CHAPTER I

INTRODUCTION

1.1 NASAL DRUG DELIVERY

1.1.1. History

The history of nasal drug delivery dates back to earlier topical applications of drugs intended for local effects. Nasal therapy also called ‘Nasya karma’ has been recognized form of treatment in the Ayurvedic system of Indian medicines. The early 1980s saw the introduction of nasal route as a promising systemic delivery alternative to other conventional drug delivery routes \[1\]. For many years, drugs have been administered intranasally for their local effect on the mucosa (e.g. Antihistamines, decongestant, vasoconstrictors and antibiotics). In more recent years many drugs have been shown to achieve a better systemic bioavailability by self medication through the nasal route than by oral administration. Some of them have been shown to duplicate the plasma profile as i.v. administration. More recently the intranasal route has aroused increasing interest as means of the systemic administration of vaccine, hormones, peptides and certain other drugs \[2\]. In addition, intranasal drug delivery enables dose reduction, rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, and fewer side effects. For nasal drug delivery various systems such as: nasal spray, nasal pumps, gels, micro emulsion, suspensions, powders and thermo reversible mucoadhesive gels have been studied. In China, extracts of aloe wood and sandalwood were used for treating emesis by inhalation through the nasal route. For several years, medicaments have been applied nasally for their local pharmacological effects on the mucosa as well as systemic approaches \[3, 4\]. This route of drug delivery was considered by traditional systems of medicine such as Unani, Ayurvedic as well as Persian. It was extensively considered by medieval Persian physicians and was presented as an important route of drug administration especially for neurologic disorders \[5\]. The nasal delivery seems to be a favorable way to circumvent the obstacles for blood-brain barrier (BBB) allowing the direct drug delivery in the bio phase of central nervous system (CNS) active compounds. The nasal route has also been used to administer tobacco by nasal snuffing \[6\]. The major problems with nasal delivery are the mucociliary clearance, which reduces the residence time of nasally applied dosage forms and the poor nasal permeability of many drugs \[7\]. Several alternative strategies have been employed to overcome these limitations. Bioadhesive polymers, for example, can be
used to achieve long residence time on nasal mucosa which results in higher concentration gradient and subsequent increased absorption of the drugs [8]. Polymers may widen the tight junctions producing absorption enhancing effect [9].

1.1.2 Nasal Route
Nasal route is easily accessible, convenient, and a reliable with a porous endothelial membrane and a highly vascularized epithelium that provides a rapid absorption of compounds into the systemic circulation, avoiding the hepatic first pass elimination. In addition, intranasal drug delivery enables dose reduction, rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, and fewer side effects [1,3]. 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 noninvasiveness, self-administration, patient comfort, and patient compliance, which are the hurdles in intravenous drug therapy [10]. It was reported that lipophilic drugs are generally well absorbed from the nasal cavity with pharmacokinetic profiles, which are often identical to those obtained after an intravenous injection with a bioavailability approaching 100% [1]. These drug delivery systems have the ability to control the rate of drug clearance from the nasal cavity as well as protect the drug from enzymatic degradation in nasal secretions. The mechanisms and effectiveness of the drug delivery systems are described in order to guide the development of specific and effective therapies for the future development of peptide preparation and other drugs that otherwise should be administered parenterally [11]. For instance, localised nasal drug delivery is usually used to treat conditions related to the nasal cavity, such as congestion, rhinitis, sinusitis and related allergic conditions. A diverse range of drugs including corticosteroids, anti-histamines, anti-cholinergic and vasoconstrictors can be administered locally. In recent years, achieving a systemic drug action using the nose as the entry portal into the body has received more attention [12,13].

1.1.3 Advantages of nasal drug delivery system [14,15]
- Absorption of drug is rapid via highly vascularised mucosa.
- Availability of large nasal mucosal surface area for dose absorption.
- Onset of action is rapid.
- Non invasive and easy for administration.
- Bypass the BBB.
INTRODUCTION

- Degradation of drug observed in GIT is avoided.
- Hepatic first pass metabolism is absent.
- Nasal bioavailability of small drug molecules is good.
- Unsuitable drug candidates for oral route can be successfully given via nasal route.
- Alternate to parenteral route especially for proteins and peptides.
- Convenient route for the patient on long term therapy.

1.1.4 Disadvantages of nasal drug delivery system\textsuperscript{[14,15]}

- Delivery volume in nasal cavity is restricted to 25–200 μL.
- Adversely affected by pathological conditions.
- Large interspecies variability is observed in this route.
- Normal defense mechanisms like mucociliary Clearance and ciliary beating affects the permeability of drug.
- Limited understanding of mechanisms and less developed models at this stage.
- Systemic toxicity occurring due to absorption enhancers is yet not established.
- Smaller absorption surface compared with GIT.
- Possibility of nasal irritation hence inconvenient compared with oral route.
- Enzymatic barrier to permeability of drug.

1.1.5 Anatomy of the Nose and Nasal cavity

The nasal passage is 12-14 cm deep and runs from the nasal vestibule to the nasopharynx. It has three main regions; vestibular, respiratory and olfactory regions. The nose has a volume of 16-19 cm\textsuperscript{3} and a surface area of approximately 180 cm\textsuperscript{2} with two cavities (i.e. nostrils) separated by the nasal septum\textsuperscript{[16]}. 

Illustrations 1: Anatomy of the human nasal cavity
The human nose differs in its anatomy and morphology between different racial and ethnic groups. The nose can be divided into the external portion which is in fact termed as the nose and the internal portions being the nasal cavities (nasal fossae; cavum nasi). The nose is the only visible part of the respiratory system, protruding from the face, and lying in between the forehead and the upper lip\textsuperscript{[17]}.

It is made up of a bony section and a cartilaginous section. The bony section is located in the superior half and contains a pair of nasal bones sitting together side by side, separated in the middle and fused posteriorly by the medial plates of the cheekbones (maxilla bones). The cartilaginous section is located in the inferior half, consisting of flexible cartilages in the anterior, caudal portion of the nose. The cartilages are connected to each other and to the bones by a tough fibrous membrane. At the base of the nose are two openings called the nostrils (anterior nares, sing. naris) and are separated by nasal septum cartilage (columna). The nostrils are a portal for air and particulates to enter the nasal cavity. Its shape can be very elliptical to circular varying between people from different ethnicity and race\textsuperscript{[17]}.

