Chapter 1

INTRODUCTION
# CHAPTER 1

## INTRODUCTION

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INTRODUCTION

Pharmaceutical dosage form development is the combination of an art as well as a science with the sole objective to produce a dosage form that is efficacious, patient friendly, stable, economical and delivers the drug as close as possible to the intended target with minimal adverse effects. Conventional forms of drug administration, in many cases, have been supplanted by the advent of novel drug delivery systems. The pharmaceutical companies are presently seeking innovative dosage forms by way of novel drug delivery systems as they represent strategic tool for expanding markets and indications, extending product life cycles and generating newer opportunities\(^1\). NDDS is no longer a theory. It is a reality and this is illustrated by the fact that around 13% of the current global pharmaceutical market is accounted for NDDS. Among the NDDS, transmucosal drug delivery market recorded second highest growth in the last five years with 171% whereas overall market growth stands at 106\(^2\).

Rapid developments in the field of molecular biology and gene technology resulted in generation of many new drugs in large number including peptides, proteins, polysaccharides, nucleic acids and other molecules possessing superior pharmacological efficacy and site specificity. But, the main impediment for oral delivery of these drugs is their inadequate oral absorption due to extensive presystemic
metabolism and instability in acidic environment. As a result, the full therapeutic potential of many drugs cannot be realized; hence administration through highly expensive and less patient friendly parenteral route is inevitable. Further, parenteral route is most hazardous due to incidences of anaphylaxis, extravasations and infection risk. Serious drawbacks associated with parenteral route and poor drug bioavailabilities have led to investigate new alternative non-invasive drug delivery systems.

1.1. Transmucosal drug delivery:

Transepithelial drug delivery across skin or absorptive mucosa seems to offer many benefits such as improved bioavailability and, hence possible to lower drug doses, thereby less dose-related side effects than the oral route. In comparison, transmucosal delivery systems exhibit a faster delivery than do transdermal delivery systems. Also, delivery occurs in a tissue that is more permeable than skin and is less variable between patients, resulting in minimal inter subject variability. In addition, these systems could potentially be used to deliver drugs that exhibit poor and variable bioavailability due to significant hepatic first-pass metabolism. The absorptive mucosa include nasal, sublingual, palatal, gingival, nasal, pulmonary, rectal, vaginal and ocular routes. In pulmonary delivery, despite the enormous surface area available for absorption, the major challenge is the reproducible placement of drug in the alveolar region due to the mucociliary clearance, hence not suitable for sustained delivery.
Vaginal, rectal and ocular mucosae offer many advantages, but poor patient compliance making them a feasible site for local applications rather than for systemic use. Sublingual mucosa is more permeable but not suitable for retentive delivery\(^5\).

On the other hand, nasal region offers added advantages in being highly vascular that has the ability to deliver even to the blood brain barrier. It also promotes rapid bioavailability, with subsequent almost immediate onset of pharmacological effect, whereby the drug enters the systemic circulation directly, bypassing the gastrointestinal tract and the first pass effect in the liver. In order to achieve optimal noninvasive delivery through nasal route the properties of the active compound and other properties of the formulation have to be considered. Moreover the dosage form must be able to be retained in the nasal cavity during the duration of therapy. The presence of mucus layer provides a unique opportunity for prolonged drug delivery via the development of mucoadhesive dosage forms. This novel approach has been explored to provide drug delivery via ophthalmic, nasal, gastrointestinal, vaginal and rectal routes. Mucoadhesive nasal gels have been a debatable subject for development of drug delivery technology to achieve desired dosage form.

Previous works concentrated on synthetic mucoadhesive materials that were not biocompatible and biodegradable. Efforts have been made by recent researches to establish the application of natural mucoadhesive materials from edible vegetables, fruits and seeds to
increase contact period with the nasal mucosa for proper release of the drug from the formulation. These materials possess a challenge to synthetic materials because of the extended contact time, edibility and biocompatibility. This investigative work was intended to establish the application of natural mucoadhesive agents from edible sources to overcome allergic and physiological interactions in the long term drug delivery. The present research envisages development and evaluation of mucoadhesive nasal drug delivery using CARVEDILOL as model drug.

Carvedilol (C_{24}H_{26}N_{2}O_{4}), the β-adrenergic blocking agent with α1-blocking activity is indicated in the treatment of mild to moderate congestive heart failure (CHF) and is used for treating high blood pressure. Carvedilol can reduce the risk of a second heart attack by 40% and increase survival among patients with congestive heart failure. For high blood pressure and congestive heart failure, the dose may range from 3.25mg twice daily to a maximum of 25mg twice daily. Poor oral bioavailability (25-35%), low molecular weight (406.5 g/mol), low dose (3.25 mg), lipophilic log PC value (3.967) with the elimination half-life of 7 to 10 hours makes it suitable for nasal delivery.

Pharmaceutical companies have looked increasingly towards drug delivery for help in lifecycle management of drugs on the market and with promising yet hard-to-deliver drugs. The drug delivery market is currently valued at US$50 billion (or 12.5% of the global pharmaceutical market) and has reached US$110 billion by 2006.
Nasal delivery commands the fourth position in market share, with about US$3 billion in sales, following oral controlled release, pulmonary and parenteral delivery routes. However, the potential for growth in this sector is extensive, in the successful delivery of proteins and peptides as an alternative to parenteral delivery. Currently, many nasal drug products on the market are indicated for the treatment of local disease such as allergic rhinitis and systemically acting drugs on the market in different therapeutic categories, with a growing number of products in the pipeline. There are many reasons for this change, including improved patient compliance (elimination of needles), avoidance of first-pass metabolism and rapid onset of action. Migraine is a key area where a nasal system has provided rapid relief, avoidance of taking an oral formulation while nauseated, and pain-free administration circumventing the need for an injection. Other therapeutic areas where nasal delivery could provide an alternative to current dosage forms are crisis situations (seizure and heart attack), erectile dysfunction, pain management, motion sickness and psychotropic drugs. Furthermore, nasal administration is used therapeutically for the systemic absorption of drugs in a variety of indications, such as sumatriptan for migraine, the antidiuretic desmopressin for the treatment of diabetes insipidus, oxytocin for stimulation of breast milk ejection, etc. Other drugs still in the research and development pipeline, which have potential for administration nasally, include vitamin B₁₂ or hydroxocobalamine,
various benzodiazepines\textsuperscript{12} and the dopamine agonist apomorphine for patients with Parkinsonism\textsuperscript{13}.

