INTRANASAL DRUG DELIVERY SYSTEM: A REVIEW

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ABSTRACT
Intranasal drug delivery is now recognized to be a useful and reliable alternative to oral and parenteral routes. Nasal drug delivery system is advance drug delivery for both topical and systemic therapies. The nasal route having suitable anatomical structure for systemic delivery of drug molecules with high permeability, high vasculature and low enzymatic environment. It provide passage of drug directly to the targeted organ or systemic circulation by avoiding first pass degradation of drug because of this low dose is used for any drug. Route is also suitable for protein and peptide delivery. This review will focus on anatomy of nasal, factor affecting nasal drug delivery and various bioavailability barriers in nasal drug delivery and the strategies to improve the bioavailability of nasal dosage forms.

Keywords: Bioavailability, mucociliary clearance, nasal drug delivery, permeation enhancer, protein and peptides, blood brain barrier.

INTRODUCTION
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] 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. [1,2]

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 [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. The nasal route has received great attention as a route for vaccination. 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. Intranasal immunization may lead to the development of local, as well as systemic immunity. 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.

Benefits of Nasal Drug Delivery are Large Surface Area, Avoids First Pass Effect, Porous Endothelium, High Total Blood Flow, Rapid Drug Onset, Potential for Direct CNS Delivery, Avoidance of Needles.

Intranasal drug delivery is now recognized to be a useful and reliable alternative to oral and parenteral routes. Undoubtedly, the intranasal administration of medicines for the symptomatic relief and prevention or treatment of topical nasal conditions has been widely used for a long period of time. However, recently, the nasal mucosa has seriously emerged as a therapeutically viable route for the systemic drug delivery. In general, among the primary targets for intranasal administration are pharmacologically active compounds with poor stability in gastrointestinal fluids, poor intestinal absorption and/or extensive hepatic first-pass elimination, such as peptides, proteins and polar drugs. The nasal delivery seems to be a favourable way to circumvent the obstacles for blood-brain barrier [BBB] allowing the direct drug delivery in the biophase of central nervous system [CNS]-active compounds. It has also been considered to the administration of vaccines.

Despite its advantages, the nasal drug administration presents some limitations that must be considered during the discovery of new chemical entities intended for nasal therapy as well as during the development of nasal formulations. First of all, in addition to physicochemical properties of drugs and characteristics of their final formulations, a variety of physiological and pathological conditions related to nasal mucosa may also compromise the extent of nasal drug absorption and therapy efficacy.

**NASAL CAVITY – ANATOMY**
BARRIERS TO NASAL DRUG DELIVERY

A large number of factors influenced therapeutic efficacy as well as toxicity of nasally administered drug product. A well designed pre-formulation program is essential for development of nasal dosage forms to overcome various barriers associated with nasal drug delivery.\[^{17}\]

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.\[^{18,19}\]

NASAL CAVITY: ANATOMY, PHYSIOLOGY

In humans and other animal species the major functions of the nasal cavity are breathing and olfaction. However, it also affords an important protective activity once it filters, heat and humidify the inhaled air before reaching the lowest airways.\[^{20}\] Nasal cavity is lined with mucus layer and hairs which are involved in those functions, trapping inhaled particles and pathogens. Moreover, resonance of produced sounds, mucociliary clearance MMC, immunological activities and metabolism of endogenous substances are also essential functions of nasal structures.\[^{21}\]

Anatomic and histological characteristics of the different areas of nasal cavity are such that allow these functions to be performed optimally. Thus, anatomically, human nasal cavity fills the space between the base of the skull and the roof of the mouth; above, it is supported by the ethmoid bones and, laterally, by the ethmoid, maxillary and inferior conchae bones.\[^{21,22,23}\] The human nasal cavity has a total volume of 15-20 mL and a total surface area of approximately 150 cm\(^2\).\[^{24}\]

It is divided by middle [or nasal] septum into two symmetrical halves, each one opening at the face through nostrils and extending posterior to the nasopharynx. Both symmetrical halves consist of four areas [nasal vestibule, atrium, respiratory region and olfactory region] that are distinguished according to their anatomic and histological characteristics.\[^{25}\]

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. 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. 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. [22] In contrast, the strong interaction between molecules containing cationic group have low permeability than anionic group of compounds. Still large molecular compounds, particulate systems [microspheres and nanoparticles] and ionic compounds have been observed to diffuse through the mucus. [26,27]

