INTRANASAL CHITOSAN MICROSPHERES FOR THE TREATMENT OF EPILEPSY

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ABSTRACT
Epilepsy is a chronic medical condition with many social facets. Till date a number of drugs are available to treat various types of epilepsies, these drugs act by one or more mechanisms to treat this psychiatric disorder. A significant percentage of patients with epilepsy continue to experience seizures despite aggressive treatment with one or more antiepileptic drugs, which lead to an unmet clinical need of more effective and less toxic anti-epileptic drugs. Although the pharmacological treatment of epilepsy has made remarkable breakthroughs but there are certain Limitations of current pharmacotherapy in epilepsy. In this context, alternative administration strategies that provide a more efficient delivery of anti-epileptic agents to the brain are urgently needed. The intranasal administration as a means of delivering therapeutic agents preferentially to the brain has recently gained significant interest. Particulate carrier technology offers a valuable advance for intra nasal drug delivery systems by the introduction of carriers such as microspheres, emulsions, liposomes etc. Chitosan is one of the most promising polymers because of its nontoxic, polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties. The strong mucoadhesive property of chitosan is most important for drug delivery through the mucosal routes. To facilitate the mucoadhesion in intranasal administration several drug delivery systems have been recently developed, which increase the contact time of drug to mucous membrane and enhance its absorption.

KEYWORDS: Chitosan, Mucoadhesive microsphere, Intranasal administration, Epilepsy.

INTRODUCTION
Epilepsy is a chronic medical condition with many social facets. The terms convulsive disorder, seizure disorder, and cerebral seizures are used synonymously with epilepsy. These all refer to repeated paroxysmal episodes of brain dysfunction marked by stereotyped modifications in behavior. Therefore, epilepsy can be defined as a heterogeneous biomedical disorder, with enormous differences in etiology and clinical features, resulting in irregular episodic bursts of electrical activity in certain neurons, which may spread to the whole brain. It is one of the most common but serious neurological disorders; affecting approximately 70 million people of all age worldwide.[1] Around 80% of people with epilepsy belong to developing countries, where epilepsy remains a major public health problem, not only because of its health implications but also for its social, cultural, psychological and economic connotations. In India about 10 million people are suffering from epilepsy.[2]

Classification of Epilepsy
Epilepsy cannot be explained as one condition, but is a varied family of disorders, having in common an abnormally increased predisposition to seizures.[3] The classification of epilepsy was standardized with adoption of the International League Against Epilepsy Classification of Epileptic Seizures, in 1981.[4,5]

Partial Seizure
Partial (or focal) seizures are conceptualized as originating at some point within networks limited to one hemisphere. These are further classified into:

Simple partial seizure
Simple partial seizures are not associated with alteration of consciousness, because they begin in a small, discrete area of brain. Only one neurologic modality is affected during the seizure, and resulting symptoms depend on the area from which it arises. Patients describe auras as the feeling experienced before a seizure; however, an aura is merely a simple partial seizure.

Complex partial seizures
Complex partial seizures are associated with alteration but not loss of consciousness. The patient is awake and staring blankly, but is not responsive to external stimuli.
Complex partial seizures can arise from any region of the brain, but most commonly arise in the temporal lobe, followed by the frontal lobe. These seizures may be accompanied by automatism that is repetitive and purposeless movements. Typical oral automatisms include lip smacking, chewing, swallowing, and gulping. Typical hand automatisms include hand wringing, patting, rubbing or squeezing, and manipulation of clothes or bed covers.\(^5\)

**Generalized Seizures**

Generalized seizures are conceptualized as originating at some point within and rapidly engaging bilaterally distributed networks.\(^4\)

These are further classified into following subcategories.

**Tonic-clonic (primary tonic-clonic)**

Grand mal seizures begin with anatomic phase of whole-body stiffening, followed by a clonic phase of repetitive contractions. Tongue biting and urinary incontinence are common with generalized tonic-clonic seizures. These seizures last 2 to 3 minutes and are followed by a period of confusion or complete unresponsiveness for at least another few minutes.\(^5, 6\)

**Absence seizures**

Absence seizures also known as petit mal seizures are manifested as brief (1-10 s) episodes of staring and unresponsiveness. Most often there are no other manifestations, but episodes that last more than 7 to 10 seconds can be associated with eye blinking or with oral or manual automatisms. They are similar to complex partial seizures in that both are characterized by staring unresponsiveness, but the clinical situation in which they arise often enables differentiation.\(^5, 6\)

**Myoclonic seizures**

Myoclonic seizures are brief, lightning-like muscular jerks. The most common signs are bilateral hand or arm jerks, although these seizures can affect any body region. Not all myoclonic movements are seizures, only cortical myoclonic movements are considered seizures.\(^5, 6\)

**Clonic seizures**

These seizures consist of only the clonic phase of generalized tonic-clonic seizures.\(^5, 6\)

**Tonic seizures**

These seizures consist of only the tonic phase of generalized tonic-clonic seizures.\(^5, 6\)

**Atonic seizures**

Atonic seizures are manifested as sudden loss of muscle tone and subsequent falling or dropping to the floor unprotected. They are referred to as drop attacks, and often cause injury as part of multiple seizure types resulting from severe epilepsy.\(^5, 6\)

**Antiepileptic Drugs (AEDs)**

Till date a number of drugs are available to treat various types of epilepsies, these drugs act by one or more mechanisms to treat this psychiatric disorder. Several classes of AEDs are available to treat various types of epilepsies (Table 1). A significant percentage of patients with epilepsy continue to experience seizures despite aggressive treatment with one or more AEDs, which lead to an unmet clinical need of more effective and less toxic anti-epileptic drugs.

