# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction:

The Blood Brain Barrier has limited penetration properties which are governed by various factors like molecular size, lipophilicity and a variety of ATP- dependant transport systems. Out of these transport systems, there are some efflux transporters which further put a stop to the entry of many lipophilic compounds(Graff & Pollack, 2005) . Thus, Central Nervous System (CNS) drug delivery faces many obstacles after systemic administration. The nasal route is known to deliver drugs across the Blood Brain Barrier (BBB). However, the window of nasal route for brain delivery is not entirely open. The Blood Brain Barrier may allow passage to few drugs administered using the nasal route while some proteins like P- glycoprotein (P-gp), limit the accessibility to the brain. Thus, Central Nervous System (CNS) drug delivery faces many obstacles after systemic administration. Hence alternate strategies need to be explored for exploiting the nasal route to improve total brain uptake and maximize central pharmacologic effects of CNS drugs.

After the drug is administered through the nose, there can be three possible routes that may be followed.

(1) Entry into the systemic circulation directly from the nasal mucosa (Minn et al., 2002) .
(2) Entry into the olfactory bulb via axonal transport along neurons.
(3) Direct entry into the brain (Chow, Chen, & Matsuura, 1999).

While considering the nasal to CNS route, a drug may be carried along the olfactory neurons by intracellular axonal transport to the olfactory bulb. The neuronal cells would take the drug by endocytosis with subsequent transport to the Central Nervous System. This route is followed by some particulate proteins (Thorne, Emory, Ala, & Frey, 1995) . The transport of drug beyond the olfactory bulb to the CNS sites is unclear. Also, the route is slow and hence does not explain the rapid appearance of some solutes in the brain and/ or cerebrospinal fluid following nasal administration (Illum et al., 2002) . Another possibility could be the drug entering into the olfactory epithelium at a point other than the affector neuron. Alternatively, a solute may diffuse

into the cerebrospinal fluid, which is in the perineural space, surrounding the brain. This may not be a pharmacologically viable option also; the diffusion of the drug through the cerebrospinal fluid into brain tissue would be against the flow of CSF (Chow et al., 1999) . Nevertheless, measurable drug concentrations have been observed in cerebrospinal fluid following nasal administration of many drugs.

Recent studies show that the present global nasal drug delivery market is valued at US$ 7 billion. It is not as saturated as the oral drug delivery market and shows a two-digit annual growth rate. Intranasal Drug Delivery Market in United States is projected to reach US$ 7.3 Billion by the year 2022 (New Report by Global Industry Analysts, Inc.). The market has huge potential to accommodate not only intranasal drugs for local infections and chronic allergies, but also other emerging therapeutics areas, which are expected to strengthen their foothold in the marketplace. These emerging areas include the expansion of therapeutic applications from the delivery of conventional local therapeutic drugs to the delivery of systemic therapeutic drugs and also the development of Central Nervous System drugs for nasal delivery, which will help the market score huge gains in the upcoming years (www.researchandmarkets.com).

Nanoparticles have been highly exploited for controlled and site – specific delivery of drugs. The site specific delivery of drugs using nanoparticles has shown promising results for the of various diseases including cancer and human immunodeficiency virus infection (Banerjee, Mitra, Kumar Singh, Kumar Sharma, & Maitra, 2002; Friese, Seiller, Quack, Lorenz, & Kreuter, 2000; S. K. Jain et al., 2008; Mitra, Gaur, Ghosh, & Maitra, 2001) . As compared with the bulk material, the formulated nanoparticles have a high surface /volume ratio and thereby result in a reduction in the dose as well as the frequency of administration of the drug with a consequent increase in the patient compliance (Wilson et al., 2010) . Due to the aforementioned properties, nanoparticles are also faring well in delivering drugs to brain (Banerjee et al., 2002).

A wide array of polymers and manufacturing techniques have been developed over a period of time, since the advent of nanoparticles. Polymeric nanoparticles demonstrate

more success in delivering active to organs like brain. Some of these include poly (lactic acid), poly (lactic acid) – poly (glycolic acid), poly (butylcyanoacrylate), polystyrene. Also, the phenomenon of surface modification to prevent immune response has been incorporated (Peracchia et al., 1999).