Illustrations 2: Lateral view of the external nose showing the cartilage and bone structure
Illustrations 3: Structure of the internal nasal cavity

The vestibular region is located at the front opening of the nasal passages which filters out particles from the inhaled air. However, drug delivery and absorption in this region is least important. This area is covered with hairs which filter the air to prevent airborne particles entering the respiratory system. The respiratory area is large with a high degree of vascularity and a surface area of about 130 cm$^2$. In this region the majority of drug absorption occurs. It is lined with pseudostratified columnar epithelium and covered with a dense layer of mucus which moves towards the posterior apertures of the nasal cavity because of the ciliary rhythmatric movements. The olfactory region is important for transporting the drug to brain and cerebrospinal fluid and has a surface area of about 15 cm$^2$. It is made of thick connective tissue and lamina propria, into which the olfactory epithelium rests. The thickness of nasal mucosa ranges between 2 and 4 mm. The epithelium cells line the nasal passage and are covered by a mucus layer 5μm in thickness which traps unwanted particles. The mucous secretion consists of water (95%), mucin (2%), salts (1%), proteins (1%) such as albumin, immunoglobulin, lysozyme, and lactoferrin, and lipids (1%). IgA, IgE and IgG are also present in the mucous secretion. The pH of the nasal secretion is ranged from 5 to 6.5. Ciliary action is responsible for clearing the mucus layer from the nasal cavity and mucus is renewed 4 - 6 times per hour. The mucus moves through the nose at a rate of 5-6 mm/min.
1.1.6 Nasal Cavity Variations and Diseases

Variations and diseases in the human anatomy are vast and this section introduces the reader to some of the possible variations to the nasal cavity that has an effect on the air and particle respiration. This theme is carried throughout the subsequent sections on variations and diseases of the other parts of the respiratory pathway (pharynx, larynx, and tracheobronchial and lung airway). There have been a large number of studies indicating that morphological variation of the human nose is found among populations from different eco-geographical locations through adaptation to climate. For example, in cold or dry environments the nose has a large external protrusion, small constricted nostrils, and is tall and narrow (leptorrhine nose). The nasal cross-sectional area is smaller to facilitate heat and moisture exchange. For hot or moist environments, the nose has a small external protrusion, large flaring nostrils, and is short and broad (platyrhine nose) in comparison to leptorrhine noses. The cross-sectional area is greater which reduces the heat transfer during exhalation. The nasal index, which compares the width of the base of the nose with the height of the nose, (e.g. Index = (width × 100)/height) is used as a way to determine the nose type. A low index (< 70) indicates a narrow nose and is considered as leptorrhine, and a high index > 85 is considered platyrhine. In between 70 and 85 the nose is considered messorhine. Other morphological differences include differences between males and females, and also one’s age (child, adult, elderly) [17].

Illustrations 4: Nasal width and height definition used for the nasal index definition. Typical nostril shape for leptorrhine and platyrhine shaped nostrils
1.1.7 Applications of nasal delivery

- **Local effects**
  The nose is exploited to treat regional disorders at relatively low effective doses with less systemic effects. Low molecular weight water-soluble or hydrophobic drugs are used to treat local pathological conditions in the nose. For example, Azelastine is a rapid acting antihistamine, mainly acting as an antagonist on H1- receptors, and as a mast cell stabilizer available as a nasal spray. Beclometasone is an anti-inflammatory corticosteroid used to reduce inflammation and local allergy. It is a well-established drug for the treatment of allergic rhinitis. Nasal decongestants such as oxymetazoline are also administered via the nose for treating common colds.

- **Systemic effects**
  Nasal delivery is convenient for acid labile drugs, proteins and peptides when rapid action is required such as in migraine relief. Nasal delivery offers a rapid action and efficient drug absorption compared to oral and intravenous delivery. Most protein and peptide drugs have low bioavailability (1–2%) due to their high molecular weight and polarity, causing poor absorption through the nasal mucosal membranes. In contrast, the bioavailability of progesterone and propranolol via nasal epithelium is comparable to parenteral administration. Lower bioavailability can be improved by using absorption enhancers in the formulations, thus prolonging the contact time of the drug with the mucous membranes using bioadhesive agents. A significant change in the relative bioavailability of isosorbide dinitrate was observed using 0.1% N-succinyl chitosan as absorption enhancer (69.85%) compared to the 0.5% chitosan (55.36%) and control groups (43.32%) in rats. Steyn and co-workers have reported that the bioavailability of recombinant human growth hormone was increased significantly after nasal delivery in combination with N-trimethyl chitosan chloride as an absorption enhancer used in pheroid technology.

- **Vaccines delivery**
  Vaccines and their applications via nose to treat respiratory infections have been investigated. The localization of immune system components in the mucosal membrane means that the respiratory epithelium is the first defence line in the body against infections. Nasal mucosa is further enriched by lymphoid tissue. It enhances the systemic levels of specific immunoglobulin G and nasal secretary immunoglobulin A and the local immune responses which provide additional protection against invading microbes. Nasal mucosa is advantageous for
immunization due to its permeability, low enzymatic activity and accommodation of the nose-associated lymphoid tissue (NALT) \[^{31}\]. The delivery of vaccine via the nose represents a convenient needle-free procedure for vaccination. Furthermore, the nose-associated lymphoid tissue (NALT) is an effective immune system \[^{33}\].

Nasal vaccines that have been investigated include influenza A and B \[^{34}\], proteasome-influenza \[^{35}\] adenovirus-vectored influenza \[^{36}\], attenuated respiratory syncytial virus and parainfluenza 3 virus \[^{37}\].

Commercially available nasal vaccines include nasal spray of Human influenza vaccine (FluMist®) and nasal drop of Equine influenza vaccine (Flu Avert®) manufactured by Medlmmune Inc. and Heska respectively.

- **CNS delivery**

The intranasal route is promising for the delivery of drugs to the brain via the exploitation of the olfactory neuroepithelium in the nose, possible pathways to transfer drugs from nose to the brain. This strategy has been considered for the treatment of Alzheimer’s disease, brain tumours, epilepsy, pain and sleep disorders. Delivery of nerve growth factor to the brain in rodents has been reported \[^{38,39}\] and in humans studies insulin \[^{40}\] and proteins have been directly transferred through olfactory path to the CNS via nasal cavity \[^{41}\].

Successfully transnasal delivery 0.5mg/kg of siRNA to the CNS with highly brain concentration compared to the other tissue has been reported by Malhotra co-workers to treat neurological disorders using peptide-tagged PEGylated chitosan nanoparticles formulations to deliver siRNA via nose \[^{42}\].

Recent publications that investigated brain targeting via the nose are summarized in Table 1.

<table>
<thead>
<tr>
<th>Drug Molecule</th>
<th>Purpose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>siRNA</td>
<td>Treatment of Neurological Disorder</td>
<td>39</td>
</tr>
<tr>
<td>Levodopa</td>
<td>Treatment of Parkinson’s Disease</td>
<td>22</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>Prevent and control seizure</td>
<td>40</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>Treatment or Prevention of Alzheimer’s Disease</td>
<td>41</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>Treatment of Depression</td>
<td>42</td>
</tr>
</tbody>
</table>
Illustrations 5: (a) Nose to brain pathway (Adapted from Olson, 2008), (b) a schematic illustration of the possible drug molecule transfer delivered nasally. (---) indicates limited substrate delivery via this route, and (?) indicates the exact pathway is unclear.

1.2 MECHANISM OF DRUG ABSORPTION

Many drugs having high water solubility have poor permeability across nasal epithelia and may present insufficient bioavailability. To enhance their permeation and bioavailability permeation enhancers are frequently employed \[43\]. In principle, permeation enhancers induce reversible modifications on the structure of the epithelial barrier. Although the exact mechanism of drug absorption/permeation enhancement is not well known, it is widely accepted that these materials modify the permeability of epithelial cell layer by modifying the phospholipid bilayer \[44\].
Different types of absorption/permeation enhancers are enlisted in Table 2 with their possible mechanism of action. The principle step in the absorption of a drug from the nasal cavity is the passage through the mucus. Fine particles easily pass through the mucus layer; however, large particles may find some difficulties. Mucus contains mucin, a protein with the potential to bind with solutes and thus affect the diffusion process. Structural changes can occur within the mucus layer as a result of environmental or physiological changes. Subsequent to a drug’s passage through the mucus, there are numerous mechanisms for absorption through the mucosa. These include transcellular or simple diffusion across the membrane, paracellular transport via movement between cell and transcytosis by vesicle carriers. Several mechanisms have been proposed, but paracellular and transcellular routes dominate. Paracellular transport is slow and passive. There is an inverse correlation between intranasal absorption and the molecular weight of water-soluble compounds. Poor bioavailability was reported for drugs with a molecular weight greater than 1000 Daltons. The second mechanism involves transport through a lipoidal route that is also known as the transcellular process and is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drugs also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions. Obstacles to drug absorption are potential metabolism before reaching the systemic circulation and inadequate residence time in the nasal cavity.

