Chapter 2: Literature review

2. LITERATURE REVIEW

A large number of therapeutic agents are found to be ineffective in the treatment of cerebral diseases due to their inability to effectively and efficiently delivered and sustained within the brain. Therefore, scientists are exploring new novel approaches to encounter this problem so that delivery of the drugs can be restricted to the brain and central nervous system. Despite of enormous research, patients suffering from fatal and/or debilitating central nervous system (CNS) diseases, such as epilepsy, migraine, brain tumors, HIV encephalopathy, cerebrovascular diseases and neurodegenerative disorders far outnumber of those are victimized from several types of depression (Misra et al 2003). The clinical failure of much potentially effective therapeutics is often not due to a lack of drug potency but mainly due to shortcomings in the delivery approach for delivering the drug to treat the diseases. Treating CNS diseases is challenging and a daunting task because a variety of formidable obstacles often impede drug delivery to the brain and spinal cord (Misra et al 2003).

2.1 Central Nervous System

Central nervous system and brain are one of the complex systems in human body. The presence of blood-brain barrier (BBB) is the major bottle neck in delivering drugs to the brain (Brightman 1992, Lo et al 2001) (Figure 2.1). Drugs used against CNS diseases should reach the brain via the blood compartment must pass the BBB. Therefore, the BBB is a predominant rate limiting barrier in brain targeted drug delivery systems. The function of BBB is dynamically regulated by various cells present at the level of BBB (Pardridge et al 1991). The transport mechanisms though BBB and physicochemical properties of the drug molecules are pertinent and must be considered while designing drug delivery system for treatment of the brain or CNS diseases.

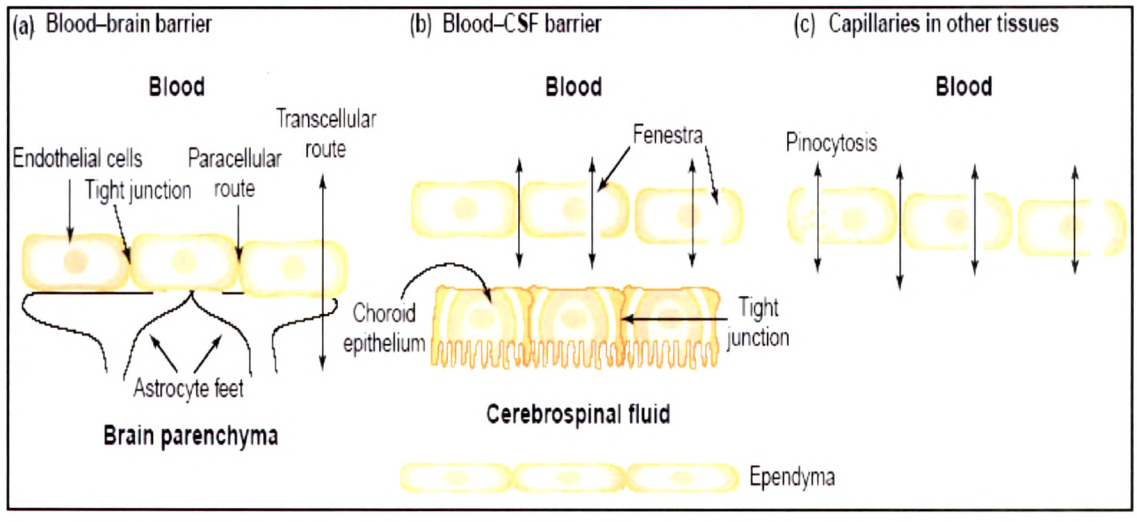
2.2 Barriers to CNS Drug Delivery

A. Blood Brain Barrier:

The BBB is a membranous barrier separates the brain from the surrounding circulating blood (Begley 1996, Schlossauer et al, 2002). Because of different structure of brain capillary compare to other tissues, it provides permeability barrier to most of the penetrants from extra cellular fluid in brain tissue. Micro vessels make up approximately 95% of the total surface area of the blood-brain-barrier (BBB), is the

principal route by which molecules reach the brain. This barrier is very efficient and makes the brain practically inaccessible for lipid- insoluble compounds such as polar molecules and ions. In brain capillaries, the principle route of transport takes place through trans-cellular mechanism only. Therefore, only lipid-soluble solutes can freely penetrates through the capillary endothelial membrane and may cross the BBB passively.

Figure 2.1: Barriers to brain delivery



The capillaries present in brain are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight epithelium, similar in nature to this barrier, is also found in other organs (skin, bladder, colon and lung) (Lo et al 2001). The tight junctures between endothelial cells results in a very high trans-endothelial electric resistance of 1500-2000 Ω cm^2 compared to 3-33 Ω cm^2 of other tissues which reduces the aqueous based paracellular diffusion observed in other tissues (Nabeshima et al 1975, Brightman 1968).

On the other hand, certain classes of drugs like benzodiazepines such as diazepam have been used as sedative-hypnotic agents, because these lipophilic drugs readily cross the BBB. However, the BBB transport of an immunosuppressive agent, cyclosporine A, which is more lipophilic than diazepam, is highly restricted. Similarly, almost all of the lipophilic anticancer agents such as doxorubicin, epipodophylotoxin and Vinca alkaloids (e.g., vincristine and vinblastine) hardly enter

the brain, causing difficulty in the treatment of brain tumors. Although levodopa, which is useful for treatment of Parkinson's disease, is very hydrophilic, it can readily penetrate the BBB. The other problem encountered with BBB is enzymatic degradation. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles. BBB enzymes also recognize and rapidly degrade most peptides, including naturally occurring neuropeptides (Brownless et al 1993, Witt et al 2001).

B. Brain Cerebrospinal Fluid Barrier

The other barrier that a systemically administered drug encounters before entering the CNS is known as the blood-cerebrospinal fluid barrier (BCB). The choroid plexus and the arachnoid membrane act as a barrier between the blood and CSF. Brain is covered by double layered structure called arachnoid membrane. Passage of substances from the blood through the arachnoid membrane is prevented by tight junctions (Nabesimha et al 1975). The arachnoid membrane is generally impermeable to hydrophilic substances (Brightman 1968, Saito 1983).

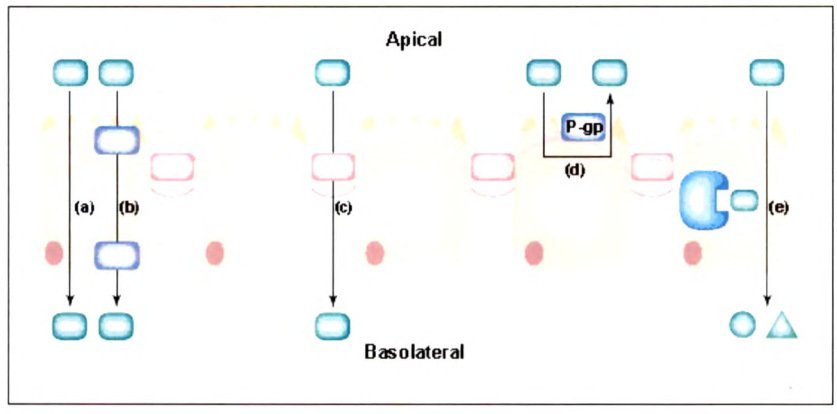
C. Brain Tumor Barrier

Intracranial drug delivery is even more challenging when the target is a CNS tumor. In CNS malignancies where the BBB is significantly compromised, a variety of physiological barriers common to all solid tumors inhibit drug delivery via the cardiovascular system. Drug delivery to neoplastic cells in a solid tumor is compromised by a heterogeneous distribution of microvasculature throughout the tumor interstitial, which leads to spatially inconsistent drug delivery. Furthermore, as a tumor grows large, the vascular surface area decreases, leading to a reduction in trans-vascular exchange of blood-borne molecules. At the same time, intra-capillary distance increases, leading to a greater diffusional requirement for drug delivery to neoplastic cells and due to high interstitial tumor pressure and the associated peri-tumoral edema leads to increase in hydrostatic pressure in the normal brain parenchyma adjacent to the tumor. As a result, the cerebral microvasculature in these tumor adjacent regions of normal brain may be even less permeable to drugs than normal brain endothelium, leading to exceptionally low extra-tumoral interstitial drug

concentrations. Brain tumors may also disrupt BBB, but these are also local and non homogeneous disruptions.

D. Efflux Transporters

Figure 2.2: Efflux transporters at Blood Brain Barrier



A thorough understanding of the two way transport mechanisms uptake and efflux through BBB is of great importance in targeting drugs to the brains or to minimize the unwanted adverse effects some therapeutically active molecules. The efflux mechanisms in the CNS are passive or active (Figure 2.2). Active efflux from the CNS via specific transporters may often reduce the measured penetration of drug at the BBB to levels that are lower than might be predicted from the physicochemical properties of the drug, for example, its lipid solubility. Recently much attention has been focused on multi-drug transporters; multi-drug resistance protein (MRP), P-glycoprotein (P_{gp}) and the multi-specific organic anion transporter (MOAT), which belong to the members of the ABC cassette (ATP-binding cassette) of transport protein (Cole et al 1992, Taylor 2002).

2.3 Strategies for enhanced CNS drug delivery

1 **Lipophilic analogs:** CNS penetration is favored by low molecular weight, lack of ionization at physiological pH and lipohilicity. Octanol/water partition coefficient, log $P_{o/w}$ is very commonly acceptable and convenient approach to predict lipophilicity of any system (Buchwald et al 2002). However, log $P_{o/w}$ alone seems to have a very limited application in predicting brain/blood concentration ratios but in order to reach near to success it is essential that

combinations with other parameters like capillary membrane permeability first pass metabolism and volume distribution (Van de Waterbeemd et al 2001, Lewis et al 2002, Lin et al 1997).

2 **Prodrugs:** Prodrugs are pharmacologically inactive compound that result from transient chemical modifications of biologically active species. After administration, the prodrug, by virtue of its improved characteristics, is brought closer to the receptor site and is maintained there for longer period of time. Here it gets converted to the active form usually via a single activation step.

3 **Receptor mediated transport:** The receptor transport is mainly based on the formation of chimeric peptides by conjugation of the drugs that has to be delivered to a transport vector that undergoes BBB-transport via receptor or via absorptive-mediated transcytosis (Pardridge et al 2002). This approach is intended to provide brain delivery of large peptides. Since this approach involves stoichiometry, only limited number of molecules fit in to this category (Misra et al 2003).

4. **Chemical drug delivery:** They are inactive chemical derivative of a drug obtained by one or more chemical modification so that the newly attached moiety are monomolecular units and provide a site specific delivery of drug through multistep enzymatic transformation (Misra et al 2003).

5. **BBB disruption:** One of the approaches to circumvent the dense microvasculature of the brain is by delivery using a transient osmotic opening. Hyperosmolar substance like mannitol, arabinose is likely to cause disruption of BBB due to migration of water from endothelial cells to capillaries, which in turn cause shrinkage of the cells and results in intracellular gaps (Miller et al 2002). The approach was resulted and breaks down the self defense mechanism of the brain and leaves it vulnerable. The other approaches are BBB disruption using use of labradimil which has selectivity for bradykinn B_2 receptor and Ultrasound induced mild hyperthermia which can be controlled and localized to a small volume within the tissue. The former approach may

lead to membrane permeability due to hyperthermia and the later one is under consideration and at a considerable distance from practical application (Chow et al 2002).

6. **Nanoparticles:** Nanoparticles has been employed as a delivery system for compounds like dalargin, kyotorphin, loperamide and doxorubicin in some animals (Kreuter et al 2001, Kreuter et al 2002). The probable mechanism could be endocytic uptake or transcytosis. The particles are usually 10 to 100 mm diameter, made from natural or artificial polymers; drugs are bound in form of solid solution or dispersion.
7. **Cell penetrating peptides:** Recently this approach has been employed by scientists and several peptides like Tat derived peptides, Transportan, Penetratin etc. have been found to translocate across the plasma membrane of eukaryotic cells, but even can be used for intracellular, and may be even transcellular, transport of large cargo macromolecules. For example, Tat fragments that are part of the cell-membrane transduction domain of the human immunodeficiency virus (HIV) have been shown in animal studies to provide enhanced brain delivery (Schwarze et al 1999, Rouselle et al 2000, Aarts et al 2002).

8. **Intracerebral delivery:** BBB can be successfully bypassed using the most direct and invasive approach like intracerebral delivery of broad class of drugs using traditional and novel drug delivery system based dosage forms like injectables controlled release polymers (Langer et al 1991, Brem et al 2000) / microspheres (Benoit et al 2000, Illum et al 1988, Blork et al 1990, Edman et al 1992) or eventually microencapsulated recombinant cells. The basic impediment is very limited and slow diffusion within the brain due to very compact, tightly packed brain cells having limited interstitial space and unusually tortuous pathways.

9. **Intracerebroventricular delivery:** Cerebrospinal fluid is in direct communication with the interstitial fluid of the brain, to the major extent alternative invasive strategy to bypass BBB is to deliver drugs directly into cerebral ventricles. The drug penetration is hindered by slow diffusion

especially with the human brain is one of the serious drawback. Moreover, rapid ventricular CSF clearance renders the delivery system equivalent to slow intravenous infusion (Misra *et al* 2003).

10. **Intranasal delivery:** Intranasal delivery is being gaining a remarkable importance for CNS targeting. Nasal mucosa is having connection with CNS through intraneuronal or extraneuronal pathways.

    i) Intraneuronal - It involves internalization into primary neurons of the olfactory epithelium, followed by distribution into other CNS areas.

    ii) Extraneuronal - It involves absorption across the nasal epithelium to submucosa,
    followed by direct access to CSF or extra cellular transport within perineuronal channels
    into CNS.

2.4 Intranasal delivery for brain targeting

Many drugs are not being effectively and efficiently delivered using conventional drug delivery approach to brain or central nervous system (CNS) due to its complexity. Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation. Realization of nose-to-brain transport and the therapeutic viability of this route can be traced from the ancient times and has been investigated for rapid and effective transport in the last two decades. Various models have been designed and studied by scientists to establish the qualitative and quantitative transport through nasal mucosa to brain. The development of nasal drug products for brain targeting is still faced with enormous challenges. A better understanding in terms of properties of the drug candidate, nose-to-brain transport mechanism, and transport to and within the brain is of utmost importance.

For a drug to act centrally when delivered via the intranasal route must traverse effectively and rapidly across the nasal mucosa and from a kinetic point of view the nose is a complex organ with drug deposition, clearance and absorption occurring concurrently (Balmer et al 2005). Hence, to understand the mechanisms and pathways of drug transport to the brain following intranasal administration, an understanding of the anatomy and physiology of nasal cavity is crucial.

