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Nasal *in-situ* Transferosomes as a Platform Technology for Nose-to-Brain Drug Delivery

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ABSTRACT

The blood-brain barrier (BBB) represents a major obstacle in the therapeutic management of disorders related to the central nervous system (CNS), blocking the access of almost 98% of small molecule medications and practically all large molecules. Intranasal drug administration represents a viable alternative to overcome the BBB, based on its ability to deliver directly from the nasal cavities into the brain using the routes of olfactory and trigeminal nerves, offering a direct nose-to-brain drug delivery pathway. Despite its advantages, intranasal drug delivery remains constrained by factors such as quick clearance, short retention and poor permeation of the drugs through biological membranes, to address these challenges, the *in-situ* gelling systems containing mucoadhesive polymers have been developed. Nasal *in-situ* transferosomal gels offer a novel formulation technique that incorporates the benefits of both transferosomal vesicular drug delivery systems and stimuli-responsive gel matrices for brain targeting.

Keywords: Transferosomes, *in-situ* gel, Intranasal, Nose-to-brain, Blood-brain barrier, Vesicular drug delivery, Mucoadhesive systems, Brain targeting.

1.INTRODUCTION

The central nervous system is one of the most challenging pharmacological and therapeutic areas for effective drug delivery. Despite the widespread prevalence of large number of neurological and psychiatric disorders affecting a significant portion of the population worldwide effective and successful pharmacological intervention in the CNS continues to be limited due to the high selectivity of the blood-brain barrier (BBB)[1,2]. The BBB is formed by specialized endothelial cells reinforced by tight junctions, Pericytes and astrocytic endfeet which restricts the passage of most of the small molecules and all macromolecular therapeutics from the systemic circulation into the brain parenchyma[3]. Conventional oral and parenteral routes therefore often fail to achieve therapeutic drug concentration at the target site of CNS without inducing systemic side effects of toxicity due to high dose requirements.

In recent years, the development in the pharmaceutical drug delivery system and the modern advances in pharmacology led to the alternative routes of drug delivery to the brain. Intranasal administration has particular attention because the nasal cavity shares a unique anatomical proximity to the brain, offering direct neural pathways namely the olfactory and trigeminal nerve pathway, through which drugs may be

transported to the CNS and bypassing the BBB [4,5]. This route is non-invasive, rapid in onset, avoids hepatic first-pass metabolism, and can be self-administered, offering distinct advantages over intrathecal or intracerebroventricular injection strategies.

However, the nasal route also has many disadvantages. The nasal epithelium is protected by a mucus layer that acts as a physicochemical barrier, mucociliary clearance removes deposited substances within 15–30 minutes, enzymatic degradation by nasal mucosal enzymes reduces drug bioavailability, and the drug should have sufficient lipophilic property for transcellular permeation which often conflicts with the aqueous solubility requirements for effective mucosal absorption [6,7]. These challenges necessitate the need for development of novel drug delivery systems capable of overcoming these multiple barriers simultaneously.

Transferosomes, first described by Cevc and Blume in 1992, represents a class of ultra-deformable lipid vesicles which differ from conventional liposomes by their ability to squeeze through pores that are significantly smaller than their own diameter under osmotic or hydrostatic gradients[8]. This deformability is caused by the incorporation of edge activators such as single-chain amphiphilic surfactants that destabilize the lipid bilayer membranes into the phospholipid matrix. When transferosomes are combined with *in-situ* gel forming polymers that undergo sol-to-gel transition upon exposure to the nasal environment (triggered by temperature change, ionic strength, or pH shift), transferosome vesicles can be delivered in a low-viscosity liquid that gels *in-situ*, thereby dramatically extending nasal residence time and improving drug contact with the olfactory epithelium [9,10].

The concept of nasal *in-situ* transferosomal gels thus represents a combination of multiple advanced drug delivery principles: the biophysical deformability of elastic vesicles, the mucoadhesive retention of stimuli-responsive polymers, and the anatomical advantages of the olfactory-trigeminal neural network. This review aims to provide an exhaustive analysis of this platform technology, tracing its scientific foundations, formulation science, mechanistic basis, therapeutic applications, and future directions.

2. THE NASAL CAVITY: ANATOMY, PHYSIOLOGY, AND DRUG ABSORPTION

2.1 Structural Organization of the Nasal Cavity

The human nasal cavity is a paired, air-filled space bounded superiorly by the cribriform plate of the ethmoid bone, inferiorly by the hard palate, laterally by the lateral nasal wall bearing three turbinates (conchae), and anteriorly by the nares. The nasal septum divides the cavity into two roughly symmetrical halves. The total surface area of the nasal cavity approximates 150–180 cm^2, of which the olfactory epithelium, located in the superior portion of the cavity covering an area of approximately 2–10 cm^2 per side, is of high importance for nose-to-brain drug delivery[11].

The nasal cavity is functionally divided into three regions: the vestibular region, the respiratory region, and the olfactory region. The vestibular region, located immediately posterior to the nares and is lined with stratified squamous epithelium, and offers limited drug absorption capacity. The respiratory region, constituting most of the nasal cavity surface, is lined by pseudostratified ciliated columnar epithelium interspersed with goblet cells and is richly vascularized, allowing for systemic absorption of drugs. The olfactory region, though occupying a smaller area in the roof of the cavity, is the critical portal for direct nose-to-brain transport[12].

The olfactory epithelium consists of three main cell types: bipolar olfactory sensory neurons (OSNs), supporting sustentacular cells, and the basal cells. The axons of OSNs project through the cribriform plate foramina as the olfactory nerve (cranial nerve I) and into the olfactory bulb,

establishing a direct neuronal connection between the nasal mucosa and the CNS. The olfactory mucosa is unmyelinated in its peripheral portion, and the olfactory neurons lack tight junctions comparable to those of the BBB, making them relatively permeable to lipophilic and even some certain hydrophilic molecules[13].

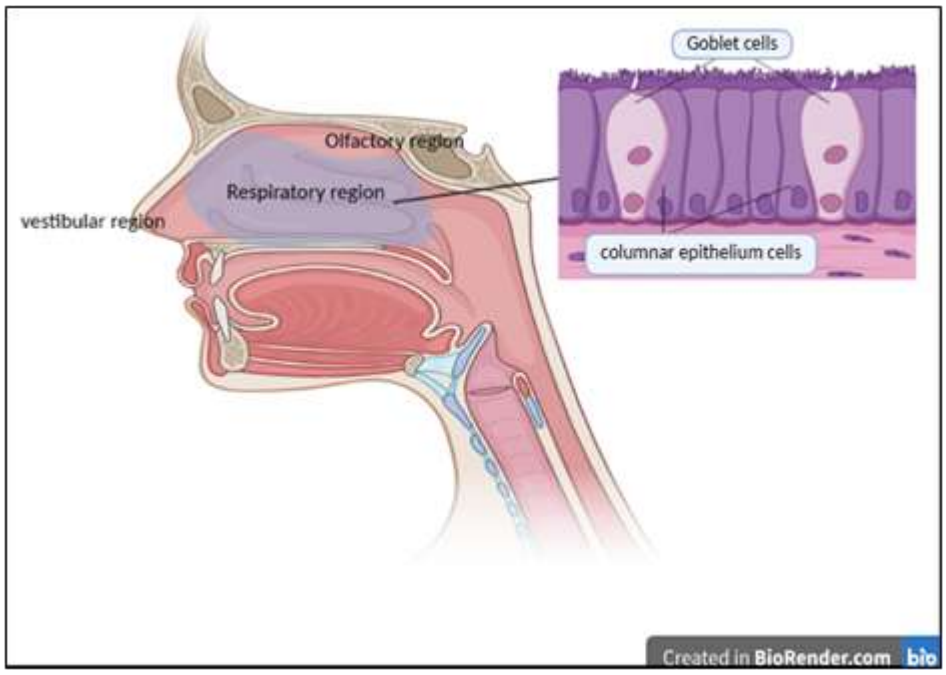

Figure 1: - Structural organization of nasal cavities

2.2 The Olfactory Pathway for Nose-to-Brain Drug Transport

Drug transport along the olfactory pathway involves two principal mechanisms which include intraneuronal (axonal) transport and extra neuronal (paracellular/transcellular) transport. Intraneuronal transport refers to the movement of drugs or drug-loaded nanoparticles along the axonal cytoplasm of olfactory neurons by endocytic uptake at the dendritic knob or cell body, followed by retrograde axonal transport to the olfactory bulb. This process is relatively slow, with transit times of hours to days, though it delivers drug directly into the CNS with no systemic exposure[14].

Extraneuronal or paracellular transport is considerably faster and is more significant for most drug delivery systems. It involves persorption (physical passage) through the olfactory epithelial junctions, movement through the perineural space surrounding olfactory nerve bundles within the lamina propria, and transport through the submucosal lymphatics and perivascular spaces associated with olfactory nerve fascicles into the subarachnoid space and olfactory bulb. The ensheathing cells that wrap olfactory axon bundles olfactory ensheathing cells (OECs) create a permeable interface compared to CNS myelin, facilitating drug entry [15].

Drug molecules or nanocarriers deposited on the olfactory epithelium may therefore be absorbed by endocytosis into olfactory neurons, taken up by OECs and transported paracellularly, or traverse the perineural space by bulk flow. Once in the olfactory bulb, drugs may distribute into deeper brain structures including the olfactory cortex, hippocampus, amygdala, and cerebrospinal fluid (CSF), from which widespread CNS distribution can occur [16,17].