**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. [28] The olfactory cells contain bipolar neurons and act as peripheral receptors and first-order ganglion cells. The nasal respiratory epithelium consists of loosely packed cells with high permeability and vasculature. [29] The permeability of environmental toxins is restricted by nasal epithelium. [30] 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. 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. 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. [31] 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. [32] 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.\[33\]

**MUCOCILIARY CLEARANCE**

Nasal mucociliary clearance is the most important physiological barrier, which reduces the nasal residential time of drugs and/or dosage forms.\[34\] 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.\[35\] 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.\[36\] 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.\[37\] 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.\[38\] 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.\[39\]

The effect of drug on mucociliary movement was extensively studied. Antihistaminic drugs, beta blockers, general anesthetics, cocaine arrest mucociliary clearance.\[40\] Moreover, correlation between reduction in mucociliary clearance rate and concentration of drug has been established. Cholinergic drugs, beta adrenergic agonists, and surfactants increase the mucociliary clearance and reduce nasal residential time;\[41\] 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.\[42\] The preservatives, such as chlorobutol and hydroxybenzoates, cause reversible inhibition of mucociliary clearance. 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.\[43\] Benzalkonium chloride is the extensively studied preservative in nasal formulation.\[44\] Long term administration of corticosteroid formulation containing 0.01 and 0.02% benzalkonium chloride in humans showed no significant reduction in mucociliary clearance rate.\[45\]

Nasal permeation enhancers are widely used to improve the bioavailability of large molecular compounds such as proteins and peptides.\[46\] Sodium taurohydrofusidate, a
well known permeation enhancer has been found to inhibit the mucociliary clearance at concentration of 0.3% and higher. [47] Other permeation enhancers such as laureth-9, deoxycholate, glycholate, and taurocholate have been shown to cause mild inhibition of mucociliary clearance. [48] Cyclodextrins and their derivatives are widely employed as permeation enhancers, particularly for poorly water soluble lipophilic compounds. [49] Some of the beta cyclodextrin derivatives like methylated beta cyclodextrin have also been found to be effective absorption enhancers for peptide molecules. [50] Mucociliary clearance rate was inhibited by formulation containing 2-4% methylated beta cyclodextrin. [51] 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. [51,52] 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. [53] A large variety of hydrophilic and amphiphilic compounds are detoxified through active Pgp mediated efflux transport in nasal mucosa. [54] 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. [55] Pgps have certain role in reducing CNS permeability of nasally administered drugs in olfactory epithelium. The maximum drug uptake and high CSF drug concentration was achieved with formulation incorporated with Pgp efflux inhibitors such as rifampin. The low level of chlorpheniramine and chlorcyclizine in CSF was most likely due to the presence of efflux transport system in olfactory epithelium. [56] 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.

DRUG ABSORPTION THROUGH THE NASAL MUCOSA
The first step in the absorption of drugs from the nasal cavity is passage through the mucus. Small uncharged particles easily pass through this layer. However, larger particles may find it more difficult to cross. After a drug passage through the mucus, there are several mechanisms for absorption through the mucosa. [57]
Factors influencing absorption are related to nasal physiology, physicochemical characteristics of drugs and formulation aspects.\[58\]

**a) Physicochemical properties of drugs**

- Molecular weight and size
- Solubility and dissolution
- Partition coefficient and pKa
- Chemical form
- Particle size and morphology

**b) Formulation properties**

- Types of dosage forms and delivery system
- Drug concentration, dose and dose volume
- Physical form of formulation
- Formulation pH
- Formulation osmolarity
- Formulation excipients

**c) Biological factors**

- Nasal blood flow

Enzymatic activity in the nose
- Physical condition of the nasal mucosa

**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.\[59\] 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.\[60\] The effect of three different concentrations of cetirizine on clinical efficacy was studied in patients.\[61\] 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.\[62\] 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. 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 - 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. 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. 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. The dosage form deposited in posterior chamber of nasal cavity will be eliminated by nasal mucociliary clearance and hence show low bioavailability. 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. 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.\[70\] 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.\[71\] 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.\[72-75\]