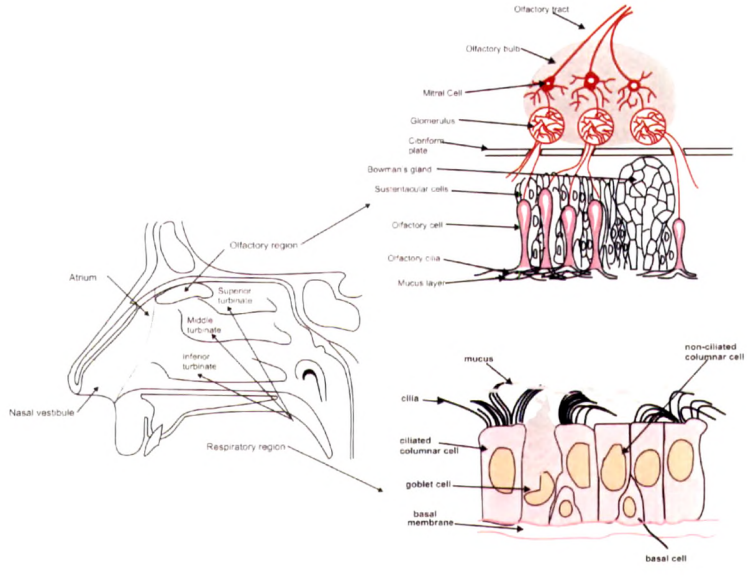
2.4.1 The nose

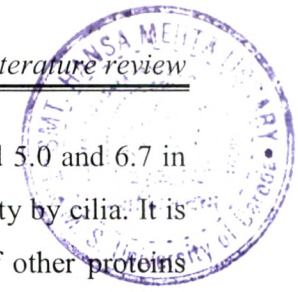
The detailed anatomy and physiology of the human nose is described in various textbooks. However, this segment discusses the important anatomical and physiological features of the nasal cavity from the intranasal drug delivery point of view (Jones 2001, Pires et al 2009). Our nose accomplishes two main functions, the olfaction i.e. the sense of smell and the filtration, heating and humidification of the inhaled air before reaching the lower airways. The nasal septum divides the human nose into two symmetrical nasal cavities lined with 2-4 mm mucosa and each having a surface area of approximately 75 cm^2 and a volume of around 7.5 mL (Talegaonkar et al 2004, Misra et al 2005, Pires et al 2009).

A solute can be deposited at one or more of the three anatomically distinct regions namely the vestibular, the respiratory or the olfactory, in each of the nostrils, following intranasal administration (Figure 2.3). The vestibular region is located at the anterior of each nasal cavity having a total area of around 0.6 cm^2 and contains nasal hairs (or vibrissae) for filtering out the air borne particles. The vestibular region contains the "nasal valve", the narrowest fragment in the respiratory tract accounting for 80% of the nasal resistance and 50% of the total airway resistances. It is composed of transitional squamous epithelium devoid of ciliated cells primarily responsible for the filtration of the inhaled air and is of minimal interest with respect to the intranasal drug absorption (Misra et al 2005, Pires et al 2009). The respiratory region is the largest having a total surface area of about 130 cm^2 with highest degree of vascularity and is mainly responsible for systemic drug absorption across nasal mucosa. It includes three folded structures namely the superior turbinate, the middle turbinate and the inferior turbinate projecting from lateral wall of each of the nasal cavity and are lined with respiratory epithelium composed of ciliated and non-ciliated columnar cells, globet cells and basal cells (Figure 2.3). Both ciliated and non-ciliated columnar cells have numerous microvilli (about 300-400 per cell) increasing the total surface area of respiratory epithelium available for drug absorption (Arora et al 2002). About 5-15% of the mucosal cells in the turbinates are goblet cells which secrete mucin forming a mucus layer on the nasal epithelium which is renewed every 15-20 minutes. Basal cells are located adjacent to the basal lamina on the basolateral side of the epithelium with lamina propria located beneath the blood vessel, nerve, and gland rich basal lamina. The respiratory mucosa also is rich in dentritic cells important for the

local immune response (IgA) (Brandtzaeg 2003). The olfactory region is located posteriorly on the roof of each nasal cavity just beneath the cribriform plate of the ethmoid bone which separates the two nasal cavities from the brain. In humans the olfactory region is of about 10 cm^2, just 10% of the total nasal area of 150cm^2 as opposed to about 50% in rodents (Misra et al 2005, Pires et al 2009). The olfactory tissue is often yellow in contrast to the surrounding pink tissue and plays an important role in the transportation of drugs to the CNS. The olfactory epithelial layer predominantly contains three types of cell the olfactory neural cells, the supporting cells (or sustentacular cells) and the basal cells. Basal cells are the progenitor cells that also provide mechanical support by adhering to the other cells. The olfactory neural cells or the axons are un-myelinated and interspaced between the supporting cells (Figure 2.3). They originate at the olfactory bulb in the CNS and terminate at the apical surface of the olfactory epithelium. The lamina propria consituting the olfactory epithelium is located beneath the epithelial layers and contains the blood vessels, mucus secreting acinar glands (Bowman's glands), nasal lymphatics, and a neuronal supply consisting of olfactory axon bundles, autonomic nerve fibers and the maxillary branch of the trigeminal nerve (Misra et al 2005, Pires et al 2009). These nerves are responsible for the transport of drugs from the nasal cavity to the CNS along the neural pathway.

Figure 2.3: Anatomy and physiology of human nose





The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 and 6.7 in children, which entraps the particles and is cleared from the nasal cavity by cilia. It is composed of approximately 95% water, 2% mucin, 1% salts, 1% of other proteins such as albumin, immunoglobulins, lysozymes and lactoferrin, and <1% lipids, and moves through the nose at an approximate rate of 5 to 6 mm/min resulting in particle clearance within the nose every 15 to 20 minutes (Arora et al 2002, Misra et al 2005, Pires et al 2009). In addition, numerous enzymes like cytochrome P450 enzyme isoforms (CYP1A, CYP2A, and CYP2E), carboxylesterases and glutathione S-transferases are found in nasal cavity (Minn et al 2002, Ding et al 2003).

2.4.2 Nasal Drug Delivery - Advantages/Disadvantages

Intranasal drug delivery is noninvasive compared to other routes of administration and offers advantages like rapid drug absorption, quick onset of action, avoidance of first pass metabolism and systemic dilution (Arora et al 2002, Misra et al 2005, Pires et al 2009). It is convenient, patient friendly, and prevents risk of gastrointestinal tract irritation. Moreover, with intranasal delivery self medication is possible and provides bioavailability profiles identical to intravenous administration. Also, intranasal route delivers the drug directly to the brain circumventing the BBB and reducing its distribution to the non-target sites. Thus, by maximizing the therapeutic index and reducing toxicity, intranasal drug delivery may allow drugs to be delivered in low doses resulting in cost effectiveness (Wermeling et al 2002). Moreover, intranasal route can be used to deliver vaccines to the lymphatic tissues inducing an immune response at distant mucosal sites (Isaka et al 2001). Nasal delivery offers minimum degradation of peptide drugs when compared to oral administration. Also, intranasal delivery does not require any modification of the therapeutic agent being delivered. However, nasal drug delivery suffers from limitations like low dose (usually not more than 25 mg per dose) and low administrable volume (25-150 µL per nostril), particularly when compounds have restricted aqueous solubility or stability. Lipophilicity of the drug is also a prerequisite as influences its uptake across the nasal mucosa. With nose to brain drug delivery, olfactory region constitutes only 10 % of the total area available for drug absorption and the concentration achievable in different regions of the brain or spinal cord varies with each agent (Talegaonkar et al 2004). The other disadvantages associated with intranasal drug delivery include active drug degradation by the nasal mucosal enzymes, decreased drug absorption with

increasing molecular weight, the possibility of drug associated mucosal irritation, frequent use may result in mucosal damage (e.g. infection, anosmia) and the large variability in drug deposition caused by local nasal infections, such as common cold (Arora et al 2002, Talegaonkar et al 2004).

2.4.3 Mechanisms of nasal drug absorption

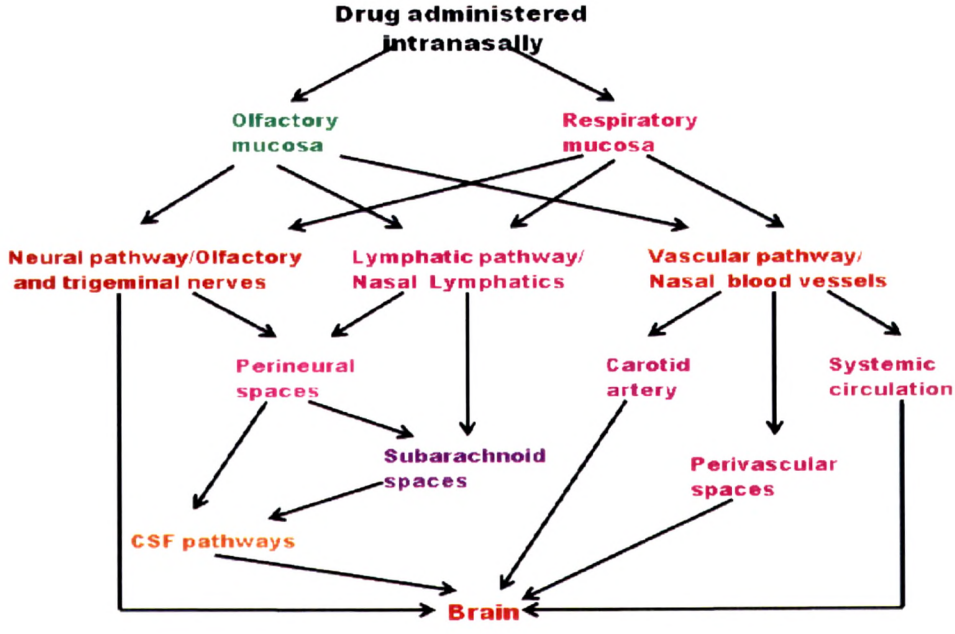
Several mechanisms have been proposed to demonstrate the transnasal absorption of drugs into the brain. However, the paracellular and the transcellular mechanisms are considered predominantly. The paracellular/extracellular mechanism is a passive and slow aqueous route of transport through the intercellular tight junctions or the open clefts of the epithelial cells of the nasal mucosa. In nose to brain transport of drug the paracellular mechanism involves two extracellular routes first across the olfactory neurons and the second across the trigeminal nerve (Jones et al 1997, Ying 2008). After reaching the olfactory bulb or the trigeminal region the therapeutics may enter other brain regions by simple diffusion, facilitated by an arterial pulsation driven perivascular pump. Paracellular mechanism demonstrates an inverse log-log correlation between intranasal absorption and the molecular weight of polar compounds. Poor bioavailability has been observed for drugs having molecular weight greater than 1000 Daltons irrespective of their lipophilicity. Agents like chitosan have also been tried to manipulate the tight junctions between the nasal epithelial cells to facilitate transnasal drug absorption (Gavini et al 2005).

The second transcellular/intracellular mechanism entails transport through a lipoidal route by either receptor mediated endocytosis or passive diffusion or fluid phase endocytosis (Illum 2002, Talegaonkar et al 2004). This mechanism accounts for the transnasal absorption of both small and large lipophilic molecules. Hence, transcellular drug uptake is mainly a function of the lipophilic nature of the drug compound with highly lipophilic drugs being expected to have rapid/complete transnasal uptake. However, the transcellular mechanism is a slow process taking hours for nasally administered drugs to reach the olfactory bulb via the intercellular axonal transport by processes like endocytosis within the olfactory neurons (Talegaonkar et al 2004, Misra et al 2005, Dhuria et al 2010).

2.4.4 Intranasal pathways of drug transport to the brain

Various pathways have been reported to justify nose to brain drug delivery (Figure 2.4). However, a combination of these pathways is responsible for the delivery of therapeutics to the brain following intranasal administration, although one pathway may predominate, depending on the properties of the therapeutic, the formulation, and the delivery device used (Dhuria et al 2010).

Figure 2.4: Pathways of nose to brain drug delivery



A. *Neural pathways*

The neural connections which the olfactory and the trigeminal nerves provide between the nasal mucosa and the brain offer a unique pathway for the nose to brain delivery of therapeutics (Talegaonkar et al 2004). This neural pathway may involve either an intraneuronal/transcellular or extraneuronal/paracellular route or both for drug delivery to the brain. The intraneuronal pathway is slow and involves axonal transport of drugs to the different brain regions. While the extraneuronal pathway delivers drugs directly to the brain within minutes (Misra et al 2005, Dhuria et al 2010).

Olfactory neural pathways originate in the olfactory region at the roof of nasal cavity, with the olfactory neurons being scattered among the supporting cells (sustentacular cells), the microvillar cells, and the basal cells. Olfactory nerve pathways have been

demonstrated by several researchers evidenced by a high concentration of fluorescent tracers in the olfactory bulbs following nasal administrations (Jansson et al 2002). The dendrites of olfactory neurons extend into the mucous layer of the olfactory epithelium, while axons of these bipolar neurons extend centrally through the lamina propria and through perforations in the cribriform plate of the ethmoid bone. The axons of olfactory neurons pass through the subarachnoid space containing CSF and terminate on mitral cells in the olfactory bulbs. From the olfactory bulb, neural projections extend to multiple brain regions including the olfactory tract, anterior olfactory nucleus, piriform cortex, amygdala, and hypothalamus. While, the trigeminal nerve innervates the respiratory and olfactory epithelium of the nasal passages and enters the brain in the pons (Clerico et al 2003). A segment of trigeminal nerve also terminates in the olfactory bulbs (Dhuria et al 2010). It conveys sensory information from the nasal cavity, the oral cavity, the eyelids, and the cornea, to the CNS via the ophthalmic, the maxillary, or the mandibular division of the trigeminal nerve. Branches from the ophthalmic division of the trigeminal nerve provide innervation to the dorsal nasal mucosa and the anterior nose, while branches of the maxillary division provide innervations to the lateral walls (the turbinates) of the nasal mucosa. Nose to brain drug delivery along the trigeminal pathways was first clearly demonstrated for 125I-IGF-I, where high levels of radioactivity were observed in the trigeminal nerve branches, trigeminal ganglion, pons, and olfactory bulb (Thorne et al 2004). Since, one portion of the trigeminal neural pathway enters the brain through the cribriform plate alongside the olfactory pathway, it is difficult to distinguish whether an intranasally administered drug reaches the olfactory bulb and other brain areas via the olfactory or the trigeminal pathway or both.