2.3 THE TRIGEMINAL PATHWAY

The trigeminal nerve provides sensory innervation to the nasal mucosa through its ophthalmic and maxillary branches. The trigeminal pathway offers a complementary and more expansive nose-to-brain route

compared to the olfactory pathway, as trigeminal fibers innervate both the olfactory and respiratory regions of the nasal mucosa, effectively extending the drug accessible surface area for direct CNS transport [18].

Trigeminal nerve endings in the nasal mucosa can internalize drug or nanoparticles by receptor-mediated or fluid-phase endocytosis. These are then transported by retrograde axonal transport along the trigeminal nerve fibers to the trigeminal ganglion (Gasserian ganglion) and to the trigeminal brainstem nuclei (trigeminal nucleus caudalis), which is anatomically contiguous with the spinal trigeminal tract and maintains connectivity with the pons, medulla, and higher brain centers [19]. This pathway is particularly relevant for drugs targeting brainstem nuclei as in the treatment of migraine and pain modulation[20].

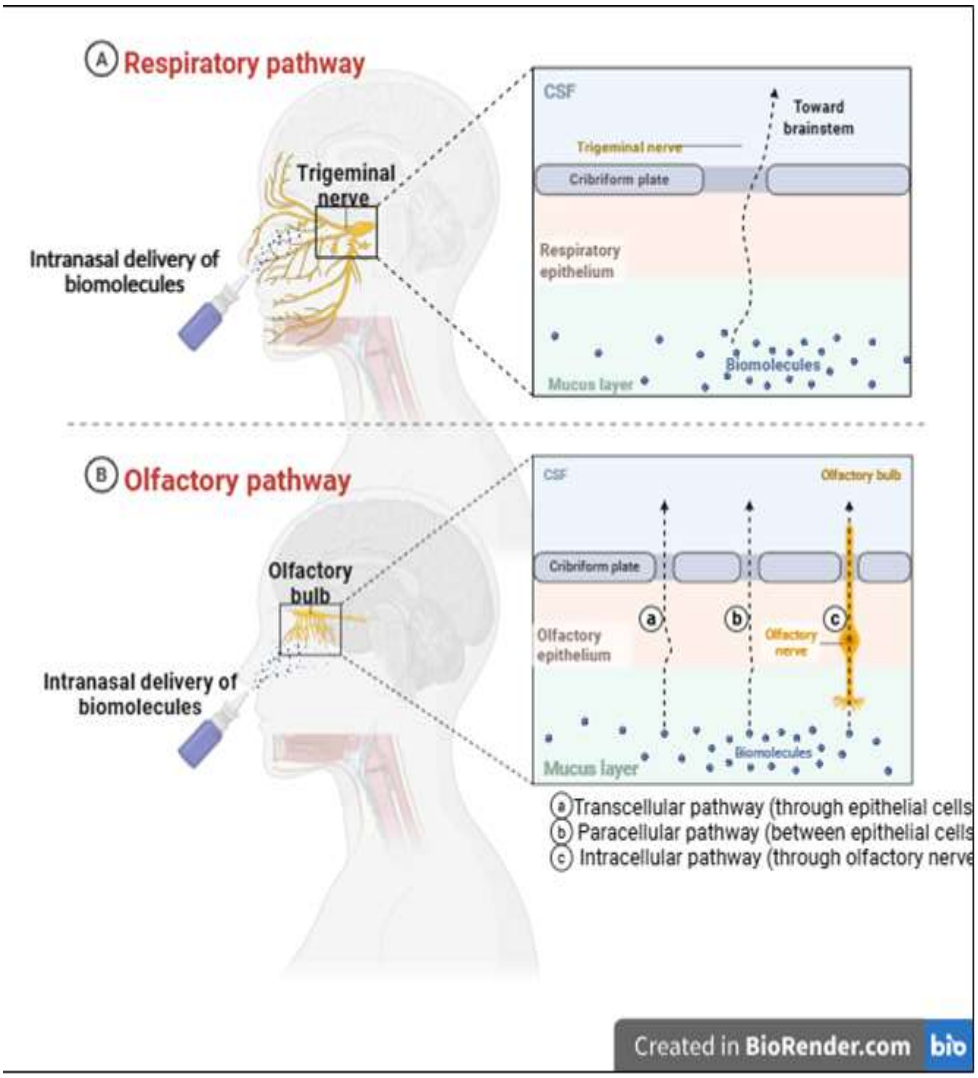

Figure 2: - The respiratory and olfactory pathway of nose to brain drug delivery

2.4 The Mucosal Barrier and Challenges for Nasal Drug Delivery

The nasal mucus is a viscoelastic gel composed of high molecular weight mucin glycoproteins (predominantly MUC5AC and MUC5B secreted by goblet cells and submucosal glands), water (approximately 95%), salts (sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), bicarbonate ions (HCO_3^-), and phosphate salts (PO_4^{3-})), lipids, proteins, and immunoglobulins[21]. The mucus layer in the nasal cavity is approximately 10–15 μm

thick and is renewed every 10–15 minutes by mucociliary clearance, which transports mucus and entrapped particles posteriorly toward the nasopharynx at a rate of approximately 5–6 mm/min [22].

Mucociliary clearance represents the physiological barrier limiting nasal drug residence time. For conventional liquid nasal formulations, effective nasal residence time is limited to 15–30 minutes, severely limiting absorption. Additionally, the mucus layer acts as a size-dependent sieve, particles larger than 500 nm may be trapped by mucus mesh, while smaller, lipophilic molecules or nanoparticles may penetrate toward the epithelial surface [23].

Enzymatic barriers in the nasal cavity include cytochrome P450 enzymes (CYP1A1, CYP2B6, CYP3A4), esterases, and proteases expressed by sustentacular cells and in the mucus, which can metabolize drug molecules before they reach the epithelium. The pH of nasal secretions ranges from approximately 5.5 to 6.5, which may influence the ionization and solubility of drug molecules. Tight junctions between nasal epithelial cells restrict paracellular transport, though these are comparatively less restrictive than BBB tight junctions [24,25].

3. TRANSFEROSOMES: STRUCTURAL PRINCIPLES AND BIOPHYSICS

3.1 Historical Development and Classification

The concept of ultra-deformable vesicles was first introduced by Gregor Cevc and Gabriele Blume in their landmark 1992 paper in Biochimica et Biophysica Acta, which described the preparation of Transfersome vesicles comprising of phosphatidylcholine (a phospholipid) and sodium cholate (an edge activator) capable of penetrating through the biological membranes[8]. Unlike conventional liposomes, which are relatively rigid spherical bilayer vesicles with a fixed phospholipid composition, transferosomes possessed sufficient membrane flexibility to deform and squeeze through pores far smaller than their diameter, driven by osmotic or hydrostatic gradients across biological membranes[26].

The term 'transferosome' derives from the Latin word 'transferre' (to carry across) and the Greek word 'soma' (body), reflecting the fundamental property of these vesicles to carry drug across biological barriers. Over the subsequent years, considerable research has expanded the transferosome concept into multiple subtypes including ethosomes (containing high concentrations of ethanol), cubosomes, pharmacosomes, transethosomes (combining ethanol and edge activators), and most recently stimulus-responsive deformable vesicles incorporating temperature-sensitive or pH-sensitive lipids [27,28.]

For nasal and nose-to-brain applications, classical transferosomes composed of phospholipid and an edge activator in an aqueous dispersion represent the most extensively investigated formulation type, though transethosomes incorporating 20–45% ethanol have also been explored for their additional membrane-perturbing and penetration-enhancing properties.

3.2 Composition of Transferosomes

A standard transferosomal formulation comprises three essential components: a phospholipid backbone, an edge activator, and an aqueous phase. Other components include co-solvents, antioxidants, drug molecules, and polymer matrices for gel preparation.

Phospholipids: The most common phospholipids used to prepare transferosomes are soy phosphatidylcholine (SPC), egg phosphatidylcholine (EPC), hydrogenated soy phosphatidylcholine (HSPC), and distearoylphosphatidylcholine (DSPC). The hydrophilic head groups of these phospholipids face the inner and outer aqueous phases, while the hydrophobic tails form the hydrophobic core of the membrane. Lecithin (a mix of phospholipids like phosphatidylcholine, phosphatidylethanolamine,

phosphatidylinositol, and phosphatidic acid, along with smaller amounts of phosphatidylserine) is most commonly used instead of purified phosphatidylcholine because it is cost effective and biocompatible compared to other phospholipids. The level of lipid saturation affects how fluid the membrane is. Unsaturated phospholipids make membranes that are more fluid and flexible, while saturated phospholipids make membranes that are more rigid and gel-like[29].

Edge Activators: The edge activator is the main component of transferosomes. These are single-chain amphiphilic surfactants that incorporate into the phospholipid bilayer and produce membrane deformability by reducing membrane resistance to bending and facilitating local transient pore formation. The most extensively used edge activators include sodium cholate (SC), sodium deoxycholate (SDC), Span 60 (sorbitan monostearate), Span 80 (sorbitan monooleate), Tween 20 (polysorbate 20), Tween 80 (polysorbate 80), Brij 30, Brij 35, and didecyldimethylammonium bromide [30,31]. The edge activator concentration relative to the phospholipid is critical at concentrations below approximately 10 mol%, deformability is enhanced without disrupting the bilayer, above approximately 30–40 mol%, the bilayer may be solubilized into mixed micelles, destroying vesicle structure. Optimal edge activator-to-phospholipid molar ratios are typically in the range of 1:9 to 3:7[32].

