CHAPTER 2

INTRODUCTION
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2.1 Central Nervous System

The brain is a delicate organ, and evolution built very effective ways to protect it. Unfortunately, the same mechanism that protects brain against chemicals can also frustrate therapeutic inventions. Despite several advances in brain research, brain and central nervous system disorders such as brain tumors, HIV encephalopathy, epilepsy, cerebrovascular diseases and neurodegenerative disorders, remain the world’s leading cause of disability, and account for more hospitalizations and prolonged care than all other diseases. Only 3-5% of CNS drug candidates that enter phase I clinical trials are successfully launched in market compared to approximately 10% for all other compounds [1]. The high failure rate of drug candidates during all stages of the drug development process is a critical issue for both economic and treatment reasons. It has been shown that the major factor leading to the failure of new chemical entities (NCEs) during drug development does not necessarily result from a lack of drug activity but is the lack of permeation of drug through various barriers which prevents the NCE from reaching its target specifically brain.

2.1.1 Barriers to CNS Delivery:

1. Blood Brain Barrier: The drug discovery process for drugs that target the central nervous system suffers from a very high rate of failure due to the presence of the blood–brain barrier, which limits the entry of drug into the brain. Blood Brain Barrier (BBB) is a major rate limiting barrier in brain targeted drug delivery system. The capillaries present in brain are lined with a layer of special endothelial cells that lack fenestration and are sealed with tight epithelium. The specificity of the endothelial cells comprising the BBB compared to the endothelial cells in the rest of the body is based on their organization. Cerebral endothelial cells are connected by intercellular proteins, occludins, claudins and junctional adhesion molecules, together with cytoplasmic accessory proteins, including zonula occludens-1 (ZO-1), ZO-2, ZO-3. Others are transmembrane proteins that are responsible for the formation of tight junctions (TJs) that seal the paracellular pathway and make the brain nearly inaccessible to polar compounds that are
not the substrates of specific transporters. The TJs in cerebral capillaries are approximately 50 to 100 times tighter than the TJs in peripheral capillaries and thus results in very high transendothelial electric resistance of 1500-2000Ω cm⁻² compared to 3-33 Ω cm⁻² of other tissues. In brain capillaries, the principle route of transport takes place through transcellular mechanism. Thus, only lipid soluble solutes can freely penetrate through the BBB. Moreover, the BBB also acts as a metabolic barrier due to the presence of numerous enzymes, including peptidases, γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), nucleotidases, cytochromes P450 (CYP450) and monoamine oxidase (MAO). These enzymes can metabolise potentially harmful drugs to inactive CNS compounds, convert an inactive drug to its active CNS metabolite or degrade them into metabolites [2, 3]. Moreover, BBB endothelial cells have very limited pinocytic vessels (3-6 per μm³) compared to peripheral endothelium (82-93 per μm³) which leads to very limited pinocytic transcellular transport. Many transmembrane proteins are expressed on the luminal and abluminal membranes of the endothelium to transport nutrients and to eliminate waste products of metabolism. In particular, proteins, such as GLUT-1 (glucose transporters), transport polar nutrients; Na-ATPase and K-ATPase transport sodium and potassium ions respectively; insulin or transferrin receptors transport proteins and organic anion transporting proteins (OATP). These transporters all play important roles in the maintenance of cerebral equilibrium. The BBB is further reinforced by a high concentration of efflux transporters, such as P-gp/ABCB1 or BCRP P-glycoprotein (Pgp), in the luminal membranes of the cerebral capillary endothelium [3-5].

2. Blood-Cerebrospinal Fluid Barrier (BCB): The other barrier that a systemically administered drug has to cross before entering the brain is known as Blood-Cerebrospinal Fluid Barrier. Physiologically, the BCB is found in the epithelium of the choroids plexus, arranged in a manner that reduces the entry of molecules and cells into the CSF. The choroid plexus and the arachnoid membrane in combination act as the barriers between the blood and CSF. Further, the BCB also have an active organic acid transporter system in the choroids plexus, which is capable of removing CSF-borne organic acids into the blood. As a result a variety of therapeutic agents such as the antibiotic penicillin, the anti-neoplastic agent methotrexate, and the antiviral agent zidovudine are actively removed from the CSF [1, 6].
3. **Blood tumor barrier:** Intracranial drug delivery is more challenging if the target is CNS tumor. In CNS malignancies the BBB is significantly compromised but variety of physiological barriers common to all solid tumors reduces the drug delivery via the systemic circulation.

