Expert Opinion

Chitosan-based hydrogels for nasal drug delivery: from inserts to nanoparticles

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Importance of the field: Chitosan represents a multifunctional polymer, featuring both mucoadhesive and permeation-enhancing properties and therefore is a widely studied excipient for mucosal drug delivery. As regards nasal administration, chitosans have been used for the preparation of gels, solid inserts, powders and nanoparticles in which a three-dimensional network can be recognized.

Areas covered in this review: This review provides a discussion of the different nasal dosage forms based on chitosan hydrogels. In the first section intranasal delivery is discuss as a useful tool for non-invasive administration of drugs intended for local or systemic treatments. Then chitosan-based hydrogels are described with a focus on their mucoadhesive and permeation-enhancing ability as well as their capacity of controlled drug release. Finally, a detailed discussion regarding several examples of the different nasal dosage forms is reported, including considerations on *in vitro, ex vivo* and *in vivo* studies.

What the reader will gain: Summary and discussion of recent data on the different pharmaceutical forms based on chitosan hydrogels could be of interest to researchers dealing with nasal drug delivery.

Take home message: The aim of this review is to stimulate further investigations in order to achieve the collection of harmonized data and concrete clinical perspectives.

Keywords: chitosan, delivery systems, hydrogels, nasal delivery

Expert Opin. Drug Deliv. (2010) 7(7):811-828

1. Nasal drug administration

Nasal drug administration has been used extensively as a route for local and systemic delivery of numerous therapeutic compounds [1]. In fact, intranasal administration is non-invasive, painless, does not require a sterile preparation, and the drug can be easily and readily administered [2]. Intranasal administration of drugs is the logical choice for the treatment of local nasal disorders. In fact, relatively low doses are effective, minimizing the systemic toxic effects of oral and parenteral routes [3]. Examples of nasally administrated drugs commercially available are decongestants for cold symptoms, antihistamines and corticosteroids for rhinosinusitis [4-10]. Intranasal administration also represents an alternative to oral and intravascular routes for systemic drug delivery [11]. The more relevant advantages of the nasal cavity [12] as administration site are the large surface area available for drug absorption owing to the presence of numerous microvilli, the high vascularization of the subepithelial layer, the avoidance of hepatic first-pass metabolism of drugs and the possibility of direct transport of drug to the brain. Some commercially available nasal formulations, intended for systemic delivery, contain drugs such as analgesics, cardiovascular drugs,

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10.1517/17425247.2010.495981 © 2010 Informa UK Ltd ISSN 1742-5247

Article highlights.

- Chitosan-based hydrogels are polymeric crosslinked networks that are capable of absorbing and retaining considerable amounts of water without dissolving, and show mucoadhesive and permeation-enhancing ability as well as their capacity of controlled drug release.
- There are several examples of nasal dosage forms based on chitosan hydrogels such as gels, solid inserts, powders and nanoparticles, which require different technological approaches to be produced and suitable devices to be administered intranasally.
- A comparison between data from different research groups seems to be impracticable, owing to the presence of experimental differences such as the characteristics of the CH used, the origin of the loaded drug, the choice of the crosslinking molecule and the final composition of the formulation as well as the different *in vitro, ex vivo* and *in vivo* methods and the various experimental conditions used.
- There is a lack of human data on drug bioavailability following intranasal administration of CH-based hydrogels and, more generally, of delivery systems unlike traditional liquid formulations.

This box summarizes key points contained in the article.

anti-inflammatory agents and antiviral drugs. It is evident that physicochemical properties of the administered drug strongly influence its absorption through the nasal mucosa. The nasal district for optimal permeation of active substances corresponds to the respiratory region. Lipophilic drugs are absorbed well in the respiratory region, whereas polar drugs and particularly high-molecular-mass molecules (peptides, proteins and nucleic acids) are able to pass across the epithelium only in low amounts. The passage across the epithelium may occur by a transcellular route exploiting concentration gradients, receptor-mediated transport or vesicular transport mechanisms, or by a paracellular route through the tight junctions between the cells. In general, polar drugs with molecular masses < 1 kDa can pass the membrane using the paracellular mechanism, whereas high-molecular-mass compounds are absorbed through the nasal membrane using an endocytic transport process [13].

1.1 Delivery of high-molecular-mass molecules

Nasal drug administration for systemic effects has been widely studied [14] for the protein hormone insulin. Several works report the development of innovative delivery systems for insulin nasal administration and describe improved drug bioavailability in different animal species. Insulin is a polar hormone with a molecular mass of ~ 6000 Da, which can be significantly metabolized in the lumen of the nasal cavity or during the passage through the nasal epithelial barrier, owing to the presence of proteolytic enzymes. Drug delivery strategies are focused mainly on the improvement of insulin stability at the administration site and on the possibility of enhancing its absorption by means of promoters and by prolonging the permanence of the applied dosage form in the nasal cavity. In fact, another factor of importance for drug absorption through the nasal mucosa, especially for drugs that are not easily absorbed, is the combined action of mucus layer and cilia called mucociliary clearance, which represents the physiological defence of the respiratory tract against exogenous inhaled materials.

It is widely known that most of the invading pathogens enter the body through mucosal surfaces, hence nasal mucosa can be a good candidate for nasal vaccines [15,16]. The nasal mucosa has received great attention because it offers the possibility to obtain both mucosal (IgA) and systemic (IgG) immune responses and, moreover, vaccines can be delivered to the nasal-associated lymphoid tissue (NALT) with minimal dilution. Nasal mucosa can also induce good secretory immune responses at other mucosal sites, such as the intestine, lung and vagina. Despite numerous nasal delivery systems for vaccines being under investigation and the first influenza vaccine spray for human use (FluMist™, MedImmune, Inc., Gaithersburg, MD, USA) being approved in the US, nasal vaccine delivery systems present many disadvantages, such as low immunogenicity, toxicity, vaccine protein instabilities and complex production processes. Therefore, effective nasal vaccines require potent mucosal adjuvants and delivery systems able to protect the antigens and enhance their immunogenicity [17,18].

1.2 Nose-to-brain delivery

Several diseases such as schizophrenia, Parkinson's disease, Alzheimer's disease, meningitis and migraine require delivery of drugs to the brain. After nasal application, many drugs may be transported across the nasal membrane into the systemic circulation, reaching the brain and the cerebrospinal fluid (CSF) only after crossing the blood–brain barrier (systemic pathway). This route avoids hepatic/gastrointestinal first-pass effects, and therefore may increase drug bioavailability, but appears to be utilized only by sufficiently lipophilic molecules or molecules able to exploit a specific transport mechanism. Alternatively to the systemic absorption, the nasal pathway from the nose to the central nervous system (CNS) involves direct transport into the brain tissue or the CSF by transport across the olfactory region of the nasal cavity (olfactory pathway) [19,20]. Despite only < 0.1% of the administrated drug having been shown to be transported directly to the brain from the nasal cavity, numerous drug delivery systems have been evaluated to increase drug permeability across the olfactory epithelium. Among the different nasal formulations, nanoparticles [21] have been shown to provide significant improvement of drug absorption across the membrane barrier. However, as reported by Illum [22], it is not clear whether nanoparticles can be selectively taken into the olfactory epithelial cells and transported to the brain or the small particle size and the large total surface area favor drug transfer from the formulation into the surrounding mucosa.

