Chapter 2

Literature Review

2.1 CNS DISORDERS

2.1.1 EPILEPSY

Epilepsy represents the most common serious neurological condition. The lifetime total prevalence of the number of people in a population who have ever had epilepsy lies between 2 and 5% Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures (CEP 1993; Blume et al 2001). These seizures are transient signs and/or symptoms of abnormal, excessive or synchronous neuronal activity in the brain. Epilepsy is usually controlled, but not cured, with medication. There are over 40 different types of epilepsy (WHO 2001), each presenting with its own unique combination of seizure type, typical age of onset, EEG findings, treatment, and prognosis. The most widespread classification of the epilepsies divides epilepsy syndromes by location or distribution of seizures and by cause (Fisher et al 2005). Epilepsy is a paroxysmal electrical disturbance of cerebral neurons which can cause a disorder of:

(1) The motor system with tonic seizures, tonic-clonic seizures, myoclonic seizures, atonic attacks and automatisms.

(2) The sensory system with visual, hearing, smell and somatosensory auras.

(3) Behaviour with feelings of anxiety and fear

(4) Consciousness of several seconds duration with Petit Mal (simple absence) or 10 to 45minutes with Grand Mal (tonic-clonic) seizures.

(5) Autonomic functions with pallor, tachycardia, blood pressure and pupillary abnormalities.

Definition and classification of seizures

The "Commission on classifications and terminology of the international league against epilepsy"classified seizures as follows

1. Partial seizures:
   - 1.1 Simple partial seizures (with motor signs, somatosensory or special sensory symptoms, autonomic or psychic symptoms)
   - 1.2 Complex partial seizures (simple partial followed by a disorder of consciousness or with disorder of consciousness from onset of seizures)
   - 1.3 Partial seizures with secondary generalisation.

2. Generalized seizures (Absence, myoclonic, clonic, tonic, tonic-clonic or atonic).
3. Other (Unclassifiable i.e., situational)

Primary generalised epilepsies share a number of features: (1) The absence of an aura (2) Geneticcontribution, possibly autosomal dominant or polygenic, with a lower threshold for convulsions and age specific penetrance (3) Triad of generalised grand mal, absence and myoclonic seizures (4) EEG 3Hz spike and wave trait (5) Photosensitivity (6) Characteristic clinical course

Partial epilepsy is characterized by the presence of an initial clinical symptom or sign as an aura or electrical disturbance signifying the focal cerebral origin. Simple partial seizures demonstrate an aura without disturbance of consciousness. With complex partial seizures a disturbance of consciousness is characteristically found. Secondary generalisation refers to the development of tonic-clonic seizures following upon partial seizures.

Complex partial seizures are characterized by: (1) Preceding simple partial aura (2) Disturbance of consciousness (3) Duration usually longer than 30 seconds up to several minutes (4) Automatism of a series of involuntary movements i.e., chewing or swallowing movements, ambulatory movements, simple verbal sounds, simple stereotyped hand movements (5) EEG focal epileptiform activity in temporal or frontal lobe.

Typical or Petit Mal absences usually occur in childhood or adolescence and are characterised by: (1) Sudden onset (2) Vacant staring of the eyes with interruption of consciousness (3) Interruption of continuing psychological activity (4) Short duration less than 10 seconds in 85% of attacks, usually less than 30 seconds (5) Upward rotation of eyes (6) Clear consciousness immediately after the attack (7) With increasing duration of attacks clonic, atonic and tonic as well as autonomic components are observed (8) EEG 3 Hz spike wave activity.

Defining epilepsy as disease or syndrome

The "Commission on classification and terminology of the International League against Epilepsy"1993 classified epilepsy as follows:

1. Partial epilepsy syndromes

   Idiopathic age related onset (i.e., benign childhood epilepsy with centrotemporal

spikes) and Symptomatic (i.e., syndromes of temporal, frontal,parietal and occipital origin).

2. Generalised epilepsy syndromes

   Idiopathic with age related onset (i.e., absence, myoclonic, tonic clonic seizures), Cryptogenic or symptomatic (i.e., West syndrome, Lennox-Gastaut syndrome) and Symptomatic generalised epilepsy syndromes (i.e., diffuse metabolic encephalopathy).

3. Other (i.e., situation related seizures, fever convulsions).

Treatment:

Curently there are 19 medications approved by the Food and Drug Administration for the use of treatment of epileptic seizures in the US: carbamazepine (common US brand name Tegretol), clorazepate (Tranxene) clonazepam (Klonopin), ethosuximide (Zarontin), felbamate (Felbatol), fosphenytoin (Cerebyx), gabapentin (Neurontin), lamotrigine (Lamictal), levetiracetam (Keppra), oxcarbazepine (Trileptal), phenobarbital (Luminal), phenytoin (Dilantin), pregabalin (Lyrica), primidone (Mysoline), tiagabine (Gabitril), topiramate (Topamax), valproate semisodium (Depakote), valproic acid (Depakene), and zonisamide (Zonegran). Most of these appeared after 1990.

Medications commonly available outside the US but still labelled as "investigational" within the US are clobazam (Frisium) and vigabatrin (Sabril). Medications currently under clinical trial under the supervision of the FDA include retigabine, brivaracetam, and seletracetam. Other drugs are commonly used to abort an active seizure or interrupt a seizure flurry; these include diazepam (Valium, Diastat) and lorazepam (Ativan). Drugs used only in the treatment of refractory status epilepticus include paraldehyde (Paral), midazolam (Versed), and pentobarbital (Nembutal).

Some anticonvulsant medications do not have primary FDA-approved uses in epilepsy but are used in limited trials, remain in rare use in difficult cases, have limited "grandfather" status, are bound to particular severe epilepsies, or are under current investigation. These include acetazolamide (Diamox), progesterone,

adrenocorticotropic hormone (ACTH, Acthar), various corticotropic steroid hormones (prednisone), or bromide.Mechanism of drug action and choice for seizure type:

1. *AEDs that influence sodium channels*

   These drugs appear to be effective against generalised tonic clonic and partial seizures. carbamazepine, phenytoin, valproic acid and lamotrigine as well as to a lesser extent barbiturates and benzodiazepines appear to act at this site. Gabapentin and oxcarbazepine also have an action at this site

2. *AEDs that influence GABA receptors*

   Drugs that enhance GABA receptor inhibition tend to be effective against myoclonic seizures (MacDondald, 1994). Valporate, benzodiazepines and barbiturates work at this site

3. *AEDs that reduce low threshold T-type calcium currents*

   Drugs that are effective against generalised absence seizures appear to reduce low threshold (T-type) calcium currents. Valporate work at this level

4. *Gabapentin*: The mechanism of action of Gabapentin is unknown

2.1.2 STROKE

Stroke can be subdivided into 2 categories, ischemic and hemorrhagic. Ischemic strokes are more prevalent than hemorrhagic, making up approximately 87% of all cases, and have been the target of most drug trials (Rosamond et al 2007). Cerebral hypoxia/ischemia can be caused by a broad spectrum of diseases that affect the cardiovascular system and/or the respiratory system. Hypoxia generally refers to a lack of oxygen in any part of the body. In a neurological context, it refers to a reduction of oxygen to the brain despite adequate amounts of blood.

There are four types of disorders to consider: focal cerebral ischemia, global cerebral ischemia, diffuse cerebral hypoxia, and cerebral infarction.

i **Focal cerebral ischemia:** Focal cerebral ischemia (FCI) is often results from a blood clot in the brain. The blood flow in the affected area is reduced. The reduction could be severe or mild but usually FCI causes irreversible injury to sensitive neurons. The clinical signs and symptoms last approximately 15–30 minutes.

ii **Global cerebral ischemia:** Global cerebral ischemia (GCI) is a serious

condition caused by ventricular fibrillation or cardiac asystole, which stops all blood flow to the brain. If the GCI lasts more than five to ten minutes, then it is likely the person will have suffered a loss of consciousness that makes recovery doubtful.

iii **Diffuse cerebral hypoxia:** Diffuse cerebral hypoxia (DCH) is limited to conditions that cause mild to moderate hypoxemia, or low arterial-oxygen content due to deficient blood oxygenation. Pure cerebral hypoxia causes cerebral dysfunction but not irreversible brain damage. Pure cerebral hypoxia can occur due to pulmonary disease, altitude sickness, or severe anemia.

iv **Cerebral infarction:** Cerebral infarction (CI) is a severe condition caused by a focal vascular occlusion in an area of the brain. This causes an area of destruction resulting from a lack of oxygen delivery.

Pathophysiology of Ischemic stroke:

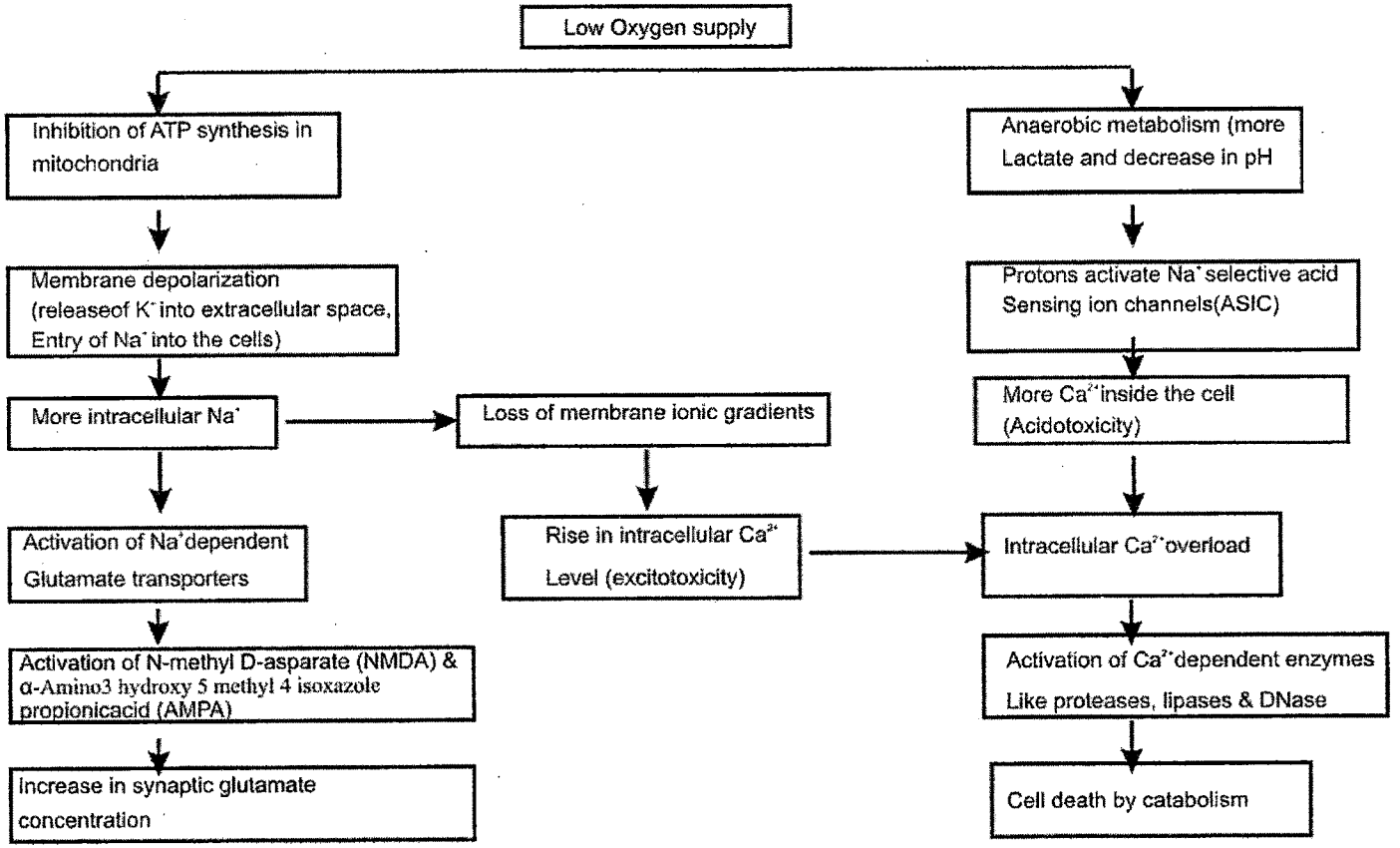
A thrombosis, an embolism or systemic hypo-perfusion, all of which result in a restriction of blood flow to the brain, can cause an ischemic stroke, which results in insufficient oxygen and glucose delivery to support cellular homoestasis. This elicits multiple processes that lead to cell death: excitotoxicity (Olney et al 1969), acidotoxicity (Simon 2006) and ionic imbalance (Caplan 2000), peri-infarct depolarization (Gonzalez et al 1992), oxidative and nitrative stress (Halliwell 1994), inflammation (Bruce et al 1996; Nawashiro et al 1997) and apoptosis (Gonzalez et al 2006).

Each of the above pathophysiological processes has a distinct time frame, some occurring over minutes, others over hours and days, causing injury to neurons, glia and endothelial cells. Within the core of the ischemic area, where blood flow is most severely restricted, excitotoxic and necrotic cell death occurs within minutes. In the periphery of the ischemic area, where collateral blood flow can buffer the full effects of the stroke, the degree of ischemia and the timing of reperfusion determine the outcome for individual cells. In this ischemic penumbra cell death occurs less rapidly via mechanisms such as apoptosis and inflammation (Gonzalez et al. 2006).

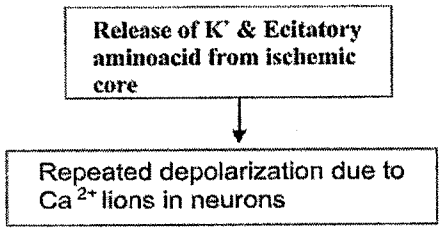
A detailed mechanism of ischemic brain damage has summarized below (Doyle et al 2008). The biochemical events involving these mechanisms are schematically shown in Fig.2.1

Fig 2.1 Mechanisms of ischemic brain damage

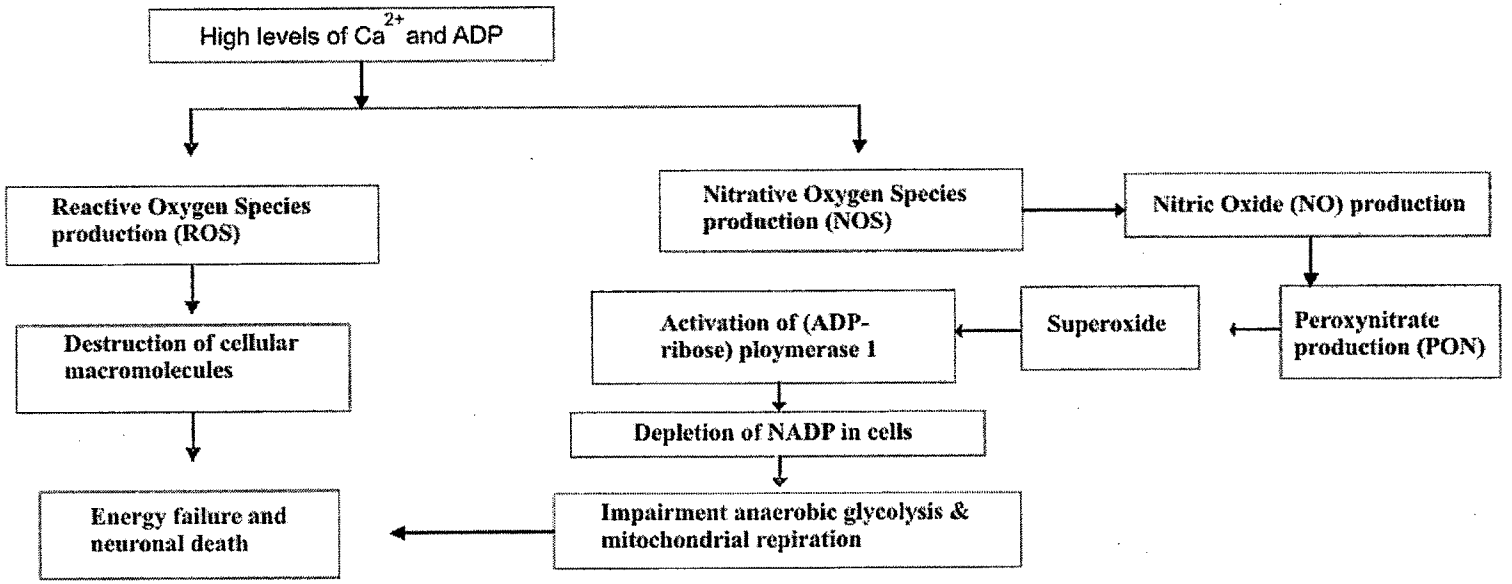
I Excitotoxicity, ii. Acidotoxicity iii. Ionicimbalance