B. *Vascular pathways*

The therapeutics can also be transported transnasally to the brain through the blood vessels supplying the nasal cavity, and from systemic circulation following nasal administration. Initially, the intranasal route was utilized to deliver drugs to the systemic circulation through absorption into the capillary blood vessels underlying the nasal mucosa. The nasal mucosa is highly vascularised receiving blood supply from branches of both the internal and external carotid artery, including branches of the facial artery and maxillary artery.(Clerico et al 2003). The olfactory mucosa receives blood from the anterior and posterior ethmoidal artery (small branches of the

ophthalmic artery), whereas the respiratory mucosa receives blood from the sphenopalatine artery (a branch of the maxillary artery). The relative density of blood vessels is greater in the respiratory mucosa than the olfactory mucosa, making the former an ideal region for absorption of drugs into the systemic circulation. The respiratory region is a combination of continuous and fenestrated endothelium, allowing the egress of both small and large molecules into the blood and subsequent transport across the BBB to the brain. This is especially true for small lipophilic drugs which more easily enter the blood stream and cross the BBB compared to large hydrophilic therapeutics such as peptides and proteins. It is also possible that the therapeutics rather than being distributed throughout the systemic circulation, enter the venous blood supply in the nasal passages to be rapidly transferred to the carotid arterial blood supplying the brain and spinal cord, a process known as counter-current transfer (Dhuria et al 2010).

The drugs can also enter the brain by bulk flow through the perivascular spaces in the nasal passages or after reaching the brain parenchyma, to be distributed throughout the brain. Perivascular spaces act as a lymphatic system for the brain, where neuron derived substances are cleared from brain interstitial fluid by entering perivascular channels associated with cerebral blood vessels. Increasing number of evidences suggest this pathway involving perivascular channels associated with blood vessels, as a potential nose to brain drug transport mechanism (Zhang et al 1992). Perivascular transport is a bulk flow mechanism rather than diffusion alone and the arterial pulsations are also a driving force for the perivascular transport. Several intranasal studies demonstrate high levels of therapeutics in the walls of cerebral blood vessels and carotid arteries, even after removal of blood by saline perfusion, suggesting that intranasally delivered drugs gain access to the perivascular spaces (Thorne et al 2004).

C. CSF pathways

Pathways that connect the subarachnoid space containing CSF, perineural spaces encompassing olfactory nerves, and the nasal lymphatics, important for CSF circulation and drainage, provide access for intranasally administered therapeutics to the CSF and other areas of the brain. The CSF is produced by secretion at the four choroid plexi, especially at the fourth and lateral ventricles. CSF is a secretory fluid

produced by the choroid plexi to cushion the brain (Illum 2000). Numerous studies report that tracers injected into the CSF in the cerebral ventricles or subarachnoid space drain to the underside of the olfactory bulbs into channels associated with olfactory nerves traversing the cribriform plate, and reach the nasal lymphatic system and cervical lymph nodes (Kida et al 1993). Thus, CSF flows along the olfactory axon bundles between the cribriform plate of the skull and the olfactory submucosa in the roof of the nasal cavity. Intranasally administered drugs can take these same pathways moving from the nasal passages to the CSF into the brain interstitial spaces and perivascular spaces for distribution throughout the brain. Several intranasal studies demonstrate that drugs gain direct access to the CSF from the nasal cavity, followed by subsequent distribution to the brain and spinal cord, with the transport being dependent on the lipophilicity, molecular weight, and degree of ionization of drug molecules (Dhanda et al 2005).

D. *Lymphatic pathways*

For several years the principle of CSF production via the choroid plexus and its absorption via the arachnoid villi to the cerebral venous sinuses had remained widely accepted. However in the last two decades, there have been few reports describing the presence of a functional and anatomical connection between the extracranial lymphatics (nasal submucosal and cervical lymphatics) and the subarachnoid space via the perineural spaces and the cribriform plate (Johnston et al 2005). The nasal submucosal layer consists of a dense vascular network that leads to systemic circulation, and a dense network of lymphatics, that communicates directly with the subarachnoid space. The nasal submucosal lymphatics lead directly to the subarachnoid space via a perineural route to the cribriform plate. Nasal lymphatics offer a direct shortcut to the subarachnoid space and have been proposed as a potential pathway for the invasion of several pathogens such as S. pneumoniae, N. meningitis or H. influenzae responsible for bacterial meningitis (Filippidis et al 2009).

2.4.5 Factors affecting transnasal drug absorption

Due to the peculiar anatomy and physiology of the nasal cavity, the deposition of drug and deposition area is mainly dependant on the delivery system and the delivery device (Vidgren et al 1998). The selection of the delivery system depends on the drug used, therapeutic indication, patient population, and the marketing preferences. Thus,

a variety of factors influence the transnasal absorption of drugs. However, all these factors are correlated with each other with biological factors dependent on the formulation related factors, while the formulation related factors being dependent on the drug related factors. Some of these factors are discussed in this section and should be taken into account while studying and designing a new nasal formulation.

A. *Biological factors*

Various strategies have been tried till date to manipulate the nasal structural features so as to increase drug absorption transnasally. However, these are not suitable during chronic applications as can result in undesirable adverse effects due to alterations in the normal physiology of the nasal cavity.

1. Structural characteristics

The nose can be anatomically divided into nasal vestibule, atrium/septum, respiratory area, olfactory region and the nasopharnyx from drug delivery point of view (figure2.3). The nasal septum divides the nasal cavity along the centre into two halves each dominated by the three tubinates: inferior, middle and superior turbinate (Arora et al 2002). The vestibular region comprising the keratinized stratified squamous epithelium is the least important from nasal drug delivery point of view. The respiratory area consists of the basal cells, goblet cells and the ciliated and non-ciliated columnar cells having 300-400 microvilli per cell. This is the largest with highest degree of vascularity responsible for systemic drug absorption. The olfactory region comprising the olfactory neural cells, the supporting cells and the basal cells responsible for CNS drug absorption across the nasal mucosa. Thus the type of cells, density and number of cells present in the specific nasal regions influence the drug absorption following intranasal administration. Various authors report the use of absorption enhancers in combination with drugs to increase the permeation of these compounds by one or more mechanisms such as increasing the membrane fluidity, decreasing the viscosity of the nasal mucus, inhibiting the proteolytic or other mucosal enzymes, disrupting the tight junctions, increasing the paracellular or transcellular transport, etc or a combination there of. Apart from this, mucoadhesive dosage forms have also been reported to increase the permeation of compounds administered intranasally.

2. Enzymatic degradation or biochemical features

Drugs administered nasally though avoid extensive metabolism in the gastrointestinal tract and first-pass metabolism in the liver may be susceptible to the enzymes of the nasal mucosa presenting a significant barrier to the systemic drug absorption. These include oxidative and conjugative enzymes (e.g., glucuronyl transferase and glutathione transferase), cytochrome P450, carboxy esterase, aldehyde dehydrogenase, carbonic anhydrase, exopeptidases and endopeptidases (e.g., aminopeptidase, carboxypeptidase, trypsin like activities, and cathepsins), etc (Arora et al 2002). These enzymes degrade the drugs within the nasal mucosa causing a pseudo-first-pass effect impeding the nasal drug absorption. Nasal proteases and peptidases have been implicated in a poor absorption of peptidic drugs, such as calcitonin, insulin, LHRH and desmopressin. However, the nasal route is still being considered superior to the oral delivery of these proteinaceous drugs. Similarly, nasally administered decongestants, alcohols, nicotine and cocaine have been reported to be metabolised by the nasal P450-dependent monoxygenase. Various approaches such as the use of protease and peptidase inhibitors like bacitracin, amastatin, boroleucin and puromycin, have been reported to improve the nasal absorption of LHRH peptides, leucin–enkephalin and human growth hormones. Prodrugs, have also been reported to increase the nasal stability and permeation of compounds, like esters of steroids (e.g. beclomethasone dipropionate monohydrate), cromoglycic acid (charged prodrug), and some peptides and amino acids (e.g. desmopressin acetate and L-tyrosine).

3. Blood supply and neuronal regulation

The nasal cavity is highly vascularized due to the presence of venous sinusoids and arteriovenous anastomosis important for the heating and humidification of the inspired air and nasal resistance. Nasal cycles of congestion (increased blood supply) on parasympathetic stimulation and relaxation (decreased blood supply) on sympathetic stimulation regulate the amount of drug absorbed, respectively (Arora et al 2002). Based on a study in dogs (anaesthetized with pentobarbitone) electrical stimulation of the parasympathetic nerves innervating the nasal mucosa resulted in increased drug permeation due to an increase in nasal blood flow and nasal secretion.

4. Site and area of nasal cavity exposed

Deposition of the nasal formulation in the anterior of the nose provides a longer nasal residence time and is an area of low permeability. While, the posterior portion of the nose provides a shorter residence time for the nasal formulation but demonstrates high transmucosal drug absorption. Also, the method of administration and the properties of the formulation eventually determine the deposition site (Arora et al 2002). In a study on 40 mg progesterone ointment higher bioavailability was observed when ointment was applied to both the nostrils than when applied to only one nostril demonstrating that the amount of drug absorbed increases as the area of mucus membrane exposed increases. However, from nose to brain drug delivery point of view, greater the drug is deposited at the olfactory region the more it is absorbed into the brain.

5. Transporters and efflux systems

An active research area in the field of intranasal drug delivery is the study of the various transporter systems present in the nasal tissue and their effects on the absorption of drugs into systemic circulation and/or brain. Presently, multidrug resistance transporters have been identified in the human nasal respiratory and olfactory mucosa, which may be influence the transport of a wide variety of hydrophobic and amphiphilic drugs transnasally. P-gp, an ATP dependent efflux transporter exists in the apical area of ciliated epithelial cells and in the submucosal vessels of the human olfactory region (Graff et al 2005). Several studies demonstrate that Pgp plays an important role in preventing active influx of drugs from the nasal mucosa systemic circulation and/or brain.

6. Nasal secretions

The various mucosal and submucosal glands secrete nasal mucus that forms a continuous layer of 5 μm on the nasal mucosa. Approximately 1.5–2 l ml of mucus is produced daily and exists as a double layer consisting of a watery hypophase, adjacent to the epithelial surface, in which the cilia beat and a more viscous gel like epiphase which is moved forward by the beating cilia. Both the composition and the viscosity of nasal secretions affect intranasal drug absorption with mucus composition affecting the drug solubility while viscosity altering the time of contact of the drug with the nasal mucosa. The nasal secretions are composed of 90% water, 2% mucin, 1% salts,

1% proteins (mainly albumin, immunoglobulins, lysozyme, lactoferrin, etc.) and lipids (Arora et al 2002). Thus, a drug should have appropriate physicochemical properties for dissolution in nasal secretions so as to permeate across nasal mucosa. The use of water-soluble analogues of investigational drugs via the nasal route has shown to increase drug absorption. It has been reported that alteration in the viscosity of either the hypophase or the epiphase of nasal mucus affects ciliary beating influencing the time of contact of drug with the nasal mucosa, and hence, its absorption.

7. Nasal cycle

Various studies revealed that the circardian rhythms affect both the frequency of nasal cycle and the secretion and clearance rates of nasal mucus. The frequency of nasal cycle has been reported to be high in the daytime compared with that in the early morning and at night. Similarly, various studies reveal that the production and clearance rates of the nasal secretions are reduced at night and thus affect the nasal drug absorption (Arora P *et al* 2002).

8. pH of the nasal secretions

The pH of the nasal secretions varies between 5.5-6.5 in adults and 5.0–7.0 in infants. A drug will be absorbed better when the nasal mucus pH is lower than the drug's pKa as the drug will be present predominantly in an unionized form (Arora et al 2002). Thus, a change in the pH of nasal secretions can affect the drug ionization altering the amount of drug absorbed transnasally. Since, the pH of the nasal mucus can alter the pH of the formulation and vice-versa, the pH of a formulation should ideally be 4.5 to 6.5 with adequate buffering capacity.

9. Mucociliary clearance

The nasal mucociliary clearance is an important clearance mechanism to remove foreign particles such as dust, allergens and bacteria, trapped on the mucus blanket during inhalation. The drug absorption is influenced by the contact time between the drug and the nasal mucosa. The mucociliary clearance is inversely related to the residence (contact) time and thus, inversely proportional to the absorption of drugs administered (Arora et al 2002). Various strategies have been used to prolong the residence time of the drug in the nasal cavity such as using bioadhesive polymers like

chitosan or polycarbophils or increasing the viscosity of the formulation. Nasal mucociliary clearance is also stimulated or inhibited by drugs, excipients, preservatives and / or absorption enhancers and thus affects the nasal drug absorption.

10. Pathological conditions

Local nasal infections such as the common cold, rhinitis and nasal polyposis are usually associated with mucociliary dysfunctioning, hyper or hypo secretions, and nasal mucosal irritation, which can influence transnasal drug absorption (Arora et al 2002). Many drugs have been screened and classified as cilio-friendly or cilio-inhibitory offering a valuable tool in the design of safe nasal drugs.

11. Environmental factors

Temperatures nearby 24°C cause a moderate reduction in the rate of mucociliary clearance. However, linear increase in ciliary beat frequency occurs with increase in temperature (Arora et al 2002).

B. Formulation related factors

A nasal formulation usually consists of the drug, a vehicle, and the excipients. The physicochemical properties of the drug as well as the formulation are imperative from the formulation design point of view.