Illustrations 6: Olfactory route to improve brain targeting of drug and mechanism of improved drug delivery
1.3 FACTORS INFLUENCING NASAL DRUG ABSORPTION

There are various factors that affect the systemic bioavailability of drugs that are administered through the nasal route. The factors can be affecting to the physiochemical properties of the drug, formulation factors, physiological and anatomical factor.

1.3.1 Physicochemical properties of drugs

Some physicochemical properties of drugs (molecular weight, lipophilicity, pKa, stability and solubility) can influence nasal absorption.

**Molecular weight, lipophilicity and pKa**

Lipophilic drugs such as propranolol, progesterone and fentanyl are well absorbed from the nasal cavity, exhibiting pharmacokinetic profiles similar to those obtained after intravenous administration. These drugs are absorbed quickly and efficiently across the nasal membrane via transcellular mechanisms. This observation is true for lipophilic compounds having molecular weight lower than 1 kDa. The extend of nasal absorption of lipophilic drugs bigger than 1 kDa is significantly reduced. On the other hand, the rate and degree of nasal absorption of polar drugs is low and highly dependent of the molecular weight. Drug absorption is expected to be diminished with decrease lipophilicity because the nasal membrane is lipophilic. Thus we can say that polar drugs may not easily transport across nasal membrane. Whenever lipophilicity is too high, the drug permeation through the wall may be reduced because drug does not dissolve easily in the aqueous environment of nasal cavity.

**Stability**

Biological, chemical and physical drug stability studies are a major consideration in all process during the development of new drug formulations. The biological stability of nasally administered drugs may reduce due to the metabolism of drugs by defensive enzymatic mechanisms by nasal cavity. To overcome this difficulty a variety of strategies may be followed, mainly through the use of prodrugs and enzymatic inhibitors.

**Solubility**

For drug absorption, drug dissolution is a pre-requisite because molecularly disperse form of a drug may cross the biomembranes. Therefore the drug must be dissolved in the nasal cavity fluid before absorption. Drug allowed enough contact with the nasal mucosa which may show slow absorption. Drugs with poorly soluble in water may
require high doses hence can cause a problem. The problem can be overcome by enhancing drug solubility using various techniques.

1.3.2 Effect of drug formulation

Viscosity
Formulation with higher viscosity has a better contact time thus increases the absorption. At the same time, high viscosity enhanced the permeability of drugs.

pH
The pKa of drug and pH at the absorption site plays important role in absorption of drug through nasal route. Thus the stability can achieve by proper selection of pH of formulation. However, the pH of formulation should be near on human nasal mucosa (5.0‐6.5) to prevent the sneezing.

Pharmaceutical form
Nasal drops are the simplest and the most convenient nasal pharmaceutical dosage form, but the exact amount of drug delivered is not easily quantified and often results in overdose. Moreover, rapid nasal drainage can occur when using this dosage form. Instead of powder sprays solution and suspension sprays are preferred because powder spray may cause nasal mucosa irritation. Nowadays nasal gel has been developed for accurate drug delivery. This increases the nasal absorption by enhancing the drug residence time and diminishing MCC.

Pharmaceutical excipients
In nasal formulations pharmaceutical excipients are selected accordingly to their functions. The most commonly used excipients are Solubilizers, buffer components, antioxidants, preservatives, humectants, and gelling/viscosifying agents.

1.3.3 Physiological and anatomical factor

Blood flow
Rich supply of blood and a large surface area make the nasal mucosa an optimal location for drug absorption. Nasal absorption of drugs is influenced by blood flow rate, as it increases the amount of drug that passes through the membrane and hence reaching the general circulation. Several studies were made to evaluate this influence. For example, Kao et al. stated that nasal absorption of dopamine was relatively slow and incomplete probably due to its own vasoconstrictor effect. From above observations, it was concluded that vasoconstriction decreases nasal drug absorption by diminishing the blood flow.
Mucociliary clearance

Mucociliary clearance (MCC) also referred to as Mucociliary apparatus it is the self-clearing mechanism of the bronchi. Nasal mucus layer defend the respiratory tract by preventing the lungs from foreign substances, pathogens and particles carried by inhaled air. These agents adhere to the mucus layer and transported to the gastrointestinal tract. Above elimination is designated MCC and it influences significantly the nasal drug absorption. The MCC system has been described as a “conveyer belt” wherein cilia provide the driving force whereas mucus acts as a sticky fluid that collects and disposes foreign particles. Hence MCC efficiency depends on the length, density and beat frequency of cilia as well as the amount and viscoelastic properties of mucus. MCC may increased by all factors that increase mucus production, decrease mucus viscosity or increased ciliary beat frequency. In physiological conditions, mucus is transported at a rate of 5 mm/min and its transit time in human nasal cavity is reported to be 15-20 min. The values which are not within the range these references are abnormal and suggestive of impaired MCC. From the above discussion we can say that the residence time of the drugs in nasal mucosa increased and hence permeation may be enhanced when MCC decreases. When MCC increases permeation rate of drug is decreased. MCC does not work properly in the following pathological conditions.

Enzymatic degradation

Internasally administration of drugs avoids gastrointestinal and hepatic first-pass effect. Drugs may be metabolized in lumen of nasal cavity due to the presence of a broad range of metabolic enzymes in nasal tissues. Some examples of enzyme which may play role in enzymatic degradation of drugs are carboxyl esterase, aldehyde dehydrogenases, epoxide hydrolases, glutathione S-transferases and Cytochrome P450 isoenzymes have been found in nasal epithelial cells. The proteolytic enzymes (amino peptidases and proteases) were also found and they play an important role in degradation of calcitonin, insulin and desmopressin. The pharmacokinetic and pharmacodynamic profile of drugs administered through nasal route may be affected by xenobiotic metabolizing enzymes.

Transporters and efflux systems

The absorption of drugs into systemic circulation and CNS through nasal route is of great interest. Multidrug resistance transporters have been identified which may be involved in the transportation of hydrophobic and amphiphilic drugs. The apical area
of ciliated epithelial cells and sub mucosal vessels of the human olfactory region contain P-gp is an efflux transporter which plays an important role in avoiding the influx of drugs from nasal membrane.

![Factors interfering with drug delivery via the nasal route](image)

**Figure 1: Factors Influencing Nasal Drug Absorption**

### 1.4 DOSAGE FORM AND DEVICES USED FOR NASAL DELIVERY

The selection of dosage form depends upon the drug being used, proposed indication, patient population.

#### 1.4.1 Nasal Drops

Nasal drops are the most traditional nasal devices for intranasal administrations of liquids owing to their low manufacturing cost. The main disadvantages of nasal drop devices are lack of precision in the administered dose and the risk of contamination during treatment. An issue that is overlooked is the special expertise required during use of nasal drops. For maximum benefit the patient should keep the nostril uppermost and the administration angle at 90° angle (angle between subject’s head and nasal drop device) while instilling the dose. The head is swirled from side to side after application of drops to nostrils [49].