Most of the clinically active AEDs decrease membrane excitability by interacting with neurotransmitter receptors or ion channels. These appear to act on Na\(^+\) channels, Ca\(^{2+}\) channels, K\(^+\) channels, GABA\(_A\) receptor and glutamatergic receptors.\(^7\) Majority of AEDs act through multiple mechanisms. Primarily, benzodiazepines (clonazepam, diazepam and clobazam) and barbiturates (phenobarbitone and primidone) enhance GABA\(_A\) receptor-mediated inhibition. Phenytin, carbamazepine, oxcarbazepine valproate and zonisamide decreases high-frequency repetitive firing of action potential by enhancing Na\(^+\) channel inactivation. Ethosuximide, valproate and zonisamide reduces a low threshold Ca\(^{2+}\) channel (T type) current. Gabapentin binds to a high-affinity site on neuronal membranes in a restricted regional distribution of the CNS. This binding site is related to an active transport process of gabapentin into neurons, this drug act by increasing GABA release. Lamotrigine decreases sustained high-frequency repetitive firing of voltage-gated Na\(^+\) action potentials that may result in a preferential decrease in the release of presynaptic glutamate. Vigabatrin irreversibly inhibits GABA aminotransaminase, which is involved in enzymatic degradation of GABA, thereby producing greater available pools of presynaptic GABA for release in central synapses.\(^8\) Tiagabine act by blocking GABA uptake. Topiramate has multiple sites of action, including Na\(^+\) channels, GABA receptors and glutamate (AMPA) receptors.\(^9\) There are certain newly developed AEDs like, retigabine act by opening potassium channels and modulating GABA\(_A\) receptor, ganaxolone act as a potent positive modulator of GABA and rufinamide enhance Na\(^+\)-channel inactivation. Levetiracetam, seletaracetam and brivaracetam inhibits glutamate release. Lacosamide and felbamate act as NMDA antagonist, whereas talampanel act as AMPA receptor antagonist.\(^7, 10\) (Figure.1).
Figure 1: Mechanisms of action of antiepileptic agents. Drugs modulate both inhibitory (left-hand side) and excitatory (right-hand side) nerve terminals. Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; GABA, γ-aminobutyric acid; GAT-1, GABA transporter 1; SV2A, synaptic vesicle glycoprotein 2A.

Limitations of current pharmacotherapy in epilepsy

Although the pharmacological treatment of epilepsy has made remarkable breakthroughs with the availability of more than 20 different antiepileptic drugs, it has fallen short of expectations, as up to one-third of the patients continuing to experience seizures or unacceptable medication-related side-effects.\cite{11,12} Indeed, pharmacoresistance has been assumed as one of the major causes underlying the failure of the anticonvulsant therapy and it is commonly associated with an increased risk of morbidity and mortality.\cite{13,14} Even though exact mechanism that developed resistance to antiepileptic drug have not been completely understood, various efflux transporters such as P-glycoprotein and members of the multidrug resistant-associated proteins are particularly upregulated in blood brain barrier (BBB) of epileptic patients.\cite{15-17} This overexpression hampers the penetration of several antiepileptic drugs into the brain, limiting the amount of the drug that efficiently reaches the therapeutic target, and consequently, it may lead to the reduction or even complete loss of pharmacological activity of AEs.\cite{15,18}

In this context, alternative administration strategies that provide a more efficient delivery of anti-epileptic agents to the brain are urgently needed. The intranasal (IN) administration as a means of delivering therapeutic agents preferentially to the brain has recently gained significant interest.\cite{19} In fact, the unique anatomical connection between the nasal cavity and the CNS provides a great opportunity for drugs administered by the IN route to reach a quick and easy access to the brain through pathways along the olfactory and trigeminal nerves innervating the nasal passages.\cite{20} Accordingly, IN delivery could be a favorable and promising alternative route to improve CNS targeting of anti-epileptic agents.

