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TRANSFERSOMES: NOVEL APPROACH FOR INTRANASAL DELIVERY

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ABSTRACT

Nasal route is highly promising for the delivery of drugs exerting local effects in the nose or for therapeutic molecules having systemic or CNS effect. This is attributed to the fact that the nasal epithelium is highly vascularized and permeable, which ensures rapid absorption of the drug. The limitation of short residence time of the formulations in the nose and poor bioavailability of hydrophilic drugs could be overcome by the inclusion of transfersomes. Transfersomes are ultra-flexible lipid bilayer vesicles that have the ability to penetrate the skin. It consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties resulting from the incorporation of "edge activators" into the vesicular membrane. Its ability to cross the membranes has been intensively studied as a result of their elasticity. It enhances the penetration of most of the low as well as high molecular weight drugs. Can be evaluated by entrapment efficiency, vesicle shape, size and diameter; etc. The use of transfersomes carrier results in delivery of high concentration of active agents through the nose, regulated by system composition and their physical characteristics. Thus, this novel technique has got a great potential for overcoming current problems faced by the conventional techniques. However, the attention to study the effect of transferosomes elasticity on brain delivery by the nasal route has not been yet studied.

KEYWORDS: Transfersomes, Elasticity, Intranasal Delivery.

INTRODUCTION

The oral route is the most convenient and popular route for drug delivery. Despite the popularity of oral route alternative routes such as trans-mucosal delivery have been extensively investigated for drugs lacking effective systemic absorption via the gastrointestinal tract (GIT), therapeutic agents having chemical instability in the GIT fluids, drugs that undergo first-pass hepatic deactivation and therapeutic molecules which cause GIT adverse effects. Alternative routes have been investigated such as intra-nasal and parenteral in order to achieve faster and higher drug absorption and hence offering improved drug bioavailability, enhanced therapeutic effect and promoted patient compliance. Significant enhancement in the drug absorption following nasal administration compared to oral delivery has been demonstrated (Dhakar et al., 2011). However, for improvement of intranasal drug absorption with molecules larger than 1000 Daltons, permeation enhancers are needed in the formulation (Ozsoy et al., 2009).

Nasal delivery is appropriate for administration of drugs to treat local nasal diseases such as sinusitis and allergic rhinitis since low doses are sufficient to provide therapeutic effects with low systemic side effects. In addition, nasal delivery might be suitable for drugs which are effective in low doses and have low oral bioavailability (Dhakar et al., 2011). The rate of absorption, and the pharmacokinetic properties of small drug molecules used for systemic therapeutic effects, administration via the nose are comparable to the parenteral route due to the nature of the nasal pathway which provide a large surface area, rich vascularity and drug high permeability (Illum, 2003; Dhakar et al., 2011). Nasal delivery is also applicable in emergency cases (Bitter et al., 2011).

Despite the nose has lower surface area compared to the lung, it provides an efficient site for systemic absorption particularly for drugs with low water solubility. Many drugs do not reach the brain following oral or parenteral administration due to the blood-brain barrier (BBB). The olfactory region of the nose is not covered by the blood brain barrier, hence evasion the BBB following nasal administration might be possible. This approach is effective for drug targeting to the brain.

Table No. 1: Advantage and limitations of nasal route (Behl et al., 1998; Singh et al., 2012).

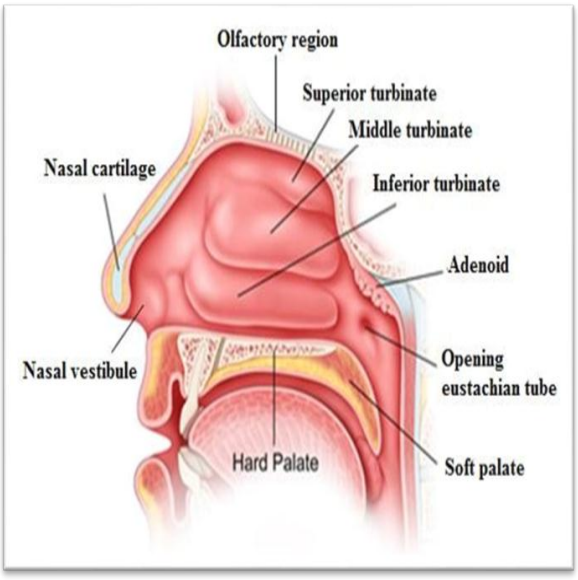
ADVANTAGES	LIMITATIONS
Suitable for drugs that are acid labile in the stomach	Volume that can be delivered into nasal cavity is restricted to 25–200 µl
Applicable for drugs that undergo extensive hepatic first-pass effect	High molecular weight compounds cannot be delivered through this route (mass cut off ≈1 kDa)
Rapid drug absorption and onset of action	Adversely affected by pathological conditions of the nose
Offers higher drug bioavailability, thus lower doses of drug are needed	Large interspecies and patient to patient variabilities are observed when using this route
Offers large surface area for drug absorption (approximately about 150 cm_2)	Normal defense mechanisms like mucociliary clearance can affect the absorption of drug
No particular skills or expertise are required for nasal drug administration	Local enzymes in the nasal cavity might degrade some drugs
Direct transportation of drug to the systemic circulation or CNS is possible	Local side effects like irritation might be happen
Offers lower risk of overdosing	Small surface area compared to the GIT
Needle-free and patient friendly	Nasal congestion from colds and flues may interfere with efficient drug delivery via this route
Offers induction of immune response when used for vaccine delivery	Frequent delivery of drugs may cause mucosal damage, hence patient becomes liable to microbial invasion through the nasal epithelium

Transferosomes are ultra-flexible lipid bilayer vesicles that have the ability to penetrate the skin intactly. Each transferosomes consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties resulting from the incorporation of "edge activators" into the vesicular membrane.