Drug Incorporation: Drug molecules may be incorporated into different regions of the transferosomal structure depending on their physicochemical properties. Hydrophilic drugs are encapsulated within the aqueous core made up of distilled water or aqueous buffer systems; lipophilic drugs are incorporated into the hydrophobic bilayer membrane; and amphiphilic molecules may partition at the lipid-water interface. The encapsulation efficiency (EE%) and drug loading capacity are key formulation parameters that depend on the drug's log P, molecular weight, charge, and the lipid-to-drug ratio [33].

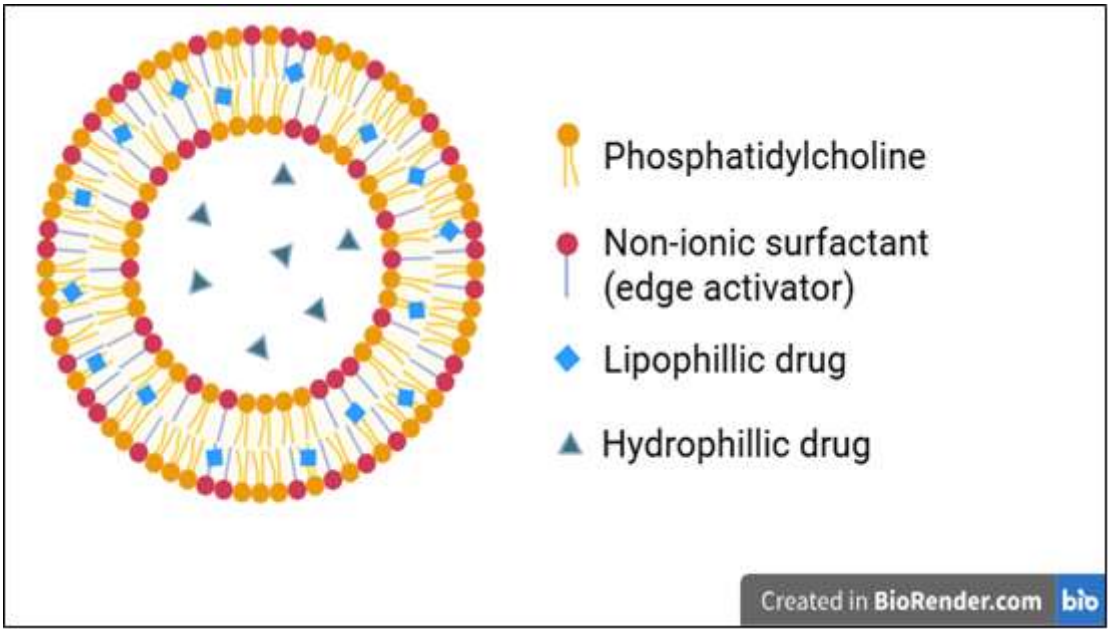

Figure 3: -structure and composition of Transferosome

3.3 Mechanism of Deformability

The deformability of transferosomal membranes is governed by the thermodynamic and mechanical properties of the mixed lipid-surfactant bilayer. In a pure phosphatidylcholine bilayer at physiological temperature, the bending modulus (κ) is approximately 10–30 k_BT

(defined as the product of the Boltzmann constant(k_B) and absolute temperature (T) conferring significant rigidity. Incorporation of an edge activator with a large head group and single acyl chain creating a geometric mismatch with the cylindrical phospholipid molecules introduces regions of high membrane curvature stress and reduces the bending modulus to values approaching 1–5 k_BT, making the membrane far more susceptible to deformation [34].

Under osmotic stress (when a transferosome encounters a dehydrated tissue surface) or hydrostatic pressure, the vesicle is driven to minimize free energy by deforming rather than retaining its spherical shape. The edge activator molecules preferentially localize at regions of high membrane curvature (the pore edges), stabilizing transient pores and facilitating the squeezing of vesicle contents and lipid bilayer through constricted aqueous channels. This process is largely elastic and reversible, allowing the vesicle to return to a spherical configuration after traversing the pore [35].

Mathematical modelling of transferosome deformability has employed the Helfrich theory of membrane elasticity, augmented by terms accounting for surface tension and spontaneous curvature contributed by asymmetrically distributed edge activator molecules. The critical penetration pressure (P^*) required for a transferosome of diameter 'd ' to traverse a pore of diameter $d_p$is estimated as"

$$P^* = \frac{4\kappa(d/d_p)^2}{d_p^2}$$

Where $d_p$is the diameter of the pore or constricted channel through which the transferosome passes. This relationship highlights the strong benefit of reducing the membrane bending modulus κ"[34,36].

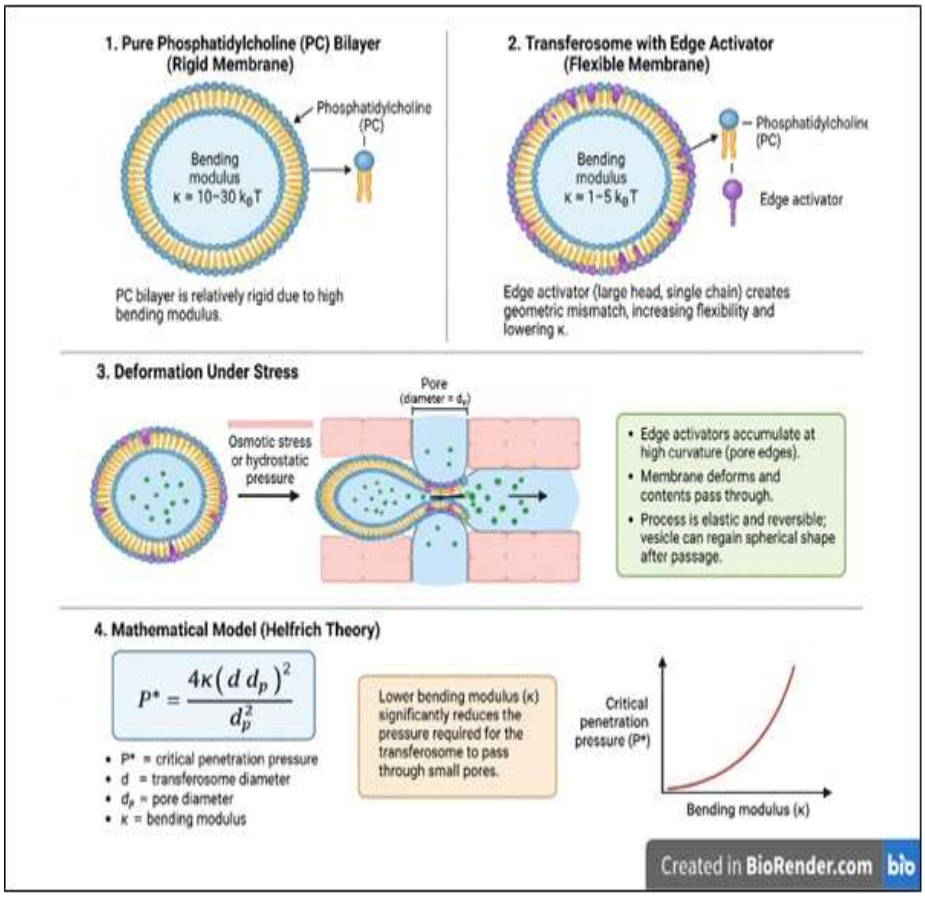

Figure 4: -Mechanism of deformability of transferosomes

4. TRANSFEROSOME PREPARATION METHODS

The different methods of preparing transferosomes are as follows

4.1 Thin Film Hydration (TFH). The thin film hydration method is the most widely employed method for transferosome preparation in research settings. Phospholipid and edge activator are dissolved in a volatile organic solvent (typically chloroform, chloroform/methanol, or ethanol) in a round-bottom flask. The solvent is removed by rotary evaporation under reduced pressure at a temperature above the lipid phase transition temperature (typically 40–60°C for common phospholipids), depositing a thin, uniform lipid film on the flask wall. The film is then hydrated with an aqueous phase (buffer or drug solution) at the same temperature, and the resulting multilamellar vesicles (MLVs) are downsized by probe sonication or membrane extrusion [37].

4.2 Ethanol Injection Method. An ethanolic solution of phospholipid and edge activator is rapidly injected (glass syringe or a Hamilton micro syringe fitted with a fine stainless-steel needle, typically in the range of 22–27 gauge or a 1–5 mL syringe is usually employed for laboratory-scale transferosome preparation to allow controlled, dropwise injection into the aqueous phase under stirring) into an aqueous phase under vigorous magnetic or mechanical stirring. The sudden solvent dilution causes rapid vesicle self-assembly. This method produces smaller vesicles (100–300 nm) with narrower size distributions than TFH without requiring sonication and is more amenable to scale-up. The residual ethanol must be removed by dialysis or evaporation. This method is particularly well-suited for nasal transferosome preparation as it avoids the need for high-energy processes that may degrade sensitive drug molecules [38].