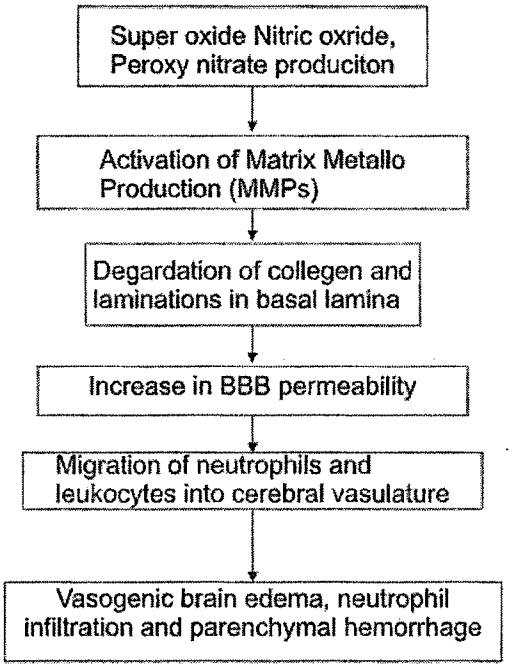


4. Perinfract depolarisation

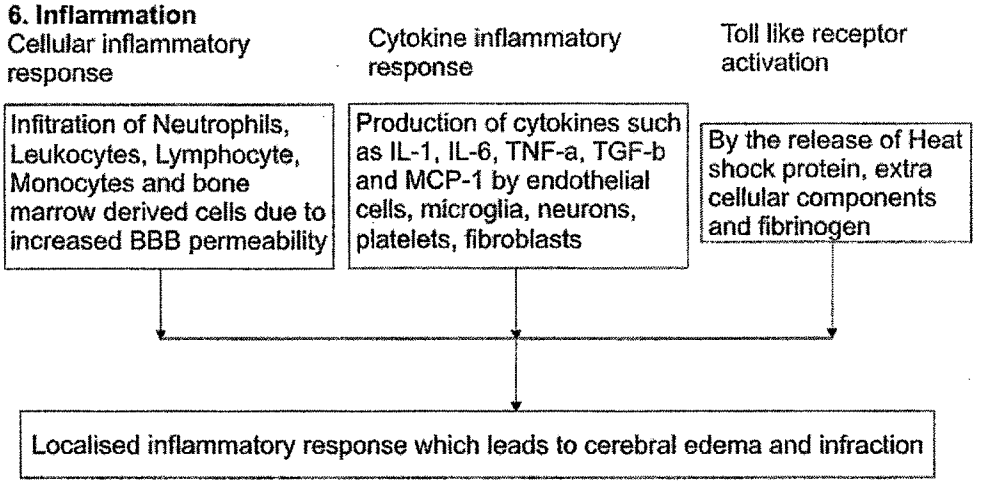


5. Oxidative and Nitrative stress





6. Inflammation



7.Apotopsis:

Programmed cell death, or "cell suicide"; a form of cell death in which a controlled sequence of events (or program) leads to the elimination of cells without releasing harmful substances into the surrounding area. Many types of cell damage can trigger apoptosis. Triggers of apoptosis include oxygen free radicals, death receptor ligation, DNA damage, protease activation and ionic imbalance. Apoptosis refers only to the structural changes cells go through, and programmed cell death refers to the complete underlying process, but the terms are often used synonymously.

Treatment :

Patients with acute stroke should have computed tomography of the brain to distinguish ischemic from hemorrhagic stroke. This separation is vital because subsequent investigations and treatment differ for the 2 types. Neuroimaging will also identify conditions that mimic stroke and can help predict outcome. Ideally, imaging should be performed soon after admission. Magnetic resonance imaging of the brain may eventually replace computed tomography because it not only identifies stroke anatomy but can also assess blood flow and perfusion in the brain, detect whether lesions are new or old, and identify carotid artery stenosis.

Despite the tremendous mortality and morbidity of stroke, treatment options remain limited. Many pathophysiological key mechanisms of cerebral ischemia have been identified in recent years, but drug treatment targeting one or a few of these mechanisms has failed to improve clinical outcome after stroke. The most plausible reason for this failure is the multiplicity of mechanisms involved in causing neuronal damage during ischemia. Drugs targeting a multimodal mode of action could potentially overcome this dilemma and have recently been shown to provide remarkable benefit in preclinical studies. The only drug approved for the acute phase of ischemic stroke is recombinant tissue plasminogen activator (rtPA) - which, however, predominantly acts by targeting a single mechanism, the lysis of the intravascular clot. Antiplatelet,

antihypertensive, and lipid-lowering therapies are approved for secondary stroke prevention. Animal experimental studies confirmed that antiplatelet, antihypertensive and lipid-lowering drugs exert multimodal actions including neuroprotective and neuroregenerative properties.

Cerebral ischemia is currently treated with

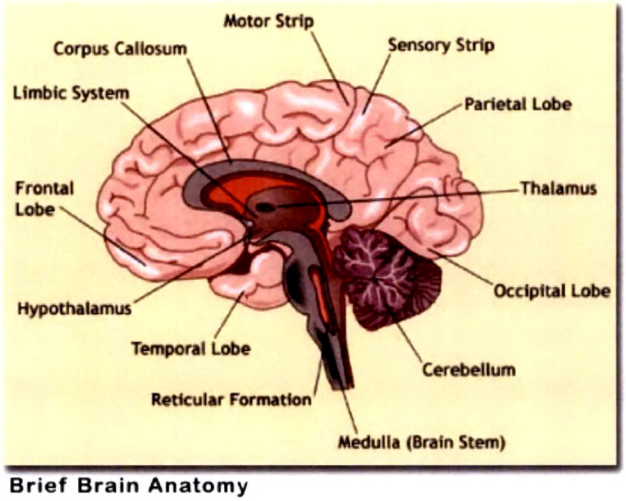
1. Clot busters and thrombolytic agents: Tissue plasminagen activator should be given within 3 hours and gives only slight benefit. The treatment continues with oral antiplatelet agents. This treatment cannot be used for hemorrhagic stroke.
2. Nootropic drugs: smart drugs, memory enhancers, and cognitive enhancers, are drugs, supplements, nutraceuticals, and functional foods that are purported to improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration.[1][2] Typically, nootropics are thought to work by altering the availability of the brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth. However the efficacy of nootropic substances in most cases has not been conclusively determined. This is complicated by the difficulty of defining and quantifying cognition and intelligence. Nicergoline or hydergine seems to be more promising.
3. The dispiriting list of failures includes calcium and sodium channel blockers: (e.g. nimodipine), NMDA-receptor antagonists (e.g. selfotel), drugs that inhibit glutamate release (e.g. lobeluzole) and various free radical scavengers (e.g. tirilazad).

2.2. CENTRAL NERVOUS SYSTEM

2.2.1 ANATOMY AND PHYSIOLOGY OF BRAIN

The major regions of the brain are the cerebral hemispheres, diencephalon, brain stem and cerebellum (Fig2.2).

Fig 2.2 Anatomical regions of brain



Cerebral hemispheres:-

The cerebral hemispheres located on the most superior part of the brain, are separated by the longitudinal fissure. They make up approximately 83% of total brain mass and are collectively referred to as the cerebrum. Major regions of cerebral hemispheres are presented in fig. The cerebral cortex constitutes a 2-4 mm thick grey matter surface layer and, because of its many convolutions, accounts for about 40% of total brain mass. It is responsible for conscious behavior and contains three different functional areas: the motor areas, sensory areas and association areas. Located internally are the white matter, responsible for communication between cerebral areas and between the cerebral cortex and lower regions of the CNS, as well as the basal nuclei (or basal ganglia), involved in controlling muscular movements.

Diencephalon:-

The diencephalon is located centrally within the forebrain. It consists of the thalamus, hypothalamus and epithalamus, which together enclose the third ventricle. The thalamus acts as a grouping and relay station for sensory inputs ascending to the sensory cortex and association areas. It also mediates motor activities, cortical arousal and memories. The hypothalamus, by controlling the autonomic (involuntary) nervous

system, is responsible for maintaining the body's homeostatic balance. Moreover it forms a part of the limbic system, the 'emotional' brain. The epithalamus consists of the pineal gland and the CSF producing choroid plexus.

Brain stem:-

The brain stem is similarly structured as the spinal cord: it consists of grey matter surrounded by white matter fiber tracts. Its major regions are the midbrain, pons and medulla oblongata. The midbrain, which surrounds the cerebral aqueduct, provides fibre pathways between higher and lower brain centers, contains visual and auditory reflex and subcortical motor centers. The pons is mainly a conduction region, but its nuclei also contribute to the regulation of respiration and cranial nerves. The medulla oblongata takes an important role as an autonomic reflex centre involved in maintaining body homeostasis. In particular, nuclei in the medulla regulate respiratory rhythm, heart rate, blood pressure and several cranial nerves. Moreover, it provides conduction pathways between the inferior spinal cord and higher brain centers.

Cerebellum:-

The cerebellum, which is located dorsal to the pons and medulla, accounts for about 11% of total brain mass. Like the cerebrum, it has a thin outer cortex of grey matter, internal white matter, and small, deeply situated, paired masses (nuclei) of grey matter. The cerebellum processes impulses received from the cerebral cortex, various brain stem nuclei and sensory receptors in order to appropriately control skeletal muscle contraction, thus giving smooth, coordinated movements.

2.2.2 DRUG DELIVERY TO THE CENTRAL NERVOUS SYSTEM (Misra et al 2005)

The brain is a delicate organ, and evolution built very efficient ways to protect it. Unfortunately, the same mechanism protect it against intrusive chemicals can also frustrate therapeutic interventions. Despite enormous advances in brain research, brain and central nervous system disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. The major problem in drug delivery to brain is the presence of the BBB. Drugs that are effective against diseases in the CNS and reach brain via the blood compartment must pass the BBB.

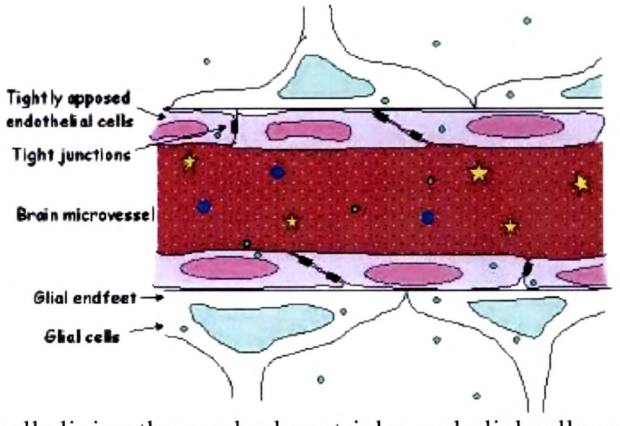
Barriers to CNS deliver

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.

i. Blood brain barrier:

It is now well established that the BBB is a unique membranous barrier that tightly segregates the brain from the circulating blood. The CNS consists of blood capillaries which are structurally different from the blood capillaries in other tissues; these structural differences result a permeability barrier between the blood within brain capillaries and the extra cellular fluid in brain tissue. 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 300nm of endothelial cytosol (Illum 2004). Capillaries of the vertebrate brain and spinal cord lack the small pores that allow rapid movement of solutes from circulation into other organs; these capillaries are lined with a layer of special endothelial cells that lack fenestrations and are sealed with tight junctions. These tight endothelium junctions can be 100 times tighter than junctions of other capillary endothelium (Butte et al 1990) Tight epithelium, similar in nature to this barrier, is also found in other organs (skin, bladder, colon, and lung). This permeability barrier, comprising, the brain capillary endothelium, is known as the BBB (Fig 2.3).

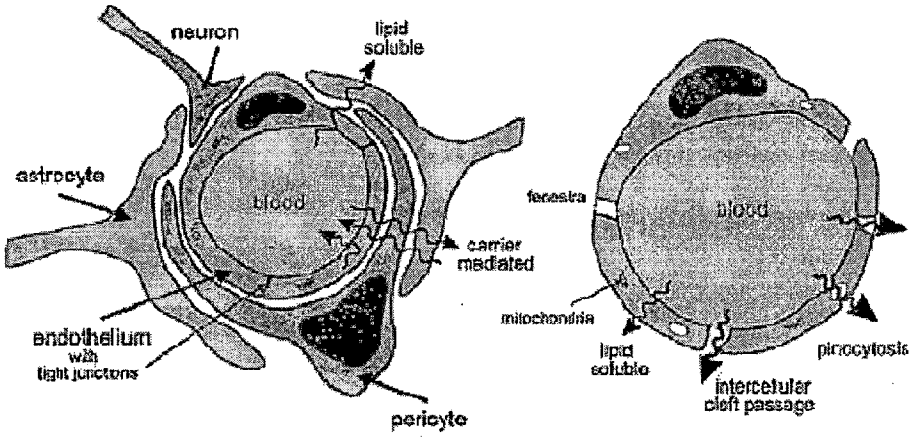
Fig 2.3 Schematic representation of BBB



Ependymal cells lining the cerebral ventricles and glial cells are of three types. Astrocytes from the structural frame work for the neurons and control their biochemical environment. Astrocytes foot processes or limbs that spread out and

abutting one other, encapsulate the capillaries are closely associated with the blood vessels to form the BBB (Fig.2.4). Oligodendrocytes are responsible for the formation and maintenance of the myelin sheath, which surrounds axons and is essential for the fast transmission of action potentials by salutatory conduction. Microglias are blood derived mononuclear macrophages. The tight junctions between endothelial cells result in a very high trans-endothelial electrical resistance of 1500-2000 Ωcm^2 compared to 3-33 Ωcm^2 of other tissues which reduces the aqueous based para-cellular diffusion that is observed in other organs.

Fig 2.4 Schematic comparison between brain (left) and general (right) capillaries



Some regions of the CNS do not express the classical BBB capillary endothelial cells, but have micro-vessels similar to those of periphery. These areas are adjacent to the ventricles of the brain and are termed the circumventricular organs (CVOs). The CVOs include the choroid plexus, the median eminence, neurohypophysis, subfornical organ, subcommisaral organ and the area postrema. Though in the CVO brain regions the capillaries are more permeable to solutes, the epithelial cells of the choroid plexus and the tanycytes of other regions from right junctions prevent transport from the aluminal extracellular fluid (ECF) to the brain ECF. The choroid plexus may be of importance when considering the transport of peptide drugs, because it is the major site of cerebrospinal fluid (CSF) production and both the CSF freely exchange.

The BBB also has an additional enzymatic aspect. 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.

Finally, the BBB is further reinforced by a high concentration of P-glycoprotein (Pgp), active-drug-efflux-transporter protein in the luminal membranes of the cerebral capillary endothelium. This efflux transporter actively removes a broad range of drug molecules from the endothelial cell cytoplasm before they cross into the brain parenchyma. Figure-1 gives a schematic representation of all these BBB properties using a comparison between brain and general capillaries.

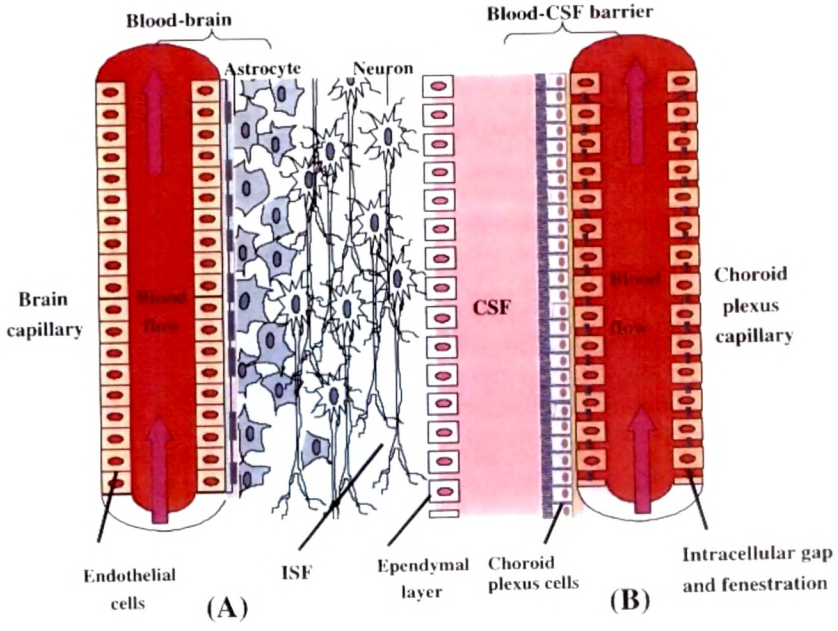
ii. Blood-cerebrospinal fluid barrier

The second barrier that 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 choroid 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 at the barriers between the blood and CSF. On the external surface of the brain, the ependymal cells fold over on to 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 Blood-CSF barrier is largely passive. The choroid plexus forms the CSF and actively regulates the concentration of molecules in the CSF. The choroid plexus consists of highly vascularized, "cauliflower – like" masses of pia mater tissue that dip into pockets formed by ependymal cells (Fig 2.5). The preponderance of choroid 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. However these epithelial-like cells have shown a low resistance as compared the cerebral endothelial cells, approximately 200Ωcm^2, between blood and CSF.