**i) Drops delivered with a pipette**

In this type of nasal drops, the quantity of dose administered depends on pulling a volume of the formulation into a glass dropper followed by placing the tip of the dropper into the nostril and squeezing the rubber top to release the formulation as
drops \[50\]. Clinical efficacy of fluticasone formulation using single dose pipette has been reported to be significantly higher than that using a nasal pump in patients with nasal polyps \[51\].

\textbf{ii) Squeeze bottles}

Squeezing bottles and nasal drops with pipette are recommended for exerting local therapeutic effects such as those used for delivery of liquid nasal decongestant formulations. Squeezing a plastic bottle causes the release of air inside the bottle from a narrow orifice. This generates a small spray volume into the frontal region of the nostril. The drawback of this device is air pulled back into the container following the release of the dose which may pull part of the nasal secretions into the bottle, resulting in contamination of the formulation. The droplet size and dose accuracy are strongly dependent on the force applied on the plastic bottle to release the medication resulting in poor control over the delivered dose. Thus, this device is not recommended for use in children \[52\].

![Figure 2: (a) Nasal drops with pipette (www.adelphi-hp.com), and (b) nasal squeeze bottles](image)

\textbf{1.4.2 Nasal Sprays}

Nasal spray system consists of a chamber, a piston and an operating actuator. Unlike nasal drops, nasal spray generates precise doses (25 - 200μl) per spray due to the presence of pumps and actuators. \textit{In vitro} studies have shown nasal sprays to produce consistent doses and reproducible plume geometry. Particle size of the generated drops, spray pattern, dose accuracy are affected by the formulation properties such as thixotropic behaviour, viscosity and surface tension. The applied force, orifice size, design of the pump and formulation can all affect the droplet size and plume geometry.
of the spray [50] Doses are delivered by nasal sprays either by using metered-dose spray pumps or pressurized metered dose inhalers (pMDIs).

**i) Metered- dose spray pumps**

Spray pumps operate by replacing the generated liquid spray with air. Hence, preservatives are needed to prevent microbial contamination of the formulation. Priming and repriming are required before starting the delivery and when keeping it for a long time to maintain consistency of the delivered dose. Different types of spray systems have been developed by manufacturers to overcome the risk of contamination and minimise irritation caused by inclusion of preservatives in the formulations. This was achieved either by designing a movable piston, collapsible bag or compressed air (carbon dioxide or nitrogen) to replace the vacuum created by the emitted volume. The pump prevents air from being pulled into the container during the generation of the spray [52]. These systems generate reproducible spray regardless of the position of the device and spraying angle, offering convenience for use by children and patients confined to bed [50].

![Illustrations 7: (a) Airless nasal device with collapsible bag (Bag on valve system) and (b) airless nasal device with sliding piston](image)

Another system is preservative-free and operates by employing an aseptic filtration system, hence the air is pulled into the container while dose is emitted and filtered, resulting in prevention of microbial contamination [52]. Single and double-dose spray device is more useful than metered dose spray pumps for drugs that have narrow therapeutic index. Examples of this system are Zomig® (Zolmitriptan) nasal spray and Migranal® (dihydroergotamine mesylate, USP) nasal spray which are used for the treatment of migraine headaches [50].
ii) Nasal pressurized metered-dose inhalers
Nasal pressurized metered-dose inhalers (pMDI) systems have been used for eliciting local therapeutic effects in the nose. The major sites of dose deposition are the anterior non ciliated regions of the nasal vestibule and anterior parts of the narrow nasal valve. Chlороfluorocarbon propellants cause irritation and dryness to the nasal mucosa. Moreover, the speeds of particles are much higher than those emitted from spray pumps. For these reasons, chlороfluorocarbon propellants have been replaced by hydrofluoroalkane propellants \[^{50}\]. Examples of pMDIs are budesonide and beclometasone dipropionate, which are used for the treatment of allergic rhinitis.

1.4.3 Nasal Powders
Preparation of chemically unstable drugs as powders enhance their stability profile compared to their solutions or dispersions. Powder dosage form is suitable for nonpeptide and peptide drugs with no preservatives or freeze storage being required. Residence of the powdered drug in the nose can be improved compared to liquid formulations and patient compliance might also be enhanced, especially with drugs having unpleasant odour or taste. However, when preparing nasal powders several factors need to be considered. These are solubility of the drug, particle size of the powdered formulation, aerodynamic properties of the particles and the possible irritation of the drug or excipients on the nasal epithelium \[^{52}\]. Ideally, particles larger than 10μm are likely to deposit in the nasal passages, whilst particles larger than 0.5 μm filtered out in the nose \[^{53}\]. Depending on the bulk density of the powder, it is possible to administer up to 50 mg of the formulation, whilst the maximum volume of liquid administration for each nostril in one time is 150 μl \[^{54}\]. Powder formulations
consisting of water insoluble and non-swellable drug carriers improve the bioavailability of hydrophilic drug molecules, showed systemic bioavailability of the elcatonin based on calcium carbonate in rabbits and rats increased after nasal administration compared to the liquid form. Optimization of particle size and morphology for nasal formulations may reduce the risk of mucosal irritation and enhance the deposition \cite{55}. The mucosal irritation, difficulty and cost of the formulation due to optimization of particle size and morphology are major limitations for development of powdered nasal formulations \cite{56}. Insufflators are convenient and simple devices for nasal administration of powders \cite{52}. The most commonly used devices for delivery of nasal powders are nasal insufflators.

i) **Nasal insufflators for experimental purposes**

Nasal insufflators or powder aerosolizers are tube-like containers in which powdered drug formulations can be stored. Alternatively, these tubes are connected with syringes or air pump to blow the powder into the nostril.

Illustrations 9: (a) Penn-Century Dry Powder Insufflator\textsuperscript{TM} - Model DP-4 connected with commercial syringe, (b) Dry Powder Insufflator\textsuperscript{TM} Air Pump Assembly ii) Bi-Directional\textsuperscript{TM} nasal insufflators
Bi-Directional™ nasal insufflators have been developed by OptiNose breath-powered nasal delivery technology. This design consists of mouthpiece and nosepiece. The breathing force carries particles from prefilled gelatin capsule with powder doses into nostril, while soft palate separates the nose from the throat. This device offers potential particle distribution into deep part of the nose, resulting in minimized lung deposition and enhanced powder targeting of the drug to the sinus ostia\(^{[57]}\).

Sumatriptan powder is an example that is currently in phase III clinical trials using the Bi-Directional™ nasal insufflator to treat acute migraine.

iii) Miat® monodose nasal insufflator

Miat® monodose nasal insufflator has been developed by Miat S.P.A Milan, Italy and consists of compressible pump, a revolving chamber with a grip tab, a nozzle, a two-push- button (pin) and a protective cap. Upon squeezing the pump the compressible compartment creates a stream of air that passes through an inlet puncture in the capsule made by two opposite pins, resulting in particle delivery to the nostril by means of air stream\(^{[58]}\).

The delivery of powder dose from the monodose insufflator is evaluated by the weight of capsule before and after each puffing\(^{[59]}\).