2. Chitosan hydrogels

2.1 Chitosan

The polymer derived by the partial deacetylation of chitin is known as chitosan (CH), a cationic linear polysaccharide composed mainly of glucosamine and *N*-acetylglucosamine (**Figure 1**). Chitin is present in shells of crustacea such as shrimp, lobsters, prawns and king crabs, and CH itself forms the body wall of most fungi, molds and yeasts [23-25]. CH can be obtained in a great range of molecular masses (low < 50 kDa, medium 50 – 150 kDa, high > 150 kDa), degrees of deacetylation (40 – 98%) and viscosities. It is insoluble at alkaline and neutral pH values, whereas at acidic pH values, the amino groups are protonated (pK_a 6.3), improving its solubility. Various counterion acidic moieties can be used to obtain water-soluble salts (i.e., chloride, glutamate, acetate, lactate, citrate) with particular properties [26]. Biocompatibility, biodegradability and, in addition, non-toxicity [27,28], are the main properties of CH and make this natural polymer a versatile material for application in biomedical and pharmaceutical fields. Moreover, CH is considered as a potential structural material in hydrogels owing to its ability to form irreversible or reversible networks under specific conditions.

2.2 Crosslinked chitosan

Hydrogels can be defined polymeric crosslinked networks that have a large number of hydrophilic sites and are capable of absorbing and retaining considerable amounts of water without dissolving. As reported by Berger *et al.* [29,30], CH hydrogels can be classified in chemically and physically crosslinked networks. Chemical hydrogels are formed by irreversible covalent links, whereas physical hydrogels are formed by various reversible non-covalent links. Chemical CH hydrogels can be obtained by: (i) crosslinking with CH itself; (ii) crosslinking with CH itself in the presence of a non-reactive polymer; and (iii) a crosslinking reaction between CH chains and the reactive chains of another type of polymer. In these hydrogels, networks are mainly formed by covalent bonds derived by the reaction between the amino groups of CH and the reactive functional groups (at least two) of a suitable crosslinking agent such as dialdehydes, oxalic acid or genipin; eventually other secondary interactions can be found in the hydrogel network. Physical CH hydrogels can be simply formed by polymer dissolution in acidic aqueous media containing monoprotic acids (i.e., hydrochloric and acetic), over concentrations suitable to guarantee the formation of intermolecular interactions and thus a three-dimensional network (entangled hydrogels). Moreover, ionic interactions with negatively charged multifunctional entities (small inorganic and organic ions or ionic polymers) can induce the formation of ionically crosslinked hydrogels. Finally, physical hydrogels can be obtained through secondary interactions of CH chains with polymers such as poly(vinyl alcohol), poly(vinyl pyrrolidone) and poly(ethylene glycol), or through the use of CH derivatives with pendant chains capable of mutual interactions (graft-type hydrogels).

Physical and chemical CH hydrogels present numerous advantages and disadvantages. Chemical hydrogels are permanent, robust and less rapidly degradable networks owing to the presence of covalent crosslinks. On the other hand, for these hydrogels an efficient control of drug release is difficult to achieve in basic conditions, owing to their tendency to swell only in acidic media. Moreover, most crosslinking agents (formaldehyde, epoxy compounds, dialdehyde) are toxic and must be removed before *in vivo* application. Despite physical hydrogels showing weak mechanical properties and uncontrolled dissolution, their ease of production, the good biocompatibility and pH-sensitive swelling/release properties in acidic, but also alkaline environment, make them potent candidates for medical and pharmaceutical applications.

Chemical characteristics (CH molecular mass and deacetylation degree, kind of crosslinker and crosslinking density) can, however, influence the ability of CH hydrogels to swell and control the release of the entrapped drug. Along with chemical characteristics, environmental conditions of the administration site (pH, temperature, ionic strength, volume of water available, presence of enzymatic activity), the choice of dosage form and the nature of the loaded active molecule can modify further the final behavior of hydrogels. As regards nasal drug delivery, the performance of CH hydrogels depends on their ability not only to control drug release, but also to interact with mucus and mucosal epithelium (mucoadhesiveness and permeation-enhancing ability).

3. Chitosan hydrogels as multifunctional excipients for nasal delivery

3.1 Mucoadhesion properties

The term mucoadhesion can be used to describe the attachment of natural or synthetic polymers to a mucosal surface on the basis of attractive and repulsive molecular forces. The mucoadhesion phenomenon has been explained by different theories such as electronic, adsorption, wetting and diffusion theories [31]. In general, three sequential steps characterize the mucoadhesion phenomenon: first, suitable wetting of the biological substrate and swelling of the polymer should induce an intimate contact of the polymer with the tissue; second, interpenetration of the polymer chains and consequently entanglement between the polymer and the mucin chains should be attained; and finally, the formation of weak chemical bonds should be possible [32].

Environmental conditions of the site of administration and physiological variables such as mucus turnover and disease conditions can influence polymer mucoadhesiveness. In particular, environmental pH is extremely important for polymers, whose swelling ability and interaction with the mucus layer depend strongly on the charge and ionization. In the case of nasal administration of CH hydrogels, environmental pH values of ~ 6.0 [33] allow polymer ionization and produce a polymeric conformation and chain mobility suitable for hydration and swelling of the network. These events, along

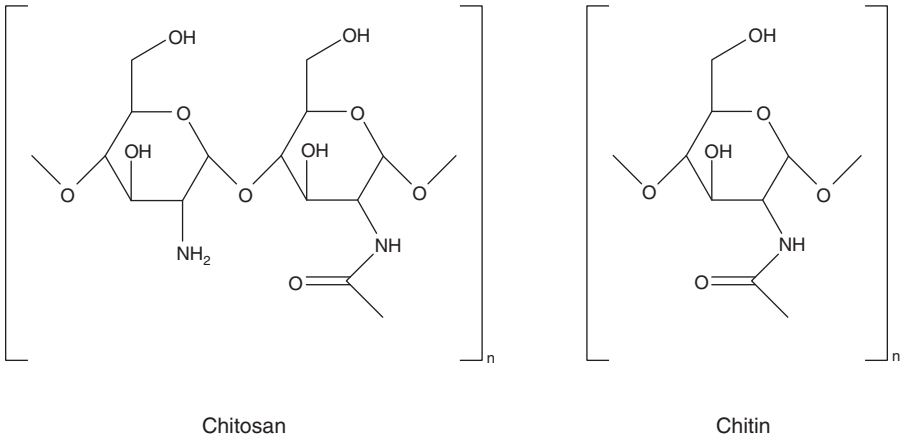

Figure 1. Chemical structure of chitin and chitosan.

with the formation of ionic interactions between the positively charged amino groups (pK_a 6.3) and the anionic moieties of mucin sialic acid (pK_a 2.6) and the formation of secondary bonds (mainly H-bonding and van der Waals attractions), guarantee CH mucoadhesiveness. Factors such as CH molecular mass and deacetylation degree as well as kind of crosslinker and crosslinking density can influence mucoadhesion properties of the hydrogel. In particular, mucoadhesiveness seems to be directly related to the number of free amino groups [26], which can vary for different CH deacetylation degrees and different crosslinking densities. Finally, as mucoadhesion requires suitable free chain length for physical entanglement to occur and availability of multiple sites for mucin attachment [34], CH molecular mass is another important factor that must be considered. In general, higher molecular masses promote stronger mucoadhesion, but the optimum molecular mass also depends on the flexibility and the conformation of CH chains, which must interpenetrate with mucus. Therefore, mucoadhesiveness of high-molecular-mass CH can be reduced by extensive crosslinking or by poor deacetylation degree [35]. Several CH derivatives have been widely studied in order to improve nasal mucoadhesiveness. For example, the immobilization of thiol groups on the primary amino groups of chitosan [36] has been shown to increase its ability to interact with nasal mucosal epithelium owing to the formation of disulfide bonds within the cysteine-rich subdomains of mucus glycoproteins [37]. Finally, as regards the different nasal delivery systems based on CH hydrogels, mucoadhesion can also be influenced by the physical state of the formulation (liquid, semisolid or solid) and by the presence of entrapped drugs able to interact with CH chains. In fact, despite CH having been shown to inhibit transiently the mucociliary clearance [38], this mechanism removes the applied dosage form from the absorption site less or more rapidly according to CH chain availability for interaction with the mucosal epithelium.