In addition, the BCB is fortified by an active organic acid transporter system in the choroid 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. 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.

Fig 2.5 (A) Blood Brain Barrier (B) Blood cerebrospinal fluid Barrier



(ISF- Interstitial fluid, CSF- Cerebro spinal fluid)

iii. Blood-tumor barrier

Intracranial drug delivery is even more challenging when the target is a VNS tumor. The presence of the BBB in the microvasculature of CNS tumors has clinical consequences. For example, even when primary and secondary systemic tumors respond to chemotherapeutic agents delivered via the cardiovascular system, intracranial metastases often continue to grow. In CNS malignancies where the BBB is significantly compromised, a variety of physiological barriers common to all solid tumors inhibit drug delivery via the cardiovascular system. Drug delivery to neoplastic cells in a solid tumor is compromised by a heterogeneous distribution of microvasculature throughout the tumor interstitial, which leads to spatially inconsistent drug delivery. Furthermore, as a tumor grows large, the vascular surface area decreases, leading to a reduction in trans-vascular exchange of blood-borne molecules. At the same time, intra-capillary distance increases, leading to greater diffusion requirements for drug delivery to neoplastic cells and due to high interstitial tumor pressure and the associated peri-turmoral edema leads to increase in hydrostatic pressure in the normal brain parenchyma adjacent to the tumor. As a result the cerebral microvasculature in these tumor adjacent regions of normal brain may be even less permeable to drugs than normal brain endothelium leading to exceptionally low extra - tumoral interstitial drug concentrations. Brain tumors may also disrupt BBB, but these are also local and nonhomogeneous disruptions.

In conclusion, the delivery of drugs to the CNS via the cardiovascular system is often precluded by a variety of formidable barriers including the BBB, the BCB and the BTB.

2.2.3 STRATEGIES FOR ENHANCED CNS DRUG DELIVERY (Misra et al, 2005)

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

reach near to success it is essential that combinations with other parameters like capillary membrane permeability first pass metabolism and volume distribution.

2. **Prodrugs:** Prodrugs are pharmacologically inactive compound that result from transient chemical modifications of biologically active species. After administration, the prodrug by virtue of its improved characteristics, is brought closer to the receptor site and is maintained there for longer period of time. Here it gets converted to the active form usually via a single activation step.
3. **Receptor mediated transport:** The receptor transport is mainly based on the formation of chimeric peptides by conjugation of the drugs that has to be delivered to a transport vector that undergoes BBB-transport via receptor or may be via absorptive – mediated transcytosis. This approach is intended to provide brain delivery of large peptides. Since this approach involves stoichiometry, only limited number of molecules fit in to this category.
4. **Chemical drug delivery:** They are inactive chemical derivative of a drug obtained by one or more chemical modification so that the newly attached moiety are monomolecular units and provide a site specific delivery of drug through multistep enzymatic transformation.
5. **BBB disruption (Osmotic BBBD, Biochemical BBBD and Ultrasound-induced disruption):** One of the approaches to circumvent the dense microvasculature of the brain is by delivery using a transient osmotic opening. Hyperosmolar substance like mannitol, arabinose is likely to cause disruption of BBB due to migration of water from endothelial cells to capillaries, which in turn cause shrinkage of the cells and results in intracellular gaps. The approach was resulted and breaks down the self defense mechanism of the brain and leaves it vulnerable. The other approaches are BBB disruption using use of labradimil which has selectivity for bradykinn B_2 receptor and Ultrasound induced mild hyperthermia which can be controlled and localized to a small volume within the tissue. The former approach may lead to membrane permeability due to hyperthermia and the later one is under consideration and at a considerable distance from practical application.
6. **Biodegradable polymer Wafers, Microspheres and Nanoparticles:** Polymeric or lipid-based devices that can deliver drug molecules at defined rates for specific

periods of time are now making a tremendous impact in clinical medicine. Drug delivery directly to the brain interstitium using polyanhydride wafers can circumvent the BBB and release unprecedented levels of drug directly to an intracranial target in a sustained fashion for extended periods of time. The fate of a drug delivered to the brain interstitium from the biodegradable polymer wafer was predicted by a mathematical model based on (a) rates of drug transport via diffusion and fluid convection; (b) rates of elimination from the brain via degradation, metabolism and permeation through capillary networks; and (c) rates of local binding and internalization. Such models are used to predict the intracranial drug concentrations that result from BCNU-loaded pCPP:SA (1,3 bis-para-carboxy phenoxy propane:sebacic acid) wafers as well as other drug-polymer combinations, paving the way for the rational design of drugs specifically for intracranial polymeric delivery. Conjugation of a polymerically delivered chemotherapeutic agent to a water-soluble macromolecule increases drug penetration into the brain by increasing the period of drug retention in brain tissue (Dang et al 19994). Hanes et al 1997 have recently developed IL-2-loaded biodegradable polymer microspheres for local cytokine delivery to improve the immunotherapeutic approach to brain tumor treatment. Nanoparticles have been employed as a delivery system for compounds like dalargin, kyotorphin, loperamide and doxorubicin in some animals. The probable mechanism could be endocytic uptake or transcytosis. The particles are usually 10 to 100 mm diameter, made from natural or artificial polymers; drugs are bound in form of solid solution or dispersion.

7. **Cell-penetrating peptides:** Recently this approach has been employed by scientists and several peptides like tat derived peptides, transportan, penetratin etc. have been found to translocate across the plasma membrane of eukaryotic cells, but even can be used for intracellular, and may be even transcellular, transport of large cargo macromolecules. For example, tat fragments that are part of the cell-membrane transduction domain of the human immunodeficiency virus (HIV) have been shown in animal studies to provide enhanced brain delivery.
8. **Molecular packaging:** Delivering the peptides like enkephalin, TRH (thyrotropin-releasing hormone), and kyotorphin analogs through the BBB is an even more complex problem because they can be rapidly inactivated by

ubiquitous peptidases (Bodor et al 1992; Brownlees et al 1993). Three issues are to be solved simultaneously to enhance penetration through BBB. They are, to enhance passive transport by increasing the lipophilicity, assure enzymatic stability to prevent premature degradation, and exploit the lock-in mechanism to provide targeting. This complex approach is known as molecular packaging strategy, where the peptide unit is part of a bulky molecule, dominated by groups that direct BBB penetration and prevent recognition by peptidases. In general, a brain targeter packaged peptide delivery system contains a red-ox targeter (T), a spacer function (S), consisting of strategically used amino acids to ensure timely removal of the charged targeter from the peptide, the peptide itself (P) and a bulky lipophilic moiety (L) attached through an ester bond or sometimes through a C-terminal adjuster (A) at the carboxy terminal to enhance lipid solubility and to disguise the peptide nature of the molecule. The first successful delivery with a package was for Tyr-D-Ala-Gly-Phe-D-Leu (DADLE), an analogue of leucine enkephalin, a naturally occurring linear pentapeptide (Tyr-Gly-Gly-Phe-Leu) that binds to opioid receptors. A similar strategy was used to deliver a thyrotropin-releasing hormone (TRH) analogue to the CNS (Prokai et al 1996). These analogues are potential agents for treating neurodegenerative disorders such as Alzheimer's disease.

9. Alternative routes for CNS drug delivery

i. **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 / microspheres 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.

ii. **Intracerebroventricular delivery:** Cerebrospinal fluid is in direct communication with the interstitial fluid of the brain, to the major extent alternative invasive strategy to bypass BBB is to deliver drugs directly into cerebral ventricles. The drug penetration is hindered by slow diffusion especially with the human brain is one of the serious drawback. Moreover, rapid ventricular

CSF clearance renders the delivery system equivalent to slow intravenous infusion.

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

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

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

iv. **Interstitial delivery:** This route of administration bypasses BBB. High CNS drug concentrations can be obtained with minimal systemic exposure and toxicity. Intracranial drug concentrations can be sustained, which is crucial in the treatment of many neurodegenerative disorders and for the antitumor efficacy of many chemotherapeutic agents. Ommaya reservoir, infusaid pump, MiniMed PIMS system and Medtronic SynchroMed system are some of the systems, which have been developed for delivering drugs directly to the brain interstitium. Until recently the most widely used method has been the interstitial injection or infusion of drugs using an ommaya reservoir or implantable pump. The adaptation of the ommaya reservoir to achieve interstitial drug delivery simply involves placing the outlet catheter directly in the intracranial target area. This technique has often been applied to neurooncological patients in whom the outlet catheter is placed in the resection cavity following surgical de-bulking of a brain tumor. Chemotherapeutic agents can be periodically injected into the subcutaneous reservoir and then delivered directly to the tumor bed. This technique, however, does not achieve truly continuous drug delivery.

The ommaya reservoir or infusion pumps have thus far been used in various clinical trials with brain tumor patients to interstitially deliver the chemotherapeutic agents BCNU or its analogs, methotrexate, adriamycin, bleomycin, ß uodeoxyuridine, cisplatin, and interleukin 2(IL-2). In most of these studies the intratumoral drug concentrations were often high, and the side effects of the therapy were mild. The success of these techniques is limited by catheter

clogging or blocking by tissue debris, inadequate distribution throughout the tumor, and a high degree of burden to the patient.

10. Biotechnological approaches:

i **Gene therapy** has also been attempted to deliver drugs to the CNS. Prior to implantation, cells will be genetically modified to synthesize and release specific therapeutic agents. The therapeutic potential of this technique in the treatment of brain tumor was demonstrated. The utility of non-neuronal cells for therapeutic protein delivery to the CNS has been reviewed recently by Snyder et al 1997. The survival of foreign tissue grafts may be improved by advancements in techniques for culturing distinct cell types. Co-grafted cells engineered to release neurotropic factors with cells engineered to release therapeutic proteins may enhance the survival and development of foreign tissue. Direct application of protein-based therapeutics to the brain could soon include variations of diphtheria toxin to combat refractory gliobastomas and engineered anti-apoptotic factor (FNK) with powerful cytoprotective activity, to protect against ischemia (Cohen et al 2003; Asoh et al 2002). As for neurodegeneration, one seemingly attractive new therapy has been the use of growth factors, such as glial-derived neurotrophic factor (GDNF) as a potential means of reducing the depletion of certain key population of cells lost in Alzheimer's or Parkinson's diseases (Alexi et al 1997; Susan 2005).

ii. Antisense drug delivery is another recent technology in CNS drug delivery. Peptide nucleic acids (PNAs) are antisense oligonucleotides containing a polypeptide backbone. Receptor mediated transcytosis has been exploited to promote PNA delivery to the CNS. For example, the attachment of PNAs to the anti-transferrin (OX26) receptor antibodies has been shown to increase the brain uptake of the PNAs, with out loss of the ability of the PNAs to hybridize to target mRNA (Banks 2001).

2.3 INTRANASAL DELIVERY FOR BRAIN TARGETING

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

For 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 intranasal route can deliver therapeutic agents to the brain bypassing the BBB. Absorption of drug across the olfactory region of the nose provides a unique feature and superior option to target drugs to brain. Many scientists have reported evidence of nose-to-brain transport. Many previously abandoned potent CNS drug candidates promise to become successful CNS therapeutic drugs vial intranasal delivery. Recently, several nasal formulations, such as ergotamine (Novartis), sumatriptan (GlaxoSmithKline), and zolmitriptan (AstraZeneca) have been marketed to treat migraine. Scientists have also focused their research toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as epilepsy, migraine, emesis, depression and erectile dysfunction.

The investigation till date has attracted researchers to place the intranasal 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.

2.3.1 NASAL ANATOMY AND PHYSIOLOGY

The human nasal cavity has a total volume of about 16 to 19 ml, and a total surface area of about 180 cm^2, and is divided into two nasal cavities via the septum. The volume of each cavity is approximately 7.5 ml, having a surface area around 75 cm^2. Post drug administration into the nasal cavity, a solute can be deposited at one or more of here anatomically distinct regions, the vestibular, respiratory and olfactory region (Fig.2.6).

The vestibular region: The vestibular region is located at the opening of nasal passages 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.

The respiratory region: The respiratory region is the largest having the highest degree of vascularity and is mainly responsible for systemic drug absorption.

The olfactory region: 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. Human olfactory region comprises of thick connective tissue lamina propria, upon which rests the olfactory epithelium. The olfactory epithelium is situated between the trans-nasal septum and the lateral wall of each side of the two nasal cavities and just below the cribriform plate of ethmoid bone separating the nasal cavity from the cranial cavity. Lamina propria has axons, bowans bundle and blood vessels whereas epithelium consists of three different cells i.e. basal cells, supporting cells and olfactory receptor cells. Neurons are interspersed between supporting cells. The olfactory receptor cells are bipolar neurons with a single dendritic and extending from the cell body to the free apical surface where it ends in an olfactory knob carrying non-motile cilia, which extend above the epithelium. Neurons are 5-6 cells thick and at the basal end neuron tapers into slender non-myelated axon that joins with other axons into a bundle to form glomeruli (fillia olfactoria) in lamina propria region surrounded by glial cells and CSR), and penetrates into the cranial cavity through small holes in the cribriform plate (Fig 2.7).

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 minutes (chein and chang 1987). The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 to 6.7 in children. 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 minutes. Numerous enzymes for instance, cytochrome P450 enzymes isoforms (CYP1A, CYP2A and CYP2E), carboxylesterases and glutathione S-transferases are found in nasal cavity (Lewis et al 1994; Lewis et al 2002; Krishna et al 1995)

Fig 2.6 Nasal vascular supply

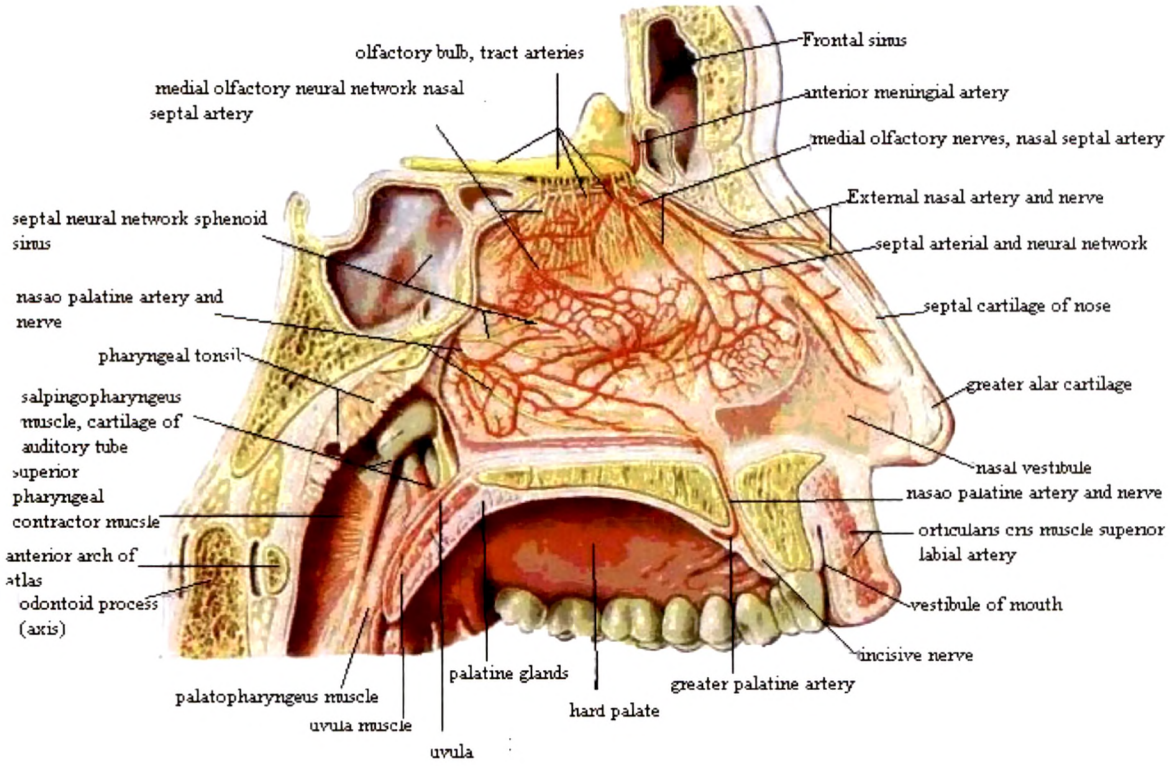
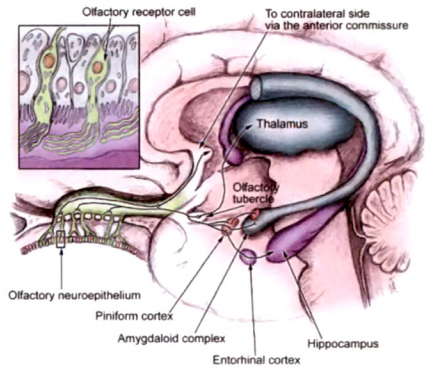


Fig 2.7 Olfactory tract to brain



2.3.2 MECHANISM OF NOSE TO BRAIN DRUG TRANSPORT

It is important to examine the pathway/mechanisms (Fisher et al 1985; Wheatley et al 1988; Tengamnuay et al 1988) involved prior to addressing the possibilities to improve transnasal uptake by the brain. The olfactory region is known to be the portal for a drug substance to enter from nose-to-brain following nasal absorption. Thus, transport across the olfactory epithelium is the predominant concern for brain targeted intranasal 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. The nasal drug delivery to he CNS is thought to involve either an ***intraneuronal*** or ***extraneuronal pathway*** (Thorne RG et al 2001; BormLange JT et al 2002).

