CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

A large number of therapeutic agents are found to be ineffective in the treatment of cerebral diseases due to their inability to effectively and efficiently delivered and 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 and neurodegenerative disorders and far outnumber of those are victimized from several types of depression (Misra et al., 2003). The clinical failure of much potentially effective therapeutics is often not due to a lack of drug potency but mainly due to shortcomings in the delivery approach for delivering the drug to treat the diseases. Treating CNS diseases is challenging and a daunting task because a variety of formidable obstacles often impede drug delivery to the brain and spinal cord (Misra et al., 2003).

2.1 Central Nervous System

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

2.2 Barriers to CNS Drug Delivery

Blood Brain Barrier:

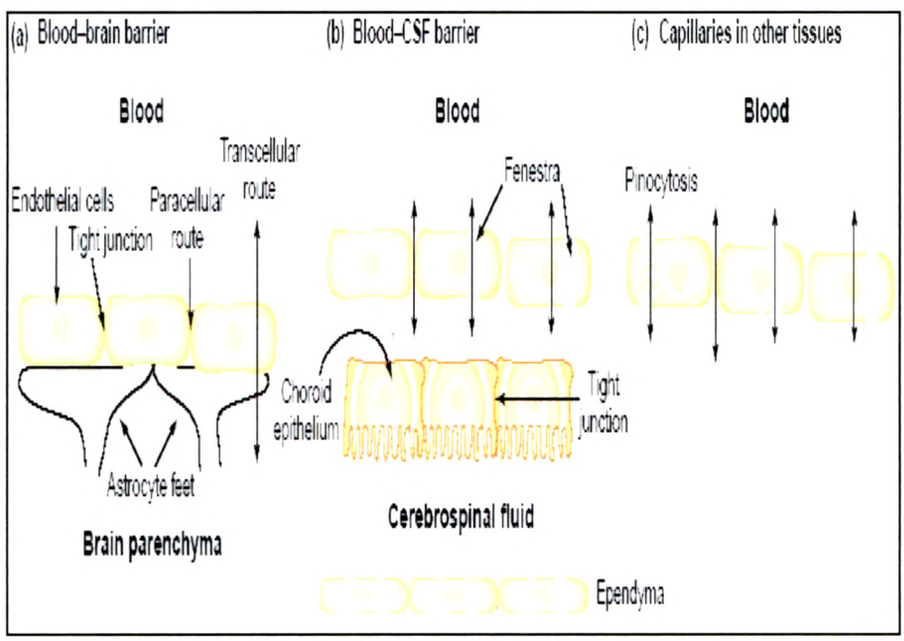
The BBB is a membranous barrier separates the brain from the surrounding circulating blood (Begley D J., 1996; Schlossauer B et al., 2002). Because of different structure of brain capillary compare to other tissues, it provides permeability barrier to most of the penetrants from extra cellular fluid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

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 et al., 1975; Brightman M W, 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 et al., 1993; Witt K A et al., 2001).

Figure 2.1 : Barriers to brain delivery



Brain Cerebrospinal Fluid Barrier

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

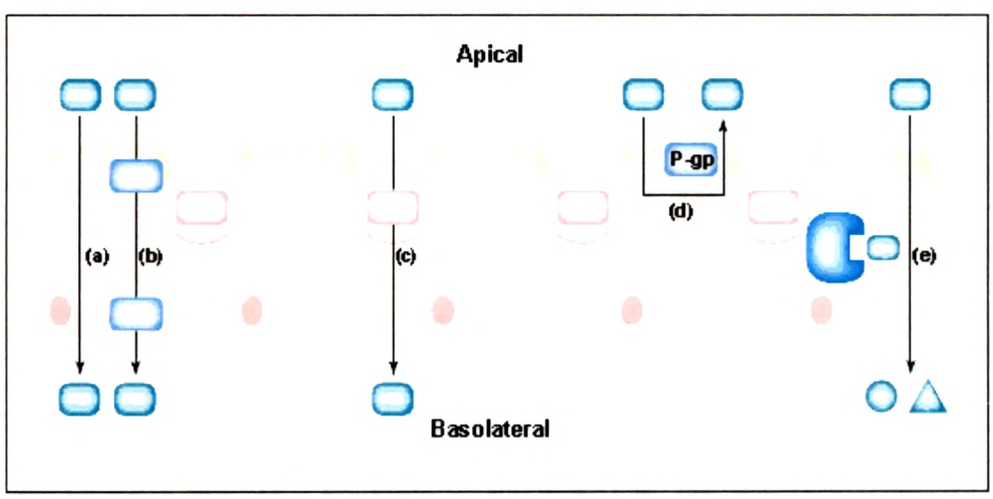
Brain Tumor Barrier

Intracranial drug delivery is even more challenging when the target is a CNS tumor. In CNS malignancies where the BBB is significantly compromised, a variety of physiological

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

Efflux Transporters

Figure 2.2: Efflux transporters at Blood Brain Barrier



A thorough understanding of the two way transport mechanisms uptake and efflux through BBB is of great importance in targeting drugs to the brains or to minimize the unwanted adverse effects some therapeutically active molecules. The efflux mechanisms in the CNS are passive or active. 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 S P C et al., 1992; Taylor E M., 2002).

2.3 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 S P et al., 1989; Hansch 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 et al., 2001; Lin J H et al., 1997; Lewis D F V., 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 (Olendorf W H et al., 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 W M et al., 1990).

Brain uptake can be positively correlated with lipid solubility or negatively correlated with hydrogen bonding (Cornford E M et al., 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.

2.4 Drug delivery approaches for brain-targeting

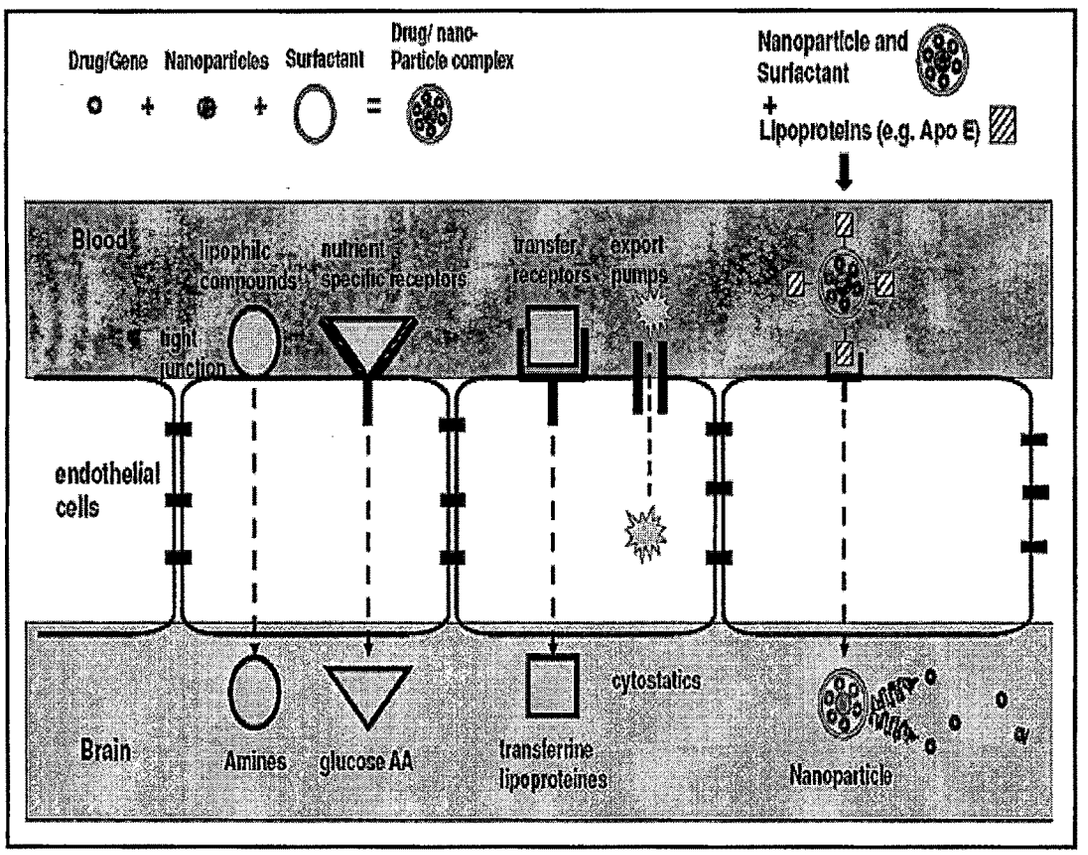
1. **Significance of Lipophilicity:** Octanol/Water partition coefficient , log $P_{o/w}$ (Buchwald P et al., 2002) is very commonly acceptable and convenient approach to predict lipophillicity and relative lipophillicity of any system. However, log $P_{o/w}$ alone seems to have a very limited application 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 et al., 2001; Lewis D F V et al., 2002; Lin J H et al., 1997)
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 et al., 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.
3. **Intracerebral Delivery:** BBB can be successfully bypassed using the most direct and invasive approach like intracerebral delivery of broad class of drugs using traditional and novel drug delivery system based dosage forms like injectables, controlled release polymers (Langer R et al., 1991; Brem H et al., 2000)/microspheres (Benoit J P et al., 2000; Illum L et al., 1988; Blork E et al., 1990; Edman P et al., 1992) or eventually microencapsulated recombinant cells. The basic impediment is very limited and slow diffusion within the brain due to very compact, tightly packed brain cells having limited interstitial space and unusually tortuous pathways.
4. **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.

