

Review Article

Multifunctional approaches of cubosome-integrated thermoresponsive gels for intranasal brain targeting: Innovations, therapeutic potential, and challenges

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ABSTRACT

Neurodegenerative disorders, such as cognitive disabilities and dementia, have nowadays become a global burden, distressing millions of elderly people worldwide. It is characterized by progressive loss of neurons in the central nervous system, affecting higher cortical centers and ultimately impacting the social life of the patients. Conventional treatment approaches to such neurological complications primarily involve systemic drug delivery through either oral or parenteral routes of administration. However, limited brain bioavailability due to the restrictive properties of the blood-brain barrier, systemic side effects, biotransformation of the drug, and limited ability to maintain therapeutic concentrations in the CNS pose a challenge in targeted therapy. In recent years, cubosomes, nanostructured lipid-based carriers of an internally bi-continuous cubic phase, have emerged as a novel drug delivery system, which are capable of encapsulating both hydrophilic and lipophilic drugs. These unique structured nanocarriers possess favourable physical properties, including high surface area and thermodynamic stability, which make them a potential tool for controlled and targeted drug delivery systems. With intranasal administration, cubosomes offer the ability to bypass the BBB due to the use of the olfactory and trigeminal neural pathways, achieving direct brain targeting of the therapeutics. Thus, this review aims to provide an overview of the nose-to-brain transport mechanisms, based on the anatomical and physiological basis following intranasal delivery of cubosomes integrated into thermoresponsive *in situ* gels. The review also focuses on their potential therapeutic applications in the treatment of neurodegenerative diseases. In addition, the review also presents formulation challenges and strategies to address them, providing insight into the future possibilities of cubosome-based gels as a new non-invasive brain-targeted therapy.

1. Introduction

Neurodegenerative disorders are mainly caused by the gradual loss of neurons and synaptic connections in the central nervous system (CNS). They typically occur at a later age and usually lead to early death [1]. Stroke, neonatal encephalopathy, dementia, meningitis, autism spectrum disorder, migraine, diabetic neuropathy, spinal cord injury, and epilepsy are the major neuropathological complications that play a crucial role in reducing the life span of the population. As per WHO reports, CNS-related disorders, including disability and premature death, have increased by 18 % since 1990. Additionally, Alzheimer's disease, the most common neurologic disorder associated with

long-term cognitive impairment, is known to affect 46.8 million people over the globe [2]. Alternatively, there will be 1,238,000 people suffering from the second most prevalent neurologic disorder, Parkinson's disease, in the US by 2030 [3]. While 10 million new cases are reported every year, currently, 55 million individuals have dementia globally (WHO dementia, 2025), and over 50 million people suffer from epilepsy (WHO epilepsy, 2024) [4].

Conventional drug delivery systems follow the 'one gene, one drug, one disease' philosophy, which may not be effective in developing strategies for disease-modifying therapies. Drugs that target a single protein are prone to resistance because even a single mutation in the target active site can significantly lower the compound's binding affinity

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and efficacy [5]. Other developed treatments, such as neurotrophin mimetics and peptidomimetics, stem cell replacement therapy, and immunotherapy, show promise in treating neurological disorders by promoting neuron growth, maintenance, and neuroplasticity [6–8]. However, they remain unsuccessful in stopping the progression of neurodegenerative diseases due to challenges of crossing the blood-brain barrier (BBB) and associated significant side effects; the precise efficacy of these therapies remains uncertain, leading to decreased survival rates [9,10].

Therefore, the drawbacks of traditional therapies, including low BBB permeability, quick systemic elimination, and lack of specificity, have rendered treatment for neurodegenerative diseases highly challenging [1,11]. Such challenges mandate the development of advanced nanocarrier-based drug delivery systems that enhance the efficacy of the therapeutic agents. Among them, cubosomes, characterised by a bicontinuous cubic phase, are exceptional possibilities because they have high drug capacity, biocompatibility, and can be engineered to provide prolonged and controlled drug release [12,13]. In addition, the incorporation of cubosomes into thermoresponsive gels enhances nasal mucosal adhesion and extends residence time, making this formulation approach very promising for targeted and effective treatment of neurodegenerative diseases [14–16].

Furthermore, the article describes the pathological basis of neurological disorders, the BBB as an obstacle for drug transport, the advantages of the nasal route in brain targeting, nasal anatomy, transport mechanisms, therapeutic benefits, structural properties of cubosomes and cubosome-based gels, and their mechanism of brain targeting. The review also elaborates on the current limitations of intranasal delivery of cubosomal formulations while highlighting how *in situ* cubosomal gels can effectively overcome them.

2. General basis and pathological considerations involved in the treatment of neurological disorders

The majority of neurological disorders in humans are caused by alterations in protein expression in the brain and other peripheral organs, which are often associated with selective neuronal loss, brain atrophy, neuroinflammation, and dysfunction [17]. The accumulation of proteins, such as tau, α -synuclein, transactive response DNA-binding protein 43, prion protein, and amyloid- β , at the neuromuscular junctions or in the cell body is a major hallmark for therapeutic targets [18]. Various risk factors contribute to neurodegeneration, which causes axonal, vascular, and cellular injury over time, ultimately leading to persistent neuronal damage and cell death by altering cellular, molecular, and metabolic functions [19]. Genetic polymorphisms influence immune-related neurodegeneration, i.e., major histocompatibility complex class II expression, phagocytosis, complement activation, microglial activation, and haematopoiesis [20]. Infectious agents can trigger neuroinflammation, negatively affecting the BBB and activating CNS immune cells, which causes excessive inflammation, harming the neurons and glial cells [20,21]. Lifestyle factors like diet containing high sugar, high-fat, cholesterol, and addictions to tobacco and alcohol, along with factors like ageing and oxidative stress, can trigger the progression of neural damage. These factors frequently exacerbate cognitive impairment by encouraging oxidative damage and inflammation in the brain [22].

To treat neurodegenerative disorders, the nose-to-brain route is considered a non-invasive technique for the quick transport of medications from the nasal cavity to the brain and bloodstream [23,24] by avoiding first-pass metabolism, degradation of active pharmaceutical ingredients, and delaying partial absorption in the gastrointestinal route, and increasing bioavailability [25]. The nasal mucosa also provides an ideal environment for quick drug absorption due to its high permeability, wide vasculature, and comparatively low enzymatic activity [26].

Cubosomes are self-assembled lipid-based cubic-shaped lyotropic

liquid crystalline nano-colloidal delivery systems. They are transparent, isotropic, non-toxic, physically stable in excess water, and have a high inner surface area, allowing for enhanced drug loading and stability within biological environments, enhancing cell uptake. The highly structured internal design of cubosomes increases viscosity, promoting effective therapeutic uptake, sustained release, and improved stability. These make cubosomes a promising lipid-based drug delivery technique for *in vivo* applications [27]. They have attracted attention as a drug delivery system due to their biocompatibility, ability to encapsulate both lipophilic and hydrophilic drugs, and offer the potential for reversible, on-demand release, providing a notable advantage over traditional liposome-based systems [28,29]. However, these cubosomal formulations possess the drawback of rapid mucociliary clearance from the nasal cavity, resulting in a brief residence time, as they are less viscous. Therefore, the nasal cavity limits the absorption of therapeutic molecules of our interest. This can be overcome by formulating a mucoadhesive *in situ* nasal gel. They enhance the nasal retention time, increase the penetration rate, and improve nasal absorption [30]. Additionally, these mucoadhesive *in situ* gels remain in solution state before intranasal administration, which can be readily applied over the nasal palate, enabling precise medication dosing. Once instilled, it comes into contact with the stimuli at the application site (e.g., body temperature (35–37 °C)) and it transits from a liquid state (at storage conditions) to a gel due to the stimuli at the application site, i.e., the nasal temperature [31]. Those temperature-stimuli-responsive components are named as thermoresponsive. These are characterized by thermoresponsive behaviour that permits temperature-dependent reversible structural transformations within the internal bicontinuous cubic phase [32]. This occurs as lipid molecules, such as monoolein or phytantriol, which constitute the cubic lattice, respond to temperature changes by rearranging their positions, inducing changes in lipid bilayer curvature and packing parameters. These transitions can also result in different lattice symmetries (field of view phases Pn3m to Im3m or Ia3d phases) and diffusion routes for molecules encapsulated in the cubosomes [33]. Additionally, these structural alterations triggered by temperature can be harnessed for controlled and site-specific drug release, where, following an increase in local or physiological temperature, aqueous channels expand or the mesophase destabilizes and release can be achieved in a controlled manner [34]. This thermoresponsiveness, which enables remote-triggered drug release properties, is a unique and promising perspective in cubosome development as a stimuli-responsive nanocarrier system for various applications.

3. Blood-brain barrier as a critical obstacle in brain targeting

The BBB is an active and selective boundary that physically separates the CNS from the peripheral circulation, playing an important role in controlling the movement of molecules and cells. Thereby, it protects the brain from the harmful substances present in the circulating blood in the periphery while allowing essential nutrients to pass through. It maintains a specialized microenvironment required for brain homeostasis [35]. The basic structure of the BBB is composed of endothelial cells (EC), which form the innermost layer of the blood vessels and are enveloped by cellular components such as pericytes and astroglial foot processes, creating a continuous stratum that acts as a barrier between blood vessels and brain tissue. They help in regulating vascular stability, permeability, and limiting paracellular transport. EC is associated with the basement membrane, connected by tight junction proteins that regulate cell-to-cell adhesion, providing structural support to the BBB, along with specialized transport mechanisms and pinocytotic vesicles for molecular exchange. Astrocyte end-feet with the basal lamina are essential for signaling and BBB preservation. It has a high trans-endothelial electrical resistance of 1500–2000 Ω cm², compared to 3–30 Ω cm² across most peripheral microvessels, which prevents ions from moving freely. A space known as the Virchow-Robin space exists around penetrating vessels and venules, where a gap between endothelial cells

and brain tissue accommodates perivascular macrophages that contribute to the immune functions of the CNS [36]. This highly integrated network of cells that supports neuronal activity, maintains the BBB, and controls cerebral blood flow is referred to as the “neuro-vascular unit” [37].

The delivery of pharmaceutical formulations to the brain is one of the most challenging tasks in drug development due to two primary factors. First, the anatomical and physiological characteristics of the BBB severely limit the transport of most drugs, especially hydrophilic compounds, making it challenging to treat CNS disorders. Second, many drugs have insufficient physicochemical properties, such as poor solubility, which restricts their bioavailability and therapeutic activity in the brain [38]. BBB only permits specific molecules to flow across easily, while others need specific transport pathways. Lipophilic, low molecular weight (<400–600 Da), and positively charged compounds can passively penetrate the BBB through simple diffusion, which is fueled by concentration gradients. Electrochemical or solute concentration gradients, along with solute carrier proteins, enable movement of polar and necessary molecules, such as glucose and amino acids, without the use of ATP. On the other hand, ATP-binding cassette transporters, such as P-glycoprotein and breast cancer-related protein, actively use ATP energy to pump out big, lipid-soluble molecules, which frequently restricts drugs from reaching the brain. Conversely, receptor-mediated transcytosis is a mechanism used by large macromolecules such as insulin, transferrin, and leptin, where binding to endothelial cell receptors triggers vesicular transport into the brain. These systems work together to control what enters and leaves the brain, protecting and maintaining its equilibrium [39]. Given the limitations imposed by the BBB, alternative non-invasive routes have gained attention, with the intranasal pathway emerging as a promising strategy to bypass this barrier for efficient brain targeting.

4. Intranasal route bypassing the blood–brain barrier for efficient brain targeting

Intranasal administration is a non-invasive way to deliver therapeutics via application in the nasal cavity to the brain and systemic circulation without crossing the BBB. It helps bypass the BBB, directly delivering the drug to the CNS, eliminating the side effects caused by systemic delivery [40]. To fully understand its potential in neurological therapy, the following sections will explore the anatomy of the nasal cavity, the underlying mechanisms of nose-to-brain drug transport, and the applications of this route in the treatment of neurodegenerative disorders.

4.1. Nasal anatomy and pathways for brain targeting

The basic structure of the nasal cavity consists of two symmetrical cavities divided by the septum, which are covered with a layer of mucosa and have a total absorption area of 150–160 cm². The exterior portion of the nose is composed of bone, cartilage, and skin, which together form an appendage on the face that encircles only the nostrils and one-third of the nasal cavity. The nasal and oral cavity is separated by the presence of the palatine bone. The nasal cavity is further divided into three distinct regions: the vestibular region, the olfactory region, and the respiratory region. The vestibular region is the most anterior part, located at the nostril opening, composed of squamous epithelial cells and a few ciliated cells. It is poorly vascularized with a surface area of 0.6 cm², and contains nasal hair, which filters the inhaled air and captures dust, pollen, and other particles, preventing them from entering the lungs. This defence mechanism aids in avoiding allergies and respiratory infections [41].

Ten percent of the nasal epithelium surface area consists of the olfactory region. It is made up of a pseudostratified columnar epithelium that mediates scent and is situated on the nasal cavity's upper portion [42]. It is composed of olfactory neural cells, the sustentacular or

supporting cells, and the basal cells. Unmyelinated neurons, also known as olfactory neural cells (axons), are situated between supporting cells from the olfactory bulb to the apical surface of the olfactory neuro-epithelium. They pick up odours and send signals to the CNS. Tight junctions connect the basal, microvillar, and supporting cells that support these neurons (Fig. 1). As neural progenitors, basal cells constantly replace olfactory neurones, causing the mucosa to become somewhat “leaky” and allowing transfer from the nose to the brain. To get to the mitral cells in the olfactory bulbs, olfactory nerve axons must cross the cribriform plate. Following intranasal delivery, the olfactory bulbs subsequently transmit signals to several brain areas, facilitating intra- and perineural trafficking [40].

The respiratory region, with an area of about 130 cm², is the most vascularized and has the largest surface area. The posterior part covers the lateral wall and is made up of four principal cells: goblet cells, ciliated, non-ciliated, and basal cells. Goblet cells secrete mucin to form mucus, an essential component to act as a barrier against numerous molecules and dust particles that must pass through the mucus layer to reach the epithelial surface [43]. Cuboidal-shaped basal cells serve as progenitors as they can proliferate and differentiate into specialized cells, such as ciliated cells and goblet cells, found in the epithelium. They are connected to the basement membrane by hemidesmosomes. Ciliated and goblet cells adhere to the basal lamina through the attachment site provided by basal cells. It also helps restore the barrier function by healing the nasal epithelium from damage or injury. Numerous microvilli found in ciliated cells aid in clearing the airway of mucus and removing particulates [44]. Projections (maxillary and ophthalmic branches) of the trigeminal nerve innervate the respiratory area. The unique cellular structure and mucosal barrier of the respiratory epithelium, along with its regenerative capacity and ciliary action, facilitate effective drug absorption and clearance, making it a promising site for therapeutic drug delivery, especially via the intranasal route [45].

4.2. Mechanisms of nose-to-brain transport

The intranasal pathway is a safe and non-invasive route that allows rapid drug delivery to the target site, making it convenient method for drug administration. As discussed, the highly vascularized nasal mucosal epithelium, porous endothelial membrane, ready accessibility, and large surface area enable this intranasal route for rapid drug absorption and faster therapeutic effect. It is also known to protect sensitive drugs, such as peptides, from enzymatic degradation, also avoids hepatic first-pass and gut wall metabolisms, which helps in improving bioavailability. Also, it allows the administration of many therapeutic agents by forming suitable formulations, for example, intranasal *in-situ* gel, which allows hydrophilic drugs to reach the CNS. Patients can self-medicate, which is convenient for both the patient and the healthcare provider [46,47].

4.2.1. Olfactory pathway

The olfactory pathway provides a special and straightforward avenue for medication administration from the nasal cavity to the brain by bypassing the BBB. Through mechanisms, such as endocytosis or pinocytosis, medications are taken up by olfactory sensory neurons found in the olfactory epithelium. Following this, the axonal transport mechanisms carry it intracellularly up the axons of these neurons to the brain's olfactory bulb [43]. The medication enters the cerebrospinal fluid or diffuses into the surrounding brain tissue from the olfactory bulb. For the direct delivery of therapeutic medicines to the CNS, particularly for the treatment of neurodegenerative disorders, this transport pathway is extremely helpful [48].

4.2.2. Trigeminal pathway

The trigeminal nerve is one of the main cranial nerves (V) that innervate the nasal cavity. Its branches extend to the nasal mucosa, face,

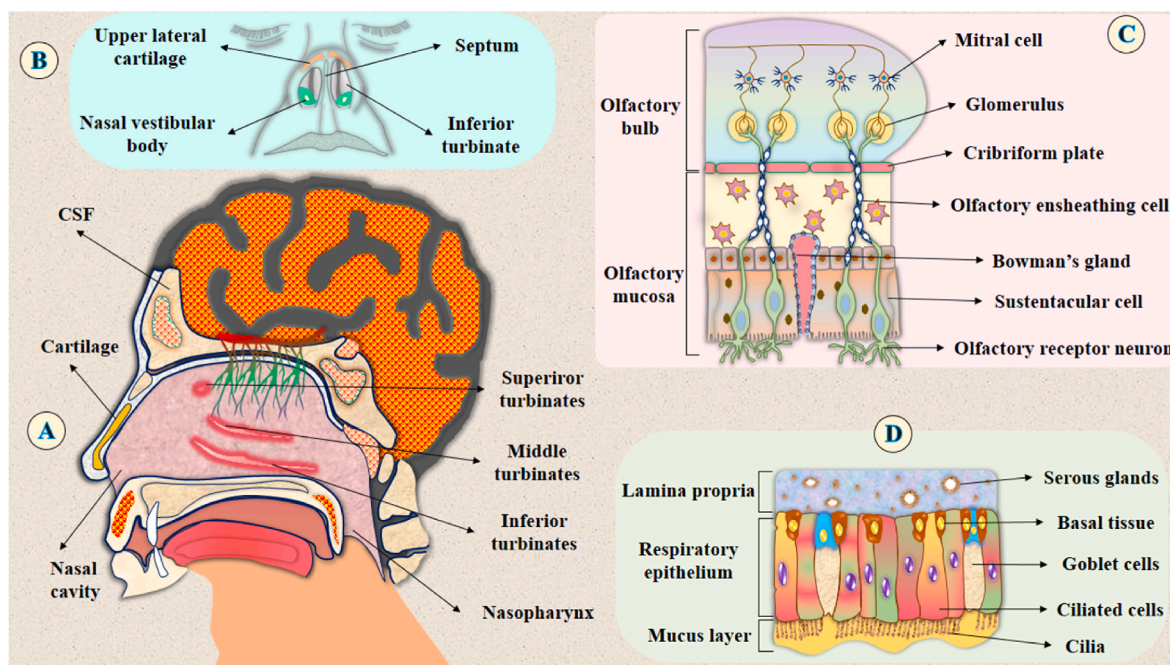


Fig. 1. Human nasal cavity anatomy relevant to nose-to-brain delivery; a) Structural anatomy of the human nasal cavity, showing the squamous mucosa in the vestibule, respiratory mucosa across the turbinates, and the olfactory region below the cribriform plate; b) Vestibular region anatomy; c) Olfactory system highlighting pseudostratified epithelium with sustentacular cells, olfactory neurons, and Bowman's glands; olfactory neurons transmit signals to the olfactory bulb with support from ensheathing glial cells; d) Respiratory mucosa composed of ciliated epithelium, goblet cells, serous glands, and lamina propria beneath a protective mucus layer [43].

and even some parts of the brainstem. It possesses both sensory and motor components. Nerve endings of the trigeminal nerve, particularly in the respiratory epithelium of the nasal cavity, absorb the drug that is administered intranasally and are used in drug transport. These nerve fibres can then carry the drug molecules directly to the brainstem and other CNS regions [38].

