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REVIEW

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Advances in mucoadhesive and mucus-penetrating materials, nano-formulations, and *in situ* gelling systems for nasal drug delivery

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ABSTRACT

Introduction: Intranasal drug delivery is increasingly valued not only for local therapy but also as a noninvasive route that can bypass the blood – brain barrier, enabling rapid treatment of neurological and systemic diseases. However, mucociliary clearance and limited epithelial absorption often reduce residence time and bioavailability, creating a need for more effective formulation strategies. Mucoadhesive and mucus-penetrating systems are among the most promising approaches.

Areas covered: This review summarizes nasal anatomical and physiological features that govern interactions between formulations and the mucosa. It overviews representative intranasal dosage forms (liquids, powders, gels, films, *in situ* gelling systems, and nano-formulations). Polymers used as mucoadhesive agents are classified into first- and second-generation materials, which enhance adhesion through hydrogen bonding, electrostatic interactions, or covalent attachment. The review also highlights polymers applied to nanoparticle surfaces to facilitate diffusion through mucus and improve epithelial access. Finally, methods to evaluate mucoadhesion and toxicity are outlined, including alternative *in vitro* and *in vivo* models.

Expert opinion: Recent advances have expanded nasal delivery options, particularly for nose-to-brain targeting. Yet translation remains limited by insufficient validation, long-term safety uncertainties, and repeated-dose effects. Future progress requires balancing adhesion with penetration, robust toxicology, and integration of innovative polymers with optimized devices.

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Mucus; mucoadhesion; mucus penetration; intranasal delivery; nose-to-brain delivery; brain targeting

1. Introduction

In recent years, local drug delivery approaches have gained increased attention, not only for treating diseases at the site of administration but also as alternative routes for systemic drug delivery and targeted therapy to specific organs [1,2]. Among these, the intranasal route has attracted considerable attention [3–5]. In the literature, the terms 'nasal drug delivery' and 'intranasal drug delivery' are used interchangeably to describe administration into the nasal cavity.

Traditionally, intranasal administration has been widely used for the treatment of various types of rhinitis, including infectious and allergic forms [6–8]. In such cases, it facilitates the effective delivery of vasoconstrictors, corticosteroids, and antihistamines [9,10]. However, the advantages of this route extend far beyond local applications. Notably, the intranasal pathway provides a unique opportunity to deliver drugs directly from the nasal cavity to the brain, offering a means to bypass the blood-brain barrier (BBB) [11–19]. This capability opens promising avenues for the development of treatments targeting neurodegenerative diseases, which are often limited by the difficulty of delivering therapeutics to the central nervous system (CNS) [20–24]. Additionally, it reduces the likelihood of systemic side effects and circumvents hepatic first pass metabolism which contributes to relatively high bioavailability [12,25,26]. The intranasal route has been shown to produce a rapid onset of therapeutic action, particularly in brain targeted delivery, with effects observable within the first five minutes post administration [27–29]. This rapid CNS access is also exploited illicitly, as many individuals who misuse psychoactive substances favor intranasal administration for its immediate effects. In addition to its pharmacokinetic benefits, intranasal administration offers several practical advantages [30–32]. Moreover, being noninvasive and easy to use, the intranasal route provides an effective alternative, particularly when intramuscular or intravenous administration is contraindicated or not technically feasible [27,33,34]. Additionally, this route of administration is also favorable for pediatric patients, as it avoids difficulties associated with swallowing oral dosage forms and reduces exposure to unpleasant tastes [35,36].

These attributes not only enhance therapeutic efficiency but also contribute to improved patient adherence, as an essential factor in the success of long-term treatments [37,38]. By simplifying the treatment process and improving tolerability, this route of delivery may also contribute to a reduction in overall healthcare expenditures.

Nevertheless, challenges such as the rapid clearance of drug substances from the nasal mucosal surface and the limited absorption area require further optimization of

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Article highlights

- The nasal route is a promising, patient-friendly option for systemic and nose-to-brain delivery, but outcomes are highly deposition-dependent.
- Rapid progress in mucoadhesive and mucus-penetrating materials, nanoformulations, and *in situ* gelling systems is expanding the intranasal delivery toolbox.
- Powders and *in situ* gels are practical, scalable approaches to extend nasal residence and enable sustained release, but translation requires consistent *in vivo* performance and repeated-dose tolerability.
- Covalently interacting mucoadhesives can markedly improve retention, but intranasal use needs robust *in vivo* efficacy and repeated-dose safety data, given potential sensitization risks.
- Mucus-penetrating nanoparticles may be most transformative for nose-to-brain delivery by directly addressing the mucus barrier.

delivery systems [39–42]. Additionally, several limitations exist, including limited dosing volume, anatomical and physiological variability among patients, and the potential for affecting olfactory function [41,43,44].

Thus, a comprehensive understanding of the physiological processes occurring within the nasal cavity has provided a foundation for the development of dosage forms with enhanced mucoadhesive and mucus-penetrating properties, aimed at overcoming these limitations. Specifically, the development of mucoadhesive drug delivery systems is based on establishing physicochemical interactions with the mucus layer or the nasal mucosa. Conversely, mucus-penetrating systems rely on the incorporation of permeability enhancers or permeability enhancing-polymers that facilitate the efficient transport of molecules across the mucosal barrier. Both mechanisms can potentially increase drug residence time or transport at mucosal surfaces, resulting in greater bioavailability, a faster onset of action, reduced required doses, and the possibility of delivering larger biomolecules. In this context, the anatomical and physiological characteristics of the nasal cavity relevant to intranasal drug administration and these two approaches used to improve mucosal drug delivery will be discussed in more detail below.

2. Anatomy and physiology of the nasal cavity

The advantages of the intranasal route of administration are largely attributed to the unique anatomical and physiological features of the nose [45,46]. This multifunctional sensory organ consists of an external portion, which is covered by skin and exhibits a triangular pyramidal configuration, and an internal nasal cavity with an estimated surface area of approximately 150–160 cm^2 [39,47]. The nasal cavity itself is divided by the nasal septum, forming the paired nasal fossae, and can extend up to 14 cm in length, running from the external nares (nostrils) to the nasopharynx [48–50]. Additionally, it is conventionally divided into distinct regions: the vestibule, atrium, the respiratory region and the olfactory region [42]. The epithelial lining of the nasal cavity includes respiratory, squamous, olfactory, and transitional cell types [51].

One of the first regions of the nasal cavity to come into contact with the external environment is the vestibule. It provides resistance to adverse environmental conditions, primarily due to its lining of keratin-coated stratified squamous epithelial layer containing nasal hairs (vibrissae), along with sebaceous and sweat glands, that enable effective filtration of airborne particles [52]. Following this, the airflow passes into a narrow and confined region called the atrium. Beyond the atrium lies the respiratory region of the nasal cavity, distinguished by an extensive surface area and the presence of the inferior, middle, and superior nasal conchae [53–55]. These structures collectively promote complex airflow patterns, thereby enhancing the interaction between inhaled air and the mucosal lining of the cavity [56,57]. This richly vascularized area is lined with ciliated pseudostratified columnar epithelium, supported by the respiratory epithelial layer, the underlying basal lamina, and the propria. Numerous mucous glands within this region aid in the humidification and filtration of inhaled air [51,58].

Adjacent to the respiratory region lies the olfactory area, which is a relatively small region, consists of the mucosal lining, olfactory cilia, olfactory epithelial cells, submucosal layer and olfactory gland. The olfactory epithelium is lined with pseudostratified columnar epithelium composed of various cell types, including basal cells, olfactory cells, sustentacular cells, trigeminal cells, and olfactory neurons [59]. This area is considered as one of the primary entry points to the CNS due to the presence of an extensive network of olfactory neurons, lymphatic vessels, and capillaries [49,60].

Thus, the anatomical and physiological features of the nasal cavity enable efficient drug absorption, particularly in the respiratory region. Meanwhile, the olfactory region provides a potential route for direct delivery to the CNS (Figure 1). In both areas, the nasal mucosa plays a critical role in drug absorption. Approximately 90% of the nasal cavity is covered by nasal mucosa, which is composed of respiratory epithelium supported by the basal lamina and the lamina propria, which plays a key role in protecting the respiratory tract from pathogens and irritants. In addition to its defensive function, the mucosa provides epithelial lubrication, facilitates the interaction of gases and nutrients, and prevents dehydration of the surface by moisturizing it [51].

Furthermore, the nasal mucosa is actively involved in the primary self-clearing mechanism of the respiratory system, known as mucociliary clearance. Owing to these properties, the mucosal surface plays a major role in intranasal drug delivery. In this regard, detailed studies of the mucosa provide opportunities to overcome challenges and have enabled a better understanding of strategies for improving the intranasal drug delivery pathway.

Notably, the nasal mucosa comprises an epithelial layer overlaid by a mucus, which functions as a viscoelastic biological gel approximately 5 µm thick [61]. This viscoelastic cover on the epithelium layer comprises a highly entangled polymeric network that functions as a selective mesh-like barrier for different molecules [62]. These topological entanglements are formed by a combination of covalent and non-covalent interactions, facilitated by hydrophobic effects, hydrogen bonding, and electrostatic attractions.

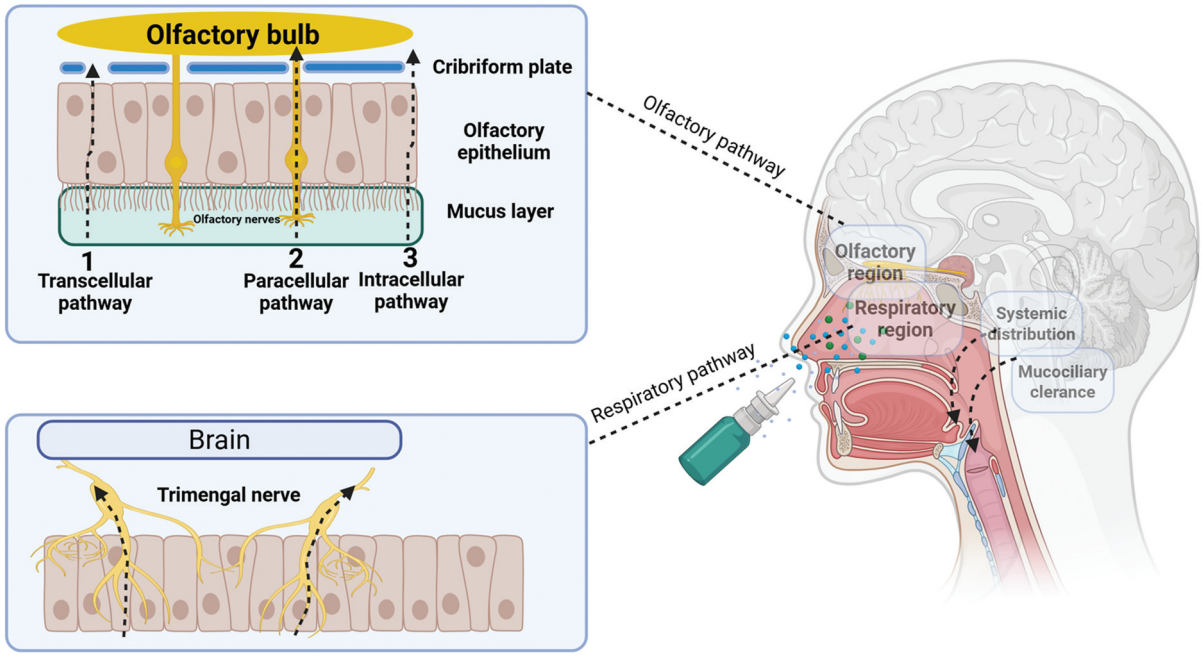

Figure 1. Schematic illustration of drug delivery to the brain via the nasal route of administration. Created in BioRender. Lopez Vidal, L. (2026) https://BioRender.com/r32n2qc.