A drug can cross the olfactory path by one or more mechanism/pathways. 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 C et al., 1987) and neuronal transport.

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 intranasal absorption and the molecular weight of water soluble compounds. Compounds, which are highly hydrophilic in nature and/or of low molecular weight, are most appropriate for paracellular transport. A sharp reduction in absorption and poor bioavailability was observed for the drugs having molecular weight greater than 1000 Da. Moreover, drugs can also cross cell membranes by a carrier – mediated active transport route. For example, chitosan, a natural biopolymer from shellfish, stretches and opens up the tight junctions between epithelial cells to facilitate drug transport.

The transcellular transport mechanisms / pathways (Illum 2000; Illum 2003) mainly encompass 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 transcellular route. Highly lipophilic drugs are

expected to have rapid/complete transnasal uptake. The olfactory neuron cells facilitate the drug transport principally to the olfactory bulb.

Fig 2.8 Nose to brain transport routes

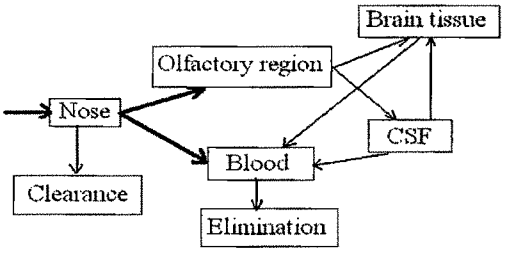


Table 2.1 Nose-to-brain transport of drug molecules and possible pathways

S.No.	Pathways	Molecules
1.	Nasal mucosa →sensory nerve cells of olfactory epithelium → subarachoid space → blood stream	Albmin
2.	Nasal mucosa→ olfactory nerve fiber	Amino acids
3.	Nasopharyngeal epithelium→ lymphatic→ cervical lymphatic vessel→ blood vessel	Rabbit virulent type III pneumococci
4.	Nasal mucosa→ cerebrospinal fluid and serum	Dopamine, Estradiol
5.	Nasal mucosa→ olfactory neurons→ brain and CSF	Estradiol, Neutropic virus and poliomyelitis virus.
6.	Nasal membrane→ olfactory dendrites→ nervous system→ supporting cells in the olfactory mucosa→ sub mucosal blood vascular system	Norethisterone, Progesterone
7.	Nasal membrane→ peripheral circulation and CSF→ CNS	Norethisterone
8.	Nasal mucosa→ peripheral and cranial nerves→ CNS	Herpes virus encephalitis
9.	Nasal mucosa→ cranial nerve→ CNS	Herpes virus simplex
10.	Nasal mucosa→ trigeminal and olfactory pathways→ CNS	Mouse passage strain of herpes virus
11.	Nasal mucosa→ sub mucous lymphatic→ cervical lymphatic pathway→ CNS	Vaccina virus
12	Nasopharynx→ cervical lymph	Water

Advantages of intranasal drug delivery

- Non – invasive, rapid and comfortable
- Bypasses the BBB and targets the CNS, reducing systemic exposure and thus systemic exposure and thus systemic side effects.
- Does not require nay modification of the therapeutic agent being delivered neurological and psychiatric disorders.
- Rich vasculature and highly permeable structure of the nasal mucosa greatly enhance drug absorption.
- Problem of degradation of peptide drugs in minimized up to a certain extent.
- Easy accessibility to blood capillaries
- Avoid destruction in the gastrointestinal tract, hepatic first pass metabolism and increased bioavailability.

Limitations of intranasal drug delivery

- Concentration achievable in different regions of the brain and spinal cord varies with each agent.
- Delivery is expected to decrease with increasing molecular weight of drug.
- Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.
- Nasal congestion due to cold or allergies may interfere with this method of delivery.
- Frequent use of this route may result in mucosal damage.

2.3.3 FACTORS AFFECTING BRAIN-TARGETED NASAL DELIVERY SYSTEMS

Some of the physicochemical, formulation and physiological factors are imperative and must be considered prior to designing intranasal delivery for brain targeting. Some of the physicochemical factors are chemical form, polymorphism, particle size, solubility and most importantly molecular weight. Moreover several other factors like formulation factors in addition to physiological factors 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.

I. Physicochemical properties of drugs:

i **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 also 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.

ii ***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.

iii ***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. The apparent cut-off point for molecular weight is approximately 1,000 with molecules less than 1,000 having better absorption. Shape is also important. Linear molecules have lower absorption than cyclic – shaped molecules. Additionally, particles should be larger than 10 mm, and otherwise the drug may be deposited in the lungs. Hydrophilicity has been found to decrease drug bioavailability.

iv ***Particle Size:*** It has been reported that particle sizes greater than 10μm 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.

v ***Solubility & dissolution Rate:*** Drug solubility and dissolution rates are important factor sin 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.

II. Formulation factors:

i ***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. Also, volume and concentration are 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.

- 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. 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.

ii ***Buffer Capacity:*** Nasal formulations are generally administered in small volumes ranging from 25 to 200μL with 100 μL being the most common dose volume. Hence, nasal secretions may alter the pH of the administrated dose. This can affects the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH in-situ.

iii ***Osmolarity:*** Drug absorption can be affected by tonicity of 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.

iv ***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 et al 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 excipients which gels in the presence of ions, such as pectin or gellan gum, 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 get. 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.

v ***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.

vi ***Preservatives:*** Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. Van De Donk et al 1980 have shown that mercury containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in the nasal systems.

vii ***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.

viii ***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 intranasal 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.

ix ***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.

x ***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 nose. Absorption enhancers may be required when a drug exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by amino peptidases.

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

- 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 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, when given intranasally 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.

III Physiological factors:

i ***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. The method of administration and properties of formulation determine the deposition site.

ii ***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. 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.

iii ***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. 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. A prolonged residence time in the nasal cavity may also be achieved by using bioadhesive polymers, microspheres, chitosan and polycarbophil or by increasing the viscosity of the formulation. 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.

iv ***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.

- v ***Effect of Pathological Condition:*** Intranasal pathologies such as allergic rhinitis, infections, or pervious nasal surgery may affect the nasal mucociliary transport process and/or capacity for nasal absorption. During the common cold, the efficiency of an intranasal 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.

2.3.4 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 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:

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 advantages 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. Micro spheres may provide more prolonged contact with the nasal mucosa and thus enhance absorption. Microspheres for nasal applications have prepared using biocompatible materials, such as hyaluronic acid ester (Illum et al 1994a)starch, albumin, dextran and gelatin. However, their toxicity / irritancy should be evaluated. It was hypothesized 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 one of the most simple and convenient systems developed for nasal delivery. The main disadvantage of this system is the lack of the 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 200 μL. The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly.

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.

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.

2.3.5 ANIMAL MODELS FOR EVALUATION OF NASAL DRUG ABSORPTION STUDIES:

Nasal absorption studies can be evaluated using two animal models vis. (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 following sections.

***In vivo* nasal absorption model**

The surgical preparation of rat for *in vivo* nasal absorption study carried out by anaesthetizing the rat by intra peritoneal injection of sodium phenobarbital. An incision is made in the neck and the trachea is canulated using polyethylene tube. Another tube is inserted through the esophagus towards the posterior region of nasal cavity. The passage of the naso-palatine tract is sealed so that the drug solution does not get drained from the nasal cavity through mouth. The drug solution is delivered to nasal cavity through nostril or through the polyethylene canula. The blood samples are collected from the femoral vein. The drug will be transported through nasal cavity to systemic circulation or to other organs/tissues only as all the possible outlets are blocked.

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 anasthetized 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 at 37 ^{0} C with aid of heating pad. The blood samples are collected using an indwelling catheter from the marginal car vein or artery as per the experimental protocol. Rabbit model has several advantages as 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)

Dog model: 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 IV injection of sodium thiopental and maintained with sodium Phenobarbital. A positive pressure pump provides ventilation through a cuffed endotracheal tube. The temperature is maintained 37 ^{0} 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 used for studying nasal absorption of metkephamid and few other drugs.

Monkey model: Monkey (approximately 8kg) 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 Phenobarbital. The head of the monkey is held in the upright position and drug solution is administered in each nostril. Post drug administration, monkey is placed in a supine position in a metabolism chair for 5 to 10 minutes. 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.

***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^{0}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, phenobarbial, secobarbital, 1-tyrosine, Propranolol hydrochloride, polyethylene glycol 4000 etc. Rabbit model can also be employed for studying *ex vivo* nasal absorption of drugs.

2.4 INTRANASAL DELIVERY OF PEPTIDES

Oral administration of peptides is impossible because of gastrointestinal enzymatic degradation and hepatic first-pass effects. Increasing evidence suggests that the intranasal route of administration may be an attractive and convenient option for the delivery of certain compounds to the brain. In fact, several peptides, including luteinizing-hormone-releasing hormone, oxytocin, calcitonin, and vasopressin, are routinely administered intranasally in clinical practice, and other peptides, including insulin, glucagon, growth hormone, growth hormone-releasing hormone, and somatostatin, are currently under investigation (Pontiroli AE 1998). The efficacy of peptide / protein following nasal administration is highly dependent on the molecular structure and size of the drug. Respiratory epithelial cells are capable of absorbing peptide/ protein by a vesicular transport mechanism, which is then transferred to the extracellular space, and subsequently taken up by the submucosal vascular network (Stratford and Lee 1986). IlLum 1992 reviewed factors affecting the nasal absorption and delivery of peptides. 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.

Ghigo et al 1996 studied Hexarelin (HEX), a GHRP growth hormone - releasing peptides on healthy elderly subjects and concluded that chronic but intermittent treatment with hexarelin administered either by intranasal or oral route, does not desensitize the GH response to the peptide but it was observed that the increased levels of IGF-I and IGFBP-3. Investigational studies in human provided the evidence of direct delivery of macromolecules to the CNS following nasal administration. CNS effects of intranasal corticotrophin-releasing hormone (CRH) without altering plasma cortisol or CRH levels have been demonstrated (Kern et al 1997). Pihoker et al 1997 studied the effect of growth hormone-releasing peptide (GHRP)-2 is a synthetic six amino acid peptide that is a potent GH secretagogue on fifteen children with short stature.participated in this study. The children were administered intranasal GHRP-2, 5–15 μg/kg, twice a day for 3 months and concluded that intranasal GHRP-2 administration was well tolerated, and produced a modest but significant increase in growth velocity. Smolnik et al 1999 studied the

effects of neuropeptides like -adrenocorticotropin (ACTH 4-10) and melanocyte stimulating hormone in human volunteers following nasal solution of the peptides. The peptides are known to be most potent regulators of neurobehavioral functions in animals and found to weaken even related brain potential of selective attention. Eventhough the mechanisms by which the peptide absorbed were elucidated, the plasma profile and behavioural studies confirmed the transportation of peptide across nasal administration. Perras et al (1999a) have reported that intranasal delivery of growth releasing hormone (GHRH) not only increased rapid eye movement sleep and slow wave sleep in humans, but also decreased growth hormone. Mayer K et al 1990 reported that intranasal delivery of peptide T found benefical in the treatment of of Painful Peripheral Neuropathy of AIDS and the clinical trail was approved by US FDA for the phase II study in 1999.

Gozes et al 2000 studied the neuroprotective effect of Activity-dependent neurotrophic factor (ADNF) in rodents through intranasal administration. They assessed neuroprotection after intranasal administration of ADNF-9 and NAP to rats treated with the cholinotoxin ethylcholine aziridium, bioavailability and pharmacokinetics after intranasal adinistration and showed significant improvements in short- term spatial memory, as assessed in a water maze, after daily intranasal administration of 1mg of peptide (ADNF-9) per animal. 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 15minutes following intranasal administration (Oh et al 2001). The higher distribution of plasmid to the brain after intranasal administration indicates that the nasal administration might be a promising route for the delivery of therapeutic genes to the brain with reduced side effecs in the other organs.

Hruz et al 2001 studied the intranaslly administered nonapeptide delta sleep-inducing peptide (DSIP) in reduction of physical and psychological withdrawal symptoms in opioid-dependent subjects. They conducted a double-blind cross-over protocol that included 3 sessions with intranasal drug administration (placebo [saline],

5 μg DSIP/kg body weight and 50 μg DSIP/kg body weight) according to a Latin square design and found that nasally administered DSIP were shown to increase P300 which is a sensitive neuropharmacological index and concluded that the intranasal application of DSIP may be a promising route of administration for further clinical trials in opioid withdrawal.

Liu et al (2001) have investigated intranasal administration of insulin like growth factor-1 (IGF-1) circumvents the BBB and protects against focal cerebral ischemic damage. The study confirmed that IGF-1 does not cross BBB efficiently however can be delivered to brain directly by intranasal administration. Recent evidence of direct nose to brain transport 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 showed the enhanced mucosal immunoglobulin response of intranasal adenoviral vector human immunodeficiency virus vaccine and it's localization in CNS. Biodistribution of recombinant adrenovirus (rADV) vectors administered through the intranasal route relieved infection of CNS, especially in the olfactory bulb, possibly via retrograde transport by olfactory neurons in nasal epithelium.

Earlier, Gantz and colleagues 2007 conducted a randomized, 2-wk, single-blind placebo run-in study which was followed by a 3 months double-blind, placebo-controlled trial to test the tolerability, safety and efficacy of PYY (gastrointestinal hormone, peptide YY) in 133 obese patients. In this study, two doses of PYY3-36 were administered as an intranasal spray before breakfast, lunch and dinner, which effectively generated PYY plasma levels similar to previous reports in rodents. No effects on body weight, the primary endpoint of the study, were observed compared to the control group in humans and concluded that the currently used preclinical rodent models may not be far off as a predictor for human efficacy and toxicity of metabolic drugs. Dhuria et al 2008 investigated the pharmacokinetics of intranasaly and intravenously administered neuropeptide (hypocretin-1, HC) in anesthetized rats, and found that tissue-to-blood concentration ratios after intranasal administration were significantly greater in all brain regions over 2 h compared to intravenous administration and concluded that approximately 80% of the area under the brain concentration-time curve following intranasal administration was due to direct

transport from the nasal passages.

Nonaka et al 2008 studied the uptake of radioactively iodinated GALP (I-GALP by brain regions and peripheral tissues of after intranasal, intravenous and intra cerebro ventricle administration in the treatment of obesity and related conditions and found that combining I-GALP with cyclodextrins increased brain uptake approximately 3-fold and concluded that intranasal administration is an effective route of administration for the delivery of GALP to the brain and that targeting among brain regions may be possible with the use of various cyclodextrins. Minmin Ma et al 2008 reported that the Intranasal treatment of transforming growth factor (TGF-1) showed significant improvement in neurological function and reduction of infarct volume induced by middle cerebral artery occlusion in mice compared with control animals and may have therapeutic potential for cerebrovascular disorders.

Inozemtseva et al 2008 synthesisied psychotropic active heptapeptide Selank (Thr–Lys–Pro–Arg–Pro–Gly–Pro) and found that the intranasal administration of selank regulated brain derived neurotrophic factor (BDNF) which inturn regulate the hippocampal functions, mainly the memory formation. Onyuksel et al 2008 showed that biocompatible and biodegradable sterically stabilized phospholipid micelles (SSM) loaded with vasoactive intestinal peptide (VIP) has anti-amyloid efficacy and intra-nasal SSM-VIP confers protection in transgenic TgCRND8 mice model of Alzheimer's disease. Tinna M. Ross et al 2008 investigated the delivery of intranasal peptoid CHIR5585, an antagonist of the urokinase plasminogen activator receptor (uPAR) to the CNS of anesthetized male rats by gamma counting and by autoradiography and found that intranasal administration resulted in significant delivery throughout the CNS.