Biotechnology research is developing a whole range of therapeutic protein products that may greatly benefit from nasal administration in addition to many currently marketed drugs as ideal intranasal delivery candidates. Although substantial growth has occurred in this delivery area in recent years, the popularity of systemic intranasal drug delivery is expected to rise alarmingly in the near future\textsuperscript{14}.

The replacement of injection therapy by "non-parenteral application routes" is an area of intensive research efforts. Alternatives to be considered are the following: nasal, pulmonal, sublingual, rectal and transdermal (iontophoretic) delivery systems. From a biopharmaceutical point of view, the avoidance of liver first-pass effects and a higher bioavailability compared to the oral administration are to be mentioned. The nasal application of peptides is the only route of administration from this list of non-parenteral delivery systems, which has gained regulatory approval so far\textsuperscript{15}. The rapid onset of action and relatively high bioavailabilities favor this route of application.
1.2. Anatomy and physiology of nose

Breathing and olfaction are the prime functions of the nasal cavity in humans and animals. Physiologically, the structure and function of this cavity are also related to the resonance of produced sounds, the filtration of particles, mucociliary clearance, immunological activities, and heating and humidification of the inspired air before it reaches the lungs.\(^\text{16}\)

The human nose is divided into two symmetrical halves by the median septum; a central partition of bone and cartilage; each half opens to the face through the nostrils and extends posteriorly to the nasopharynx.\(^\text{17}\) The volume of each cavity is approximately 7.5 ml and has a surface area around 75 cm\(^2\). The nasal vestibule is the most anterior part of the nasal cavity; it is adjacent to the atrium, the intermediate region. The respiratory region occupies most of the nasal cavity and its turbinates or conchae considerably increase the surface area. In humans, the inferior, middle and superior turbinate are attached to the lateral wall, while a more complex\(^\text{18}\). (Fig. No.1.1)

The human nasal cavity has a total volume of 15-20 ml and a total surface area of approximately 150 cm\(^2\), of which the respiratory region covers about 85%. The olfactory region in humans covers about 2-10 cm\(^2\) on the roof of the respiratory region.\(^\text{19}\)
Fig. No. 1.1 Saggital section of the human nasal cavity, showing (A) the nasal vestibule, (B) atrium, (C1) respiratory area: inferior, (C2) middle and (C3) superior turbinate, (D) olfactory region, and (E) nasopharynx.

The nasal passage, which runs from the nasal vestibule, i.e., nasal valve, to the nasopharynx, has a depth of approximately 12-14 cm. The lining is ciliated, highly vascular, and rich in mucus glands and goblet cells. The blanket of nasal mucus is transported in a posterior direction by the synchronized beat of the cilia. An individual cilium is approximately 5 μm in length 0.2 μm in diameter and moves at a frequency of about 20 beats per second.

The rate of diffusion of a nasal preparation through the mucus blanket and its rate of clearance from the nasal cavity may be influenced by the physiochemical properties of the formulation vehicle, the particle size and surface charge of a drug, and any
additives incorporated\textsuperscript{21}. Nasal secretions in the adults have a normal pH in the range of 5.5-6.5 and often contain a variety of enzymes.

The vehicle for nasal formulations and mode of application can be optimized to deliver drugs to the absorptive turbinate region. Several factors should be considered in the optimization of nasal drug delivery:

I. Methods and techniques of administration.

II. Site of deposition,

III. Rate of clearance, and

IV. Minimization of any pathological conditions.

Absorption promotors have long been used to achieve a better systemic bioavailability of nasally administered drugs\textsuperscript{22}. However, the long-term use of an absorption promoter and the chronic used a promotor containing nasal formulation could affect the biochemical and biophysical characteristics and the functions of nasal mucosa and thus the efficiency of transnasal permeation. Therefore, the local toxic effects, the possibility of antibody formation and the tolerance potential of nasal formulations all need to be evaluated.

