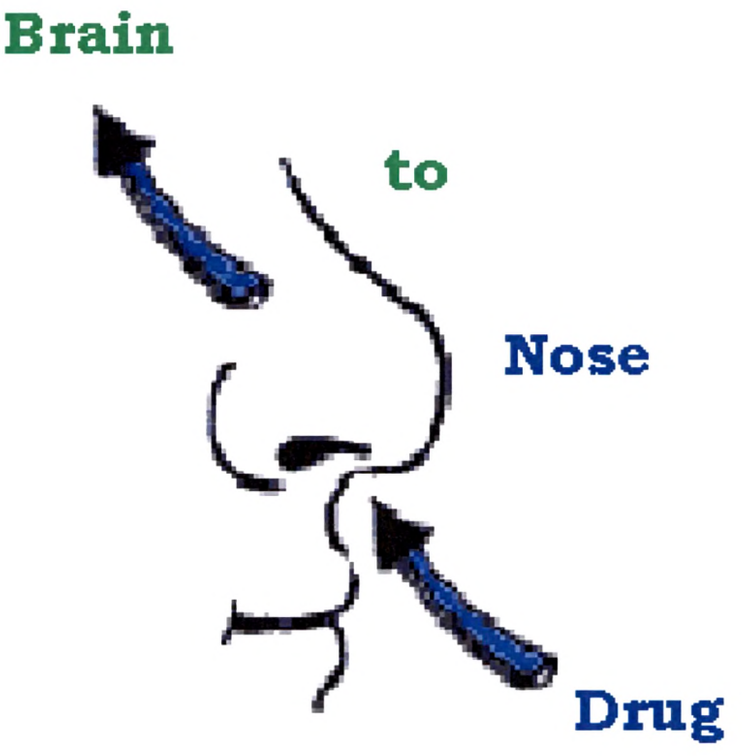


CHAPTER 2

REVIEW OF LITERATURE

2.1 Central Nervous System Disorders and Drug Market

Despite enormous advances in brain research, brain and central nervous system (CNS) disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. Patients suffering from fatal and/or devastating CNS disorders, such as neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD) epilepsy, migraine, brain tumors, HIV encephalopathy, and cerebrovascular diseases, far outnumber of those are victimized from several types of systemic cancers and heart disease (Misra et al. 2003). Beyond loss of life, this broad category of disorders can have an overwhelming effect on the quality of life for the surviving patient and can lead to serous social and economic burdens on society. CNS disorders contribute to as much as 35% of the disease burden in the seven major pharmaceutical markets (US, Japan, France, Germany, Italy, Spain and UK) as measured in terms of daily-adjusted life years. The worldwide patient population with CNS disorders is steadily rising, both in terms of prevalence and in terms of treatment, driven by an aging population, improving diagnostic techniques, increasing physician and patient awareness and a gradual shift away from the social stigma traditionally attached to many psychiatric conditions. The CNS disorders could increase their share of the total global burden of disability and mortality from 10.5% in 1990 to 15% in 2020 (a larger proportionate increase than even cardiovascular disease) as reported by the 1990 Global Burden of Disease Study (www.who.int accessed August 12, 2002). Thus, the treatment of CNS disorders is the greatest challenge and largest potential growth sector of the pharmaceutical industries. A large number of therapeutic agents are found to be ineffective in the treatment of CNS disorders because of variety of formidable obstacles in effective drug delivery and maintenance of therapeutic concentrations in CNS for prolonged period (Vyas et al. 2005a). Frequently, the molecule is too large or has polar functional groups and the blood-brain barrier (BBB) limits its access to the CNS (Talegaonkar and Mishra 2004). The delivery of drugs to CNS is a challenge in the treatment of CNS disorders (Graff and Pollack 2005). The clinical failure of most of compounds active in CNS disorders is often not due to a lack of drug efficacy but mainly due to shortcomings in the drug delivery approach. The method of delivering a drug to the CNS has an impact on the drug's commercial potential. Thus, the market of CNS drug delivery technology is directly linked to the CNS drug market. General methods that can enhance drug delivery to the brain are, therefore, of great interest. Hence, scientists are

exploring the novel approaches so that delivery of the drugs can be enhanced and/or restricted to the brain and CNS.

2.2 Alzheimer's Disease: Prevalence, Etiology and Treatment

Numerous demographic and social changes are occurring for the first time in the history of many parts of the world. There are proportionally more elder people today because of increased life expectancy. The world's older population is expected to grow considerably. In 2050, there will be more than three times as many people age 65 and older as there are today. Reduced fertility has, at the same time, resulted in a decrease in the number of younger people available to take care of elderly people. One of the issues related to aging population is their mental health, which historically has been ignored in many parts of the world. Potentially, large numbers of elderly people are in need of mental health services. Psychiatric and neurological disorders account for 5 of the top ten leading causes of disability worldwide as reported by the 1990 Global Burden of Disease Study. Projections show that psychiatric and neurological conditions could increase their share of the total global burden of disability and mortality from 10.5% in 1990 to 15% in 2020, a larger proportionate increase than even cardiovascular disease (www.who.int accessed August 12, 2002). In geriatric population all over the world, dementia has emerged as a major health problem (Luthra et al. 2004) and experts currently believe that 60% of cases of dementia are due to AD (Redwood 1999). AD is now the fourth leading cause of death in adults and almost 8 million individuals world wide are suffering from it. AD is the fastest growing CNS indication in terms of prevalence and by 2010 it is forecast that AD will afflict about 15 million individuals. The onset of AD is usually after 65 years of age, though earlier onset is not uncommon. As the age advances, incidence of AD roughly doubles every 5 years. By the age 85, almost half of all people are afflicted (The World Health Report 2001; Alzheimer's disease 2002; Lahiri et al. 2002). This has obvious implications for the total number of individuals living with this disorder as life expectancy increases in the population. Unless effective methods for prevention and treatment for AD are developed, this disorder will reach epidemic proportion afflicting an estimated 22 million individuals world wide within 50 years (Alzheimer's disease 2002; Lahiri et al. 2002). Over the last 3 years, the value of the AD market has grown by an average of 19% per year. Growth has been driven by increasing availability and reimbursement of Alzheimer's drugs. There are considerable opportunities within these markets due to an ageing population and the high unmet clinical need, particularly within the late stage of disease.

Alzheimer's disease and its etiology:

Alzheimer's disease is a highly disabling neuropsychiatric disorder characterized by an irreversible deterioration of memory and intellectual behavior. While the etiology of AD remains unknown, evidence has been presented that the hippocampus (an essential brain structure for memory and learning) is one of the principal areas affected by AD (Marx 1991). A specific loss of cholinergic neurons and deficits of choline acetyltransferase have been suggested to play a major role in the primary cognitive symptoms of the disease. Decreased central cholinergic activity has received major attention from investigators in search of biochemical approach that supports a pharmacotherapy for the disease. Inhibition of acetylcholinesterase is a promising approach and the most common method under investigation for the treatment of AD (Giacobini 1993). AD was first studied, identified and described by Dr. Alois Alzheimer in early 1900s (Thirumalai 2002; Fight Alzheimer's disease 2003). It is a progressive neurodegenerative brain disorder characterized by progressive decline of cognitive functions such as memory, thinking, comprehension, calculation, language, learning capacity and judgment, affecting the personal activities of daily living. As a result, patient becomes a stranger in his own family (Thirumalai 2002).

For the treatment of AD currently FDA approved acetylcholine esterase inhibitors, tacrine (Cognex®), donepezil (Aricept®), galantamine (Reminyl®), and rivastigmine (Exelon®) and N-methyl-D-aspartate (NMDA) receptor antagonist memantine (Namenda® (memantine) are available in the market. Cholinesterase inhibitors alleviate inhibit the action of acetylcholine esterase, the enzyme responsible for the destruction of acetylcholine in the brain. Memantine, a noncompetitive NMDA receptor antagonist, exhibits dual mechanism of action. At the receptor level, it produces improved neurotransmission and activation of neurons by modulating the glutaminergic neurotransmission system and reducing glutamate release. However, when glutamate release is excessive, memantine inhibits the excitatory action of glutamate by antagonizing NMDA receptors. The drug thus blocks NMDA receptors from excessive glutaminergic stimulation and prevents an increase in calcium influx; it subsequently results in decreased cell death and alleviates symptoms of AD (Ho and Chagan 2004). All these products currently available for the management of AD are administered by oral route.

2.3 Central Nervous System

Central nervous system and brain are one of the complex systems in human body. The brain is a delicate organ, and evolution built very efficient ways to protect it. The same mechanisms that protect it against intrusive chemicals can also frustrate therapeutic interventions. The delivery of drugs to CNS is a challenge in the treatment of CNS disorders. The presence of BBB is the major bottle neck in delivering drugs to the brain (Brightman 1992; Lo et al. 2001). Drugs may be administered directly into the CNS or administered systematically (e.g., by intravenous injection) for targeted action in the CNS. Systemically, administered drugs for CNS disorders must pass the BBB to reach the brain/CNS. Almost, 100% of large molecule drugs and >98% of small molecule drugs do not cross the BBB (Pardridge 1991). Thus, 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 1991). The transport mechanisms through 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.

2.3.1 Central Nervous System Barriers:

The failure of systemically delivered drugs to effectively treat many CNS diseases can be rationalized by considering a number of barriers that inhibit drug delivery to the CNS. The CNS was protected by barrier layers at three key sites, the endothelium of brain parenchymal blood vessels (i.e. BBB), the choroid plexus epithelium (i.e. blood-cerebrospinal fluid (CSF) barrier (BCB)), and the arachnoid epithelium of the meninges. (Figure 2.1) (Abbott 2004).

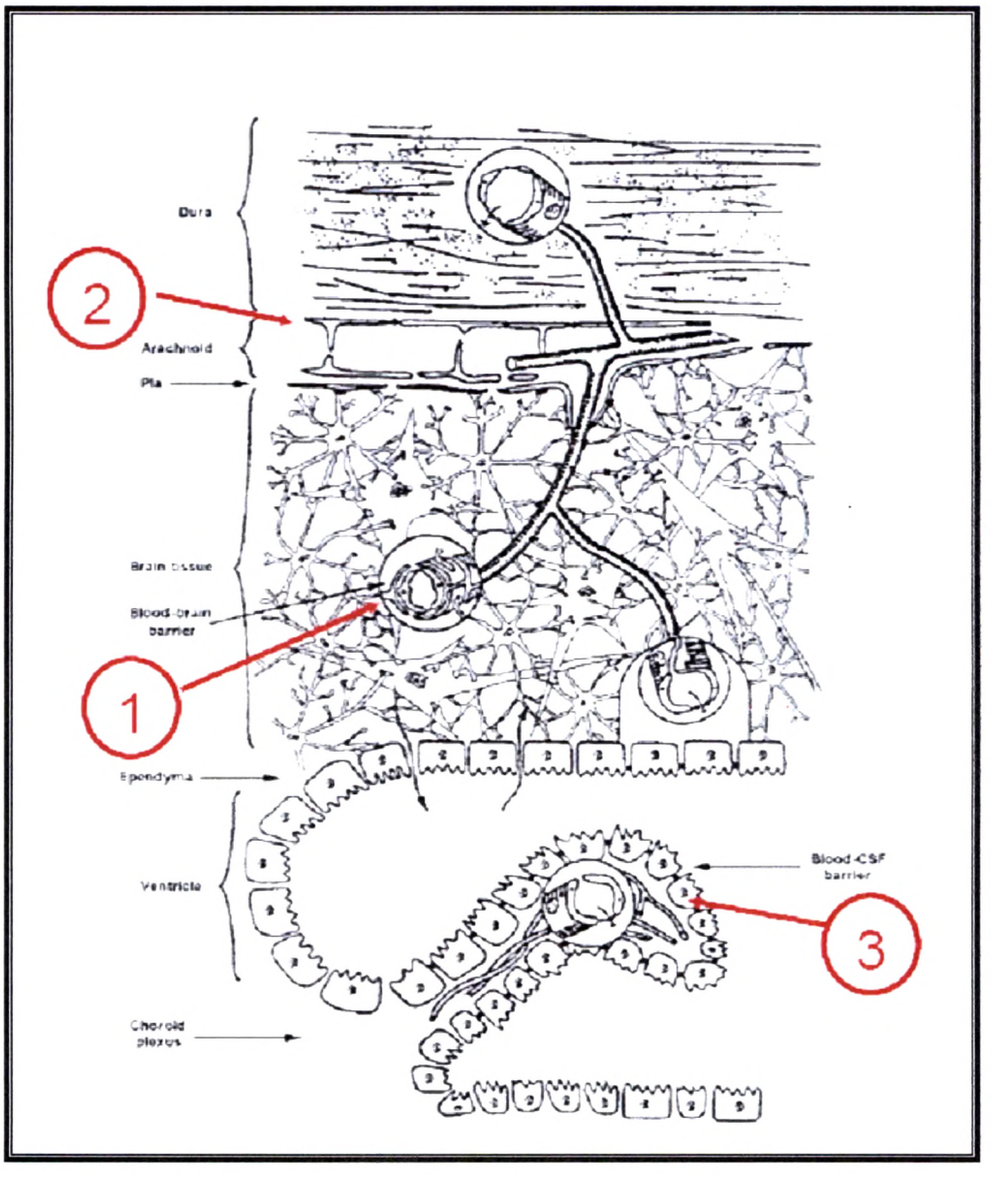


Figure 2.1 Location of barrier sites in the CNS. Barriers are present at three main sites: 1) the brain endothelium forming the blood-brain barrier (BBB), 2) the arachnoid epithelium forming the middle layer of the meninges, and 3) the choroid plexus epithelium which secretes cerebrospinal fluid (CSF) forming blood-cerebrospinal fluid barrier (BCB). In each site, the physical barrier is caused by tight junctions that reduce the permeability of the paracellular (intercellular cleft) pathway.

2.3.1.1 Blood-Brain Barrier:

The BBB is a system of layers of cells at the cerebral capillary endothelium, which are connected by tight junctions (zonulae occludens) and separate the brain from the blood (Rapoport 1976; Begley 1996; Lo et al. 2001; Schlossauer and Steuer 2002). These tight endothelium junctions can be 100 times tighter than junctions of other capillary endothelium (Butte et al. 1990). In addition each brain capillary is composed of two lipid membranes (the luminal membrane facing the blood and the anti-luminal membrane, facing the brain) separated by 300 nm of endothelial cytosol (Illum 2004). 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 BBB, is the principal route by which molecules reach the brain. Thus, the barrier has many properties similar to a continuous cell membrane, allowing lipid-soluble molecules transport across the membrane where hydrophilic solutes demonstrate minimal permeation (Smith 1990). The BBB impedes the use, for example, of many of the newer genetically engineered drugs, such as humane recombinant neurotrophic factors and other therapeutic agents that can protect brain cells from damage and promote nerve repair. 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 PD, is very hydrophilic, it can readily penetrate the BBB. A thorough understanding of the two way transport mechanisms through BBB is of great importance in targeting drugs to the brains or to minimize the unwanted adverse effects some therapeutically active molecules. 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). 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 and Williams 1993; Witt et al. 2001).

2.3.1.2 Blood-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). Since the CSF can exchange molecules with the interstitial fluid of the brain parenchyma, the passage of blood-borne molecules into the CSF is also carefully regulated by the BCB. Physiologically, the BCB is found in the epithelium of the choroids plexus, which are arranged in a manner that limits the passage of molecules and cells into the CSF. The choroid plexus and the arachnoid membrane act together as a barrier between the blood and CSF. Brain is covered by double layered structure called arachnoid membrane. On the external surface of the brain the ependymal cells fold over onto themselves to form a double layered structure, which lies between the dura and pia, this is called the arachnoid membrane. Within the double layer is the subarachnoid space, which participates in CSF drainage. Passage of substances from the blood through the arachnoid membrane is prevented by tight junctions (Nabeshima et al. 1975). The arachnoid membrane is generally impermeable to hydrophilic substances and its role is forming the BCB is largely passive. The choroid plexus forms the CSF and actively regulates the concentration of molecules in the CSF. The choroid plexus consist of highly vascularized, "cauliflower like" masses of pia mater tissue that dip into pockets formed by ependymal cells. The preponderance of choroids plexus is distributed throughout the fourth ventricle near the base of the brain and in the lateral ventricles inside the right and left cerebral hemispheres. The cells of the choroidal epithelium are modified and have epithelial characteristics. These ependymal cells have microvilli on the CSF side, basolateral interdigitations, and abundant mitochondria. The ependymal cells, which line the ventricles, form a continuous sheet around the choroid plexus. While the capillaries of the choroid plexus are fenestrated, non-continuous and have gaps between the capillary endothelial cells allowing the free-movement of small molecules, the adjacent choroidal epithelial cells form tight junctions preventing most macromolecules from effectively passing into the CSF from the blood (Brightman 1968). However, these epithelial- like cells have shown a low resistance as compared the cerebral endothelial cells, approximately 200 $\Omega.cm^2$, between blood and CSF (Saito and Wright 1983). In addition,

the BCB is fortified by an active organic acid transporter system in the choroids plexus capable of driving CSF-borne organic acids into the blood. As a result a variety of therapeutic organic acids such as the antibiotic penicillin, the anti-neoplastic agent methotrexate, and the antiviral agent zidovudine are actively removed from the CSF and therefore inhibited from diffusing into the brain parenchyma. Furthermore, substantial inconsistencies often exist between the composition of the CSF and interstitial fluid of the brain parenchyma, suggesting the presence of what is sometimes called the CSF-brain barrier (Pardridge 1988). This barrier is attributed to the insurmountable diffusion distances required for equilibration between the CSF and the brain interstitial fluid. Therefore, entry into the CSF does not guarantee a drug's penetration into the brain.

2.3.2 Physicochemical Factors that Influence Brain Uptake:

Therapeutic activity in form of biological response is a measure of brain uptake. But this biological activity mainly depends on rate of transfer from blood to brain, or distribution between blood and brain, and interaction between drug and targeted receptors in the brain. If these two factors cannot be distinguished, then it is impossible to use biological activity as a measure of either rate or equilibrium transfer.

As on to date, in new chemical entity (NCE) designing program the lipophilic factor (log $P_{o/w}$) is still used as an informative tool for CNS targeting (Gupta 1989; Hansch et al. 1995). Increase in lipophilic factor with the intent to improve membrane permeability might not only make chemical handling difficult, but also leads to increase in the volume of distribution and tends to affect all other pharmacokinetic parameters (Lin and Lu 1997; Van de Waterbeemd et al. 2001). Furthermore, increasing lipophilicity tends to increase the rate of oxidative metabolism by cytochrome P450 and other enzymes (Van de Waterbeemd et al. 2001; Lewis and Dickins 2002). Hence, to improve bioavailability, the effects of lipophilicity on membrane permeability and first pass metabolism have to be balanced.

The various experiments measuring tools of brain uptake such as brain uptake index (Oldendorf 1970), Permeability-surface area product (PS), Permeability coefficient (PC) are widely utilized. Based on the relationship between the octanol / water partition coefficient (PC, log $P_{o/w}$) divided by the square root of the molecular weight of molecules and the BBB permeability coefficient (PS), therapeutic substrates can be classified in three different classes: (a) substrates exhibiting a good correlation, (b) substrates

exhibiting a greater PS value than indicated by their lipophilicity, and (c) substrates exhibiting a smaller PS value than indicated by their lipophilicity (the molecular weight of substrates greater than 400 Da, cut off for BBB passage regardless of lipophilicity (Levin 1980)). The transport mechanism for groups (a) and (b) is passive diffusion and facilitated transport, respectively (Pardridge et al. 1990).

Brain uptake can be positively correlated with lipid solubility or negatively correlated with hydrogen bonding (Cornford and Oldendorf 1986). The extent to which a compound forms hydrogen bonds is vital for its ability to permeate endothelial cell membranes. The higher the hydrogen bonding potential, lower the uptake into the brain. By reducing the hydrogen bonding potential for a congeneric series of steroid hormones, there was a log increase in uptake with each removal of hydrogen bond pairs. The correlation of blood-brain distribution coefficients (as log BB *in-vivo* and *in-vitro* values) using hydrogen bonding descriptors are available (Abraham et al. 1994) but are not very similar to correlations for log PS. Hence the factors that influence blood-brain distribution are not quantitatively the same as those that influence brain perfusion. So it is vitally important when discussing brain uptake to specify what measure of brain uptake is being used. A variety of *in silico* models (Sippl 2002) and *in vitro* permeability assays (de Boer and Gaillard 2002) have been developed in an attempt to characterize and predict BBB permeability and integrate such prediction in the early phases of drug development, together with various other considerations (Kerns 2001; Mertsch and Maas 2002; Buchwald and Bodor 2002).

2.3.3 Drug delivery approaches for brain-targeting:

To circumvent the multitude of barriers inhibiting CNS penetration by potential therapeutic agents, numerous drug delivery strategies have been developed (Habgood et al. 2000; Thorne and Frey 2001; Lo et al. 2001; Witt et al. 2001; Siegal and zylber-Katz 2002; Filmore 2002).

1. **Lipophilic analogs:** CNS penetration is favored by low molecular weight, lack of ionization at physiological pH, and lipophilicity (Pardridge 1988). Octanol/Water partition coefficient (log $P_{o/w}$) (Buchwald and Bodor 2002) is very commonly acceptable and convenient approach to predict lipophilicity and relative lipophilicity of any system. 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

Table 2.1 Prodrugs approved by USFDA in recent past and presently available on the market

No.	Drug	Approval
1	Valacyclovir	Valtrex® , the L-valine ester prodrug of Acyclovir approved by USFDA in 1995 for treatment of herpes Zoster
2	Fosphenytoin Sodium	Cerebyx® , a phosphooxy methyl prodrug of phenytoin approved in 1996 for the treatment of epilepsy
3	Famciclovir	Famvir®, a prodrug of pencyclovir approved by USFDA in September 1997 for treatment of herpes simplex)
4	Oseltamivir phosphate	Tamiflu™, an ethyl ester prodrug of oseltamivir carboxylate inhibitor of influenza A and B, approved by USFDA in October 1999
5	Basalzide disodium	Colazal™, a sulfa-free prodrug of 5 amino salicylic acid approved by USFDA in July 2000 for treatment of moderate active ulcerative colitis
6	Valganciclovir hydrochloride	Valcyte™, an L-valyl ester prodrug of ganciclovir approved by USFDA in march 2001 for the treatment of cytomegalovirus retinitis in AIDS patient.

2.4 Intranasal Drug Delivery

Many drugs are not being effectively and efficiently delivered using conventional drug delivery approach to brain or CNS due to its complexity. The brain and the CNS both have limited accessibility to blood compartment due to a number of barriers. Many advanced and effective approaches to brain delivery of drugs have emerged in recent years. 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/trigeminal pathway 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. One of the first to demonstrate the presence of the olfactory pathway for non-microbial and non-viral agents was Faber (1937), who placed Prussian blue dye in nasal cavity of rabbits and observed the dye in the perineural space of the olfactory nerve and in the subarachnoid space of the brain. 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 some time the BBB has impeded the development of many potentially interesting CNS drug candidates due to their poor distribution into the CNS. Owing to the unique connection of the nose and the CNS, the i.n. route can deliver therapeutic agents to the brain bypassing the BBB (Frey 2002). Absorption of drug across the olfactory region of the nose provides a unique feature and superior option to target drugs to brain (Dominique and Gilles 1993). When administered nasally to the rat, some drugs resulted in CSF and olfactory bulb drug levels considerably higher than those following intravenous administration (Sakane et al. 1991; Sakane et al. 1994; Hussain 1998; Chow et al. 1999; Wang et al. 2006). Direct delivery of a wide variety of therapeutic agents to the CNS following i.n. administration, as well as the therapeutic benefit of i.n. drug treatments, has been demonstrated in mice, rats, primates, and humans by various groups (Thorne et al. 2001; Frey 2002; Born et al. 2002; Dhanda et al. 2005). Table 2.2 gives an overview of some of the recent studies performed on humans. Currently, several drugs are under clinical trials for assessing nose to brain delivery. For instance, i.n. apomorphine is being developed for the treatment of male erectile dysfunction (Kendirci and Hellstrom

2004; Nastech Inc.) and cholecystokinin is undergoing clinical trails for the treatment of obesity.

Many previously abandoned potent CNS drug candidates promise to become successful CNS therapeutic drugs via i.n. delivery. Recently, several nasal formulations, such as ergotamine (Novartis), sumatriptan (GlaxoSmithKline), and zolmitriptan (AstraZeneca) have been marketed to treat migraine (Vyas et al. 2005a). Scientists have also focused their research toward i.n. administration for drug delivery to the brain, especially for the treatment of diseases, such as, AD, PD, dementia, epilepsy, migraine, emesis, depression and erectile dysfunction.

