temperature for 60 minutes. The generated vesicles are allowed to swell for two to three hours at room temperature. After being sonicated for 15-20 minutes to reduce their size, the produced vesicles are then placed in storage at 40°C.
- **Reverse phase evaporation technique:**[14] In a clean beaker, a specified quantity of lipids (cholesterol and soy lecithin) is ingested. Then, Tween 80 is added as a surfactant to the same beaker and is dissolved in a 3:1 solvent solution of ether and chloroform. Lipids and surfactants are kept in a 1:1 ratio. The beaker is left at room temperature for 24 hours in order to form the thin film. The final thin film is then sonicated by a probe sonicator for 5 minutes at ambient temperature in 2 ml of phosphate buffer saline (pH 6.4). The film is hydrated in 2 ml of phosphate buffer saline (pH 6.4) using sodium deoxycholate as an edge deactivator, and then further sonicated for 10 min to obtain highly deformable vesicles as transferosomal suspensions

containing drug. Formulated transferosomal suspensions are passed through Whatman® filter paper (No.40).

- **Modified hand shaking technique:**[15] The drug, lecithin (PC), and edge activator are dissolved in a 1:1 mixture of ethanol and chloroform. By evaporating organic solvent while shaking hands above the lipid transition temperature (43°C), organic solvent is eliminated. Rotation caused a thin lipid coating to develop on the flask wall. For the solvent to completely evaporate, the thin film is left on all night. The film is then gently shaken for 15 minutes at the appropriate temperature while being hydrated with phosphate buffer (pH 6.4). Up to an hour at 2-8°C, the transfersome suspension continued to hydrate.
- **Reverse evaporation-Vortexing sonication technique:**[16] Drug is dissolved in a (2:1) chloroform/methanol solution together with a surfactant and soy lecithin, and the mixture is vortexed for 10 minutes. In a desiccator, the resultant mixture is let to dry thinly for 24 hours at room temperature. With the exception of oleic acid, which is added with soya lecithin, the obtained film is hydrated with 10 ml of simulated nasal fluid (SNF, pH 5.5) containing permeation enhancer (PE). The resulting vesicles are then allowed to swell for two hours at room temperature before being vortexed for 20 minutes. The produced big vesicles are extruded through a 0.2-m Sartorius membrane filter three times to further reduce their size after being sonicated by a probe sonicator for 20 min. The vesicles are separated using a cooling centrifuge for 3 h at 20,000 rpm at 4°C, then washed with simulated nasal fluid.
- **Suspension homogenization process:**[17] This method involves combining an appropriate amount of an edge-active chemical, such as sodium cholate, with an ethanolic soybean phosphatidylcholine solution. A total lipid concentration is produced by mixing this prepared solution with Triethanolamine-HCl buffer. The resulting suspension undergoes two or three cycles of sonication, freezing, and thawing.
- **Centrifugation process:**[18] In this procedure, alcohol is used to dissolve the medication, surfactants, and phospholipids. The solvent is then eliminated using rotary evaporation at 40°C under reduced pressure. In vacuum, the last remnants of the solvent are eliminated. Centrifuging at 60 rpm for an hour at room temperature hydrates the deposited lipid layer with the proper buffer. The resultant vesicles swell for two hours at room temperature. The resulting multi-lamellar lipid vesicles are sonicated again at room temperature.
- **Aqueous lipid suspension process:** The vehicles used in this procedure have a predetermined Drug-to-Lipid Ratio between 1/4 and 1/9. The composition is favoured based on the specific formulation type. In contrast to the typical phosphatidylcholine vesicles in the fluid phase, this would guarantee the great flexibility of the vesicle membrane. In particular, soyphosphatidylcholine is used to create vesicles with a size distribution that has a standard deviation of roughly 30% and a range of 100–200 nm. The lipids could be suspended in an aqueous phase in which the medication is dissolved to create this formulation.
- **High pressure homogenization technique:**[19] In PBS or distilled water including alcohol, the phospholipids, edge activator, and drug are equally disseminated before being concurrently swirled and ultrasonically shaken. The mixture is then shaken intermittently with ultrasonic energy. A high-pressure homogenizer is subsequently used to homogenise the resulting mixture. The transfersomes are then kept in the proper conditions for storage.
- **Ethanol injection method:**[20] The phospholipid, edge activator, and lipophilic drug are each dissolved in ethanol with magnetic stirring for the appropriate amount of time, until a clear solution is obtained, to create the organic phase. The water-soluble materials are dissolved in the phosphate buffer to create the aqueous phase. This stage allows for the introduction of hydrophilic drugs. The temperature of both solutions is raised to 45–50^{O}C. After that, the aqueous solution is continuously stirred while the ethanolic phospholipid solution is added dropwise. The process of removing ethanol involves putting the resulting dispersion into a vacuum evaporator and then sonicating it to reduce the particle size.
- **Freeze thaw method:**[21] Multilamellar vesicles are subjected to this method's alternating cycles of extremely low temperature for freezing and very high temperature exposure. The gear-up suspension is put in a tube and submerged for 30 seconds in a nitrogen bath at around 30 degrees Celsius. It is subjected to a high temperature in a water bath after it has frozen. There are eight to nine repetitions of this course.