Nasal drug delivery system

Nasal delivery is traditionally used for administration of drugs to treat local nasal diseases such as sinusitis and allergic rhinitis since low doses are sufficient to provide therapeutic effects with low systemic side effects. In addition, nasal delivery might be suitable for drugs which are effective in low doses and have low oral bioavailability.\cite{21} The rate of absorption, and the pharmacokinetic properties of small drug molecules used for systemic therapeutic effects, administration via the nasal pathway are comparable to the parenteral route as it provide a large surface area, rich vascularity and drug high permeability.\cite{22}

It provides an efficient site for systemic absorption particularly for drugs with low water solubility.\cite{23} Many drugs do not reach the brain following oral or parenteral administration due to the BBB. The olfactory region of the nose is not covered by the BBB, hence evasion of the BBB following nasal administration might be possible. This approach is effective for drug targeting to the brain.\cite{24} Furthermore, the nose is attractive route for vaccine delivery for immunization.\cite{25}
Advantage and limitations of nasal route

Advantages
- Suitable for drugs that are acid labile in the stomach
- Applicable for drugs that undergo extensive hepatic first-pass effect
- Rapid drug absorption and onset of action
- Offers higher drug bioavailability, thus lower doses of drug are needed
- Offers large surface area for drug absorption
- No particular skills or expertise are required for nasal drug administration
- Direct transportation of drug to the systemic circulation or CNS is possible
- Offers lower risk of overdosing
- Needle-free and patient friendly
- Offers induction of immune response when used for vaccine delivery.

Limitations
- Volume that can be delivered into nasal cavity is restricted to 25–200 μl
- High molecular weight compounds cannot be delivered
- Adversely affected by pathological conditions of the nose
- Large interspecies and patient to patient variability
- Mucociliary clearance can affect the absorption of drug
- Local enzymes in the nasal cavity might degrade some drugs
- Local side effects like irritation
- Nasal congestion from colds and flues may interfere with drug delivery
- Frequent delivery of drugs may cause mucosal damage.

Anatomy of the nose
The nasal passage is 12-14 cm deep and runs from the nasal vestibule to the nasopharynx. It has three main regions; vestibular, respiratory and olfactory regions (Figure 2). The nose has a volume of 16-19 cm³ and a surface area of approximately 180 cm² with two cavities (i.e. nostrils) separated by the nasal septum. The vestibular region is located at the front opening of the nasal passages which filters out particles from the inhaled air. However, drug delivery and absorption in this region is least important. This area is covered with hairs which filter the air to prevent airborne particles entering the respiratory system. The respiratory area is large with a high degree of vascularity and a surface area of about 130 cm². In this region the majority of drug absorption occurs. It is lined with pseudo stratified columnar epithelium and covered with a dense layer of mucus which moves towards the posterior apertures of the nasal cavity because of the ciliary rhythmic movements. The olfactory region is important for transporting the drug to brain and cerebrospinal fluid and has a surface area of about 15 cm². It is made of thick connective tissue and lamina propria, into which the olfactory epithelium rests. The thickness of nasal mucosa ranges between 2 and 4 mm. The epithelium cells line the nasal passage and are covered by a mucus layer 5μm in thickness which traps unwanted particles. The mucous secretion consists of water (95%), mucin (2%), salts (1%), proteins (1%) such as albumin, immunoglobulin, lysozyme, and lactoferrin, and lipids (1%). IgA, IgE and IgG are also present in the mucous secretion. The pH of the nasal secretion is ranged from 5 to 6.5. Ciliary action is responsible for clearing the mucus layer from the nasal cavity and mucus is renewed 4 - 6 times per hour. The mucus moves through the nose at a rate of 5-6 mm/min.

Figure 2: Anatomy of the nasal cavity: vestibule, inferior, middle and superior turbinates; olfactive region and nasopharynx. Drug deposition following intranasal administration mainly occurs in the respiratory zone around the inferior turbinate. Partial obstruction of the nasal cavity by the turbinates prevents at least in part the deposition on the olfactory epithelium and on the nasopharynx.
Applications of nasal delivery

Local effects
Low molecular weight water-soluble or hydrophobic drugs are used to treat local pathological conditions in the nose. For example, Azelastine\textsuperscript{29} is a rapid acting antihistamine, mainly act as amast cell stabilizer available and available in nasal spray. Beclometasone\textsuperscript{30} is an anti-inflammatory corticosteroid used to reduce inflammation and local allergy. It is a well-established drug for the treatment of allergic rhinitis. Nasal decongestants such as oxymetazoline are also administered via the nose for treating common colds.\textsuperscript{31,32}

Systemic effects
Nasal delivery is convenient for acid labile drugs, proteins and peptides when rapid action is required such as in migraine relief.\textsuperscript{33} Nasal delivery offers a rapid action and efficient drug absorption compared to oral and intravenous delivery.\textsuperscript{34} Most protein and peptide drugs have low bioavailability (1–2\%) due to their high molecular weight and polarity, causing poor absorption through the nasal mucosal membranes. In contrast, the bioavailability of progesterone and propranolol via nasal epithelium is comparable to parenteral administration.\textsuperscript{35} Lower bioavailability can be improved by using absorption enhancers in the formulations, thus prolonging the contact time of the drug with the mucous membranes using bioadhesive agents. The bioavailability of recombinant human growth hormone was increased significantly after nasal delivery in combination with N-trimethyl chitosan chloride as an absorption enhancer.\textsuperscript{36}