Although the most researched route for transport from the nose to the brain is the olfactory one, which primarily targets the olfactory bulb and forebrain, the trigeminal pathway offers a backup channel that is particularly helpful in situations where the olfactory region is inaccessible or impaired [43]. It also projects to the brainstem, pons, and spinal cord, enabling medications to reach deeper or different CNS regions. According to previous research, the trigeminal route may provide a quicker delivery to specific brain areas, particularly those linked to pain pathways, seizure control, or sleep regulation. All these characteristics enable the utilisation of the trigeminal pathway for targeting brainstem illnesses, migraines, trigeminal neuralgia, and even neurodegenerative diseases more precisely [49].

5. Cubosomes: An advanced nanocarrier system

Cubosomes, bicontinuous cubic phases, size ranging from 10 to 500 nm in diameter, are lyotropic liquid crystalline phases made up of a lipid and a polar solvent like water. Liquid crystals possess properties of both solid crystals and isotropic liquids; they flow like liquids but have molecules that are oriented like solids. Their special capacity to maintain thermodynamic stability in excess solvent, whereas other systems tend to dissociate, makes them superior to other nanoformulations [50]. They appear slightly spherical and contain a three-dimensional honey-combed (cavernous) structure, resembling a tightly packed structure formed by two reciprocally intertwined but non-communicating internal networks of aqueous channels separated by a lipid bilayer [50].

Inverted bicontinuous cubic phases are a type of Infinite Periodic Minimal Surfaces (IPMSs). These surfaces are characterized by connected saddle-like shapes that have zero mean curvature at any point,

and the lipid monolayers are draped on either side of these surfaces, which contain their terminal methyl groups. The diamond IPMS D-surface is associated with the Pn3m phase, where the water channels are tetrahedrally interlinked in a 4 by 4 arrangement. The gyroid IPMS G-surface is present within Im3m phase, where the water channels are joined 6 by 6, whereas the primitive(P)-surface is embedded in the Ia3d phase, where the aqueous channels are coplanarly joined 3 by 3 arrangements [51].

5.1. Structure, types, and physicochemical properties of cubosomes

Cubosomes can be classified into three main types based on their internal cubic phase structure: Im3m (primitive), Pn3m (double diamond), and Ia3d (gyroid), which were confirmed using X-ray scattering. The Im3m cubic phase, also known as the primitive bicontinuous cubic phase, is the most basic of the three main cubosome structures [52]. There is one periodic minimal surface that compartmentalizes the internal space into two channels that are continuous and do not overlap. The surface has cubic symmetry; the lattice (minimum surface) represents straight axes with low curvature, usually the Schwarz P surface. Although less frequently observed than other types, Im3m can form given specific lipid compositions and water content. Structurally, the Im3m phase has lower curvature, which will influence the loading and release (Fig. 2a) characteristics of the encapsulated materials [53]. While the Im3m has the simplest geometry, and therefore, the lowest surface area compared to other cubic phases, it is less desirable for applications that need high drug loading capability, but could be preferable for delivering some hydrophilic or amphiphilic molecules with less structure because they can be compartmentalized unfashionably [54].

The Pn3m cubic phase, or double diamond phase, is one of the most frequently observed internal structures in cubosomes. In its packing, there are two non-overlapping and interpenetrating networks of water channel structures separated by a continuous lipid bilayer structure arranged in a diamond-like lattice (Fig. 2b). The geometry of the phase is based on the Schwarz D surface, which is more complex and offers

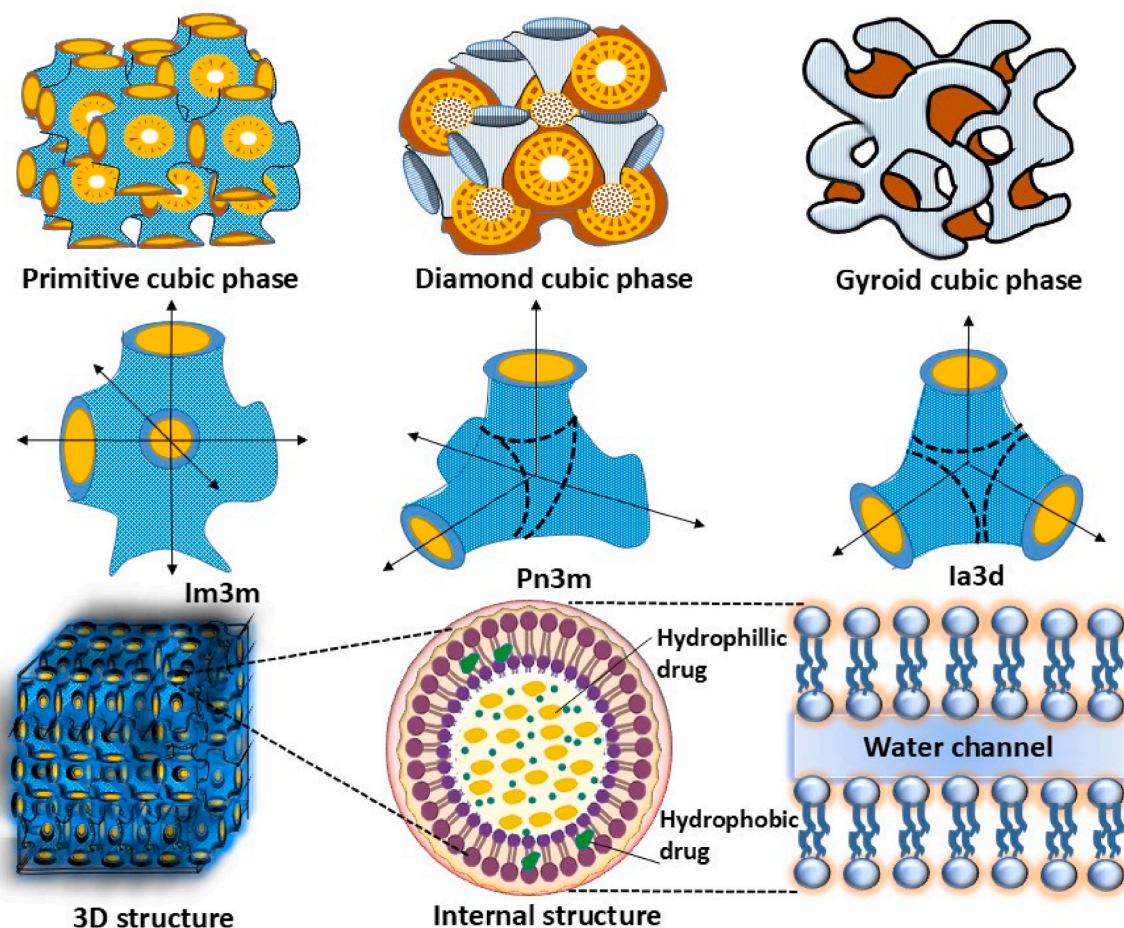


Fig. 2. Schematic representation of the three primary types of cubosomes based on their internal cubic phase structures; a) Im3m (Primitive Cubic Phase) showing two non-intersecting, straight aqueous channels separated by a simple minimal lipid bilayer surface; b) Pn3m (Double Diamond Phase) depicting an intricate network of diamond-shaped interpenetrating water channels organized by the Schwarz D surface; and c) Ia3d (Gyroid Phase) characterized by a highly curved, continuous lipid bilayer forming a gyroid minimal surface, separating two symmetrical, co-continuous helical water networks.

greater internal surface area than the Im3m phase [55]. The high level of curvature and interconnectivity of the water channels provides great encapsulation potential for a wide range of hydrophilic drugs, peptides, proteins, and other biologically active molecules; furthermore, the high internal surface area also offers controlled and sustained release properties [56]. The Pn3m structure tends to form in a range of processes, including monoolein-water systems under physiological conditions, making it highly relevant for pharmaceutical and biomedical applications. The symmetry and organization of this phase can also contribute to stability and robustness in biological environments.

The Ia3d cubic phase, also known as the gyroid phase, is the most complex and thermodynamically stable cubosome structure. As demonstrated by Schwarz G, in this cubical phase, the surface dividing the internal space using a highly curved lipid bilayer into two non-intersecting continuous water channel networks that form a gyroidal (helical) surface (Fig. 2c) [52]. This inherently high curvature and unique structure result in a triply periodic minimal surface with no straight lines, which maximizes both, surface area and symmetry. Because of the high internal complexity of the Ia3d cubic phase, this phase is effective for both hydrophilic and hydrophobic entrapment and targetable applications that require sustained circulation (release kinetics) [57]. The interconnected gyroid channels offer efficient pathways for diffusion, making this phase ideal for sustained and/or controlled passive drug release. Due to its mechanical stability and ability to retain its structure in variable temperature and pH conditions, the Ia3d structure is a major area of research for vaccines, anticancer,

and gene therapy carriers [58].

Among the three, the P-type emerges at the polymer-influenced surface, whereas D-type is common inside cubosomes and associated with GMOs. Homogenization favours the P-type structure, while coarse cubosomes display D-type morphology [59]. The polymer contributions or other factors are probably the cause of the morphological alteration. D-type is dominant over P-type at a lower concentration (3 %) of polymer because of the preferred location of polymer on the particle surface.

5.2. Methods of preparation and characterization

Basic components used in the formulation of cubosomes are amphiphilic lipids and surfactants [60]. The primary approaches of cubosomal preparation include the top-down and bottom-up methods; other methods used for fabrication are solvent evaporation, spray drying, hydrotrope method, and the melt dispersion emulsification process. The descriptions of these methods are mentioned in Table 1, along with their advantages and drawbacks. Dispersion of these nanoparticles produced in the cubosomes formation by ultrasonication, probe sonication, and high-pressure homogenization [61] (Table 1).

5.3. Method of preparation of cubosome-based gel

Following the successful formulation of cubosomal dispersions, these nanosystems are often incorporated into suitable gel matrices to

Table 1

Comparative summary of various preparative strategies of cubosomes.

Preparation Technique	Description	Advantages	Disadvantages	References
Top-down method (High-Energy Dispersion Method)	Used when the lipid is GMO Steps: • Heating the lipid polymer and adding a suitable stabilizer to the lipid to form bulk viscous cubic aggregates. • Followed by high-energy homogenization to create a fine dispersion.	The product remains stable for up to a year	Not suitable for large-scale production involving temperature-sensitive bioactive agents like peptides and proteins	[62,63]
Bottom-up approach (Solvent Dilution method)	Used when the lipid is phytantriol and requires relatively low energy input. • Uses a hydrotrope and stabilizer in excess water to form cubosomes. • The hydrotrope solubilizes water-insoluble lipids in aqueous media.	• Offers long-term stability through uniform stabilizer dispersion • Ideal for incorporating temperature-sensitive agents	Tend to contain a lot of water inside, which can make it difficult for water-soluble medications to be entrapped	[64]
Spray-drying method (Atomization and Solvent evaporation)	Used for producing powdered cubosomes. • Organic solvents that evaporate upon exposure to hot air are used. Steps: Lipid-surfactant solvent mixture: • Lipid phase: dissolving the lipid and stabilizer. • Aqueous phase: hydrophilic solid carriers like dextran and sorbitol. • Both mixtures to be combined under continuous stirring, resulting in the formation of a low-viscosity emulsion • Further atomization needs to be done into fine droplets using a spray nozzle, along with exposure to hot air to get cubosomes as dry powder particles.	Formulated powder is stable and easy to store. This process is suitable for industries as it is simple, economical, and easy to scale up	Energy-intensive process and not suitable for thermosensitive API	[65]
Solvent evaporation method (Homogenization or ultrasonication)	Use of high-energy sonication for homogenization makes it different from spray-drying. Steps: • Organic phase: dissolving the lipid in an organic solvent. • Added dropwise into the aqueous phase, containing Pluronic, on a magnetic stirrer at constant temperature. • The drug can be incorporated either in the organic or aqueous phase, depending on its solubility. • Stirring gradually evaporates the volatile organic solvent, which needs to be further homogenized using a homogenizer or an ultrasonicator to form fine-dispersed cubosomes.	• Formation of uniform-sized particles and encapsulating a wide range of bioactive agents while maintaining the structural integrity • Requires careful handling of organic solvents and temperature-sensitive compounds	Possible existence of leftover organic solvents in the completed cubosome formulation may cause toxicity.	[66]
Melt dispersion emulsification method	Steps: • Amphiphilic lipid needs to be melted on a hot plate magnetic stirrer with continuous stirring at 1500 rpm and 60 °C. • Surfactant, such as Poloxamer 407 (P407), needs to be added and mixed properly. • Stabilizers, such as PVA, Tween 80, Chremophor EL, Chremophor RH, Transcutal P, are to be dissolved in water to form an aqueous phase, which was added dropwise to the lipid mixture. Stirring needs to be continued for 2 h. • The drug needs to be dissolved either in the lipid or aqueous mixture, depending on its solubility. • Stable cubosomal dispersions will be formed by emulsifying the resulting mixture in a homogenizer.	Efficient incorporation of both hydrophilic and hydrophobic drugs	Melting involves elevated temperatures, which may cause degradation of thermosensitive drugs.	[67]
Hydrotrope method (Vortexing and stirring)	Similar to the Bottom-up approach. Steps: • Aqueous phase of Pluronic needs to be added dropwise into lipid-surfactant mixture prepared by melting amphiphilic lipid and dispersing it into the hydrotrope, i.e., ethanol or chloroform, under continuous stirring. • Ternary phase diagrams of the lipid, hydrotrope, and water will be constructed to determine the ideal dilution conditions for cubosome formation. • Vortex and magnetic stirrer needs to be used as low-energy input devices for formulating the cubosomes, which will further homogenized	Useful for incorporating both hydrophilic and lipophilic drugs and maintaining structural integrity	It can be difficult to achieve a constant and even distribution of particle sizes.	[68]

(continued on next page)

Table 1 (continued)

Preparation Technique	Description	Advantages	Disadvantages	References
Microfluidic Method	using high pressure homogenizer for achieving uniformly sized fine particles. Use of microfluidic chips or devices that precisely control the mixing of lipid, stabilizer, and aqueous phases in microscale channels. Steps: - Lipid and aqueous solutions need to be put into microfluidic channels. - Controlled mixing allows self-assembly of cubosomes in real-time. Size and composition can be tuned by adjusting flow rate and concentration.	Precise control over particle size and size distribution. Scalable with parallelization Can encapsulate sensitive compounds gently	Requires specialized equipment Not yet widely used in industry	[69]
Double Emulsion Method (W/O/W)	Involves forming a water-in-oil-in-water (W/O/W) emulsion. The inner aqueous phase may contain the drug, surrounded by lipid, which will then be dispersed in an external aqueous phase. Steps: - Form W1/O emulsion (e.g., water-in-oil, with hydrophilic drug inside water). - Disperse W1/O into external water phase (W2) → forms W1/O/W2. Lipids phase self-assemble into cubosomes during emulsification	Suitable for entrapping hydrophilic drugs Allows dual drug loading (hydrophilic and lipophilic)	Complexity in process Stability issues with double emulsions	[70]
High-Pressure Homogenization Without Heating	A purely mechanical method using repeated high-pressure shearing (e.g., 10,000–20,000 psi) to break down bulk lipid into cubosomes, without heating or organic solvents. Steps: - Mix the bulk cubic phase with stabilizer and water - Pass through high-pressure homogenizer at room temperature multiple times - Breaks down the bulk into uniform nanostructures.	Avoids heat and solvents, safer for thermosensitive agents Industrially scalable	High equipment cost May not be suitable for very viscous systems	[71]
Supercritical Fluid Method	Involves the use of supercritical CO₂ as a solvent or co-solvent to form cubosomes by dissolving lipids and drugs, followed by depressurization Steps: - Lipids need to be dissolved in supercritical CO₂ with/without co-solvents. - Rapid depressurization will lead to nanoparticle formation - Cubosomes are recovered after solvent removal.	Solvent-free or minimal solvent residue Eco-friendly method	Requires precise control of pressure and temperature Limited scalability currently	[72]
Freeze-Drying (Lyophilization) with Cryoprotectants	Not a primary preparation method but a post-processing technique to convert cubosome dispersions into powders, improving shelf life. Cubosomes will be freeze-dried using cryoprotectants like trehalose or mannitol. Steps: - Prepare cubosome dispersion using any method. - Cryoprotectants (e.g., trehalose, mannitol) will be added. - Lyophilization needs to be followed to obtain dry cubosome powder - To be reconstituted before use.	Improves long-term stability and transport Reconstitution retains structural integrity	Requires careful formulation to prevent structural collapse	[73]

enhance their stability, residence time, and therapeutic efficacy, particularly for localized and intranasal delivery [82]. Some methods of formulating cubosomal gels are discussed below.

5.3.1. Cold method for thermoresponsive polymers

In this technique, pre-formed cubosomal dispersions are incorporated into a cold aqueous solution of a thermosensitive polymer (e.g., P407) at 4–8 °C while stirring. The mixture is then refrigerated until it is able to flow freely at room temperature, but the solution becomes a gel at physiological temperatures. Utilizing a cold incorporation method preserves the important nanoscale architecture of the cubosomes, maintains stability of labile drugs against heat, and allows the gel matrix to impart *in-situ* gelation, thereby increasing the residence time at the administration site. For example, a freshly prepared fluoxetine-loaded cubosomal *in situ* gel prepared using the cold method with P407, showed improved nasal residence and brain bioavailability [83].

