REVIEW ARTICLE

Bioavailability of intranasal drug delivery system

Satish Balakrishna Bhise, Adhikrao Vyankatrao Yadav, Amelia Makrand Avachat, Rajkumar Malayandi

Biopharmaceutical Research Group, Department of Biopharmaceutics, Government College of Pharmacy, Karad, India

Nasal drug delivery system offers lucrative way of drug delivery of both topical and systemic therapies. The high permeability, high vasculature and low enzymatic environment of nasal cavity are well suitable for systemic delivery of drug molecules via nose. The noninvasiveness and self administrative nature of nasal delivery also attracts the formulation scientists to deliver protein and peptide compounds. Despite of all the advantages of nasal drug delivery, the bioavailability of nasally administered products, especially for protein and peptide molecules, is affected by many barriers such as physiological barriers, physicochemical barriers, and formulation barriers. This review will focus on the various bioavailability barriers in nasal drug delivery and the strategies to improve the bioavailability of nasal dosage forms.

Key words: *Bioavailability, mucociliary clearance, nasal drug delivery, permeation enhancer, protein and peptides*

INTRODUCTION

The administration of drugs via nose is not a novel approach for drug delivery. In ancient days, nasal drug delivery was used for the systemic administration of psychotherapeutic compounds and other similar substances.[1] But in modern pharmaceutics, nasal delivery is considered as a route of choice for local effect rather than systemic effect. Delivery of drugs via nose for maintenance therapy of nasal allergy, sinusitis, nasal congestion, and nasal infections is a routine practice. However, there has been a great deal of research in investigating nose as a potential route for systemic therapies for both conventional as well as protein and peptide molecules.[2] Advent of biotechnology, molecular biology, and pharmacology provided a lot of endogenous protein and peptide molecules for therapeutic use, the delivery of such molecules is possible through nasal drug delivery. Recent interest in nasal delivery of conventional molecules reflects the desire on behalf of the pharmaceutical companies to extend the life span of drugs in the face of generic completion by delivering them via novel route. The greater permeability of nasal mucosa with large surface area affords a rapid onset of therapeutic effect. The low metabolic environment of nose has potential to overcome the limitation of oral route and duplicate the benefit of intravenous administration. In addition to that, nasal administration minimizes the lag time associated with oral drug delivery and offers non-invasiveness, self-administration, patient comfort, and patient compliance, which are the hurdles in intravenous drug therapy. The interesting advantage of nasal drug delivery is the possibility of targeting central nervous system (CNS) by bypassing blood brain barrier[3] (BBB). The drugs absorbed nasally via olfactory epithelium are reported to enter in olfactory neurons and supporting cells and subsequently into the brain, which reduced not only the systemic toxicity of centrally acting drugs but also enhanced therapeutic efficacy.[4] The nasal route has received great attention as a route for vaccination.[5] Nasal delivery of suitable antigen along with proper adjuvant to the nasal associated lymphoid tissue (NALT) has potential to induce humoral and cell mediated immunity. Nasal route is the route of choice for rapid mass immunization in developing countries and disaster area.[6] Intranasal immunization may lead to the development of local, as well as systemic immunity.[7] Despite having large number of advantages, bioavailability of nasal dosage form is hindered by various physicochemical, physiological and formulation factors. Many authors have reviewed various aspects of nasal drug delivery system.[8] However, there is no single review addressing the bioavailability of intranasal drug delivery system. The present review will focuses on the various barriers to nasal drug delivery systems and strategies to improve the bioavailability.

Address for correspondence:
Dr. S B Bhise, Government College of Pharmacy, Karad, Satara - 415 124, Maharashtra, India. E-mail: rajkumar_bpharm@yahoo.com

BARRIERS TO NASAL DRUG DELIVERY

Intranasal drug delivery is considered as a lucrative route of drug delivery system for formulation scientist because of its easy and simple formulation strategies. A large number of factors influenced therapeutic efficacy

as well as toxicity of nasally administered drug product.[9] A well designed pre-formulation program is essential for development of nasal dosage forms to overcome various barriers associated with nasal drug delivery. Clinical trials of nasal dosage forms are a costly affair; hence, if formulation fails to satisfy the regulatory criteria in bio-studies, it is not only a great financial loss but also loss of time for the pharmaceutical company. To avoid such unfavorable situations, various factors such as safety, efficacy, bioavailability, toxicity, and stability of dosage forms need to be established during formulation development. A large number of factors serve as barrier for systemic bioavailability of nasal drug product,[10] which have been listed in Table 1.

Physiological barriers

Nasal mucus

Airway mucus is composed of primarily water (95%), mucus glycoprotein (2%), other proteins including albumin, immunoglobulin, and lysozyme (1%), inorganic salts and lipids. Mucus glycoprotein, well known as mucin is the major component of the mucus. This compound is primarily responsible for the viscoelastic properties of mucus. The visco-elastic properties also depend on the percentage content of mucin, water, and other ions. The pH of the nasal secretion also determines the viscoelastic properties of mucus. It is important to consider the interaction between the drug and mucus. There may be a chance of change in viscoelastic properties due to drug - mucus interaction that could be effectively utilized to improve the bioavailability of nasal dosage forms. The mode of transportation of drug molecule across the mucus barrier is principally based on drug diffusion mechanism. The drug diffusion across the nasal mucus is governed by factors such as molecular weight of drug, viscosity of mucus, surface charge, drug-mucus interaction, etc.[11] Small unionized molecules readily cross the mucosal barrier. Sialic acid, which contains anionic carboxylic group, repels the anionic molecule and hence it reduces the transport of such drugs.[12] In contrast, the strong interaction between molecules containing cationic group have low permeability than anionic group of compounds.[13] Still large molecular compounds, particulate systems (microspheres and nanoparticles) and ionic compounds have been observed to diffuse through the mucus.[14] Therefore, better understanding of the factors influencing the diffusion through mucus is presently lacking [Table 1]. If at all, the drug transportation through mucus membrane and mucus-drug interaction, is established, it will be helpful for formulation scientist to optimize the formulation for bioavailability enhancement. Various nasal mucosal barriers are presented in Figure 1.

Nasal epithelium

The nasal membrane can be classified into olfactory and nonolfactory epithelia. The olfactory epithelium is pseudostratified columnar in type, and consists of specialized olfactory cells, supporting cells, and both serous and mucous glands, whereas the nonolfactory epithelium is a highly vascular tissue covered by a ciliated pseudostratified columnar epithelium.[15] The olfactory cells contain bipolar neurons and act as peripheral receptors and first-order ganglion cells.[16] The nasal respiratory epithelium consists of loosely packed cells with high permeability and vasculature [Figure 2]. The permeability of environmental toxins is restricted by nasal epithelium.[17] Nasal absorption is achieved by different mode of transportations such as passive diffusion, carrier mediated transport, and transcytosis. However, nasal absorption was hindered by efflux transporters such as glycoprotein [Figure 3]. Low molecular lipophilic compounds rapidly get permeated through nasal mucosa. For example, the bioavailability of nasal absorption of ondansetron was comparable with intravenous route in rats.[18] This study revealed complete and rapid absorption of drugs through nasal epithelium. Various reasons are suggested for high permeability of nasal mucosa including high vasculature, non keratinized epithelium, low metabolic activity and high perfusion rate.[19] Acidic environment of stomach, intestinal bacteria, and digestive enzymes present in the gastrointestinal tract (GIT) hinder the bioavailability of orally administered drugs. In case of nasal cavity, slightly acidic pH, sterile environment, and low enzymatic activity offers higher bioavailability of certain substance such as propranolol, metoclopramide, nifedipine, apomorphine, midazolam than oral drug delivery.[20] However, nasal epithelium acts as a barrier for high molecular compounds such as desmopressin, insulin, human growth hormone, etc. The tight junctions of epithelium act as barrier for such macromolecular compounds. The degree of ionization plays an important role in permeability as well as bioavailability of drugs through nasal membrane. The influence of effect of drug permeability on degree of ionization is described else where in this article.

Mucociliary clearance

Nasal mucociliary clearance is the most important physiological barrier, which reduces the nasal residential time of drugs and/or dosage forms.[21] Bioavailability of nasal dosage form depends on the residential time of the drug in the nasal cavity. The nasal mucociliary clearance system transports the mucus layer that covers the nasal epithelium towards the nasopharynx by ciliary beating. In true sense, mucociliary clearance is one of the defense mechanism of the respiratory tract to protect the body against any noxious material that is inhaled.[22] Ciliated mucous cells present in the nasal mucosal membrane are responsible for mucociliary clearance. Nasal clearance proceeds at an average rate of 5-6 mm/min.[23] Nasal mucociliary clearance carries the airway secretion backward to the nasopharynx. This material is dispatched by wiping action of the palate to the stomach, periodically through swallowing.[24] A large number of factors influence the nasal mucociliary clearance, which include nasal pathophysiology, temperature and moisture of inhaled air, drugs, pollutants, and pH of the nasal secretion.[25] A wide variety of methods are used to determine the mucociliary clearance.[26] Different

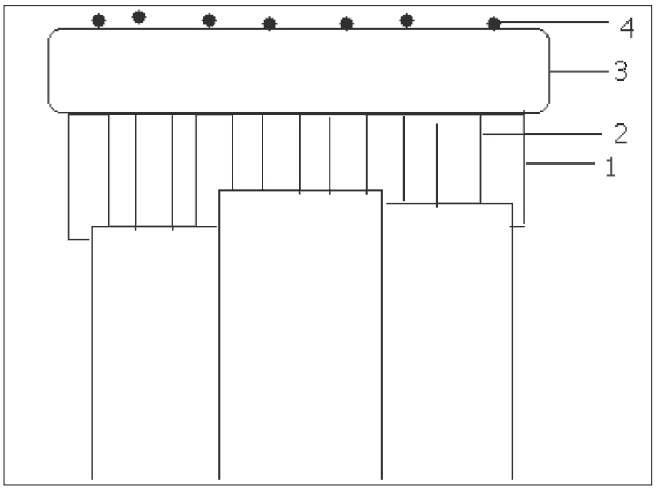

Figure 1: Nasal mucosal barriers 1. sol layer 2. cilia 3. gel layer 4. particles

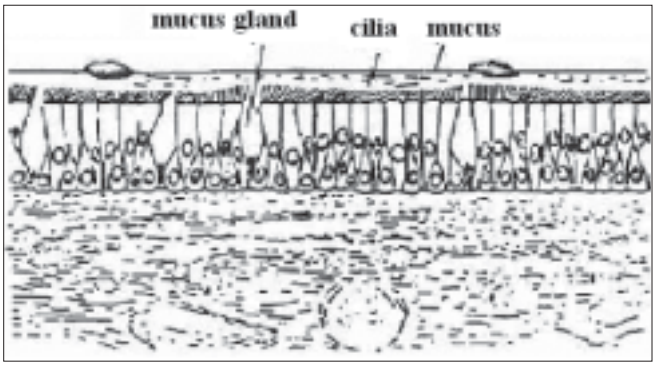

Figure 2: Structure of nasal respiratory mucosa

features of mucociliary clearance such as total clearance, mucous flow rate, and mucociliary transit time (MTT) have been used to determine the nasal mucociliary clearance rate and hence nasal residential time of the dosage form.[27]