5. **Intranasal delivery:** Intranasal delivery is being gaining a remarkable importance for CNS targeting. Nasal mucosa is having connection with CNS through intraneuronal or extraneuronal pathways. The drug/formulation applied on the nasal mucosa may follow either intraneuronal or extraneuronal or both pathways (Thorne R G et al., 1994; Born J et al., 2002).
    (i) Intraneuronal - It involves internalization into primary neurons of the olfactory epithelium, followed by distribution into other CNS areas.
    (ii) Extraneuronal - It involves absorption across the nasal epithelium to submucosa, followed by direct access to CSF or extracellular transport within perineuronal channels into CNS.

    The limitations and problems encountered in Nasal deliveries are enzymatic degradation, low pH nasal epithelium, nasal irritation possibilities, large variability in terms of nasal pathology, large molecular weight of active compounds and low lipophillicity etc. (Behl C R et al., 1998).
6. **BBB Disruption:** One of the approaches to circumvent the dense microvasculature of the brain is was by delivery using a transient osmotic opening. Hyperosmolar substances like mannitol, arabinose is likely to cause disruption of BBB (Miller G et al., 2002) due to migration of water from endothelial cells to capillaries which in turn cause shrinkage of the cells and results in intracellular gaps. The approach was resulted and breaks down the self defense mechanism of the brain and leaves it vulnerable. The other approaches are BBB disruption using use of labradimil which has selectivity for bradykinin B_2 receptor and Ultrasound-induced mild hyperthermia which can be controlled and localized to a small volume within the tissue. The former approach may lead to membrane permeability due to hyperthermia and the later one is under consideration and at a considerable distance from practical application (Chow C W et al., 2002).

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



7. **Nanoparticles:** Nanoparticles has been employed as a delivery system for compounds like dalargin, kyotorphin, loperamide and doxorubicin in some animals (Kreuter J et al., 2001; Kreuter J et al., 2002). The probable mechanism could be endocytic uptake or transcytosis. The particles are usually 10 to 1000 nm diameter, made from natural or artificial polymers; drugs are bound in form of solid solution or dispersion.
8. **Receptor mediated transport:** The receptor transport is mainly based on the formation of chimeric peptides by conjugation of the drugs that has to be delivered to a transport vector that undergoes BBB-transport via receptor. Or may be via absorptive-mediated transcytosis (Pardridge W M et al., 2002). This approach is intended to provide brain delivery of large peptides. Since this approach involves stoichiometry, only limited number of molecules can fit in to this category.

9. **Cell-penetrating peptides:** Recently this approach has been employed by scientists and several peptides like Tat derived peptides, Transportan, Penetratin etc have been found to translocate across the plasma membrane of eukaryotic cells, but even can be used for intracellular, and may be even transcellular, transport of large cargo macromolecules. For example, Tat fragments that are part of the cell-membrane transduction domain of the human immunodeficiency virus (HIV) have been shown in animal studies to provide enhanced brain delivery (Schwarze et al., 1999; Rouselle C et al., 2000; Aarts M et al., 2002).
10. **Prodrugs:** Prodrugs are the compounds which are pharmacologically inactive and they undergo metabolic transformation within the body to pharmacologically active compounds (effective drugs). Prodrug approach has a slow start initially with certain disappointments but it has gained importance within last decade. (Misra et. al.,2003)

2.5 Nanoparticles for delivery across BBB

Nanoparticles are the colloidal particles ranging in size from 1-1000 nm. Nanoparticles have also been used as transport vectors delivery of many drugs to brain. As such nanoparticles are rapidly scavenged up by the reticuloendothelial system leading to very short circulation time. Surface modified nanoparticles consisting of colloidal polymer particles of poly-butylcyanoacrylate with the desired drug absorbed onto the surface and then coated with polysorbate 80 are prevented from opsonization and uptake by reticuloendothelial system and thus enhancing the circulation time in the blood. Nanoparticles have been used as a vector for delivery of hexapeptide dalargin (an enkephalin analog). The most probable mechanism for delivery of drugs via nanoparticles to brain seems to be endocytosis by the blood capillary endothelial cells following adsorption of blood plasma components, most likely apolipoprotein E (apo E), after intravenous injection. These particles interact with the Low Density Lipoproteins (LDL) receptors on the endothelial cells and then get internalized. After internalization by the brain capillary endothelial cells, the drug releases in these cells by desorption or degradation of the nanoparticles and diffuses into the residual brain. Alternatively, transport may occur by transcytosis of the nanoparticles with drug across the endothelial cells. One more probable mechanism is the increase in the contact time of the nanoparticles with the membrane. In addition to these processes, it is reported that non ionic

surfactants are able to inhibit the P-gp efflux pump. This inhibition could contribute to the brain transport properties of the nanoparticles. Even solid lipid nanoparticles with surface modified properties are used for successful delivery of drugs to brain. (Christophe O.J., 2005, Kreuter J., 2005)

Polymeric nanoparticles have been proposed as interesting colloidal systems that allow the enhancement of therapeutic efficacy and reduction of toxicity of large variety of drugs. PEGylated PHDCA (n-hexadecylcyanoacrylate) nanoparticles made by PEGyalated amphiphilic copolymer penetrate into the brain to a larger extent than all the other tested nanoparticle formulations, without inducing any modification of the BBB permeability. And the result defines two important requirements to take into account in the design of adequate brain delivery systems, long-circulating properties of the carrier and appropriate surface characteristics to permit interactions with endothelial cells. Other than cyanoacrylates, biodegradable polymers such as poly (ethylene glycol)-poly (lactide), poly (lactic-co-glycolic acid), poly (methylmethacrylate), poly (Lactic Acid) and block copolymers, chitosan, albumin can also be used for the preparation of the nanoparticles as vectors for the drug delivery to brain. (Kreuter J. 2001)