3.2 Chitosan as permeation/absorption enhancer

Permeation enhancers are generally used to improve drug bioavailability, providing reversible modifications of nasal epithelium. Low-molecular-mass promoters are generally absorbed through nasal mucosa and can exert undesirable systemic effects, whereas polymeric promoters (high-molecular-mass molecules) show reduced systemic toxicity as they are not absorbed. Moreover, the mechanism of action of the promoter and thus its local safety must be taken into consideration as it can reduce epithelia cell viability and ciliary function. Among polymeric promoters, CH has been found to be substantially safe on nasal epithelium, even if some adverse effects on the epithelial cells have been described and correlated to its high deacetylation degrees and charge density [39]. When applied on the nasal mucosal surface, CH shows penetration enhancement properties owing to its ability to translocate the proteins ZO-1 and occludin from plasma membranes to cytoplasm [40] and to promote a partial alteration of cytoskeleton (redistribution of F-actin) [41], thus opening tight junction complexes. Several studies have reported that only protonated CH is able to open tight junctions, facilitating paracellular transport of drugs [42]. As reported previously, this cationic polymer presents a great degree of ionization at nasal pH values, but its chemical characteristics, such as molecular mass and deacetylation degree, can influence its absorption-enhancing properties. In general, increased molecular mass and deacetylation degree have been shown to improve CH ability to open tight junctions [43]. Thiolated and quaternized CH derivatives have been reported to enhance the permeability of the nasal epithelium [39]. In particular, *N*-trimethyl CH, which has been found to be more soluble in neutral and alkaline environments than CH owing to the presence of fixed positive charges, shows considerable permeation-enhancing ability as a function of its quaternization degree. Also thiolated CH derivatives, along with improved mucoadhesive properties, have

demonstrated greater absorption-enhancing properties than CH itself. Finally, it must be underlined that permeation-enhancing ability of CH hydrogels, as well as mucoadhesion capacity, depends on CH chains' availability for interaction with nasal mucosal epithelium and therefore factors that can influence CH mucoadhesion can also modify its absorption-enhancing properties.

3.3 Control of release

The intranasal route can be used for either acute or chronic therapy over a wide range of lengths of treatment and frequency of administration [44]. Drug release must be modulated according to the therapeutic requests and can be suitably controlled by means of hydrogel-type formulations. After administration in the nasal cavity, CH hydrogels can release the entrapped drug by different mechanisms. Phenomena that can be present during drug release from hydrogel formulations are drug diffusion in the gelled network and hydrolytic or enzymatic degradation of the polymer. In the case of solid dosage forms it is evident that the swelling of the crosslinked polymer is a key factor that contributes to determining the kinetic release of drug [45]. The ability of CH hydrogels to swell in water is mainly related to the presence of hydrophilic groups in the polymeric chains, to the nature of the crosslinker and to the extent of crosslinking. However, environmental conditions and interaction between CH hydrogel and the loaded drug can strongly influence hydrogel ability to hydrate and swell. When drug diffusion into the hydrogel occurs much faster than hydrogel distention, the release kinetic is said to be swelling-controlled. Moreover, the physicochemical characteristics of the loaded molecule (molecular mass, logP, pK_a) can influence the diffusion rate through the network mesh in the swollen state. In the case of CH hydrogel, particularly ionically crosslinked CH, the presence of free positive charges offers the possibility for interaction with negatively charged drugs, thus influencing the swelling and release behavior of the formulation. The environment-sensitive swelling and release behavior of CH hydrogels can be taken into consideration when a versatile drug delivery system is needed for pharmaceutical application. In particular, thermoreversible CH hydrogels have been studied for improving the patient compliance owing to their ability to form transient gel or liquid states depending on the environmental temperature. In fact, these new hydrogels can be useful for nasal drug delivery as they can be easily administered in the nasal cavity as liquids but can be converted into gels at the absorption site. After sol-gel transition at physiological temperature, drug release from thermoreversible CH hydrogels is governed by diffusion of the drug from the gelled matrix [46].

4. Pharmaceutical dosage forms

There are many recent reviews and papers concerning CH pharmaceutical dosage forms for nasal administration of therapeutics. The choice of CH and CH derivatives as excipients for nasal dosage forms is a result of its mucoadhesive properties, enhancing effect ability and its capacity to control drug release. Along with these attractive properties, this natural source polymer can be crosslinked, allowing relatively low cytotoxic and biodegradable hydrogels to be obtained. Chemically or physically crosslinked CH, in which a three-dimensional network can be recognized, can be directly administered in the nasal cavity as gels or suspensions of nanoparticles, but also as solids or liquids able to reproduce gels *in situ*. As regards the physical state of CH-based dosage forms, the following can be considered: (i) liquid formulations such as suspensions of nanoparticles and solutions that can be transformed into gels after administration in the nasal cavity; (ii) semisolid formulations such as gels; and (iii) solid formulations such as powders and inserts that can be transformed into gels after nasal administration. All these formulations require different technological approaches to be produced and suitable devices to be administered intranasally.

4.1 Liquid formulations

Liquid formulations, such as solutions or suspensions of nanoparticles, can be easily prepared and administered through metered dose nasal actuator systems in small volumes. One of the major problems associated with liquid formulations is the chemical and physical stability of the preparation due to the presence of water; the addition of antimicrobial preservatives can be useful but sometimes they may cause irritation of the nasal mucosa, particularly in chronic use. Finally, when the viscosity of these formulations exceeds 500 mPa·s, administration in the nasal cavity can be difficult, although greater mucoadhesion and longer residence time can be obtained.

4.1.1 *Solutions*

As Desbrieres [47] showed, low concentrations of CH solutions show a Newtonian behavior, but increasing the polymer concentration leads to the appearance of a non-Newtonian behavior. This can be explained as being a result of the formation of intermolecular interactions and thus a three-dimensional network, characteristic of entangled hydrogels. Of course the rheological behavior of CH solutions can be influenced by polymer concentration, but also by the molecular mass and the deacetylation degree as well as the temperature. CH solutions have been investigated for nasal administration of insulin (CH molecular mass ~ 100 kDa, 85% deacetylated; concentration < 1.5%, w/v) [48], buspirone (CH hydrochloride 85% deacetylated; concentration < 1%, w/v) [49] and nerve growth factor (CH molecular mass ~ 250 kDa, 75 – 80% deacetylated; concentration < 0.5%, w/v) [50]. Although this review deals exclusively with CH hydrogels in which a three-dimensional network can be recognized, these papers underline the importance of solutions prepared at low CH concentration in improving drug absorption through nasal mucosa (*in vivo* absorption

studies in rats) and favoring brain uptake by means of the olfactory pathway (*in vivo* brain uptake studies in rats).

On the other hand, some CH solutions can be transformed into gels within the desired tissue or body cavity as a result of secondary hydrophobic or hydrogen bonding interactions between polysaccharide chains. Several molecules have been used to accelerate the gelation of CH solutions: β-glycerophosphate as well as polyols or polyoses. An advantage of *in situ* forming formulations is that they can be easily dropped or sprayed into the nasal cavity and spread on the mucosa in a solution state and can be converted into gels under physiological conditions, in response to stimuli such as a temperature variation (temperature-sensitive formulations). However, as observed Schuetz *et al.* [51], storage stability is a critical point concerning the development of commercially viable temperature-sensitive hydrogels if biopolymer interactions cannot be impeded or slowed down during the storage period. Wu *et al.* prepared a thermosensitive hydrogel based on quaternized chitosan and poly(ethylene glycol) for nasal delivery of insulin, using α-β-glycerophosphate as gelling agent [52]. In particular, they found that application of loaded hydrogels in the nasal cavity of rats provided a decrease of blood glucose concentration (40 – 50% of initial blood glucose concentration) for at least 4 – 5 h after administration, without provoking apparent cytoxicity. **Table 1** summarizes CH solutions used for nasal drug delivery.