The ability of transferosomes to cross the skin as a result of their elasticity has been intensively studied. However, the attention to study the effect of transferosomes elasticity on brain delivery by the nasal route has not been yet studied. Hence the aim of the present study is to investigate the presence of possible correlation between vesicle elasticity and the amount of drug reaching the brain through nasal route.

Anatomy of the nose

The nasal passage is 12-14 cm deep and runs from the nasal vestibule to the nasopharnyx. It has three main regions; vestibular, respiratory and olfactory regions. The nose has a volume of 16-19 cm^3 and a surface area of approximately 180 cm^2 with two cavities (i.e. nostrils) separated by the nasal septum. The vestibular region is located at the front opening of the nasal passages which filters out particles from the inhaled air. However, drug delivery and absorption in this region is least important. This area is covered with hairs which filter the air to prevent air- borne particles entering the respiratory system. The respiratory area is large with a high degree of vascularity and a surface area of about 130 cm^2. In this region the majority of drug absorption occurs.



It is lined with pseudo-stratified columnar epithelium and covered with a dense layer of mucus which moves towards the posterior apertures of the nasal cavity because of the ciliary rhythmatic movements. The olfactory region is important for transporting the drug to brain and cerebro-spinal fluid and has a surface area of about 15 cm^2. It is made of thick connective tissue and lamina propria, into which the olfactory epithelium rests. The thickness of nasal mucosa ranges between 2 and 4 mm. The epithelium cells line the nasal passage and are covered by a mucus layer 5µm in thickness which traps unwanted particles. The mucous secretion consists of water (95%), mucin (2%), salts (1%), proteins (1%) such as albumin, immunoglobulin, lysozyme and lactoferrin, and lipids (1%). IgA, IgE and IgG are also present in the mucous secretion (Ozsoy et al., 2009). The pH of the nasal secretion is ranged from 5 to 6.5 (Ugwoke et al.,

2005). Ciliary action is responsible for clearing the mucus layer from the nasal cavity and mucus is renewed 4 - 6 times per hour. The mucus moves through the nose at a rate of 5-6 mm/min (Graf, 1986). The characteristics of human nasal epithelium are summarized in Table.

Table No. 2: Human nasal epithelium characteristics (Pires et al. 2009).

Nasal regions	Histology of nasal epithelium	Functions	Surface area	Blood supply (i.e.vascularity)	Permeability
Vestibule	Stratified squamous & keratinized epithelial cells with nasal vibrissae	Support & protection	≈ 0.6 cm^2	Low	Poor
Atrium	Stratified squamous cells & pseudostratified cells	Support	---	Low	Reduced
Respiratory Region	Columnar non-ciliated cells	Support to nasal function	≈ 130 c m^2	Very high	Good
	Columnar ciliated cells	Clearance of particles			
	Globet cells	Secretes mucus			
	Basal cells	Progenitors of other cell types			
Olfactory Region	Sustentacular cells,	Support bears synthetic olfactory	≈ 15 cm^2	High	Direct path to CNS
	Receptor cells	Olfaction sensation			
	Basal cells	Progenitors of other cell type			

Applications of nasal delivery

i) Local effects

The nose is exploited to treat regional disorders at relatively low effective doses with less systemic effects. Low molecular weight water-soluble or hydrophobic drugs are used to treat local pathological conditions in the nose. For example, Beclometasone (Ghimire et al., 2007) is an anti-inflammatory corticosteroid used to reduce inflammation and local allergy. It is a well-established drug for the treatment of allergic rhinitis. Nasal decongestants such as oxymetazoline are also administered via the nose for treating common colds (Pires et al., 2009).

ii) Systemic effects

Nasal delivery is convenient for acid labile drugs, proteins and peptides when rapid action is required such as in migraine relief. Nasal delivery offers a rapid action and efficient drug absorption compared to oral and intravenous delivery. Most protein and peptide drugs have low bioavailability (1–2%) due to their high molecular weight and polarity, causing poor absorption through the nasal mucosal membranes. In contrast, the bioavailability of progesterone and propranolol via nasal epithelium is comparable to parenteral administration (O'Hagan and Illum, 1990). Lower bioavailability can be improved by using absorption enhancers in the formulations, thus prolonging the contact time of the drug with the mucous membranes using bioadhesive agents. A significant change in the relative bioavailability of isosorbide dinitrate was observed using 0.1% N-succinyl chitosan as absorption enhancer (69.85%) compared to the 0.5% chitosan (55.36%) and control groups (43.32%) in rats (Na et al., 2013). Steyn and co-workers have reported that the bioavailability of recombinant human growth hormone was increased significantly after nasal delivery in combination with N-trimethyl chitosan chloride as an absorption enhancer used in pheroid technology (Steyn et al., 2010).

