CHAPTER 2

LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 Pain, Neuropathic Pain and Drug Delivery

Pathophysiological nociceptive pain occurs when the tissue is inflamed or injured. This pain may appear as spontaneous pain (pain in the absence of any intentional stimulation) and/or as hyperalgesia and/or allodynia. Hyperalgesia is a higher pain intensity that is felt upon noxious stimulation and allodynia is the occurrence of pain that is elicited by stimuli that are normally below the pain threshold. While nociceptive pain is elicited by noxious stimulation of the sensory endings in the tissue, neuropathic pain results from injury or disease of neurons in the peripheral or central nervous system (Schaible HG and Richter F, 2004)[7]. Neuropathic pain is not a single entity or diagnosis. Rather it represents a variety of syndromes and a heterogeneous group of etiologically different diseases sharing certain Neutrogena signs and symptoms. The main features of neuropathic pain are a combination of symptoms and signs such as, pain in areas with sensory loss, hyper excitability, evoked stimulus dependent pain, ongoing stimulus independent pain, sympathetic involvement and summation of pain.

2.1.1 Pain: Anatomy & Physiology

Nociception is conveyed from the periphery to the brain at three Levels: the peripheral nociceptor, the spinal cord, and the supra-spinal (brain) levels. Physiological pain is produced by stimulation of high threshold thermo or mechanical nociceptors, which transmit via fast conducting myelinated A delta fibres. These enter the dorsal horn of the spinal cord and synapse at laminae I and V.

Pathophysiological pain originates from stimulation of the high threshold polymodal nociceptors, free nerve endings, present in all tissues. The nociceptors respond to chemical (bradykinin, histamine, eicosanoids like prostaglandins, leukotrienes, thromboxanes; site of action of NSAIDS, substance P, 5HT & opiod peptides;site of action of opiod drugs) mechanical, and thermal stimuli and are transmitted via slow conducting unmyelinated C fibres. These synapse at laminae II and III, substantia gelatinosa, of the dorsal horn. The second order neurons are either nociceptive specific (substantia gelatinosa) or wide dynamic range neurons in laminae V and VI that respond to a wide range of noxious and non-noxious input.

Both pathways ascend up the spinal cord via the spinothalamic tracts to the thalamus, which synapse and project on to the somatosensory cortex. Inhibitory inter-neurons in the substantia gelatinosa prevent activation of the dorsal root ganglia. Inter neurons can be activated by A

beta and inhibited by A beta and C fibre activity. Pain can be 'gated-out' by stimulating the large A beta fibres in the painful area. This is the working mechanism behind transcutaneous electrical nerve stimulation. The descending inhibition pathways originate at the level of the cortex and thalamus, and descend via the brainstem (periaqueductal grey) and the dorsal columns to terminate at the dorsal horn of the spinal cord. The primary afferent neurotransmitters involved in both fast component and slow component pain are the excitatory amino acids (L Glutamate, ATP, and Neuropeptides (Substance P). L Glutamate has receptors such as NMDA, AMPA, LAP4 which can be blocked by a variety of drugs. In the spinal cord, the modulators of pain transmission include opioid peptides, biogenic amines

In the CNS, periaqueductal grey area, the main neurotransmitters are opioid peptides, through GABA, & excitatory amino acids. Peripheral tissue injury provokes two kinds of modification in the responsiveness of the nervous system:

a) Peripheral sensitization: a reduction in the threshold of nociceptor afferent peripheral terminals

b) Central sensitization: an activity, dependent increase in the excitability of spinal neurons. Direct injury to tissues and nerves excite central pain pathways via spinal afferent pathways. Studies have demonstrated that a noxious stimulus strong enough to cause peripheral tissue damage might produce prolonged changes in processing of afferent information in the dorsal horn neurons of the spinal cord and CNS, including decreased pain threshold (*hyperalgesia*), increased pain to normal stimulation, and increased duration of response to brief stimulation (*allodynia*). This is called central sensitization.

2.1.2 Need for pain control and management

Pain perception is now known to be one of a group of linked bodily responses triggered by nociception. During acute pain, these responses include secretion of "stress"-related hormones that promote tissue breakdown, energy mobilization, and fluid retention; cardiovascular responses such as tachycardia, hypertension, ischemia, and ventricular arrhythmias; slowing of peristalsis; and immune impairment. These adverse responses take place even in unconscious patients undergoing surgery while under general anesthesia. In the conscious patient, poor respiratory effort due to acute pain can further impair pulmonary function following upper abdominal surgery and encourage immobility with ensuing deep venous thrombosis. Intriguing studies link inadequate analgesia to accelerated experimental tumor growth, suggesting that "pain can kill." Thus, "pain" is more than just suffering. It is linked with specific physiological responses that cascade to increase pain intensity and

extent. Analgesia can subdue these responses and potentially improve overall patient outcome and shorten hospital stay.

Post operative pain can affect all organ systems and includes:

Respiratory – reduced cough, atelectasis, sputum retention and hypoxemia.

Cardiovascular – increased myocardial oxygen consumption and ischemia.

Gastrointestinal – decreased gastric emptying, reduced gut motility and constipation.

Genitourinary – urinary retention

Neuroendocrine – hyperglycemia ,protein catabolism and sodium retention.

Musculoskeletal – reduced mobility, pressure sores and increased risk of deep vein thrombosis.

Psychological – anxiety and fatigue

2.1.3 Pain management and drug delivery

To live everyday with any type of pain is extremely adverse experience that challenges every fiber of an individual's being. (Francesca F et al., 2007; Schaible HG and Richter F, 2004). The pain disorder brings very high direct and indirect costs to patients and society in terms of suffering and lost productivity. Pain is able to alter a patient's quality of life by interfering with mood, sleep and emotional wellbeing. Patients are not frequently receiving the appropriate drug therapy with inadequate drug dosing and are frequently dissatisfied with treatment (Colombo B et al., 2006). Despite array of advanced strategies, predominantly opioid based techniques are still limited by side-effects such as vomiting, nausea, itching, urinary retention respiratory and cardiovascular effects. Neuropathic pain varies in intensity but when it is severe it is one of the most difficult pain syndromes to treat, "resistant" to what would be considered high doses of opioids; hence the idea that neuropathic pain is resistant to opioids. Treatment of pain is based on drugs able to reduce hyperexcitability i.e. the effect is measured either in terms of a reduction in pain intensity and pain relief or considering patient satisfaction (Schwatzman RJ et al., 2001).

Targeted drug delivery system is required for a large number of drugs to overcome the problems of insufficient bioavailability of drug at site of action and associated higher systemic toxicity of drug. Many diseases like cancer, multiple sclerosis, herpes zoster infection, accidental surgeries and diabetes patients suffer from different types of pain. The clinical failure of the potential effective therapy is of not due to lack of drug efficacy but rather due to shortcoming in method by which drug is being delivered. A large number of

therapeutic agents are found to be ineffective in the treatment of cerebral diseases due to their inability to effectively and efficiently delivered and sustain within the brain. Therefore, scientists are exploring the novel approaches to encounter this problem so that delivery of the drugs can be restricted to the brain and central nervous system. Despite of enormous research, patients suffering from fatal and/or debilitating central nervous system (CNS) diseases, such as epilepsy, migraine, brain tumors, HIV encephalopathy, cerebrovascular diseases, dipression and neurodegenerative disorders (Misra A et al., 2003). Treating CNS diseases is challenging and a daunting task because a variety of formidable obstacles often impede drug delivery to the brain and spinal cord (Misra A et al., 2005).

2.2 Brain anatomy, physiology and barriers to drug delivery

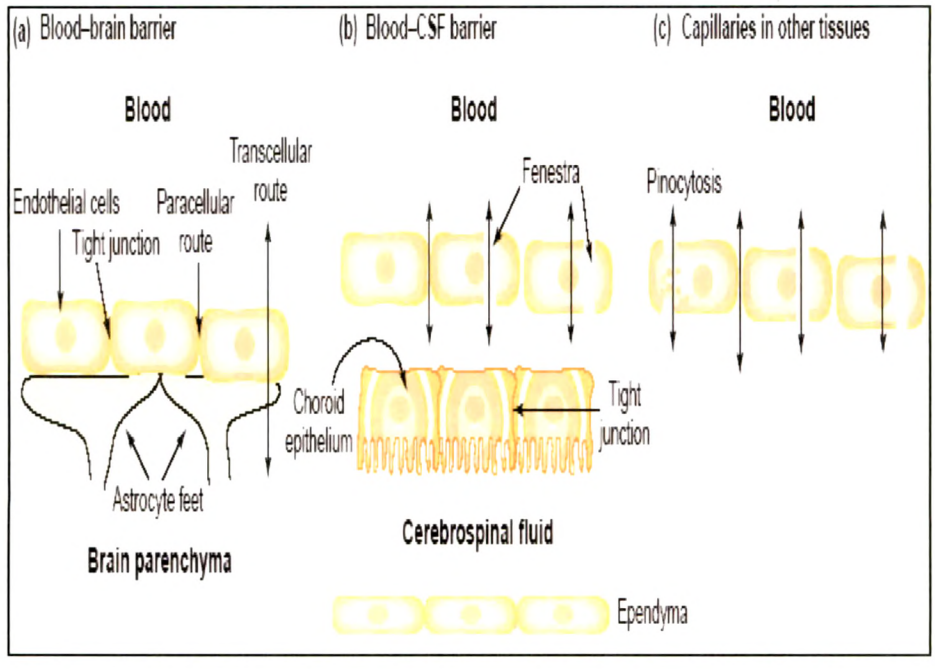
The brain is an integral part of the central nervous system, acting as a major regulating and communicating organ to maintain the body's homeostasis in response to changes in both the external and internal environment. The primary cells of the brain include nerve cells (neurons) and supporting glial cells (Nolte J, 2002). The three major subdivisions of the brain are the cerebrum, cerebellum, and the brain stem.

The cerebrum is the largest section and is easily divided into the right and left hemispheres along the mid-sagittal plane. These hemispheres are made up of the cerebral cortex, which is responsible for functions such as language and information processing. Brain tissue is protected externally by the skull, which constrains the volume and regulates intracranial tissue pressure (Mokri B, 2001). However, more so than in any other tissue, getting drugs into the brain is much more difficult than getting drugs out of the brain due to presence of Blood-Brain Barrier (BBB). Internally, the BBB greatly limits permeability and transport across the endothelial cell membranes of the blood vessels. Elimination of drugs from the brain is facilitated by mechanisms such as multidrug resistance transporters that actively extrude drug across the BBB (Golden PL and Pollack GM, 2003) and a cerebrospinal fluid (CSF) turnover rate that is higher than the lymphatic drainage rate found in most peripheral tissues (Davson H and Segal MB, 1996). The production, flow, and drainage of the extracellular fluid (ECF) significantly affect the efficacy of brain drug delivery.

2.2.1 Barriers to CNS Drug Delivery

Barriers to CNS delivery are diagrametically shown in Fig. 2.1 and discussed below.

Figure 2.1: Barriers to brain delivery



2.2.1.1 Blood Brain Barrier

The BBB is a membranous barrier separates the brain from the surrounding circulating blood (Begley DJ, 1996; Schlossauer B and Steuer H, 2002). Because of different structure of brain capillary compare to other tissues, it provides permeability barrier to most of the penetrants from extra cellular fluid in brain tissue. Micro vessels make up approximately 95 % of the total surface area of the blood-brain-barrier (BBB), is the principal route by which molecules reach the brain. This barrier is very efficient and makes the brain practically inaccessible for lipid- insoluble compounds such as polar molecules and ions. In brain capillaries, the principle route of transport takes place through trans-cellular mechanism only. Therefore, only lipid-soluble solutes can freely penetrates through the capillary endothelial membrane and may cross the BBB passively.

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

based paracellular diffusion observed in other tissues (Nabeshima S et al., 1975; Brightman MW, 1968).

On the other hand, certain classes of drugs like benzodiazepines such as diazepam have been used as sedative-hypnotic agents, because these lipophilic drugs readily cross the BBB. However, the BBB transport of an immunosuppressive agent, cyclosporine A, which is more lipophilic than diazepam, is highly restricted. Similarly, almost all of the lipophilic anticancer agents such as doxorubicin, epipodophylotoxin and Vinca alkaloids (e.g., vincristine and vinblastine) hardly enter the brain, causing difficulty in the treatment of brain tumors. Although levodopa, which is useful for treatment of Parkinson's disease, is very hydrophilic, it can readily penetrate the BBB. The other problem encountered with BBB is enzymatic degradation. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles. BBB enzymes also recognize and rapidly degrade most peptides, including naturally occurring neuropeptides (Brownless J and Williams CH, 1993; Witt KA et al., 2001).