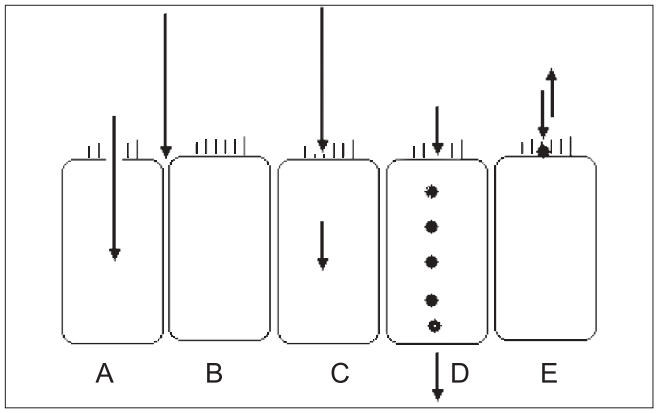

Figure 3: A. Transport of drugs across nasal epithelium transcellular passive diffusion, B. paracellular passive diffusion, C. Carrier mediated, D. transcytosis, E. efflux transport

Total clearance of the deposited dose is monitored by the clearance of radio labeled dosage form, which is measured by gamma camera. Mucus flow rate is measured by the transport time or speed of marker placed on the nasal mucosa. The continuous monitoring of the nasal epithelium by gamma camera provides the transport time of the dosage form. The nasal MTT can also be measured in healthy human subjects by sodium saccharin method or dye method.[28] The time required sensing the sweet taste or appearance of dye in pharyngeal area after nasal administration of sodium saccharin or dye is measured. It has been established that MTT in healthy human volunteers is between 2.5 and 25.0 min.[28]

The effect of drug on mucociliary movement was extensively studied.[29-32] Antihistaminic drugs, beta blockers, general anesthetics, cocaine arrest mucociliary clearance.[33] Moreover, correlation between reduction in mucociliary clearance rate and concentration of drug has been established.[34] Cholinergic drugs, beta adrenergic agonists, and surfactants increase the

Table 1: Barriers in nasal drug product development

Nasal barriers	Factors to be considered
I. Physiological barrier	
a. Nasal mucus	Viscosity, pH of mucus and drug/dosage form-mucus interaction
b. Nasal epithelial barrier	Molecular weight, ionization constant and mode of transport
c. Mucociliary clearance	Nasal residential time and nature of dosage form
d. Pathophysiology	Volume of nasal secretion and permeability of epithelium
e. Nasal metabolism	Nature of the molecules (e.g., protein and peptides)
f. Efflux transport system	Nature of drug molecule and duration of therapy
II. Physicochemical barriers	
a. Drug solubility and dissolution	Nature of dosage form, dose, pKa, and polymorphism
b. Molecular weight and size	Less bioavailability with molecular weight more than 1000
c. Compound lipophilicity	Affects the nose to blood and nose to brain absorption
d. pH and pKa	Unionized pH favors for absorption
III. Formulation factors	
a. Drug concentration, dose, and volume	High concentration for better bioavailability, maximum dose in Minimum vehicle (less than 200 µl)
b. Osmolarity	Isotonic solution prevents epithelial damage and toxicity
c. Site of deposition	Site of deposition based on viscosity, position of head, volume, delivery device, deposition at anterior chamber prolong the nasal residential time

mucociliary clearance and reduce nasal residential time;[35] thereby it reduces the bioavailability of drugs. Nasal drug formulations for topical or systemic drug delivery usually contain preservatives. The effect of preservatives on nasal mucociliary clearance has been extensively studied.[36] The preservatives, such as chlorobutol and hydroxybenzoates, cause reversible inhibition of mucociliary clearance.[37] Preservatives such as cresol, chlorocresol, and phenyl mercuric salts showed irreversible inhibition of mucociliary clearance, whereas nasal mucociliary transport was not altered by benzalkonium chloride.[38] Benzalkonium chloride is the extensively studied preservative in nasal formulation.[39] Long term administration of corticosteroid formulation containing 0.01 and 0.02% benzalkonium chloride in humans showed no significant reduction in mucociliary clearance rate.[40]

Nasal permeation enhancers are widely used to improve the bioavailability of large molecular compounds such as proteins and peptides.[41] Sodium taurohydrofusidate, a well known permeation enhancer has been found to inhibit the mucociliary clearance at concentration of 0.3% and higher.[42] Other permeation enhancers such as laureth-9, deoxycholate, glycholate, and taurocholate have been shown to cause mild inhibition of mucociliary clearance.[43] Cyclodextrins and their derivatives are widely employed as permeation enhancers, particularly for poorly water soluble lipophilic compounds.[44] Some of the beta cyclodextrin derivatives like methylated beta cyclodextrin have also been found to be effective absorption enhancers for peptide molecules.[45] Mucociliary clearance rate was inhibited by formulation containing 2-4% methylated beta cyclodextrin.[46] Mucociliary clearance should neither be interfered by drugs nor excipients, because it is an important physiological defense mechanism. The main goal of preformulation program along with preclinical scientist is to screen the suitability of drug for nasal administration and to screen the ideal excipients for nasal formulation, which will retain the normal physiological mucociliary clearance.

Nasal pathophysiology

The influence of physiology of abnormal nose on bioavailability of nasal drug products has not been studied in sufficient details. The most frequent and common disease associated with nose is rhinitis, hence pathology of rhinitis and its influence in drug bioavailability is briefly discussed in this section. Rhinitis is classified as allergic rhinitis and common cold. Allergic rhinitis is the allergic airway disease, which affects 10% of population.[47] Numerous allergens are constantly present in our environment, and any one of them can cause allergic rhinitis. In this condition, air borne allergic particles including pollens, molds, animal allergens are deposited on the nasal mucosa. Allergic rhinitis, is also known as hay fever, may be acute, seasonal or chronic and perennial. Allergic rhinitis is characterized by hypersecretion, itching, and sneezing. Histamine is the most important allergic mediator, causing the symptoms associated with allergic rhinitis. Histamine, which is released on the mucosal surface, has shown more allergic reactions than injected into nasal mucosa.[47] Apart from histamine, other arachidonic acid metabolites such as leukotrienes and prostaglandins also play little role in allergic manifestations.[47] The epithelial damage caused by allergic rhinitis is due to inflammation and infiltration of eosinophils. An increased level of albumin and other plasma proteins in nasal fluid was found in allergic rhinitis.[47] Earlier studies have reported the increased transport and bioavailability of macromolecular substances due to the infiltration of protein substances.[48] However, recent study by Person[49] and coworkers has clearly shown that the intranasal absorption of macromolecules is not increased in allergic rhinitis. Common cold is a disease of nasal cavity caused by viral infection.[49] In viral rhinitis, the mucosa is edematous and infilltered by neutrophils and mononuclear cells. The epithelial damage, abundant increase in mucous secretion and high vascular permeability associated with viral rhinitis are hypothetically favorable for nasal drug absorption.[50] However, introduction of nasal dosage form as an external stimulus increases the nasal mucus secretion and leads to drainage of dosage forms and hence reduced bioavailability.[51] There is little data on the effect of common cold on pharmacokinetics of intranasal drug delivery system. Future research in nasal pathophysiology and its impact on bioavailability of intranasal drug delivery system will provide better understanding of this subject in near future.

Nasal metabolism

Although nasal secretions consist of enzymes, they do not have a significant effect on the extent of absorption of most of compounds except protein and peptide molecules.[52] The low metabolic environment offers nasal drug delivery as a lucrative route for both conventional and protein molecules. Nasal bioavailability of a significant number of drugs such as progesterone, testosterone, estradiol, naloxane, propranolol, and butorpheanol is almost 100%,[53] whereas the oral bioavailability of the above mentioned drugs is almost nil except propranolol, which has oral bioavailability ranging from 20 to 30%.[54] Presystemic metabolism is the major rate limiting step in the bioavailability of oral drug product; which is particularly true for highly lipophilic compounds.[55] The nasal administration of these compounds resulted in complete absorption because of a) the rate of absorption was very fast, hence enzymatic exposure time is very short and b) the level of enzymes in nasal cavity is very low and can be easily saturated with drugs.[56] Although nose provides low metabolic environment, metabolism of drugs in nasal cavity is considered as a major barrier for bioavailability of nasal dosage forms, especially for protein and peptide molecules.[57] The cytochrome P450 enzymes present in the nasal mucosa is second only to those present in liver, if expressed per gram tissue.[58] Cytochrome P450 has broad substrate specificity and catalyzes NADPH dependent mono oxygenation of lipophilic compounds.[59] A number of compounds like phenacetin have been shown to undergo metabolism in the nasal cavity by this enzyme. Cytochrome P 450 is present in

both respiratory and olfactory mucosa and hence it reduces both nose to blood and nose to brain transport of drugs.[60] Low bioavailability of protein and peptide drugs is evident in presence of various proteolytic enzymes such as exopeptidase (mono and diamino peptidase) and endopeptidase (serine, cysteine, and aspartic peptidase).[61] These enzymes are able to cleave peptide and protein molecules at their C and N termini and at the internal peptide bond, respectively.[61] In case of peptides, however, hydrolysis by peptidase plays an important role in their nasal bioavailability. A classical example is extensive and rapid hydrolysis of pentapeptide in the nasal cavity of rats.[61]

P glycoprotein efflux transport

Drug absorption may be hindered by efflux transporters such as P-glycoproteins (Pgps). Basically, Pgps are a group of glycosylated membrane proteins found in the epithelial cells of small intestine and other body tissues. Multi drug resistance (MDR) genes present in the humans, coding for Pgps has been found in the human nasal respiratory mucosa.[62] A large variety of hydrophilic and amphiphilic compounds are detoxified through active Pgp mediated efflux transport in nasal mucosa.[63] Topical administration of steroids in nasal cavity may increase the expression of Pgps in the respiratory epithelium and hence affect the bioavailability of nasal dosage forms.[64] Pgps have certain role in reducing CNS permeability of nasally administered drugs in olfactory epthelium. The maximum drug uptake and high CSF drug concentration was achieved with formulation incorporated with Pgp efflux inhibitors such as rifampin.[65] The low level of chlorpheniramine and chlorcyclizine in CSF was most likely due to the presence of efflux transport system in olfactory epithelium.[66] The physiological barrier of Pgp efflux system in nasal drug delivery not only affects the peripheral drug concentration, but also certainly affects the brain drug concentration of centrally acting drugs. The efflux transport system plays a vital role in detoxification and hence more studies are required for incorporating Pgp efflux transport inhibitors in formulation to improve the bioavailability of nasal dosage forms.

