Chapter One

INTRODUCTION

& OVERVIEW
1.1. Problem Statement and Proposed Approach

"Existing antiepileptic drugs are associated with dose and unwanted exposure related side effects, which can be mitigated by the use of alternative antiepileptic drugs; however the major obstacle in the delivery of these agents to the brain is constraints of blood brain barrier. Hence present research work has been designed to develop a nanotechnology based formulation of a nonconventional antiepileptic drug. Intranasal route has been selected to target the drug at the site of action with improved efficacy & compliance."

1.2. Background Information

Brain is a delicate organ, isolated from general circulation and characterized by the presence of relatively impermeable endothelial cells with tight junctions, enzymatic activity and the presence of active efflux transporter mechanisms. These formidable obstacles often block the delivery of neurotherapeutic agents to the brain across the blood-brain barrier (BBB). Although several promising molecules have the potential in the in vitro settings but lack of in vivo response is probably because the molecule cannot reach the brain in a sufficient concentration. Delivery of a neurotherapeutic agent across the BBB is a major limitation in the treatment of central nervous system (CNS) disorders and CNS infections (Baratchi et al, 2009).

Nanoconstructs are materials and devices that have a functional organization in at least one dimension on the nanometer (one billionth of a meter) scale, ranging from a few to about 100 nanometers. Nanoconstructs aimed at biologic applications and medicine in general, and neuroscience in particular, are designed fundamentally to interface and interact with cells and their tissues at the molecular level. The potential of nanotechnological applications to biology and medicine arise from the fact that they exhibit bulk mesoscale and macroscale chemical and/or physical properties that are unique to the engineered material or device and not necessarily possessed by the molecules alone. This supports the development of nanoconstructs that can potentially carry out multiple specific functions at once or in a predefined sequence, which is an important property for the clinically successful delivery of neurotherapeutic agents and other molecules to the central nervous system (Silva, 2008).

Use of alternative route to CNS drug delivery of drugs is one of the strategies under exploration, to avoid systemic drug exposure by retaining drug concentration in brain
area only. An alternative CNS drug delivery strategy that has received relatively little attention is the intranasal route. Drugs delivered intranasally are transported along olfactory sensory neurons to yield significant concentrations in the CSF and olfactory bulb. Nasal medication delivery takes a middle path between slow onset oral medications and invasive, highly skilled delivery of intravenous medications. Medication deposited on the highly vascular nasal mucosa may be rapidly absorbed into the blood stream and cerebral spinal fluid (CSF), achieving therapeutic drug levels more quickly and predictably than oral medications while avoiding needles. This results in therapeutic drug levels and effective treatment of seizures, pain, anxiety, hypoglycemia, opiate overdose, epistaxis (bloody noses), etc. without the need to give a shot or a pill. In recent studies, intranasal administration of wheat germ agglutinin horseradish peroxidase resulted in a mean olfactory bulb concentration in the nanomolar range. In theory, this strategy could be effective in the delivery of therapeutic proteins such as brain-delivered neurotropic factor (BDNF) to the olfactory bulb as a treatment for Alzheimer's disease (Thorne et al., 1995). The nasal drug delivery to the CNS is thought to involve either an intraneuronal or extraneuronal pathway (Thorne & Frey, 2001). Recent evidence of direct nose-to-brain transport (Illum, 2002) and direct access to CSF of three neuropeptides bypassing the bloodstream has been shown in human trials, despite the inherent difficulties in delivery (Born et al., 2002). The difficulties that have to be overcome include an enzymatically active, low pH 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 this method is that it is non-invasive relative to other strategies.

1.3. Epilepsy and Antiepileptic Drugs

Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsy is the world’s most common serious neurologic disorder, affecting approximately 50 million individuals worldwide, with 25% unable to be controlled despite treatment. Epilepsy is a chronic disorder of the cerebral cortex with various etiologies characterized by recurrent seizures due to an excessive paroxysmal discharge of cortical neurons, associated with a variety of semiologic manifestations. A single seizure and acute symptomatic seizures during an acute illness or acute injury therefore are not synonymous with epilepsy. Epilepsy is
thus defined as a disorder in which repeated seizures arise spontaneously in the same patient. Having more than two seizures carries a high risk of ongoing seizures, and though some patients with single seizures have a high risk, epilepsy is arguably evident when two unprovoked seizures occur. For many people seizures represent not only a clinical disorder that requires medical care, but also a condition with an important stigmatizing social impact.