iii) Vaccines delivery

Vaccines and their applications via nose to treat respiratory infections have been investigated. The localization of immune system components in the mucosal membrane means that the respiratory epithelium is the first defence line in the body against infections (Bitter et al., 2011). Nasal mucosa is further enriched by lymphoid tissue. It enhances the systemic levels of specific immunoglobulin G and nasal secretary immunoglobulin A and the local immune responses which provide additional protection against invading microbes. Nasal mucosa is advantageous for immunization due to its permeability, low enzymatic activity and accommodation of the nose-associated lymphoid tissue (NALT) (Bitter et al., 2011). The delivery of vaccine via the nose represents a convenient needle-free procedure for vaccination. Furthermore, the nose-associated lymphoid tissue (NALT) is an effective immune system (Brandtzaeg, 2011). Nasal vaccines that have been investigated include influenza A and B (Huang et al. 2004). Commercially available nasal vaccines include nasal spray of Human influenza vaccine (FluMist®) and nasal drop of Equine influenza vaccine (Flu Avert®) manufactured by Medlmmune Inc. and Heska respectively.

iv) CNS delivery

The intranasal route is promising for the delivery of drugs to the brain via the exploitation of the olfactory neuroepithelium in the nose (Figure 1.4a), possible pathways to transfer drugs from nose to the brain have been explained by Illum, (2000) (Figure 1.4b). This strategy has been considered for the treatment of Alzheimer's disease, brain tumours, epilepsy, pain and sleep disorders (Pires et al.,

2009). Delivery of nerve growth factor to the brain in rodents has been reported (Frey et al., 1997; Chen et al., 1998) and in humans studies insulin and proteins (Pietrowsky et al., 1996) have been directly transferred through olfactory path to the CNS via nasal cavity. Successfully transnasal delivery 0.5mg/kg of siRNA to the CNS with highly brain concentration compared to the other tissue has been reported by Malhotra co-workers to treat neurological disorders using peptide-tagged PEGylated chitosan nanoparticles formulations to deliver siRNA via nose.

Pathway of drug from brain to nose

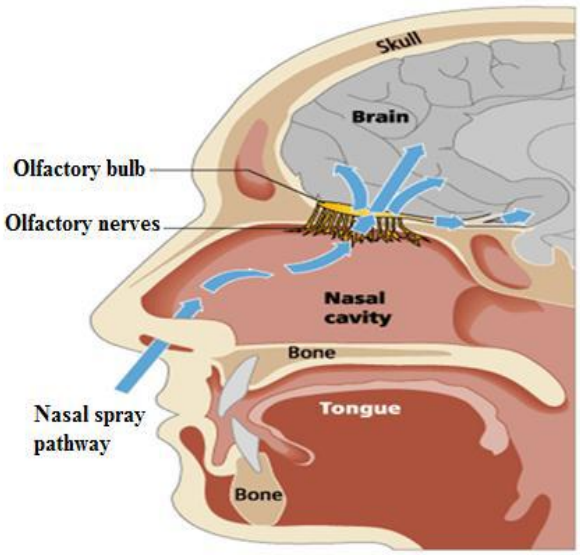


Figure 2: (a) Nose to brain pathway

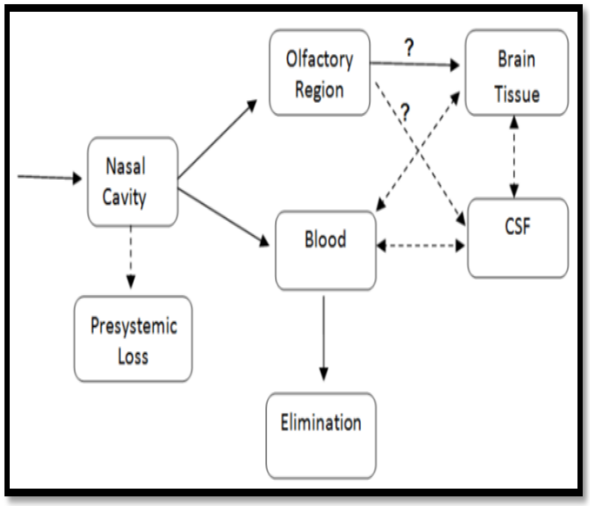


Figure: 2(b) A schematic illustration of the possible drug molecule transfer delivered nasally.

(---) indicates limited substrate delivery via this route
(?) Indicates the exact pathway is unclear (Illum, 2000).

Mechanisms of drug transport following intranasal administration

The drug must pass through the mucus layer of the nasal cavity for absorption to take place. Uncharged molecules pass through the mucus much more readily than charged molecules. Two main mechanisms of drug absorption through nasal mucosa have been proposed: paracellular absorption and transcellular absorption (Figure).

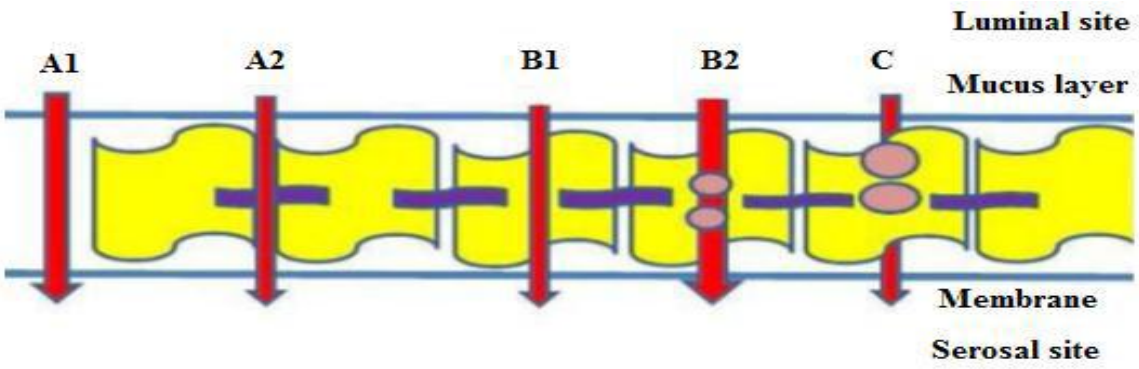


Figure 3: Mechanisms of drug transport across epithelial cells.
[A1] Intercellular spaces [A2] Tight junctions
[B1] Passive diffusion [B2] Active transport
[C] Transcytosis (Adapted from Kushwaha et al., 2011).