The investigations till date have attracted researchers to place the i.n. drug delivery option under the microscope. Nevertheless, it is imperative to understand the uptake of drug across the nasal mucosa. From a kinetic point of view, nose is a complex organ since three different processes, such as disposition, clearance, and absorption of drugs, simultaneously occur inside nasal cavity. For effective absorption of drugs across nasal mucosa, it is essential to comprehend the nasal anatomy and related physiological features of the nose.

2.4.1 Nasal Anatomy and Physiology of the Nose:

The human nasal cavity extends from the nasal vestibule to the nasopharynx. Internal dimensions of the nasal cavity are 12 cm from the tip of the nose to nasopharynx, 4-5 cm from the floor of the cavity to the cribriform plate (Lang and Vath 1989). The nasal cavity has a total volume of about 15 to 20 mL, and a total surface area of about 150 cm^2, and is divided into two halves of approximately equal size via the septum (Mygind 1979; Mygind and Anggard 1984). The volume of each cavity is approximately 7.5 mL, having a surface area around 75 cm^2 (Mygind and Anggard 1984; Illum 2000). Post drug administration into the nasal cavity, a solute can be deposited at one or more of three anatomically distinct regions, the vestibular, respiratory or olfactory region.

The vestibular region (10-20 cm^2) is located at the opening of nasal passages (Schipper et al. 1991; Mathison et al. 1998) and is responsible for filtering out the air borne particles. It is considered to be the least important of the three regions with regard to drug absorption (Merkus 2001). The respiratory region is the largest (about 130 cm^2) having the highest degree of vascularity and is mainly responsible for drug absorption. The olfactory region

is of about 10 cm^2 in surface area, and it plays a vital role in transportation of drugs to the brain and the CSF. The three distinct anatomical regions present in the nasal cavity and its cross sectional sketch is shown in (Figure 2.2).

The epithelium of the nasal passage is covered by a mucus layer, which entraps particles. The mucus layer is cleared from the nasal cavity by cilia, and is renewed every 10 to 15 min (Chein and Chang 1987). The mucus 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 min. The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 and 6.7 in children (Hehar et al. 1999). Numerous enzymes (Chein and Chang 1985; Chein and Chang 1987; Chein et al. 1989; Reed 1993) for instance, cytochrome P450, enzyme isoforms (CYP1A, CYP2A, and CYP2E), carboxylesterases and glutathione S-transferases are found in nasal cavity (Lewis et al. 1991; Lewis et al. 1994; Krishna et al. 1995).

essential that combinations with other parameters like capillary membrane permeability, first pass metabolism and volume of distribution (Lin and Lu 1997; Van de Waterbeemd et al. 2001; Lewis and Dickins 2002) is to be used.

2. **Prodrugs:** Brain uptake of drugs can be improved via prodrug formation (Bodor and Kaminski1987). Prodrugs are the compounds which are pharmacologically inactive and they undergo metabolic transformation within the body to pharmacologically active compounds (effective drugs). After administration, the prodrug, by virtue of its improved characteristics, is brought closer to the receptor site and is maintained there for longer periods of time. Here it gets converted to the active form, usually via a single activating step. For example, esterification or amidation of hydroxy-, amino-, or carboxylic acid- containing drugs, may greatly enhance lipid solubility and, hence, entry into the brain. Once in the CNS, hydrolysis of the modifying group will release the active compound. Prodrug approach has a slow start initially with certain disappointments but it has gained importance within last decade. Certain prodrugs which have been successfully studied and have reached up to the level of marketable product are shown in Table 2.1 (Bodor and Buchwald 2003).

3. **Chemical Drug Delivery:** Chemical drug delivery systems (CDDS) represent novel and systematic ways of targeting active biological molecules to specific target sites or organs based on predictable enzymatic activation. They are inactive chemical derivatives of a drug obtained by one or more chemical modifications so that the newly attached moieties are monomolecular units (generally comparable in size to the original molecule) and provide a site-specific or site-enhanced delivery of the drug through multi-step enzymatic and/or chemical transformations. Undoubtedly, the concept evolved from the prodrug concept, but became essentially different by the introduction of multi-step activation and target moieties. Brain-targeting chemical delivery systems represent just one of the most developed classes of CDDS. Using the general CDDS concept, successful deliveries have been achieved to the brain, to the eye, and to the lung (Bodor and Buchwald 1997).

4. **Receptor mediated transport:** The receptor mediated transport to the brain is mainly based on the formation of chimeric peptides by conjugation of the non-transportable drugs that has to be delivered, to a transport vector that undergoes BBB-transport via receptor mediated transcytosis (Pardridge 1986) or may be via absorptive-mediated

transcytosis (Tamai et al. 1997). This approach is intended to provide brain delivery of large peptides. Since this approach involves stoichiometry, only limited number of molecules can fit in to this category.

5. **Nanoparticles:** Nanoparticles has been employed as a delivery system for compounds like dalargin, kyotorphin, loperamide and doxorubicin in some animals (Kreuter 2001; Kreuter 2002). The probable mechanism could be endocytic uptake or transcytosis. The particles are usually 10 to 100 nm diameter, made from natural or artificial polymers; drugs are bound in form of solid solution or dispersion.

6. **BBB Disruption:** One of the approaches for enhanced CNS drug delivery involves the systemic administration of drugs in conjunction with transient BBB disruption. Theoretically, with the BBB weakened, systemically administered drugs can undergo enhanced extravasation rates in the cerebral endothelium, leading to increased parenchymal drug concentrations. A variety of techniques that transiently disrupt the BBB have been investigated; however, albeit physiologically interesting, many are unacceptably toxic and therefore not clinically useful. These include the infusion of solvents such as dimethyl sulfoxide or ethanol and metals such as aluminium; X-irradiation; and the induction of pathological conditions including hypertension, hypercapnia, hypoxia or ischemia. The mechanisms responsible for BBB disruption with some of these techniques are not well understood. Another approach is osmotic BBB disruption. Intracarotid injection of hyperosmolar substances like mannitol, arabinose is likely to cause disruption of BBB (Hiesiger et al. 1986; Miller 2002) due to migration of water from endothelial cells to capillaries which in turn cause shrinkage of the cells and results in intracellular gaps. However, osmotic disruption breaks down the self defense mechanism of the brain and leaves it vulnerable to damage or infection from all circulating chemicals or toxins. The other approaches are BBB disruption using use of labradimil which has selectivity for bradykinin 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 (Cho et al. 2002).

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 1991; Brem and Gabikian 2000)/microspheres (Benoit et al. 2000; Bjork and Edman 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:** CSF 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 and is one of the serious drawbacks. Moreover, rapid ventricular CSF clearance renders the delivery system equivalent to slow intravenous infusion.
10. **Intranasal delivery:** Intranasal (i.n.) delivery is being gaining a remarkable importance for CNS targeting. Nasal mucosa is having connection with CNS through intraneuronal or extraneuronal pathways. The drug/formulation applied on the nasal mucosa may follow either intraneuronal or extraneuronal or both pathways (Born et al. 2002; Thorne et al. 2004; Illum 2004).
    (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 extracellular transport within perineuronal channels into CNS.

    The limitations and problems encountered in Nasal deliveries are enzymatic degradation, low pH nasal epithelium, nasal irritation possibilities, large variability in

terms of nasal pathology, large molecular weight of active compounds and low lipophilicity etc. (Behl et al. 1998).

Table 2.2 Studies indicative of nose-to-brain transport in man

• **Functional evidence of facilitated transport to the brain**		
No.	**Drugs**	**Reference number**
1	Arginine-Vasopressin (n=15)	Pietrowsky et al. 1996a
2	Cholecystokinin-8 (n=20)	Pietrowsky et al. 1996b
3	Corticotropin releasing hormone (n==11)	Kern et al. 1997
4	Angiotensin II (n=12)	Dread et al. 1998
5	Growth hormone releasing hormone (n=23)	Perras et al. 1999a
6	Vasopressin (n=26)	Perras et al. 1999b
7	Insulin (n=18)	Kern et al. 1999
8	Adrenocorticotropin 4 -10 (n=54)	Smolnik et al. 1999
9	Adrenocorticotropin 4 -10 (n=60)	Smolnik et al. 2000
10	Insulin (n=12)	Kern et al. 2001
11	Insulin (n=20)	Hallaschimd et al. 2004
12	Insulin (n=38)	Benedict et al. 2004
13	Insulin (n=61)	Reger et al. 2006
• **Direct evidence of nose-to-brain uptake**		
14	99mTc-DPTA-hyaluronidase (n=2)	Okuyama 1997
• **Direct evidence of nose of CSF uptake**		
15	Insulin (n=8)	Fehm et al. 2000
16	Adrenocorticotropin 4 -10 (n=5)	Fehm et al. 2001
17	Apomorphine (n=5)	Quay 2001
18	Melanocortin/Vasopressin/Insulin (n=36)	Born et al. 2002
19	Melatonin/hydroxycobalamin (n=2)	Merkus 2003
20	Melanocortin(4-10) (n=13)	Hallaschimd et al. 2006

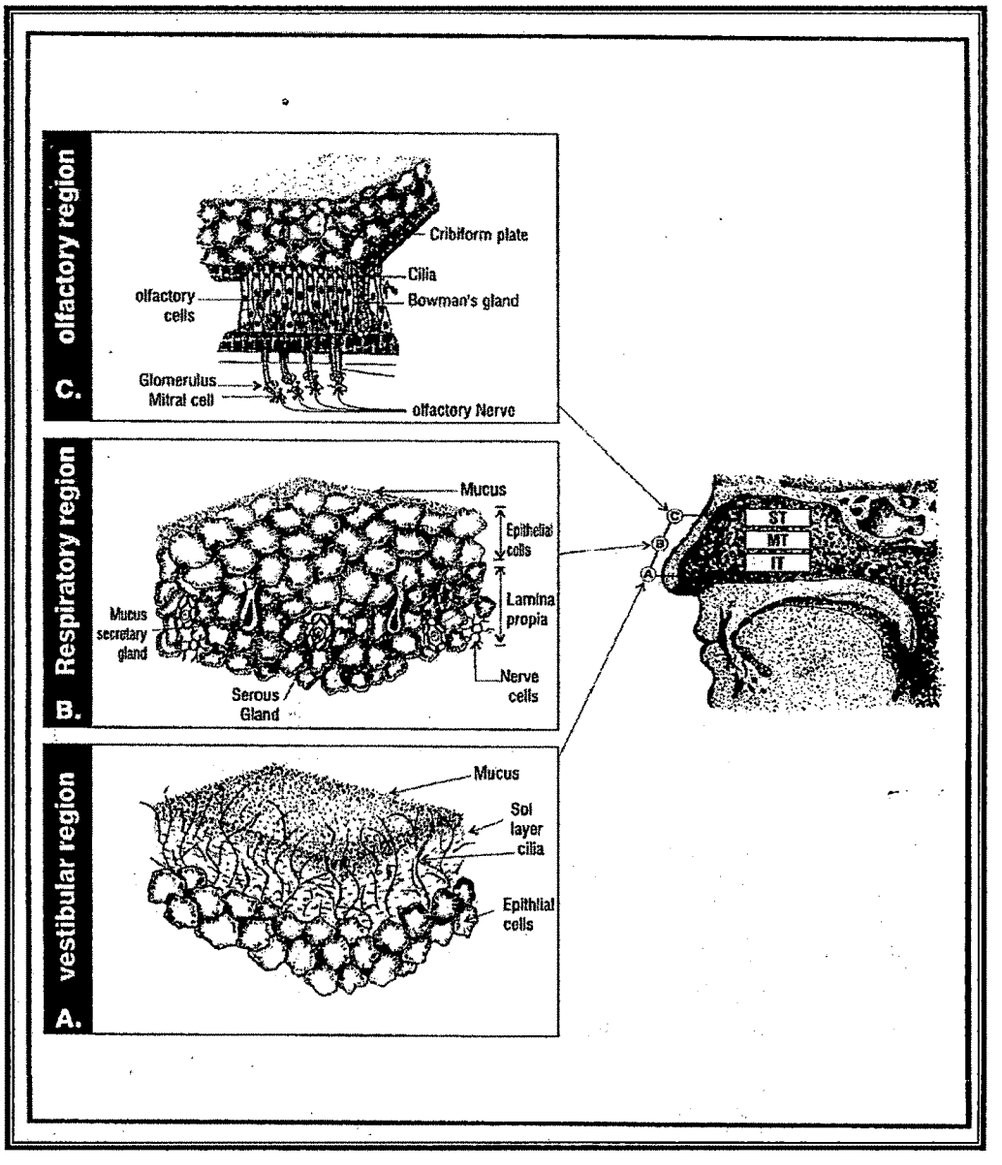


Figure 2.2 Anatomy of nose: cross sectional sketch illustrating (A) the vestibular, (B) the respiratory, and (C) the olfactory region.

2.4.2 Intranasal Drug Delivery – Advantages/Disadvantages:

Intranasal drug delivery is a non-invasive route of administration and offers several advantages such as rapid absorption and bioavailability profiles identical to intravenous administration. Nasal delivery route is convenient, patient friendly, and also prevents risk of gastrointestinal tract irritation. Furthermore, drugs delivered through the intranasal route, also to some extent, avoid a systemic dilution effect and first pass metabolism (Vyas et al. 2005a). Moreover, it offers self medication options to manage emergency situations (Lianli et al. 2002). Intranasal drug delivery delivers the drug directly to the brain by circumventing BBB and reduces drug delivery to non targeted sites. Direct transport of could result rapid and/or higher uptake in brain. This may lead to the administration of lower doses and in turn can reduce toxicity. However, the few limitations of i.n. delivery are low dose/volume especially when compounds have less aqueous solubility are difficult to formulate. High lipophilicity and preferably low molecular weight of drug are the prerequisites as it could influence the uptake across nasal mucosa. The other drawbacks associated with i.n. drug delivery that have to be overcome includes active degradation or alteration by enzyme, low pH of nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold. Drug compounds devoid of offensive/pungent odor/aroma and non-irritant nature are highly desirable to facilitate dosage form design for i.n. drug delivery systems.

2.4.3 Mechanisms of Transnasal Transport to the Brain:

It is important to examine the pathway/mechanisms (Fisher et al. 1985; Wheatley et al. 1988; Tengamnuay and Mitra 1988) involved prior to addressing the possibilities to improve transnasal uptake by the brain. After i.n. administration drug can reach the brain via multiple transport pathways (Figure 2.3), including: (1) olfactory neuronal pathway, (2) extraneuronal olfactory epithelial pathway, (3) trigeminal nerve pathway, and (4) systemic pathway (Mathison et al. 1998; Thorne et al. 2004).

In the olfactory neuronal pathway drug is internalized into the olfactory neurons, followed by axonal transport to the brain. In the extraneuronal olfactory epithelial pathway, the drug reaches the brain parenchymal tissue, and/or CSF by the extracellular routes, most likely perineural channels (Thorne and Frey 2001). The transport of dugs via

the olfactory nerve pathway is slow (drugs take hours/days to reach the brain) when compared to the extraneuronal olfactory epithelial pathway wherein the drug reaches the brain within minutes (Thorne et al. 1995; Thorne and Frey 2001). From drug distribution studies, it appears that drug traveling along the olfactory neural pathway distributes into rostral brain structures including the olfactory bulb, anterior olfactory nucleus, frontal cortex, and hippocampus. In addition, drug traveling along the trigeminal nerve distributes into caudal brain structures including upper cervical spinal cord, midbrain, pons, and hypothalamus (Hanson and Frey 2007). The trigeminal nerve innervates the nasal mucosa as well and has been shown to provide an additional pathway for nose-to-brain drug delivery. Olfactory epithelial deposition may also involve drug delivery via the trigeminal nerve, although there is no data at present to support this mechanism Although, evidence exists for the direct delivery of drugs to the brain via the olfactory and trigeminal pathways, the overall mass transport to the brain for each pathway has yet to be elucidated. The systemic pathway involves entry of the drug into the blood circulation from various regions of the nose and subsequent passage across the BBB. The first three pathways are direct pathway to CNS from nose, while the fourth pathway constitutes an indirect pathway via the blood circulation. Following drug administration by i.n. route, one of more of the aforementioned pathways may simultaneously contribute to the delivery of drugs to the CNS. Designing approaches to optimize direct nose-to-brain delivery while minimizing drug entry into the blood would be beneficial for drugs with systemic side effects.

The olfactory region is well known to be the portal for a drug substance to enter from nose-to-brain following nasal absorption and transport across the olfactory epithelium is the predominant concern for brain targeted i.n. delivery. Nasal mucosa and subarachnoid space; lymphatic plexus located in nasal mucosa and subarachnoid space along with perineural sheaths in olfactory nerve filaments and subarachnoid space appears to have communications between them. A drug can cross the olfactory path by one or more mechanism/pathways (Fisher et al. 1987; Jones et al. 1997). These include paracellular transport by movement of drug through interstitial space of cells, transcellular or simple diffusion across the membrane or receptor/fluid phase mediated endocytosis and transcytosis by vesicle carrier (McMartin et al. 1987) and neuronal transport. These three mechanisms mentioned are described in this section (below).

The paracellular transport mechanism/route is slow and passive. It mainly uses an aqueous mode of transport. Usually, the drug passes through the tight junctions and the open clefts of the epithelial cells present in the nasal mucosa. There is an inverse log-log correlation between i.n. absorption and the molecular weight of water-soluble compounds. Compounds which are highly hydrophilic in nature and/or low molecular weight are most appropriate for paracellular transport. A sharp reduction in absorption and poor bioavailability were observed for the drugs having molecular weight greater than 1000 Da (McMartin et al. 1987; Chein et al. 1987). Moreover, drugs can also cross cell membranes by a carrier-mediated active transport route.

The transcellular transport mechanism/pathway (Illum 2003; Illum 2000) mainly encompasses transport via a lipoidal route. The drug can be transported across the nasal mucosa/epithelium by either receptor mediated endocytosis or passive diffusion or fluid phase endocytosis. Small lipophilic compounds or larger molecules usually are transported by a transcellular route. The transport across nasal mucosa is mainly a function of the lipophilic nature of a drug compound. Highly lipophilic drugs are expected to have rapid/complete transnasal uptake.

Potential nose-to-brain transport pathways followed by several drug molecules are recorded in Table 2.3 and possible drug transport routes are depicted in Figure 2.4.

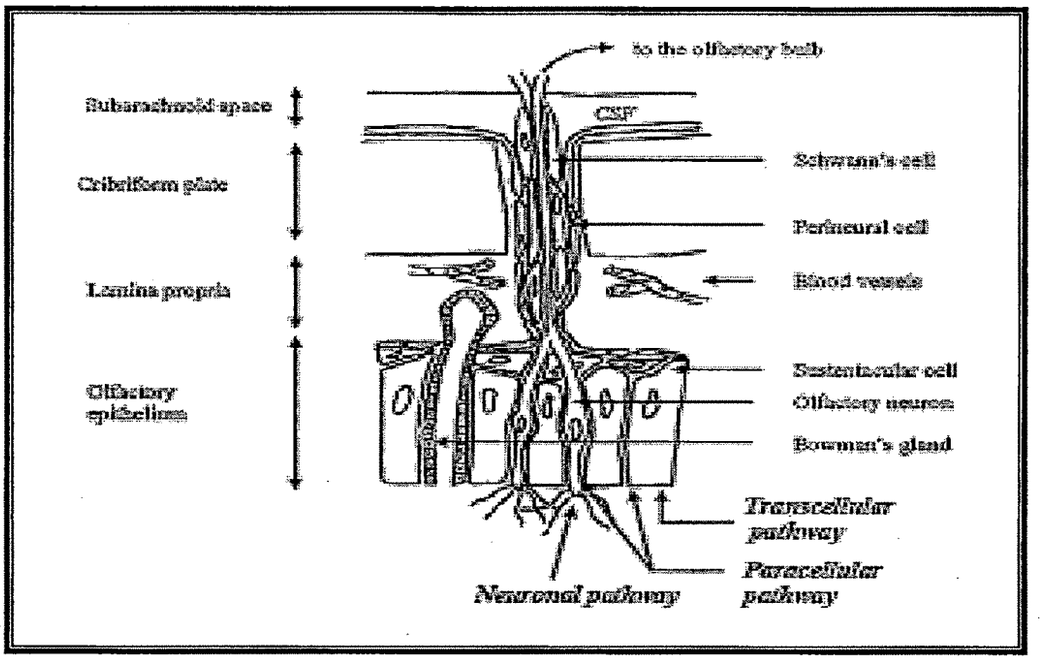


Figure 2.3 Mechanisms of Transnasal Transport to the Brain.

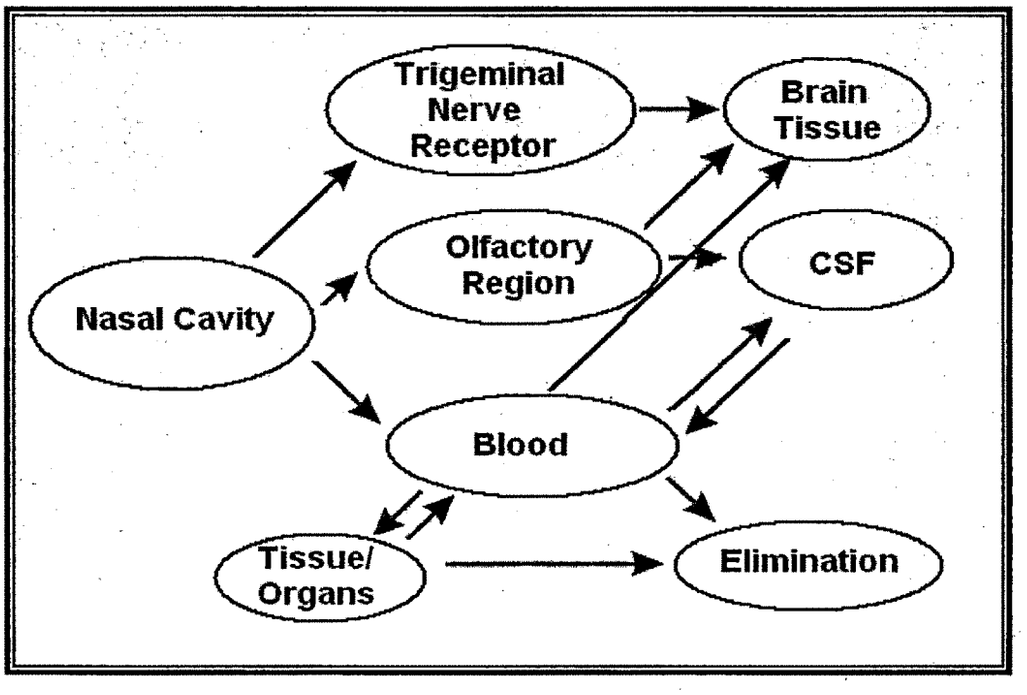


Figure 2.4 Possible transport routes: nasal mucosa to brain/CNS.

Table 2.3 Nose-to-brain transport of drug molecules and possible pathways

Pathways	Molecules
Nasal mucosa→sensory nerve cells of olfactory epithelium→subarachnoid space→blood stream	Albumin
Nasal mucosa→olfactory nerve fiber	Amino acids
Nasopharyngeal epithelium→lymphatic→cervical lymphatic vessel→blood vessel	Rabbit virulent type III Pneumococci
Nasal mucosa→cerebrospinal fluid and serum	Dopamine, Estradiol
Nasal mucosa→olfactory neurons→brain and CSF	Estradiol, Neutropic virus and poliomyelitis virus.
Nasal membrane→olfactory dendrites→nervous system→supporting cells in the olfactory mucosa→sub mucosal blood vascular system	Norethisterone, Progesterone
Nasal membrane→peripheral circulation and CSF→CNS	Norethisterone
Nasal mucosa→peripheral and cranial nerves→CNS	Herpes virus encephalitis
Nasal mucosa→cranial nerve→CNS	Herpes virus simplex
Nasal mucosa→ trigeminal and olfactory pathways→CNS	Mouse passage strain of herpes virus
Nasal mucosa→sub mucous lymphatic→cervical lymphatic pathway→CNS	Vaccina virus
Nasopharynx→cervical lymph	Water

2.4.4 Factors Affecting Brain-Targeted Nasal Delivery Systems:

In addition, dosage form design also plays a key role in altering pharmacokinetics and bioavailability following i.n. administration. Figure 2.5 illustrates different delivery systems, devices and various dosage forms for i.n. drug delivery.