Anticonvulsants are more accurately called antiepileptic drugs (abbreviated "AEDs"), sometimes referred to as antiseizure drugs. The ultimate objective of Anti-Epileptic Drug (AED) therapy is to eliminate seizures without subjecting the patients to side effects. The mainstay of treatment of epilepsy is anticonvulsant medications. Often, anticonvulsant medication treatment will be lifelong and can have major effects on quality of life. The choice among anticonvulsants and their effectiveness differs by epilepsy syndrome. Mechanisms, effectiveness for particular epilepsy syndromes, and side-effects differ among the individual anticonvulsant medications. Currently there are 20 medications approved by the Food and Drug Administration for the use of treatment of epileptic seizure: carbamazepine (Tegretol), clorazepate (Tranxene), clonazepam (Klonopin), ethosuximide (Zarontin), felbamate (Felbatol), fosphenytoin (Cerebyx), gabapentin (Neurontin), lacosamide (Vimpat), lamotrigine (Lamictal), levetiracetam (Keppra), oxcarbazepine (Trileptal), phenobarbital (Luminal), phenytoin (Dilantin), pregabalin (Lyrica), primidone (Mysoline), tiagabine (Gabitril), topiramate (Topamax), valproate semisodium (Depakote), valproic acid (Depakene), and zonisamide (Zonegran). Most of these appeared after 1990. Medications commonly available outside the US but still labelled as "investigational" within the US are clobazam (Frisium) and vigabatrin (Sabril). Medications currently under clinical trial under the supervision of the FDA include retigabine, brivaracetam, and seletracetam (http://www.epilepsy.org). Figure 1 illustrates below the major mechanisms of anticonvulsant action of different AEDs.
Epilepsy being a CNS centered disorder every AED drug or molecule has to present at site of action at optimum level to produce efficacy however, major hurdle in this movement is BBB. Current oral epilepsy therapy has the regrettable tendency to distribute a medication to wide regions of the brain and body, including where it is not needed to stop seizures, and where it may produce adverse effects. Many options currently exist for oral medication to treat epilepsy, but none are ideal. Adequate control with acceptable side effects is achieved only in approximately two out of three patients. When patients present with acute seizures, oral medication is not an option, and establishment of intravenous access may entail delays. The rapidity by which a medication can be delivered to the systemic circulation and then to the brain plays a significant role in reducing the time needed to treat seizures and reduce opportunity for damage to the CNS (Wermeling, 2009). Therefore, to potentially obtain a better therapeutic-to-toxic ratio, interest has been expressed in alternative routes to treat seizures e.g. rectal, dermal, nasal, buccal, inhaled or direct delivery to CNS. Each route has its own limitations and advantages with respect to dose, drug property, pharmacokinetics, and final efficacy either immediate or sustained (Schmidt, 2009).
Moreover, none of the presently available drug is effective for over all forms of epilepsies. Therefore, there is still a need for exploring newer drug molecules, which should be effective and on the other hand should have minimal toxicity.

Accumulating evidence indicates that amiloride (a potassium-sparing diuretic) exerts the anticonvulsant action in various in vivo and in vitro experiments and the intracellular acidification appears to be a primary mechanism for its anticonvulsant action (Jarogniew et al., 2009).

1.4. Rationale and Hypothesis of the Proposed Work

A significant number of people continue to have seizures regularly despite taking medication. Also, AEDs are notorious for their side effects that may vary from gastrointestinal complaints, to problems in cognition, mood and behaviour or severe hypersensitivity reactions due to inherent AED property or unwanted exposure related to routinely used route of administration. It is estimated that in fact in one third of patients the treatment goal as described above cannot be achieved: patients keep getting seizures or experience bothersome side effects.

Keeping these facts in mind it is therefore important that research on the alternative AEDs, with their safe and effective delivery by implying appropriate route of administration like intranasal route for instant and direct delivery to brain needs to be carried out.

Amiloride, a sodium hydrogen exchanger (NHE) inhibitor, has shown anticonvulsant potential in both in vitro (Sokolova et al., 1992) and recent in vivo studies (Ali et al., 2004, 2005). Recently some researchers have developed liposomal formulations of amiloride (Ali et al., 2007; Alam et al., 2008) with an improved anticonvulsant action in animal models of epilepsy as compared to free drug.

Hence present hypothesis deals with the novel nanoconstruct formulation development of the amiloride for its effective delivery to the brain by implying suitable non-invasive route of administration technique for the better management of epilepsy.
1.5. Aim and Objectives of the Proposed Work

The main aim of the present work was to ‘design and develop nanoconstructs for targeted drug delivery of neurotherapeutic agents’ that is easy to apply in form of nasal drops, but can still effectively deliver drug to the brain by overcoming blood brain barrier via intranasal route for the treatment of epilepsy.

The specific objectives of the present work were:

- To establish proof of concept (Chapter 3)
- To perform drug profiling and preformulation studies of selected drug (Chapter 4)
- To develop and validate analytical methods for estimation of selected drug as bulk and in formulation (Chapter 5)
- To develop, optimize and characterize nanoconstruct formulation (Chapter 6)
- To perform stability studies of developed formulation (Chapter 7)
- To perform radiolabelling and in-vivo studies (Chapter 8)
- To perform safety studies of the developed formulation (Chapter 9)