2.5 DELIVERY SYSTEM BASED APPROACHES FOR INTRANASAL DELIVERY OF DRUGS

Various approaches have been tried to achieve higher CNS delivery through nasal route by using absorption enhancers, prodrug and the delivery systems. 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 AM et al.2002 have reported targeted brain delivery of 17-beta-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 estra-diol in CSF and have a significant value in treatment of Alzheimer's disease. Similarly, Kao HD 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 bio availability 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 Parkinson's disease with many advantages such as improved bioavailability, reduced side effects and potentially enhanced CNS drug delivery. Lian Li et al. 2002 reported rapid onset intranasal delivery of diazepam using ethyl-laurate-based microemulsion. At 2 mg/kg dose, the maximum drug plasma concentration was arrived within 2-3 min, and the bioavailability (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 epilepticus. Different delivery systems like microemulsion, mucoadhesive microemulsion, nanoparticles have gained importance because of their capability as an effective drug delivery system in brain targeting. Microemulsion based drug delivery systems were shown to facilitate effective and larger extent of drug transport across nasal mucosa to the brain. Vyas et al (2005, 2006a, 2006b) have reported rapid and larger extend of drug transport into rat brain following intranasal administration of mucoadhesive microemulsions of zolmitriptan, sumatriptan and clonazepam. These studies were further supported by other researchers Jogani et al 2007; and Mukesh et al 2007. The mucoadhesive system for nasal route is preferred because of longer residential time of delivery system at the site of administration and possible enhanced transport mechanism by the mucoadhesive agents. Different delivery systems used for the brain targeting through in route were listed in Table 2.2.

Table 2.2 Different intranasal delivery systems for brain targeting

Drug	Indication/delivery system	Outcome	Reference
Insulin like growth factor	Protection against cerebral ischemic damage/ Solution	Direct nose to brain transport was noticed	Thorne et al 2004
Vasoactive intestinal peptides (VIP)	Multi-functional CNS neuropeptide	VIP was successfully delivered by i.n	Dufes et al 2003
Lidocaine	Anasthesia (Solution)	Substantial absorption following intranasal delivery	Chou et al 1998
Insulin	Prevention of obesity (Solution)	App. 5-fold higher concentration in the brain	Gizurarson et al 1996, 1997
Sodium fluorescein	Solution	40% higher brain concentrations	Bagger et al 2004
Raltitrexed	antineoplastic (Solution)	99% of RTX content within 360 min in the brain	Wang et al 2006
5-fluorouracil	Solution	higher concentration in cerebral cortex following in than iv	Sakane T *et al* 1999
Benoylecgonine	Solution	significant increase in brain level for prolonged time	Chow et al 2001
Ergoloid mesylate	Solution	brain-targeting without first-pass metabolism	Chen et al 2008
Carbamazepine	Anti-epileptic (Chitosan microspheres	Increased amount of drug absorbed with chitosan glutamate	Gavini et al 2005
Insulin	Prevention of obesity (Microspheres	Increment in absorption & synergestic effect was noticed	Tengamnuay 1990
Desmopressin	Nocturnal uresis Cationic liposomes)	Enhancement of anti-diuresis	Terada et al 2005
Nicotine	Smoking cessation Proliposomes)	Sustained levels of nicotine	Jung et al 2000
Nimodipine	Anti-hypertensive (Microemulsions)	Improved bioavailability in brain	Zhang et al 2004

Diazepam	Mild sedative /(Microemulsions)	Rapid-on-set of action following intranasal administration	Lian Li et al 2002
Zolmitriptan	Treatment of migraine (Mucoadhesive microemulsion)	Increase in brain targeting efficiency wasobserved	Vyas et al 2005
Sumatriptan	Treatment of migraine (Mucoadhesive microemulsion)	larger extent of transport into the rat brain	Vyas et al 2006
Clonazepam	Treatment of epilepsy (Mucoadhesive microemulsion)	Higher brain uptake	Vyas et al 2006
Tacrine	Treatment of Alzheimer's disease (Mucoadhesive microemulsion)	Rapid and larger extent of transport into the rat brain	Jogani et al 2007
Respiridone	Treatment of Alzheimer's disease (Mucoadhesive microemulsion)		Mukesh et al 2007
Progesterone	viscous castor oil mixture/ enhancement of dopaminiergic activity	Effective targeting the brain than the s.c. route	de Souza Silva et al 2008
Estradiol	Cyclodextirn complexes	direct transport fro nasal cavity into CSF	Wang et al 2006
Huperzine A	Treatment of Alzheimer's disease /Insitu gel	Higher concentration in brain parts than IV route.	Zhao et al 2007
$MnCl_2$	Radiolabeled $^{54}MnCl_2$ aerosol system	olfactory transport to brain	Brenneman et al 2000
Estradiol	Chitosan nanoparticles	Significantly improved transport CNS than inclusion complex.	Wang et al 2008
Nimodipine	MPEG-PLA nano particles	High AUC in brain and CSF	Zhang et al 2006
coumarin	Lectin-conjugated PEG–PLA nanoparticles	2 folds in different brain tissues	Xiaoling Gao et al 2006

2.6 MICROEMULSIONS

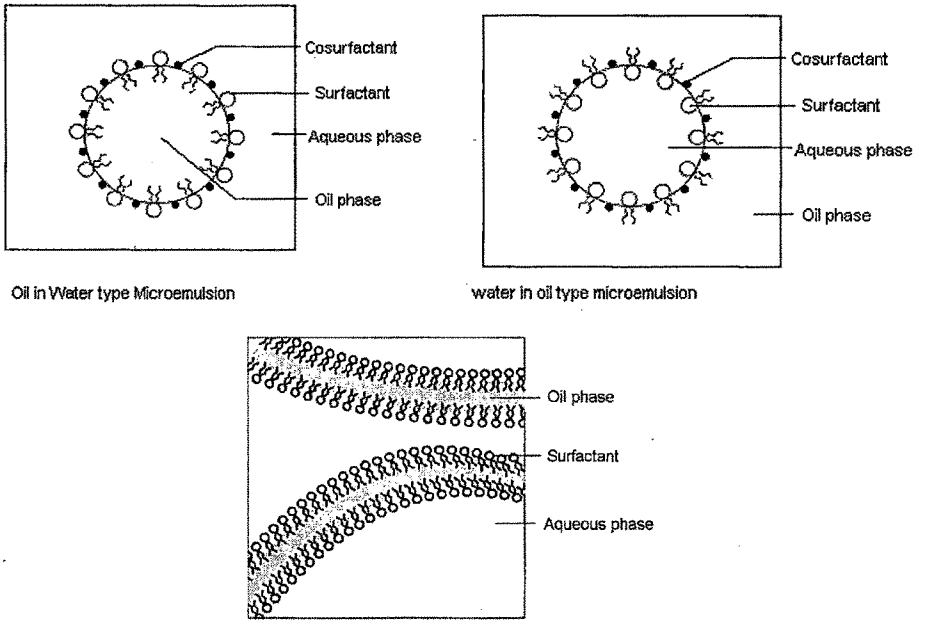
Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multi component fluids composed of oil, water and surfactant (usually in conjunction with a cosurfactant). The droplets in a microemulsion are in the range of 1 nm-100 nm in diameter. It is well established that dispersed particles having diameter less than one-fourth ($1/4^{th}$) the wavelength of visible light, i.e. less than approximately 120 nm, do not refract light and therefore microemulsions appear transparent to the eye. The basic difference between emulsions and microemulsions is that emulsions exhibit excellent kinetic stability but they are thermodynamically unstable as compared to microemulsions. The microemulsion concept was introduced as early in the 1943 by Hoar & Schulman who generated clear single phase solution by titrating a milky emulsion with hexanol. Schulman et al. introduced the term microemulsion for this system in 1959. In recent years microemulsions have attracted a great deal of attention because of their biocompatibility, biodegradability, ease of preparation and handling and most importantly solubilization capacity for both water and oil soluble drugs. The differences between emulsions and microemulsions are enlisted in the following table 2.3:

Table 2.3 Differences between emulsions and microemulsions

Characteristics	Emulsion	Microemulsion
Droplet size	100-100,000 nm	10-100 nm
Phase	Two	One
Appearance	Opaque	Transparent
Proportion of dispersed phase	30-60%	23-40% without corresponding to increase in viscosity
Energy requirement	Requires large energy input at the time of preparation	Forms spontaneously, so no energy requirement
Stability	Theoretically stable but thermodynamically unstable	Kinetically unstable but thermodynamically stable
Surfactant concentration	2-3% by weight	>6% by weight

Microemulsions have various textures such as oil droplets in water, water droplets in oil, bi continuous mixtures (Fig 2.9). ordered droplets or lamellar mixtures with a wide range of phase equilibria among them and with excess oil and/or water phases. This great variety is governed by variations in the composition of the whole system and in the structure of the interfacial layers.

Fig. 2.9 O/W type, W/O type and bicontinuous microemulsion



The rationale for developing and using medicated microemulsions is listed in Table 2.4.

Table 2.4 Rationale for developing and using Medicated Microemulsions

Reason	Drug Examples
Solubilization of poorly water soluble drugs	Diazepam,VitaminA,VitaminE,Dexamethasone palmitate
Solubilization of hydrolytically susceptible compounds	Lomustine, Physostigmine salicyalate
Reduction of irritation, pain or toxicity of intravenously administered drugs	Diazepam
Potential for sustained release dosage forms	Barbiturates
Site specific drug delivery to various organs	Cytotoxic drugs

2.6.1 THEORIES OF MICROEMULSION FORMULATION

Three different approaches have been proposed to explain microemulsion formation and the stability aspects. However, no single theory explains all aspects of microemulsion formation but each has its own significance in understanding of microemulsion formation. The important features of the microemulsion are thermodynamic stability,optical transparency, large overall interfacial area (about 100 m^2 /mL), variety of structures like ordered droplets on lamellar mixtures with wide range of phase equilibria with excess oil/water phases, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.

Thermodynamics of microemulsion:

The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, it lowers the interfacial tension till CMC (Critical Micelle Concentration), after which micelles are formed. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system. In many cases, the interfacial tension is not yet ultra low when the CMC is reached. It has been studied and observed by Schulman and workers that the addition of a co surfactant (medium sized alcohol or amine) to the system reduces the interfacial tension virtually to zero and further addition of a surfactant (where γ is zero) leads to negative interfacial tension.

i **Mixed Film theories:**

The relatively large entropy of mixing of droplets and continuous medium explains the spontaneous formation of microemulsion. Schulman (Hoar and Schulman, 1942; Schulman & Strokenius, 1959) emphasized the importance of the interfacial film. They considered that the spontaneous formation of microemulsion droplets was due to the formation of a complex film at the oil-water interface by the surfactant and cosurfactant. This caused a reduction in oil-water interfacial tension to very low values (from close to zero to negative) which is represented by following equation.

$\gamma_i = \gamma_{0/w}^{-\pi i}$

Where, $\gamma_{0/w}$= Oil-water interfacial tension without the film present

$^{\pi i}$= Spreading pressure

γ_i=interfacial tension

Mechanism of curvature of a duplex film:

The interfacial film should be curved to form small droplets and to explain both the stability of the system and bending of the interface. A flat duplex film would be under stress because of the different tension and spreading of pressure on either side of it. The reduction of this tension gradient by equalizing the two surface pressures and tensions is the driving force for the film curvature. Both sides of the interface expand spontaneously with penetration of oil and co surfactant until the pressures become equal. The side with higher tension would be concave and would envelop the liquid on that side, making it an internal phase. It is generally easier to expand the oil side of an interface than the waterside i.e. by penetration of the oil or co surfactant into the hydrocarbon chain area hence W/O microemulsion can be formed easily than O/W microemulsion (Tadros, 1983)

ii Solubilization theories

The group of Shinoda (Shinoda and Kunieda, 1973; Shinoda and Lindman, 1987; Friberg and Venable, 1983; Friberg and Lapcynska, 1976; Friberg and Buraczewska, 1977; Rance and Friberg, 1977) considered microemulsion to be thermodynamically stable mono phasic solution of water-swollen (W/O) or oil swollen (O/W) spherical micelles. Rance and Friberg (1977) illustrated the relation between reverse micelles and W/O microemulsion with the help of phase diagrams. The inverse micelle region of ternary system i.e. water, pentanol and sodium dodecyl sulphate (SDS) is composed of water solubilized reverse micelles of SDS in pentanol. Addition of O-xylene up to 50% gives rise to transparent W/O region containing a maximum of 28% water with 5 % pentanol and 6% surfactant (i.e. microemulsions). The quaternary phase diagram constructed on adding p-xylene shows relationship of these areas to the isotropic inverse micellar phase. These four component systems could be prepared by adding hydrocarbon directly to the inverse micellar phase by titration. Thus the

system mainly consists of swollen inverse micelle rather than small emulsion droplets (Shinoda and Kunieda, 1973; Rance & Friberg, 1977.

iii Thermodynamic theories:

This theory explains the formation of microemulsion even in the absence of cosurfactant. For microemulsion to form spontaneously, the free energy is

$\Delta G = \gamma \Delta A$

Where, ΔG= Free energy

Γ= Interfacial tension

ΔA=Inverse in surface area

Thermodynamic theory takes into account entropy of droplets mixing and thermal fluctuations at the interface giving interfacial bending instability. (Ruckenstein and Chi, 1975; Ruckenstein and Krishnan, 1979; Ruckenstein and Krishnan, 1980; and Overbeek 1978 considered that microemulsion formation is entropically driven and they emphasized the calculation of entropy term. The dispersion of droplets in the continuous phase increases the entropy of the system and produces a negative free energy change, which is not very important for large droplet macro emulsions but significantly important for very small droplets as in microemulsions. Ruckenstein and Chi, 1981 gave the equation for free energy (ΔG_m) in microemulsion formation.

$\Delta G_m = \Delta G_1 + \Delta G_2 + \Delta G_3$

Where, ΔG_m= Free energy

ΔG_1= Interfacial energy

ΔG_2= Free energy of inter droplet interactions

ΔG_3= Entropy for dispersion of droplets in continuous medium

Later it was shown that accumulation of the surfactant and cosurfactant at the interface results in a decrease in chemical potential generating an additional negative free energy change called as dilution effect (Ruckenstein, 1981). This theory explained the role of cosurfactant and salt in a microemulsion formed with ionic surfactants. The cosurfactant produces an additional dilution effect and decreases interfacial tension further. The addition of salts to system containing ionic surfactants causes similar effects, because it shields the electric field produced by the adsorbed ionic surfactant the adsorption of large amount of surfactant.

2.6.2 FACTORS AFFECTING THE TYPE OF MICROEMULSION AND PHASE BEHAVIOUR OF THE MICROEMULSION

Type of microemulsion

The formation of oil or water swollen microemulsion depends on the packing ratio, property of surfactant, oil phase, temperature, chain length, type and nature of cosurfactant.

Packing Ratio:

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

Critical Packing (c.p.p.) =V/ (a*l)

Where, V-Volume of surfactant molecule

a = Head-group surface area

l =length

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

Property of surfactant, oil phase and temperature:

The type of emulsion, to a large extent, depends on the nature of surfactant; Gerbacia & Rosano (1973) observed that the interfacial tension could be temporarily reduced due to diffusion of cosurfactant through the interface. Microemulsion is formed by the combination of dispersion and stabilization processes. The dispersion process involves a transient reduction of interfacial tension to nearly zero or negative value at which the interface expands to form fine dispersed droplets. Subsequently, they absorb more surfactant until the bulk phase is depleted enough to bring the value of interfacial tension positive. The interfacial film of alcohol and surfactant initiates the stabilization process. Stability of O/W emulsion system can be controlled by the

interfacial charge. If the diffuse double layer at the interface is compressed by high concentration of counter ions, water in oil microemulsions are formed. Type of surfactant also determines type of microemulsion formed. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these groups, which are a measure of the differential tendency of water to swell head group and of oil to swell the tail area are important for specific formulation when estimating the surfactant HLB in a particular system.

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils, such as alkanes, penetrate the lipophilic group region largely than long chain alkanes and swelling of this region to a great extent results in an increased negative curvature. Temperature is extremely important in determining the effective head group size of non ionic surfactants. Winsor studied the effect of temperature on the type of microemulsion formed. For the given amount of components in ternary system with nonionic surfactant, oil, and water, at relatively low temperatures, type I system (an oil in water with excess oil) is formed. At intermediate temperature type III system (microemulsion with excess of both oil and water) is present. At relatively higher temperature type II (water in oil microemulsion with excess water) system exist (Winsor PA, 1954, 1968)

The chain length, type and nature of cosurfactant:

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

Phase behavior of microemulsion:

Salinity: At low salinity, the droplet size of O/W microemulsion increases. This corresponds to increase in the solubilization of oil and this is best characterized by increase in light scattering. As salinity further increases, the system becomes bi continuous over an intermediate salinity range. The microemulsion remains oil continuous with the drop size decreasing with increasing salinity which causes complete phase transition.

