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New Approaches to Improve the Intranasal Absorption of Insulin

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Abstract

Insulin is essential for type 1 and advanced type 2 diabetics to maintain blood glucose levels and prolong lives. Due to its large molecular weight and short half-life, it has been usually administered subcutaneously accompanied with side effects such as the possibility of hypoglycemia episodes, weight gain, pain, local tissue necrosis, infection, nerve damage and inadequate post meal glucose control. In order to overcome these limitations, alternative delivery routes of insulin are expected to provide better safety and compliance for the patient. Non-invasive insulin delivery system represents one of the most challenging goals for pharmaceutical industry. Nasal insulin delivery has been extensively studied as an alternative to subcutaneous injection for the treatment of diabetes. The pharmacokinetic profile of nasal insulin is similar to that obtained by intravenous injection. Nasal drug administration has been used as an alternative route for the local or systemic availability of drugs restricted to intravenous administration. This is due to the large surface area, porous endothelial membrane, high total blood flow, the avoidance of first-pass metabolism and ready accessibility. The nasal administration of drugs, including numerous compound, peptide and protein drugs, for systemic medication has been widely investigated in recent years. Drugs are cleared rapidly from the nasal cavity after intranasal administration, resulting in rapid systemic drug absorption. This review describes the main barriers preventing nasal insulin absorption and special attention is given to new approaches to improve the intranasal absorption of insulin, including the application of new safe absorption enhancers and the use of appropriate delivery systems. It seems that bioadhesive delivery systems or water-insoluble powders with absorption enhancers are the most promising methods for intranasal delivery of insulin.

Keywords: Insulin, Nasal drug administration, Hypoglycemia episodes, Pharmacokinetic profile

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Introduction

Diabetes mellitus is a disorder of glucose regulation characterized by accumulation of glucose in the blood [1, 2]. In 2013, 382 million people throughout the world suffered from diabetes and this number is estimated to be 592 million by 2035 [3, 4]. Insulin therapy is essential in the treatment of patients with insulin-dependent diabetes (type 1) and for many patients with non-insulin-dependent diabetes (type 2) [5, 6]. As a standard administration method, patients with type 1 must self administer insulin subcutaneously once or multiple times per day. Insulin treatment is also required during the later stages of type 2 diabetes to maintain glycemic control [7-9]. Frequent subcutaneous injections are always associated with pain, tenderness, local tissue necrosis, microbial contamination, and nerve damage [10-12]. In recent decades, a number of alternative insulin delivery methods involving micro- or nanotechnologies have been developed to overcome the limitations and drawbacks of conventional delivery [13-17] Particularly methods that can achieve non-invasive administration [13, 18]. Long-term release [19] or closed-loop based smart delivery [17, 20] are highly desirable. They hold great potential to significantly improve the quality of life for diabetics. The main alternatives studied for insulin delivery include nasal, pulmonary, dermal, rectal and oral routes [21], as shown in Fig. 1. Pulmonary insulin application led to a rapid absorption of insulin across the mucosa. In 2006, the Federal Drug Administration approved the first commercially available pulmonary inhaled insulin, Exubera. The relative bioavailability was low (approximately 10%), however, the dose of inhaled insulin must be ten times higher than the dose applied subcutaneously to induce a comparable metabolic effect. Moreover, the long-term consequences of the inhalation of insulin (i.e. the development of insulin antibodies, changes in lung function and lung safety) were raised during clinical development. Because of these problems, Exubera failed to gain acceptance from both patients and physicians and was withdrawn from the market in October 2007 Dermal insulin application does not result in a reproducible and sufficient transfer of insulin across the highly efficient skin barrier. The dream of an 'insulin tablet' has not become reality because of the low permeability of insulin through the gastrointestinal mucosa and susceptibility to chemical and enzymatic degradation in the gastrointestinal tract. By contrast, intranasal insulin therapy has considerable potential for controlling post-prandial hyperglycemia in the treatment of both

