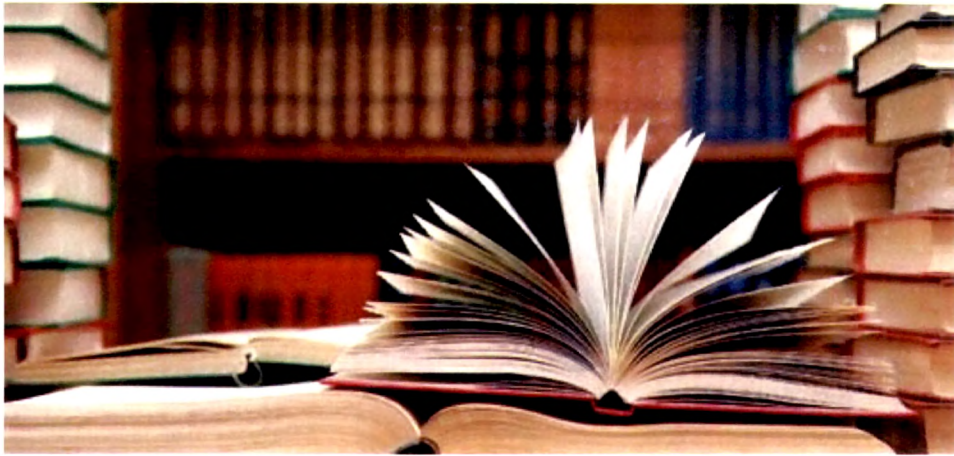


# *Chapter 2*

## *Literature review*



## **2.1 Pain (Bonica et al., 1979)**

### **Defining Pain**

Pain is a perception that signals the individual that tissue damage has occurred or may be occurring. It is subjective and very complex. The processes in the body that are involved in the perception of pain are called "nociception." Basic and clinical research during the past 50 years has confirmed that there are many mechanisms involved in nociception.

### **Classifying Pain**

Pain can be "acute" or "chronic." Acute pain lasts a short time, or is expected to be over soon. The time frame may be as brief as seconds or as long as weeks. Chronic pain may be defined as pain that lasts beyond the healing of an injury, continues for a period of several months or longer, or occurs frequently for at least months. To develop the best treatment strategies, health care professionals also classify pain based on its characteristics, its cause, or the mechanisms in the body or the mind that are probably involved in sustaining it. One common classification based on mechanisms distinguishes pain into categories called "nociceptive," "neuropathic," and "psychogenic."

### **Types of Pain**

**Nociceptive Pain:** Nociceptive pain is believed to be caused by the ongoing activation of pain receptors in either the surface or deep tissues of the body.

There are two types: somatic pain and "visceral" pain.

"Somatic" pain is caused by injury to skin, muscles, bone, joint, and connective tissues. Deep somatic pain is usually described as dull or aching, and localized in one area. Somatic pain from injury to the skin or the tissues just below it often is sharper and may have a burning or pricking quality. Somatic pain often involves inflammation of injured tissue. Although inflammation is a normal response of the body to injury, and is essential for healing, inflammation that does not disappear with time can result in a chronically painful disease. The joint pain caused by rheumatoid arthritis may be considered an example of this type of somatic nociceptive pain.

"Visceral" pain refers to pain that originates from ongoing injury to the internal organs or the tissues that support them. When the injured tissue is a hollow structure, like the intestine or the gall bladder, the pain often is poorly localized and cramping. When the injured structure is not a hollow organ, the pain may be pressure-like, deep, and stabbing.

**Neuropathic Pain:** Neuropathic pain is believed to be caused by changes in the nervous system that sustain pain even after an injury heals. In most cases, the injury that starts the pain involves the peripheral nerves or the central nervous system itself. It can be associated with trauma or with many different types of diseases, such as diabetes. There are many neuropathic pain syndromes, such as diabetic neuropathy, trigeminal neuralgia, postherpetic neuralgia ("shingles"), post-stroke pain, and complex regional pain syndromes (also called reflex sympathetic dystrophy or "RSD" and causalgia). Some patients who get neuropathic pain describe it as bizarre, unfamiliar pain, which may be burning or like electricity. The pain may be associated with sensitivity of the skin.

**Psychogenic Pain:** Most patients with chronic pain have some degree of psychological disturbance. Patients may be anxious or depressed, or have trouble coping. Psychological distress may not only be a consequence of the pain, but may also contribute to the pain itself. "Psychogenic" pain is a simple label for all kinds of pain that can be best explained by psychological problems.

This close relationship between pain and psychological distress means that all patients with chronic pain should have an assessment of these psychological factors, and psychological treatments should be considered an important aspect of pain therapy. In some cases, psychological problems appear to be a main cause of the pain. This does not mean that the person is not actually experiencing the pain. Rather, the patient is truly suffering but the main cause somehow relates to the emotions, or to learning, or to some other psychological process. Although doctors sometimes encounter patients who pretend to be in pain (some can be called malingerers), this appears to be a rare occurrence. Most patients with pain that appears to be determined primarily by psychological processes are hurting just like those who have pain associated with a clear injury to the body.

Sometimes, psychogenic pain occurs in the absence of any identifiable disease in the body. More often, there is a physical problem but the psychological cause for the pain is believed to be the major cause for the pain. The various types of psychogenic pain can be diagnosed using a classification developed by psychiatrists (the Diagnostic and Statistical Manual of the American Psychiatric Association).

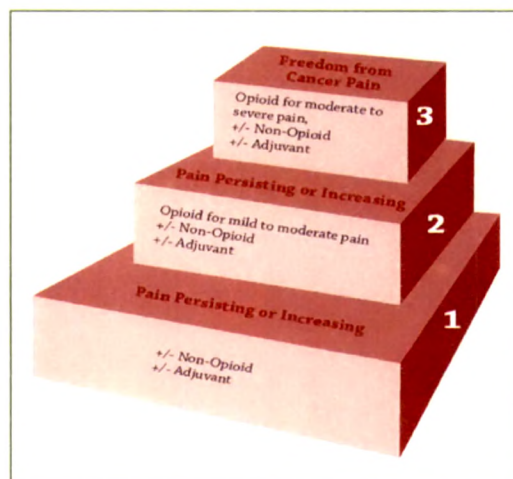
### **General Principles of Pain Management**

Pain results not only from a physical sensation but also from many contributing psychosocial factors. The treatment of pain, therefore, should include medicinal and

non medicinal stratagems. When choosing a medicinal strategy, remember to proceed as the World Health Organization describes: 1) by the ladder, 2) by the mouth, and 3) by the clock whenever possible.

**By the ladder.** Figure 2.1 are useful guides for starting a patient on analgesics. The key decision here is assessing the severity of the patient's pain.

**By the mouth.** Oral administration is the easiest route for an alert patient. The patient is allowed a role in his or her own care and can feel empowered by this participation. Intravenous or intramuscular routes of administration are useful for patients in excruciating pain or who are vomiting, but these routes are more difficult to administer, can cause pain themselves, and require more frequent dosing because of more rapid metabolism. Transdermal administration is effective in patients who cannot take medicines by mouth. There is a delay of 12-24 h until therapeutic levels are reached once a patch is placed, and drug continues to be released for up to 24 h once the patch is removed. The titration of the medicine should occur only at 72-h intervals. A patient's skin must also be clean, hairless, and dry. Profuse sweating can impair adhesion and alter absorption rates. Many analgesic drugs are available as rectal suppositories. This mode of delivery has a role to play in pain control, but absorption of drugs by the rectal route can be inconsistent. Rectal medications should not be used in patients with neutropenia (low white blood cell levels) or thrombocytopenia (low platelet counts) because of the risk of infection and bleeding.



**Figure 2.1: WHO pain Ladder (from WHO Guidelines**  
**(<http://www.who.int/cancer/palliative/painladder/en/>)**



### Problem associated in the cure of pain

While acute—particularly post-traumatic and post surgical pain can be treated satisfactorily with available analgesics in most cases—chronic inflammatory and neuropathic pain often responds only poorly. The identification of novel targets for analgesic therapy is therefore a major focus in current pharmacological research. Chronic pain states are frequently accompanied by an increased pain sensitivity, which can appear as hyperalgesia, an increased sensitivity to noxious stimuli, or allodynia, a painful sensation of usually innocuous stimuli (Figure 2.2).

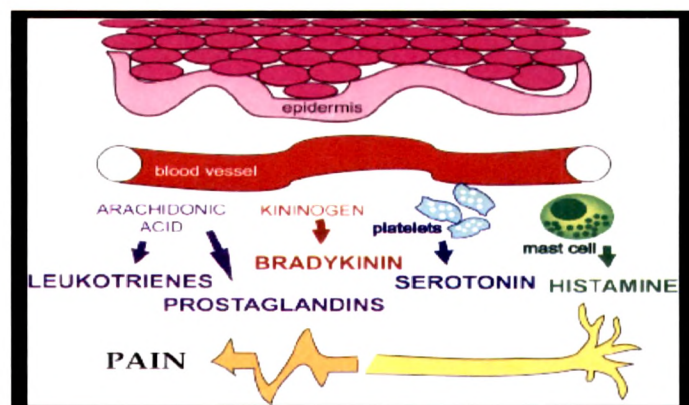


Figure 2.2: Pain pathway

### 2.2. Migraine

Migraine headache is an episodic headache disorder. It is a common condition with a prevalence of 17.6% in females and 5.7% in males. An American Migraine Study estimated that 23 million persons older than 12 years of age have severe migraine headaches; however, this condition is under-treated and under-diagnosed worldwide. Not all headache sufferers seek medical attention, but those who do generally consult family practitioners, internists or pediatricians, ophthalmologists, and neurologists. The social and economic effects of migraine are staggering--perhaps \$2 to \$17.2 billion is lost in productivity per year. The treatment of migraine has not only medical but also serious economic and social implications. Thus, primary-care physicians should be well versed in the diagnosis and treatment of migraine. Rational migraine treatment necessitates an accurate diagnosis, identification and removal of potential triggering factors, non-pharmacological and if needed pharmacological intervention. Both the avoidance of migraine trigger factors and the use of non-pharmacological therapies have a part to play in overall migraine management. Effective management

also includes establishing realistic expectations, patient reassurance, and education (Capobianco et al., 1996).

### **Two types of migraines**

While there are many variations, there are two main types of Migraines

- Migraine without aura (previously called common migraine). Almost 80 percent of migraine sufferers have this type of migraine.
- Migraine with aura (previously called classic migraine). This type of migraine announces itself about a half-hour before the onset of head pain with an aura. Aura is usually a visual disturbance that lasts about 15 minutes. If you have a visual aura, the most common aura type, you may see flashing lights, bright spots, or zigzag lines; or you may temporarily lose part of your vision. Other types of auras may include numbness or tingling in the hand, tongue, or side of the face, or weakness in one arm.

### **Symptoms in Migraine**

Any attempt to explain the pathophysiology of migraine has to account for the following components of the attack:

#### **Premonitory Symptoms (Aura)**

The aura may last 20 to 30 minutes and may include one or more of the following:

- Mood changes (commonly a sense of elation associated with hyperactivity)
- Increased appetite (particularly for sweet foods).
- Excessive yawning may precede migraine by as long as 24 hours, on at least some occasions, in about one third of migraine patients.
- Blindspots (scotomata) or visual field cuts may have distinctive scintillations or fortification patterns around them. Typically, the scotomata clear as the headache appears.
- Sensory hyperacuity (light may be perceived as dazzling or may provoke pain, sounds may appear unnaturally loud, and smells may be more intense during (or even before) the headache phase).

#### **Focal Neurological Symptoms**

- These neurological symptoms may arise from the cerebral cortex, brain stem, or cerebellum and may anticipate the onset of headache as in the prodromal phase of classic migraine or may appear during the headache phase.
- Focal neurological symptoms of classic migraine, whether arising as a prodrome or developing during the headache phase, are accompanied by diminished cortical perfusion of the appropriate part of the opposite cerebral hemisphere. On some

occasions a wave of hypo-perfusion may advance slowly over the cortex in association with a slow march of visual or other neurological symptoms whereas on other occasions it may persist as a local or diffuse cortical oligemia. It is clear that the presence or absence of headache does not depend on changes in cerebral blood flow.

### **Headache**

- It is unilateral in two thirds of patients. It commonly starts as a dull ache at the occipito-nuchal junction, or in one temple and then spreads over that side of the head or the whole head or may remain localized as a "bar of pain" extending from the eye to the occiput. The pain is usually constant and unremitting but assumes a pulsatile or throbbing quality when severe, it may consistently affect the same side of the head or may move from side to side, even in the one migraine episode. Pain may radiate down the neck to the shoulder or, in some cases, to the arms and even the leg on the same side of the body, suggesting that the spinothalamic tract has collaborated with trigeminal pathways in the production of pain.
- The frontal branches of the superficial temporal artery become distended in about one third of patients, venous engorgement may be seen, and heat loss increases from the affected area. Most patients appear pale and "dark under the eyes" as the headache worsens, although exceptional patients flush before or during the attack. Sensitivity of the scalp to touch and muscular hyperalgesia may develop during, and outlast, the headache phase.
- However headache of migraine is not necessarily associated with vascular pulsation, dilation of extra-cranial arteries or increased cerebral perfusion.

### **Gastrointestinal symptoms**

- Nausea sometimes precedes the onset of headache but commonly evolves as the attack progresses and may culminate in vomiting. Diarrhea is associated in about 20 % of patients. Such gastrointestinal symptoms are mediated by an enzyme, dopamine beta-hydroxylase (DBH) that is the final enzyme in the synthesis of noradrenaline.
- **Pain-Sensitive Cranial Structure**
- The foundation for any study of the causes and treatments of headache is knowledge of the pain-sensitive structures and pain-conducting pathways within the cranium.
- All available evidence supports an orderly somatotopic representation of the supratentorial pain-sensitive meningeal and vascular structures within the trigeminal system. Pain sensation from posterior fossa structures is carried centrally by the vagus nerve, the upper three cervical nerves, and possibly by trigeminal afferents as well.

### **The Origin of Migraine Headache**

The bones of the skull and brain substance are insensitive to pain because they lack pain sensitive nerve fibers. Pain is referred to the frontotemporal area of the skull, from the following structures:

- The dura.
- The intracranial segment of the internal carotid artery.
- The proximal few centimetres of the anterior and middle cerebral arteries.
- A portion of the cerebral veins and venous sinuses.
- The middle meningeal artery.
- The superficial temporal artery.

The previously mentioned structures contain pain sensitive nerves with the nociceptors at their ends. The latter can be stimulated by stress, muscular tension, dilated blood vessels and other triggers of headache. Once stimulated, the nociceptor sends a message up the length of the nerve fiber to the nerve cells in the brain, signalling that a part of the body hurts. In periarterial fluid sampled during migraine headache, a polypeptide was found, named "neurokinin". This bradykinin-like substance was postulated to set up a sterile inflammatory response in the vessel, which became pain-sensitive, and is responsible for the transmission of the pain impulse to the brain nerve cells.

Platelets aggregation takes place in subgroups of migraine patients and may be a factor in the vascular thrombosis of "complicated migraine". However the blood platelets in most patients seem to be remarkably normal and their role in migraine is probably limited to aggregation in some instances and to serotonin releases which potentiate the pain-producing effect of bradykinin. Dilation of scalp arteries in this area contributes to the intensity of headache, and

compression of the temporal artery eases the pain. Platelet serotonin content increases before migraine attacks and falls during the headache phase in most migraine patients. A serotonin-releasing factor was found present in the blood during migraine headache. The main metabolite of serotonin, 5-hydroxyindoleacetic acid, is excreted in excess in the urine of some patients during migraine attacks. However it seems unlikely that the amount of serotonin released from platelets during migraine headache would be sufficient to cause any vascular constriction, but it may possibly combine with bradykinin to render the arterial wall



sensitive to painful dilation. It has been postulated that free fatty acids might be responsible for the release of serotonin from blood platelets in migraine. Blood histamine is significantly increased after migraine headache. It is claimed that liberated histamine might contribute to the vascular component of migraine. Prostaglandins, long-chain unsaturated fatty acids derived from arachidonic acid, have potent constrictor and dilator effects. During migraine headache, plasma levels of PGE1 do not alter, but the level of PGE2-like substances has been shown to fall significantly, in contrast with its elevation found in cluster headache.

### **The Mechanisms of Migraine**

Hypotheses for the mechanisms of many aspects of migraine have been extensively studied (Welch et al., 1997). The aura symptoms are, most likely caused by a mechanism similar to spreading excitation and depression (Spierings et al., 1995). It has been believed that migraine attack is a specific reaction pattern to an episode of focal cerebral hypoxia. This hypothesis holds that any type of focal brain hypoxia (and thus not only a vasospasm) may provoke a migraine attack. Indeed, as hypoxia is a result of an imbalance between energy supply and energy use, the former can be decreased and/or the latter be increased. Spreading cortical depression, leading to the aura, is believed to be a consequence of brain hypoxia occurring in classical migraine. There are no genuine differences between classical and common migraine, according to the cerebral hypoxia theory. The latter theory may improve our understanding of the mode of action of antimigraine drugs. Certain calcium entry blockers have a direct protective effect on brain hypoxia, but some other pharmacotherapeutic approaches may also prevent cerebral hypoxia via an effect on brain metabolism, vasomotion or platelet behaviour (Amery et al., 1985). It has been postulated that the classic migraine is both spreading cortical depression and localized ischemia linked in a vicious cycle by potassium induced vasoconstriction. The cycle can be initiated by any event that raises the local cortical ECF (extracellular fluid) potassium concentration to approximately 20 mM. Such an event could be a localized burst of activity of a group of cells, localized metabolic impairment, or a transient reduction in blood flow to a region of the cortex. Once this level of potassium concentration is reached, it may result in localized depolarization of neurons, releasing more potassium into the ECF. As the concentration continues to rise, vasoconstriction becomes more intense, perpetuating the cycle that results in localized depression of cortical neuronal activity and ischemia. The condition is propagated to adjacent

regions of the cortex by diffusion and glial-mediated spread of potassium (Young et al., 1992).

Neuronal hyperexcitability between attacks may be due to:

- Mitochondrial disorder.
- Magnesium deficiency.
- Abnormality of presynaptic calcium channels.

Like many others neurological diseases, mitochondrial involvement, by means of abnormalities in cerebral oxidative metabolism, may play a role in migraine (Lanteri-Minet et al., 1996). The importance of magnesium in the pathogenesis of migraine headaches is clearly established by a large number of clinical and experimental studies. However, the precise role of various effects of low magnesium levels in the development of migraines remains to be discovered. Magnesium concentration has an effect on:

- Serotonin receptors.
- Nitric oxide synthesis and release.
- A variety of migraine related receptors and neurotransmitters.