Advantages of nanoparticulate drug delivery systems:

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

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

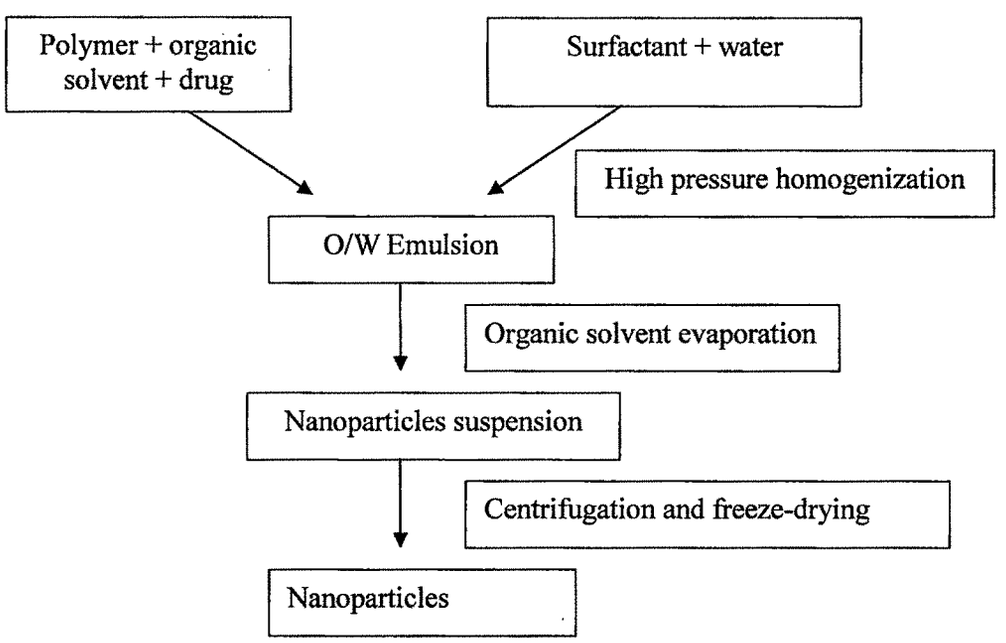
1. **Dispersion polymerization**: Dispersion polymerization starts with monomer, an initiator, solvent in which the formed polymer is insoluble, and a polymeric stabilizer. Polymer forms in the continuous phase and precipitates into a new particle phase stabilized by the polymeric stabilizer. Small particles 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.

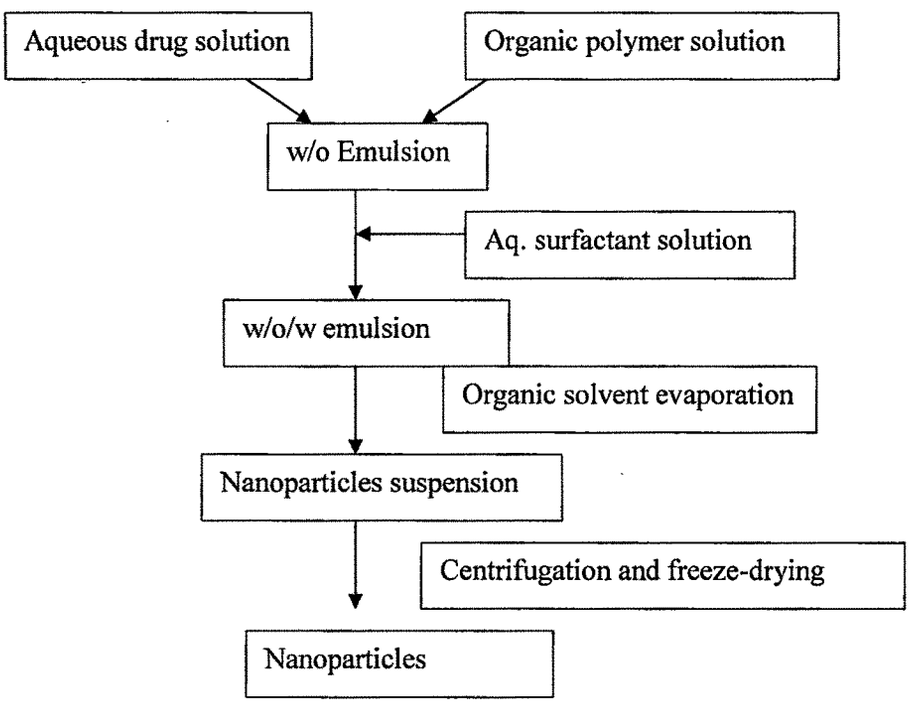
B. Nanoparticles prepared from preformed polymers: Several techniques have been suggested to prepare the biodegradable polymeric nanoparticles from preformed polymers such as poly (D,L-lactide) (PLA), poly (D,L-glycolide) (PLG) and poly (D,L-lactide-co-glycolide) (PLGA). The basic methodologies of the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

1. Emulsion-evaporation:

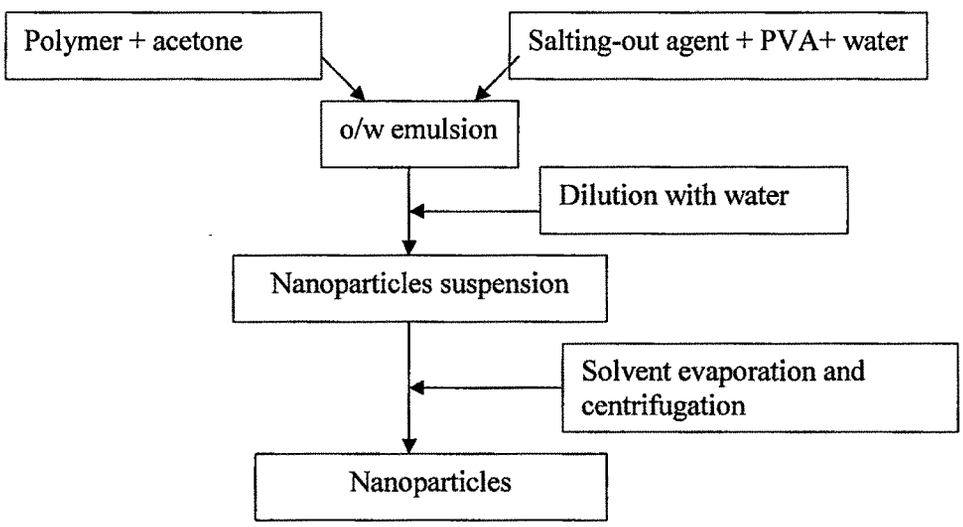
This is one of the most frequently used methods. The preformed polymer and drug are dissolved in a water-immiscible organic solvent, which is then emulsified in an aqueous solution containing stabilizer. The emulsification is brought about by subsequent exposure to a high energy source such as high pressure 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

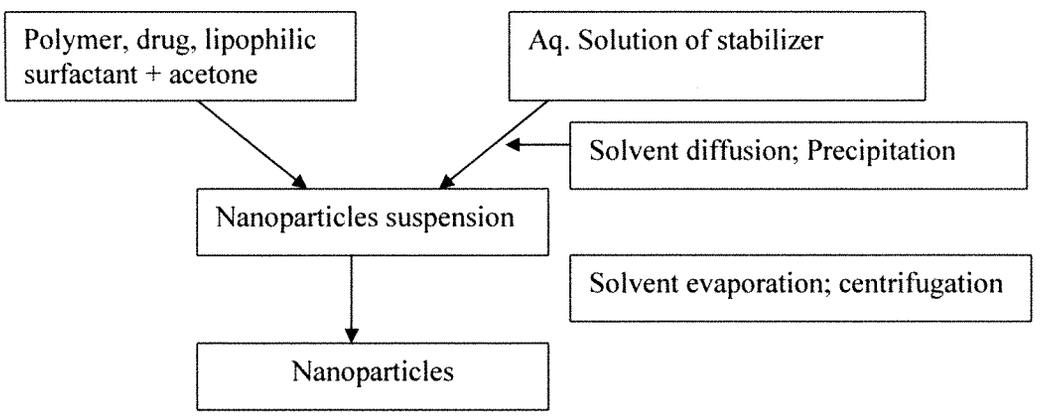


3. **Salting –out**: This technique involves the addition of polymer and drug solution in a slightly water-miscible solvent such as acetone to an aqueous solution containing the salting out agent and a colloidal stabilizer under vigorous mechanical stirring. When this o/w emulsion is diluted with a sufficient 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 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 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 et al., 2007, Fessi et al, 1989)

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, 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.