2.2.1.2 Brain Cerebrospinal Fluid Barrier

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

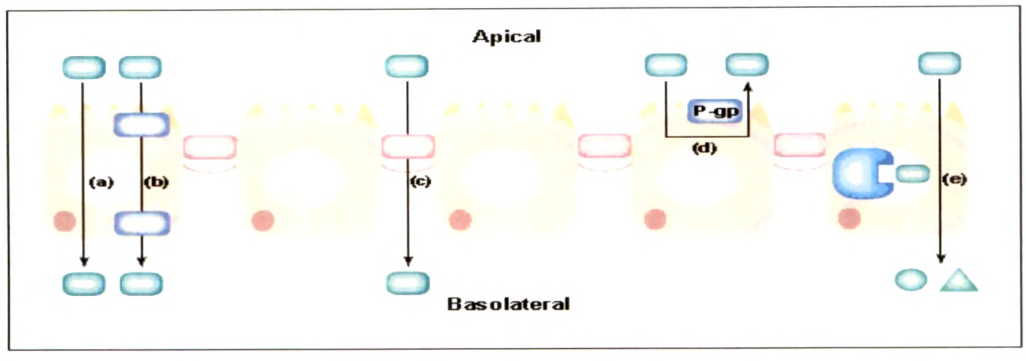
2.2.1.3 Efflux Transporters

A thorough understanding of the two way transport mechanisms uptake and efflux through BBB is of great importance in targeting drugs to the brains or to minimize the unwanted adverse effects some therapeutically active molecules.

The efflux mechanisms in the CNS are passive or active. Active efflux from the CNS via specific transporters may often reduce the measured penetration of drug at the BBB to levels that are lower than might be predicted from the physicochemical properties of the drug, for example, its lipid solubility. Recently much attention has been focused on multi-drug transporters; multi-drug resistance protein (MRP), P-glycoprotein (P_{gp}) and the multi-specific

organic anion transporter (MOAT), which belong to the members of the ABC cassette (ATP-binding cassette) of transport protein (Cole SPC et al., 1992; Taylor EM, 2002).

Figure 2.2: Efflux transporters at Blood Brain Barrier



2.2.2 Physicochemical factors responsible for brain uptake

Therapeutic activity in form of Biological response is a measure of brain uptake. But this biological activity mainly depends on rate of transfer from blood to brain, or distribution between blood and brain and interaction between drug and targeted receptors in the brain.

As on to date, in NCE designing program the lipophilic factor (log *P*) is still used as an informative tool for CNS targeting (Gupta SP, 1989; Hansch C et al., 1995). Increase in lipophilic factor with the intent to improve membrane permeability may leads to increase in the volume of distribution and tends to affect all other pharmacokinetic parameters including rate of oxidative metabolism by cytochrome P450 (Van de Waterbeemd H et al., 2001; Lin JH and Lu AY, 1997; Lewis DFV and Dickins M, 2002). Hence, the optimum balance is required between improved bioavailability and first pass metabolism.

The various experiments measuring tools of brain uptake such as brain uptake index (Oldendorf WH, 1970), Permeability-surface area product (PS), Permeability coefficient (PC) are widely utilized. Based on the relationship between the octanol/ water partition coefficient (PC, log *P*), molecular weight of molecules and the BBB permeability coefficient (PS), therapeutic substrates can be classified in three different classes: (a) substrates exhibiting a good correlation, (b) substrates exhibiting a greater PS value than indicated by their lipophilicity, and (c) substrates exhibiting a smaller PS value than indicated by their lipophilicity (the molecular weight of substrates greater than 400 Da, cut off for BBB passage). The transport mechanism for groups (a) and (b) is passive diffusion and facilitated transport, respectively (Pardridge WM et al., 1990).

Brain uptake can be positively correlated with lipid solubility or negatively correlated with hydrogen bonding (Cornford EM and Oldendorf WH, 1986). The higher the hydrogen bonding potential, lower the uptake into the brain. By reducing the hydrogen bonding potential for a congeneric series of steroid hormones, there was a log increase in uptake with each removal of hydrogen bond pairs.

2.3 Strategies for drug delivery to the brain

Researchers have utilized various strategies to increase CNS drug delivery of hydrophilic and large molecular weight drugs. Fig. 2.3 shows various transport routes across Bloo Brain Barrier (BBB). Opening the tight junctions at the Blood Brain Barrier (BBB) has been done by artificially creating osmotic pressure and the administration of bradykinin analogs. Junctional opening of the BBB enables paracellular CNS drug delivery across the barrier. However, opening the barrier by either mechanism may allow CNS entry of toxins and unwanted molecules, potentially resulting in significant damage. Increasing lipid solubility of drug may significantly alter pharmacokinetic parameters such that clearance and half-life become undesirable.

Another alternative for brain delivery is utilization of the native carriers expressed at the BBB Carriers, also known as transporters; deliver essential hydrophilic and large compounds across the BBB such as choline, purines, and amino acids. Though they are an attractive means of CNS delivery, the drugs must have carrier-mediated specificity, thus limiting their molecular characteristics. The use of targeted nanocarriers such as drug-polymer conjugates, nanoparticles, liposomes and other colloidal systems leads to achieving site specific targeting ability, thereby improving therapeutic index and reduction in side effects (Lockman PR et al., 2002).

2.3.1 Conventional Strategies

2.3.1.1 Significance of Lipophilicity

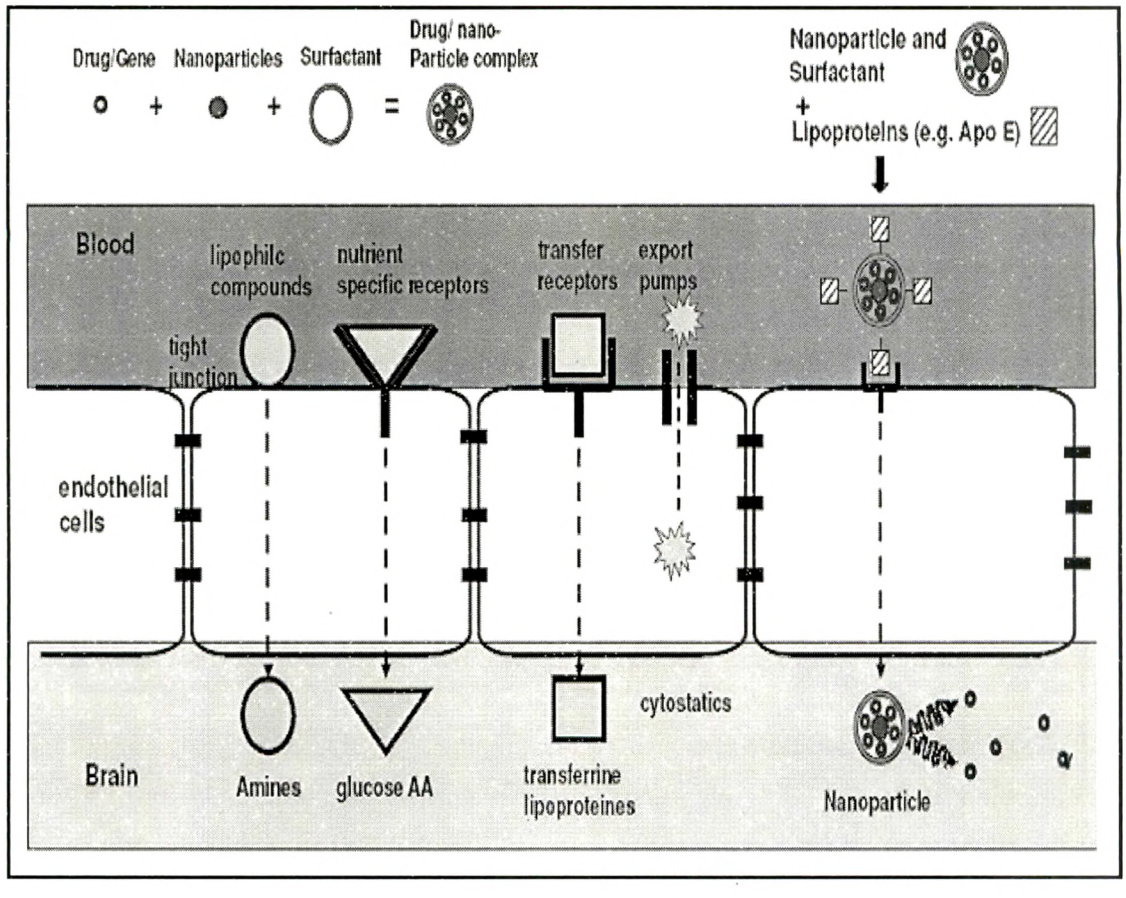
Octanol/Water partition coefficient , log $P_{o/w}$ (Buchwald P and Bodor N, 2002) is very commonly acceptable and convenient approach to predict lipophillicity and relative lipophillicity of any system. However, log $P_{o/w}$ alone seems to have a very limited application in predicting brain/blood concentration 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 of distribution (Van de Waterbeemd H et al., 2001; Lewis DFV and Dickins M, 2002; Lin JH and Lu AY, 1997)

2.3.1.2 Site targeting index and targeting enhancement factors

For CNS-targeted drug delivery system, it is of utmost important to quantitatively assess the site-targeting effectiveness (Bodor N and Buchwald P, 2003). A site targeting index (STI) could be defined as ratio between the area under concentration-time curve (AUC) for the concentration of drug itself at the targeted site, and that at a systemic site for example blood or plasma. STI gives an accurate indication and true scenario about how effectively the desirable active therapeutic agent is actually delivered to its intended site.

Figure 2.3: Transport routes across the blood–brain barrier (BBB)



2.3.1.3 BBB Disruption

Artificially opening the BBB by administration of hyper osmotic agents, vasoactive molecules or by various alkylglycerols is well established (Neuwelt EA et al., 1991; Rapoport SI, 2000). When a hypertonic solution of 25% mannitol is introduced via a cannula into the carotid artery (in humans at a rate of 4–8 ml/second), for approximately 30 seconds the BBB

remains open for 30 minutes, Following this treatment, a drug can be administered via the same catheter and will freely enter the CNS. The proposed cellular mechanism behind osmotic disruption of the BBB involves the physical pulling apart/breaking of tight junctions due to the shrinkage of cerebral endothelial cells and expansion of the blood volume caused by the addition of the hyperosmotic agent (Kern W et al., 1999).

Several endogenous pro-inflammatory vasoactive agents, such as bradykinin, histamine, nitric oxide, and various leukotrienes, are known to induce increases in BBB permeability in a concentration and time dependent manner. These vasoactive compounds are characteristically ultra-short-acting due to either rapid deactivation through metabolic processes or tachyphylaxis at the receptor signal transduction level (Borlongan CV, 2003).

The use of alkylglycerols to increase BBB permeability of anticancer agents has been examined in a rat glioma tumor model (Erdlenbruch BSC et al., 2003).

2.3.1.4 Intra-cerebral administration

An obvious way of circumventing the BBB is to inject a drug directly into the brain, intraventricularly or intrathecally into the subarachnoid space. These methods have a number of drawbacks. If an injected volume or a solid implant is rapidly introduced into brain tissue it will certainly damage an area of brain (Krewson CE et al., 1995; Yan Q et al., 1994).
This approach is invasive, requiring a craniotomy in which a small hole is drilled in the head for drug administration into the brain. An advantage of this approach is that a wide range of compounds and formulations can be considered for administration. Thus, both large and small-molecule therapeutics can be delivered, either alone or in various polymer formulations, to achieve sustained release.

2.3.1.5 Intra-parenchymal delivery

Intra-parenchymal drug delivery involves the placement of a needle directly into the target parenchymal space for bolus injection or continuous infusion into the interstitial space. This technique has been used to deliver both particulate drug carriers and small molecule therapeutics. When bolus injection is used, the resulting drug distribution throughout the tissue is primarily a result of concentration dependent diffusion. Thus, in order to achieve and maintain therapeutic drug levels in a large tissue region, it may become necessary to use high concentrations of the drug, which can quickly lead to dose-limiting neuro-toxicity. For this reason, direct injection is more efficacious for the delivery of small molecule therapeutics compared to particulate drug carriers (Kawakami K et al., 2004).

2.3.1.6 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.

2.4.1.7 Pro-drugs and chemical delivery systems

Prodrugs are the compounds which are pharmacologically inactive and they undergo metabolic transformation within the body to pharmacologically active compounds (effective drugs). After administration, the prodrug, by virtue of its improved characteristics, is brought closer to the receptor site and is maintained there for longer periods of time. Here it gets converted to the active form, usually via a single activating step.

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

No.	Drug	Approval
1	Valacyclovir	**Valtrex®**, the L-valine ester of Acyclovir approved by USFDA in 1995 for treatment of Herpes Zoster
2	Fosphenytoin sodium	**Cerebyx®**, a phosphooxy methyl prodrug of Phenytoin approved by USFDA in 1996 for treatment of Epilepsy.
3	Famciclovir	**Famvir®**, a prodrug of Penciclovir approved by USFDA in 1997 for treatment of Herpes Simplex.
4	Oseltamivir phosphate	**Tamiflu™**, an ethyl ester prodrug of Oseltamivir carboxylate inhibitor of influenza A and B, approved y USFDA in October 1999.
5	Basalzide disodium	**Colazal™**, a sulfa-free prodrug of 5 amino salicylic acid approved by USFDA in 20005 for treatment of moderate active ulcerative colitis.
6	Valganciclovir hydrochloride	**Valcyte™**, an L- valyl ester prodrug of Ganciclovir approved by USFDA in 2001 for the treatment of Cytomegalovirus retinitis in AIDS patient.