5.3.2. Dispersion method for mucoadhesive gels

In this approach, the mucoadhesive polymer, which is capable of forming a gel (Carbopol, hydroxypropyl methylcellulose (HPMC), or chitosan), is first exposed to water in a stirring environment. Thereafter, the previously incorporated cubosomal dispersion is slowly added to the gel composition to promote addition while maintaining the desired pH (for example, to pH 6 or 7 using triethanolamine with Carbopol), optimum viscosity and mucoadhesive strength. For example, cubosomal gel formulations using Carbopol 934 create a gel formulation that retains the nano-architecture of cubosomes while optimizing mucus membrane adhesion, allowing for sustained drug release, and protection of sensitive tissues, suitable for topical application [71].

5.3.3. Hybrid method (Thermo-mucoadhesive gel)

The hybrid thermosensitive mucoadhesive approach synergistically combines the advantages of temperature-triggered gelation and enhanced mucosal adhesion, offering superior localization and sustained drug delivery. In this method, a thermoresponsive polymer, such

as P407, is blended with a mucoadhesive polymer, such as chitosan, Carbopol, or HPMC, to create a polymeric base exhibiting dual functionality. The preformed cubosomal dispersion is gently incorporated into the cold polymeric matrix under continuous stirring to ensure homogeneity and preserve the integrity of the cubosomal characteristics. The resulting formulation could be stored under refrigeration to maintain its solution state before administration. Upon exposure to physiological temperature, the system undergoes a rapid sol-to-gel transition, forming a viscoelastic gel that adheres firmly to the mucosal surface. This dual mechanism enhances the residence time, drug permeation, and therapeutic efficacy while minimizing systemic loss [84].

5.3.4. Ion-sensitive gelation

The ion-activated or *in situ* gelation method utilizes the inherent ionic responsiveness characteristics of certain polymers, such as gellan gum, sodium alginate, or pectin, to achieve localized gel formation upon administration. In this technique, the cubosomal dispersion is uniformly blended with an ion-sensitive polymeric solution under mild stirring. Upon exposure to physiological ionic environments, such as the presence of Ca^{2+} , Na^{+} , or K^{+} ions in nasal or ocular fluids, the formulation rapidly transitions from a liquid to a gel state through ion-mediated cross-linking. This mechanism enables controlled *in situ* gelation at the target site, forming a stable depot that retains cubosomes and ensures sustained, localized drug release. The encapsulated cubosomes maintain their internal nanostructure within the gel matrix, providing a protective microenvironment for the drug and enhancing permeation across biological barriers [85].

5.4. Characterization parameters

The characterization of cubosomes is crucial for establishing their stability, efficacy, and their use as a drug delivery system, especially as a brain-targeted delivery system [86]. In general, the relevant characterization parameters include particle size, polydispersity index (PDI), and zeta potential, which are routinely assessed using dynamic light scattering (DLS). Atomic force microscopy (AFM) is a high-resolution nanometer-scale imaging system that characterizes cubosomal dispersions in their unsaturated hydrated state, without the need for staining or coating. This directly visualizes surface morphology, size, shape, and aggregation behaviour, providing detailed topography information that outperforms DLS [87]. AFM operates in tapping and force spectroscopy modes, minimizing deformation of soft lipid particles, while still quantifying mechanical properties, such as elasticity and adhesion. AFM is highly effective in monitoring morphological stability and topographical alterations during drug release, as well as in studying protein or peptide interactions on the surface of cubosomes, all under physiological conditions [88]. Cubosomes must have an optimal particle size (typically 100–300 nm) to sufficiently penetrate mucosal surfaces and be taken up by neuronal cells. A particle PDI of <0.3 represents particles with a narrow size range, which is important to ensure the reproducibility of their performance [89]. Zeta potential values reflect surface charge (in mV) and represent electrostatic stability. Moderate to high zeta potential values, typically above ± 30 mV, suggest moderate to good electrostatic repulsion, which allows colloidal dispersion stability. For morphological characterization electron microscopy (EM) is used, which can take the form of transmission electron microscopy (TEM) or cryo-TEM, to visualize the internal bicontinuous cubic phase and confirm the presence of nanoparticles with the appropriate nanostructure. To verify entrapped drug percentage, which is necessary to achieve proper dosing and sustained release, entrapment efficiency (EE %), i.e., the amount of drug incorporated into the cubosomes, needs to be determined. To assess the internal structure and behaviour of lipids, small-angle X-ray scattering (SAXS), and differential scanning calorimetry (DSC) to study thermal properties of the lipids, can be used [90]. To enhance inference regarding the behaviour of the formulation, *in vitro* drug release, pH, and viscosity measures (particularly for cubosomal

gels), as well as mucoadhesion can be determined. Taken together, the characterization methods are crucial for understanding the behaviour of the formulation under physiological conditions after application and optimizing cubosomal systems for intranasal delivery targeted to the brain.

6. Mechanism of drug absorption and brain penetration via cubosomal gels

Cubosomal gels are valuable for delivering therapeutic agents to the brain because they possess unique physicochemical properties and multifaceted capabilities [53]. These systems effectively leverage the advantages of cubosomes and the longevity of bioadhesive gels, which offer significant improvements in drug absorption via the nasal route. The mechanisms of their success provide a great deal of complexity and include improved mucosal adhesion, enhanced permeation across biological barriers, extended drug release, and targeted delivery to brain tissues (Fig. 3). These areas can be understood in detail under the sub-headings below.

6.1. Mucoadhesion and penetration enhancement

Cubosomal gels are highly effective for mucosal drug delivery as a result of an optimal combination of both nanoscale cubosomes and a bioadhesive gel matrix. Mucoadhesion is defined as the ability of a formulation to adhere to mucosal surfaces, which is fundamental for increasing the residence time of the drug in a particular area of administration. Increasing residence time is critical for systems designed to deliver drugs intranasally for brain targeting [91]. Generally, cubosomal gels use mucoadhesive polymers (chitosan, Carbopol, HPMC, etc.) to interact with mucins in nasal mucus employing hydrogen bonding, ionic bonds, and van der Waals forces [92]. This will help anchor the formulation at the mucosal surface and prevent mucociliary clearance, allowing for sufficient drug absorption time [93].

Penetration enhancement is an equally important feature of cubosomal gels, additionally supporting effective drug absorption and brain targeting. The cubosomes in the gel are typically composed of amphiphilic lipids (e.g., GMO or phytantriol), which can provide a bicontinuous cubic phase, capable of fusing with biological membranes. The lipidic construct of the gel may also temporarily disrupt or fluidize the mucosal epithelium, thereby enhancing drug transport via both, paracellular and transcellular routes. Moreover, cubosomes are relatively small in size (typically 100–300 nm), which allows them to more easily penetrate epithelial barriers and also be taken up by endocytosis [94]. The formulation typically contains surfactants and stabilizers, such as P407 or Tween 80, which are known to alter tight junctions, thereby further enhancing permeability. This, in addition to the mucoadhesion effects allowing for prolonged retention and a membrane-penetrative mechanism, enables improved drug absorption and direct nose-to-brain transport through the nasal olfactory and trigeminal nerve pathways, thus bypassing the BBB [95].

6.2. Sustained release and targeting efficiency

Cubosomal gels have a structure that enables prolonged drug delivery and sustained release times. This is important for maintaining drug levels in brain tissue, given the time required to deliver an effective concentration. Presence of bicontinuous cubic phase in cubosomes consisting of lipid bilayers forming an internal aqueous channel [57]. This design enables a tortuous diffusion pathway for the drugs, reducing release rates of both hydrophilic and lipophilic drugs within the cubosome. When cubosomes are incorporated into a gel matrix, the gel properties (increased viscosity and polymeric network) reduce drug diffusion further, as there is a double-controlled release mechanism: from the cubosome and gel [34]. Maintaining a prolonged release profile over time, cubosomal delivery approach can reduce the dosing

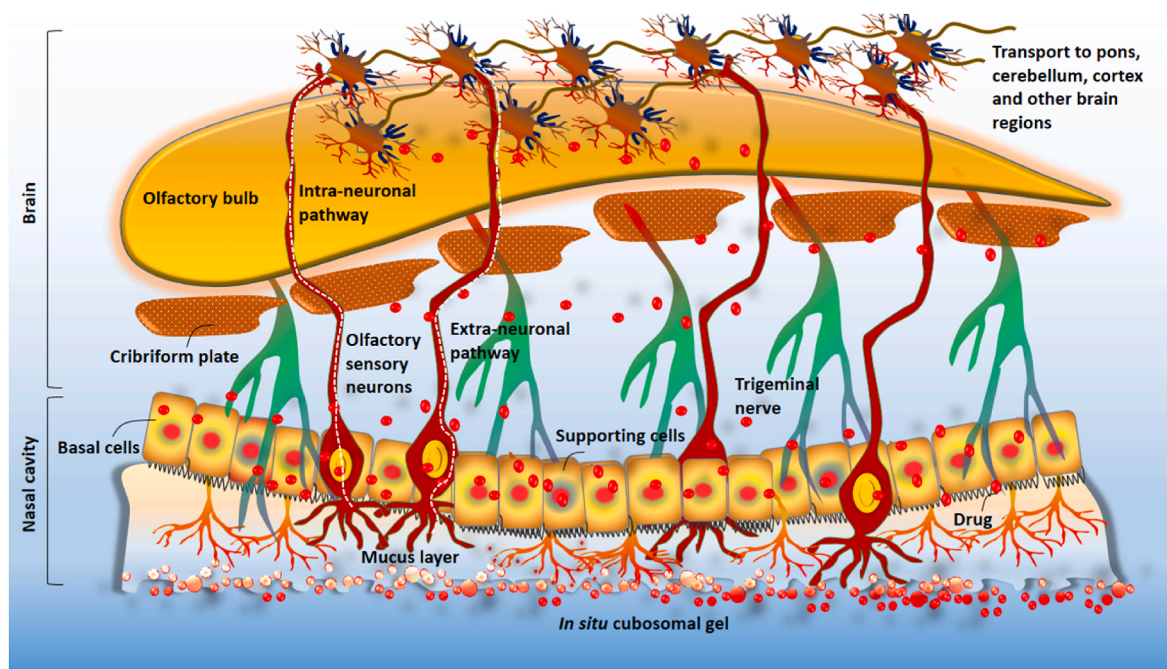


Fig. 3. Mechanism of nose-to-brain delivery via *in situ* cubosomal gel system; The drug-loaded gel, when administered to the nasal cavity, interacts with the olfactory epithelium and the mucus layer. The delivery system uses both intraneuronal and extraneuronal pathways. Intra-neuronal transmission is through olfactory sensory neurons and the olfactory bulb, whereby drugs can go directly to the brain. The extra-neuronal route goes paracellularly and transcellularly via supporting cells within the trigeminal nerve to various parts of the brain (for example, the pons, cerebellum, and cortex). The figure shows the involvement of nasal epithelial components (the basal cell, supporting cell, and sensory neurons), the cribriform plate, and the two different pathways to improve the efficacy of brain-targeted drug delivery via cubosomal systems.

frequency, increase patient compliance, and maintain a steady-state concentration of the drug at the target site, especially in chronic neurological conditions.

In terms of targeting efficiency, cubosomal gels can achieve both passive and active targeting to the brain. Passive targeting occurs primarily through the mucoadhesive and permeation-enhancing properties of the gel, which allows direct drug delivery to the brain via intranasal administration [96]. The direct nose-to-brain pathway helps maximize delivery efficiency by avoiding systemic circulation and crossing the BBB, leading to greater total local concentrations of drugs in the CNS [63]. Active targeting can be further enhanced by functionalizing the cubosome surface with ligands, such as transferrin, lactoferritin, or specific peptides that bind to receptors on the surface of brain endothelial or neuronal cells [91]. The ligand-receptor interaction further enhances cellular uptake and enables site-specific drug delivery. Therefore, sustained release property, coupled with targeted delivery mechanisms, facilitates cubosomal gels to enhance CNS targeting and subsequent therapeutic efficacy, while minimizing systemic side effects and off-target distributions [97].

6.3. Efficient nose-to-brain delivery

The BBB poses one of the largest limitations in treating CNS disorders. The BBB is a highly complex and selective semipermeable membrane boundary that limits the entry of most therapeutic agents into the systemic circulation of the brain [36]. The barrier is defined by tight junctions between the endothelium and pericytes as well as astrocytic end-feet, making it impenetrable to large, hydrophilic, or poorly lipophilic molecules. As a result, many drugs can fail to reach therapeutic concentrations within the brain when administered through conventional systemic routes.

When the cubosomal gel formulations are applied intranasally, the presence of the mucoadhesive gel provides prolonged contact with the nasal mucosa, allowing the encapsulated drug to be absorbed by the

neuronal routes [64]. As discussed, the olfactory region provides the opportunity to transport therapeutic agents entrapped in nanoparticles to the olfactory bulb, allowing the drugs to be distributed further into the CNS [97]. Similarly, the trigeminal nerve pathways that innervate the respiratory mucosa can provide another opportunity to access the brainstem and spinal cord.

The cubosomal structure advantages this transport due to its nanometer scale (100–300 nm, typically) and lipidic nature, which mimics biological membranes and is taken up by cells through either endocytosis or passive diffusion. Additionally, through the lipid bilayer of cubosomes fusing with neuronal membranes, the drug can be delivered intracellularly [65]. Direct delivery via this route not only avoids first-pass hepatic metabolism and systemic circulation but also produces rapid therapeutic action in the brain. It is also noteworthy that cubosomal gels are a valuable option for the delivery of therapies for neurological disorders such as Alzheimer's disease, epilepsy, Parkinson's disease, and brain tumors [66].

6.4. Biocompatibility and safety profile

In addition to their advanced delivery capabilities, cubosomal gels offer an excellent biocompatibility and safety profile, which is critical for clinical applications, especially those involving chronic administration or sensitive delivery routes such as the nasal cavity. The core components of cubosomes, commonly GMO or phytantriol, are lipids that are generally recognized as safe (GRAS) by regulatory agencies [67]. From a regulatory and translational standpoint, the use of GRAS excipients, scalable manufacturing processes, and consistent safety data across *in vitro*, *ex vivo*, and *in vivo* models strengthens the clinical promise of cubosomal gels. These lipids are biodegradable and mimic the structure of endogenous lipids in the body, thereby reducing the risk of immunogenicity or cytotoxicity. This makes them suitable for chronic and repeated administration without compromising tissue integrity or systemic health.

The gel matrix used in cubosomal systems directly interacts with the nasal mucosa and is typically composed of mucoadhesive polymers such as Carbopol, HPMC, or chitosan, which are non-toxic and well tolerated, and are extensively used in FDA-approved pharmaceutical formulations. These polymers not only enhance mucoadhesion but also help modulate the release profile of the drug by improving absorption and protecting the mucosal lining from chemical and mechanical damage [68]. Importantly, these materials are non-irritating to the nasal mucosa and suitable for repeated or long-term use. Long-term use of these formulations has not been linked to any genotoxic or carcinogenic effects, nor has it had a substantial impact on haematological or biochemical parameters, according to toxicological assessments.

When compared to other nanoformulations, such as nanoemulsions, liposomes, or solid lipid nanoparticles (SLNs), cubosomes provide a more thermodynamically stable structure with lower risk of phase separation, aggregation, or premature drug release. Their non-ionic and pH-tolerant nature makes cubosomes particularly suitable for the nasal environment, which can be affected by disease or individual variability [32].

Furthermore, the sustained release profile of cubosomal gels minimizes the occurrence of peak-through fluctuations in drug levels, which often lead to toxicity or therapeutic failure. The slow and controlled diffusion of the drug from the cubosome core through the gel matrix, ensures that the concentration remains within the therapeutic window for extended durations [98]. This contributes to enhanced efficacy and reduced dosing frequency, ultimately improving patient compliance. Overall, the formulation components, physicochemical stability, and pharmacokinetic advantages make cubosomal gels a safe and promising carrier system for brain-targeted drug delivery. Therefore, this novel formulation approach enhances effectiveness and reduces dosing frequency, improving patient acceptability.

6.5. Comparison of cubosomes with other novel formulations

Recently, a number of nanocarriers, including niosomes, SLNs, dendrimers, and liposomes, have been studied for nose-to-brain delivery of drugs as they can cross the BBB and increase the bioavailability in the CNS [57,99,100]. Niosomes, which consist of non-ionic surfactants, cholesterol and charge inducers, have shown excellent biocompatibility. These niosomes are able to encapsulate hydrophilic and lipophilic forms of drugs, making them suitable for mucosal application. Their surfactant components help briefly open nasal epithelial tight junctions to increase paracellular and transcytotic transport [101]. However, their long-term physical stability can be compromised by vesicle aggregation, and scale-up reproducibility can be difficult [102]. SLNs, however, are a solid lipid that can offer protection from degradation and sustained drug release within the solid matrix. They also have biocompatibility and can maintain drug stability [103]. However, they have a low drug loading capacity and drugs can be expelled from SLNs due to lipid recrystallization during storage [104]. Cubosomes have bicontinuous cubic phase structures that contain more stable internal architecture, which resists phase polymorphism and aggregation, leading to enhanced encapsulation and sustained release characteristics. Their thermodynamically stable nanostructure, high surface area, and ability to maintain colloidal stability under physiological conditions give them an advantage over both niosomes and SLNs for consistent and reproducible nasal drug delivery [105].

Dendrimers and liposomes have also shown the potential for brain-targeted nanodelivery. However, cubosomes have advantages in terms of safety, scalability and performance. Dendrimers possess complex branched and monodisperse macromolecular architecture, allowing for precise surface modifications for ligand attachment, and targeted delivery [106]. Dendrimers can be cumbersome and costly to synthesize. Due to concerns about cytotoxicity (especially with cationic dendrimers) and limited drug-loading capacity of larger or hydrophobic molecules these disadvantages must be weighed into their design [107].

Dendrimers are well studied for BBB targeting by passive and active mechanisms (e.g. ligand-mediated receptor targeting with transferrin, lactoferrin, or apolipoprotein E).