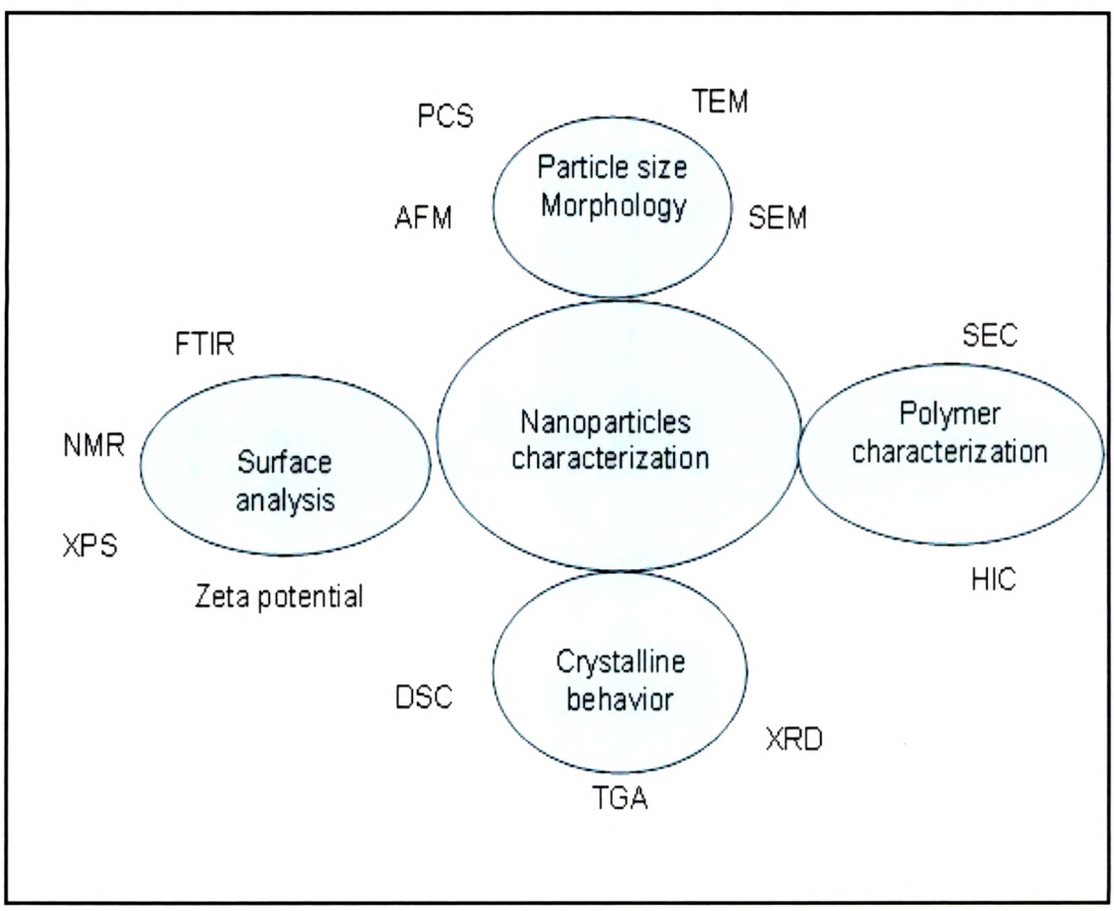
TECHNIQUES FOR CHARACTERIZATION OF NANOPARTICLES

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

There are many sensitive techniques for characterizing nanoparticles, depending upon the parameter being looked at; laser light scattering (LLS) or photon correlation spectroscopy (PCS) for particle size and size distribution; scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) for morphological properties; X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR),a and nuclear magnetic resonance spectroscopy (NMR) for surface chemistry; and differential scanning calorimetry (DSC) for thermal properties (Fig

below). Parameters such as density, molecular weight, and crystallinity affect release and degradation properties, where as surface charge, hydrophilicity, and hydrophobicity significantly influence interaction with the biological environment.

Figure 2.4: Techniques to characterize nanoparticles



Particle size and Morphology: Nanoparticle size is critical not only in determining its release and degradation behaviour (Dunne M et al, 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.

Surface chemistry analysis: X-ray photon spectroscopy, Fourier transform infrared spectroscopy, 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 et al, 2004) (DSC) and differential thermal analysis (DTA) (Oh I et al, 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 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 et al, 2001)

Hydrophobicity: Hydrophobicity determines he 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. Nanoparticles, 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.

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.

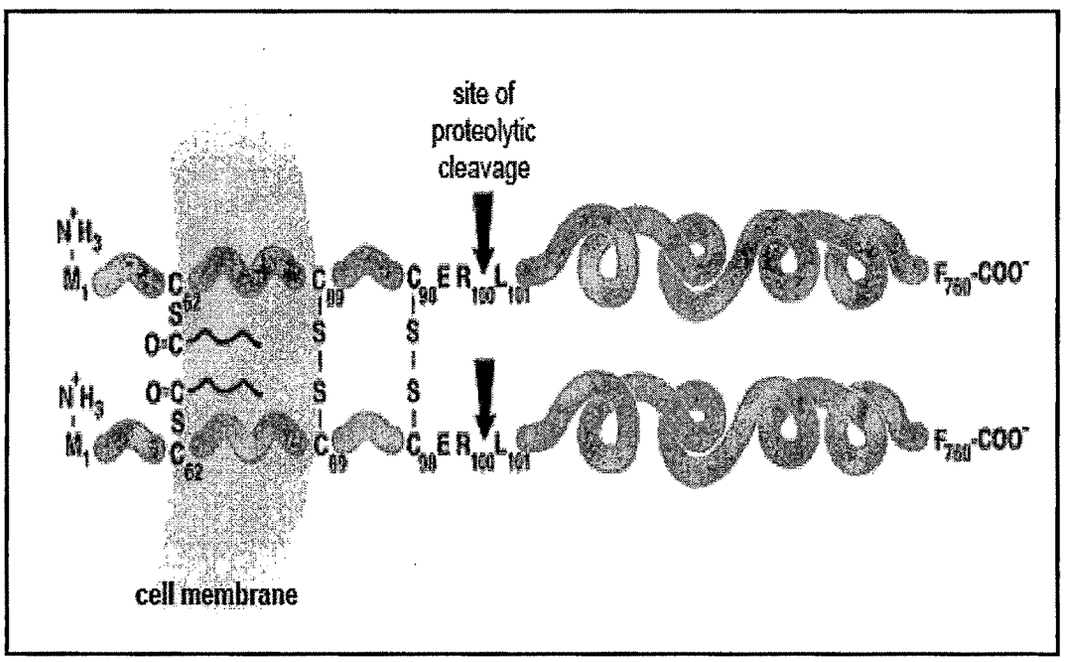
2.6 Receptor mediated transport through transferrin receptors

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., 2000). The TfR is expressed mainly on hepatocytes, erythrocytes, intestinal cells, monocytes, as well as on endothelial cells of the BBB (Ponka P, 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 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, 1999, Huebers H A et al. 1987).