For example morphine has a very low brain uptake; replacing one hydroxyl group of morphine with methyl group to form codeine increases the lipophilicity. On further lipidization, adding two acetyl groups to the molecule to form heroin (or di-acetyl-morphine), increases the brain uptake to very significant levels. Heroin is rapidly metabolized to

morphine in the brain, which interacts with the opiod receptor (Bodor N and Buchwald P, 2003). Morphine being relatively polar becomes effectively locked into the brain as it cannot diffuse back out across the BBB. This lock-in principle is a major feature of the prodrug approach to CNS delivery. Certain prodrugs which have been successfully studied and have reached up to the level of marketable product are shown in Table 2.1 (Bodor N and Buchwald P, 2003).

2.3.2 Novel Strategies

2.3.2.1 Nanoparticles as suitable vectors

The use of Nanoconstructs may have a distinct advantage over the previous approaches to circumvent the BBB. Nanoparticulate delivery system with surface modification directly targeting the brain would offer enormous advantages including:

- Ability to cross the BBB
- Bypass the multidrug resistance drug transporters of brain endothelial cells, which limit the therapeutic drug concentration in the brain.
- Minimizing peripheral drug side-effects (Toxicity).
- Minimizing hepatic first pass metabolism.
- Avoidance of RES (Reticuloendothelial system).
- Prolonged circulation time.
- Amenable to small molecules, peptides proteins or nucleic acids. (Misra A et al., 2003)

Colloidal drug carriers (Nanoconstructs) used for brain targeting mainly include micelles, emulsions, liposomes and nanoparticles (nanospheres and nanocapsules). But out of these only liposomes and nanoparticles have been largely exploited for brain drug delivery. The aim in using colloidal carriers is generally to increase the specificity towards cells or tissues, to improve the bioavailability of drugs by increasing their diffusion through biological membranes and/or to protect them against enzyme inactivation. Moreover, the colloidal systems allow access across the BBB of non-transportable drugs by masking their physico-chemical characteristics through their encapsulation in these systems.

The fate of colloidal particles after intravenous administration is determined by a combination of biological and physico-chemical events that need to be considered in the design of efficient drug carrier systems. After intravenous administration, all colloidal systems, indeed, dramatically interact with plasma proteins, especially with immunoglobulins, albumin, the elements of the complement, fibronectin, etc. This process,

known as "opsonization" is crucial in dictating the subsequent fate of the administered colloidal particles. Thus, colloidal particles that present hydrophobic surface properties are efficiently coated with plasma components and rapidly removed from the circulation, since the macrophages of the liver and the spleen own their specific receptors for these opsonins. However, colloidal particles that are small and hydrophilic enough can escape, at least partially, from the opsonization process and consequently remain in the circulation for a relatively prolonged period of time (Owens III DE and Peppas NA, 2006). Additionally, the concept of "steric hindrance" has been applied to avoid the deposition of plasma proteins either by adsorbing at the surface of the colloids some surfactant molecules (such as copolymers of polyoxyethylene and polyoxypropylene) or by providing a sterical stability by the direct chemical link of polyethyleneglycol (PEG) at the surface of the particles (Peracchia MT et al., 1999). In addition, active targeting can be achieved by the attachment of a specific ligand (such as a monoclonal antibody) onto the surface of the colloidal particle, preferentially at the end of the PEG molecules since the targeted colloidal particles will be much more efficient if they are also sterically stabilized.

Polymeric nanoparticles

Polymeric nanoparticles (NPs) are made from natural or artificial polymers in the sizes ranging from 10 to 1000 nm. Various biodegradable polymers such as poly (lactic acid) (PLA), poly (lactic-coglycolic acid) (PLGA), poly (ε-caprolactone) (PCL), chitosan, poly (alkylcyanoacrylate) (PACA), poly (lysine) and poly (aspartic acid) (PAsp) are used to compose the core matrix. The term nanoparticle is somewhat general since it does not take into account the morphological and structural organization of the polymer. In this respect, "nanosphere" is used to identify a nanoparticle system with a matrix character and constituted by a solid core with a dense polymeric network. In contrast, "nanocapsules" are formed by a thin polymeric envelope surrounding an oil-filled cavity. Nanocapsules may, thus, be considered as a "reservoir" system. Practically, the nanoparticles have a size around 200 nm and the drugs or other molecules may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. These systems are attractive because the methods of preparation are generally simple and easy to scale-up.

The advantage of using nanoparticles for drug delivery results from their two basic properties. Firstly, due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. Secondly, the use of biodegradable materials for nanoparticles preparation, allows

sustained drug release at the targeted site over a period of days or even weeks after injection (Vinogradov SV et al., 2003).

Kreuter showed when dalargin or loperamide were adsorbed onto the surface of poly (butylcyanoacrylate) (PBCA) nanoparticles further coated with the detergent, Polysorbate-80 (Tween 80), a pronounced analgesic effect was obtained. *In-vivo* experiments in mice have clearly shown that the analgesic effect of dalargin was obtained only when the drug was pre-adsorbed onto the nanoparticles, whereas a single mixture of dalargin and PBCA nanoparticles did not show any analgesic effect. It has been reported that apolipoproteins (APO) could be involved in the brain penetration of PBCA nanoparticles coated with Polysorbate-80 (Kreuter J et al., 2002). A study has been performed using PBCA nanoparticles loaded with dalargin/loperamide and coated with the APO-A, B, C, E or J (with or without precoating with Polysorbate-80). In these conditions, only dalargin/loperamide-PBCA nanoparticles coated with polysorbate-80 and/or with APO-B or APO-E were able to achieve an anti-nociceptive effect. No anti-nociceptive effect was seen after coating with the other apolipoproteins. Thus, it is suggested that the Polysorbate-80 could act as an anchor for APO-B and APO-E, at the surface of the nanoparticles which are then be able to interact with LDL receptor, before being taken up by the receptor-mediated endocytosis (Kreuter J et al., 2002).

Body distribution of nanoparticles after intravenous injection

After intravenous injection, nanoparticles, like other colloidal carriers, are taken up by the reticuloendothelial system (RES). The intravenously injected colloids mainly distribute into the RES organs liver (60-90 % of the injected dose), spleen (2-10 %), lungs (3-20 %) and a low amount (>1 %) into the bone marrow (Kreuter J, 1983). It has been acknowledged for over a decade (Kreuter J, 1983; Singer et al. 1969) that coating of the injected colloid particulates with serum components - opsonins - precedes the uptake by the RES and that opsonization process strongly facilitates or even is necessary to induce phagocytosis of the particles. The surface properties of the particles seem to influence strongly the spectrum of the adsorbed serum components. The adsorption of the serum components, mainly proteins, is competitive and is found to be only partially reversible or even irreversible. The hydrophobic bounding was the predominant force in the adhesion of the protein to foreign surfaces. These hydrophobic interactions seem to occur due to the unfolding of the proteins that reveals the hydrophobic interior. This process optimizes hydrophobic interactions and increases the entropy of the adsorbed material. The opsonins coat around the injected particulates then seems to trigger their uptake by cells of the RES (Van Oss CJ et al., 1984;

Van Oss CJ, 1978). Modification of surface properties of nanoparticles is therefore necessary for targeting nanoparticles to non RES organs.

2.3.2.2 Drug Delivery to the brain via endogenous BBB transporters

BBB has a very high resistance owing to the tight junctions, which cement adjacent endothelial cells together. Due to the presence of the tight junctions, there is no para-cellular pathway for solute distribution into brain interstitial fluid from blood. Circulating molecules can only gain access to brain interstitium via a transcellular route through the brain capillary endothelial membranes. In the absence of the lipid-mediated pathway, circulating molecules may gain access to brain only via transport on certain endogenous transport systems within the brain capillary endothelium. These endogenous transporters have an affinity for both small molecules and large molecules and can be broadly classified into three categories: 1) Carrier mediated transport (CMT); and 2) Receptor-mediated transport (RMT) 3) Active efflux transport (AET).

Carrier Mediated Transport (CMT)

To meet the metabolic needs of the brain, the capillary endothelial cells that form the BBB express many selective carrier/transport systems for delivering essential nutrients from the blood to the brain. An approach to increasing the transcellular passage of drugs across the BBB into the brain is to design drugs that structurally resemble or can be linked to endogenous compounds that are transported into brain by the carriers or transporters expressed in the brain microvessel endothelial cells (Audus KL et al., 1992). CMT systems for hexoses, lactic acid, phenylalanine, arginine, choline, adenosine, and adenine, are present on the luminal membrane of the brain capillary endothelium.

Receptor Mediated Transport (RMT)

Receptor mediated endocytosis is a process where a solute will bind to its cognate cell membrane receptor to elicit either a constitutive (class I) or ligand-stimulated (class II) internalisation. Constitutive (class I) receptor mediated endocytosis will result from continual plasma membrane turnover with the receptor internalized within an endocytic vesicle even in the absence of ligand, e.g. transferrin receptor or low-density lipoprotein (LDL) receptor. In class II receptor mediated endocytosis, the ligand binding to its receptor triggers the internalisation, e.g. insulin like growth factor (IGF) binding to their respective receptors.

Active efflux transport (AET)

The major active efflux transporters responsible for the removal of the drug from brain to blood are P-glycoprotein (Pgp), Multidrug Resistance–Associated Protein (MRP), and breast cancer resistance protein (BCRP). These transporters are part of the larger ATP binding cassette (ABC) family of proteins that remove a wide variety of compounds from the cell through an ATP-dependent active transport process (Borges- walmsley et al. 2003).

Targeting of Nanoparticles via Transferrin conjugation

Transferrin (Tf) is a structurally related class of metal-binding glycoproteins of approximately 80 kDa in size whose primary function is the binding and transportation of non-heme iron through the blood to cells through transferrin receptors (TfR). The capillaries of the brain have been well known to have relatively high levels of Transferrin receptors (TfR), and since the blood brain barrier (BBB) effectively excludes many therapeutic drugs, including almost all peptide and protein-based therapeutics, targeting of the BBB's TfR for drug delivery is an attractive strategy. Transferrin is non-toxic and non-immunogenic for the use in the drug delivery. The transferrin conjugated to the nanoparticle surface, leads to receptor based endocytosis across the blood brain barrier, leading to the enhanced brain concentrations, where the nanoparticles release the drug in a sustained manner (Mishra V et al., 2006; Soni V et al., 2008).

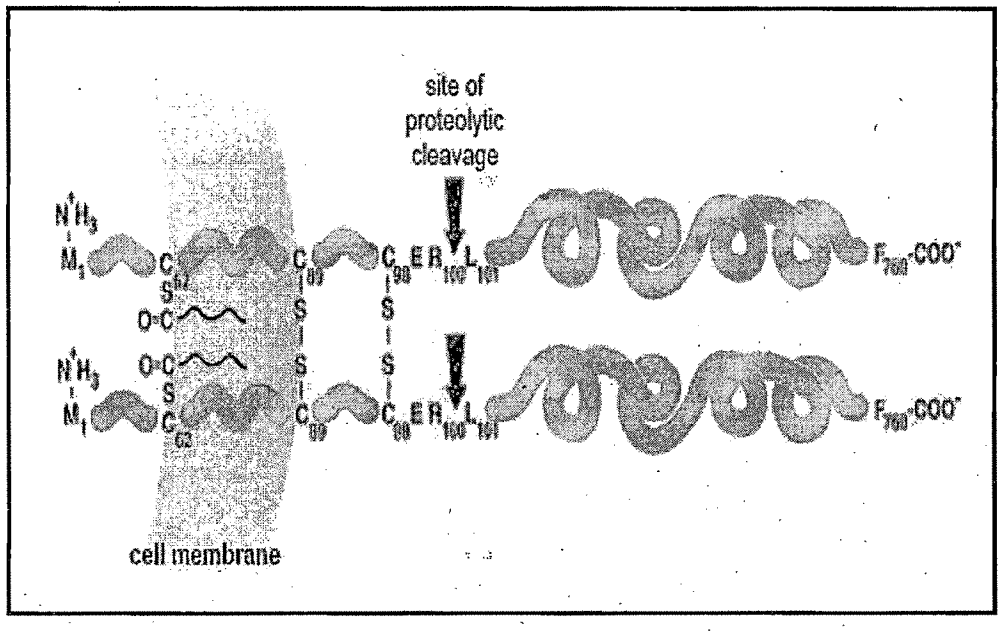
2.4 Receptor mediated transport through Transferrin receptors

2.4.1 The transferrin receptor

The transferrin receptor (TfR) is a transmembrane glycoprotein consisting of two 90 kDa subunits. A disulfide bridge links these subunits and each subunit can bind one transferrin (Tf) molecule (Moos T and Morgan EH, 2000). The TfR is expressed mainly on hepatocytes, erythrocytes, intestinal cells, monocytes, as well as on endothelial cells of the BBB (Ponka P and Lok, CN, 1999). Furthermore, in the brain the TfR is expressed on choroid plexus epithelial cells and neurons. The TfR mediates cellular uptake of iron bound to transferrin (Tf). The Schematic representation of the transferrin receptor is shown in Fig. 2.4.