4.1.2 Suspensions of nanoparticles

Recently, nanosized networks of chemically or physically crosslinked polymers [53-55] have become highly popular owing to their improved potential uses in medicine and pharmacy [56]. In particular, they can be useful because of their ability to protect drugs and macromolecules from degradation in biological media, cross biological barriers and deliver therapeutics to a target site with following controlled release. Further, they have a large surface area that can be used as a conjugation surface to specialize hydrogels for specific targets [57] and can be responsible for the rapid response to physical and biological variations in environmental conditions such as temperature, pH, ionic strength changes or the presence of specific antigens. In fact, because of their small particle size [58], nanoparticles can swell to many times their original dimension or collapse into a compact mass immediately after the appearance of external stimuli. Other essential aspects to take into account are the simplicity of formulation, the prolonged storage of drug formulations in the lyophilized form and the good dispersion stability of the particles. In particular, the stability of nanoparticles in media with conditions of ionic strength and pH similar to those found in biological environments, depends, to a great extent, on the electrical state of the particles [58,59]. Generally, nanosized particles show lower drug loading efficiency than microsized particles; however, the amount of loaded drug can be strongly influenced by its nature, and in the case of hydrogel particles it is generally lower for lipophilic molecules than for hydrophilic molecules.

Polysaccharide-based nanoparticles represent a very promising drug delivery platform, particularly for transmucosal delivery of bioactive molecules [60]. Among the polysaccharides used to this end, CH is known because of its mucoadhesiveness, biocompatibility, low toxicity and biodegradability, and also for its capacity to associate and release therapeutic molecules in their bioactive form, as well as to enhance drug transport across well-organized epithelial barriers. Different methods have been used to prepare CH nanoparticles, including the water-in-oil emulsion method, emulsion-droplet coalescence method, reverse micellar method, desolvation method, ionotropic gelation, self-assembly and complex coacervation [61-64]. Most CH nanoparticles of hydrogel consistency, intended for nasal administration, are obtained by ionic gelation, self-assembly and complex coacervation. These methods offer many advantages, such as simple and mild preparation procedure without the use of organic solvent, heat or vigorous agitation, and thus can be applicable to broad categories of drugs, including sensitive biomolecules. In general, the basis molecular parameters of CH, such as molecular mass and degree of deacetylation, can influence particle size and surface charge [65], whereas drug physicochemical characteristics, such as pK_a and solubility, can affect the entrapment efficiency.

Several works have described the use of CH nanoparticles for insulin nasal delivery. Fernandez-Urrusuno and coworkers prepared insulin-loaded nanoparticles by ionotropic gelation of CH with tripolyphosphate anions [66] and confirmed their ability to enhance the insulin nasal absorption by *in vivo* animal studies (conscious rabbit model). In particular, they found that CH nanoparticles enhance the nasal absorption of insulin to a greater extent than an aqueous solution of CH and they observed that the amount and molecular mass of CH do not have a significant effect on insulin response. The same authors [67] showed that lyophilized insulin-loaded CH nanoparticles can be easily suspended, again preserving their efficacy in lowering plasma glucose levels in rabbits, following intranasal administration. On the contrary, Dyer *et al.* [68] showed that similar nanoparticles do not improve the absorption-enhancing effect of CH with respect to a CH solution or a powder form and that CH powder is the most effective formulation for nasal delivery of insulin in the sheep model. More recently, Wang and co-authors [69] investigated CH-*N*-acetyl-L-cysteine nanoparticles, prepared by gelation with tripolyphosphate, for the nasal delivery of insulin. They reported that the physicochemical properties of the nanoparticles are affected by the number of thiol groups present and that intranasal administration of CH-*N*-acetyl-L-cysteine nanoparticles in rats enhances the absorption of insulin with respect to nanoparticles prepared with unmodified CH and a control insulin solution. In a recent paper Mao *et al.* [70] showed that insulin nanocomplexes prepared by self-assembly [71] between insulin and CH derivatives (PEGylated trimethyl-CH) do not seem to enhance nasal permeation in diabetic rats, with respect to a simple insulin

Table 1. Summary of CH solutions for nasal drug delivery.

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Solution (CH conc. < 1.5%, w/v)	CH Mol. mass ~ 100 kDa, DD 85%	CH hydrochloric acid	Dissolution	Porcine zinc insulin	*In vitro* permeation (rabbit nasal mucosa) and *in vivo* bioavailabilty (rats)	[48]
Solution (CH conc. < 1.0%, w/v)	CH hydrochloride DD 85%	CH hydrochloride	Dissolution	Buspirone hydrochloride	*In vivo* bioavailabilty (rats) and *in vivo* brain uptake (rats) by gamma scintigraphy	[49]
Solution (CH conc. < 0.5%, w/v)	CH Mol. mass ~ 250 kDa, DD 75 – 80%	CH acetic acid	Dissolution	Nerve growth factor	*In vitro* permeation (bovine olfactory epithelium) and *in vivo* brain uptake (rats) by microdialysis probe	[50]
Solution (CH conc. 0.5%, w/v)	CH glutamate Mol. mass 205 kDa	CH glutamate	Dissolution	Human zinc insulin	*In vivo* bioavailability (rats and sheep)	[68]
In situ gelling solution (CH conc. 3.6%)	CH Mol. mass > 780 kDa, DD 89% HT CH chloride QD ~ 50%	HT CH chloride poly(ethylene glycol) α-β-glycerophosphate lactic acid	Dissolution	Recombinant human insulin	*In vitro* release, *in vivo* uptake in nasal epithelial cells (rats), *in vivo* viability of nasal epithelial cells (rats) and *in vivo* bioavailabilty (rats)	[52]

CH: Chitosan; DD: Deacetylation degree; HT CH chloride: *N*-[(2-hydroxy-3-trimethylammonium) propyl] CH chloride; Mol. mass: Molecular mass; QD: Quaternization degree.

solution. CH nanoparticles for insulin nasal delivery have also been prepared by ionotropic gelation with tripolyphosphate and concomitant complexation with sodium alginate [72]. These nanoparticles have the capacity to enhance insulin absorption after nasal administration to conscious rabbits, with respect to a control solution of drug. Moreover, the results indicate that the presence of alginate in the nanoparticles leads to a prolonged hypoglycemic response for up to 5 h. A new type of nanoparticle [73] consisting of CH and sulfobutylether-β-cyclodextrin or carboximethyl-β-cyclodextrin seems to be a promising nanocarrier for nasal delivery of insulin. The nanoparticles show permeation-enhancing properties, and are able to enter the nasal mucosa and to transport insulin across the nasal barrier, leading to a significant decrease in the plasma glucose levels (*in vivo* bioavailability studies in rats). Two excellent reviews from Illum [18] and Mistry and co-workers [21] underline the lack of literature data confirming the transmucosal movement of nanoparticles in the nasal epithelia, and report that most studies are limited to comparing nanoparticle formulations with solution of drug alone, without investigating further the actual mechanism of enhancement of drug permeation through nasal mucosa.