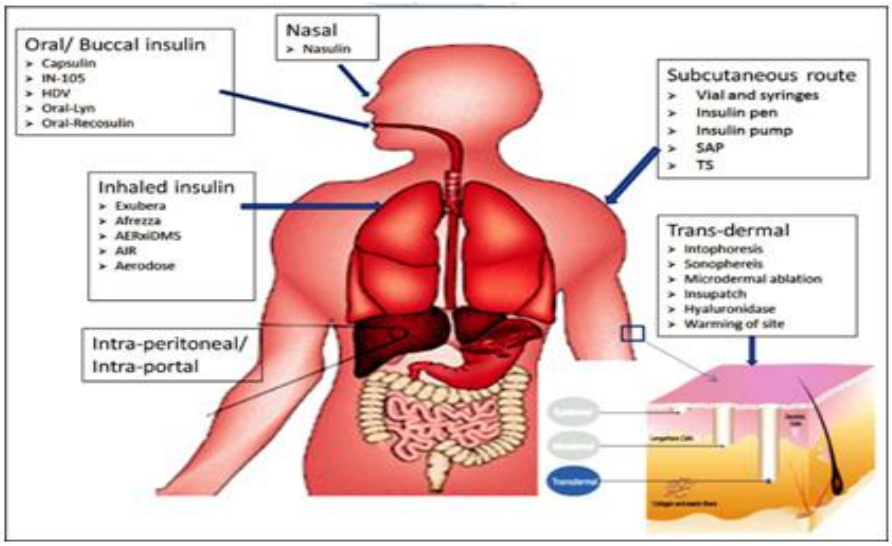

Fig. 1 Various routes for insulin administration

IDDM and NIDDM, based on its advantages (described in the following section), and is attracting more and more attention. However, effective insulin absorption via the nasal route is improbable without the help of absorption enhancers and/or prolonging the residence time of the drug formulation in the nasal cavity. In recent years the nasal route has gained importance as a non-invasive drug application route for the following reasons: the nose has a large surface area available for drug absorption due to the coverage of the epithelial surface by numerous microvilli, the subepithelial layer is highly vascularized, the venous blood from the nose passes directly into the systemic circulation and therefore avoids the loss of drug by first-pass metabolism in the liver, it offers lower doses, more rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, fewer side effects, high total blood flow per cm3 , porous endothelial basement membrane, it is easily accessible and drug is delivered directly to the brain along the olfactory nerves [22-24]. However the primary

function of the nose is olfaction, it heats and humidifies inspired air and also filters airborne particulates [25]. This review article provides general information on the nasal passage routes of drugs and improving the passage through the nasal mucosa, and discusses the barriers that prevent nasal insulin absorption and new strategies to improve its intranasal absorption.

Nasal Mucosa and Enhancing Drug Passage through the Nasal Route

Nasal route

Compared to other biological membranes the nasal mucosa is a rather porous and thin endothelial basal membrane. It also has a rapid blood flow with a highly vascularized epithelial layer and a vast absorption area (150 cm^2) with microvilli in epithelial cells. Due to these characteristics, it offers many advantages such as fast absorption of drugs, rapid action and low risk of overdose [26-28]. Among the major disadvantages of the nasal route are the limited application volume (25-250 µL), the difficulty of high molecular weight drugs (>1,000 Da) to pass through the nasal mucosa, the presence of pathological conditions, mucocilliary drug clearance, enzymatic barriers and irritation of the nasal mucosa [27, 29]. The nasal route consists of three functional areas, namely the vestibular, respiratory and olfactory areas [26,27-30]. Particularly the respiratory area, with its rich vascularity and vast surface area, is where the drugs are absorbed to the greatest extent. Retention of nasally-applied drugs in this area is subject to various factors such as particle size of the drug, density, shape and hygroscopicity, respiration and the presence of pathologic conditions in the nasal cavity. While particles larger than 10 µm can be accumulated in the respiratory area via respiration, those smaller than 5 µm are inhaled and reach the lungs and those smaller than 0.5 µm are exhaled [31, 32]. The nasal respiratory mucosa is covered with mucus. The mucus is 5 µm thick and has a viscous gel on the upper part and an aqueous sol layer on the lower part [28]. The mucosal secretion contains 95% water, 2% mucin, 1% salts, 1% albumin, immunoglobulin, lysozyme, lactoferrin and other proteins and 1% lipids. Nasal mucus also contains IgA, IgE and IgG. Nasal epithelia are covered with a new mucus layer approximately every 10 minutes [33]. All components in the air inhaled from outside via the respiratory channel adhere to the mucus in the nasal cavity or are dissolved in the mucus and pushed to the nasopharynx to be thrown into to the gastrointestinal channel. The clearance of the mucus and the components that are adsorbed/dissolved to the gastrointestinal tract is named as mucocilliary clearance [26]. Epithelial cells have thin, hair-like structures or cilia on the surface. Every cell has approximately 300 cilia. The mucus layer makes a fluctuating movement together with the underlying cilia. Nasally-applied drugs are cleared from the nose within a half-life of approximately 21 minutes with this movement [30].