The expression level of the TfR depends on the level of iron supply and rate of cell proliferation. For example, in malignant cells an elevated level of TfR expression is found. This is caused by the high iron requirements for malignant growth (Ponka P and Lok, CN, 1999; Huebers HA and finch CA, 1987).

Figure 2.4: Schematic representation of the transferrin receptor



The iron concentration determines TfR synthesis and expression via an iron-responsive element (IRE) in the mRNA of the TfR (Kuhn LC, 1991; Casey JL et al., 1989). This IRE is also found in the mRNA of ferritin, a protein that can store iron . In cases of low iron concentrations, a socalled IRE binding protein stabilises the mRNA of the TfR, which can therefore be translated. The mRNA of ferritin is in low-iron situations less stable and is therefore translated to a lesser extent.

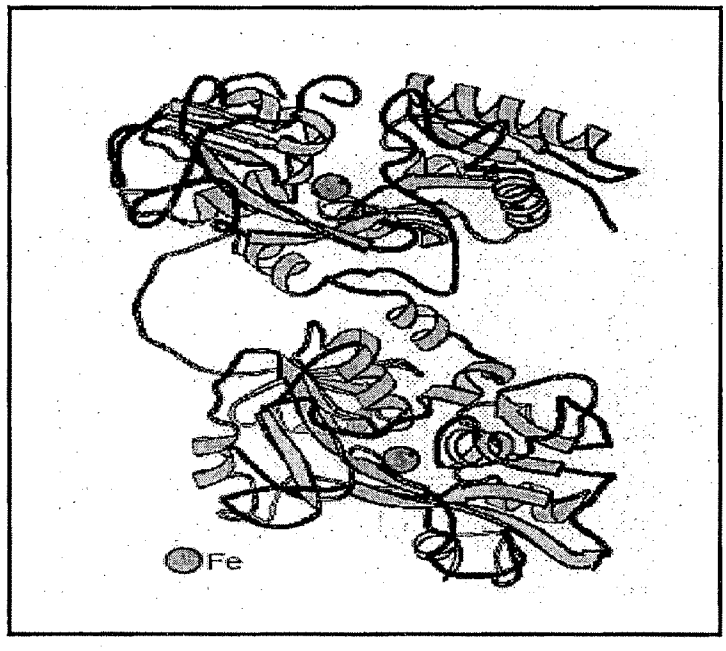
Recently, a second TfR (TfR-2) has been identified (Trinder D and Baker E, 2003), which does not contain IRE in its mRNA. TfR-2 is differentially distributed from TfR and has a 25-fold lower affinity for Tf. Finally, a soluble or serum TfR is present in the circulation (Kohgo Y et al., 1986). During the process of recycling of the TfR, some receptors are shed, in which case they appear in truncated form in the blood circulation (Shih YJ et al., 1990). It has been shown that serum TfR to ferritin ratios has significant predictive value for differentiating iron deficiency anaemia from non-iron deficiency anaemia (Kohgo Y et al., 2002).

2.4.2 Transferrin

Tf, the natural occurring ligand for the TfR, is a member of the family of Fe-binding glycoproteins, which also includes lactoferrin, melanotransferrin and ovotransferrin. Plasma Tf is mainly synthesised in the liver, but similar proteins are also synthesized in the brain, testes, and mammary glands. In the brain, Tf mRNA has been found in choroid plexus epithelial cells, oligodendrocytes, astrocytes, and neurons. However, the oligodendrocytes

appear to be the major source of brain-derived Tf. Furthermore, Tf in the brain is found in neurons and BCEC, although this Tf is probably derived from the extracellular fluid, blood plasma, and brain interstitial fluid by receptor-mediated endocytosis (Moos T and Morgan EH, 2000). Fig. 2.5 shows structure of human transferrin. Tf is a single chain, 80 kDa protein, which is folded into two lobes. Each lobe of the Tf molecule can bind one iron ion, a binding that is virtually irreversible at physiological pH. Iron is being released as the pH is lowered to values below 6.5.

Figure 2.5: Structure of human transferrin



In plasma and other extracellular fluids, Tf is present as a mixture of iron free (apo-Tf), monoferric Tf, and diferric Tf (holo-Tf). The relative abundance of each form depends on the concentrations of iron and Tf. Tf can also bind other metals, such as aluminum, cadmium, manganese or copper, albeit with lower affinity. It has not been determined yet whether the binding of these metals has physiological significance.

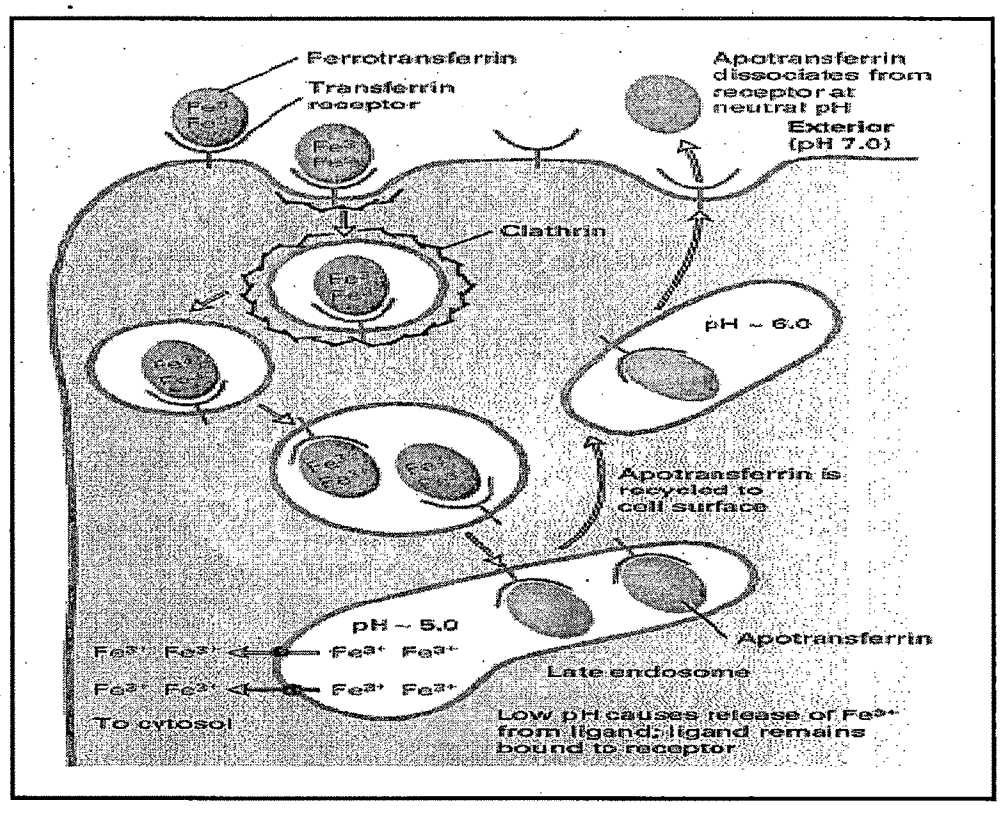
2.4.3 Lactoferrin

Lactoferrin (Lf), a mammalian cationic iron-binding glycoprotein belonging to the transferrin (Tf) family, consists of a polypeptide chain of about 690 amino acids folded into two globular lobes, each of which contains one iron-binding site. Lf is a basic, positively charged protein with an isoelectric pointof 8.0–8.5 (Nuijens JH, 1996).

It .is expressed and secreted by glandular epithelial cells and is abundant in milk of some species such as humans, rats, and mice (Suzuki YA and Lonnerdal B, 2004; Ward PP et al., 2002). It is becoming increasingly evident that Lf is a multifunctional protein to which several physiological roles have been attributed. These include regulation of iron homeostasis, anti-microbial infections, anti-inflammatory activity, regulation of cellular growth, inhibition of platelet aggregation and enhancement of immune function (Brock JH, 2002; Ward PP et al., 2002). Lf receptors are suggested to have pivotal roles for mediating multiple biological activities of Lf (Suzuki YA and Lonnerdal B, 2002, Suzuki YA, 2005). Specific receptors for Lf have been identified in intestinal epithelial cells and human fetal small intestine (Suzuki YA and Lonnerdal B, 2002). Lf receptor (LfR) has been demonstrated existing on the BBB in different species and involved in Lf transport across the BBB in vitro and in vivo (Fillebeen C et al. 1999; Suzuki YA, 2005; Huang RQ, 2007). Recently, Ji et al. compared the brain uptake of Lf with that of Tf and OX-26, and the results showed that the uptake of Lf was much higher than the other two (Ji B et al., 2006).

2.4.4 Transferrin receptor (TfR) – transferrin interaction

Figure 2.6: TfR internalisation upon binding of Tf to its receptor



Upon binding of Tf to its receptor, as shown in Fig. 2.6, the receptor-ligand complex is endocytosed via clathrin-coated vesicles. Subsequently, the endosomes that are formed are acidified to approximately pH 5.5. At low pH Fe3+ is released from Tf, and transported to the cytosol via the divalent metal transporter 1 (DMT-1) (Li H and Qian ZM, 2002; Moos T and Morgan EH, 2004). The remaining apo- Tf has a high affinity for the TfR at low pH and is recycled back to the luminal side of the BCEC. At physiological pH apo-Tf is released from the TfR and is able to acquire iron again. The intracellular Fe3+ can be stored in ferritin, or it can be used for mitochondrial activity (Li H and Qian ZM, 2002). Furthermore, Fe3+ can be exocytosed at the abluminal side, probably via ferroportin-1, hephaestin and/or hephaestin independent export systems (Qian ZM et al., 2002). There is also a second mechanism proposed in which diferric Tf crosses the BBB. Huwyler J and Pardridge WM (1998) have shown that the TfR is present on the abluminal membrane of BCEC (Huwyler J and Pardridge WM, 1998). Further research by Zhang Y and Pardridge WM, 2001 has revealed that Tf is rapidly effluxed from the brain and that the efflux of apo-Tf exceeds that of holo-Tf (Zhang Y and Pardridge WM, 2001). These results indicate that there is a bi-directional transport of Tf. However, it has been shown that the iron transport across the BBB exceeds the Tf transport. Therefore, the mechanism in which Tf returns to the luminal side after releasing Fe3+ intracellularly, is considered the most likely.

Many groups across the globe have worked on delivering transferrin and lactoferrin conjugated nanocarriers for brain targeting. The receptor-mediated endocytosis of Tf from blood to brain is well documented (Visser CC et al. 2004; Hatakeyama H et al. 2004; Ulbrich K et al. 2009; Changa J et al. 2009). However there are only few citations from different authors signifying the role of Lf as brain delivery vector (Hu K et al. 2009; Huang RQ et al. 2007). Hence, delivering the drugs after incorporation into polymeric nanoparticles and conjugating with Tf and Lf shows good potential for delivering drugs to brain.

2.5 Nanoparticles

2.5.1 Techniques for preparation of nanoparticles

Nanoparticles can be obtained by polymerization of monomers entrapping the drug molecules or from preformed polymers.

A. Nanoparticles prepared by polymerization process

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

1. Dispersion polymerization

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

2. Emulsion polymerization

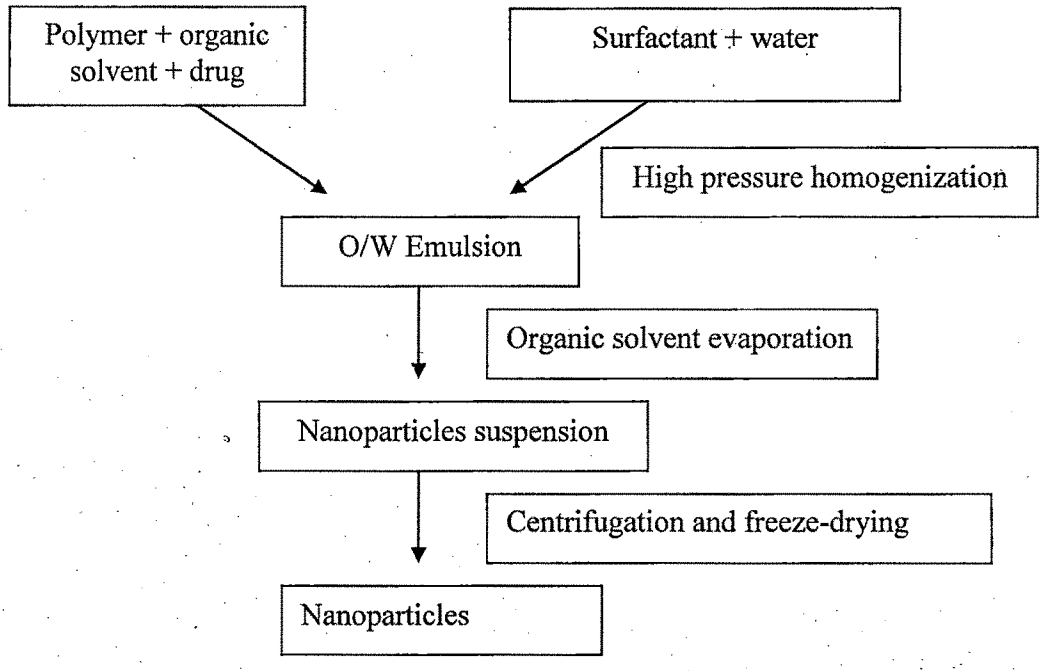
In this technique the monomer is emulsified in non-solvent containing surfactant, which leads to the formation of monomer swollen micelles and stabilized monomer droplets. The polymerization is performed in the presence of initiator. Emulsion polymerization may be performed using either organic or aqueous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