Brain uptake of drug can be positively correlated with lipophilicity or negatively correlated with hydrogen bonding. Increase in lipophilic factor improve membrane permeability improves BBB permeation and brain uptake. But very high lipophilicity also leads to increase in the volume of distribution and affect other pharmacokinetic parameters including rate of oxidative metabolism by cytochrome P450. Thus, the optimum balance is required [1].

Therapeutic action in form of pharmacological response is a measure of brain uptake. This biological activity depends on rate of transfer from blood to brain or distribution between blood and brain and interaction between drug and targeted receptors in brain. The different experimental measuring tools for brain uptake such as brain uptake index, permeability surface area product, permeability coefficient are widely used [7, 8].

Drugs used against CNS diseases should reach the brain via the blood compartment and must also pass above mentioned barriers particularly the Blood Brain Barrier (BBB). Frequently the molecule is too large or has a polar function group and thus the above said barriers limit its access to CNS. To overcome the barriers various drug delivery strategies have been developed.
2.1.2 Drug delivery strategies for CNS Targeting:

Numerous drug delivery strategies have been developed to overcome the several of barriers inhibiting CNS penetration by potential therapeutic agents. These alterations can be divided into following three categories: manipulating drugs, disrupting the BBB and finding alternative routes for drug delivery.

1. **Drug Manipulations:** This strategy includes change in parent molecule in a manner that favored brain uptake. This manipulation mainly includes use of lipophilic analogs, prodrugs, chemical drug delivery system and carrier/ vector mediated drug delivery system. For example, dopamine, a drug for Parkinson’s disease, cannot cross the BBB and enter the CNS. Carbonylation of dopamine known as L-DOPA allows the active transport of the inactive prodrug form through the BBB. After the prodrug has entered the brain, DOPA decarboxylase activates L-Dopa into active dopamine [1, 9].

2. **Disruption of BBB:** One of the approaches to circumvent the dense microvasculature of the brain is by delivering drug moiety using transient osmotic opening. Hyperosmolar substances like mannitol, arabinose is likely to cause destruction of BBB because to migration of water from endothelial cells to capillaries which in turn cause shrinkage of cells and results in intracellular gaps. A somewhat safer technique involves the systemic delivery of the convulsant drug, Metrazol, which temporary improves the BBB permeability while causing seizures. Simultaneous administration of the anticonvulsant pentobarbital blocks seizing while allowing BBBD to persist. Recently, new and potentially safer biochemical techniques have been developed to disrupt the BBB. Selective opening of brain tumor capillaries (the blood-tumor barrier), by the intracarotid infusion of leukotriene C4 was achieved without concomitant alteration of the adjacent BBB [10, 11].

3. **Inhibition of Efflux Transporters:** The presence of efflux transporters at the BBB reduces the entry of many CNS drugs into the brain. In HIV treatment, the most efficient drugs, such as abacavir and efavirenz, are substrates of the ABC transporters. Hence, a useful strategy for these types of drugs is to inhibit efflux transporter activity or saturate these transporters with substrates that have higher affinity than the drug. This strategy is widely used in HIV multi-therapy and improves the intracerebral concentration of HIV protease inhibitors. However, this
strategy may have several drawbacks because this strategy will allow the penetration of other xenobiotics [1].

4. **Drug Delivery with Nanocarriers:** Liposomes, polymeric nanoparticles, solid lipid nanoparticles and micelles are all nanocarriers and have garnered great interest in recent pharmaceutical research. Drug delivery is a method of bypassing poor solubility, poor permeability or poor bioavailability by incorporating the compound of interest into phospholipidic, polymeric or inorganic vesicles. For example, immunoliposomes, grafted with OX26 monoclonal antibody are able to recognize transferrin receptor at the BBB, which transport it through a rat BBB model via endocytosis [1, 12].

5. **Alternative Routes to CNS Drug Delivery:** Despite of previous two techniques, many potentially effective drug molecules still cannot cross into the brain parenchyma at therapeutic levels. Thus, above mentioned strategies aim to improve the CNS penetration of drugs delivered via the circulatory system; the result is higher drug concentration throughout the entire body and frequently leads to systemic side effects. A third class of strategies aimed at enhancing CNS penetration of drug molecules is composed of delivery methodologies that do not depend on systemic circulation [1].