Passage routes of drugs via nasal mucosa

The passage of drugs via the nasal mucosa is mainly achieved in three ways, which are paracellular, transcellular and transcytotic (Fig. 2) [34, 35]. The first route is the paracellular transport, which is associated with the intercellular spaces and tight junctions. Paracellular transport is an important route particularly for absorption of peptides and proteins, so it has been reported that the paracellular route should be reversibly opened to enhance nasal absorption of peptides, and mucosal absorption increases due to the hydrophilic characteristic of drugs [36, 37]. The second passage route is the transcellular route which is achieved with passive diffusion or active transport mechanism. It is important in absorption of lipophilic molecules or the molecules that are recognized by the membrane (active carrier transport) [26, 29, 34]. The third passage route is transcytosis. Here, the particle is taken into a vesicle and transferred to the cell. Finally, it is accumulated in the interstitial space [38, 39].

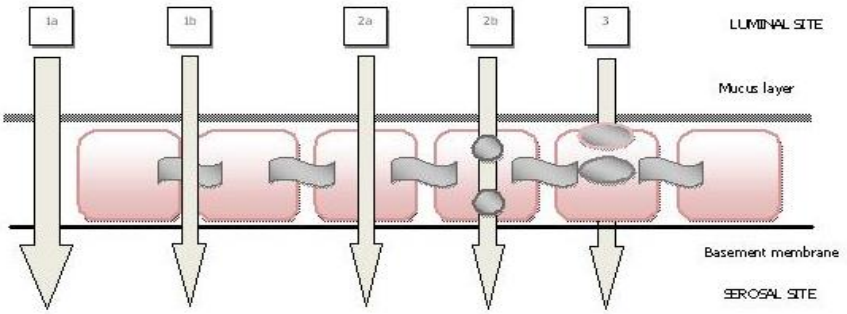

Fig. 2 (1) Paracellular route (1a) intercellular spaces, (1b) tight junctions, (2) transcellular route (2a) passive diffusion, (2b) active transport, (3) transcytosis (Source from Ref. [47]).

Strategies and perspectives to enhance the intranasal absorption of insulin

Among the factors affecting the absorption of drugs through the nasal mucosa and their bioavailability are the physico-chemical properties (ionization, lipophilicity, etc.) of the drug, surface charge and hydrophobicity as well as the molecular size, which is the most important factor [38]. Hydrophilic drugs with low molecular weight are absorbed via the nasal mucosa at a rate that is almost comparable to intravenous application [40, 41]. Nevertheless, the nasal mucosa is an obstacle for the passage of large molecules, particularly for those above 1,000 Da in size [38], and therefore, the nasal bioavailability of hydrophilic peptides and proteins is usually less than 1% [42]. This low bioavailability of these drugs is associated with the weak mucosal membrane permeability and the presence of proteolitic enzymatic activity in the nasal mucosa. Another reason is the mucocilliary clearance, through which they are rapidly removed from the nasal area [43]. To overcome these disadvantages, different approaches have been proposed to enhance nasal absorption of molecules with peptide and protein structures [44-46], which can be summarized as follows:

- ✓ Modification of the chemical structure of the insulin to increase metabolic stability and/or membrane permeability or chemical modification by Protecting insulin from proteolytic degradation and improving the permeation characteristics of insulin
- ✓ There are oxidative, conjugative enzymes and exopeptidases and endopeptidases in the nasal cavity. This vast diversity of enzymes leads to a pseudo first pass effect which hinders the absorption of protein peptide drugs. The drug may be applied with enzyme inhibitors to protect them from the activity of these enzymes in the mucosa or using enzyme inhibitors
- ✓ Inclusion of absorption enhancers (such as bile salts and surfactants, fusidic acid derivates, phosphatidylcholines and cyclodextrines) to enhance the passage of drugs with polar structure through nasal mucosa or Using absorption enhancers. Ex Cationic polymers, Chitosan and its derivatives, Poly-L-arginine, Cationized gelatin, Cell-penetrating peptides, Cyclodextrins, Tight junction modulators, Tight junction modulating lipids, Tight junction modulating peptides, Nitric oxide donors, N-acetyl-L-cysteine.
- ✓ Development of novel formulations including drug carrier systems (liposomes, lipid emulsions, niosomes, nano- and micro-particles) or Using delivery systems with increased retention time. Ex Bioadhesive microsphere delivery system, Bioadhesive powders, Gel preparations, Insoluble powder formulations using nanoparticles [47].

One of the preferred approaches among these is the nano- and micro-particulate systems, which are prepared primarily with mucoadhesive polymers to provide sufficient retention time of the drug for absorption in the nasal cavity. Particulate systems facilitate the passage of peptide and protein structured drugs through the nasal mucosa and protect them from enzymatic activity by increasing the retention time of the drug in the nasal cavity, establishing tight contact between the nasal mucosa and the drug, providing localization of the drug at high concentrations, and opening the tight junctions between the epithelial cells.

Nano-/ micro-particulate systems

Nano- and micro-particles are matrix systems where the drug is dispersed in the polymeric material. These particles are produced with different encapsulation methods, including spray-drying, solvent evaporation and phase separation [48-50]. In nano- and micro-particulate carrier systems, the drug is loaded via either incorporation with the system or its adsorption on the particulate system. Drug is released from the particles through certain mechanisms, which are: (a) release from the particle surface, (b) diffusion of the drug from the swollen polymer matrix, or (c) drug release through the erosion of polymers [51].

The nano- and micro-particulate systems, which are prepared for nasal systemic effect of macromolecules, generally use degradable starch, dextran, chitosan, microcrystalline cellulose (MCC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), carbomer, and wax-like maize starch, gelatin polymers [52]. The mucoadhesive properties of these systems are an important factor in their retention and action in the nasal mucosa. Chitosan, which is a positively charged polymer with a strong mucoadhesive property, is frequently used in nasal application of macromolecules [53, 54]. Mucoadhesion is achieved by the ionic interaction of positively charged amine groups of D-glucosamine units of chitosan with negatively charged sialic acid groups of musin or other negatively charged groups of the mucosal membrane [55]. The effect of chitosan that enhances penetration has been associated with its mucoadhesive property as well as its ability to transiently open the tight junctions in the nasal mucosa. It has been reported that chitosan does not lead to any histological changes in the nasal mucosa [56-58]. Among the polymers used particularly in nasal application of antigens are poly(L-lactic acid) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA). These polymers have been approved by the Food and Drug Administration (FDA) and they are transformed into lactic and/or glycolic acid

in the body. Mucoadhesive polymers like alginate and Sephadex®, poly (vinyl alcohol) and chitosan are used together to increase mucoadhesiveness of PLA and PLGA polymers [59-61].

Insulin

Insulin is a peptide hormone consisting of 51 amino acids whose molecular weight is 6 kDa. Since its first discovery in 1922 to the present, numerous non-invasive routes have been tried to improve insulin treatment and the quality of life of the patients suffering from diabetes mellitus. The nasal route for insulin delivery has been one of the mostly studied alternative routes due to its advantages. Some of these studies are summarized in Table 1. As seen in the table, since insulin has a high molecular weight, numerous formulation approaches have been tried to improve its absorption through the nasal mucosa. Among these approaches are carrier systems such as powders, microspheres and nanoparticles most of which are prepared with mucoadhesive polymers. Another approach is the use of permeation enhancers with different structures to overcome the barrier characteristic of the nasal mucosa.