Firstly, the paracellular route is energy independent and occurs by drug passing through the aqueous spaces between the cells via a slow passive diffusion. In general, as the molecular size of the drug increases the intranasal absorption via this route decreases. For example, a drug with a molecular weight greater than 1 kDa has poor systemic bioavailability following nasal administration (McMartin et al., 1987) and bioavailability of these molecules can be enhanced using absorption enhancers (Ozsoy et al., 2009). Zhang et al. (2005) demonstrated that the systemic absorption of large molecular weight of recombinant hirudin-2 (rHV2) (6900 Daltons) improved intranasally by including absorption enhancer (e.g. chitosan 0.5%, hydroxypropyl-β- cyclodextrin 5%, or ammonium glycyrrhizinate 1%) into the formulation.

Secondly, transcellular absorption mechanism is applicable to lipophilic drugs, which are readily absorbed by diffusion through the epithelial cellular membranes of the nose. Transcellular transport of drugs might be carrier mediated or may involve opening of tight junctions for drug absorption. Excipients used in nasal formulations such as chitosan opens

the tight junctions between cells and thus the drug transportation from nasal cavity to the systemic circulation is facilitated (Dodane et al., 1999).

Thirdly, substances in the nasal mucosa particularly in the olfactory mucosa include: Pglycoprotein, organic cation transporter, dopamine transporter and amino acid transporters. These transporters transfer amino acids, amines and cations. This mechanism is known as carrier mediated processes.

Fourthly, the mechanism for drug transportortation is endocytosis. In this mechanism materials are engulfed into the cell. The uptake of particles by nasal epithelial cells is mediated by the M cells. Endocytosis is the predominant mechanism for transporting compounds that have molecular weights higher than 1000 Da such as proteins, peptides (Costantino et al., 2007), polypeptides and polypeptide-coated nanospheres in the range of 500 nm. It has been reported that the absorption of polar molecules (Illum, 2000) and larger peptides) are greatly improved by the incorporation of absorbing enhancers into the formulation such as surfactants (e.g. sodium laurylsulfate), enzyme inhibitors (e.g. bestatin), bile salts (e.g. sodium deoxycholate), chitosan, and cyclodextrins (Davis and Illum, 2003; Jadhav et al., 2007).

Permeation enhancers accelerate the rate of transportation of hydrophilic and larger protein and peptide molecules through mucosal membranes. A number of parameters control the safety and efficacy of nasal permeation enhancer, such as enzymatic activities of Cytochrome P450 isoenzymes in the nose, mucociliary clearance, duration of contact between formulation and nasal mucosa. Absorption enhancers reversibly change the nasal mucosa, effectively increasing the drug absorption and do not cause local or systemic irritation or toxicity and their action is reversible.

Factors affecting nasal drugs delivery

For applications in brain targeting, local delivery and systemic delivery of drugs several factors should be considered prior to designing intranasal formulations. These are the physicochemical properties of drug, excipients to be included in formulation and the physiological condition of the nasal cavity (Figure) (Behl et al., 1998; Dhakar et al., 2011).