Various techniques that are employed for the preparation of nanoparticles can be broadly classified into emulsion polymerization, dispersion polymerization, interfacial polymerization/denaturation and desolvation. By emulsion polymerization method, the monomer is dispersed in aqueous solution as a uniform emulsion and stabilized by the surfactants. The surfactants facilitate emulsification of the monomer into the aqueous phase by decreasing surface tension at the monomer – water interface. High-torque mechanical stirring brings the aqueous and organic phases together by emulsification or homogenization. Polyalkylcyanoacrylate nanoparticles have been polymerized by this method. In addition, denaturation and desolvation have been used to produce polymeric nanoparticles (Reis, Neufeld, Ribeiro, & Veiga, 2006).

The size, surface properties as well as charge of the prepared nanoparticles are important characteristics. Nanoparticles with a size greater than 100 nm are easily captured by Kupffer cells or other phagocytic cell populations which thus restrict their biodistribution (E Weisse). Hydrophilic nanoparticles with particle size less than 100 nm have been reported to avoid opsonization with a consequent prolongation of duration of action as well as enhanced targeting to drug specific sites. Nanoparticles of size ranging from 15 nm to 50 nm have greater probability of passing the Blood Brain Barrier than nanoparticles of larger size (S. K. Jain et al., 2008).

Almeida et al evaluated in an *in vivo* study the transport of carboxylated polystyrene particles administered through the nasal route (Almeida, Alpar, & Brown, 1993). Chitosan nanoparticles produced by ionic gelation method have been reported by Wang et al to deliver estradiol intranasally. The *in vivo* studies indicated successful nose to brain delivery (X. Wang, He, Leng, & Tang, 2006). Coumarin – loaded PEG – poly(lactic acid) nanoparticles were administered intranasally by Gao et al. they found a 2 – fold increase in coumarin in the olfactory bulb, olfactory tract, cerebrum and cerebellum (Gao et al., 2007) .

Alzheimer's disease is a progressive neurodegenerative disorder which includes a variety of conditions. These conditions may either be sporadic and / or familial, characterized by the persistent loss of neuronal activity. Alzheimer's disease affects 24.3 million people worldwide and becomes one of the most severe socio – economical and medical burden all over the world (Ferri et al., 2005) . The disease burden of Alzheimer's goes beyond the cost of medicines / therapy. The most common outcome of Alzheimer's is dementia. Much of the costs have been due to informal care or direct societal care. In India, the total societal cost of dementia was estimated to be Rs. 206.11 billion for the year 2012. The leading market areas for Alzheimer's are US, Europe and Japan. However, the future leading markets are anticipated to be located in China, India and the South Asian and Western Pacific countries. This is because the fastest growth of elderly population is taking placing in these areas. By 2022, the Alzheimer's market in India will be close to $ 13.1 million (Data, 2013).

Alzheimer's disease is characterized by memory dysfunction, loss of lexical access, spatial and temporal disorientation and impairment of judgment. The symptomatic course of disease is generally 5 or more years; although a 7-year preclinical period of stepwise decline in memory and attention span has been described (Linn et al., 1995). Synapse loss also occurs and has been shown to be the best correlate of cognitive decline (R. D. Terry et al., 1991). This multifactorial disorder combines both genetic and non-genetic components. The major non genetic risk factors are advanced age, diabetes, obesity, trauma, or cardiovascular diseases. The genetic mutations share common biochemical pathways that include the altered production of the amyloid –β with an overabundance of the $A\beta_{1-42}$ fragment, the principal constituent of senile plaques. The major genetic risk factor for the sporadic form of Alzheimer's is the inheritance of the ε4 allele of apolipoprotein E, a gene located on chromosome 19q13. The apolipoprotein allele can affect the rate of progression of the disease, the extent of the neuronal cell loss, cholinergic activity, and accumulation of amyloid plaques in hippocampus and cortical areas and total Aβ production and deposition in the brain of Alzheimer's subjects (Sahni, Doggui, et al., 2011).

During the past two decades, one of the foremost challenges in health research was to understand better the cause(s) of Alzheimer's for the development of safe and effective pharmacological treatments. Irrespective of the form of therapy, the current approaches provide only temporary symptomatic relief, improve cognitive function, but do slow the long term progression of this disorder with several side effects. Moreover, these treatments have a modest effect on the progression of Alzheimer's from mild cognitive impairment to disabling dementia and death.

Currently available treatments for Alzheimer's disease are designed to address the symptoms only. A new genre of agents for the disease, are also targeting the underlying pathology, particularly with the aim of reducing amyloid load in the brain, in the hope of modifying the disease process. One of the popular approaches using the above mentioned concept is the immunotherapy approach. This involves passive immunization using monoclonal antibodies, directed at known epitopes. This would ensure the likelihood of treated patients achieving an adequate level of antibody response.