In comparison, cubosomes provide a self-assembled, thermodynamically stable system with an extensive ability to entrap any type of therapeutic molecules, including peptides, proteins, and hydrophobic drugs. Due to their unique structural stability and ability to be mucoadhesive and responsive to physiological conditions (e.g. temperature) they can also provide sustained, site-specific drug delivery to the brain directly via the nasal route [105]. Cubosomes and liposomes, though both lipid-based nanocarriers, differ fundamentally in their internal organization and drug delivery capabilities. Liposomes consist of concentric phospholipid bilayers enclosing discrete aqueous compartments, while cubosomes possess a bicontinuous cubic liquid crystalline structure comprising interpenetrating aqueous channels within a lipid matrix [99]. This unique nonlamellar geometry of cubosomes offers higher surface area, greater encapsulation versatility for hydrophilic, hydrophobic, and amphiphilic drugs, and improved structural stability under physiological conditions. Additionally cubosomes exhibit slower and more controlled drug release profiles, enhanced resistance to dilution and degradation, and superior potential for ligand conjugation and mucoadhesive delivery [99]. Supporting the data, a comparison between the cubosomes and liposomes was studied by Chang et al., where cubosomes exhibited superior structural stability and encapsulation efficiency upon loading with curcumin, maintaining consistent particle size and morphology even at higher drug concentrations. In contrast, liposomes showed significant aggregation and increased polydispersity, indicating reduced stability and less efficient curcumin incorporation, thereby highlighting the robustness and enhanced delivery potential of cubosomes [108]. Collectively, cubosomes represent an entirely new nanocarrier class with distinct advantages over other conventional and novel systems for safe, efficient and targeted brain delivery.

6.6. Active targeting and conjugation of cubosomes with various proteins and peptides for focused delivery

Active targeting mechanisms are one of the promising strategies to enhance the specificity and efficacy of cubosome-based drug delivery systems, particularly for the brain-targeted delivery via the intranasal route. Passive targeting relies on the physicochemical properties of nanoparticles and physiological transport mechanisms [71]. Cubosomes with nano size and high surface area have an enhanced permeability and retention effect in active targeting, which further improves precision by guiding the nanocarriers directly to specific cell types or tissues [34]. This can be achieved by functionalizing the surface of cubosomes with targeting ligands, such as proteins, peptides, antibodies, or aptamers that have high affinity for receptors overexpressed on target cells. This ligand-mediated recognition significantly improves the accumulation of therapeutics at the desired site while minimizing systemic exposure and off-target effects. Proteins and peptides are preferably selected for targeting moieties because they exhibit high specificity and affinity towards target receptors, they are biocompatible and biodegradable, and offer ease of synthesis and modification [71].

Cubosomes can be functionalized using a diverse range of targeting moieties, including transferrin, lactoferrin, apolipoprotein E (ApoE), and peptide ligands, such as TAT, Angiopoietin-2, and RGD peptides, all of which have been shown to facilitate receptor-mediated transcytosis [109]. These ligands enable cubosomes to replicate the normal routes of transport, thus enabling improved drug delivery to the CNS. The surface conjugation of biomolecules to cubosomes typically occurs through covalent coupling (e.g., carbodiimide chemistry), electrostatic interactions, or adsorption techniques to achieve stable and consistent biomolecule attachment to the cubosome surface without disrupting morphology and stability of the cubosomal nanostructure [110]. Additionally, these targeting cubosomal systems demonstrated more cellular uptake and intracellular trafficking, ultimately achieving improved

therapeutic effects in neurological diseases and disorders [91]. For example, transferrin or lactoferrin-conjugated cubosomes have been shown to increase brain bioavailability using mechanisms of endocytosis. However, cell-penetrating peptides, like TAT, have been shown to facilitate translocation across membranes and neuronal uptake. The combination of the mucoadhesive and thermoresponsive properties of cubosomal gels, along with active targeting using ligand-mediated approaches, provides a strong base for developing reliable, sustained and precise drugs delivery to the brain [111].

In another study, the functionalized cubosomes demonstrated a crucial role in enhancing targeted drug delivery by enabling efficient transport of therapeutic agents across the BBB and improving accumulation at tumor sites. In the cited study, Angiopep-2 (Ang2)-functionalized cubosomes demonstrated superior BBB penetration and selective uptake by glioblastoma cells through receptor-mediated transcytosis, leading to increased intracellular drug delivery and enhanced cytotoxic efficacy. Similar to the previously reported result, this targeted approach minimizes systemic toxicity and maximizes therapeutic concentration within the brain, underscoring the potential of ligand-functionalized cubosomes for precise and effective treatment of glioblastoma multiforme (GBM) [109]. Similarly, hyaluronic acid functionalized cubosomes specifically targeted CD44-overexpressing tumor cells, enhancing cellular uptake and inducing selective apoptotic cell death while sparing healthy cells. This targeted approach not only improved therapeutic efficacy by increasing local drug concentration at the tumor site but also minimized off-target toxicity [110]. Therefore, the conjugation of cubosomes with functional proteins and peptides represents a major advance in the design of next-generation nanocarriers for neurotherapeutic applications.

7. Cubosomes and cubosome-based gels as a delivery system in neurodegenerative diseases

Properties of *in-situ* thermoresponsive intranasal gels, such as improved nasal retention time, focused therapeutic effects, liquid at storage temperature conditions and formation of a gel after administration, when exposed to body (nasal) temperature [98], help in accurate dosing by reducing dosing frequency and improving drug absorption and drug bioavailability, especially useful for treating neurological and psychiatric diseases [34]. Due to their unique architecture, biocompatibility, and ability to bypass the BBB via intranasal delivery, cubosomes offer a promising strategy to deliver therapeutic agents precisely to affected brain regions (Table 2). Some applications backed by research are listed below.

7.1. Alzheimer's disease

Alzheimer's disease is characterized by a progressive decline in cognitive function due to the accumulation of extracellular amyloid-beta (A β) plaques, hyperphosphorylated tau knots, loss of cholinergic neurons, and neuroinflammation [112]. One of the main barriers to the development of therapeutics for Alzheimer's disease is that most indeed are restricted in their access to the BBB. As cubosomes provide an exceptional delivery platform to encapsulate both hydrophilic and hydrophobic drug molecules, protecting them from enzymatic degradation, reducing rates of systemic metabolism, and having an unlimited capacity to hold drugs due to their lipidic nanostructure and internal surface area, they enhance both bioavailability and rate of release, and decrease dosing frequency, lowering peripheral side effects. Traditional drugs for the treatment of Alzheimer's disease, like donepezil, rivastigmine, and galantamine, which are commonly referred to as acetylcholinesterase (AChE) inhibitors, have shown the aforementioned advantages when incorporated into cubosomes [80]. Additionally, natural polyphenols, like curcumin and resveratrol, which possess anti-amyloid and antioxidant properties, have been found to exhibit increased solubility and stability through cubosomal encapsulation

[113]. Sophisticated strategies have been used to modify cubosomes for those that prefer intranasal (due to convenience and ease of dosing) delivery, preventing a drug from being restricted to the BBB via the olfactory and trigeminal nerves for drug transport to the hippocampus and cortex.

A recent study revealed that cubosomal (T-cub) drug delivery dramatically improved the oral bioavailability and brain-targeting ability of piperine by overcoming its hydrophobicity and first-pass metabolism. Due to their sustained release profiles and method to pass through the BBB via Tween 80, the T-cubs led to significant cognitive enhancement in rats with induced cognitive impairment. Despite the results observed in cognitive impairment studies, the T-cubs reduced neuroinflammation and apoptosis as indicated in a follow-up biochemical analysis. Meanwhile, the toxicity studies showed that it was safe to consume [114]. In another study, cubosomes functionalized with non-immunogenic odorless lectin (OL) improved nose-to-brain transport of therapeutic peptides, such as S14G-HN, in mouse models. The study employed an A β _{25–35}-induced intrahippocampal injection model in SD rats to mimic cholinergic neurodegeneration and cognitive impairment characteristic of Alzheimer's disease (Fig. 4). This *in vivo* model enabled evaluation of therapeutic efficacy through behavioural (Morris water maze) and biochemical (AChE activity) assessments. The OL-modified cubosome system securely targeted the brain, increased the rate of drug uptake, and resulted in a more significant increase in cognitive function and neuroprotection, providing a safe and efficacious system for peptide delivery for Alzheimer's disease [80].

7.2. Glioblastoma multiforme

GBM represents an aggressive and fatal form of brain malignancy that is characterized by rapid cell proliferation, diffuse infiltration of surrounding tissues, and resistance to conventional treatments [115]. Several molecular targets implicated in GBM include the epidermal growth factor receptor (EGFR), phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling pathway, vascular endothelial growth factor (VEGF), and the protein O6-methylguanine-DNA methyltransferase, indicating involvement in DNA repair. Mutations or amplification of EGFR (e.g., EGFRvIII variant) and overexpression of VEGF stimulate tumor growth, promote angiogenesis, and confer resistance to therapeutic interventions targeting tumors [116]. Therefore, targeting the pathways signaling to these molecules is vital for the success of GBM therapeutics. Cubosomal-based delivery systems are a novel way to potentially increase the brain bioavailability of chemotherapeutics and molecular inhibitors by a passive diffusion mechanism that circumvents the BBB and enables localized, sustained, and targeted delivery to GBM aberrant cells, producing negligible systemic toxicity.

A study reveals that AT101-loaded GMO cubosomes achieve a high entrapment efficiency (97.7 %) and provide a sustained drug release of approximately 35 % over 72 h. Using NMR diffusometry, the drug was shown to be tightly associated with the cubosomal lipid matrix. Importantly, these cubosomes exhibited enhanced cellular uptake and deep penetration into 3D tumor spheroids of GBM. They also induced significant cytoskeletal rearrangement in GBM cells, specifically actin fiber shortening, leading to greater cytotoxicity compared to free AT101, while sparing normal brain cells [117].

7.3. Oxidative stress-related neurodegeneration

Oxidative stress-related neurodegeneration involves excessive production of ROS, leading to cellular damage, mitochondrial dysfunction, and apoptosis of neuronal cells. This mechanism is implicated in the progression of several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, GBM, etc. [118]. Key molecular targets include the Nrf2/ARE pathway (antioxidant defense), MAPK cascade (stress signaling), NF- κ B (inflammation), and caspases (apoptosis mediators) [119]. Cubosomes offer a promising platform for targeted

Table 2

Characteristics and therapeutic outcomes of cubosome and cubosome-based gel formulations.

S. no	Type of formulation	Components	Target	Formulation Characteristic parameters	Outcome	References
1	Cubosome	Lipid- GMO Surfactant- PF127 Stabilizer- Ethanol Drug- Agmatine	Epilepsy	PS (nm) = 270.4 PDI = 0.219 ZP (mV) = −12 EE % = 88.92 ± 0.33	Agmatine-loaded cubosomes were produced using Pluronic F17 for intranasal delivery for the treatment of epilepsy. Compared to the traditional intraperitoneal administration of diazepam, the nasal distribution of agmatine cubosomes decreased the length of convulsions and expedited their onset. It also enhanced absorption and subsequent bioavailability compared to conventionally marketed formulations.	[74]
2	Cubosome	Lipid- GMO Surfactant- P407 Drug- Verapamil	Brain	PS = 105.9 to 253.20 PDI = 0.31 to 0.47 ZP = −23.63 to −35.12 EE = 24.13 to 80.18	Verapamil-loaded cubosomes were prepared using the melt dispersion method. Increased sustained release, efficacy, and nasal permeation were reported, which makes it a preferred choice of drug delivery systems for brain targeting.	[75]
3	Cubosomal <i>In-situ</i> Gel	Lipid- GMO Surfactant- P407 Drug- Benzalkonium chloride	migraine	PS = 76 to 147 ZP = +12 EE = 78 to 81	By using <i>Samanea saman</i> seed gum, sumatriptan succinate <i>in situ</i> nasal gel was developed for mucoadhesion. Optimized formulation showed suitable particle size, PDI, and high entrapment efficiency. Initially, it exhibited burst release followed by sustained drug release of 70 %. Cmax and AUC were significantly higher with nasal administration compared to intravenous. These findings suggested cubosomal dispersion as a potential brain delivery method due to improved nasal mucosa penetration and brain targeting.	[31]
4	Cubosomal <i>In-situ</i> Gel	Lipid- GMO Surfactant- P407 Stabilizer- PVA Drug- Almotriptan malate (ALM)	Brain	PS = 177.15 ± 7.85 PDI = 0.36 ± 2.55 ZP = −21.26 ± 0.19 EE = 72.58 ± 8.82	ALM-loaded thermoresponsive <i>in situ</i> cubosomal gel was prepared and optimized to get the desired characteristic parameters. <i>In-situ</i> gel formulation was found to be thermodynamically stable and showed better permeation. Based on these findings, cubosomal gel was concluded as a novel approach for ALM brain targeting	[76]
5	Cubosomal <i>In-situ</i> Gel	Lipid- GMO Surfactant- P407 Stabilizer- Tween 80 Drug- Granisetron hydrochloride (GS)	Brain (Emesis)	PS = 165.8 to 397.6 ZP = −29.7 to −24.5 EE = 35.4 to 68.5	To increase the residence time on the nasal mucosa and improve the physical stability of the optimized cubosomal dispersion, it was formulated as an <i>in situ</i> thermoresponsive intranasal gel. The histopathological analysis revealed that the optimized cubosomal gel is safe and tolerable. Additionally, it exhibited considerably better GS penetration across the nasal membrane, higher bioavailability, and better brain distribution after intranasal delivery when compared to a drug solution.	[77]
6	Cubosomal <i>In-situ</i> Gel	Lipid- GMO Surfactant- PF127 Stabilizer- Tween 80 Drug- risperidone	Brain	PS = 160.43 to 456.94 PDI = 0.222 to 0.578 ZP = −29.8 to −22.4 EE = 9.61 to 54.42	For physical stabilization, <i>in situ</i> gel was created using the optimal cubosomal dispersion formula with the lowest PS, PDI, and highest ZP and EE. For the gelation of the optimized dispersion recipe, Polyox outperformed gellan gum. After being applied to the sheep's nasal mucosa, the optimized cubosomal gel was determined to be both safe and biocompatible.	[78]
7	cubosomal thermos-gel	Lipid- GMO Surfactant- P407 Stabilizer- PVA Drug- Saquinavir mesylate (SQ)	Brain	PS = 100 to 250 ZP = −38 EE = 55 to 85	The optimization of SQ-loaded cubosomal dispersion by using Box Behnken design, where the effect of monoolein, P407, and PVA was studied as independent variables. Cubosomal formulation was prepared and evaluated according to the quadratic response model, which was obtained by evaluating particle size, entrapment efficiency, gelation temperature, and stability index as responses.	[79]
8	Cubosomal <i>in-situ</i> gel	Lipid- GMO-GTP Surfactant- PF127-PF68 Drug- Duloxetine HCl	Brain	PS = 145.5 to 515.9 PDI = 0.3 to 1 ZP = 1.6 to 10.6 EE = 93.1 to 99.7	Duloxetine HCl-loaded cubosomal <i>in situ</i> intranasal gel with suitable characteristics was formulated for brain targeting. Cubosomal gel formulation showed better <i>in vivo</i> biodistribution in plasma, enhanced bioavailability, and better instillation in the nasal cavity as compared to intranasal solution.	[27]
9	Cubosome	Lipid- GMO Surfactant- PF127 Stabilizer- MAL-PEG-OA Drug- Odorranalectin (OL)	Brain (Alzheimer's disease)	PS 100 to 120 ZP = −14.05 EE = 65.97	Various peptides and proteins of therapeutic significance for brain disorders are prone to enzymatic hydrolysis <i>in vivo</i> and have limited capacity to pass the BBB. A surface-engineering protocol for PEGylated cubosomes with targeting molecules was prepared to ensure the delivery across the BBB and enhance learning deficits in Alzheimer's disease rat models. OL, the smallest lectin known, with very low immunogenicity, loaded cubosomes provide a new, efficient, and non-invasive platform for brain-targeted drug delivery, especially for biotechnological therapeutics like peptides, proteins, and DNA.	[80]
10	Cubosomal <i>In-situ</i> Gel	Lipid- GMO-GTP Stabilizer- P407, P188 Surfactant- Myrj 59 Drug- Repaglinide (REP)	(anti-diabetic)	PS = 170 to 233 PDI = 0.139 to 0.387 ZP = 4.9 to 7.77 EE = 93.2 to 95.66	Myrj 59 stabilised cubosomal dispersion and <i>in situ</i> gel ensures successful delivery of REP via the intranasal route. The physicochemical characterisation and <i>in vitro</i> release studies showed a favourable PS morphological cubic structure, good entrapment efficiency, and prolonged drug release. Furthermore, examining the potential use of IN or IP routes for administering REP for the treatment of diabetes demonstrated that REP-loaded cubosomes and <i>in situ</i> gel had a stronger antidiabetic impact than IV REP solution	[81]