1.2.1. The mucosa

The humans nostrils are covered by skin; the anterior nasal cavity is lined with stratified squamous and transitional epithelium, and the highly vascular respiratory epithelium is mostly ciliated, columnar, and stratified\textsuperscript{23}. The respiratory epithelium comprises five
main cell types: ciliated and non-ciliated columnar cells, goblet cells, basal cells and low numbers of neurosecretory cells in the basement membrane (Fig. No.1.2). Approximately 20% of the total numbers of cells in the lower turbinate area are ciliated cells, with about 100 fine projections or cilia on each apical surface, which are used to transport the mucus towards the nasopharynx\textsuperscript{24}. Each columnar cell, both ciliated and non-ciliated, is covered with about 300 microvilli, which help to enlarge the surface area. The non-ciliated cells, which line around 60-70% of the respiratory mucosa, have high metabolic activity and are involved in fluid transport in and out of the cells. The goblet cells, which cover approximately 10% of the mucosa in the turbinate area, contain numerous secretory granules. Basal cells are precursors of columnar and goblet cells that are poorly differentiated and do not reach the apical side of the mucosa. It is believed that they have the ability to replace other cell types after differentiation. The olfactory mucosa is a pseudostratified columnar epithelium which covers the superior region of the human nasal cavity, and is composed of supporting cells, basal cells, microvillar cells and the typical receptor or olfactory cells\textsuperscript{25}. The basal lamina or basement membrane is situated between the epithelium and the lamina propria. The lamina propria is a loose type of connective tissue containing glands, subepithelial cells\textsuperscript{26}, and vascular and nervous tissue that is situated adjacent to the underlying skeletal structures.
Nasal secretions originate mostly from submucosal glands, but are also contributed to by goblet cells and transudate from plasma. Mucus is composed of water (95%), glycoproteins (2%), albumin, immunoglobulins, lysozyme, lactoferrin and other proteins (1%), inorganic salts (1%) and lipids (<1%). Despite their low proportions, it is the glycoproteins that provide mucus with its characteristic viscoelastic properties\textsuperscript{27, 28}. The mucus layer is divided into the lower, low viscosity layer with a thickness slightly less than the length of the cilia, i.e. 5-10 µm, and the more viscous upper layer of about 0.5-5 µm thickness. The average baseline human nasal pH is approximately 6.3, with large inter- and intra subject variations.
1.3. Mechanisms and Pathways of Nasal Drug Delivery

1.3.1. Mechanisms

The mechanisms of nasal drug delivery was investigated using an octapeptide, and horseradish peroxidase, a protein molecule; two mechanisms of transport are involved, a fast rate, which is lipophilicity dependent, and a slower rate, which is sensitive to the variation of molecular weight (Fig. No.1.3). The results of the nasal absorption are inconsistent with the nonspecific diffusion of penetrant molecules through the aqueous channels between the nasal mucosa cells, which impose a molecular size-dependent nasal permeability\textsuperscript{29}. These data, combined with other literature results, indicated that a good systemic bioavailability can be achieved for molecules with a molecular weight of up to 1000 Daltons when no enhancer is used; with the assistance of enhancers, a good bioavailability can be extended to a molecular weight of at least 6000 Daltons\textsuperscript{30}.

Water-soluble compounds, such as sodium cromoglycate, were found well absorbed. Their nasal absorption is likely to be dependent upon diffusion through aqueous channels ( pores). The molecular size of such a compound is a determinant for the rate of nasal absorption.
Fig. No. 1.3. Fate of drugs on nasal administration.

1.3.2. Pathways

The olfactory epithelium is known to be a portal for a substance to enter the central nervous system (CNS) and the peripheral circulation following nasal absorption. In addition, the nasopharynx has been shown to act as the portal of entry for viruses that induce some common viral diseases. i.e. musks, the common cold, smallpox, chickenpox and poliomyelitis. There appears to be communication between the nasal cavity and the subarachnoid space, between the lymphatic plexus in the nasal mucosa and the subarachnoid space, as well as between the perineural sheath, in the olfactory nerve filaments.
and the subarachnoid space. The process of drug transport across the nasal membrane involves either the diffusion or drug molecules through the pore channels in the nasal mucosa or participation or some nonpassive pathways before they reach the bloodstream\textsuperscript{32}.

1.4. Factors influencing the absorption of drugs across the nasal epithelium

Drug transport across the nasal epithelium is assumed to occur by one or more of the following mechanisms: transcellular passive diffusion, paracellular passive diffusion, carrier-mediated absorption and secretion and absorption through transcytosis\textsuperscript{33} (Fig. No. 1.4). Expression of efflux transporter proteins was first known to mediate multidrug resistance (MDR) in tumor cells, but may also result in reduced absorption of substrate compounds in various normal cells and epithelia. The factors influencing nasal absorption are related to nasal physiology, the physico-chemical characteristics of the compound and the properties of the specific drug formulation\textsuperscript{34}. 
Fig. No.1.4 Potential drug transport mechanisms across the nasal epithelium: transcellular passive diffusion (A), paracellular passive diffusion (B), carrier-mediated transport (C), absorption through transcytosis (D) and efflux transport (E).

1.4.1. Physiological factors/ barriers

1.4.1.1. Mucociliary clearance

Mucociliary clearance involves the combined actions of the mucus layer and the cilia, and is an important factor in the physiological defense of the respiratory tract against inhaled hazardous particles. The composition, function and clinical aspects of nasal mucus have been widely reviewed. It is assumed that the speed of mucociliary clearance in healthy humans is about 5 mm, although this is easily influenced by pharmaceutical excipients, airborne irritants or diseases\textsuperscript{35,36}. The tips of the cilia are in contact with and transport the superficial viscoelastic mucus layer towards the
nasopharynx, while the less viscous lower layer of the mucus is relatively stationary. Several workers, using various in vitro or in vivo methods, have investigated ciliary beat frequency in order to evaluate the effects of drugs or formulation additives or of infections in the upper airways on the mucociliary system\textsuperscript{37}. The cilia beat in a coordinated fashion, with a frequency of approximately 10 Hz, when measured in in vitro studies on human nasal cilia.

### 1.4.1.2. Protective barriers

The first step in the absorption of drugs from the nasal cavity is passage through the mucus. Uncharged substances with small molecular weight can easily pass through this layer. However, larger or charged particles may find it more difficult to cross. Mucin, the principal protein in the mucus, has the potential to bind to solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes such as pH, temperature, etc\textsuperscript{38}. The nasal membrane is a physical barrier and the mucociliary clearance is a temporal barrier to drug absorption across the nasal epithelium.