Some of the physicochemical, formulation and physiological factors are imperative and must be considered prior to designing i.n. delivery for brain targeting. As shown in Figure 2.6, some of the physicochemical factors are chemical form (Huang et al. 1985), polymorphism, particle size, solubility and most importantly molecular weight (Agarwal and Mishra 1999; Corbo et al. 1989). Moreover, several other factors like formulation factors (Dahlin and Bjork 2000) and physiological factors (Kroll et al. 1998; Morimoto et al. 2001; Schipper et al. 1995) are also having decisive repercussion on the *in vivo* result/performance of the product and in turn influence the uptake of drug at targeted site. Some of the imperative physicochemical, formulation and biological factors are described.

2.4.4.1 Physicochemical properties of drugs:

Chemical form

The chemical form of a drug is important in determining absorption. For example, conversion of the drug into a salt or ester form can alter its absorption. Huang et al. (1985) studied the effect of structural modification of drug on absorption. It was observed that *in-situ* nasal absorption of carboxylic acid esters of L-Tyrosine was significantly greater than that of L-Tyrosine.

Polymorphism

Polymorphism is known to affect the dissolution rate and solubility of drugs and thus their absorption through biological membranes. It is therefore advisable to study the polymorphic stability and purity of drugs for nasal powders and/or suspensions.

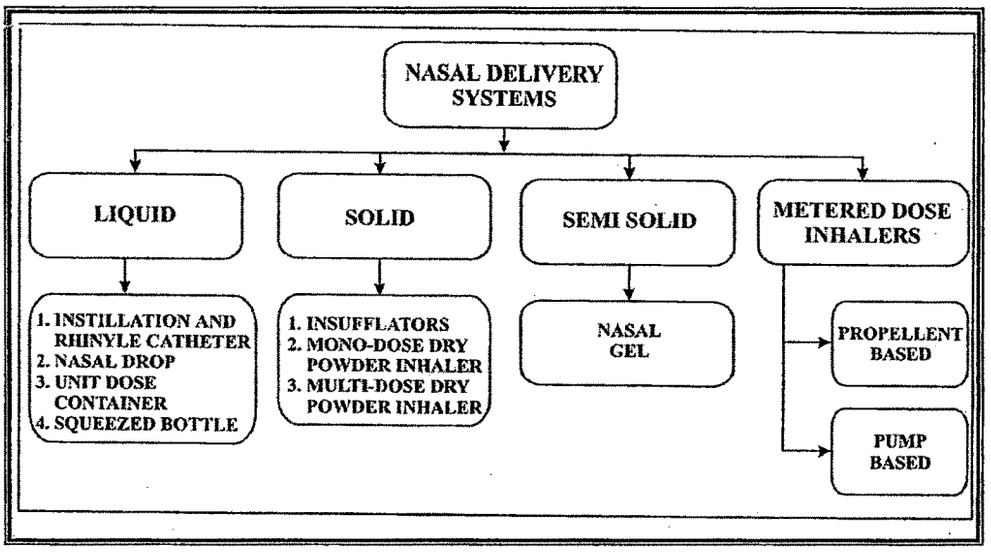


Figure 2.5 Nasal Delivery devices and dosage forms

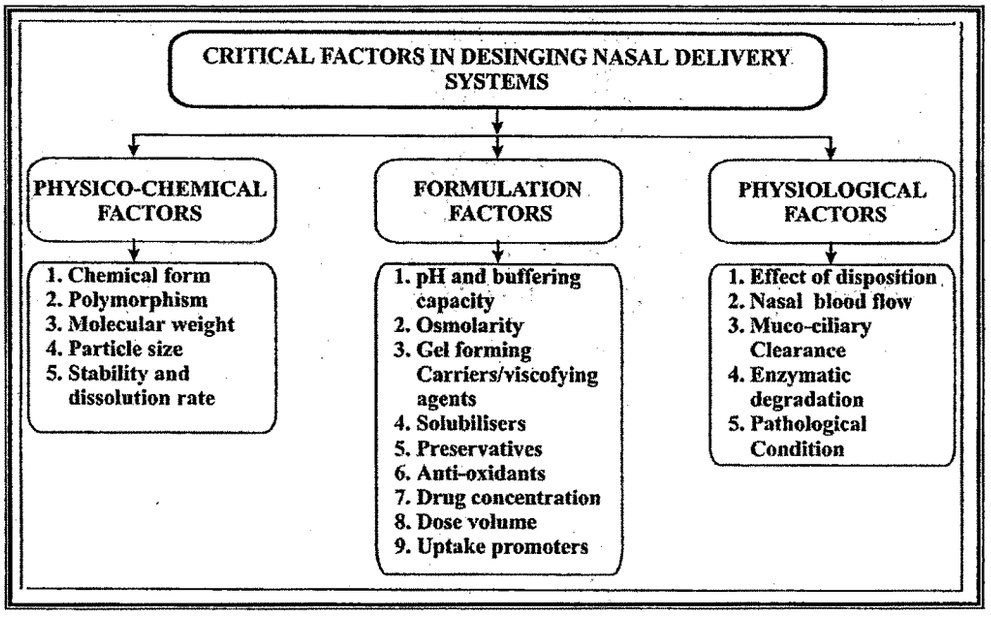


Figure 2.6 Critical physicochemical factors need to be considered prior to designing intranasal drug delivery systems

Molecular Weight

A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 Da. Absorption decreases significantly if the molecular weight is greater than 1000 Da except with the use of absorption enhancers. Nasal drug absorption is affected by molecular weight, size, formulation pH, pKa of molecule, and delivery volume among other formulation characteristics. Molecular weight still presents the best correlation to absorption (Fisher et al. 1987). The apparent cut-off point for molecular weight is approximately 1,000 Da, with molecules less than 1,000 Da having better absorption. Shape is also important. Linear molecules have lower absorption than cyclic-shaped molecules. Additionally, particles should be larger than 10 μm, and otherwise the drug may be deposited in the lungs (Jones et al. 1997). Hydrophilicity has also been found to decrease drug bioavailability (Corbo et al. 1989).

Particle Size

It has been reported that particle sizes greater than 10 μm are deposited in the nasal cavity. Particles that are 2 to 10 μm can be retained in the lungs and particles of less than 1 μm are exhaled (Fry and Black 1973; Chein and Chang 1987).

Solubility & Dissolution Rate

Drug solubility and dissolution rates are important factors in determining nasal absorption from powders and suspensions. The particles deposited in the nasal cavity need to be dissolved prior to absorption. If a drug remains as particles or is cleared away, no absorption occurs.

2.4.4.2 Formulation factors:

pH of the formulation

Another formulation factor important for absorption is pH. Both the pH of the nasal cavity and pKa of a particular drug need to be considered to optimize systemic absorption. Nasal irritation is minimized when products are delivered with a pH range of 4.5 to 6.5 (Conley 1994). Also, volume and concentration is important to consider. The delivery volume is limited by the size of the nasal cavity. An upper limit of 25 mg/dose and a volume of 25 to 150 μL/nostril have been suggested.

The pH of a nasal formulation is important for the following reasons:

- To avoid irritation of nasal mucosa;

- To allow the drug to be available in unionized form for absorption;
- To prevent growth of pathogenic bacteria in the nasal passage;
- To maintain functionality of excipients such as preservatives; and
- To sustain normal physiological ciliary movement.

Lysozyme is found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection (Thompson 1940). It is therefore advisable to keep the formulation at a pH of 4.5 to 6.5 keeping in mind the physicochemical properties of the drug as drugs are absorbed in the unionized form.

Buffer Capacity

Nasal formulations are generally administered in small volumes ranging from 25 to 150 µL with 100 µL being the most common dose volume. Hence, nasal secretions may alter the pH of the administrated dose. This can affect the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH *in-situ*.

Osmolarity

Drug absorption can be affected by tonicity of the formulation. Shrinkage of epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity. Low pH has a similar effect as that of a hypertonic solution.

Gelling/Viscosity building agents or gel-forming carriers

Pennington et al. (1988) studied that increase in solution viscosity may provide a means of prolonging the therapeutic effect of nasal preparations. Suzuki and Makino (1999) showed that a drug carrier such as hydroxypropyl cellulose was effective for improving the absorption of low molecular weight drugs but did not produce the same effect for high molecular weight peptides. Use of a combination of carriers is often recommended from a safety (nasal irritancy) point of view.

For gelling to occur in the nasal cavity with a liquid composition comprising an excipient which gels in the presence of ions, such as pectin or gellan gum (Achari et al 2002), it is likely to be necessary to add monovalent and/or divalent cations to the composition so that it is close to the point of electrolyte induced gelation. When such a composition is administered to the nasal cavity, the endogenous cations present in the nasal fluids will

cause the mobile liquid composition to gel. In other words, the ionic strength of the composition is kept sufficiently low to obtain a low viscosity formulation that is easy to administer, but sufficiently high to ensure gelation once administered into the nasal cavity where gelation will take place due to the presence of cations in the nasal fluids.

Solubilizers

Aqueous solubility of drug is always a limitation for nasal drug delivery in solution. 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 (Gattefosse bulletin 1997). Other options include the use of surfactants or cyclodextrins such as HP–β-cyclodextrin that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers. In such cases, their impact on nasal irritancy should be considered.

Preservatives

Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride (Hellan and Graf 1995; Bernstein 2000), phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations (Hillardal 1985). Van De Donk et al. (1980; 1982) have shown that mercury-containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in nasal systems.

Antioxidants

A small quantity of antioxidants may be required to prevent drug oxidation. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxyl toluene and tocopherol. Usually, antioxidants do not affect drug absorption or cause nasal irritation. Chemical/physical interaction of antioxidants and preservatives with drugs, excipients, manufacturing equipment and packaging components should be considered as part of the formulation development program.

Humectants

Many allergic and chronic diseases are often connected with crusts and drying of mucous membrane. Certain preservatives/ antioxidants among other excipients are also likely to cause nasal irritation especially when used in higher quantities. Adequate i.n. moisture is essential for preventing dehydration. Therefore, humectants can be added especially in

gel-based nasal products. Humectants avoid nasal irritation and are not likely to affect drug absorption. Common examples include glycerin, sorbitol and mannitol.

Drug Concentration, Dose & Dose Volume

Drug concentration, dose and volume of administration are three interrelated parameters that impact the performance of the nasal delivery performance. Nasal absorption of L-Tyrosine was shown to increase with drug concentration in nasal perfusion experiments. However, in another study (Hirai et al. 1981), Aminopyrine was found to absorb at a constant rate as a function of concentration. In contrast, absorption of salicylic acid was found to decline with concentration. This decline may be attributed to permanent damage of nasal mucosae cells.

Role of Absorption Enhancers

In typical scenarios where desired absorption profile is not attained by the nasal product, the use of absorption enhancers is recommended. The selection of absorption enhancers is based upon their acceptability by regulatory agencies and their impact on the physiological functioning of the nose. Absorption enhancers may be required when a drug exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by aminopeptidases.

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

- Inhibit enzyme activity;
- Reduce mucus viscosity or elasticity;
- Decrease mucociliary clearance;
- Open tight junctions; and
- Solubilize or stabilize the drug.

Absorption enhancers are generally classified as physical and chemical enhancers. Chemical enhancers act by destructing the nasal mucosa very often in an irreversible way, whereas physical enhancers affect nasal clearance reversibly by forming a gel. The enhancing effect continues until the gel is swallowed. Examples of chemical enhancers are chelating agents, fatty acids, bile acid salts, surfactants, and preservatives. Osmolarity and pH may accelerate the enhancing effect.

One major area of focus has been the incorporation of absorption enhancers to increase bioavailability. Examples of enhancing agents are surfactants, glycosides, cyclodextrins,

and glycols. Absorption enhancers improve absorption through many different mechanisms, such as increasing membrane fluidity, increasing nasal blood flow, decreasing mucus viscosity, and enzyme inhibition. A classic example of a polypeptide compound with low (~3%) nasal bioavailability is calcitonin. Calcitonin has 32 amino acids in length and is approximately 3,500 Da (Morimoto et al. 2001; Novartis Pharma Inc. 1999), when given i.n. to rats and rabbits using a number of different cyclodextrins, its absorption, as measured by decrease in serum calcium concentrations, was significant in comparison to the formulation without additive and thus, demonstrating the usefulness of absorption enhancers (Schipper et al. 1995).

2.4.4.3 Physiological factors:

Effect of Deposition on Absorption

Deposition of the formulation in the anterior portion of the nose provides a longer nasal residence time. The anterior portion of the nose is an area of low permeability while posterior portion of the nose, where the drug permeability is generally higher, provides shorter residence time (Arora et al. 2002). The method of administration and properties of the formulation determine the deposition site.

Nasal blood flow

Nasal mucosal membrane is very rich in vasculature and plays a vital role in the thermal regulation and humidification of the inhaled air (Kroll et al. 1998). Turbinate and septum has dense network of erectile cavernous tissues. The network is rich in vasculature and it is excellent membrane for drug absorption. The blood flow and therefore the drug absorption will depend upon the vasoconstriction and vasodilatation of the blood vessels.

Effect of Mucociliary Clearance

It is important that the integrity of the nasal clearance mechanism is maintained to perform normal physiological functions such as the removal of dust, allergens and bacteria. The ciliary activity is the driving force of the secretory transport in the nose to constantly remove particles that are trapped on the mucus blanket during inhalation (Figure 2.7). The absorption of drugs is influenced by the residence (contact) time between the drug and the epithelial tissue. The mucociliary clearance is inversely related to the residence time and therefore inversely proportional to the absorption of drugs administered (Martin et al. 1998). A prolonged residence time in the nasal cavity may

also be achieved by using bioadhesive polymers like chitosan and polycarbophil or by increasing the viscosity of the formulation (Ugwoke et al. 2001). Nasal mucociliary clearance can also be stimulated or inhibited by drugs, excipients, preservatives and/or absorption enhancers and thus affect drug delivery to the absorption site (Martin et al. 1997).

Effect of Enzymatic Activity

Several enzymes that are present in the nasal mucosa might affect the stability of drugs. For example, proteins and peptides are subjected to degradation by proteases and amino-peptidase at the mucosal membrane. The level of amino-peptidase present is much lower than that in the gastrointestinal tract. Peptides may also form complexes with immunoglobulin (Igs) in the nasal cavity leading to an increase in the molecular weight and a reduction of permeability (Mathison et al. 1998).

Effect of Pathological Condition

Intranasal pathologies such as allergic rhinitis, infections, or previous nasal surgery may affect the nasal mucociliary transport process and/or capacity for nasal absorption. During the common cold, the efficiency of an i.n. medication is often compromised. Nasal clearance is reduced in insulin-dependent diabetes. Nasal pathology can also alter mucosal pH and thus affect absorption of drugs.

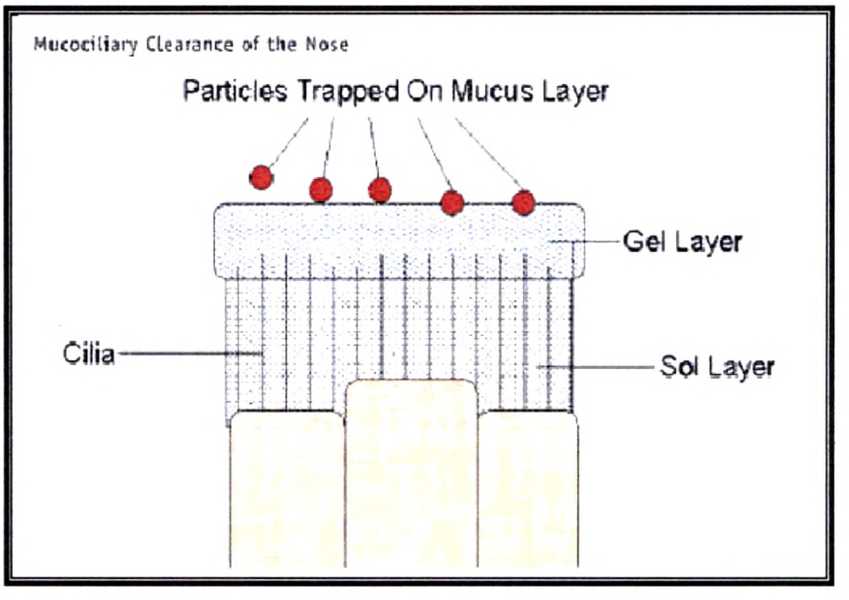


Figure 2.7 Mucociliary clearance of the nose

2.4.5 Approaches for Drug Deposition in the Olfactory Epithelium:

As discussed above the deposition of drug in the nasal cavity greatly influence the drug absorption. For i.n. administered drugs, it is desired to get deposited in the olfactory epithelium/olfactory mucosa to reach the brain. The olfactory epithelium is located at the very top of the nasal cavity under the cribriform plate of the ethmoid bone. Due to restrictive nasal anatomy, only a small fraction of the inhaled air reaches the olfactory region (Dhanda et al. 2005). However, odorants can reach the olfactory mucosa due to their volatile nature and high diffusivities. Airborne solutes as well as drug administered in conventional nasal sprays are less likely to access the olfactory region due to their nonvolatile nature and low diffusivities. Therefore, there is a need to develop novel delivery systems for the olfactory deposition of drugs. Table 2.4 lists some of novel strategies and devices for deposition in the olfactory epithelium after i.n. administration.

Table 2.4 Approaches for drug deposition in the olfactory epithelium

Strategy/Device (Company)	Description	Advantages	Disadvantages	References
Particle Orientation: Instill drop/spray in the Praying-to-Mecca position	The patient kneels and bends forward on the floor with the top of the head touching the ground and nose facing upwards.	Deposition in the central regions and potentially in the olfactory region. Little or no exposure to the floor of the nasal cavity	Aukward patient position	Kubba et al. 2000; Benninger et al. 2004
Device: Optinose (Optinose, Inc.)	Bi-directional nasal delivery technique	Maximizes nasal deposition while minimizing lung deposition. Minimizes taste disturbances.	Use may be difficult for patients with breathing disorders	Djupesland 2004
Device: DirectHaler (DirectHaler A/S, Denmark)	Bi-directional technique for dry powders	Prevents lung deposition. Minimizes taste disturbances.	Not suitable for administration of liquid aerosols. Use may be difficult for patients with breathing disorders.	www.directhaler.com
Device: ViaNase (Kurve Technologies)	Controlled particle dispersion technology	Delivery to all nasal areas including paranasal sinus and olfactory region. Clearance of particles from nose is claimed to be slower	The actual technological principles are unclear.	www.kurvetech.com/pdf/ViaNaseDataSheet4.pdf

Table 2.4 Approaches for drug deposition in the olfactory epithelium (contd...)

Strategy/Device (Company)	Description	Advantages	Disadvantages	References
Device	Solid-state chip-based ink-jet dispersion technology	Can be used for dispensing a mixture of drugs	Yet to be fully developed.	Hayes et al. 2003
Device: Iontophoresis/ Phonophoresis (Electro-transport with chemical permeation enhancers)	-	Efficient control of the rate of drug delivery	Discomfort and local adverse effects due to electrical, thermal, and mechanical damage are possible.	Lerner 2002
Particle Size	10-30 μm particles/ droplets <10 μm particles/ droplets <10 nm particles/ droplets	Deposited almost completely in the nose. Greater deposition in the posterior regions of the nose. Greater nasal deposition due to turbulent diffusion.	Deposited primarily in the anterior portion of the nose. Lung deposition increases with reducing size.	Cheng et al. 1991; Keck et al. 2000
Spray Pattern/ Plume angle	Narrow spray plume angles	Deeper deposition within the nose	-	Cheng et al. 2001

2.4.6 Nasal Dosage Forms:

Due to typical anatomy and physiology of the nasal cavity, with non-ciliated part of nasal cavity and a ciliated region in the more posterior part of the nose, the site of deposition is extremely important for mucociliary clearance and in turn resident time of the formulation in nose; the most critical parameter for drug absorption. The deposition and deposition area are mainly a function of delivery system and delivery device. It predominantly affects many factors such as mode of administration, particle size of the formulation, velocity of the delivered particles, spray angle and cone.

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

2.4.6.1 Liquid dosage forms:

Nasal Emulsions & Ointments

Nasal emulsions and ointments have not been studied in detail as other nasal delivery systems. They offer 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.

Specialized Delivery System

Microsphere technology is one of the specialized systems becoming popular for designing nasal products. Microspheres may provide more prolonged contact with the nasal mucosa and thus enhance absorption. Microspheres for nasal applications have been prepared using biocompatible materials, such as starch, hyaluronic acid ester (Illum et al. 1994a), albumin, dextran and gelatin (Morimoto et al. 2001; Bjork and Edman 1990) however, their toxicity/irritancy should be evaluated. It was hypothesized (Edman et al. 1992) that in the presence of starch microspheres, the nasal mucosa is dehydrated due to moisture uptake by the micro spheres. This results in reversible "shrinkage" of the cells, providing a temporary physical separation of the tight (intercellular) junctions that increases the absorption of drugs.

Nasal Drops

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

Nasal Sprays

Both solution and suspension formulations can be formulated into nasal sprays. Due to the availability of metered dose pumps and actuators, a nasal spray can deliver an exact dose from 25 to 150 µL. The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly.

2.4.6.2 Semi solid dosage forms:

Nasal Gels

Nasal gels are high-viscosity thickened solutions or suspensions. Until the recent development of precise dosing devices, there was not much interest in this system. The advantages of a nasal gel include the reduction of post-nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients and target delivery to mucosa for better absorption. Vitamin B_{12} gel has been recently developed as a prescription product.

2.4.6.3 Solid dosage forms:

Nasal Powders

This dosage form may be developed if solution and suspension dosage forms cannot be developed e.g., due to lack of drug stability. The advantages to the nasal powder dosage form are the absence of preservative and superior stability of the formulation. However, the suitability of the powder formulation is dependent on the solubility, particle size, aerodynamic properties and nasal irritancy of the active drug and/or excipients. Local application of drug is another advantage of this system but nasal mucosa irritancy and metered dose delivery are some of the challenges for formulation scientists and device manufacturers.

2.4.7 Delivery of Proteins/Peptides to CNS through the Nose:

Investigational studies in human have provided evidence of direct delivery of macromolecules to the CNS following nasal administration. CNS effects of intranasal corticotropin-releasing hormone (CRH) without altering plasma cortisol or CRH levels have been demonstrated (Kern et al. 1997). Perras et al. (1999a) have reported that intranasal delivery of growth hormone-releasing hormone (GHRH) not only increased rapid eye movement sleep and slow wave sleep in humans, but also decreased growth hormone.

The efficacy of peptide/protein following nasal administration is highly dependent on the molecular structure and size of the drugs. Respiratory epithelial cells are capable of absorbing peptide/protein by a vesicular transport mechanism, which is then transferred to the extracellular spaces, and subsequently taken up by the submucosal vascular network (Stratford and Lee 1986). In recent studies, intranasal administration of wheat germ agglutinin horseradish peroxidase resulted in a mean olfactory bulb concentration in the nanomolar range. Vajdy and O'Hagan (2001) reported that after nasal administration of DNA plasmids, the level of plasmid in the brain was 3.9 to 4.8 times higher than the plasmid concentration in the lungs and spleen. It was also found that the plasmid DNA reached the brain within 15 min following intranasal administration (Oh et al. 2001). The higher distribution of plasmid to the brain after intranasal administration indicates that nasal administration might be a promising route for the delivery of therapeutic genes to the brain with reduced side-effects in the other organs. Recent evidence of direct nose-to-brain transport (Illum 2004) and direct access to CSF of three neuropeptides bypassing the bloodstream has been demonstrated in human trials, despite the inherent difficulties in delivery (Born et al. 2002).

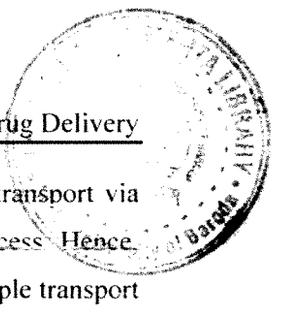
Lemiale et al. (2003) have studied the enhanced mucosal immunoglobulin response of intranasal adrenoviral vector human immunodeficiency virus vaccine and it's localization in CNS. Biodistribution of recombinant adrenovirus (rADV) vectors administered through intranasal route revealed infection of CNS, specifically in the olfactory bulb, possibly via retrograde transport by olfactory neurons in nasal epithelium. Dragphia et al. (1995) have demonstrated gene delivery in rat CNS via nasal instillation. It was noticed that mitral cells from olfactory bulb, locus coeruleus and area postrema expressed β-galactocidase for 12 days and could be useful for gene therapy of disease affecting different CNS structures.

Liu et al. (2001) have investigated intranasal administration of insulin like growth factor-I (IGF-I) circumvent the BBB and protects against focal cerebral ischemic damage. The study confirmed that IGF-I does not cross BBB efficiently however, can be delivered to brain directly by intranasal administration.