CH nanospheres have been evaluated extensively as vaccine carriers. Vila *et al.* [74] prepared hydrophobic particles coated with hydrophilic polymers such as PEG or CH and particles made solely of CH to evaluate their ability to deliver vaccines across the nasal and intestinal mucosa. In particular, CH nanoparticles obtained by ionotropic gelation with tripolyphosphate were found to be stable on incubation with lysozyme and more efficient, following intranasal administration to conscious mice, at improving the local and systemic immune responses to tetanus toxoid, with respect to an antigen solution. The same authors [75] evaluated the potential utility of different molecular mass CH (23, 38 and 70 kDa) nanoparticles (ionotropic gelation with tripolyphosphate) as long-term nasal vaccine carriers. Following intranasal administration to mice, nanoparticles loaded with tetanus toxoid show an increasing and long-lasting humoral immune response and a significantly higher mucosal response at 6 months post-administration than that obtained for the fluid vaccine. Moreover, different molecular masses of CH were not found to affect nanoparticle ability to provide improved access to the associated antigen to the immune system. CH nanoparticles [76], prepared by the desolvation method with sodium sulfate and loaded by adsorption with ovalbumin, were administered intranasally in rats, inducing a significantly higher immune response (IgG and IgA increase) compared with a control formulation containing ovalbumin and CH. Finally, Amidi *et al.* [77] prepared *N*-trimethyl-CH nanoparticles by ionic crosslinking (with or without ovalbumin) with tripolyphosphate. They performed *in vivo* uptake studies in a rat model, in which the transport of fluorescent nanoparticles across the nasal mucosa was confirmed by confocal laser scanning microscopy. This work confirms that nanoparticles

can be internalized by the nasal epithelial cells and transported to submucosal layer and makes these new particles promising carriers for nasal delivery of high-molecular-mass molecules such as vaccines. Nasal mucosal immunization can also be achieved by the use of plasmid DNA-loaded CH nanoparticles, due to CH's ability to form nanosized ionic complexes with the negatively charged phosphate groups of nucleic acids. Iqbal and co-workers [78] reported that intranasal immunization with plasmid DNA formulated with CH induced peptide- and virus-specific cytotoxic T-lymphocyte responses in mice that were comparable to those induced by means of intradermal immunization. Moreover, Khatri *et al.* [79] investigated the preparation of CH nanoparticles by complex coacervation for nasal mucosal immunization against hepatitis B and showed their potential in producing humoral (both systemic and mucosal) and cellular immune responses on nasal administration in mice.

The use of CH nanoparticles for nasal delivery has also been proposed for small molecules such as estradiol [80]. Estradiol CH nanoparticles were prepared (ionotropic gelation of CH with tripolyphosphate anions) with the aim of reducing nasal mucociliary clearance and enhancing estradiol permeation, thus improving its bioavailability, especially for brain targeting. It was found that estradiol levels in plasma and CSF after intranasal administration are significantly lower and higher, respectively, than those obtained after intravenous injection and that estradiol can arrive at the brain tissue earlier following intranasal administration (*in vivo* bioavailability and brain uptake studies in rats). This work underlines the importance of CH nanoparticles for nose-to-brain delivery and confirms that these non-toxic and mucoadhesive vehicles are able to improve drug access to the brain. **Table 2** summarizes CH nanoparticle suspensions used for nasal drug delivery.

4.2 Semisolid formulations

Gels allow intimate contact with the mucosa surface, prolonging the residence time and improving drug bioavailability with respect to solutions. Moreover, CH gels can be easily prepared by polymer solubilization in acidic aqueous media starting from suitable concentrations. Nevertheless, these semisolid formulations can be difficult to administer because of their high viscosity and are unable to provide an efficient control of drug release owing to their tendency to dissolve.

Varshosaz *et al.* [81] studied the nasal release of insulin from different CH gels obtained with two CH concentrations (2 and 4% in acetic acid solution) and molecular masses (150 and 400 kDa) and with two permeation enhancers (lecithin and ethylendiamine tetra-acetic acid). They found that the gel composed of 2% CH (400 kDa) and ethylendiamine tetra-acetic acid, which shows good control of drug release and great mucoadhesive properties, can increase insulin absorption, thus reducing glucose level with respect to the intravenous route (*in vivo* studies in rats). An interesting recent work from Alsarra *et al.* [82] underlines the disadvantages of the use of chemical CH hydrogels when the crosslinking agent is potentially toxic. The authors evaluated different mucoadhesive polymeric hydrogels (poly-*N*-vinyl-2-pyrrolidone, chitosan and carbopol) for nasal delivery of acyclovir. In particular, they found that CH crosslinked with glutaraldehyde provides mild inflammation, loss of ciliary processes and congestion of vessels of respiratory epithelium, whereas nasal mucosal damages are less severe in the presence of poly-*N*-vinyl-2-pyrrolidone crosslinked by γ-irradiation (*in vivo* histopathological investigation, sheep nasal mucosa). Of course this behavior can be correlated to the use of glutaraldehyde as crosslinking agent during CH hydrogel production. **Table 3** summarizes CH gels used for nasal drug delivery.

4.3 Solid formulations

Solid formulations can be obtained by means of different technological approaches, in various forms such as powders and inserts. When these formulations are based on crosslinked CH, they can be transformed into gels after administration in the nasal cavity. They have advantages over liquid formulations, such as larger amounts of drug per dose delivered, higher drug concentration on the mucosa, longer absorption time in the nasal cavity and great chemical and physical stability during product distribution and storage.

4.3.1 Powders

Most CH solid formulations for intranasal administration consist of powders > 1 μm in size (microparticles) that can be produced by various methods (i.e., spray-drying, spray-freeze-drying, emulsion crosslinking and emulsification/solvent evaporation). Of course several factors, such as the nature of the active molecule, particle size, geometry and morphology requirement and residual toxicity associated with the final product, must be evaluated for the selection of a suitable preparative method. Moreover, particle size distribution, shape and density can influence the site and amount of particle deposition in the nasal cavity [83]. Although particles with diameter > 10 μm are more likely to be effectively deposited in the nasal cavity, the physiological nose ability to filter the inspired air and prevent particles from entering the lungs allows the collection of particles > 0.5 μm in the nose as well [84]. Hence, fine microparticles with mean diameter < 10 μm, as well as particles with larger diameter, can be suitable for nasal administration. However, the site of deposition and the deposition area depend on several parameters that are also related to the delivery device. For these reasons, the choice of an appropriate administration device is extremely important and must be considered carefully [85].

Powders, granules or agglomerates can be obtained from drug/excipient solutions, suspensions or emulsions by spray-drying. This technique consists of the drying of atomized droplets in a stream of hot air. CH microparticles can be obtained by dissolving the polymer in aqueous acidic solution in the presence of active molecules (solubilized or dispersed),

Table 2. Summary of CH nanoparticle suspensions for nasal drug delivery.