1. Physicochemical properties of Drugs

The rate and extent of drug absorption post intranasal administration depends on the following physicochemical characteristics of the drug.

a. Lipophilicity

A linear correlation has been reported between lipophilicity and drug permeation using several compounds transnasally. Although the nasal mucosa possesses some hydrophilic character, it is primarily lipophilic in nature with lipophilic agents like alprenolol and propranolol absorbed well across nasal mucosa in contrast to the hydrophilic molecule metoprolol. Lipophilic compounds readily cross the nasal mucosa via the transcellular route by partitioning into the lipid (bilayer) of the cell membrane and diffusing into the cytoplasm (Arora et al 2002). A number of lipophilic drugs such as progesterone, naloxone, buprenorphine, barbiturates, testosterone and

17α-ethinyloestradiol, have been reported to be almost completely absorbed transnasally in animal models.

b. Chemical form

The chemical form of a drug is important in determining its permeation across mucosal surfaces. Huang et al studied the effect of structural modification of L-Tyrosine on its transnasal absorption with L-Tyrosine carboxylic acid ester having significantly greater absorption than L-Tyrosine (Huang et al 1985). Thus, chemical modification of the drug into a salt or an ester form can alter its absorption.

c. Polymorphism

Polymorphism is known to affect the solubility and the dissolution rate of drugs and thus, their absorption across biological membranes (Doelker 2002). It is therefore desirable to study the polymorphic stability and purity of drugs especially for nasal powders and/or suspensions.

d. Solubility and dissolution rate

The drug solubility and dissolution rates are pertinent factors in determining transmucosal absorption from nasal powders and suspensions. The particles deposited on the nasal mucosa need to be dissolved prior to be absorbed transnasally. If a drug remains as particles or is cleared then no absorption takes place.

e. Molecular weight

The molecular weight and lipophilicity of a drug act together to determine its permeation transnasally. An inversely proportional correlation has been reported between the transnasal permeation of drugs and their molecular weight up to 300 daltons. However, the absorption decreases significantly if the molecular weight is greater than 1,000 daltons except with the use of absorption enhancers (Arora et al 2002). Lipophilic agents demonstrate a direct relationship between the molecular weight and drug permeation whereas hydrophilic molecules depict an inverse relationship. The permeation of drugs less than 300 daltons is not significantly influenced by their physicochemical properties and mostly permeates by paracellular mechanism. But, the permeation rate is highly sensitive to molecular size for drugs having molecular weight greater than 300 Da.

f. Partition coefficient and pKa

As stated by the pH partition theory, unionized species are absorbed better compared to the ionized species and the same is true with the nasal drug absorption. Jiang et al. have demonstrated a quantitative relationship between the partition coefficient and the nasal absorption constant (Jiang et al 1997). However, for weak acids or bases, the mucosal absorption has been reported to be altered by the pH of the environment. The nasal permeation of molecules like salicylic acid and aminopyrine has been observed to be highly dependent on their degree of ionization. While, the absorption rate increased with the increase in pH for aminopyrine, substantial deviations were observed for salicylic acid. The authors suggested that perhaps a different transport pathway, along with the lipoidal pathway, existed for salicylic acid. For benzoic acid, highest degree of absorption was observed when in unionized form (pH 2.5, 44%), but even at 99.9% ionization (pH 7.19) 13% absorption was observed (Huang et al 1985). Also, the rate of absorption was calculated to be four times faster for the unionized species than for the ionized. Thus, the results of these depict partition coefficient as a major factor governing nasal drug absorption.

g. Shape

Shape is also important. Linear molecules have lesser absorption than cyclic-shaped molecules (McMartin et al 1987).

2. Physicochemical properties of formulation

The following physicochemical properties of the formulation determine the rate and extent of drug absorption following intranasal administration.

a pH and Mucosal Irritancy

Both the pH of the formulation and the nasal cavity affect the permeation of a drug across nasal by altering the degree of drug ionization. The pH of the formulation should be adjusted near to the nasal physiological pH i.e.4.5–6.5 to avoid nasal irritation (Arora et al 2002). This pH range also prevents any bacterial growth as lysozymes in the nasal secretions are active at acidic pH and ensures efficient transmucosal permeation for weakly acidic or basic drugs as drugs are absorbed in the unionized form.

b Buffer capacity

Nasal formulations are generally administered in volumes ranging from 25 to 300μL (Arora et al 2002). Hence, nasal secretions can alter the pH of the administered formulation. This in turn affects the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain its pH in–situ.

c. Osmolarity

Drug absorption can be affected by tonicity of the formulation. Ohwaki et al. studied the effect of formulation osmolarity on the absorption of secretin in rats (Ohwaki et al 1985). Shrinkage of the epithelial cells was been observed in the presence of hypertonic solutions resulting in maximal secretin absorption. The results were further confirmed when sorbitol was used as an osmoregulatory agent. Hypertonic saline solutions cause structural changes in mucosal membranes and are also known to inhibit or decrease ciliary activity. Low pH has a similar effect on cells as hypertonic solutions. Therefore, isotonic solutions are usually preferred for administration.

d. Viscosity

A study by Jansson et al. revealed that increase in the viscosity of the formulation increases the amount of drug absorbed by prolonging the contact time between the drug and the nasal mucosa and thus, the time for drug permeation (Jansson et al 2002). At the same time, viscous formulations interfere with the normal physiological functions like ciliary beating or mucociliary clearance and thus alter the transnasal absoption of drugs. The use of a combination of "generally regarded as safe" (GRAS) carriers is often recommended from a safety (nasal irritancy) point of view.

e. Drug distribution

Drug distribution in the nasal cavity is an important factor that affects the efficiency of nasal absorption. The mode of drug administration and the posture during administartion affect this distribution, which in turn can help determine the extent of absorption of a drug (Arora et al 2002). Nasal deposition of particles is related to the individual's nasal resistance to airflow. With nasal breathing, nearly all the particles having an aerodynamic size of 10-20 mm are deposited on the nasal mucosa.

f. Dosage form

The type of nasal dosage form used also influence the transnasal drug absoption. Nasal drops though are the simplest and the most convenient dosage form cannot deliver an exact volume of formulation resulting in drug overdose (Arora et al 2002). Moreover, postnasal drip and anterior leakage is another problem associated with nasal drops. Solution and suspension sprays are preferred over powder sprays because powders may cause mucosal irritation by drying the mucus membrane. Recently, metered-dose gel devices have been developed that accurately deliver drug formulation. Gels reduce the rapid nasal drainage of the formulation localizing it within the nasal cavity for a longer period of time. A limited amount of work has been reported on the use of ointments and emulsions as nasal formulations. Specialized systems such as microemulsions, microspheres (using chitosan, carbopol 934P and lactose, liposomes, proliposomes, nanoparticles, films and niosomes have also been reported for nasal. These offer a better possibility of drug permeation by providing an intimate and prolonged contact time between the drug and the nasal mucosa. Promising results using these novel delivery systems have been reported for several agents like gentamicin, fluorescein 5′-isothiocyanate (FITC)–dextran and insulin.

g. Formulation excipients

A variety of excipients such as solubilizers, preservatives, antioxidants, etc. are found in nasal formulations. Although they are responsible for several nasal irritations, antioxidants, preservatives, humectants and flavoring or taste masking agents are not expected to alter nasal drug absorption (Arora et al 2002).

I Solubilizers

The aqueous solubility of a drug is often a limitation for nasal solutions. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol® (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol® (saturated polyglycolyzed C_8-C_{10} glyceride) can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as hydroxypropyl–beta-cyclodextrin that serve as biocompatible solubilizers and stabilizers in combination with lipophilic absorption enhancers. In such cases, effect of the solubilizers on nasal irritancy should be evaluated.

II Preservatives

Most nasal formulations are water-based and, therefore, require preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzyl alcohol are some of the commonly used preservatives in nasal formulations.

III Antioxidants

Antioxidants may also be required to improve the stability of drugs prone to oxidation. Commonly used antioxidants include sodium metabisulfite, sodium bisulfite, butylated hydroxytoluene and tocopherol.

IV Humectants

Many allergic and chronic diseases can be connected with crusts and drying of the mucous membranes. Preservatives/antioxidants, among other excipients, are likely to cause nasal irritation especially when used in large quantities. Adequate intranasal moisture is essential for preventing dehydration. Common examples of humectants include glycerin, sorbitol and mannitol.

V Drug Concentration, Required Dose, and Dose Volume

Drug concentration, dose and volume of administration are three interrelated parameters that act together affecting the performance of the nasal delivery system. Nasal absorption of L-tyrosine has been shown to increase with drug concentration in nasal perfusion experiments. While, in another study aminopyrine was found to absorb at a constant rate as a function of concentration (Arora et al 2002). The volume that can be delivered to the nasal cavity is restricted to 25- 150 µl. Different approaches have been explored to use this volume effectively including the use of solubilizers, gelling, or viscofying agents.

VI Role of Absorption Enhancers

The selection of absorption enhancers is based upon their acceptability by regulatory agencies and their impact on nasal physiological function. Absorption enhancers improve drug permeation either by altering the physicochemical properties of the drug such as its solubility, partition coefficient, etc. or by altering the structure of nasal mucosa. Absorption enhancers may be required when a drug exhibits poor membrane

permeability, large molecular size, lack of lipophilicity and susceptibility to enzymatic degradation (Arora et al 2002).

C. Delivery device related factors

Different types of devices are used to deliver formulations intranasally. Both the size and the site and pattern of deposition affect the transnasal permeation of drugs.

1. Size of the droplet or powder

The size of the droplet produced depends on the shape and size of the device used. If the particle size produced is less than 10μm the particles will be deposited in the upper respiratory tract, whereas if the particles are less than 0.5μm they will be exhaled. Particles or droplets with size between 5–7μm will be retained in the nasal cavity and subsequently permeated (Arora et al 2002).

2. Site and pattern of deposition

The site and pattern of drug deposition is affected by the formulation composition, the dosage form (liquid, viscous, semisolid, solid), the delivery device used, the design of actuators and adapters, and the administration technique (Arora et al 2002). The permeability of the deposition site and the area of nasal cavity exposed affect the drug absorption. These factors also determine the retention time of the drug in the nasal cavity.

2.4.6 Intranasal formulations for transnasal drug delivery

Nasal cavity consists of a non-ciliated anterior part and a ciliated posterior region, Hence, the site of deposition is extremely important for mucociliary clearance and in turn residence time which can regulate drug absorption, of the administered formulation. The deposition and deposition area are mainly a function of the delivery system and delivery device. Different dosage forms and their application to deliver the drugs to the central nervous system following intranasal drug delivery are discussed in this section.

A. Liquid Dosage Forms

Liquid dosage forms either in form of soluble, suspended or colloidal systems are normally used for formulating nasal delivery systems.

1 Nasal Drops

Nasal drops are one of the most simple and convenient systems developed for nasal delivery. These can be either solution or suspension formulations. The main disadvantage of this system is the lack of dose precision whereby 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 (Vidgren et al 1998).

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 anywhere from 25 to 200 μL (Newman et al 1988). The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly.

3 Nasal Emulsions and microemulsions, liposomes and nanoparticles

Although intranasal emulsions have not been studied extensively, a large number of data exist to demonstrate the efficacy of intranasal microemulsions (Vyas et al 2006). The large surface area available for absorption with microemulsions depicts an advantage over emulsions. Nasal emulsions and microemulsions offer the advantages for local application mainly due to their viscosity. One of the major disadvantages is poor patient acceptability. The physical stability of emulsion formulations and precise delivery are some of the main formulation issues. Nanoparticles may ensure an improved transnasal drug delivery to the brain since they are able to protect the encapsulated drug from biological and/or chemical degradation, and extracellular transport by P-gp efflux proteins. This eventually increases the brain concentrations of the drug. Their small diameter potentially allows nanoparticles to be transported transcellularly via the various endocytic pathways through the olfactory neurones to the brain. Surface modification of the nanoparticles can also be tried to achieve targeted nose-to-brain drug delivery. These can be applied both in suspension or powder form.

B *Semi-Solid Dosage Forms*

Semi-solid systems include nasal gels, ointments and liquid systems containing temperature or pH sensitive polymers that gel at respective physiological temperature or pH of the nasal cavity. These systems ensure longer residence time within the nasal mucosa due to their semi-solid consistency.

1 Nasal Gels

Nasal gels are thickened solutions or suspensions of drug in a highly viscous polymer base. Until the recent development of precise dosing devices, there was not much interest in this system. The advantages of a nasal gel include longer residence time with in the nasal cavity due to its high viscosity, minimised drug wastage due to reduced post-nasal dripping and anterior leakage, reduction of taste impact due to reduced post-nasal dripping, reduction of irritation by using soothing/emollient excipients, and better drug absorption by offering intimate contact between the drug and the nasal mucosa. Thermoreversible gels using temperature sensitive polymers like poloxamers and pH sensitive gels employing pH sensitive polymers like polycarbophils have also been reported and offer advantage of more accurate dosing over conventional gels.

C *Solid Dosage Forms*

Solid dosage forms though not very popular with intranasal drug delivery are more suitable for pulmonary drug delivery and similar applications. However, these systems pose the problem of mucosal irritation by drying of the nasal mucosa.

1 Nasal Powders

Powder dosage forms may be developed if solution and suspension dosage forms cannot be developed, mainly due to lack of drug stability. The advantages of a nasal powder dosage form are the absence of preservative and superior stability of the drug in 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. An additional advantage of this system is local application of drug, but nasal mucosa irritancy and metered dose delivery are some of the challenges for formulation scientists and device manufacturers who are interested in powder

dosage forms. Apart from plain drug various delivery systems like, microspheres, nanoparticles, liposomes, etc can be formulated as nasal powders.

D *Novel Formulation Approaches for Intranasal Drug Delivery*

In order to formulate a nasal formulation with desirable performance and commercial attributes, the drug properties, delivery system and nasal physiology should all be considered and understood from the early stages of product development. It is advisable to focus on maximizing the residence time and ensuring efficient absorption of drug.

1 Mucoadhesive solutions:

Mucoadhesive solutions comprising mucoadhesive polymers like chitosan, cellulose polymers, polycarbophils, poloxamers, etc. have been reported to enhance drug permeation transnasally (Michael et al 2005). These systems being viscous and mucoadhesive provide longer residence time for better drug absorption. Illum et al have reported an enhancement in the absorption of insulin across the nasal mucosa of rat and sheep using cationic chitosan based nasal solution. Numerous studies have demonstrated that chitosan and their derivatives are effective and safe absorption enhancers to improve mucosa delivery of hydrophilic macromolecules such as peptides and protein drugs. However, these systems suffer from post-nasal dripping and anterior leakage when compared to gels or powder formulations.

2 Microspheres:

Microspheres, including mucoadhesive microspheres, are novel systems that are becoming increasingly popular with nasal drug delivery. Microspheres may provide prolonged contact with the nasal mucosa enhancing the rate and extent of drug absorption (Michael et al 2005). Microspheres or for nasal applications are usually prepared using biocompatible materials, such as starch, albumin, dextran, hyaluronic acid, chitosan and gelatine, hydroxypropyl methylcellulose, carbopol 934P and various combinations of these polymers. These polymers on absorbing nasal secretions form a gel-like layer which is slowly cleared from the nasal cavity. However, the toxicity of the polymer on the nasal mucosa cells should be critically evaluated.

3 Microparticulates

Microparticulates similar to microspheres constitute an efficient dosage form for the in situ gelling nasal drug delivery (Michael et al 2005).

4 Microemulsions

Microemulsions are optically isotropic and thermodynamically stable multicomponent fluids composed of oil, water and surfactant. Of the various dosage forms used intranasally microemulsions offer several advantages like high solubilization of lipophilic drugs, thermodynamic stability, easy to prepare and handle, stabilization of hydrolytically susceptible compounds and provide large surface area for better drug absorption. A mucoadhesive microemulsion, consisting of polymers like carbomers or chitosan, in addition to the advantages of a microemulsion will provide longer residence time in the nasal cavity, depicting rapid and complete absorption of drugs (Vyas et al 2006).