B. Nanoparticles prepared from preformed polymers

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

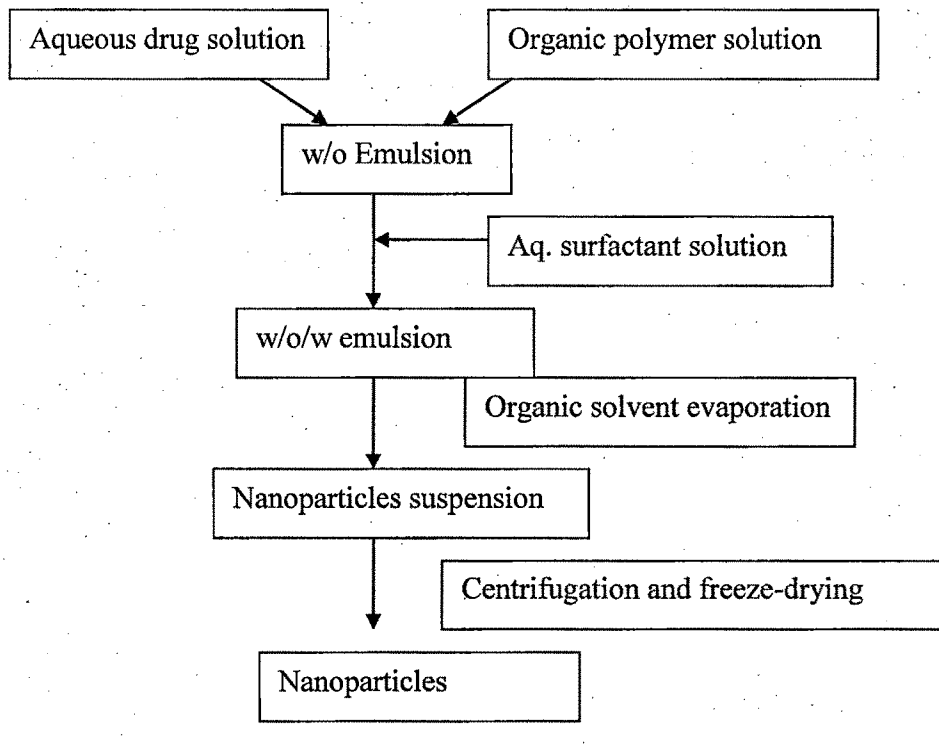
1. Emulsion-evaporation

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



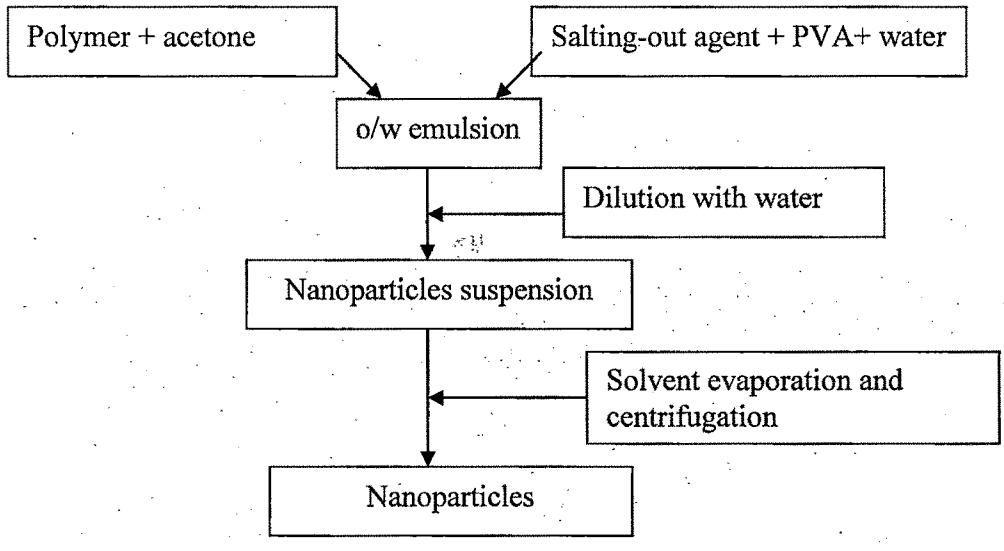
2. Double-emulsion evaporation

This procedure is used to encapsulate hydrophilic drugs and proteins.



3. Salting –out

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



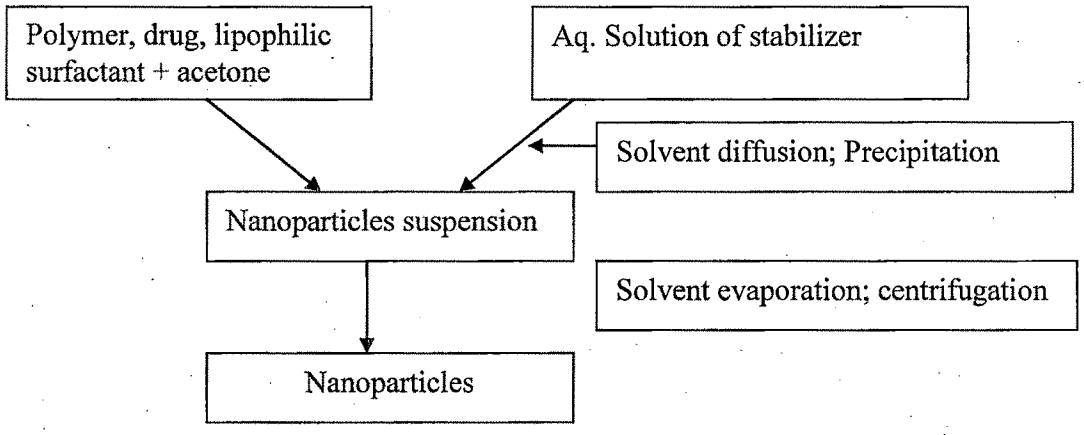
4. Emulsification-diffusion

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

5. Solvent displacement/Nanoprecipitation

This method is usually employed to incorporate lipophilic drugs into the carriers based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water from a lipophilic solution. Nanoprecipitation technique is simple, easy to execute and provides uniform particle size with narrow particle size distribution. This method is based on the interfacial deposition of a polymer following diffusion of a

semi-polar and miscible solvent in the aqueous medium in the presence of a surfactant/stabilizer.



The nanoparticle preparation process by nanoprecipitation method, apparently simple, may involve complex interfacial hydrodynamic phenomena. The origin of the mechanism of nanosphere formation could be explained in terms of interfacial turbulence or spontaneous agitation of the interface between two unequilibrated liquid phases, involving flow, diffusion and surface processes. (Marangoni effect) It is suggested by Derakhshandeh K et al., 2007 that the interfacial turbulence is caused by localized lowering of the interfacial tension where the organic phase undergoes rapid and erratic pulsations or "kicks" each of which is quickly damped out by viscous drag.

The molecular mechanism of interfacial turbulence could be explained by the continuous formation of eddies of solvent (e.g. acetone) at the interface. Such eddies originate either during drop formation or in thermal inequality in the system. Thus, once the process has started, movements associated with previous kicks change the pressure inside the solvent by increasing the surface pressure inside the solvent or decreasing the interfacial tension. Thus, if the solvent droplets formed contain polymer, these will tend to aggregate and form nanoparticles because of continuous diffusion of solvents and because of the presence of a nonsolvent medium (Guerrero DQ et al., 1998). Of the various factors, solute transfer out of the phase of higher viscosity, concentration gradients near the interface and interfacial tension sensitive to solute concentration are the most important factors. The presence of surfactant/stabilizer may markedly complicate the situation since they act to suppress interfacial flow and the rapid diffusion of acetone to the aqueous phase. The main advantage of surfactants in process is the instantaneous and reproducible formation of nanometric, monodispersed nanospheres. (Derakhshandeh K et al., 2007; Fessi H et al., 1989)

2.5.2 Stability of Nanoparticles

Freeze-drying is one of the well established methods for the preservation of unstable molecules over long periods of time. (Corveleyn S and Remon JP, 1996; Diminsky D et al., 1999; Li B et al., 2000) Most studies have shown a good preservation of the physicochemical properties of the particles when the lyoprotectant was employed in a sufficient concentration.

2.5.3 Techniques for Characterization of Nanoparticles

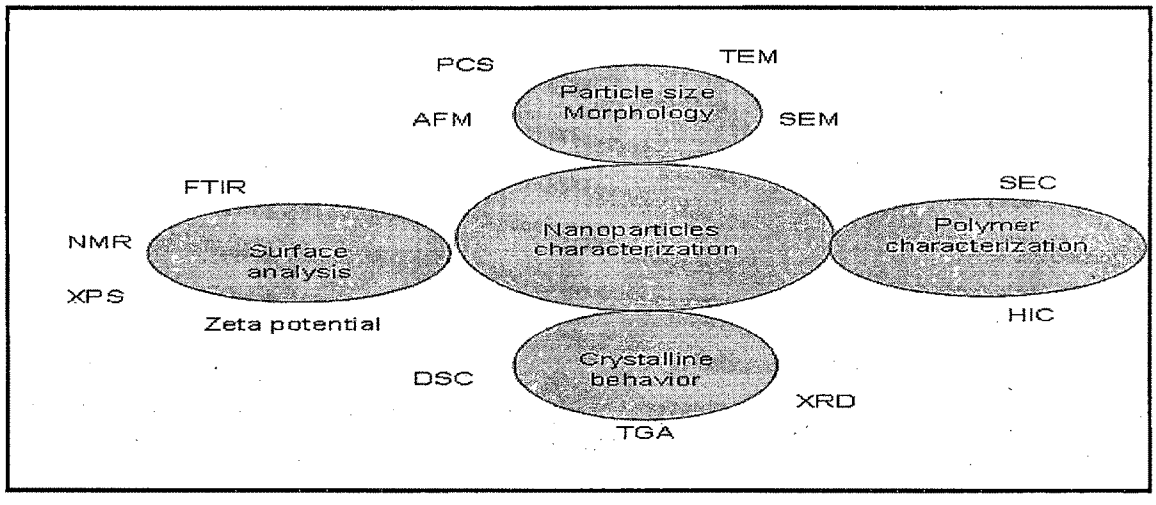
Characterization of the nanoparticle carrier systems to thoroughly understand the properties is essential before putting them to pharmaceutical application. After preparation, nanoparticles are characterized at two levels. The physicochemical characterization consists of the evaluation of the particle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

There are many sensitive techniques for characterizing nanoparticles, depending upon the parameter being looked at; laser light scattering (LLS) or photon correlation spectroscopy (PCS) for particle size and size distribution; scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for morphological properties; X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR),a and nuclear magnetic resonance spectroscopy (NMR) for surface chemistry; and differential scanning calorimetry (DSC) for thermal properties (Fig 2.7 shows this diagrammatically). Parameters such as density, molecular weight, and crystallinity affect release and degradation properties, where as surface charge, hydrophilicity, and hydrophobicity significantly influence interaction with the biological environment.

Particle size and Morphology

Nanoparticle size is critical not only in determining its release and degradation behaviour (Dunne M, 2000) but also in determining the efficacy of the therapeutic agent by affecting tissue penetration or even intracellular uptake. Particle size can be determined by Photon correlation spectroscopy, Scanning electron microscopy, Transmission electron microscopy, Atomic force microscopy.

Figure 2.7: Techniques to characterize nanoparticles



Surface chemistry analysis

X-ray photon spectroscopy, Fourier transform infrared spectroscopy and Nuclear magnetic spectroscopy are the techniques employed to analyze the surface chemistry.

Crystallinity

The physical state of both the drug and the polymer are determined because this will have an influence on the in vitro and in vivo release characteristics of the drug. The crystalline behaviour of polymeric nanoparticles is studied using X-ray diffraction and thermo-analytical methods such as differential scanning calorimetry (Ubrich N, 2004) (DSC) and differential thermal analysis (DTA) (Oh I, 1999). DSC and X-ray diffraction techniques are often combined to get useful information on the structural characteristics of both drugs and polymers.

Surface charge

Zeta potential is measure of the surface charge of the nanoparticles. The zeta potential value can influence particle stability and mucoadhesion as well as intracellular trafficking of nanoparticles as a function of pH. High zeta potential values, either positive or negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The extent of surface hydrophilicity can then be predicted from the values of zeta potential (Soppimath et al, 2001). Surface charge is generally detrmined by well-known electrophoresis method with the help of zetasizer (Panagi Z, 2001)

Hydrophobicity

Hydrophobicity determines the distribution of nanoparticles in the body after administration. Hydrophilic particles tend to remain in the blood for a longer time. Hydrophilicity is detrmined by water contant amgle measurements or hydrophobic interaction chromatography (Kreuter J, 1994)

Entrapment efficiency and drug release studies

The encapsulation efficiency depends on the preparation method, nanoparticle size, molecular weight and nature of the polymer and drug. It is mostly measured by Spectrophotometry and HPLC. HPLC assay analyses, size exclusion chromatography, and spectroscopic measurements are used to monitor drug release from nanoparticles. All of these techniques require separation of liberated drug from nanoparticle dispersion.