*Illustrations 10:* (a) Optinose Bi-Directional™ nasal insufflators (b) Miat® monodose nasal insufflator.

iv) Monopowder P® insufflator

The Monopowder P® insufflator developed by Valois Dispray, France consists of a pump, a nasal adaptor and a powder reservoir. The piston actuates a stream of air to
expel the powder into the nasal pathway \[^{60}\]. Deposition and particle size of the delivered powder depends on formulations and actuator performance. Moreover, a multi-dose dry powder device has been developed to improve patient compliance \[^{52}\]. For example, the multi dose system of budesonide powder has been marketed by Astrazeneca, to treat allergic rhinitis.

1.4.4 Liquid formulations

Liquid dosage forms are usually aqueous solutions, suspensions or emulsions, however, formulations made with oily or alcoholic vehicles are also available. Humidifying effect is important for nasal mucosa as allergy and irritation are mainly related to dryness of the mucus membrane. The main drawbacks of liquid dosage forms are:

(i) The microbial growth is possible, necessitating the use of preservatives.

(ii) Long-term use of liquid dosage forms may interfere with nasal mucociliary function and cause irritation due to the presence of preservatives in the nasal formulations.

(iii) Short half-life of the drug because of its liability for degradation.

(iv) Probability of dripping out of the liquid dose from the nostril following administration. Liquid dosage forms are easily removed to the posterior part of the respiratory tract by mucociliary clearance \[^{61}\].

1.4.5 Nasal Gels

Pharmaceutical gels are semisolid preparations in which the drug is dispersed in a polymeric matrix. Gels have several advantages including:

(i) Reduced mucociliary clearance owing to the high viscosity of gels.

(ii) Masked unpleasant taste of the formulation due to reduced accessibility of the formulation to the nasopharynx.

(iii) Reduced loss of the formulation from nostril while breathing or sneezing.

(iv) Minimized irritation due to the soothing effect of excipients used in nasal gel.

(v) Enhanced contact between the drug and nasal mucosa, which may enhance the absorption profile of the drug.
1.5 RECENT APPROACHES IN BRAIN TARGET VIA OLFACTORY ROUTE [62]

i) Invasive approach
Invasive approaches deliver drug to the brain by mechanically breaching the BBB and are summarized below:

a) Intra-cerebro-ventricular (ICV) infusion: It has been reported that the concentration of a drug in the brain is only 1–2% of the CSF concentration at just 1–2 mm from the surface. The drug eventually distributes to the general circulation, where the drug then enters the brain parenchyma following transport across the BBB. This result is similar to a slow intravenous infusion rather than a direct administration of drugs into the brain. Pharmacologic effects can be seen after ICV administration, if the target receptors of the drug (for example, opioid peptides) are located near the ependymal surface of the brain.

b) Convection-enhanced delivery (CED): The general principle of CED involves the stereotactically guided insertion of a small-caliber catheter into the brain parenchyma. Through this catheter, infusate is actively pumped into the brain parenchyma and penetrates in the interstitial space.

c) Intra-cerebral injection or use of implants: Both the bolus injection of chemotherapy agents and the placement of a biodegradable, chemotherapeutic impregnated, wafer into a tumour resection cavity, rely on the principle of diffusion to drive the drug into the infiltrated brain have demonstrated the presence of high drug concentration (0.5–3.5 mM for carmustine, 0.2–1 mM for paclitaxel) within the first 3 mm from the polymer implants in monkeys; significant concentrations (0.4 μM for carmustine, 0.6 μM for paclitaxel) were measured up to approx. 5 cm from the implant as long as 30 days after implantation.

d) Disruption of the BBB:
Disruption of the BBB can open access of the brain to components in the blood by making the tight junction between the endothelial cells of the brain capillaries leaky. Different techniques are used to disrupt the tight junctions:

Osmotic disruption: The osmotic shock causes endothelial cells to shrink, thereby disrupting the tight junctions. Intracarotid administration of a hypertonic mannitol solution with subsequent administration of drugs can increase drug concentration in brain and tumour tissue to reach therapeutic concentration.
MRI-guided focused ultrasound BBB disruption technique: Ultrasound has been shown to be capable of BBB disruption. The combination of microbubbles (preformed microbubbles of ultrasound contrast agent, optison, with a diameter of 2–6 μm which is injected into the blood stream before exposures to ultrasound). This technique has been shown to increase the distribution of Herceptin in brain tissue by 50% in a mice model.

ii) Pharmacological approach

The pharmacological approach to crossing the BBB is based on the observation that some molecules freely enter the brain, e.g. alcohol nicotine and benzodiazepine. This ability to passively cross the BBB depends on the molecular size being less than 500 D), charge (low hydrogen bonding capabilities) and lipophilicity (the more lipophilic, the better the transport). This approach consists of modifying, through medicinal chemistry, a molecule that is known to be active against a CNS target to enable it to penetrate the BBB. Modification of drugs through a reduction in the relative number of polar groups increases the transfer of a drug across the BBB. Lipid carriers have been used for transport, and there are successful examples of both these approaches. Modification of antioxidants with pyrrolopyrimidines increases their ability to access target cells within the CNS. Enhanced delivery of ganciclovir to the brain was observed by covalently attaching 1-methyl-1,4-dihydronicotinate to an hydroxymethyl group. Fatty acid such as N-docosahexaenoyl (DHA) have been incorporated in small drugs to increase their brain uptake. Incorporation of low molecular mass drugs into pluronic micelles can increase drug solubility and drug stability, and can improve drug pharmacokinetics and biodistribution. Polymeric micelles have been utilized for delivery of CNS drugs across the BBB, and for oral delivery of drugs and tumour-specific delivery of antineoplastic agents.

iii) Physiological approaches

The brain requires essential substances for metabolism and survival, such as glucose, insulin, growth hormone, low density lipoprotein (LDL), etc. These substances are recognized by specific receptors or transport mechanisms, resulting in specific transport into the brain. Since almost every neuron in the brain is perfused by its own capillary as a result of the small distance separating capillaries (on average 40 μm) and the brain's very high perfusion rate. Therefore, the most effective way of delivering neuroactive drugs is via transporters or internalizing receptors on these capillaries.
1.6 MICROEMULSIONS AS A DRUG DELIVERY SYSTEM

Oil and water are immiscible. They separate into two phases when mixed, each saturated with traces of the other component \[63\]. An attempt to combine the two phases requires energy input to establish water-oil contacts that would replace the water-water and oil-oil contacts. The interfacial tension between bulk oil and water can be as high as 30-50 dynes/cm \[64\]. To overcome this, surfactants can be used. Surfactants are surface-active molecules. They contain water-loving (hydrophilic) and oil-loving (lipophilic) moieties \[65\]. Because of this characteristic, they tend to adsorb at the water-oil interface. An emulsion is formed when a small amount of an appropriate surfactant is mechanically agitated with the oil and water. This results in a two-phase dispersion where one phase exists as droplets coated by surfactant that is dispersed throughout the continuous, other phase. These emulsions are milky or turbid in appearance due to the fact that the droplet sizes range from .1 to 1 micron in diameter \[65\]. As a general rule, the type of surfactant used in the system determines which phase is continuous. If the surfactant is hydrophilic, then oil will be emulsified in droplets throughout a continuous water phase. The opposite is true for more lipophilic surfactants. Water will be emulsified in droplets that are dispersed throughout a continuous oil phase in this case \[66\]. Emulsions are kinetically stable, but are ultimately thermodynamically unstable. Over time, they will begin to separate back into their two phases. The droplets will merge together, and the dispersed phase will sediment (cream) \[65\]. At this point, they degrade back into bulk phases of pure oil and pure water with some of the surfactant dissolved in preferentially in one of the two \[64\].