5 Liposomes and proliposomes

Liposomes and proliposomes have been delivered by various routes. Positively charged liposomes possess maximum bioadhesion prolonging the residence time within the nasal cavity thereby improving the bioavailability (Iwanaqa et al 2000).

6 Nanoparticles

Polymeric nanoparticles are efficient carriers for the transnasal absorption of drugs and proteins. Chitosan based nanoparticles can enhance nose-to-brain delivery of drugs compared to equivalent drug solutions formulations due to the protection of the drug from degradation and/or efflux back into the nasal cavity (Dhuria et al 2010). They have also reviewed the various transport pathways and future strategies for delivering drugs across nasal mucosa in the form of nanoparticles.

7 Thermoreversible and pH-sensitive gels

Thermoreversible nasal gels comprised of temperature sensitive polymers like poloxamers and pH-sensitive gels consisting pH sensitive polymers like polycarbophils have been reported to enhance drug permeation transnasally (Vyas et al 2006).

8 Micelles

Micelles are formed by the self-assembly of amphiphilic block copolymers in aqueous solutions and are of great interest with respect to drug delivery applications.The drugs are physically entrapped in the core of the block copolymeric micelles and transported at concentrations that can exceed their intrinsic water- solubility. The hydrophilic blocks also form hydrogen bonds with the aqueous surroundings to form a tight shell around the micellar core protecting the contents of the hydrophobic core effectively from hydrolysis and enzymatic degradation.

9 Nasal inserts

The nasal inserts serve as a novel, new, bioadhesive, solid dosage form for the prolonged systemic drug delivery via the nasal route. The principle involves imbibition of the nasal fluid from the mucosa after administration and to form a gel in the nasal cavity to avoid nasal irritation. The resulting gel adheres to the mucosa due to its bioadhesive properties, acting as release controlling matrix allowing sustained drug delivery. Due to dissolution of the gel and/or mucociliary clearance, there is no need for the removal of the insert mechanically after it is depleted of drug. The in-situ gelling nasal inserts are usually prepared by lyophilisation of aqueous solutions of drug, polymer as carrier and other excipients if required. The sponge-like structure of in-situ gelling nasal inserts is an important parameter to ensure rapid hydration and gelation of the inserts at the nasal mucosa. The drug release from nasal inserts is a complex phenomenon of water penetration, relaxation of the polymer chains, swelling and spreading of the insert, dissolution of the water-soluble polymer and drug, interactions of the drug and carrier, and drug diffusion through the rehydrated insert.

2.5 Nanoparticles for transnasal drug delivery

Nanoparticles are the colloidal particles ranging in size from 1-1000 nm. Polymeric nanoparticles are efficient carriers for the transnasal absorption of drugs and proteins. Illum et al demonstrated that chitosan based nanoparticles can enhance nose-to-brain delivery of drugs compared to equivalent drug solutions formulations due to the protection of the drug from degradation and/or efflux back into the nasal cavity (Mistry et al 2009). They have also reviewed the various transport pathways and future strategies for delivering drugs across nasal mucosa in the form of nanoparticles.

Advantages of nanoparticulate drug delivery systems:

- Nanoparticles deliver drugs to the brain that normally do not cross the blood-brain barrier.
- NPs reduce the peripheral side effects of the drugs that cross the BBB by increasing the relative dose of drugs reaching the brain.
- NPs provide the prolonged release of drugs at the site of action.

2.5.1 Techniques for preparation of nanoparticles

Nanoparticles can be obtained by polymerization of monomers entrapping the drug molecules or from preformed polymers (Quintanar-Guerrero et al 1998).

A. Nanoparticles prepared by polymerization process:

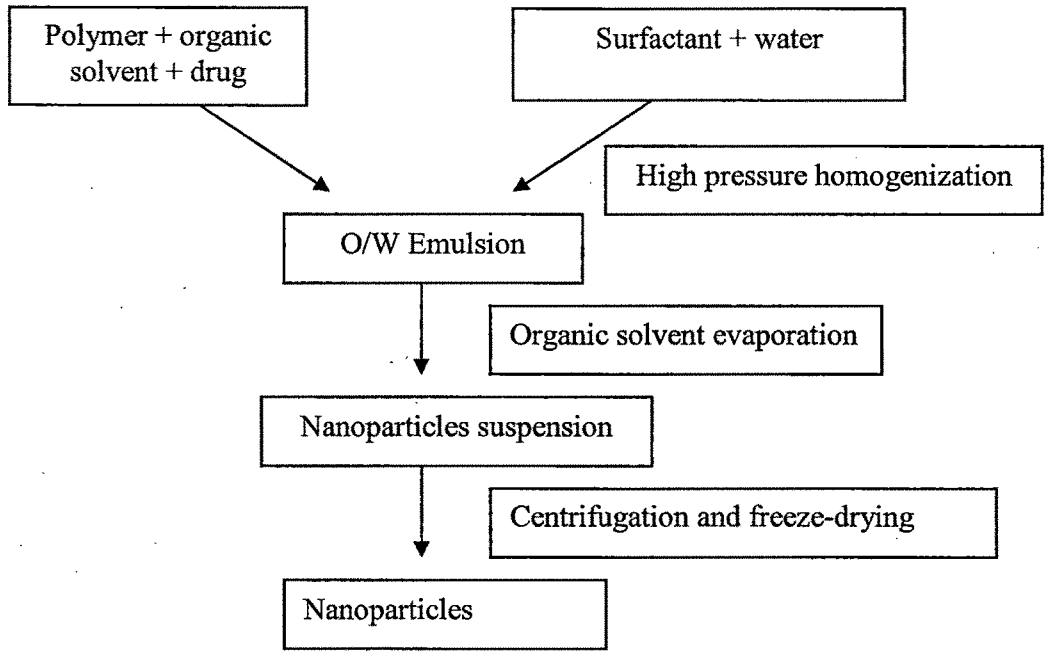
Two types of polymerization processes have been adopted to prepare polymeric nanoparticles:

1. Dispersion polymerization: Dispersion polymerization starts with monomer, an initiator, solvent in which the formed polymer is insoluble, and a polymeric stabilizer. Polymer forms in the continuous phase and precipitates into a new particle phase stabilized by the polymeric stabilizer. Small particles are formed by aggregation of growing polymer chains precipitating from the continuous phase as these chins exceed a critical chain length. Coalescence of these precursor particles with themselves and with their aggregates results in the formation of stable colloidal particles, which occurs when sufficient stabilizer covers the particles.

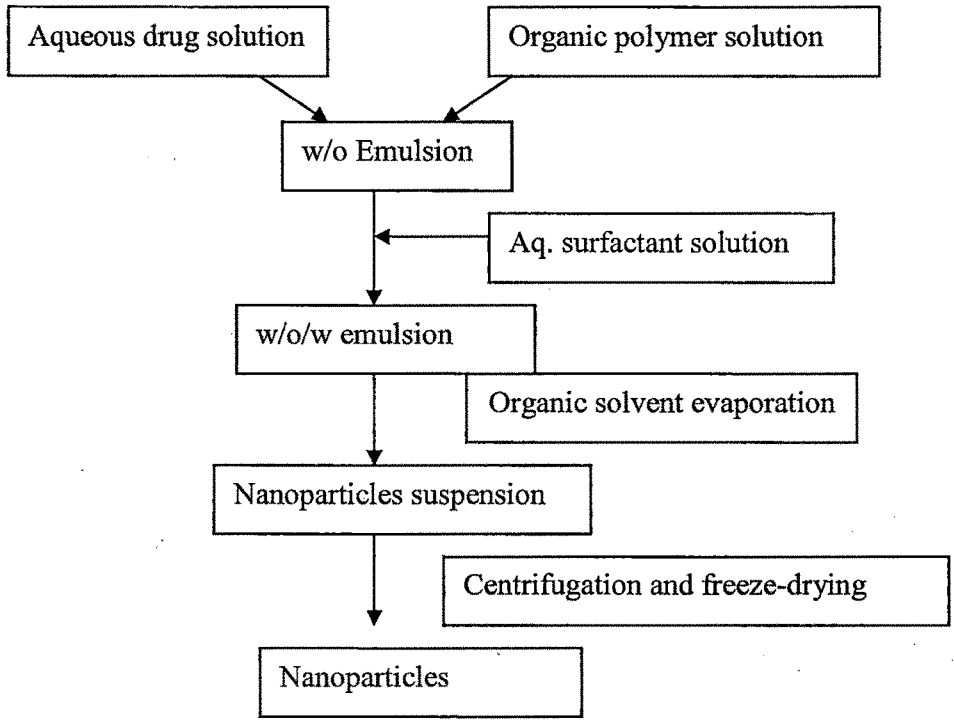
2. Emulsion polymerization: In this technique the monomer is emulsified in non-solvent containing surfactant, which leads to the formation of monomer swollen micelles and stabilized monomer droplets. The polymerization is performed in the presence of initiator. Emulsion polymerization may be performed using either organic or aqueous media as continuous phase. Poly (methyl methacrylate), poly (alkyl cyanoacrylate), acrylic copolymer, polystyrene, poly(vinyl pyridine) and polyacrolen nanoparticles are prepared by emulsion polymerization technique.

B. Nanoparticles prepared from preformed polymers: Several techniques have been suggested to prepare the biodegradable polymeric nanoparticles from preformed polymers such as poly (D,L-lactide) (PLA), poly (D,L-glycolide) (PLG) and poly (D,L-lactide-co-glycolide) (PLGA). The basic methodologies of the commonly used preparation methods are as follows:

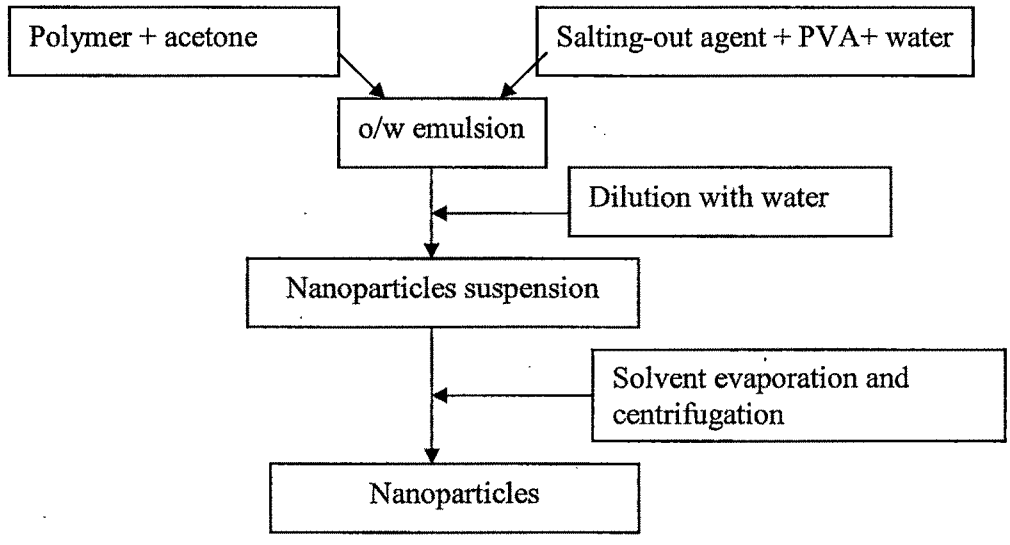
1. Emulsion-evaporation: This is one of the most frequently used methods. The preformed polymer and drug are dissolved in a water-immiscible organic solvent, which is then emulsified in an aqueous solution containing stabilizer. The emulsification is brought about by subsequent exposure to a high energy source such as high pressure homogenizer. The organic phase is evaporated under reduced pressure resulting into formation of nanoparticles, which are then collected by ultracentrifugation, washed and lyophilized for storage.



2. Double-emulsion evaporation: This procedure is used to encapsulate hydrophilic drugs and proteins

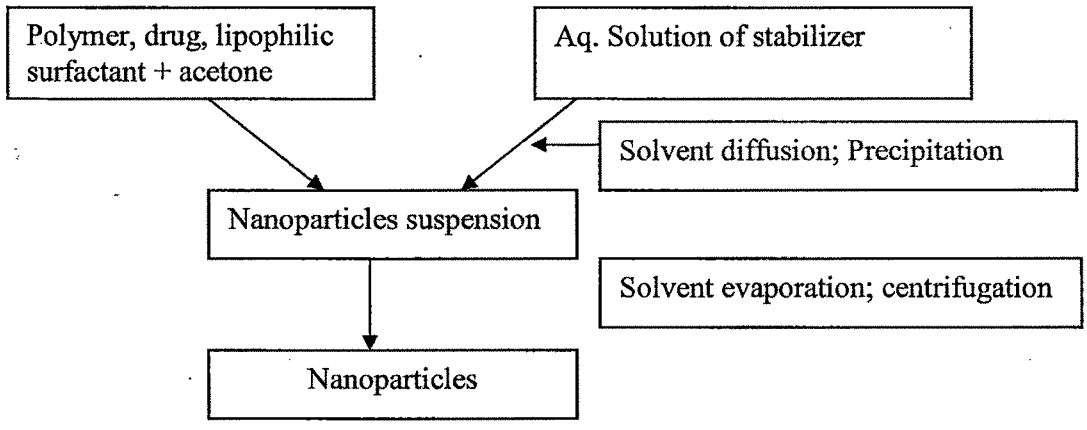


3. Salting-out: This technique involves the addition of polymer and drug solution in a slightly water-miscible solvent such as acetone to an aqueous solution containing the salting out agent and a colloidal stabilizer under vigorous mechanical stirring. When this o/w emulsion is diluted with a sufficient volume of water, it induces the formation of nanoparticles by enhancing the diffusion of acetone into the aqueous phase.



4. Emulsification-diffusion: This method is derived from the salting-out procedure. It involves adding of a polymer solution, in partially water miscible solvent (such as ethyl acetate, benzyl alcohol, propylene carbonate) presaturated with water, to an aqueous solution containing stabilizer under vigorous stirring. The subsequent addition of water to the system destabilizes the equilibrium between the two phases and causes the solvent to diffuse into the external phase, resulting in reduction of the interfacial tension and in nanoparticle formation.

5. Solvent displacement/Nanoprecipitation: This method is usually employed to incorporate lipophilic drugs into the carriers based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution.