The available evidence suggests that up to 50% of patients during an acute migraine attack have lowered levels of ionized magnesium. Infusion of magnesium results in a rapid and sustained relief of an acute migraine in such patients. Two double-blind studies suggest that chronic oral magnesium supplementation may also reduce the frequency of migraine headaches (Mauskop et al., 1998). Increased tissue levels of taurine, as well as increased extracellular magnesium, could be expected to:

- Dampen neuronal hyperexcitation.
- Counteract vasospasm.
- Increase tolerance to focal hypoxia.
- Stabilize platelets.
- Taurine may also lessen sympathetic outflow.

Thus it is reasonable to speculate that supplemental magnesium taurate will have preventive value in the treatment of migraine.

#### **Treatment of pain and migraine:**

Opioids remain the mainstay for the management of pain like post operative pain. Opioids are the first-line drugs for the treatment of moderate to severe pain but opioids must be administered with care. The effective dose required varies from individual to individual. As a result, the dose used must be titrated to avoid dose-

related adverse side effects as well as inadequate analgesia. As with all analgesics, the effects of treatment must be constantly monitored, and changes in therapy should be made as indicated. Unfortunately, opioid-induced adverse side effects are common and can be serious. Common side effects of opioids include nausea, vomiting, sedation, and pruritus. Less common but more serious side effects include respiratory depression and sometimes cardiac arrest. Clearly, patient care includes appropriate monitoring and therapy for these events. In an effort to improve pain control and decrease the incidence and severity of drug-induced adverse side effects, many clinicians have introduced the use of non-opioids drugs like Tizanidine HCl and Cyclobenzaprine HCl for the management of pain (Ashburn et al., 1994).

### **2.3 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.

#### **2.3.1. 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. 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. 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 (Figure 2.3).

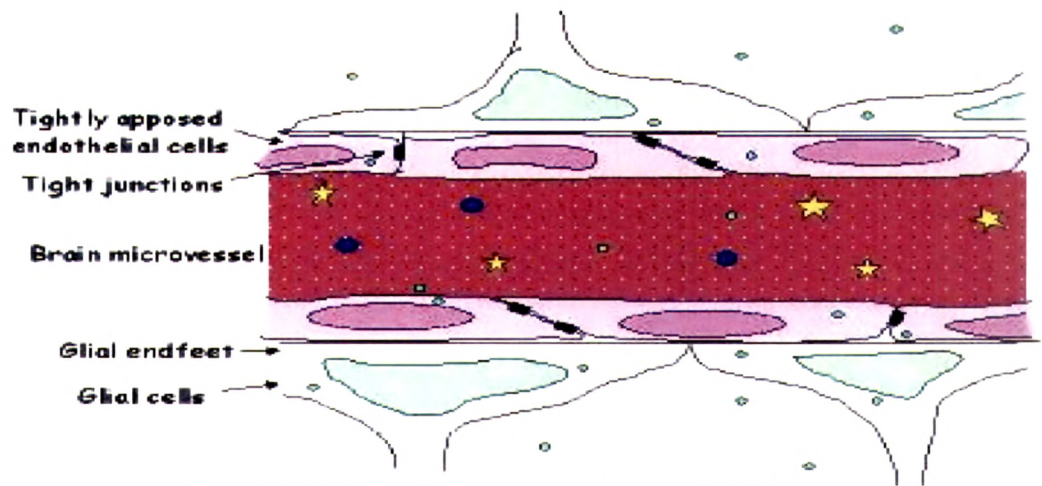


Figure 2.3: Schematic representation of BBB

Ependymal cells lining the cerebral ventricles and glial cells are of three types. Astrocytes form the structural framework for the neurons and control their biochemical environment. Astrocyte foot processes or limbs that spread out and abutting one another, encapsulate the capillaries and are closely associated with the blood vessels to form the BBB (Figure 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. Microglia are blood-derived mononuclear macrophages. The tight junctions between endothelial cells result in a very high trans-endothelial electrical resistance of 1500-2000  $\Omega\text{cm}^2$  compared to 3-33  $\Omega\text{cm}^2$  of other tissues which reduces the aqueous-based paracellular diffusion that is observed in other organs.

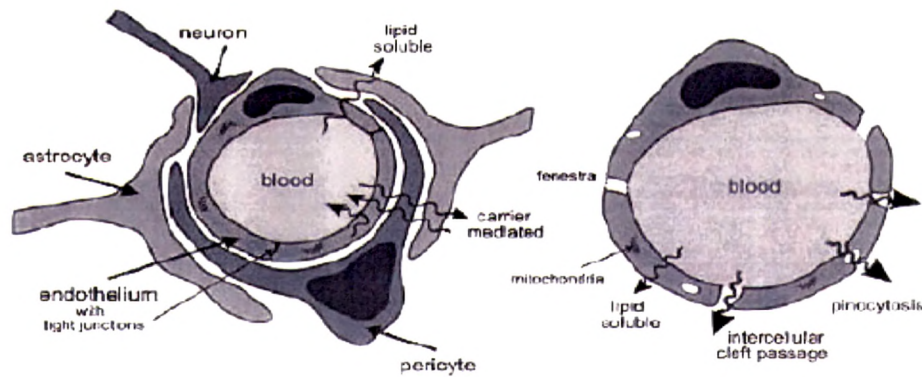


Figure 2.4: Schematic comparison between brain (left) and general (right) capillaries



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, subcommisural 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 form tight junctions prevent transport from the luminal 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.

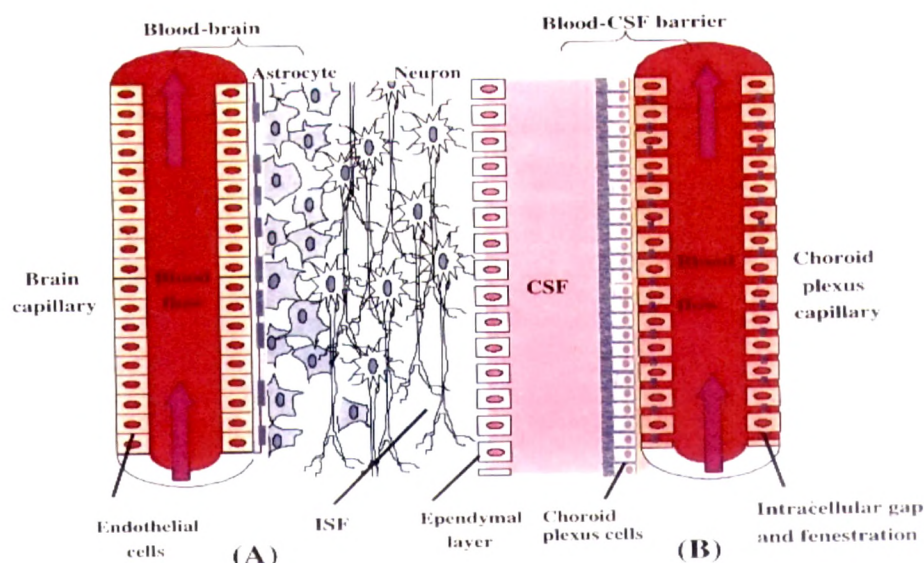
## **ii. Blood-cerebrospinal fluid barrier**

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. The arachnoid



membrane is generally impermeable to hydrophilic substances, and its role in 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 (Figure 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  $200\Omega\text{cm}^2$ , 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.



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

**Figure 2.5: (A) Blood Brain Barrier (B) Blood cerebrospinal fluid Barrier**  
**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 tumour 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-tumoral edema leads to increase in hydrostatic pressure in the normal brain parenchyma adjacent to the tumor. As a result the cerebral microvasculature in these tumor adjacent regions of normal brain may be even less permeable to drugs than normal brain endothelium leading to exceptionally low extra – tumoral interstitial drug concentrations. Brain tumors may also disrupt BBB, but these are also local and 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.

### **2.3.2. 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 lipophilicity. 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 moieties 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 defence mechanism of the brain and leaves it vulnerable. The other approaches are BBB disruption using use of labradimil which has selectivity for bradykinn B<sub>2</sub> 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. *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 nm 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,  $\beta$  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 glioblastomas 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 et al., 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 et al., 2001).

#### **2.4. Intranasal drug deliver for brain targeting**

In recent years, the nasal mucosa has been considered as an administration route to achieve faster and higher level of drug absorption. The richly supplied vascular nature of the nasal mucosa coupled with its high drug permeation makes the nasal route of administration attractive for centrally acting drugs, which 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 (Smith et al., 1958).

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. The world market has seen an increasing number of systemically acting drugs being marketed as nasal formulations. For example, sumatriptan (GlaxoSmithKline, <http://www.gsk.com>), zolmitriptan (AstraZeneca, [http:// www.astrazeneca.com](http://www.astrazeneca.com)), ergotamine (Novartis, <http://www.novartis.com>), butorphanol (Bristol- MyersSquibb, <http://www.bms.com>), all with the indication for treatment of migraine, where a rapid onset of action is beneficial; estradiol (Servier, <http://www.servier.com>), where an improved bioavailability as compared to oral delivery has been achieved; and desmopressin (Ferring, <http://www.ferring.se>), buserelin (Aventis, <http://www.aventis.com>) and calcitonin (Novartis), all peptides normally only administered by injection because of low membrane permeability and susceptibility to degradation by enzymes in the gastrointestinal tract. A range of companies specializing in the development of innovative nasal delivery systems and formulation problems has come to the fore: Nastech (<http://www.nastech.com>), Britannia Pharmaceuticals (<http://www.britannia-pharm.co.uk>), Intranasal Technologies (<http://www.intranasal.com>), Bentley Pharmaceuticals (<http://www.bentleypharm.com>) and West Pharmaceutical Services

(<http://www.westdrugdelivery.com>) are actively developing novel nasal formulations for conventional generic drugs (e.g. apomorphine, triptans, morphine, midazolam, fentanyl, non-steroid anti-inflammatory drugs), as well as for peptides and proteins (e.g. leuprolide, parathyroid hormone, insulin, interferon) in situations where the nasal route would be beneficial for the therapeutic efficacy of the drug.

Scientists have also focused their research toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as pain, 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 (Lisbeth Illum et al., 2002).

#### **2.4.1. 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<sup>2</sup>, 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<sup>2</sup>. 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 (Figure.2.6).

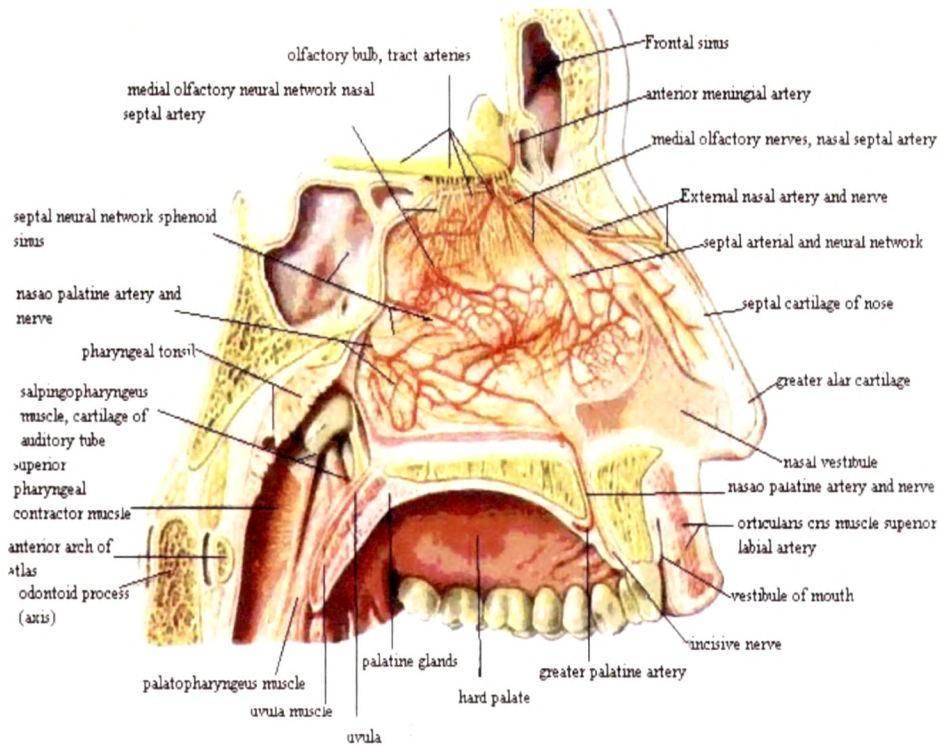
**The vestibular region:-** The vestibular region is located at the opening of nasal passages and is responsible for filtering out the air borne particles. It is considered to be the least important of the three regions with regard to drug absorption.

**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<sup>2</sup> 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, bowmans bundle and blood vessels whereas epithelium

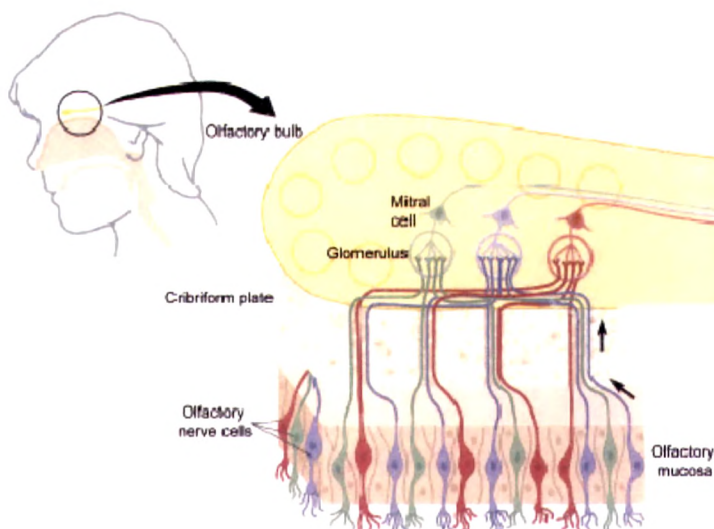
cribriform plate (Figure 2.7).

transferases are found in nasal cavity.



**Figure 2.6: Nasal vascular supply**



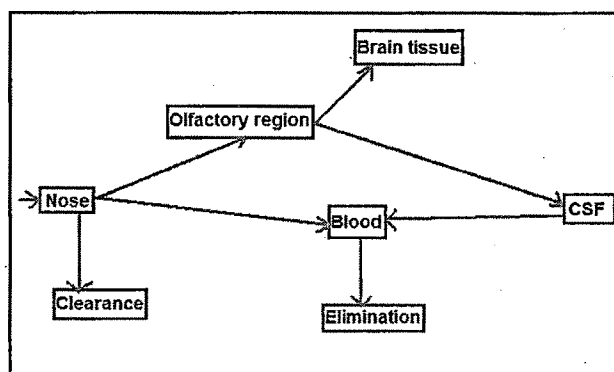


**Figure 2.7: Location of the olfactory bulb, the olfactory mucosa and the olfactory nerve cells in humans Modified picture from the Nobel Prize official homepage (Nobelprize.org)**

#### 2.4.2. 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 (figure 2.8). 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 the CNS is thought to involve either an *intraneuronal* or *extraneuronal pathway* (Thorne et al., 2001, BormLange 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 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 mechanism / pathway 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.



**Figure 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	Albumin
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 (Illum et al., 2003)**

- 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 is 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 (Illum et al., 2003)**

- 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.

#### **2.4.3 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:**

- 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.
- 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  $\mu$ m, 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 10  $\mu$ m are deposited in the nasal cavity. Particles that are 2 to 10  $\mu$ m can be retained in the lungs and particles of less than 1  $\mu$ m are exhaled.
- v **Solubility & dissolution Rate:** Drug solubility and dissolution rates are important factor in determining nasal absorption from powders and suspensions. The particles deposited in the nasal cavity need to be dissolved prior to absorption. If a drug remains as particles or is cleared away, no absorption occurs.

## 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 is important to consider. The delivery volume is limited by the size of the nasal cavity. An upper limit of 25 mg/dose and a volume of 25 to 150 ml/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 200  $\mu$ L with 100  $\mu$ L being the most common dose volume. Hence, nasal secretions may alter the pH of the administered dose. This can affect the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH in-situ.
- 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 gel. In other words, the ionic strength of the composition is kept sufficiently low to obtain a low viscosity formulation that is easy to administer, but sufficiently high to ensure gelation once administered into the nasal cavity where gelation will take place due to the presence of cations in the nasal fluids.

- 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 polyglycolized C<sub>8</sub>-C<sub>10</sub> 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- $\beta$ -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 aminopeptidases.

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 previous 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.

#### **2.4.4. 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 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  $\mu\text{L}$ . 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<sub>12</sub> 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.

#### **2.4.5. Animal Models for Evaluation of Nasal Drug Absorption Studies**

Nasal absorption studies can be evaluated using two animal models viz. (1) whole animal or in vivo model and (2) isolated organ perfusion or ex vivo model. The models are commonly employed as per the needs of experiment. These models are described in the sections 2.2.10.1 onwards.



**1) Whole animal or in vivo model Rat model:**

The surgical preparation of rat for in vivo nasal absorption study carried out by anaesthetizing the rat by intraperitoneal injection of sodium Phenobarbital. An incision is made in the neck and the trachea is cannulated using polyethylene tube. Another tube is inserted through the esophagus towards the posterior region of nasal cavity. The passage of the nasopalatine tract is sealed so that the drug solution does not get drained from the nasal cavity through mouth. The drug solution is delivered to nasal cavity through nostril or through the polyethylene cannula. 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 anaesthetized by intramuscular injection of a combination of ketamine or xylene. The drug solution is sprayed in form of nasal spray into each nostril. The head of the rabbit is upheld in upright position. During the study, rabbits are allowed to breathe naturally through the nostrils. The body temperature of the rabbits shall be maintained at 37 °C with aid of heating pad. The blood samples are collected using an indwelling catheter from the marginal ear vein or artery as per the experimental protocol (Corbo et al., 1998).

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 i.v. 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 °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 (Lee et al., 1991).

**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 (Visor et al., 1987).

**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.

## **2) Ex-vivo nasal perfusion models**

Surgical preparation is the same as defined under in vivo rat model. During perfusion studies, a funnel is placed between the nose and reservoir to minimize the loss of drug solution. The drug solution is filled in reservoir and temperature is maintained at 37 ° C. The drug solution is circulated using peristaltic pump. The drug solution is dripped on the nostril and collected via funnel to the reservoir. The drug solution in the reservoir is stirred constantly and circulated for a predetermined time period as per the design of the protocol. The amount of drug transported cross the nasal cavity is back calculated from the concentration of drug remained in the reservoir. One of the drawbacks of this model is that unstable drugs may lead to incorrect results. This model is used to determine the nasal absorption of salicylic acid, aminopyrine, phenol red, phenobarbital, secobarbital, 1-tyrosine, Propranolol hydrochloride, polyethylene glycol 4000 etc. Rabbit model can also be employed for studying ex vivo nasal absorption of drugs (Chien et al., 1985).