2.4.8 Delivery of Non-Peptide Molecules to the CNS:

Many small molecules have been shown to be transported directly to the brain and/or CSF from the nasal cavity. The properties of small molecules, including size and lipophilicity affect delivery to the CNS following intranasal delivery (Kublik and Vidgren 1998; Wermling et al. 2003). Small molecular drugs, such as cocaine (Chow et al. 1999) and benzoylecgonine (Chow et al 2001), local anesthetics (Bagger and Bechgaard 2004), dihydroergotamine (Aelling et al. 1986) and dopamine (Dahlin et al. 2000) have been shown to reach the CNS via the olfactory pathway in animals. This has been reviewed by Illum (2004) and Mathison et al. (1998). Dorman et al. (2002) have studied the olfactory transport of inhaled manganese phosphate into rat brain. The study concluded that olfactory route contributes to manganese delivery to the rat olfactory bulb and tubercle. Anand Kumar et al. (1974) and David et al. (1981) have demonstrated intranasal delivery of estrogen and progesterone respectively, to the CSF. Considerable efforts have been made by the scientists in exploring the prospects for brain targeting following intranasal administration. Sakane et al. (1999) have investigated the transportation of 5-fluorouracil (5-FU) following intranasal, nasal perfusion and intravenous drug delivery. The results revealed that the concentration of 5-FU was significantly higher in cerebral cortex following intranasal administration in cerebral cortex. A significant amount of 5-fluorouracil was transported across nasal cavity to the brain via CSF. It was also concluded that intranasal delivery of hydrophilic drug to brain is practical. Char et al. (1992) have evaluated the potential of intranasal delivery of [14C] dextromethorphan hydrochloride (DM) in rat brain. The study revealed uptake of DM in brain following intranasal route was 65.9% when compared to intravenous route. It was documented that the nasal route is a viable alternative to the parenteral route for DM administration.

In mice, it is recently shown that dopamine reached the right olfactory bulb after nasal administration into the right nostril and after 4 h the concentration in the right olfactory bulb was 27 times higher than the left olfactory bulb (Dahlin et al. 2000). Following intravenous administration, the uptake into brain was low. Moreover, micro radiography of the olfactory region of the rat showed the presence of drug molecules along the



olfactory neuron bundles. This can either indicate transneural transport or transport via CSF surrounding the bundles. However, transneural transport is a slow process. Hence, the drug might be crossing the olfactory region by means of one and/or multiple transport mechanisms to reach into CSF and olfactory bulb.

In another study (Wang et al. 2003), Methotrexate has shown preferential transfer into parts of the CNS directly from the nasal cavity compared to intravenous administration. The author concluded that the olfactory epithelium and the olfactory bulb were the essential gateways for this direct pathway and that the methotrexate after a single intranasal administration has a promising and durable therapeutic effect against certain CNS tumors.

Bergstrom et al. (2002) have also reported the transport of picolinic acid along the olfactory pathways after administration via intranasal and intravenous routes in mice. Autoradiography demonstrated rapid uptake of radioactivity in the olfactory nerve layer and in the ipsilateral olfactory bulb following intranasal administration. The study also suggested that intact neuroepithelium is a prerequisite for uptake of picolinic acid in olfactory bulb. Picolinic acid meets the structural requirement and transfers along the olfactory pathways to brain. Increased interest in brain targeting of drugs having limited ability to pass through the BBB was also documented. A study carried out by Jansson and Bjork (2002), *in vivo* olfactory uptake and transfer using fluorescein-dextran (FD3) was demonstrated and visualized. The study showed transcellular absorption across olfactory epithelium after the intranasal administration of 3kDa FD3. Significant uptake by olfactory bulb was also noticed within 15 min. FD3 transfer in connective tissue surrounding the olfactory nerve bundles to the olfactory bulb of brain was also evidenced. The study concluded higher amounts were found in turbinate as compared to nasal septum. Studies have also shown that drugs such as L-NAME (Sippel et al. 1999) and cocaine (at the lower end of the lipophilicity scale) (Chow et al. 1999) have a higher CSF and olfactory bulb concentration after nasal administration than that obtained after parenteral administration. Sakane et al. (1991) reported that following intranasal administration of the antibiotic cephalexin to rats, higher CSF concentration was reached at 15 min., but it declined to approximately half that concentration at 30 min. Because cephalexin does not cross the BBB well and because CSF concentration was 166-fold higher after intranasal administration than after systemic administration in spite of similar blood levels, it was concluded that cephalexin entered the CSF directly from the nasal

cavity. Using a series of fluorescein isothiocyanate-labeled dextrans (FITC-dextran) with increasing molecular weights, it was found that dextrans with molecular weights of up to 20,000 Da could be transported directly from the nasal cavity of rats into the CSF (Chen et al. 1998). The concentration of the FITC-dextrans in the CSF increased with decreasing molecular weight. These FITC-dextrans were not found in the CSF after intravenous administration. Similarly, a comparison of the brain olfactory bulb concentrations achieved 30 min after intranasal administration of 7.4 nM dopamine (153 Daltons) with those obtained after intranasal administration of 7.4 nM nerve growth factor (NGF) (26,500 Daltons) (Dahl et al. 1997; Thorntorn-Manning et al. 1997) to rats, revealed a five-fold higher delivery of the lower molecular weight dopamine. Comparing the percentages of the original dose remaining in the brain 30 to 45 min after intranasal administration of dopamine (0.12%) (Dahlin et al. 2001) and NGF (0.023%) (Chen et al. 1998) in rodents revealed a similar difference. In addition, with most small molecules, a significantly higher molar dose can be delivered intranasally than with larger protein or DNA therapeutic agents.

Ishikawa et al. (2001) reported that powder formulation of calcitonin utilizing CaCO3 improves the nasal bioavailability by increasing residence time in the nasal cavity and thus enhances the systemic bioavailability. Recently Bergstrom et al. (2002) studied the uptake of picolinic acid (PA) in the brain. [3H]PA was administered via unilateral nasal instillation or i.v. injection to mice. Autoradiography demonstrated rapid uptake of radioactivity in the olfactory nerve layer and in the ipsilateral olfactory bulb following nasal instillation, which was maintained at a high level even after 4 h. On the other hand i.v. injection of [3H]PA demonstrated selective uptake and retention of radioactivity in the olfactory bulb. Hussain et al. (2002) have found that intranasal administration of folic acid effectively results in complete and rapid absorption into the CNS. This provides a method of rapidly and reliably delivering folic acid, alone or in combination with other compounds, to the systemic circulation to produce a beneficial effect in the treatment or prevention of AD and stroke.

2.4.9 Approaches Used for Improved CNS Delivery through Nose:

Various approaches have been tried to achieve higher CNS delivery through nasal route. A study by Gwak et al. (2003) has shown that the analgesic effect of intranasal enkephelins is significantly higher when administered with aid of absorption enhancers.

Al-Ghananeem et al. (2002) have reported targeted brain delivery of 17-β-estradiol via administration of water soluble prodrugs and absorption was fast following intranasal delivery of these prodrugs. These drugs are capable of producing high concentration of estradiol in CSF and have a significant value in treatment of AD. Similarly, Kao et al. (2000) have investigated during their study that water soluble prodrugs of L-dopa can be delivered specifically to CNS via intranasal administration. Absorption was rapid following intranasal delivery and bioavailability was approximately 90%. Olfactory bulb and CSF concentration of L-dopa was significantly high. It was concluded that prodrugs of L-dopa can be successfully used for PD with many advantages such as improved bioavailability, reduced side effects and potentially enhanced CNS drug delivery. Illum et al. (2002) have studied the effect of chitosan-morphine nasal formulation vis-à-vis slow i.v. infusion of morphine in healthy volunteers who reported sedation at the earliest time point after nasal administration compared with i.v. administration. This suggests that after nasal administration morphine may be able to reach CNS more rapidly than after i.v. administration. Lianli et al. (2002) reported rapid onset intranasal delivery of diazepam using ethyl-laurate-based microemulsion. At a 2 mg/kg dose, the maximum drug plasma concentration was arrived within 2-3 min, and the bio-availability (0-2 h) after nasal spray compared with i.v injection was about 50%. The results suggest that this approach may be helpful during emergency treatment of status epileptics. Zhang et al. (2004) and Zhang et al. (2006) have evaluated nimodipine (NM) loaded microemulsion system and MPEG-PLA nanoparticles respectively, for direct delivery of NM to brain after intranasal administration. Significantly higher ratios of AUC in different brain tissues and cerebrospinal fluid to that in plasma was obtained after nasal administration compared to i.v. administration of microemulsion and nanoparticles. These results confirmed that a fraction of NM could be transported directly into the brain via the olfactory pathway after nasal delivery and suggested potential of microemulsion and nanoparticulate delivery systems for improving the efficacy of the direct nose to brain transport for the drugs. Vyas et al. (2005b, 2006a, 2006b) have reported rapid and larger extent of drug transport of into rat brain following intranasal administration of mucoadhesive microemulsions of zolmitriptan, sumatriptan and clonazepam respectively.

2.4.10 Animal Models for Evaluation of Nasal Absorption Studies:

Nasal absorption studies can be evaluated using two animal models viz. (1) whole animal or *in vivo* model and (2) isolated organ perfusion or *ex vivo* model. The models are commonly employed as per the needs of experiment. These models are described in the sections 2.4.10.1 and 2.4.10.2 onwards.

2.4.10.1 *In vivo* nasal absorption model:

Rat Model

Based on studies with many compounds, it has been established that the rat is an excellent animal model to study nasal absorption of drugs. For most non-peptide drugs the results obtained in the rat can very accurately predict the absorption profiles of the drugs in humans. The surgical preparation of rat for *in vivo* nasal absorption study carried out by anaesthetizing the rat by intraperitoneal injection of sodium pentobarbital. An incision is made in the neck and trachea is cannulated using polyethylene tube. Another tube is inserted through the esophagus towards the posterior region of nasal cavity. The passage of the nasopalatine tract is sealed so that the drug solution does not get drained from the nasal cavity through mouth. The drug solution delivered through nasal cavity through nostril or through the polyethylene cannula. The blood samples are collected from the femoral vein (Chein et al. 1989). The drug will be transported through nasal cavity to systemic circulation or to other organs/tissues only as all the possible outlets are blocked.

Mice Model

Mice are also widely used as an animal model to study nasal absorption of drugs. The mice were anaesthetized by intraperitoneal injection of sodium pentobarbital. The drug solution is sprayed in form of nasal spray into each nostril. The head of the mice is upheld in upright position. Using mice model, the drugs mentioned below (Table 2.5) were studied for nose-to-brain transport studies.

Rabbit model

Rabbits weighing approximately 3 kg are either anaesthetized or maintained in a conscious state depending on the need of an experiment.

The rabbits are anaesthetized by intramuscular injection of a combination of ketamine or xylene. The drug solution is sprayed in form of nasal spray into each nostril. The head of the rabbit is upheld in upright position. During the study, rabbits are allowed to breathe naturally through the nostrils. The body temperature of the rabbits shall be maintained 37 °C with aid of heating pad. The blood samples are collected using an indwelling catheter

from the marginal ear vein or artery as per the experimental protocol. Rabbit model has several advantages are stated below.

⇒ Relatively cheap, easily available and does not require dedicated laboratory facility

⇒ Permits extrapolation of the data when studied using larger animal such as monkey

⇒ Due to larger blood volume (approx. 300 mL), it allows frequent sampling (1 to 2 mL)

The rabbit model has been reported to use to study nasal absorption of controlled release formulation of progesterone.

Dog model

The brief model for *in vivo* dog model for nasal absorption studies is described as below. The dog is either anaesthetized or maintained in the conscious stage depending on the purpose of the experiment. In the anaesthetized model, the dog is anaesthetized using i.v. injection of sodium thiopental and maintained with sodium pentobarbital. A positive pressure pump provides ventilation through a cuffed endotracheal tube. The temperature is maintained 37 °C with aid of heating pad. The blood samples are collected from the jugular vein according to the design of experimental protocol.

The dog model has been used to study nasal absorption of propranolol, insulin and few other drugs.

Sheep model

The *in vivo* sheep model for nasal drug delivery is similar to that discussed for dog model. Male in-house bred sheep are selected devoid of nasal diseases. The sheep model has been found to be used to study nasal absorption of metkephamid and few other drugs.

Monkey model

The *in vivo* monkey model for nasal absorption studies is defined as mentioned below. Monkey (approximately 8 kg) is anaesthetized, tranquilized or maintained in the conscious stage as per the protocol of the experiment. The monkey is tranquilized by intramuscular injection of ketamine hydrochloride or anaesthetized by intravenous injection of sodium pentobarbital. The head of the monkey is held in the upright position and drug solution is administered n each nostril. Post drug administration, monkey is placed in a supine position in a metabolism chair for 5 to 10 min. Throughout the study, monkey is allowed to breathe naturally through the nostrils. The blood samples are

collected via an indwelling catheter mounted in the vein as per the design of protocol. The monkey model has been used in studying the nasal absorption of insulin, leutinizing releasing hormone and nicardipine etc.

2.4.10.2 *Ex-vivo* nasal perfusion models:

Surgical preparation is the same as defined under *in vivo* rat model. During perfusion studies, a funnel is placed between the nose and reservoir to minimize the loss of drug solution. The drug solution is filled in reservoir and temperature is maintained at 37 °C. The drug solution is circulated using peristaltic pump. The drug solution is dripped on the nostril and collected via funnel to the reservoir. The drug solution in the reservoir is stirred constantly and circulated for a predetermined time period as per the design of the protocol. The amount of drug transported cross the nasal cavity is back calculated from the concentration of drug remained in the reservoir. One of the drawbacks of this model is that unstable drugs may lead to incorrect results. This model is used to determine the nasal absorption of salicylic acid, aminopyrine, phenol red, Phenobarbital, secobarbital, l-tyrosine, Propranolol hydrochloride, polyethylene glycol 4000 etc. Rabbit model can also be employed for studying *ex vivo* nasal absorption of drugs.

2.4.11 Marketed and Investigational Products:

Many products are already on the market and many more drugs are under investigation for intranasal delivery. Biopharmaceutical data and some of the marketed and investigational pharmaceuticals are summarized in Table 2.6, Table 2.7 and Table 2.8.

Table 2.5 Example of drug substances for which mice model is explored for nose-to-brain transport studies

Sr. No.	Drugs
1	Activity dependent neuroprotective protein (ADNP) like peptide (NAP)
2	Basic Fibroblast Growth Factor
3	Heparin-binding Epidermal Growth Factor
4	Exendin
5	Hypocretin-1
6	Kainic Acid
7	Morphine
8	1-methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine
9	Picolinic Acid
10	Dopamine

Table 2.6 Pharmacokinetic data of potential brain-targeted drugs

Drugs	**Animal Model**	**T_{max} (min)**	**% Relative bioavailability**
Buprenorphine	Rat	2 – 5	95
Cocaine	Human	15 – 60	-
Dopamine	Rhesus monkey	15	-
Diazepam	Humans	60	72-84
Ergotamine tartarate	Rat	20	62-65.4
Hydralazine	Rat	10 – 30	83-127
Lorazepam	Humans	30-240	51
Naloxone	Rat	20	101
Propranolol	Rat Human Dog	<2 <2 <2	100 109 103
Verapamil	Dog	5	37
Metaclopromide hydrochloride	Rat	23.3	87.21

Table 2.7 List of marketed nasal products for brain-targeting

Product	Drug	Indication	Approval Date	Manufacturer
Stadol NS®	Butorphanol tartarate (10mg/mL, 1 mg/spray)	Management of pain and migraine	08/08/2001 12/03/2002	ESI Lederle Roxane Labs.
Stimate NS	Desmopressin acetate (0.01%)	Haemophillia A	-	Rhone Poulenc Rorer
Syneral® Nasal Solution	Nafarelin acetate	Central precocious puberty	-	Roche Laboratories
Migranal	DHE-45 Dihydroergotamine	Migraine	31/07/2002	Xcel Pharm.
DDAVP® Nasal Spray	Desmopressin acetate	Prevention of polydipsia and polyurea	04/09/2003 12/11/2003	Ferring Pharm. Aventis Pharm.
Zomig Nasal Spray	Zolmitriptan (2.5 and 5 mg/spray)	Migraine	30/09/2003	AstraZeneca

Table 2.8 List of investigational drug substances for brain-targeting

No.	Drug	Status	Indication	Reference
1	Scopolamine hydrobromide	Human studies	Prevention of nausea and vomiting by motion sickness	Tonndorf et al. 1953
2	Buprenorphine hydrochloride	Human studies	Relief of moderate to severe pain	Frills and Brewster 1989
3	Chlorpheniramine maleate	Human studies	Antihistaminic agent	Secher et al. 1982
4	Chlorphenpyridamine maleate	Human studies	Antihistaminic agent	Schaffer and Seidmon 1952
5	Prophenpyridamine maleate	Human studies	Antihistaminic agent	Schaffer and Seidmon 1952
6	Clonazepam	Human studies	Treatment of petit mal, akinetic and myoclonic seizers	Schols-Hendrinks et al. 1995
7	Diphenhydramine hydrochloride	Human studies	Antihistaminic and antitussive agent	Gaffey et al. 1987
8	Doxylamine succinate	Human studies	Antidepressant	Romeo et al. 1996
9	Ergotamine tartarate	Human studies	Treatment of migraine	Aelling et al. 1986

Table 2.8 List of investigational drug substances for brain-targeting (Contd...)

No.	Drug	Status	Indication	Reference
10	Metoclopramide hydrochloride	Human studies	Antiemetic	Citron et al. 1987
11	Midazolam	Human studies	Preoperative sedation, General anesthetic	Fukuta et al. 1993
12	Neostigmine bromide	Human studies	Myasthenia gravis	Sghirlanzoni et al. 1992
13	Nicotine	Product recently approved in USA and UK	Management of smoking cessation	Johansson et al. 1991
14	Propranolol hydrochloride	Human studies	Management of hypertension and angina pectoris	Hussain et al. 1980
15	Sufentil citrate	Human studies	Analgesic agent	Haynes et al. 1993
16	Vasopressin	Human studies	Polydipsia, polyurea and dehydration with diabetes insipidus	Dashe et al. 1964

Many sophisticated and effective approaches to CNS drug delivery have emerged in recent years. Direct transport of drugs through the olfactory pathway to the CNS has generated immense interest in devising strategies and methodologies to exploit this approach as a portico for CNS drug delivery. However, numerous factors work in tandem which determines the efficiency of drug delivery. The problems arise due to the physiological status in terms of nasal function and accompanying pathologies and pharmaceutical challenges with respect to CNS drug delivery, i.e., low bioavailability, local irritation and toxicity upon long-term usage. Synthesis of more lipophilic analogues, enzyme inhibitors, permeation enhancers, colloidal and bio-adhesive novel drug delivery modalities could help to eliminate few of the problems to some extent. Few formulations have already been successfully marketed and many are under phase I/II/III clinical stages. The emergence of peptide and protein moieties in the therapeutic scene has certainly heightened the scientific and industrial attention to rediscover the potential of this route of drug delivery. It is needless to say that the nasal route with all its inherent advantages has been heralded as the most promising means for the delivery of drugs to the CNS in the near future.

2.5 Microemulsions

Microemulsions (ME) are clear, thermodynamically stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. These systems are currently of interest to the pharmaceutical scientist because of their considerable potential to act as drug delivery vehicles by incorporating a wide range of drug molecules (Lawrence and Rees 2000). Hoar and Schulman (1943) introduced the concept of ME in 1940's. The concept was debated and redefined as a "system of water, oil and amphiphile which is single, optically isotropic and thermodynamically stable liquid solution. The key difference between ME and emulsion is their kinetic and thermodynamic stability. The differences are enlisted in following Table 2.9 (Shinoda and Lindman 1987).

Microemulsions in broader terms, doesn't contain any microstructure and it includes system that are co-solvents i.e. system wherein the constituents or components are molecularly dispersed. However, most researchers in the field agree that for a ME to be formed it is important that the system contains some definite microstructure. In other words there is a definite boundary between the oil and the water phases at which surfactant is located. It is imperative to consider the structure and properties of surfactants located at the interface between oil and water phases. The conventional surfactants

comprise of a polar head group region and a non-polar tail region. The non-polar region is having larger volumes particularly in case of ionic surfactants. On dispersal in water, surfactants self-associate into a variety of equilibrium phases, the nature of which stems directly from the interplay of the various inter and intra-molecular forces as well as entropy considerations. Surfactants also self-associate in non-aqueous solvents, particularly apolar liquids such as alkanes. In this case, the orientation of the surfactant molecules is reversed compared to those adopted in aqueous solution. This reorientation serves to optimize the solvation requirements of the surfactant and minimizes the free energy of the system overall. When surfactants are incorporated into immiscible mixtures of oil and water, the surfactant molecules can locate at the oil/water interface which is thermodynamically very favorable. A number of phases can result which may be structured on the microscopic or macroscopic scale, one example of a phase structured on the microscopic scale is an optically isotropic ME phase. Figure 2.8 shows the variety of such microscopic and macroscopic arrangements of surfactants in presence of water, oil or combinations of all three. Figure 2.9 illustrates the three different types of ME systems which may result due to arrangement of surfactant between the oil and water interface depending upon the composition. It can be seen while the three structures shown are quite different, in each there is an interfacial surfactant in monolayer separating the oil and water domains. Note that while the oil-in-water (o/w) and water-in-oil (w/o) droplets are represented in Figure 2.9 as spheres, they may be asymmetric in shape, frequently adopting the shape of a prolate ellipsoid. The presence of o/w ME droplets is likely to be a feature in ME where the volume fraction of oil is low. Conversely, w/o droplets are likely when the volume fraction of water is low and systems where the amounts of water and oil are similar, a bicontinuous ME may result. In the latter case, both oil and water exist as a continuous phase in the presence of a continuously fluctuating surfactant-stabilized interface with a net curvature of zero.

Table 2.9 Comparison of emulsions and microemulsions

Sr. No.	Emulsions	Microemulsions
1	Kinetically stable formulations	May or may not be kinetically stable
2	Thermodynamically unstable	Thermodynamically stable
3	Appearance is cloudy	Single, isotropic, clear or translucent solutions
4	Requires large energy input at the time of preparation	Do not require any energy

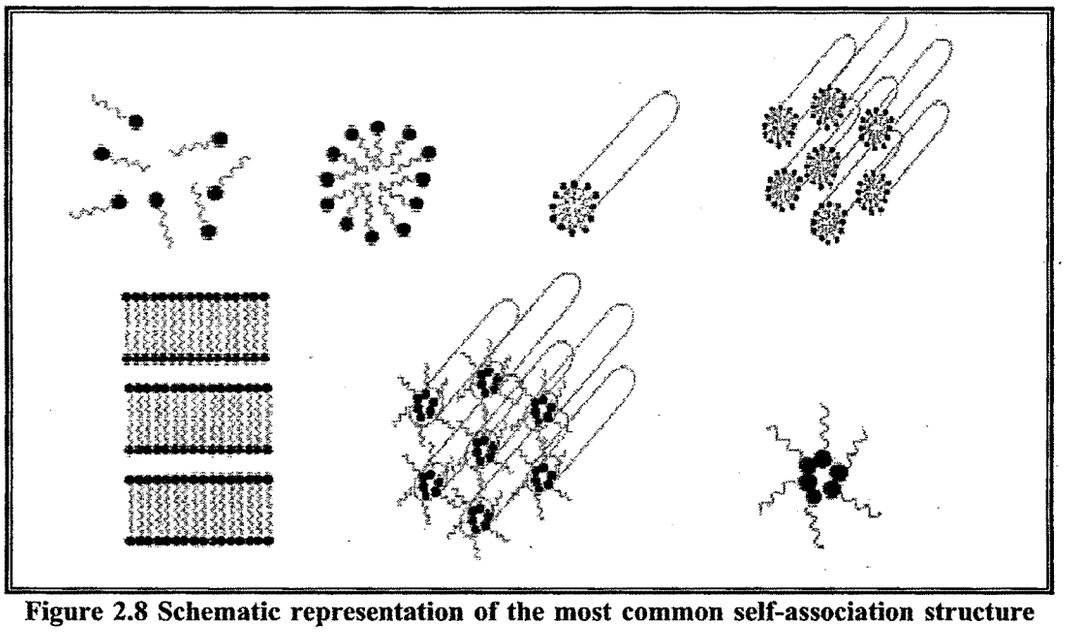


Figure 2.8 Schematic representation of the most common self-association structure resulting by the association of surfactant with oil, water or combinations thereof.