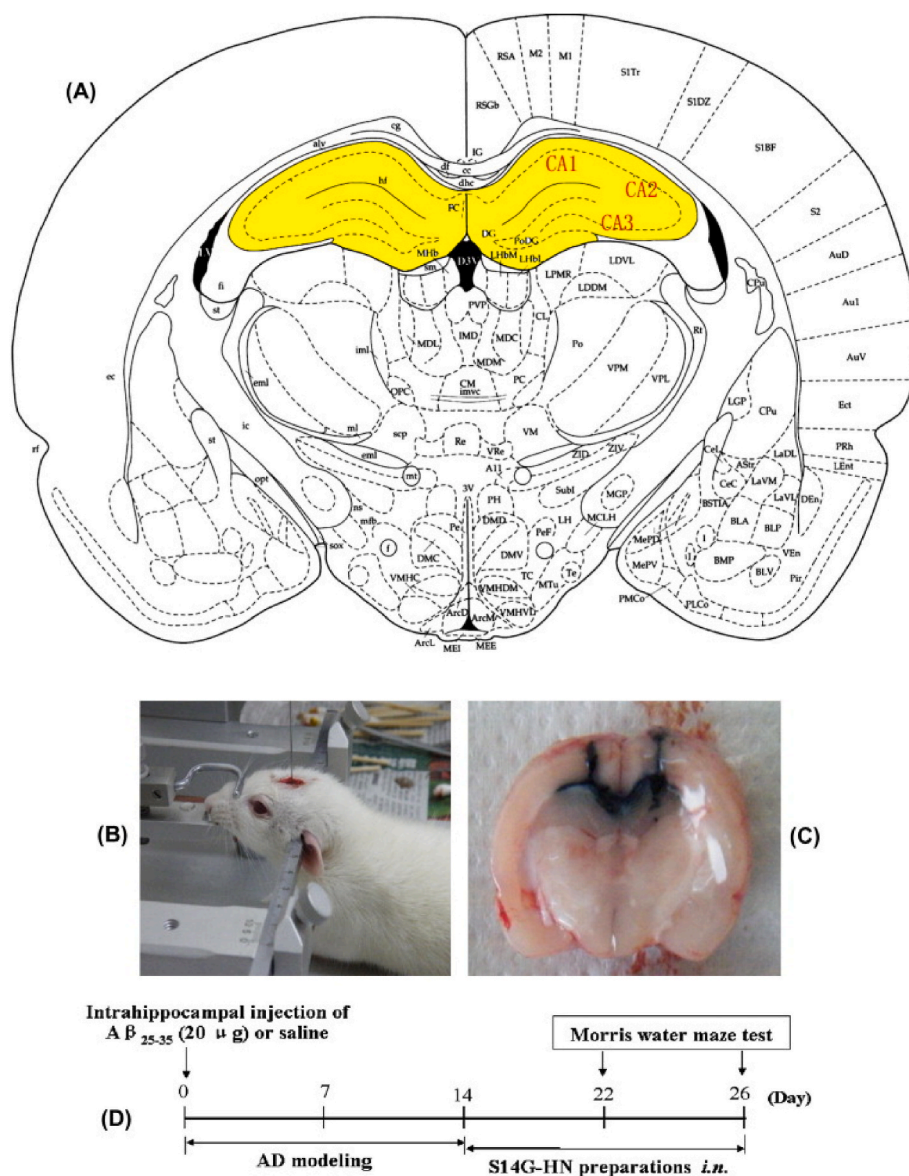


Fig. 4. Schematic illustration of the intra-hippocampal injection procedure and schedule of the Morris water maze test; A) The region targeted in the hippocampus (highlighted yellow) based upon the Paxinos brain atlas; B) the animals were properly oriented in a stereotaxic frame to perform an accurate injection intra-hippocampal injection; C) the injection site is shown with Indian ink (shown black); and D) the experimental timeline depicting the timing of the intrahippocampal injection and the behavioral evaluation using the Morris water maze test [80]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

delivery of antioxidant compounds, such as curcumin, thereby enhancing their bioavailability and neuroprotective efficacy by reducing ROS accumulation and preserving neuronal viability. Supporting the fact, a study highlights the development of cubosomes co-loaded with curcumin and fish oil (FO) as a neuroprotective strategy against oxidative stress-induced neuronal damage. The study further showed that cubosomes can successfully co-encapsulate both curcumin and FO in liquid crystalline nanostructure, as characterized by SAXS and QELS, with curcumin exhibiting an enhanced encapsulation efficiency, even better than reported PLGA-based nanoparticles. In H_2O_2 -exposed SH-SY5Y cells, the formulation significantly reduced ROS levels and apoptotic cell death. These findings suggest cubosomes as effective carriers for phytopharmaceuticals in neuroregenerative therapies [120].

7.4. Migraine

Migraine is a neurovascular disorder that is characterized by

recurrent, pulsating headaches, as well as nausea and heightened sensitivity to light [121]. Molecular targets related to disease pathology include Calcitonin Gene-Related Peptide (CGRP), 5-HT_{1B/1D} receptors, and voltage-gated calcium channels, such as CACNA1A, TRPV1, PACAP, and BDNF. They are also contributors to pain-signaling pathways and neuroinflammation [122]. Cubosomes can improve brain delivery of anti-migraine drugs, such as triptans or CGRP antagonists, through either intranasal or intravenous routes of administration, thereby bypassing first-pass metabolism and increasing the duration of efficacy, therapeutic availability, and onset of action.

This evidence is supported by a study that reported the development of a cubosomal *in situ* nasal gel using *Samanea saman* seed gum as a new mucoadhesive, a particle size of ~129 nm, entrapment efficiency of ~79 %, and a release of ~70 %. Furthermore, biodistribution *in vivo* studies in rats demonstrated that the use of a nasal formulation resulted in a 2.5-fold higher brain area under the concentration-time curve (AUC) and higher maximum plasma concentration (C_{max}) of

sumatriptan succinate compared to administration via intravenous delivery. The data indicated better nasal mucosal permeation and efficient brain targeting, indicating that this cubosomal nasal gel may be a useful approach for managing migraine using a CNS route [31].

7.5. Schizophrenia

Schizophrenia is a long-term neuropsychiatric disorder that is characterized by hallucinations, delusions, and cognitive impairment [123]. Schizophrenia is a disorder with dysregulation of the dopamine D2, glutamate NMDA, serotonin 5-HT_{2A}, and GABAergic signalling pathways. Within this disorder, there has been increased interest in the pathways involving protein DISC1, neuregulin-1, and the pathway involving brain-derived neurotrophic factor (BDNF) [124]. Cubosome delivery systems may increase CNS availability for the delivery of poorly soluble antipsychotics and allow for sustained and targeted release/deposition of antipsychotics to decrease adverse effects from antipsychotic medication.

In this context, a recent report on chitosan-stabilised cubosomal nanoparticles for intranasal delivery of paliperidone palmitate features high mucoaffinity ($\Delta ZP \sim 20.9$ mV) and an exceptional encapsulation efficiency (99.7 %), making delivery and transportation from nose-to-brain effective. *In vivo* studies using a 3D-printed nasal cast demonstrated greater than 50 % deposition in the olfactory region, which is crucial for producing direct brain targets essential for the treatment of schizophrenia. The results demonstrated the range of effective permeability across nasal epithelial cells, supporting its non-invasive use and sustained release of antipsychotics as a delivery strategy [125]. Another study proposed a haloperidol-loaded cubosomal *in-situ* nasal gel using *Artocarpus heterophyllus* (jackfruit) mucilage, as a new natural mucoadhesive for potential enhancement of drug retention and permeation through the nasal mucosa. The optimal formulation (H7) reached a high encapsulation efficiency value (78.8 %), a desirable particle size (~ 156 nm), and acceptable mucoadhesive capacity compared to synthetic mucoadhesives, specifically Carbopol P934. *Ex vivo* studies using sheep nasal mucosa demonstrated significantly better HPD permeation when utilized through the proposed nasal delivery system, indicating potential success for nose-to-brain transport in treating schizophrenia. The results showed the feasibility of the natural-polymer-based cubogel system for non-invasive targeted administration of antipsychotics to the CNS [126].

7.6. Epilepsy

Epilepsy is a neurological disorder defined by various recurrent seizures resulting from abnormal electrical activity in the brain. Presently, important development targets for epilepsy include voltage-gated sodium channels (e.g., SCN1A), GABA-A receptors, glutamate AMPA/NMDA receptors, and T-type calcium channels. Mutations have been identified in genes including LGI1 and KCNQ2 [127]. Cubosomes can maintain sustained release and targeted delivery of anti-seizure medications. This unique delivery method might allow for proper medication levels for prolonged periods without fluctuating on either side to alleviate seizure incidents.

In a study, lamotrigine-loaded cubosomal *in-situ* nasal gel (cubogel) was developed to enhance delivery to the brain to address poor oral bioavailability and low BBB penetration. The optimized cubosomes (116–197 nm) were incorporated into a thermosensitive gel using P407, ensuring the medication has a prolonged residence time in the nasal cavity and sustained delivery. *In vivo*, the LTG cubogel significantly enhanced the anticonvulsant efficacy in the pilocarpine-induced chronic epilepsy model, while elevating brain levels of GABA, serotonin, and antioxidant capacity, and suppressing excitatory neurotransmitters (dopamine, Ca²⁺, acetylcholine), as well as inflammatory mediators (CRP and GFAP). Confocal laser scanning microscopy images revealed that both RhB-loaded cubosomal dispersion and cubogel provided

significantly greater nasal mucosal penetration than RhB solution. The cubosomal formulations exhibited greater green fluorescence, which indicates enhanced uptake of RhB from the formulation. The cubogel exhibited the most intense fluorescence overall, indicating superior permeability compared to the other formulations (Fig. 5). The LTG cubogel was superior to both free drug and cubosomes on their own, and demonstrates a great opportunity for the treatment of epilepsy in a targeted and non-invasive manner [15].

In another study, results showed that agmatine-loaded cubosomes, administered intranasally, significantly improved the therapeutic response in a pentylenetetrazole-induced epilepsy model. The optimized formulation (F7) provided high entrapment efficiency (88.92 %), favourable particle size (~ 270 nm), sustained drug release (>95 % over 12 h), and an overall prolonged therapeutic effect. Agmatine cubosomes exhibited longer onset and shorter seizure durations (611 s and 16 s, respectively) compared to intraperitoneal diazepam, indicating better neuroprotective and anticonvulsant efficacy. Overall, it was concluded that cubosomal nose-to-brain delivery evaded the BBB to produce a non-invasive formulation and targeted delivery for epilepsy treatment with better drug bioavailability and fewer systemic side effects [74].

7.7. Miscellaneous

Cubosomal formulations, owing to their unique nanostructured lipid matrix, have demonstrated superior therapeutic efficacy across various pathological conditions by enhancing drug solubility, stability, and targeted delivery [128]. Studies have shown their effectiveness in improving brain bioavailability for antiemetic therapy (granisetron), enhancing anti-inflammatory action in topical pain management (felbinac), enabling neuroprotection in glaucoma via targeted retinal delivery (LM22A-4), and facilitating transdermal chemo-photothermal therapy in melanoma [129]. These versatile systems offer sustained release, improved permeation, and site-specific drug accumulation, making them a promising platform for advanced drug delivery.

A study reported that granisetron-loaded cubosomes incorporated into a thermosensitive *in situ* nasal gel significantly enhanced the management of chemotherapy-induced emesis by facilitating direct nose-to-brain delivery, thereby bypassing the BBB. The optimized cubosomal system demonstrated high entrapment efficiency, sustained drug release, and enhanced mucosal permeation, resulting in increased brain bioavailability compared to conventional formulations. Moreover, histopathological evaluations confirmed the safety and biocompatibility of the formulation on the nasal mucosa. These findings suggest that the cubosomal *in situ* gel provides a non-invasive and effective strategy for delivering granisetron to the brain, offering superior antiemetic control during chemotherapy [77].

Another study reported that felbinac-loaded cubosomes were effectively formulated into a hydrogel (cubogel) for the topical treatment of inflammatory pain, demonstrating superior performance over conventional gels. The cubosomal system enabled high drug entrapment, sustained release, and enhanced skin permeation, resulting in prolonged anti-inflammatory activity up to 24 h post-application. Compared to standard felbinac gel, the cubosomal gel significantly reduced paw edema and inflammatory cell infiltration, as confirmed by histopathological analysis. The cubic nanostructure of cubosomes enables targeted and localized drug delivery, thereby improving therapeutic efficacy and reducing dosing frequency in inflammatory conditions [130].

Furthermore, Chen et al. reported that using cubosomes targeted with doxorubicin and indocyanine green represents a novel approach to treating melanoma, enabling a synergistic chemo-photothermal therapy as shown in Fig. 6. The cubosome nanostructure selects for lipid bilayer arrangement that optimizes transdermal drug permeation across the stratum corneum, which can be one of the major barriers to skin drug delivery. Doxorubicin and indocyanine green were also able to spatially localize at the tumor site, allowing for better accumulation of the therapeutic. These cubosomes exhibited pH- and heat-responsive drug

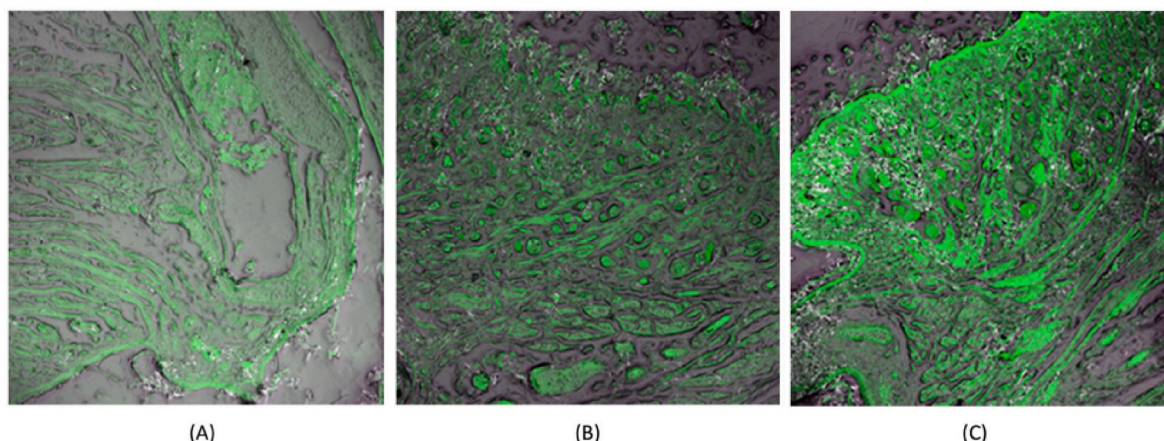


Fig. 5. Confocal laser scanning microscopy images of rats' nasal mucosal membrane regions, 2h after intranasal administration of: (A) RhB solution, (B) RhB-loaded cubosomes "F4", and (C) RhB-loaded cubogel "G3" formulations [15].

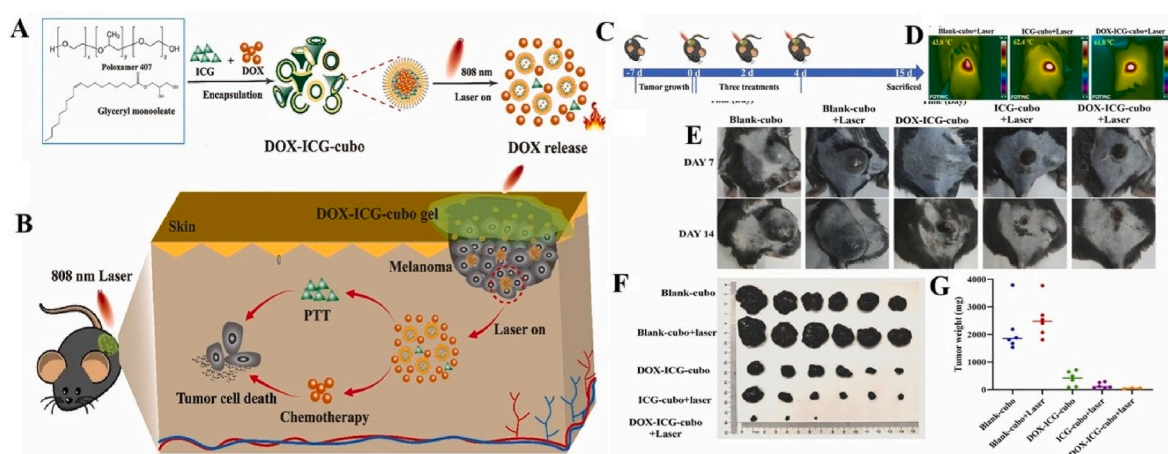


Fig. 6. (A) Formulation process of doxorubicin -indocyanine green cubosomes (cubo); (B) Pictorial presentation of developed cubosomal gel and its effect on melanoma upon exposure to photothermal therapy, and chemotherapy; (C) Anti-tumor pre-clinical studies in B16F10-bearing C57BL/6 mice; (D) Infrared images post transdermal application of blank cubo, ICG cubo, and doxorubicin-indocyanine cubosomes proceeded by laser irradiation 808 nm at 1.5cm² for 10 min; (E) Day 7 and 14 images of tumor treated with three different cubo; (F) images of tumor post treatment; (G) respective weights of tumor after different treatment [131]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

release, ensuring controlled delivery under tumor-specific conditions. Moreover, their high photothermal conversion and biocompatibility significantly improved *in vivo* anti-melanoma efficacy, highlighting cubosomes as a potent nanocarrier system for aggressive skin cancers like melanoma [131].

Overall, cubosomal drug delivery systems represent a highly versatile and efficient nanoplatform capable of overcoming biological barriers, enhancing therapeutic outcomes, and offering targeted, sustained delivery across a wide spectrum of diseases. However, this delivery system faces a few critical challenges due to various formulation-related and physicochemical factors, which are explained in the connecting section.

8. Challenges of developing cubosomes for intranasal application

Despite promising drug delivery systems, *in situ* thermoresponsive cubosomal intranasal gels have many issues affecting their stability, formulation, and clinical use. Selecting a suitable thermoresponsive polymer that guarantees the best sol-gel transition under physiological conditions (32–37 °C) is a significant challenge. Furthermore, because these nanostructures are prone to aggregation or phase transitions,

which may impact drug loading and release, cubosomal stability is a concern. To prevent destabilization, it is also essential to ensure that cubosomes and thermoresponsive polymers are compatible [132]. Another issue is drug loading efficiency, as some medications have low encapsulation and suboptimal sustained release due to their poor solubility or compatibility with cubosomal lipids [133]. Furthermore, mucoadhesion and mucociliary clearance greatly impact drug retention and absorption. The presence of nasal mucus barrier may impede drug penetration and formulation may be eliminated due to rapid mucociliary clearance before adequate absorption occurs. Achieving efficient BBB penetration is another crucial obstacle. Molecular size and lipophilicity limitations make it challenging to ensure sufficient drug transport into the brain, even though the intranasal route circumvents the BBB by using the olfactory and trigeminal pathways. Some of these challenges faced with the cubosome-based delivery systems are briefly discussed in the later sections.

8.1. Aggregation and fusion

Cubosomal formulation is a dispersion of nanoparticles; individual cubosomes may clump together because of insufficient steric or electrostatic stabilization, high ionic strength, or suboptimal storage

conditions, and cause aggregation. Aggregation affects the colloidal stability, increases particle size, increases PDI, and reduces the efficacy of the delivery system. Sometimes, two cubosomes may merge, disrupting the internal structure and leading to the leakage of encapsulated substance. This may be caused by inadequate surfactant coverage, pH, ionic charges, and mechanical stress handling. Aggregation and fusion limit the self-life and functional integrity of cubosomal formulations [134].

8.2. Polymorphic transitions

Structural changes in the internal lipidic organisation, typically transitions between mesophases such as cubic, hexagonal, and lamellar or micellar phases, are referred to as a polymorphic transition. The mesophase structure of cubosomes is highly sensitive to environmental factors, like temperature, pH, ionic strength, and incorporation of drugs, which can induce a polymorphic transition. These transitions affect the formulation of cubosomes, stability, and can also alter the release profile [135].