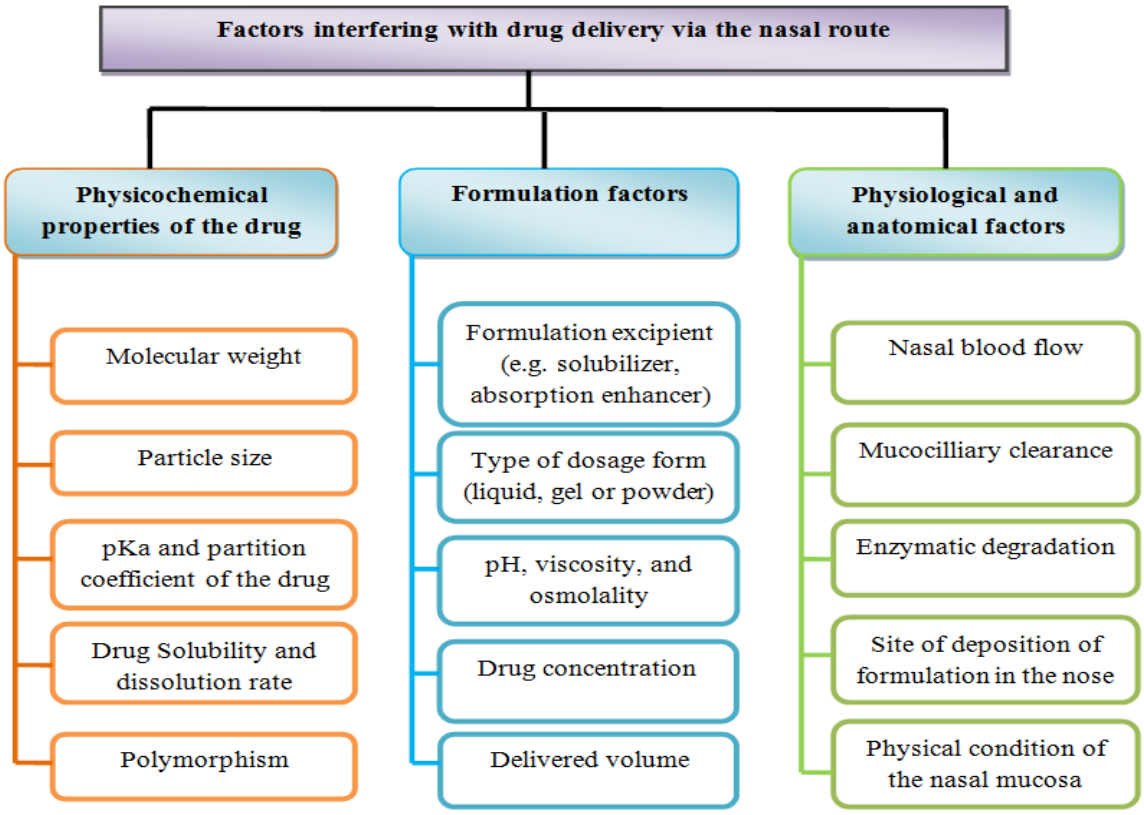


Figure 4: Barriers interfering with drug delivery via the nasal route (Behl et al., 1998; Dhakar et al., 2011).

Transfersomes.

Transfersomes are variety of elastic or deformable vesicles that was initially introduced in the early 1990's by Gregor Cevc. The main barrier and rate limiting step for diffusion of drugs across the skin, stratum corneum. Recent advances in modulating vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues. These have resulted in the design of two novel vesicular carriers, ethosomes and ultraflexible lipid based transferosomes.

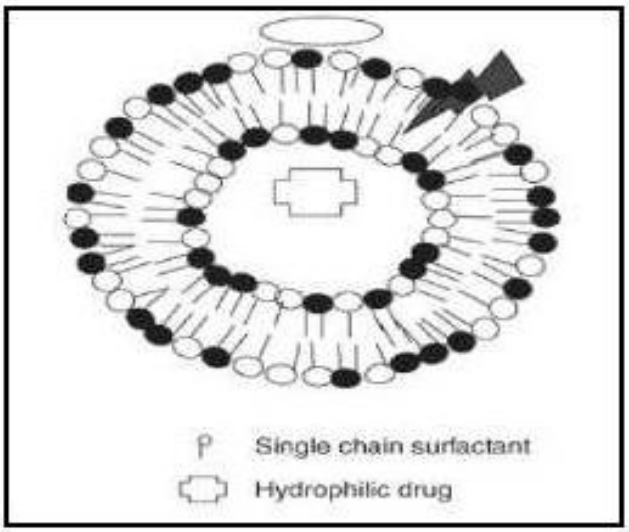


Figure 5: Deformable transfersomes vesicle.

Transfersomes are ultradeformable vesicles possessing an aqueous core encircled by the complex lipid bilayer. Local composition and Interdependency of shape of the bilayer makes the vesicle both self-regulating and self-optimizing. Due to their deformability, they are good candidates for the non-delivery of small, medium and large sized one's. Transferosomes can distort and pass through narrow constriction (5-10 times less than their own diameter) without measurable loss of their elasticity which can be achieved by mixing suitable surface active agents in the proper ratios. The resulting elasticity of transfersomal membrane reduces the danger of entire vesicle rupture in the skin and allows transferosomes to follow natural water gradient when applied under non-occlusive condition across the epidermis. They overcome the skin permeation by squeezing themselves alongside the intracellular lipids of the sratum corneum. The elevated and self-optimizing deformability of distinctive composite transferosomes which are adaptable to ambient stress allow the ultra-deformable transferosomes to change its membrane composition locally and irreversibly, when it is pressed against or attracted into narrow pore. The transfersomes components that maintain tough deformation preferentially gather while the less adaptable molecules, lowers the energetic cost of membrane deformation and permits the resulting highly flexible particles, first enter and pass through the pore rapidly and efficiently. Peptides like insulin, bovine serum albumin, vaccines can be delivered. Delivery systems which offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable improves physiological and pharmacological response is transdermal drug delivery systems are available for the treatment of various diseases such as cardiovascular disease, Parkinson's disease, Alzheimer's disease, depression, anxiety and postmenopausal bone loss and urinary incontinence.

Table No 5: Comparison of transfersomes with different vesicles

S.no	Method	Advantage	Disadvantage
1	Liposomes	Phospholipid vesicle, Biocompatible, Biodegradable	Less skin penetration less stable
2	Proliposome	Phospholipid vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of vesicles
3	Physical methods (e.g. iontophoresis)	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
4	Niosomes	Non-ionic surfactants vesicles	Less skin penetration easy handling But will not reach up to deeper skin layer
5	Proniosomes	Greater stability, Will convert into noisome insitu, stable	Less skin penetration easy handling But will not reach up to deeper skin layer
6	Transfersomes and Protransfersomes	More stable, high penetration due to high deformability, biocompatible & biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reach upto deeper skin layers.	None, but for some limitations

Salient features

- Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can shows drug molecules with wide range of solubility.
- Transfersomes can deform and pass through narrow constriction without any measurable loss, this high deformability gives better penetration of intact vesicles.
- Transfersomes can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, antifungal, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein and albumin.
- Transfersomes are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes.
- Transfersomes have high entrapment efficiency and in case of lipophilic drug near to 90%.
- Transfersomes protect the encapsulated drug from metabolic degradation.
- They act as depot, releasing their contents slowly and

gradually.
- They can be used for both systemic as well as topical delivery of drug.
- Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives.