The mucus is composed predominantly of water (approximately 98%) and primarily consists of glycosylated proteins called mucins, along with electrolytes, lipid molecules, cellular debris, and other proteins [61,63]. These mucins are glycoproteins with carbohydrate chains linked to a protein core via O-glycosidic bonds, rich in sugars and polymerized through disulfide bridges, which influence the rheological properties of mucus. Mucin forms a three-dimensional network in aqueous solution. The presence of negatively charged polysaccharide side chains contributes to the stabilization of this structure through electrostatic repulsion, complementing the existing hydrophobic interactions within the system. This matrix is sensitive to environmental conditions, particularly ionic strength and changes in pH. In this regard, mucin exhibits different properties depending on the environmental conditions [64]. Consequently, molecular dysregulation of the components may lead to alterations in the physical properties of mucus, contributing to the development of various pathological conditions [65]. Additionally, the rheological properties of mucus have a significant impact on the efficiency of mucociliary clearance [66]. At the initial stage, mucus must maintain a gel-like state; however, a transition to more viscous properties markedly impedes its transport and removal. This is particularly critical in the context of mucociliary clearance, a mechanism by which the mucus layer is propelled toward the nasopharynx through the coordinated beating of cilia extending from the apical surface of epithelial cells (Figure 2).

Owing to this flow, the elimination half-life of administered drugs is approximately 21 minutes, which limits their absorption [67]. Another factor affecting drug absorption is the restricted permeability of molecules, as well as properties such as surface charge and hydrophobicity [67]. Notably, the preferred transport routes are largely determined by the balance between lipophilicity and hydrophilicity. Specifically, hydrophilic drugs are primarily absorbed via the paracellular pathway, which involves the movement of compounds through the gaps between cells. In contrast, lipophilic drugs are more efficiently transported through the transcellular pathway, whereby drugs pass directly through the cells

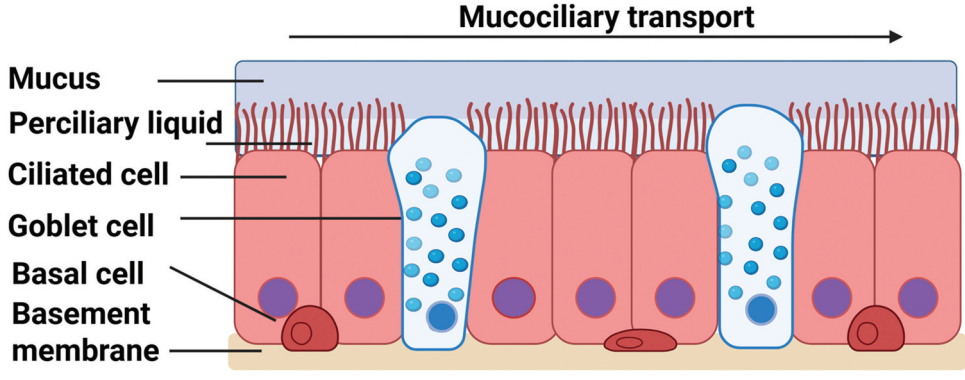

Figure 2. Schematic illustration of mucociliary transport. Created in BioRender. Lopez Vidal, L. (2026) https://BioRender.com/eehb0bv.

[53]. In this context, molecular size is also closely related: molecules with a molecular weight above 1 kDa exhibit poor absorption, whereas molecules around 300 Da are absorbed rapidly [59]. Regarding the delivery of drugs directly to the CNS, the transport mechanisms involved in the direct delivery of substances from the nasal cavity to the brain comprise several possible pathways [68]. In particular, these include transport via branches of the trigeminal nerve, which traverse the pons and the cribriform plate and reach specific regions of the brain. Another potential route involves the olfactory nerve, utilizing both transcellular and paracellular pathways [69]. The transcellular pathway entails drug transport through cells via receptors, transporters, or vesicular mechanisms, whereas the paracellular pathway involves passage through tight junctions or the intercellular spaces between supporting cells and neurons [70].

Thus, given these constraints in intranasal delivery and the potential to deliver drugs directly to the CNS, approaches that prolong mucosal contact, particularly mucoadhesive dosage forms, have gained significant attention.

3. Mucoadhesive dosage forms

Considerable interest in the mucoadhesion approach has emerged due to its potential to optimize localized drug delivery by maintaining therapeutic materials directly on the mucosal surface [71]. First introduced around 1947, the concept highlighted the application of dental adhesive with gum tragacanth for oral penicillin delivery, and later, in 1983, the use of viscous gels and mucoadhesive tablets for application to the oral mucosa was proposed [72,73]. While the importance of designing materials with strong mucoadhesive properties has long been recognized, the past two decades have witnessed remarkable and accelerated advancements in mucoadhesive materials and drug delivery systems [74]. Mucoadhesion is the ability of materials to bond with the mucosal surface, involving a series of physicochemical processes between the drug delivery system and the human mucosa [75]. Typically, such systems include excipients based on polymers capable of interacting with the mucus layer or the nasal mucosa. Chemical interactions may involve the formation of covalent bonds with mucosal components such as mucin glycoproteins, non-covalent interaction through the hydrogen bonds, as well as electrostatic forces. Meanwhile, physical interactions are primarily based on the interpenetration of polymer chains with mucus or the wetting of the mucosal surface. The mechanism of mucoadhesion is generally divided into two stages: adsorption and consolidation. In the first stage, initial contact is established primarily through wetting of the mucosal surface. The second stage involves the formation of actual adhesive interactions between the material and the mucus. In this regard, the selection of mucoadhesive materials for intranasal drug delivery tends to favor hydrophilic macromolecules capable of forming hydrogen bonds and containing multiple functional groups [76]. Building on these principles, a wide range of mucoadhesive intranasal drug formulations have been developed to date, including solutions, powders, gels, films, and both micro- and nanoscale formulations (Table 1).

Table 1. Examples of dosage forms used for intranasal delivery.

Dosage form	Therapeutic agent	Therapeutic objective	Advantages	Disadvantages	References
Nasal spray	Montelukast sodium, strain of *Lactiplantibacillus plantarum*, probiotics	Local treatment, suitable for systemic and CNS delivery	Easy, convenient self-administration, precise dosing, rapid absorption, widely accepted	Limited volume, can irritate nasal mucosa, requires proper technique	[77–79]
Nasal Powder	Vaccines, dimethyl fumarate	Local treatment, suitable for vaccines and peptides, CNS delivery	No preservatives needed, high stability for heat/moisture-sensitive drug, strong mucosal adhesion	Special devices required, difficult for some patients to use	[80,81]
Nasal Gel	Phenobarbital sodium, methotrexate	Local treatment, CNS delivery	Long residence time, reduced dripping, effective for sustained release, less irritation than sprays	Harder to apply evenly, may feel uncomfortable or thick, limited acceptance in some patients	[82,83]
Nasal Films	Donepezil hydrochloride, anti-inflammatory drug	Suitable for peptides, proteins, CNS delivery	Sustained/controlled release, excellent mucoadhesion, long residence time	Insertion may feel uncomfortable, lower patient acceptance, harder to manufacture	[84,85]
In Situ Gel	Rizatriptan, mirtazapine, sumatriptan, darunavir	Local treatment, systemic and CNS delivery	Easy administration as liquid, forms gel for prolonged release, reduces dripping and improves bioavailability	Increased viscosity may feel uncomfortable, requires specific polymers responsive to pH or temperature	[86–89]
Microparticles/ Microspheres	Sumatriptan succinate, diltiazem hydrochloride, budesonide	Local treatment, suitable for peptides/ proteins	Prolonged residence time, controlled release, improved stability	Risk of nasal irritation, larger particles may reduce comfort, complex manufacturing	[90–92]
Nanoparticles	Carmustine, ropinirole hydrochloride, meloxicam	Suitable for peptides/ proteins, prospect for sensitive drug, effective for CNS targeting	Enhances permeation across nasal mucosa, can bypass BBB,	Potential toxicity concerns, requires careful characterization, complex manufacturing	[93–95]
Nanoemulsions	Meloxicam, pramlintide and insulin, antimicrobial formulations	Effective for lipophilic drugs, CNS delivery	Enhances brain delivery, stable system	Can cause irritation, requires surfactants	[96–99]

3.1. Mucoadhesive liquid dosage forms

Intranasal administration of pharmaceutical solutions represents one of the most traditional drug delivery approaches. After administration nasal solutions are distributed across the mucosal surface, forming a thin aqueous film that promotes the dissolution of the drug and facilitates its absorption through the nasal epithelium. Substantial experience has been accumulated in the application of such formulations for the delivery of antihistamines, vasoconstrictors, vaccines, corticosteroids, and isotonic saline solutions. Moreover, extensive experience has been gained with intranasal vaccines. For instance, studies by Hashimoto et al. [100] demonstrated that modulating osmolarity, using an adenovirus-based formulation as a model, can significantly enhance immunogenicity.

More recently, attention has shifted toward the use of intranasal solutions containing mucoadhesive pharmaceutical excipients as promising carriers for drug delivery to the CNS [3,39]. Among these, intranasal formulations of synthetic peptide analogs of antidiuretic hormone, such as desmopressin, have been developed as a nasal spray and are currently used in the treatment of diabetes insipidus [101]. However, one of the major limitations of intranasal liquid formulations is their restricted dosing capacity, because only small volumes can be comfortably administered, and their reduced stability compared with other dosage forms, particularly intranasal powders [102]. These limitations can be mitigated by using mucoadhesive polymers to improve drug retention and absorption efficiency and by including appropriate preservatives to enhance formulation stability.

3.2. Mucoadhesive powder formulations

A comprehensive understanding of the physicochemical processes occurring within the nasal mucosa, combined with the optimization of formulation and processing parameters, has facilitated the development of mucoadhesive powders for intranasal administration [103,104]. This strategy includes prolonging the retention time of the formulation in the nasal cavity by enhancing the viscosity of the mucus through water uptake by dry powder particles upon deposition on the mucosal surface. To support effective self-administration, various pharmaceutical devices have been developed to deliver powders precisely and reproducibly into the nasal cavity (Figure 3).

In the study reported by Fransen et al. [105], mucoadhesive powder formulations were developed using sodium starch glycolate and partially pregelatinized maize starch. These formulations were evaluated in the presence of a hydrophobic additive. The results indicated that the swelling behavior of sodium starch glycolate was delayed when the hydrophobic component was incorporated, whereas carriers based on partially pregelatinized starch showed no significant change in response to the hydrophobic additive. Mucoadhesive formulations for nasal powders were investigated by Trenkel and Scherließ [106]. It was demonstrated that materials with long polymer chains and a negative charge contribute to prolonged residence time within the nasal cavity. Specifically, rheological analyses revealed that carboxymethylcellulose and pectin form highly viscous coatings on particles, which remain stable for up to 15 minutes. Furthermore, adhesion studies conducted on agar-mucin gels indicated an enhancement of mucoadhesive properties for hydroxypropyl methylcellulose and pectin. Other studies have demonstrated the feasibility of developing intranasal vaccines in a powder form using carboxymethylcellulose, sodium alginate, chitosan, and gelatin as mucoadhesive agents [80]. The antiparkinsonian drug levodopa has been investigated in combination with mucoadhesive polymers formulated as nasal powders. A chitosan-cysteine conjugate was synthesized and employed as a mucoadhesive polymer to enhance nasal retention and drug absorption [102]. The formulation containing a higher concentration of the chitosan-cysteine conjugate demonstrated slower drug release after 3 minutes, with a release of $47.5 \pm 12.4\%$, compared with $97.5 \pm 11.5\%$ in the control formulation containing only levodopa. Formulations containing ten-fold and two-fold lower amounts of the conjugate exhibited intermediate release values of $76.2 \pm 11.5\%$ and $92.7 \pm 7.4\%$, respectively.

3.3. Mucoadhesive films

Mucoadhesive films represent one of the notable dosage forms for nasal drug delivery. When administered into the nasal cavity, these formulations adhere to the mucosal surface through non-covalent interactions between the polymers and mucin glycoproteins. This results in the formation of a soft, elastic layer on the mucosa, which – depending on the formulation – can provide therapeutic, protective, or moisturizing effects [107]. Fast dissolving intranasal films incorporating hydroxypropyl methylcellulose and polyvinyl alcohol have been developed as a novel platform for insulin delivery [108]. Drug release occurred over 30 minutes, during which a threefold increase in glycerol content reduced the extent of release, likely due to increased viscosity of the formulation, whereas initial and twofold increases produced no significant differences. However, despite some reports in the literature on the use of mucoadhesive films for nasal drug delivery, this type of dosage form is not particularly convenient for administration.