1.6.1 Types of Microemulsion

Microemulsions are thermodynamically stable, but are only found under carefully defined conditions \[65,77\]. One way to characterize these systems is by whether the domains are in droplets or continuous \[82\]. Characterizing the systems in this way results in three types of microemulsions: oil-in-water (o/w), water-in-oil (w/o), and bicontinuous. Generally, one would assume that whichever phase was a larger volume would be the continuous phase, but this is not always the case. Oil-in-water microemulsions are droplets of oil surrounded by a surfactant (and possibly co-surfactant) film that forms the internal phase distributed in water, which is the continuous phase. This type of microemulsion generally has a larger interaction volume than the w/o microemulsions \[79\]. The monolayer of surfactant forms the
interfacial film that is oriented in a “positive” curve, where the polar head-groups face the continuous water phase and the lipophilic tails face into the oil droplets [64]. The o/w systems are interesting because they enable a hydrophobic drug to be more soluble in an aqueous based system, by solubilizing it in the internal oil droplets. Most drugs tend to favor small/medium molecular volume oils as opposed to hydrocarbon oils due to the polarity of the poorly water-soluble drugs. An o/w drug delivery tends to be straightforward when compared to w/o microemulsions. This is the result of the droplet structure of o/w microemulsions being retained on dilution with the biological aqueous phase [79]. Water-in-oil microemulsions are made up of droplets of water surrounded by an oil continuous phase. These are generally known as “reverse-micelles”, where the polar headgroups of the surfactant are facing into the droplets of water, with the fatty acid tails facing into the oil phase. This type of droplet is usually seen when the volume fraction of water is low, although the type of surfactant also impacts this as well [79]. A w/o microemulsion used orally or parenterally may be destabilized by the aqueous biological system. The biological system increases the phase volume of the internal phase, eventually leading to a “percolation phenomenon” where phase separation or phase inversion occurs [80,81]. When the amount of water and oil present are similar, a bicontinuous microemulsion system may result. In this case, both water and oil exist as a continuous phase. Irregular channels of oil and water are intertwined, resulting in what looks like a “sponge-phase” [65,82,83]. Transitions from o/w to w/o microemulsions may pass through this bicontinuous state. Bicontinuous microemulsions, as mentioned before, may show non-Newtonian flow and plasticity. These properties make them especially useful for topical delivery of drugs or for intravenous administration, where upon dilution with aqueous biological fluids, forms an o/w microemulsion [79, 84-88].

![Figure 3: Schematic representation of the most commonly encountered self-association structures in water, oil or a combination](image-url)
1.6.2 Surfactant Use in Microemulsions

Surfactants are molecules that typically contain a polar head group and an apolar tail. They are surface-active and microstructure-forming molecules with a strong chemical dipole \(^{[65]}\). They can be ionic (cationic or anionic), nonionic, or ionic. Surfactant molecules self-associate due to various inter- and intra-molecular forces as well as entropy considerations. All of these serve to optimize the free-energy overall. For example, when surfactant is mixed with oil and water, they accumulate at the oil/water interface, because it is thermodynamically favorable \(^{[79]}\). The surfactant molecules can arrange themselves in a variety of shapes. They can form spherical micelles, rod-shaped micelles, a hexagonal phase (consisting of rod-shaped micelles), lamellar (sheet) phases, reverse micelles, or hexagonal reverse micelles. At low concentrations of dispersed (internal) phase, spherical, isolated droplets are present in the microemulsions. At higher dispersed phase concentrations, the final structures depend on the interaction between droplets. If they are repulsive, no droplet overlap will be produced due to colliding droplets. If attractive interactions are present, multiple droplets may collide and form other structures \(^{[63]}\). The hydrophilic-lipophilic balance (HLB) of the surfactant can be taken into account to try to rationalize the surfactant’s behavior. It is generally accepted that a surfactant with HLB from 3-6 will favor the formation of water-in-oil (w/o) microemulsions, whereas surfactants with HLB from 8-18 are preferred for oil-in-water (o/w) microemulsions \(^{[79]}\). It must be noted, though, that microemulsions are only obtained under certain carefully defined conditions, and the HLB of the

Figure 4: Schematic representation of the three most commonly encountered microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion.
surfactant can only be used as a starting point in the selection of components that will form a microemulsion. Another method used to relate the type of surfactant to the structures it forms is through the critical packing parameter (CPP). This, like HLB, is an empirical approach since there are many other factors that impact the final structures found in microemulsions. The CPP is a measure of the surfactant’s preferred geometry, and therefore can be used to predict the type of structure that possibly will be formed. The CPP can be calculated by dividing the partial molar volume of the hydrophobic part of the surfactant by the product of the optimal head group area and length of the surfactant tail [79]. Surfactants that are “cone- shaped” where the tail group or head group is much larger than the other will tend to accumulate at curved interfaces resulting in micelles. Surfactants that are more “block- shaped” where tail group and head group are similar in size and the CPP values are close to one end to form worm-like micelles or lamellar structures. Values of CPP greater than one indicate that the head groups are much larger, resulting in w/o microemulsion systems. The opposite is true for CPP values less than one. They generally produce o/w microemulsion systems. Values for CPP around one indicate the possible formation of lamellar phases [79]. Regardless of the surfactant chosen for the microemulsion formulation, it must be able to lower the interfacial tension to an extremely small value. This aids the dispersion process, providing a flexible film that readily surrounds droplets of the internal phase while still having appropriate lipophilic character to provide a curvature at the interfacial region [70].

1.6.3 Nonionic Surfactants
Most nonionic surfactants are structurally similar to ionic surfactants, except for the fact that with ionic surfactants, the head group is uncharged. Because there are no electrostatic charges from the head groups, the interactions between these nonionic head groups are dominated by steric and osmotic forces [88]. Cosurfactants are generally not needed to form microemulsions with nonionics. This is due to the fact that pure specimens of nonionic’s usually are made up of mixtures of slightly varying chain length [89]. Ethoxylated alcohols are the most common nonionic surfactants [65]. These alcohols contain a wide-ranging degree of ethoxylation, where ethylene oxide is added to fatty acids to make them more water-soluble. They are considered “amphiphiles”, with an oil- loving hydrocarbon tail group and a water loving ethoxylated alcohol group [69]. Nonionic surfactants show good biological acceptance [90]. They are able to form microemulsions that are insensitive to pH and electrolyte
Examples of nonionic surfactants include polyoxyethylene surfactants, such as Brij 35, or sugar esters, such as sorbitan monooleate (Span 80). Polyoxyethylene sorbitan monooleate (Tween 80) and polyoxyethylene sorbitan monolaurate (Tween 20) appear safe and acceptable for oral and parenteral use. Polysorbates are partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5 or 4 miles of ethylene oxide for each mole of sorbitol and its anhydrides. These vary in size due to a mixture of molecules and are considered hydrophilic nonionic surfactants. Sorbitans are partial esters of sorbitol and its mono and dianhydrides with fatty acids. These are considered lipophilic nonionic surfactants.

Nonionic surfactants that contain sugar hydrophilic groups, such as alkylpolyglucoside surfactants, and sucrose ester surfactants are very hydrophilic and form temperature-insensitive microemulsions with the addition of alcohol. Alkanol amides and polyamines are the primary nitrogen-based nonionic surfactant types.