Figure 2.5: Schematic representation of the transferrin receptor



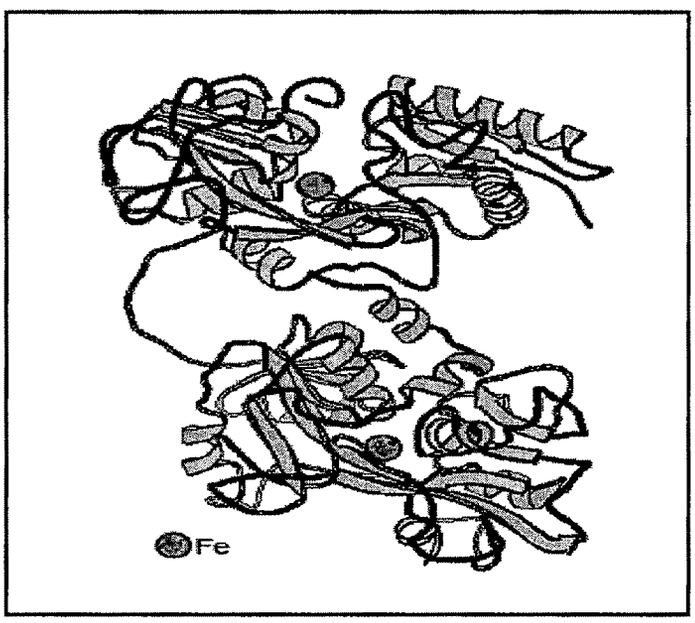
The iron concentration determines TfR synthesis and expression via an iron-responsive element (IRE) in the mRNA of the TfR (Kuhn L C., 1991, Casey J L et. al, 1989). This IRE is also found in the mRNA of ferritin, a protein that can store iron . In cases of low iron concentrations, a socalled IRE binding 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, 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 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 Y J et. al., 1990). It has been shown that serum TfR to ferritin ratios have significant predictive value for differentiating iron deficiency anaemia from non-iron deficiency anaemia (Kohgo Y., 2002).

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., 2000).

Figure 2.6: Structure of human transferrin

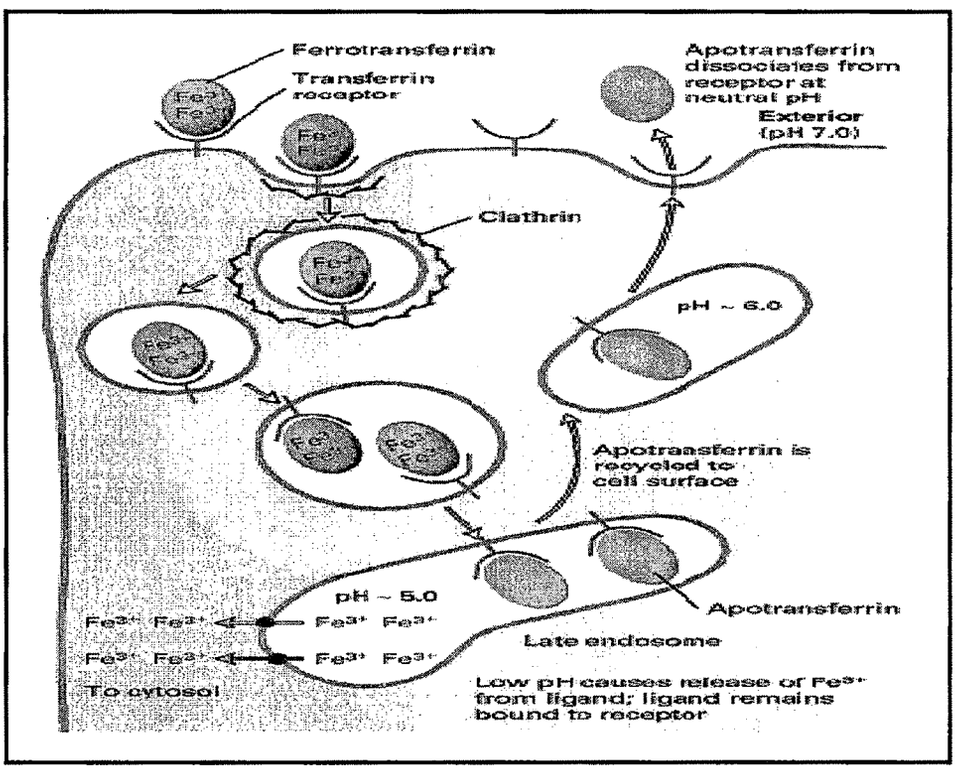


Tf is a single chain, 80 kDa protein, which is folded into two lobes. Each lobe of the Tf molecule can bind one iron ion, a binding that is virtually irreversible at physiological pH. Iron is being released as the pH is lowered to values below 6.5. In plasma and other extracellular fluids, Tf is present 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.

Transferrin receptor (TfR) – transferrin interaction

Figure 2.7: TfR internalisation upon binding of Tf to its receptor



Upon binding of Tf to its receptor, 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 Z M 2002, Moos T, 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 Z M 2002). Furthermore, Fe3+ can be exocytosed at the abluminal side, probably via ferroportin-1, hephaestin and/or hephaestin independent export systems (Qian Z

M., 2002). There is also a second mechanism proposed in which diferric Tf crosses the BBB. Huwyler and Pardridge (1998) have shown that the TfR is present on the abluminal membrane of BCEC (Huwyler and Pardridge 1998). Further research by Zhang and Pardridge 2001 has revealed that Tf is rapidly effluxed from the brain and that the efflux of apo-Tf exceeds that of holo-Tf (Zhang and Pardridge 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 conjugated nanocarriers for brain targeting. Mishra V. et. al, 2006, have successfully transported azidothymidine to brain , after incorporation in albumin nanoparticles and conjugating with transferrin. Soni V. et al, 2008, have shown the improved brain uptake of 5-fluorouracil in the form of Tf conjugated liposomes. Other groups have shown the enhanced invitro intracellular uptake of Tf conjugated nanocarriers.

Hence, delivering the drugs after incorporation into polymeric nanoparticles and conjugating with Tf shows good potential for delivering drugs to brain.

2.7 Intranasal Drug Delivery

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

For some time the BBB has impeded the development of many potentially interesting CNS drug candidates due to their poor distribution into the CNS. Owing to the unique connection of the nose and the CNS, the intranasal route can deliver therapeutic agents to the brain bypassing the BBB (Frey W H et al., 2002). Absorption of drug across the olfactory region of the nose provides a unique feature and superior option to target drugs to brain (Dominique D et al., 1993). When administered nasally to the rat, some drugs resulted in CSF and olfactory bulb drug levels considerably higher than those following intravenous administration (Hussain A A et al., 1998; Chow H S et al., 1999; Sakane T et al., 1994). Evidence of nose-to-brain transport has been reported by many scientists.

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 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.