The small size of films makes their insertion into the nostrils challenging, especially for self-administration, and difficulties in achieving proper placement may compromise reproducibility of dosing and patient compliance. In addition, films can be prone to folding, sticking to the applicator or fingers, and causing discomfort during insertion, which further limits their practical utility compared with sprays, drops, or powder-based systems.

3.4. Mucoadhesive gels

Compared with films, mucoadhesive gels are generally more convenient for nasal administration. Their semi-solid nature allows easy application into the nostrils without the handling difficulties associated with thin films, such as folding or misplacement. Once applied, gels spread over the mucosal surface, ensuring intimate contact and prolonged residence time. They are dispersed systems typically composed of both low- and high-molecular-weight components. Owing to their network structure and tailored composition, gels can generate a soft, elastic, and mucoadhesive layer on the mucosal surface,

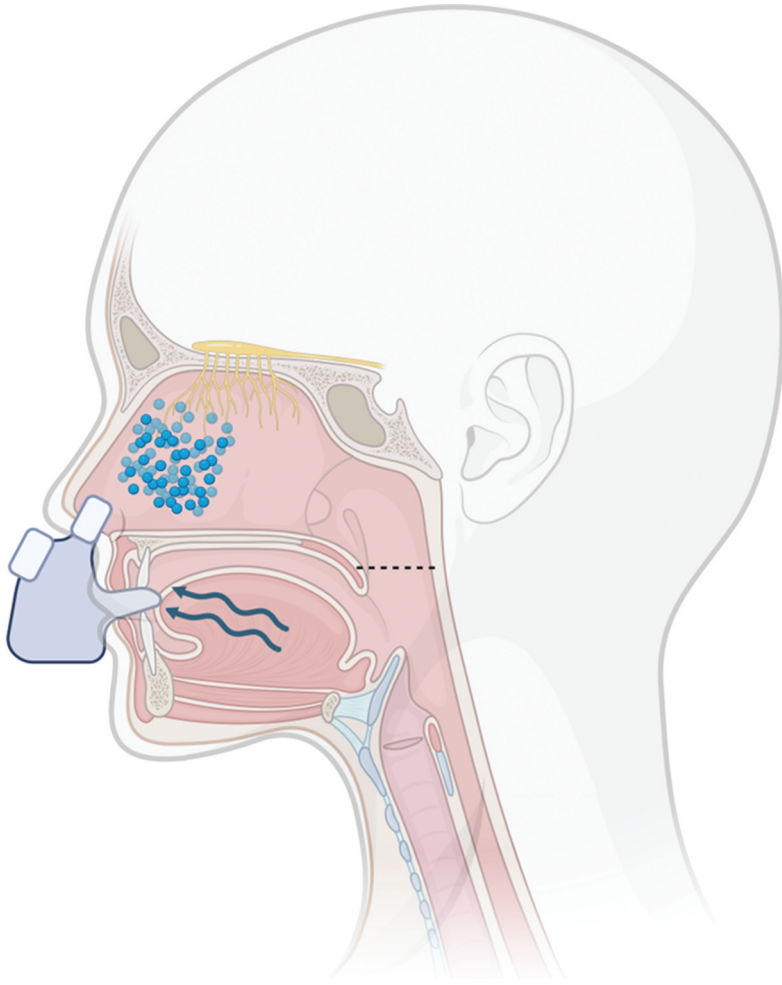

Figure 3. Illustration of a nasal device intended for self-administration of powder formulations. The device delivers a powder plume into the nasal cavity, where particles deposit primarily on the respiratory and olfactory mucosa. The illustration also indicates the typical airflow direction and the anatomical regions relevant for intranasal drug delivery. Created in BioRender. Lopez Vidal, L. (2026) https://BioRender.com/8hgt7q6.

thereby enhancing drug residence time and local effects, much like films. Chettupalli et al. [109] developed intranasal liposomal gels loaded with zolmitriptan for migraine therapy. Gelation was achieved using hydrophilic polymers such as Carbopol 934, Poloxamer 407, and hydroxypropyl methylcellulose K100, resulting in sustained drug release for up to 36 hours.

Recently, Tahir et al. [110] designed nano-transfersomes to enhance drug penetration and subsequently incorporated these vesicular carriers into a chitosan-based gel. The nano-transferosomal gel exhibited prolonged drug release compared with nano-transfersomes alone.

However, the application of these dosage forms in intranasal drug delivery is constrained by the relatively small surface area of the nasal cavity, which limits the total amount of drug that can be effectively absorbed. This poses a particular challenge for drugs requiring higher doses or sustained therapeutic levels. To address these limitations, various formulation strategies have been explored, among which *in situ* gel systems are especially promising.

3.5. *Mucoadhesive* in situ *gel systems*

In situ gelling systems are administered in a liquid or semi-liquid state and undergo gelation directly on the mucosal surface in response to stimuli such as temperature, pH, or ion concentration [111]. The resulting gel prolongs residence time at the site of administration, enhancing drug retention and bioavailability. This strategy is particularly advantageous for intranasal drug delivery and has therefore been the focus of numerous recent studies. For example, Tohamey et al. [112] reported that the permeation rate of aripiprazole, a drug used in the treatment of schizophrenia, reached 32.79 ± 1.25 $*10^{-4}$ μm/h from an *in situ* gelling system prepared with chitosan and hydroxypropyl cellulose, compared with only 4.35 ± 0.84 $*10^{-4}$ μm/h for an intranasal drug suspension. In another study [113], mucoadhesive polymers such as Carbopol 974P, sodium alginate, and sodium carboxymethylcellulose were employed to develop an intranasal *in situ* gelling delivery system for almotriptan malate, an

antimigraine drug. This approach not only enhanced the mucoadhesive properties of the formulation but also allowed modulation of the gelation temperature. An *in situ* gelling hydrogel was also developed for the intranasal delivery of an antipsychotic peptide, employing nanoparticles composed of oxidized starch and carboxymethyl chitosan [114]. Interestingly, the most highly crosslinked hydrogels, where the proportion of carboxymethyl chitosan that of the less crosslinked formulations, demonstrated a faster drug release rate. This effect was attributed to their greater swelling capacity and the specific interactions between polymer components. In addition, mucoadhesive *in situ* gel systems have been actively investigated for the intranasal delivery of metoclopramide hydrochloride. Formulations incorporating Carbopol 934P, hydroxypropyl cellulose, polyvinyl alcohol, and chitosan as mucoadhesive agents exhibited sustained drug release, underscoring the versatility of such systems for achieving prolonged therapeutic effects [115].

In our opinion, *in situ* gelling systems represent one of the most promising strategies for intranasal administration. They are convenient to use, as their liquid nature at the time of administration allows uniform spreading across the nasal cavity, while the subsequent gelation ensures prolonged retention. This combination leads to improved drug absorption and enhanced bioavailability.

3.6. Mucoadhesive micro- and nano-formulations

Particulate formulations are becoming an increasingly common approach for intranasal drug delivery. Several microparticulate products are already commercially available, while nano-formulations are emerging as a particularly promising trend in this field.

Nanoscale drug delivery systems offer several advantages, including their small particle size and the ability to tailor physicochemical properties. These features facilitate both passive transport (via simple diffusion) and active mechanisms such as receptor-mediated transport and carrier-based delivery, thereby helping to overcome the BBB [116]. For example, lipid nanoparticles can reach the brain through passive diffusion, amino acid-based carriers via active transport, and other molecules through receptor-mediated pathways [117–120]. As a result, a wide range of micro- and nanoscale formulations has been developed to improve delivery efficiency.

Mucoadhesive nanoemulsions were developed for the intranasal delivery of rotigotine as an antiparkinsonian agent [121]. Particularly, incorporation of a 1% chitosan blend yielded droplets of approximately 130 nm in size. The optimized rotigotine nanoemulsion achieved a drug release of about 89%, whereas the chitosan-containing formulation showed a release of around 70%. These findings were consistent with *ex vivo* studies on excised tissue, which demonstrated penetration rates of approximately 85% for the optimized formulation and 65% for chitosan-containing system.

Another study conducted by Palshetkar et al. [122] involved the development of a nanoemulsion of the antipsychotic drug iloperidone. In this study, the wet-milling method was used to prepare a nanosuspension composed of the drug, Poloxamer 188, Methocel K15M, and gellan gum. The resulting formulation had an average particle size of about 268 nm and showed improved permeation through the nasal mucosa, along with enhanced motor activity in *in vivo* studies following intranasal administration compared with oral suspensions.

The wet milling method was also employed in the study by Kakad et al. [123], which demonstrated the feasibility of preparing nanosuspensions of antiviral drugs for the treatment of neuro-AIDS. Additionally, the authors used high-speed and high-pressure homogenization and compared the properties of the drug nanoemulsions obtained using these three techniques. Ritonavir and lopinavir were selected as the neuro-AIDS drugs. The nanosuspensions of ritonavir and lopinavir produced by high-pressure homogenization exhibited optimal properties, with particle sizes of approximately 125 nm and 83 nm, respectively. The study noted that reducing particle size enhances rapid drug absorption in experiments on mucosal permeation using goats nasal mucosa.

Furthermore, mucoadhesive chitosan microparticles crosslinked with D,L-glyceraldehyde, with an average size of approximately 4.77 µm, were developed for intranasal vaccination. Histological analysis confirmed adhesion to and penetration of the nasal mucosa within 15 minutes [124]. Additionally, an innovative nanoparticle formulation employing the cationic mucoadhesive polymer Eudragit® RL 100 for rivastigmine delivery was proposed by Kalra et al. [125] After 8 hours, nanoparticle penetration through the nasal mucosa was approximately 38% higher than that of the rivastigmine solution, while drug release from the nanoparticles was about 36% greater than the control over the same period. In another study [126], nanostructured mucoadhesive carriers were developed for the treatment of bacterial infections by incorporating 0.2% sodium alginate into niosomes and nanoparticles to enhance mucoadhesion. These systems showed prolonged retention on sheep nasal mucosa, with niosomes and nanoparticles exhibiting residence times of 10.0 ± 0.5 and 12.0 ± 0.2 hours, respectively. In the study reported by Azrak et al. [127], niosomal carriers were explored for the intranasal delivery of carvedilol, used for the treatment of cardiovascular diseases. To enhance mucoadhesive properties, chitosan was incorporated into the formulation. Interestingly, a marked increase in mucoadhesion was observed in the carvedilol-loaded niosomal systems, which the authors tentatively attributed to the positive surface charge of the formulation resulting from the presence of chitosan. Mucoadhesive nanoemulsions were developed for the delivery of the anticancer drug luteolin, with a 0.25% chitosan coating incorporated to enhance their mucoadhesive properties. The resulting nanoemulsions had an average particle size of approximately 68 nm and demonstrated significantly improved nasal mucosal penetration, with over 95% permeation observed after 90 minutes in contrast to less than 10% for the control formulation [128].

The Uchegbu group investigated N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycolchitosan (GCPQ), an amphiphilic polysaccharide derivative capable of self-assembling into nanoparticles, for nose-to-brain delivery of leucine^5-enkephalin hydrochloride and levodopa. The authors highlighted the mucoadhesive properties of GCPQ as a key

factor enhancing drug retention in the nasal cavity and improving bioavailability in the brain [129,130].

Thus, across the different mucoadhesive mechanisms and nasal formulations developed to exploit them, a wide range of polymers has been employed, including natural polysaccharides, semi-synthetic derivatives, and fully synthetic polymers [131]. These materials generally meet one or more essential criteria for effective mucoadhesion, such as hydrophilicity, the presence of ionic charges (positive or negative), high molecular weight, and functional groups capable of forming strong intermolecular hydrogen bonds.