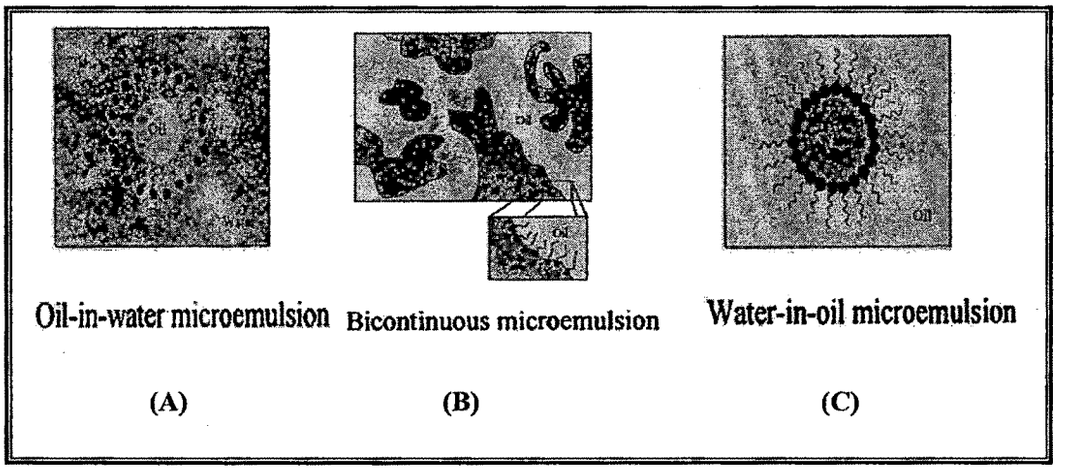


Figure 2.9 Schematic representations of three most commonly observed microemulsion structures (A) oil-in-water, (B) bicontinuous, and (C) water-in-oil microemulsion.

2.5.1 Microemulsion Formation and Phase Behavior:

2.5.1.1. Theories of Microemulsion Formation:

Three approaches can be used to explain the ME formation and stability. These are (1) interfacial or mixed film theories (Schulman et al. 1959; Prince et al. 1967), (2) solubilization theories (Shinoda Kunieda 1973; Shinoda and Friberg 1975), and (3) thermodynamic treatments (Ruckenstein and Chi 1975; Ruckenstein et al. 1980).

The simplified thermodynamic rationalization describing the ME formation can be explained as mentioned below. The free energy of ME formation can be considered to depend on the extent to which surfactant lowers the surface tension of oil-water interface and the change in the entropy of the system such that,

$$\Delta G_f = \gamma \Delta A - T\Delta S$$

Where, ΔG_f - the free energy of formation,

γ - the surface tension of oil-water interface,

ΔA - the change in interfacial area on microemulsification,

ΔS - the change in entropy of the system which is effectively dispersion entropy,

T - the temperature.

When a ME is formed, usually the change in ΔA is very large due to formation of large number of small droplets. Originally workers proposed that in order for a ME to be formed a (transient) the negative value of γ was required, it is now recognized that while value of γ is positive at all times, it is very small (of the order of fractions of mN/m), and is offset by the entropic component. The dominant favorable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, there are also expected to be favorable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus, a negative free energy of formation is achieved when large reduction in the surface tension is accompanied by significant favorable entropic change. In such cases, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable. Several factors determine the formation of w/o or o/w ME. Intuitively, it can be summarized that the most likely ME is that in which the phase with smaller volume fraction forms the droplets and this by no means can be considered the exclusive case. By their very nature, o/w ME droplets generally have larger effective interaction volume than w/o droplets. In the case

of ionic surfactants this is attributable to the presence of an electrical double layer at the surface of the o/w droplet which introduces a strong repulsive term. For o/w ME stabilized by a non-surfactant, although there is hydration shell associated with the polar head groups, the predominant repulsive factor can be attributable to steric interactions. It is also imperative to note that arrangement of surfactant at the interface with high curvature is always easier option for example small droplets, if the surfactant tails extend outwards into continuous oil phase. This also is entropically more favorable as the hydrocarbon tails have more directional freedom which in turn result tends to lower interfacial tension for w/o ME as compare to o/w ME and making their preparation a more easy process. Therefore, it should be noted that while ME are thermodynamically stable, there may be kinetic barriers to their formation. As a consequence, the order of addition of components may impact on the ease of preparation, and in some cases mechanical agitation or the input of heat will assist more rapid microemulsification.

2.5.1.2 Phase Behavior:

The relationship between the phase behavior and its composition can be captured with the aid of phase diagram. Compositional variables can be studied as a function of temperature and pressure, although the exception of ME prepared using supercritical and near critical solvents or with aid of chlorofluorocarbon and HFA propellants are studied under the ambient pressure conditions. The phase behavior of simple ME system comprising of an oil, water and surfactant can be studied with the aid of ternary phase diagram in which each corner of the phase diagram represents 100% of that particular phase component. However, in most pharmaceutical preparations, the ME contains drug and/or co-surfactant as additional components. The co-surfactant is also amphiphilic with an affinity for both the oil and aqueous phases and partitions to an appreciable extent into the surfactant interfacial monolayer present at the oil-water interface. The co-surfactant does not necessarily be capable of forming association structures in its own right. A wide variety of surfactants can function as co-surfactants such as non-ionic surfactants, alcohols, alkanoic acids, alkanoids and alkyl amines. Reports in the literature reveal that the few studies conducted wherein, large numbers of drug molecules were found to have surface active properties and can influence the phase behavior (Shinoda et al. 1991). In the case where four or more components are investigated, pseudo ternary phase diagrams are used where a corner will typically represent a binary mixture of two components such as surfactant/co-surfactant, water/drug or oil/drug. The number of different phases present

for a particular mixture can be visually assessed. Microstructural features can also be investigated with a wide variety of techniques. A schematic pseudo ternary phase diagram illustrating these features is represented in Figure 2.10.

Constructing phase diagrams is time consuming, particularly when the aim is to delineate a phase boundary, as the time taken for the system to equilibrate can be greatly increased as the phase boundary is approached. Heat and sonication are often used, especially when the system contains non ionic surfactants to speed up the process. The procedure most commonly employed is to prepare a series of pseudo binary compositions and titrate with the third component, evaluating the mixture after each addition. Care must be exercised to ensure that the observations are not made on the metastable systems. However, time constraints impose physical limit on the length of time for which systems can be left to equilibrate and consequently elimination of metastable state. In practice, centrifugation can be useful to speed up any separation. Outside the ME region, particularly for compositions close to the oil-water binary axis, there is insufficient surfactant to facilitate the formation of a single ME phase. In this case multiple phases may exist, the complexity of which increases with increase in the number of components in the mixture. Within this region, and indeed other multiphase regions of the ternary phase diagram, ME can exist in equilibrium with excess water or an oil phase. This multiphase systems can be described using the Winsor classification system. In the Winsor classification system, the one phase ME that is generally explored as drug delivery systems is known as Winsor IV systems.

Transition between the various phases mapped out in this phase diagrams can be driven by the further addition of one of the components, addition of new component such as electrolyte, or by changing the temperature. Transitions from w/o to o/w ME may occur via a number of different structural states including bicontinuous, lamellar and also to multiphase systems. Microemulsions stabilized by non-ionic surfactants, especially those based on polyoxyethylene are very susceptible to temperature because a decrease in surfactant solubility occurs with increasing temperature, and as a result systems stabilized by non-ionic surfactants or mixtures thereof often have characteristic phase inversion temperatures (PITs), with the PIT of the ME varying with a range of experimental factors including the amount and the nature of oil present and the nature of surfactant(s) present.

2.5.1.3 The Role of Surfactant:

The selection criterion of surfactant is also pertinent issue and shall be reviewed critically prior to designing ME (Barnes et al. 1988; Skodvin et al. 1993; Olla et al. 1993). The single phase ME systems can be classified as Winsor IV. The surfactants used to stabilize such systems may be (1) non-ionic, (2) zwitterionic, (3) cationic, or (4) anionic surfactants. Combination of these, particularly ionic and non-ionic can be very effective at increasing the extent of the ME region. Examples of non-ionic surfactants are polyoxyethylene surfactants (Brij 35) or sugar esters (span 80). Phospholipids are the example of zwitterionic surfactant which exhibits excellent biocompatibility. Soya-lecithin and egg-lecithin are commercially available may contain diacylphosphatidylcholine and its major constituent. Quaternary ammonium alkyl salts from one of the best known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide (CTAB), and twin tailed surfactant didodecyammonium bromide (DDAB) amongst the well known. The most widely used anionic surfactant is probably sodium bis-2-ethylhexylsulphosuccinate (AOT) which is twin tailed and is particularly effective stabilizer of w/o ME. Attempts have been made to rationalize the surfactant behavior in terms of hydrophile-lipophile balance (HLB) as well as critical packing parameter. Both approaches are fairly empirical but shall be employed as guide for selection of surfactant. The HLB takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecule. It is generally accepted that the low HLB surfactants (3-6) are favored for formation of w/o ME whereas, high HLB surfactants (8-18) are preferred for the formation of o/w ME systems. Ionic surfactants such as sodium dodecyl sulphate which have HLBs greater than 20, often require co-surfactants to reduce their HLB value within the required range for ME formation (Carlfors et al. 1991).

In most cases, single-chain surfactants alone are unable to reduce the oil /water interfacial tension sufficiently to enable a ME to form. Medium chain length alcohols which are commonly added as co-surfactants, have the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface thereby increasing the entropy of the system (Attwood 1994; Eccleston 1994; Tenjarla 1999). Medium chain length alcohols also increase the mobility of the hydrocarbon tail and also allow greater penetration of the oil into this region. Furthermore, any alcohol present may also influence the solubility properties of the aqueous and oily phases due to its partitioning between these phases. It

has also been suggested that some oils, for example the ethyl esters of fatty acids, also act as 'co-surfactants' by penetrating the hydrophobic chain region of the surfactant monolayer. All of the aforementioned mechanisms are considered to facilitate ME formation. In the case of ME stabilized by ionic surfactants, the addition of alkanols also serves to reduce repulsive interactions between the charged head groups. A number of double chain surfactants such as AOT and DDAB are able to form ME without the aid of co-surfactants. These surfactants are characterized by having small head groups in comparison to their hydrocarbon tails. Phosphatidylcholine or lecithin is also a twin-tailed surfactant, but in this case it is generally necessary to include a co-surfactant in order to disrupt the lamellar structures which characterize its biological behavior. Thus, medium chain alcohols have been successfully used as co-surfactants for the formation of lecithin-based ME. Interestingly w/o ME have been prepared using short diacyl chain lecithins and small molecular volume oils where it is possible that the small molecular volume oils penetrate the hydrophobic chain region thereby facilitating ME formation.

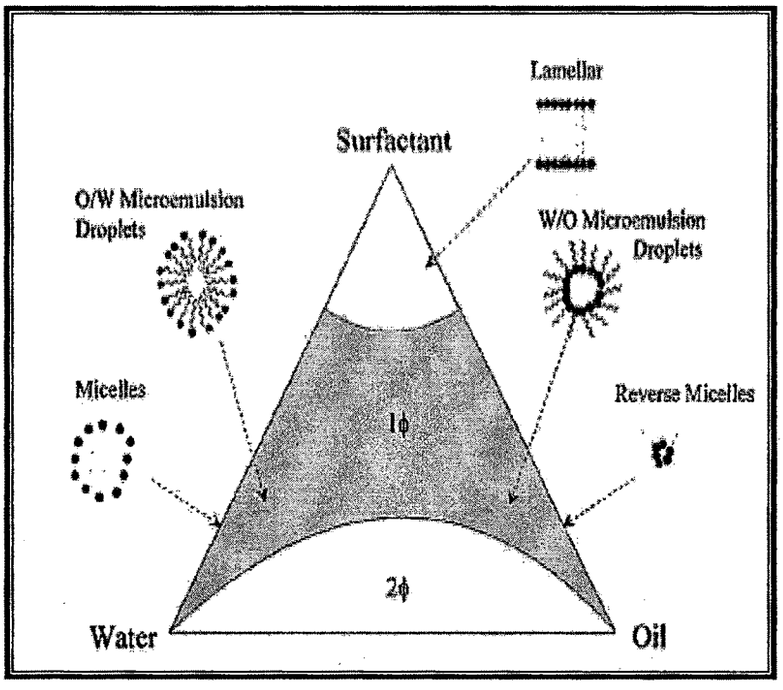


Figure 2.10 A hypothetical Pseudo ternary phase diagram depicting various microstructural features observed during microemulsion formation.

2.5.2 Microemulsion Characterization:

The release of drug(s) from ME system is a function of the microstructure formation within the ME. In contrast to the ease of preparation of ME, it is difficult to characterize microstructure of a ME (Khoshinevis et al. 1997). However, the knowledge is essential for their successful scaling up for commercial production and exploitation. Microemulsions have been evaluated with a variety of techniques over the years but set of appropriate methods are required in order to fully characterize these systems. At macroscopic levels viscosity, conductivity and dielectric methods provide useful information. For instance, viscosity measurements may provide indication regarding the presence of rod-like or worm-like reverse micelles (Angelico 1998). Conductivity measurements may provide useful information regarding the type of ME for example oil-continuous or water-continuous (Yu and Neuman 1995). It may also provide means of monitoring percolation or phase-inversion phenomena. Dielectric measurements are powerful tool to probe the structural and dynamic features of ME system. The isoelectric nature and optical clarity makes their study by spectrophotometric techniques particularly in comparison to macroemulsions. Pulse field gradient NMR has been successfully used measure self-diffusion coefficients of the various components and yields information on the mobility and microenvironment. Scattering methods have also been proven invaluable in elucidation of ME structure. It includes method such as dynamic and static light scattering, small-angle neutron scattering (SANS) (Bolzinger et al. 1999) and small-angle X-ray scattering (SAXS) (Kaler and Prager 1982; Kahlweit et al. 1987). Tabony et al. (1986) have identified presence of cubic phase in bicontinuous ME region using scattering methods. These techniques have been found extremely useful in the development of ME models such as cubic random cell and disordered open connected models. Neutron scattering methods using contrast variation have been used to investigate the nature of the oil penetration into the interfacial surfactant monolayer of ME (Eastoe et al. 1996). Freeze-fractured electron microscopy has been used to study ME structure. Freeze-fracture electron microscopy has also been used to study ME structure, however extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts (Vinson et al. 1991).

A potentially serious limitation of these methods lies in the requirement to dilute ME systems in order to eliminate particle-particle interactions. It is therefore imperative to work with a method works on high dispersed phase concentration. In spite of above

mentioned complications, much of the work reported in the pharmaceutical literature has been conducted using concentrated ME systems. For the most part where particle size is obtained using photon correlation spectroscopy, the measurement quoted remains uncorrected.

2.5.3 Microemulsion Optimization:

Optimization techniques for the dosage forms such as tablets, capsules and injectables have been extensively studied, while, few optimization techniques such as titration technique and pseudo-ternary phase diagram have been reported for optimization of ME (Lawrence and Rees 2000). Microemulsions can be optimized using titration method or by constructing a pseudo-ternary phase diagrams at different surfactant: co-surfactant levels. Combinations of both the approaches are frequently used to systematically optimize the ME formulation. Pseudo-ternary phase diagram is usually used to comprehensively study the ME region and its phase behavior, although construction of phase diagram is expensive and time consuming exercise (Lawrence and Rees 2000).

2.5.4 Microemulsion-based Formulation in Nasal Drug Delivery:

ME has generated considerable amount of interest in as a potential drug delivery systems. The major advantages associated with ME as delivery systems are their thermodynamic stability, optical clarity and ease of preparation. The existence of micro-domains of different polarity within the single phase system enables and offers freedom to formulator to incorporate both water soluble and oil soluble drugs. The viscosity of ME can be tailored as per the need of application or in some instances through incorporation of specific gelling agents such as carbopol or gelatin (Trotta et al. 1997; Kantaria et al. 1999). The attraction of o/w ME lies in their ability to incorporate hydrophobic drugs into the non-polar oil phase thereby enhancing their solubility. However, most drugs are not soluble in hydrocarbon oils, rather the polarity of the majority of poorly water-soluble drugs favor their solubilization in small/medium molecular volume oils such as tributyrin or Miglyol 812. In fact formulating a drug in a hydrocarbon o/w ME may offer no advantages in terms of solubilization over the corresponding micelle. The dispersal of drug as a solution in nanometer-sized droplets enhances the rate of dissolution into contacting aqueous phase and *in vivo* generally results in increase in drug bioavailability. It is also noteworthy that the use of o/w ME in drug delivery is more straightforward than in case of w/o ME. This is because of the droplet structure of o/w ME is often retained on dilution by biological aqueous phase, thereby permitting oral as well as parenteral

administration. Consequently, oral drug delivery of labile drug is the focus of growing attention; particularly many of the new therapeutic agents in the development are hydrophilic drugs such as peptides or oligonucleotides. Hydrophilic drugs of this kind may be incorporated into the dispersed phase of w/o ME where they are afforded some protection from enzymatic degradation. In addition, the presence of surfactant and in some cases co-surfactant, for example medium chain triglycerides in many cases serve to increase membrane permeability thereby increasing the drug uptake. Similarly, ME gels also have been found some applications in the area of transdermal drug delivery.

2.6 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 1980s. 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 (Lee et al. 2000). Furthermore, some mucoadhesive polymers can modulate the permeability of epithelial cells by partially opening tight junctions (Borchard et al. 1996; Schipper et al. 1997). Carbopol 934 increase paracellular transport which is caused by the cells being depleted of extracellular Ca^{+2} since Carbopol has a high binding affinity for Ca^{+2} (Borchard et al. 1996; Luessen et al. 1995). Carbopol polymers also inhibit enzymes (Bai et al. 1995) and this is also a result of the strong binding affinity of Carbopol for Ca^{+2}, which depletes the enzymes of calcium ions (Luessen 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. Mucoadhesives can not 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 (Lehr 2000). 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.

2.6.1 Mechanisms and Theory of Bioadhesion:

The mechanisms by which 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:

1. The electronic theory assumes that the different electronic structures of the mucoadhesive and the biological material result in electron transfer upon contact. This results in the formation of electrical double layer at the interface and adhesion occurs due to attractive forces across the double layer.
2. The adsorption 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.
3. 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.
4. The diffusion theory states that interpenetration and entanglement of polymer chains are responsible for mucoadhesion. The more structurally similar a 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.
5. 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.

The bioadhesive properties of a wide range of materials have been evaluated over the last decade and synthetic polymers such as carbopol and polycarbophil display excellent adhesion when tested *in vitro*. However, in *in vivo*, such performance may not be replicated, which explains why relatively few bioadhesive delivery systems have become commercially available. Furthermore, as with any formulation excipient, bioadhesives have the potential of inducing biological toxicity. The mucoadhesive properties of naturally occurring polymers such as hyaluronan (HA) has previously been investigated by Pritchard et al. (1996) for various grades of esterified and non-esterified HA using *in vitro* weight detachment studies and frog palate studies. The authors concluded that non-esterified HA had superior mucoadhesive properties compared to their esterified counterparts. Another naturally occurring polymer that has been of much interest over the past decade is chitosan, owing to its good biocompatibility, non-toxicity and biodegradability. In addition to its mucoadhesive properties, chitosan has been shown to enhance drug absorption through tight junctions via the paracellular route (Borchard et al. 1996).

2.6.2 Chitosan:

In the last decades, the intranasal delivery of a few classes of drugs such as peptides, proteins, morphine and anti-migraine etc. have undergone significant improvement in the delivering these compounds in the systemic circulation or to restricted sites. Delivering drug compounds efficiently using surfactants and permeation enhancers has been a classical approach; however, the role of mucoadhesive agents can not be neglected. Chitosan is a deacetylated chitin, the second most abundant polysaccharide existing in the world. Chitosan is a positively charged polysaccharide and soluble below pH 7. Chitosan is cationic compound due to presence of amino group; it binds with the mucosal cells which possesses net negative charge due to presence of amino groups. The amino group of chitosan interacts with sialic groups present within the mucin of mucosal cells which causes bioadhesion and reduces rapid clearance. Illum (1992; 1999) reported the use of chitosan in intranasal drug delivery system for facilitating absorption. Subsequent to that, Illum et al. (1994b) also reported that chitosan facilitates absorption of drugs such as salmon calcitonin and insulin across the sheep and rat nasal mucosa respectively. Artursson et al. (1994) revealed that the mucoadhesion properties of chitosan may be attributed to its bioadhesive properties and ability to open up the tight junctions located between the mucosal cells. The similar observations have been reported by Schipper et al. (1997) and Dodane et al. (1999). Lehr et al. (1992) have studied the mechanism of opening up of tight junctions, they found that the possible linkage may be attributed to interaction between amino groups of D-glucosamine and sialic acid groups present in the mucin. Later they have reported that the interaction is adjusted to yield optimum interaction when the pH of the formulation is adjusted to trigger the maximum ionization of sialic groups and amino groups. A study conducted by He et al. (1998) revealed that chitosan microspheres have excellent bonding properties with intestinal mucosal cells. Apsden et al. (1997) evaluated the effect of chitosan on mucociliary clearance on frog palate model (*ex-vivo*) and human nasal turbinate tissue. Chitosan was found to transiently decrease the mucociliary clearance which retains the innate physiology after removal of chitosan.

Recently, Soane et al. (1999) evaluated the nasal clearance characteristics of chitosan solutions and microspheres in humans. It was noticed that the clearance (t-half) for chitosan solution was 41 min whereas, for microspheres it was 84 min as compared to control sample wherein the clearance (t-half) was 21 min.

Chitosan also has dramatic effect with respect to improved nasal absorption due to its property of opening the tight junctions which leads to paracellular mode of transport. This has been demonstrated using CaCO-2 models by Kotze et al. (1998). The time to open up of tight junctions could be as rapid as 15 min which allows the compounds such as growth hormones (Mol. weight 20,000 Da) to transport across the lumen of nasal mucosa. It was also reported that chitosan improves bioavailability between 5- and 10-fold by increasing transport across the nasal mucosa. The role of chit san can be found from the literature wherein compounds such as Desmopressin, insulin, leuprolide acetate, salmon calcitonin, PTH, CCK-8 have been found to rapidly transverse across nasal mucosa. Roon et al. (1999) have investigated the role of chitosan in the treatment of migraine (alniditan) and analgesic agent (morphine) in ovine model. Chitosan is a compound with high molecular weight which is not absorbed by the body. The compound is generally regarded as safe (GRAS) and non toxic in nature to cilia. Therefore, in recent times chitosan has been explored as a tool for drug delivery including intranasal drug delivery by a number of scientists.

2.6.3 Carbomer:

The polyacrylic acid derivatives (carbomers) such as carbopol and polycarbophil are being widely explored as mucoadhesive drug delivery systems to enhance/improve the bioavailability. Number of research publications can be cited from the literature delineating the mucoadhesive properties of carbomer derivatives. Vidgren et al. (1991) have demonstrated the *in vivo* nasal mucoadhesion performance of carbopol. A polyacrylic acid gel bioadhesive system has been found to improve the adhesion and increased mucosal transport and absorption of insulin and calcitonin in rats. Insulin was administered via intranasal route to rabbits along with drum-dried waxy maize starch (DDWM) or maltodextrin with different DE values and carbopol. The bioavailability of formulations containing carbopol DDWM-974P (5 to 10%) was significantly higher as compared to maltodextrin-carbopol 974P mixtures. The bioavailability of the powder formulation containing DDWM and 10% w/w carbopol P was found to be 14.4% as compared to 5% w/w carbopol P containing powder formulation. *In vitro* mucoadhesive performance has been studied by many scientists (Mortazavi et al. 1992; LueBen et al. 1994; Park and Robinson 1984).

2.7 Radiolabeling

Reports from the literature indicated usefulness of radiolabeled formulation to study biodistribution and brain scintigraphy imaging in animals. An understanding about the brain targeting efficiency of ME, its distribution to various organs/tissues can be achieved by direct coupling with technetium-99m (^{99m}Tc). Technetium-99m is a radionuclide of choice because of its unique properties like visualization of coupled complex in brain and extremely low levels can be detected using the radiolabeled formulations. Many studies have been carried out by the scientists to conclude and investigate the diseases associated with the brain and CNS. A few reviews are represented below to indicate the usefulness of the radiolabeling technique for investigating transport and scintigraphy images of the brain and CNS.

Interictal brain ^{99m}Tc-HMPAO SPECT (Single photon emission computed tomography) study in chronic epilepsy was conducted by Jha et al. (1998) and the results indicated that HMPAO SPECT is more sensitive than CT scan and EEG, in localizing an epileptogenic focus in cases of chronic epilepsy.

Tourtauchaux et al. performed ^{99m}Tc-HMPAO SPECT to evaluate the clinical efficacy of 12 weeks of tacrine therapy on regional cerebral blood flow in patients with probable AD. The authors found the use of ^{99m}Tc-HMPAO SPECT analysis helpful to confirm diagnosis and to follow the evolution of disease under treatment by tacrine.