8.3. Sterilization-related problems

Cubosomes have a highly ordered cubic structure, which enhances drug loading and facilitates the delivery of drugs in the body. These require high purity and safety standards. Sterilization is a crucial step in eliminating all forms of microbes to ensure medical and pharmaceutical safety [136]. However, the complex and sensitive structure of cubosomes makes them difficult to sterilize using standard methods, like heat, radiation, or filtration, as these may disrupt the internal structure and alter their properties. For example, autoclaving can degrade the lipids and internal structure due to heat [137].

8.4. Limited information on biodistribution, metabolism, and clearance

Cubosomes are primarily used for targeted delivery because of their lipid composition and customizable surface properties. They rapidly distribute throughout the body once administered, and their biodistribution plays a critical role in determining the therapeutic efficacy and safety [99]. However, they often get absorbed by the mononuclear phagocyte system, which causes them to accumulate in organs like the liver, spleen, and lungs. Many preclinical studies have found localization within these organs upon intravenous administration. Particle size, surface charge, lipid constitution, and use of surface stabilizers also affect biodistribution. Surface modification with polyethylene glycol (PEGylation) has been found to decrease short-term clearance and increase circulation time, resulting in more desirable profiles. However, existing *in vivo* studies do not provide much insight about complete biodistribution dynamics, especially in comparison to other nanodelivery systems [138]. The precise metabolic processes of cubosomes are currently being studied. Furthermore, more research is needed to clarify their clearance processes, whether they involve hepatic metabolism, renal excretion, or biodegradation [139]. Optimizing cubosome formulations for safety, efficacy, and targeted distribution in drug delivery applications requires more understanding of the pharmacokinetic characteristics [140].

8.5. Challenges faced due to biological interaction, protein corona formation, and mucosal barrier penetration

A key issue with all cubosome-based delivery systems in biological environments is their complex interactions with biological fluids, particularly via the formation of a protein corona. A protein corona is a layer of adsorbed plasma proteins that spontaneously forms on the surface of nanoparticles after systemic administration [34]. Biologically, the protein corona can significantly change the physicochemical properties of cubosomes, including surface charge, hydrophobicity, and

steric profile, and subsequently impact biodistribution, cellular uptake, and biological recognition (both immune and non-immune). In some cases, opsonization and clearance of the cubosomes occur through the mononuclear phagocyte system, potentially leading to loss of their circulation and therapeutic efficacy. For delivery by mucosal routes (e.g., intranasal or intravaginal), an additional hurdle exists due to the presence of the mucus barrier. Cubosomes need to traverse the viscoelastic mucin layer without being trapped or degraded [34]. The ability of cubosomes to penetrate this barrier is highly dependent upon their size, surface charge, and hydrophilicity. For example, particles with a dense PEG coating may show greater mucus penetration as the density of the PEG reduces adhesive interactions. However, it remains challenging to achieve the optimal balance between mucus-penetrating ability and effective cellular uptake, as modifications to improve one can often reduce the other [91]. These biological interactions require precise surface engineering and detailed *in vivo* assessment to confirm that cubosomes can consistently reach and act on their intended target sites.

8.6. Challenges faced for clinical and translational progress

Despite the remarkable advancements in the design, optimization, and preclinical evaluation of cubosome-based drug delivery systems, their translation into clinical applications remains in its nascent stages. To date, no cubosome-specific formulations have reached advanced clinical trials, and there are currently no active or completed human studies registered on platforms such as [ClinicalTrials.gov](https://clinicaltrials.gov) or the WHO International Clinical Trials Registry. This translational gap primarily arises from challenges related to large-scale manufacturing, long-term stability, and regulatory standardization of these complex lipid nanostructures. Ongoing research efforts are focused on addressing critical translational barriers, such as scale-up reproducibility, batch uniformity, and long-term storage stability through innovations in microfluidic fabrication, solid-state stabilization and lyophilization techniques. Once these challenges are systematically resolved, cubosomes are expected to progress rapidly toward regulatory approval and clinical deployment as a next-generation nanocarrier platform for precision drug delivery.

9. Overcoming the challenges through the incorporation of cubosomes into *in situ* cubosome-based gel formulations

Cubosome-based drug delivery systems present several unique challenges, including aggregation, polymorphic transitions, formation of a protein corona, and the inherent limitations of the mucosal barrier, as discussed above [32]. To address these points, the incorporation of cubosomes into thermoresponsive *in situ* gel systems is especially promising due to the delicate nature of the nasal cavity, its favourable conditions for brain drug delivery, and the ability to stabilize the nanoparticles in the gel system [31]. The *in situ* gel will act as a physical stabilizer, creating a defined microenvironment and system that limits the aggregation and subsequent fusion of the cubosomes by reducing the neutron energy and number of collisions, thereby limiting microparticle formation [141]. The proper selection of thermoresponsive polymers like P407 or blends of P407 with polymer (i.e. HPMC, and/or Carbopol) will allow for a rapid phase change of sol–gel above the physiological temperature range (32–37 °C), from fluid to a gel state, without exceeding a given gelation time in a fluid state [142]. Once in the gel, it reduces the chance of being expelled and allows diffusion of the active ingredient to be sustained in a new diffusion matrix composed of both the cubosome and the hydrogel network [143]. Furthermore, a gel at the mucosal site improves mucoadhesion, engenders a limited rate of mucociliary clearance and increases the envelope for drug absorption, thereby increasing the time available for absorption of the encapsulated drug.

In addition, because the gel would protect cubosomes from direct protein interactions by acting as a barrier, it could reduce the risk of

forming protein's corona. This is especially the case for local applications, such as those in the nasal cavity, where the likelihood of systemic exposure is limited at the site of application. To improve penetration through the BBB, we could incorporate surface engineering via either PEGylation of the stabilizers or conjugation with a ligand (e.g., lactoferrin, transferrin) to enhance permeability through mucus and receptor-mediated uptake to the CNS via the trigeminal or olfactory pathways [144]. Cubosomal lipids must be compatible with gelling agents, hence it is critical to perform preformulation studies on their rheological behaviour, phase state, and thermodynamic stability to understand and anticipate any potential unwanted polymorphic transitions. Enhancing drug loading and entrapment for poorly soluble or hydrophobic actives can be achieved through co-solubilization approaches, such as lipid modifications or the introduction of solubility enhancers [32]. Overall, these optimizations of cubosomal *in situ* gel systems, adopted from novel use of bi-phasic systems, provide a robust and practical mechanism to overcome biological, physicochemical, and clinical limitations, thus paving the way for enhanced intranasal routes of administration with potential for targeting the brain and improved therapeutic outcomes.

10. Conclusion

Intranasal cubosome-based gel formulations showcase a revolutionary advancement in the treatment of neurodegenerative diseases. These systems facilitate direct delivery of both hydrophilic and lipophilic drugs to the CNS via olfactory and trigeminal pathways, circumventing the BBB, increasing bioavailability, and reducing systemic side effects. The incorporation of thermoresponsive polymers (such as P407) along with mucoadhesive polymers (HPMC) enhances naso-mucosal adhesion, prolongs residence time, and ensures sol-to-gel transition at physiological temperature, optimizing both drug retention and penetration. This dual-phase system not only mitigates enzymatic degradation and mucociliary clearance but also improves bioavailability, neuronal uptake, and therapeutic outcomes across complex CNS pathologies.

Cubosome-based gel formulations have shown promise in a range of CNS conditions, including Alzheimer's disease, Parkinson's, epilepsy, schizophrenia, and glioblastoma, as demonstrated by several preclinical studies. Functionalized cubosomes with ligands, including transferrin or lectins, exhibit receptor-mediated endocytosis to allow for active targeting of the diseased neural area. *In vivo* studies have demonstrated enhanced CNS localization, prolonged duration neuroprotection, and decreased levels of inflammatory biomarkers, providing evidence of efficacy across multiple neurological models. Their high encapsulation efficiency, controlled drug release, and ability to tune their surface properties greatly exceed those of standard formulations. In contrast, there are still challenges related to formulation and biological interaction, such as aggregation, polymorphic transitions, and protein corona formation. However, the cubosomal gel matrix effectively addresses and stabilizes nanoparticle dispersion, shields against biological interactions, and supports consistent release behaviour. Thus, cubosomal *in situ* gels offer a multi-mechanistic and customizable approach for precision CNS therapy.

Future work should remain focused on clinical translation, including toxicology testing, more complex pharmacodynamic modeling, and human studies. Advances in ligand-functionalized cubosomes, PEGylation techniques, and stimuli-responsive systems specifically may further enhance targeting and site-specific delivery to the brain. Furthermore, understanding *in vivo* biodistribution, metabolism, and long-term safety will be essential to establish cubosome-based gels as a standard approach to non-invasive neurotherapeutics.

CRediT authorship contribution statement

Jyotika Das: Writing – original draft, Methodology, Data curation. **Varnita Karmakar:** Writing – original draft, Methodology, Data

curation. **Manisha Pandey:** Writing – review & editing, Writing – original draft, Investigation. **Neha Jain:** Writing – review & editing, Writing – original draft, Investigation. **Bapi Gorain:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- [1] D.G. Gadhave, V.V. Sugandhi, S.K. Jha, S.N. Nangare, G. Gupta, S.K. Singh, K. Dua, H. Cho, P.M. Hansbro, K.R. Paudel, Neurodegenerative disorders: mechanisms of degeneration and therapeutic approaches with their clinical relevance, *Ageing Res. Rev.* 99 (2024) 102357, <https://doi.org/10.1016/J.ARR.2024.102357>.
- [2] G. Hu, L. Yang, Y. Cai, F. Niu, F. Mezzacappa, S. Callen, H.S. Fox, S. Buch, Emerging roles of extracellular vesicles in neurodegenerative disorders: focus on HIV-associated neurological complications, *Cell Death Dis.* 7 (2016) 1–12, <https://doi.org/10.1038/cddis.2016.336>.
- [3] J.R. Deliz, C.M. Tanner, P. Gonzalez-Latapi, Epidemiology of parkinson's disease: an update, *Curr. Neurol. Neurosci. Rep.* 24 (2024) 163–179, <https://doi.org/10.1007/s11910-024-01339-w>.
- [4] V. Karmakar, B. Gorain, Potential molecular pathways of angiotensin receptor blockers in the brain toward cognitive improvement in dementia, *Drug Discov. Today* 29 (2024) 103850, <https://doi.org/10.1016/J.DRUDIS.2023.103850>.
- [5] C.J. Van Der Schyf, The use of multi-target drugs in the treatment of neurodegenerative diseases, *Expert Rev. Clin. Pharmacol.* 4 (2011) 293–298, <https://doi.org/10.1586/ecp.11.13>.
- [6] H. Ahmadian-Moghadam, M.S. Sadat-Shirazi, M.R. Zarrindast, Therapeutic potential of stem cells for treatment of neurodegenerative diseases, *Biotechnol. Lett.* 42 (2020) 1073–1101, <https://doi.org/10.1007/s10529-020-02886-1>.
- [7] D. Madhubala, R. Mahato, M.R. Khan, A. Bala, A.K. Mukherjee, Neurotrophin peptidomimetics for the treatment of neurodegenerative diseases, *Drug Discov. Today* 29 (2024) 104156, <https://doi.org/10.1016/j.drudis.2024.104156>.
- [8] A. Mukherjee, S. Biswas, I. Roy, Immunotherapy: an emerging treatment option for neurodegenerative diseases, *Drug Discov. Today* 29 (2024) 103974, <https://doi.org/10.1016/j.drudis.2024.103974>.
- [9] R.N.L. Lamprey, B. Chaulagain, R. Trivedi, A. Gothwal, B. Layek, J. Singh, A review of the common neurodegenerative disorders: current therapeutic approaches and the potential role of nanotherapeutics, *Int. J. Mol. Sci.* 23 (2022) 1851, <https://doi.org/10.3390/IJMS23031851>.
- [10] E. Passeri, K. Elkhoury, M. Morsink, K. Broersen, M. Linder, A. Tamayol, C. Malaplate, F.T. Yen, E. Arab-Tehrany, Alzheimer's disease: treatment strategies and their limitations, *Int. J. Mol. Sci.* 23 (2022) 13954, <https://doi.org/10.3390/IJMS232213954>.
- [11] K. Shashikant, M. Sanjana, K. Gayatri, G. Rakhi, Navigating neurodegenerative disorders: a comprehensive review of current and emerging therapies for neurodegenerative disorders, *J. Neurosci. Neurol. Disord.* 8 (2024) 33–46, <https://doi.org/10.29328/JOURNAL.JNND.1001095>.
- [12] S.A. Vahab, A. Nair, D. Raj, G.P. Akhil, P.P. Sreelakshmi, V.S. Kumar, Cubosomes as versatile lipid nanocarriers for neurological disorder therapeutics: a comprehensive review, *Naunyn-Schmiedeberg's Arch Pharmacol* 397 (2024) 3729–3746, <https://doi.org/10.1007/S00210-023-02879-7>.
- [13] Kasturi Pangarkar, Cubosomes: a frontier in nanotechnology for enhanced drug delivery, *Int. J. Pharm. Sci.* 2 (2024) 27–36, <https://doi.org/10.5281/ZENODO.14024554>.
- [14] S. Kakulade, D. Kulkarni, K. Moravkar, S. Zambad, A. Tekade, S. Chalikwar, Development, characterization and pharmacokinetic evaluation of selegiline HCl loaded cubosomal thermoreversible mucoadhesive gel for nose to brain delivery, *J. Drug Deliv. Sci. Technol.* 100 (2024) 106111, <https://doi.org/10.1016/J.JDDST.2024.106111>.
- [15] A.M. Mohsen, A.A.A. Salama, M.H. Asfour, Cubosome-based thermosensitive *in situ* gelling system for intranasal administration of lamotrigine with enhanced antiepileptic efficacy, *Pharmaceut. Dev. Technol.* 28 (2023) 520–534, <https://doi.org/10.1080/10837450.2023.2216755>.
- [16] N.R. Rarokar, S.D. Saoji, N.A. Raut, J.B. Taksande, P.B. Khedekar, V.S. Dave, Nanostructured cubosomes in a thermoresponsive depot system: an alternative approach for the controlled delivery of docetaxel, *AAPS PharmSciTech* 17 (2015) 436, <https://doi.org/10.1208/S12249-015-0369-Y>.
- [17] S.H.W. Scheres, B. Ryskeldi-Falcon, M. Goedert, Molecular pathology of neurodegenerative diseases by cryo-EM of amyloids, *Nature* 621 (2023) 701–710, <https://doi.org/10.1038/s41586-023-06437-2>.