2.5.4 Nanoparticles conjugated to ligands in Receptor mediated transport systems

Receptor-mediated transport is responsible for the trafficking of larger molecules such as insulin and transferrin. In this system, a molecule binds a receptor expressed on the endothelial cell. When there is a "match," it is endocytosed into the cell and transported via vesicles to the basolateral membrane, thereby gaining access to the CNS. Receptor-mediated transport has been manipulated to allow anticancer agents to "piggyback" through the BBB with an agent that binds to a target receptor on the BBB endothelium. Although promising, particularly for delivery of large agents such as antibodies and genes, this technique is limited by transport systems that have low affinity and capacity, such that basal levels of the endogenous substrate may interfere with binding of engineered ligands. Peptides and proteins can undergo transport to the brain via RMT. Examples of receptors involved in RMT are the insulin receptor, the transferrin receptor, and the transporters for low-density lipoprotein, leptin and insulin-like growth factors. In general, RMT occurs in 3 steps: receptor-mediated endocytosis of the compound at the luminal (blood) side, movement through the endothelial cytoplasm, and exocytosis at the abluminal (brain) side of the brain capillary endothelium.

2.6 Intranasal Drug Delivery

An alternative CNS drug delivery strategy that has received relatively little attention is the intranasal route. Intranasal route of drug delivery delivers the drug rapidly and directly to CNS by circumventing the BBB. (Lianli Li et al., 2002) The administration of nanoparticles

through the intranasal route may not require the any ligand attachment or any surface modifier for delivery to brain.

Advantages of intranasal route for brain delivery:

- Noninvasive route for delivery of drug to brain.
- Delivers drug directly to brain bypassing BBB and reduces drug delivery to non targeted sites.
- Direct transport of drugs to the brain may lead to the administration of lower doses and reduce the toxicity.
- Systemic dilution effect and first pass metabolism can be avoided.

Intranasal administration is a promising approach for rapid-onset delivery of medications to the CNS, bypassing the BBB. However, there are also limitations. One of the biggest limitations is insufficient drug absorption through the nasal mucosa. Many drug candidates cannot be developed for the nasal route because they are not absorbed well enough to produce therapeutic effects. Another constraint concern in nasal administration is that a small administration volume is required, beyond which the formulation will be drained out into the pharynx and swallowed.

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

The investigations till date have 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.

The difficulties that have to be overcome include active degradation or alteration by enzyme, low pH of nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold. An obvious advantage of

intranasal route is that it is noninvasive relative to other routes of administration. Intranasal drug delivery delivers the drug directly to the brain by circumventing BBB and reduces drug delivery to non targeted sites. Direct transport of drugs to the brain may lead to the administration of lower doses and in turn can reduce toxicity. Systemic dilution effect and first pass metabolism are also avoided. Direct transport could result rapid and/or higher uptake in brain, which provides an alternative option of self-medication in management of emergencies (Lianli Li et al., 2002). However, the few limitations of intranasal delivery are low dose/volume especially when compounds have less aqueous solubility are difficult to formulate. High lipophilicity and preferably low molecular weight of drug are the prerequisites as it could influence the uptake across nasal mucosa. Drug compounds devoid of offensive/pungent odor/aroma and non-irritant nature are highly desirable to facilitate dosage form design for intranasal drug delivery systems. (Misra A et al., 2005)

2.6.1 Mechanisms of Transnasal Transport to the Brain

It is important to examine the pathway/mechanisms (Fisher AN et al., 1987; Wheatley MA et al., 1988; Tengamnuay P and Mitra AK, 1988) involved prior to addressing the possibilities to improve transnasal uptake by the brain. The olfactory region is known to be the portal for a drug substance to enter from nose-to-brain following nasal absorption. Thus, transport across the olfactory epithelium is the predominant concern for brain targeted intranasal delivery. Nasal mucosa and subarachnoid space; lymphatic plexus located in nasal mucosa and subarachnoid space along with perineural sheaths in olfactory nerve filaments and subarachnoid space appears to have communications between them. The nasal drug delivery to the CNS is thought to involve either an intraneuronal or extraneuronal pathway (Thorne RG and Frey WH, 2001; BormLange JT and Kern W, 2002).

A drug can cross the olfactory path by one or more mechanism/pathways (Fisher AN et al., 1987; Jones NS et al., 1997). These include paracellular transport by movement of drug through interstitial space of cells, transcellular or simple diffusion across the membrane or receptor/fluid phase mediated endocytosis and transcytosis by vesicle carrier (Mc Martin C et al., 1987) and neuronal transport. These three mechanisms mentioned are described in this section (below).

The paracellular transport mechanism/route is slow and passive. It mainly uses an aqueous mode of transport. Usually, the drug passes through the tight junctions and the open clefts of the epithelial cells present in the nasal mucosa. There is an inverse log-log correlation between intranasal absorption and the molecular weight of water-soluble compounds.

Compounds which are highly hydrophilic in nature and/or low molecular weight are most appropriate for paracellular transport. A sharp reduction in absorption and poor bioavailability were observed for the drugs having molecular weight greater than 1000 Da (Mc Martin C et al., 1987; Chien YW and Chang S, 1987). 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 (Illum L, 2003; Illum L, 2000) mainly encompasses transport via a lipoidal route. The drug can be transported across the nasal mucosa/epithelium by either receptor mediated endocytosis or passive diffusion or fluid phase endocytosis. Small lipophilic compounds or larger molecules usually are transported by a transcellular route. The transport across nasal mucosa is mainly a function of the lipophilic nature of a drug compound. Highly lipophilic drugs are expected to have rapid/complete transnasal uptake.

The neuronal transport of drug can take place via intercellular axonal transport. The olfactory neuron cells facilitate the drug transport principally to the olfactory bulb.

Potential nose-to-brain transport pathways followed by several drug molecules are recorded in Table 2.2 and possible drug transport routes are depicted in Fig. 2.8.

Figure 2.8: Possible transport pathways: nasal mucosa to brain/CNS

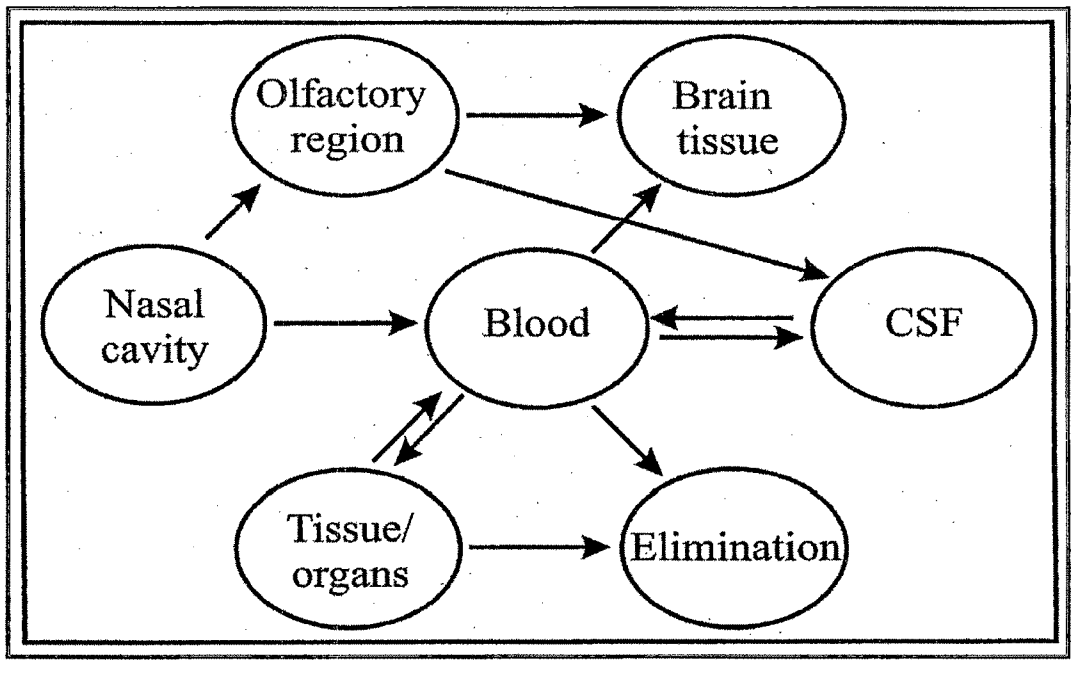


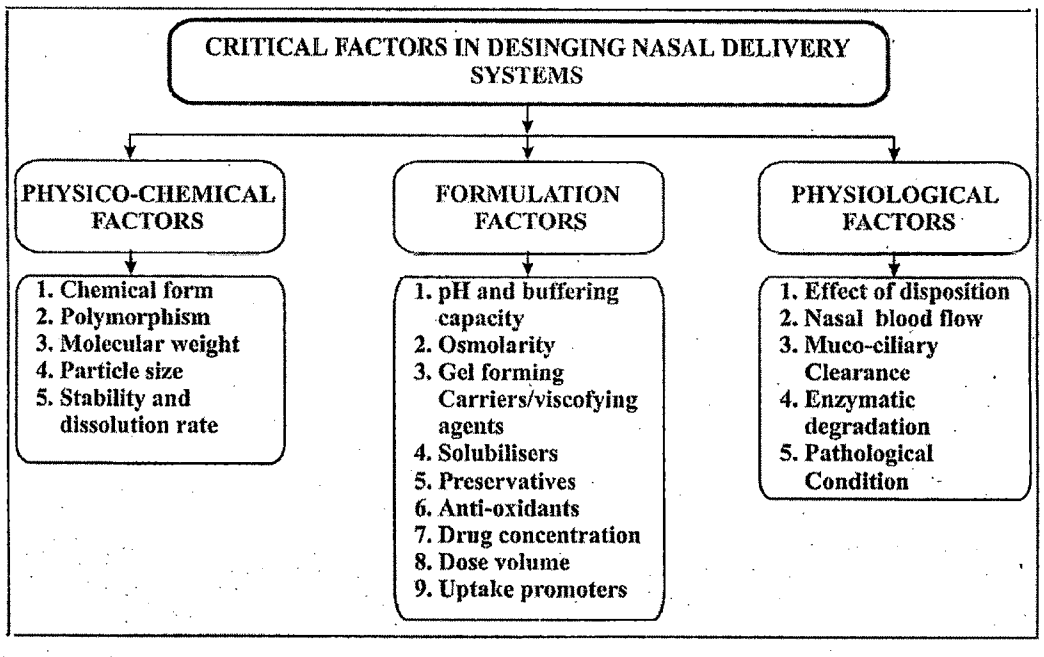
Table 2.2: Nose-to-brain transport of drug molecules and possible pathways

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

2.6.2 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. As shown in (Figure 2.9), some of the physicochemical factors are Chemical form (Huang C et al., 1985), polymorphism, particle size, solubility and most importantly molecular weight (Agrawal V and Mishra B, 1995; Corbo D et al., 1989). Moreover, several other factors like formulation factors (Perras B et al., 1999; Dahlin M and Bjork E, 2000) in addition to physiological factors (Kroll RA and Neuwelt EA , 1998; Morimoto K et al., 2001; Novartis Pharma Inc., 1999; Schipper NM et al., 1995) are also having decisive repercussion on the *in vivo* result/performance of the product and in turn influence the uptake of drug at targeted site. Some of the imperative physicochemical, formulation and biological factors are described.

Figure 2.9: Critical physicochemical factors need to be considered prior to designing intranasal drug delivery systems



2.7 Microemulsion and Nanoemulsion

2.7.1 Microemulsion

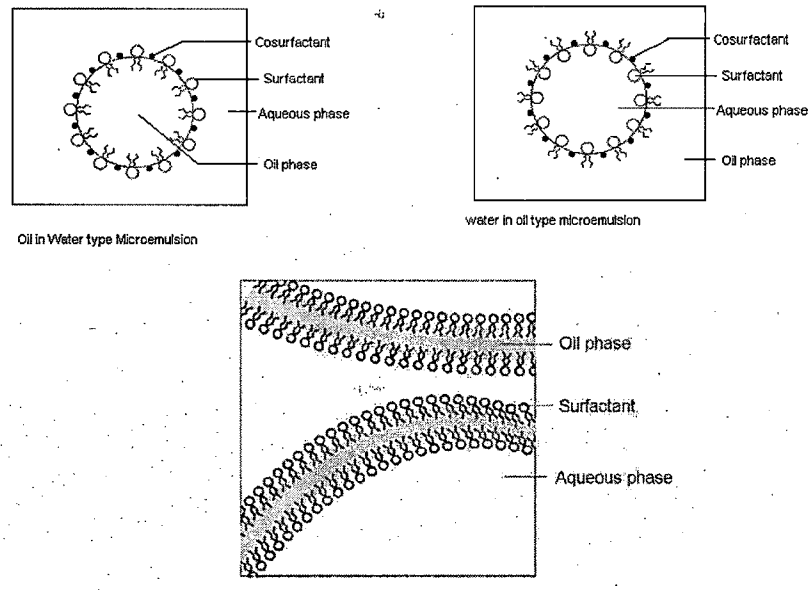
Among the novel systems for brain delivery through intranasal route, microemulsions gained considerable interest for their simple formulation with more stability and optical clarity and efficient to across the biological membranes, biocompatibility, biodegradability, easy to prepare and handle and most importantly solubilization capacity for both water and oil soluble drugs. Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multicomponent fluids composed of oil, water and surfactant (usually in conjunction with a co-surfactant).