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Nanoparticle suspension	CH hydrochloride Mol. mass$_1$ < 50 kDa, DD$_1$ 87%; mol. mass$_2$ 130 kDa, DD$_2$ > 70%	CH hydrochloride pentasodium tripolyphosphate	Ionotropic gelation	Bovine insulin	*In vitro* release and *in vivo* bioavailabilty (albino rabbits)	[66]
Nanoparticle suspension	CH hydrochloride Mol. mass 130 kDa, DD$_2$ > 70%	CH hydrochloride pentasodium tripolyphosphate	Ionotropic gelation	Bovine insulin	*In vitro* release and *in vivo* bioavailabilty (albino rabbits)	[66]
Nanoparticle suspension	CH glutamate Mol. mass 205 kDa	CH glutamate pentasodium tripolyphosphate	Ionotropic gelation	Human zinc insulin	*In vivo* bioavailability (rats and sheep)	[68]
Nanoparticle suspension	CH Mol. mass 20 kDa, DD 86% CH-*N*-acetyl-L-cysteine free thiol groups 60 – 97%	CH or CH-*N*-acetyl-L-cysteine pentasodium tripolyphosphate	Ionotropic gelation	Insulin	*In vitro* mucoadhesion, *in vitro* swelling, *in vitro* release and *in vivo* bioavailability (rats)	[69]
Nanocomplex suspension	CH Mol. mass$_1$ 400 kDa, DD$_1$ 85.1%; mol. mass$_2$ 100 kDa, DD$_2$ 85.4%; mol. mass$_3$ 50 kDa, DD$_3$ 89.9% Trimethyl CH QD$_2$ 39.3%; QD$_3$ 39.6% PEGylated trimethyl CH GR$_2$ 58.5 or 66.1%; GR$_3$ 59.2 or 64.9%	CH or Trimethyl CH or PEGylated trimethyl CH	Self-assembly	Insulin	*In vitro* release, *in vitro* cell uptake and permeation (Caco-2) and *in vivo* bioavailability (rats)	[70]
Nanoparticle suspension	CH hydrochloride Mol. mass ~ 119 kDa, DD ~ 86%	CH hydrochloride sodium alginate pentasodium tripolyphosphate	Ionotropic gelation/ complexation	Insulin from bovine pancreas	*In vitro* release and *in vivo* bioavailability (rabbits)	[72]
Nanoparticle suspension	CH hydrochloride Mol. mass 110 kDa, DD 86%	CH hydrochloride SB-β-CD or CM-β-CD ± pentasodium tripolyphosphate	Ionotropic gelation	Insulin from bovine pancreas	*In vivo* cell uptake (rat nasal epithelial cells) and *in vivo* bioavailability (rats)	[73]
Nanoparticle suspension	CH hydrochloride Mol. mass > 50 kDa, DD 87%	CH hydrochloride pentasodium tripolyphosphate	Ionotropic gelation	Tetanus toxoid	*In vivo* bioavailability (rats) and biodistribution (lymph nodes) and *in vivo* immunization (IgG and IgA, mice)	[74]
Nanoparticle suspension	CH hydrochloride Mol. mass$_1$ 23 kDa, DD$_1$ 85.1%; mol. mass$_2$ 38 kDa, DD$_2$ 85.4%; mol. mass$_3$ 70 kDa, DD$_3$ 89.9%	CH hydrochloride pentasodium tripolyphosphate	Ionotropic gelation	Tetanus toxoid	*In vitro* release and *in vivo* immunization (IgG and IgA, mice)	[75]
Nanoparticle suspension	CH Mol. mass$_1$ 10 kDa, DD$_1$ ~ 80%; mol. mass$_2$ 100 kDa, DD$_2$ ~ 80%; mol. mass$_3$ 500 kDa, DD$_3$ ~ 80%	CH acetic acid sodium sulfate	Desolvation method	Ovalbumin or cholera toxin	*In vivo* immunization (IgG and IgA, rats)	[76]

CH: Chitosan; CM-β-CD: Carboximethyl-β-cyclodextrin; DD: Deacetylation degree; GR: Graft ratio; Mol. mass: Molecular mass; QD: Quaternization degree; SB-β-CD: Sulfobutylether-β-cyclodextrin.

Table 2. Summary of CH nanoparticle suspensions for nasal drug delivery (continued).

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Nanoparticle suspension	CH Mol. mass 177 kDa, DD 93% Trimethyl CH QD 25%	Trimethyl CH pentasodium tripolyphosphate	Ionotropic gelation	Ovalbumin	*In vitro* release, *in vitro* cell viability (Calu-3 cells) and *in vivo* uptake (rat nasal epithelial cells)	[77]
Nanoparticle suspension	CH Mol. mass 400 kDa, DD 85%	CH acetic acid	Complex coacervation	Plasmid DNA	*In vitro* cell transfection (HeLa cells) and *in vivo* immunization (IgG and IgA, mice)	[78]
Nanoparticle suspension	CH hydrochloride Mol. mass 110 kDa, DD 87%	CH hydrochloride	Complex coacervation	Plasmid DNA	*In vivo* immunization (mice)	[79]
Nanoparticle suspension	CH Mol. mass 50 kDa, DD 95%	CH acetic acid pentasodium tripolyphosphate	Ionotropic gelation	Estradiol	*In vivo* bioavailability (rats) and *in vivo* brain uptake (rats) by microdialysis probe	[80]

CH: Chitosan; CM-β-CD: Carboximethyl-β-cyclodextrin; DD: Deacetylation degree; GR: Graft ratio; Mol. mass: Molecular mass; QD: Quaternization degree; SB-β-CD: Sulfobutylether-β-cyclodextrin.

and eventually an appropriate crosslinking agent, and subsequently atomizing and drying this solution or dispersion. Particles are formed immediately after solvent evaporation due to the stream of hot air [86]. Particles size can be modulated by varying nozzle size, spray flow rate, atomization pressure, inlet air temperature, as well as CH concentration, nature and extent of the crosslinking agent, if present. Gavini *et al.* [87] used a spray-drying method to prepare metoclopramide-loaded microspheres composed of CH hydrochloride, sodium alginate, or a combination of the two polysaccharides. The morphology, swelling behavior, mucoadhesive properties, drug release from microparticles and *ex vivo* drug permeation (sheep nasal mucosa) were evaluated. All spray-dried microparticles show good mucoadhesive properties, whereas release, swelling and permeation behavior are found to be influenced by particle composition. In particular, the formation of chitosan/alginate complex led to control of drug release from microparticles. The same authors [88] obtained spray-dried metoclopramide-loaded microparticles based on 5-methyl-pyrrolidinone-CH. They compared these innovative microparticles with particles made by unmodified CH and showed that CH derivatives allow greater mucoadhesiveness, less swelling capacity and more prolonged *ex vivo* permeation profile (sheep nasal mucosa) to be obtained. Interestingly, they found that these microparticles are able to provide gelling systems in the range of pH tested (5.5 – 7.4), owing to the formation of ionic interactions between the positive charge of 5-methyl-pyrrolidinone-CH and the negative charge of anions present in the aqueous buffer. The anions act as crosslinkers between the polymeric chains and provide the *in situ* formation of ionically crosslinked hydrogels. Several works have reported the preparation of microparticles based on CH salts, such as hydrochloride and glutamate, obtained by the spray-drying method without the use of crosslinking agents or anionic polymers as conterions. Despite swelling or water uptake studies not having been performed, some authors have reported release/dissolution studies underlying the formation of gels or viscous layers able to control drug diffusion from the microparticles [89-91]. In fact, a high-concentrate CH layer can be obtained around the particle, leading to the formation of entangled hydrogels in which the drug slowly diffuses. On the contrary, CH salts have been found to act as dissolution rate enhancer of drugs poorly soluble in water. In fact, Gavini *et al.* [92] described the improvement of carbamazepine dissolution rate with respect to the pure drug. This behavior can be due to drug amorphization during the spray-drying process used for microparticle production.

Spray-freeze-drying consists of nebulizing an aqueous solution, containing drug and polymer, into a cryogenic medium (liquid nitrogen), thus generating a dispersion of frozen particles that can be subsequently dried in a lyophilizer. This procedure allows the production of large porous and uniform particles suitable for nasal delivery. Garmise *et al.* [93] used this innovative technique to obtain dry powder

Table 3. Summary of CH gels for nasal drug delivery.