Characteristics of Transferosomes

Precise and reproducible quality control measures are required to access the Transfersomes formulation's *in vitro* and *in vivo* behaviour.

Morphological Study:[22] Optical microscopy with a 45x resolution is used to confirm the vesicle development. The development of vesicles is detected in the dry thin film of Transferosomal suspension that has been spread on a glass slide and fixed by drying at room temperature. A digital camera is used to take microphotos of the transferosome under the microscope. Using a scanning electron microscope, the precise surface characteristics of the chosen transferosome formulation are seen.

Mean Vesicle Diameter and Zeta Potential Measurement:[23] Using the Malvern Zeta Sizer, dynamic light scattering (DLS) is used to evaluate the

average vesicle size and size distribution of drug-loaded transferosomes. As assessed by electrophoretic mobility, the Zeta potential (Surface Charge), which shows the stability of the transferosomes, can be characterised as an electrokinetic potential. Malvern Zeta Sizer is used to assess the relevant zeta potential after diluting the sample with water.

Determination of Percentage Entrapment Efficiency by Centrifugation Method[24]

Determination of unentrapped drug: Two centrifugal tubes each containing 10 ml of transferosomal material are spun at 15,000 rpm for an hour at 40°C using a Remi cooling centrifuge. The crystal-clear supernatant is decanted, and the precipitate that formed was mixed with 5 ml of phosphate buffer with a 6.4 pH for 30 minutes under comparable circumstances. To verify that all of the unentrapped medication is removed, the suspension is decanted and the procedure is repeated with the addition of 5 ml of phosphate buffer. The amount of medication that is not entrapped is calculated at 376nm.

Determination of entrapped drug: For the purpose of determining the amount of medication that is entrapped, 2ml of transferosomal solution is combined with 2ml of 10% triton x-100 solution and 2ml of 6.4 pH phosphate buffer. This mixture is then centrifuged at 15,000 rpm for 30 minutes at 40°C. Vacuum filtering is used to filter the contents through a 0.22 membrane filter. A UV spectrophotometer is used to evaluate the filter at 376nm with the appropriate dilutions.

$$\% \text{ Entrapment efficiency} = \frac{W-S}{W} \times 100$$

Where, W is the amount of entrapped and unentrapped drug.
S is the amount of unentrapped drug.

Number of Vesicles Per Cubic mm:[25] The optimization of the transfersome composition and other process variables depends on this parameter. Unsonicated transferosomal formulations are diluted five times using 0.9% sodium chloride. This sample is examined using an optical microscope and a hemocytometer. By using an optical microscope, the transfersomes with vesicles larger than 100nm can be seen. The number of transfersomes in small squares are counted and calculated using the following formula:

Total number of transferosomes per cubic mm

$$= \frac{(\textit{Total number of transferosomes counted} \times \textit{Dilution factor} \times 4000)}{\textit{Total number of squares counted}}$$

Degree of Deformability or Permeation Measurement:[26] This is an important characteristic since it affects how well the transferosomal formulation penetrates the body. Pure water is the standard employed in this inquiry. A sequence of microporous filters with known pore sizes between 50 and 400 nanometers are used to filter the solution. DLS measurements are utilised to capture the particle size and size distribution after each pass.