### 1.4.1.3. Enzymes

While nasal administration of drugs does avoid first pass hepatic metabolism, there is a broad range of metabolic enzymes situated in the nasal mucosa which can limit then bioavailability of some drugs, especially those containing peptides or proteins. Among
the enzymes present are the oxidative Phase I enzymes (e.g. cytochrome P-450 enzymes), non-oxidative enzymes, conjugative phase II enzymes and proteolytic enzymes such as endo-and exo-peptidases. The nasal enzyme population and/or activities vary extensively among different species\textsuperscript{39}. However, the level of activity seems to be lower for nasal enzymes than for those in the gastrointestinal tract or liver, on the basis of the amount of tissue involved.

1.4.1.4. Nasal pathophysiology

Nasal polyposis atrophic rhinitis and severe vasomotor rhinitis can reduce the capacity of nasal absorption of such drugs as cerulein. The common cold or any pathological conditions involving mucociliary dysfunctions can greatly affect the rate of nasal clearance and subsequently the therapeutic efficacy of drugs administered intranasally. Various pathophysiological changes, such as the common cold, seasonal rhinitis, nasal polyps and cancer, may also alter absorption from the nasal cavity in different ways, although this has not yet been thoroughly investigated\textsuperscript{40}.

1.5. Fundamentals of nasal absorption

1.5.1. Physical and chemical parameters

The physicochemical characteristics of the administered drug, which can influence nasal absorption, include molecular weight, solubility, dissolution/permeation rate, charge, partition coefficient,
pKa, particle size and the presence of polymorphism\textsuperscript{41}. An inverse relationship between molecular weight and percent absorption has been reported by Donovan et al\textsuperscript{41} based on studies on polyethylene glycol of different molecular weights. These data are supported by the results of animal studies compiled with literature data, indicated good bioavailability for compounds with molecular weights up to 1000 dalton in formulations without adjuvant. However, contrary to the findings of Donovan et al\textsuperscript{41} no difference in absorption characteristics between gastrointestinal and nasal mucosa was found in rats. Accordingly, mechanisms other than the suggested aqueous pores between cells of the nasal mucosa might be involved in the absorption of large molecules. Other studies have demonstrated that hydrophobicity is an important factor in nasal drug delivery, in contrast to studies on quaternary ammonium compounds where a decrease in absorption was found with increased lipophilicity and molecular weight.

1.5.1.1. Effect of molecular weight

It has been reported that nasal absorption falls off sharply for a drug molecule with a molecular weight of greater than 1000 Daltons; oral absorption declines even more steeply when the molecular weight goes beyond 400 Daltons\textsuperscript{42}. When the nasal absorption of a wide range of water-soluble compounds with different molecular weights, like insulin, dextran, and p-aminohippuric acid, was studied in Wister rat\textsuperscript{43}, the results indicated that a good linear correlation exists
between the log (percentage of drug absorbed nasally) and the log (molecular weight), suggesting the participation of aqueous channels in the nasal absorption of water-soluble molecules.

1.5.1.2. Partition coefficient

The partition coefficient is a ratio of concentrations of un-ionized compound between the two or more solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is to be adjusted such that the predominant form of the compound is un-ionized. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log $P$

$$\log P_{oct/wat} = \log \left( \frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{un-ionized water}}} \right)$$

1.5.1.3. Effect of perfusion rate

When ex vivo nasal perfusion technique, the nasal administration of various drugs been evaluated showed that as the perfusion rate increases the nasal absorption is first increased and then reaches a plateau level that is independent of the rate of perfusion (>2 ml/min).

1.5.1.4. Effect of perfusate volume

As the volume of the perfusate solution increases, the first-order disappearance rate of drugs from the perfusion solution has been observed to decrease\textsuperscript{44}. Results from studies using drugs with
different molecular structures suggested that the intrinsic rate constant varies from one drug to another\textsuperscript{45}.

1.5.1.5. Effect of solution pH

When the effect of the pH of a perfusion solution on nasal absorption was examined, it was found that the extent of absorption is pH dependent, which is higher at a pH lower than the pK\textsubscript{a} and decreases as the pH increases beyond the pK\textsubscript{a}\textsuperscript{46}. The rate of nasal absorption decreased as the pH increased owing to the ionization of the penetrant molecule. A good linear relationship was found to exist between the absorption rate constant of hydralazine and the fraction of its undissociated species calculated from the pK\textsubscript{a} value\textsuperscript{47}. The nasal absorption rate of decanoic, octanoic, and hexanoic acids was also found to be pH dependent and reached a maximum at pH 4.5, beyond which it decreased steadily as the solution became more acidic or basic\textsuperscript{48}.

The variation in solution pH was also observed to affect the nasal absorption of peptide-based drugs, such as insulin. For example, the reduction in plasma glucose levels was noted to depend upon the pH of an insulin solution administered intranasally\textsuperscript{49}. At pH 6.1 only a slight hypoglycemic effect was attained, whereas at pH 3.1 a reduction of about 55\% in the glucose level was achieved. The insulin molecule is known to have an isoelectric point at pH 5.4 and becomes positively charged at a pH lower than its isoelectric pH and is negatively charged at a pH higher than its isoelectric pH.
1.5.1.6. Effect of drug lipophilicity

The effect of lipophilicity on the extent of nasal absorption using a series of barbiturates at pH 6.0, at which the barbiturates (pKₐ = 7.6) exist entirely in the undissociated form⁴⁶. Only a fourfold change in the nasal absorption was noted between pentobarbital and barbital, even though the magnitude of their partition coefficients was different by as much as 40 times. Furthermore, it was also observed that the great difference in the partition coefficient between propranolol and 1-tyrosine resulted in only a very small variation in the rate constant of nasal absorption⁴⁶. Results from nasal delivery studies of a series of progestational steroids, which varied in their hydrophilicity, in ovariectomized rabbits, demonstrated that the partition coefficient determined in an octanol-water system does not predict well the permeation behavior of these progestational steroids across the nasal mucosa⁵⁰. The results however, showed that the systemic bioavailability of progesterone and its hydroxy derivatives correlates well with the partition coefficient determined in nasal mucosa-buffer system. This observation indicates that trans nasal permeation behavior cannot be predicted by the lipophilicity measured in a simple octanol-water system.