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Gel (CH conc. 2 or 4%)	CH Mol. mass$_1$ 150 kDa Mol. mass$_2$ 400 kDa	CH acetic acid ± lecithin ± EDTA	Dissolution	Human zinc insulin	*In vitro* release, *in vitro* mucoadhesion and *in vivo* bioavailability (rats)	[81]
Gel (CH conc. 2%)	CH Mol. mass 103.2 kDa, DD 76.6%	CH lactic acid glutaraldehyde	Dissolution and chemical crosslinking	Acyclovir	*ex vivo* mucoadhesion (sheep nasal mucosa), *in vitro* release and *in vivo* histopathological investigation (sheep nasal mucosa)	[82]

CH: Chitosan; DD: Deacetylation degree; EDTA: Ethylendiamine tetra-acetic acid; Mol. mass: Molecular mass.

formulation for nasal delivery of influenza vaccine. They found that dry powder based on CH as mucoadhesive excipient is more stable with respect to liquid formulations. Moreover, *in vivo* studies in rats demonstrated that powder formulation can increase the residence time in the nasal cavity and improve serum and mucosal antibody response.

In the emulsion crosslinking method, a CH aqueous solution is emulsified in an oil phase, thus producing a water-in-oil emulsion, which can be stabilized using an appropriate surfactant. The aqueous droplets containing CH are than hardened using a crosslinking agent such as glutaraldehyde. Finally, microparticles are filtered, washed and dried [94]. As the size of aqueous droplets can be controlled by mean of surfactant concentration and speed of stirring during the formation of emulsion, particle size can be easily modified. However, the nature and the extent of crosslinking agent can also influence particle size. Sankar *et al.* [95] produced CH microspheres for intranasal delivery of pentazocine. They evaluated the influence of parameters such as particle size and loading efficiency on swelling and bioadhesion capacity and drug release characteristics as well as pentazocine bioavailability during *in vivo* studies in a rabbit model. In particular, they observed a controlled and sustained drug release and an improved bioavalability with respect to intravenous, oral and nasal administration of a solution of pure drug. Jain *et al.* [96] prepared mucoadhesive CH microspheres by an emulsion crosslinking method (glutaraldehyde) and studied the effects of drug, citric acid and permeation enhancer concentration on their physicochemical and functional properties. In particular, the *in vivo* performance (bioavailability in rabbits) of microspheres revealed a prolonged and controlled release of salbutamol. The same authors [97] described the use of similar microspheres for nasal delivery of insulin. *In vivo* studies (bioavailability in rabbits) show that glutaraldehyde crosslinked microspheres provide greater reduction of blood glucose level than citric acid crosslinked microspheres, showing a prolonged and controlled release of drug.

The emulsification/solvent evaporation method involves different steps and the use of surfactants to stabilize the emulsion, generally a water-in-oil emulsion. In particular, the emulsification of a polymer solution containing the active molecule is followed by particle hardening through solvent evaporation and polymer precipitation. During the emulsification process, homogenization, sonication or whirl mixing produced microdroplets, which are responsible for microparticles' size distribution. Lim and co-workers obtained microparticle formulations consisting of hyaluronic acid, CH and a combination of the two polysaccharides by this technique [98,99]. They performed *in vitro* and *in vivo* (bioavailability in rabbits) studies for gentamicin delivery in the nasal cavity and found a synergistic effect in combining hyaluronic acid and CH to form microparticles able to obtain a high bioavailability and prolonged release of the model drug. **Table 4** summarizes CH powders used for nasal drug delivery.

4.3.2 Inserts

Nasal inserts are solid formulations that can be administrered in the posterior of the nasal cavity [100,101] where they can be transformed rapidly into gels, thus avoiding the foreign body sensation. With respect to powders, for which precision of dosage is difficult to achieve, these new nasal systems are able to deliver an exact dose of drug into the nasal cavity. Owing to CH mucoadhesive properties, nasal inserts based on this polymer can adhere to the mucosa and prevent rapid clearance of the drug, thus increasing its residence time. Moreover, good control of drug release can be achieved through the crosslinked CH's ability to form a gelled network in which the drug can diffuse. Finally, there is no need to remove the inserts mechanically from the nasal cavity because, after gel formation, they can be easily eliminated towards the nasopharynx by the mucociliary clearence. As nasal inserts are generally obtained by freeze-drying, which consists of sublimation of the frozen water yielding the formation of pores or channels in the polymer, they are characterized by a sponge-like structure. The sponge-like structure of nasal inserts is extremely important to ensure rapid rehydration and gelation at the administration site.

Several works have reported the use of nasal inserts based on a well-known mucoadhesive gelling polymer such as

Table 4. Summary of CH powders for nasal drug delivery.

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Powder	CH glutamate Mol. mass 205 kDa	CH glutamate	Blending in a mortar mill	Human zinc insulin	*In vivo* bioavailability (sheep)	[68]
Microspheres	CH hydrochloride Mol. mass 160 kDa, DD 86%	CH hydrochloride ± sodium alginate	Spray-drying	Metoclopramide hydrochloride	*In vivo* swelling, *in vitro* mucoadhesion, *in vitro* release and *ex vivo* permeation (sheep nasal mucosa)	[87]
Microspheres	CH food grade 90 Methylpyrrolidinone CH	CH acetic acid or methylpyrrolidinone CH	Spray-drying	Metoclopramide hydrochloride	*In vitro* swelling, *in vitro* mucoadhesion, *in vitro* release and *ex vivo* permeation (sheep nasal mucosa)	[88]
Microspheres	CH Mol. mass$_1$ 40 kDa, DD$_1$ ~ 96%; mol. mass$_2$ 480 kDa, DD$_2$ ~ 96%; mol. mass$_3$ 850 kDa, DD$_3$ ~ 96%	CH hydrochloric acid	Spray-drying	Methotrexate	*In vitro* mucoadhesion, *in vitro* release and *in vitro* nasal ciliotoxicity (toad palate model)	[89]
Microparticles	CH Mol. mass 150 kDa, DD > 85% CH glutamate Mol. mass$_1$ < 200 kDa, DD$_1$ 75 – 90%; mol. mass$_2$ 200 – 600 kDa, DD$_2$ 75 – 90%	CH or CH glutamate acetic acid	Spray-drying	Zolmitriptan	*In vitro* dissolution	[90]
Microspheres	CH hydrochloride Mol. mass 160 kDa, DD 86%	CH hydrochloride	Spray-drying	*N*-cyclopentiladenosine	*In vitro* and *ex vivo* mucoadhesion (sheep nasal mucosa), *in vitro* dissolution and release, *ex vivo* permeation (sheep nasal mucosa) and *in vivo* bioavailability and brain distribution (rats)	[91]
Microspheres	CH hydrochloride Mol. mass 160 kDa, DD 86% CH glutamate	CH hydrochloride or CH glutamate	Spray-drying	Carbamazepine	*In vitro* release and *in vivo* bioavailability (sheep)	[92]
Dry powder	CH Mol. mass 161 kDa, DD 92%	CH	Spray-freeze-drying	Inactivated influenza virus	*In vitro* release studies and *in vivo* bioavailability studies (*rats*)	[93]
Microspheres	CH	CH acetic acid glutaraldehyde	Emulsion crosslinking	Pentazocine	*In vitro* swelling, *in vitro* bioadhesion (rabbit small intestine), *in vitro* release and *in vivo* bioavailability (rabbits)	[95]
Microspheres	CH	CH acetic acid glutaraldehyde ± citric acid ± sodium taurocholate	Emulsion crosslinking	Salbutamol	*In vitro* mucoadhesion, *in vitro* release and *in vitro* permeation (she goat intestinal mucosa) and *in vivo* bioavailability (rabbits)	[96]
Microspheres	CH	CH acetic acid glutaraldehyde or citric acid	Emulsion crosslinking	Insulin	*In vitro* swelling, *ex vivo* mucoadhesion (she goat intestinal mucosa), *in vitro* release, *ex vivo* permeation (she goat intestinal mucosa) and *in vivo* bioavailability (rabbits)	[97]
Microspheres	CH DD 85% CH hydroglutamate Mol. mass 180 – 230 kDa	CH or CH hydroglutamate ± hyaluronic acid	Emulsification/ solvent evaporation	Gentamicin sulfate	*In vitro* release, *ex vivo* mucoadhesion (frog palate model) and *in vivo* bioavailability (rabbits)	[98] [99]

CH: Chitosan; DD: Deacetylation degree; Mol. mass: Molecular mass.