Stein et al. (1999) have studied SPECT of the brain with ^{99m}Tc-HMPAO during sumatriptan challenge in obsessive-compulsive disorder: investigating the functional role of serotonin autoreceptor revealed that ^{99m}Tc-HMPAO SPECT of the brain was found satisfactory to conclude the studies.

Direct ^{99m}Tc labeling of monoclonal antibodies has been demonstrated by Garron et al. (1991). The study revealed that the development of fast, reliable analytical methods has made possible the qualitative and quantitative assessment of technetium species generated by the radiolabeling process. Labeling stability is determined by competition of the ^{99m}Tc-antibody bond with three ligands, Chelex 100 (a metal chelate-type resin), free DTPA solution and 1% HSA solution. Very good ^{99m}Tc-antibody stability is obtained with activated IgG (IgGa) and Fab' fragment, which makes these substances possible candidates for immunoscintigraphy use.

Radiolabeling with ^{99m}Tc to study high-capacity and low-capacity biochemicals were performed by Eckelman (1995) and has shown the labeling of biochemicals with ^{99m}Tc for use with easily saturated sites, e.g., receptors and enzymes, is considered. Finally, attention is given to factors that affect the preparation of high specific activity, high affinity ^{99m}Tc-labeled biochemicals. In the study, a brief review of the history of the development of ^{99m}Tc-labeled radiopharmaceuticals, the use of technetium chelates in high-capacity systems is discussed. The latter are used in the study of five organ systems, the kidneys, liver, bone, brain, and heart. The chemical characterization of ^{99m}Tc complexes is also reviewed, followed by discussion of the various approaches to the labeling of proteins with direct labeling, the preformed chelate approach, and the antibody chelator conjugate approach. Capala et al. (1997) have demonstrated radiolabeling of epidermal growth factor (EGF) with ^{99m}Tc and *in vivo* localization following intracerebral injection in to normal and glioma-bearing rats. These studies are the first to describe a method for radiolabeling EGF with ^{99m}Tc and to detect it by external scintigraphy in the brains of tumor-bearing rats.

2.8 Physicochemical Properties and Analytical Profiles of Drugs

The drugs used to carry out the research were Tacrine and Donepezil. Literature citation reveals number of different methods for determination of these compounds in parent form, diffusion medium and biological fluids/tissues/organs. A few methods based on which the active ingredient, formulations and biological fluids/tissues/organs may be useful to carry out proposed research work are discussed.

2.8.1 Tacrine:

Tacrine hydrochloride (CAS#1684-40-8), (1,2,3,4-tetrahydro-9-aminoacridine monohydrochloride), a potent, centrally active, reversible cholinesterase inhibitor, was the first drug approved by the USFDA in 1993 for treating the symptoms of mild to moderate AD (Small 1992; Davis & Powchik 1995; Giacobini 1998). The chemical structure of tacrine HCl is shown in Figure 2.11 and physiochemical properties are summarized in Table 2.10 (Clarke's Analysis of Drugs and Poisons; Martin Dale, The Complete Drug Reference).

Presently tacrine is available in the market as oral capsule dosage forms. The recommended initial adult dosage of tacrine is 10 mg four times daily. This dosage should be maintained for at least 4 weeks with monitoring of serum aminotransferase concentrations every other week beginning 4 weeks after tacrine therapy is initiated. It is important that dosage escalation *not* be attempted during this period because of the possibility of delayed-onset liver function abnormalities. Following this initial 4 weeks, dosage then may be increased to 20 mg four times daily, provided there is no clinically important evidence of elevation in serum aminotransferase concentrations and the patient is otherwise tolerating therapy with the drug. Depending on patient tolerance, further escalations in dosage can be made in 40-mg daily increments (divided into 4 doses daily) at 4-week intervals up to a maximum of 160 mg daily (40 mg 4 times daily). The highest tolerated dosage should be administered since cognitive improvement is more likely to occur at higher dosages. If there is no improvement in clinical status after 3–6 months of tacrine therapy, most clinicians would discontinue the drug. Tacrine is well absored from gastro intestinal tract afte oral administration. However, peroral administration of tacrine is associated with low bioavailability (~17%), extensive hepatic first pass effect, rapid clearance from the systemic circulation, a short elimination half life (2-4 hrs) (Telting-

Diaz & Lunte 1993), large inter individual differences (Hartvig et al 1990; Lou et al 1996), a reversible dose dependent hepatotoxicity and peripheral cholinergic side effects (O'Brien et al 1991; Farlow et al 1992; Sathyan et al 1995). Its clinical uses have been limited due to associated cholinergic, hepatic, and gastrointestinal adverse reactions (Abramowicz 1993; Qizilbash et al 2000; Yang et al 2001). A recent study had shown that gastrointestinal side effects, such as diarrhea, anorexia, dyspepsia, and abdominal pain, and raised serum liver enzymes were the major reasons for its withdrawal (Qizilbash et al 2000).

Table 2.10 Physicochemical properties of Tacrine HCl

Name of API	Tacrine Hydrochloride
Chemical Name	1,2,3,4-tetrahydro-9-aminoacridine monohydrochloride
Formula	$C_{13}H_{14}N_2.HCl$
Molecular weight	234.7
Appearance, color, taste	Pale yellow, crystalline powder, bitter
Melting range	melts between 283°C and 284°C
Solubility	Soluble in water, methanol, 0.1N HCl, acetate buffer (pH 4.0), phosphate buffer (pH 7.0 to 7.4), dimethylsulfoxide (DMSO), ethanol, and propylene glycol. Sparingly soluble in linoleic acid and PEG 400.
Partition Coefficient	2.71
Dissociation constant	9.95

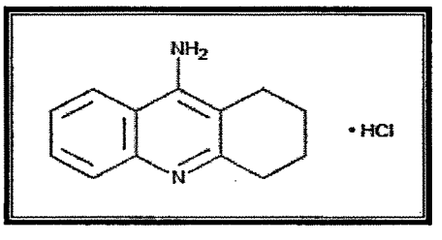


Figure 2.11 Chemical structure of Tacrine HCl

Literature citation reveals various analytical methods for estimation of tacrine and its formulations and a few methods are described in this section.

Hsieh et al (1983) have reported HPLC method for determination of tacrine in human rat tissues.

Park et al. (1986) have described the C18 Bond Elut columns and an HPLC/fluorometry methodology for the isolation and quantitation of tacrine at therapeutic levels in serum from human subjects.

Forsyth et al. (1988) have reported reversed phase HPLC method with fluorimetric detection for determination of tacrine in human serum after extraction with chloroform.

Ekman et al. (1989) and Hsu et al (1990) have reported HPLC method for determination of tacrine and its metabolites in plasma.

Aparico et al. (1998) have reported spectrofluorimetric method to determine tacrine in human serum and capsule dosage form. The method allows the determination of 1-70 ng/mL of tacrine in aqueous solutions containing acetic acid-sodium acetate buffer (pH 5.6) with $\lambda_{excitation}$ 242 nm and $\lambda_{emission}$ 362 nm.

Hansen et al. (1998) developed a method for simultaneous quantitation of tacrine and its metabolites in human plasma and urine. The method was based on simple one-step liquid–liquid extraction with ethyl acetate followed by isocratic, reversed-phase high-performance liquid chromatography and fluorescence detection (excitation: 330 nm and emission: 365 nm).

Vargas et al. (1998) have developed capillary zone electrophoresis method for the determination of tacrine, 7-methoxytacrine and their basic metabolites in pharmaceutical and biological samples (urine and serum). Separation of all compounds by capillary zone electrophoresis was carried out using a 46.6 cm untreated fused-silica capillary applying 20 kV separation voltage using 50 mM phosphate buffer of pH 2.8 for tacrine and its metabolite and of pH 7.8 for 7-methoxytacrine and its metabolite as background electrolyte. Tacrine and its metabolite were separated in less than 4 min while 7-methoxytacrine and its metabolite were separated in less than 7 min. The detection limits obtained were 3 ppb and 4 ppb in aqueous solutions; 50 ppb and 47 ppb for the determination in urine (diluted 1:10); 52 ppb and 56 ppb for determination in deproteinized serum samples for tacrine and 7-methoxytacrine respectively.

Jaskari et al. (2000) have employed HPLC method with a mobile phase, acetonitrile: triethylamine: deionized water at pH 6.5 (22:1:77) for the analysis of tacrine during *in vitro* drug release studies and permeation across human skin from ion exchange fiber.

Bollo et al. (2000) have developed differential pulse polarographic method for quantitative determination of tacrine in pharmaceuticals. Tacrine was electrochemically reduced and oxidized in aqueous medium and linear relation between the peak current and the tacrine concentration was used to analyze the tacrine in capsule dosage form.

Yang et al. (2001) have employed UV-Visible spectrophotometric method for the analysis tacrine during *in vitro* release studies from poly (D,L-lactide-co-glycolide) microspheres.

Vihola et al. (2002) have also employed HPLC method for the studying the drug release characteristic from the thermally responsive polymer nanoparticles composed of poly(*N*-vinylcaprolactam).

Jiang et al. (2003) developed reverse-phase HPLC method for the simultaneous determination of tacrine and its prodrug (N-butyramide tacrine) in mouse plasma and brain homogenate. The method involves deprotienisation and subsequent detection at 240 nm using gradient solvent system of methanol in 0.1 M phosphate buffer solution (pH 6.1).

2.8.2 Donepezil:

Donepezil hydrochloride is chemically 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride used widely for the palliative treatment of mild to moderate dementia of the Alzheimer's type. The chemical structure of donepezil HCl is shown in Figure 2.12 and physiochemical properties are summarized in Table 2.11 (Clarke's Analysis of Drugs and Poisons; Martin Dale, The Complete Drug Reference).

Presently donepezil is available in the market as oral film coated or orally disintegrating tablets in the strength of 5 and 10 mg. The recommended adult dosage of doenpezil HCl is 5 mg once daily at bedtime, increased if necessary after one month to 10 mg daily. Donepezil HCl is well absorbed from the gastrointestinal tract, maximum plasma concentrations being achieved within 3 to 4 h. It is about 95% bound to plasma proteins, mainly albumin. It undergoes partial metabolism via the cytochrome P450 isoenzyme CYP3A4, and to a lesser extent by CYP2D6, to 4 major metabolites. Over 10 days, about 57% of a single dose is recovered from the urine as metabolites, and about 15% from the faeces; 17% of the drug remains unchanged and is excreted in urine; 28% remains unrecovered suggesting accumulation. The elimination half-life is about 70 h. Steady-state concentrations are achieved within 3 weeks of the start of therapy

Table 2.11 Physicochemical properties of Donepezil HCl

Name of API	Donepezil HCl
Chemical Name	2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one monohydrochloride
Formula	$C_{24}H_{29}NO_3$, HCl
Molecular weight	416
Appearance, color, odor	A white to off white solid powder
Melting point	224°C
Solubility	Soluble water; chloroform, glacial acetic acid
Dissociation constant	8.90

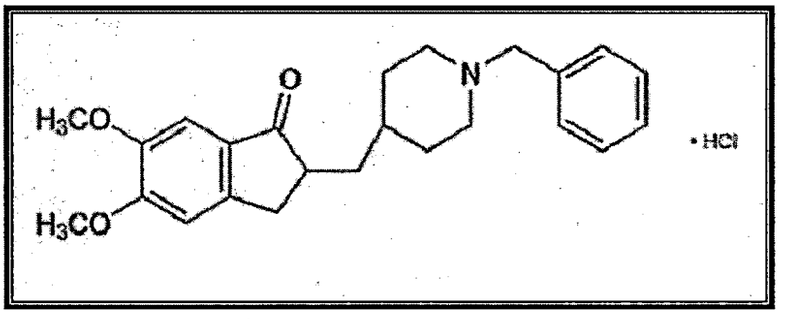


Figure 2.12 Chemical structure of Donepezil HCl

Literature citation reveals various analytical methods for estimation of tacrine and its formulations and a few methods are described in this section.

Matsui et al. (1999) have developed a rapid, sensitive and enantioselective LC–MS–MS method using deuterium-labeled internal standard for the simultaneous quantitative determination of donepezil enantiomers in human plasma without interconversion during clean-up process and measurement. The method involves use of an avidin column for the separation of donepezil enantiomers, and their detection by MS–MS without interference from its metabolites and plasma constituents.

Gotti et al. (2001) have reported capillary zone electrophoresis for the analysis and enantioresolution of donepezil in pharmaceuticals.

Yasui-Furukori et al. (2002) have described a simple and sensitive HPLC method with UV absorbance detection for the quantification of donepezil in human plasma. The method involves alkalinization of sample with 0.5 ml of NaOH (0.1 *M*) and extraction of the test compound from 1 ml of plasma using isopropanol–hexane (3:97, v/v). The organic phase was back-extracted with 75 µl of HCl (0.1 *M*) and analyzed by HPLC. The mobile phase consisted of phosphate buffer (0.02 *M*, pH 4.6), perchloric acid (6 *M*) and acetonitrile (59.5:0.5:40, v/v) and was delivered at a flow-rate of 1.0 ml/min at 40 °C. The peak was detected using a UV detector set at 315 nm, and the total time for a chromatographic separation was 8 min. The method was validated for the concentration range 3–90 ng/ml. Mean recoveries were 89–98%. Intra- and inter-day relative standard deviations were less than 7.3 and 7.6%, respectively, at the concentrations ranging from 3 to 90 ng/ml. The method shows good specificity with respect to commonly prescribed psychotropic drugs, and it could be successfully applied for pharmacokinetic studies and therapeutic drug monitoring.

Pappa et al. (2002) have developed a stability-indicating HPLC method for determination of donepezil hydrochloride in tablets. The HPLC method was performed with a reversed phase C_{18} column, detection at 268 nm and a mixture of methanol, phosphate buffer 0.02 M and triethylamine (50:50:0.5) as mobile phase. The peak of donepezil was obtained at retention time of 9 min.

Lu et al. (2003) and Lu et al. (2004) have developed liquid chromatographic method coupled with electrospray ionization-mass spectrometry for the determination of donepezil in human plasma. Method involves extraction of the alkalized plasma by isopropyl alcohol-n-hexane (3:97, v/v) and use of loratadine as the internal standard.

Solutes are separated on a C_{18} column with a mobile phase of methanol-acetate buffer (pH 4.0) (80:20, v/v). Detection is performed with a time-of-flight mass spectrometer equipped with an electrospray ionization source operated in the positive-ionization mode. The linear calibration curve is obtained in the concentration range 0.1-15 ng/mL. The limit of quantitation is 0.1 ng/mL.

Shiraishi et al. (2005) have measured plasma donepezil concentration by LC/MS/MS method. Electrospray ionization-MS/MS was carried out on mass spectrometer equipped with a LC system. The spectrometer was set to admit the protonated molecules $[M+H]^+$ at m/z 380 (donepezil) and m/z 394 (internal standard; (R,S)-1-benzyl-4-[2-[(5,6-dimethoxy-1-indanon)-2-yl]-ethyl]piperidine hydrochloride), with monitoring of the product ions at m/z 91 (donepezil) and m/z 91 (internal standard).

Radwan et al. (2006) have described a new precise, sensitive and accurate stereoselective HPLC method for the simultaneous determination of donepezil enantiomers in tablets and rat plasma with enough sensitivity. Enantiomeric resolution was achieved on a cellulose tris (3,5-dimethylphenyl carbamate) column known as Chiralcel OD, with UV detection at 268 nm, and the mobile phase consisted of n-hexane, isopropanol and triethylamine (87:12.9:0.1). Using the chromatographic conditions described, donepezil enantiomers were well resolved with mean retention times of 12.8 and 16.3 min, respectively. Linear response ($r > 0.994$) was observed over the range of 0.05-2 µg/ml of donepezil enantiomers, with detection limit of 20 ng/ml. The proposed method was found to be suitable and accurate for the quantitative determination of donepezil enantiomers in tablets.

Visible spectorphotometric method using orange G dye for quantification of donepezil HCl in tablet formulation has been reported Pillai and Singhvi (2006). In this method solution of donepezil HCl was reacted with orange G dye solution in potassium chloride-hydrochloric acid buffer (pH 1.2) for 10 min to form the colored complex. The colored complex was than extracted with chloroform and analyzed using UV-Visible spectrophotometer at 482 nm.

Abbas et al. (2006) have developed and validated stability indicating methods for the determination of donepezil HCl according to ICH guidelines. Authors have described three spectrophotometric methods using zero order, first order and second order spectra. The absorbance was measured at 315 nm for zero order while the amplitude was measured at 332.1nm for first order and 340 nm for second order spectra using deionized

water as a solvent. Donepezil HCl can be determined in the presence of up to 70% of its oxidative degradate using zero order, 80% using first order and 90% using second order spectra. The linearity range was found to be 8-56 μg/mL for all three spectra. The suggested methods were applied for the analysis of donepezil HCl in both powder and tablet form. Also, a spectrofluorimetric method depending on measuring the native fluorescence of donepezil HCl in deionized water using $\lambda_{excitation}$ 226 nm and $\lambda_{emission}$ 391 nm is suggested. The linearity range was found to be 0.32-3.20 μg/mL using this method and donepezil HCl was determined in the presence of up to 90% of its oxidative degradate. This spectrofluorimetric method was applied for the analysis of donepezil HCl in tablet form as well as in human plasma. The authors have also reported another method which depends on use TLC separation of donepezil HCl from its oxidative degradate and then determining donepezil HCl spectrodensitometrically. The mobile phase was methanol: chloroform: 25% ammonia (16: 64: 0.1 by volume). The linearity range was found to be 2-15 μg/spot.

Nakashima et al. (2006) have developed HPLC method with fluorescence detection for determination of donepezil in plasma and microdialysate samples. The method involves rapid isocratic separation of donepezil by a short C_{30} column and use of (±)-2-[(1-benzyl-piperidine-4-yl)ethyl]-5,6-dimethoxyindan-1-one hydrochloride as an internal standard. The eluate was monitored at 390 nm with an excitation at 325 nm. The method was successfully applied for monitoring of donepezil levels in rat plasma, blood and brain microdialysates and patient plasma.

2.9 References

Aarts M, Liu Y, Liu L. Treatment of ischemic brain damage by perturbing NMDA receptor-PSD-95 protein interactions. *Science* **2002**; 298:846-50

Abbas SS, Fayez YM, Abdel Fattah LE. Stability Indicating Methods for Determination of Donepezil Hydrochloride According to ICH Guidelines. *Chem Pharm Bull* **2006;** 54(10):1447-1450.

Abbott NJ. Dynamics of CNS barriers: evolution, differentiation and modulation. *Cell Molec Neurobiol* **2004**; 25:5-23.

Abraham MH, Chadha HS, Mitchell RC. Hydrogen bonding. 33. Factors that influence the distribution of solutes between blood and brain. *J Pharm Sci* **1994**; 83:1257-1268.

Abramowicz M. Tacrine for Alzheimer's disease. *Med Lett Drugs Ther* **1993;** 35:87-88.

Achari RG, Ahmed S, Behl CR, deMeireles JC, Liu R, Romeo VD, Sileno AP. Nasal delivery of apomorphine. Nastech Pharma Inc. **2002**; *United States Patent 20020193397.*

Aelling W, Rosenthaler J. Venoconstrictor effects of dihydroergotamine after intranasal and intramuscular administration. *Eur J Clin Pharmacol* **1986**; 30:581-584.

Agarwal V, Mishra B. Recent trends in drug delivery systems: intranasal drug delivery, *Ind J Exper Biol* **1999**; 37:6-16.

Al-Ghananeem AM, Traboulsi AA, Dittert LW, Hussain AA. Targeted brain delivery of 17 beta-estradiol via nasally administered water soluble prodrugs. *AAPS Pharm Sci Tech* **2002**; 3(1):E5.

Alzheimer's Disease, March 2002.

Anand Kumar TC, David GFX, Umberkoman B, Saini KD. Uptake of radioactivity by body fluids and tissues in rhesus monkeys after intravenous injection or intranasal spray of tritium-labeled estradiol and progesterone. *Curr Sci* **1974**; 43:435-439.

Angelico R, Palazzo G, Colafemmina G, Cirkle PA, Guistini M, Ceglie A. Water diffusion and head group mobility in polymer-like reversed micelles: evidence of a sphere-to-rod-to-sphere transition. *J Phys Chem B* **1998;** 2883-2889.

Aparico I, Bello MA, Callejon M, Jimenez JC. Guiraum A. Spectrofluorimetric determination of tacrine in pharmaceuticals and spiked human serum. *Analyst* **1998**; 123:1575-1576.

Apsden TJ, Mason JDT, Jones N, Lowe J, Skaugrud O, Illum L. Chitosan as a nasal delivery system: the effect of chitosan on in vitro and in vivo mucociliary transport rates. *J Pharm Sci* **1997**; 509-513.

Arora P, Sharma S, Garg S. Permeability issues in Nasal Drug Delivery. *Drug Deliv Tech* **2002**; 7(18).

Artursson P, Lindmark T, Davis SS, Illum L. Effect of chitosan on the permeability of the monolayers of intestinal epithelial cells (Caco-2). *Pharm Res* **1994**; 1358-1361.

Attwood D. Microemulsions. In: Kreuter J. (Ed.), Colloidal Drug Delivery Systems, Dekker, New York, **1994**; 31-71.

Bagger M, Bechgaard E. A microdialysis model to examine nasal drug delivery and olfactory absorption in rats using lidocaine hydrochloride as a model drug. *Int J Pharm* **2004**; 269:311-322.

Bai JPF, Chang LL, Guo JH. Effects of polyacrylic polymers on the lumenal proteolysis of peptide drugs in the colon. *J Pharm Sci* **1995**; 84:1291-1294.

Barnes IS, Hyde ST, Ninham BW, Derial PJ, Drifford M, Warr GG, Zemb TN. The disordered opened connected model of microemulsions. *Progr Colloid Polym Sci* **1988**; 76:90-95.

Begley DJ. The blood–brain barrier: principles for targeting peptides and drugs to the central nervous system. *J Pharm Pharmacol* **1996**; 48:136-146.

Behl CR, Pimplaskar HK, Sileno AP, deMeireles J, Romeo VD. Effects of physiochemical properties and other factors on systemic nasal drug delivery. *Adv Drug Delivery Rev* **1998**; 29:89-116.

Benedict C, Hallschmid M, Hatke A, Schultes B, Fehm HL, Born J, Kern W. Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* **2004**; 29:1326-1334.

Benninger MS, Hadley JA, Osguthorpe JD, Marple BF, Leopold DA, Derebery MJ, Hannley M. Techniques of intranasal steroid use. *Otolaryngol Head Neck Surg* **2004**; 130(1):5-24.

Benoit JP, Faisant N, Venier-Julienne MC. Development of microspheres for neurological disorders: from basics to clinical applications. *J Control Release* **2000**; 65:285-96.

Bergstrom U, Franzen A, Eriksson C, Lindh C, Brittebo EB. Drug targeting to the brain: transfer of picolinic acid along the olfactory pathways. *J Drug Target* **2002**; 10(6):469-478.

Bernstein IL. Is the use of benzalkonium chloride as a preservative for nasal formulations a safety concern? A cautionary note based on compromised mucocilliary transport. *J Allergy Clin Immunol* **2000**; 105:39-44.

Bjork E, Edman P. Characterization of degradable starch microspheres as a nasal delivery system for drugs. *Int J Pharm* **1990**; 62:187-192.

Bodor N, Buchwald P. Brain-Targeted drug Delivery: Experiences to date. *Am J Drug Deliv* **2003**; 1(1):13-26.

Bodor N, Buchwald P. Drug targeting via retrometabolic approaches. *Pharmacol Ther* **1997**; 76:1-27.

Bodor N, Kaminski JJ. Prodrugs and site-specific chemical delivery systems. *Annu Rep Med Chem* **1987**; 22:303-313.

Bollo S, Muñoz L, Núñez-Vergara LJ, Squella JA. Electrochemical Characterization of Tacrine, an Antialzheimer's Disease Drug, and Its Determination in Pharmaceuticals. *Electroanalysis* **2000**; 12(5):376-382.

Bolzinger MA, Thevenin MA, Grossiord JL, Poelman. Characterization of sucrose ester microemulsion by freeze fracture electron micrograph and small angle neutron scattering experiments. *Langmuir* **1999**; 15:2307-2315.

Borchard G, Lueben HL, deBoer AG, Verhoef JC, Lehr CM, Junginger HE. The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: Effect of chitosan-glutamate and carbomer on epithelial tight junctions in vitro. *J Control Release* **1996**; 39:131-138.

Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* **2002**; 5(6):514-516.

Brem H, Gabikian P. Biodegradable polymer implants to treat brain tumors. *J Control Release* **2000**; 65:285-296.