1.5.1.7. Effect of drug concentration

The effect of a variation in the drug concentration in the perfusion solution for nasal absorption was studied by monitoring the disappearance of 1-tyrosine and the formation of 1-tyrosine using the
ex vivo nasal perfusion technique in the rat. It was found that the nasal absorption of 1-tyrosine depends upon its concentration since the formation of 1-tyrosine is dependent upon the initial concentration of 1-tyrosine46.

1.6. Distribution and deposition

The distribution or drug (or formulation) delivered intranasally in the nasal cavity is one of the important factors that could affect the efficiency of nasal absorption51. The mode of administration could influence the distribution of a drug in the nasal cavity which in turn determines the efficiency of its absorption. Using a cast of a human nose, the distribution of drug following intranasal administration by different types of nasal delivery systems, including nose drops, nasal gels, a plastic bottle nebulizer, an atomized pump and a metered-dose pressurized aerosol, was evaluated and a significant difference was demonstrated52. Among the systems evaluated the atomized pump was found to be the best nasal delivery system because it delivered a constant dose and achieved a very uniform distribution on the nasal mucosa. The results also suggested that the use of a large volume of a dilute solution is preferable to a small volume of a concentrated solution. A simulated nasal cavity made of acryl resin was also developed for studying the distribution of drug particles in the nasal cavity53. No significant difference was noted among the gas, liquid, and Powder-type preparations. The highest concentration was usually detected in the anterior portion of the middle turbinate.
The nasal deposition of particles is also related to an individual's nasal resistance to airflow\textsuperscript{54}. With nasal breathing nearly all particles having an aerodynamic size of 10-20μm are often found to be deposited on nasal mucosa\textsuperscript{55}. One should avoid deposition in both the poorly absorptive stratified epithelium of the anterior atrium and in the posterior nasopharyngeal region, which leads to drug loss to the stomach by swallowing. Insoluble particles, if deposited in the main nasal passage, are likely to be transported posteriorly by ciliary movement and dispatched to the stomach. If the drug is introduced as a vapor or a gel as a soluble particle it firstly diffuses into the lining secretions and then be absorbed from there into the microcirculation.

The deposition of aerosols in the respiratory tract is also a function of particle size as well as respiratory patterns\textsuperscript{56}. The density, shape, and hygroscopicity of the particles and pathological conditions in the nasal passage influence the deposition of particles, whereas particle size distribution determines the site of deposition and affects the subsequent biological responses. A uniform distribution throughout the nasal mucosa could be achieved by delivering the drug particles from a new nasal spray using a pressurized gas propellant. A metered-dose delivery system developed for the nasal delivery of flunisolide a synthetic fluorinated corticosteroid was assessed and found to provide a consistent dose delivery and spray pattern that affects the deposition of droplets in the nasal cavity\textsuperscript{57}. 
Among the mechanisms usually taken into consideration when one assesses the deposition of particles in the respiratory tract, i.e., inertia, sedimentation, and diffusion, inertial deposition was found to be a dominant mechanism in nasal deposition. Particles with an aerodynamic diameter of 50 μm or greater do not enter the nasal passage. It was demonstrated that 60% of aerosolized particles with an aerodynamic diameter of 2-20 μm are deposited in the anterior region of the nostrils\(^{58-60}\). The site of drug deposition within the nasal cavity depends upon the type of delivery system used and the technique of administration applied. It was found that following administration as nasal drops and nasal gels, greater coverage of the nasal walls is achieved which is independent of the volume administered (over the range 0.1-0.75 ml)\(^{61,62}\). The particles, once deposited at the anterior region of the nasal cavity may be again conveyed posteriorly by inhaled air, ciliary movement, and/or diffusion in the mucous layer.

The regional deposition of drugs discharged from a pressurized aerosol product and a metered-pump spray product was compared in a model nose\(^{62}\). The results indicated that these two products produce no significant difference in regional deposition. In addition, most of the drug in each case was observed to deposit in the anterior region of the nose by inertia impaction, with little nasal penetration of the drug. The anteriorly deposited drug can be spread backward by mucociliary flow and general surface flow. The initial distribution and subsequent
clearance of aerosol discharged from a nasal pump spray was recently studied\textsuperscript{63}. These results also showed that aerosol is concentrated mainly in the anterior region of the nose, but the area of deposition varies from one subject to another. On the average, 56\% of the dose was retained at the initial site of deposition 30 min after administration; the remaining 44\% of the dose was cleared to the nasopharynx. Whaley et al\textsuperscript{62} developed a method to expose the nasal cavity with radiolabelled aerosol without exposure of the remainder of the respiratory tract. The results of these studies suggested that the efficiency of deposition is at 15 ± 2\% of the inhaled activity; the maximum deposition is noted to occur in the anterior third of the nasal cavity (which contains 78 ± 4\% of the total radioactivity deposited). On the other hand the middle third of the nasal cavity receives 13 ± 3\% and the posterior third 9 ± 2\% of the deposited radioactivity respectively.

A mathematical model was recently developed to describe the rate processes involved in the deposition of drug delivered into the human nasal cavity by a delivery system\textsuperscript{64}. The model contains a series of parallel first-order rate processes consisting of the convective transport of drug and carrier by fluid flow, mucocilial clearance and peristalsis, and drug decomposition, as well as a series of sequential irreversible first-order rate processes consisting of the release and absorption of the drug before its appearance in the systemic circulation. Stimulation using this model showed that the use of a
bioadhesion technique could improve bioavailability and, in the meantime, reduces the variability in absorption that could result from removal of the drug from the nasal cavity by sniffing, blowing or wiping the nose.