Physicochemical properties of drug molecules

Drug solubility and dissolution rate

In general, drug solubility and dissolution rate is the most studied biopharmaceutical factor,[67] as far as bioavailability of dosage form is concerned. Solubility of the drug or dosage form is the first prerequisite for absorption and bioavailability of powder dosage form. The fluid available for dissolution of drug particles in nasal mucosa is very less, when compared with gastrointestinal fluids in oral drug delivery.[68] Saturation solubility of the drug in given nasal physiological pH is very important physicochemical parameter, which determines the rate and extent of absorption of nasal dosage form. However, most of the nasal drug products are either in solution form or gel form, so that influence of drug dissolution on bioavailability of nasal drug product is not extensively studied at this point of time. Superior stability of solid powder dosage forms, controlled release mode and its ease of manufacturing may provide number of solid nasal dosage forms to the market in near future.

Molecular weight and size

Absorption through nasal mucosa is apparently inversely related to the molecular weight of drug molecules. In the absence of permeation enhancer, nasal absorption is sharply reduced for drugs with molecular weight over 1000 Da.[69] The log linear correlation was established between percent drug absorbed and molecular weight.[70] However, mode of transportation of the molecule is equally important for bioavailability of nasal dosage forms. For drugs, which undergo passive diffusion, lipophilicity is more essential than its hydrophilicity. The active transport system requires prolonged nasal residential time for better bioavailability rather than low molecular weight.[71] Fisher and coworker[72] studied the effect of molecular weight of water soluble compounds on nasal absorption in rats. 4-Oxo-4H-1-benzopyran-2-carboxylic acid, paramaino hippuric acid, sodium cromoglycate, inulin, and dextran of molecular weights 190, 194, 512, 5200, and 70000, respectively were used in this study. A 368 fold increase in molecular weight resulted in a 43 fold decrease in the nasal absorption of these compounds. Another study indicated the molecular weight dependency of nasal and oral absorption of polyethylene glycol, 600, 1000, and 2000 in rats.[73] The results showed an inverse relationship between percent drug absorption and molecular weight. The leaky nature of nasal mucosa allows to permeate the conventional molecules (molecular weight less than 1000 Da) and hence provides better absorption and bioavailability than other routes of drug delivery such as sublingual, subcutaneous, and oral drug delivery systems.[72-74] The comparative human pharmacokinetics of nasal, oral, and injection (intramuscular) dosing of morphine was studied.[75] The data illustrated that intranasal dosing achieves a similar fast drug onset (T_{max} ~ 15 min) as compared with intramuscular dosing and is much faster than oral dosing (T_{max} ~ 50 min).[75] Apomorphine is another classical example, where intranasal apomorphine absorption is as rapid as subcutaneous injection. The rapid onset of intranasal apomorphine is more relevant and essential for either erectile dysfunction or Parkinson's disease.[76]

Lipophilic and low molecular weight conventional molecules have better absorption and bioavailability in nasal route than any other non invasive route of drug delivery. However, the real challenges for formulation scientist are in developing intranasal dosage form for large molecular protein and peptide drugs.[77] Nasal epithelial cells are interconnected on the apical side with series of narrow belt like structures. These structures are called as Tight Junctions (TJs). TJs serve as a dynamic, regulatable, semipermeable diffusion barrier between epithelial cells. TJ barriers allow interchangeability of small ions between apical and lateral side of the cell

membrane. TJs act as a barrier for bioavailability of intranasal dosage form of protein and peptide molecules. Many protein and peptide molecules including buserelin, octreotide and desmopressin, and salmon calcitonin showed better intranasal bioavailability than oral drug delivery.[78] Even though permeability of large molecular compounds is a rate limiting factor in the bioavailability of intranasal dosage form, low metabolic activity and non invasive nature of nasal drug delivery system stimulated the researchers to work on the development of intranasal dosage form for peptide and protein molecules.

Compound lipophilicity

Absorption of drug substances through biological membrane may be dependent on the hydrophilic lipophilic balance (HLB) of the compounds.[79] Large number of studies has been carried out, for evaluating the effect of lipophilicity on nasal absorption of conventional drug molecules using rat as an animal model. It was found that nasal absorption of drug was increased with increasing lipophilicity of the compounds.[80] Nasal absorption of steroids was directly correlated with lipophilicity of drug molecules and was found to be independent of pH.[81] Corbo and coworker investigated nasal absorption of progesterone and its derivatives.[82] The results showed increased nasal absorption with increasing lipophilicity of the progesterone derivatives. An interesting study, which demonstrates the relationship between lipophilicity and absorption was carried out in acyclovir prodrugs using rat as an animal model.[83] Increased order of nasal absorption of acyclovir prodrugs was observed based on their lipophilicity, whereas parent acyclovir showed no apparent absorption. The authors further concluded that nasal absorption was more for low molecular weight compounds with moderate lipophilicity than high molecular weight compounds with high lipophilicity. Nasal absorption of drugs through olfactory bulb has received more attention in recent days.[84] The lipophilicity of the drug molecule is the first prerequisite for centrally acting drugs. However, direct nose to brain pathway allows the transportation of drug without sufficient lipophilicity.[85] The influence of lipophilicity on drug absorption to CNS was studied using sulpha drugs in rat model.[86] The mean cerebrospinal fluid drug concentration was found to increase with lipophilicity of sulpha drugs. The nasal absorption of L-Tyrosine and its prodrugs were studied to explore the effect of structural modification on drug absorption.[87] It was observed that carboxylic ester derivatives of L-Tyrosine were absorbed 4-10 times faster than parent drug. This study concludes that increase in the rate and extent of absorption of drug from nasal mucosa is not only because of compound lipophilicity but also due to lack of anionic moiety in the structure of carboxylic ester derivatives.

pH, pKa, and ionization constant

The pH partition theory explains the process of drug absorption from biological membrane and its distribution across biological membrane. Since most of the compounds available for therapeutic use are of either weakly acidic or weakly basic in nature, their degree of ionization depends on the pH of nasal secretion, which is between 4.5 and 6.5. Thus pH is essential for physiological defense mechanism against microbial organisms. Lysozyme is present in nasal secretion, which is responsible for destroying certain microorganism at acidic pH. Lysozyme inactivation at alkaline condition leads to nasal infection and hence it affects the nasal mucociliary clearance and bioavailability of nasal formulations.[88] The amount of drug that exists in unionized form in nasal cavity is a function of pka of the drug molecule and pH of nasal secretion. As nasal secretion pH is a fairly constant in healthy human subjects, pKa of drug molecule determines the degree of ionization and hence bioavailability of nasally administered drugs.[89] The effect of pH on nasal absorption of drugs was investigated at different pH values.[90] The pH, at which drugs are available in unionized form is favorable for absorption of drugs and hence it improves the bioavailability of nasal dosage form. Studies at pH ranging from 2.0 to 7.1 of the nasal absorption of benzoic acid were reported.[90] High degree of absorption was found to be non ionized form (pH 2.5, 44%) but even at 99.9% ionization (pH 7.19), 13% absorption was observed. The rate of absorption was four times faster for non ionized species when compared with ionized form of benzoic acid. However, manipulation of micro environmental pH of nasal mucosa is not advisable, because acidic pH causes nasal irritation followed by increase in the nasal mucociliary clearance rate, whereas alkaline pH leads to the microbial susceptibility and infection. Therefore, it is advised to maintain the nasal formulation pH in the physiological range of 4.5 to 6.5.

Formulation barriers

Drug concentration, dose, and dose volume

Nasal absorption was shown to increase with certain drug substances, particularly, where concentration gradient plays an important role in drug absorption. *Ex vivo* experiments in rats demonstrated the effect of drug concentration on nasal absorption. Nasal absorption of l-tyrosyl -L- tyrosine was found to increase with increasing concentration of the drug. However, few experiments showed different effects of drug concentration on the absorption of drugs, for example, the absorption of aminopyrine from rat nasal mucosa was constant as a function of its concentration.[91] Interestingly, nasal absorption of salicylic acid was decreased with increasing concentration of administered drug. Low absorption of high concentration of salicylic acid was lined with its nasal epithelial toxicity and nasal membrane resistance.[92] The effect of three different concentrations of cetrizine on clinical efficacy was studied in patients.[93] The clinical efficacy was improved with drug concentration up to only 0.125%. Moreover, the clinical efficacy has been declined in the higher drug concentration of 0.250%. From the above studies one cannot judge the effect of drug concentration on absorption and bioavailability. However, the absorption

of drug and its concentration cannot be correlated with the mechanism of drug absorption from the nasal mucosa. If the drug undergoes passive diffusion, it should obey linear relationship between drug concentration and absorption. But this is not true because various factors such as quantity and nature of mucus, mucociliary clearance, membrane resistance are the membrane transport of drugs through nasal cavity. The effect of dose on nasal absorption was studied with large number of compounds such as secretin, calcitonin, desmopressin, etc.[94] Higher nasal absorption was observed with high concentration of dose. Nasal cavity has capacity to retain limited volume of the administered dose, beyond which formulation will drain out of the nasal cavity. Higher drug concentration was administered with high volume of dose that may not increase the drug absorption through nasal cavity. The ideal dosage form for better absorption should possess dose volume around 25 µl - 150 µl. The maximum dose is soluble in minimum quantity of liquid dosage form (less than 200 µl), the higher drug concentration definitely leads to high absorption and hence bioavailability.

Osmolarity of dosage form

The effect of formulation osmolarity on nasal absorption was studied using secretin as a model drug in rats.[95] The results indicated profound influence of osmolarity on nasal drug absorption. The nasal drug absorption has been affected by the sodium chloride concentration in the formulation. Maximum drug absorption was observed with 0.462 M sodium chloride concentration. High concentration of sodium chloride not only leads to higher bioavailability but also toxicity to nasal epithelial cells.[96] The maintenance of isotonicity of the formulation will reduce the nasal epithelial cell damage and hence it will reduce the toxicity of the nasal formulation. The effects of formulation tonicity on nasal absorption and bioavailability have not been extensively studied. More research in this area is required to optimize the tonicity modifiers in nasal formulation.