Nanoprecipitation technique is simple, easy to execute and provides uniform particle size with narrow particle size distribution. This method is based on the interfacial deposition of a polymer following diffusion of a semi-polar and miscible solvent in the aqueous medium in the presence of a surfactant/stabilizer. The nanoparticle preparation process by nanoprecipitation method, apparently simple, may involve complex interfacial hydrodynamic phenomena.

Stability of Nanoparticles:

Freeze-drying is one of the well established methods for the preservation of unstable molecules over long periods of time. (Corveleyn et al 1996, Diminsky et al 1999, Li et al 2000). Most studies have shown a good preservation of the physicochemical

properties of the particles when the lyoprotectant was employed in a sufficient concentration.

2.5.2 Techniques for characterization of nanoparticles

Characterization of the nanoparticle carrier systems to thoroughly understand the properties is essential before putting them to pharmaceutical application. After preparation, nanoparticles are characterized at two levels. The physicochemical characterization consists of the evaluation of the particle size, size distribution, and surface properties (composition, charge, hydrophobicity) of the nanoparticles. The biopharmaceutical characterization includes measurements of drug encapsulation, in vitro drug release rates, and in vivo studies revealing biodistribution, bioavailability, and efficacy of the drug.

There are many sensitive techniques for characterizing nanoparticles, depending upon the parameter being looked at; laser light scattering (LLS) or photon correlation spectroscopy (PCS) for particle size and size distribution; scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for morphological properties; X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR),a and nuclear magnetic resonance spectroscopy (NMR) for surface chemistry; and differential scanning calorimetry (DSC) for thermal properties (Fig below). Parameters such as density, molecular weight, and crystallinity affect release and degradation properties, where as surface charge, hydrophilicity, and hydrophobicity significantly influence interaction with the biological environment.

2.5.3 Nanoparticles conjugated to ligands for receptor mediated transcytosis

Receptor-mediated transport is responsible for the trafficking of larger molecules such as insulin and transferrin. In this system, a molecule binds a receptor expressed on the endothelial cell. When there is a "match," it is endocytosed into the cell and transported via vesicles to the basolateral membrane, thereby gaining access to the CNS. Receptor-mediated transport has been manipulated to allow anticancer agents to "piggyback" through the BBB with an agent that binds to a target receptor on the BBB endothelium. Although promising, particularly for delivery of large agents such as antibodies and genes, this technique is limited by transport systems that have low

affinity and capacity, such that basal levels of the endogenous substrate may interfere with binding of engineered ligands. Peptides and proteins can undergo transport to the brain via RMT. Examples of receptors involved in RMT are the insulin receptor, the transferrin receptor, and the transporters for low-density lipoprotein, leptin and insulin-like growth factors.

In humans, HIR are highly expressed in organs like kidney, thymus, brain, heart and stomach and to a lesser extend in placenta, liver and skeletal muscles. The brain regions where HIR are expressed are (Hopkins et al 1997, Schulingkamp et al 2000):-

Maximum	choroid plexus,
High	**external plexiform layer of olfactory bulb.**
Moderate to high	structures of the limbic system and hypothalamus particularly the lateral septum, amygdala, subiculum, hippocampus, mammillary body, and arcuate nucleus.
Moderate	regions of cerebral cortex and cerebellum
Moderate to low	brainstem and midbrain structures.
Low	circumventricular organs and thalamus.

The brain regions where rat transferrin receptors (TFR) are expressed are (Hill et al 1985):-

Maximum	cerebral cortex.
High	regions of cerebral cortex and cerebellum
Moderate to high	mesolimbic system, parietal and frontal cortex, amygdaloid body and brain stem.
Moderate	hypothalamus, subthalamus, **olfactory bulb** and cerebellar cortex
Moderate to low	thalamus, occulomotor nucleus, substantia nigra, amygdala and trigeminal nucleus
Low	mamillary body, subfornical organs, and visual cortex

2.6 Microemulsions for transnasal drug delivery

Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multicomponent fluids composed of oil, water and

surfactant (usually in conjunction with a cosurfactant). The droplets in a microemulsion are in the range of 1nm-100nm in diameter. It is well established that dispersed particles having diameter less than one-forth ($1/4^{th}$) the wavelength of visible light, i.e. less than approximately 120 nm, do not refract light and therefore microemulsions appear transparent to the eye (Lawrence et al 2000). The basic difference between emulsions and microemulsions are that emulsions exhibit excellent kinetic stability but are thermodynamically unstable when compared to microemulsions. The differences between emulsions and microemulsions are enlisted in table 2.1.

Table 2.1 Differences between emulsions and microemulsions

Characteristics	Emulsion	Microemulsion
Droplet size	100-100,000nm	10-100nm
Phases	Two	One
Appearance	Opaque	Transparent and isotropic
Proportion of dispersed phase	30-60%	20-40% without corresponding increase in viscosity
Energy requirement	Requires large energy input at the time of preparation	Forms spontaneously so no energy requirement
Stability	Kinetically stable but thermodynamically unstable	Kinetically unstable but thermodynamically stable
Surfactant concentration	2-3% by weight	>6% by weight

The microemulsions concept was introduced as early in the 1943 by Hoar & Schulman who generated clear single-phase solution by titrating a milky emulsion with hexanol. Schulman *et al* introduced the term microemulsion for this system in 1959. In recent years microemulsions have attracted a great deal of attention because of their biocompatibility, biodegradability, easy to prepare and handle and most importantly solubilization capacity for both water and oil soluble drugs. The various rationales for developing and using medicated microemulsions are listed in table 2.2.

Table 2.2 Rationale for developing and using medicated microemulsions

Reason	Drug examples
Solubilization of poorly water soluble drugs	Diazepam, vit.A, vit.E, dexamethasone palmitate.
Stabilization of hydrolytically susceptible compounds	Lomustine, physostigmine salicylate
Reduction of irritation, pain or toxicity of intravenously administered drugs	Diazepam

Potential for sustained release dosage forms	Barbiturates
Site specific drug delivery to various organs	Cytotoxic agents

Microemulsions can have various textures, such as oil droplets in water, water droplets in oil, bicontinuous mixtures, ordered droplets or lamellar mixtures with a wide range of phase equilibria among them and with excess oil and/or water phases. This great variety is governed by variations in the composition of the whole system and in the structure of the interfacial layers.

2.6.1 Factors influencing type of microemulsion formed (Lawrence et al 2000)

The formation of oil swollen, water swollen or microemulsion will depend on the following factors:

A. Packing Ratio

B. Property of surfactant, oil phase and temperature

C. The chain length, type and nature of co surfactant.

A Packing Ratio

The HLB of surfactant determines the type of microemulsion through its influence on molecular packing and film curvature. The analysis of film curvature for surfactant associations leading to microemulsion formation has been explained by Israclachvili, Mitchell & Ninham (1976) and Mitchell & Ninham (1981) in terms of packing ratio, also called as critical packing parameter.

Critical packing parameter (c.p.p.) = V/(a*1)

Where, V – Volume of surfactant molecule

a = Head-group surface area

l = Length.

If c.p.p. has value between 0 and 1 interface curves toward water (positive curvature) and o/w systems are favored, but when c.p.p is greater than 1, interface curves spontaneously toward oil (negative curvature) so w/o microemulsions are favored. At zero curvature, when the HLB is balanced (p is equivalent to 1), then either bicontinuos or lamellar structures may form according to the rigidity of the film (zero curvature).

B Property of surfactant, oil phase and temperature

The type of emulsion, to a large extent, depends on the nature of surfactant. Gerbacia & Rosano (1973) observed that the interfacial tension could be temporarily reduced due to the diffusion of cosurfactant through the interface. Microemulsion is formed by a combination of dispersion and stabilization process.

The dispersion process involves a transient reduction of interfacial tension to near zero or negative value at which the interface expands to form fine dispersed droplets. Subsequently these absorb more surfactant until the bulk phase is depleted enough to bring the value of interfacial tension positive. The interfacial film of alcohol and surfactant initiates the stabilization process, it is considered that the stability of o/w system can be controlled by the interfacial charge. If the diffuse double layer at the interface is compressed by high concentration of counter ions, water in oil microemulsions are formed.

Type of surfactant also determines type of microemulsion formed. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these group, which are a measure of the differential tendency of water to swell head group and of oil to swell the tail area are important for specific formulation when estimating the surfactant HLB in a particular system.

Temperature is extremely important in determining the effective head group size of nonionic surfactants. At low temperature, they are hydrophilic and form normal o/w system. At higher temperature, they are lipophilic and form w/o systems. At an intermediate temperature, microemulsion coexists with excess water and oil phases and forms bicontinuos structure.

The oil component also influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils, such as alkanes, penetrate the lipophilic group region to a great extent than long chain alkanes. And so swelling of this region to a great extent results in an increased negative curvature.

C The chain length, type and nature of cosurfactant

Alcohols are widely used as a cosurfactant in microemulsions. Addition of shorter chain cosurfactant (e.g. ethyl alcohol) gives positive curvature effect, as alcohol swells the head region more than tail region so, it becomes more hydrophilic and o/w type is favored, while longer chain cosurfactant (e.g. cetyl alcohol) favors w/o type by alcohol swelling more in chain region than head region.

2.6.2 Formation of microemulsion and phase behaviour

When water oil and surfactants are mixed, microemulsions are only one of a number of association structures including ordinary emulsions, micellar and mesomorphic phases of various concentrations such as lamellar, hexagonal, cubic and various gels and oily dispersions which can form depending on the chemical nature and concentration of each of the components at prevailing temperature and pressure. Preparation of a stable, isotropic homogeneous transparent nontoxic microemulsion requires consideration of a number of variables. A number of trials have to be carried out to study the effect of each variable. This requires considerable amount of time and labour, which can be best reduced by constructing phase diagram. Phase diagrams help to find microemulsion region in ternary or quarternary system and also minimum amount of surfactant for microemulsion formation (Lawrence et al 2000).

A Ternary System

The phase behavior of surfactant - oil - water (s/o/w) is best reported by using ternary diagram. Here, two independent composition variables are sufficient, since third one is complement to 100%. The phase diagram allows one to determine ratio of oil:water; surfactant – consurfactant at the boundary of microemulsion region. To plot the composition of four component systems, a regular tetrahedron composed fixed and varying the other three or by using a constant ratio of two components (surfactant and cosurfactant or cosolvent). It also shows that single phase or multiphase regions of microemulsion domain are near the center of diagram in areas containing large amounts of surfactant that is toxic. The phase behaviour of surfactants, which form microemulsions in absence of cosurfactant, can be completely represented by a ternary diagram.

B Winsor's phase diagram

Winsor (1947, 1954) reported the relationship between the phase behaviour of amphiphile-oil-water and nature of the different components of ternary system. And he classified the phase behavior into four classes: Type-I, Type-II, Type-III, and Type-IV.

Winsor's type I system consists of a lower phase o/w microemulsion coexisting with excess oil.

Winsor's type II diagram consists of an upper phase w/o microemulsions in equilibrium with excess water.

Winsor's type III diagrams system forms when the surfactants are concentrated in a surfactant rich bicontinuos middle phase, which coexists with both oil and water.

Winsor's type IV shows single phase with oil, water and surfactant, which are homogeneously mixed.

C Quarternary phase diagram

Microemulsions are generally quarternary systems. To study their phase behavior, pseudoternary phase diagram consisting of the oil-water amphiphile is commonly drawn in which amphiphile is surfactant / cosurfactant ratio. Optimizing done by using pseudoternary diagram is not accurate. Therefore, it is better to use quarternary phase diagram for such system.

2.6.3 Formulation of microemulsions

Microemulsions are isotropic systems, which are difficult to formulation than ordinary emulsions because their formulation is a highly specific process involving spontaneous interactions among the constituent molecules. Generally the microemulsion formulation involves following components.

a. An oil phase: Toluene, cyclohexane, mineral or vegetable oils, silicone oils or esters of fatty acids etc. have been widely investigated as oil components.

b. An aqueous phase: The aqueous phase may contain hydrophilic active ingredients and preservatives. Buffer solutions have been utilized as aqueous phase by some researchers.

c. A primary surfactant: The surfactants are generally ionic, nonionic or amphoteric. The surfactants chosen are generally from the non-ionic group because of their good cutaneous tolerance. Only for specific cases, amphoters are being investigated.

d. Secondary surfactant (cosurfactant): The cosurfactants originally used were short chain fatty alcohols (pentane, hexanol, benzyl alcohol). These are most often polyols, esters of polyols, derivatives of glycerol and organic acids. Their main purpose is to make the interfacial film fluid by wedging themselves between the surfactant molecules.

2.6.4 Methods for construction of phase diagram (Lawrence et al 2000)

Quarternary phase diagram should be constructed to define the extent and nature of the microemulsion regions and surrounding regions. For that several methods can be used. In one method, a large number of samples of different composition must be prepared. The microemulsion region is identified by its isotropic nature and low viscosity. Other regions can be identified by their characteristic optical structure. These diagrams are complicated and time consuming to prepare and provide a major drawback in the evaluation of a wide range of surfactant, cosurfactant and other components.

In another method, microemulsion region can be located by titration. At a constant ratio of SAA/ CoS, various combinations of oil and SAA/CoS are produced. The water is added drop by drop. After the addition of each drop, the mixture is stirred and examined through a crossed polarized filter. The appearance (transparence, opalescence, and isotropy) is recorded along with the number of phases. Thus, an appropriate delineation of the boundaries can be obtained in which it is possible to refine through the production of compositions point-by-point beginning with the four basic components.

The original method for construction of phase diagram developed by Bowcott and Schulman (1955) can be used for preparation of microemulsion. In this, adding the oil-surfactant mixture to some of the aqueous phase in a temperature-controlled container with agitation makes a coarse macroemulsion as a first step, the system is then titrated with cosurfactant until clarity is obtained and then diluted with water to give a microemulsion of the desired concentration.

Rosano et al. (1988) suggested a simple routine for rapid evaluation of components for their suitability in microemulsions without construction of phase diagram. In this

also coarse emulsion is prepared and titrated to clarity with the chosen cosurfactant. The minimum concentration of surfactant required to cover the interface is calculated. If the system does not clarify after adding the cosurfactant in an amount equivalent to the primary surfactant, the system is considered unacceptable, and first the cosurfactant and finally the oil is changed in a logical manner.

2.6.5 Characterization of microemulsions (Vyas et al 2006)

The determination of microemulsion structure is difficult, although it is important for the successful commercial exploitation of microemulsions as a drug delivery system.