## 2.5. Novel nasal delivery systems

### Chitosan and other chitosan positively charged derivatives, its use in the pharmaceutical field

In recent years, the polysaccharide material, chitosan has attracted much interest as a nasal delivery system that is able to efficiently deliver polar drugs (including peptides) to the systemic circulation and provide therapeutically relevant bioavailability. Chitosan is produced, by a process of deacetylation, from the chitin found in crustacean shells. The resultant free amino groups enable the formation of positively charged chitosan salts with organic and inorganic acids.

#### 2.5.1. Chitosan

Chitosan (poly[  $\beta$  -(1-4)-2-amino-2-deoxy-D-glucopyranose]) is a biodegradable cationic polysaccharide produced by partial deacetylation of chitin derived from naturally occurring crustacean shells. The molecular formula is  $C_6H_{11}O_4N$  and its structure of chitin and chitosan are shown in Figure 2.9. The polymer is comprised of copolymers of glucosamine and N-acetyl glucosamine. The term chitosan embraces a series of polymers that vary in molecular weight (from approximately 10,000 to 1 million Dalton) and degree of deacetylation (in the range of 50-95%). Since chitosan displays mucoadhesive properties, strong permeation enhancing capabilities for hydrophilic compounds and a safe toxicity profile (Singla et al., 2001), it has received considerable attention as a novel excipient in drug delivery system and has been included in the European Pharmacopoeia since 2002. Its applications are summarized in Table 2.2.

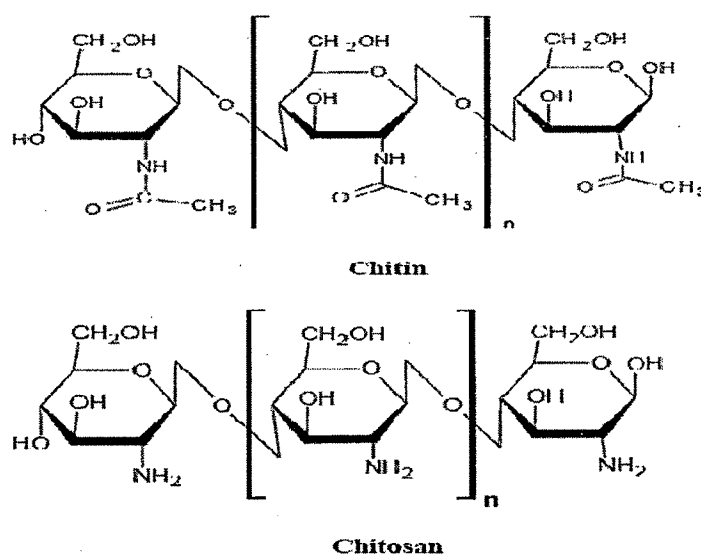


Figure 2.9: Structure of chitin and chitosan

Table 2.2: Application of chitosan in the pharmaceutical field

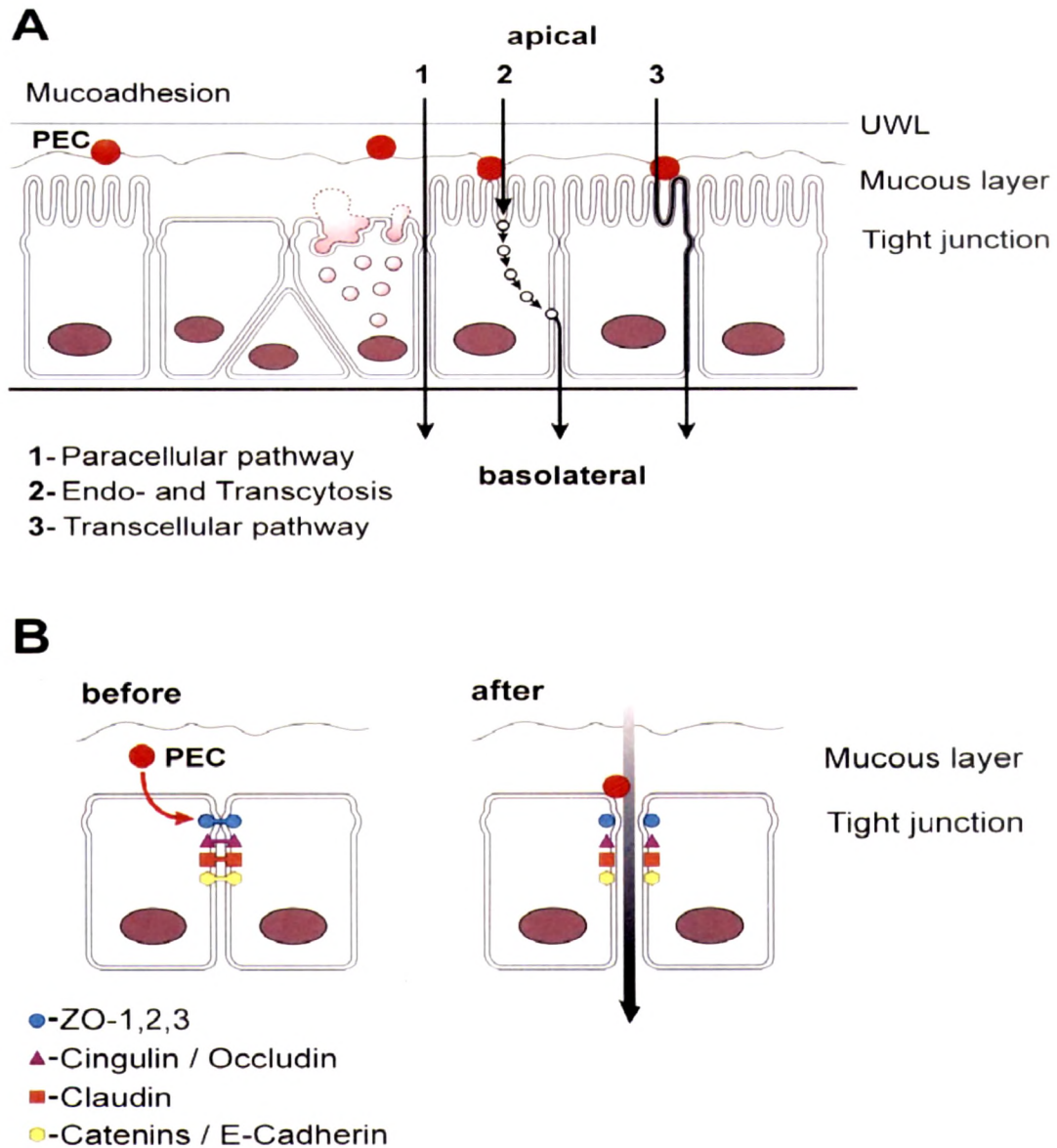
Conventional formulations	Novel formulations
Direct compression tablet Controlled release matrix tablet Wet granulation Gels Films Emulsions Wetting agent Coating agent Microspheres and microcapsules Targeting	Bioadhesion Transmucosal drug transport Vaccine delivery Non-viral DNA delivery

Despite its biocompatibility, the uses of chitosan in biomedical fields are limited by its poor solubility in physiological media. Chitosan has an apparent pKa value between 5.5 and 6.5 and upon dissolution in acid media the amino groups of the polymer are protonated rendering the molecule positively charged. At neutral and alkaline pH, most chitosan molecules lose their charge and precipitate from solution. To improve the poor water-solubility of chitosan at physiological pH, several derivatives have been studied. For example, the modification of chitosan by quaternization of the amino groups (Sieval et al., 1998, Le Dung et a., 1994), N-carboxymethylation (Muzzarelli et al., 1982) and PEGylation (Saito et al., 1997, Ohya et al., 2000) have been reported. Moreover, chitosan was used to modify the surface of poly (D, L-lactic acid) (PDLA) in order to enhance its cell affinity (Cai et al., 2002).

**Mechanism**

The absorption promoting effect of chitosan has been found to be a combination of mucoadhesion and a transient opening of the tight junctions in the mucosal cell membrane, as shown in Figure 2.10. The mucoadhesive properties of chitosan are a consequence of an interaction between the positively charged chitosan and negatively charged sialic acid groups on the mucin. Such interactions encourage prolonged contact time between the drug substance and the absorptive surface, thereby permitting the absorption of drug molecules via the paracellular (Figure 2.10A, 1), transcellular pathway (Figure 2.10A, 3), or through endo- and transcytosis (Figure 2.10A, 2). Generally, only small, hydrophilic molecules with a molecular weight below 500 g/mol are able to penetrate via a paracellular pathway through the tight

junction. Most compounds are absorbed via a transcellular pathway through the cell membrane of the epithelial cells due to the high surface area. Endo- and transcytosis is characterized by the engulfment of the extracellular material, followed by the pinching of the membrane vesicles from the plasma membrane (Figure 2.10A, 2). Adsorptive endocytosis of chitosan nanoparticles has previously been demonstrated (Huang et al., 1999).



**A. Mucoadhesion.**

**B. Opening the tight junction.**

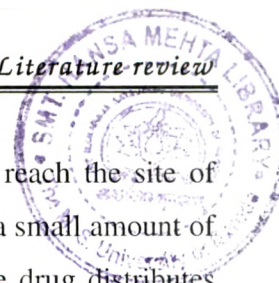
**Figure 2.10: Schematic overview of the mechanism of chitosan as a permeation enhancer**

Tight junctions are located between apical and basolateral domains in epithelial cells, and appear as a continuous apical belt around the cell periphery. They regulate the passage of molecules across these natural barriers. Large molecular weight drugs need to pass through these tissue barriers in order to reach their targeted sites. As part of the body's normal activity, tight junctions selectively open and close in response to various signals both inside and outside the cells. This permits the passage of large molecules across the tight junction barrier. On a molecular level, tight junctions consist of proteins, for example, claudins, occludins and junctional adhesion molecules. Such molecules are anchored in the membranes of two adjacent cells and interact with one other to hold the cells together, preventing other molecules from passing between them. In intact tight junctions, these proteins are strongly associated with the plasma membrane (Dodane et al., 1999). However under conditions precluding tight junction formation, these proteins appear to be relocated from the membrane into other cellular compartments. It has been shown in both cell culture (Caco-2) and animal models that chitosan is able to induce a transient opening of tight junctions, thus increasing membrane permeability particularly to polar drugs, including peptides and proteins (Dodane et al., 1999). The opening of the tight junctions has been demonstrated by a loss of ZO-1 proteins and occludins from the cytosolic and membrane fractions into the cytoskeletal fraction (Schipper et al., 1987, Smith et al., 2004).

### **Toxicity**

Although a number of investigations have been performed to elucidate the cytotoxicity of chitosan, the results were controversial. A series of toxicity studies indicated that chitosan was toxic, with the extent of toxicity being dependent upon the molecular weight, degree of deacetylation and salt form (Sgouras et al., 1990, Carreño-Gómez et al., 1997). By contrast, other investigations have suggested that the toxicity of chitosan is negligible, with experiments investigating the effects on cilia beat frequency (CBF) in guinea pigs after 28 days application, effect on mucociliary clearance rates on human nasal tissue and effect on nasal membranes in rats (Aspden et al., 1996, Aspden et al., 1997). A ten-day acute toxicity study in rabbits showed neither macroscopic nor microscopic effects on organs or tissues, and oral toxicity of chitosan was reported to be 16 g/kg body weight (LD<sub>50</sub>). The mucociliary clearance rate in man, measured by a saccharine clearance test, was found to be unaffected after daily nasal application of chitosan (Soane et al., 1999).





The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site, while the majority of the drug distributes throughout the rest of the body in accordance with its physicochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task. One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles. The idea of using submicron drug delivery systems for drug targeting was conceived and developed after Paul Ehrlich originally proposed the idea of tiny drug-loaded magic bullets over a hundred year ago (Kumar et al., 1996).

Among these carriers, liposomes and micro/nanoparticles have been the most extensively investigated. Liposomes present some technological limitations including poor reproducibility and stability, and low drug entrapment efficiency. Nevertheless, several low molecular weight drugs are now commercially available which employ this technology. Polymeric nanoparticles, which possess a better reproducibility and stability profiles than liposomes, have been proposed as alternative drug carriers that overcome many of these problems. Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process: nanospheres and nanocapsules (Allemann et al., 1993).

Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces (Figure 2.11). Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed onto their exterior (Figure 2.11). The term “nanoparticles” is adopted because it is often very difficult to unambiguously establish whether these particles are of a matrix or a membrane type. Nanoparticles not only have potential as drug delivery carriers as they offer non-invasive routes of administration such as oral, nasal and ocular routes, but also show to be good adjuvant for vaccines. Despite these advantages, there is no ideal nanoparticles system available. Most of nanoparticles prepared from water-insoluble polymers are involved heat, organic solvent or high shear force that can be harmful to

the drug stability. Moreover, some preparation methods such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force.

Among water-soluble polymers available, chitosan is one of the most extensively studied. This is because chitosan possesses some ideal properties of polymeric carriers for nanoparticles (Table 2.3) such as biocompatible, biodegradable, nontoxic, and inexpensive. Furthermore, it possesses positive charge and exhibits absorption enhancing effect. These properties render chitosan a very attractive material as a drug delivery carrier. In the last two decades, chitosan nanoparticles (chitosan NP) have been extensively developed and explored for pharmaceutical applications.

#### **Preparation method**

Over the past 30 years, chitosan NP preparation technique has been developed based on chitosan microparticles technology. There are at least four methods available: ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex. The most widely developed methods are ionotropic gelation and self assemble polyelectrolytes. These methods offer many advantages such as simple and mild preparation method without the use of organic solvent or high shear force. Thus, they would be applicable to a broad categories of drugs including macromolecules which notorious as labile drugs. In general, the factors found to affect nanoparticles formation including particle size and surface charge are molecular weight and degree of deacetylation of chitosan. The entrapment efficiency is found to be dependent on the pKa and solubility of entrapped drugs. The drug is mostly found to be associated with chitosan via electrostatic interaction, hydrogen bonding, and hydrophobic interaction.

Table 2.3: Criteria for ideal polymeric carriers for nanoparticles & nanoparticles delivery systems

Polymeric carriers	Nanoparticles delivery systems
<ul style="list-style-type: none"><li>• Easy to synthesize and characterize</li><li>• Inexpensive</li><li>• Biocompatible</li><li>• Biodegradable</li><li>• Non-immunogenic</li><li>• Non-toxic</li><li>• Water soluble</li></ul>	<ul style="list-style-type: none"><li>• Simple and inexpensive to manufacture and scale-up</li><li>• No heat, high shear forces or organic solvents involved in their preparation process</li><li>• Reproducible and stable</li><li>• Applicable to a broad category of drugs; small molecules, proteins and polynucleotides</li><li>• Ability to lyophilize</li><li>• Stable after administration</li><li>• Non-toxic</li></ul>

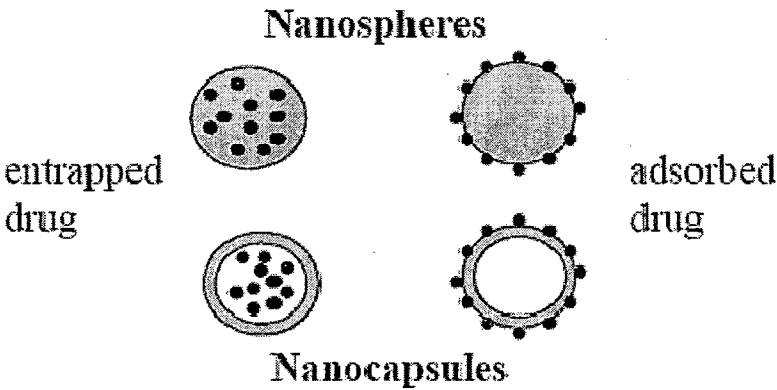
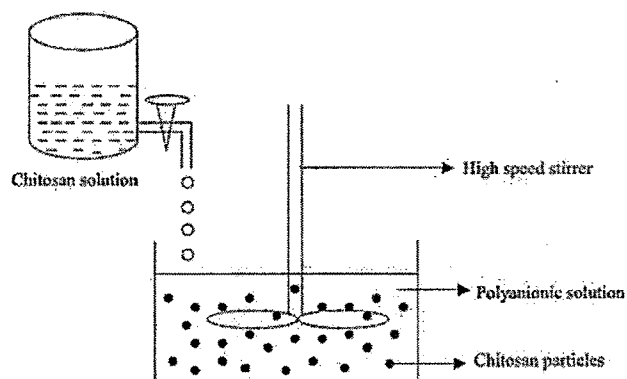


Figure 2.11: Various type of drug loaded nanoparticles (Allemann et al. 1993)

**Ionotropic gelation**

Chitosan NP prepared by ionotropic gelation technique was first reported by *Calvo et al.*, (1997b) and has been widely examined and developed (Janes et al., 2001, Pan et al., 2002). The mechanism of chitosan nanoparticles formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate (Bodmeier et al., 1989). This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and

surface charge of particles can be modified by varying the ratio of chitosan and stabilizer (Calvo *et al.*, 1997a) (Figure 2.12).



**Figure 2.12: Preparation method of chitosan nanoparticles using Ionotropic gelation method (Agnihotri *et al.*, 2004)**

#### **Microemulsion method**

Chitosan NP prepared by microemulsion technique was first developed by Maitra *et al.* (1999). This technique is based on formation of chitosan nanoparticles in the aqueous core of reverse micellar droplets and subsequently cross-linked through glutaraldehyde. In this method, a surfactant was dissolved in N-hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring at room temperature. Nanoparticles were formed in the presence of surfactant. The system was stirred overnight to complete the cross-linking process, which the free amine group of chitosan conjugate with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The yields obtained were the cross-linked chitosan NP and excess surfactant. The excess surfactant was then removed by precipitate with  $\text{CaCl}_2$  and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alter the degree of cross-linking. Nevertheless, some disadvantages exist such as the use of organic solvent, time-consuming preparation process, and complexity in the washing step.

#### **Emulsification solvent diffusion method**

El-Shabouri reported chitosan NPs prepared by emulsion solvent diffusion method, (El-Shabouri *et al.*, 2002) which originally developed by Niwa *et al.* (1993) employing

PLGA (Niwa *et al.*, 1993). This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon injection an organic phase into chitosan solution containing a stabilizing agent (i.e. poloxamer) under mechanical stirring, follow by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanoparticles preparation.

#### **Polyelectrolyte complex (PEC)**

Polyelectrolyte complex or self assemble polyelectrolyte is a term to describe complexes formed by self-assembly of the cationic charged polymer and plasmid DNA. Mechanism of PEC formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also possess this property. Generally, this technique offers simple and mild preparation method without harsh conditions involved. The nanoparticles spontaneously formed after addition of DNA solution into chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature (Erbacher *et al.*, 1998). The complexes size can be varied from 50 nm to 700 nm.