Deposition site of dosage form

The site of deposition of nasal formulation in nasal cavity is an important factor for absorption and bioavailability of nasal dosage forms. In general, deposition of formulation in anterior portion of the nose provides a prolonged nasal residential time and better absorption.[97] The dosage form deposited in posterior chamber of nasal cavity will be eliminated by nasal mucociliary clearance and hence show low bioavailability.[97] The site of deposition and deposition pattern of liquid dosage form is dependent on the delivery device, mode of administration, physicochemical properties of drug molecules.[98] Nasal drops, traditionally used in formulations suffer from short nasal residential time. The correlation between dosage form deposition and bioavailability of and/or therapeutic efficacy is not very easy to establish. Large number of factors such as position of head, viscosity, delivery device, tonicity, and volume of the dosage form affects the absorption and bioavailability of nasal drops. Powder nasal dosage forms are rarely used in clinical applications. The nasal residential time of powder dosage form is almost equal to that of liquid dosage form. However, nasal residential time of powder dosage form can be increased with usage of bioadhesive polymers such as carbopol, poly carbophil, etc.[99] Low clearance rate of nasal powder dosage form has shown better patient compliance, especially in children if the taste and smell of dosage form is unacceptable. Aerodynamic properties of the powder dosage form determine the deposition pattern in the nose. Furthermore, powder properties such as particle size and shape, density and flow characteristics and dosage form delivery devices have an influence on the distribution of drug in nose and hence it affects the absorption and bioavailability.[100] The deposition of the gel in the nasal cavity depends on the mode of administration, the nasal gel has poor spreading abilities due to its high viscosity and hence it shows narrow distribution area in nasal cavity.[101] The deposition gel of the nasal cavity and its influence in bioavailability is not sufficiently explored and large number of studies is required to conclude a firm decision.

STRATEGIES TO IMPROVE BIOAVAILABILITY

A wide number of formulation strategies are made available to improve the bioavailability of nasal dosage forms. The basic underlying mechanisms for bioavailability enhancement are as follows (i) incorporating nasal permeation enhancers to improve the absorption, (ii) usage of enzyme inhibitors to eliminate nasal metabolism, (iii) formulation of mucoadhesive dosage forms to improve the nasal residential time, and (iv) prodrug approach for optimizing favorable physicochemical properties. Any one of the approach or combination of two or more strategies is widely used to improve the bioavailability of nasal formulations. While developing novel nasal dosage form with enhanced bioavailability, range of factors such as dose, dosing frequency, duration of therapy, short term and long term toxicity of drugs and excipients, therapeutic indication, cost and type of patients to be treated should be considered to achieve both safety as well as efficacy of nasal formulations. Various strategies used to improve the bioavailability of nasal drug products are listed in Table 2.

Enzyme inhibitors

A number of studies have described the role of enzyme inhibitors on bioavailability of nasal formulations.[102,103] Particularly, enzyme inhibitors are essential components of formulation, while developing a dosage form for protein and peptide molecules. Mostly peptidase and protease inhibitors are widely used to improve the bioavailability of protein and peptide molecules. Enzymatic activity can also be reduced by addition of enzyme inhibitors such as bestatin, amastatin, boroleucin, borovaline, aprotinin, and trypsin inhibitors.[104] The absorption enhancers such as bile salts and fusidic acid derivatives exert enzyme inhibition and hence enhance the absorption and bioavailability.[105]

Table 2: Strategies to improve nasal bioavailability

1.	Nasal enzyme inhibitors e.g., bestatin, amastatin, boroleucine, fusidic acids, and bile salts
2.	Nasal permeation enhancers e.g., cyclodextrins, surfactants, saponins, fusidic acids, and phospholipids
3.	Prodrug approach e.g., cyclic prodrug, esters, and derivatization of C and N termini
4.	Nasal mucoadhesives in nasal drug delivery e.g., carbopol, polycarbophil, cellulose derivatives, lecithin, and chitosan
5.	Particulate drug delivery e.g., microspheres, nanoparticles, and liposome

The analgesic activity of leucine enkephalin and its analogue was investigated with and without enzyme inhibition. The addition of azelaic acid (1%) and thiomersal gave the maximum analgesic activity in mice.[106] Nasal bioavailability can also be increased by PEGylation, synthesis of prodrugs and analogues. PEGylated salmon calcitonin showed strong resistance against enzymatic degradation and hence highest bioavailability was achieved.[107] Chemical modification of salmon calcitonin to elcatonin (S-S bond is replaced by C-N bond) showed better bioavailability than salmon calcitonin.[108] Moreover, the addition of endocytic inhibitor did not change the bioavailability of elcatonin; indicating the enzymatic resistance of modified calcitonin. However, chemical modification, prodrugs and analogues requires clinical studies for regulatory approval. Clinical studies are costly and time consuming and there is no guarantee for success of drug product, so these methods for enzyme inhibition are considered as non-lucrative approach for pharmaceutical companies.

Nasal permeation enhancers

Permeation enhancers have been employed for improving the absorption of poorly absorbed and large molecular weight compounds. Complete mechanism of drug absorption enhancement through nasal mucosa is not known. However, various mechanisms such as increase in the membrane fluidity, creating transient hydrophilic pores, decreasing the viscosity of mucous layer and opening up of tight junctions are the proposed mechanisms of permeation enhancers, which improve the bioavailability of nasal dosage forms.[109] Some of the permeation enhancers like bile salts and fusidic acid derivatives can also inhibit the enzymatic activity in the membrane, thereby improving bioavailability. Even though nasal permeation enhancers can improve the therapeutic efficacy of drug products, its toxicity should be considered while developing dosage form. One of the most common and frequently reported problems with permeations enhancer is the nasal irritation during administration of nasal dosage form.[110] The ideal characteristics of nasal permeation enhancers are as follows:

a) It should be pharmacologically inert.
b) It should be non-allergic, non-toxic, and non-irritating.
c) It should be highly potent.
d) It should be compatible with a wide variety of drugs and excipients.
e) It should be odorless, tasteless, and colorless.
f) It should be inexpensive and readily available in highest purity.
g) It should be accepted by many regulatory agencies all around the world.

The permeation enhancer not only can lead to improvement in the absorption and bioavailability but also provides uniform dosing efficacy. Their non-specific action, long-term toxicity and nasal irritation are the major hurdles, which affects the clinical applicability of permeation enhancers in the development of nasal dosage form. The different excipients used in nasal formulation including permeation enhancers are classified in Table 3.

Cyclodextrins act as a solublizer and permeation enhancer for nasal drug delivery and they are well tolerated in humans.[111] Amongst cyclodextrins, beta cyclodextrin is being considered to have a Generally Recognized As Safe (GRAS) status. All other cyclodextrins are experimental material at this time. Schipper and coworkers studied the efficacy of beta cyclodextrin as permeation enhancer for nasal drug delivery of insulin.[112] The administration of insulin in a 5% solution of dimethyl beta cyclodextrin did not enhance the absorption of insulin in rabbits, whereas powder dosage form significantly enhances the bioavailability of insulin in rabbits.[112] Several compounds such as calcitonin, cortisone, diazepam, and naproxen have been investigated for their nasal bioavailability enhancement using cyclodextrin as the permeation enhancer.[113] Surfactants are the most effective permeation enhancers, but the issues like nasal irritation, epithelial toxicity, and ciliostatic activity are the barriers for usage of surfactant as a nasal permeation enhancer. The extent of nasal absorption of insulin from nebulizer spray was observed to be pH dependent indicating maximum absorption at acidic pH. However, nasal absorption of insulin with surfactants like saponins, BL-9 and glycolate was significantly increased even at acidic pH, which correlated with hypoglycemic effect.[113] Comparative pharmacokinetics of intranasal delivery of salmon calcitonin was studied with various surfactants. A 10-fold increase in serum calcitonin levels over the control group (calcitonin without surfactant) was observed in formulations incorporated with surfactants.[113] Laureth-9 was used as a permeation enhancer to improve the intranasal bioavailability of insulin.[113] The bile salts are believed to improve the bioavailability by both solublization of insulin and by direct effect of surfactant on the cell membrane.

A fusidic acid derivative, primarily sodium taurodihydrofusidate (STDHF) was widely used as a nasal permeation enhancer to improve the nasal bioavailability. STDHF was developed as anti

Table 3: Excipients used in nasal dosage forms

Pharmaceutical excipients	Type of dosage form	Comments
Permeation enhancers e.g., 1. Cyclodextrins 2. Fusidic acid derivatives 3. Phosphatidylcholines 4. Microspheres and liposomes 5. Bile salts and surfactants	Powders, gels, solutions	Improve the bioavailability of drugs particularly molecular weight above 1000 Da Causes nasal epithelial toxicity
Solvents e.g., ethanol, polyethylene, propylene glycols, etc.	Gels, solutions	To increase the concentration of drug in vehicle and reduce the dose volume. May act as a permeation enhancer
Viscosity modifiers e.g., cellulose derivatives	Gels, solutions	To improve nasal residential time
Mucoadhesive polymers e.g., carbopol, polycarbophil, cellulose derivatives, lecithin, and chitosan	Powders, gels, solutions	To improve the nasal residential time
Preservatives e.g., benzalkonium chloride	Gels, solutions	To maintain sterility of dosage form, may alter the nasal residential time
Enzyme inhibitors e.g., bestatin, amastatin, boroleucine, fusidic acids, and bile salts	Powders, gels, solutions	To improve the bioavailability of protein and peptides
Tonicity modifiers/buffers e.g. sodium chloride, citrate buffer	Solutions	To avoid the nasal epithelial toxicity

bacterial agent and hence its short term and long term toxicity was well established.[114] The physicochemical properties of fusidic acid and its derivatives are similar to that of bile salts. STDHF was extensively used as a transnasal permeation enhancer for protein and peptide molecules. Bioavailability of insulin was found to be 18% and 5% with 1% of STDHF in rats and rabbits, respectively.[115] The 21-fold increase in nasal bioavailability of human growth hormone with 0.5% STDHF was observed in comparative pharmacokinetic studies in sheep.[116] The nasal bioavailability of protein and peptide molecules such as insulin, calcitonin, human growth hormone, and octreotide using STDHF as permeation enhancer showed increase in the bioavailability and also showed the safety of the STDHF as a permeation enhancer.[117] Phospholipids are surface active compounds, which are found in both animal cells as well as plant cells. Several researchers have explored the efficacy of these compounds as nasal permeation enhancer. Lysophosphatidylcholine (LPC) is the most extensively studied phospholipid as a nasal permeation enhancer. The enhanced intranasal bioavailability of insulin and human growth hormone was observed with various animal models such as rat, rabbit, and sheep.[118] The absorption of biosynthetic human growth hormone was studied using various permeation enhancers in rats. The relative bioavailability of 25.8% was observed with LPC, whereas other permeation enhancers required high concentration to show similar effect.[119] Didecanoyl-L-phosphatidylcholine (DDPC) was the other important permeation enhancer, belonging to the phospholipid family. The pharmacokinetic studies of growth hormone were studied using different concentrations of DDPC as a permeation enhancer. The results concluded that increasing relative concentration of DDPC increases the absorption of nasally administered growth hormone in animal models. Similar study was carried out to explore the impact of DDPC on bioavailability of human growth hormone. Significant absorption enhancement was observed with DDPC.[120] However, nasal necrosis was observed in contrast to human studies. Nasal bioavailability enhancement of growth hormone by DDPC was due the enhanced transport by transcellular route through ciliated cells. In either case more studies are required to determine the toxic effect of DDPC on nasal mucosa.