Based on their mechanisms of interaction with the mucosal surface, mucoadhesive polymers are generally classified into two generations [132]. First-generation polymers adhere non-specifically through physical entanglement and weak intermolecular forces such as electrostatic interactions, hydrogen bonding, and van der Waals forces. In contrast, second-generation polymers are chemically modified to introduce functional groups that enable specific binding to mucin glycoproteins, thereby enhancing both the strength and selectivity of adhesion. Both approaches to mucoadhesion are discussed below.

3.6.1. First generation mucoadhesive polymers

This group of polymers is typically categorized into neutral, amphoteric, anionic, and cationic types. The latter two categories generally demonstrate stronger mucoadhesive properties, while neutral polymers tend to have comparatively lower interaction strength with mucin. In this case, mucoadhesion is mainly driven by the diffusion of polymers and components into the mucosal layer, leading to the formation of an interpenetrating network.

The assumption that anionic polymers may be less suitable as mucoadhesive materials due to potential repulsion from the anionic nature of mucin glycoproteins is not justified. These polymers are widely used in the design of intranasal drug delivery systems because they contain carboxyl groups that interact with the oligosaccharide chains of mucin, particularly sialic acid and sulfate residues. Additionally, the increased charge density contributes positively to adhesion [133,134]. One of the highly valued anionic synthetic polymers is poly (acrylic acid) (PAA), synthesized through various methods such as free radical polymerization, atom transfer radical polymerization, plasma polymerization, or reversible addition-fragmentation chain transfer polymerization [135]. Through crosslinking of PAA with different agents, additional commonly used materials in the design of mucoadhesive drug delivery systems named Carbopol have been obtained [136]. In particular, intranasal nanoparticles incorporating PAA as a mucoadhesive agent have been developed to enhance the bioavailability of galantamine hydrobromide for the treatment of Alzheimer's disease [137]. An insulin gel composed of Carbopol 934P and hydroxypropyl methylcellulose K4M was developed for intranasal delivery, demonstrating sustained release *in vitro*. The gel exhibited a cumulative insulin release of 90.38% ± 4.15% after 5 hours, compared with 96.88% ± 4.23% release from simple insulin solution after 4 hours [138]. Another well-known polymer from this group is a naturally derived polysaccharide, sodium alginate, which is obtained from various sources of algae, particularly brown seaweed [139,140]. In the work reported by Hussein et al. [141], sodium alginate-based microparticles were developed for the controlled release of the antiparkinsonian drug ropinirole hydrochloride via intranasal administration. It was found that increasing the sodium alginate content in the microparticles significantly prolonged the drug release time: with a high polymer content, the release lasted approximately 60 minutes, whereas with a lower content, it was around 1 minute. Another anionic polymer of semi-synthetic nature, derived from the chemical modification of plant-based cellulose and known as carboxymethylcellulose (CMC), is widely used in the development of mucoadhesive systems. For example, Ugwoke et al. [142] have investigated the CMC-based formulation for intranasal delivery of apomorphine. The obtained data demonstrated the absence of toxicity upon nasal administration, as well as the potential use of such systems in the treatment of acute diseases; however, further studies are needed for long-term disease management.

In turn, cationic polymers exhibit strong mucoadhesive properties due to electrostatic interactions between their positively charged groups and the negatively charged mucin components of the mucosal surface. This principle formed the basis of the earliest attempts to develop mucoadhesive drug delivery systems. Undoubtedly, one of the most prominent representatives of this group is chitosan, a natural polymer obtained by the deacetylation of chitin, which enables the formation of free amino groups [143,144]. Additionally, it has been proposed that hydrogen bond formation with chitosan also contributes to its mucoadhesive properties [145]. In the context of intranasal drug delivery, a mucoadhesive gel based on chitosan was developed, into which nano-ethosomes containing sumatriptan (used for the treatment of migraine) were incorporated. The resulting gel demonstrated a mucoadhesive strength of 6533 ± 150 dyne/cm^2 and showed sustained drug release from the nano-ethosomal within the gel, in comparison to nano-ethosomal and an oral drug solution [146]. Cationic polymers that possess a strong positive charge and contain amino groups also include polyethyleneimine (PEI), which is obtained through polymerization [147]. The use of polyethyleneimine as a mucoadhesive material has been applied in the development of intranasal vaccine delivery systems. Immunological analysis showed that the developed liposomes with PEI effectively stimulated both local (mucosal) and systemic immune responses [148]. Synthetic cationic polymers, including poly(L-lysine), poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA), and poly(allylamine), are also promising materials for the design of mucoadhesive intranasal drug delivery systems of cationic nature. Notably, all of the aforementioned polymers are being increasingly explored for chemical modification to introduce functional groups that can further enhance their mucoadhesive properties.

A particularly understudied group of polymers is amphoteric polyelectrolytes, which contain both negatively and positively charged groups within the same macromolecule. Naturally occurring examples include proteins, which are polyampholytes by nature. Until recently, these polymers were thought to exhibit poor mucoadhesive properties due to self-neutralization of their charges. However, Fu et al. [149]

demonstrated that the mucoadhesive behavior of polyampholytes is strongly influenced by the solution pH in relation to their isoelectric point. When the environmental pH is below the isoelectric point, the macromolecules acquire a net positive charge, which can result in strong mucoadhesive interactions.

3.6.2. Second generation mucoadhesive polymers enhanced by reactive functional groups

A major breakthrough in the field of mucoadhesion came in 1999, when Bernkop-Schnürch and colleagues introduced thiol-containing polymers, or 'thiomers,' which can form covalent disulfide bonds with cysteine residues on mucosal surfaces under physiological conditions [150,151]. The formation of these covalent bonds accounts for the markedly enhanced mucoadhesive properties of thiolated polymers compared with conventional materials, leading to significantly prolonged residence on the mucosal surface and improved formulation retention. The presence of free thiol groups is therefore essential for these interactions to take place [152]. Among the most widely used polymer conjugates in this field are cysteine derivatives of PAA, PVA, alginate, gelatin, karaya gum, xanthan gum, gellan gum, and chitosan [153]. This approach has yielded several promising conjugates for use in mucoadhesive drug delivery systems [154–156].

Notably, certain conjugates have shown strong potential for intranasal administration of therapeutic agents. For example, Millotti et al. [157] developed thiolated PAA microparticles for the intranasal delivery of exenatide. The thiolated formulation released about 50% of the drug within the first 10 minutes, compared with 85% release from the control, indicating a slower and more controlled release profile. Moreover, permeation studies on nasal mucosal tissue demonstrated significantly enhanced drug penetration for the thiolated formulation, underscoring its promise for intranasal delivery of peptide drugs. Netsomboon et al. [158] developed preactivated thiolated poly(acrylic acid) formulations for the intranasal delivery of apomorphine. The degree of pre-activation was controlled by employing polymers of different molecular weights. This approach increased mucosal penetration by approximately 2.1- to 2.8-fold compared with unmodified apomorphine, depending on polymer molecular weight, and significantly enhanced overall drug permeability.

Thiolated chitosan was used to develop a gel incorporating duloxetine proniosomes. The thiolated gel demonstrated a more sustained drug release profile, with around 54% of the drug released over 8 hours, in contrast to 71% released from the control formulation. In addition, the thiomer-based gel showed a 1.86-fold increase in mucosal permeability on goat mucosal surface compared with the control [159]. In the study of Patel et al. [160], thiolated nanoparticles were developed for the delivery of tizanidine hydrochloride, a drug used to treat muscle spasms, headaches, and back pain. The resulting nanoparticles demonstrated enhanced permeation through the RPMI 2650 cell monolayer, which represents nasal epithelial cells. Specifically, chitosan nanoparticles and thiolated chitosan nanoparticles showed permeation increases of 13- and 29-fold, respectively, compared with the drug solution.

An alternative approach for improving mucoadhesion was proposed by Davidovich-Pinhas and Bianco-Peled [161,162], demonstrating the potential use of acrylate groups to interact with the sulfhydryl moieties of mucin via Michael-type addition reactions, thereby opening new avenues for the development of mucoadhesive drug delivery systems. In particular, the presence of a double bond in the acrylate group enables the formation of covalent linkages with thiol groups of cysteine residues. The introduction of acrylate groups has facilitated the development of mucoadhesive drug delivery systems using polymers such as cellulose derivatives [163,164], chitosan [165] and alginate [161]. Building upon this concept, we developed an acryloylated carrier for intranasal drug delivery, aimed at enhancing mucoadhesive properties and improving drug residence time on the nasal mucosa. To achieve this, we utilized pharmaceutical-grade copolymers manufactured by the German company Evonik Ind. and marketed under the trademark Eudragit®. Specifically, the polycationic Eudragit® EPO was chemically modified using acryloyl chloride. The resulting acryloylated Eudragit® EPO demonstrated no signs of toxicity in a slug mucosal irritation assay. Furthermore, an *ex vivo* study conducted using sheep nasal mucosa revealed improved retention of the acryloylated Eudragit® EPO solution on the nasal mucosa compared with the control solution FITC-dextran and Eudragit® EPO. This enhanced retention is attributed to the ability of acryloyl groups to form covalent bonds with thiols present in mucins, contributing to prolonged residence time and potentially facilitating improved intranasal drug delivery [166].

Beyond the incorporation of acryloyl groups into polymers, functionalization with methacryloyl groups is another promising strategy, as these groups can also form covalent bonds with thiol functionalities present in mucin [167]. The introduction of methacryloyl groups has been used with various polymers, including chitosan to produce microporous matrices, gellan gum for tissue engineering applications, and gelatin to form hydrogels via transdermal photopolymerization [168–170]. Among these, chitosan was chemically modified through a reaction with methacrylic anhydride to enhance its mucoadhesive properties, and its effectiveness on mucosal tissues was demonstrated [171,172]. Building on this approach, methacrylated derivatives of hydroxypropyl methylcellulose (HPMC) and gellan gum were also developed as potential candidates for improving mucoadhesive drug delivery systems [173,174]. For intranasal mucoadhesive drug delivery, methacrylated poly(2-ethyl-2-oxazoline) was developed through partial hydrolysis to obtain poly[(2-ethyl-2-oxazoline)-co-ethyleneimine], followed by subsequent reaction with methacrylic anhydride. Cytotoxicity studies on the HEK293 cell line demonstrated cell viability above 90% in the group treated with the polymers containing methacryloyl groups, indicating valuable biocompatibility. Mucoadhesion testing on nasal mucosa showed statistically significant retention on the mucosal surface comparable to cationic chitosan, thereby confirming the enhanced mucoadhesive properties of the modified polymer [175].

In addition to acryloyl and methacryloyl groups, the introduction of crotonyl groups has recently emerged as a promising strategy to enhance mucoadhesion. Like other functional groups, crotonyl moieties can form covalent bonds with thiol residues in mucin, thereby strengthening adhesion. Vanukuru et al. [176] synthesized crotonylated chitosan via N-acylation with crotonic anhydride, alongside methacrylated chitosan, and evaluated their properties. Both modified polymers demonstrated improved mucoadhesive performance for intranasal drug delivery. Peak detachment force and total work of adhesion were significantly higher for the functionalized chitosans compared with unmodified chitosan, and *ex vivo* studies on freshly excised sheep nasal tissue confirmed prolonged retention of up to 20 minutes. These findings underscore the potential of crotonylated and methacrylated chitosans as effective mucoadhesive carriers for intranasal delivery.

The strategy of introducing adhesive functional groups has also led to another promising approach, pioneered by the Khutoryanskiy group, involving the incorporation of maleimide functionalities for mucoadhesive drug delivery [177,178]. This approach introduces maleimide groups into polymers, enabling covalent bonding with cysteine thiol residues in mucin, similar to earlier thiol-based strategies. The mucoadhesive potential of maleimide groups has been demonstrated in diverse delivery systems, including polyvinylpyrrolidone (PVP) nanogels, polyethylene glycol – modified alginate, and liposomes. These maleimide-functionalized platforms exhibited enhanced adhesion to multiple mucosal surfaces, highlighting their promise for effective drug delivery across different mucosal tissues [179,180]. PEGylated liposomes functionalized with maleimide groups were proposed for intranasal delivery to enhance both permeability and mucoadhesion. The resulting liposomes, approximately 90 nm in size, showed prolonged retention on the nasal mucosa compared with FITC – dextran [179,181].