Vaccines delivery
Nasal mucosa is enriched by lymphoid tissue. It enhances the systemic levels of specific immunoglobulin G and nasal secretary immunoglobulin A and the local immune responses which provide additional protection against invading microbes.\textsuperscript{37} Nasal mucosa is advantageous for immunization due to its permeability, low enzymatic activity and accommodation of the nose-associated lymphoid tissue (NALT).\textsuperscript{38} The delivery of vaccine via the nose represents a convenient needle-free procedure for vaccination. Furthermore, NALT is an effective immune system.\textsuperscript{39} Nasal vaccines that have been investigated include influenza A and B,\textsuperscript{40} proteasome-influenza,\textsuperscript{41} adenovirus-vectored influenza,\textsuperscript{42} attenuated respiratory syncytial virus and parainfluenza 3 virus.\textsuperscript{43}

CNS intranasal route
The intranasal route is promising for the delivery of drugs to the brain via olfactory neuroepithelium.\textsuperscript{44} This strategy has been considered for the treatment of Alzheimer’s disease, brain tumors, epilepsy, pain and sleep disorders.\textsuperscript{45} Delivery of nerve growth factor to the brain in rodents has been reported,\textsuperscript{45,46} and in humans studies insulin\textsuperscript{47} and proteins\textsuperscript{48} have been directly transferred through olfactory path to the CNS via nasal cavity. Successfully trans-nasal delivery 0.5mg/kg of siRNA to the CNS with high brain concentration compared to the other tissue has been used to treat neurological disorders using chitosan nanoparticles formulations to deliver siRNA.\textsuperscript{49}

Mechanisms of drug transport following intranasal administration
The drug is expected to pass through the mucus layer of the nasal cavity for efficient absorption. Uncharged molecules pass through the mucus much more readily than charged molecules. Two main mechanisms of drug absorption through nasal mucosa have been proposed: paracellular absorption and transcellular absorption. Firstly, the paracellular route is energy independent and occurs by drug passing through the aqueous spaces between the cells via a slow passive diffusion. In general, as the molecular size of the drug increases the intranasal absorption via this route decreases.

For example, a drug with a molecular weight greater than 1 kDa has poor systemic bioavailability following nasal administration and bioavailability of these molecules can be enhanced using absorption enhancers.\textsuperscript{50} The systemic absorption of large molecular weight of recombinant hirudin-2 (6900 Daltons) improved intranasally by combination with absorption enhancer (e.g. chitosan 0.5\%, hydroxypropyl-\(\beta\)-cyclodextrin 5\%, or ammonium glycyrrhizinate 1\%) into the formulation.\textsuperscript{50}

Transcellular absorption mechanism is applicable to lipophilic drugs, which are readily absorbed by diffusion through the epithelial cellular membranes of the nose. Transcellular transport of drugs might be carrier mediated or may involve opening of tight junctions for drug absorption. Excipients used in nasal formulations such as chitosan opens the tight junctions between cells and thus the drug transportation from nasal cavity to the systemic circulation is facilitated.\textsuperscript{51,52}

Substances in the nasal mucosa particularly in the olfactory mucosa include: P-glycoprotein, organic cation transporter, dopamine transporter, and amino acid transporters. These transporters transfer amino acids, amines and cations.\textsuperscript{53-55} This mechanism is known as carrier mediated processes.

The mechanism for drug transportation is endocytosis. In this mechanism materials are engulfed into the cell. Endocytosis is the predominant mechanism for transporting compounds that have molecular weights higher than 1000 Da such as proteins, peptides,\textsuperscript{56} polypeptides and polypeptide-coated nanospheres in the range of 500 nm.\textsuperscript{57} It has been reported that the absorption of polar molecules\textsuperscript{44} and larger peptides\textsuperscript{47} are greatly improved by the incorporation of absorbing enhancers into the formulation such as surfactants (e.g. sodium laurylsulfate), enzyme inhibitors (e.g. bestatin), bile salts (e.g. sodium deoxycholate), chitosan, and cyclodextrins.\textsuperscript{58,59}
Permeation enhancers accelerate the rate of transportation of hydrophilic and larger protein and peptide molecules through mucosal membranes. A number of parameters control the safety and efficacy of nasal permeation enhancer, such as enzymatic activities of Cytochrome P450 iso-enzymes in the nose, mucociliary clearance, duration of contact between formulation and nasal mucosa. Absorption enhancers reversibly change the nasal mucosa, effectively increasing the drug absorption and do not cause local or systemic irritation or toxicity and their action is reversible.

**Mucociliary clearance**

Mucociliary clearance (MCC) is an essential defense mechanism for the elimination of foreign materials, pathogens and particles from the nose. Materials are trapped in the mucus layer and are subsequently transported by ciliary beating to the nasopharynx and eventually to the gastrointestinal tract. This process reduces the nasal absorption of drugs. Overcoming the MCC and increasing the residence time of drug in the nasal cavity are obtained by incorporation of mucoadhesive agents into formulation, which may enhance nasal absorption. The clearance half-life of radio labeled material in liquid was 15 minutes from the nasal cavity using conscious sheep. By contrast, using bio-adhesive chitosan solutions the half-life was 43 min and when employing bio-adhesive chitosan microspheres the half-life exceeded 110 min. Chitosan may decrease MCC, hence increasing contact time of the delivery system with nasal mucosa, which enhances the drug bioavailability.