1.6.4 Ionic Surfactants

The use of ionic surfactants can be fairly limited in general pharmaceutical dosage forms. A large majority of ionic surfactants do not form balanced microemulsions without the addition of another component. These additives are required because the head group of the ionic surfactants are generally substantially more hydrophilic than poly(ethylene oxide) moieties. The salts or co-surfactants shift the overall HLB into the optimal range for microemulsion formulation. The addition of co-surfactants will be discussed in a later section. Ionic surfactants can be cationic, anionic, or zwitterionic. Cationic surfactants generally fall into the class of quaternary ammonium alkyl salts. Alkylammonium halides and tetra-alkylammonium halides are the most numerous in this class. Alkyl ammonium halides are excellent hydrogen bond donors and interact strongly with water. The most well known examples from the cationic surfactant class are hexadecyltrimethyl-ammonium bromide (HDTAB) and di dodecyl ammonium bromide (DDAB). Although less numerous, phosphorous can be quaternarized with alkyl groups to create alkyl phosphonium cationic surfactants as well. Alkali alkanoates, also known as soaps, are the most common anionic surfactants. The anionic charge in these surfactants comes from the ionized carboxyl group. Dioctyl sodium sulfosuccinate (DOSS) is the most widely studied anionic surfactant. It has twin tails and is a particularly good
stabilizer of w/o microemulsions \(^{[79]}\). Zwitterionic surfactants, which contain both negatively and positively charged groups, form microemulsions upon the addition of co-surfactants. Phospholipids, such as lecithin, are common zwitterionic surfactants. Unlike other ionic surfactants, which are somewhat toxic, these show excellent biocompatibility. This is most likely due to the fact that lecithin is obtained naturally from soybean or egg, which contains diacylphosphatidylcholine as the major constituent \(^{[65,79]}\). Another important class of zwitterionic surfactants to note is the betaines, such as alkylbetaines, amidoalkylbetaines, and heterocyclic betaines \(^{[88]}\).

1.6.5 Microemulsion Use
Micro emulsions have many inherent properties, which make them interesting for a variety of applications. They are thermodynamically stable theoretically allowing for infinite shelf-life, are translucent, and contain small microstructures \(^{[65]}\). To date, microemulsion use shows great potential in a wide variety of areas including enhanced oil recovery, cutting oils, drug delivery, detergency, and lubrication \(^{[77,78]}\).

1.6.6 Preparation Method
- **Selection of oil phase:**
  The selection of oil phase is done based on solubility. The oils which are selected is castor oil and turpentine oil. As turpentine oil is counter irritant castor oil is used.
- **Selection of surfactant and co-surfactant**
  The criteria for the selection of surfactant were its HLB value, drug solubility and non-toxic nature. Surfactants like Tween-20, and Tween-80 are used. Above method was carried out for the selection of surfactants. Cosurfactants were selected based on their ability to form stable and clear microemulsion at a minimum concentration. Based on this cosurfactants like Polyethylene Glycol 400 (PEG 400) and Glycerol are used.
- **Preparation of microemulsion**
  Microemulsion was prepared by water titration method. Predetermined amount of the drug was dissolved in the required quantity of oil. Surfactant and cosurfactant in a fixed ratio (1:1,2:1,3:1& 4:1) were added to it. Finally the above mixture was titrated by distilled water with continuous stirring until a transparent and homogenous microemulsion is produced. After preparing microemulsion the further size of the globule can be reduced by the use of sonicater \(^{[92]}\).
1.6.7 Characteristics of Microemulsions

If a surfactant that possesses balanced hydrophilic and lipophilic properties is used in the right concentration, a different oil and water system will be produced. The system is still an emulsion, but exhibits some characteristics that are different from the milky emulsions discussed previously. These new systems are called “microemulsions”. The interfacial tension between phases, amount of energy required for formation, droplet sizes, and visual appearance are only a few of the differences seen when comparing emulsions to microemulsions. Microemulsions are in many respects small-scale emulsions. They are fragile systems in the sense that certain surfactants in specific concentrations are needed for microemulsion formation [71,72].

This increased surface area would ultimately influence the transport properties of a drug [74]. The free energy of the system is minimized by the compensation of surface energy by dispersion entropy. The flexible interfacial film results in droplet sizes that fall in a range of 10-100 nm in diameter for microemulsion systems [65,68,71].

Although these systems are formed spontaneously, the driving forces are small and may possibly take time to reach equilibrium [70].

This is a dynamic process. There is diffusion of molecules within the microstructures and there are fluctuations in the curvature of the surfactant film. These droplets diffuse through the continuous phase while kinetics of the collision, merging, and separation of droplets occur [65,72,73]. With droplet sizes in the nanometer range, microemulsions are optically transparent and are considered to be solutions [67,74,75]. They are homogeneous on a macroscopic scale, but are heterogeneous on a molecular scale [63].

Microemulsions usually exhibit low viscosities and Newtonian flow characteristics. Their flow will remain constant when subjected to a variety of shear rates. Bicontinuous formulations may show some non-Newtonian flow and plasticity [72].

Microemulsion viscosity is close to that of water, even at high droplet concentrations. The microstructure is constantly changing, making these very dynamic systems with reversible droplet coalescence [1].

To study the different properties of microemulsions, a variety of techniques are usually employed. Light scattering, x-ray diffraction, ultracentrifugation, electrical conductivity, and viscosity measurements have been widely used [76].
1.7 MICROSPHERES AS A DRUG DELIVERY SYSTEM