The droplets in a micro emulsion are in the range of 1nm-100nm in diameter. The dispersal of drug as a solution in nano meter-sized droplets enhances the rate of dissolution into contacting aqueous phase and in vivo generally results in increase in drug bioavailability. In addition, the presence of surfactant and in some cases co-surfactant, for example medium chain triglycerides in many cases serve to increase membrane permeability thereby increasing the drug uptake.

Microemulsions have various textures such as oil droplets in water, water droplets in oil, bi continuous mixtures (Fig 2.10). ordered droplets or lamellar mixtures with a wide range of phase equilibria among them and with excess oil and/or water phases. This great variety is

governed by variations in the composition of the whole system and in the structure of the interfacial layers.

Fig. 2.10: O/W type, W/O type and bicontinuous microemulsion



2.7.1.1 Thermodynamics of microemulsion

The important features of the microemulsion are thermodynamic stability,optical transparency, large overall interfacial area (about 100 m^2 /ml), variety of structures like ordered droplets on lamellar mixtures with wide range of phase equilibria with excess oil/water phases, low interfacial tension and increased solubilization of oil/water dispersed phase. Microemulsion requires more surfactant than emulsion to stabilize a large overall interfacial area.

The interfacial tension between the oil and water can be lowered by the addition and adsorption of surfactant. When the surfactant concentration is increased further, if lowers the interfacial tension till CMC (Critical Micelle Concentration), after which micelles are formed. This negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. The large interfacial area formed may divide itself into a large number of closed shells around small droplets of either oil in water or water in oil and further decrease the free energy of the system.

2.7.1.2 Factors affecting the type of microemulsion

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

Packing Ratio

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

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

Where, V=Volume of surfactant molecule

a = Head-group surface area

l =length

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

Property of surfactant, oil phase and temperature

The type of emulsion, to a large extent, depends on the nature of surfactant; Gerbacia E & Rosano HL (1973) observed that the interfacial tension could be temporarily reduced due to diffusion of cosurfactant through the interface. Microemulsion is formed by the combination of dispersion and stabilization processes. The dispersion process involves a transient reduction of interfacial tension to nearly zero or negative value at which the interface expands to form fine dispersed droplets. Subsequently, these absorb more surfactant until the bulk phase is depleted enough to bring the value of interfacial tension positive. Type of surfactant also determines type of microemulsion formed. Surfactant contains hydrophilic head group and lipophilic tail group. The areas of these groups, which are a measure of the differential tendency of water to swell head group and of oil to swell the tail area are important for specific formulation when estimating the surfactant HLB in a particular system.

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils, such as alkanes, penetrate the lipophilic group region largely than long chain alkanes and swelling of this region to a great extent results in an increased negative curvature. Temperature is extremely important in determining the effective head group size of non ionic surfactants. Winsor studied the effect of temperature on the type of microemulsion formed. For the given amount of components in

ternary system with nonionic surfactant, oil, and water, at relatively low temperatures, type I system (an oil in water with excess oil) is formed. At intermediate temperature type III system (microemulsion with excess of both oil and water) is present. At relatively higher temperature type II (water in oil microemulsion with excess water) system exist (Winsor PA 1954, 1968).

The chain length, type and nature of cosurfactant

Alcohols are widely used as a cosurfactant in microemulsions. Addition of shorter chain cosurfactant (eg. Ethyl alcohol) gives positive curvature effect, as alcohol swells the head region more than tail region and o/w type is favoured. While longer chain cosurfactant (eg. Cetyl alcohol) favours w/o type by alcohol swelling more in tail region than head region.

2.7.1.3 Factors affecting phase behavior of microemulsion

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

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

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

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

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

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

2.7.1.4 Ternary Phase Diagrams

The phase behaviour of surfactant-oil-water (S/O/W) is best reported by using ternary diagram. Here, two independent composition variables are sufficient, since third one is complement to 100% (Fig 2.12). The phase diagram allows one to determine ratio of oil: water, surfactant-cosurfactant at the boundary of microemulsion region. To plot the composition of four component systems, a regular tetrahedron composed by fixing and varying the other three or by using a constant ratio of two components (surfactant and cosurfactant or co solvent). Fig. 2.12 shows the pseudo ternary diagram at constant surfactant to cosurfactant ratio. It also shows that single phase or multiphase regions of microemulsion domain are near the centre of diagram in areas containing large amounts of surfactant that is toxic. The phase behaviour of surfactants, which form microemulsions in absence of cosurfactant, can be completely represented by ternary diagram.

Winsor's regions: Winsor PA (1954) reported the relationship between the phase behaviour of amphiphiles-oil-water and nature of the different components of ternary system. Different regions of a phase diagram are shown in Fig. 2.11

Figure 2.11: Different regions of phase diagram

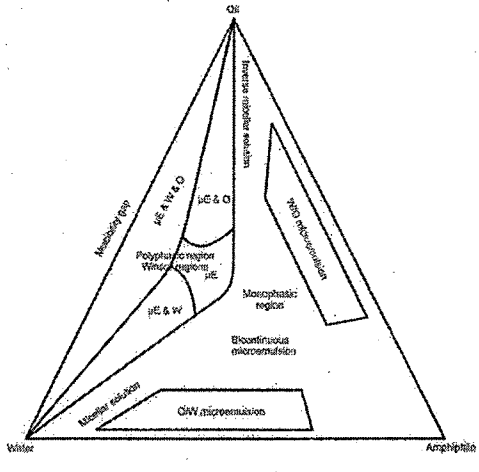
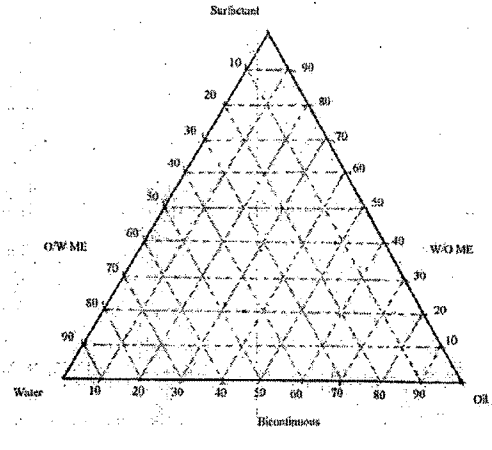


Figure 2.12: Ternary system



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

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

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

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

Methods for constructing Phase diagram

Phase diagrams should be constructed to define the extent and nature of the microemulsion regions and surrounding regions.

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

2.7.1.5 Formulation of microemulsions

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

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

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

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

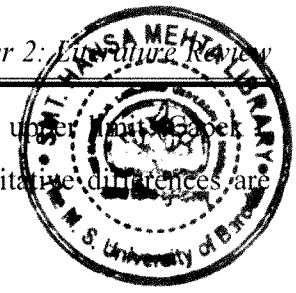
d) Secondary surfactant (cosurfactant): co surfactants originally used were short chain fatty alcohols (pentanol, hexanol, benzyl alcohol). These are most often polyols, esters of polyols, derivatives of glycerol and organic acids. Their main purpose is to make the inter facial film fluid by wedging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

2.7.2 Comparision Microemulsion Vs Nanoemulsion

There are two major misunderstandings in the literature regarding nano-emulsions. One arises from their similarities tomicroemulsions. Nano-emulsions are emulsions with an extremely small droplet size (Solans C et al., 2003) which can overlap those of microemulsions. The definition of emulsions by the International Union of Pure and Applied Chemistry (IUPAC) states: "In an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid" (IUPAC, Manual of colloid science, 1972). Obviously, microemulsions are excluded from this definition if the word "dispersed" is interpreted as non-equilibrium and opposite to "solubilized", a term that can be applied to microemulsions and micellar systems. Therefore, there is a fundamental difference between microemulsions and nanoemulsions: microemulsions are equilibrium systems (i.e. thermodynamically stable), while nano-emulsions are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases. Nevertheless, nano-emulsions may possess a relatively high kinetic stability, even for several years (Solans C et al., 2003). The other source of misunderstandings about nano-emulsions is related to the method of preparation. For instance, in Ref. (Mason TJ et al., 2006) a review on nano-emulsions, only emulsions with droplet size in the nanometer range obtained by shear methods are considered as nano-emulsions. According to these authors, emulsions with extremely small droplet size (i.e. nano-emulsions) obtained by the so-called condensation methods (e.g. phase inversion temperature (PIT) or composition (PIC) methods, self- emulsifying methods, etc.) should not be considered as nano-emulsions.

2.7.3 Nanoemulsion

As a summary of this point, nano-emulsions are emulsions (non-equilibrium systems, defined according to (Solans C et al., 2003)) with a remarkable small droplet size (in the nanometer range, e.g. 20– 200 nm), regardless of the preparation method. Evidently the size range may



vary depending on the authors. Some authors consider 500 nm as the upper limit (Tadros T et al., 2004) In any case, the size limit is not a key issue because no qualitative differences are established by droplet size.

2.7.3.1 Formulation of Nanoemulsion

Nano-emulsions, being non-equilibrium systems, cannot be formed spontaneously. Consequently, energy input, generally from mechanical devices or from the chemical potential of the components, is required. Nano-emulsion formation by the so-called dispersion or high-energy emulsification methods is generally achieved using high- shear stirring, high-pressure homogenizers and ultrasound generators. It has been shown that the apparatus supplying the available energy in the shortest time and having the most homogeneous flow produces the smallest sizes [12]. High-pressure homogenizers meet these requirements. Therefore, they are the most widely used emulsifying machines to prepare nano-emulsions.

Resently, Ultrasonication method has been explored for preparation of Nanoemulsion. (Kentish S et al., 2008) Studies to date comparing ultrasonic emulsification with rotor–stator dispersing have found ultrasound to be competitive or even superior in terms of droplet size and energy efficiency (Abismail B et al., 1999, Tadros T et al., 2004). Microfluidization has been found to be more efficient than ultrasound, but less practicable with respect to production cost, equipment contamination and aseptic processing (Abismail B et al., 1999). Comparing mechanical agitation to ultrasound at low frequency, Tadros T et al. (2004) found that for a given desired diameter, the surfactant amount required was reduced, energy consumption (through heat loss) was lower and the ultrasonic emulsions were less polydisperse and more stable.

Ultrasonic emulsification is believed to occur through two mechanisms. Firstly, the application of an acoustic field produces interfacial waves which become unstable, eventually resulting in the eruption of the oil phase into the water medium in the form of droplets. (Li MK and Fogler HS, 1978a) Secondly, the application of low frequency ultrasound causes acoustic cavitation, that is, the formation and subsequent collapse of microbubbles by the pressure fluctuations of a simple sound wave. Each bubble collapse (an implosion on a microscopic scale) event causes extreme levels of highly localized turbulence. The turbulent micro-implosions act as a very effective method of breaking up primary droplets of dispersed oil into droplets of sub-micron size. (Li MK and Fogler HS, 1978b)

Lipid NE of drug prepared using Ultrasonic technique constituted of one or combination of emulsifiers. For the preparation of o/w NE, aqueous and oil phases were separately prepared. The aqueous phase consisted of water and hydrophilic surfactant. The oil phase consisted of oil, lipophilic surfactant and drug. The two phases were heated separately to 50–60 °C. Then oil phase was gradually added to the aqueous phase with constant stirring on a magnetic stirrer at this temperature, to get coarse emulsion. The resultant coarse emulsion was sonicated using sonicator.

2.7.3.2 Stability of Nanoemulsion

The main limitation for developing applications for nano-emulsions is their stability. Although practically all papers on nano-emulsions indicate that nano-emulsions can be stable even by years, the small droplet size makes nano-emulsions break by the Ostwald ripening mechanism (Taylor P, 1995) in time periods which pose a great limitation for developing applications different than nanoparticle preparation. In fact, only an extremely low solubility of the dispersed phase, as presented by silicone oils, would give the stability needed for most of the applications.

Pharmacy is the field where more direct applications of nano-emulsions are proposed. Many of them consist in self-emulsifying systems, so the stability problem is solved by using the nano-emulsions short after their preparation. Self-emulsifying nano-emulsions for parenteral application (Kelmann NG et al., 2007) or oral application (Nielsen FS et al., 2007) have been dsescribed.

2.7.4 Characterization of Microemulsion and Nanoemulsion

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

Phase Behaviour Studies

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

phases as a function of composition variables and temperature. They also allow comparison of the efficiency of different surfactant for given application.

Dynamic light scattering

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

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

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

Electron microscopic study

The microemulsion/Nanoemulsion can be characterized by electron microscopic techniques eventhough although the high liability of the samples and the possibility of artifacts, electron microscopy is used to study microstructure/nanostructure. The ME/NE systems are observed under microscope either followed by chemical or thermal fixation methods. But the thermal fixation method, especially freeze fracture electron microscopy has also been used to study structure; in which extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts. It has been reported that other than CRYO-TEM, the direct observation of the ME/NE over the grid followed by normal air drying is also an useful tool in the study of structure and it's size analysis (Shafiq S et al., 2007).