#### **2.5.2. Thiolated Chitosans: Novel Polymers for Mucoadhesive Drug Delivery**

Mucoadhesion can be defined as the ability of synthetic or biological macromolecules to adhere to mucosal tissues such as the mucosa of the small intestine. Since the early 1980s, the concept of mucoadhesion has gained considerable interest in pharmaceutical technology. If this concept can reach its full potential, it might open the door for novel, highly efficient dosage forms especially for oral drug delivery. Mucoadhesive drug delivery systems promise several advantages that arise from localization at a given target site, prolonged residence time at the site of drug absorption, and an intensified contact with the mucosa increasing the drug concentration gradient. Hence, uptake and consequently bioavailability of the drug may be increased and frequency of dosing reduced with the result that patient compliance is improved. Various natural and synthetic polymers have been discovered as mucoadhesive excipients. Their mucoadhesive properties can be explained by their interaction with the glycoproteins of the mucus, based mainly on

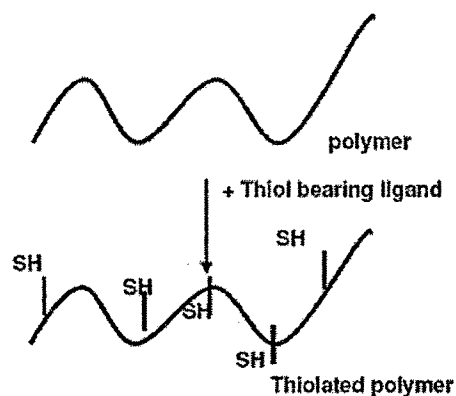
non-covalent bonds such as ionic interactions, hydrogen bonds and van der Waals forces (Thanou et al., 2000).

Chitosan has been studied as a biomaterial and as a pharmaceutical excipient for drug delivery, because of its favourable biological properties such as biodegradability and low toxicity (Illum et al., 1998). Moreover, its ability to facilitate paracellular transport of drugs across mucosal barriers (Borchard et al., 1996, Lee et al., 1995). Because of its complimentary properties, such as enzymatic biodegradability, non-toxicity and biocompatibility chitosan has received considerable interest as a novel excipient in drug delivery systems, and has been included in the European Pharmacopoeia since 2002. In spite of its reported successes, a major downside of chitosan is that it is insoluble at physiological pH, whereas it is soluble and active as an absorption enhancer only in its protonated form in acidic environments (Kotze et al., 1999).

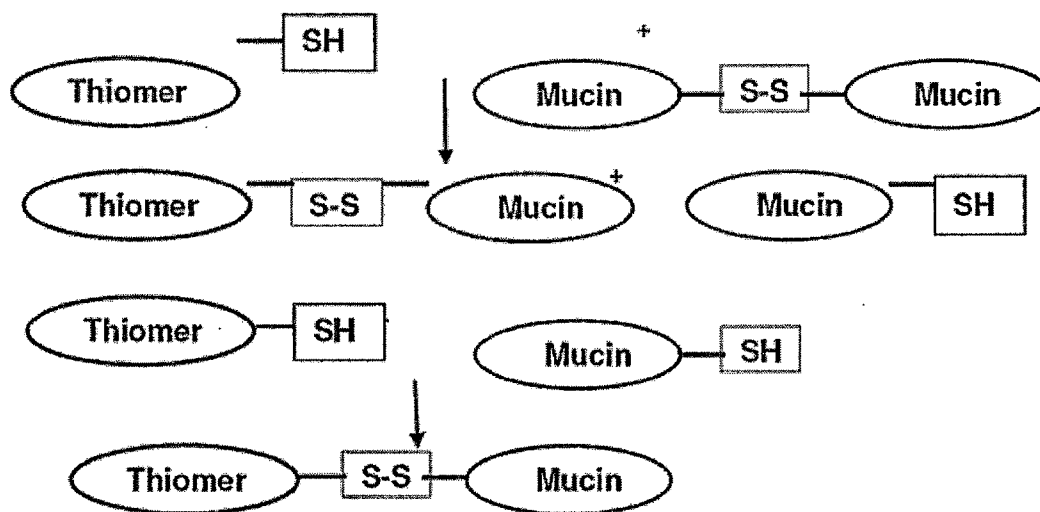
Recently, it has been shown that polymers with thiol groups provide much higher adhesive properties than polymers generally considered to be mucoadhesive. The enhancement of mucoadhesion can be explained by the formation of covalent bonds between the polymer and the mucus layer which are stronger than non-covalent bonds. These thiolated polymers (see Figure 2.13), known as *thiomers*, interact with cysteine rich subdomains of mucus glycoproteins via disulfide exchange reactions or via simple oxidation process as shown in Figure. 2.14. To further enhance the solubility of chitosan and to improve its mucoadhesive and/or permeation enhancing properties, various derivatives such as trimethylated chitosan, (Thanou et al., 2000) mono-N-carboxymethyl chitosan. (Thanou et al., 2001), N-sulfochitosan (Baumann et al., 2001) and chitosan-EDTA conjugates (Andreas et al., 1998) were developed. A further modification is based on the immobilization of thiol bearing moieties on the polymeric backbone of chitosan. To date, three different thiolated chitosan derivatives have been synthesized: chitosan-thioglycolic acid conjugates (Andreas et al., 2001, Hornof et al., 2003) chitosan-cysteine conjugates (Andreas et al., 1999) and chitosan-4-thio-butyl-amidine (chitosan- TBA) conjugates. These thiolated chitosans have numerous advantageous features in comparison to unmodified chitosan, such as significantly improved mucoadhesive and permeation enhancing properties (Andreas et al., 1999, Andreas et al 2003., Roldo et al., 2004, Langoth et al., 2004). The strong cohesive properties of thiolated chitosans make them highly suitable excipients for controlled drug release dosage forms (Andreas et al., 2003, Kast et al., 2003).



Moreover, solutions of thiolated chitosans display *in situ* gelling properties at physiological pH values (Hornof et al., 2003). It is the aim of this review to provide an overview about different thiolated chitosan derivatives that have been synthesized so far as well as their characterization and optimization utilizing various *in vitro* test systems. The performance of thiolated chitosan in *in vivo* studies, providing proof of their applicability in peroral peptide delivery systems, will be discussed as well.



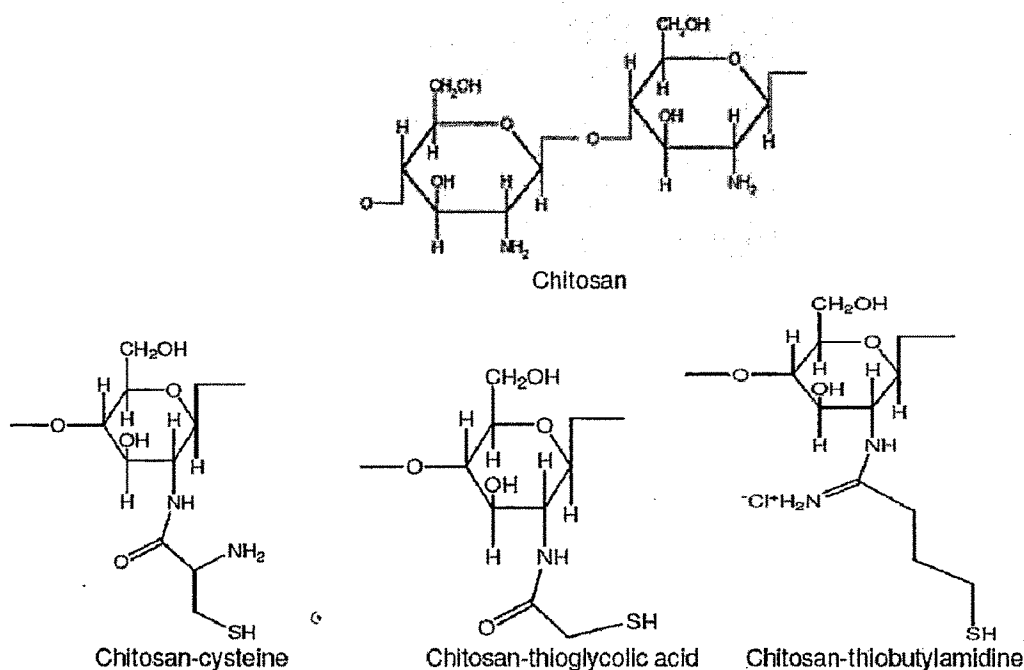
**Figure 2.13: Simple diagrammatic representation of preparing thiolated polymer** (Sreenivas et al., 2008)



**Figure 2.14: Mechanism of disulphide bond formation between thiomers and mucus glycoproteins (Mucins)** (Sreenivas et al., 2008)

**Synthesis of thiolated chitosan**

The primary amino group at the 2-position (Figure 2.15) of the glucosamine subunit of chitosan is the main target for the immobilization of thiol groups. As shown in Figure 2.15 sulfhydryl bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds. In case of the formation of amide bonds the carboxylic acid group of the ligands cysteine and thioglycolic acid reacts with the primary amino group of chitosan mediated by a water soluble carbodiimide (Kast et al., 2001). The formation of disulfide bonds by air oxidation during synthesis is avoided by performing the process at a pH below 5. At this pH-range the concentration of thiolate anions, representing the reactive form for oxidation of thiol groups, is low, and the formation of disulfide bonds can be almost excluded. Alternatively, the coupling reaction can be performed under inert conditions. In the case of the formation of amidine bonds, 2-iminothiolane is used as a coupling reagent (Andreas et al., 2003). It offers the advantage of a simple one step coupling reaction. In addition, the thiol group of the reagent is protected against oxidation because of the chemical structure of the reagent. Orientating studies with all these thiolated chitosans showed that a degree of modification of 25–250 mmol thiol groups per gram chitosan leads to the highest improvement in the mucoadhesive and permeation enhancing properties. The amount of immobilized thiol groups in reduced and oxidized form can be determined via Ellman's reagent (Hornof et al., 2003) with and without previous quantitative reduction of disulfide bonds with borohydride (Leitner et al., 2003).



**Figure 2.15: Structure of chitosan and thiolated chitosan** (Sreenivas et al., 2008)

### Properties of Thiolated Chitosan

#### Mucoadhesive properties

The improved mucoadhesive properties of thiolated chitosans are explained by the formation of covalent bonds between thiol groups of the polymer and cysteine rich subdomains of glycoproteins in the mucus layer (Leitner et al., 2003). These covalent bonds are supposedly stronger than noncovalent bonds such as ionic interactions of chitosan with anionic substructures of the mucus layer. This theory was supported by the results of tensile studies with tablets of thiolated chitosans which demonstrated a positive correlation between the degree of modification with thiol bearing moieties and the adhesive properties of the polymer (Roldo et al., 2004). These findings were confirmed by another *in vitro* mucoadhesion test system where the time of adhesion of tablets on intestinal mucosa was determined. The contact time of the thiolated chitosan derivatives increased with increasing amounts of immobilized thiol groups (Andreas et al., 2003). With chitosan-thioglycolic acid conjugates a 5–10-fold increase in mucoadhesion in comparison to unmodified chitosan was achieved. The mucoadhesive properties of chitosan-TBA (chitosan-4-thio-butyl-amidine) conjugates were even further improved. One explanation for this phenomenon can be given by the theory that chitosan-TBA conjugates additionally increased mucoadhesive

properties due to improved ionic interactions between the additional cationic amidine substructure of the conjugate (see Figure 2.15) and anionic substructures within the mucus layer. Tensile studies with chitosan-TBA conjugates of low, medium and high molecular mass (150, 400 and 600 kDa) furthermore indicated that medium molecular mass thiolated chitosans displayed relatively, the highest mucoadhesiveness. Utilizing a medium molecular mass chitosan-TBA conjugate displaying 264 mM thiol groups per gram polymer led to a more than 100-fold improvement in mucoadhesion compared to unmodified chitosan. This represents the greatest progress made so far in the development of mucoadhesive polymers (Roldo et al., 2004).

#### **Permeation enhancing effect**

In 1994 Illum et al showed the permeation enhancing capabilities of chitosan for the first time. Chitosan is able to enhance the paracellular route of absorption, which is important for the transport of hydrophilic compounds such as therapeutic peptides and antisense oligonucleotides across the membrane. Various studies carried out on Caco-2 cell monolayers demonstrated a significant decrease in the transepithelial electrical resistance after the addition of chitosan (Artursson et al., 1994). The mechanism underlying this permeation enhancing effect seems to be based on the positive charges of the polymer which interact with the cell membrane resulting in a structural reorganization of tight junction-associated proteins (Schipper et al., 1997). In the presence of the mucus layer, however, this permeation enhancing effect is comparatively lower, as chitosan cannot reach the epithelium because of size limited diffusion and/or competitive charge interactions with mucin (Schipper et al., 1999). Nevertheless, these results obtained on Caco-2 cell monolayers could be confirmed by *in vivo* studies, showing an enhanced intestinal absorption of the peptide drug, buserelin, in rats due to the co-administration of chitosan hydrochloride (Luessen et al., 1996). The permeation enhancing effect of chitosan can be greatly improved by the immobilization of thiol groups. The effect of thiolated chitosans has been shown in various permeation studies in Ussing type chambers using freshly excised intestinal mucosa (Andreas et al., 1999). The uptake of fluorescence-labelled bacitracin, for instance, was improved 1.6-fold utilizing 0.5% of chitosan-cysteine conjugate instead of unmodified chitosan (Andreas et al., 1999). In another study, the permeation enhancing effect of chitosan-TBA, in comparison to the permeation enhancing effect of unmodified chitosan, was shown. The uptake of the cationic marker compound, rhodamine-123 was 3-fold higher in the presence of thiolated chitosan than in

unmodified chitosan (Langoth et al., 2004). The likely mechanism responsible for this improved permeation enhancement has been attributed to the inhibition of the protein, tyrosine phosphatase. This enzyme seems to be involved in the opening and closing process of the tight junctions. Tyrosine phosphatase is responsible for the dephosphorylation of tyrosine subunits of occludin, representing an essential transmembrane protein of the tight junctions. When these tyrosine subunits of occludin are dephosphorylated, the tight junctions are closed. In contrast, when these tyrosine subunits are phosphorylated, the tight junctions are opened. The inhibition of tyrosine phosphatase by compounds such as phenylarsine oxide, pervanadate or reduced glutathione leads consequently to phosphorylation and opening of the tight junctions. In contrast to the stable but toxic tyrosine phosphatase inhibitors phenylarsine oxide and pervanadate, the inhibitory effect of glutathione is lower as it is rapidly oxidized on the cell surface, losing its inhibitory activity (Grafstrom et al., 1980). Due to the combination of reduced glutathione with thiolated chitosans, however, the oxidation of the inhibitor on the membrane can be restricted, as thiomers are capable of reducing oxidized glutathione (Clausen et al., 2002).

**Mechanisms being responsible for improved mucoadhesion** (Andreas et al., 2005)

#### **Formation of disulfide bonds with the mucus gel layer**

The formation of disulfide bonds between the thiomers and the mucus gel layer takes place either via thiol/disulfide exchange reactions (1) or via a simple oxidation process of free thiol groups (2). The different types of mucus glycoproteins or designated mucins exhibiting cysteine-rich subdomains have been reviewed previously. Generally there are no mucosal surfaces in which mucins with cysteine rich subdomains are not present. In contrast to noncovalent bonds disulfide bonds are not influenced by factors such as ionic strength and pH. Velocity and extent of disulfide bond formation depends on the concentration of thiolate anions representing the reactive form for thiol/disulfide exchange reactions and oxidation processes. The concentration of thiolate anions in turn depends on:

**The pKa value of the thiol group.** In dependence on the polymer backbone and the chemical structure of the ligand, more or less reactive thiomers can be designed. Thiol groups of the chitosan–thiobutylamidine conjugate, for instance, exhibit a pKa value of 9.9, whereas the pKa value of the thiol groups of poly(acrylate)–cysteine conjugates is 8.35.

**The pH of the thiomers.** As only ionic thiomers are used, they all display a high buffer capacity. The buffer capacity of a sodium poly(acrylate) matrix tablet, for instance, can be compared with that of an at least 25 M acetate buffer. As all charged groups remain concentrated on the polymeric network a kind of microclimate can be established. The reactivity of thiol groups can consequently be controlled by adjusting the pH of the polymer to a certain level. The higher the pH is adjusted, the more reactive are the thiol groups and vice versa.

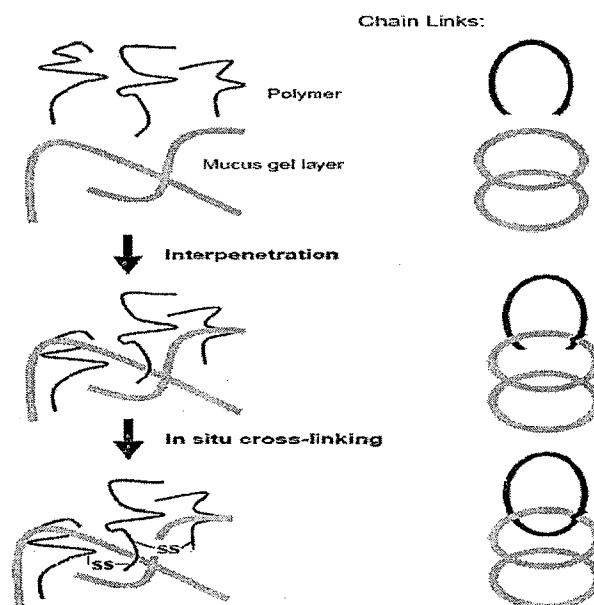
**The pH of the surrounding medium.** The reactivity of thiol groups inside the polymeric network is mainly controlled by the pH of the thiomers, whereas the reactivity on the surface of the polymer is more controlled by the pH of the surrounding medium. As the mucus gel layer being close to the epithelium has a pH around 7, thiol groups penetrating into the mucus are always sufficiently reactive.

Evidence for the formation of covalent bonds between thiomers and the mucus gel layer has been provided recently. *Leitner et al (2003)* could show by four different methods including rheological, diffusion, gel permeation and certain mucoadhesion studies the formation of disulfide bonds between thiolated polymers and mucus glycoproteins. In another publication it was also shown that mucin can be effectively bound to thiolated polyacrylate, while it is not at all bound to unmodified polyacrylate. Due to the addition of the disulfide bond breaker dithiothreitol already immobilized mucin could be completely removed from the thiolated polymer

#### **In situ cross-linking process**

Another likely mechanism being responsible for the improved mucoadhesive properties of thiomers is based on their in situ cross-linking properties. During and after the interpenetration process, which could be verified for mucoadhesive polymers such as poly(acrylic acid) recently, disulfide bonds are formed within the thiomers themselves leading to additional anchors via chaining up with the mucus gel layer. The mechanism is illustrated in Figure 2.16.