Microspheres are solid spherical particles ranging in size from 1 to 1000 μm. They are spherical free flowing particles comprising proteins or synthetic polymers. The microspheres are free flowing powders including proteins or synthetic polymers, which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microspheres matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through particle matrix have the potential for the controlled release of the drug. They are made up of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products. Delivery of drug into the brain for central nervous system (CNS) disease require treatment, however, such route of delivery is very problematic. Therapeutic effect of the drug can be described by achieving the desired concentration of drug in blood or tissue for a prolonged period. Hence, it is a reliable means to deliver a drug to a target site with specificity and in a controlled manner. Microsphere used as a not controlled release but for targeted therapy also so it offers certain advantages over the conventional release dosage form for those drugs having a first pass metabolism. All these drawbacks of conventional delivery system necessitate the development of controlled drug delivery system. Delivery of drug to CNS is problematic for drugs having a hydrophilic in nature and having a high molecular weight because of impervious nature of Blood-Brain Barriers (BBB). Parkinsonism disease is a leading cause of neurological disability and is the second most common progressive neurodegenerative disorder. The effect of the Parkinsonism disease reaches 1-2% in people over the age of 50. It has no gender preference and has a worldwide distribution. The symptoms of Parkinsonism disease are largely related to progressive loss of dopamine in the basal ganglia. While enormous progress has been made regarding our understanding of the pathogenic mechanisms of neurological diseases, there are only a small number of effective drugs for treating this illness. A key obstacle for developing an effective treatment for neurological diseases is the blockage of drug entrance into the CNS by the BBB. <2% of all small molecule-drugs and virtually no large molecule drugs can cross the BBB. Therefore, it is of critical significance to search drug delivery strategies that can effectively deliver the drugs to...
CNS [96]. Recent developments in nasal drug delivery have suggested intranasal administration as a safe and acceptable route for brain targeting, especially for drugs with biological effects on the CNS and limited blood-brain permeability [97]. The nose to brain delivery would be beneficial in therapeutic situations where a rapid and/or specific targeting of drugs to the brain is required. Conditions such as Parkinson’s disease, Alzheimer’s disease or pain would be benefited from the development of nasal delivery systems, which will increase the fraction of drug that reaches the CNS after nasal delivery. The olfactory region located at the upper remote parts of the nasal passages offers the potential for certain compounds to circumvent the BBB and enter into the brain [98]. Intranasal delivery also offers the advantage of simple administration, cost effectiveness and convenience. This novel delivery method allows drugs, therapeutic proteins, polynucleotides, and viral vectors that do not normally cross the BBB to be delivered to the CNS. In addition, intranasal targeting of drugs to the CNS avoids first pass elimination by the liver allowing a lower therapeutic drug dose and fewer systemic side effects. Delivery from the nose to the CNS occurs within minutes along both the olfactory and trigeminal nerves. Delivery occurs by an extracellular route and does not require that the drugs bind to any receptor or undergo axonal transport [99-104]. Ropinirole, a recent introduction in the clinical treatment of Parkinson’s disease, suffers with the problems of low oral bioavailability and frequent dosing [105]. It is an orally administered non-ergoline dopamine agonist, and chemically it is hydrochloride (HCL) salt of 4-[2-(dipropyl amino) ethyl]-1, 3-dihydro-2H-indol-2-one with a molecular weight of 296.84. It is having short half-life (4-6 hrs) and low bioavailability (35%) due to extensive first pass metabolism [106]. The usual dose is 3-9 mg daily and has to be taken in three divided doses due to a short half-life of the drug [107]. Microspheres are solid spherical particles ranging from 1 to 1000 μm. Bioadhesive microspheres give more residence time to facilitate absorption through nasal mucosa against nasal mucociliary clearance [93]. The treatment of all neurodegenerative diseases is a big challenge because of the numerous protective barriers surrounding the CNS. The targeting of drug to the CNS, for the therapeutic advancement of neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease can be done by administering the drug formulation such as nanoparticle, liposome, microemulsion, and microsphere, which can cross the BBB or by delivering the drug formulation through intranasal route which can bypass the BBB [108]. Nasal delivery of drugs targeting the CNS is currently an area of great
interest. In addition to “nose to brain delivery” intranasal drugs can enter through a "nose to systemic circulation to brain" pathway. In this case, it is necessary for the drug to readily permeate the BBB from the circulation. For this to be achieved the drug must exhibit satisfactory passive or active transport across the light junction barriers of the BBB\(^{109}\).

1.7.1 Applications of Microspheres\(^{110}\)

Some of the applications of microencapsulation can be described in detail as given below:

- Prolonged release dosage forms. The microsphere drug can be administered, as microsphere is perhaps most useful for the preparation of tablets, capsules or parenteral dosage forms.
- Microsphere can be used to prepare enteric-coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.
- It can be used to mask the taste of bitter drugs.
- It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat. Microsphere does not yet provide a perfect barrier for materials, which degrade in the presence of oxygen, moisture or heat, however a great degree of protection against these elements can be provided. For example, vitamin A and K have been shown to be protected from moisture and oxygen through microsphere.
- Microsphere can be used to decrease the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.
- Microsphere has also been used to decrease potential danger of handling of toxic or noxious substances. The toxicity occurred due to handling of fumigants, herbicides, insecticides and pesticides have been advantageously decreased after microencapsulation.
- The hygroscopic properties of many core materials may be reduced by microsphere.
- Many drugs have been microsphere to reduce gastric irritation.

A key obstacle for developing effective treatment for neurological diseases is the blockage of drug entrance into the CNS by the BBB. Less than 2% of all small molecule-drugs and virtually no large molecule-drugs can cross the BBB. Therefore, it is of critical significance to search drug delivery strategies that can effectively
deliver the drugs to CNS \[111\]. Recent developments in nasal drug delivery have suggested intranasal administration as a safe and acceptable route for brain targeting, especially for drugs with biological effects on the central nerves system (CNS) and limited blood–brain permeability\[112\].

![Diagram](image)

**Illustrations 11:** a) Position of olfactory bulb with respect to brain an nasal cavity, b) Different pathways for reaching the brain after intranasal administration

The nose to brain delivery would be beneficial in therapeutic situations where a rapid and/or specific targeting of drugs to the brain is required. Conditions such as Parkinson’s disease, Alzheimer’s disease or pain would be benefited from the development of nasal delivery systems, which will increase the fraction of drug that reach the CNS after nasal delivery. The olfactory region located at the upper remote parts of the nasal passages offers the potential for certain compounds to circumvent the blood-brain barrier and enter into the brain \[113\]. Intranasal delivery also offers the advantage of simple administration, cost effectiveness and convenient. This novel delivery method allows drugs, therapeutic proteins, polynucleotides and viral vectors that do not normally cross the BBB to be delivered to the CNS. Additionally, intranasal targeting of drugs to the CNS avoids first pass elimination by the liver allowing a lower therapeutic drug dose and fewer systemic side effects. Delivery from the nose to the CNS occurs within minutes along both the olfactory and trigeminal nerves. Delivery occurs by an extracellular route and does not require that the drugs bind to any receptor or undergo axonal transport \[114-119\]. Microspheres are solid spherical particles ranging in size from 1-1000μm. Bioadhesive microspheres give
more residence time to facilitate absorption through nasal mucosa against nasal mucociliary clearance\textsuperscript{120}.

### 1.7.2 Preparation of microspheres

There are many methods used for preparation of microspheres. Among these methods, spray drying is a common technology for manufacturing dry powders, granules and agglomerates from drug-excipient solutions or suspension. It involves atomization followed by drying and deposition of powders in collecting vessel\textsuperscript{121}. The first stage involves supplying a liquid feed dispersed through an atomizer into fine droplets in inert gas in the drying chamber. The large surface area promotes rapid solvent evaporation. Dried particles are passed over to a cyclone compartment for separation and a narrow particle size distribution (1 - 5 μm) is yielded depending on the spray drying parameters. Various methods have been used to prepare microspheres by spray drying. Microspheres made of water-insoluble polymer polylactic acid (PLA) or poly(lactide coglycolide) (PLGA) were used to prepare paclitaxel loaded microspheres\textsuperscript{122} ketotifen loaded microspheres, and for the encapsulation of water-soluble materials such 5-Fluorouracil\textsuperscript{123}. However, water-soluble polymers such as chitosan glutamate and sodium alginate were used to prepare zolmitriptan loaded microspheres\textsuperscript{124} and levocetirizine dihydrochloride loaded microspheres\textsuperscript{125}. Spray drying involves dissolving the polymer in a suitable solvent with the drug dispersed in the polymeric solution. The solvent is then evaporated in the drying chamber to form the dried microspheres.

![Figure 5: General methods used for microspheres preparation](image-url)
1.7.3 Characterization of Microsphere

Particle size and shape
The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM).

Electron spectroscopy for chemical analysis
The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA).

Density determination
The density of the microspheres can be measured by using a multi volume pycnometer.

Isoelectric point
The micro electrophoresis is used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined.

Angle of contact
The angle of contact is measured to determine the wetting property of a micro particulate carrier. In vitro methods
Release studies for different type of microspheres are carried out by using different suitable dissolution media, mostly by rotating paddle apparatus (USP / BP).

Drug entrapment efficiency
Drug entrapment efficiency can be calculated using following equation, %

\[
\text{Entrapment} = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100
\]

Swelling index
The swelling index of the microsphere was calculated by using the formula, Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 100 \text{[126]}.