4.3 Sonication and Extrusion. Following hydration by thin film hydration or ethanol injection method, the crude vesicle dispersion is subjected to size reduction and homogenization. Probe sonication (20–40 kHz, 1–10 min) or bath sonication can reduce vesicle size to 100–250 nm, however, probe sonication may introduce metallic contamination and degrade heat-sensitive molecules. Extrusion through polycarbonate membranes (100–400 nm pore size) using an extruder device provides precisely controlled vesicle size and narrow polydispersity and is the preferred method for transferosome preparation[39].

4.4 Supercritical Fluid Technology. Supercritical CO_2 ($scCO_2$) technology has been explored as a greener alternative for transferosome preparation, offering advantages of solvent-free processing, narrow size distributions, and ease of scale-up. The PGSS (particles from gas-saturated solutions) and DELOS (depressurisation of an expanded liquid organic solution) methods, using $scCO_2$ as an anti-solvent or propellant, can produce transferosomes with controlled size and excellent reproducibility, though these techniques require specialised high-pressure equipment[40].

5. INCORPORATION OF TRANSFEROSOMES INTO *IN-SITU* GEL MATRIX

Once the transferosomal dispersion has been prepared and characterized it is incorporated into the *in-situ* gelling matrix by a straightforward mixing procedure, maintaining the temperature below the gel transition point to prevent early gelation. For thermosensitive systems, all polymer dissolution and mixing is performed at 4°C (cold method), and transferosome dispersion is added to the cold polymer solution with gentle mixing[41]. For ion-activated systems (gellan gum, sodium alginate), the transferosome dispersion is mixed with the polymer solution at room temperature. The final formulation must be assessed for homogeneity and vesicle integrity after incorporation[42].

The concentration of polymer must be carefully determined and optimized in the presence of transferosomes, as lipid vesicles may interact with polymer chains and alter the sol-gel transition temperature. Commonly, the gelation temperature of poloxamer-based transferosomal gels is 1–3°C lower than polymer-alone controls, attributed to hydrophobic interactions between poloxamer segments and lipid acyl chains. Conversely, the gel strength may also be altered by the particulate reinforcement effect of vesicles within the polymer network[43].

6. PHYSICOCHEMICAL CHARACTERIZATION OF NASAL *IN-SITU* TRANSFEROSOMAL GELS

6.1 Vesicle Characterization

Characterizing vesicles is a crucial step in the process of determining the physical, chemical, and in-vitro performance properties of transferosomal products. The principal points of concern for vesicle characterization and their respective analytical methods include:

Vesicle Size and Size Distribution: one of the major and widely used methods for determining the hydrodynamic size and polydispersity index of vesicular dispersions is dynamic light scattering (DLS, photon correlation spectroscopy). Nasal transferosomes that can be considered acceptable generally have average diameters of 100 to 400 nm (150-250 nm being the best range for olfactory permeation), and PDI values below 0. 3, which shows a good degree of monodispersity. Nanoparticle tracking analysis (NTA) measures sizes of individual particles and can determine particle concentrations, so it is highly useful in supplementing DLS which is limited to providing information on polydisperse formulations only. Also, transmission electron microscopy (TEM), cryo-TEM, and atomic force microscopy (AFM) are approaches that allow one to have a look at the vesicle to check its shape and get some idea about its structural integrity[44].

Zeta Potential: Zeta potential (ζ) is the measure of the electrokinetic charge of the vesicle's surface, which primarily determines the stability of a colloidal system (its resistance to aggregation) and the properties of the vesicle to penetrate mucus. Transferosomes formed by PC (Phosphatidylcholine) and bile salt edge activators generally have negative zeta potentials ranging from -15 to -45 mV, which is due to the presence of anionic sulfonate/carboxylate head groups of the bile salt. The very negative charges (less than -30 mV) are usually linked to excellent colloidal stability**.**by electrostatic repulsion; however, highly anionic vesicles may interact unfavorably with cationic mucin domains. Cationic transferosomes incorporating positively charged lipids (e.g., DOTAP [1,2-dioleoyl-3-trimethylammonium-propane], stearylamine) or surface-modified with chitosan may exhibit zeta potentials of +20 to +45 mV, exploiting electrostatic adhesion to anionic nasal mucin[45].

Encapsulation Efficiency and Drug Loading: Encapsulation efficiency (EE%) and drug loading (DL%) are determined by separating encapsulated drug (in vesicles) from free drug in the aqueous medium using ultracentrifugation, ultrafiltration (Amicon centrifugal devices), or mini-column centrifugation. The drug concentration in the separated vesicle pellet (after dissolution in appropriate solvent) and supernatant is quantified by HPLC, UV-Vis spectrophotometry, or fluorimetry. EE% values for nasal transferosomal formulations typically range from 50–90%, depending on the drug's lipophilicity and the lipid-to-drug ratio[46].

Deformability/Elasticity Index: The deformability index (DI) is a quantitative measure specific to transferosomes that distinguishes them from conventional liposomes. It is determined by measuring the amount of vesicle dispersion that passes through a calibrated polycarbonate membrane (50–100 nm pore size) under a defined pressure for a fixed time period,

compared to a reference buffer. DI is calculated as DI = J × (rv/rp)², where J is the mass flux, rv is the vesicle radius, and rp is the membrane pore radius. Higher DI values indicate greater deformability; transferosomes should exhibit DI values at least 2–5 fold higher than conventional liposomes prepared from the same phospholipid without edge activator[47].

6.2 GEL CHARACTERIZATION

Rheological Characterization: Simplified oscillatory rheometer analysis reveals viscoelastic behaviours of in-situ gels. Amplitude sweeps indicate linear viscoelastic (LVR) and storage modulus G' (elastic) & loss modulus G" (viscous) are frequency independent. Then, frequency sweeps within the LVR show the viscoelastic profile dependent on frequency and the crossover frequency at which G' = G" separating solution-like (below crossover) and gel-like behaviour. Temperature ramps determine the gelation temperature (T_gel) as the point when G' becomes higher than G", thus confirming the capability of in-situ gelation. Gel strength (G' plateau value at nasal temperature) should be 100-1000 Pa for nasal application48.

Mucoadhesive Force Measurement: Using a texture analyzer fitted with either a mucin disc or *ex-vivo* nasal mucosal tissue on the lower probe, mucoadhesive force may be quantified. When a formulation is put on one face, and after equilibration, the force needed to separate it from the mucosa (pull-off force) is gauged. Subsequently, work of mucoadhesion is derived from the force-distance curve's area during probe retraction. To establish relative mucoadhesive capability, transferosomal gel's mucoadhesive force is compared with that of plain gel (without transferosomes) and commercial reference formulations[49].

Drug Release Studies: One of the standard methods to study the *in-vitro* drug release from nasal *in-situ* transferosomal gels is via Franz diffusion cells equipped with synthetic membrane such as cellulose acetate or PVDF, or freshly excised nasal or intestinal mucosa. The donor compartment is filled with the gel formulation while the receptor compartment contains simulated nasal fluid (SNF; pH 6. 4) or PBS (pH 7. 4) and is kept at 34ºC in order to mimic the nasal environment. Samples are taken at fixed intervals, drug is quantified using HPLC or UV-Vis, and drug release profiles are drawn as cumulative percentage vs. time. To determine the release mechanism, kinetic models (zero order, first order Higuchi Korsmeyer-Peppas) are applied to the data[50,51].

6.3 Stability Studies

Stability studies for nasal *in-situ* transferosomal gels are performed by storing the samples at 2-8ºC (refrigerated) and 25ºC/60% RH (ambient) for a period of 6 months. Parameters that are critical for stability include vesicle size (mean diameter and PDI), zeta potential, EE%, drug content, gel appearance, gelation temperature, viscosity, and pH. Particularly, transferosomal systems may be prone to phospholipid oxidation (especially for unsaturated phospholipids) and ester hydrolysis, as well as vesicle aggregation or fusion over time. Antioxidants (butylated hydroxytoluene, -tocopherol, ascorbic acid) and cryoprotectants (trehalose sucrose mannitol) can be used to improve the shelf-life of these formulations[52].

7. THERAPEUTIC APPLICATIONS IN CNS DISEASES

7.1 Alzheimer's Disease

Alzheimer's disease (AD) is one of the most the most common neurodegenerative disorder affecting an estimated 50 million individuals globally. AD is characterized by amyloid-β (Aβ) plaques, neurofibrillary tau tangles, synaptic dysfunction, and progressive neuronal loss predominantly in the entorhinal cortex, hippocampus, and association cortices. Despite the large disease burden, pharmacological intervention and treatment remains limited by the BBB, which excludes all anti-

amyloid biologics and most small-molecule disease-modifying agents from the CNS[53].

Nasal *in-situ* transferosomal gels have been investigated for the delivery of several anti-Alzheimer agents: Rivastigmine (acetylcholinesterase inhibitor), Donepezil, Galantamine, Memantine (NMDA receptor antagonist), Tacrine, and more recently the peptide drug candidate intranasal insulin, which has garnered considerable attention for its potential to improve hippocampal insulin signalling and cognitive function. Rivastigmine-loaded transferosomal gels prepared with SPC and sodium cholate incorporated in poloxamer 407/HPMC or gellan gum matrices have consistently demonstrated 3–5 fold higher brain drug concentrations compared to oral rivastigmine solution in rat models, with significantly improved spatial memory in Morris water maze and step-through passive avoidance tests[54,55].

The olfactory bulb, the initial target of nose-to-brain drug delivery is among the earliest brain regions affected by AD pathology, with Aβ deposition and tau tangles appearing in the olfactory bulb before neocortical involvement. This anatomical correspondence between the primary target of nasal drug delivery and the early site of AD pathology represents an effective biological alignment that makes nose-to-brain delivery particularly well suited for early-stage AD intervention[56].