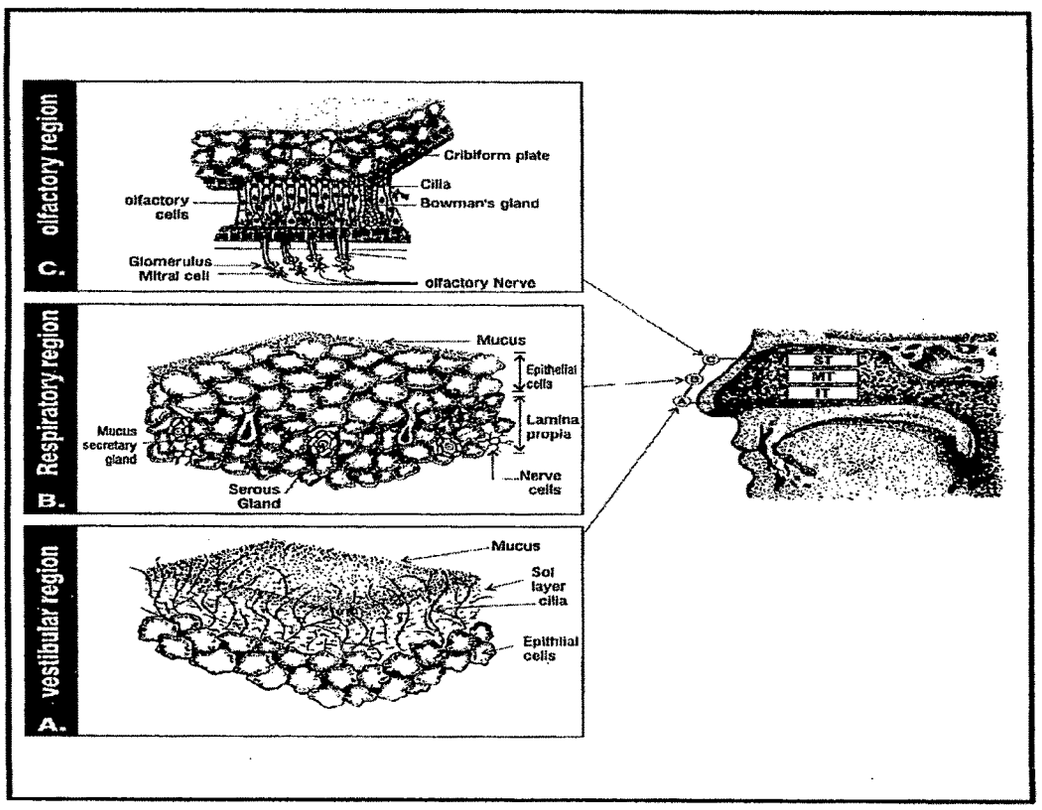
The investigations till date have attracted researchers to place the intranasal drug delivery option under the microscope. Nevertheless, it is imperative to understand the uptake of drug across the nasal mucosa. From a kinetic point of view, nose is a complex organ since three different processes, such 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.

Nasal Anatomy and Physiology of the Nose

The human nasal cavity has a total volume of about 16 to 19 mL, and a total surface area of about 180 cm^2, and is divided into two nasal cavities via the septum (Mygind N et al., 1984). The volume of each cavity is approximately 7.5 mL, having a surface area around 75 cm^2 (Mygind N et al., 1984; Illum Lisbeth., 2000). Post drug administration into the nasal cavity, a solute can be deposited at one or more of three anatomically distinct regions, the vestibular, respiratory or olfactory region.

The vestibular region is located at the opening of nasal passages (Schipper N G M et al., 1991; Mathison S et al., 1998) and is responsible for filtering out the air borne particles. It is considered to be the least important of the three regions with regard to drug absorption (Merkus P et al., 2001). The respiratory region is the largest having the highest degree of vascularity and is mainly responsible for drug absorption. The olfactory region is of about 10 cm^2 in surface area, and it plays a vital role in transportation of drugs to the brain and the CSF. The three distinct anatomical regions present in the nasal cavity and its cross sectional sketch is shown in Figure below

Figure 2.8: Anatomy of nose: cross sectional sketch illustrating (A) the vestibular, (B) the respiratory, and (C) the olfactory region.



The epithelium of the nasal passage is covered by a mucus layer, which entraps particles. The mucus layer is cleared from the nasal cavity by cilia, and is renewed every 10 to 15 minutes (Chein Y W et al., 1987). The pH of the mucosal secretions ranges from 5.5 to 6.5 in adults and 5.0 and 6.7 in children (Hehar S S et al., 1999), which entraps particles and is cleared from the nasal cavity by cilia. The mucus moves through the nose at an approximate rate of 5 to 6 mm/min. resulting in particle clearance within the nose every 15 to 20 minutes. Numerous enzymes (Chein Y W et al., 1989; Chein Y W et al., 1987; Reed C J et al., 1993) for instance, cytochrome P450, enzyme isoforms (CYP1A, CYP2A, and CYP2E), carboxylesterases and glutathione S-transferases are found in nasal cavity.

Intranasal Drug Delivery - Merits/Demerits

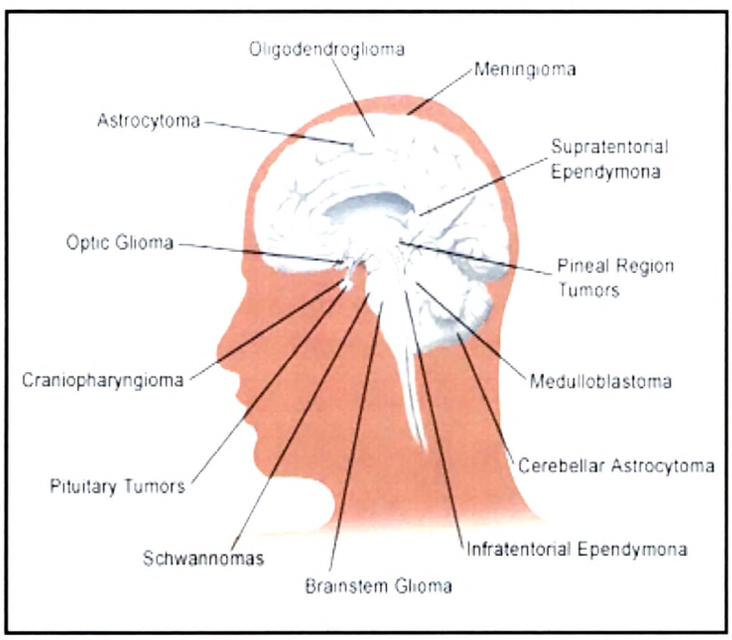
The difficulties that have to be overcome include active degradation or alteration by enzyme, low pH of nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold. An obvious advantage of intranasal route is that it is 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. 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 et. al., 2005)

2.8 Brain Tumor

Brain tumor is a mass of cells that have grown and multiplied uncontrollably. Primary brain tumors originate in brain and may be benign (with definite boundaries) or malignant (spread to other parts of body). Metastatic (or secondary) brain tumors come from cancer cells in another part of the body. The diseased cells spread to the brain by moving through the bloodstream.

Gliomas (astrocytoma) are the most common primary brain tumors. Forty to sixty percent of the primary brain tumors are gliomas. They originate from the star shaped glial cells (astrocytes) that support nerve cells. There are many types of gliomas:

Figure 2.9.: Types of glioma tumors



- **Astrocytoma**—The tumor arises from star-shaped glial cells called astrocytes. In adults, astrocytomas most often arise in the cerebrum. In children, they occur in the brain stem, the cerebrum, and the cerebellum. A grade III astrocytoma is sometimes called an anaplastic astrocytoma. A grade IV astrocytoma is usually called a glioblastoma multiforme.
- **Brain stem glioma**—The tumor occurs in the lowest part of the brain. Brain stem gliomas most often are diagnosed in young children and middle-aged adults.
- **Ependymoma**—The tumor arises from cells that line the ventricles or the central canal of the spinal cord. They are most commonly found in children and young adults.
- **Oligodendroglioma**—This rare tumor arises from cells that make the fatty substance that covers and protects nerves. These tumors usually occur in the cerebrum. They grow slowly and usually do not spread into surrounding brain tissue. They are most common in middle-aged adults.