MCC can also be influenced by targeting the region of drug deposition in the nose. Posterior part of the nose is more ciliated than its anterior part; therefore the clearance of the drug deposited posteriorly might be faster than the drug deposited in the anterior part of the nose. For example, nasal sprays deposit drugs mainly in the anterior sections of the nasal cavity, leading to slower clearance than nasal drops which are directly instilled into the deeper regions of the nose.

**Mucoadhesive drug delivery systems**

The concept of mucoadhesion (bio-adhesion) has been traditionally used to increase the residence time of formulations at the site of absorption for local or systemic therapeutic effects. Mucoadhesive agents are synthetic or natural polymers which adhere to biological or mucosal surfaces due to strong interactions between polymer and the mucus.

Mucoadhesive systems are designed to decrease the mucociliary movement, mucosal enzymatic activity and to open the tight junctions to enhance permeation of drug through the epithelial tissue. Mucoadhesive systems thus improve therapeutic outcomes by allowing the drug at the site of action for extended period of time, target the drug to the specific tissue and control the drug release. It also decreased frequency of drug administration and improves patient compliance.

A number of theories explain the mechanisms involved in the interactions between polymers and mucus. An optimal degree of polarity is required for the polymer to wet and swell sufficiently when contacting the mucus layer and interpenetration between mucus and polymer chains should take place to achieve bioadhesion. To facilitate the mucoadhesion in intra-nasal administration several drug delivery systems have been recently developed, which increase the contact time of drug to mucous membrane and enhance its absorption.

**Mucoadhesive microspheres**

Particulate carrier technology offers a valuable advance for intra nasal drug delivery systems by the introduction of carriers such as microspheres, emulsions, liposomes etc. Microspheres are polymeric spheres in the size range of 1 - 1000 μm. Due to their high surface area, microspheres can interact with mucin and increase residence time of the formulation with epithelial cells. Microparticle carriers protect drug from the enzymatic or chemical degradation in vivo. Particulate bio-adhesive systems may accommodate the drug and release it over a long period of time, resulting in reduced dosing frequency. Mucoadhesive microspheres have the ability to swell and form viscous gels upon contact with mucous layer retaining the drug for extended time at the site of absorption. Microspheres which constitute a significant part of these particulate delivery systems have several useful attributes. The most valuable property of microspheres is to increase the residence time of drug at the mucosal surface in nasal cavity. It has been reported that microspheres exert a direct effect on the nasal mucosa because the epithelial cells dehydrate causing the tight junction to separate by absorbing water from mucus and swelling. The increased residence time of microspheres at the mucosal surface may facilitate the increased uptake of drug formulation incorporated with the microspheres by more increased contact time between the vaccine and the mucosal membrane. The microspheres with a mucoadhesive property can offer additional advantages that may help to prolong residence time and improve uptake of drug incorporated with them. The other advantages of mucoadhesive drug delivery systems include bioavailability improvement of drugs, absorption enhancement of macromolecules and prolonged residence time at the site of application. In general, the mucoadhesive nature, which increases the time of attachment at the absorption site, the easy availability of free amino group for cross-linking, ease of fabrication of polymeric particles without using hazardous solvents, the cationic nature that permits ionic cross-linking with multivalent anions, and finally the ability to control the release of the administered drug makes chitosan the polymer of choice for developing the polymeric particle.
Chitosan as a bio-adhesive enhancer

Chitosan is a natural polysaccharide consisting of glucosamine and N-acetyl glucosamine. It can be derived through the partial deacetylation of chitin, the major compound of exoksetons in crustaceans. The term chitosan refers to a series of polymers with different molecular weights, viscosity, degrees of deacetylation, pKa, etc. The molecular unit of chitosan has one amino group and two hydroxyl groups that are potentially capable of reacting with an acidic medium. The amino group in chitosan has a pKa value of ~6.5; hence, chitosan is positively charged and is soluble in an acidic solution with a charge density that is dependent on the pH and the degree of deacetylation. The presence of an amino group in chitosan enables it to chemically react with anionic systems, thereby resulting in the modification of the physicochemical characteristics of such combinations.\(^{73}\)