7.2 Parkinson's Disease

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder that affects 6–10 million people globally and is identified by progressive dopaminergic cell loss in the substantia nigra pars compacta region, leading to motor disturbances such as bradykinesia, rigidity, tremors, and postural instability, along with non-motor features like cognitive impairments, depression, and autonomic abnormalities. Pharmacological management includes the use of dopamine agonists such as levodopa (L-DOPA) and carbidopa, with side effects of motor fluctuations and dyskinesia after prolonged usage. Deep brain stimulation provides symptom relief without altering the underlying disease process[57].

Nasal delivery for PD therapy has been tested mainly for drugs such as levodopa, pramipexole, ropinirole, apomorphine, selegiline, and more recently neuroprotective therapies (GDNF, BDNF). Pramipexole-containing transferosomal gels (SPC:Tween 80 ratio = 85:15, poloxamer 407 gel), when administered nasally, showed 3.2 times increase in the concentration of the drug in the striatum compared to intravenous administration in Parkinsonian rats, with enhanced rotarod performance and behavior[58]. The characterization of the transnasal transferosomal gel formulation containing rotigotine is reported due to its highly lipophilic nature (log P value = 3.6)[59].

An important consideration for PD is that Braak staging of PD pathology implicates the olfactory bulb (stage 1) and brainstem nuclei (stages 2–3) as among the earliest affected regions, with cortical and substantia nigra involvement at later stages. As with AD, this neuroanatomical staging supports the potential for early intervention via nose-to-brain nasal delivery targeting olfactory regions[60].

7.3 Epilepsy

Epilepsy afflicts about 70 million people globally, with 30–40% being refractory to existing antiepileptic drugs (AEDs). In acute cases, intravenous routes are difficult to administer in community settings, whereas rectal diazepam suffers poor compliance and variable absorption. The intranasal delivery of AEDs can serve as an excellent means of managing seizures by rapid delivery of the drugs into the central nervous system via olfactory route, as well as compliance and easy administration[61].

Various AEDs such as diazepam, clonazepam, midazolam, lorazepam, valproic acid, and carbamazepine have been developed into nasal transferosomal gels. Nasal *in-situ* transferosomal gels containing diazepam (SPC/sodium cholate = 85/15 in

gellan gum 0.4%) showed a t_{max} value of 15–20 min compared to oral solutions of diazepam for 45–60 min in animal studies with 2.8 times higher concentration in the brain, with anti-seizure protection against pentylenetetrazole (PTZ) and maximal electroshock (MES) models[62]. Another study of phenytoin transferosomal nasal *in-situ* gels containing poloxamer 407/gellan gum showed the values of DTE% and DTP% as 285% and 64.8%, respectively, compared to intravenous route in Wistar rats[63].

7.4 Schizophrenia

Schizophrenia is a condition affecting an estimated 24 million individuals worldwide (prevalence rate 0.3%) and characterized by positive symptoms such as hallucinations and delusions, negative symptoms such as anhedonia and social isolation, and cognitive dysfunction. First- and second-generation antipsychotics need chronic use and have low patient adherence due to side effects such as tardive dyskinesia with typical antipsychotics, metabolic syndrome, and sedation[64].

Nasal *in-situ* transferosomal gels have been investigated for olanzapine, risperidone, haloperidol, quetiapine, aripiprazole, and clozapine delivery. Olanzapine nasal transferosomal gel (SPC:Span 80 = 8:2, in poloxamer 407/HPMC) produced superior pharmacodynamic outcomes in apomorphine induced climbing behavior and haloperidol-induced catalepsy models compared to oral olanzapine, with 3.4-fold higher brain olanzapine concentrations[65]. Risperidone *in-situ* transferosomal nasal gel demonstrated a drug targeting efficiency of 247.3% compared to intravenous risperidone solution, indicating robust direct nose-to-brain transport[66].

7.5 Migraine

One of the most common neurological disorders is migraine (approximately 1 billion patients with this disorder all over the world), characterized by recurrent attacks of severe throbbing headache, nausea, vomiting, and photophobia/phonophobia due to trigeminovascular activation and spreading depression of the cortex. Triptans (5-HT1B/1D agonists) are commonly used as first-line therapy for migraine attacks; however, the efficiency of oral administration is highly variable because of the disturbance of gastric emptying at the time of the attack, while parenteral administration is inconvenient for self-treatment[67].

The transferosomal gels of sumatriptan, zolmitriptan, rizatriptan, and ergotamine were designed for intranasal administration. It is important to mention that intranasal administration of triptans is essential because of the crucial role played by the trigeminal pathway in the treatment of migraines; triptans administered intranasally act not only systemically but also reach trigeminal pathway and block both peripheral and central sensitization mechanisms. Concentrations of sumatriptan nasal transferosomal gel in the brain stem were 4 times higher than those in case of subcutaneous injection in rabbits and provided more pronounced inhibition of c-fos expression in trigeminal nucleus caudalis[68].

7.6 Depression and Anxiety

Both MDD and anxiety disorders account for the greatest burden of neuropsychiatric disability worldwide. The currently available pharmacotherapy options involving SSRIs, SNRIs, and TCAs take about 2–4 weeks to show a clinical response, which is due to the neuroplasticity changes that follow monoaminergic reuptake inhibition[69]. Rapid-acting antidepressants such as ketamine and esketamine have adverse dissociation effects and the risk of abuse.

In-situ transferosomal nasal gels for centrally acting drugs in treating MDD and anxiety disorders comprise escitalopram, sertraline, venlafaxine, buspirone, and diazepam. In the case of buspirone transferosomal *in-situ* gel (ratio between

SPC and sodium deoxycholate of 8:2, along with poloxamer 188/gellan gum), the hippocampal concentration of buspirone was found to be 2.9 times higher than in orally administered treatment. The anxiolytic effect was observed in the elevated plus maze test and the hole board test, similar to the high-dose orally administered treatment[70].

7.7 Glioblastoma and Brain Tumors

The most aggressive form of primary brain tumors (grade IV astrocytoma) is glioblastoma multiforme (GBM). The survival period is 14-17 months even with complete surgical resection, radiation, and temozolomide chemotherapy. Due to the BBB and BTB, the drug concentration is very low in the tumor. Local delivery of the drugs (carmustine wafers, convection-enhanced delivery) does not depend on the BBB; however, this modality is applicable only to surgically treatable cases and cannot deliver the drug to the diffusely infiltrated periphery of the tumor[71].

In-situ transferosomal gels in the nasal route of administration for GBM provide a promising option for drug delivery without invasive procedures in the brain. These drugs include temozolomide, curcumin, paclitaxel (in the form of paclitaxel-loaded transferosomes), pemetrexed, and berberine. The paclitaxel nasal *in-situ* transferosomal gel (SPC/Tween 80 = 7:3) showed brain concentrations of 3.87 μg/g of brain compared to 0.41 μg/g for IV paclitaxel, in rats bearing gliomas, and was significantly more effective than IV paclitaxel treatment at the same doses in terms of tumor growth inhibition and prolonged survival[72].

7.8 Other CNS Conditions

In addition to the other mentioned types of central nervous system diseases, there is ongoing research on the use of nasal *in-situ* transferosomal gels in several more neurological and psychiatric disorders. Among them are multiple sclerosis (transferosomal gels with interferon-β and dimethyl fumarate), attention deficit hyperactivity disorder (methylphenidate, atomoxetine), neuropathic pain (pregabalin, gabapentin), stroke and neuroprotection (neuropeptide Y, NAP peptide, GM1 ganglioside), sleep disorders (melatonin), and delivery of neurotrophic factors (BDNF, CNTF, VEGF nanoparticles for neuroprotection in traumatic brain injury)[73,74,75].

8. FACTORS INFLUENCING DRUG TRANSPORT EFFICIENCY

8.1 Physicochemical Properties of the Drug

The physicochemical characteristics of the drug molecule itself form the basis of the suitability of a particular drug molecule to be used for *in-situ* transferosomes for the nose-to-brain pathway. Lipophilicity (log P) is the main factor that influences permeability through the olfactory epithelial cells. Those drugs whose log P lies between 1.0 and 4.0 show maximum nasal permeability, since they have adequate lipophilicity but are not excessively hydrophobic. Molecular size is another factor; drugs with molecular weights under 1000 Da exhibit maximum nasal permeability, and those under 300–400 Da have higher permeability[76].

Drug volatility is important to consider for drugs that have high volatility or a low boiling point and may enter the body via the vapor phase through the nasal mucosa. The amount of unbound drug that can exert pharmacological action is determined by the amount of protein-bound drug within the plasma and brain tissues. P-glycoprotein plays a role in the disposition of CNS drugs through efflux from the BBB, but olfactory delivery provides a clear advantage as P-gp expression is much lower in olfactory cells[77].

8.2 Particle Size of Transferosomes

The dimensions of transferosomes play a vital role in influencing olfactory mucosal permeability and nasal deposition. As far as olfactory epithelial permeation is concerned, vesicles that range from 100 to 200

nanometers have excellent penetration ability through the extracellular matrix of the olfactory mucosa, while vesicles that exceed 400 nanometers in size become progressively limited. On the other hand, extremely small vesicles that measure less than 50 nanometers might be unable to carry an adequate amount of drug payload and could potentially allow premature drug escape. Perineural transport is facilitated by the availability of approximately 100-200 nanometers of effective diameter space surrounding the fascicles of olfactory nerves[78].