**Figure 2.16: Schematic presentation of improved mucoadhesion by an insitu cross-linking in comparison to chain links (Andreas et al., 2005)**

It is similar to the mechanism on which the adhesive property of most adhesive is based on, i.e. a penetration of the adhesive into a certain surface structure followed by a stabilization process of the adhesive. In case of superglues, for instance, monomeric cyanoacrylates penetrate into raw surfaces followed by a polymerization process. Thiolated polymers display in situ gelling properties due to the oxidation of thiol groups at physiological pH-values, which results in the formation of inter- and intramolecular disulfide bonds. The in situ gelling behaviour of thiomers was characterized in vitro by rheological measurements. The sol–gel transition of thiolated chitosan, for instance, was completed at pH 5.5 after 2 h, when highly cross linked gels were formed. In parallel, a significant decrease in the thiol group content of the polymers was observed, indicating the formation of disulfide bonds. The rheological properties of unmodified chitosan remained constant over the whole observation period. Rheological investigation of thiolated chitosans furthermore demonstrated a clear correlation between the total amount of polymer-linked thiol groups and the increase in elasticity of the formed gel. The more thiol groups were immobilized on chitosan, the higher was the increase in elastic modulus  $G'$  in solutions of thiolated chitosan. These in situ gelling properties are in particular of interest for liquid or semisolid vaginal, nasal and ocular formulations, which should stabilize themselves once, applied on the site of drug delivery.

## **Future Trends**

### **Non-invasive peptide delivery**

The incorporation of peptide drugs exhibiting a cationic net charge in anionic mucoadhesive polymers on the one hand leads to a strong reduction in the mucoadhesive properties and, on the other hand, may hinder drug release as a result of strong ionic interactions between the therapeutic ingredient and the polymeric network. Consequently, cationic therapeutic peptides or peptidomimetics such as calcitonin or desmopressin need to be embedded in cationic or non-ionic mucoadhesive polymers. As non-ionic polymers cannot provide sufficient high mucoadhesion and thiolated chitosans display comparatively the highest mucoadhesive properties among cationic polymers, this type of thiomers seems to be a favourable tool for the oral administration of cationic hydrophilic macromolecules. Apart from oral delivery systems thiolated chitosans seem to be useful also for other non-invasive routes of peptide drug administration. In particular, the nasal, vaginal, buccal and ocular mucosae are interesting targets.

### **Production of micro- and nano-particles**

Microparticles based on chitosan disintegrate very rapidly unless they are combined with multivalent anionic compounds such as sodium sulfate (Lubben et al., 2001) or alginate leading to stabilization by an ionic cross-linking process. Due to the addition of such multivalent anionic compounds, however, the mucoadhesive properties of chitosan are strongly reduced. In contrast, microparticles that are based on thiolated chitosan do not disintegrate. Because of the formation of disulfide bonds within the polymeric network, microparticles are strongly stabilized. Consequently, a controlled drug release out of thiolated chitosan microparticles can be provided. In contrast to the addition of multivalent anionic compounds, the immobilization of thiol groups on chitosan leads to strongly improved mucoadhesive properties.

### **Tissue engineering**

A further interesting application of thiolated chitosans is their use in tissue engineering. The expanding field of tissue engineering applications has accelerated the need for materials which are tissue compatible, biodegradable and with mechanical properties similar to the target tissues. Biodegradable and biocompatible polymers have been attractive candidates for scaffolding materials because they degrade as the new tissues are formed, eventually without inflammatory reactions or toxic degradation. Further, studies in this direction were performed with L-929 mouse

fibroblasts seeded onto chitosan thioglycolic acid sheets. The results of this study showed that thiolated chitosan can provide a porous scaffold structure guaranteeing cell anchorage, proliferation and tissue formation in three dimensions. (Ma et al., 2001). Due to the *in situ* gelling properties, it seems possible to provide a certain shape of the scaffold material by pouring a liquid thiolated chitosan (Kast et al., 2003) cell suspension in a mold. Furthermore, liquid polymer cell suspensions may be applied by injection forming semi-solid scaffolds at the site of tissue damage. Since low concentrated aqueous solutions of thiolated chitosan remain liquid when stored under inert conditions and are rapidly gels under access of oxygen, they seem to be promising candidates for such applications.

### **Coating of stents**

Another promising application of thiolated chitosans is their use as coating material for stents. Polymer-coated drug-eluting stents are a potential technique to achieve high local tissue concentrations of an effective drug at the precise site and at the time of vessel injury. First orientating studies demonstrated that by simply dipping the stent in a thiolated chitosan solution and drying it on air, a stable coating could be achieved. During the drying process, cross-linking of chitosan by the formation of disulfide bonds due to air oxidation, takes place. The polymeric network is thereby stabilized on the stent. The chitosan coating should allow sustained release of incorporated drugs such as anti-inflammatory agents or agents avoiding cell proliferation. Recently, it was shown that stents can be successfully coated with thiolated poly(acrylic acid) and that sustained release of a model peptide drug out of this thiomeric coating can be provided. Similar results can be expected for thiolated chitosans but have to be verified by ongoing studies.

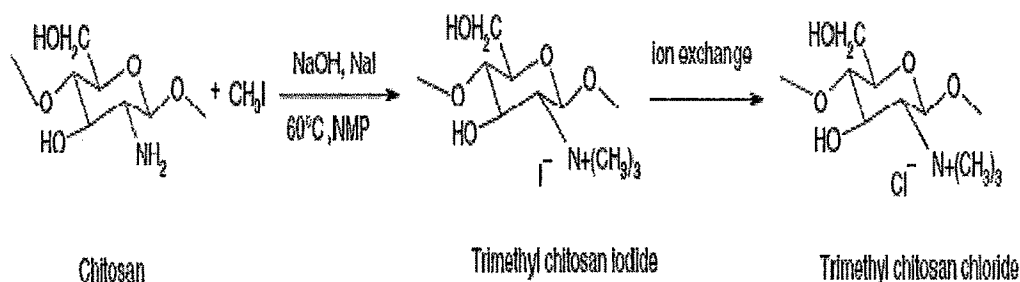
### **2.5.3. Trimethyl chitosan and its applications in drug delivery**

Chitosan in its protonated form facilitates the paracellular transport of hydrophilic drugs by combination of bioadhesion and a transient widening of the tight junctions in the membrane. Although it is incapable of enhancing absorption across the nasal mucosa. Kotze *et al* put forward the hypothesis that polymers such as unmodified chitosan with a primary amino group may not be the optimal ones in opening tight junctions, but the polymers or derivatives with different substituent, different basicities, or different charged densities will have the same or even increased efficacy in this respect (Kotze' et al., 1999). Kotze' *et al* further stated in this regard that there was a need for chitosan derivatives with increased solubility, especially at neutral and

basic pH values, for use as absorption enhancers aimed at the delivery of therapeutic compounds in the more basic environments (Kotze' et al., 1998, Kotze' et al., 1997). Attempts to boost up the positive charge on the polymer chain appear to be consistent with this rationale. The quaternization of amino group with simplest alkyl group i.e. methyl is the unambiguous step towards comprehending the hypothesis.

### Synthesis of trimethyl chitosan

Quaternization (methylation) of amino groups in chitosan can be achieved with methyl iodide at elevated temperature in strong alkaline environment to bind the acid being generated during the reaction taking place and to avoid protonation of the unreacted primary amino groups (Figure 2.17). The degree of quaternization (DQ) can be altered by increasing the number of reaction steps or by increasing the reaction time or by controlling the reaction steps or by using different deacetylation grades of chitosan. At higher degrees of quaternization however evidence of O-methylation on the 3 and 6 hydroxyl groups of chitosan is found. In general, O-methylation led to less soluble products. It is desirable hence to prepare trimethyl chitosan (TMC) polymers with a high DQ but with a low degree of O-methylation.



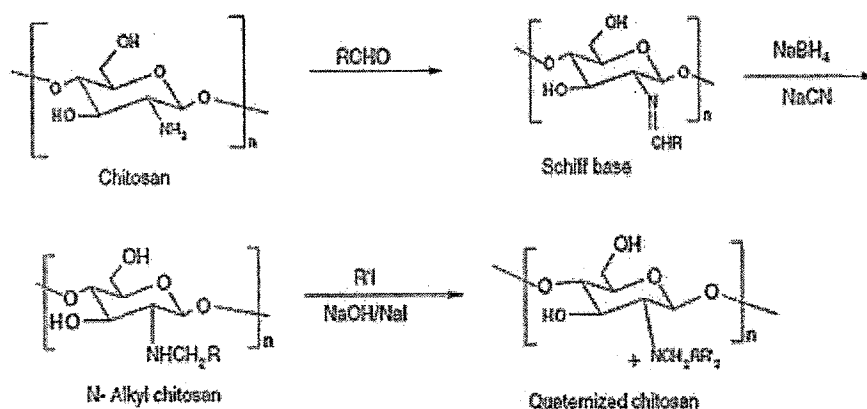
**Figure 2.17: Synthesis of trimethyl chitosan** (Mourya et al., 2009)

The synthesis of N,N,N-trimethyl chitosan was reported by Domard *et al* (1986) based on the dispersion of 5 g chitosan in 250 ml N-methyl-2 pyrrolidinone reacting with CH<sub>3</sub>I and NaOH (chitosan:CH<sub>3</sub>I:NaOH in molar ratio 1:15:2) for 3 h at 36°C (Domard et al., 1986). This method however caused extensive depolymerization of chitosan. The process was modified by Le Dung with respect to the ratio of reactants (chitosan:CH<sub>3</sub>I : NaOH in molar ratio 1:15:3.45) to reduce polymer degradation and control the different parameters affecting quaternization. The 1H-NMR examination however suggests that such procedure would mainly result in dimethylated polymer with only 10–15% of quaternization. Sieval *et al* (1998) modified the process with

respect to the solvent/reagent addition sequence and reported one step and two step syntheses. In one step synthesis, chitosan was dispersed in NMP with CH<sub>3</sub>I, and NaI and then the mixture was made alkaline by adding aqueous NaOH solution (Sieval et al., 1998). In two step synthesis, chitosan was dispersed in aqueous NaOH with NaI and then CH<sub>3</sub>I mixed with NMP was added. The resultant product was washed with ethanol and ether and subjected to methylation again but with less quantity of CH<sub>3</sub>I this time. Dimethylation is significantly decreased by repeating the basic reaction. To get the higher degree of substitution *Snyman et al (2002)* carried out the reaction in repetition of one, two, three or four times with same or different quantity of CH<sub>3</sub>I: the starting polymer for each subsequent step was the product of previous reaction step washed with ethanol (*Snyman et al., 2002*). The modifications were done by *Hamman et al (2001)* and employed by *Polnok et al (2004)* for varying the number of reaction steps and the type of base (*Hamman et al., 2001, Polnok et al., 2004*). The bases used were NaOH and dimethylaminopyridine along with NMP. The degrees of quaternization of TMC polymers obtained from the processes using dimethylaminopyridine as the base were lower than those using sodium hydroxide. The polymer degradation also was lower. This may be explained by the weaker alkaline properties of dimethylaminopyridine compared to sodium hydroxide. A combination of the two bases did not reduce polymer degradation, while the DQ was limited to relatively low values (12.5–34.4%). The attempts to increase the DQ by increasing the number of steps are accompanied by O-methylation. Moreover an increased number of reaction steps decreased dimethylation also. However an extended duration of reaction increased both DQ and dimethylation (*Jintapattanakit et al., 2008*). *Runarsson et al (2007)* changed the solvent system to DMF/H<sub>2</sub>O mixture (50:50) and performed the reaction without the aid of a catalyst-sodium iodide (*Runarsson et al., 2007*). This significantly reduced O-methylation since DMF/water seems to lowers the reactivity of the hydroxyl group enough to keep the O-methylation down. The DQ, however, was always low in the materials obtained. The DQ varied from 0 to 74% depending on the reaction conditions accompanied by monomethylation, dimethylation and O-methylation (chitosan:CH<sub>3</sub>I:NaOH in molar ratio 1: 6 or 12:1.5 to 9, time 0.5 to 48 h, temperature 21, 50, 75 °C). Based on this solvent system recently they also claimed to get high degree of substitution 81 to 88% by ‘one pot’ synthesis procedure (chitosan:CH<sub>3</sub>I:NaOH in molar ratio 1:6:3, time 48 h, room temperature) (*Ru´narsson et al., 2008*). They suggested protection group

strategy for more selective N-quaternization (sequence of N-phtahloylation, O-tritylation, N-deprotection, N-methylation and O-deprotection). The exchange of counter ion iodide with chloride was done finally by dissolving the quaternized polymer in a small quantity of water followed by the addition of HCl in methanol or by dissolving in NaCl solution. The exchange can be achieved by dialysis too against NaCl solution and water. All these methods of methylation make use of methyl iodide which despite being efficient is a highly volatile, carcinogenic and expensive reagent. In addition it offers limited control over a perilously chemical reaction. In an attempt to overcome these disadvantages, an alternative sequence for the synthesis of chitosan quaternized derivatives is proposed by *Britto et al (2006)* using dimethylsulfate as the reactive agent wherein the polymer in solution of NaOH and NaCl is mixed and refluxed with methylating agent at RT or at 70°C (de Britto et al., 2006). Here also the quaternization intensity was time and temperature dependent. The undesirable O-methylation and polymeric degradation were observed to take place for the reaction also. Other synthetic strategies have been reported to produce TMC derivatives but are not as widely used as the Domard reaction. One such method utilizes sequence of formation of Schiff's base and reduction reported by *Muzzarelli et al (1985)* (Figure 2.18) (*Muzzarelli et al., 1985*). The trimethylation up to 60% of the amine groups could be accomplished by Schiff's base formation with formaldehyde, followed by reduction with sodium borohydride and quaternization in alkaline condition with methyl iodide. This two-step method likely prevents chain scission and deacetylation of remaining N-acetyl groups, and might result in TMC without O-methylation. With this, quaternization with different alkyl groups is also possible as in synthesis of N-diethylmethylchitosan (*Avadi et al., 2005*) N-N-propyl-N,N dimethyl chitosan and N-furfuryl-N,N-dimethyl chitosan (*Jia et al., 2001*), N-butyl N,N dimethyl chitosan (*Ignatova et al., 2007*) and N-phenyl or N-(substituted phenyl) N,N-dimethyl chitosan (*Guo et al., 2007*). In an attempt to synthesize O-methyl free TMC, *Verheul et al (2008)* synthesized dimethylated chitosan first and quaternized it (*Verheul et al., 2008*). The procedure was based on the method of *Muzzarelli et al 1985*, with modifications in solvent and reducing agent system as use of a formic acid-formaldehyde methylation and quaternization by  $\text{CH}_3\text{I}$  in NMP without assistance of catalyst.





**Figure 2.18: Alkylation of chitosan followed by quaternization** (Mourya et al., 2009)

### Physicochemical properties of trimethyl chitosan

TMC proved to be a derivative of chitosan with superior solubility and basicity, even at low degrees of quaternization, compared to chitosan and salts. The chitosan and salts are only soluble in acidic pH levels. Even at these low pH levels, it was difficult to prepare 1.5% (w/v) solutions due to the high viscosity of the solutions. TMC, even with a DQ as low as 10%, on the other hand is soluble either in acidic, basic or neutral medium (pH range 1–9 up to 10% w/v concentration). The highest solubility is reported with TMC of an intermediate DQ (40%) regardless of DD and molecular weight (Jintapattanakit et al., 2008). The increase in solubility was attributed to the replacement of the primary amino group on the C-2 position of chitosan with quaternary amino groups. The absolute molecular weights, radius and polydispersity of a range of TMC polymers with different degrees of quaternization (22.1, 36.3, 48.0 and 59.2%) were determined with size exclusion chromatography (SEC) and multi-angle laser light scattering (MALLS). The absolute molecular weight of the TMC polymers decreased with an increase in the DQ. The respective absolute molecular weights measured for each of the polymers were 2.02, 1.95, 1.66 and 1.43 g/mol. It should be noted that the molecular weight of the polymer chain increases during the reductive methylation process due to the addition of methyl groups to the amino group of the repeating monomers. However, a net decrease in the absolute molecular weight is observed due to degradation of the polymer chain caused by exposure to the reaction conditions such as the strong alkaline environment and elevated experimental temperatures during the synthesis (Snyman et al., 2002). The intrinsic viscosity, as an

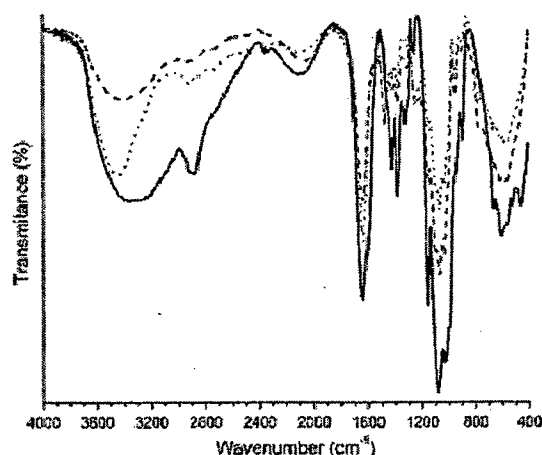
indication of molecular weight, also decreases with an increase in the DQ of the polymer. Like the native chitosan, TMC has the mucoadhesive properties (Cardile et al., 2008). The intrinsic mucoadhesivity of TMC was found to be lower than the chitosan salts, chitosan hydrochloride and glutamate, but if compared to the reference polymer, pectin, TMC possessed superior mucoadhesive properties (Snyman et al., 2003). The mucoadhesive properties of TMC with different DQ have been explored but the results are controversial. *Sandri et al (2005)* have reported the increase in mucoadhesive properties towards buccal mucosa with increase in DQ in the study of fluorescein isothiocyanate dextran (MW 4,400 Da) as a model drug (Sandri et al., 2005). On the other hand Synman et al., had found that the mucoadhesive properties of TMC decreased with an increase in DQ between 22.1 and 48.8% (Snyman et al., 2002). This may be due to the presence of fixed positive charges and their interaction with the negative sialic groups on the mucus protein structure. The decrease in mucoadhesion with an increase in the DQ may be explained by changes in the conformation of the respective TMC polymers due to interactions between the fixed positive charges on the C-2 position of each polymer. These interactions may force the polymer to change its conformation with a decrease in polymer-chain flexibility. Furthermore, steric effects caused by the attached methyl groups may also hide the positive charges on the amino groups. This decrease in flexibility and screening effect influences both the rate and amount of charge exchange between the negatively charged sialic groups of the mucus and the fixed positive charge of the TMC polymers and the interpenetration into the mucus layer with a subsequent lower mucoadhesivity.