Recently, maleimide-functionalized poly(*N*-(2-hydroxypropyl) methacrylamide) has been investigated for intranasal delivery [182]. Mucoadhesive properties increased with the number of maleimide groups introduced into the polymer. Interestingly, a control experiment with glycol chitosan showed no statistically significant difference compared with derivatives containing the lowest maleimide content. These results confirm that covalent bonding between thiols and maleimides enhances mucoadhesion. Importantly, no cytotoxicity was observed in cell studies, regardless of maleimide content. Thus, maleimide derivatives of poly(*N*-(2-hydroxypropyl)methacrylamide) represent a promising strategy for improving intranasal mucoadhesion.

One strategy for enhancing the mucoadhesive properties of polymers involves the introduction of catechol groups through chemical modification. The mucoadhesion of such derivatives is attributed to covalent interactions formed when catechols oxidize to o-quinones, which subsequently react with amino and thiol residues. The adhesive role of catechols was first recognized in the early 1980s by Waite and colleagues, who identified them as key components of the adhesive proteins in marine mussels [183]. In a recent study, Deng et al. [184] synthesized a mucoadhesive chitosan – catechin conjugate for use in a nasal vaccine delivery system. This formulation enabled a high local concentration of porcine epidemic diarrhea virus antigen on the nasal mucosa of mice, a key factor in initiating a robust mucosal immune response.

Hunter et al. [185] developed Pickering nanoemulsions stabilized with aldehyde-functionalized nanoparticles and demonstrated improved retention on sheep nasal mucosa *ex vivo* compared with nanoparticles lacking aldehyde groups. The enhanced mucoadhesive properties were attributed to the ability of aldehyde groups to form imine bonds with amine groups on the mucosal surface through Schiff base chemistry.

Zhang et al. [186] reported the development of phenylboronic acid-functionalized glycopolymeric nanoparticles for nasal delivery of biomacromolecules, using insulin as a model protein. The phenylboronic acid groups can form dynamic covalent bonds with 1,2-diols in mucin, conferring strong mucoadhesive properties. These properties were confirmed through experiments measuring mucin adsorption onto the nanoparticle surface. *In vivo* studies in rats further demonstrated that the nanoparticles enhanced insulin absorption across the nasal mucosal barrier without causing mucosal irritation.

Thus, diverse polymer functionalization strategies – such as the introduction of thiol, acryloyl, methacryloyl, crotonoyl, maleimide, catechol, and aldehyde groups – have shown significant potential to enhance mucoadhesive properties in intranasal drug delivery. These approaches are summarized in Figure 4, which illustrates the underlying mechanisms responsible for the improved adhesion.

Recent advances have significantly expanded the range of mucoadhesive systems designed to form covalent bonds with mucosal surfaces. These materials often show higher mucoadhesive strength and longer nasal residence times, which may translate into improved drug bioavailability. However, most studies to date report only preliminary biocompatibility or toxicology testing. Progressing these modified excipients will require more rigorous evaluation under repeated and prolonged exposure to address potential long-term safety concerns. Cook and Shorthouse [76] caution that covalently reactive ('reactive') mucoadhesives may present an elevated sensitization risk and should therefore be assessed early in development. They describe such systems as inherently higher risk for compatibility issues and sensitization, recommending prioritized hazard assessment in collaboration with specialist toxicologists who have the necessary facilities and materials. They further argue that any residual risk is best justified only where reactive mucoadhesives enable therapies for serious conditions that cannot be effectively treated using currently available excipients, providing a clear clinical and economic rationale for progression to human studies.

4. Mucus-penetrating systems

In addition to using mucoadhesive polymers or enhancing the mucoadhesive properties of polymers, another strategy to improve drug delivery across mucus involves the use of mucus-penetrating systems that facilitate the transport of molecules through the mucosal barrier. In this context, two main approaches have been identified. The first relies on inert materials that penetrate the mucus layer via passive diffusion without interacting with the mucosal surface. The second

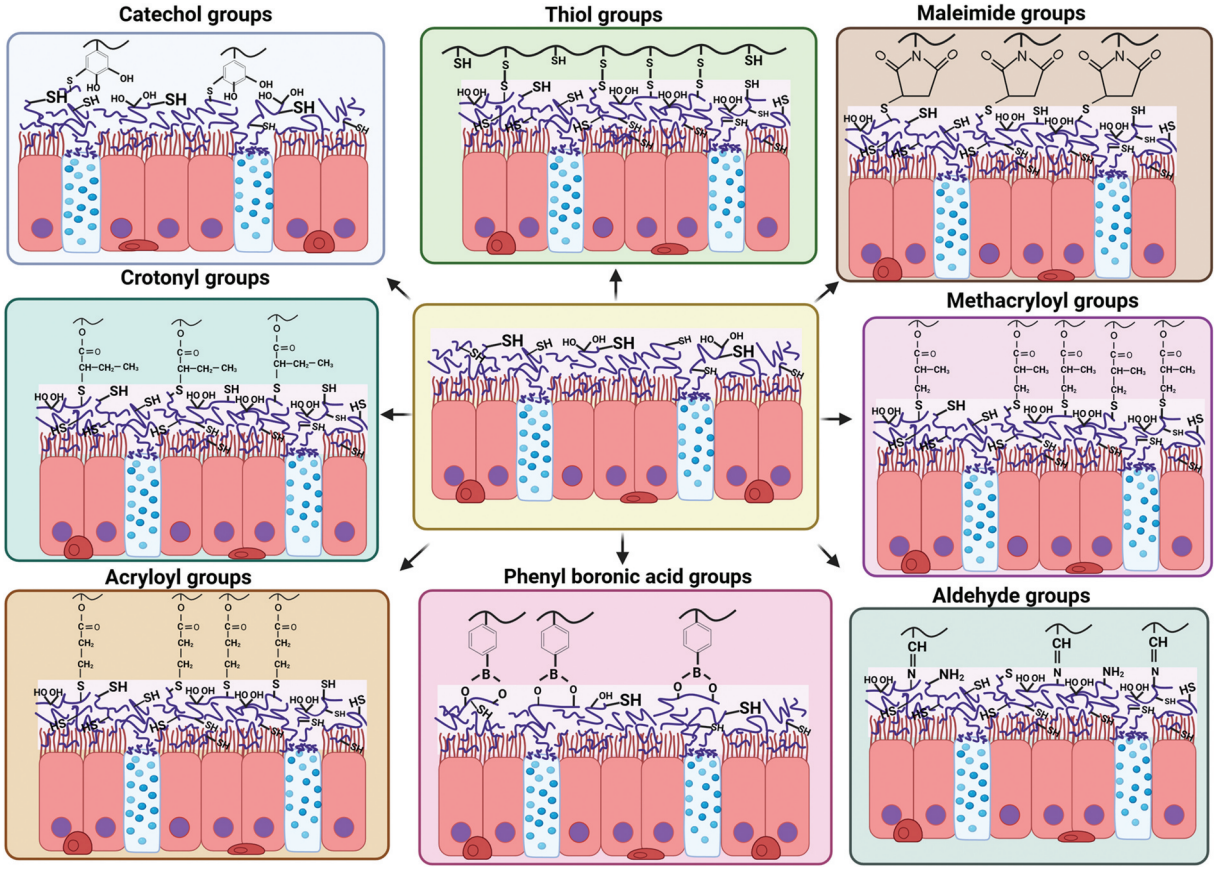

Figure 4. Schematic illustration of underlying mechanisms responsible for the improved mucoadhesion. Created in BioRender. Lopez Vidal, L. (2026) https://BioRender.com/gbk8mwh.

approach focuses on systems capable of altering mucus properties through physicochemical interactions [187,188].

One particularly promising development in the first approach has been reported by the group of Hanes [189,190], who improved the mucosal penetration of nanoparticles by modifying their surfaces with polyethylene glycol (PEG). PEG, a hydrophilic and nonionic polymer, contains two terminal hydroxyl groups that enable the formation of either branched or linear molecular architectures. The terminal hydroxyl groups can be modified with a range of ligands, thereby enabling their functionalization and allowing for precise modulation of polymer properties [191]. Such modifications not only improve mucosal penetration but may also enhance mucoadhesion through the incorporation of specific functional groups [192,193]. By coating nanoparticles with PEG, a neutral and hydrophilic shell is formed, which significantly reduces electrostatic interactions with the mucosal environment, thereby enhancing nanoparticle diffusion through mucus barriers [194]. Moreover, by adjusting the molecular weight of the polymer, it is possible to fine-tune the balance between mucoadhesive and mucus-penetrating properties [190,195,196].

PEGylation is a valuable technique in the development of nanoparticle-based drug delivery systems, not only because it enhances particle stability by reducing aggregation, but also because it prevents opsonization in the bloodstream and decreases uptake by the mononuclear phagocyte system, which is crucial for the clearance kinetics of PEGylated particles [197–201]. Owing to their advantageous characteristics, these systems have attracted considerable interest in the field of intranasal drug delivery, particularly for targeting the CNS. In particular, in the work by Junior et al. [202] PEGylated polycaprolactone nanoparticles were investigated for the intranasal delivery of bexarotene directly to the brain. The administration of PEGylated nanoparticles resulted in drug concentrations in the brain that were three-fold and two-fold higher, respectively, compared with the non-PEGylated drug dispersion over a specified time period. Furthermore, rapid mucus penetration was observed, with penetration efficiencies of 98.8% and 99.5% for nanoparticles PEGylated at 5% and 10%, respectively. These findings highlight the potential of PEGylation to enhance both drug bioavailability and mucosal permeability in intranasal delivery systems.

In work by Bazargani et al. [203] PEGylated solid lipid nanoparticles (SLNs) with varying degrees of PEGylation were synthesized and subsequently loaded with the antiretroviral drugs elvitegravir and atazanavir. The PEGylated particles demonstrated enhanced penetration: as the PEGylation degree increased to 5%, 10%, and 15%, the amount of coumarin used as a particle marker, penetrating the system increased by 1.6-, 3.15-, and 5.83-fold, respectively, compared with the non-PEGylated formulation, as assessed using a Transwell system. Moreover, particle aggregation and adhesion within mucus decreased proportionally with higher

PEGylation levels. This presents a promising strategy for improving intranasal drug delivery systems for antiretroviral therapy.

For antiretroviral therapy, PEGylated polylactic acid nanoparticles loaded with zidovudine were developed for intranasal administration. Prolonged drug release was demonstrated for samples containing PEG. Moreover, pharmacokinetic studies showed that PEGylated nanoparticles extended the drug half-life up to 7 hours. The presence of PEG was shown to influence the mucosal layer by preventing aggregation of nanoparticles in the mucus and potentially facilitating their penetration through the nasal mucus [204].

Various types of methoxy polyethylene glycol were investigated for conjugation with chitosan to reduce its toxicity and improve macromolecular permeability across the nasal mucosa. A marked decrease in the cytotoxicity of chitosan was observed following PEGylation, with the toxicity of the conjugates depending on the extent of PEGylation. Furthermore, the permeability of the PEGylated conjugates was up to five times greater than that of conventional chitosan [205].

Porfiryeva et al. [28] designed and evaluated two types of nanoparticles for nasal delivery of haloperidol, both prepared via interpolyelectrolyte complexation between cationic Eudragit® EPO (EPO) and anionic Eudragit® L 100–55. The first type, obtained directly from commercially available copolymers, exhibited mucoadhesion. In contrast, the second type was produced by PEGylating Eudragit® L 100–55 prior to complexation, resulting in nanoparticles with reduced mucoadhesion and enhanced mucus penetration. Both systems were loaded with haloperidol, a model antipsychotic, and tested *in vivo* in rats. Haloperidol induces catalepsy, assessed here by measuring the time rats maintained a slightly uncomfortable 'lecturer' posture after nasal administration. The PEGylated nanoparticles showed superior penetration through sheep nasal mucosa compared with non-PEGylated controls. *In vivo*, catalepsy was more pronounced within the first 10 minutes for the PEGylated formulation (178 ± 5 s at 10 min and 174 ± 5 s at 20 min) than for the unmodified complex (59 ± 49 s and 108 ± 61 s, respectively), as illustrated in Figure 5. While PEGylation is widely used to enhance mucosal permeability and pharmacological performance, alternative polymer modification strategies have also been investigated to further improve delivery efficiency and stability [206].