Acetylcholine esterase inhibitors are the most successful class of therapeutic agents to decrease the progression of Alzheimer's disease. Out of those, Galantamine is approved by the USA FDA and the European Medicines Agency for the symptomatic treatment of Alzheimer's disease due to its ability to moderate acetylcholinesterase inhibition in the CNS. Its potentiating effects are derived from allosteric interaction with nicotinic acetylcholine receptors that enhance the sensitivity of the receptors to acetylcholine. Galantamine is commercially available as tablets and oral suspension. However, when administered via the oral route, it leads to severe nausea and vomiting because of its motor and evacuative function on the intestinal tissues (De Caro, V Giandalia, Siragusa MG, Campisi G, & L, 2009) . Additionally, recent reports have shown that Galantamine also has anti amyloid activity (Matharu et al., 2009). But to fully exploit its potential, it has to be delivered properly and that implies surpassing the Blood Brain Barrier (BBB). Thus, an alternate route and in addition probably the delivery system needs to be thought upon.

Another aspect that is worth investigating is immunization against Alzheimer's. Passive immunization involves administering antibodies that are able to enter the Central Nervous System, to induce the clearance of pre existing amyloid aggregates. It has previously been shown that monoclonal antibodies (mAbs) may stabilize antigen conformation against incorrect folding and recognize an incompletely folded epitope, inducing native conformation in a partially unfolded protein. Such mAbs interact with strategic sites where protein unfolding is initiated, thereby stabilizing the protein and preventing further aggregation(Carlson & Yarmush, 1992; Solomon & Schwartz, 1995) . Depending upon the region where the antibody would act on the aggregated peptide, the antibodies are classified as C – terminal targeted, N – terminal targeted, the central region of the amyloid peptide or at a particular epitope at the oligomer. Bapineuzumab is a therapeutic antibody that binds to and clears beta amyloid peptide. It could become the first biologic agent to reach the market for treating Alzheimer's disease. It is a N – terminus anti amyloid humanized monoclonal antibody.

However, antibodies have limited access to the brain due to BBB. Only 0.1 % of an intravenous antibody dose was shown to pass via BBB into the brain (Banks et al., 2002). Bapineuzumab is a therapeutic antibody that binds to and clears beta amyloid peptide. It could become the first biologic agent to reach the market for treating Alzheimer's disease. It is a N – terminus anti amyloid humanized monoclonal antibody.

Thus, though Monoclonal antibodies provide a promising option to put a halt to the progression of the disease, they have the same limitation of improper delivery to the actual site of action. Therefore the main challenge would be to effectively deliver the active / antibody choosing an appropriate route and delivery system.

For addressing the challenge, employing nanoparticulate delivery system via the nasal route seems to be a desirable solution.

### 1.2 Objective and Rationale

The objective of this work was to develop nanoparticulate drug delivery platform which would be employed to carry an acetylcholine esterase inhibitor (Galantamine

hydrobromide) and a monoclonal antibody (Bapineuzumab) to be administered via the nasal route.

Such an endeavour is expected not only to reduce side effects of orally administered acetylcholinesterase inhibitor but also improve its targeting to the site of action i.e brain. In addition, this investigation would open avenues towards possibility of immunization utilizing Bapineuzumab against Alzheimer's disease via the nasal route.

**1.3 Plan of work:**

**I$^{st}$ year:**

1. Literature Survey.
2. Procurement of chemicals, reagents and drugs.
3. Analytical method Selection / Development
4. Preformulation studies.
5. Formulation development of nanoparticles containing Galantamine HBr .
6. Formulation development of nanoparticles containing Bapineuzumab .

**II$^{nd}$ year:**

7. Optimization of formulations by varying various formulation & process variables.
8. Characterization of drug loaded and antibody loaded nanoparticles:
   - Particle size analysis [DLS].
   - Transmission electron microscopy [TEM].
   - Zeta potential.
   - % Entrapment efficiency.
   - Solid-state analysis [DSC, XRD].
   - Stability studies.

**III$^{rd}$ year:**

9. Evaluation of formulations:

   *In-Vitro Studies:*

   - Drug release from the formulation using *in-vitro* model/ technique

   *Ex-vivo* diffusion studies

- Cytotoxicity study of nanoparticles by using cell lines.

*In-vivo* Studies – Pharamacokinetics and biodistribution studies.

## 1.4 References


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