Table 5. Summary of CH inserts for nasal drug delivery.

Type of dosage form	Chitosan characteristics	Hydrogel network composition	Method of preparation	Loaded drug	*In vitro, ex vivo* and *in vivo* studies	Ref.
Inserts	CH glutamate	CH glutamate	Freeze-drying	Oxymetazoline hydrochloride	*In vitro* water uptake, *in vitro* bioadhesion and *in vitro* release	[105]
Inserts	CH Mol. $mass_1$ 150 kDa, DD_1 97%; mol. $mass_2$ 600 kDa, DD_2 90%	CH acetic acid hyaluronic acid	Freeze-drying	Insulin or vancomycin	*In vitro* water uptake, *in vitro* mucoadhesion and *in vitro* release	[106]
Inserts	CH Mol. mass 150 kDa, DD 97%	CH acetic acid pectin	Freeze-drying	Chlorpromazine hydrochloride	*In vitro* water uptake, *in vitro* mucoadhesion, *in vitro* release and *ex vivo* permeation (sheep nasal mucosa)	[107]

CH: Chitosan; DD: Deacetylation degree; Mol. Mass: Molecular mass.

hydroxypropyl methylcellulose [102-104]. Bertram and Bodmeier reported the formulation of nasal inserts [105] based on different polymers comprising CH glutamate. They found that the type of polymer chosen for insert preparation influences the mechanical properties of the inserts, their water uptake behavior and bioadhesion potential and their ability to control drug release. *In vitro* studies revealed that CH is able to form a thin film on mucosal substrates (agar/mucin gel), owing to its opposite charge to mucin and agar, but it is less efficient at prolonging the contact time with the mucosa with respect to carrageenan, Carbopol and other negatively charged polymers. The authors suggest that the good bioadhesion abiliy of these polymers is related to a good balance between available hydrogen bonding sites and an open expanded conformation. In a recent work, Luppi *et al.* [106] investigated the use of nasal inserts based on CH/hyaluronate complexes for peptide and protein delivery. CH/hyaluronate polyelectrolyte complexes were obtained in different preparative conditions and used for the preparation of lyophilized cone-like shaped inserts loaded with vancomycin or insulin. Drug release from *in situ* gelling systems is a complex phenomenon of water uptake, polymer chain relaxation, swelling, drug–polymer interactions, drug dissolution and diffusion through the rehydrated insert. The results indicate that the selection of suitable conditions for preparation of the complexes allows modulation of insert functional properties. Moreover, the water uptake ability of the different complexes can be influenced by the presence of insulin or vancomycin in nasal inserts, owing to the possibility of ionic interactions between drugs and polyelectrolyte complexes. On the contrary, insulin and vancomycin release from the gelled inserts seemed to be affected by the molecular mass of the drug but not by the presence of drug–complex interactions. In another work, Luppi [107] described the use of CH/pectin polyelectrolyte complex for the formulation of chlorpromazine-loaded nasal inserts. The results show that higher amounts of pectin in the complexes, with respect to higher amounts of CH, produced a more evident porous structure of the nasal inserts, improving water uptake ability and mucoadhesion capacity. Finally, the presence of increasing amounts of pectin allows interaction with chlorpromazine hydrochloride, inducing the formation of less hydratable inserts, thus prolonging drug release and permeation. These investigations contribute to verifying the formation of polyelectrolyte complexes between CH and anionic polysaccharides (hyaluronic acid and pectin) at pH values in the vicinity of the pK_a interval of the two counterions and to confirming their potential use for nasal drug delivery. **Table 5** summarizes CH inserts used for nasal drug delivery.

5. Expert opinion

CH hydrogels can be obtained in various dosage forms with different physical state with the aim of improving the therapeutic efficacy of nasally administered drugs. The characteristic three-dimensional network of hydrogels plays an important role and can guarantee the suitable performance of the final dosage form when applied in the nasal cavity. In fact, the kind of crosslinker, the entity and the nature of the crosslinking can drastically modify CH hydrogels' mucoadhesive and permeation-enhancing properties as well as their ability to control the release of the entrapped drug. As can be observed from the tables in this review, the most commonly used crosslinkers for nasal CH hydrogels are pentasodium tripolyphosphate, especially for nanoparticles preparation, and anionic polymers such as sodium alginate, pectin and hyaluronic acid, useful for obtaining nanoparticles, microparticles, or inserts. Moreover, numerous CH salts (hydrochloride, acetate, glutamate, lactate and citrate) are used for the formulation of semisolid and solid entangled hydrogels. In general, most works report a particular dosage form (nanoparticles rather than microparticles or gels) based on a particular CH

hydrogel for nasal delivery of a specific drug. In these studies design parameters relative to the technological approach and to the composition of the dosage form are modified, with the aim of obtaining the best performing formulation. However, these studies are often limited to comparing the dosage form with a solution of drug alone, without taking into account the importance of a control solution, containing drug and CH, for an in-depth investigation of the mechanism that stands on the basis of the results. Moreover, there is a lack of literature data regarding the evaluation of different dosage forms containing the same drug and based on the same crosslinked CH hydrogel. A comparison between data from different research groups seems to be impractical, owing to the presence of experimental differences such as the characteristics of the CH used, the origin of the loaded drug (porcine zinc insulin rather than bovine insulin or recombinant human insulin), the choice of the crosslinking molecule and the final composition of the formulation. In this review it is also evident that the evaluations that are generally performed include water uptake/swelling studies, release/dissolution studies, mucoadhesion studies, permeation studies, uptake studies in nasal epithelial cells, viability studies of nasal epithelial cells and bioavailabilty studies; other more specific investigations are related to the particular loaded drug (immunization studies for vaccines or brain uptake studies by microdialysis probe for nose-to-brain drug delivery). Of course, several studies have also been performed to obtain a physicochemical characterization of the dosage form in order to have a more comprehensive discussion of the results. However, other critical points that must be taken into consideration when data obtained by different laboratories need to be compared are the different *in vitro, ex vivo* and *in vivo* methods and the various experimental conditions used. In particular, *in vivo* studies are generally performed on animals of different species (mice, rat, rabbit and sheep).

Along with the above considerations, it must be underlined that, despite several nasal formulations being commercially available for systemic delivery of low-molecular-mass drugs, there is actually only one product on the market for nasal delivery of macromolecules (salmon calcitonin, Miacalcin®, Novartis) [108]. In fact, systemic bioavailability of high-molecular-mass molecules through the nasal route is dramatically dependent on the presence of permeation promoters. Several studies in animal models show that CH hydrogels are promising and relatively safe enhancer excipients owing to their ability to modulate nasal epithelial permeability and to prolong the residence time of the formulation in the nasal cavity [2,14]. However, it is evident that there is a lack of human data on drug bioavailability following intranasal administration of CH hydrogels and, more generally, of delivery systems unlike liquid formulations containing promoters, such as bile salts and phospholipids. Nevertheless, in the authors' opinion there is enough scientific evidence for the potentiality of CH and CH hydrogels in nasal drug delivery and this contribution is intended to stimulate further investigations in order to achieve collection of harmonized data and concrete clinical perspectives.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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