Alcohol concentration: When alcohol is used as a cosurfactant in microemulsion, increasing the concentration of low molecular weight alcohol leads to the phase transition from W/O to bi continuous and ultimately to O/W type microemulsion. The vice versa transition is visible in case of high molecular weight alcohol.

Surfactant hydrophobic chain length: The increase in length of hydrophobic chain length of the surfactant shows the change of O/W microemulsion to W/O via bi continuous phase.

pH: Change in pH influences the microemulsions which contain pH sensitive surfactants especially of carboxylic acids and amines change the phase behaviour from W/O to O/W by increasing the pH.

Nature of oil: Increase in the aromaticity of oil leads to phase transition from O/W to W/O and is opposite to that of increase in the oil alkane carbon number.

Ionic strength: As the ionic strength increases the system passes from O/W microemulsion in equilibrium with excess oil to the middle phase and finally to W/O microemulsion in equilibrium with excess water.

2.6.3 FORMATION OF MICROEMULSION AND PHASE BEHAVIOUR

When water, oil and surfactants are mixed, microemulsions are one of a numerous association structures including ordinary emulsions, micellar and mesomorphic phases of various concentrations such as lamellar, hexagonal and cubic. Various gels and oily dispersions can form depending on the chemical nature and concentration of each of the components at prevailing temperature and pressure. Preparation of a stable, isotropic homogeneous, transparent, non toxic microemulsion requires consideration of a number of variables. Construction of phase diagrams reduces a number of trials and labour. Phase diagrams help to find the microemulsion region in ternary or quaternary system and also help to determine the minimum amount of surfactant for microemulsion formation.

Phase Diagrams:

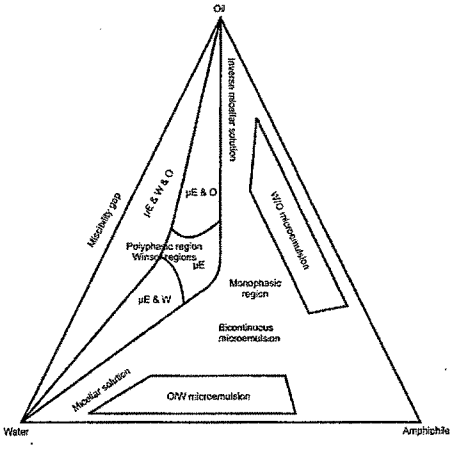
Ternary systems

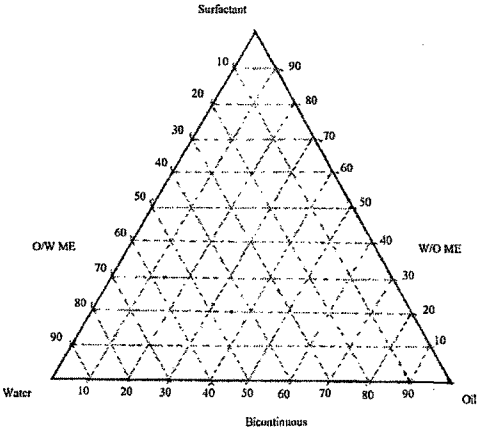
The phase behaviour of surfactant-oil-water (S/O/W) is best reported by using ternary diagram. Here, two independent composition variables are sufficient, since third one is complement to 100% (Fig 2.10). The phase diagram allows one to determine ratio of oil: water, surfactant-cosurfactant at the boundary of microemulsion region. To plot

the composition of four component systems, a regular tetrahedron composed by fixing and varying the other three or by using a constant ratio of two components (surfactant and cosurfactant or co solvent). Fig. 2.10 shows the pseudo ternary diagram at constant surfactant to cosurfactant ratio. It also shows that single phase or multiphase regions of microemulsion domain are near the centre of diagram in areas containing large amounts of surfactant that is toxic. The phase behavior of surfactants, which form microemulsions in absence of cosurfactant, can be completely represented by ternary diagram.

Winsor's regions: Winsor (1954) reported the relationship between the phase behaviour of amphiphiles-oil-water and nature of the different components of ternary system. Different regions of a phase diagram are shown in Fig. 2.9

Fig. 2.9 Different regions of phase diagram **Fig. 2.10 Ternary system**





Winsor I: The microemulsion composition corresponding to Winsor I is characterized by two phase, the lower oil/water(O/W) microemulsion phase in equilibrium with excess oil;

Winsor II: The microemulsion composition corresponding to Winsor II is characterized by very low interfacial tension and maximal solubilization of oil and water for a given quantity of surfactant. Since, in this phase, microemulsion coexists with both excess phases and no one can distinguish the dispersed phase from the continuous phase.

Winsor III: This phase comprises of three phases, middle microemulsion phase (O/W plus W/O, called bicontinuous) in equilibrium with upper excess oil and lower water.

Winsor IV: Microemulsions can be distinguished from the micelles by its inner core swollen with oil. The microemulsion structure depends on the chemical composition, temperature and concentration of the constituents.

Different surfactants stabilize different microstructures, because of their aggregation pattern in a particular medium leading to a system with a minimum free energy and thermodynamically stable. Even though the spherical micelles are considered to have minimal water-hydrocarbon contact area for a given volume, the inter micellar free energy and the impossibility of the existence of voids in the hydrophobic region leads to other amphiphillic assemblies like cylinders and planes. They are organized in the form of liquid crystalline phases or liquid isotropic phases. A wide variety of surfactant molecules obeys the geometric rules embodied in the packing parameter. In concentrated aqueous solutions, amphiphiles exist as 1.lamellar phase with two configuration (planar and continuous lamellar phase) 2.hexagonal phase (surfactant molecules aggregate into circular cylinder micelles that pack onto the hexagonal lattice). 3.cubic phases 4.nematic phases. In dilute solution they exist as worm or thread like micelles, anomalous isotropic (sponge) like structure and vesicles.

Quaternary Phase Diagrams

Microemulsion is generally quaternary system. To study their phase behavior, pseudo ternary phase diagram consisting of the oil-water amphiphiles is commonly drawn in which amphiphile is in surfactant/ cosurfactant ratio. Optimization done by using pseudo ternary diagram is not an accurate method. Hence, it is better to use quaternary phase diagram for such system.

Methods for constructing Phase diagram:

Quaternary phase diagrams should be constructed to define the extent and nature of the microemulsion regions and surrounding regions. Several methods can be used to achieve the same. In one method, a large number of samples of different composition must be prepared. The microemulsion region is identified by its isotropic nature and low viscosity. Other regions can be identified by their characteristic optical structure (Shinoda and Friberg, 1986). These diagrams are complicated and time consuming to prepare and provide a major drawback in the evaluation of a wide range of surfactant, co surfactant and other components.

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

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

Rosano et al (1988) suggested a simple routine test for rapid evaluation of components for their stability in microemulsions without construction of phase diagram. In this also coarse emulsion is prepared and titrated to clarity with the chosen cosurfactant. The minimum concentration of surfactant required to cover the interface is calculated. If the system does not clarify after adding the cosurfactant in an amount equivalent to the primary surfactant, the system is considered to be unacceptable and first the cosurfactant and finally the oil is changed in a logical manner.

Formulation of microemulsions:

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

a) *Oil Phase*: Toluene, Cyclohexane, mineral oil or vegetable oils, silicone oils or esters of fatty acids etc. have been widely investigated as oil components.

b) *Aqueous phase*: Aqueous phase may contain hydrophilic active ingredients and preservatives. Some workers have utilized buffer solutions as aqueous phase.

c) *Primary surfactant*: The surfactants are generally ionic, non ionic or amphoteric. The surfactants chosen are generally for the non ionic group because of their good cutaneous tolerance. Only for specific cases, amphoteric surfactants are being investigated.

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

2.6.4 CHARACTERIZATION OF MICROEMULSIONS

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

Phase Behaviour Studies:

Visual observations, phase contrast microscopy and Freeze Fracture transmission electron microscopy can differentiate microemulsions from liquid crystals and coarse emulsions. Clear isotropic single phase systems are identified as microemulsions whereas opaque systems showing bifringence when viewed by cross polarized microscopy may be taken as liquid crystalline system. Coarse emulsions are identified as consisting of two phases when viewed by phase contrast microscopy and showing no bifringence under a cross polarizer. Phase behaviour studies provide information about the boundaries of different phases as a function of composition variables and temperature. They also allow comparison of the efficiency of different surfactant for given application.

Scattering techniques for microemulsions characterization:

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

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

Where, θ is the scattering and λ the wavelength of the radiation. The lower limit of size that can be measured with these techniques is about 2 nm. The upper limit is

about 100 nm for SANS and SAXS and up to a few micrometers for light scattering. These methods are very valuable for obtaining quantitative information on the size, shape and dynamics of the components. The major drawback of these techniques is that samples need to be diluted in order to reduce interparticulate interaction. This dilution can modify the structure and the composition of the pseudo phases. Nevertheless, successful determinations are carried out using a dilution technique that maintains the identity of droplets.

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

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

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

Where, k is Boltzmann constant, T is the absolute temperature and $\acute{\eta}$ is the viscosity of the medium.

Nuclear Magnetic Resonance Studies:

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

Electron Microscopic Studies:

The microemulsion can be characterized by electron microscopic techniques eventhough high liability of the samples and the possibility of artifacts, electron microscopy is used to study microstructure. The microemulsion systems are observed under microscope either followed by chemical or thermal fixation methods. But the

thermal fixation method, especially freeze fracture electron microscopy has also been used to study microemulsion structure; in which extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts. It has been reported that other than CRYO-TEM, the direct observation of the microemulsion over the grid followed by normal air drying is also an useful tool in the study of microstructure and size analysis (Sheikh Shafiq et al 2007).

Interfacial tension and electrical conductivity measurements:

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

Rheological properties and viscosity measurements:

In general microemulsions have low viscosity and exhibit Newtonian flow behaviour. At very high shear rates shear thinning is observed. Viscosity data are helpful in determining the shape of the corresponding aggregates or extract information regarding the interaction potential between the droplets. Even though microemulsions of bi continuous structure possess highly interconnected structure, they show newtonian flow with low viscosity because of their very short structural relaxation time (less than 1 millisecond). When there is transition from a droplet structure to a bi continuous structure, viscosity of the system increases. Viscosity measurements can indicate the presence of rod like or work like reverse micelle. Viscosity measurements as a function of volume fraction have been used to determine the hydrodynamic radius of droplets as well as interactions between the droplets and

deviations from spherical shape by fitting the results to appropriate model (e.g. for microemulsions showing newtonian behavior, Einstein's equation for the relative viscosity can be used to calculate the hydrodynamic volume of the particles.

Stability studies

The stability of the micro emulsion has been assessed by conducting long term stability study and accelerated stability studies. In long term stability study, the system is kept at room temperature and refrigeration temperature. Over the time period micro emulsion systems are evaluated for their size, zeta potential, assay, pH, viscosity and conductivity. On long term study, the activation energy for the system and shelf life of the system may be calculated as like other conventional delivery system (Nornoo et al 2007).

Accelerated stability studies are the essential tools to study the thermodynamic stability of micro emulsions. It can be done by centrifugation, heating/cooling cycle and freeze/ thaw cycles.

1. In the centrifugation, the system is subjected to centrifugation at 822 gm for 30 minutes and followed by the observation for phase separation
2. The Heating / Cooling cycle of keeping the system at 4°C and 45°C for not less than 48 hours at each stage.
3. Freeze/ Thaw cycles of micro emulsion can be done between - 21°C and 25°C or between 5°C and 10°C (Sheikh Shafiq et al 2007).

Pharmaceutical applications of microemulsions:

Microemulsions are explored as delivery system in various routes like oral, topical, transdermal, vaginal, parenteral, ocular and pulmonary drug delivery. Microemulsions are used as sustained release dosage form, colloidal carrier systems and delivery system for proteins and peptide. This delivery system can be tailored for site specific drug delivery and drug targeting. Fluorocarbon based microemulsions are used as plasma substitute.

2.6.5 INTRANASAL DRUG DELIVERY BY MICROEMULSIONS:

Turgul et al 1992 suggested that emulsion formulations containing membrane adjuvants such as oleic acid and monoolein can be used to enhance the nasal delivery of low-bioavailable, lipid-soluble drugs. They showed that the emulsions were capable delivery system for nasal delivery of peptide drugs like *O*-(*N*-morpholino-carbonyl-3-L-phenylaspartyl-L-leucinamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane(rennin inhibitor).

Lianli et al 2002 first studied the suitability of the microemulsion as drug delivery system for intranasal use and reported that an ethyl laurate-based microemulsion system with Tween 80 as surfactant, propylene glycol and ethanol as cosolvents for intranasal delivery of diazepam has been found to show 50% bioavailability of intravenous injection. Zhang et al 2004 studied the brain uptake of nimodipine by intranasal administration of nonionic surfactant based microemulsion and found three folds higher uptake of nimodipine and higher ratios of AUC in brain tissues and cerebrospinal fluid to that in plasma. Microemulsion formulations of clonazepam incorporated with mucoadhesive agents exhibited faster onset of action followed by prolonged duration of action in treatment of status epilepticus. Brain/blood uptake ratio of clonazepam at 0.5 hr. of administration of clonazepam microemulsion, clonazepam mucoadhesive microemulsion, clonazepam solution and intravenous administration of clonazepam microemulsion exhibited 0.5, 0.67, 0.48 and 0.13 respectively indicating more effective brain targeting following intranasal administration and best brain targeting with Clonazepam microemulsion. (Vyas and Misra, 2006). Microemulsion based systems are currently under investigation for their possible role in brain targeting in neurodegenerative disorders like Alzheimer's disease and Parkinsonism. (Jogani and Misra, 2007) studied microemulsion /mucoadhesive microemulsion of tacrine, assessed its pharmacokinetic and pharmacodynamic performances for brain targeting and for improvement in memory in scopolamine-induced amnesic mice. The results demonstrated rapid and larger extent of transport of tacrine into the mice brain and faster regain of memory loss in scopolamine-induced amnesic mice after intranasal microemulsion administration. In another studies, Vyas et al (2005, 2006a) have reported rapid and larger extend of drug transport into rat brain following intranasal administration of mucoadhesive microemulsions of zolmitriptan and sumatriptan. Mukesh et al 2007 studied the IN delivery of risperidone and concluded that significant quantity of risperidone was quickly and effectively delivered to the brain by intranasal administration of mucoadhesive nanoemulsion of risperidone. Botner et al 2008investigated that the micoemulsion system on nasal administration was capable of delivering insulin in diabetic rabbits with 41.82% of absolute bioavailability. Elshafeey 2009 showed that the intranasal delivery of microemulsion of sildenafil citrate showed shorter T_{max} and higher AUC compared to the oral tablets in rabbits and higher relative bioavailability of sildenafil citrate.

2.7 MUCOADHESIVE AGENTS

Mucoadhesive dosage forms that can stick to the site of application / absorption have attracted considerable interest since the idea was first introduced early in the 1980's. The advantages of mucoadhesive formulations include: **(i)** prolonged residence time at the site of drug absorption, and **(ii)** better contact with the underlying mucosa so that the diffusion path of the drug to the epithelium is shorter (Lee et al 2000). Furthermore, some mucoadhesive polymers can modulate the permeability of epithelial cells by partially opening tight junctions (Borchard G et al., 1996; Schipper NGM et al., 1997). Carbopol increases paracellular transport which is caused by the cells being depleted of extracellular Ca^{+2} since Carbopol polymers also inhibit enzymes and this is also a result of the strong binding affinity of Carbopol for Ca^{+2}, which depletes the enzymes of calcium ions (Luessen HL et al 1995).

Mucoadhesive polymers interact with glycoproteins in the mucus layer that covers mucosal epithelial surfaces in the body, and popular routes in which mucoadhesive materials are used are the nasal, ocular, buccal, vaginal, rectal and the oral route. Mucoadhesive 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 CM 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.

Mechanism and theory of bioadhesion :

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

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

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

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-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 hyaluronic acid (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 (Inez et al 2001). Illum (1994b) reported the use of chitosan in intranasal drug delivery system for facilitating absorption. Lehr et al. 1992 have studied the mechanism of opening up of the tight junctions, they found that the possible linkage may be attributed due to interaction between amino groups of D- glucosamine and sialic acid groups present in the mucin.

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. 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 (Morimoto et al 1985). Insulin was administered via intranasal route to rabbits along with drum-dried waxy maize starch (DDWM) or maltodextrin 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 formulations 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). Carbopol has been also shown to be a useful mucoadhesive polymer in drug delivery system (Ishida M et al, 1982; Akiyama Y et al, 1995).