Table 1 Studies on nasal insulin formulations

Polymers/ Others	Delivery system	Enhancer	Animal model	Results	Ref.
Degradable starch	Microspheres	LFC	Sheep	While the relative bioavailability of insulin from microspheres was10.7%, addition of enhancer to the formulation, bioavailability of insulin was increased to 31.5%.	62
Soluble starch	Powder and Microspheres	-	Rats	A comparison between microspheres and starch powders (mw 11000 and 25000) indicated that the insoluble starch of mw25000 and the microspheres reduced the plasma glucose level to the same extent. Besides water soluble starch powder (mw 11000) did not change the plasma glucose level.	63
Crosslinked starch and Dextran	Microspheres	Epichlorohydrin	Rats	The effect on the glucose level of insulin from starch and dextran microspheres was rapid and maximum decrease in plasma glucose level was achieved in 30-40 minutes. The effect of starch microspheres was found more efficient than that of dextran microspheres to decrease blood glucose level.	64
Starch-Carbopol®974P and maltodextrin - Carbopol® 974P	Freeze-dried powder	-	Rabbits	The nasal bioavailability achieved with the application of Starch-Carbopol® 974P powder was significantly higher than that of the maltodextrin-Carbopol® 974P mixtures.	65
Starch	Microspheres	Bile salt derivatives (LFC, GDC, STDF)	Sheep	Bioadhesive starch microspheres have improved transport of insulin across nasal membrane in the presence of absorption enhancers. Addition of enhancer to the microspheres has increased insulin absorption than that of absorption enhancer in solution.	66
Amioca® starch and Carbopol® 974P	Powder		Rabbits	Following nasal single-dose application of a physical mixture of Amioca® starch and Carbopol®974P (9/1) the bioavailability of insulin has been found to be more than 10%.	67
Crosslinked starch	Nanoparticles	SGC, LFC	Rats	A rapid hypoglycemic effect has been observed with nasal application of nanoparticles. It has been emphasized that the release of insulin from nanoparticles can be modified by adjusting the degree of cross- has significantly increased with combination of permeation enhancers and nanoparticles.	68
Dextran	Microspheres	-	Rats	Microspheres with insulin on the surface were more effective in promoting insulin absorption than those with insulin distributed within the dextran matrix.	69
Anionic resin(SPS), nonionic resins (PAE, SDBC) and cationic resin (CA)	Powder	-	Rabbits	Nasal administration of insulin mixed with anionic resin caused a rapid increase of the plasma insulin level, while nasal administration of insulin alone caused little increase Nonionic resin (SDBC) showed similar enhancement in nasal insulin absorption in contrast, the other nonionic resin and cationic resin did not improve insülin absorption.	70
Hyaluronic acid ester	Microspheres	-	Sheep	Average relative bioavailability of insulin from microspheres was calculated as 11% when compared with insulin administered by subcutaneous route.	71
Chitosan	Nanoparticles	-	Rabbits	The freeze-dried formulation of insulin-loaded chitosan nanoparticles has led to a greater decrease in plasma glucose level when compared to the insulin chitosan solution.	72
Cross linked chitosan	Nanoparticles	-	Rats	Microspheres containing chitosan and ascorbyl palmitate caused a 67% reduction of blood glucose compared to intravenous route and absolute bioavailability of insulin was found as 44%.	73
Thiolated chitosan	Nanoparticles	-	Rats	Insulin-loaded thiolated chitosan microspheres let to more than 1.5- fold higher bioavailability and more than 7-fold higher pharmacological efficacy than unmodified chitosan microspheres.	74
chitosan	Nanoparticles	CM-β-CD	-	The fast release of insulin from chitosan/CM-β-CD nanoparticles was observed (84-97% insulin within15 min.).	75
chitosan	Nanoparticles	-	Rats	Nanoparticles containing insulin have increased the pharmacodynamic activity of the drug. The synthesis of gold nanoparticles prepared by using chitosan has used a new method, and therefore, the surface properties of chitosan were improved for binding of biomolecules.	76
chitosan	Nanoparticles	NAC	Rats	Nasal administration of chitosan- NAC nanoparticles increased the insulin absorption compare to unmodified chitosan nanoparticles and control insulin solution.	77

chitosan	Nanoparticles	SBE-β-CD CM-β-CD	Rabbits	The nanoparticles have reversibly increased the transepithel resistance of the cells and increased the membrane permeability in in-vitro cell culture studies. Nasal application of fluorescence-loaded nanoparticles to rats has proved their ability to pass through nasal mucosa. In conclusion, insulin-loaded nanoparticles have decreased the plasma glucose level(more than 35% reduction).	78
Aminated gelatin	Microspheres	-	Rats	Aminated gelatin microspheres have significantly increased the nasal absorption of insulin when administered in dry formulation but no significant hypoglycemic effect was observed when given as a suspension.	79, 80