- **Intraventricular/Intrathecal Route:** One strategy for bypassing the BBB and improves CNS uptake that has been studied extensively both in laboratory and in clinical trials is the intralumbar injection or intreventricular infusion of drugs directly into the CSF. When compared to vascular drug delivery, intra-CSF drug administration theoretically has several advantages. Intra-CSF administration bypasses the BCB and results in immediate high CSF drug concentrations. Since, the drug is somewhat contained within the CNS, a smaller dose can be used, potentially minimizing systemic adverse effects. However, this delivery method has not achieved its theoretical potential for several reasons. These include a slow rate of drug distribution within the CSF and higher in intracranial pressure associated with fluid injection or infusion into small ventricular volumes. It results in to high clinical occurrence of hemorrhage, CSF leaks, and neurotoxicity and CNS infections [13].

- **Interstitial Delivery:** The most direct way of circumventing the BBB is to deliver drugs directly to the brain interstitium. By directing agents to an intracranial target, interstitial drug
delivery can theoretically yield high CNS drug levels with minimal systemic toxicity. Furthermore, with this strategy, intracranial drug concentrations can be extended, which is crucial in treatment with many chemotherapeutic agents. Drug delivery directly to the brain interstitium can be achieved by injections, pumps, catheters, biodegradable wafers [13].

- Intranasal delivery: The impermeable nature of blood brain barrier is always a challenge for successful delivery of drugs to central nervous system. Intranasal delivery is gaining a remarkable importance in CNS targeting due to various advantages. Realization of nose-to-brain transport and the therapeutic viability of this route can be traced from ancient times and has been investigated for rapid and effective transport in the last two decades [14].
2.2 INTRA NASAL DELIVERY

Intranasal drug delivery is one of the focused delivery option for brain targeting as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation. From the ancient times, Nasal route has received attention for the mankind. Nasal therapy, also called “NASAYA KARMA”, has been recognized form of treatment in the Ayurvedic system of Indian medicine. Fig. 1 represents fate of drug after nasal administration. Due to the unique connection of the nose and brain, the intranasal route can deliver therapeutic agents to the brain bypassing the BBB [15].

![Diagram of drug transport from nasal cavity to brain](image)

**Figure 1 Direct transport of drug from nasal cavity to brain**

Absorption of drug across the olfactory region of the nose provides unique feature and superior option to target drugs to brain. Evidence of nose-to-brain transport has been reported by many scientists. Many previously rejected potent CNS drug candidates promise to become successful CNS therapeutic drugs via intranasal delivery. Due to this, the investigations till have attracted researchers to keep the intranasal drug delivery option under the microscope. Nevertheless, it is important to understand the uptake of drug across the nasal mucosa [16].

2.2.1 Nasal Anatomy and Physiology:

The human nasal cavity has a total volume of about 16-19 ml and total surface area of about 180 cm². It is divided into two nasal cavities via septum. The nasal cavity consists of three
anatomically distinct regions, the vestibular, respiratory or olfactory region. Post drug administration into the nasal cavity, a solute can be deposited at one or more of three anatomically distinct regions. Drug will have to cross the olfactory membrane and also the arachnoid membrane surrounding the arachnoid space containing the cerebrospinal fluid in before reaching CNS from the nasal cavity[16] (Fig. 2).

Fig 2 Human nasal cavity, showing the nasal vestibule (A), Atrium (B), Respiratory area: Inferior (C1), Middle (C2) and superior (C3) turbinate, Olfactory region (D) and Nasophrynx (E)

The major portion of nasal cavity is respiratory region that is having area of about $130\text{cm}^2$ and the three turbinates are also present in this region. There are four types of cells present in the respiratory epithelium that are ciliated, non-ciliated, and basal and goblet cells. The respiratory region is having the highest degree of vascularity and is mainly responsible for drug absorption. The olfactory region has just minor portion in nasal cavity round about $10-20\text{cm}^2$. It is to be found in the roof of nasal cavity & on the upper part of the nasal septum [17-18].

The receptors for sense of smell are present in olfactory region. This olfactory region bypasses the blood brain barrier and drug can directly reaches to CNS. The free entry is there in between
the nasal submucosal interstitial space & olfactory perinueronal space, which is continuous with a subarachnoid extension that surroundings the olfactory nerve. In humans, the olfactory region is present on the roof of nasal cavity and separates cranial cavity and present below the cribiform plate of the ethmoid bone [14, 19].