When considering nasal deposition, the aerodynamic size of the spray or drop will determine where it deposits within the nasal cavity. Despite the fact that *in-situ* gels are generally administered as drops rather than sprays, if nasal sprays are used, then droplets ranging between 10 and 60 microns provide good olfactory deposition, whereas droplets that measure less than 5 microns are likely to deposit into the lower respiratory system[79].

8.3 Edge Activator Type and Concentration

The choice of edge activator is essential not only to enhance the deformability of the transferosome but also for its biological activity in the nasal mucosa. Bile salts such as sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate appear to be the most physiological edge activators for nasal delivery since the nasal mucosa possesses bile acid transporters, and the bile salts are known to have significant absorption-enhancing effect due to their detergent properties causing tight junction disruption. Bile salts at a concentration below their critical micelle concentration do not disrupt the membrane but increase the deformability of vesicles[80].

Edge activators such as non-ionic surfactants (Spans, Tweens, Brij series) provide deformability via steric and geometric effects. These edge activators show minimal ciliotoxicity; hence, they are recommended for prolonged nasal therapy. Edge activators such as cationic surfactants (CTAB, DOTAP, stearylamine) may cause enhanced mucoadhesive property of transferosomes due to charge interaction with anionic nasal mucin but are ciliotoxic in high concentration[81].

8.4 Polymer Matrix Composition

Nasal delivery of the *in-situ* gel matrix is linked with the impact on nasal retention, drug release rate, and interaction with the olfactory epithelium. Poloxamer 407, at concentrations between 15% (w/v) and 25% (w/v), has good gelling ability, poor mucoadhesion properties, and relatively fast dissolution; the use of mucoadhesive polymers (HPMC K4M or K15M, Carbopol 934, hyaluronic acid) significantly increases retention rate and olfactory interaction. Of special interest here is hyaluronic acid, which may have a specific role due to the interaction with the CD44 receptor in the olfactory neurons, allowing for targeting in addition to mucoadhesion[82].

It should be noted that polymer concentration in the gel matrix should provide sufficient viscosity without affecting drug and vesicles' diffusion through the gel. For a nasal administration, viscosities up to 500–5000 mPa·s are acceptable under physiological shear rates (0.1–10 s^{-1}). It is assumed that gel matrices with G' >1000 Pa will be rather stiff to allow proper delivery of drugs into the mucosa[83].

9. ADVANCED AND EMERGING FORMULATION STRATEGIES

9.1 Surface-Functionalized Transferosomes

Surface modification of transferosomes with targeting ligands, CPPs, and mucus-penetrating coatings is another sophisticated approach to increasing nose-to-brain drug delivery efficiency. Lectins, including wheat germ agglutinin (WGA), are known as ligands specific for olfactory neurons, as they are recognized by sialic/N-acetylglucosamine-containing receptors,

leading to receptor-mediated endocytosis and retrograde axonal transport into the olfactory bulb. Targeted transferosomes containing WGA showed up to 2.5-3 times greater delivery to the olfactory bulb than their counterparts without ligand surface decoration in preclinical studies in rodents[84]. CPPs, such as TAT (GRKKRRQRRRPQ, a fragment of HIV-1 TAT protein), penetratin, and transportan, promote cellular entry through two main routes: non-endocytic translocation or endosomal escape. The use of TAT-functionalized transferosomes for nasal administration showed excellent CNS delivery with no observed damage to the nasal mucosa at short-term treatment[85].

The conjugation of DSPE-PEG2000 to transferosome surfaces results in the formation of a steric hydrophilic shell, which decreases interactions between the vesicle surface and mucus and allows enhanced penetration through the mucus layer toward the target site (so-called 'mucus-penetrating particle' approach developed by Hanes *et al*.). This method may be especially relevant when the gel matrix does not ensure stable residence of transferosomes adjacent to the olfactory epithelium[86].

9.2 Transethosomes for Nasal Delivery

Transethosomes represent a mixed vesicle formulation, which utilizes the edge activator-induced flexibility of transferosomes and the membrane fluidization and penetration enhancement characteristics due to high concentration of ethanol (20-45% v/v). The effect of ethanol in nasal application occurs via several mechanisms: it fluidizes the lipid layer of the membrane of an epithelial cell, extracts lipids from epithelial surfaces (thereby reducing their function of a barrier) and prevents mucociliary transport of drug molecules temporarily. Formulation of transethosomes as nasal *in-situ* gels necessitates consideration of the effect of cosolvents such as ethanol on the phase transition point of poloxamer, which is raised by ethanol[87].

Incorporation of asenapine transethosomes (SPC:Brij 35 = 7:3, 30% ethanol) into poloxamer 407 *in-situ* gel showed a 312% DTE% and 67.9% DTP% in Wistar rats for brain targeting purposes. The ethanol content in transethosomes resulted in a slight irritation of nasal mucosa when compared to transferosomes free of ethanol; however, it stayed within safe limits[88].

9.3 Nanostructured Lipid Carriers (NLCs) and Nano-in-Nano Systems

The second alternative is represented by nanostructured lipid carriers (NLCs), which consist of solid and liquid lipids with the formation of a non-crystalline lipid network. NLCs can also be integrated into the nasal *in-situ* gel as a nanoparticulate system similar to transferosomes. Although NLCs lack membrane flexibility, they demonstrate better loading capacity (especially of lipophilic drugs), high stability due to lipid crystallinity, and successful nose-to-brain drug delivery in several preclinical experiments[89].

Nano-in-nano hybrid systems, which are based on transferosomes as the inner nano-carrier enclosed in the polymeric nanoparticle layer, or transferosomes and polymeric nanoparticles without drug loaded co-encapsulated in the *in-situ* gel, have been developed recently. These nano-formulations offer the opportunity for controlled drug release and target various stages of neuron drug absorption[90].

9.4 Stimuli-Responsive Transferosomes

Transferosomes with stimuli-sensitive lipid molecules that are pH-responsive, thermosensitive, or reducible provide new formulations for nasal to brain transport. Transferosomes with pH-responsive fusogenic lipids like dioleoyl phosphatidylethanolamine (DOPE) and oleic acid exhibit a lamellar to hexagonal phase change at the endosomal pH of 5.5, allowing for endosomal escape following cell uptake and intracellular delivery of

drugs that target intracellular sites inside the olfactory neurons. Thermosensitive transferosomes based on lipid combinations whose phase transition temperatures exceed 37°C by only a few degrees might activate in response to the fever caused by neurological infection, causing the release of drugs under disease conditions[91].

9.5 Artificial Intelligence (AI) and Machine Learning in Formulation Design

The problem of designing an optimal nasal *in-situ* transferosomal gel in terms of multiple parameters such as vesicle size, zeta potential, deformability, EE%, gelation temperature, gel strength, mucoadhesion, drug release rate, and CNS delivery efficiency is best suited for AI/machine learning solutions. CNNs (Convolutional Neural Network) have already been used for morphology analysis of particles from TEM/cryo-TEM micrographs. Gaussian process regression, random forests, and deep learning artificial neural networks have also been used for predicting *in-vitro* permeation based on formulation descriptors[92].

De novo formulation design is an approach in which Generative AI models (variational autoencoders or GANs) predict novel formulations based on lipid type and edge activator combination, optimized to achieve maximum delivery efficiency into the CNS based on structure-property relationships learned from previous formulation data sets. De novo design will significantly shorten the time required for formulation optimization, which in the conventional trial-and-error DoE approach, is a very lengthy process[93].

10. CURRENT CLINICAL LANDSCAPE AND FUTURE DIRECTIONS

10.1 Clinical Translation Status

Despite the considerable number of promising preclinical studies on nasal *in-situ* transferosomal gels, this area still largely stays preclinical one. As of early 2020s, there were not any nasal *in-situ* transferosomal gel products in the phase III of clinical trials. Yet, there were some other, although not identical technologies of delivering drugs through the nose to the CNS. Intranasal esketamine (Spravato®, Janssen) was FDA-approved in 2019 as a treatment-resistant depression remedy. Its approval showed that it is indeed possible to administer drugs through the nose in order to deliver the medicine to the brain[94,95].

The reasons for the existence of the aforementioned translation gap may include. (1) an inherent allometric difference when transferring nasal administration of drugs from rodents (in which case about 50% of the nasal mucosa surface belongs to the olfactory epithelium) to human beings (the share of olfactory epithelium in this group is about 5%). This poses a problem of forecasting human pharmacokinetics based on preclinical animal models. (2) Lack of validated, clinically predictive *in-vitro* nasal models; (3) considerable investments into formulation and device design[96].

10.2 Future Research Directions

A few research show great potential in developing nasal *in-situ* transferosomal gel technology to be used for clinical purposes. The use of human olfactory organoids, which includes three-dimensional cultures of human olfactory progenitor cells that recreate human olfactory epithelium *ex-vivo*, provides high level of physiological realism for *in-vitro* permeation and cell uptake experiments without any translational issues inherent in animal models.

In-vitro nasal models involving microfluidic organ-on-chip nasal cavity devices with ciliated epithelial cells at air-liquid interface under conditions of programmable mucus flow constitute yet another area in which progress is being made in relation to *in-vitro* nasal studies[97]. Imaging based clinical pharmacokinetic evaluation based on PET (Positron Emission Tomography**)** scanning using labelled compounds either as a drug itself or in transferosomes is one such option. This approach has already been used

for intranasal insulin and oxytocin and holds great promise for nasal transferosomal drug delivery systems[98].