The symptoms of glioma are headaches, seizures or convulsions, difficulties in thinking or speaking, behavioral changes, loss of balance and vision changes. Many patients develop increased intracranial pressure. The causes for brain tumor are not known. Environmental agents, familial tendencies, viral causes, and other possibilities are under investigation.

The diagnosis of the brain tumors is usually done by the use of Computerized Axial Tomographer (CAT), Magnetic Resonance Imager (MRI), Positron Emission Tomography (PET) & Radionucleide (RN) Scan. The treatment modalities for brain tumor include surgery, radiation therapy, chemotherapy or a combination of either of these treatment options.

2.9 Drug Profile

2.9.1 Etoposide

Description

a. **Category:** Anticancer (epipodophylotoxin).

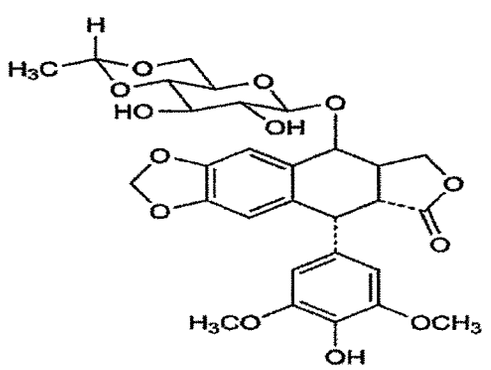
b. **Generic Name:** Etoposide.

c. **Marketed preparations available:** Celltop; Eposin; Exitop; Lastet; Toposar; Vepesid; Vepeside in the form of capsules and injection to be given by infusion.

d. **Empirical formula:** $C_{29}H_{32}O_{13}$.

e. **Molecular weight:** 588.6

f. **Structure:**



g. **Appearance:** It is white or almost white crystalline powder.

h. **Physical properties**

1) **Solubility:** Practically insoluble or very slightly soluble in water; slightly soluble in ethanol, in chloroform, in dichloromethane, in ethyl acetate, and in methylene chloride; sparingly soluble in methanol.

i. **Mechanism of action:** It inhibits DNA topoisomerase II, thereby inhibiting DNA synthesis. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G_2 phases.

j. **Analytical methods:** Following are the various analytical methods described in the literatures for the determination of Etoposide.

1. **High Performance Liquid Chromatrography**:

(i) Column: phenyl (300 × 3.9 mm, 10 μm). Mobile phase: acetonitrile:water:glacial acetic acid (35:64:1), 1.0 mL/min flow rate. Fluorescence detection (λ_{ex}=288 nm, λ_{em}=328 nm). Internal standard (IS): teniposide. Retention times: etoposide 6.5 min; IS, 18 min.

(ii) Column: ODS Hypersil Si-10 (300 × 4 mm i.d., 10 μm). Mobile phase: methanol with 1% NHOH: methylene chloride (2:98), flow rate 2 mL/min. UV detection (λ=254 nm). Retention time: 4.6 min

2 **UV spectrophotometry:** Detected by UV spectroscopy in methanol at 283nm and ethanol at 284nm.

k. **Pharmacokinetics**

Interpatient variability	Bioavailability	
Oral Absorption	Dose-dependent; absorption decreases as etoposide dose increases; mean 50%. daily doses greater than 200 mg should be divided (BID) absorption does not appear to be altered by food or changes in stomach pH and emptying; however manufacturer recommends drug be taken on an empty stomach.	
	Time to peak plasma concentration	1-1.5 h
Distribution	Detected in saliva, liver, spleen, kidney, myometrium, healthy brain tissue, and brain tumour tissue, minimally in pleural fluid	
	Cross blood brain barrier	in low and variable concentrations
	Volume of distribution	7-17 L/m^2, 32% of body weight
	Plasma protein binding	95%
Metabolism	Hepatic biotransformation	
Excretion	Fecal and urinary excretion	
	Urine	44-60% (67% of that unchanged)
	Feces	up to 16% (as unchanged drug and metabolites)
	Biliary	≤6%
	Terminal half life	7 h (range, 3-12)
	Clearance	19-28 mL/min/m^2

l. **Adverse effects:** Etoposide causes allergic reations, congestive heart failure. Etoposide causes hypotension upon injection. Hence it is given in the form of infusion over a period of 30mins.

m. **Indications:** Small cell lung cancer, Refractory testicular tumors. It crosses blood brain barrier in low and variable concentrations and hence not effectively used in brain tumors.

n. **Dosage** (No particular established dose for brain tumors)

1) Intravenous:

Testicular cancer: In combination with other approved chemotherapeutic agents ranges from 50 – 100mg/m^2/day on day 1 through 5-100mg/m^2/day on day 1, 3, 5.

Small Cell Lung Cancer: In combination with other approved chemotherapeutic agents ranges from 35mg/m^2/day for 4days to 50mg/m^2/day for 5 days.

2) Oral:

Orally the dose of etoposide is twice that of the IV dose rounded to nearest 50mg.

2.9.2 Temozolomide

Description

a. **Category:** Anticancer (alkylating agent).

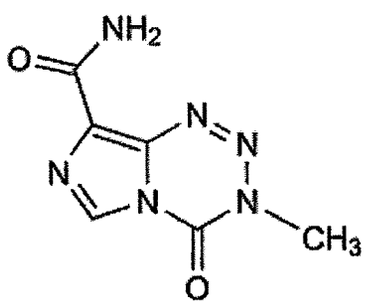
b. **Generic Name:** Temozolomide.

c. **Marketed preparations available:** Temodal, Temodar in the form of capsules.

d. **Empirical formula:** $C_6H_6N_6O_2$.

e. **Molecular weight:** 194.2

f. **Structure:**



g. **Appearance:** It is light tan to pink powder.

h. **Physical properties**

Solubility: Slightly soluble in acetonitrile and water, very slightly soluble in methanol and ethanol.

i. **Mechanism of action:** Temozolomide undergoes rapid chemical conversion at physiologic pH to the active compound, monomethyl triazeno imidazole carboxamide (MTIC). The cytotoxicity of MTIC is thought to be due primarily to methylation of DNA at the O^6 position of guanine. Both temozolomide and dacarbazine are prodrugs of MTIC. Unlike dacarbazine, temozolomide does not require metabolic activation by the cytochrome P450.

j. **Analytical methods:** Following are the various analytical methods described in the literatures for the determination of Temozolomide.

1. **High Performance Liquid Chromatrography**:

(i) Column: C18 (150 × 4.6 mm i.d., 5 μm). Mobile phase: aqueous acetic acid (0.1%):acetonitrile (90:10), flow rate 1.0 mL/min. Internal standard (IS): ethazolastone. UV detection (λ = 316 nm). Retention times: temozolomide, 2.7 min; IS, 5.0 min

(ii) Column: RP HP ODS (Hypersil, 100 × 4.6 mm i.d., 5 μm); (guard) HP ODS (Hypersil, 20 × 4.0 mm i.d., 5 μm). Mobile phase: methanol:acetic acid (0.5%) (10:90), flow rate 1.0 mL/min. UV detection (λ = 330 nm). Internal Standard: ethazolastone. Retention times: 3.2 min; IS, 7.4 min