Table No. 6: Different additives used in formulation of transfersomes.

S.No	Class	Example	Use
1	Phospholipids	Soya phosphatidyl choline, egg phosphatidyl choline, dipalmitoylphosphatidyl choline	Vesicles forming component
2	Surfactants	Sod.cholate, Sod.deoxycholate, Tween-80, Span- 80, Tween 20	Vesicles forming component
3	solvents	Ethanol, methanol, isopropyl alcohol, chloroform	As a solvent
4	Buffering agent	Saline phosphate buffer (pH 6.4), phosphate buffer pH 7.4	As a hydrating medium
5	Dye	Rhodamine-123 Rhodamine-DHPE Fluorescein- DHPE Nile-red	For CSLM study

Methods of preparation of transfersomes (Venkatesh et al, 2014; Nautiyal et al, 2015)

A. Thin film hydration technique is employed for the preparation of Transfersomes which comprised of three steps.

1. A thin film is prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in volatile organic solvent (chloroform, methanol). Organic solvent is then evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50°C for dipalmitoyl phosphatidyl choline) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight.
2. A prepared thin film is hydrated with buffer (pH 6.5) by rotation at 60 rpm for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature.
3. To prepare small vesicles, resulting vesicles were sonicated at room temperature or 50°C for 30 min. Using a bath sonicator or vortex shaker or probe sonicated at 4°C for 30 min. The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

B. Modified hand shaking, lipid film hydration technique is also founded for the preparation of Transfersomes which comprised following steps:

1. Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent
2. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension further hydrated up to 1 hour at 2-8°C.

Optimization of formulation containing transfersomesS[13]

There are various process variables which could affect the preparation and properties of the transfersomes. The preparation procedure was accordingly optimized and validated. The process variables are depending upon the procedure involved for manufacturing of formulation. The preparation of transfersomes involves various process variables such as,

- Lecithin: surfactant ratio
- Effect of various solvents
- Effect of various surfactants
- Hydration medium

Optimization was done by selecting entrapment efficiency of drug. During the preparation of a particular system, the other variables were kept constant.

POLYMERS EMPLOYED FOR THE DEVELOPMENT OF TRANSFEROSOMES.

- **LECITHIN-** a yellow-brownish fatty substances occurring in animal and plant tissues composed of phosphoric acid, choline, fatty acids, glycerol, glycolipids, triglycerides and phospholipids. Available from sources of soybean, milk, marine sources, rapeseed, cottonseed and sunflower. Phosphatidylcholine dissolve in ethanol. Lecithin is a source of choline an essential nutrient. It can be totally metabolized by humans.

Applications

- Acts as wetting, stabilizing, choline enrichment carrier.
- Good dispersing agent
- Helps in emulsification and encapsulation
- Acts as catalyst, color intensifying agent.
- Good stabilizing and suspending agent.
- Eliminates foam in water based paints.
- Used as an anti-sludge additive in motor lubricants. Anti-gumming agent in gasoline, spreading agent, textile, rubber industries.

➢ **SOYAPHOSPHATIDYL CHOLINE**

Phosphatidyl cholines are a class of phospholipid that incorporates choline as a head group. They are major component of biological membranes and can be easily obtained. It is a major constituent of cell membranes and pulmonary surfactant is more commonly found in outer leaflet of a cell membrane, transported between membranes by phosphatidyl choline transfer protein. Phospholipid composed of choline head group and glycerophoric acid with fatty acids. Phospholipase decatalyzes the phosphatidyl choline to form phosphatidic acid and releasing the soluble choline head group into the cytosol. Phosphatidyl choline supplementation slows down aging related processes and improves brain functioning and memory but does not benefit in the patients in dementia.

USES: Used in the cure of inflammatory bowel disease.

➢ **DISTEROYL PHOSPHATIDYL CHOLINE-** Appears like a solid substance and stable. Incompatible with strong oxidizing agents. Vesicles formed by sonication of saturated chain phosphatidyl cholines in aqueous media have been used extensively as a model for the lipid component in the plasma membrane. The rate of loss of small vesicles and information about the structures to which the small vesicles are converted can be obtained from sedimentation velocity experiments. The kinetic behavior of small disteroyl phosphatidyl choline vesicles is examined. Small single bilayer vesicles are unstable at all temperatures. The vesicles size distributions changed as a function of time at all temperatures below the phase transition temperatures but constant at transition temperature and above. It is to be stored at -20°c.

➢ **DIPALMITOYL PHOSPHATIDYLCHOLINE-** Phospholipid consisting of two palmitic acids. It is the major constituent of pulmonary surfactant. Synthesized mainly through remodeling of phosphatidyl choline.1, 2-dipalmitoyl-sn-glycero-3-PC is a zwitter ionic phosphoglyceride that can be used for the preparation of liposomal monolayer. The extent of incorporation of the enzyme glutamyl transpeptidase in erythrocyte membranes was five times higher when proteoliposomes were prepared from L-DPPC as compared to control. L-DPPC incorporated vesicles have potential in establishing active immunotherapy with the antigens.