Several polymer classes have been explored as alternatives to PEGylation for enhancing nanoparticle mucus penetration across various transmucosal delivery routes [206]. In a study by Ways et al. [207] chitosan was conjugated with short-chain PEG, poly(2-hydroxyethyl acrylate), poly(2-ethyl-2-oxazoline), or poly(*N*-vinylpyrrolidone), followed by crosslinking of the resulting graft copolymers with sodium tripolyphosphate. All nanoparticle formulations demonstrated increased diffusivity in mucin solutions *in vitro* and improved penetration into sheep nasal mucosa *ex vivo*.

Decoration of nanoparticles with short-chain PEG or other neutral polymers imparts mucus-penetrating properties by reducing their ability to engage in adhesive interactions with mucins. In this respect, mucus penetration and mucoadhesion are opposing phenomena: strong polymer – mucin interactions promote mucoadhesion and surface retention, whereas the absence of such interactions allows particles to diffuse more freely through the mucus layer. Each strategy offers distinct advantages for nose-to-brain delivery. Mucoadhesion prolongs residence time at the nasal epithelium and can enhance absorption of small molecules by maintaining a high local concentration, but strong adhesion may impede transport toward the olfactory region. Conversely, mucus-penetrating systems can reach deeper nasal regions more efficiently and may improve uptake into olfactory pathways, yet their rapid mobility can reduce epithelial contact time and diminish retention.

In addition to polymer-based approaches, several biochemical agents and penetration enhancers have been investigated

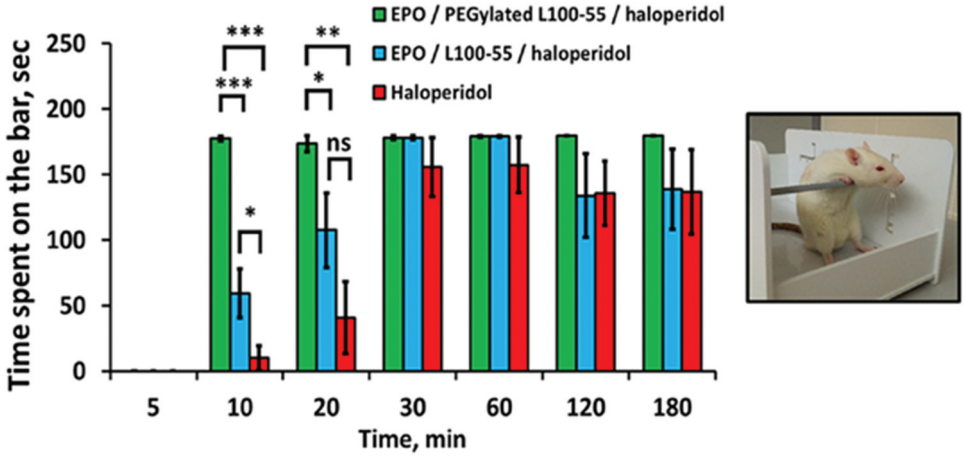

Figure 5. *In vivo* catalepsy test in rats caused by haloperidol (1 mg/kg, nasal), EPO/L100-55/haloperidol (1 mg/kg, nasal) and EPO/PEGylated L100-55/haloperidol (1 mg/kg, nasal) ($n = 6$, mean ± SD, '*' represents $p < 0.05$). Inset: exemplar image of rat on elevated rod displaying catalepsy effect. Reprinted from [28] with permission from Elsevier.

for their capacity to transiently reduce mucus viscosity or modulate mucosal interactions, thereby promoting improved transport through the mucus layer. One of the approaches to improving transmucosal drug delivery is the use of cell-penetrating peptides (CPP). Polyarginines are one of the most commonly used CPPs that have demonstrated high efficiency in transporting protein drugs [208]. The positive charge from guanidinium groups enables polyarginines to effectively engage in electrostatic interactions with the anionic oligosaccharide fragments of mucins and proteoglycans [209].

Khafagy et al. [210] used CPPs for delivering the hormone leptin, which regulates appetite, via intranasal administration. CPPs were bound to the peptide drug through intermolecular interactions for direct delivery to the brain via the nasal route. As a result, formulation of L-penetratin increased the relative nasal bioavailability of leptin to approximately 43%. Moreover, even a single intranasal dose of the formulation significantly increased leptin concentration in the brain, particularly in the anterior region. Thus, this combination may be considered as a potential approach for obesity treatment through intranasal administration.

Another approach to enhance penetration through the mucus is the use of mucolytic agents that break down mucoglycoprotein structures, thereby facilitating access to the mucus layer. One notable example in this context is *N*-acetyl-L-cysteine (NAC), which disrupts disulfide bonds within mucoglycoproteins, leading to mucus liquefaction and improved drug accessibility [211]. Additionally, the viscoelastic properties of mucus can be regulated by various enzymes. In particular, it has been demonstrated that nattokinase reduces mucus viscosity and promotes the reduction of nasal polyp tissue [212]. In the context of polymer-based drug delivery systems, developments have included the formulation of nanoparticles composed of poly(acrylic acid) loaded with papain. In this system, papain acts on the viscoelastic properties of mucus by cleaving its mucoglycoprotein components. Mucus samples exposed to papain formulations showed substantial reduction in viscosity, whereas untreated mucus and its mixture with PAA particles retained nearly 70% of their initial rheological properties [213].

Low-molecular-weight penetration enhancers are also widely used in mucosal and topical drug delivery to facilitate transport across biological membranes. Such strategies are particularly common in transdermal and transcorneal delivery, where intrinsic membrane permeability is very low [214,215]. Chemical enhancers – including cyclodextrins, chelating agents, bile salts and surfactants – can reversibly modulate epithelial barrier integrity and thereby improve drug penetration. Similar classes of penetration enhancers have also been explored in nasal drug delivery. For example, Merkus et al. [216] investigated the absorption-enhancing effects of several cyclodextrins (CDs) on intranasally administered insulin in rats. Coadministration of 5% (w/v) dimethyl-β-cyclodextrin (DMβCD) produced a remarkably high insulin bioavailability of 108.9 ± 36.4% relative to intravenous administration, accompanied by a profound reduction in blood glucose levels to approximately 25% of baseline. In contrast, coadministration of 5% (w/v) α-cyclodextrin yielded a bioavailability of 27.7 ± 11.5% and reduced blood glucose to around 50% of the initial value. The rate of insulin absorption- and the associated hypoglycemic response – was notably slower with α-CD than with DMβCD.

Thus, different methods are used for penetration through the mucus, with mucus-penetrating nanoparticles effectively overcoming the mucosal barrier to enhance local drug delivery and improve therapeutic efficacy.

5. Testing methods

The development of novel drug delivery systems for transmucosal administration requires a thorough evaluation of their properties, including interactions with mucosal surfaces and potential toxicological effects. This involves methodologies for assessing formulation adhesion to mucosal tissues, penetration through mucus, and overall biocompatibility. Accordingly, the following sections will provide an overview of the currently available methods for evaluating mucoadhesive properties, mucosal diffusion and penetration, as well as the experimental approaches used to investigate the toxicity of various intranasal drug delivery systems.

5.1. Nasal mucoadhesion

5.1.1. Rheology

One of the most commonly used methods for evaluating mucoadhesive interactions is the study of polymer rheology. This technique is based on assessing the strength of interactions between polymers and mucosal systems. It involves simulating the interaction between polymers and mucin solutions. Using a rheometer, the viscosity and elasticity of both the complete system and its individual components are measured. Subsequent calculations allow for the determination of system viscosity and, ultimately, the estimation of mucoadhesive strength [75].

In addition, rheological analysis is undoubtedly a valuable tool for understanding the viscoelastic properties of formulations under physiologically relevant conditions. By simulating specific temperatures, it is possible to evaluate changes in both the storage modulus (G′) and the loss modulus (G″) of the system. For example, the rheological properties of a nasal spray based on an emulsion gel were investigated by Sailer et al. [217] It was shown that temperature variations significantly affected the viscoelastic structure of the spray. Although structural recovery was observed, it was slightly reduced at 34°C. Nevertheless, the viscosity remained sufficiently high to ensure adequate retention of the formulation within the nasal cavity and to prevent leakage.

One of the limitations of the rheological approaches applicable specifically to the nasal drug delivery is the unavailability of nasal mucins. In the majority of studies, researchers use surrogates such as porcine gastric mucin that is commercially available.

5.1.2. Tensile (detachment) method

The method is based on tensile testing, in which the pharmaceutical formulation is brought into contact with mucus and

the force required to break the resulting adhesive bond is measured. This approach is widely regarded as one of the most convenient and frequently employed techniques for assessing mucoadhesion. Its major advantage lies in the high level of control it offers over experimental variables such as applied force, elongation rate, and quantitative readout. In addition, tensile testing is versatile, allowing the evaluation of a broad range of pharmaceutical formulations and compositions, including both soft and solid dosage forms. Although it does not fully replicate the dynamic nature of the *in vivo* environment or capture the complexity of biological interactions between mucus and the formulation, it nevertheless provides a robust and reliable tool for preliminary mucoadhesion evaluation.

These tests are typically performed using specialized instruments such as texture analyzers or universal testing machines. These devices not only enable the quantification of adhesive and rheological properties of the system under investigation but also allow modulation of physicochemical parameters to mimic different physiological conditions. The experiments evaluating mucoadhesive properties of formulations can be organized in several configurations, as shown in Figure 6: (1) the dosage form may be directly attached to the mobile probe of a texture analyzer and then brought into contact with the mucosal tissue; (2) the mucosal tissue may be affixed to the probe and subsequently contacted with the formulation; or (3) the formulation may be sandwiched between two pieces of mucosal tissue, which are respectively attached to the probe and used as the substrate. Tensile tests typically allow determination of the maximum force required to detach a dosage form from the mucosal surface, as well as the total work of adhesion, which is calculated as the area under the force – distance curve. A related variant of this method can be carried out using a specialized balance. Its operating principle is similar to that of tensile testing, as it measures the vertical force required to separate the mucus from the formulation [218].

In Alami-Milani et al. [82] study, this design was used to investigate intranasal hydrogel systems with mucoadhesive and thermosensitive properties. The mucoadhesion study showed that the adhesive strength correlated with the concentration of PEG in the system. Specifically, samples with a high concentration of PEG exhibited lower mucoadhesion; a possible explanation of this was interactions with hydroxyl groups and the presence of sorbitol in the formulation.

5.1.3. Flow-through method

One of the most widely used methods for assessing mucoadhesion allows the evaluation of diverse drug formulations, including micro- and nanoparticles. It typically employs freshly excised animal mucosal tissue as a substrate, which is irrigated with physiologically relevant fluid to simulate natural conditions. This approach was originally proposed by Rao and Buri [219]. In mucoadhesion studies, the formulation is typically applied to freshly excised mucosal tissue mounted on a specialized support. A controlled flow of biological fluid is then introduced to mimic physiological wash-off conditions. At defined time intervals, the amount of formulation retained on the mucosal surface can be quantified using spectrophotometric or fluorescence techniques, or by chromatographic methods such as HPLC. To ensure physiological relevance, these experiments are best conducted in incubators that maintain body temperature.

This method was further optimized and extensively applied by the Khutoryanskiy research group to evaluate mucoadhesive formulations across multiple routes of mucosal administration, including ocular, gastrointestinal, nasal, vaginal, intravesical, and oromucosal delivery [172,220–223]. Incorporation of fluorescent markers into the samples enabled simultaneous assessment of both mucoadhesive and mucus-penetrating properties. In these studies, formulations were applied to excised nasal mucosa mounted at a defined angle within an incubator and gradually washed off in a controlled dropwise manner. At predetermined intervals, the tissue was imaged using fluorescence microscopy, and subsequent image analysis allowed quantification of formulation retention time on the mucosal surface.