Rheological properties and viscosity measurements

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

Stability studies

The stability of the micro emulsion has been assessed by conducting long term stability study and accelerated stability studies. In long term stability study, the system is kept at room temperature and refrigeration temperature. Over the time period ME/NE systems are evaluated for their size, zeta potential, assay, pH, viscosity and conductivity. On long term study, the activation energy for the system and shelf life of the system may be calculated as like other conventional delivery system. (Nornoo AO and Chow DS, 2008)

Accelerated stability studies are the essential tools to study the thermodynamic stability of micro emulsions. It can be done by centrifugation, heating/cooling cycle and freeze/thaw cycles. NE are not subjected to freeze/thaw cycle.

1. In the centrifugation, the system is subjected to centrifugation at 10000 rpm for 30 minutes and followed by the observation for phase separation
2. The Heating / Cooling cycle (six) of keeping the system at 4°C and 45°C for not less than 48 hours at each stage.
3. Freeze/ Thaw cycles of micro emulsion can be done between - 21°C and 25°C or between 5°C and 10°C (Shafiq S et al., 2007).

2.7.5 Pharmaceutical applications of Microemulsion and nanoemulsion

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

Regarding applications, nano-emulsions were firstly developed, and used for a long time, to obtain nanoparticles by polymerization (Ugelstad J et al., 1973), the so-called miniemulsion polymerization method, and more recently to obtain solid lipid nanoparticles (Müller RH et al., 2007), and ceramic particles (Porras M et al., 2005). At present, new applications are being developed to use nano-emulsions as consumer products. Nano-emulsion formulations are studied for their application as nanocarriers which allow the treatment of a variety of diseases. The following are examples of the most recent proposals of drugs solubilised in nano-emulsions for disease treatments: anticonvulsant (Kelmann NG et al., 2007), antihypertensive (Shafiq S et al., 2007) antibiotic (Date AA and Nagarkenser MS, 2007); antinflammatory applied through skin (Calderilla-Fajardo SB et al., 2006). There are reports on: drugs solubilised in nano-emulsions for HIV/AIDS therapy (Vyas TK et al., 2008);

cancer therapy investigated by solubilizing the drug in a cholesterol rich nano-emulsion (Dias M et al., 2007); and application of anthrax vaccine through W/O nanoemulsions (Bielinska AU et al., 2007). Special magnetic nano-emulsions are also being studied for medicine applications (Primo FL et al., 2007).

2.8 Drug Profile

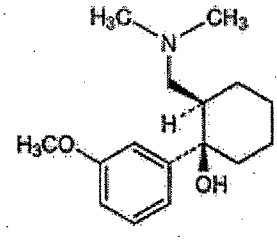
2.8.1 Tramadol

Description

- **Synonym:** Tramadol HCl
- **Drug Category:** Analgesics, Analgesics- Opioid
- **Chemical IUPAC Name:** (1R,2R)-2-(dimethylaminomethyl)-1-(3methoxyphenyl) cyclohexan-1-ol
- **Chemical Formula:** $C_{16}H_{25}NO_2$
- **Brand Names:** Ultram, Crispin, Ralivia Flashtab, Tramal, Tridural, Zydol
- **Indication:** Indicated in the treatment of moderate to severe pain.
- **Route:** Oral, IV, IM, rectal, sublingual, buccal, intranasal
- **Dosage Forms:** Tablet, extended release tablet

Physiochemical properties

- **Molecular Weight:** 263.3752 g/mol
- **Melting Point:** 84-86°C
- **LogP:** 2.4
- **Chemical Structure:**



Pharmacology: Tramadol, a centrally-acting analgesic, exists as a racemic mixture of the *trans* isomer, with important differences in binding, activity, and metabolism associated with the two enantiomers. Although Tramadol is a synthetic analog of codeine, it has a significantly lower affinity for opioid receptors than codeine. Tramadol is used to treat postoperative, dental, cancer, and acute musculosketetal pain and as an adjuvant to NSAID therapy in patients with osteoarthritis.

Mechanism of Action: Tramadol and its O-desmethyl metabolite (M1) are selective, weak OP3-receptor agonists. Opiate receptors are coupled with G-protein receptors and function as both positive and negative regulators of synaptic transmission via G-proteins that activate effector proteins. As the effector system is adenylate cyclase and cAMP located at the inner surface of the plasma membrane, opioids decrease intracellular cAMP by inhibiting adenylate cyclase. Subsequently, the release of nociceptive neurotransmitters such as substance P, GABA, dopamine, acetylcholine and noradrenaline is inhibited. The analgesic properties of Tramadol can be attributed to norepinephrine and serotonin reuptake blockade in the CNS, which inhibits pain transmission in the spinal cord. The (+) enantiomer has higher affinity for the OP3 receptor and preferentially inhibits serotonin uptake and enhances serotonin release. The (-) enantiomer preferentially inhibits norepinephrine reuptake by stimulating alpha(2)-adrenergic receptors.

Tramadol has inhibitory actions on the 5-HT_{2C} receptor. Antagonism of 5-HT_{2C} could be partially responsible for tramadol's reducing effect on depressive and obsessive-compulsive symptoms in patients with pain and co-morbid neurological illnesses.[55] The overall analgesic profile of tramadol supports intermediate pain especially chronic states, is slightly less effective for acute pain than hydrocodone, but more effective than codeine.

Pharmacodynamic: Tramadol, a centrally acting synthetic opioid analgesic. Although its mode of action is not completely understood, from animal tests, at least two complementary mechanisms appear applicable: binding of parent and M1 metabolite to μ-opioid receptors and weak inhibition of re-uptake of norepinephrine and serotonin.

Opioid activity is due to both low affinity binding of the parent compound and higher affinity binding of the O-demethylated metabolite M1 to μ-opioid receptors. In animal models, M1 is up to 6 times more potent than tramadol in producing analgesia and 200 times more potent in μ-opioid binding. Tramadol-induced analgesia is only partially antagonized by the opiate antagonist naloxone in several animal tests. The relative contribution of both tramadol and M1 to human analgesia is dependent upon the plasma concentrations of each compound. Tramadol has been shown to inhibit reuptake of norepinephrine and serotonin *in vitro,* as have some other opioid analgesics.

Pharmacokinetic

Absorption: Racemic tramadol is rapidly and almost completely absorbed after oral administration. The mean absolute bioavailability of a 100 mg oral dose is approximately 68%.The mean peak plasma concentration of racemic tramadol and M1 occurs at two and three hours, respectively, after administration in healthy adults.

Distribution: The volume of distribution of tramadol was 2.6 and 2.9 liters/kg in male and female subjects, respectively, following a 100 mg intravenous dose. The binding of tramadol to human plasma proteins is approximately 20% and binding also appears to be independent of concentration up to 10 μg/mL. Saturation of plasma protein binding occurs only at concentrations outside the clinically relevant range.

Metabolism: Tramadol is extensively metabolized after oral administration by a number of pathways, including CYP2D6 and CYP3A4, as well as by conjugation of parent and metabolites. Approximately 30% of the dose is excreted in the urine as unchanged drug, whereas 60% of the dose is excreted as metabolites. The remainder is excreted either as unidentified or as unextractable metabolites. The major metabolic pathways appear to be *N*- and *O*-demethylation and glucuronidation or sulfation in the liver. One metabolite (*O*-desmethyltramadol, denoted M1) is pharmacologically active in animal models. Formation of M1 is dependent on CYP2D6 and as such is subject to inhibition, which may affect the therapeutic response.

Elimination: Tramadol is eliminated primarily through metabolism by the liver and the metabolites are eliminated primarily by the kidneys. The mean terminal plasma elimination half-lives of racemic tramadol and racemic M1 are 6.3 ± 1.4 and 7.4 ± 1.4 hours, respectively. The plasma elimination half-life of racemic tramadol increased from approximately six hours to seven hours upon multiple dosing.

Toxicity: LD_{50}=350mg/kg (orally in mice)

Side effects: A The most commonly reported adverse drug reactions are nausea, vomiting, sweating and constipation. Drowsiness is reported, although it is less of an issue than for non-synthetic opioids. Other side effects are dizziness, headache, diarrhoea, dry mouth, fatigue, seizure.

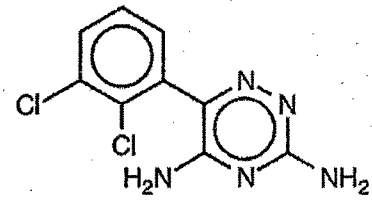
2.8.2 Lamotrigine HCl

Description

- **Synonym:** Lamotrigine
- **Drug Category:** Analgesics, Anticonvulsants
- **Chemical IUPAC Name:** 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine
- **Chemical Formula:** $C_9H_7Cl_2N_5$
- **Brand Names:** Lamictal , Lamictal Cd
- **Indication:** Partial seizures, Primary generalized tonic-clonic seizures
  Generalized seizures of Lennox-Gastaut syndrome
  For treatment of neuropathic pain
- **Route:** Oral
- **Dosage Forms:** Tablet, chewable tablet

Physiochemical properties

- **Molecular Weight:** 256.091 g/mol
- **Melting Point:** 216-218 °C
- **Solubility:** very slightly soluble in water (0.17 mg/mL at 25°C)
- **LogP:** 1.497
- **Chemical Structure:**



Pharmacology: Lamotrigine, an antiepileptic drug (AED) of the phenyltriazine class, is chemically unrelated to existing antiepileptic drugs. Lamotrigine is also used in the treatment of depression and bipolar disorder. Lamotrigine is thought to exert its anticonvulsant effect by stabilizing presynaptic neuronal membranes. Lamotrigine inhibits sodium currents by selectively binding to the inactivated state of the sodium channel and subsequently suppresses the release of the excilatory amino acid, glutamate.

Mechanism of Action: One proposed mechanism of action of Lamotrigine, the relevance of which remains to be established in humans, involves an effect on sodium channels. *in vitro* pharmacological studies suggest that lamotrigine inhibits voltage-sensitive sodium channels,

thereby stabilizing neuronal membranes and consequently modulating presynaptic transmitter release of excitatory amino acids (e.g., glutamate and aspartate).

Pharmacokinetic

Absorption: Lamotrigine is rapidly and completely absorbed after oral administration with negligible first-pass metabolism (absolute bioavailability is 98%). The bioavailability is not affected by food. Peak plasma concentrations occur anywhere from 1.4 to 4.8 hours following drug administration. The lamotrigine chewable/dispersible tablets were found to be equivalent, whether they were administered as dispersed in water, chewed and swallowed, or swallowed as whole, to the lamotrigine compressed tablets in terms of rate and extent of absorption. In terms of rate and extent of absorption, lamotrigine orally disintegrating tablets whether disintegrated in the mouth or swallowed whole with water were equivalent to the lamotrigine compressed tablets swallowed with water.

Distribution: Estimates of the mean apparent volume of distribution (Vd/F) of lamotrigine following oral administration ranged from 0.9 to 1.3 L/kg. Vd/F is independent of dose and is similar following single and multiple doses in both patients with epilepsy and in healthy volunteers. Data from in vitro studies indicate that lamotrigine is approximately 55% bound to human plasma proteins at plasma lamotrigine concentrations from 1 to 10 mcg/ml (10 mcg/ml is 4 to 6 times the trough plasma concentration observed in the controlled efficacy trials). Because lamotrigine is not highly bound to plasma proteins, clinically significant interactions with other drugs through competition for protein binding sites are unlikely. The binding of lamotrigine to lasma proteins did not change in the presence of therapeutic concentrations of phenytoin, phenobarbital, or valproate. Lamotrigine did not displace other AEDs (carbamazepine, phenytoin, phenobarbital) from protein binding sites.

Metabolism: Lamotrigine is metabolized predominantly by glucuronic acid conjugation; the major metabolite is an inactive 2-N-glucuronide conjugate. After oral administration of 240 mg of 14 C-lamotrigine (15 μCi) to 6 healthy volunteers, 94% was recovered in the urine and 2% was recovered in the feces. The radioactivity in the urine consisted of unchanged lamotrigine (10%), the 2-N-glucuronide (76%), a 5-N-glucuronide (10%), a 2-N-methyl metabolite (0.14%), and other unidentified minor metabolites (4%).

Elimination: . 94% of an orally administered dose of LTG is eliminated in the urine. Elimination half life is 24.1 ± 5.7 h

Toxicity: LD_{50}=250 (mg/kg) (in rat, mice)

Adverse effects: The most commonly observed adverse experiences associated with the use of adjunctive therapy with lamotrigine (incidence of at least 10%) were dizziness, headache, diplopia, somnolence, ataxia, nausea and asthenia. Other advers effects are serious skin rashes including Stevens-Johnson syndrome and toxic epidermal necrolysis, hypersensitivity reactions, acute multiorgan failure, blood dyscrasias, suicidal behavior and ideation, withdrawal seizures, status epilepticus, sudden unexplained death in epilepsy

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