➢ **CHOLESTEROL-** A sterol or modified steroid which is a lipid molecule biosynthesized by animal cells to maintain integrity and fluidity. It enables no need of cell membrane, ability to change its shape, able to move by the animals. Cholesterol serves as precursor for vitamin D, steroid hormones, bile acids, it is the principal sterol synthesized by humans. Most of ingested cholesterol is esterified and poorly absorbed. The cholesterol modulates membrane fluidity over a range of physiological temperatures**.** The trans confirmation of tetracycline ring decrease fluidity but side chain is rigid and planar**.** In neurons a myelin sheath is derived from Schwann cellmembrane, providing insulation for many conducting impulses. Cholesterol is slightly soluble in water, insoluble in blood it transported in blood stream through lipoproteins. High levels of cholesterol termed as hypercholesterolemia. Low levels of cholesterol results hypocholesterolemia.

➢ **DEOXYCHOLIC ACID-** Also known as deoxycholate, cholanoic acid. It is one of the metabolic byproducts and secondary bile acids of intestinal bacteria. The two primary bile acids secreted by the liver are cholic acid and chenodeoxycholic acid. Soluble in alcohol and acetic acid. Comprises active component DCA used in the treating inflammations and enhances immune system. It can be used as immunostimulant.

Uses

- Used for research purposes in studying liposomes, lipid bilayers, and biological membranes.
- Used in the production if high density lipoproteins.
- Used in healing of local inflammations

Applications

- Deoxycholic acid used in the emulsification of fats for the absorption in the intestine.
- Used in the prevention of gallstones.
- Used for mesotherapy injections along with phosphatidyl choline.
- Used as emulsifier in food industry.
- Deoxycholates and bile acid derivatives used for incorporation in nanotechnology.

➢ **TWEEN 80-** Nonionic surfactant and emulsifier used in foods and cosmetics. Also known as polysorbate 80. It is derived from polyethoxylated sorbitan and oleic acid. It is introduced above CMC. Amber coloured viscous liquid. It is not carcinogenic. It is soluble in ethanol, cottonseed oil.

USES

- As an emulsifier in the preparation of food products.
- Used to identify the phenotype of strain.
- Used in icecream as it increases the resistance of melting
- Prevents milk proteins from coating the fat droplets.
- Used as surfactant in soaps and cosmetics.
- Used as a solubilizer in mouthwash.
- Used as an excipient to stabilize aqueous formulations of parenteral preparations and emulsifier in antiarrhythmic amiodarone.

➢ **TWEEN 20-** Clear yellow to yellow green viscous liquid. It is a surfactant whose stability and relative non toxicity used as a detergent and emulsifier in scientific pharmaceutical applications. It is a derivative of polyoxyethylene derivative of sorbitan monolaurate.

APPLICATIONS

- Used as wetting agent.
- It has a broad sense of application in biotechnical sciences
- As a washing agent in immunoassays.
- Saturate binding sites.
- Stabilize proteins.
- Used as excipient to stabilize emulsions and suspensions.

➢ **SPAN 20-** also known as sorbiton monolaurate. It is a mixture of partial esters of sorbitol and its mono and dianhydrates with edible lauric acid. It is an amber coloured oily viscous liquid, light cream to tan beads or flakes or a hard, waxy solid with a slight odour. Soluble in hot and cold water.

➢ **SPAN 80-** Also known as sorbiton oleate. It is a nonionic surfactant. Highest skin permeation can be achieved if we use 10 % span 80. Cationic lipid vesicles were developed of mainly of span 80. Microemulsions can be formulated by span 80 as lipophilic linker. Addition of increased concentrations of span 80 to suspensions caused marked changes in stability. Span 80 served as a coaservating agent in microspheres.

➢ **ETHANOL:** Also called as ethyl alcohol, pure alcohol phytoactive drug. It is a volatile, colorless liquid that has a slight odor. Produced by fermenting sugars with yeast. It causes alcohol intoxication. Ethanol's hydroxyl group is able to participate in hydrogen bonding rendering it more viscous and less volatile than polar organic compounds such as propane. It has a complex mode of action and affects multiple systems in brain.

USES

- Used in scents, thermometer as a solvent.
- Intended for flavoring, coloring and medicines
- As motor fuels & fuel additive, rocket fuels.
- As it dissolves hydrophobic flavoring compounds, used as beverages in cooking.

➢ **METHANOL:** Also known as methyl alcohol, wood alcohol, simplest alcohol, light, volatile, colorless, flammable liquid. It is produced naturally in the anaerobic metabolism. It is produced in anaerobic metabolism of bacteria. At room temperature it is used as polar liquid, antifreeze, solvent, fuel, denaturant for ethanol. If ingested in large quantities it metabolized in to formic acid or formate salts.

Toxicity: Methanol has toxicity, if ingested 10ml of methanol can break down into formic acid causing permanent blindness. It may be fatal due to its CNS depressant activity.

USES

- Used as a solvent for laboratory purposes especially for UV spectroscopy, HPLC LCMS due to its low UV cut off.
- Used as fuel in German military rockets.
- Used as denaturating agent in polyacrylamide gel electrophoresis
- Methanol used as fuel in camping and boating stoves.

➢ **CHLOROFORM:** A colorless, sweet smelling, dense liquid trihalomethane which is considered as hazardous.it is soluble in water, benzene, acetone, DMSO, diethyl ether, oils, ethanol. It is estimated that over 90% of atmospheric chloroform is of natural origin. It is produced by brown seaweeds, red seaweeds and green seaweeds. Chloroform can be produced by heating a mixture of chlorine and chloromethane or methane.