Brightman M. Ultrastructure of brain endothelium, in Bradbury MWB (ed) Physiology and pharmacology of the blood-brain barrier. *Handbook of experimental pharmacology*, **1992;** 103, Springer-Verlag, Berlin. 1-22.

Brightman MW. The intracerebral movement of proteins injected into blood and cerebrospinal fluid of mice. *Prog Brain Res* **1968;** 29:19-40.

Brownless J, Williams CH. Peptidases, peptides and the mammalian blood-brain barrier. *J Neuroche* **1993;** 60:1089-1096.

Buchwald P, Bodor N. Computer-aided drug design: the role of quantitative structure property, structure-activity, and structure-metabolism relationships (QSPR, QSAR, QSMR). *Drugs Future* **2002;** 27:577-588.

Butte AM, Jones HC, Abbot NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats; a development study. *J Physiol* **1990;** 429:47-62.

Capala J, Barth RF, Bailey MQ, Fenstermarker RA, Marek MJ, Rhodes BA. Radiolabeling of epidermal growth factor with ^{99m}Tc and *in vivo* localization following intracerebral injection in to normal and glioma bearing rats. *Bioconjug Chem* **1997;** 8:289-295.

Carlfors J, Blute I, Schmidt V. Lidocaine in microemulsion – a dermal delivery system. *J Disp Sci Technol* **1991;** 12:467-482.

Char H, Kumar S, Patel S, Piemontese D, Iqbal K, Malick AW, Salvador RA, Behl CR. Nasal delivery of [14C] dextromethorphan hydrochloride in rats: levels in plasma and brain. *J Pharm Sci* **1992;** 81(8):750-752.

Chen XQ, Fawcett JR, Rahman YE, Ala TA, Frey WH. Delivery of nerve growth factor to the brain via the olfactory pathway. *J Alzheimer's Dis* **1998;** 1:35-44.

Cheng YS, Guilmette RA, Simpson SQ, Cheng KH, Swift DL. Nasal deposition of ultrafine particles in human volunteers and its relationship to airway geometry. *Aerosol Science & Technol* **1991;** 25(3):274-291.

Cheng YS, Holmes TD, Gao J, Guilmette RA, Li S, Surakitbanharn Y, Rowlings C. Characterization of nasal spray pumps and deposition pattern in a replica of the human nasal airway. *J Aerosol Med* **2001;** 14(2):267-280.

Chien YW, Chang S. **1985.** In Transnasal Systemic Medications: Fundamental Concepts and Biomedical assessments (Elsevier science publishers, Amsterdam, 1985) 1-99.

Chien YW, Chang S. Intranasal drug delivery for systemic medication. *Crit Rev Ther Drug Carrier Syst* **1987**; 4:67-194.

Chien YW, Su KSE, Chang S. **1989.** Nasal Systemic Drug Delivery, Marcel Dekker, Inc, New York. 1-38.

Cho CW, Liu Y, Cobb WN. Ultrasound-induced mild hyperthermia as a novel approach to increase drug uptake in brain micro-vessel endothelial cells. *Pharm Res* **2002;** 19:1123-1129.

Chow HS, Anavy N, Villalobos A. Direct nose-brain transport of benzylecgonine following intranasal administration in rats. *J Pharm Sci* **2001**; 90(11):1729-1735.

Chow HS, Chen Z, Matsuura GT. Direct transport of cocaine from the nasal cavity to the brain following intranasal cocaine administration in rats. *J Pharm Sci* **1999;** 88(8):754-758.

Citron M, Reynolds J, Karla J, Kay B, Nathan K, Jaffe N, Miller F. Pharmacokinetic comparison of intranasal, oral and intramuscular metaclopramide in healthy volunteers. *Cancer Treat Rep* **1987;** 71:317-319.

Clarke's Analysis of Drugs and Poisons. Moffat AC, Osselton MD, Widdop B, Galichet LY. (Eds), 3rd Edition, London, UK: Pharmaceutical Press.

Cole SPC, Bhardwaj G, Gerlach JH, McKemzie JG, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG. Over expression of a transporter gene in a multi-drug-resistant human lung cancer cell line. *Science* **1992;** 258:1650-1654.

Conley SF. Comparative trial of acceptability of beclomethasone dipropionate and a new formulation of flunisolide. *Ann Allergy* **1994;** 72:829-532.

Corbo D, Liu J, Chien YW. Drug absorption through mucosal membranes: effect of mucosal route and penetrant hydrophilicity. *Pharm Res* **1989**; 6:848-852.

Cornford EM, Oldendorf WH. Epilepsy and the blood– brain barrier. *Adv Neurol* **1986;** 4:787–812.

Dahl AR, Lewis J`L. Respiratory tract uptake of inhalants and metabolism of xenobiotics. *Ann Rev Pharmacol Toxicol* **1993;** 32: 383-407.

Dahlin M, Bergman U, Jansson B, Bjork E, Brittebo E. Transfer of dopamine in the olfactory pathway following nasal administration in mice. *Pharm Res* **2000;** 17:737-742.

Dahlin M, Björk E. Nasal absorption of (S)-UH-301 and its transport into the cerebrospinal fluid of rats. Int J Pharm **2000;** 195:197-205.

Dahlin M, Jansson B, Bjork E. Levels of dopamine in blood and brain following nasal administration to rats. *Eur J Pharm Sci* **2001;** 14:75-80.

Dashe AM, Kleeman CR, Czaczkes JW, Rubinoff H, Spears I. Synthetic vasopressin nasal spray in treatment of diabetes insipidus. *J Am Med Assoc* **1964;** 190:1069.

David GF, Puri CP, Anand Kumar TC. Bioavailability of progesterone enhanced by intranasal spraying. *Experentia* **1981;** 37:533-534.

Davis KL, Powchik P. Tacrine. *The Lancet* **1995;** 345:625-630.

de Boer AG, Gaillard PJ. In vitro models of blood-brain barrier: when to use which? *Curr Med Chem* **2002;** 2:203-209.

Dhanda DS, Frey WH II, Leopold D, Kompella UB. Approaches for drug deposition in the human olfactory epithelium. *Drug Deliv Technol* **2005;** 5:64-72.

Djupesland PG. Nasal delivery device. **2004;** *United States Patent 6,715,485.*

Dodane V, Amin Khan M, Merwin JR. Effect of chitosan on epithelial permeability and structure. *Int J Pharm* **1999;** 182(1):21-32.

Dominique D, Gilles P. Nasal administration: a tool for tomorrow's systemic administration of drugs. *Drug Develop Indus Pharmacy* **1993;** 19(1&2):101-122.

Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC, Wong BA. Olfactory transport: a direct route of delivery of inhaled manganese phosphate to the rat brain. *J Toxicol Environ Health A* **2002;** 65(20):1493-1511.

Dragphia R, Caillaud C, Manicom R, Pavirani A, Kahn A, Poenaru L. Gene delivery into the central nervous system by nasal instillation in rats. *Gene Ther* **1995;** 2(6):418-423.

Dread I, Willeke K, Pietrowky R, Born J, Fehm HL. Intranasal angiotensin II directly influences central nervous regulation of blood pressure. *Am J Hypertens* **1998;** 11:971-977.

Eastoe J, Hetherington KJ, Sharpe D, Dong J, Heenan RK, Steytler D. Mixing of alkanes with surfactant monolayers in microemulsions. *Langmuir* **1996;** 12:3876-3880.

Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC. (Eds.), Encyclopedia of Pharmaceutical Technology, Vol. 9, Marcel Dekker, New York, **1994**; 375-421.

Eckelman WC. Radiolabeling with technetium-99m to study high-capacity and low-capacity biochemical systems. *Eur J Nucl Med* **1995**; 22:249-263.

Edman P, Bjork E, Ryden I. Microspheres as nasal delivery system for peptide drugs. *J Control Release* **1992;** 21:165-172.

Ekman L, Lindström B, Roxin P. Determination of tacrine and its 1-hydroxy metabolite in plasma using column liquid chromatography with ultraviolet detection. *J Chromatogr B: Biomed Sci Appl* **1989**; 494:397-402.

Faber WF. The nasal mucosa and subarachnoid space. *Am J Anat* **1937**; 62:121-148.

Farlow M, Gracon SI, Hershey LA, Lewis KW, Sadowsky CH, Dolan-Ureno J. A controlled trial of tacrine in Alzheimer's disease. *J Am Med Assoc* **1992;** 268:2523-2529.

Fehm HL, Perras B, Smolnik R, Kern W, Born J. Manipulating neuropeptidnergic pathway in humans. A novel approach to neuropharmacology. *Eur J Pharmacol* **2000;** 405:43-54.

Fehm HL, Smolnik R, Kern W, Mcgreger GP, Bickel U, Born J. The melanocortin Melanocyto-stimulating hormone/adrenocorticotropin 4-10 decreases body fat in humans. *J Clin Endocrinol Meta* **2001;** 86:1144-1148.

Fight Alzheimer's Disease, **2003**, www.androidworld.com/prod62.htm.

Filmore D. Breeching the blood-drain barrier. *Modern Drug Discov* **2002;** 5:22-27.

Fisher AN, Brown K, Davis SS, Parr GD, Smith DA. The effect of molecular size on the nasal absorption of water-soluble compounds in the albino rat. *J Pharm Pharmacol* **1987**; 39:357-362.

Fisher AN, Brown K, Davis SS, Parr GD, Smith DA. *J Pharm Pharmacol* **1985**; 37:38-41.

Forsyth DR, Ford JM, Truman CA, Roberts CJC, Wilcock GK. Determination of tacrine hydrochloride in human serum by chloroform extraction, reversed-phase high-performance liquid chromatography and fluorimetric detection. *J Chromatogr B: Biomed Sci Appl* **1988**; 433:352-358.

Frey WH II. Bypassing the blood–brain barrier to deliver therapeutic agents to the brain and spinal cord. *Drug Deliv Tech* **2002**; 2:46-49.

Frills, Brewster D. The systemic availability of buprenorphine administered by nasal spray. *J Pharm Pharmacol* **1989**; 41:803-805.

Fry FA, Black A. Regional deposition and clearance of particles in the human nose. *J Aerosol Sci* **1973**; 4(2):113-116.

Fukuta R, Braham H, Yanase N, Atsumi K, Kurosu. The sedative effects of intranasal midazolam administration in the dental treatment of patients with mental disabilities. Part I. The effect of 0.2 mg/kg dose. *J Clin Pediatr Dent* **1993**; 17:231-237.

Gaffey M, Gwaltney J, Sastre A, Dressler W, Sorrentino J, Hayden F. Intranasally and orally administered antihistamine treatment of experimental rhinovirus colds. *Am Rev Resp Dis* **1987**; 136:556-560.

Garron JY, Moinereau M, Pasqualini R, Saccavini JC. Direct 99mTc labeling of monoclonal antibodies: Radiolabeling and in-vitro stability. *Int J Rad Appl Intru* **1991**; 18:695-703.

Gattefosse Bulletin. New lipidic systems enhancing the bioavailability of problem drugs. **1997**; 1-81

Giacobini E. Cholinesterase inhibitors for Alzheimer's disease therapy: from tacrine to future applications. *Neurochem Int* **1998**; 32:413-419.

Giacobini E. Pharmacotherapy of Alzheimer's disease: new drugs and novel therapy. In: Corain B, Iqbal K, Nicolini M, et al, eds. *Alzheimer's Disease: Advances in Clinical and Basic Research.* New York: Wiley; **1993**:529-538.

Giacobini E. Pharmacotherapy of Alzheimer's disease: new drugs and novel therapy. In: Corain, B., Iqbal, K., Nicolini, M., Winblad, B., Wisniewski, H., Zatta, P. (eds)

Alzheimer's Disease: Advances in Clinical and Basic Research. **1993;** New York: Wiley, pp. 529-538.

Gotti R, Cavrini V, Pomponio R, Andrisano V. Analysis and enantioresolution of donepezil by capillary electrophoresis. *J Pharm Biomed Anal* **2001**; 24(5-6):863-870.

Graff CL, Pollack GM. Nasal drug delivery: Potential for targeted central nervous system delivery. *J Pharm Sci* **2005;** 94:1187-1195.

Gupta SP. QSAR studies on drugs acting at the central nervous system. *Chem Rev* **1989;** 89:1765-1800.

Gwak HS, Cho YM, Chun IK. Analgesic effects of intra-nasal enkephelins. *J Pharm Pharmacol* **2003;** 55(9):1207-1212.

Habgood MD, Begley DJ, Abbott NJ. Determinants of passive drug entry into the central nervous system. *Cell Mol Neurobiol* **2000;** 20:231-253.

Hallén H, Graf P. Benzalkonium chloride in nasal decongestive sprays has a long-lasting adverse effect on the nasal mucosal of healthy volunteers. *Clin Exper Allergy* **1995;** 25:401-405.

Hallschmid M, Benedict C, Schultes B, Fehm HL, Born J, Kern W. Intranasal insulin reduces body fat in men but not in women. *Diabetes* **2004;** 53:3024-3029.

Hallschmid M, Smolnik R, McGregor G, Born J, Fehm HL. Overweight humans are resistant to the weight-reducing effects of melanocortin4–10. *J Clin Endocrinol Metab* **2006;** 91:522-525.

Hansch C, Leo A, Hoekman D. Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. *American Chemical Society* **1995;** Washington, DC.

Hanson LR, Frey WH II. Strategies for Intranasal Delivery of Therapeutics for the Prevention and Treatment of NeuroAIDS. *J Neuroimmune Pharm* **2007**; 2:81-86

Hartvig P, Askmark H, Aquilonius SM, Wiklund L, Lindstorm B. Clinical pharmacokinetics of intravenous and oral 9-amino-l,2,3,4-tetrahydroacridine, tacrine. *Eur J Clin Pharmacol* **1990;** 38:259-263.

Hayes DJ, Frederickson CJ, Wallace DB. Devices for presenting airborne materials to the nose. **2001**; *United States Patent 6,325,475.*

Haynes G, Brahen N, Hill H. Plasma sufentanil concentration after intranasal administration to pediatric outpatients. *Can J Anesth* **1993**; 40:286-288.

He P, Davis SS, Illum L. In vitro evaluation of mucoadhesive properties chitosan microspheres. *Int J Pharm* **1998**; 75-88.

Hehar SS, Mason JDT, Stephen AB, Washington N, Jones NS, Jackson SJ, Bush D. Twenty-four hour ambulatory nasal pH monitoring. *Clin Otolaryngol* **1999**; 24:24-25.

Hiesiger EM, Voorhies RM, Basler GA, Lipschutz LE, Posner, Shapiro WR. Opening the blood–brain and blood–tumor barriers in experimental rat brain tumors: the effect of intracarotid hyperosmolar mannitol on capillary permeability and blood flow. *Ann Neurol* **1986**; 19:50–59.

Hillardal G. Adverse reaction to locally applied preservatives in nose drops. **1985**; 47: 278-279.

Hirai S, Yashika T, Matsuzawa T, Mima H. Absorption of drugs from the nasal mucosa of rat. *Int J Pharm.* **1981**; 7:317-325.

Ho Y, Chagan L. Memantine: A New Treatment Option for Patients with Moderate-to-Severe Alzheimer's Disease. *P&T* **2004**; 29(3): 162-165.

Hoar TP, Schulman JH. Transparent water-in-oil dispersions: the oleopathic hydro-micelle. *Nature* **1943**; 152:102-103.

Hsieh JYK, Yang RK, Davis KL. High-performance liquid chromatographic determination of tetrahydroaminoacridine in human and rat tissues using a rapid Sep-Pak C_{18} extraction. *J Chromatogr B: Biomed Sci Appl* **1983**; 274:388-392.

Hsu RS, Dileo EM, Chesson SM. High-performance liquid chromatography for the determination of tacrine and its metabolites in plasma. *J Chromatogr B: Biomed Sci Appl* **1990**; 530:170-176.

Huang C, Kimura R, Nassar A, Hussain A. Mechanism of nasal drug absorption of drug II: absorption of L-tyrosine and the effect of structural modification on its absorption. *J Pharm Sci* **1985**; 74:298-1301.

Hussain AA, Dittert LW, Traboulsi A. Brain delivery of folic acid for the prevention of Alzheimer's disease and stroke. **2002**; *United States Patent 6,369,058.*

Hussain AA, Foster T, Hirai S, Kashihara T, Batenhorst R, Jone M. Nasal absorption of Propranolol in humans. *J Pharm Sci* **1980;** 69:1240-1243.

Hussain AA. Intranasal drug administration delivery. *Adv Drug Del Rev* **1998;** 29:39-49.

Illum L, Farraj NF, Davis SS. Chitosan as a novel nasal delivery system for peptide drugs. *Pharm Res* **1994b;** 6(2):186-189.

Illum L, Farraj NF, Fisher AN, Gill I, Miglietta M, Benedetti LM. Hyaluronic acid ester microspheres as a nasal delivery system for insulin. *J Control Release* **1994a**; 29(1-2):133-141.

Illum L, Watts P, Fisher AN, Hinchcliffe M, Norbury H, Jabbal-Gill I. Intranasal delivery of morphine. *J Pharmacol Exp Ther* **2002;** 301:391-400.

Illum L. **1992.** Nasal Delivery of peptides: Factors affecting nasal absorption. In: Topics in Pharmaceutical Sciences. In: Crommelin DJA, Midha KK. (Eds), Medpharm, GmbH, Stuttgart. 77-82.

Illum L. **1999**. Bioadhesive formulations for nasal peptide delivery. In: Mathiowitz E, Chickering-III DE, Lehr C. (Eds), Bioadhesive Drug Delivery Systems, New York, Marcel Dekker. 507-541.

Illum L. Is nose-to-brain transport of drugs in man a reality? *J Pharm Pharmacol* **2004**; 56:3-17.

Illum L. Nasal Drug Delivery – possibilities, problems and solutions. *J Control Release* **2003;** 87:187-198.

Illum L. Nasal Drug Delivery: new developments and strategies. *Drug Discovery Today* **2002;** 7:1184-1189.

Illum L. Transport of drugs from the nasal cavity to the central nervous system. *Eur J Pharm Sci* **2000;** 11:1-18.

Ishikawa F, Katsura M, Tamai I, Tsuji A. Improved nasal bioavailability of calcitonin by insoluble powder formulation. *Int J Pharm* **2001;** 224:105-114.

Jansson B, Bjork E. Visualization of in vivo olfactory uptake and transfer using fluorescein dextran. *J Drug Target* **2002;** 10(5):379-386.

Jaskari T, Vuorio M, Kontturi K, Urtti A, Manzanares JA, Hirvonen J. Controlled transdermal iontophoresis by ion-exchange fiber. *J Control Release* **2000**; 67:179-190.

Jha SK, Dougall P, Behari M, Ahuja GK. Interictal brain 99mTc-HMPAO SPECT study in chronic epilepsy. *J Assoc Physicians India* **1998**; 46(5):438-441.

Jiang Y, Zhang Y, Zhang ZR. Simultaneous determination of *N*-butyramide-tacrine and tacrine in mouse plasma and brain homogenate by high-performance liquid chromatography with a simple gradient solvent system. *J Chromatography B* **2003**; 791:285-290.

Johansson PO, Bende M, Carlsson T, Gunnarasson P. Absolute bioavailability of nicotine applied to different nasal regions. *Eur J Clin Pharmacol* **1991**; 41:585-588.

Jones NS, Quraishi S, Mason JDT. The nasal delivery of systemic drugs. *Int J Clin Pract* **1997**; 51:308-311.

Kahlweit M, Strey R, Haase D, Kunieda H, Schmeling T, Faulhaber B, Borkovec M, Eicke H-F, Busse G, Eggers F, Funck TH, Richmann H, Magid L, Soderman O, Stilbs P, Winkler J, Dittrich A, Jahn W. How to study microemulsions. *J Colloid Interface Sci* **1987**; 118:436-453.

Kaler EW, Prager S. A model of dynamic scattering by microemulsions. *J Colloid Interface Sci* **1982**; 86:359-369.

Kantaria S, Rees GD, Lawrence JM. Gelatin-stabilized microemulsion-based organogels: rheology and application in iontophoretic transdermal drug delivery. *J Control Release* **1999**; 60:355-365.

Kao HD, Traboulsi A, Itoh S, Dittert LW, Hussain A. Enhancement of the systemic and CNS specific delivery of L-dopa by the nasal administration of its water soluble prodrugs. *Pharm Res* **2000**; 17(8):978-984.

Keck T, Leiacker R, Klotz M, Lindemann J, Riechelmann H, Rettinger G. Detection of particles within the nasal airways during respiration. *Eur Arch Otorhinolaryngol* **2000**; 257(9):493-497.

Kendirci M, Hellstrom WJG. Intranasal apomorphine. *IDrugs* **2004**; 7(5):483-488.

Kern W, Born J, Schreiber H, Fehm HL. Central nervous system effects of intranasally administered insulin during euglycemia in men. *Diabetes* **1999**; 48(3):557-563.

Kern W. Peters A. Fruehwald-schultes B, Deinnger E, Born J, Fehm HL. Improving influence of insulin on cognitive functions in human. *Neuroendocrinolgy* **2001**; 74:270-280.

Kern W, Schiefer B, Schwarzenburg J, Stange EF, Born J, Fehm HL. Evidence for central nervous effects of corticotropin-releasing hormone on gastric acid secretion in humans. *Neuroendocrinology* **1997**; 65:291-298.

Kerns EH. Hightroughput physicochemical profiling for drug discovery. *J Pharm Sci* **2001**; 90:1838-1858.

Khoshnevis P, Mortazavi SA, Lawrence MJ, Aboofazil R. *In vitro* release of sodium salicylate from water-in-oil phospholipids microemulsions. *J Pharm Pharmacol* **1997**; 49(S4):47.

Kotze AF, Leeuw BJ, Luessen HL, Boer AG, Verhoef JC, Junginger HE. Comparison of the effect of different chitosan salts and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2). *J. Controlled Release* **1998**; *51*(1):35-46.

Kreuter J. Nanoparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev* **2001**; 47:65-81.

Kreuter J. Transport of drugs across the blood-brain barrier by nanoparticles. *Curr Med Chem* **2002**; 2:241-249.

Krishna NSR, Getchell TV, Awasthi YC, Dhooper N, Getchell ML. Age- and gender-related trends in the expression of glutathione S-transferases in human nasal mucosa. *Ann Otol Rhin Laryng* **1995**; 104:812-822.

Kroll RA, Neuwelt EA. Outwitting the blood-brain barrier for therapeutic purposes: osmotic openings and other means. *Neurosurgery* **1998**; 42:1083-1099.

Kubba H, Spinou E, Robertson A. The effect of head position on the distribution of drops within the nose. *Am J Rhinol* **2000**; 14(2):83-86.

Kublik H, Vidgren MT. Nasal delivery systems and their effect on deposition and absorption *Adv Drug Del Rev* **1998**; 29:157-177.

Lahiri DK, Farlow MR, Greig NH, Sambamurti K. Current drug targets for Alzheimer's disease treatment. *Drug Development Research* **2002**; 56:267-281.

Lang J, Vath H. The distance between the ducts nasolacrimus and the canalis palatinus major on the sidewall of the nasal septum and the distance between the ostium pharyngeum tubae auditivae and canalis palatinus major and the upper side of the soft palate. *Anat Anz* **1989**; 168(4):315-320

Langer R. Polymer implants for drug delivery in brain. *J Control Release* **1991**; 16:53-60.

Lawrence JM, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev* **2000**; 45:89-121.

Lee JW, Park JH, Robinson JR. Bioadhesive-based dosage forms: the next generation. *J Pharm Sci* **2000**; 89:850-866.

Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int J Pharm* **1992**; 78:43–48.

Lehr CM. Lectin-mediated drug delivery: The second generation of bioadhesives. *J Control Release* **2000**; 65:19-29.

Lemiale F, Kong WP, Akyurek LM, Ling X, Huang Y, Chakrabarti BK, Eckhaus M, Nabel GJ. Enhanced mucosal immunoglobulin A response of intranasal adenoviral vector human immunodeficiency virus vaccine and localization in the central nervous system. *J Virol* **2003**; 77(18):10078-10087.

Lerner EN. Administering pharmaceuticals to the mammalian central nervous system. **2002**; *United States Patent 6,410,046.*

Levin VA. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J Med Chem* **1980**; 23:682-684.

Lewis DFV, Dickins M. Substrate SAR's in human P450s. *Drug Discov Today* **2002**; 7:918-925.

Lewis JL, Nikula KJ, Novak R, Dahl AE. Comparative localization of carboxylesterase in F344 rat, beagle dog and human nasal tissue. *Anat Rec* **1994**; 239:55-64.

Lewis JL, Rhoades CE, Gervasi PG, Griffith WC, Dahl AR. The cyanide-metabolizing enzyme rhodanese in human nasal respiratory mucosa. *Toxicol Appl Pharmacol* **1991**; 108:114-120.