Chitosan and its derivatives are widely used in nasal drug delivery system as permeation enhancer.[121] Strong cationic nature of chitosan promotes interaction with sialic acid present in mucosa and hence it opens the TJs of nasal epithelial cells. Chitosan formulations were extensively studied not only for delivery of drugs but also for vaccines. Chitosan increased the serum IgG titer after administration of diphtheria vaccine.[122] It was observed that chitosan formulation was superior to non chitosan formulations in inducing antibody levels. In another study, chitosan powder dosage form was considered as superior nasal dosage form, which not only increased the bioavailability of insulin but also improved the stability of insulin both in nasal environment.[123] In addition to chitosan, its derivatives are widely used in drug delivery applications especially in nasal drug delivery system. N-trimethyl chitosan chloride, a quaternary ammonium salt of chitosan improves the nasal abosprtion of (^{14}C)-mannitol in rats.[124] However, the degree of trimethylation plays an important role in absorption enhancement property.

Prodrug approach

Prodrug approaches, historically, have been used to improve the pharmaceutical properties such as solubility, taste, odor, stability, etc. In recent days, designing of prodrug is used to improve the physicochemical properties such as solubility and compound lipophilicity to overcome the pharmacokinetic demerits associated with drug molecules. However, prodrug approach has also been used to reduce the presystemic metabolism and chemical decomposition. The basic principle associated with prodrug is to cover the undesired functional group(s) with another functional group, which usually are referred as promoiety. Designing prodrug for improving the nasal bioavailability is one of the lucrative approaches especially for protein and peptide molecules. The ideal prodrug for bioavailability enhancement of proteins and peptides would exhibit enhanced membrane permeability along with increased enzymatic stability. After crossing both enzymatic and membrane barrier, the prodrug should undergo enzymatic transformation to release the parent molecule.

Designing of cyclic prodrug using C and N terminal ends reduced the metabolic degradation caused by exopeptidase. A recent study involved synthesis of cyclic hexapeptide to improve the enzymatic stability and permeability through biological membranes.[125] It showed increased permeability of cyclic prodrug than parent molecule. Derivatization is another lucrative approach for synthesis of prodrug to improve bioavailability of drug molecules especially for peptide molecules. Derivatization could be possible in C terminal amide group, N terminal amide group and phenol group in various peptide molecules. The nasal absorption enhancement of L-Tyrosine was achieved by structural modification.[126] It was observed that carboxylic ester derivatives have enhanced absorption than their parent molecule. The enamine derivatives were prepared to improve the absorption of peptide molecules such as angiotensin II, bradykinin, carulein, carnosine, enkephalin, vasopressin, and calcitonin.[127] These agents showed absorption enhancement with prodrug approach. The research interest and applicability of prodrug concept is not only restricted to protein and peptide molecules but also extends to conventional molecules. Nasal absorption of non absorbable hydrophilic compound, acyclovir was achieved by synthesizing a series of aliphatic ester prodrugs.[128] The ester prodrugs showed consistent increase in lipophilicity with corresponding decrease in aqueous solubility and increase in absorption through rat nasal mucosa. The conversion kinetics of ester derivatives of acyclovir to parent molecule is due to the presence of respiratory carboxylesterase in nasal respiratory epithelium.[128]

Nasal mucoadhesive drug delivery system

Although nasal enzyme inhibition and incorporation of permeation enhancers are the two popular approaches, these approaches hinder the normal physiological process and hence are prone to cause toxicity of nasal mucosa. Designing bioadhesive drug delivery system is a novel approach in nasal drug delivery, which enhances the nasal residential time of the drug molecule and hence enhances the absorption and bioavailability of nasally administered drug products. Bioadhesion is the ability of synthetic or natural material to adhere to a biological tissue or membrane for a prolonged period of time. Bioadhesive drug delivery implies attachment of drug delivery system to a specific biological tissue, which increases the local residential time of the delivery system. If biological tissue is covered by mucus, the attachment of drug delivery system to the mucus is called as mucoadhesive drug delivery system. Mucoadhesive system is the ideal choice of drug delivery system for systemic nasal drug delivery because it improves the nasal residential time. Intimate contact of drug delivery system to the nasal mucosa not only prolongs the duration of action but also increases extent of absorption. Pharmaceutical excipients which improve the mucoadhesion are called as mucoadhesive materials. The mucoadhesive synthetic and natural polymers are called as first generation mucoadhesive material. Apart from these polymers, lecithin a new second generation promising mucoadhesive material is widely used in drug delivery systems.[129] Lecithin is a non immunogenic compound, basically constructed by protein or glycoprotein moiety capable of specific and reversible binding with mucin and other carbohydrate moiety.

Mucoadhesive drug delivery has been used to improve the therapeutic efficacy of local as well as systemic drug delivery. The bioavailability of nasally administered drugs was improved with all kinds of therapeutic substances such as small organic molecules, antibiotics, vaccines, DNA, proteins, and other macro molecules.[130] Intranasal bioavailability of aqueous solution of apomorphine was found to be 45%.[131] The nasal bioavailability of apomorphine is rate limited by drainage of aqueous solution through nasopharynx and rapid oxidation in aqueous solution. Highest nasal bioavailability of 98% of apomorphine was achieved by using mucoadhesive polymer like polyacrylic acid, carbopol, and carboxymethylcellulose.[132] Nasal mucoadhesive gels are the lucrative ways of improving the nasal residential time. Chitin and chitosan nasal gel formulations were prepared using indomethacin and papaverine as model drugs.[99] Chitin nasal gel increased the nasal residential time of both drugs than nasal powder dosage form.[133] Similar study showed that cationic chitosan was fairly mucoadhesive in comparison to polycarbophil as a reference substance. Nasal absorption of nifidipine gel has been studied in rats.[134] Nasal administration of PEG/carbopol system resulted in rapid absorption and high C_{max}. However, the rapid rate of elimination was also found in the same study. Plasma concentration of nifedipine after nasal administration in aqueous carbopol gel formulation was very low.[134] The usage of PEG in nasal formulation should be carefully optimized, because it is irritant to nasal mucosa if concentration exceeds 10%. The effect of insulin loaded polycarbophil gel on nasal bioavailability was studied

in animal models.[135] Low concentration of polymer favors absorption and hence better bioavailability. Carbopol, pluronic, chitosan and its derivatives, polycarbophil and cellulose derivatives are widely studied as gelling agents in nasal drug delivery.[136] Mucoadhesive powder dosage form not only offered increased nasal residential time but also reduced the oxidation of apomorphine in nasal cavity. In addition to apomorphine, other small molecular weight compounds including budenoside, caffeine, ketorolac, nicotine, pentazocine, and ondansetron have been characterized for nasal administration with mucoadhesives.[137]

The airway diseases such as rhinitis and asthma are commonly associated with inflammation. The systemic administration of steroidal drugs produces low drug concentration at the targeted site. Moreover, it has been associated with systemic toxicity such as immunosuppression, fluid retention, and hyperacidity. Nasal delivery of steroid is one of the meaningful approaches to improve the therapeutic efficacy with minimum systemic toxicity. However, nasal residential time of such dosage form determines the duration of action; nasal mucoadhesive dosage form improves the duration of action of steroidal drugs along with patient compliance. Petersen and coworkers[138] studied the pharmacokinetics of budenoside loaded bioadhesive grafted copolymers of polymethacrylic acid and polyethylene glycol. The result stated that the bioadhesive drug delivery system offered relatively quick absorption (T_{max} ~ 45 min) with steady state plasma drug concentration that lasted more than 8 h. Continuous release of the drug could be possible because carboxylic group of polymers strongly adhered to the epithelial mucosa. The physical interaction of mucoadhesion may be due to hydrogen bonding at acidic pH.[138] Nasal mucoadhesive vaccine loaded microparticles induced both systemic as well as mucosal immunity. Vila and coworkers studied the nasal immunization of tetanus toxoid by encapsulating and administration in the form of PEG coated polyacetic acid mucoadhesive nanospheres.[139] The results indicated high level of tetanus toxoid in the blood as compared to non bioadhesive nasal drug delivery system. The protein and peptide molecules bioavailability is rate limited by short nasal residential time of the formulation in nasal cavity, which impaired the uptake of the macromolecules from nasal epithelial cells. Insulin is one of the most widely studied proteins with respect to nasal delivery using mucoadhesive dosage forms.[103,140] The strategies adopted to improve the nasal bioavailability of insulin are mucoadhesive micropartcles and nanoparticles, mucoadhesive gels and powders. Mucoadhesive polymers also act as permeation enhancer by opening the TJs of the nasal epithelium and hence it improves the bioavailability. New generation mucoadhesive polymers such as chitosan derivatives and polycarbophil derivatives are in developmental stage. If these polymers get regulatory approval, we can expect few nasal mucoadhesive drug delivery systems in market.