USES

- The major use of chloroform today is the production of chlorofluoromethane which is a major precursor to tetrafluoroethylene. Chlorofluoromethylene used as popular refrigerant.
- Used as a solvent in laboratory as it is unreactive miscible with organic liquids not flammable and conveniently volatile
- Used as a reagent for dichlorocarbene CCl_2 group which affects ortho formylation of activated aromated rings. Phenols and aldehydes in riemer tiemann reaction.
- Used as anaesthetic as it depresses CNS system.
- Increases the movement of K+ ions through the potassium channels in nerve cells.

Characterization of transfersomes

❖ **Entrapment Efficiency:** The entrapment efficiency is expressed as the percentage entrapment of the drug added. It is determined by first separation of the un-entrapped drug by use of mini- column centrifugation method. After centrifugation, the vesicles were disrupted using 0.1% Triton X-100 or 50% n-propanol. The entrapment efficiency is expressed as: Entrapment efficiency= (amount entrapped/ total amount added) ×100.

❖ **Vesicle Diameter**: Vesicle diameter can be determined using photon correlation spectroscopy or dynamic light scattering (DLS) method. Samples were prepared in distilled water, filtered through a 0.2 mm membrane filter and diluted with filtered saline and then size measurement done by using photon correlation spectroscopy or dynamic light scattering (DLS) measurements.

❖ **Vesicle Shape & Type:** Transfersomes vesicles can be visualized by TEM, phase contrast microscopy, etc. The stability of vesicle can be determined by assessing the size and structure of vesicles over

time. Mean size is measured by DLS and structural changes are observed by TEM.

- **Number of Vesicle per cubic mm:** This is an important parameter for optimizing the composition and other process variables. Nonsonicated transfersome formulations are diluted five times with 0.9% sodium chloride solution. Haemocytometer and optical microscope can then be used for further study.

The Transfersomes in 80 small squares are counted and calculated using the following formula: Total number of Transfersomes per cubic mm = Total number of Transfersomes counted × dilution factor × 4000.

- **Confocal Scanning Laser Microscopy (CSLM) Study:** Conventional light microscopy and electron microscopy both face problem of fixation, sectioning and staining of the skin samples. Often the structures to be examined are actually incompatible with the corresponding processing techniques; these give rise to misinterpretation, but can be minimized by Confocal Scanning Laser Microscopy (CSLM). In this technique lipophilic fluorescence markers are incorporated into the transfersomes and the light emitted by these markers used for investigating the mechanism of penetration of transfersomes across the skin for determining histological organization of the skin (epidermal columns, interdigitation), shapes and architecture of the skin penetration pathways for comparison and differentiation of the mechanism of penetration of transfersomes with liposomes, niosomes and micelles.

- **Degree of Deformability or Permeability Measurement:** In the case of transfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Transfersomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting transfersomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements.

- **Drug Content:** The drug content can be determined using a modified high performance liquid chromatography method (HPLC) method using a UV detector, column oven, auto sample, pump, and computerized analysis program.

- **Occlusion Effect**: Occlusion of skin is considered to be helpful for permeation of drug in case of traditional topical preparations. But the same proves to be detrimental for elastic vesicles. Hydrotaxis (movement in the direction) of water is the major driving force for permeation of vesicles through the skin, from its relatively dry surface to water rich deeper regions. Occlusion affects hydration forces as it prevents evaporation of water from skin.

- **In Vitro Drug Released:** In vitro drug release study is performed for determining the permeation rate. Time needed to attain steady state permeation and the permeation flux at steady state and the information from in-vitro studies are used to optimize the formulation before more expensive in vivo studies are performed. For determining drug release, transfersomes suspension is incubated at 32°C and samples are taken at different times and the free drug is separated by mini column centrifugation. The amount of drug released is then calculated indirectly from the amount of drug entrapped at zero times as the initial amount (100% entrapped and 0% released).

- **Turbidity measurement:** Turbidity of drug in aqueous solution can be measured using nephelometer.

- **Surface charge and charge density:** Surface charge and charge density of transfersomes can be determined using zetasizer.

- **Stability Studies:** Transfersomes stability was determined at 4°C and 37°C by TEM visualization and DLS size measurement at different time intervals (30, 45, and 60 days), following vesicles preparation.

Applications

Table No. 7: Application of transfersomes

S.No	Name of drug	Inference
1	Curcumin	Better permeation for anti-inflammatory activity
2	Indinavir sulfate	Improved influx for activity against acquired immune deficiency syndrome (AIDS)
3	Ketoprofen	Improved penetration for anti-inflammatory activity
4	Insulin	Induce therapeutically significant hypoglycemia with good efficacy and reproducibility
5	Capsaicin	Increase skin penetration
6	Colchicine	Increase skin penetration
7	Vincristine	Increase entrapment efficiency & skin permeation.

8	Interferon-α	Efficient delivery means controlled release. Overcome stability problem.
9	Norgesterol	Improved transdermal flux
10	Tamoxifen	Improved transdermal flux
11	Methotrexate	Improved transdermal flux
12	Oestradiol	Improved transdermal flux
13	Tetracaine, Lignocain	Suitable means for the noninvasive treatment of local pain on direct topical drug application.
14	Corticosteroids	Improved site specificity and overall drug safety.
15	Hydrocortisone	Biologically active at dose several times lower than currently used formulation.
16	Triamcinolone acetonide	Used for both local and systemic delivery.
17	Human serum albumin	Antibody titer is similar or even slightly higher than subcutaneous injection.
18	Stavudine	Improved the in vitro skin delivery of Stavudine for antiretroviral activity
19	Tetanus toxoid	For transdermal immunization

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