Lianli Li, Nandi I, Kim KH. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal administration of diazepam. *Int J Pharm* **2002;** 237:77-85.

Lin JH, Lu AY. Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol Rev* **1997;** 49:403-449.

Liu XF, Fawcett JR, Thorne RG, DeFor TA, Frey WH II. Intranasal administration of insulin-like growth factor-I bypasses the blood-brain barrier and protects against focal cerebral ischemic damage. *J Neurol Sci* **2001;** 187(1-2):91-97.

Lo EH, Singhal AB, Torchilin VP, Abbott NJ. Drug delivery to damaged brain. *Brain Res Rev* **2001;** 38:140-148.

Lou G, Montgomery PR, Sitar DS. Bioavailability and pharmacokinetic disposition of tacrine in elderly patients with Alzheimer's disease. *J Psychiatry Neurosci* **1996;** 21:334-339.

Lu Y, Wen H, Li W, Chi Y, Zhang Z. Determination of donepezil hydrochloride (E2020) in plasma by liquid chromatography-mass spectrometry and its application to pharmacokinetic studies in healthy, young, Chinese subjects. *J Chromatogr Sci* **2004**; 42(5):234-237.

Lu YH, Wen HM, Li W, Chi YM, Zhang ZX. Determination of donepezil in human plasma by HPLC-MS. *Yao Xue Xue Bao* **2003**; 38(3):203-206.

LueBen HL, Lehr CM, Rentel C-O, Noach ABJ, de Boer AG, Verhoef JC, Junginger HE. Bioadhesive polymers for the peroral delivery of peptide drugs. *J Control Release* **1994**; 29:329-338.

Luessen HL, Verhoef JC, Borchard G, Lehr CM, deBoer AG, Junginger HE. Mucoadhesive polymers in peroral peptide drug delivery. II. Carbomer and polycarbophil are potent inhibitors of the intestinal proteolytic enzyme trypsin. *Pharm Res* **1995;** 12:1293-1298.

Luthra K, Tripathi M, Grover R, Dwivedi M, Kumar A, Dey AB. Apolipoprotein E Gene polymorphism in Indian patients with Alzheimer's disease and vascular dementia. *Dementia and Geriatric Cognitive Disorders* **2004;** 17:132-135.

Martin E, Schipper NGM, Verhoef JC, Merkus FWHM. Nasal mucociliary clearance as a factor in nasal drug delivery. *Adv Drug Deliv Rev* **1998;** 29:13-38.

Martin E, Verhoef JC, Cullander C, Romeijn SG, Nagelkerke JF, Merkus FWHM. Confocal laser scanning microscopic visualization of the transport of dextrans after nasal administration to rats: effects of absorption enhancers. *Pharm Res* **1997**; 14:631-637.

Martindale, The complete Drug Reference, Thirty second edition, 1999: 450-452.

Marx J. Alzheimer's research moves to mice. *Science* **1991**; 253:266-267.

Mathison S, Nagilla R, Kompella UB. Nasal route for direct delivery of solutes to the central nervous system: fact or fiction? *J Drug Target* **1998**; 5:415-441.

Matsui K, Oda Y, Nakata H, Yoshimura T. Simultaneous determination of donepezil (aricept®) enantiomers in human plasma by liquid chromatography–electrospray tandem mass spectrometry *J Chromatogr B: Biomed Sci Appl* **1999**; 729:147-155.

McMartin C, Hutchinson LEF, Hyde R, Peters GE. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J Pharm Sci* **1987**; 76:535-540.

Merkus P, Guchelaar HJ, Bosch DA, Merkus FWHM. Direct access of drugs to the human brain after intranasal drug administration. *Neurology* **2003**; 60:1669-1671.

Merkus P. Nose-to-brain, management forum, Nasal Drug delivery symposium, London. **2001**; 26-27th March.

Mertsch K, Maas J. Blood-brain barrier penetration and drug development from an industrial point of view. *Curr Med Chem* **2002**; 2:189-209.

Miller G. Breaking down barriers. *Science* **2002**; 297:1116-1118.

Misra Ambikanandan, Ganesh S, Shahiwala AA, Shah SP. Drug delivery to the central nervous system: a review. *J Pharm Pharm Sci* **2003**; 6(2):252-273.

Morimoto K, Katsumata H, Yabuta T, Iwanaga K, Kakemi M, Tabata Y, Ikada Y. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. *Eur J Pharm Sci* **2001**; 13:179-185.

Mortazavi SA, Carpenter BG, Smart JD. An investigation of the rheological behaviour of the mucoadhesive/mucosal interface. *Int J Pharm* **1992**; 83:221-225.

Mygind N, Änggård A. Anatomy and physiology of the nose – pathophysiologic alterations in allergic rhinitis. *Clin Rev Allergy* **1984**; 2:173-188.

Mygind N. **1979.** Nasal Allergy, 2nd Ed., Blackwell, Oxford: 260-262.

Nabeshima S, Reese TS, Landis DM, Brightman MW. Junctions in the meninges and marginal glia. *J Comp Neurol* **1975;** 164(2):127-169.

Nakashima K, Itoh K, Kono M, Nakashima MN, Wada M. Determination of donepezil hydrochloride in human and rat plasma, blood and brain microdialysates by HPLC with a short C_{30} column *J Pharm Biomed Anal* **2006;** 41:201-206.

Novartis Pharmaceuticals Corporation, New Jersey; Miacalcin® (calcitonin-salmon). **1999**; Nasal Spray product information.

O'Brien JT, Eagger S, Levy R. Effects of tetrahydroaminoacridine on liver function in patients with Alzheimer's disease. *Age Ageing* **1991;** 20:129-131.

Oh YK, Kim JP, Hwang TS, Ko JJ, Kim JM, Yang JS. Nasal absorption and biodistribution of plasmid DNA: an alternative route of DNA vaccine delivery. *Vaccine* **2001;** 19:4519-4525.

Okuyama S. The fist attempt at radioisotopic evaluation of the integrity of nose-brain barrier. *Life Sci* **1997;** 60:1881-1884.

Oldendorf WH. Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res* **1970;** 24:1629-1639.

Olla M, Monduzzi M, Ambrosone M. Microemulsions and emulsions in DDAB/water/oil systems. *Colloids Surfaces A: Physicochem Eng Aspects* **1999;** 160:23-36.

Pappa H, Farrú R, Vilanova PO, Palacios M, Pizzorno MT. A new HPLC method to determine Donepezil hydrochloride in tablets. *J Pharm Biomed Anal* **2002;** 27:177-182.

Pardridge WM, Triguero D, Yang J, Cancilla PA. Comparison of in-vitro and in-vivo models of drug transcytosis through blood-brain barrier. *J Pharm Exp Ther* **1990;** 253:884-891.

Pardridge WM. **1991.** Peptide drug delivery to the brain. Raven Press, New York, U.S.A.

Pardridge WM. Recent advances in blood brain-barrier transport. *Annu Rev Pharmacol Toxicol* **1988;** 28:25-39.

Pardridge WM. Receptor-mediated peptide transport through the blood–brain barrier. *Endocrine Rev* **1986;** 7:314-330.

Park K, Robinson R. Bioadhesive polymers as platforms for oral controlled drug delivery: Method to study bioadhesion. *Int J Pharm* **1984**; 19:107-127.

Park TH, Tachiki KH, Summers WK, Kling D, Fitten J, Perryman K, Spidell K, Kling AS. Isolation and the fluorometric, high-performance liquid chromatographic determination of tacrine. *Anal Biochem* **1986**; 159(2):358-362.

Pennington AK, Ratcliffe JH, Wilson CG, Hardy JG. The influence of solution viscosity on nasal spray deposition and clearance. *Int J Pharm* **1988;** 43:221-224.

Perras B, Marshall L, Kohler G, Born J, Fehm HL. Sleep and endocrine changes after intranasal administration of growth hormone –releasing hormone in young and aged humans. *Psychoneuroendocrinilogy* **1999a;** 24:743-757.

Perras B, Pannenberg H, Marchell L, Pietrowsy R, Bom J, Fehm HL. Beneficial treatment of age related sleep disturbances with prolonged intranasal vasopressin. *J Clin Psychopharmacol* **1999b;** 19:28-36.

Pietrowsky R, Struben C, Molle M, Fehm HL, Born J. Brain potential changes after intranasal vs. intravenous administration of vasopressin: Evidence for a direct nose-brain pathway for peptide effects in humans. *Biol Psychiatry***1996a;** 39(5):332-340.

Pietrowsky R, Thiemann A, Kern W, Fehm HL, Born J. A nose to brain pathway for psychotropic peptides: Evidence from a brain evoked potential study with cholecystokinin. *Psychoneuroendocrinology***1996b;** 21:559-572.

Pillai S, Singhvi I. Application of Orange G dye for quantitation of Citalopram Hydrobromide, Donepezil Hydrochloride and Rabeprazole Sodium from Tablet Formulation. *Ind J Pharm Sci* **2006**; 68(5):682-684.

Prince LM. A theory of aqueous emulsion. I. Negative interfacial tension at the oil/water interface. *J Colloid Interface Sci* **1967;** 23:165-173.

Pritchard K, Lansley AB, Martin GP, Helliwell M, Marriott C, Benedetti LM. Evaluation of the bioadhesive properties of hyaluronan derivatives: detachment weight and mucociliary transport rate studies. *Int J Pharm* **1996**; 129:137-145.

Qizilbash N, Birks J, Lopez-Arrieta J, Lewington S, Szeto S. Tacrine for Alzheimer's disease. *Cochrane Database of Syst Rev* **2000;** 3:CD000202.

Quay SC. Successful delivery of apomorphine to the brain following intranasal administration demonstrated in clinical study. *PR Newswire* **2001;** July 18th.

Radwan MA, Abdine HH, Al-Quadeb BT, Aboul-Enein HY, Nakashima K. Stereoselective HPLC assay of donepezil enantiomers with UV detection and its application to pharmacokinetics in rats. *J Chromatogr B Analyt Technol Biomed Life Sci* **2006;** 830(1):114-119.

Rapoport SI. Blood brain barrier in physiology and medicine. New York: Raven Press; **1976.**

Redwood H. Alzheimer's disease and other forms of Dementia: The balance between medical treatment and caregiving. *Health and Age,* November 29, **1999.**

Reed CJ. Drug metabolism in the nasal cavity: relevance to toxicology. *Drug Metab Rev* **1993;** 25:173-205.

Reger MA, Watson GS, FreyWH2nd, Baker LD, Cholerton B, Keeling ML, Belongia DA, Fishel MA, Plymate SR, Schellenberg GD, Cherrier MM, Craft S. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiol Aging* **2006;** 27:451-458

Romeo VD, Sileno A, Pimplaskar HK, deMeireles J, Gries WJ, Behl CR. Intranasal absorption of doxylamine succinate in healthy volunteers. **1996;** In Preparation.

Roon KI, Soons PA, Uitendaal MP, de Beukelaar F, Ferrari MD. Pharmacokinetic profile of alniditan nasal spray during and outside migraine attacks. *Br J Clin Pharmacol* **1999;** 47(3):285-290.

Rouselle C, Clair P, Lefauconnier JM. New advances in the transport of doxorubicin through the blood-brain barrier by a peptide vector-mediated strategy. *Mol Pharmacol* **2000;** 57:679-86.

Ruckenstein E, Chi JC. Stability of microemulsions. *J Chem Soc Faraday Trans* **1975;** 71:1690-1707.

Ruckenstein E, Krishnan R. Effect of electrolytes and mixtures of surfactants on the oil–water interfacial tension and their role in formation of microemulsions. *J Colloid Interface Sci* **1980;** 76:201-211.

Saito Y, Wright EM. Bicarbonate transport across the frog choroid plexus and its control by cyclic nucleotides. *J Physiol* **1983;** 336:635-648.

Sakane T, Akizuki M, Yamashita S. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the dissociation of the drug. *J Pham Pharmacol* **1994;** 46(5):378-379.

Sakane T, Akizuki M, Yoshida M. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. *J Pharm Pharmacol* **1991;** 43(6):449-451.

Sakane T, Yamashita S, Yata N, Sezaki H. Transnasal delivery of 5-fluorouracil to the brain in the rat. *J Drug target* **1999;** 7(3):233-240.

Sathyan G, Ritschel WA, Hussain AS. Transdermal delivery of tacrine: I, Identification of suitable delivery vehicle. *Int J Pharm* **1995;** 114:75-83.

Schaffer EE, Seidmon. The intranasal use of prophenpyridamine maleate and chlorprophepyridamine maleate in allergic rhinitis. *Ann Allergy* **1952;** 10:194.

Schipper NGM, Olsson S, Hoogstraate JA, deBoer AG, Vårum KM, Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs 2: Mechanism of absorption enhancement. *Pharm Res* **1997;** 14:923-929.

Schipper NGM, Verhoef JC, Merkus FWHM. The nasal mucocilliary clearance: relevance to nasal drug delivery. *Pharm Res* **1991;** 8:807-814.

Schipper NGM, Verhoef JC, Romeijn G, Merkus FWHM. Methylated cyclodextrins are able to improve the nasal absorption of salmon calcitonin, *Calcif Tissue Int* **1995;** 56:280-282.

Schlossauer B, Steuer H. Comparative anatomy, physiology and in vitro models of the blood-brain and blood-retina barrier. *Curr Med Chem* **2002;** 2:175-186.

Schols-Hendrinks, Lohman J, Janknegt R, Korten J, Merkus F. Absorption of clonazepam after intranasal and buccal administration. *Br J Clin Pharmacol* **1995;** 39:449-451.

Schwarze SR, Ho A, Vocero-Akbani A. In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* **1999;** 285:1569-1572.

Secher C, Krikegaard J, Borum P, Mansson A, Osterhammel P, Mygind N. Significance of H1 and H2 receptors in the human nose: rationale for the topical use of combined antihistamine preparations. *Allergy Clin Immunol* **1982;** 70:211-218.

Sghirlanzoni D, Pareyson C, Benvenuti G, Cei V, Cosi M, Lombardi M, Nicora R, Ricciardi, Cornelio F. Efficacy of intranasal administration of norstigmine in myasthenic patients. *J Neurol* **1992;** 239:165-169.

Shinoda K, Araki M, Sadaghiani M, Khan A, Lindman B. Lecithin based microemulsions: phase behavior and microstructure. *J Phys Chem* **1991;** 95:989-993.

Shinoda K, Friberg S. Microemulsions. Colloidal aspects. *Adv Colloid Interface Sci* **1975;** 4:281-300.

Shinoda K, Kunieda H. Conditions to produce so-called microemulsions. Factors to increase the mutual solubility of oil and water by solubilizer. *J colloid Interface Sci* **1973;** 42:381-387.

Shinoda K, Lindman B. Organised surfactant systems: microemulsions. *Langmuir* **1987;** 3:135-149.

Shiraishi T, Kikuchi T, Fukushi K, Shinotoh H, Nagatsuka S, Tanaka N, Ota T, Sato K, Hirano H, Tanada S, Iyo M, Irie T. Estimation of Plasma IC50 of Donepezil Hydrochloride for Brain Acetylcholinesterase Inhibition in Monkey Using N-[^{11}C]methylpiperidin-4-yl Acetate ([11C]MP4A) and PET. *Neuropsychopharmacology* **2005**; 30:2154-2161.

Siegal T, zylber-Katz E. Strategies for increasing drug delivery to the brain: focus on brain lymphoma. *Clin Pharmacokinet* **2002;** 41:171-186.

Sippel JM, Giraud GD, Holden WE. Nasal administration of the nitric oxide synthase inhibitor L-NAME-induces daytime somnolence. *Sleep* **1999;** 22:786-788.

Sippl W. Computational approaches for the prediction of blood-brain barrier permeation. *Curr Med Chem* **2002;** 2:212-227.

Skodvin T, Sjoblem J, Saeten JO, Gestblom B. Solubilization of drugs in microemulsions as studied by dielectric spectroscopy. *J Colloid Interfcae Sci* **1993;** 155:392-401.

Small GW. Tacrine for treating Alzheimer's disease. *J Am Med Assoc* **1992;** 268:2564-2565.

Smith QR. Advances in Neurology. In: Wurtman R, editors. Alzheimer's Diseases. New York: Raven Press; **1990.** Vol. 51. p. 217-222.

Smolnik R, Molle M, Fehm HL, Born J, Brain potentials and attention after acute and subchronic intranasal administration of ACTH 4-10 and desacetyl-alpha-MSH in humans. *Neuroendocrinology* **1999**; 70:63-72.

Smolnik R, Perras B, Molle M, Fehm HL, Born J, Event-related brain potentials and working memory function in healthy humans after single-dose and prolonged intranasal administration of adrenocorticotropin 4-10 and desacetyl-alpha-melanocyte stimulating hormone. *J Clin Psychopharmacol* **2000**; 20:445-454.

Soane RJ, Frier M, Perkins AC, Jones NS, Davis SS, Illum L. Evaluation of the clearance of bioadhesive systems in humans. *Int J Pharm* **1999**; 178:55-65.

Stein DJ, Van-Heerden B, Wessels CJ, Van-Kardenburg, Warwick J, Wasserman HJ. Single photon emission computed tomography of the brain with Tc-99m HMPAO during sumatriptan challenge in obsessive-compulsive disorder: investigating the functional role of the serotonin auto-receptor. *Prog Neuropsychopharmacol Biol Psychiatry* **1999**; 23(6):1079-1099.

Stratford RE, Lee VH. Aminopeptidase activity in homogenates of various absorptive mucosas in albino rabbit: Implication in peptide delivery. *Int J Pharm* **1986**; 30:73-82.

Suzuki Y, Makino Y. Mucosal drug delivery using cellulose derivatives as a functional polymer *J Control Release* **1999**; 62:101-107.

Tabony J. Formation of cubic structures in microemulsions containing equal volumes of water and oil. *Nature* **1986**; 319:400.

Talegaonkar S, Mishra PR. Intranasal delivery: An approach to bypass the blood brain barrier. *Indian J Pharmacol* June **2004**; 36(3):140-147.

Tamai I, Sai Y, Kobayashi H, Kamata M, Wakamiya T, Tsuji A. Structure-internalization relationship for adsorptive-mediated endocytosis of basic peptides at the blood–brain barrier. *J Pharmacol Exp Ther* **1997**; 280:410-415.

Taylor EM. The impact of efflux transporters in the brain on the development of drugs for CNS disorders. *Clin Pharmacokinet* **2002**; 41:81-92.

Telting-Diaz M, Lunte CE. Distribution of tacrine across the blood-brain barrier in awake, free moving rats using in vivo microdialysis sampling. *Pharm Res* **1993**; 10:44-48.

Tengamnuay P, Mitra AK. Transport of tyrosine and phenylalanine across the rat nasal mucosa. *Life Sci* **1988;** 43:585-593.

Tenjarla S. Microemulsions: an overview and pharmaceutical applications. *Crit Rev Therapeutic Drug Carrier Systems* **1999;** 16:461-521.

The World Health Report, **2001.**

Thirumalai MS, A Stranger in the Family: Alzheimer's Disease and Language, *Language in India,* **2002**, Volume 1.

Thompson R. Lysozyme and its relation to antibacterial properties of various tissues and secretions. *Arch Pathol* **1940;** 30:1096.

Thorne RG, Emory CR, Ala TA, Frey WH. Quantitative analysis of the olfactory pathway for drug delivery to the brain. *Brain Res* **1995;** 692:278-282.

Thorne RG, Frey WH II. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet* **2001;** 40:907-946.

Thorne RG, Pronk GJ, Padmanabhan V, Frey WH II Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience* **2004;** 127:481-496

Thornton-Manning JR, Dahl AR. Metabolic capacity of nasal tissue interspecies comparisons of xenobiotic-metabolizing enzymes. *Mut Res* **1997;** 380:43-59.

Tonndorf J, Hyde RW, Chinn HI, Lett JE. Absorption form nasal mucosa membrane: systemic effect of hyoscine following intranasal administration. *Ann Otol Rhinol Laryngol* **1953;** 62:630

Tourtauchaux R, Jalenques I, Mesras D, Maublant J. Regional cerebral perfusion by Tc-99m HMPAO SPECT before and after tacrine in patients with Alzheimer's disease. Neurosis- Personality disorders and eating disorder; old age psychiatry, 367s.

Trotta M, Morel S, Gasco MR. Effect of oil phase composition on the skin permeation of felodipine from (o/w) microemulsions. *Pharmazie* **1997;** 52:50-53.

Ugwoke MI, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *J Pharm Pharmacol* **2001;** 53:3-22.

Vajdy M, O'Hagan DT. Microparticles for intranasal immunization. *Adv Drug Deliv Rev* **2001**; 51:127-141.

Van de Donk HJM, Merkus FWHM. Decreases in ciliary beat frequency due to intranasal administration of propranolol. *J Pharm Sci* **1982**; 71:595-596.

Van de Donk HJM, Muller Plantema IP, Zuidema J, Merkus FWHM. The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. *Rhinology* **1980**; 18:119-130.

Van de Waterbeemd H, Smith DA, Beaumont K. Property-based design: optimization of drug absorption and pharmacokinetics. *J Med Chem* **2001**; 44:1313-1333.

Vargas MG, Havel J, Patocka J. Capillary zone electrophoretic determination of some drugs against Alzheimer's disease. *J chromatography* 1998, 802, 1:121-128.

Vihola H, Laukkanen A, Hirvonen J, Tenhu H. Binding and release of drugs into and from thermosensitive poly(*N*-vinyl caprolactam) nanoparticles. *Eur J Pharm Sci* **2002**; 16:69-74.

Vinson PK, Sheehan JG, Miller WG, Scriven LE, Davis HT. Viewing microemulsions with freeze-fracture transmission electron microscopy. *J Phys Chem* **1991**; 95:2546-2550.

Vyas TK, Babbar AK, Sharma RK, Misra A. Intranasal mucoadhesive microemulsion of zolmitriptan: Preliminary studies on brain-targeting. *J Drug Target* **2005b**; 13(5):317-324.

Vyas TK, Babbar AK, Sharma RK, Singh S, Misra A. Intranasal Mucoadhesive Microemulsions of Clonazepam: Preliminary Studies on Brain Targeting. *J Pharm Sci* **2006b**, 95(3):570-580.

Vyas TK, Babbar AK, Sharma RK, Singh S, Misra A. Preliminary Brain Targeting Studies of Intranasal Mucoadhesive Microemulsions of Sumatriptan. *AAPS PharmSciTech* **2006a**; 7(1):Article 8.

Vyas TK, Shahiwala A, Marathe S, Misra AN. Intranasal Drug Delivery for Brain Targeting. *Current Drug Delivery* **2005a**; 2(2):164-175.

Wang D, Gao Y, Yun L. Study on brain targeting of raltitrexed following intranasal administration in rats. *Cancer Chemother Pharmacol* **2006**; 57:97-104.

Wang F, Jiang X, Lu W. Profiles of methotrexate in blood and CSF following intranasal and intravenous administration to rats. *Int J Pharm* **2003;** 263(1-2):1-7.

Wermeling DP, Miller JL, Rudy AC. Systematic Intranasal Drug Delivery: Concepts and Applications. *Drug Delivery Technology* **2003.**

Wheatley MA, Dent J, Wheeldon EB, Smith PL. Nasal drug delivery: An in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. *J Control Release* **1988;** 8:167-177.

Witt KA, Gillespie TJ, Huber JD, Egleton RD, Davis TP. Peptide drug modifications to enhance bioavailability and blood-brain barrier permeability. *Peptides* **2001;** 22:2329-2343.

Yang Q, Williams D, Owusu-Ababio G, Ebube NK, Habib MJ. Controlled release tacrine delivery system for the treatment of Alzheimer's disease. *Drug Delivery* **2001;** 8:93-98.

Yasui-Furukori N, Furuya R, Takahata T, Tateishi T. Determination of donepezil, an acetylcholinesterase inhibitor, in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection. *J Chromatography B: Analyt Technol Biomed Life Sci* **2002**; 768(2):261-265.

Yu ZJ, Neuman RD. Reversed micellar solution-to-bicontinuous microemulsion transition in sodium bis(2-ehtylhexyl) phosphate/n-heptane/water system. *Langmuir* **1995;** 11:1081-1086.

Zhang Q, Jiang X, Jiang W, Lu W, Su L, Shi Z. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation of the targeting efficiency to brain. *Int J Pharm* **2004**; 275:85-96.

Zhang Q, Zha L, Zhang Y, Jiang W, Lu W, Shi Z, Jiagn X, Fu S. The brain targeting efficiency following nasally applied MPEG-PLA nanoparticles in rats. *J Drug Target* **2006**; 14(5):281–290.