2. **UV spectrophotometry:** Detected by UV spectroscopy in ethanol at 327nm

k. **Pharmacokinetics**

Interpatient variability	minimal intrapatient and interpatient variability	
Oral Absorption	Rapidly and completely absorbed, with 100% bioavailability. Food delays absorption but is clinically insignificant. Consistency of administration with respect to food is recommended.	
	time to peak plasma concentration	1 h, increased to 2.3 h after high fat meal
Distribution	extensive tissue distribution; equilibrium between plasma and ascitic fluid reached after 2 h	
	cross blood brain barrier?	9-29% of serum concentration
	volume of distribution	15-18 L/m^2 (oral) 0.4 ± 0.1 L/kg (intravenous)
	plasma protein binding	10-20%
Metabolism	rapid, spontaneous, pH-dependent formation of MTIC	
	active metabolite(s)	MTIC
	inactive metabolite(s)	amino imidazole carboxamide (AIC), temozolomide acid metabolite (TMA)
Excretion	renal or hepatic clearance play a minor role in elimination	
	urine	5-10% unchanged over 24 h, remainder as AIC or unidentified polar metabolites. 38% recovered over 7 days (6% unchanged, 12% as AIC, 2% as TMA, 17% as unidentified polar metabolites).
	terminal half life	temozolomide: 1.8 h (oral) 92 ± 14 min (intravenous) MTIC: 1.5-1.8 h
	clearance	115 mL/min/m^2 (87-155 mL/min/m^2) (oral). 220 ± 48 mL/min (intravenous)

l. **Adverse effects:** Haematologic toxicity is dose-limiting with values of 1 g/m^2 and 1.25 g/m^2 being associated. Myelosuppression and thrombocytopenia are also dose-limiting.

m. **Indications:** Brain tumors

n. **Dosage**

150 mg/m^2 (range 100-200 mg/m^2) PO once daily for 5 consecutive days starting on day 1 for a cycle of 4 weeks

POLYMER REVIEW

Poly(D,L-lactide-co-glycolide)

Nonproprietary Names:

Poly(lactide-co-glycolide)

Functional Category:

Bioabsorbable, Biocompatible, Biodegradable material

Trade Names:

Purasorb PLG (PURAC, USA.)

Medisorb 5050 DL (Alkermes Inc.)

Resomer RG 502 (Boehringer Ingelheim, Germany)

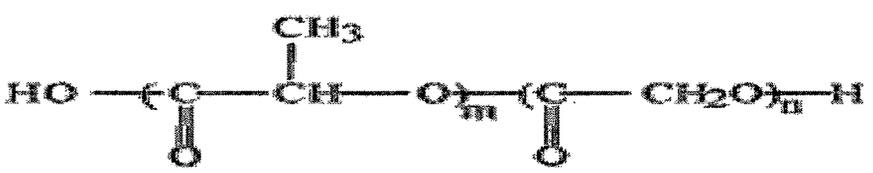
Lactel 5050 DL PLGA (Birmingham Polymers Inc.)

Chemical Name and CAS Registry Number:

CAS Number: 26780-50-7

Chemical Name: Poly(D,L-lactide-co-glycolide)

Structrural Formula:



Chemical structure of PLGA polymer. The "m" component represents lactic acid and "n" component represents glycolic acid.

Method of Manufacture:

Generally, aliphatic polyesters like PLGA can be synthesized via polycondensation of hydrocarboxylic acids and catalytic ring-opening polymerization of lactones. Ring opening polymerization is preferred because polyesters with high molecular weights can be produced. Moreover, the dehydration of hydrocarboxylic acids to form lactones does not have to be carried to a high degree of completion. Lactones can easily be purified owing to the

differences of their physical and chemical properties from those of the corresponding hydrocarboxylic acid.

Description:

PLGA polymers are a group of synthesized copolymers of Lactic and Glycolic acid. They are nontoxic, and can be easily fabricated into variety of novel devices such as rods, screws, nails and cylinders. The polymers are available commercially in varying molecular weight and molecular ratio of Lactic and Glycolic acid. Co-monomer ratio of lactic acid and glycolic acid for PLGA range from 85:15 to 50:50.

Typical Properties:

Description : White or nearly white amorphous powder

Odor: Odorless

Inherent Viscosity: 0.45dl/g 0.1% in chloroform, 25 ^{o}c.

Glass Transition Temp: 50 ^{0}C

Solubility: Methylene Chloride, Ethyl Acetate, Tetrahydrofuran, Acetone, Chloroform

Polymer Composition: 51:49 molar ratio of D,L lactide : glycolide

Acid Number: 9mg KOH/gm

Polymer composition and crystallinity play important roles in the solubility of these aliphatic polyesters. The crystalline homopolymers of glycolic acid are soluble only in strong solvents like hexafluoroisopropanol. The crystalline homopolymers of lactic acid also do not have good solubility in most organic solvents. However, amorphous polymers of D,L-lactic acid and co-polymers of lactic acid and glycolic acid are soluble in many organic solvents.

Stability and Storage Conditions:

Aliphatic polyesters like PLGA easily susceptible to hydrolysis in the presence of moisture. Hence they should be properly stored preferably refrigerated at around 4 ^{o}c or below. It is necessary to allow the polymers to reach room temperature before opening the container.

After the original package has been opened, it is recommended to re-purge the package with high-purity nitrogen prior to resealing.

Safety:

PLGA and homopolymers of D,L-lactic acid and glycolic acid are used in parenteral pharmaceutical formulations and are regarded as biodegradable, biocompatible and bioabsorbable materials. Their biodegradation products are nontoxic, non-carcinogenic and non-teratogenic. In general, these polymers exhibit very little hazard.

Handling Precautions:

Observe normal precautions appropriate to the circumstances and quantity of material handled. Contact with eyes, skin and clothing, and breathing the dust of the polymers should be avoided. PLGA produces acid materials such as hydroxyacetic acid and/or lactic acid in the presence of moisture; thus contact with materials that will react with acids, especially in moist conditions, should be avoided.

Regulatory Status:

GRAS listed.

Applications in Pharmaceutical Formulation or technology:

In aqueous environment, PLGA undergoes hydrolytic degradation, through cleavage of the ester linkages, into nontoxic hydrocarboxylic acids. Eventually, they are degraded to carbon dioxide and water via the citric acid cycle. Owing to their reputation as safe materials and their biodegradability, they are primarily used as biocompatible and biodegradable polymers for formulation of many types of implantable and injectable drug delivery systems for both human and veterinary use. Examples of implantable delivery system include rods, cylinders, tubing, films, fibres, pellets, and beads. Examples of injectable drug delivery system include microcapsules, microspheres, nanoparticles, and liquid injectable controlled release systems. The rate of biodegradation and drug release characteristics from these polymers can be controlled by changing the physic-chemical properties of the polymers, such as crystallinity, hydrophobicity, co-polymer ratio, and polymer molecular weight.

Biodegradation and toxicity of PLGA:

The PLGA copolymers degrade in the body by hydrolytic cleavage of ester linkage to lactic and glycolic acid. These monomers are easily metabolized in the body via Kreb's cycle and eliminated as carbon dioxide and water. The degradation process of polymers both in vivo and in vitro is affected by several factors, including preparation method; the presence of low molecular weight compounds, size, shape and morphology; intrinsic properties of the polymer (molecular weight, chemical structure hydrophobicity, crystallinity, and glass transition temperature of the polymer); physicochemical parameters (pH, temperature and ionic strength of the environment); site of implantation and mechanism of hydrolysis.

Bulk erosion is the main degradation pathway for PLGA copolymer. A three-phase mechanism for PLGA biodegradation has been proposed . Initially, a significant decrease in molecular weight of polymer is observed, with no appreciable weight loss and no soluble monomer products formed after random chain scission. This phase is followed by a decrease in molecular weight with rapid loss of mass and formation of soluble mono and oligomeric products. Finally, soluble monomer products are formed from soluble oligomer fragments, resulting in completer polymer degradation

PLGA copolymer biodegradation products are formed at a very slow rate and hence do not affect normal cell function. These polymers have been tested for toxicity and safety in extensive animal studies and are currently being used in humans for resorbable sutures, bone implants and screws, and contraceptive implants.

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