1.7. Formulation aspects

Formulation factors that should be taken into consideration to obtain successful nasal absorption of drugs include the concentration of the drug, the dose and volume of administration, the pH, viscosity and osmolarity. Moreover, different excipients, including preservatives and absorption enhancers, are also likely to alter the bioavailability. In addition, the dosage forms (drops, sprays, gels, powders, etc.), the administration technique (inhalation, mechanically assisted, etc.) and the device used will also affect the level of absorption.

1.8.1. Delivery systems

The selection of delivery system depends upon the drug being used, proposed indication, patient population and last but not least, marketing preferences. Some of these delivery systems and their important features are summarized below.

1.8.1.1. Nasal drops

Nasal drops are one of the most simple and convenient systems developed for nasal delivery. The main disadvantage of this system is the lack of dose precision and therefore nasal drops may not be suitable for prescription products. It has been reported that nasal
drops deposit human serum albumin in the nostrils more efficiently than nasal sprays.

1.8.1.2. Nasal sprays

Both solution and suspension formulations can be formulated into nasal sprays. Due to the availability of metered dose pumps and actuators, a nasal spray can deliver an exact dose from 25 to 200 µL. The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly.

1.8.1.3. Nasal powders

The advantages to the nasal powder dosage form are the absence of preservative and superior stability of the formulation. However, the suitability of the powder formulation is dependent on the solubility, particle size, aerodynamic properties and nasal irritancy of the active drug and/or excipients. Local application of drug is another advantage of this system but nasal mucosa irritancy and metered dose delivery are some of the challenges for formulation scientists and device manufacturers.

1.8.1.4. Nasal gels

Many pre-clinical and clinical studies with potent proteins, peptides and DNA delivered through nasal route have been completed and demonstrated that efficacy can be achieved systemically. However administration of many of these formulations to the nasal region suffers from a serious drawback due to its inability to retain at the nasal region. Therefore a platform that holds the formulation at the
requisite site of application is an important argument today. Of all, the bioadhesive polymers has gained great interest especially the mucoadhesive agents that have the capacity to form secondary bonds as well as entanglement with the mucin molecules thereby forming a platform over the nasal mucosa\textsuperscript{68}. However most of these mucoadhesive polymers are synthetic in nature and have questionable biodegradability. A need for natural mucoadhesive agent with proven mucoadhesive properties, biocompatible and biodegradable is an area of extensive research efforts.

Nasal gels in brief may be defined as high-viscosity thickened solutions or suspensions with or without mucoadhesive agents. Until the recent development of precise dosing devices, there was not much interest in this system. The advantages of a nasal gel include the reduction of post-nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients and target delivery to mucosa for better absorption.

Recent approaches to enhance the transnasal delivery of drugs applied various bioadhesive polymers, such as methylcellulose, carboxy methylcellulose, hydroxypropylcellulose, or polyacrylic acid\textsuperscript{69}. The enhancement of nasal drug absorption by bioadhesives presumably results from the increase in the residence time of drug in the nasal cavity and a higher local drug concentration in the mucus lining on the nasal mucosal surface. One of the properties of a
bioadhesive polymer is its ability to swell by absorbing water from the mucous layer in the nasal cavity and thereby forming a gel-like layer in which the polymer forms a bond with the glycoprotein chains of the mucin. However most of these mucoadhesive polymers are synthetic in nature and are not biodegradable. A need for natural mucoadhesive agent with proven mucoadhesive properties, biocompatible and biodegradable is an area of extensive research efforts.

1.9. Bio/mucoadhesion

Bioadhesion is the phenomenon in which a synthetic or natural macromolecule adheres to a biological tissue, which can be either an epithelial surface or the mucus layer covering a tissue and are held together for extended periods of time by interfacial forces. It is a complex phenomenon and several steps have been suggested in mucoadhesive bond formation. The first step is the spreading, wetting and dissolution of mucoadhesive polymer at the interface. The second step is the mechanical or physical entanglement between the polymer and the mucus, resulting in an inter-penetration layer. The next step is the result of chemical interactions, such as covalent and ionic bonds, hydrogen bonding and Van der Waal’s interactions. Hydrogen bonds and hydrophobic interactions are the most desirable on developing mucoadhesive systems, since strong primary bonds (e.g. covalent bonds and ionic bonds) could cause irreversible damage of mucosal surface.
Mechanisms of polymer adherence to mucosal surfaces have not yet been fully understood and five theories have been proposed for the mucoadhesion. It is unlikely that a single, universal theory will account for all types of adhesion observed. These theories include the adsorption, diffusion, wetting, fracture and electronic theories. The ‘adsorption theory’ states that interfacial chemical bonds are formed upon initial contact between mucosal surface and the mucoadhesive polymer. In the ‘diffusion theory’, it has been suggested that after initial contact between the mucosal surface and the mucoadhesive polymer, a physically entangled network between the polymer and the mucus is formed. The ‘wetting theory’ is based on the ability of the polymer to spread on biological surfaces. This theory is generally applicable to liquid bioadhesive systems. The ‘fracture theory’ is related to the force required for the separation of polymers from the mucus below. According to the ‘electronic theory’, electron transfer occurs between mucosal surface and the mucoadhesive polymer as a result of their different electronic properties. Electrostatic interactions with the negatively charged mucin surface contribute to the formation of an intermediate inter-diffusion network.68

1.9.1. **Ideal characteristics of mucoadhesive dosage forms**

The ideal characteristics of mucoadhesive dosage forms containing bioadhesive polymers are 72-80

1. Localization in specified regions to improve and enhance bioavailability of drugs.
2. Prolonged residence time to permit once daily dosing so that patient compliance can be improved.