An example of the fluorescence-based flow-through method for evaluating nasal retention of polymeric formulations is shown in Figure 7. In this experiment, 1 mg/mL solutions of glycol chitosan, poly(*N*-(2-hydroxypropyl)

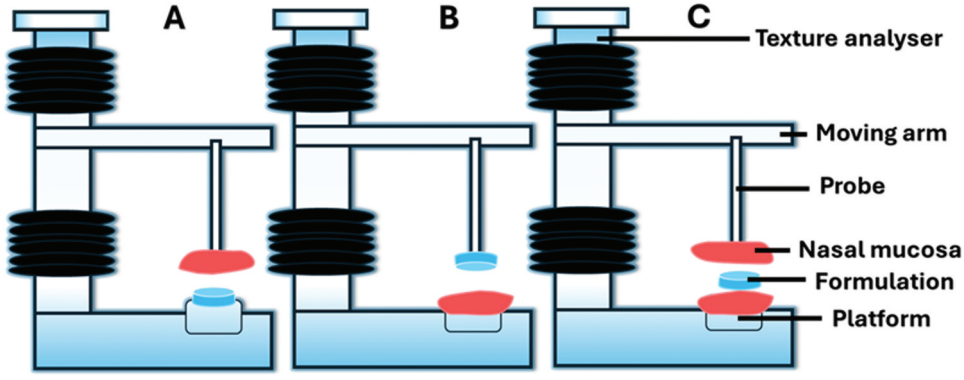

Figure 6. Schematic representation of tensile testing configurations used for the assessment of mucoadhesion: (A) mucosal tissue attached to the probe and brought into contact with the formulation; (B) the dosage form attached to the probe and applied to mucosal tissue; and (C) the formulation positioned between two pieces of mucosal tissue attached to the probe and substrate.

methacrylamide) (PHPMA), and its maleimide-containing derivatives with 25.2% and 11.5% substitution (PHPMA-Mi25 and PHPMA-Mi11), each supplemented with 0.05 mg/mL sodium fluorescein, were applied to sheep nasal mucosa. A 0.05 mg/mL sodium fluorescein solution alone was used as a control. The tissues were subsequently washed with artificial nasal fluid, and retention was assessed by fluorescence imaging. Glycol chitosan was used as a positive control in these experiments due to its well-established strong mucoadhesive properties. Polymeric derivatives of PHPMA containing maleimide groups demonstrated superior retention on the nasal mucosa, attributable to the ability of maleimide moieties to form covalent bonds with mucin thiol groups.

The method is straightforward and convenient, offering flexibility in adjusting formulation composition, the type and volume of biological fluid applied, and the choice of mucosal tissue. However, it does not fully replicate physiological conditions. Limitations include the need to periodically remove the specimen for imaging, which interrupts the wash-off process, and the challenge of maintaining experimental consistency when repositioning tissue within the microscope and incubator. Shan et al. [177] applied this method to assess the mucoadhesive properties of chemically modified poly(2-ethyl-2-oxazoline) on excised sheep nasal mucosa. The tissue was perfused with 1 mL of artificial nasal fluid at 0.43 mL/min using a syringe pump, with images captured at 0, 5, 10, 20, 30, 40, 50, and 60 minutes. The modified polymers demonstrated a marked increase in mucoadhesion compared with their unmodified counterparts.

In the study by Matarazzo [224], the flow-through method was used to evaluate the mucoadhesive properties of an intranasal drug delivery system based on nanostructured carriers for cannabidiol. Mucosal tissue was mounted on a longitudinally halved cylindrical tube fixed at a 45° angle and exposed to simulated nasal fluid flow. Drug retention on the mucosal surface was quantified over time by HPLC, revealing that retention depended on the type of delivery system employed.

The flow-through method can be used to estimate the time or volume of artificial nasal fluid required to wash away part or all of a formulation from the mucosal surface. This approach allows direct comparison of the retention behavior of different formulations under controlled, physiologically relevant flow conditions.

5.1.4. Rotating cylinder

Mucoadhesive properties can also be assessed using the rotating cylinder method, originally proposed by Bernkop-Schnürch and colleagues [182]. In this approach, dosage forms are placed on freshly excised mucosal tissue fixed to a cylinder, which is then immersed in a dissolution tester and rotated while the time to detachment or dissolution is recorded. This method enables the simulation of polymer adhesion and cohesion under physiologically relevant media. A key advantage is the ability to control shear stress and generate quantitative adhesion data, with measurements performed dynamically to capture changes in adhesive properties over time. Like the tensile method, it can be applied to a wide range of dosage forms, from soft formulations to solid tablets. However, limitations include the risk of sample damage under shear and the influence of multiple experimental variables that must be carefully considered when interpreting results. Specifically, polymer discs prepared from gellan gum derivatives were placed on mucosal tissue and fixed onto the cylinder. The results demonstrated that certain aminated derivatives exhibited up to a 14-fold increase in residence time compared with the unmodified polymer [225].

In the study by Laffleur [226], the rotating cylinder method was used to assess the mucoadhesive properties of thiolated hyaluronic acid. Polymeric discs were applied to freshly excised sheep nasal mucosa mounted on a cylinder and immersed in artificial nasal fluid, and the detachment time was recorded as a measure of adhesion. Thiolated hyaluronic acid showed markedly enhanced mucoadhesion, with a detachment time of approximately 24 hours compared with only 7 hours for the unmodified polymer.

One limitation of the rotating cylinder method, particularly when applied to the evaluation of formulations for nasal drug delivery, is the requirement for relatively large volumes of artificial nasal fluid. This can create conditions that deviate from the physiological environment of the nasal cavity, where fluid volumes are limited. As a result, the test may underestimate the retention of mucoadhesive formulations or fail to accurately reproduce the dynamic clearance processes occurring *in vivo*.

5.1.5. Comparative performance across mucoadhesive systems and assessment methods

Across the *in vitro*, *ex vivo* and *in vivo* models discussed above, some recurring behaviors emerge. However, direct 'ranking' of polymers across studies is inherently constrained by differences in mucin source and concentration, tissue type, hydration state, contact time/pressure, and the methodology/endpoint used to assess mucoadhesion. Comparisons are therefore most informative when made within a given study (e.g. relative to an unmodified polymer or a non-mucoadhesive control) and should then be interpreted in the context and limitations of the specific model. This point is particularly relevant for rheology-based 'synergism' approaches, where the calculated interaction term depends strongly on experimental conditions and parameter choice [227]. Solution-based *in vitro* assays using commercially sourced or freshly isolated mucins (e.g. rheology/rheological synergism, turbidimetric titration, or isothermal titration calorimetry) primarily probe polymer – mucin interactions and associated changes in cohesive/adhesive strength under tightly controlled conditions. Ionic polymers such as chitosan, linear and weakly cross-linked carboxylated polymers commonly show stronger responses in these assays (e.g. greater rheological synergism or more pronounced mucin aggregation) than nonionic comparators [228–230]. Covalently reactive systems, such as thiolated polymers, often produce larger shifts in these readouts because covalent bonding can add an additional binding mode beyond physical interactions [231]. *Ex vivo* retention/adhesion/wash-off models incorporate

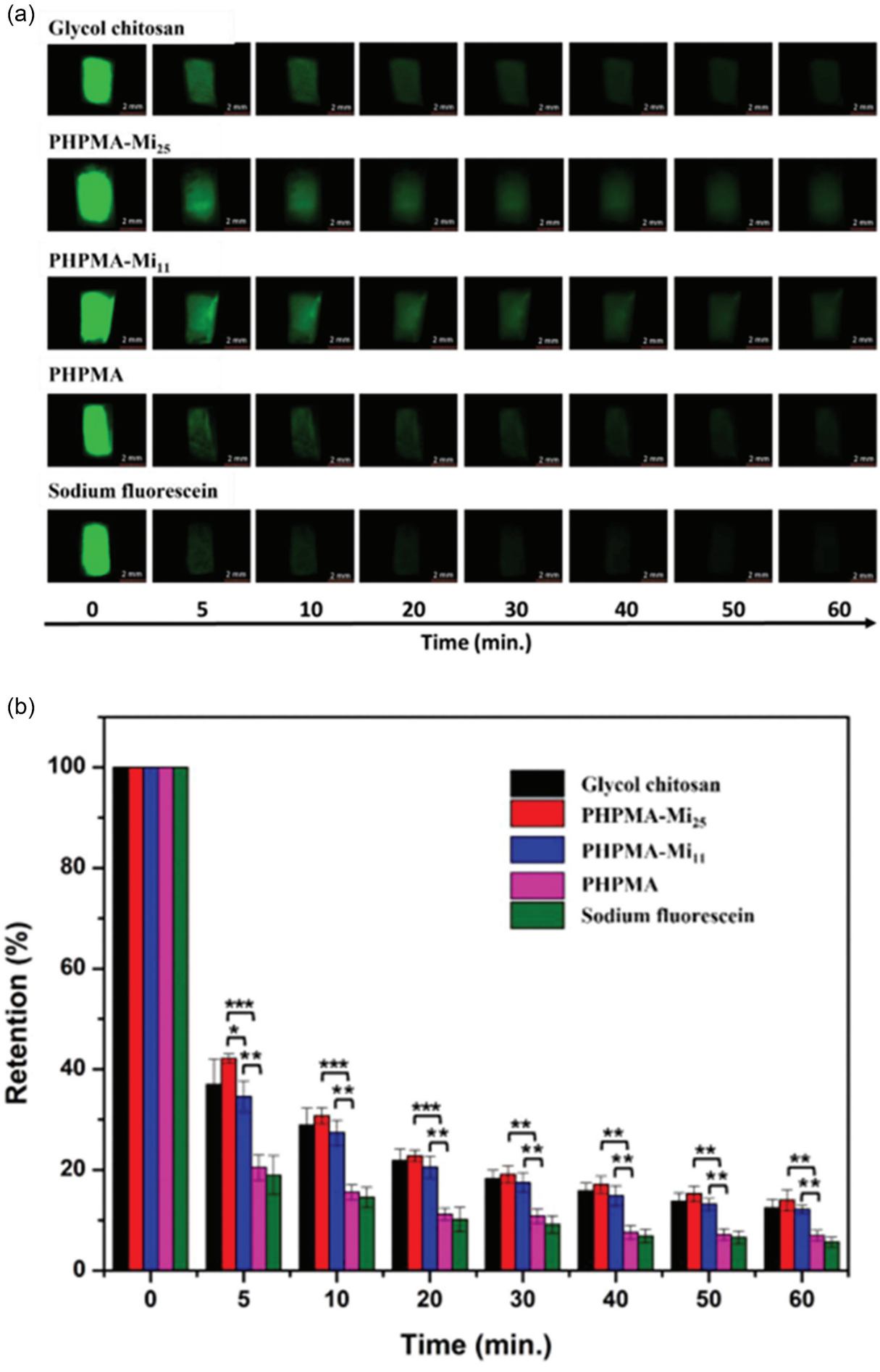

Figure 7. Retention of fluorescent formulations on sheep nasal mucosa *ex vivo*. (a) Fluorescence images showing retention of 1 mg/mL glycol chitosan, PHPMA,

a mucosal surface and native mucus layer, and therefore begin to capture formulation spreading, hydration dynamics and polymer mobility on tissue. Under these conditions, ionic polymers typically show stronger retention/adhesion than nonionic analogues, while covalently binding systems often provide a further increase in performance [139,177]. *In vivo* studies integrate multiple physiological and behavioral factors that cannot always be tightly controlled, often resulting in greater variability. Consequently, *in vivo* outcomes do not necessarily mirror in vitro rankings, and formulations that appear more adhesive *in vitro* do not always translate into superior *in vivo* performance. In addition, excessively strong adhesion or gel formation at the nasal surface may impede drug diffusion through mucus and reduce access to olfactory pathways, which can be counterproductive when the goal is nose-to-brain delivery [28,232].

5.2. Toxicity studies

In addition to evaluating mucoadhesion, it is essential to assess the toxicity of developed formulations, especially when chemical modification of polymers introduces additional functional groups. A traditional approach in this context is the assessment toxicity to cells.