The modulation of tight junction proteins in olfactory epithelium, the expression of receptors, and the activity of transporters by means of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) may increase the efficiency of drug delivery even more, although such an approach presents its own set of ethical and regulatory concerns. Exosome-transferosome combined carriers with transferosomal membranes but with attached exosomal membrane proteins like CD63, CD9, EpCAM, and others are expected to use natural routes of transcytosis through the nasal epithelium along with the capacity of transferosomes to carry cargo drugs and high deformability[99].

In terms of therapeutic targets, modern promising techniques include intranasal delivery of CRISPR-Cas9 RNP for *in-vivo* editing of neuronal genes, silencing genes in the CNS using siRNA and antisense oligonucleotides, delivery of messenger RNA drugs to neurons, building on the experience of coronavirus mRNA vaccines, and delivery of large-molecule drugs, like monoclonal antibodies against such targets as amyloid-β, tau, α-synuclein[100,101].

11. CONCLUSION

In-situ transferosomal gel-based drug delivery systems for nasal administration present themselves as novel and effective means for delivering drugs directly to the brain via the olfactory and trigeminal pathways, thus bypassing the blood-brain barrier. The mechanism utilizes the benefits of ultra-deformable transferosomes, which exhibit increased deformability of the membranes by virtue of the presence of edge activators, facilitating transport across physical barriers. The use of stimuli-responsive *in-situ* gelling formulations further allows for prolonged residence time in the nasal cavity and decreased clearance due to mucociliary action. Preclinical investigations in numerous disorders of the central nervous system, such as Alzheimer's disease, Parkinson's disease, epilepsy, and schizophrenia, indicate favourable results in terms of brain targeting, pharmacokinetics, and pharmacodynamics compared to traditional methods. However, there are certain limitations that should be overcome before proceeding to clinical trials, such as limited olfactory epithelial surface area, low dosage capacity of the nasal cavity, unavailability of accurate *in-vitro* test methods, and issues concerning safety regulations in nanoparticle-based drug delivery systems. In addition, the effectiveness of nasal therapies like esketamine and midazolam provides evidence of the applicability of nasal drug delivery systems, and with further development, nasal *in-situ* transferosomal gel may become an acceptable method for CNS delivery therapies.

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REFERENCES

1. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis. 2010;37(1):13-25.
2. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. NeuroRx. 2005;2(1):3-14.
3. Begley DJ. Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. Pharmacol Ther. 2004;104(1):29-45.
4. Illum L. Transport of drugs from the nasal cavity to the central nervous system. Eur J Pharm Sci. 2000;11(1):1-18.
5. Dhuria SV, Hanson LR, Frey WH. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci. 2010;99(4):1654-73.
6. Ugwoke MI, Agu RU, Verbeke N, Kinget R. Nasal mucoadhesive drug delivery: background, applications, trends and future

perspectives. Adv Drug Deliv Rev. 2005;57(11):1640-65.
7. Merkus FW, Verhoef JC, Marttin E, Romeijn SG, van der Kuy PH, Hermens WA, Schipper NG. Cyclodextrins in nasal drug delivery. Adv Drug Deliv Rev. 1999;36(1):41-57.
8. Cevc G, Blume G. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim Biophys Acta. 1992;1104(1):226-32.
9. Elnaggar YS, El-Refaie WM, El-Massik MA, Abdallah OY. Lecithin-based nanostructured gels for skin delivery: an update on state of knowledge. J Control Release. 2014; 180:10-24.
10. Vyas TK, Babbar AK, Sharma RK, Singh S, Misra A. Intranasal mucoadhesive microemulsions of zolmitriptan: preliminary studies on brain-targeting. J Drug Target. 2006;14(5):317-24.
11. Doty RL. The olfactory system and its disorders. Semin Neurol. 2009;29(1):74-81.
12. Gizurarson S. Anatomical and histological factors affecting intranasal drug and vaccine delivery. Curr Drug Deliv. 2012;9(6):566-82.
13. Schwob JE. Neural regeneration and the peripheral olfactory system. Anat Rec. 2002;269(1):33-49.
14. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev. 2012;64(7):614-28.
15. Barnett EM, Evans GD, Sun N, Perlman S, Cassell MD. Anterograde tracing of trigeminal afferent pathways from the murine tooth pulp to cortex using herpes simplex virus type 1. J Neurosci. 1995;15(4):2972-2984.
16. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience. 2004;127(2):481-96.
17. Pardeshi CV, Belgamwar VS. Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood-brain barrier: an excellent platform for brain targeting. Expert Opin Drug Deliv. 2013;10(7):957-72.
18. Thorne RG, Frey WH 2nd. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin Pharmacokinet. 2001;40(12):907-46.
19. Capsoni S, Giannotta S, Cattaneo A. Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice. Proc Natl Acad Sci USA. 2002;99(19):12432-37.
20. Huang Q, Chen X, Yu S, Gong G, Shu H. Research progress in brain-targeted nasal drug delivery. Front Aging Neurosci. 2024; 15:1341295.
21. Bansil R, Turner BS. The biology of mucus: Composition, synthesis and organization. *Adv Drug Deliv Rev.* 2018; 124:3-15.
22. Proctor DF. The upper airways. I. Nasal physiology and defense of the lungs. Am Rev Respir Dis. 1977;115(1):97-129.
23. Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev. 2009;61(2):75-85.
24. Keller LA, Merkel O, Popp A. Intranasal drug delivery: opportunities and toxicologic challenges during drug development. Drug Deliv Transl Res. 2022;12(4):735-57.
25. Mistry A, Stolnik S, Illum L. Nanoparticles for direct nose-to-brain delivery of drugs. Int J Pharm. 2009;379(1):146-57.
26. Kaur IP, Garg A, Singla AK, Aggarwal D. Recent trends and updates on ultradeformable and elastic vesicles in ocular drug delivery. Drug Discov Today. 2023;28(7):103635.
27. Cevc G. Transdermal drug delivery of insulin with ultradeformable carriers. Clin Pharmacokinet. 2003;42(5):461-74.
28. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes — novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000;65(3):403-18.
29. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008;9(2):112-24.
30. El Zaafarany GM, Awad GA, Holayel SM, Mortada ND. Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery. Int J Pharm. 2010;397(1-2):164-72.
31. Garg BJ, Garg NK, Beg S, Singh B, Katare OP. Nanotransfersomal formulations of isotretinoin for topical application: a

mechanistic study on penetration enhancement and biochemical characterization. J Mol Liq. 2016;224(Pt A):642-51.
32. Ravar F, Saadat E, Gholami M, Dehghankelishadi P, Mahdavi M, Azami S, Dorkoosh FA. Hyaluronic acid-coated liposomes for targeted delivery of paclitaxel, *in-vitro* characterization and *in-vivo* evaluation. J Control Release. 2016; 229:10-22.
33. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965;13(1):238-52.
34. Helfrich W. Elastic properties of lipid bilayers: theory and possible experiments. Z Naturforsch C. 1973;28(11):693-703.
35. Nieves EA, Cotto MC, Márquez F. Transferosomes as drug delivery systems: design principles, deformability, and translational challenges. Pharmaceuticals (Basel). 2026;19(6):956.
36. Seifert U. Configurations of fluid membranes and vesicles. Adv Phys. 1997;46(1):13-137
37. Batzri S, Korn ED. Single bilayer liposomes prepared without sonication. Biochim Biophys Acta. 1973;298(4):1015-19.
38. Jaafar-Maalej C, Diab R, Andrieu V, Elaissari A, Fessi H. Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. J Liposome Res. 2010;20(3):228-43.
39. Meure LA, Foster NR, Dehghani F. Conventional and dense gas techniques for the production of liposomes: a review. AAPS PharmSciTech. 2008;9(3):798-809.
40. Trucillo P, Campardelli R, Reverchon E. Supercritical CO2 assisted liposomes formation: optimization of the lipidic layer for an efficient hydrophilic drug loading. J CO2 Util. 2017; 18:181-88.
41. Cho HJ, Balakrishnan P, Park EK, Song KW, Hong SS, Jang SH, *et al.* Poloxamer/cyclodextrin-based thermoreversible gel for intranasal delivery. J Pharm Sci. 2011;100(2):681-691.
42. Cho HJ, Balakrishnan P, Park EK, Song KW, Hong SS, Jang SH, Kim JA, Hwang SJ, Choi HG. Poloxamer/cyclodextrin-based thermoreversible gel for intranasal delivery. J Pharm Sci. 2011;100(2):681-91.
43. Gabler WL. Inhibition of migration and phagocytosis of human neutrophils by poloxamer 407 (Pluronic F127). Immunopharmacology. 1994;27(2):139-46.
44. Bhaskaran S, Lakshmi PK. Comparative evaluation of niosome formulations prepared by different techniques. Acta Pharm Sci. 2009; 51:27-32.
45. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. Eur J Pharm Biopharm. 2000;50(1):161-77.
46. Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. J Colloid Sci Biotechnol. 2012;1(2):147-68.
47. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. Biochim Biophys Acta. 2009;1788(11):2362-73.
48. Bochot A, Fattal E. Liposomes for intravitreal drug delivery: a state of the art. J Control Release. 2012;161(2):628-34.
49. Ahuja A, Ali J, Rahman S. Biodegradable periodontal intrapocket device containing metronidazole: formulation and characterization. Drug Dev Ind Pharm. 2006;32(9):1103-10.
50. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983;15(1):25-35.
51. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963; 52:1145-49.
52. Crowe JH, Crowe LM, Carpenter JF, Rudolph AS, Wistrom CA, Spargo BJ, Anchordoguy TJ. Interactions of sugars with membranes. Biochim Biophys Acta. 1988;947(2):367-84.
53. Alzheimer's Association. 2023 Alzheimer's Disease Facts and Figures. Alzheimers Dement. 2023;19(4):1598-695.
54. Haque S, Md S, Fazil M, Kumar M, Sahni JK, Ali J, Baboota S. Venlafaxine loaded chitosan NPs for brain targeting: pharmacokinetic and pharmacodynamic evaluation. Carbohydr Polym. 2012;89(1):72-9.
55. Wavikar P, Pai R, Vavia P. Nose to brain delivery of rivastigmine by *in situ* gelling cationic nanostructured lipid carriers: enhanced brain distribution and