**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.\[76,77\]

**ENZYME INHIBITORS**

A number of studies have described the role of enzyme inhibitors on bioavailability of nasal formulations.\[78,80\] 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.\[81\] The absorption enhancers such as bile salts and fusidic acid derivatives exert enzyme inhibition and hence enhance the absorption and bioavailability.\[82\] 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.\[83\] 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.\[84\] Chemical modification of salmon calcitonin to elcatonin [S-S bond is replaced by C-N bond] showed better bioavailability than salmon calcitonin.\[85\] 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.\[86-88]\n
**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.\[89\] 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.\[90\] 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.\[91\] 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. Cyclodextrins act as a solublizer and permeation enhancer for nasal drug delivery and they are well tolerated in humans.\[92\] 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.\[93\] 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.\[94\] Several compounds such as calcitonin, cortisone, diazepam, and naproxen have been investigated for their nasal bioavailability enhancement using cyclodextrin as the permeation enhancer.\[95\] 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.\[96\] 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.\[97\] Laureth-9 was used as a permeation enhancer to improve the intranasal bioavailability of insulin. The bile salts are believed to improve the bioavailability by both solubilization of insulin and by direct effect of surfactant on the cell membrane.\[98\]

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 bacterial agent and hence its short term and long term toxicity was well established.\[99\] 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.\[100\] The 21-fold increase in nasal bioavailability of human growth hormone with 0.5% STDHF was observed in comparative pharmacokinetic studies in sheep.\[101\] 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.\[102\] 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.\[103\] 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.\[104\] 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. However, nasal necrosis was observed in contrast to human studies. Nasal bioavailability enhancement of growth hormone by DDPC was due to 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.

NASAL MUCAOADHESIVE 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. 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. Intranasal bioavailability of aqueous solution of apomorphine was found to be 45%. 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. 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. 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. \[114\] 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. \[115\] 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. \[116\] 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 \[117\] studied the pharmacokinetics of budenoside loaded bioadhesive grafted copolymers of poly(methacrylic acid and polyethylene glycol. The result stated that the bioadhesive drug delivery system offered relatively quick absorption \(T_{\text{max}} \sim 45\) 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. \[118\] 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. \[119\] 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. \[120\] The strategies adopted to improve the nasal bioavailability of insulin are mucoadhesive microparticles 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.\textsuperscript{[121]}

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.\textsuperscript{[122]} 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.\textsuperscript{[123]} 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.\textsuperscript{[124]} 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.\textsuperscript{[125]} 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 \{lysophosphatidylcholine\} incorporated microsphere.\textsuperscript{[126]} 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.\textsuperscript{[127]} 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.\textsuperscript{[128]} 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 glycocholate as a permeation enhancer.\textsuperscript{[129]} The comparative pharmacokinetics in rats showed high permeability of liposome pretreated with permeation enhancer than solution containing the same quantity of permeation enhancer.\textsuperscript{[130]} 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.\textsuperscript{[131]}

**NASAL VACCINES**

Nasal mucosa is the first site of contact with inhaled antigens\textsuperscript{[132]} and, therefore, its use for vaccination, especially against respiratory infections, has been extensively evaluated. In fact, nasal vaccination is a promising alternative to the classic parenteral route, because it is able to enhance the systemic levels of specific immunoglobulin G and nasal secretory immunoglobulin A\textsuperscript{[133]}. In upper airways, the systemic and local immunological responses are mainly mediated by the nasal associated lymphoid tissue situated underneath the nasal epithelium. The nasal associated lymphoid tissue is composed of agglomerates of dendritic cells, T cells and B cells which are involved in the initiation and execution of immune responses\textsuperscript{[134]}. Examples of the human efficacy of intranasal vaccines include those against influenza A and B virus, proteosoma-influenza, adenovirus-vectored influenza, group B meningococcal native\textsuperscript{[135]}, attenuated respiratory syncytial virus and parainfluenza 3 virus. However, human nasal vaccination is not restricted to the upper airways affections. After nasal immunization secretory immunoglobulin A can also be detected in other mucosal secretions, which may be important against virus transmitted through other mucosal sites, such as human immunodeficiency virus and hepatitis B virus\textsuperscript{[136]}.

**CONCLUSION**

A well designed pre-formulation program is essential for development of nasal dosage forms to overcome various barriers associated with nasal drug delivery. Factors influencing absorption are related to nasal physiology, physicochemical characteristics of drugs and formulation aspects. For study of drug concentration for nasal can be determine using Ex vivo experiments in rats. 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. A wide number of formulation strategies are made available to improve the bioavailability of nasal dosage forms. However, human nasal vaccination is not restricted to the upper airways affections.

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