3. Optimum contact with absorbing surface to permit modification of tissue permeability, to inhibit enzyme activity or to suppress mucus production, also for a high concentration gradient between delivery systems and absorbing membrane is achieved.

4. To deliver agents locally for the purpose of modulating antigency

5. The strong interaction between the polymers and the mucosal lining of the tissue helps to increase the contact time and permits localization on essential tissue when modification of tissue permeability is important for delivering peptides, proteins and ionized species.

**1.9.2. Factors affecting mucoadhesion**

**1.9.2.1. Polymer released forms**

**1.9.2.1.1. Molecular weight**

The optimum molecular weight for maximum mucoadhesion depends upon the type of mucoadhesive polymer and tissue. Numerous studies have identified that there is a certain molecular weight at which bioadhesive is at a maximum. The interpenetration of polymer molecules is favorable for low molecular weight polymers whereas entanglement favors for high molecular weight polymers. The
optimum molecular weight for the maximum bioadhesion depends on the type of polymer.

According to Gurny et.al\textsuperscript{81} it seems that the bioadhesive forces increases with the molecular weight of bioadhesive polymer up to 100,000 and that beyond this level there is not much effect.

1.9.2.1.2. Concentration of active polymer

Bremercker\textsuperscript{82} reported that there is an optimum concentration of polymer corresponding to the best bioadhesives. In highly concentrated systems, the bioadhesive strength drops significantly. Duchare et.al\textsuperscript{83} for solid dosage forms showed that the polymer concentration is responsible for the stronger bioadhesion.

1.9.2.1.3. Flexibility of polymer chains

Flexibility is important for interpenetration and entanglement. As water-soluble polymer becomes cross-linked, the mobility of the individual polymer chain decreases. As the cross linking density increases the effective length of the chain, which can penetrate into mucus layer, decreases even further and mucoadhesive strength is decreased.

1.9.2.1.4. Spatial confirmation

Spatial confirmation of a molecule is also important. Despite a high molecular weight of 19,500,000 for dextrans, they have adhesive strength similar to that of polyethyleneglycol, which has a molecular weight of 200,000. The helical conformation of electrons may shield
many adhesively active groups, primarily responsible for adhesion unlike PEG polymers that have a linear conformation.

1.9.2.2. Environment related factors

1.9.2.2.1. pH

pH can influence charge on the surface of mucus as well as certain ionizable mucoadhesive polymers. Mucus will have a different charge density depending on pH because of differences in dissociation of functional groups on the carbohydrate moiety and amino acids of polypeptide backbone. Some studies have shown that the pH of the medium is important for the degree of hydration of cross linked polyacrylic acid showing consistently increased hydration from pH 4 through pH 7, and then a decrease as alkalinity and ionic strength increases. For example polycarbophil shows maximum adhesive strength at pH 3 and gradually decreases as the pH increases up to 5.

1.9.2.2.2. Initial contact time

The initial contact time between mucoadhesive and the mucus layer determines the extent of swelling and the interpenetration of polymer chains. Although with the initial pressure the initial contact time can dramatically affect the performance of a system the mucoadhesive strength increases as the initial contact time increases84.
1.9.2.2.3. **Secretion of the model substrate surface**

Since physical and biological changes may occur in the mucus gels on tissues under experimental conditions, the variability of biological substrate should be confirmed by examining properties such as permeability, electro physiology, or histology. Such studies may be necessary before and after preparing the *in vitro/ex vivo* tests using tissues.

1.9.2.2.4. **Swelling**

Swelling depends both on polymer concentration and on water presence. When swelling is too great, decrease in bioadhesion occurs; such phenomena must not occur too early, in order to exhibit to a sufficient action of the bioadhesive system.

1.10. **Measurement of mucoadhesion**

Several test methods have been reported in the literature. These tests are necessarily not only to screen a large number of mucoadhesives, but also to study their mechanisms. Peppas et.al reported that these tests are also important during the design and development of a mucoadhesive system as they ensure compatibility, physical and mechanical stability, surface analysis and bioadhesive bond strength. The test methods can broadly be classified into two major categories. Most *in vitro/ex vivo* methods are based on the measurements of either shear of tensile stress or *in vivo* methods using endoscopy, gamma scintigraphy. Some of the methods commonly employed for mucoadhesion determination are:
1.10.1. Fluorescent probe method:

Park and Robinson\textsuperscript{84} studied polymer interaction with the conjunctival epithelial cell membrane using fluorescent probes. In this method the membrane lipid bilayered and membrane proteins were labeled with pyrene and fluorescein isothiocyanate, respectively. The cells were mixed with the mucoadhesive agents and changes in fluorescence spectra were monitored. This gave a direct indication of polymer binding and its influence on polymer adhesion.

1.10.2. Mechanical spectroscopic method:

Kerr et.al\textsuperscript{86}, used to investigate the interaction between glycoproteins gels and polyacrylic acid by mechanical spectroscopic method. Mortazavi et.al\textsuperscript{87} used similar methods to investigate the effect of carbopol 934P on rheological behavior of mucus gel, role of mucus and effect of various factors such as ionic concentration, polymer molecular weight and its concentration and the introduction of anionic, cationic and neutral polymers on mucoadhesive mucus interface.

1.10.3. Colloidal gold staining method:

Park et.al\textsuperscript{88}, proposed the colloidal gold staining technique for the study of bioadhesion. The technique employed red colloidal gold particles, which were stabilized by the adsorbed mucin molecules (mucin-gold conjugates). Upon interaction with mucin gold conjugates; bioadhesive hydro gels developed a red color on the surface. The
interaction between them is quantified either by the measurement of the intensity of the red color on the hydrogel surface or by measurement of decrease in the concentration of the conjugates from the absorbance change at 525nm.