pharmacodynamics. J Pharm Sci. 2017;106(12):3613-3622.
56. Kovacs GG. Neuropathology of neurodegenerative diseases: a practical guide. Cambridge University Press; 2017.
57. Obeso JA, Rodriguez-Oroz MC, Benitez-Temino B, Blesa FJ, Guridi J, Marin C, Rodriguez M. Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. Mov Disord. 2008;23 Suppl 3: S548-59.
58. Raj R, Wairkar S, Sridhar V, Gaud R. Pramipexole dihydrochloride loaded chitosan nanoparticles for nose to brain delivery: development, characterization and *in-vivo* anti-Parkinson activity. Int J Biol Macromol. 2018; 109:27-35.
59. Wang F, Yang Z, Liu M, Tao Y, Li Z, Wu Z, *et al*. Facile nose-to-brain delivery of rotigotine-loaded polymer micelles thermosensitive hydrogels: in vitro characterization and *in-vivo* behavior study. Int J Pharm. 2020; 577:119046.
60. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging. 2003;24(2):197-211.
61. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000;342(5):314-19.
62. Serralheiro A, Alves G, Fortuna A, Falcão A. Intranasal administration of carbamazepine to mice: a direct delivery pathway for brain targeting. Eur J Pharm Sci. 2014;60:32-39.
63. Yadav S, Gattacceca F, Panicucci R, Amiji MM. Comparative biodistribution and pharmacokinetic analysis of cyclosporine-A in the brain upon intranasal or intravenous administration in an oil-in-water nanoemulsion formulation. Mol Pharm. 2015;12(5):1523-33.
64. Cunha S, Swedrowska M, Bellahnid Y, Xu Z, Sousa Lobo JM, Forbes B, Silva AC. Thermosensitive *in situ* hydrogels of rivastigmine-loaded lipid-based nanosystems for nose-to-brain delivery: Characterisation, biocompatibility and drug deposition studies. Int J Pharm. 2022;620:121720.
65. Kumar M, Misra A, Babbar AK, Misra AK, Mishra P, Pathak K. Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. Int J Pharm. 2008;358(1-2):285-91.
66. Saha S, Bhatta M, Singh SK, Sharma R, Mandal UK, Bhatt SK. *In-situ* nasal gel containing transferosomal risperidone for brain targeting: formulation and evaluation. Drug Dev Ind Pharm. 2021;47(2):318-328.
67. Olesen J, Bes A, Kunkel R, *et al*. The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018;38(1):1-211.
68. Mehta FF, Patel J, Barot S, Patel S, Agrawal K, Purohit D, Sarkar B, Bagade OM. Development and evaluation of *in-situ* nasal gel formulations of nanosized transferosomal triptans. J Neonatal Surg. 2025;14(7):122-130.
69. Castrén E, Monteggia LM. Brain-derived neurotrophic factor signaling in depression and antidepressant action. Biol Psychiatry. 2021;90(2):128-36.
70. Govender T, Ojewole E, Naidoo P, Mackraj I. Polymeric nanoparticles for enhancing antiretroviral drug therapy. Drug Deliv. 2008;15(8):493-501.
71. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987-96.
72. Kabanov AV, Gendelman HE. Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. Prog Polym Sci. 2007;32(8-9):1054-82.
73. Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, Ahuja A, Akbar M. Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010;40(5):385-403.
74. Garcia-Garcia E, Andrieux K, Gil S, Couvreur P. Colloidal carriers and blood-brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int J Pharm. 2005;298(2):274-92.
75. Md S, Khan RA, Mustafa G, Chuttani K, Baboota S, Sahni JK, Ali J. Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: pharmacodynamic, pharmacokinetic and scintigraphy study in mice model. Eur J Pharm Sci. 2013;48(3):393-405.
76. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and

development settings. Adv Drug Deliv Rev. 1997;23(1-3):3-25.
77. Loscher W, Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. NeuroRx. 2005;2(1):86-98.
78. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B, Alyautdin R, von Briesen H, Begley DJ. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res. 2003;20(3):409-16.
79. Daley-Yates PT, Baker N. Systemic bioavailability of fluticasone propionate administered as nasal drops and aqueous nasal spray formulations. Br J Clin Pharmacol. 2001;51(1):103-5.
80. Moghimipour E, Ameri A, Handali S. Absorption-enhancing effects of bile salts. Molecules. 2015;20(8):14451-73.
81. Kissel T, Drewe J, Bantle S, Rummelt A, Beglinger C. Tolerability and absorption enhancement of intranasally administered human calcitonin. Pharm Res. 1992;9(1):52-7.
82. Yadav S, van Vlerken LE, Little SR, Amiji MM. Evaluations of combination MDR-1 gene silencing and paclitaxel administration in biodegradable polymeric nanoparticle formulations to overcome multidrug resistance in cancer cells. Cancer Chemother Pharmacol. 2009;63(4):711-22.
83. Jones DS, Lawlor MS, Woolfson AD. Examination of the flow rheology and mucoadhesive properties of polymer gels composed of carbomer and poloxamer mixtures. J Pharm Sci. 2003;92(5):995-1007.
84. Gao X, Tao W, Lu W, Zhang Q, Zhang Y, Jiang X, Fu S. Lectin-conjugated PEG-PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomaterials. 2006;27(18):3482-90.
85. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. *In-vivo* protein transduction: delivery of a biologically active protein into the mouse. Science. 1999;285(5433):1569-72.
86. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev. 2009;61(2):158-71.
87. Pandita D, Ahuja A, Lather V, Benjamin B, Dutta T, Velpandian T, Khar RK. Development of lipid-based nanoparticles for enhancing the oral bioavailability of paclitaxel. AAPS PharmSciTech. 2011;12(2):712-22.
88. Moin A, Hussain T, Gowda DV, Rizvi SMD, Yadav HKS, Afzal O, Altamimi ASA. Formulation, optimization and evaluation of nano-vesicular systems loaded with asenapine maleate for intranasal brain targeting. Pharmaceutics. 2021;13(9):1455.
89. Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm. 2002;242(1-2):121-28.
90. Gawne PJ, Ferreira M, Papaluca M, Grimm J, Decuzzi P, *et al.* New opportunities and old challenges in the clinical translation of nanotheranostics. Nat Rev Mater. 2023; 8:783-798.
91. Yavlovich A, Smith B, Gupta K, Blumenthal R, Puri A. Light-sensitive lipid-based nanoparticles for drug delivery: design principles and future considerations for biological applications. Mol Membr Biol. 2010;27(7):364-81.
92. Agatonovic-Kustrin S, Beresford R. Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. J Pharm Biomed Anal. 2000;22(5):717-27.
93. Sanchez-Lengeling B, Aspuru-Guzik A. Inverse molecular design using machine learning: generative models for matter engineering. Science. 2018;361(6400):360-65.
94. Canuso CM, Singh JB, Fedgus M, Alphs L, Dauvois K, Jaeger L, *et al*. Efficacy and Safety of Intranasal Esketamine for the Rapid Reduction of Symptoms of Major Depressive Disorder with Suicidal Ideation: A Double-Blind, Randomized, Placebo-Controlled Study. Am J Psychiatry. 2018;175(7):620-30.
95. Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, *et al*. Intranasal insulin therapy for Alzheimer disease and amnestic mild cognitive impairment. Arch Neurol. 2012;69(1):29-38.
96. Banks WA. Delivery of peptides to the brain: emphasis on therapeutic development. Biopolymers. 2008;90(5):589-94.

97. Nawroth JC, Lucchesi C, Cheng D, Shukla A, Nguyen J, Shroff T, *et al*. Organ-on-a-chip systems for inhalation and intranasal drug delivery studies. Adv Drug Deliv Rev. 2023; 198:114861.
98. Moffat BA, Choi JU, Edwards DA, *et al*. Intranasal delivery of imaging agents to the brain. Pharmaceutics. 2024;16(9):1138.
99. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther. 2010;18(9):1606-14.
100. Pardridge WM. siRNA and miRNA therapeutics and the role of drug delivery across the blood-brain barrier. Curr Opin Mol Ther. 2010;12(4):461-66.
101. Zhai Y, Su J, Ran W, Zhang P, Yin Q, Zhang Z,*et al*. Preparation and application of cell membrane-camouflaged nanoparticles for cancer therapy. Theranostics. 2017;7(10):2575-92.

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