2.8 NASAL GEL

Morimoto et al 1985 first prepared carbopol based nasal gels and studied the release of drugs in the animal models. The effect of polyacrylic acid gel on the nasal absorption of insulin and calcitonin was investigated in rats and it was found that after nasal administration of insulin, its absorption from 0.15 w/v polyacrylic acid gel is greater than with 1% w/v gel. There would be an optimum concentration and possibly an optimum viscosity for the polyacrylic acid gel base. Morimoto et al 1987 investigated the effect of other absorption enhancers in the nasal carbopol gels. Nasal absorption of nifedipine from gel preparations, PEG 400, aqueous carbopol gel and carbopol PEG has been studied in rats. Nasal administration of nifedipine in PEG resulted in rapid absorption and high C_{max}; however, the elimination of nifedipine from plasma was very rapid. The plasma concentration of nifedipine after nasal administration in aqueous carbopol gel formulation was very low. The use of PEG 400 in high concentration in humans should be considered carefully because PEG 400 is known to cause nasal irritation in concentrations higher than 10%. Toung et al 1998 attempted osseous reconstruction using critical-size facial defect in the Sprague-Dawley rat with type I collagen gel augmented with insulin-like growth factor 1 (IGF-1) and concluded that Type I collagen gel augmented with IGF-1 results in a significant increase in healing of a nasal critical-size defect in a rodent model. Mossad 2003 assessed the ability of zinc nasal gel to shorten the duration and reduce the severity of the common cold in healthy adults and found that Zincum gluconicum nasal gel reduced symptom severity of the common cold in healthy adults, when started within 24-48 h of the onset of illness. Abdolhossein et al. 2004 investigated the nasal absorption of insulin from a carbopol-based nasal gel spray in rabbits and found that insulin gel formulation produced a significant hypoglycemic response as compared with insulin nasal solution and suggested that the carbopol gel promoted the nasal absorption of insulin in rabbit model. D'Souza et al 2005 studied the in vitro release and hypoglycemic activity of insulin from nasal gel composed of carbopol and hydroxypropyl methylcellulose in animal model and healthy human volunteers and reported that the use of bioadhesive nasal gel containing insulin not only promoted the prolonged contact between the drug and the absorptive sites in the nasal cavity but also facilitated direct absorption of medicament through the nasal mucosa. Rita et al 2006 developed intranasal delivery systems of sumatriptan using thermoreversible

polymer Pluronic F127 (PF127) and mucoadhesive polymer Carbopol 934P (C934P) and reported that the PF127 gel formulation of sumatriptan with in situ gelling and mucoadhesive properties with increased permeation rate is promising for prolonging nasal residence time and thereby nasal absorption. Varshosaz et al 2006 studied *in vitro* release of insulin and the toxicity of 4 absorption enhancers: saponin, sodium deoxycholate, ethylendiamine tetra-Acetic Acid (EDTA) and lecithin from low and medium molecular weight of chitosan based gel and found that insulin was released by a zero-order kinetic from the gels and the gel of 2% medium molecular weight of chitosan with EDTA caused increase in insulin absorption and reduction the glucose level by as much as 46% of the intravenous route. Jie Wu et al 2007 prepared a thermosensitive hydrogel by simple mixing *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) and poly(ethylene glycol) (PEG) with a small amount of β-glycerophosphate (β-GP) for intranasal administration of insulin and studied the absorption of fluorescein isothiocyanate (FITC)-labeled insulin in rat nasal cavity from the formulation by confocal laser scanning microscopy. They showed that HTCC–PEG–GP formulation can be used as nasal drug delivery system to improve the absorption of hydrophilic macromolecular drugs. Czapp et al 2008 studied the transport of drug through nasal route from carbopol - HPMC based gel. They investigated the anticonvulsant activity of Phenobarbital in fully kindled rats and concluded that intranasal administration of phenobarbital in rats was associated with efficient brain penetration rates allowing to achieve therapeutic concentrations. Buddenberg et al 2008 studied the brain uptake of dopamine from a viscous galenic castor-oil based gel through intra-nasal drug administration and concluded that intra-nasally applied dopamine can act on the central nervous system by entering the brain via the nose-brain pathway and making this kind of application procedure is a promising alternative for targeting the brain for treating disorders involving mesocortical and/or nigrostriatal dopaminergic disturbances. Different mucoadhesive agents were shown for their potential for the nasal delivery of the peptides which was reviewed by Illum 1999.

2.9 RADIOLABELING

2.9.1 Labeling with ^{99m}Tc

More than 80% of radiopharmaceuticals used in nuclear medicine are ^{99m}Tc - labeled compounds due to favorable physical and radiation characteristics of ^{99m}Tc. The 6 h physical half life and the little amount of electron emission permit the administration of millicurie (mCi) amounts of ^{99m}Tc radioactivity without significant radiation dose to the patient. In addition, the monochromatic 140 keV photons are readily collimated to give images of superior spatial resolution. Further more, ^{99m}Tc is readily available in a sterile, pyrogen free, and carrier-free state from ^{99}Mo - ^{99m}Tc generators (Saha, 2004).

2.9.2 Chemistry of Technetium

Technetium is a transition metal of silvery grey color belonging to group VIIB (Mn, Tc, Re) and has a half-life of 2.1×10^5 years. The electronic structure of the neutral technetium atom is $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 3d^6\ 3d^{10}\ 4s^2\ 4p^6\ 4d^6\ 5s^1$. Technetium can exist in eight oxidation states, namely 1- to 7+, which result from the loss of a given number of electrons from the 4d and 5s orbitals or gain of an electron to the 4d orbital. The stability of these oxidation states depends on the types of ligands and chemical environment. The 7+ and 4+ states are most stable and exist in oxides, sulfides, halides and pertechnetates.

2.9.3 Reduction of ^{99m}Tc

The chemical form of ^{99m}Tc available from the Moly generator is sodium pertechnetate (^{99m}Tc-$NaTcO_4$). The pertechnetate ion, $^{99m}TcO_4^-$, having the oxidation state 7+ or ^{99m}Tc, resembles the permanganate ion, MnO_4^-, and the perrhenate ion, ReO_4^-. It has a configuration of a pyramidal tetrahedron with Tc^{7+} located at the center and four oxygen atoms at the apex and corners of the pyramid. Chemically $^{99m}TcO_4^-$ is a rather non reactive species and does not label any compound by direct addition. In ^{99m}Tc – labeling of many compounds, prior reduction of ^{99m}Tc from the 7+ state to a lower oxidation state is required. Various reducing agents that have been used are stannous chloride ($SnCl_2.2H_2O$), stannous citrate, stannous tartrate, concentrated HCl, sodium borohydride ($NaBH_4$), dithionite and ferrous sulphate. Among these, stannous chloride is the most common used reducing agent in most preparations of ^{99m}Tc – labeled compounds.

The chemical reactions that occur in the reduction of technetium by stannous chloride in acidic medium can be stated as follows:

$$3\,Sn^{2+} \longleftrightarrow 3\,Sn^{4+} + 6e^- \quad \text{..... 1}$$

$$2\,^{99m}TcO_4^- + 16\,H^+ + 6e^- \longleftrightarrow 2\,^{99m}Tc^{4+} + 8\,H_2O \quad \text{..... 2}$$

Adding the above two equations, one has

$$2\,^{99m}TcO_4^- + 16\,H^+ + 3\,Sn^{2+} \longleftrightarrow 2\,^{99m}Tc^{4+} + 3\,Sn^{4+} + 8\,H_2O \quad \text{..... 3}$$

Equation 9.2 indicates that $^{99m}Tc^{7+}$has been reduced to $^{99m}Tc^{4+}$. Other states such as $^{99m}Tc^{3+}$ and $^{99m}Tc^{5+}$ may be formed under different physicochemical conditions. It may also be possible for a mixture of these species to be present in a given preparation. Experiments with millimolar quantities of ^{99m}Tc have shown that Sn^{2+} reduces ^{99}Tc to the 5+ state and then slowly to the 4+ state in citrate buffer at pH 7. Technetium-99 is reduced to the 4+ state by Sn^{2+} in concentrated HCl.

The amount of ^{99m}Tc atoms in the ^{99m}Tc-eluate is very small ($\approx 10^{-9}$ M), and therefore only a minimal amount of Sn^{2+} is required for reduction of such a small quantity of ^{99m}Tc; however enough Sn^{2+} is added to ensure complete reduction. The ratio of Sn^{2+} ions to ^{99m}Tc atoms may be as large as 10^6. The reduced ^{99m}Tc species are chemically reactive and combine with wide variety of compounds bearing chemical groups like –COOH, -OH, $-NH_2$, and –SH (Saha, 2005).

2.9.4 Gamma Scintigraphy

Gamma scintigraphy provides a noninvasive method to 'see' the *in vivo* fate of a pharmaceutical dosage form. The power of the technique is realized fully by correlating deposition and transit of the delivery system with resulting systemic drug levels. Since their inception 25 years ago, these methods have evolved into the preferred technology for evaluation of the *in vivo* behavior of drug delivery systems. Gamma scintigraphy is a technique by which the transit of a dosage form through its intended site of delivery can be noninvasively imaged *in vivo* via the judicious introduction of an appropriate short-lived, gamma-emitting radioisotope. The observed transit of the dosage form is then correlated with the rate and extent of drug absorption using human subjects or an appropriate animal model. Gamma scintigraphy has proven to be of great value in assisting product development as well as in the testing of marketed products. In a typical scintigraphic procedure, radiated photons from the labeled dosage form or an anatomical site pass through a collimator and strike the sodium-iodide crystal of a gamma camera. The resultant flash of light is subsequently detected by photomultiplier tubes. The analog signal is then digitized and this permits quantitative image processing (Digenis et al, 1998).

2.10 DRUGS PROFILE

2.10.1 CLOBAZAM

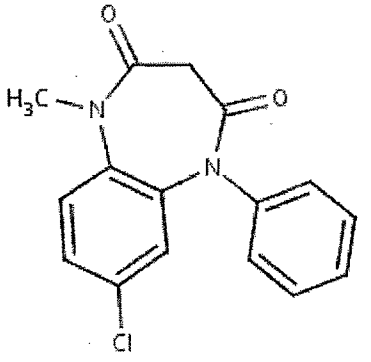
CAS number : 22316-47-8

Molecular weight : 300.74

Chemical name : *7-Chloro-1,5-dihydro-1-methyl-5-phenyl-1,5-benzodiazepine- 2,4(3H)-dione*

Moecular formula : $C_{16}H_{13}ClN_2O_2$

Molecular structure :



Appearance : White, almost white crystalline powder

Solubility : Slightly soluble in water, freely soluble in methylene chloride and sparingly soluble in alcohol. Experimental solubility in water is 188 mg/L. Predicted water solubility is 1.64e-01 mg/ml

Log P value (predicted) : 2.14

Melting range : 166-168°C

Dosage available : 10 mg oral tablet

Dose:

Treatment of anxiety: The usual anxiolytic dose for adults and adolescents over 15 years of age is 20-30 mg daily in divided doses or as a single dose given at night. Doses up to

60 mg daily have been used in the treatment of adult in-patients with severe anxiety.

Treatment of epilepsy in association with one or more other anticonvulsants: In epilepsy a starting dose of 20-30 mg/day is recommended, increasing as necessary up to a maximum of 60 mg daily.

Pharmacokinetics:

Absorption: Clobazam is rapidly and extensively absorbed following oral administration, with the bioavailability close to 87%. Concomitant administration of alcohol increases it's bioavailability by 50%. Food may slow the rate of absorption but not the extent. It is not influenced by age and sex. Distribution: Clobazam binds in approximately in large amounts with plasma proteins (85%). Peak plasma concentration occurs after 1-4 hours of oral ingestion (Brogden et al 1980). Metabolism: The two important chemical changes that clobazam undergoes during metabolism are dealkylation and hydroxylation and main metabolites being N-desmethyl clobazam, 4-Hydroxy clobazam and N-desmethyl 4 hydroxy clobazam (Guberman et al 1990). Among all these metabolites, N-desmethyl clobazam is pharmacologically active showing pharmacological profile similar to that of parent drug (Brogden et al 1980). While the half life of clobazam has been reported to be about 48 hours, N-desmethyl clobazam possess a longer half life of about 72 hours. Moreover N-desmethyl clobazam is accumulated during long term treatment achieving concentration levels upto 10 times greater than clobazam and therefore it may be an important factor in both therapeutic and toxic responses. Excretion: It is mainly excreted in urine (87-91%) and accumulation is expected in impaired renal functions (Guberman et al 1990 and Bun et al 1990).

Indications: Clobazam is used for adjunctive therapy in complex partial seizures, certain types of status epilepticus, specifically the myoclonic, myoclonic-absent, simple partial, complex partial, and tonic varieties, and non-status absence seizures (Gastaut et al 1984). It is also approved for treatment of anxiety. In India, clobazam (Frisium, Aventis Pharma India, Ltd.) is approved for use as an adjunctive therapy in epilepsy and in acute and chronic anxiety. In addition to epilepsy and severe anxiety, clobazam is also approved as an adjunctive agent in schizophrenia and other psychotic disorders.

Mechanism of action: Clobazam and its active metabolite, N-desmethyl clobazam (norclobazam), work by enhancing GABA-activated chloride currents at $GABA_A$-receptor-coupled Cl^- channels. It was also reported that these effects were inhibited by the GABA antagonist flumazenil.

Adverse effects: Benzodiazepines have the drawback, particularly after long term use, of causing rebound seizures upon abrupt or over-rapid discontinuation of therapy

forming part of the benzodiazepine withdrawal syndrome. Common side effects are ataxia, somnolence, diplopia and dysarthria.

Drug interactions: Alcohol increases bioavailability of clobazam by 50% and cimetidine increases the pharmacological effects of clobazam.

Clobazam's possible useful role in serial or cluster seizures, catamenial seizures and in an emergency

Patients who have regular clusters of generalized tonic-clonic or partial seizures may benefit from taking a single dose of clobazam 30 mg prophylactically immediately after the first seizure (Brodie, 1990). Clobazam may be useful in the treatment of catamenial seizures given at night for 7 - 10 days starting with 10 mg nocte with increments up to 40 mg, if required. The advantages of clobazam as an anticonvulsant include: (1) High therapeutic index, (2) Broad spectrum of action across a wide range of seizure types, (3) Dosage once daily with its long half-life, (4) Safety, (5) Few drug interactions. Adding clobazam to another drug as adjunctive therapy may be regarded as rational polypharmacy where a drug with a different structure and mechanism of action is added to achieve a combined pharmacodynamic effect (Trimble, 1998). Tolerance to the anticonvulsant effect may develop in approximately one third of patients usually within 3 months of therapy (Trimble, 1998). Clobazam may be used to treat or abort a bout of nonconvulsive status.

2.10.2 CLOPIDOGREL BISULPHATE

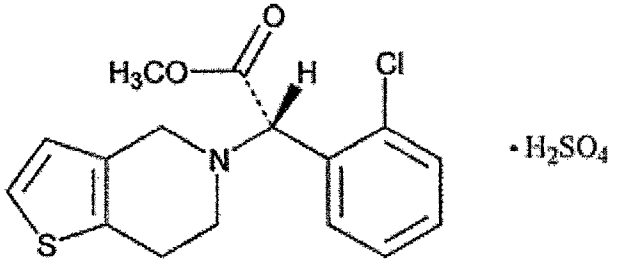
CAS number : 120202-66-6

Molecular weight : 419.90 9 (Free base 321.82)

Chemical name : Thieno[3,2-c]pyridine-5(4H)-acetic-acid, α-(2-chloro phenyl)-6,7-dihydro-, methyl ester,(S)-,sulfate (1:1).

Moecular formula : $C_{16}H_{16}ClNO_2S{\cdot}H_2SO_4$

Molecular structure :



Appearance	:	white to off-white powder.
Dissociation constant pK_a	:	4.55
Partition co-efficient	:	About 3.9 at pH 7.4 in a water/octanol medium
Melting range	:	About 176.8°C using differential scanning calorimetry
Solubility:		Clopidogrel bisulfate is practically insoluble in water at neutral pH but freely soluble at pH 1 and has aqueous solubility of 50.78 mg/ml. It also dissolves freely in methanol, sparingly in methylene chloride and is practically insoluble in ethyl ether.

pH and Effect on UV Absorbance:

At pH 2	:	UV max. abs. = 271 and 278 nm
		UV min. abs. = 259 and 275 nm
At pH 7	:	UV max. abs. = 269 and 276 nm
		UV min. abs. = 266 and 274 nm
At pH 9	:	UV max. abs. = 269 and 276 nm
		UV min. abs. = 266 and 274 nm

Dosage available : 75 mg oral tablet

Dose: Recommended Dose

Stroke, Myocardial ischemia or Established Peripheral Arterial Disease:

The recommend- ded dose of clopidogrel bisulphate is 75 mg once daily long term with or without food.