- [18] G.G. Kovacs, Molecular pathology of neurodegenerative diseases: principles and practice, *J. Clin. Pathol.* 72 (2019) 725–735, <https://doi.org/10.1136/jclinpath-2019-205952>.
- [19] S.K. Lotz, B.M. Blackhurst, K.L. Reagin, K.E. Funk, Microbial infections are a risk factor for neurodegenerative diseases, *Front. Cell. Neurosci.* 15 (2021) 691136, <https://doi.org/10.3389/fncel.2021.691136>.
- [20] P.L. McGeer, S. Itagaki, E.G. McGeer, Expression of the histocompatibility glycoprotein HLA-DR in neurological disease, *Acta Neuropathol.* 76 (1988) 550–557, <https://doi.org/10.1007/BF00689592>.
- [21] A.T. Aborode, R.O. Adesola, G.Y. Scott, V.A. Adepoju, O.D. Akan, A. Ogunyemi, A.P.-G. Adetunji, F.Q. Arogundade, D.K. Somuah, A.O. Emmanuel, B. Ameh, I. S. Oluwaseun, A.I. Osu, V.C. Jonathan, C.S. Amadi, M.V. Oladayo, A.F. Matthew, I.A. Onifade, S. Fakorede, A.A. Bakre, Role of blood-brain barrier in bacterial translocation, *Neuroscience* 580 (2025) 99–114, <https://doi.org/10.1016/J.NEUROSCIENCE.2025.06.037>.
- [22] A. Popa-Wagner, D. Dumitrascu, B. Capitanescu, E. Petcu, R. Surugiu, W.H. Fang, D.A. Dumbrava, Dietary habits, lifestyle factors and neurodegenerative diseases, *Neural Regen. Res.* 15 (2020) 394–400, <https://doi.org/10.4103/1673-5374.266045>.
- [23] S. Chakraborty, V. Karmakar, K. Chatterjee, A. Chatterjee, M. Dwivedi, B. Gorain, Chitosan nanoparticle-mediated nose-to-brain delivery of naringenin: attenuating memory decline in experimental animals via behavioural assessment and modulation of biochemical parameters, *Int. J. Biol. Macromol.* 286 (2025) 138336, <https://doi.org/10.1016/j.ijbiomac.2024.138336>.
- [24] D. Psimadas, P. Georgoulis, V. Valotassiou, G. Loudos, Molecular nanomedicine towards cancer, *J. Pharmacol. Sci.* 101 (2012) 2271–2280, <https://doi.org/10.1002/jps>.
- [25] U.C. Galgatte, A.B. Kumbhar, P.D. Chaudhari, Development of in situ gel for nasal delivery: design, optimization, in vitro and in vivo evaluation, *Drug Deliv.* 21 (2014) 62–73, <https://doi.org/10.3109/10717544.2013.849778>.
- [26] S. Adwan, T. Obeidi, F. Al-Akayleh, Chitosan nanoparticles embedded in situ gel for nasal delivery of imipramine hydrochloride: short-term stage development and controlled release evaluation, *Polymers (Basel)* 16 (2024) 3062, <https://doi.org/10.3390/polym16213062>.
- [27] F.M. Elsenosy, G.A. Abdelbary, A.H. Elshafeey, I. Elsayed, A.R. Fares, Brain targeting of duloxetine HCL via intranasal delivery of loaded cubosomal gel: in vitro characterization, ex vivo permeation, and in vivo biodistribution studies, *Int. J. Nanomed.* 15 (2020) 9517–9537, <https://doi.org/10.2147/IJN.S277352>.
- [28] S.S. Garg, A. Vyas, V.K. Arivarasam, J. Gupta, Cubosomes: an emerging nanodrug delivery platform for anti-diabetic medications, *J. Drug Deliv. Sci. Technol.* 97 (2024) 105808, <https://doi.org/10.1016/J.JDDST.2024.105808>.
- [29] R. Nithya, P. Jerold, K. Siram, Cubosomes of dapsone enhanced permeation across the skin, *J. Drug Deliv. Sci. Technol.* 48 (2018) 75–81, <https://doi.org/10.1016/J.JDDST.2018.09.002>.
- [30] H.A. Salama, A.A. Mahmoud, A.O. Kamel, M. Abdel Hady, G.A.S. Awad, Phospholipid based colloidal poloxamer-nanocubic vesicles for brain targeting via the nasal route, *Colloids Surf. B Biointerfaces* 100 (2012) 146–154, <https://doi.org/10.1016/j.colsurfb.2012.05.010>.
- [31] A. Tekade, P. Ghodke, A. Patange, P. Patil, Nanostructured cubosomal in situ nasal gel for the treatment of migraine, *J. Drug Deliv. Sci. Technol.* 87 (2023) 104797, <https://doi.org/10.1016/j.jddst.2023.104797>.
- [32] D. Sivasadan, M.H. Sultan, S.S. Alqahtani, S. Javed, Cubosomes in drug delivery—A comprehensive review on its structural components, preparation techniques and therapeutic applications, *Biomedicines* 11 (2023) 1114.
- [33] S.A. Gaballa, O.H. El Garhy, H. Moharram, H. Abdelkader, Preparation and evaluation of cubosomes/cubosomal gels for ocular delivery of beclomethasone dipropionate for management of uveitis, *Pharm. Res.* 37 (2020) 1–23, <https://doi.org/10.1007/S11095-020-02857-1/FIGURES/13>.
- [34] H. Umar, H.A. Wahab, A.M. Gazzali, H. Tahir, W. Ahmad, Cubosomes: design, development, and tumor-targeted drug delivery applications, *Polymers* 14 (2022) 3118, <https://doi.org/10.3390/POLYM14153118>.
- [35] J. Castillo-González, E. González-Rey, Beyond wrecking a wall: revisiting the concept of blood–brain barrier breakdown in ischemic stroke, *Neural Regen. Res.* 20 (2025) 1944–1956, <https://doi.org/10.4103/nrr.nrr-d-24-00392>.
- [36] Y. Ma, T. Dong, F. Luan, J. Yang, F. Miao, P. Wei, Interaction of major facilitator superfamily domain containing 2A with the blood–brain barrier, *Neural Regen. Res.* 20 (2024) 2133–2152, <https://doi.org/10.4103/nrr.nrr-d-24-00191>.
- [37] T. Balzano, J.A. Pineda-Pardo, N. Esteban-García, M. López-Aguirre, A. Reñares-Sebastián, I. Trigo-Damas, M. Takada, J.A. Obeso, J. Blesa, Temporal dynamics of neurovascular unit changes following blood-brain barrier opening in the putamen of non-human primates, *J. Contr. Release* 377 (2025) 116–126, <https://doi.org/10.1016/j.jconrel.2024.11.019>.
- [38] J.J. Lochhead, R.G. Thorne, Intranasal delivery of biologics to the central nervous system, *Adv. Drug Deliv. Rev.* 64 (2012) 614–628, <https://doi.org/10.1016/j.addr.2011.11.002>.
- [39] C.M. Bellettato, M. Scarpa, Possible strategies to cross the blood-brain barrier, *Ital. J. Pediatr.* 44 (2018) 131, <https://doi.org/10.1186/s13052-018-0563-0>.
- [40] L.R. Hanson, W.H. Frey II, Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease, *BMC Neurosci.* 9 (Suppl 3) (2008) S5, <https://doi.org/10.1186/1471-2202-9-S3-S5>.
- [41] I. Drath, F. Richter, M. Feja, Nose-to-brain drug delivery: from bench to bedside, *Transl. Neurodegener.* 14 (2025) 1–22, <https://doi.org/10.1186/s40035-025-00481-w>.
- [42] M. van Woensel, N. Wauthoz, R. Rosière, K. Amighi, V. Mathieu, F. Lefranc, S. W. van Gool, S. de Vleeschouwer, Formulations for intranasal delivery of pharmacological agents to combat brain disease: a new opportunity to tackle GBM? *Cancers* (2013) 1020–1048, <https://doi.org/10.3390/cancers5031020>.
- [43] A. Ghosh, A. Majie, V. Karmakar, K. Chatterjee, S. Chakraborty, M. Pandey, N. Jain, S. Roy Sarkar, A.B. Nair, B. Gorain, In-depth mechanism, challenges, and opportunities of delivering therapeutics in brain using intranasal route, *AAPS PharmSciTech* 25 (2024) 1–23, <https://doi.org/10.1208/S12249-024-02810-0>.
- [44] S. Wei, Z. Zhai, X. Kong, C. Wu, B. Zhu, Z. Zhao, X. Zhang, The review of nasal drug delivery system: the strategies to enhance the efficiency of intranasal drug delivery by improving drug absorption, *Int. J. Pharm.* 676 (2025) 125584, <https://doi.org/10.1016/j.ijpharm.2025.125584>.
- [45] A. Lofts, F. Abu-Hijleh, N. Rigg, R.K. Mishra, T. Hoare, Using the intranasal route to administer drugs to treat neurological and psychiatric illnesses: Rationale, successes, and future needs, *CNS Drugs* 36 (2022) 739–770, <https://doi.org/10.1007/S40263-022-00930-4>.
- [46] Y. Jiang, J. Zhu, G. Xu, X. Liu, Intranasal delivery of stem cells to the brain, *Expert Opin. Drug Deliv.* 8 (2011) 623–632, <https://doi.org/10.1517/17425247.2011.566267>.
- [47] S. Talegaonkar, P.R. Mishra, Intranasal delivery: an approach to bypass the blood brain barrier, *Indian J. Pharmacol.* 36 (2004) 140–147.
- [48] R.G. Thorne, C.R. Emory, T.A. Ala, W.H. Frey, Quantitative analysis of the olfactory pathway for drug delivery to the brain, *Brain Res.* 692 (1995) 278–282, [https://doi.org/10.1016/0006-8993\(95\)00637-6](https://doi.org/10.1016/0006-8993(95)00637-6).
- [49] C.V. Pardeshi, V.S. Belgamwar, 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.* 10 (2013) 957–972, <https://doi.org/10.1517/17425247.2013.790887>.
- [50] R.R. Bhosale, R.A. Osmani, B.R. Harkare, P.P. Ghodake, Cubosomes: the inimitable nanoparticulate drug carriers, *Scholars Acad. J. Pharm.* 2 (2013) 481–486.
- [51] S.P. Akhlaghi, I.R. Ribeiro, B.J. Boyd, W. Loh, Impact of preparation method and variables on the internal structure, morphology, and presence of liposomes in phytantriol-Pluronic® F127 cubosomes, *Colloids Surf. B Biointerfaces* 145 (2016) 845–853, <https://doi.org/10.1016/j.colsurfb.2016.05.091>.
- [52] I.D.M. Azmi, S.M. Moghimi, A. Yaghmur, Cubosomes and hexosomes as versatile platforms for drug delivery, *Ther. Deliv.* 6 (2015) 1347–1364, <https://doi.org/10.4155/TDE.15.81>.
- [53] A.G. Nath, P. Dubey, A. Kumar, K.K. Vaiphei, J.M. Rosenholm, K.K. Bansal, A. Gulbake, Recent advances in the use of cubosomes as drug carriers with special emphasis on topical applications, *J. Lipids* (2024), <https://doi.org/10.1155/2024/2683466>, 2024.
- [54] A.S. Palma, B.R. Casadei, M.C. Lotierzo, R.D. de Castro, L.R.S. Barbosa, A short review on the applicability and use of cubosomes as nanocarriers, *Biophys. Rev.* 15 (2023) 553–567, <https://doi.org/10.1007/s12551-023-01089-y>.
- [55] M. El Mohamad, Q. Han, A.J. Clulow, C. Cao, A. Safdar, M. Stenzel, C. J. Drummond, T.L. Greaves, J. Zhai, Regulating the structural polymorphism and protein corona composition of phytantriol-based lipid nanoparticles using choline ionic liquids, *J. Colloid Interface Sci.* 657 (2024) 841–852, <https://doi.org/10.1016/j.jcis.2023.12.005>.
- [56] M. Fornasier, K. Krautforst, J. Kulbacka, P. Jönsson, S. Murgia, U. Bazylińska, Cubosomes and hexosomes stabilized by sorbitan monooleate as biocompatible nanoplateforms against skin metastatic human melanoma, *J. Colloid Interface Sci.* 677 (2025) 842–852, <https://doi.org/10.1016/j.jcis.2024.08.126>.
- [57] H.M.G. Barriga, M.N. Holme, M.M. Stevens, Cubosomes: the next generation of smart lipid nanoparticles? *Angew. Chem. Int. Ed.* 58 (2019) 2958–2978, <https://doi.org/10.1002/anie.201804067>.
- [58] M. Chountoules, S. Pispas, I.K. Tseti, C. Demetzo, Lyotropic liquid crystalline nanostructures as drug delivery systems and vaccine platforms, *Pharmaceuticals* 15 (2022) 429, <https://doi.org/10.3390/ph15040429>.
- [59] L. Gade, B.J. Boyd, M. Malmsten, A. Heinz, Phospholipase A2 induces structural changes in phospholipid-containing cubosomes, *Colloids Surf. A Physicochem. Eng. Asp.* 714 (2025) 136604, <https://doi.org/10.1016/j.colsurfa.2025.136604>.
- [60] S. Gaballa, O. El Garhy, H. Abdelkader, Cubosomes: composition, preparation, and drug delivery applications, *J. Adv. Biomed. Pharm. Sci.* 3 (2020) 1–9, <https://doi.org/10.21608/jabps.2019.16887.1057>.
- [61] G. Garg, Shailendra Saraf, Swarnlata Saraf, Cubosomes: an overview, *Biol. Pharm. Bull.* 30 (2007) 350–353, <https://doi.org/10.1248/bpb.30.350>.
- [62] M.L. Formica, D.A. Real, M.L. Picchio, E. Catlin, R.F. Donnelly, A.J. Paredes, On a highway to the brain: a review on nose-to-brain drug delivery using nanoparticles, *Appl. Mater. Today* (2022) 101631, <https://doi.org/10.1016/j.apmt.2022.101631>.
- [63] D. Lee, T. Minko, Nanotherapeutics for nose-to-brain drug delivery: an approach to bypass the blood brain barrier, *Pharmaceutics* 13 (2021) 2049, <https://doi.org/10.3390/pharmaceutics13122049>.
- [64] Q. Huang, X. Chen, S. Yu, G. Gong, H. Shu, Research progress in brain-targeted nasal drug delivery, *Front. Aging Neurosci.* 15 (2024) 1341295, <https://doi.org/10.3389/FNAGI.2023.1341295>.
- [65] C. Oliveira, C.J.O. Ferreira, M. Sousa, J.L. Paris, R. Gaspar, B.F.B. Silva, J. A. Teixeira, P. Ferreira-santos, C.M. Botelho, A Versatile nanocarrier — cubosomes , characterization , and applications, *Nanomaterials* 12 (2022) 1–26.
- [66] Y. Wu, A. Angelova, Recent uses of lipid nanoparticles, cell-penetrating and bioactive peptides for the development of brain-targeted nanomedicines against neurodegenerative disorders, *Nanomaterials* 13 (2023) 3004, <https://doi.org/10.3390/nano13233004>.
- [67] B. Angelov, A. Angelova, M. Drechsler, V.M. Garamus, R. Mutafchieva, S. Lesieur, Identification of large channels in cationic PEGylated cubosome nanoparticles by