In addition, the free amino group in chitosan is readily available and reacts with a number of negatively charged polymers. Chitosan is one of the most promising polymers because of its nontoxic, polycationic, biocompatible, and biodegradable nature, and particularly due to its mucoadhesive and permeation-enhancing properties. The strong mucoadhesive property of chitosan is most important for drug delivery through the mucosal routes. In addition, the interaction of the positively charged chitosan with the negatively charged mucin layer and the tight junctions facilitates the paracellular transport of hydrophilic macromolecules by opening the tight junctions of the mucosal barrier.\(^{74}\) The strong mucoadhesive properties of chitosan point to its potential as a permeation enhancer for mucosal drug delivery. Nasal and oral drug delivery researches have confirmed that significantly higher amounts of macromolecules can be absorbed across the mucosal barrier after co-administration with chitosan.\(^{75}\) The absorption-enhancing effect of chitosan has been found to be due to the combination of improved mucoadhesion between the formulation and the nasal tissues and the transient effect of chitosan on the paracellular pathways. The mucociliary clearance rate can be decreased by the use of mucoadhesive polymers. Several studies showed that chitosan prolonged the residence time of nasally delivered drugs at the absorption site.\(^{62,74}\)

Last two decades witnessed an unprecedented advance in pharmacotherapy of epilepsy. Despite this pharmacological improvements around 35\% of people with epilepsy still do not respond adequately to drug therapy.\(^{75}\) Considerable efforts are shifted on decoding the complexities of treatment response for epileptic patient. The mechanisms by which AEDs penetrate the BBB and how these mechanisms might be compromised in patients with refractory epilepsy have been critically monitored.\(^{76}\) Several experimental and clinical findings showed that intrinsic or acquired overexpression of multidrug transporters in the BBB create pharmacoresistance in epilepsy because of low concentrations of AEA which is not sufficient to produce anti-epileptic effect in patients.\(^{77}\) Multidrug transporters such as P-glycoprotein and the multidrug resistance-associated proteins in the BBB are thought to act as active defense mechanism, restricting the penetration of lipophilic substances into the brain.\(^{78}\) Moreover, overexpression of drug efflux transporters in neurons and glia of the brain parenchyma further limit the effectiveness of AEDs through restricted access to intracellular target sites. These results support the theory that multiple drug resistance to AEDs involves cerebrovascular changes that hindered the achievement of appropriate drug levels in the central nervous system. At the same time, initial experimental and clinical studies indicated that coadministration of the multidrug transporters inhibitors improved seizure control in rodent intractable epilepsy models.\(^{79}\)

In view of these shortcomings of epilepsy treatment, researchers across the globe are working on development of alternative pathways that transfers antiepileptic agents into the CNS effectively and safely. Literature suggests that nanomedicine may be possible drug carriers for selective brain drug delivery via different routes. Several nanomedicines have confirmed early preclinical success for the management of CNS conditions such as epilepsy, Alzheimer’s disease, brain tumors, HIV and encephalopathy.

An additional approach to bypass the BBB is administration via nasal route. Certain neuropeptides and perhaps other drugs (sertaline, respiridone, olanzapine, rivastigmine and 17- beta-estradiol) can be delivered safely and repeatedly using intranasal nanoparticles as sustained release carriers to enhance bioavailability to clinically relevant CNS targets. In such approach thyrotrophin releasing hormone nanoparticles (TRH-NPs) have provided an alternative treatment that could significantly impede or possibly prevent epileptogenesis where more conventional therapies have not been successful. Intranasal administration of a TRH analog and nanoparticles containing TRH acutely suppressed the fully kindled seizures in a concentration-dependent manner.\(^{80}\)

In a separate study, a suitable gel formulation was designed to provide the absorption of a highly lipophilic drug, carbamazepine through nasal mucosa. Significantly high level of the drug was found in the brain following intranasal administration compared to intravenous and oral routes. These findings suggested the existence of a direct transport pathway for carbamazepine from the nasal cavity to the brain. This pathway may represent a new delivery route to the brain and central nervous system of such drugs which are needed in high and rapid concentration in the brain, especially in emergencies.\(^{81}\)

The similar pharmacokinetic profiles of carbamazepine also demonstrated by Serralheiro et al.\(^{82}\) 2014 following IN delivery. Carbamazepine quickly reaches the brain
predominantly via systemic circulation. However, the biodistribution of carbamazepine through the brain regions with higher concentrations in the olfactory bulb and frontal cortex following IN instillation, in comparison with IV injection, strongly suggests the involvement of a direct transport of carbamazepine from nose to brain.

In similar lines anticonvulsant drug, phenobarbital was efficiently delivered to the rat brain following intranasal administration. A mucoadhesive preparation of phenobarbital resulted in brain concentrations that were superior to intranasal administration. In comparison with concentrations achieved following intravenous administration of 5.4 mg/kg phenobarbital, intranasal administration of the same dosage resulted in 2.4-fold higher Cmax values in cortical areas. This study concludes that, intranasal administration of phenobarbital in rats is associated with efficient brain penetration to achieve better therapeutic concentrations.

Recently, Serralheiro et al., 2015 evaluated the pharmacokinetics of lamotrigine administered by the intranasal route to mice. The heterogeneous biodistribution of lamotrigine in different brain regions, with higher concentration levels attained in the olfactory bulb comparatively to the frontal cortex and the remaining portion of the brain, strongly suggest that lamotrigine was directly transferred to the brain via the olfactory neuronal pathway, circumventing the BBB. Therefore, it seems that intranasal route can be assumed as a suitable and valuable drug delivery strategy for the chronic treatment of epilepsy, also providing a promising alternative approach for a prospective management of pharmacoresistance.