Particulate drug delivery system

The classical approach to improve the bioavailability of nasal formulations is particulate systems such as microspheres, nanoparticles, and liposomes. The microspheres used in nasal drug delivery are water insoluble but absorb water into the sphere matrix, resulting in swelling of sphere and the formation of gel. The gel formation improves the nasal residential time and hence it improves the bioavailability. Another mechanism stated for improving nasal bioavailability is improving the nasal permeation by opening of the tight junctions of the nasal epithelium. A wide variety of materials were investigated to construct the microspheres including starch, dextran, albumin, hyaluronic acid, carbopol, chitosan, etc.[141,142] Dextran microspheres have been used as a delivery system of nicotine, insulin, and octreotide. Illum and coworkers introduced well characterized bioadhesive dextran microspheres for prolonging residence time in the nasal cavity.[142] The slowest clearance was detected for DEAE-dextran, where 60% delivery dose was present at the deposition site after 3 h. However, the microspheres did not successfully improve the bioavailability of insulin.[143] In later study, the same insulin dose administered with dextran microsphere of particle size less than 45 µm, which showed 52% decrease in plasma glucose in rats.[144] Degradable starch microspheres (DSM) are the most frequently used nasal drug delivery systems for absorption of insulin, gentamicin, human growth hormone metclopromide, cynacobalamin, and desmopressin.[26] The safety and efficacy of nasal microspheres have been demonstrated by many researchers.[26,145] Insulin loaded DSM resulted in a rapid dose dependent decrease in blood glucose. The histopathological studies in rabbits have demonstrated that DSM does not induce a series of morphological change in nasal mucosa.[144] Moreover, the DSM was well tolerated by human volunteers with absence of nasal irritation and did not cause significant changes in nasal mucociliary clearance.[146] DSM was used as a carrier for delivery of human growth hormone. DSM loaded human growth hormone was prepared with and without permeation enhancer. The relative bioavailability of human growth hormone in sheep model is 3%, whereas it increased to 14% with permeation enhancer (lysophophatidylcholine) incorporated microsphere.[147] Chitosan loaded microspheres are extensively used as drug delivery vehicle for nasal drug administration. Chitosan microspheres were fabricated to improve the bioavailability and achieve the prolonged release profile of gentamicin.[148] Chitosan microspheres showed better adhesion to nasal mucosa because of its cationic nature and opening of the TJ, hence it showed prolong release profile and improved bioavailability, respectively. Hydroxypropyl methylcellulose, gelatin, polyacrylic acids, polycarbophils, carbopol, gelatin, and albumin are the polymers widely used to formulate the microspheres for nasal drug delivery.

Liposomes have been delivered by nasal route; the amphiphilic nature of liposome is well characterized for favorable permeation of drugs through biological

membranes. The permeability of liposome entrapping insulin through nasal mucosa of rabbits has been studied with and without incorporating sodium glychoate as a permeation enhancer.[149] The comparative pharmacokinetics in rats showed high permeability of liposome pretreated with permeation enhancer than solution containing the same quantity of permeation enhancer.[149] The loading and leakage character of desmopressin loaded liposome and the effect of liposome on permeability of desmopressin on nasal mucosa was studied. High permeability of liposome was achieved than solution dosage form. In liposome formulation, cationic liposomes are prone for higher permeability than negatively charged liposomes.[149]

CONCLUSION

Nasal drug delivery has a great potential to treat both acute and chronic diseases. Last 25 years of nasal drug delivery research has provided only a handful of products in market. Most of these products are of conventional molecules rather than protein molecules. The suggested reason for drug products of protein and peptide molecule do not meet the regulatory requirements is due to low bioavailability and high toxicity of drug products incorporated with bioavailability enhancers. One of the most important factors hindering the quality of nasal drug product is inter and intra subject variability in pharmacokinetics of dosage forms. Bioavailability of nasal drug products is one of the major challenges for pharmaceutical companies to bring their product in market. The circumstances, which do not favor clinical applicability of nasal drug product is the lack of enough basic research in the area of nasal drug delivery. In contrast, pharmaceutical companies are investing a huge amount of money in the development of nasal drug products because of growing demand of nasal drug products in global pharmaceutical market. This research environment will lead to serious of adverse effects in the society in future. To avoid such backdrops, biomedical scientists, formulation researchers, pharmaceutical companies, funding agencies, and government along with regulatory bodies should pay attention to basic research in nasal drug delivery such as nasal pathophysiology, invention of new excipients to improve the nasal bioavailability, drug delivery devices, toxicodynamic studies of drugs and excipients and *in vitro* methods for nasal drug metabolism and bioavailability.

REFERENCES

1. Chein YW, Su KS, Chang SF. Nasal systemic drug delivery. 2nd ed. New York: Dekker; 1989. p. 26.
2. Kissel T, Werner U. Nasal delivery of peptides: an *in vitro* cell culture model for the investigation of transport and metabolism in human nasal epithelium. J Control Rel 1998;53:195-203.
3. Talegonkar S, Mishra PR. Intranasal delivery: An approach to by pass the blood brain barrier. Ind J Pharmacol 2004;36:140-7.
4. Kao HD. Enhanced delivery of L Dopa by the administration of its prodrug via nasal route. Ph. D thesis, University of Kentucky Lexington, Kentucky: 1995.
5. Wan Po AL, Rogers E, Shepphard M, Scott EM. Delivery system for non parental vaccines. Adv Drug Del Rev 1995;18:101-9.
6. Bryant ML, Brown P, Gurevich N, McDougall IR. Comparison of the clearance of radio-labelled nose drops and nasal spray as mucosally delivered vaccine. Nucl Med Commun 1999;20:171-4.
7. Zuercher AW, Coffin SE, Thurnheer MC, Fundova P, Cebra JJ. Nasal-associated lymphoid tissue is a mucosal inductive site for virus-specific humoral and cellular immune responses. J Immunol 2002;168:1796-803.
8. Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. J Pharm Pharmacol 2001;53:3-21.
9. Romeo VD, deMeireles JC, Gries WJ, Xia WJ, Sileno AP, Pimplaskar HK, et al. Optimization of systemic nasal drug delivery with pharmaceutical excipients. Adv Drug Deliv Rev 1998;29:117-33.
10. Michael H, Lisbeth I. Intra nasal insulin delivery and therapy. Adv Drug Del Rev 1999;35:199-234.
11. Behl CR, Pimplaskar AP, Sileno JD, Romeo VD. Effect of physicochemical and other factors on systemic nasal drug delivery. Adv Drug Deliv Rev 1997;29:89-116.
12. Kamath KR, Park K. Mucoadhesive preparation. New York: Marcel Dekkar; 2003. p. 133-63.
13. O'Hagan DT, Critchley H, Farraj NF, Fisher AN, Johansen BR, Davis SS, et al. Nasal absorption enhancers for biosynthetic human growth hormone in rats. Pharm Res 1990;7:772-6.
14. Ambruosi, A, Yamamoto H, Kreuter J. Body distribution of polysorbate-80 and doxorubicin loaded [14C] poly(butyl cyanoacrylate) nanoparticles after I.V. administration in rats. J Drug Target 2005;13:535-42.
15. Agu RU, Jorissen M, Willems T. Effects of pharmaceutical compounds on ciliary beating in human nasal epithelial cells: A comparative study of cell culture models. Pharm Res 1999;16:1380-5.
16. Charlton ST, Davis SS, Illum L. Evaluation of bioadhesive polymers as delivery systems for nose-to-brain delivery: In vitro characterization. J Control Rel 2006;61:167-82.
17. Martin M, Hutchinson LE, Hyde R, Peters GE. Analysis of structural requirements for the absorption of drugs and macromolecules from nasal cavity. J Pharm Sci 1987;76:535-40.
18. Hussain AA, Dakkuri A, Itoh S. Nasal absorption of ondansetron in rats: An alternative route of drug delivery. Cancer Chemother Pharmacol 2000;45:432-4.
19. Monterio-Riviere NA, Popp JA. Ultra structural characterization of the nasal respiratory epithelium in rats. Am J Anat 1984;169:31-43.
20. Costantino HR, Illum L, Brandt G, Johnson PH, Quay SC. Quay Intranasal delivery: Physicochemical and therapeutic aspects. Int J Pharm 2007;337:1-24.
21. Verdugo P. Goblet cells secretion and mucogenesis. Annu Rev Physiol 1990;52:157-76.
22. Clary-Meinesz CF, Cosson J, Huitorel P, Blaive B. Temperature effect on ciliary beat frequency of human nasal and tracheal ciliated cells. Biol Cell 1992;76:335-8.
23. Wanner A. Clinical aspects of mucociliary transport. Am Rev Respir Dis 1977;116:73-125.
24. Satir P, Sleigh MA. The physiology of cilia and mucociliary interactions. Annu Rev Physiol 1990;52:137-55.
25. Zhou M, Donovan M. Recovery of nasal mucosa following laureth-9 induced damage. Int J Pharm 1996;130:93-102.
26. Illum L, Farraj N, Critvhley H, Davis SS. Nasal administration of gentamycin using novel microsphere delivery system for drugs. Int J Pharm 1988;46:261-5.
27. Marttin E, Nicolaas GM, Schipper J, Coos V, Frans WH. Nasal mucociliary clearance as a factor in nasal drug delivery. Adv Drug Del Rev 1997;29:13-38.
28. Passali D, Bianchini Ciampoli M. Normal values of mucociliary transport