5.2.1. Toxicity to cells

Cell viability can be assessed using a variety of established cell lines and assays that evaluate the ability of cells to maintain metabolic activity and membrane integrity. Among these, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is one of the most widely employed. In this method, cultured cells are seeded and exposed to the test sample, followed by incubation with the MTT reagent. Subsequent spectrophotometric or microplate measurements of absorbance or fluorescence are used to quantify cell viability relative to untreated controls, thereby determining the degree of cytotoxicity. According to ISO 10993–5 guidelines for *in vitro* cytotoxicity testing, a formulation is considered non-cytotoxic if cell viability remains above 70%.

In this context, epithelial cell monolayers such as RPMI 2650, Calu-3, and 16HBE14o-representing human nasal epithelium, lung carcinoma, and normal bronchial epithelium, respectively, are commonly employed [70]. A key limitation of this approach is the need for specialized laboratory facilities and the technical challenges associated with cell culture, particularly given the sensitivity of lines such as RPMI. Nevertheless, it remains one of the most widely used methods, as it enables direct assessment of biological responses, is applicable to diverse cell types, and allows quantitative evaluation of cytotoxicity.

For example, Vanukuru et al. [176], employed the Caco-2 cell line and the MTT assay to evaluate the cytotoxicity of chitosan and its chemically modified derivatives intended for intranasal delivery. After 4 and 24 hours of exposure, approximately 70% of the cells remained viable, compared with untreated controls.

Another example of cell toxicity evaluation is provided by a study in which the MTS CellTiter 96 assay was used to assess cell proliferation. The results demonstrated no evidence of toxicity at low formulation concentrations, with cell viability remaining above 80% for the gel formulation containing methylated β-cyclodextrin [233].

5.2.2. Slug mucosal irritation test

An alternative approach for toxicity assessment is the slug mucosal irritation test, originally proposed by Remon and colleagues [234] and later modified by Khutoryanskaya et al. [235]. This method involves weighing slugs before and after exposure to test samples over defined time intervals. Under irritating conditions, slugs produce large amount of mucus, leading to measurable mass loss that serves as an indicator of mucosal irritation.

In the study by Callens et al [236], the slug mucosal irritation test was used to evaluate the toxicity of a nasal powder formulation containing Carbopol 974 and starch, alongside parallel testing on rabbit nasal mucosa. Over a four-week period, the results in rabbits showed a high level of agreement with the slug assay results. Specifically, the positive control benzalkonium chloride induced excessive mucus production and elevated levels of proteins and lactate dehydrogenase, whereas the nasal powder formulation produced no signs of irritation.

In our previous study [166], we assessed the toxicity of chemically modified acryloylated polycation Eudragit® EPO using the slug mucosal irritation test. The modified copolymers did not induce irritation, as evidenced by low levels of mucus production comparable to those of the negative control (Figure 8). In contrast, exposure to a substrate containing benzalkonium chloride elicited a pronounced irritant response, with slugs producing 28.02 ± 2.70% mucus, confirming its toxic effect. This strong irritation caused by the positive control was further supported by the characteristic yellow coloration of the mucus produced.

An important advantage of the slug mucosal irritation test is its capacity to model chronic and repetitive exposure, enabling assessment of formulations under conditions that better reflect prolonged intranasal use. Compared with conventional cell culture assays, this method also offers the benefit of testing materials in contact with the whole organism, where different cell types and tissues interact in an integrated biological context. However, the slug mucosal irritation test has practical limitations, primarily due to the limited commercial availability of slugs and the technical challenges associated with their laboratory maintenance and breeding.

PHPMA-Mi25, and PHPMA-Mi11 solutions containing 0.05 mg/mL sodium fluorescein, as well as a control solution of 0.05 mg/mL sodium fluorescein alone, after washing with artificial nasal fluid. Scale bar: 2 mm. (b) Quantitative retention of the same formulations on sheep nasal mucosa following exposure to different volumes of artificial nasal fluid (pH 5.7; $n = 3$; mean ± SD; '*' indicates $p < 0.05$). Reprinted from [182], under the terms of the Creative Commons CC-BY.

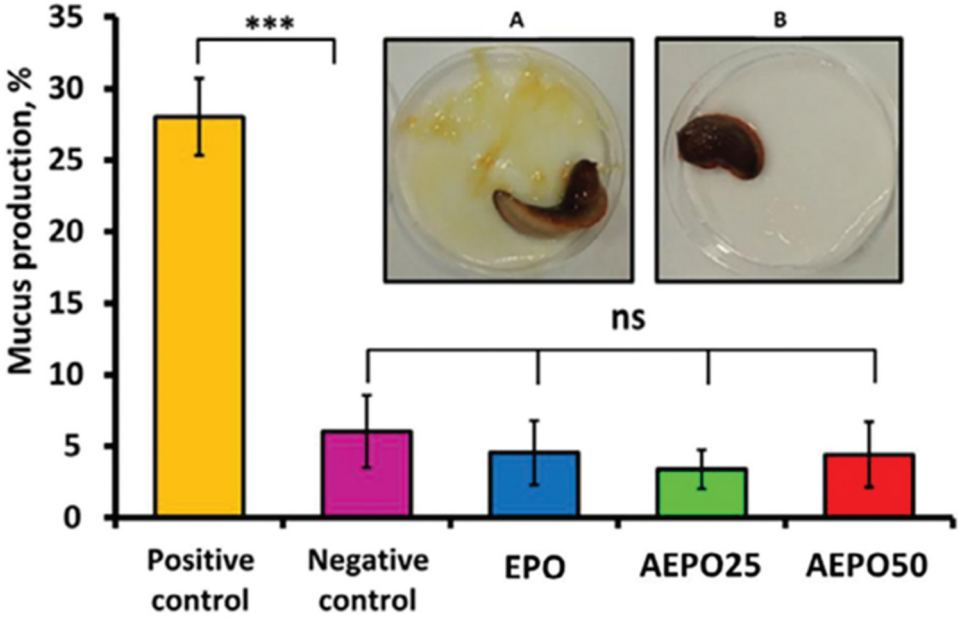

Figure 8. Mucus production by *Limax flavus* slugs following contact with solutions of 1 wt% benzalkonium chloride (positive control), artificial nasal fluid (negative control), a 0.1 wt% solution of Eudragit EPO (EPO), and solutions of two EPO derivatives with 25.1 ± 1.6% and 50.0 ± 0.8% degrees of acryloylation (AEPO25 and AEPO50) at pH 5.7. Data are presented as mean ± standard deviation ($n = 5$). Inset: representative images of *L. flavus* slugs in the positive (A) and negative (B) control experiments. Reprinted from [166] with permission from Elsevier.

5.2.3. *Planaria toxicity fluorescent assay*

Planaria are aquatic non-parasitic flatworms widely used in pharmacological and regeneration research. Their epithelia are mucosal and covered with motile cilia that closely resemble the physiological features of the nasal mucosa, making them a promising alternative model for predicting nasal irritation [237]. In a simple fluorescence-based assay, organisms are exposed to a test substance, treated with 0.1% fluorescein, immobilized in gelatin gel, and imaged by fluorescence microscopy; fluorescence intensity is then quantified using ImageJ. This method was applied to assess chemically modified chitosan derivatives intended for intranasal delivery: benzalkonium chloride (positive control) caused planarian mortality, whereas 0.1% solutions of chitosan and its derivatives induced no mortality across pH 6.0–6.8 in both water and artificial pond water after 24–72 hours, confirming an absence of acute toxicity [176]. The approach is technically straightforward, low-cost, and ethically attractive. However, its broader utility will depend on further validation against established *in vitro* and *in vivo* endpoints. Overall, planaria illustrate the growing shift toward alternative models for assessing nasal formulation toxicity, driven by the demand for more ethical and predictive methods.

6. Conclusion

Nasal drug delivery has attracted increased attention over the last decade, largely driven by the prospect of nose-to-brain delivery as a noninvasive route to treat CNS disorders. At the same time, the nasal route remains a patient-friendly option for systemic therapy, but its performance is strongly governed by formulation deposition, mucociliary clearance, and the interplay between the formulation and the mucus barrier. Recent advances in mucoadhesive and mucus-penetrating materials, nanoformulations, and *in situ* gelling systems have substantially expanded the intranasal delivery toolbox, enabling longer residence times and more controlled drug release. However, optimized intranasal delivery must balance a fundamental trade-off between improving nasal retention and maintaining efficient penetration through the mucus barrier. Translating promising concepts into reliable clinical products will therefore require consistent in vivo performance, robust repeated-dose safety/tolerability data, and better standardized, clinically relevant models and endpoints.

7. Expert opinion

The growing interest in nasal formulations is largely driven by their excellent potential for direct brain targeting. Over the past decade, a broad spectrum of approaches has been investigated for this purpose, including mucoadhesive formulations, *in situ* gelling systems, and mucus-penetrating nanoparticles.

Mucoadhesive liquid formulations provide relatively modest improvement in residence time and are ultimately limited by small dosing volumes, rapid dilution, and the need for preservatives. Powdered mucoadhesives overcome several of these limitations and represent a more promising direction. Their superior stability, higher drug-loading capacity, and gradual hydration create stronger and more sustained mucoadhesion than liquids. The emergence of modern powder-delivery devices further improves deposition accuracy. However, their tolerability under long-term repeated use

remains insufficiently characterized, and this represents a critical barrier to clinical adoption.

In situ gelling systems are more acceptable to patients than powders and provide substantially prolonged residence compared with simple mucoadhesive solutions. Their main limitations are the variability of gelation kinetics *in vivo* and the difficulty of achieving both rapid gelation and adequate spread across the nasal cavity. Nevertheless, we expect *in situ* gels to play an important role in the near term, especially for peptides and proteins.

Notable progress has been made with novel polymers capable of forming covalent bonds with mucins of the nasal epithelium. Thiolated polymers ('thiomers') have already advanced to clinical evaluation in certain drug delivery applications, demonstrating that covalent strategies can be translatable. However, other covalent chemistries proposed for mucoadhesion have not yet progressed to clinical application. For intranasal delivery specifically, robust *in vivo* studies are still required to confirm efficiency, long-term safety, and tolerability under repeated use.

Mucus-penetrating nanoparticles offer the most transformational potential for direct brain targeting because they address the fundamental physical barrier of the nasal mucus layer. PEG-coated nanoparticles have demonstrated impressive penetration profiles, but their long-term safety is uncertain due to anti-PEG immune responses. In our opinion, the development and validation of PEG alternatives (for example, poly (2-oxazolines), zwitterionic polymers, and polyvinylpyrrolidone) represent one of the most critical priorities for the field. These polymers could enable clinically viable mucus-penetrating systems without the immunological liabilities associated with PEG.

Overall, the main challenge for future progress lies in balancing retention and penetration. Strong mucoadhesion supports prolonged exposure but can hinder the movement of nanoparticles toward the olfactory region. Conversely, mucus-penetrating systems may diffuse too rapidly unless combined with controlled-release matrices. We believe that hybrid strategies, such as mucoadhesive powders incorporating mucus-penetrating nanoparticles or *in situ* gels with engineered diffusion pathways, are the most likely to achieve reliable and predictable nose-to-brain delivery.

Although nose-to-brain delivery is often discussed as a single outcome, it can arise via distinct transport routes, most commonly described as olfactory- and trigeminal-associated pathways, and the practical feasibility of each is strongly shaped by where the formulation deposits within the nasal cavity. In practice, anatomical targeting of the small posterior – superior olfactory region is challenging and highly dependent on device performance, plume/droplet characteristics and patient technique, which contributes to inter-individual variability. The efficiency of nose-to-brain drug delivery is also strongly influenced by disease state. Conditions such as rhinitis and inflammation, along with altered mucus properties and changes in mucociliary function, can markedly affect drug retention, penetration and tolerability.

Reliable clinical translation will ultimately require robust long-term safety and tolerability studies, more standardized and predictive *in vivo* models, and rigorous evaluation under repeated dosing. Progress will depend on demonstrating acceptable effects on ciliary function and nasal irritation, using practical dosing volumes, and applying clinically meaningful endpoints that distinguish local nasal benefit, systemic exposure and brain-targeting performance.

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Declarations of interest

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