A Phase Behavior Studies

Visual observations, phase contrast microscopy and freeze fracture transmission electron microscopy can be used to differentiate microemulsions from liquid crystals and coarse emulsions. Clear isotropic one-phase systems are identified as microemulsions whereas opaque systems showing bifringence when viewed by cross polarized light microscopy may be taken as liquid crystalline system. Coarse emulsions are systems identified as consisting of two phases when viewed by phase contrast microscopy and showing no bifringence under a cross polarizer.

Phase behavior studies provide information the boundaries of different phases as a function of composition variables and temperature. They also allow comparison of the efficiency of different surfactant for given application.

B Scattering techniques for microemulsions characterization

Small angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and static as well as dynamic light scattering are widely applied techniques in the study of microemulsions. In the static scattering techniques, the intensity of scattered radiation $I(q)$ is measured as a function of the scattering vector q.

$$q = (4\pi/\lambda) \sin \theta/2$$

Where, θ is the scattering angle and λ the wavelength of the radiation. The lower limit of size that can be measured with these techniques is about 2 nm. The upper limit is about 100 nm for SANS and SAXS and up to a few micrometers for light scattering. These methods are very valuable for obtaining quantitative information on the size,

shape and dynamics of the components. The major drawback of these techniques is the dilution of the sample required for the reduction of interparticular interaction. This dilution can modify the structure and the composition of the pseudo phases. Nevertheless, successful determinations have been carried out using a dilution technique that maintains the identity of droplets.

Static light scattering techniques have also been widely used to determine microemulsion droplet size and shape. In these experiments the intensity of scattered light is generally measured at various angles and for different concentrations of microemulsion droplets.

Dynamic light scattering which is also referred as photon correlation spectroscopy (PCS), is used to analyze the fluctuations in the intensity of scattering by the droplets due to Brownian motion. This technique allows the determination of z-average diffusion coefficients, D. In the absence of interparticle interactions, the hydrodynamic radius of the particles R_H, can be determined from the diffusion coefficient using the Stokes- Einstein equation

$$\mathbf{D = kT/6\pi\eta R_H}$$

Where, k is Boltzmann constant, T is the absolute temperature, and n is the viscosity of the medium.

C Nuclear magnetic resonance

The structure and dynamics of microemulsions can be studied by using nuclear magnetic resonance techniques. Self – diffusion measurements using different tracer techniques, generally radio labeling, supply information on the mobility of the components. The Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique uses the magnetic gradient on the samples and it allows simultaneous and rapid determination of the self-diffusion coefficients (in the range of 10^4 to 10^{12} m^2s^{-1}), of many components.

D Electron microscopy

The microemulsions can be characterized by using several electron microscopic techniques although the high liability of the samples and the possibility of artifacts electron microscopy used to be considered as misleading techniques in microemulsion studies. However, images showing clear evidence of the microstructure can be obtained. Freeze-fracture electron microscopy has also been used to study microemulsion structure, however, extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts.

E Interfacial tension, electrical conductivity and viscosity measurements

The formation and the properties of microemulsion can be studied by measuring the interfacial tension. Ultralow values of interfacial tension are correlated with phase behaviour, particularly the existence of surfactant phase or middle-phase microemulsions in equilibrium with aqueous and oil phases. Spinning drop apparatus can be used to measure the ultralow interfacial tension. Interfacial tensions are derived from the measurement of the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with the high-density phase. To determine the nature of the continuous phase and to detect phase inversion phenomena, the electrical conductivity measurements are highly useful. A sharp increase in conductivity in certain w/o microemulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a 'percolative behaviour' or exchange of ions between droplets before the formation of bicontinuos structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of microemulsion systems.

Viscosity measurement can indicate the presence of rod-like or work-like reverse micelle. Viscosity measurements as a function of volume fraction have been used to determine the hydrodynamic radius of droplets, as well as interactions between droplets and deviations from spherical shape by fitting the results to appropriate model (eg. For microemulsions showing Newtonian behaviour, Einstein's equation for the relative viscosity can be used to calculate the hydrodynamic volume of the particles).

2.7 Mucoadhesive Agents

Mucoadhesive dosage forms that can stick to the site of application / absorption have attracted considerable interest since the idea was first introduced early in the 1980's. The advantages of mucoadhesive formulations include: **(i)** prolonged residence time at the site of drug absorption, and **(ii)** better contact with the underlying mucosa so that the diffusion path of the drug to the epithelium is shorter. Furthermore, some mucoadhesive polymers can modulate the permeability of epithelial cells by partially opening tight junctions (Illum 2002). Carbopol 934 increase paracellular transport which is caused by the cells being depleted of extracellular Ca^{+2} since Carbopol polymers also inhibit enzymes and this is also a result of the strong binding affinity of Carbopol for Ca^{+2}, which depletes the enzymes of calcium ions (Leussan et al 1995).

Mucoadhesive polymers interact with glycoproteins in the mucus layer that covers mucosal epithelial surfaces in the body, and popular routes in which mucoadhesive materials are used are the nasal, ocular, buccal, vaginal, rectal and the oral route. Mucoadhesive cannot distinguish between adherent or shed-off mucus and this means that application through the oral route is of limited interest. Furthermore, if the mucus turnover is rapid, as it is for example, in the nose, adhesion to the mucosa might not affect the bioavailability of the drug. The rheology of the formulation might be more important in such cases. A second generation of bioadhesives, lectin-like cyto-adhesives, is now in focus (Leussan et al 1995). These bioadhesives achieve more specific mucoadhesion that is independent of mucus turnover. This class of substances will probably be most useful for the oral route, rather than the nasal / ocular routes.

2.7.1 Mechanism and theory of bioadhesion

The mechanisms by which the mucoadhesive bonds form are not completely clear. It is generally accepted that the process involves three steps **(1)** Wetting and swelling of polymer to permit intimate contact with biological tissue **(2)** interpenetration of bioadhesive polymer chains with mucin molecules leading to entanglement; and **(3)** formation of weak chemical bonds between entangled chains.

Five theories of adhesion have been developed to explain the properties of a wide range of materials including glues, adhesives and paints:

a. The electronic theory assumes that the different electronic structures of the mucoadhesive and the biological material result in electron transfer upon contact.
b. The absorption theory states that the bioadhesive bond is due to van der Waals interactions and hydrogen bonds. This is the most widely accepted theory of adhesion.
c. The wetting theory uses interfacial tension to predict the degree of spreading of, for example, a gel formulation on the mucosa, which can then be used to predict the degree of mucoadhesion.
d. The diffusion theory states that interpenetration and entanglement of polymer chains are responsible for mucoadhesion. The more structurally similar mucoadhesive is to the mucosa, the greater the mucoadhesion will be. It is believed that an interpenetration layer of 0.2 μm-0.5 μm is required to produce an effective bond.
e. The fracture theory analyzes the force required to separate two surfaces after adhesion. It is often used for calculating fracture strengths of adhesive bonds during detachment.

2.8 Cerebral ischemia or stroke

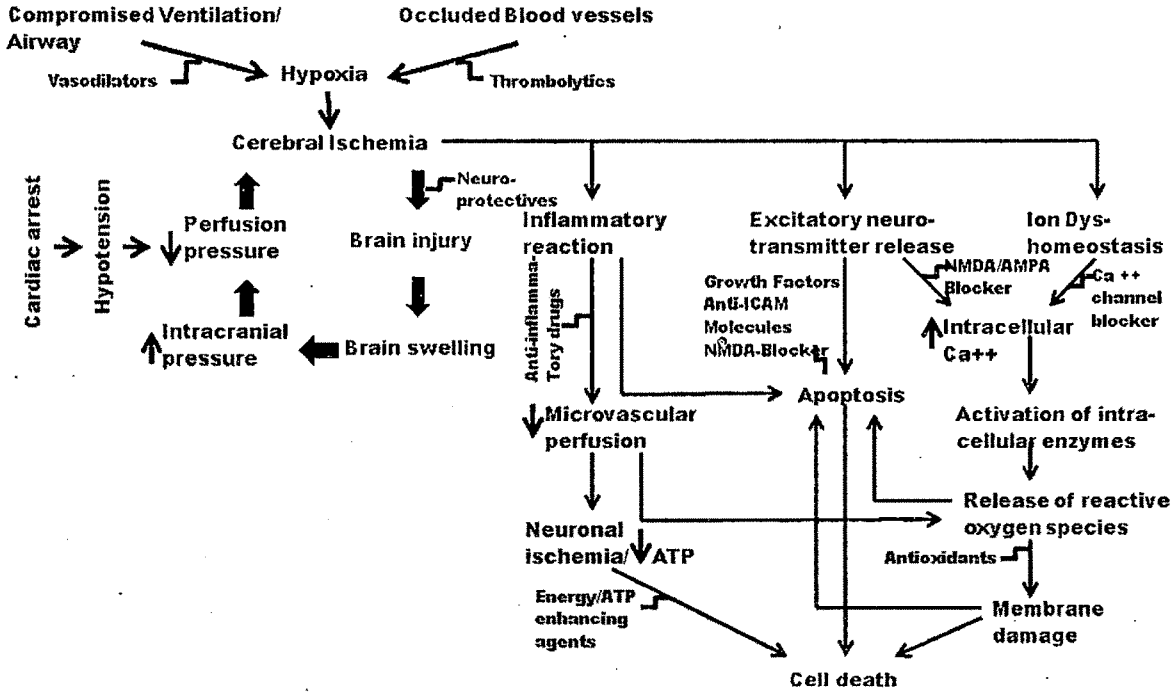
Stroke is gradual loss of brain functions due to interrupted blood supply to the brain. It is the most debilitating disorder of the brain resulting in significant neuronal death and functional loss and ranks second as the most common cause of death worldwide (Murray et al 1997). The unmodifiable risk factors for stroke include age, race, sex and family history of stroke. After the age of 55 years, each decade doubles the risk of stroke. It can be ischemic caused by an interruption of blood supply, or haemorrhagic due to rupturing of a blood vessel, gradually resulting in irreversible loss of functionality of the affected area. 87% of strokes are ischemic with the remaining being hemorrhagic (Miller et al 2009). An ischemic stroke sets off a series of ischemic mechanisms leading to the death of the affected tissue, i.e. infarction, resulting in permanent loss of related motor and sensory activities. These ischemic mechanisms include induction of anaerobic respiration, release of excitatory neurotransmitters and formation of reactive oxygen species ultimately leading to apoptosis (Doyle et al 2008). Brain tissue stops functioning if deprived of oxygen for more than 60 to 90 seconds and after approximately three hours, suffers irreversible injury leading to infarction.

Stroke being a complex condition, disconcerting various motor and sensory functions, necessitates an early treatment to confine the neuronal damage to the minimum. The only approved and validated therapy for acute ischemic stroke includes intravenous tPA within 3 hr of stroke onset and is more effective for the management of ischemic strokes than haemorrhagic (Murray et al 1997). However in May 2009, the American Heart Association (AHA) /American Stroke Association (ASA) gave nod to the use of tPA to treat acute ischemic stroke between 3 and 4.5 hours of symptom onset, but still emphasize on early medical intervention, as early the treatment provided better the outcomes (Hacke et al 2008). Various promising molecules have been unsuccessful being either too toxic or not able to cross the BBB (Fisher et al 2003).

2.8.1 Pathophysiology of ischemic stroke

Stroke pathophysiology is the study of what processes are involved in causing or are a result of a stroke. Ischemic stroke occurs because of loss of blood supply to a brain region due to either a thrombus or an embolus, initiating the ischemic cascade (Doyle KP *et al* 2008). A thrombus is usually formed around an atherosclerotic plaque narrowing the lumen of the blood vessel and reducing the blood supply to the tissue. Whilst an embolic stroke occurs when an embolus formed elsewhere in the circulatory system, typically in the heart, breaks off and enters the cerebral circulation and then occludes brain blood vessels. The final event in cerebral ischemia is the death of neurons, resulting in irreversible loss of neurologic function. Today, due to the advent of animal and tissue culture models of ischemia it has become increasingly clear that many secondary biochemical changes exacerbate brain injury in response to the initial ischemic insult (Doyle et al 2008). It is evident from various reported preclinical studies that an understanding of these secondary mechanisms, such as excitotoxicity, acidosis, inflammation, oxidative stress, peri-infarct depolarization and apoptosis, has resulted in a number of therapeutic strategies that aim to prevent these processes and decrease the resulting neuronal cell death that results from cerebral ischemia (figure 2.5).

Figure 2.5: Pathophysiology, cellular events and therapeutic interventions of ischemic stroke



The primary pathologic mechanism in stroke is the depletion of energy stores forcing brain cells to switch over to anaerobic respiration within the affected brain tissue leading to the production of less ATP and lactic acid. Lactic acid being irritant destroys cells and disrupts the normal acid-base balance in the brain. This further initiates a cascade of interrelated events, such as the failure of mitochondria, the release of excitatory neurotransmitters like glutamate, that result in cellular injury and death (Doyle et al 2008). The extracellular glutamate is normally kept low by the so-called ion pumps, powered by the concentration gradients of ions (mainly Na^+) across the cell membrane. As a result of lack of oxygen and glucose these transmembrane ion gradients run down, and glutamate transporters release more of glutamate into the extracellular space. Glutamate acts on nerve cell receptors (especially NMDA receptors), producing an influx of calcium which activates enzymes that digest cell proteins, lipids and nuclear material. Calcium influx can also lead to the failure of mitochondria causing energy deprivation and triggering apoptosis.

Ischemia also induces production of reactive oxygen species that react with and damage number of cellular and extracellular elements particularly blood vessel lining

or endothelium. The brain tissue unlike other organs is especially vulnerable to ischemia as has little respiratory reserve and dependents completely on aerobic metabolism (Doyle et al 2008). Brain tissue survival can be improved to some extent if one or more of these processes are inhibited.

2.8.2 Pharmacological approaches to the problem of cerebral ischemia or stroke

The two fundamental approaches to the development of acute stroke therapy remain reperfusion and neuroprotection. Thrombolytics are used to restore reperfusion following stroke like intravenous tPA and as far as neuroprotection is concerned, till date there is no approved therapy to manage acute ischemic stroke (Fisher et al 2003). Several molecules have been investigated for their benefit in acute ischemic stroke but failed in clinicals being either too toxic or unable to cross the BBB. However, neuroprotectives with multiple mechanisms of action, especially inhibiting platelet aggregation and having no risk of symptomatic intracranial hemorrhage, will be more beneficial than thrombolytics for ischemic stroke management, after the initial therapy with tPA has been provided (Fisher et al 2003).