Likewise Alam et al., 2015 investigated the efficacy of nanostructured lipid carriers (NLCs) to enhance the brain targeting of lamotrigine following intranasal administration. Sustained drug concentration was obtained in NLCs carrying lamotrigine after IN administration after 24 h. Scintigraphy studies proved high accumulation of drug in brain compared to its oral administration.

Mouez et al., 2014 used chitosan microspheres (21-53 μm) for delivery of verapamil nasally in rabbits and found a significantly higher bioavailability (58.6%) than when applying a simple solution of verapamil to the nasal cavity (47.8%). It should be noted that due to the lipophilicity of the molecule the difference although significant statistically is not of much therapeutic significance.

Baltzley et al., 2014 prepared olanzapine (OZ) loaded chitosan nanoparticles through ionotropic gelation of chitosan with tripolyphosphate (TPP) anions and studied in terms of their size, drug loading, and in vitro release. The OZ nanoparticles were administered IN to rabbits, and OZ plasma concentration at predetermined time points was compared to IN administration of OZ in solution. The concentrations of OZ in plasma were analyzed by ultra-performance liquid chromatography mass spectroscopy. OZ-loaded chitosan nanoparticles significantly enhanced systemic absorption with 51±11.2% absolute bioavailability as compared to 28±6.7% after IN administration of OZ solution. The results of the present study suggest that intranasal administration of OZ-loaded chitosan nanoparticles formulation could be an attractive modality for delivery of OZ systemically.

Similar results were found by Gungor et al., 2010 for nasal delivery of ondansetron. The chitosan/ondansetron microspheres showed a significant burst release, allowed the required rapid onset of the therapeutic effect and due to the controlled release of the remaining drug also afforded a sustained plasma profile.

Chitosan microspheres containing insulin (20-45 μm) were prepared by an emulsification-cross-linking process and administered to rats in a study by Varshosaz et al. 2009, and showed a 67% reduction in blood glucose level and a bioavailability of 44%. Likewise Patil et al., 2012 studied the nasal delivery of carvedilol encapsulated in chitosan microspheres produced by an identical method and found a bioavailability of 72.3% in rats. It was also shown by gamma scintigraphy that the microspheres cleared slowly from the nasal cavity, in line with the study in sheep published by Soane et al., 2001.

In addition, Nagda et al., 2011 produced chitosan microspheres by a solvent evaporation technique, with encapsulation efficiency for ketorolac of 52–78% w/w, and a particle size ranging from 14–46 μm. Morphological changes in nasal epithelia exposed to the ketorolac loaded microspheres were milder than those exposed to the drug alone and isopropyl alcohol. Sheep nasal mucosa remained intact and retained good morphology after exposure to microspheres in vitro.

Sun et al., 2009, developed methotrexate-loaded microspheres (<5 μm), employing three different (low, medium and high) molecular weights of chitosan. The medium molecular weight chitosan microspheres exhibited the strongest mucoadhesion and allowed control of the rate of release of methotrexate by modifying the drug/polymer ratios.

Gavini et al., 2008 developed a microspheres based on methyl pyrrolidone derivatized chitosan (MPC) loaded with metoclopramide. In comparison with unmodified chitosan microparticles containing drug, the size (~10μm) and the in-vitro release behavior (100% in 30 minutes) were similar, however MPC based drug containing microparticles showed better muco-adhesiveness but less swelling capability. It was also evident that the swelling capacity was very dependent on the drug content for both chitosan and derivatised
chitosan microparticles. An ex-vivo permeation study through sheep nasal mucosa showed a slower and hence prolonged permeation profile (40% at 3 hours), as compared to the pure drug (80% at 3 hours) and nonderivatised chitosan microparticles (60% at 3 hours) with the same drug content. The same group also studied the nasal delivery of carbamazepine in chitosan microspheres and found an increase in Cmax from 25 ng/ml to 800 ng/ml in sheep, when compared to delivery of pure drug in powder form.

Recently attempt was made to deliver lamotrigine; via intranasal route as mucoadhesive microspheres, developed by emulsion-solvent evaporation using chitosan as polymer cross linked by glutaraldehyde by varying stirring rate, viscosity, volume of the phases, drug polymer ratio, and % of cross linking agent. Prepared microspheres were smooth spherical shaped microparticles of size range 17 µm to 40 µm with avg. particle size of 23 µm, DSC and FTIR studies support solid solution entrapment with no interaction between drug and other ingredients. Entrapment efficiency was 75.74±0.50 mucoadhesion was 98.5% and drug release was 87.86% which conclude that chitosan based microspheres are suitable for the intranasal delivery of lamotrigine (Dave and Purohit, 2013).99

Moreover, an intranasal formulation for lorazepam, as an alternative route of drug delivery to the brain was developed by Jose et al., 201396 using chitosan as polymer. Direct transport of drugs to the brain circumventing the brain barrier following intranasal administration, provides a unique feature and better option to target brain. The presence of mucoadhesive microspheres in the gel vehicle via nasal route can achieve a dual purpose of prolonged drug release and enhanced bioavailability.