The degree of deformability is expressed as:

$$D = J \frac{rv}{rp}$$

Where,
D = Degree of deformability
J = Amount of suspension extruded within 5min
rv = Size of vesicle
rp = Barrier pore size.

Turbidity and Surface Charge Measurement[27]

a) **Turbidity:** With a nephelometer, the turbidity of any medication in aqueous solution is measured.
b) **Surface Charge and Charge Density:** To ascertain the surface charge and charge density, a zeta sizer is employed.

- **Penetration Ability:** To assess transfersome penetration, fluorescence microscopy is typically used.
- **Physical Stability:** This involves calculating the initial percentage of the drug that is entrapped in the formulation and storing transfersomes in sealed glass ampoules. For at least three months, the ampoules are stored at 4±20°C (refrigeration), 25±20 °C (room temperature), and 37±20 °C (body temperature). After 30 days, samples from each ampoule are collected and tested to identify medication leakage. The initial drug entrapment is assumed to be 100% when calculating the percent drug loss.
- **Occlusion Effect:**[28] Occlusion of the skin is thought to be beneficial for drug absorption in the case of conventional topical treatments, but it is unfavourable for elastic vesicles. In transfersomes, the primary driving force for permeation through the skin, from its comparatively dry surface to its water-rich deeper parts, is hydrotaxis (movement in the direction) of water. Occlusion influences hydration forces because it stops water from the skin from evaporating, hence research is crucial.
- **Confocal Scanning Laser Microscopy Study:** Skin sample fixation, sectioning, and staining issues arise in conventional light microscopy and electron microscopy. Confocal scanning laser microscopy (CSLM) helps reduce errors caused by misinterpretations since the structures to be investigated are frequently incompatible with the corresponding processing procedures. This approach incorporates lipophilic fluorescent markers into the transfersomes, and the light these markers emit is employed for the following purposes:

✓ To learn more about how transfersomes penetrate the skin,
✓ To identify the skin's histological organisation (epidermal columns, interdigitation) and the shapes and the architecture of the skin penetration pathways.

- ✓ To examine and contrast the ways that liposomes, niosomes, and micelles penetrate the body.

***In vitro* Drug Release Study:**[29] Using a cellophane membrane, the *in vitro* drug release patterns of all transfersomes are investigated. During the drug release research, a vertical Franz diffusion cell is employed. The cellophane membrane is installed on a diffusion cell assembly with a 2 cm^2 effective diffusion area after it had been immersed in a suitable buffer for 24 hours. The receptor fluid is stirred at 100 rpm and kept at a constant temperature of 37±0.5°C throughout the trials. The receptor compartment contained 15 ml of phosphate buffers with a particular pH. The membrane in the donor compartment received 1 ml of the prepared mixture. A 1 ml sample is taken out at regular intervals. Fresh diffusion medium is added in an equivalent volume right away.

Applications[30]

Due to the integration of phospholipids, transfersomes have the potential to increase the stability of labile pharmaceuticals and allow for the regulated release of delivered drugs. They can be easily administered by various routes such as intravenously, orally, transdermally and intranasally.

Following are some areas where this nano-vascular drug delivery system is being used:

- **Delivery of Anticancer Drugs:** In one study involved in the topical treatment of melanoma using paclitaxel-containing oligopeptide hydrogels transfersome-embedded by the thin-film dispersion method. It has been demonstrated that transfersomes made of phosphatidylcholine, tween80, and sodium deoxycholate can successfully enter tumour tissues.
- **Delivery of Anti-Inflammatory drug:** A number of research teams produced and investigated topical transfersomes including diclofenac sodium, celecoxib, mefenamic acid, and curcumin. According to research, transfersomes may increase the stability and effectiveness of anti-inflammatory medications.
- **Delivery of NSAIDs:** The use of transfersomes for transdermal administration can help treat NSAID side effects such Gastrointestinal discomfort. Transfersomes have already been used to test the efficacy of some medications, including diclofenac and ketoprofen, and the formulation of ketoprofen has already received regulatory agency approval in Switzerland.
- **Delivery of Proteins and Peptides:** With the aid of transfersomes, heavy molecules with large molecular weights can be moved across the skin with ease. For instance, mammalian skin can be used to transmit insulin and interferons like leukocytic generated interferon (INF). They have long been employed as a means of transport for various proteins and peptides.