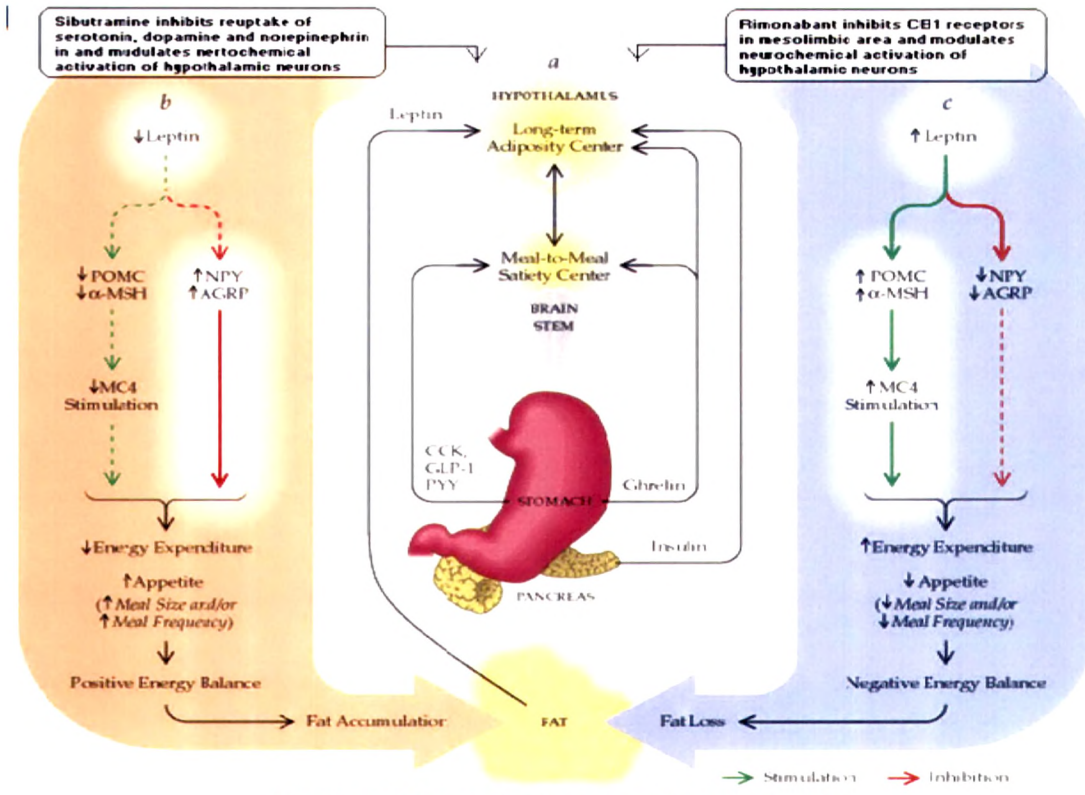
2.9 Obesity

Obesity is a growing health problem in many of the richest nations of the world and is now considered a chronic disease that is reaching epidemic proportions (Barness et al 2007). Obesity in general is defined as a state of 'excess body fat' or 'body weight that is 20% over the ideal' and is expressed in terms of 'Body Mass Index' (BMI). Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, breathing difficulties during sleep, certain types of cancer, and osteoarthritis (Haslam et al 2005). It has now been explored that the body has a homeostatic mechanism for controlling body fat and that the central nervous system (CNS) is involved. Many reviews of energy balance control contain spaghetti diagrams of interacting factors endocrines, autonomic mediators, gastrointestinal peptides, CNS transmitters, etc all impinging on the hypothalamus, which, in turn, releases mediators that act on CNS, autonomic and endocrine systems affecting food intake and energy balance (Mantzoros CS 1999).

2.9.1 Pathophysiology of obesity (Rang 2003)

The main determinant is a disturbance of the homeostatic mechanisms that control energy balance, but genetic endowment underlies this disturbance (figure 2.6). Other factors such as food intake and lack of physical activity contribute and there are also social, cultural and psychological aspects.

Figure 2.6: Homeostatic mechanisms involved in obesity



Homeostatic mechanisms involved in obesity

A Obesity as a disorder of the homeostatic control of energy balance

Since the homeostatic control of energy balance is extremely complex, it is not easy to determine what goes wrong in obesity.

Resistance to leptin (derived from the Greek *leptos* meaning thin) seems to be a characteristic of obesity. Such resistance can be caused by defects in leptin synthesis, in its carriage in the circulation, in its transport into the CNS, in leptin receptors in the

hypothalamus or in postreceptor signaling. There is some evidence that the action of a member of the family of suppressors of cytokine signaling, SOCS-3, may underlie or contribute to leptin resistance.

TNF-α, another cytokine that relays information from fat to brain, is increased in the adipose tissue of insulin-resistant-obese individuals. Another pathophysiological alteration in obesity is a reduced insulin sensitivity of muscle and fat.

Reduced function of β_3–adrenoceptors in brown adipose tissue can also be implicated, alternatively one of the proteins that uncouple oxidative phosphorylation in fat cells, UCP-2, can be dysfunctional in obese individuals.

A further cause is that alternation of function of specific transcription factors, such as the PPAR transcription factors α, β and γ, may have a role in obesity. These transcription factors regulate the gene expression of enzymes associated with lipid and glucose homeostasis, and also promote the genesis of adipose tissue. PPARγ is expressed preferentially in fat cells and synergises with another transcription factor, C/EBPα, to convert precursor cells to fat cells.

In fact the pathophysiology of obesity can involve disturbance(s) in any of the multitude of other factors involved in energy balance.

B Genetic factors and obesity

Studies in twins and in adoptees and their families indicate that from 40% to as much as 80% of the variance of BMI can be attributed to genetic factors. It is estimated that heritability is as high as 30-40% for factors relevant to energy balance such as body fat distribution resting metabolic rate, energy expenditure after overeating lipoprotein lipase activity and basal rates of lipolysis.

There are some rare cases in which obesity is the consequence of a single gene disorder, but, in general, it probably involves the interaction of many genes. Most obese subjects studied so far have not had any abnormalities in the genes for either leptin or the leptin receptor. Linkage of human obesity to genes for other factors

relevant to energy balance has been reported: a β_3–adrenoceptor and the glucocorticoid receptor.

C Food intake and obesity

The type food eaten can play a part in upsetting the energy balance. Fat has more calories per gram and it may be that the mechanisms regulating appetite react rapidly to carbohydrate and protein but slowly to fat.

Obese individuals diet to lose weight. However, when a subject reduces calorie intake, shifts into negative energy balance and loses weight, the resting metabolic rate decreases, and there is a concomitant reduction in energy expenditure. The decrease in energy expenditure appears to be largely caused by an alteration in the conversion efficiency of chemical energy to mechanical work in the skeletal muscles. This adaptation to the caloric reduction contributes to the difficulty of maintaining weight loss by diet.

D Physical exercise and obesity

It's now recognized that physical activity-i.e. increased energy expenditure- has a much more positive role in reducing fat storage and adjusting energy balance in the obese, particularly if associated with modification of the diet.

2.9.2 Pharmacological approaches to the problem of obesity

Carefully controlled diet and physical exercise are the main therapeutic approaches to obesity, but many patients may also need anti-obesity drugs. Such drugs can aim to suppress food intake, increase energy expenditure or increase lipolysis. A prodigious effort is underway by numerous pharmaceutical companies round the world to provide effective anti-obesity agents. At present, only orlistat (a pancreatic lipase inhibitor; decreases fat absorption by preventing the breakdown of dietary fat in the gastrointestinal tract) is approved for the management of obesity (Rang HP 2003). Sibutramine (originally intended to be used as an antidepressant) was a clinically approved orally administered, centrally acting anti-obesity drug and produced its therapeutic effects by norepinephrine, serotonin and dopamine reuptake inhibition (http://www.rxlist.com/meridia). It has been withdrawn due to serious peripheral side

effects such as cardiovascular disturbances, gastrointestinal disturbances, etc arising from unwanted tissue distribution. Many others are in various stages of development.

2.10 Drug Profile

2.10.1 Nicergoline

a. Category: Nootropic, vasodilator agents, adrenergic alpha-antagonists.

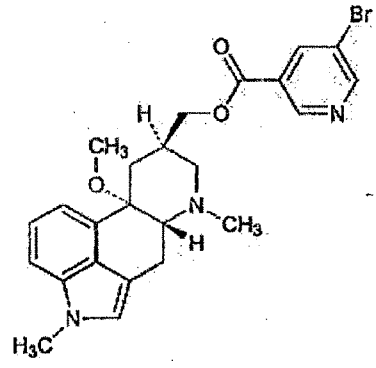
b. Generic Name: Nicergoline.

c. Marketed preparations available: Sermion, Ergotop, Dospan in the form of tablets.

d. Empirical formula: $C_{24}H_{26}BrN_3O_3$.

e. Molecular weight: 484.39

f. Structure:



g. Appearance: Nicergoline occurs as white to light yellow, crystals or crystalline powder.

h. Physical properties

Solubility: It is soluble in acetonitrile, in ethanol (99.5%) and in acetic anhydride, and

practically insoluble in water.

Melting point: 136°C.

i. Mechanism of action: Nicergoline acts by inhibiting the postsynaptic alpha(1)-adrenoceptors on vascular smooth muscle. This inhibits the vasoconstrictor effect of circulating and locally released catecholamines (epinephrine and norepinephrine), resulting in peripheral vasodilation. Therefore the mechanism of nicergoline is to increase vascular circulation in the brain, thereby enhancing the transmission of nerve signals across the nerve fibres, which secrete acetylcholine as a neural transmitter.

j. Pharmacokinetics: Nicergoline is rapidly and nearly completely absorbed after oral administration. The half-life of nicergoline was 13 - 20 hours. Nicergoline is effectively bound to plasma proteins (>90%), with higher affinity for α-acid glycoprotein than for serum albumin. Nicergoline is excreted as its metabolites predominantly in urea (approximately 80% of the total dose) and in feces (10-20%).

k. Adverse effects: severe hypotension dizziness, dyspepsia, hot flashes, skin rash, sleepiness and insomnia.

l. Indications: senile dementia, transient ischemia, macular degeneration, migraines.

m. Dosage: 4-8mg, available as 5mg, 10mg and 30mg tablets.

2.10.2 Hydergine or ergoloid mesylate

a. Category: Nootropic, vasodilator agents, adrenergic alpha-antagonists.

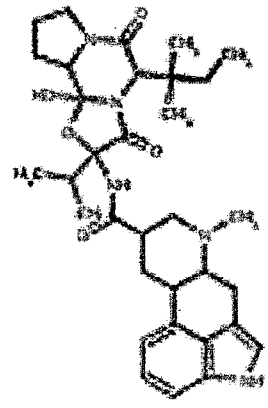
b. Generic Name: Ergoloid mesylate.

c. Marketed preparations available: Hydergine, Gerimal, Dihydroergotoxin Mesylate in the form of tablets.

d. Empirical formula: $C_{32}H_{45}N_5O_8S$

e. Molecular weight: 591.74.

f. Structure:



g. Appearance: White to off-white, microcrystalline or amorphous, practically odorless powder.

h. Physical properties

Solubility: Slightly soluble in water; soluble in methanol and in alcohol; sparingly soluble

in acetone.

i. Mechanism of action: Ergoloid mesylates act centrally, decreasing vascular tone and slowing the heart rate, and acts peripherally to block alpha-receptors. One other possible mechanism is the effect of ergoloid mesylates on neuronal cell metabolism, resulting in improved oxygen uptake and cerebral metabolism, thereby normalizing depressed neurotransmitter levels.

j. Pharmacokinetics: Rapidly but incompletely (approximately 25%) absorbed from the gastrointestinal tract. Approximately 50% of the absorbed dose is eliminated by first-pass metabolism.

k. Adverse effects: Transient nausea, vomiting, headache, blurred vision, skin rashes, nasal stuffiness, flushing of the skin, dizziness, bradycardia, orthostatic hypertension and gastric disturbances

l. Indications: Dementia

m. Dosage: 2.25-9mg daily, available as 1mg, 4.5mg tablets, 1mg/mL Liquid or 1mg liquid capsules.

2.10.3 Sibutramine hydrochloride monohydrate

a. Category: Appetite suppressant, Antidepressant.

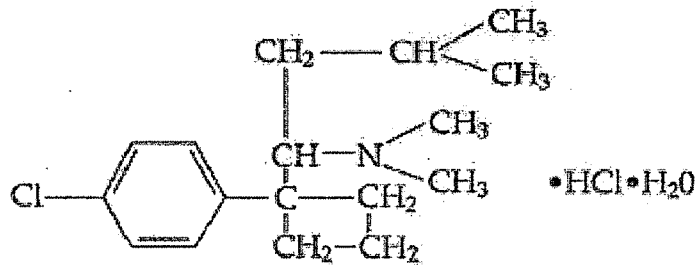
b. Generic Name: Sibutramine.

c. Marketed preparations available: Meridia, Reductil in the form of capsules.

d. Empirical formula: $C_{17}H_{29}Cl_2NO$

e. Molecular weight: 334.33

f. Structure:



g. Appearance: Sibutramine hydrochloride monohydrate is a white to cream crystalline powder.

h. Physical properties

Solubility: In water at pH 5.2: 2.9 mg/mL. Its octanol: water partition coefficient is 30.9 at pH 5.0.

i. Mechanism of action: Sibutramine exerts its pharmacological actions predominantly via its secondary (M1) and primary (M2) amine metabolites. The parent compound, sibutramine, is a potent inhibitor of serotonin and norepinephrine reuptake in vivo, but not in vitro. However, metabolites M1 and M2 inhibit the reuptake of these neurotransmitters both in vitro and in vivo. Sibutramine produces its therapeutic effects by inhibition of norepinephrine (NE), serotonin (5-hydroxytryptamine, 5-HT), and to a lesser extent, dopamine reuptake at the neuronal synapse. By inhibiting the reuptake of these neurotransmitters, sibutramine promotes a sense of satiety and decrease in appetite, thereby reducing food intake.

j. Pharmacokinetics: Rapid absorption following oral administration. Absolute bioavailability is not known, but at least 77% of a single oral dose of sibutramine is absorbed. Sibutramine is metabolized in the liver principally by the cytochrome P450 (3A4) isoenzyme, to desmethyl metabolites, M1 and M2. These active metabolites are further metabolized by hydroxylation and conjugation to pharmacologically inactive metabolites, M5 and M6.

k. Adverse effects: Cardiovascular events such as increased risk of heart attacks and strokes, dry mouth, paradoxically increased appetite, nausea, strange taste in the mouth, upset stomach, constipation, trouble sleeping, dizziness, drowsiness, menstrual cramps/pain, headache, flushing, or joint/muscle pain.

l. Indications: Obesity

m. Dosage: 5-30 mg, available as 5mg, 10mgand 15mg capsules.

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