#### **Characterization of trimethyl chitosan**

##### **FTIR**

The FTIR spectrum of TMC is obtained by dissolution of the TMC sample in deionized water and chitosan in aqueous acetic acid and casting in Petri dishes. The FTIR spectrum of TMC provides the evidence for the occurrence of methylation especially in the region 1,700–1,200  $\text{cm}^{-1}$  (Figure 2.19). The evidences are: (a) the band centered at 1,475  $\text{cm}^{-1}$  in the spectrum of TMC, which is attributed to the asymmetric angular deformation of C–H bonds of methyl groups, is absent in the spectrum of chitosan (de Britto et al., 2007) and (b) the band due to the angular deformation of N–H bond of amino groups occurs in both spectra, at 1,577  $\text{cm}^{-1}$  (1,500–1,620  $\text{cm}^{-1}$ ) for chitosan and at 1,559  $\text{cm}^{-1}$  for TMC, but it is weaker or

disappears due to the occurrence of N-methylation (Domard et al., 1986). A new peak appears at a high wave number 1,630–1,660  $\text{cm}^{-1}$  which are assigned to the quaternary ammonium salt (de Britto et al., 2007). There are peaks at about 1,415–1,430  $\text{cm}^{-1}$ , which are assigned to the characteristic absorption of N-CH<sub>3</sub>. Characteristic peaks of alcohol and second alcohol between 1,160 and 1,030  $\text{cm}^{-1}$ , if do not change, confirms the lack of the introduction of an alkyl group at C-3 and C-6 in the chitosan.



**Figure 2.19: FTIR spectra of the chitosan DD 96% (—), TMC DQ 52.5% (---) and TMC DQ 27% (.....) (Mourya et al., 2009)**

<sup>1</sup>H NMR (Sieval et al., 1998, de Britto et al., 2007)

<sup>1</sup>H NMR spectra were measured with a 300 or 600 MHz spectrometer by dissolving TMC samples in D<sub>2</sub>O at 80°C. The residual water in the NMR sample does not pose a difficulty since at 80°C this peak does not interfere with the spectrum of the polymer. The signals assigned include a peak at 3.4 ppm for quaternized amino group and a peak at 2.5 ppm for dimethyl amino group, as well as at 3.36–3.56 ppm for O-methylated group. (Apart from these, the signal of native monomers are evidenced as peaks at, 4.5–5.0 ppm for hydrogen bonded to the anomeric carbon 1; at 3.4–4.0 due to hydrogen bonded to the carbon atoms 3, 4, 5 and 6 of the glycopyranose unit; 3.18 attributed to the hydrogen atom bonded to the carbon 2 of the glycopyranose ring and ~2 corresponding to the hydrogen atoms of the methyl moieties of the acetamido groups). The NMR spectroscopy can be employed to determine the pattern of substitution as DQ, degree of dimethylation and degree of acetylation. The concept is illustrated as follows DQ%

$$\text{DQ}\% = \left[ \frac{[(\text{CH}_3)_2]}{[\text{H}]} \times \frac{1}{9} \right] \times 100$$

where DQ% is the DQ as a percentage,  $[(CH_3)_3]$  is the integral of the trimethyl amino group (quaternary amino) peak at 3.1–3.4 ppm, and  $[H]$  is the integral of the  $^1H$  peaks between 4.7 and 5.7 ppm on the  $^1H$ -NMR spectrum. The later corresponds to the hydrogen bonded to  $C_1$  of the glycoside ring. The degree of dimethylation can be calculated by equation:

$$DM\% = \left[ \frac{[(CH_3)_2]}{[H]} \times \frac{1}{6} \right] \times 100$$

where % DM is the degree of dimethylation as a percentage,  $[(CH_3)_2]$  is the integral of the dimethyl amino peak at 3.1–3.4 ppm. *Curti et al (2006)* reported a new method of calculating TMC's DQ based on the intensities of all methyl hydrogen signals on the  $^1H$  NMR spectrum that does not require the use of the  $C_1$  hydrogen signal as reference (*Curti et al., 2006*). It is important to note however, that in using this method, the intensity of the signal referent to dimethyl site is superestimated, once the signals of the  $C_2$  hydrogen in the glycoside ring and that of the dimethyl site are overlapped.

$$DQ\% = \left[ \frac{[(CH_3)_3]}{9} \times \frac{1}{S} \right] \times 100$$

$$S = \frac{[(CH_3)_3]}{9} + \frac{[(CH_3)_2]}{6} + \frac{[(NHCOCH_3)]}{3}$$

$[(NHCOCH_3)]$  is the intensity of the signal due to the hydrogen of the methyl groups of the acetamido moieties. With these parameters *Curti et al (2006)* also reported degree of acetylation of TMC with following expression (*Curti et al., 2006*).

$$DA\% = \left[ \frac{[(NHCOCH_3)]}{3} \times \frac{1}{S} \right] \times 100$$

$^1H$  NMR spectroscopy demands the use of a suitable deuterated solvent. Besides, concentrated chitosan solutions results in a very viscous solutions or are even insoluble for some derivatives, requiring measurements at 80°C in order to obtain narrow spectral line widths.

#### Solid-state CP-MAS $^{13}C$ NMR

The solid-state CP-MAS  $^{13}C$  NMR technique has mostly been applied to characterize chitosan and its derivatives; the characteristic signals can be identified for TMC. The

DQ can be calculated by following equation where,  $I_x$  is the intensity of the signal (de Britto et al., 2008).

$$\frac{I_{CH_3}}{(I_{C1} + I_{C2} + I_{C3} + I_{C4} + I_{C5} + I_{C6})/6}$$

<sup>13</sup>C NMR (Sieval et al., 1998)

<sup>13</sup>C -NMR spectra were measured in D<sub>2</sub>O at 80°C at 150 MHz. The signals assigned include the dimethylated signal at 43.7 ppm, and the trimethylated signal, at 55.1 ppm.

**Determination of degree of quaternization (DQ) by titration** (Curti et al., 2006)

The average DQ of the sample TMC was also determined from its titration with aqueous 0.1 M AgNO<sub>3</sub>. Thus, an aliquot of the aqueous solution of TMC (CP = 1.2 g/l) was transferred to a glass cell maintained at 25°C ± 0.1°C and the solution conductivity was measured upon the addition of aqueous AgNO<sub>3</sub>. The average DQ was then determined by using the expression:

$$DQ\% = \left[ \frac{M_{TMC} \times V \times [AgNO_3]}{m} \right] \times 100$$

where  $M_{TMC}$  is the molecular weight (g/mol) of the repeating unit of TMC containing the quaternized site,  $V$  (dm<sup>3</sup> or l) and  $[AgNO_3]$  (mol/dm<sup>3</sup> or mol/l) are the equivalent volume and concentration of AgNO<sub>3</sub> aqueous solution, respectively, and  $m$  (g) is the mass of TMC.

**DSC** (de Britto et al., 2004)

The DTG curves of chitosan and TMC samples with increasing DQ, respectively recorded in nitrogen atmosphere from room temperature to 500 and hydrophilicity of the polymers. The study of mass loss corresponding to the evaporation of water indicated direct dependence of water content on the presence and number of charges on the polymer chains. The second thermal event occurs in the temperature range 200–400°C for chitosan and 190–350°C for the TMC samples, and it is attributed to the thermal degradation of the polymers, including the deacetylation of chitosan and the decomposition of the substituted sites in the methylated derivatives. The complete thermal decomposition of the polymers, involving the depolymerization and pyrolytic processes, is attained at temperatures higher than 350°C and 400°C for the TMC samples and chitosan, respectively. This stage of the thermal decomposition begins at

lower temperatures for the TMC samples, showing that these derivatives are less thermally stable than the parent chitosan.

#### **Applications of trimethyl chitosan**

The nanoparticles as a nasal delivery system were prepared by ionic gelation technique with TMC and tripolyphosphate. The nanoparticles prepared had an average size of about 350 nm and a positive zeta-potential, a loading efficiency up to 95% and a loading capacity up to 50% (w/w) for model compound ovalbumin with preservation of its integrity. Release studies showed that more than 70% of the protein remained associated with the TMC nanoparticles for at least 3 h on incubation in phosphate buffer solution (pH 7.4) at 37°C. In vivo uptake studies indicated the transport of fluorescein isothiocyanatealbumin associated TMC nanoparticles across the nasal mucosa. Cytotoxicity tests with Calu-3 cells showed no toxic effects of the nanoparticles, whereas a partially reversible cilio-inhibiting effect on the ciliary beat frequency (CBF) of chicken trachea was observed (Amidi et al., 2006). The procedure of nanoparticle preparation was extended for monovalent influenza subunit H<sub>3</sub>N<sub>2</sub> antigen TMC nanoparticle.

The intranasal administered of antigen-TMC nanoparticles induced higher immune responses induced significant IgA levels in nasal washes of all mice as compared to other tested antigen formulations (Amidi et al., 2007). The protective immune responses in terms of bactericidal activity to meningococcal C conjugate vaccine was observed after intranasal immunization of mice with the LTK63 mutant plus chitosan or TMC as novel delivery platform (microparticles or powder suspensions) (Baudner et al., 2005). The analogous results were obtained for entrapment of tetanus toxoid in nanoparticles formulated with TMC by tripolyphosphate (ionic) gelation technique (loading efficacy [90%, particle size within the range of 40–400 nm], ex-vivo studies for cellular uptake of nanoparticles by J774A.1 cells and in vivo studies for adjuvant effect on nasal application (Sayın et al., 2008). The influence of DQ of TMC on level of immune induction was demonstrated with use of TMC DQ 40% and TMC DQ 60 the level of immune induction It has been demonstrated that responses as adjuvants for inducing immune responses relies upon DQ of TMC. In demonstration of such influence on the level of immune induction, TMC DQ 40% appeared to be the most potent adjuvant for intranasal administration with ovalbumin (Boonyo et al., 2007).

**Table 2.4: Application of trimethyl chitosan** (Mourya et al., 2009)

Use	Result
Nanoparticles	Self-assembled nanoparticles prepared by TMC and poly( $\gamma$ -glutamic acid) for oral delivery of insulin. Superior stability of nanoparticles in a broader pH range and sustained release profile of insulin with permeation enhancement observed
Micropartilces	Microparticles of TMC prepared by a supercritical fluid drying technique as a carrier for pulmonary delivery of insulin. Enhanced bioavailabilty of insulin observed without local adverse reactions after single administration of insulin powders
Micropartilces	Microparticles of TMC prepared by a supercritical fluid drying technique as a carrier for pulmonary delivery of diphtheria toxoid. The induction of immune response observed was equivalent to subcutaneous toxoid administration and superior for IgG2/IgG1 ratio and IgA level
Nanoparticles	Thymopentin-loaded TMC nanoparticles on oral administration show higher efficiency for ratio of lymphocyte CD4 <sup>+</sup> /CD8 than thymopentin alone
Nanocomplexes, nanoparticles	PEC and nanoparticles prepared with TMC and PEG-graft-TMC copolymer for peroral insulin delivery. Comparision of their properties suggest PEC as a potentially useful technique to achieve the objective
Absorption enhancer	Superporous hydrogel based systems developed for desmopressin with TMC which increased absorption of the drug across intestinal cells
Absorption enhancer	The combinations of TMC DQ 48 and 64%, dicarboxymethyl chitosan oligosaccharide, and chitosan lactate oligomer with monocaprin and melittin showed synergistic performance in terms of absorption across Caco-2, intestinal epithelial cells and TEER as compared to the individual absorption enhancers
Film for food application	Biocide against <i>Listeria monocytogenes</i> and <i>Salmonella typhimurium</i>



Chitosan and its derivatives are the subject of investigation since long. Despite of significant academic exploration of TMC, significant scope exists in creating IPR wealth in the area of applications of TMC. It has been used in designing gastroretentive systems due to its bioadhesive properties. An interesting application has been mentioned in using TMC as tissue permeability enhancer in fabricating implantable patch. More recently in a collaborative project of industry and academics, nanoparticles fabrication of peptide drugs using TMC is found to deliver the drug in trans-ocular and trans-nasal delivery of drug. Significant information on regulatory aspects of chitosan is still needed. Commercial exploitation of chitosan native faces significant barriers as difficulties in preparing uniformly reproducible charges in bulk quantities from various marine organisms and the high prices of the polymers. Trimethylation and quaternization by various methods further add up to cost and may forbid the wider usage of such derivatives. High cost of TMC and unavailability of vendors thus is still a constrain in developing applications.

**Table 2.5: US patents of quatrenized chitosan** (Mourya et al., 2009)

Patent	Description
US Patent 4,921,949	Process for making quaternary chitosan derivatives for cosmetic agents
US Patent 5,770,712	Crosslinked hydrogel beads from chitosan
US Patent 6,306,835	Biocidal chitosan derivatives
US Patent 7,381,716	Nanoparticles for protein drug delivery
US Patent 7,291,598	Nanoparticles for protein drug delivery
US Patent 7,282,194	Nanoparticles for protein drug delivery
US Patent 6,726,920	Implantable drug delivery patch
US Patent 6,410,046	Administering pharmaceuticals to the mammalian central nervous system
US Patent 6,328,967	Delivery system to modulate immune response
US Patent 6,207,197	Gastroretentive controlled release microspheres for improved drug delivery

## 2.6. Drug profile

### 2.6.1 Tizanidine HCl

**Chemical name:** 5-chloro-4-(2-imidazolin-2-ylamino)-2,1,3-benzothiadiazole hydrochloride.

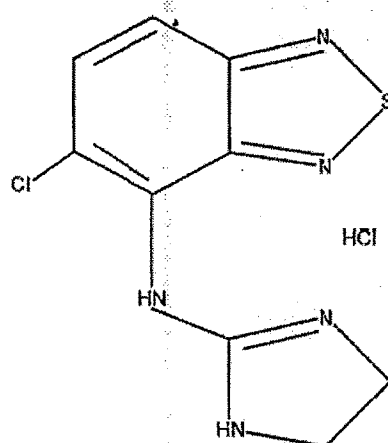
**Molecular formula:**  $C_9H_8ClN_5S \cdot HCl$

**Molecular weight:** 290.2

**Melting point:** 280 °C

**Solubility:** Tizanidine HCl is slightly soluble in water and methanol; solubility in water decreases as the pH increases.

**Description:** Tizanidine HCl hydrochloride is a centrally acting  $\alpha_2$ -adrenergic agonist. Tizanidine HCl is a white to off-white, fine crystalline powder, which is odorless or with a faint characteristic odor. Structural formula:



**Mechanism of Action:** Tizanidine HCl is an agonist at  $\alpha_2$ -adrenergic receptor sites and presumably reduces spasticity by increasing presynaptic inhibition of motor neurons. In animal models, Tizanidine HCl has no direct effect on skeletal muscle fibers or the neuromuscular junction, and no major effect on monosynaptic spinal reflexes. The effects of

Tizanidine HCl are greatest on polysynaptic pathways. The overall effect of these actions is thought to reduce facilitation of spinal motor neurons. The imidazoline chemical structure of Tizanidine HCl is related to that of the anti-hypertensive drug clonidine and other  $\alpha_2$ -adrenergic agonists. Pharmacological studies in animals show similarities between the two compounds, but Tizanidine HCl was found to have one-tenth to one-fiftieth (1/50) of the potency of clonidine in lowering blood pressure.

## **Pharmacokinetics**

### **Absorption and Distribution**

Following oral administration, Tizanidine HCl is essentially completely absorbed. The absolute oral bioavailability of Tizanidine HCl is approximately 40% (CV = 24%), due to extensive first-pass hepatic metabolism. Tizanidine HCl is extensively distributed throughout the body with a mean steady state volume of distribution of 2.4 L/kg (CV = 21%) following intravenous administration in healthy adult volunteers. Tizanidine HCl is approximately 30% bound to plasma proteins.

### **Pharmacokinetics, Metabolism and Excretion**

Tizanidine HCl has linear pharmacokinetics over a dose of 1 to 20 mg. Tizanidine HCl has a half-life of approximately 2.5 hours (CV=33%). Approximately 95% of an administered dose is metabolized. The primary cytochrome P450 isoenzyme involved in Tizanidine HCl metabolism is CYP1A2. Tizanidine HCl metabolites are not known to be active; their half-lives range from 20 to 40 hours. Following single and multiple oral dosing of <sup>14</sup>C-Tizanidine HCl, an average of 60% and 20% of total radioactivity was recovered in the urine and feces, respectively

### **Dosage and Administration:**

A single dose of 8 mg of Tizanidine HCl reduces muscle tone in patients with spasticity for a period of several hours. The effect peaks at approximately 1 to 2 hours and dissipates between 3 to 6 hours. Effects are dose-related. Although single doses of less than 8 mg have not been demonstrated to be effective in controlled clinical studies, the dose-related nature of Tizanidine HCl's common adverse events make it prudent to begin treatment with single oral doses of 4 mg. Increase the dose gradually (2 to 4 mg steps) to optimum effect (satisfactory reduction of muscle tone at a tolerated dose). The dose can be repeated at 6 to 8 hour intervals, as needed, to a maximum of three doses in 24 hours. The total daily dose should not exceed 36 mg. Experience with single doses exceeding 8 mg and daily doses exceeding 24 mg is limited. There is essentially no experience with repeated, single, daytime doses greater than 12 mg or total daily doses greater than 36 mg.

**Use:** Tizanidine HCl is a centrally acting skeletal muscle relaxant, used for the symptomatic treatment of painful muscle spasms and spasticity. Furthermore, Tizanidine HCl has analgesic properties and is used for the treatment of chronic headache, back pain and post operative pain.

## **Special Populations**

### **Age Effects**

No specific pharmacokinetic study was conducted to investigate age effects. Cross study comparison of pharmacokinetic data following single dose administration of 6 mg Tizanidine HCl showed that younger subjects cleared the drug four times faster than the elderly subjects. Tizanidine HCl has not been evaluated in children.

### **Hepatic Impairment**

The influence of hepatic impairment on the pharmacokinetics of Tizanidine HCl has not been evaluated. Because Tizanidine HCl is extensively metabolized in the liver, hepatic impairment would be expected to have significant effects on pharmacokinetics of Tizanidine HCl. Tizanidine HCl should ordinarily be avoided or used with extreme caution in this patient population.

### **Renal Impairment**

Tizanidine HCl clearance is reduced by more than 50% in elderly patients with renal insufficiency (creatinine clearance < 25 mL/min) compared to healthy elderly subjects; this would be expected to lead to a longer duration of clinical effect. Tizanidine HCl should be used with caution in renally impaired patients

### **Gender Effects**

No specific pharmacokinetic study was conducted to investigate gender effects. Retrospective analysis of pharmacokinetic data, however, following single and multiple dose administration of 4 mg Tizanidine HCl showed that gender had no effect on the pharmacokinetics of Tizanidine HCl.