1.10.4. **Viscometric method:**

Hassan\textsuperscript{89} developed a simple viscometric method to qualify mucin-polymer bioadhesive bond strength. Viscosities of 15\%w/w procine gastric mucin dispersion were measured with Brookfield’s viscometer. In absence or presence of selected neutral, anionic and cationic polymer, viscosity components and the forces of bioadhesion were calculated.

1.10.5. **Thumb test:**

It is a simple test method that can be used to identify mucoadhesion. The adhesiveness is measured by the difficulty of pulling the thumb from the adhesive as a function of the pressure and the contact time\textsuperscript{90}. Although the thumb test may not be conclusive, it provides useful information on mucoadhesive potential.

1.10.6. **Electric conductance**

Bremecker\textsuperscript{91} used modified rotational viscometer to determine electrical conductance of various semi solid mucoadhesive ointments and found that the conductance was low in the presence of adhesive substance.
1.11. Enhancement in Absorption

Generally, the absorption enhancers act via one of the following mechanisms:

1. Inhibit enzyme activity
2. Reduce mucus viscosity or elasticity
3. Decrease mucociliary clearance
4. Open tight junctions and
5. Solubilize or stabilize the drug.

Absorption enhancers are generally classified as physical and chemical enhancers. Chemical enhancers act by destructing the nasal mucosa very often in an irreversible way, whereas physical enhancers affect nasal clearance reversibly by forming a gel. The enhancing effect continues until the gel is absorbed. Examples of chemical enhancers are chelating agents, fatty acids, bile acid salts, surfactants and preservatives. Osmolarity and pH may enhance the absorption of drug. Several methods have been used to facilitate the nasal absorption of drugs:

1.11.1. Structural modification: The chemical modification of the molecular structure of a drug has been often used to modify the physicochemical properties of a drug and hence it could also be utilized to enhance the nasal absorption of a drug.

1.11.2. Salt or ester formation: The drug could be converted to form a salt or an ester for achieving better transnasal permeability, such as
formation of a salt with increased solubility or of an ester with better nasal membrane permeability.

1.11.3. **Formulation design:** Proper selection of formulation excipients could improve the stability and/or enhance the nasal absorption of drugs.

1.11.4. **Surfactants:** Incorporation of surfactants into nasal formulations could modify the permeability of nasal mucosa, which may facilitate the nasal absorption of drugs. Several approaches have been developed and successfully applied to enhance the efficiency of nasal drug delivery\(^9^3\). A major limiting factor associated with the addition of enhancers to a formulation for nasal administration is the potential toxicity to the nasal mucosa. Nasal absorption enhancers are required to be nonirritating, nontoxic and non allergenic or at least to have immediately reversible effects.

Moreover they should be potent, compatible with the drug and other excipients in the formulation and systemically inert in the concentrations used. In addition, the optimal enhancer has to be readily available. Lack of odor, taste and influence on mucociliary clearance are other important requirements for nasal drug delivery\(^9^4\). No single enhancer can be expected to fulfil all these requirements. Instead, potential enhancers have to be carefully evaluated to reach basic acceptability in enhancing ability and an overall safety profile, with regard to both local and systemic effects. Enhancers have been classified in various ways, possibly because some enhancers have
overlapping chemical properties and have been shown to possess more than one possible mechanism of action. In addition, the effects of some enhancers are only partly understood\(^95\). Surfactants and bile salts were the first enhancers to be tested and several other promoters have been investigated subsequently.

The enhancers evaluated to date appear to act by a wide range of mechanisms, including perturbation of lipid membranes, facilitation of leakage of lipids and proteins from the membranes, tight junction regulation, and chelation of Ca\(^{2+}\) ions in the cell membranes\(^96\). Sodium taurocholate and glycocholate are enhancers extensively studied which indicated in many cases indicated promising results\(^97\), although large interspecies differences in the effects of these enhancers on the nasal absorption of human growth hormone were later observed\(^98\). Moreover, studies in healthy volunteers who received the somatostatin analogue octreotide in combination with sodium salts of tauro or glycocholate showed poor local tolerability\(^99\), clearly demonstrating the difficulties in extrapolating absorption data from animals to humans\(^100\). Large interspecies differences have also been shown with dimethyl-\(\beta\)-cyclodextrin (DM\(\beta\)CD), although the cyclodextrin concept does remain promising. SLS (0.5%) has been shown to promote the absorption of fluorescein isothiocyanate dextran (FD-4, Mw 4400 Dalton) after nasal administration to rats, resulting in high early peak plasma concentrations compared to those reached with chitosan, poly-L-arginine and the other enhancers investigated.
The integrity of the tight junctions, which may be seen as barriers to the paracellular diffusion of molecules, is dependent on extracellular Ca\(^{2+}\). It is believed that the mechanism of action of EDTA includes depletion of Ca\(^{2+}\) from the tight junctional areas, thus allowing the junctions to open. This is consistent with the findings of Yamamoto et al., which demonstrated a promoting effect of EDTA on the absorption of fluorescein isothiocyanate-dextrans with various molecular weights after nasal administration to rats. Interestingly, EDTA is the only enhancer found in nasal products on the Swedish market, although the concentration used indicates that a preservative rather than an absorption-promoting effect is achieved. To summarize, this overview gives an indication of the difficulties associated with the development of absorption enhancers for nasal formulations. The low numbers of registered products containing enhancers reflects the generally restrictive view of their safety, particularly for long-term use in chronic conditions.

The first approach involves the use of various surfactants or bile salts to promote nasal absorption. Mild surfactants at low concentrations may only alter membrane structure and permeability, whereas certain surfactants at high concentrations may disrupt and even dissolve nasal membranes.