time in young subjects. Int J Pediatr Otorhinilaryngol 1985;9:151-6.
29. Hybbinette JC, Mercke U. Effect of parasympathomimetic agonists and antagonists on mucociliary activity. Acta Otolaryngol 1982;93:465-73.
30. Corssen G, Alien CR. Acetylcholine: Its significance in controlling ciliary activity of human respiratory epithelium in vitro. J Appl Physiol 1959;14:901-4.
31. Lindberg S, Khan R, Runer T. The effect of formoterol, a long acting beta (2) -adrenoreceptor agonist on mucociliary activity. Eur J Pharmacol 1995;285:275-80.
32. Hee J, Guilerm R. Discussion on smoke and mucociliary transport. Eur J Respire Dis Suppl 1985;66:86-8.
33. Donovan MD, Zhou CS. Drug effects on in vivo nasal clearance in rats. Int J Pharm 1995;116:77-86.
34. Deitmer T, Scheffler R. The effect of different preparations of nasal decongestants on ciliary beat frequency in vitro. Rhinology 1993;31:151-3.
35. Kanthakumar K, Cundell DR, Johnson M, Wills PJ, Taylor GW, Cole PJ, et al. Effect of salmeterol on human nasal epithelial cell ciliary beating: Inhibition of ciliotoxin in pyocyanin. Br J Pharmacol 1994;112:493-8.
36. Joki S, Sano J, Nuutinen P. Effect of some preservative agents on rat guinea pig tracheal human nasal ciliary beat frequency. Am J Rhinol 1996;10:181-6.
37. Batts AH, Marriott C, Martin GP, Bond DW. The effect of some preservatives in nasal preparation on mucociliary clearance. J Pharm Pharmacol 1989;41:156-9.
38. Batts AH, Marriott C, Martin GP, Wood CF, Bond SW. The effect of some preservatives in nasal preparation on mucus and ciliary components of mucociliary clearance. J Pharm Pharmacol 1990;42:145-51.
39. Batts AH, Marriott C, Martin GP, Bond DW, Greaves JL, Wilson CG. The use of radio labeled saccharin solution to monitor the effect of the preservative thiomersal benzalkonium chloride and EDTA on human nasal clearance. J Pharm Pharmcol 1991;43:180-5.
40. Ainge G, Bowles JA, McCormick SG, Richards DH, Scales MD. Lack of deleterious effect of corticosteroid sprays containing benzalkonium chloride on nasal ciliated epithelium. In vivo results in laboratory animals. Drug Invest 1994;8:127-33.
41. Hirai S, Yashiki T, Mima H. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. Int J Pharm 1981;9:173-84.
42. Hermens WA, Hooymans PM, Verhoef JC, Merkus FW. Effect of absorption enhancers on human nasal tissue ciliary movement in vivo. Pharm Res 1990;7:144-6.
43. Donovan MD, Flynn GL, Amidon GL. The molecular weight dependence of nasal absorption: The effect of absorption enhancers. Pharm Res 1990;7:808-15.
44. Hermens WA, Deurloo MJ, Romeijn SG, Verhoef JC, Merkus FW. Nasal absorption of 17 ß oestradiol by dimethyl ß cyclodextrin in rabbits and rats. Pharm Res 1990;7:500-3.
45. Schipper NG, Romeijn SG, Verhoef JC, Merkus FW. Nasal insulin delivery with dimethyl beta cyclodextrin as an absorption enhancer in rabbits: Powder more effective than liquid formulations. Pharm Res 1993;10:682-6.
46. Romeijn SG, Verhoef JC, Martin E, Merkus FW. The effect of nasal drug formulations on ciliary beating in vitro. Int J Pharm 1996;135:137-45.
47. Okudu M, Mygind N. Pathophysiological basis for topical steroid treatment in nose. 1st ed. London: Tindall; 1980. p. 23-33.
48. Liu CM, Okuda M. Injurious effect of eosinophil extract on the human nasal mucosa. Rhinology 1988;26:121-32.
49. Mygind T, Lildhold T. Nasal Polyposis. An Inflammatory Disease and its Treatment. 1st ed. Copenhagen: Munksgaard; 1997. p. 50-60.
50. Winther B, Brofeldt S, Grønborg H, Mygind N. Pathology of naturally occurring colds. Eur J Respir Dis 1983;128:345-7.
51. Toft A, Wihl JA, Toxman J, Mygind N. Double-blind comparison between beclomethasone dipropionate as aerosol and as powder in patients with nasal polyposis. Clin Allergy 1982;12:391-401.
52. Brittebo EB. Metabolism of progesterone in the nasal mucosa in mice and rats. Acta Pharmacol Toxicol 1982;51:441-5.
53. Hussain A, Hamadi S, Kagoshima M, Iseki K, Dittert L. Does increasing the lipophilicity of peptides enhance their nasal absorption. J Pharm Sci 1991;80:1180-1.
54. Hussain AA, Hirai S, Bawarshi RS. Nasal absorption of propranolol. J Pharm Sci 1979;68:1196-9.
55. Bawarshi-Nassar RN, Hussain A, Crooks PA. Nasal absorption of 17a-ethinyl estradiol in the rat. J Pharm Pharmacol 1989;41:214-5.
56. Huang CH, Kimura R, Bawarshi-Nassar R, Hussain A. Mechanism of nasal absorption of drugs I: Physical chemical parameters influencing the rate of in-situ nasal absorption of drugs in rats. J Pharm Sci 1985;74:608-11.
57. Faraj JA, Hussain A, Aramaki Y, Iseki K, Kagoshima M, Dittert LW. Mechanism of nasal absorption of drugs III: Nasal absorption of leucine enkephalin. J Pharm Sci 1990;79:768-70.
58. Pontiroli AE. Peptide hormones: Review of current and emerging uses by nasal delivery. Adv Drug Deliv Rev 1998;29:81-7.
59. Prego C, Garcia M, Torres D, Alonso MJ. Transmucosal macromolecular drug delivery. J Control Rel 2005;101:151-62.
60. van Den Berg MP, Verhoef JC, Romeijn SG, Merkus MW. Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. Eur J Pharm Biopharm 2004;58:131-5.
61. Sayani AP, Chien YW. Systemic delivery of peptides and proteins across absorptive mucosa. Crit Rev Ther Drug Carrier Syst 1996;13:85-184.
62. Potschka H, Fedrowitz M, Loscher W. P-glycoprotein and multi drug resistance-associated protein are involved in the regulation of extra cellular levels of the major antiepileptic drug carbamazepine in the brain. Neuroreport 2001;12:3557-60.
63. Wioland MA, Fleury-Feith J, Corlieu P, Commo F, Monceaux G, Lacau-St-Guily J, et al. CFTR, MDR1, and MRP1 immuno localization in normal human nasal respiratory mucosa. J Histochem Cytochem 2000;48:1215-22.
64. Henriksson G, Norlander T, Zheng X, Stierna P, Westrin KM. Expression of P-glycoprotein 170 in nasal mucosa may be increased with topical steroids. Am J Rhinol 1997;11:317-21.
65. Graff CL, Pollack GM. P-glycoprotein attenuates brain uptake of substrates after nasal instillation. Pharm Res 2003;20:1225-30.
66. Graff CL, Pollack GM. Functional evidence for P-glycoprotein at the nose-brain barrier. Pharm Res 2005;22:86-93.
67. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics A Treatise. 1st ed. New Delhi: Vallabha prakasan; 1995. p. 34
68. Jones N. The nose and para nasal sinuses physiology and anatomy. Adv Drug Deliv Rev 2001;51:5-19.
69. Fisher A, Brown K, Davis S, Parr G, Smith DA. The effect of molecular size on the nasal absorption of water soluble compounds in the albino rat. J Pharm Pharmacol 1987;39:357-62.
70. Maitani Y, Machida Y, Nagai T. Influence of molecular weight and charge on dextran and DEAE dextran in rabbits. Int J Pharm 1989;40:23-7.
71. Irwin WJ, Dwivedi AK, Holbrook PA, Dey MJ. The effect of cyclodextrins on the stability of peptides in nasal enzymatic systems. Pharm Res 1994;11:1698-703.
72. Fisher AN, Illium L, Davis SS, Schachut EH. Di iodo L tyrosine labeled dextrans as molecular size markers of nasal absorption in the rats. J Pharm Pharmacol 1992;44:550-4.
73. Donovan M, Flynn, Amidon G. Absorption of polyethylene glycols 600 through 2000: the molecular weight dependence of gastrointestinal and nasal absorption. Pharm Res 1990;7:863-8.
74. Cecilia W. Nasal drug delivery - In vitro studies on factors influencing on absorption. Ph. D thesis. Acta Universitatis, Uppasala. 2002. p. 12-4.
75. Illum L, Watts P, Fisher AN, Hinchcliffe M, Norbury H, Jabbal-Gill I, et al. Intranasal delivery of morphine. J Pharmacol Exp Ther 2002;301:391-400.
76. Costantino HR, Sileno AP, Johnson PH. Pharmacokinetic attributes of intranasal delivery: Case studies and new opportunities. Drug Deliv 2005;3:8-11.
77. Dahl R, Mygind N. Anatomy, physiology and function of the nasal cavities in health and disease. Adv Drug Deliv Rev 1998;29:3-12.
78. Greiff L, Andersson M, Svensson J, Wollmer P, Lundin S, Persson CG.