- synchrotron radiation SAXS and Cryo-TEM imaging, *Soft Matter* 11 (2015) 3686–3692, <https://doi.org/10.1039/C5SM00169B>.
- [68] R. Qaiser, F. Pervaiz, H. Shoukat, H. Yasin, H. Hanan, G. Murtaza, Mucoadhesive chitosan/polyvinylpyrrolidone-co-poly (2-acrylamide-2-methylpropane sulphonic acid) based hydrogels of captopril with adjustable properties as sustained release carrier: formulation design and toxicological evaluation, *J. Drug Deliv. Sci. Technol.* 81 (2023) 104291, <https://doi.org/10.1016/j.jddst.2023.104291>.
- [69] C.P. Pilkington, J.M. Seddon, Y. Elani, Microfluidic technologies for the synthesis and manipulation of biomimetic membranous nano-assemblies, *Phys. Chem. Chem. Phys.* 23 (2021) 3693–3706, <https://doi.org/10.1039/D0CP06226J>.
- [70] Y.S. Lee, P.J. Johnson, P.T. Robbins, R.H. Bridson, Production of nanoparticles-in-microparticles by a double emulsion method: a comprehensive study, *Eur. J. Pharm. Biopharm.* 83 (2013) 168–173, <https://doi.org/10.1016/j.ejpb.2012.10.016>.
- [71] Y. Wei, J. Zhang, Y. Zheng, Y. Gong, M. Fu, C. Liu, L. Xu, C.C. Sun, Y. Gao, S. Qian, Cubosomes with surface cross-linked chitosan exhibit sustained release and bioavailability enhancement for vinpocetine, *RSC Adv.* 9 (2019) 6287–6298, <https://doi.org/10.1039/C8RA10302J>.
- [72] H. Liu, X. Liang, Y. Peng, G. Liu, H. Cheng, Supercritical fluids: an innovative strategy for drug development, *Bioengineering* 11 (2024) 788, <https://doi.org/10.3390/BIOENGINEERING11080788>.
- [73] L. Bøge, A. Västberg, A. Umerska, H. Byssell, J. Eriksson, K. Edwards, A. Millqvist-Fureby, M. Andersson, Freeze-dried and re-hydrated liquid crystalline nanoparticles stabilized with disaccharides for drug-delivery of the plectasin derivative AP114 antimicrobial peptide, *J. Colloid Interface Sci.* 522 (2018) 126–135, <https://doi.org/10.1016/j.jcis.2018.03.062>.
- [74] D. Somavanshi, C. P. G. Sonawane, K. Pansare, Y. Ahire, V. Jadhav, Preparation and evaluation of agmatine cubosomes (lipid base nano system) nose to brain delivery for epilepsy, *African J. Biol. Sci.* 6 (2024) 2848–2863, <https://doi.org/10.48047/AFJBS.6.7.20>.
- [75] M.M. Faisal, E. Gomaa, A.E. Ibrahim, S. El Deeb, A. Al-Harrasi, T.M. Ibrahim, Verapamil-loaded cubosomes for enhancing intranasal drug delivery: development, characterization, ex vivo permeation, and brain biodistribution studies, *AAPS PharmSciTech* 25 (2024) 1–15, <https://doi.org/10.1208/S12249-024-02814-W>.
- [76] G.N. Desai, P.M. Dandagi, T.M. Kazi, Nanosized intranasal delivery of novel self-assembled cubic liquid crystals: formulation and evaluation, *J. Pharm. Innov.* 18 (2023) 934–951, <https://doi.org/10.1007/s12247-022-09695-1>.
- [77] E.M. Eissa, M.H. Elkomy, H.M. Eid, A.A. Ali, M.A.S. Abourehab, A.M. Alsubaiyel, I.A. Naguib, I. Alsalahat, A.H. Hassan, Intranasal delivery of granisetron to the brain via nanostructured cubosomes-based in situ gel for improved management of chemotherapy-induced emesis, *Pharmaceutics* 14 (2022) 1374, <https://doi.org/10.3390/PHARMACEUTICS14071374>.
- [78] F.E. Abdelrahman, I. Elsayed, M.K. Gad, A. Badr, M.I. Mohamed, Investigating the cubosomal ability for transnasal brain targeting: in vitro optimization, ex vivo permeation and in vivo biodistribution, *Int. J. Pharm.* 490 (2015) 281–291, <https://doi.org/10.1016/j.ijpharm.2015.05.064>.
- [79] K.M. Hosny, Nanosized cubosomal thermogelling dispersion loaded with saquinavir mesylate to improve its bioavailability: preparation, optimization, in vitro and in vivo evaluation, *Int. J. Nanomed.* 15 (2020) 5113–5129, <https://doi.org/10.2147/IJN.S261855>.
- [80] H. Wu, J. Li, Q. Zhang, X. Yan, L. Guo, X. Gao, M. Qiu, X. Jiang, R. Lai, H. Chen, A novel small Odorranalectin-bearing cubosomes: preparation, brain delivery and pharmacodynamic study on amyloid- β 25-35-treated rats following intranasal administration, *Eur. J. Pharm. Biopharm.* 80 (2012) 368–378, <https://doi.org/10.1016/j.ejpb.2011.10.012>.
- [81] M. Mansour, E. Elmowafy, H.A. Gad, Intranasal versus intraperitoneal myrj 59-stabilized cubosomes: a potential armamentarium of effective anti-diabetic therapy, *Colloids Surf. B Biointerfaces* 199 (2021) 111534, <https://doi.org/10.1016/j.colsurf.2020.111534>.
- [82] A. Bhageerathy, V.V. Prasanth, Formulation and evaluation of moxifloxacin hydrochloride loaded cubosomal gel for ocular delivery, *J. Drug Deliv. Therapeut.* 14 (2024) 88–99, <https://doi.org/10.22270/jddt.v14i3.6464>.
- [83] M. Nawaz, S. Hayat, U. Farooq, M.A. Iqbal, S.H. Khalid, T.W. Nee, K.Y. Khaw, R. Munir, M.U. Ijaz, Development of N-alkylated benzimidazole based cubosome hydrogel for topical treatment of burns, *RSC Adv.* 14 (2024) 32008–32020, <https://doi.org/10.1039/D4RA04816D>.
- [84] A. Sanjana, M.G. Ahmed, B.H.J. Gowda, S. Surya, Formulation and characteristic evaluation of tacrolimus cubosomal gel for vitiligo, *J. Dispersion Sci. Technol.* 45 (2024) 224–233, <https://doi.org/10.1080/01932691.2022.2139716>; WGROUP: STRING-PUBLICATION.
- [85] O. Mertins, P.D. Mathews, A. Angelova, Advances in the design of pH-sensitive cubosome liquid crystalline nanocarriers for drug delivery applications, *Nanomaterials* 10 (2020) 963, <https://doi.org/10.3390/NANO10050963>.
- [86] G. Yadav, P. Madvali, A. Jain, Cubosomes nanocarrier as novel drug delivery system : a comprehensive review, *Int. J. Pharm. Sci.* 2 (2024) 3924–3935, <https://doi.org/10.5281/zenodo.13494538>.
- [87] Q.W. Yu, Q. Ma, Y.Q. Feng, Temperature-response polymer coating for in-tube solid-phase microextraction coupled to high-performance liquid chromatography, *Talanta* 84 (2011) 1019–1025, <https://doi.org/10.1016/J.TALANTA.2011.03.003>.
- [88] E.I. Goksu, J.M. Vanegas, C.D. Blanchette, W.C. Lin, M.L. Longo, AFM for structure and dynamics of biomembranes, *Biochim. Biophys. Acta Biomembr.* 1788 (2009) 254–266, <https://doi.org/10.1016/J.BBAMEM.2008.08.021>.
- [89] M. Danaei, M. Dehghankhold, S. Ataei, F. Hasanazadeh Davarani, R. Javanmard, A. Dokhani, S. Khorasani, M.R. Mozafari, Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems, *Pharmaceutics* 10 (2018) 57, <https://doi.org/10.3390/pharmaceutics10020057>.
- [90] S.B. Rizwan, Y.D. Dong, B.J. Boyd, T. Rades, S. Hook, Characterisation of bicontinuous cubic liquid crystalline systems of phytantriol and water using cryo field emission scanning electron microscopy (cryo FESEM), *Micron* 38 (2007) 478–485, <https://doi.org/10.1016/j.micron.2006.08.003>.
- [91] P. Gawarkar-Patil, B. Mahajan, A. Pawar, V. Dhapte-Pawar, Cubosomes: evolving platform for intranasal drug delivery of neurotherapeutics, *Future J. Pharm. Sci.* 10 (2024) 91, <https://doi.org/10.1186/s43094-024-00665-7>.
- [92] B.M. Boddupalli, Z.N.K. Mohammed, R.A. Nath, D. Banji, Mucoadhesive drug delivery system: an overview, *J. Adv. Pharm. Technol. Res.* 1 (2010) 381–387, <https://doi.org/10.4103/0110-5558.76436>.
- [93] R.P. Patil, D.D. Pawara, C.S. Gudewar, A.R. Tekade, Nanostructured cubosomes in an in situ nasal gel system: an alternative approach for the controlled delivery of donepezil HCl to brain, *J. Liposome Res.* 29 (2019) 264–273, <https://doi.org/10.1080/08982104.2018.1552703>.
- [94] S.D. Kaur, Gurdeep Singh, Gurpreet Singh, K. Singhal, S. Kant, N. Bedi, Cubosomes as potential nanocarrier for drug delivery: a comprehensive review, *J. Pharm. Res. Int.* (2021) 118–135, <https://doi.org/10.9734/jpri/2021/v33i31b31698>.
- [95] T.T.L. Nguyen, V.A. Duong, Advancements in nanocarrier systems for nose-to-brain drug delivery, *Pharmaceutics* 18 (2025) 1–27, <https://doi.org/10.3390/ph18050615>.
- [96] D. Mohanty, O.A. Alsaïdan, A. Zafar, T. Doodle, J.K. Gupta, M. Yasir, A. Mohanty, M. Khalid, Development of atomoxetine-loaded NLC in situ gel for nose-to-brain delivery: optimization, in vitro, and preclinical evaluation, *Pharmaceutics* 15 (2023) 1985, <https://doi.org/10.3390/pharmaceutics15071985>.
- [97] M.L. Formica, D.A. Real, M.L. Picchio, E. Catlin, R.F. Donnelly, A.J. Paredes, On a highway to the brain: a review on nose-to-brain drug delivery using nanoparticles, *Appl. Mater. Today* 29 (2022) 101631, <https://doi.org/10.1016/J.APMT.2022.101631>.
- [98] P.B. Khedekar, Cubosomes: a vehicle for delivery of various therapeutic agents, *MOJ Toxicol.* 4 (2018) 19–21, <https://doi.org/10.15406/mojt.2018.04.00083>.
- [99] N. Attri, S. Das, J. Banerjee, S.H. Shamsuddin, S.K. Dash, A. Pramanik, Liposomes to cubosomes: the evolution of lipidic nanocarriers and their cutting-edge biomedical applications, *ACS Appl. Bio Mater.* 7 (2024) 2677–2694, <https://doi.org/10.1021/ACSAABM.4C00153>.
- [100] C.P. Costa, J.N. Moreira, J.M. Sousa Lobo, A.C. Silva, Intranasal delivery of nanostructured lipid carriers, solid lipid nanoparticles and nanoemulsions: a current overview of in vivo studies, *Acta Pharm. Sin. B* 11 (2021) 925–940, <https://doi.org/10.1016/J.APSB.2021.02.012>.
- [101] A.R. Al Jayoush, H.A.F.M. Hassan, H. Asiri, M. Jafar, R. Saeed, R. Harati, M. Haider, Niosomes for nose-to-brain delivery: a non-invasive versatile carrier system for drug delivery in neurodegenerative diseases, *J. Drug Deliv. Sci. Technol.* 89 (2023) 105007, <https://doi.org/10.1016/J.JDDST.2023.105007>.
- [102] M. Thakkar, S. Brijesh, Opportunities and challenges for niosomes as drug delivery systems, *Curr. Drug Deliv.* 13 (2016) 1275–1289, <https://doi.org/10.2174/1567201813666160328113522>.
- [103] M. Yasir, U.V.S. Sara, Solid lipid nanoparticles for nose to brain delivery of haloperidol: in vitro drug release and pharmacokinetics evaluation, *Acta Pharm. Sin. B* 4 (2014) 454–463, <https://doi.org/10.1016/J.APSB.2014.10.005>.
- [104] P. Ghasemiyeh, S. Mohammadi-Samani, Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages, *Res. Pharm. Sci.* 13 (2018) 288–303, <https://doi.org/10.4103/1735-5362.235156>.
- [105] A.H. Bhatt, H.P. Patel, P.R. Patel, H.G. Rathod, J.H. Hirawala, P.S. Shaikh, F. A. Maulvi, Cubosomes in non-oral drug delivery: advancing precision therapeutics from bench to bedside, *Int. J. Pharm.* 684 (2025) 126108, <https://doi.org/10.1016/J.IJPHARM.2025.126108>.
- [106] Y. Zhu, C. Liu, Z. Pang, Dendrimer-based drug delivery systems for brain targeting, *Biomolecules* 9 (2019) 790, <https://doi.org/10.3390/Biom9120790>.
- [107] X. Li, A. Naeem, S. Xiao, L. Hu, J. Zhang, Q. Zheng, Safety challenges and application strategies for the use of dendrimers in medicine, *Pharmaceutics* 14 (2022) 1292, <https://doi.org/10.3390/PHARMACEUTICS14061292>.
- [108] C. Chang, T.G. Meikle, C.J. Drummond, Y. Yang, C.E. Conn, Comparison of cubosomes and liposomes for the encapsulation and delivery of curcumin, *Soft Matter* 17 (2021) 3306–3313, <https://doi.org/10.1039/D0SM01655A>.
- [109] X. Cai, A. Refaat, P.Y. Gan, B. Fan, H. Yu, S.H. Thang, C.J. Drummond, N. H. Voelcker, N. Tran, J. Zhai, Angiopep-2-Functionalized lipid cubosomes for blood-brain barrier crossing and glioblastoma treatment, *ACS Appl. Mater. Interfaces* 16 (2024) 12161–12174, <https://doi.org/10.1021/ACSAMI.3C14709>.
- [110] A. Pramanik, Z. Xu, N. Ingram, P.L. Coletta, P.A. Millner, A.I.I. Tyler, T.A. Hughes, Hyaluronic-acid-tagged cubosomes deliver cytotoxics specifically to CD44-positive cancer cells, *Mol. Pharm.* 19 (2022) 4601–4611, <https://doi.org/10.1021/ACS.MOLPHARMACEUT.2C00439>.
- [111] S.M. Ghorai, A. Deep, D. Magoo, C. Gupta, N. Gupta, Cell-penetrating and targeted peptides delivery systems as potential pharmaceutical carriers for enhanced delivery across the blood–brain barrier (BBB), *Pharmaceutics* 15 (2023) 1999, <https://doi.org/10.3390/PHARMACEUTICS15071999>.
- [112] K.K. Siddappaji, S. Gopal, Molecular mechanisms in Alzheimer's disease and the impact of physical exercise with advancements in therapeutic approaches, *AIMS Neurosci* 8 (2021) 357–389, <https://doi.org/10.3934/Neuroscience.2021020>.

- [113] M. Rakotoarisoa, A. Angelova, Amphiphilic nanocarrier systems for curcumin delivery in neurodegenerative disorders, *Medicines* 5 (2018) 126, <https://doi.org/10.3390/medicines5040126>.
- [114] Y.S.R. Elnaggar, S.M. Etman, D.A. Abdelmonsif, O.Y. Abdallah, Novel piperine-loaded Tween-integrated monoolein cubosomes as brain-targeted oral nanomedicine in Alzheimer's disease: pharmaceutical, biological, and toxicological studies, *Int. J. Nanomed.* 10 (2015) 5459–5473, <https://doi.org/10.2147/IJN.S87336>.
- [115] Z. Lan, X. Li, X. Zhang, Glioblastoma: an update in pathology, molecular mechanisms and biomarkers, *Int. J. Mol. Sci.* 25 (2024) 3040, <https://doi.org/10.3390/ijms25053040>.
- [116] X. Chen, X. Zhong, F. Zhang, X. Zhou, X. Yue, X. Li, Molecular mechanisms and therapeutic targets in glioblastoma multiforme: network and single-cell analyses, *Sci. Rep.* 15 (2025) 1–16, <https://doi.org/10.1038/s41598-025-92867-z>.
- [117] D.K. Flak, V. Adamski, G. Nowaczny, K. Szutkowski, M. Synowitz, S. Jurga, J. Held-Feindt, At101-loaded cubosomes as an alternative for improved glioblastoma therapy, *Int. J. Nanomed.* 15 (2020) 7415–7431, <https://doi.org/10.2147/IJN.S265061>.
- [118] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, *Nat. Rev. Drug Discov.* 3 (2004) 205–214, <https://doi.org/10.1038/nrd1330>.
- [119] A. Singh, R. Kukreti, L. Saso, S. Kukreti, Oxidative stress: a key modulator in neurodegenerative diseases, *Molecules* 24 (2019) 1–20.
- [120] M. Rakotoarisoa, B. Angelov, V.M. Garamus, A. Angelova, Curcumin- and fish oil-loaded spongosome and cubosome nanoparticles with neuroprotective potential against H₂O₂-Induced oxidative stress in differentiated human SH-SY5Y cells, *ACS Omega* 4 (2019) 3061–3073, <https://doi.org/10.1021/acsomega.8b03101>.
- [121] E.C. Gross, M. Lisicki, D. Fischer, P.S. Sándor, J. Schoenen, The metabolic face of migraine — from pathophysiology to treatment, *Nat. Rev. Neurol.* 15 (2019) 627–643, <https://doi.org/10.1038/s41582-019-0255-4>.
- [122] J. Khan, L.I. Al Asoom, A. Al Sunni, N. Rafique, R. Latif, S. Al Saif, N.B. Almandil, D. Almohazey, S. AbdulAzeez, J.F. Borgio, Genetics, pathophysiology, diagnosis, treatment, management, and prevention of migraine, *Biomed. Pharmacother.* 139 (2021) 111557, <https://doi.org/10.1016/j.biopha.2021.111557>.
- [123] Z. Farsi, M. Sheng, Molecular mechanisms of schizophrenia: insights from human genetics, *Curr. Opin. Neurobiol.* 81 (2023) 102731, <https://doi.org/10.1016/j.conb.2023.102731>.
- [124] T. Nakamura, A. Takata, The molecular pathology of schizophrenia: an overview of existing knowledge and new directions for future research, *Mol. Psychiatr.* 28 (2023) 1868–1889, <https://doi.org/10.1038/s41380-023-02005-2>.
- [125] L. Deruyver, C. Rigaut, A. Gomez-Perez, P. Lambert, B. Haut, J. Goole, In vitro evaluation of paliperidone palmitate loaded cubosomes effective for nasal-to-brain delivery, *Int. J. Nanomed.* 18 (2023) 1085–1106, <https://doi.org/10.2147/IJN.S397650>.
- [126] A. Tekade, M. Ratnaparkhi, A. Shewale, R. Shinde, Design and assessment of in-situ nasal gel incorporated with nanostructured cubosomes for the targeted therapy of schizophrenia, *J. Res. Pharm.* 29 (2025) 947–958.
- [127] K.T. Sumadewi, S. Harkitasari, D.C. Tjandra, Biomolecular mechanisms of epileptic seizures and epilepsy: a review, *Acta Epileptol.* 5 (2023) 28, <https://doi.org/10.1186/s42494-023-00137-0>.
- [128] L. Zhang, J. Li, D. Tian, L. Sun, X. Wang, M. Tian, Theranostic combinatorial drug-loaded coated cubosomes for enhanced targeting and efficacy against cancer cells, *Cell Death Dis.* 11 (2020) 1–20, <https://doi.org/10.1038/s41419-019-2182-0>.
- [129] Y. Ding, S.H. Chow, J. Chen, A.P.L. Brun, C.M. Wu, A.P. Duff, Y. Wang, J. Song, J. H. Wang, V.H.Y. Wong, D. Zhao, T. Nishimura, T.H. Lee, C.E. Conn, H.Y. Hsu, B. V. Bui, G.S. Liu, H.H. Shen, Targeted delivery of LM22A-4 by cubosomes protects retinal ganglion cells in an experimental glaucoma model, *Acta Biomater.* 126 (2021) 433–444, <https://doi.org/10.1016/j.ACTBIO.2021.03.043>.
- [130] A.P. Pandit, V.P. Bhanushali, S.A. Patel, U.Y. Kandeekar, V.D. Kakad, T. C. Deshpande, Topical felbinac loaded cubosomal hydrogel for promising treatment of inflammatory pain, *J. Dispersion Sci. Technol.* (2024) 1–11, <https://doi.org/10.1080/01932691.2024.2440423>.
- [131] Z. Chen, Q. Huang, Y. Song, X. Feng, L. Zeng, Z. Liu, X. Hu, C. Tao, L. Wang, Y. Qi, H. Song, Cubosomes-assisted transdermal delivery of doxorubicin and indocyanine green for chemo-photothermal combination therapy of melanoma, *Biomed. Pharmacother.* 166 (2023) 115316, <https://doi.org/10.1016/j.biopha.2023.115316>.
- [132] A.S.A. Ahmed, S. Jeganath, S. Kanna, Cubosomes in transdermal drug delivery: advances, challenges, and future perspectives, *J. Neonatal Surg.* 14 (2025) 216–222.
- [133] B.J. Boyd, Characterisation of drug release from cubosomes using the pressure ultrafiltration method, *Int. J. Pharm.* 260 (2003) 239–247, [https://doi.org/10.1016/S0378-5173\(03\)00262-X](https://doi.org/10.1016/S0378-5173(03)00262-X).
- [134] P.T. Spicer, K.L. Hayden, M.L. Lynch, A. Ofori-Boateng, J.L. Burns, Novel process for producing cubic liquid crystalline nanoparticles (cubosomes), *Langmuir* 17 (2001) 5748–5756, <https://doi.org/10.1021/la010161w>.
- [135] J.S.L. Leu, J.J.X. Teoh, A.L.Q. Ling, J. Chong, Y.S. Loo, I.D. Mat Azmi, N.I. Zahid, R.J.C. Bose, T. Madheswaran, Recent advances in the development of liquid crystalline nanoparticles as drug delivery systems, *Pharmaceutics* 15 (2023) 1421, <https://doi.org/10.3390/pharmaceutics15051421>.
- [136] D. Kumari, V. Karmakar, S.P. Sisinthy, M. Pandey, N. Jain, B. Gorain, Nanoemulsion and nanoemulgel-based carriers as advanced delivery tools for the treatment of oral diseases, *Drug Deliv. Transl. Res.* 15 (2025) 1139–1155, <https://doi.org/10.1007/s13346-024-01735-2>.
- [137] A. Mandal, D. Pal, V. Agrahari, H.M. Trinh, M. Joseph, A.K. Mitra, Ocular delivery of proteins and peptides: challenges and novel formulation approaches, *Adv. Drug Deliv. Rev.* 126 (2018) 67–95, <https://doi.org/10.1016/j.addr.2018.01.008>.
- [138] S. Jeayeng, J. Kwanthongdee, R. Jitreeprasert, K. Rungnanchai, K. Naksavadi, R. Rirkkrai, V. Wongcharoenthavorn, W. Mahikul, A. Chatsirisupachai, Natural products as promising therapeutics for fine particulate matter-induced skin damage: a review of pre-clinical studies on skin inflammation and barrier dysfunction, *PeerJ* 13 (2025) e19316, <https://doi.org/10.7717/peerj.19316>.
- [139] H. Jung, M.E. King, M.L. Personick, Strategic synergy: advances in the shape control of bimetallic nanoparticles with dilute alloyed surfaces, *Curr. Opin. Colloid Interface Sci.* 40 (2019) 104–117, <https://doi.org/10.1016/j.cocis.2019.02.004>.
- [140] M.A. Batel, C.M. Barcelo Chong, C. Gatumel, H. Berthiaux, A study on the impact of shifted feeding of a segregating binary mixture in continuous powder mixing, *Powder Technol.* 452 (2025) 120564, <https://doi.org/10.1016/j.powtec.2024.120564>.
- [141] J.Y.T. Chong, X. Mulet, B.J. Boyd, C.J. Drummond, Steric stabilizers for cubic phase lyotropic liquid crystal nanodispersions (cubosomes). *Advances in Planar Lipid Bilayers and Liposomes*, first ed., Elsevier Inc, 2015 <https://doi.org/10.1016/bs.adplan.2014.11.001>.
- [142] B. Vigani, S. Rossi, G. Sandri, M.C. Bonferoni, C.M. Caramella, F. Ferrari, Recent advances in the development of in situ gelling drug delivery systems for non-parenteral administration routes, *Pharmaceutics* 12 (2020) 1–29, <https://doi.org/10.3390/pharmaceutics12090859>.
- [143] M. Nagao, A.H. Ranneh, Y. Iwao, K. Yamamoto, Y. Ikeda, Preparation of cubosomes with improved colloidal and structural stability using a gemini surfactant, *Mol. Pharm.* 20 (2023) 5066–5077, <https://doi.org/10.1021/acs.molpharmaceut.3c00378>.
- [144] S. Ding, A.I. Khan, X. Cai, Y. Song, Z. Lyu, D. Du, P. Dutta, Y. Lin, Overcoming blood–brain barrier transport: advances in nanoparticle-based drug delivery strategies, *Mater. Today* 37 (2020) 112–125, <https://doi.org/10.1016/j.mattod.2020.02.001>.