Proteins and peptides are complex biogenic molecules that are challenging to transport into the body, disintegrate in the GI tract, suffer from transdermal toxicity due to their large size, and totally degrade when administered orally. The bioavailability of proteins acquired with the use of transfersomes is essentially identical to that obtained through the subcutaneous injection of a protein suspension. When applied frequently to the epicutaneous surface, the transfersome formulation also generated potent immune responses. An illustration of this is an adjuvant immunogenic serum albumin that, in spite of numerous cutaneous obstacles, is immunologically active using injections of both proteotransfersome preparations and transfersome carriers.

Interferons, such as INF-α, have also been transported via transferosomes. INF-α is a naturally occurring protein with antiviral, antiproliferative, and some immunomodulatory properties. Transferosomes as drug delivery systems have the potential to increase the stability of labile pharmaceuticals and provide controlled release of the medication delivered.

Delivery of Corticosteroids: Due to their site specificity and general medication safety when used topically, transfersomes are employed for the delivery of corticosteroids. Transfersome-administered steroids are physiologically active at doses that are far lower than those required by other common formulation methods.

In the recent past, numerous researchers have also attempted delivery of medications to the CNS through the nose. The formulations' inadequate contact with the nasal mucosa is the main drawback of nasal route delivery, though. There have been numerous initiatives in recent years to lengthen the duration that medication formulations stay in the nasal cavity, which has increased nasal drug absorption, one among them is *in situ* gels.

IN-SITU GELS[31]

In situ means "in position" or "in its original spot" in Latin. The creation of continuous and controlled medication delivery systems has received more focus over the past 30 years. It has been extensively studied how to design polymeric systems like *in situ* gels. A liquid formulation that produces a solid or semisolid depot after administration is known as *in situ* gel formation in the context of drug delivery systems.

Systems that create *in situ* activated gels are those that change into a gel phase when subjected to physiological circumstances. The earliest mention of this novel idea of producing a gel in place dates back to the early 1980s. Gelation results from the cross-linking of polymer chains, which can be done chemically (covalent cross-linking) or non-chemically (non-covalent bond formation) *(physical cross-linking).*

In situ gel may be administered orally, ocularly, intravenously, rectally, vaginally, and via injection. The transitional state known as "gel" is made up of physically crosslinked networks of long polymer molecules, with liquid molecules being contained within a three-dimensional polymeric network that has been swelled by a solvent. This system is a gel under physiological conditions and a liquid aqueous solution prior to injection. Reproducible prolonged and sustained drug release is made possible by the *in situ* gel's biocompatibility, incredible stability, and consistent medication dosages, which improves accuracy.

The *in situ* gel drug administration methods include oral, ophthalmic, vaginal, rectal, intravenous, intraperitoneal, and others. A new dosage form called *in situ* gel has recently been used in nasal medication delivery. In contrast to other liquid nasal formulations, nasal *in situ* gels are administered as low viscosity solutions into the nasal cavity. Upon contact with the nasal mucosa, the polymer changes conformation and produces a gel, which not only extends the contact time between the drug and the absorptive sites within the cavity but also releases the drug slowly and continuously. As a result, it is particularly helpful for medications that are taken on a long-term basis. The phase transition can be induced by a shift in pH, a shift in temperature or by the presence of cations.

CONCLUSION

As an alternative route for the administration of medications and biomolecules that undergo enzymatic or acidic degradation, go through first-pass hepatic metabolism, are insufficiently absorbed in the GIT, or have unfavourably slow effects when taken orally, nasal drug delivery is a rapidly developing field. The nasal route avoids the bioavailability problems brought on by the aforementioned considerations and has the benefit of regulated drug delivery for prolonged periods of time. Patient compliance, which *in situ* gels can provide, is closely related to the success of a controlled release solution. Using polymeric *in situ* nasal gels for controlled medication release has many benefits over traditional dosage forms and is a dependable, non-invasive method of drug delivery. Furthermore, the incorporation of transferosomes makes it a more adaptable preparation for drug delivery via the nasal cavity.

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