Absorption across the nasal airway mucosa in house dust mite perennial allergic rhinitis. Clin Physiol Funct Imaging 2002;22:55-7.
79. Frey WH, Liu J, Chen X, Thorne. Delivery of 125I-NGF to the brain via the olfactory route. Drug Deliv 1997;4:87-92.
80. Ohwaki T, Ishii M, Aoki S, Tatsushi K, Kayano M. Effect of dose, pH and osmolarity on nasal absorption of secretin in rats III: In vivo membrane permeation test and determination of apparent partition coefficient of secretin. Chem Pharm Bull (Tokyo) 1989;37:3359-62.
81. Ohwaki T, Ando H, Watanabe S, Miyake Y. Effect of dose pH, osmolarity on nasal absorption of secretin In rats. J Pharm Sci 1985;74:550-2.
82. Corbo DC, Liu JC, Chein YW. Drug absorption through mucosal membranes. Effect of mucosal route and penetrant hydrophilicity. Pharm Res 1989;6:848-52.
83. Shao Z, Park GB, Krishnamurthy R, Mitra AK. The physicochemical properties, plasma enzymatic hydrolysis and nasal absorption of acyclovir 2' ester prodrugs. Pharm Res 1994;11:237-42.
84. Vyas TK, Shahiwala A, Marathe S, Misra A. Intranasal drug delivery for brain targeting. Curr Drug Deliv 2005;2:165-75.
85. Lerner EN, Van ZE, Stewart GR. Enhanced delivery of octreotide to the brain via transnasal iontophoretic administration. J Drug Target 2004;12:273-80.
86. Vyas TK, Shahiwala A, Marathe S, Misra A. Intranasal drug delivery for brain targeting. Curr Drug Deliv 2005;2:165-75.
87. Nagai T, Nishimoto Y, Nambu N, Suzuki Y, Sekine K. Powder dosage form of insulin for nasal administration. J Control Release 1984;1:15-22.
88. Hirai S, Yashika T, Matsuzawa T, Mima H. Absorption of drugs from the nasal mucosa of rat. Int J Pharm 1981;7:317-25.
89. Streisand B, Zhang J, Niu S, McJames S, Natte R, Pace NL. Buccal absorption of fentanyl is pH dependent in dogs. Anesthesiology 1995;82:759-64.
90. Huang C, Kimura R, Nassar RB, Hussain A. Mechanism of nasal absorption of drugs I: Physicochemical parameters influencing the rate of in-situ nasal absorption of drugs in rats. J Pharm Sci 1985;74:608-11.
91. Hussain A, Hamadi S, Kagoshima M, Iseki K, Dittert L. Does increasing the lipophilicity of peptides enhance their nasal absorption. J Pharm Sci 1991;80: 1180-1.
92. Brannon MD, Herron JM, Reidenberg P, Affrime MB. Lack of hypothalamic-pituitary-adrenal axis suppression with once-daily or twice-daily beclomethasone dipropionate aqueous nasal spray administered to patients with allergic rhinitis. Clin Ther 1995;17:637-47.
93. Clement P, Roovers MH, Francillon C, Dodion P. Dose ranging placebo controlled study of cetrizine nasal spray in adults with perennial allergic rhinitis. Allergy 1994;49:668-72.
94. Chiang R, Barnes R, Shoupe D, Lobo RA. Dose related changes in LH bioactivity with intranasal GnRH agonist administration. Contraception 1985;32:347-57.
95. Ohwaki K, Ando H, Kakimoto F, Uesugi K, Watanabe S, Miyake Y, et al. Effects of dose, pH, osmolarity on nasal absorption of secretin in rats: Histological aspects of the nasal mucosa in relation to the absorption variation due to the effects of pH and osmolarity. J Pharm Sci 1987;76:695-8.
96. Ohwaki K, Ando H, Watanabe S, Miyake Y. Effects of dose, pH, and osmolarity on nasal absorption of secretin in rats. J Pharm Sci 1985;74:550-2.
97. Gizurarson S, Bechgaard E. Intranasal administration of insulin to humans. Diabetes Res Clin Prac 1991;12:71-84.
98. Hardy JC, Lee SW, Wilson CG. Intranasal drug delivery by spray and drops. J Pharm Pharmacol 1985;37:294-7.
99. Illum L, Jorgensen H, Bisgaard H, Krogsgaard O, Rossing N. Bioadhesive microspheres as a potential nasal drug delivery system. Int J Pharm 1987;39:189-99.
100. Vidgren P, Vidgren M, Vainio P, Nuutinen J, Paronen P. Double-labelling technique in the evaluation of nasal mucoadhesion of disodium cromoglycate microspheres. Int J Pharm 1991;73:131-6.
101. Morimoto K, Morisaka K, Kamada A. Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. J Pharm Pharmacol 1985;37:134-6.
102. Watanabe Y, Mizufune Y, Utoguchi N, Endo K, Matsumoto M. Studies of drug delivery systems for a therapeutic agent used in osteoporosis. II. Enhanced absorption of elcatonin from nasal mucosa in rabbits. Biol Pharm Bull 1998;21:1191-4.
103. Illum L, Fisher AN, Jabbal-Gill I, Davis SS. Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides. Int J Pharm 2001;222:109-19.
104. Hussain MA, Koval CA, Shenvi AB, Aungst BJ. Recovery of rat nasal mucosa from the effects of aminopeptidase inhibitors. J Pharm Sci 1990;79:398-400.
105. Donnelly A, Kellaway IW, Taylor G, Gibson M. Absorption enhancers as tools to determine the route of nasal absorption of peptides. J Drug Target 1998;5:121-7.
106. Hussain MA, Aungst BJ. Nasal absorption of leucine enkephalin in rats and the effects of aminopeptidase inhibition as determined from the percentage of the dose unabsorbed. Pharm Res 1992;9:1362-4.
107. Barlow D, Takashi S. The design of peptide analogues for improved absorption. J Control Release 1994;29:283-91.
108. Hofstee BH. Specificity of esterase. II. Behavior of pancreatic esterase I and II toward a homologous series of N-fatty acid esters. J Biol Chem 1952;199:365-71.
109. Y.W. Chien. Transnasal Systemic Medications. 1st ed. New York: Elsevier Science Publishing Co. Inc; 1985. p. 100-6.
110. Chien YW. Novel Drug Delivery Systems. 1st ed. New York: Marcel Dekker Inc; 1992. p. 229-68.
111. Merkus FW, Verhoef JC, Marttin E, Romeijn SG, van der Kuy PH, Hermens WA, et al. Cyclodextrins in nasal drug delivery. Adv Drug Deliv Rev 1999;36:41-57.
112. Schipper NG, Hermens WA, Romeijn SG, Ver-Schiphoef J, Merkus FW. Nasal absorption of 17-b-estradiol and progesterone from a dimethyl-b-cyclodextrin inclusion formulation in rats. Int J Pharm 1990;64:61-6.
113. Aungst BJ, Rogers NL. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. Pharm Res 1988;5:305-8.
114. Merkus FW, Schipper NG, Hermens WA, Romeijn SG, Verhoef JC. Absorption enhancers in nasal drug delivery: Efficacy and safety. J Control Rel 1993;24:201-8.
115. Deurloo MJ, Hermens WA, Romeijn SG, Verhoef JC, Merkus FW. Absorption enhancement of intra nasally administered insulin by sodiumtaurodihydrofusidate (STDHF) in rabbits and rats. Pharm Res 1989;6:853-6.
116. Baldwin PA, Klingbeil CK, Grimm CJ, Longenecker JP. The effect of sodium tauro-24,25-dihydrofusidate on the nasal absorption of human growth hormone in three animal models. Pharm Res 1990;7:547-52.
117. Kissel T, Drewe J, Bantle S, Rummelt A, Beglinger C. Tolerability and absorption enhancement of intranasally administered octreotide by sodium taurodihydrofusidate in healthy subjects. Pharm Res 1992;9:52-7.
118. Fisher AN, Farraj NF, Hagan DT. Effect of L-a-lysophosphatidylcholine on the nasal absorption of human growth hormone in three animal species. Int J Pharm 1991;74:147-56.
119. Laursen T, Ovesen P, Grandjean B, Jensen S, Jørgensen JO, Illum P, et al. Nasal absorption of growth hormone in normal subjects: Studies with four different formulations. Ann Pharmacother 1994;28:845-8.
120. Agerholm C, Bastholm L, Johansen PB, Nielsen MH, Elling F. Epithelial transport and bioavailability of intranasally administered human growth hormone formulated with the absorption enhancers didecanoyl-L-a-phosphatidylcholine and a-cyclodextrin in rabbits. J Pharm Sci 1994;83:1706-11.
121. Hasegawa H, Ichinohe T, Strong P, Watanabe I, Ito S, Tamura S, et al. Protection against influenza virus infection by intranasal administration of hemagglutinin vaccine with chitin microparticles as an adjuvant. J Med Virol 2005;75:130-6.
122. Read RC, Naylor SC, Potter CW, Bond J, Jabbal-Gill I, Fisher A, et al. Effective nasal influenza vaccine delivery using chitosan. Vaccine 2005;23:4367-74.
123. Prego C, Garcia M, Torres D, Alonso MJ. Transmucosal macromolecular drug delivery. J Control Rel 2005;101:151-62.

124. Witschi C, Mrsny RJ. In vitro evaluation of microparticles and polymer gels for use as nasal platforms for protein delivery. Pharm Res 1999;16:382-90.
125. Lee VH, Yamamoto A. Penetration and enzymatic barriers of peptide and protein absorption. Adv Drug Del Rev 1990;4:171-207.
126. Huang CH, Kimura R, Nassar RB, Hussain A. Mechanism of nasal absorption of drugs, I: Physicochemical parameters influencing the rate of *in situ* nasal absorption of drugs in rats. J Pharm Sci 1985;74:608-11.
127. Rasmussen GJ, Bundgaard H. Prodrugs of peptides: 15. 4-Imidazolidinone prodrug derivatives of enkephalins to prevent aminopeptidase-catalyzed metabolism in plasma and absorptive mucosae. Int J Pharm 1991;76:113-22.
128. Beauchamp LM, Orr GF, DeMiranda P, Burnette T, Krenistsky TA. Amino acid ester prodrugs of acyclovir. Antiviral Chem Chemother 1992;3:157-64.
129. Smart JD, Nicholls TJ, Green KL, Rogers DJ, Cook JD. Lectins in drug delivery: a study of the acute local irritancy of the lectins from Solanum tuberosum and Helix pomatia. Eur J Pharm Sci 1999;9:93-8.
130. Suzuki Y, Makino Y. Mucosal drug delivery using cellulose derivatives as a functional polymer. J Control Release 1999;62:101-7.
131. Ikechukwu Ugwoke M, Sam E, Van Den Mooter G, Verbeke N, Kinget R. Nasal mucoadhesive delivery systems of the antiparkinsonian drug, apomorphine: Influence of drug-loading on in vitro and in vivo release in rabbits. Int J Pharm 1999;181:125-38.
132. Ugwoke MI, Agu RU, Vanbilloen H, Baetens J, Augustijns P, Verbeke N, et al. Scintigraphic evaluation in rabbits of nasal drug delivery systems based on carbopol 971P and carboxymethylcellulose. J Control Release 2000;68:207-14.
133. Illum L, Farraj NF, Davis SS. Chitosan as a novel nasal delivery system for peptide drugs. Pharm Res 1994;11:1186-9.
134. Kubota R, Komiyama T, Shimada H. Evaluation of the method for nifedipine administration for a rapid onset of clinical effect: A clinical study in normal volunteers. Yakugaku Zasshi 2001;121:355-64.
135. Newman SP, Steed KP, Hardy JG, Wilding IR, Hooper G, Sparrow RA. The distribution of an intranasal insulin formulation in healthy volunteers: Eåect of diåerent administration techniques. J Pharm Pharmacol 1994;46:657-60.
136. Park H, Robinson JR. Mechanism of mucoadhesion of poly(acrylic acid) hydrogels. Pharm Res 1987;4:457-64.
137. Loftsson T, Gudmundsdottir H, Sigurjonsdottir JF, Sigurdsson HH, Sigfússon SD, Másson M, *et al*. Cyclodextrin solubilization of benzodiazepines: formulation of midazolam nasal spray. Int J Pharm 2001;212:29-40.
138. Petersen H, Kullberg A, Edsbacker S, Greiff L. Nasal retention of budesonide and fluticasone in man: Formation of airway mucosal budesonide-esters in vivo. Br J Clin Pharmacol 2001;51:159-63.
139. Vila A, Sanchez A, Tobío M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002;78:15-24.
140. Dyer AM, Hinchcliffe M, Watts P, Castile J, Jabbal-Gill I, Nankervis R, *et al*. Nasal delivery of insulin using novel chitosan based formulations: A comparative study in two animal models between simple chitosan formulations and chitosan nanoparticles. Pharm Res 2002;19:998-1008.
141. Edman P, Bjork E, Ryden L. Microspheres as a nasal delivery system for peptide drugs. J Control Release 1992;21:165-72.
142. Illum L, Farraj N, Fisher A. Hyaluronic acid ester microspheres as a nasal delivery system for insulin. J Control Release 1994;29:133-41.
143. Koochaki PE. Nasal pharmaceutical composition in powder form. European Patent Application EP 571671, 1993.
144. Ryden L, Edman P. Effect of polymers and microspheres on the nasal absorption of insulin in rats. Int J Pharm 1992;83:1-10.
145. Critchley HS, Farraj D, Illum L. Nasal absorption of desmopressin in rats and in sheep: Effect of bioadhesive microsphere delivery system. J Pharm Pharmacol 1994;46:651-6.
146. Vivien N, Buri P, Balant L, Lacroix S. Nasal absorption of metoclopramide administered to man. Eur J Pharm Biopharm 1994;40:228-31.
147. Illum L, Farraj NF, Davis SS, Johansen BR, Hagan DT. Investigation of the nasal absorption of biosynthetic human growth hormone in sheep-use of a bioadhesive microsphere delivery system. Int J Pharm 1990;63:207-11.
148. Ryden L, Edman P. Effect of polymers and microspheres on the nasal absorption of insulin in rats. Int J Pharm 1992;83:1-10.
149. Chien YW, Chang SF. Intranasal drug delivery for systemic medications. Crit Rev Ther Drug Carr Syst 1987;4:67-194.

Source of Support: Nil, **Conflict of Interest:** None declared.