### **Drug Interaction:**

Tell your doctor of all prescription and non-prescription medications you may take, especially of: blood pressure drugs (e.g., water pills, clonidine, methyldopa, guanabenz, guanfacine), MAO inhibitors (e.g., linezolid, furazolidone, phenelzine, selegiline), sleep medicines, sedatives (e.g., diazepam), tranquilizers, anti-anxiety drugs, narcotic pain relievers (e.g., codeine, morphine), barbiturates (e.g., phenobarbital), certain antihistamines (e.g., diphenhydramine), other muscle relaxants (e.g., Cyclobenzaprine HCl), certain antidepressants (e.g., imipramine, amitriptyline, trazodone), phenothiazine psychiatric drugs (e.g., chlorpromazine), birth control pills, rofecoxib.

**Acetaminophen:** Tizanidine HCl delayed the T<sub>max</sub> of acetaminophen by 16 minutes. Acetaminophen did not affect the pharmacokinetics of Tizanidine HCl.

**Alcohol:** Alcohol increased the AUC of Tizanidine HCl by approximately 20% while also increasing its C<sub>max</sub> by approximately 15%. This was associated with an increase in side effects of Tizanidine HCl. The CNS depressant effects of Tizanidine HCl and alcohol are additive.

**Fluvoxamine:** Clinically significant hypotension (decreases in both systolic and diastolic pressure) has been reported with concomitant administration of fluvoxamine following single doses of 4 mg. Caution is recommended when considering concomitant use of Tizanidine HCl with other inhibitors of CYP1A2, such as, antiarrhythmics (amiodarone, mexiletine, propafenone), cimetidine, fluoroquinolones (ciprofloxacin, norfloxacin), rofecoxib, oral contraceptives, and ticlopidine.

**Oral Contraceptives:** No specific pharmacokinetic study was conducted to investigate interaction between oral contraceptives and Tizanidine HCl, but retrospective analysis of population pharmacokinetic data following single and multiple dose administration of 4 mg Tizanidine HCl showed that women concurrently taking oral contraceptives had 50% lower clearance of Tizanidine HCl than women not on oral contraceptives.

**Side Effects:** Constipation, dry mouth, drowsiness, dizziness, weakness and fatigue may occur. If any of these effects persist or worsen, contact your doctor or pharmacist promptly. To relieve dry mouth, suck on (sugarless) hard candy or ice chips, chew (sugarless) gum, drink water or use a saliva substitute. Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects.

Tizanidine HCl rarely has caused very serious (possibly fatal) liver disease. Tell your doctor immediately if you develop symptoms of liver disease, including persistent nausea, stomach/abdominal pain, dark urine, yellowing eyes/skin. A serious allergic reaction to this drug is unlikely, but seeks immediate medical attention if it occurs. Symptoms of a serious allergic reaction include: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

**Packaging and storages:** preserve in tight container and store at room temperature.

### **2.6.2 Cyclobenzaprine HCl**

**Chemical name:** 3-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine hydrochloride

**Molecular formula:** C<sub>20</sub>H<sub>21</sub>N•HCl

**Molecular weight:** 311.9

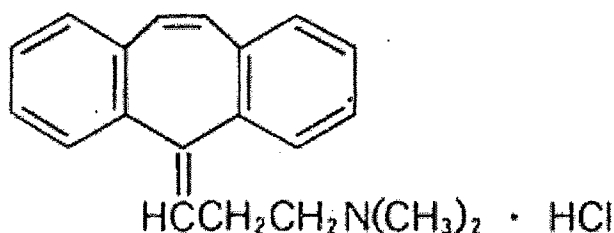
**pKa :** 8.47 at 25°C

**Melting point:** 217°C

**Solubility:** It is freely soluble in water and alcohol, sparingly soluble in isopropanol, and insoluble in hydrocarbon solvents. If aqueous solutions are made alkaline, the free base separates

**Description:** Cyclobenzaprine HCl hydrochloride is a white, crystalline tricyclic amine salt.

**Structural formula:**



**Mechanism of Action:** Cyclobenzaprine HCl relieves skeletal muscle spasm of local origin without interfering with muscle function. It is ineffective in muscle spasm due to central nervous system disease. Cyclobenzaprine HCl reduced or abolished skeletal muscle hyperactivity in several animal models. Animal studies indicate that Cyclobenzaprine HCl does not act at the neuromuscular junction or directly on skeletal muscle. Such studies show that Cyclobenzaprine HCl acts primarily within the central nervous system at brain stem as opposed to spinal cord levels, although its action on the latter may contribute to its overall skeletal muscle relaxant activity. Evidence suggests that the net effect of Cyclobenzaprine HCl is a reduction of tonic somatic motor activity, influencing both gamma ( $\gamma$ ) and alpha ( $\alpha$ ) motor systems. Pharmacological studies in animals showed a similarity between the effects of Cyclobenzaprine HCl and the structurally related tricyclic antidepressants, including reserpine antagonism, norepinephrine potentiation, potent peripheral and central anticholinergic effects, and sedation. Cyclobenzaprine HCl caused slight to moderate increase in heart rate in animals.

**Pharmacokinetics:** Estimates of mean oral bioavailability of Cyclobenzaprine HCl range from 33% to 55%. Cyclobenzaprine HCl exhibits linear pharmacokinetics over the dose range 2.5 mg to 10 mg, and is subject to enterohepatic circulation. It is highly bound to plasma proteins. Drug accumulates when dosed three times a day, reaching steady-state within 3-4 days at plasma concentrations about four-fold higher than after

a single dose. At steady state in healthy subjects receiving 10 mg t.i.d. (n=18), peak plasma concentration was 25.9 ng/mL (range, 12.8-46.1 ng/mL), and area under the concentration-time (AUC) curve over an 8-hour dosing interval was 177 ng.hr/mL (range, 80-319 ng.hr/mL). Cyclobenzaprine HCl is extensively metabolized, and is excreted primarily as glucuronides via the kidney. Cytochromes P-450 3A<sub>4</sub>, 1A<sub>2</sub>, and, to a lesser extent, 2D<sub>6</sub>, mediate N-demethylation, one of the oxidative pathways for Cyclobenzaprine HCl. Cyclobenzaprine HCl is eliminated quite slowly, with an effective half-life of 18 hours (range 8-37 hours; n=18); plasma clearance is 0.7 L/min. The plasma concentration of Cyclobenzaprine HCl is generally higher in the elderly and in patients with hepatic impairment.

**Dosage and Administration:** For most patients, the recommended dose of Cyclobenzaprine HCl is 5 mg three times a day. Based on individual patient response, the dose may be increased to 10 mg three times a day. Use of Cyclobenzaprine HCl for periods longer than two or three weeks is not recommended. Less frequent dosing should be considered for hepatically impaired or elderly patients.

**Use:** Cyclobenzaprine HCl is used together with rest and physical therapy to treat skeletal muscle conditions such as pain or injury. It is not useful for spasticity due to neurologic conditions such as cerebral palsy. Although centrally acting muscle relaxants like Cyclobenzaprine HCl are also helpful in aborting a migraine headache.

**Precautions:**

***General***

Because of its atropine-like action, Cyclobenzaprine HCl should be used with caution in patients with a history of urinary retention, angle-closure glaucoma, increased intraocular pressure, and in patients taking anticholinergic medication.

***Impaired Hepatic Function***

The plasma concentration of Cyclobenzaprine HCl is increased in patients with hepatic impairment. These patients are generally more susceptible to drugs with potentially sedating effects, including Cyclobenzaprine HCl. Cyclobenzaprine HCl should be used with caution in subjects with mild hepatic impairment starting with a 5 mg dose and titrating slowly upward. Due to the lack of data in subjects with more severe hepatic insufficiency, the use of Cyclobenzaprine HCl in subjects with moderate to severe impairment is not recommended.



***Carcinogenesis, Mutagenesis, Impairment of Fertility***

In rats treated with Cyclobenzaprine HCl for up to 67 weeks at doses of approximately 5 to 40 times the maximum recommended human dose, pale, sometimes enlarged, livers were noted and there was a dose-related hepatocyte vacuolation with lipidosis. In the higher dose groups this microscopic change was seen after 26 weeks and even earlier in rats which died prior to 26 weeks; at lower doses, the change was not seen until after 26 weeks. Cyclobenzaprine HCl did not affect the onset, incidence or distribution of neoplasia in an 81-week study in the mouse or in a 105-week study in the rat.

At oral doses of up to 10 times the human dose, Cyclobenzaprine HCl did not adversely affect the reproductive performance or fertility of male or female rats. Cyclobenzaprine HCl did not demonstrate mutagenic activity in the male mouse at dose levels of up to 20 times the human dose.

***Pregnancy***

*Pregnancy Category B:* Reproduction studies have been performed in rats, mice and rabbits at doses up to 20 times the human dose, and have revealed no evidence of impaired fertility or harm to the fetus due to Cyclobenzaprine HCl. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

***Nursing Mothers***

It is not known whether this drug is excreted in human milk. Because Cyclobenzaprine HCl is closely related to the tricyclic antidepressants, some of which are known to be excreted in human milk, caution should be exercised when Cyclobenzaprine HCl is administered to a nursing woman.

***Pediatric Use***

Safety and effectiveness of Cyclobenzaprine HCl in pediatric patients below 15 years of age have not been established.

**Drug Interaction:** Cyclobenzaprine HCl may have life-threatening interactions with MAO inhibitors. Cyclobenzaprine HCl may enhance the effects of alcohol, barbiturates, and other CNS depressants. Tricyclic antidepressants may block the antihypertensive action of guanethidine and similarly acting compounds. Tricyclic antidepressants may enhance the seizure risk in patients taking tramadol.

**Side Effects:** Abdominal pain, acid regurgitation, constipation, diarrhea, dizziness, nausea, irritability, mental acuity decreased, nervousness, upper respiratory infection, and pharyngitis, fatigue/tiredness, asthenia, nausea, constipation, dyspepsia, unpleasant taste, blurred vision, headache, nervousness, and confusion.

**Packaging and storages:** preserve in tight container and Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

## 2.7. References

Alexi, T., Venero, J.L., Hefti, F. (1997). Protective effects of neurotrophin-4/5 and transforming growth factor-alpha on striatal neuronal phenotypic degeneration after excitotoxic lesioning with quinolinic acid. *Neuroscience*, 78(1), 73-86.

Allemann, E., R. Gurny., Doelker. E.(1993). Drug-loaded nanoparticles-preparation methods and drug targeting issues. *Eur. J. Pharm. Biopharm.*, 39(5), 173-191.

Amery, W.K. (1985). Migraine and cerebral hypoxia: a hypothesis with pharmacotherapeutic implications. *Cephalalgia*, 5(2), 131-3.

Amidi, M., Romeijn, S.G., Borchard, L., Junginger, H.E., Hennink, W.E., Jiskoot, W. (2006). Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. *J. Control. Release* 111(1-2), 107-116.

Amidi, M., Romeijn, S.G., Coos Verhoef, J., Junginger, H.E., Bungener, L., Huckriede, A., Crommelin, D.J.A., Jiskoot, W. (2007). N-trimethyl chitosan (TMC) nanoparticles loaded with influenza subunit antigen for intranasal vaccination: biological properties and immunogenicity in a mouse model. *Vaccine*, 25(1), 144-153.

Andreas, B.S. (2005). Thiomers: A new generation of mucoadhesive polymers. *Advanced Drug Delivery Reviews*, 57(11), 1569– 1582.

Andreas, B.S., Brandt, U.M., Clausen, A.E. (1999). Synthesis and in vitro evaluation of chitosan-cysteine conjugates. *Sci. Pharm.*, 67, 96–208.

Andreas, B.S., Hopf, T.E. (2001). Synthesis and in vitro evaluation of chitosan-thioglycolic acid conjugates. *Sci. Pharm.*, 69, 109–118.

Andreas, B.S., Hornof, M., Zoidl, T. (2003). Thiolated polymers–thiomers: modification of chitosan with 2-iminothiolane. *Int. J. Pharm.*, 260(2), 229–237.

Andreas, B.S., Krajicek, M.E. (1998). Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan-EDTA conjugates. *J. Control. Rel.*, 50(1-3), 215–223.

Ashburn, M.A., Love, G., Pace, N.L. (1994). Respiratory-related critical events with intravenous patient-controlled analgesia. *Clin J Pain*, 10(1), 52-56.

Asoh, S., Ohsawa, I., Mori, T., Katsura, K., Hiraide, T., Katayama, Y. (2002). Protection against ischemic brain injury by protein therapeutics. *Proc Natl Acad Sci USA*, 99 (26), 17107-17112.

Aspden, T., Illum, L., Skaugrud, φ. (1996). Chitosan as a nasal delivery system: Evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. *Eur. J. Pharm. Sci.*, 4(1), 23-31.

Aspden, T., Mason, J.D.T., Jones, N.S., Lowe, J., Skaugrud, φ., Illum, L. (1997). Chitosan as a nasal delivery system: The effect of chitosan solutions on in vitro and in vivo muco-ciliary transport rates in human turbinates and volunteers. *J. Pharm. Sci.*, 86(4), 509-513.

Avadi, M.R., Jalali, A., Sadeghi, A.M.M., Shamimi, K., Bayati, K.H., Nahid, E., Dehpour, A.R., Rafiee-Tehrani, M. (2005). Diethyl methyl chitosan as an intestinal paracellular enhancer: ex vivo and in vivo studies. *Int. J. Pharm.* 293(1-2), 83-89.

Banks, W.A. (2001). Oligonucleotides targeting prion diseases. *JPharmacol Exp Ther.*, 297(3), 1113 -1121.

Baudner, B.C., Verhoef, J.C., Giuliani, M.M., Peppoloni, S., Rappuoli, R., Delgiudice, G., Junginger, H.E. (2005). Protective immune responses to meningococcal C conjugate vaccine after intranasal immunization of mice with the LTK63 mutant plus chitosan or trimethyl chitosan chloride as novel delivery platform. *J. Drug. Target.*, 13(8-9), 489-498.

Baumann, H., Faust, V. (2001). Concepts for improved regioselective placement of O-sulfo, N-sulfo, Nacetyl, and N-carboxymethyl groups in chitosan derivatives. *Carbohydr. Res.*, 331(1), 43-57.

Bodmeier, R., Chen, H.G., Paeratakul, O. (1989). A novel approach to the oral delivery of micro- or nanoparticles. *Pharm. Res.*, 6(5), 413-417.

Bodor, N. (1992). A strategy for delivering peptides into the central nervous system by sequential metabolism. *Science*, 257, 1698-700.

Bonica, J. J. (1979). "The need of taxonomy". *Pain*, 6 (3), 247-252.

Boonyo, W., Junginger, H.E., Waranuch, N., Polnok, A., Pitaksuteepong, T. (2007). Chitosan and trimethyl chitosan chloride (TMC) as adjuvants for inducing immune responses to ovalbumin in mice following nasal administration. *J. Control. Release*, 121(3), 168-175.

Borchard, G., Lueßen, H.L., de Boer, A.G., Verhoef, J.C., Lehr, C.M., Junginger, H.E. (1996). The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption: III. Effects of chitosan glutamate and carbomer on epithelial tight junctions in vitro. *J. Control. Release*, 39(2-3), 131- 138.

BornLange, J.T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L. (2002). Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci.*, 5(6), 514-516.

- Brownlees, J., Williams, C.H. (1993). Peptidases peptides and the mammalian blood-brain barrier. *J Neurochem.*, 60 (2), 793-803.
- Cai, K., Yao, K., Cui, Y., Lin, S., Yang, Z., Li, X., Xie, H., Qing, T., Luo, J. (2002). Surface modification of poly (D,L-lactic acid) with chitosan and its effects on the culture of osteoblasts in vitro. *J. Biomed. Mater. Res.*, 60 (3), 398-404.
- Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L., Alonso. M.J. (1997a). Chitosan and chitosan/ethylene oxide propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharm. Res.*, 14(10), 1431-1436.
- Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L., Alonso. M.J. (1997b). Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carrier. *J. Appl. Poly. Sci.*, 63(1), 125-132.
- Calvo, P., Vila-Jato, J.L., Alonso. M.J. (1997c). Evaluation of cationic polymer-coated nanocapsules as ocular drug carriers. *Int. J. Pharm.*, 153(1), 41-50.
- Capobianco, D.J., Cheshire, W.P., Campbell, J.K. (1996). An overview of the diagnosis and harmacologic treatment of migraine. *Mayo Clinic Proceedings*, 71(11), 1055-1066.
- Cardile, V., Frasca, G., Rizza, L., Bonina, F., Puglia, C., Barge, A., Chiambretti, N., Cravotto, G. (2008). Improved adhesion to mucosal cells of water-soluble chitosan tetraalkylammonium salts. *Int. J. Pharm.*, 362(1-2), 88-91.
- Carreño-Gómez, B., Duncan, R. (1997). Evaluation of the biological properties of soluble chitosan and chitosan microspheres. *Int. J. Pharm.*, 148 (2), 231-240.
- Chien, Y.W. (1985). *Transnasal systemic medication*, Elsevier, Amsterdam, (Y.W. Chien Ed.), 42-46.
- Clausen, A.E., Kast, C.E., Andreas, BS.(2002). The role of glutathione in the permeation enhancing effect of thiolated polymers. *Pharm. Res.*, 19(5), 602-608.
- Cohen, K.A., Liu, T., Bissonette, R., Puri, R.K., Frankel, A.E. (2003). DAB389EGF fusion protein therapy of refractory Glioblastoma Multiforme. *Curr Pharm Biotechnol*, 4(1), 39-49.
- Corbo, D.C., Huang Y.C., Chein, Y.W. (1998). Nasal delivery of progestational steroids in ovariectomized rabbits. I. Progesteron comparison of pharmacokinetic with intravenous and oral administration. *Int. J. Pharm.*, 46 (1-2), 133-139.
- Curti, E., Campana-Filho, S.P. (2006). Viscosity Behavior of Chitosan and N,N,N-Trimethylchitosan Chloride Salts in Acid-Free Aqueous Solution. *J. Macromol. Sci. A: Pure Appl. Chem*, 43(3), 555-572.
- de Britto, D., Campana-Filho, S.P. ( 2004). A kinetic study on the thermal degradation of N,N,N-trimethylchitosan. *Polym. Degrad. Stab.*, 84(2), 353-361.

de Britto, D., Assis, O.B.G. (2007). A novel method for obtaining a quaternary salt of chitosan Carbohydr. Polym. 69(2), 305-310.

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

Domard, A., Rinaudo, M., Terrassin, C. (1986). New method for the quaternization of chitosan. Int. J. Macromol., 8(2), 105-107.

Dung, P., Milas, M., Rinando, M., Desbrieres, J. (1994). Water soluble derivatives obtained by controlled chemical modification of chitosans. Carbohydrate Polymers, 24(3), 209-214.

El-Shabouri, M. H.(2002). Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. Int. J. Pharm., 249 (1-2), 101-108.