LFC = Lysophosphatidylcholine; GDC= Glycodeoxychlote; STDF = Sodium taurodihydroxyfusidate, SPS = Sodium polystrene sulphonate; PAE = Polyacrylester; SDBC = Styrene-diviniylbenzene copolymer; SGC = Sodium glycocolate. CA = Cholestramine; CM-β-CD = Carboxymethyl-β-cyclodextrin; NAC = N-acetyl-L-Cysteine; SBE-β-CD = Sulfobutylether-β-cyclodextrin.

Table 2 Comparison of different strategies for improving intranasal absorption of insulin

Strategies	Advantages	Disadvantages	Effectiveness
Chemical modification	Protects the drug against proteolytic degradation. Improves the drug's permeation across the nasal mucosa owing to increased lipophilicity	Decreased pharmacological activities of the parent peptides. Not very useful for larger peptides such as insulin	Limited effect
Use of enzyme inhibitors	Improves the stability of drugs at the absorption site	Unable to dramatically improve bioavailability in the absence of other absorption-enhancing measures Might affect the normal metabolism of the body and cause side-effects	Not very effective
Use of absorption enhancers	Improves the permeability of the epithelial cell layer based on different mechanisms	Some of the mechanisms can cause irritation and damage to the nasal mucosa	The most common and effective approach
Use of delivery systems with increased retention time (especially insoluble powder formulations)	Prolongs the intimate contact time of the formulation on the nasal mucosa by adhering to the surface of the mucus layer	-	The most promising, especially when combined with absorption enhancers
Use of nanoparticles	Protects insulin from degradation in the nasal cavity Enhances insulin intranasal absorption and controls the release	Complicated preparation process Many negative reports	With paradoxical reports

In one of these studies, aminated gelatin microspheres were prepared as nasal drug delivery system for peptide drugs [80]. In vitro studies have demonstrated that these microspheres had a significantly slower release than the native gelatine microspheres. The microspheres prepared were applied nasally to rats in the form of powders and suspensions. The effect of the aminated gelatin microspheres on enhancing absorption was found to be significantly higher in powder formulations. The aminated gelatin microspheres, due to their positive charge and mucoadhesive characteristic, are suggested as a novel carrier system for macromolecules. In another study, insulin nanoparticles were prepared by cross-linking epichlorohydrin with starch in the presence of a permeation enhancer such as sodium glycocholate or lysophosphatidylcholine [69]. These particles were nasally applied to rats. The particles containing sodium glycocholate increased the plasma insulin levels significantly. Besides, these particles produced a higher hypoglycemic effect when compared to nanoparticles that contain lysophosphatidylcholine. The effect of cell-penetrating peptides (CPPs) was evaluated on the nasal absorption of insulin [81]. CPPs have dramatically increased the nasal absorption of insulin. L-Penetratin was found to be the most effective enhancer of insulin absorption compare to other CPPs. However, increasing the D-penetratin concentration let to a decrease in the efficiency of nasal insulin absorption. In conclusion, the particulate systems and enhancers have generally improved the transport of insulin through the nasal mucosa. These findings suggest that nasal formulations of insulin could be introduced to the drug market in the future.

Conclusion

Although researchers have made great efforts to improve the nasal bioavailability of insulin (Table 2), formulations that meet clinical needs have not yet been reported. The two most important factors that hamper the absorption of insulin across the nasal mucosa are low permeability of the nasal mucosa to large molecules and rapid mucociliary clearance of formulations from the nasal cavity. Consequently, formulation design must try to overcome the two barriers – for example, by using a mucoadhesive drug delivery system or coadministration with absorption enhancers. According to previous research work, it seems that bioadhesive microsphere delivery systems or water-insoluble powders with absorption enhancers are the most promising

for nasal absorption of insulin. Future studies should focus on the screening of safe and effective absorption enhancers and the search for appropriate bioadhesive and water-insoluble excipients with a view to achieve higher nasal bioavailability of insulin.

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