Taksande et al., 2017a95 prepared and optimized LT loaded CH mucoadhesive microspheres for nasal administration employing ionic gelation method. Physicochemical investigations demonstrated that the LT microspheres showed suitable particle size for nasal administration, high encapsulation efficacy, and strong bioadhesion potential without any morphological toxicity to excised sheep nasal mucosa. In addition, permeation, across excised sheep nasal mucosa exhibited good permeability of LT loaded CH microspheres. Importantly the intranasal administration of LT microspheres delayed the onset of convulsion and offered the complete protection against the PTZ induced seizures compared to its peripheral administration. Taksande et al 2017b96 evaluated N,N,Ntrimethyl chitosan was as chemically modified derivative of chitosan for the preparation of lamotrigine loaded TMC mucoadhesive microspheres for intra-nasal administration by ionic gelation technique.

Zaripour et al., 201397 successfully synthesized TMC from chitosan by the two-step method. NMR spectrum of TMC showed 51% degree of quaternization. The synthesized TMC was used in the preparation of nanospheres loaded tetanus toxoid (TT). TMC nanospheres loaded TT was prepared under mild conditions using TPP as a polyanion cross-linker. Stable TMC nanospheres with a small size and a narrow size distribution were obtained. The TMC nanospheres have an acceptable loading capacity for proteins, and a positive surface charge, suitable to attach to nasal mucosa. The integrity of the loaded antigen was preserved. In vitro studies confirmed that this system is capable of delivering the antigen with TMC to nasal mucosa.

DuPlessis et al., 2010a98 evaluated the ability of trimethyl chitosan (TMC), with different degrees of quaternization, to increase insulin absorption in vivo following nasal and rectal administration in rats. Two batches of TMC with different degrees of quaternization (TMC-L, 12.3% quaternized and TMC-H, 61.2% quaternized) and chitosan hydrochloride were administered intranasally (0.25 and 0.5% w/v) and rectally (0.5% w/v) with insulin (4 IU/kg body weight), at a pH of 4.4 and 7.4, in rats. Blood samples were taken over a period of 2 h for measurement of blood glucose levels and plasma insulin levels. Local toxicity evaluation was done by histological examination of the nasal and rectal epithelia. At pH 4.4 all these polymers were able to increase nasal and rectal insulin absorption, compared to the control groups. However, at a pH of 7.4, only TMC-H was able to increase the nasal and rectal absorption of insulin. These results relate to the insolubility of chitosan hydrochloride at neutral pH values, while the charge density of TMC-L is still too low for any significant interaction at pH 7.4. Histological evaluation of the nasal and rectal epithelia shows no changes in the morphology of the cells after exposure to these polymers. Only slight congestion of the nasal submucosa was observed and all these polymers led to a mild increase in mucus secretion at pH 4.4. Highly quaternised TMC proves to be a potent absorption enhancer in vivo, especially at neutral pH values where chitosan salts are ineffective.
Table 1: Selections of antiepileptic drugs against epileptic seizure

<table>
<thead>
<tr>
<th>Type of Epileptic Seizure</th>
<th>Antiepileptic Drugs First Line</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Generalized Tonic-Clonic</td>
<td>Valproic acid, Lamotrigine, Topiramate</td>
<td>Zonisamide, Phenytoin, Carbamazepine, Oxcarbazepine, Phenobarbital, Primidone, Felbamate</td>
</tr>
<tr>
<td>Partial</td>
<td>Carbamazepine, Phenytoin, Lamotrigine, Oxcarbazepine, Valproic acid</td>
<td>Levetiracetam, Topiramate, Tiagabine, Zonisamide, Gabapentin, Pregabalin Phenobarbital, Primidone, Felbamate</td>
</tr>
<tr>
<td>Absence</td>
<td>Valproic acid, Ethosuximide</td>
<td>Lamotrigine, Clonazepam</td>
</tr>
<tr>
<td>Atypical Absence, Myoclonic, Atonic</td>
<td>Valproic acid, Lamotrigine, Topiramate</td>
<td>Clonazepam, Felbamate</td>
</tr>
</tbody>
</table>

CONCLUSION

Chitosan microspheres are an attractive alternative nasal delivery system for a variety of drugs for the treatment of epilepsy. The microspheres have been produced by various methods such as spray drying, ionotropic gelation or emulsification followed by crosslinking and in general been applied as a dry formulation to the nasal cavity that showed the ability to take up water and form a mucoadhesive gel. This approach overcomes the mucociliary clearance, prolongs the time required for absorption and higher drug concentration reaches to brain overall increasing therapeutic effect of antiepileptic drugs.

REFERENCES


80. Kubek MJ, Domb AJ, Veronesi MC. Attenuation of kindled seizures by intranasal delivery of