Erbacher, P., Zou, S., Steffan, A. M., Remy, J. S. (1998). Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability. Pharm. Res., 15(9), 1332-1339.

Fisher, N., Brown, K., Davis, S. S., Parr, G. D., Smith, D.A. (1987). The effect of molecular size on the nasal absorption of water soluble compounds in the albino rat. J. Pharm. Pharmacol, 39(5), 357-362.

Gattefosse bulletin. (1997). New lipidic systems enhancing the bioavailability of problem drugs, 1-81.

Grafstrom, R., Stead, A.H., Orrenius, S. (1980). Metabolism of extracellular glutathione in rat small-intestinal mucosa. Eur. J. Biochem., 106(2), 571-577.

Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., Li, P. (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan Carbohydr. Res., 342 (10), 1329-1332.

Hamman, J.H., Kotze, A.F. (2001). Effect of the type of base and number of reaction steps on the degree of quaternization and molecular weight of N-trimethyl chitosan chloride. Drug Development and Industrial Pharmacy, 27(5), 373-380.

Hornof, M.D., Kast, C.E., Andreas, B.S. (2003). In vitro evaluation of the viscoelastic behavior of chitosan -thioglycolic acid conjugates. Eur. J. Pharm. Biopharm., 55(2), 185-190.

Huang, C., Kimura, R., Nassar, A., Hussain, A. (1985). Mechanism of nasal drug absorption of drug II: absorption of L-tyrosine and the effect of structural modification on it's absorption. J Pharm Sci., 74 (12), 298-1301.

Huang, M., Ma, Z., Khor, E., Lim, L. (1999). Uptake of FITC-chitosan nanoparticles by A549 cells, Pharm. Res., 19(10), 1488-1494.

- Ignatova, M., Manolova, N., Rashkov, I.(2007). Novel antibacterial fibers of quaternized chitosan and poly(vinyl pyrrolidone) prepared by electrospinning. *Eur. Polym. J.*, 43(4), 1112-1122.
- Illum, L. (1998). Chitosan and its use as a pharmaceutical excipient. *Pharm. Res.*, 15(9), 1326–1331.
- Illum, L. (2002). Nasal drug delivery: new developments and strategies. *DDT*, 7(23), 1184-1189.
- Illum, L. (2003). Nasal drug delivery – possibilities, problems and solutions. *J Control Release*, 87(1-3), 187-98.
- Janes, K. A., Fresneau, M. P., Marazuela, A., Fabra, A., Alonso, M. J. ( 2001). Chitosan nanoparticles as delivery systems for doxorubicin. *J. Control. Release*, 73(2-3), 255-267.
- Jia, Z., Shen, D., Xu, W. (2001). Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydr. Res.*, 333(1), 1-6.
- Jintapattanakit, A., Mao, S., Kissel, T., Junyaprasert, V.B. (2008). Physicochemical properties and biocompatibility of N-trimethyl chitosan: effect of quaternization and dimethylation. *Eur. J. Pharm. Biopharm.*, 70(2), 563-571.
- Kast, C.E., Andreas, B.S. (2001). Thiolated polymers –thiomers: development and in vitro evaluation of chitosan-thioglycolic acid conjugates. *Biomaterials*, 22(17), 2345–2352.
- Kast, C.E., Frick, W., Losert, U., Andreas, B.S. (2003). Chitosan thioglycolic acid conjugate: a new scaffold material for tissue engineering?. *Int. J.Pharm.*, 256(1-2), 183–189.
- Kast, C.E., Valenta, C., Leopold, M., Andreas, B.S. (2002). Design and in vitro evaluation of a novel bioadhesive vaginal drug delivery system for clotrimazole. *J. Control. Rel.*, 81(3), 347–354.
- Kotze, A.F., Luessen, H.L., de Leeuw, B.J., de Boer, A.G., Verhoef, J.C., Junginger, H.E. (1997). N-Trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: in vitro evaluation in intestinal epithelial cells (Caco-2). *Pharm Res.*, 14(9), 1197–1202.
- Kotzé, A.F., Luessen, H.L., Thanou, M., Verhoef, J.C., de Boer, A.G., Junginger, H.E., Lehr, C.M. (1999). Chitosan and chitosan derivatives as absorption enhancers for peptide drugs. In: *Bioadhesive. Drug Delivery Systems*, edited by Mathiowitz, E., Chickering III, D.E., Lehr C.M., Marcel Dekker Inc, New York, 341–385.
- Kotzé, A.F., Luessen, H.L., Thanou, M., Verhoef, J.C., de Boer, A.G., Junginger, H.E. (1999). Chitosan for enhanced intestinal permeability: prospects for derivatives soluble in neutral and basic environments. *Eur. J. Pharm. Sci.*, 7 (2), 145-151.

Kotzé, A.F., Luessen, H.L., Thanou, M., Verhoef, J.C., de Boer, A.G., Junginger, H.E. (1999). Chitosan for enhanced intestinal permeability: prospects for derivatives soluble in neutral and basic environments. *Eur. J. Pharm. Sci.*, 7 (2), 145-151.

Kumar, V., Banker, G.S. (1996). Target-oriented drug delivery systems. In: *Modern Pharmaceutics*, edited by Banker, G. S. and Rhodes, C.T., Marcel Dekker, New York, 611.

Langoth, N., Guggi, D., Pinter, Y., Andreas, B.S. (2004). Thiolated Chitosan: In Vitro Evaluation of its Permeation Enhancing Properties. *J. Control. Rel.*, 94(1), 177-86.

Lanteri-Minet, M., Desnuelle, C. (1996). Migraine and mitochondrial dysfunction. *Revue Neurologique*, 152(4), 234-8.

Lee, K.Y., Ha, W.S., Park, W.H. (1995). Blood compatibility and biodegradability of partially N-acylated chitosan derivatives. *Biomaterials*, 16(16), 1211–1216.

Lee, W.A., Narog, B.A., Patapoff T. W., Wang, Y.J. (1991). Intranasal bioavailability of insulin powder formulation: Effect of permeation enhancer to protein ratio. *J. Pharm. Sci.*, 80 (8), 725-729.

Leitner, V.M., Marschutz, M.K., Andreas, B.S. (2003). Mucoadhesive and cohesive properties of poly (acrylic acid)-cysteine conjugates with regard to their molecular mass. *Eur. J. Pharm. Sci.*, 18(1), 89–96.

Lubben, I.M.V., Verhoef, J.C., Aelst, A.C.V., Borchard, G., Junginger, H.E. (2001). Chitosan microparticles for oral vaccination: preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. *Biomaterials*, 22(7), 687–694.

Luessen, H.L., Leeuw, B.J.D., Langemeyer, M.W., Boer, A.G.D., Verhoef, J.C., Junginger, H.E. (1996). Mucoadhesive polymers in peroral peptide drug delivery. VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. *Pharm. Res.*, 13(11), 1668–1672.

Ma, P.X., Choi, J.W. (2001). Biodegradable polymer scaffolds with well defined interconnected spherical pore network. *Tissue Eng.*, 7(1), 23–33.

Maitra, A. N., Ghosh, P. K., De, T. K., Sahoo, S. K. (1999). Process for the preparation of highly monodispersed hydrophilic polymeric nanoparticles of size less than 100 nm. US patent, 5, 874,111.

Mauskop, A., Altura, B.M. (1998). Role of magnesium in the pathogenesis and treatment of migraines. *Clinical Neuroscience*, 5(1), 24-7.

McMartin, C., Hutchinson, L.E.F., Hyde, R., Peters, G.E. (1987). Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J Pharm Sci.*, 76(7), 535-540



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

Mourya, V.K., Inamdar, N. N. (2009). Trimethyl chitosan and its applications in drug delivery. *J Mater Sci: Mater Med.*, 20(5), 1057–1079.

Muzzarelli, R.A.A., Tanfani, F. (1985). The N-permethylation of chitosan and the preparation of N-trimethyl chitosan iodide *Carbohydr. Polym.* 5(4), 297-307.

Muzzarelli, R.A.A., Tanfani, F., Emanuelli, M. (1982). N- (carboxymethylidene) chitosans and N-(carboxymethyl) chitosans: novel chelating polyampholytes obtained from chitosan glyoxylate. *Carbohydr. Res.*, 107 (2), 199-214.

Niwa, T., Takeuchi, H., Hino, T., Kunou, N., Kawashima, Y. (1993). Preparation of biodegradable nanospheres of water-soluble and insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *J. Control. Release.* 25(1-2), 89-98.

Ohya, Y., Cai, R., Nishizawa, H., Hara, K., Ouchi, T. (2000). Preparation of PEG-grafted chitosan nanoparticles as peptide drug carriers. *S.T.P. Pharma. Science*, 10 (1), 77-82.

Pan, Y., Li, Y., Zhao, H., Zheng, J., Xu, H., Wei, G., Hao, J., Cui, F. (2002). Bioadhesive polysaccharide in protein delivery system: chitosan nanoparticles improve the intestinal absorption of insulin in vivo. *Int. J. Pharm.*, 249(1-2), 139-147.

Pennington, A.K., Ratcliffe, J.H., Wilson, C.G., Hardy, J.G. (1988). The influence of solution viscosity on nasal spray deposition and clearance. *Int J Pharm.*, 43(3), 221-224.

Polnok, A., Borchard, G., Verhoef, J.C., Sarisuta, N., Junginger, H.E. (2004). Influence of methylation process on the degree of quaternization of N-trimethyl chitosan chloride. *Eur. J. Pharm. Biopharm.* 57 (1), 77-83.

Prokai, K., Tatrai, P.L., Bodor, N. (1996). Brain-targeted delivery of a leucine-enkephalin analogue by retrometabolic design. *J Med chem.*, 39 (24), 4775-4782.

Roldo, M., Hornof, M., Caliceti, P., Andreas, B.S. (2004). Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation. *Eur. J. Pharm. Biopharm.*, 57(1), 115-21.

Runarsson, O.V., Holappa, J., Jonsdottir, S., Steinsson, H., Masson, M., (2008). tert-Butyldimethylsilyl O-protected chitosan and chitooligosaccharides: useful precursors for N-modifications in common organic solvents. *Carbohydr. Polym.*, 74(15), 2576-2582.

Runarsson, O.V., Holappa, J., Nevalainen, T., Hjalmarsson, M., Jarvinen, T., Loftsson, T., Einarsson, J.M., Jonsdottir, S., Valdimarsson, M., Masson, M. (2007).

Antibacterial activity of methylated chitosan and chitooligomer derivatives: Synthesis and structure activity relationships. *Eur. Polym. J.*, 43(6), 2660-2671.

Saito, H., Wu, X., Harris, J., Hoffman, A. (1997). Graft copolymers of poly (ethylene glycol)(PEG) and chitosan. *Macromol. Rapid Commun.*, 18 (7), 547-550.

Sandri, G., Rossi, S., Bonferoni, M.C., Ferrari, F., Zambito, Y., Di Colo, G., Caramella, C. (2005). Buccal penetration enhancement properties of N-trimethyl chitosan: Influence of quaternization degree on absorption of a high molecular weight molecule. *Int J Pharm.*, 297 (1-2).146-55.

Sayin, B., Somavarapu, S., Li, X.W., Thanou, M., Sesardic, D., Alpar, H.O., Senel, S. (2008). Mono-N-carboxymethyl chitosan (MCC) and N-trimethyl chitosan (TMC) nanoparticles for non-invasive vaccine delivery. *Int. J. Pharm.* 363(1-3), 139-148.

Schipper, N.G. M., Olsson, S., Hoogstraate, J. A., deBoer, A.G., Vårum Per Artursson, K.M. (1997). Chitosans as absorption enhancers for poorly absorbable drugs 2: Mechanism of absorption enhancement. *Pharm. Res.*, 14(7), 923-929.

Schipper, N.G.M., Olsson, S., Hoogstraate, J.A., Boer, A.G.D., Varum, K.M., Artursson, P. (1997). Chitosans as absorption enhancers for poorly absorbable drugs. 2: Mechanism of absorption enhancement. *Pharm. Res.*, 14(7), 923-929.

Schipper, N.G.M., Varum, K.M., Stenberg, P., Ocklind, G., Lennernas, H., Artursson, P. (1999). Chitosans as absorption enhancers for poorly absorbable drugs. 3: Influence of mucus on absorption enhancement. *Eur. J. Pharm. Sci.*, 8(4), 335-343.

Sgouras, D., Duncan, R. (1990). Methods for the evaluation of biocompatibility of soluble synthetic polymers which have potential for biomedical use. 1 Use of the tretazolium-based colorimetric assay (MTT) as a preliminary screen for evaluation of in vitro cytotoxicity. *J. Mater. Sci. Mater. Med.*, 1, 61-68.

Sieval, A.B., Thanou, M., Kotze, A.F., Verhoef, J.C., Brussee, J., Junginger, H.E. (1998). Preparation and NMR characterization of highly substituted N-trimethyl chitosan chloride. *Carbohydrate Polymers*, 36(2), 157-165.

Sieval, A.B., Thanou, M., Kotze, A.F., Verhoef, J.C., Brussee, J., Junginger, H.E. (1998). Preparation and NMR characterization of highly substituted N-trimethyl chitosan chloride. *Carbohydrate Polymers*, 36(2-3), 157-165.

Singla, A.K., Chawla, M. (2001). Chitosan: some pharmaceutical and biological aspects-an update. *J. Pharm. Pharmacol.*, 53 (8), 1047-1067.

Agnihotri, S.A., Mallikarjuna, N.N., Aminabhavi, T.M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Controlled Release*. 100 5-28.

Smith, E.L., Hill, R.L., Borman, A. (1958). Activity of insulin degraded by aminopeptidase. *Biophys Acta*, 29(1), 207-208.

Smith, J., Wood, E., Dornish, M. (2004). Effect of chitosan on epithelial cell tight junctions. *Pharm. Res.*, 21(1), 43-49.

Snyder, E.Y., Senut, M.C. (1997). The use of non neuronal cells for gene delivery. *Neurobiol Dis.*, 4(2), 69-102.

Snyman, D., Hamman, J.H., Kotze, A.F. (2003). Evaluation of the mucoadhesive properties of N-trimethyl chitosan chloride. *Drug Dev. Ind. Pharm.*, 29(1), 61-69.

Snyman, D., Hamman, J.H., Kotze, J.S., Rollings, J.E., Kotze, A.F. (2002). The relationship between the absolute molecular weight and the degree of quaternisation of N-trimethyl chitosan chloride. *Carbohydrate Polymers*, 50(2), 145-150.

Soane, R.J., Frier, M., Perkins, A.C., Jones, N.S., Davis, S.S., Illum, L. (1999). Evaluation of the clearance characteristics of bioadhesive systems in humans. *Int. J. Pharm.*, 178(1), 55-65.

Spierings, E.L. (1995). Symptomatology and pathogenesis of migraine. *Journal of Pediatric Gastroenterology & Nutrition*, 21(1), S37-41.

Sreenivas, S.A., Pai, K.V. (2008). Thiolated Chitosans: Novel Polymers for Mucoadhesive Drug Delivery – A Review. *Tropical Journal of Pharmaceutical Research*, 7 (3), 1077-1088.

Susan, A. (2005). Biotechnology, brain and future. *Trends Biotechnol.*, 23(1), 34-41.

Suzuki, Y., Makino, Y. (1999). Mucosal drug delivery using cellulose derivatives as a functional polymer. *J Control Release*, 62(1-2), 101-107.

Tengamnuy, P., Mitra, A.K. (1988). Transport of tyrosine and phenyl alanine across the rat nasal mucosa. *Life Sci.*, 43(7), 585-593.

Thanou, M., Florea, B.I., Langemeyer, M.W., Verhoef, J.C., Junginger, H.E. N-trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug busserelin in vitro (Caco-2 cells) and in vivo (rats). *Pharm. Res.*, 17(1), 27-31.

Thanou, M., Nihot, M.T., Jansen, M., Verhoef, J.C., Junginger, J.C. (2001). Mono-N-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. *J. Pharm. Sci.*, 90(1), 38-46.

Thorne, R.G., Frey, II, W.H. (2001). Delivery of neurotropic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet.*, 40(12), 907-946.

Update. *J. Pharm. Pharmacol.*, 53(8), 1047-1067.

Van De Donk, H.J.M., Muller Plantema, I.P., Zuidema, J., Merkus, F.W.H.M. (1980). The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. *Rhinology*, 18(3), 119-130.

Verheul, R.J., Amidi, M., van der Wal, S., van Riet, E., Jiskoot, W., Hennink, W.E. (2008). Synthesis, characterization and in vitro biological properties of O-methyl free N,N,N-trimethylated chitosan. *Biomaterials*. 29(27), 3642-3649.

Visor, G.C., Thompson, S., Ling, T. (1987). Nasal absorption of calcium antagonists nicardipin in rats and rhesus monkeys. *Drug Dev. Ind. Pharm.*, 13(8), 1329-1335.

Welch, K.M. (1997). Pathogenesis of migraine. *Seminars in Neurology*, 17(4), 335-41.

Wheatly, M .A., Dent, J., Wheeldon, E.B., Smith, P.L. (1988). Nasal drug delivery: An in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancer. *J Control Release*. 8(2), 167-177.

WHO Integrated Management of Adult and Adolescent Illness. (2004). Palliative care: symptom management and end-of-life care. Available at <http://www.who.int/entity/3by5/publications/documents/en/genericpalliativecare> 082004. [Accessed January 24, 2008.]

Young, D.B., Van Vliet, B.N. (1992). Migraine with aura: a vicious cycle perpetuated by potassium-induced vasoconstriction. *Headache*, 32(1), 24-34.

<i>Chapter 2</i> .....	11
2.1 Pain .....	12
2.2. Migraine .....	15
2.3 Drug Delivery to the Central Nervous System .....	21
2.3.1. Barriers to CNS deliver .....	21
2.3.2. Strategies for Enhanced CNS Drug Delivery .....	26
2.4. Intranasal drug deliver for brain targeting .....	30
2.4.1. Nasal Anatomy and Physiology .....	32
2.4.2. Mechanism of Nose to Brain Drug Transport .....	34
2.4.3 Factors Affecting Brain-Targeted Nasal Delivery Systems .....	37
2.4.4. Nasal Dosage Forms .....	43
2.4.5. Animal Models for Evaluation of Nasal Drug Absorption Studies .....	44
2.5. Novel nasal delivery systems .....	47
2.5.1. Chitosan .....	47
2.5.2. Thiolated Chitosans: Novel Polymers for Mucoadhesive Drug Delivery .....	55
2.5.3. Trimethyl chitosan and its applications in drug delivery .....	65
2.6. Drug profile .....	77
2.6.1 Tizanidine HCl .....	77
2.6.2 Cyclobenzaprine HCl .....	80
2.7. References .....	84