Acute Coronary Syndrome: For patients with non-ST-segment elevation acute coronary syndrome (unstable angina/non-Q-wave MI), clopidogrel bisulphate should be initiated with a 300 mg loading dose and continued for long term at 75 mg once a day with aspirin (80 mg-325 mg daily).

For patients with ST-segment elevation acute myocardial infarction, the recommended dose of clopidogrel bisulphate is 75 mg once daily, administered in combination with aspirin, with or without thrombolytics. Clopidogrel bisulphate may be initiated with or without a loading dose of 300 mg.

Pharmacokinetics:

Absorption: Clopidogrel is absorbed after oral administration of repeated 75 mg clopidogrel (base), with peak plasma levels (approx. 3 mg/L) of the main circulating metabolite occurring at approximately 1 hour after dosing. The pharmacokinetics of the carboxylic acid metabolite are linear (plasma concentrations increase in proportion to dose) in the dose range of 50 to 150 mg of clopidogrel. Absorption is at least 50%, based on urinary excretion of clopidogrel-related metabolites. Administration of clopidogrel bisulphate with meals did not significantly modify the bioavailability of clopidogrel as assessed by the pharmacokinetics of the main circulating metabolite. Distribution: Clopidogrel and the main circulating metabolite bind reversibly *in vitro* to human plasma proteins (98% and 94%, respectively). The binding is non saturable *in vitro* up to a concentration of 100μg/mL. Metabolism: Clopidogrel is extensively metabolized by the liver to a pharmacodynamically active chemical moiety. The main circulating metabolite (the carboxylic acid derivative) is inactive and represents about 85% of the circulating metabolites in plasma. The relationship between platelet aggregation and the concentration of the main circulating metabolite has not been established. Excretion: Following an oral dose of ^{14}C-labeled clopidogrel in humans, approximately 50% was excreted in the urine and approximately 46% in the feces in the 5 days after dosing. The elimination half-life of the main circulating metabolite was 8 hours after single and repeated administration. Covalent binding to platelets accounted for 2% of the radiolabel with a half life of 11 days.

Indications: The role of platelets in the pathophysiology of atherosclerotic disease and atherothrombotic events has been established. Long-term prophylactic use of antiplatelet drugs has shown consistent benefit in the prevention of ischemic stroke, myocardial infarction, unstable angina, peripheral arterial disease, need for vascular bypass or angioplasty, and vascular death in patients at increased risk of such outcomes, including those with established atherosclerosis or a history of atherothrombosis. Clopidogrel bisulfate is a specific inhibitor of adenosine-diphosphate (ADP)-induced platelet aggregation.

Mechanism of action: Clopidogrel is a potent oral ADP receptor/$P2Y_{12}$ inhibitor which is present on the membrane of platelets; thereby it inhibits ADP induced platelet aggregation. It has also been reported to prevent thrombocyte aggregation by

inhibiting fibrinogen binding to activated fibrinogen receptors (GPIIb-IIIa complex) on the platelets (Dunn et al. 1984; Di Minno et al. 1985).

Adverse effects: Inhibition of platelet aggregation occurs 24-48 h following the oral intake of clopidogrel and reaches its maximum level in 3 to 5 days. Restoration of platelet functions occurs slowly within 7-14 days, after withdrawal of the drug. Adverse effects associated with clopidogrel therapy include: neutropenia (Incidence: 5/10,000), Thrombotic thrombocytopenic purpura (TTP) (Incidence: 4/1,000,000), Hemorrhage - The incidence of hemorrhage may be increased by the co-administration of aspirin (Diener et al 2004), Gastrointestinal Hemorrhage (Incidence: 2.0%) and Cerebral Hemorrhage (Incidence: 0.1 to 0.4%).

Clopidogrel and cytochrome P450:

Clopidogrel is found to be inactive *in vitro*, it needs activation by cytochrome P450 dependent pathways. Clopidogrel has to be administered by IV or oral for its activation. The short living metabolite can be produced by the 8 cytochrome P450 isozymes, in which CYP3A4 predominantly responsible for clopidogrel activation. Thomas et al 2002 showed the activation capability of 10 different cytocrome P450 isozymes on clopidogrel and rated them. Out of these ten CYP1A2, 2C9, 2C19, 2D6, 3A4, 3A5,1A1, 2A6, 2E1, 2B6 isozymes, the ascending order of activation capacity was found as 1A1, 2A6, 2B6, 2C9, 2C19, 2D6, 1A2, 2E1, 3A5 and 3A4. Since the nasal mucosa containing CYP1A, CYP2A, and CYP2E (Lewis et al 1994; Lewis et al 2002), the intranasal administration of clopidogrel may not suffer from lack of activation.

Clopidogrel in stroke prevention and Ischemic events:

Clopidogrel is a thioenopyridine-derived antiplatelet drug which has been used for the management of chronic ischemia. Platelet aggregation is important in stroke development, both in the pathogenesis of atherosclerosis and in the occurrence of acute cerebral artery occlusions (Davi et al 2007). The role and applications of antiplatelet therapy in cerebral ischemia was reviewed by Bednar et al 1999. The initial step is platelet adhesion to the arterial wall, a result of endothelial damage. Platelet adhesion is promoted by several factors, including von Willebrand factor, fibrinogen, and subendothelial collagen. Afterwards, adhesion platelets are activated mainly by thromboxane A2, thrombin, and adenosine diphosphate. This process requires the cyclooxygenase (COX)–mediated metabolism of arachidonic acid to

prostaglandin H2 (PGH2), which in turn is processed to thromboxane A2 (Savage et al 2001). Platelet activation results in platelet aggregation due to the conversion of the glycoprotein IIb/IIIa receptor to a form that binds fibrinogen and other adhesion molecules. Based on the relevance of platelet aggregation in stroke pathogenesis, its inhibition became a target for therapeutic intervention. The results of many trials that evaluated antiplatelet agents in stroke led to recommendations that all stroke patients not requiring anticoagulation should receive antiplatelet agents (ESO 2008). In a large clinical trial (CAPRIE 1996), clopidogrel reduced the composite endpoint of stroke, myocardial infarction, or vascular death compared with aspirin in high-risk patients. *In vitro* studies measuring the population spike amplitude in murine hippocampal slices after a hypoxic episode found that pretreatment with clopidogrel increased neuronal integrity, compared with control treatment, taken as indication of a neuroprotective capacity of clopidogrel. Huber et al 2005 demonstrated that there was a considerable improvement of posthypoxic population spike amplitude of hippocampal slices of mice treated with 1 mg/kg of BW of clopidogrel bisulphate before 1 hour of hypoxic induction. Clopidogrel is shown to be beneficial in prevention of ischemia reperfusion injury probably via its effects on inflammatory cells, platelets and endothelial cells. Morale H. et al 2005 reported that clopidogrel prevented the increase in malondialdehyde (MDA) level and the decrease in glutathione (GSH) level and superoxide dismutase (SOD) activity in tissue and plasma caused by ischemia reperfusion injury. Pasqualini et al 2002 reported that clopidogrel increases the perfusion of tissues by decreasing platelet and leukocyte adhesion and reducing blood viscosity in cases with peripheral artery diseases. Platelet aggregation due to increased permeability in major organs during reperfusion was reported to be prevented by clopidogrel (Jeffrey et al 1999). Thus the inhibition of platelet aggregation by preventing endothelial and leukocyte dysfunction might be one of the reperfusion injury (Dunzendorfer et al 2002). It has been demonstrated that treatment with clopidogrel decreases chemokinesis of monocytes. Pretreatment of endothelial cells with clopidogrel has been shown to decrease the priming of endothelial cells by tumor necrosis factor and neutrophil transmigration. (Dunzendorfer et al 2002). Therefore the decrease in monocyte chemokinesis and TNF level by the drug may protect the tissues during the reperfusion period.

2.10.3 INSULIN LIKE GROWTH FACTOR - 1

Insulin like growth factors (IGFs) are polypeptides with high sequence similar to insulin. Insulin-like growth factor-I (IGF-1) is a 70 amino acid neurotrophic factor with a molecular weight of 7649 Da and structural homology to proinsulin (Rinderknecht and Humbel, 1978). The major source of IGF-1 in the body is the liver (Rosen and Pollak, 1999), although it is expressed in many other tissues, including the CNS (Werther et al, 1990). Circulating IGF-1 plays a prominent role in normal growth and development by mediating the indirect effects of growth hormone, with which it has a complex relationship (Le Roith et al, 2001). This complex system/IGF axis consists of two cell surface receptors (IGF 1R and IGF 2R), two ligands (IGF-1 and IGF II), a family of six high affinity IGF binding proteins (IGF BP 1-6), as well as associated IGFBP degrading enzymes, referred to collectively as proteases. The IGF-1 and IGF-1I are mitogenic peptides that are highly homologous to each other and share structural homology with insulin. Daughaday et al 1989 elucidated that IGF-1 mRNA can be detected in many tissues, the majority of circulating IGF-1 is produced in the liver and regulated by growth hormone. Generally both IGF-1 and IGF-1I are both thought to act in an endocrine fashion and as paracrine and autocrine growth factors locally. IGF regulate cell growth and differentiation in the developing and mature brain as well as cellular DNA synthesis. In spite of high-affinity receptor for IGF-1 is found on brain capillary endothelial cells, blood-borne IGF-1 has difficulty in crossing the blood-brain barrier except in specific hypothalamic and anterior thalamic nuclei (Rein-hardt and Bondy, 1994). IGF-1 may be more efficiently transported across the blood–CSF barrier (Pulford and Ishii, 2001). But the IGF-1 binding capacity present in both blood and CSF may hinder significant transport from the bloodstream into CNS parenchyma by this route.

IGF-1 and its receptors are abundant in cerebellum. Cerebellar purkinjee cells synthesize IGF-1 and express IGF-1 receptors during their entire life (Anderson IK et al 1988). It has been investigated as a potential neuroprotective drug for the treatment of stroke and other forms of neural damage (Smith 2003). Insulin-like growth factor (IGF) treatment has been shown to have trophic and neuroprotective effects *in vitro* and *in vivo* in different lesion models. IGF-1 has potent neuroprotective effects after hypoxic-ischemic injury and global ischemia. The role of IGF-1 in focal cerebral ischemia is only partially understood. IGF-1 has been shown to protect against stroke

in rats when injected directly into the lateral ventricles. Glukman et al 1992 have demonstrated that intracerebroventricular IGF-1 significantly reduces the extent of infraction and global neuronal loss in adult rats when administered before or even 2h after transient unilateral hypoxic ischemic injury.

Intranasal IGF-1:

Liu et al 2001 first reported that the intranasal administration of insulin like growth factor can bypass the blood brain barrier and protects against focal cerebral ischemic damage. The intranasal administration of 143-150μg IGF-1/ Kg body weight to the rat model of middle cerebral artery occlusion showed the reduction in the infract volume and improved neurological function. (Liu et al 2001). Further Liu et al 2004 showed that the administration of intranasal insulin like growth factor up to 6 hours after the onset of ischemia still provided effective treatment and recovery in the focal cerebral ischemic damage in rats. Vig et al 2006 showed that intranasally administered IGF-1 partially restored expression of calbindin D28k and PKC-γ and resulted in improving calcium homeostasis and PKC-γ mediated signaling in SCA-1 purkinjee cells in SCA-1 mice. This was found to be a promising for a new therapeutic approach for SCA-1 and other cerebral ataxias. Throne et al 2004 elucidated the transport pathway of IGF-1 by ^{125}I labeled IGF-1 on nasal administration and reported that intranasally delivered IGF-1 can bypass the blood–brain barrier via olfactory and trigeminal-associated extracellular pathways to rapidly elicit biological effects at multiple sites within the brain and spinal cord.

IGF-1 in Ischemic events:

Takayuki et al 2001 reported that the neuroprotective role of insulin like growth factor –I in transient forebrain Ischemia may be due to phosphatidylinositol 3-kinase (PI3-K) activation and subsequent promotion in cell survival. Loddick et al 1998 synthesized a human IGF-1 analog [(Leu24, 59, 60, Ala31)hIGF-1] with high affinity to IGF-binding proteins by which IGF-1 was displaced in rat MCAO model and concluded that pharmacological elevation of "free" endogenous IGFs in the brain confers protection in a clinically relevant model of stroke. Because of the dramatic protection observed in the rat MCAO model, the investigation suggested that displacement of IGFs from IGFBPs in the brain is a potential treatment for stroke.

IGF treatment protects the developing or adult brain from hypoxic-ischemic injury (Gluckman et al 1992, Johnston et al 1996, Guan et al 1993 & 1996) and forebrain ischemia (Zhu et al 1994), induces myelination (McMorris et al 1993, Ye et al 1995, Roth et al 1995) and reduces neuronal death *in vitro* caused by diverse forms of injury (Tagami et al 1997; Cheng et al 1992; Galli et al 1995; Sortino et al 1996). Paradoxically, injury to the developing or adult brain is commonly associated with increases in brain IGFs as well as their associated binding proteins.

The potential of IGF-1 treatment on cerebral ischemia was further demonstrated by Schäbitz et al 2001 and concluded that continuous treatment with intraventricularly and subcutaneously administered IGF-1 achieved a long-lasting neuroprotective effect as early as 24 hours after ischemia by MCAO in rats as measured by MRI.

Sukhanov et al 2007 showed that IGF-1 suppressed the oxidative stress and decreased atherosclerosis progression. The investigation revealed that IGF-1 infusion in ApoE-null mice decreased atherosclerotic plaque progression and macrophage infiltration into lesions. Furthermore, IGF-1 decreased vascular expression of the proinflammatory cytokines interleukin-6 and tumor necrosis factor-1, reduced aortic superoxide formation and urinary 8-isoprostane levels, and increased aortic pAkt and eNOS expression and circulating endothelial progenitor cells, consistent with an anti-inflammatory, antioxidant, and prorepair effect on the vasculature.

Anti oxidant property of IGF-1 and glutathione (GSH) level:

Cantürk et al 2003 investigated the effect of IGF-1 supplementation during liver cirrhosis induced by common bile duct ligation and measured blood biochemical parameters, tissue malondialdehyde, glutathione levels and the activity of tissue antioxidant enzymes and concluded that IGF-1 treatment improves liver function and decreases oxidative liver damage and histopathological findings. Gustafsson et al 2004 investigated the role of uncoupling proteins (UCPs) in IGF-1-mediated protection from hyperglycemia-induced oxidative stress and neurodegeneration on human neuroblastoma SH-SY5Y cells. Cells were differentiated with retinoic acid for 6 days, after which exposure to 8, 30, or 60 mM glucose with or without 10 nM IGF-1 was started. After 48-72 hr, the number of neurites per cell, UCP3 protein expression, mitochondrial inner membrane potential (MMP), and intracellular levels of ROS and total glutathione were examined. This study concluded that IGF-1 treatment prevented the glucose-induced neurite degeneration and UCP3 down-regulation and IGF-1 may

protect from hyperglycemia-induced oxidative stress and neuronal injuries by regulating MMP, possibly by the involvement of UCP3. García-Fernández et al 2005 reported that the hepatoprotective and antifibrogenic effects of IGF-1 in cirrhosis are associated with a diminution of the hepatic contents of several factors all of them involved in oxidative damage indicated the potential antioxidant role of IGF-1.

Donahue et al 2006 investigated the relationship between growth hormone (GH) and insulin-like growth factor-1 (IGF-1), and age by measuring glutathione (GSH and disulfide glutathione (GSSH) levels in hippocampus and frontal cortex of young (4-month-old) and aged (30-month-old) male Fisher 344xBrown Norway rats and concluded that the age-related decline in circulating growth hormone and IGF-1 contribute to increased oxidative stress in hippocampus with age. Jallali et al 2007 tested the capacity of three growth factors with established roles in cartilage, namely insulin-like growth factor (IGF)-1, fibroblast growth factor (FGF) and transforming growth factor (TGF)-beta 1, to alter intracellular reactive oxygen species (ROS) levels on the explants of articular cartilage from young, mature, and aged rats and viability of chondrocytes following ROS stress and growth factor treatment was assessed using the live/dead cytotoxicity assay, and the activities of the antioxidant enzymes catalase (CAT), total superoxide dismutase (SOD), and glutathione peroxidase (GPX) were measured. This study concluded that IGF-1 is a potent antioxidant in mature and aged rat and human chondrocytes, protecting cells against ROS-induced cell death probably through the enhancement of the activity of the antioxidant enzyme GPX. Higashi et al 2008 conducted antioxidant studies of IGF-1 on apoE mice to characterize the potential anti-oxidant effects of IGF-1 in vascular endothelial cells and to determine a potential effect of IGF-1 on GPX expression and activity in human aortic endothelial cells and concluded that IGF-1 exerts potent anti-oxidant effects on cells, mediated by translational/post-translational increase in GPX expression and activity via a PI3K dependent pathway.

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