The olfactory epithelium is pseudo stratified columnar structure (Fig. 3). It consists of specialized olfactory cells, supporting cells, serous and mucosal glands. The non olfactory part is a vascular membrane. Its surface is covered by ciliated pseudo stratified columnar epithelium. Numerous groups of microvilli can be seen microscopically along the group of cilia. There are approximately 500 microvilli on the surface of each ciliated cell. These cells with microvilli are called goblet cells. Another type of epithelial cells is observed in the free surface of the mucous membrane. They are rounded or elongated in shape and rough on the surface. These cells are defined as squamous cells [20].
The composition of the nasal secretions is complex and it consists of a mixture of secretory materials secreted from the goblet cells, nasal glands, and lacrimal glands and a transudate from plasma. In a clean, noninfected, nonallergic, and nonirritated nose, the mucosa is covered by a thin layer of clear mucus which is secreted from the mucous and serous glands in the nasal mucosa and submucosa. A total of approximately 1500-2000 ml of mucus is produced daily, which contains 90-95 % water, 1-2 % salt and 2-3 % mucin. The mucus has a two-layer composition: The watery (sol) layer is located immediately adjacent to the mucosal surface, and the mucous (gel) layer, which is more superficial. Normal nasal secretions contain sodium, potassium, calcium as well as proteins, including albumins and immunoglobulin A (IgA) and G (IgG) [21].

2.2.2 Drug Absorption through the Nasal Mucosa

As seen with the other epithelium in the body, absorption across nasal epithelium can occur by one or combination of mechanisms. Following two mechanisms have been considered predominantly. The first mechanism involves an aqueous route of transport also known as the paracellular route. Drugs are believed to pass through the epithelium via the pores between the cells (the tight junction). This route is slow and passive and this pathway is specially suited for smaller hydrophilic molecules. Although, the tight junctions are dynamic structures that can open and close to certain extent, the size of these channels is less than 10 Å. Hence, the paracellular route will be less efficient for large molecules [19, 22].

The second mechanism involves transport through a lipoidal route that is also known as the transcellular process and is responsible for the transport of lipophilic drugs by an efficient concentration dependent passive diffusion process, by receptor or carrier mediation and by vesicular transport mechanism. This pathway is especially suited for small lipophilic molecules or large molecules. Intracellular axonal transport of drugs through olfactory neuron cells is also one of the mechanisms, responsible for transport of drugs primarily to the olfactory bulb.

Other mechanisms include organic cation transport system and organic anion Transport system. For example, Dopamine is poorly bioavailable if given orally, but if given intranasally the good effect can be seen. At least three dopamine transporters contribute in this process: dopamine
transporter (DAT; encoded by SLC6A3) and organic cation transporter1 (OCT1) and OCT2
(encoded by SLC22A1 and SLC22A2, respectively [23].
2.2.3 Advantages and limitations of nasal delivery:

The nasal delivery offers various advantages which are as follows [24-28]:

- The attractive, noninvasive, and most conceivable portals for entry to systemic circulation and central nervous system
- Non-invasive, rapid and comfortable
- Bypasses the BBB and targets the CNS which provides faster onset of action
- Avoidance of first pass metabolism by the liver
- Rich vasculature and highly permeable structure of the nasal mucosa greatly enhance drug absorption. Rapid absorption through nasal route can be achieved with quick action.
- Works for wide range of drugs.
- It facilitates the treatment of many neurological and psychiatric disorders.
- Direct transport of drugs to the brain may lead to the administration of lower doses and in turn can reduce toxicity.

Limitations of nasal delivery are as follows [24]:

- Smaller instillation volume generally 25-200 µl.
- Nasal irritation may occur
- Delivery is expected to decrease with increasing molecular weight of drug.
- Nasal congestion due to cold or allergies may interfere with this method of delivery.
- Concentration achievable in different regions of the brain and spinal cord varies with each agent.
- The normal defense mechanisms like mucociliary clearance or ciliary beating can affect the permeability of drugs.
2.2.4 Factors influencing the absorption of drugs through nasal epithelium:

Factors influencing absorption are related to nasal physiology, physicochemical characteristics of drugs and formulation aspects.

2.2.4.1 Physicochemical characteristics of drugs: Various physicochemical characteristics of drug can also affect nasal absorption of the drug.

- Molecular Weight and Size: Extent of the absorption of the drug depends on molecular weight particularly for hydrophilic compounds. Nasal route is suitable for efficient delivery of drugs up to 1000 Daltons. Absorption reduces significantly if the molecular weight is greater than 1000 Daltons except with the use of penetration enhancers [29].

- Solubility and Dissolution: Drug solubility is a major factor in determining absorption of drug through biological membranes. It not only limits the drug absorption but, it can also limit an inventor’s ability to formulate a product if the drug is not sufficiently soluble in the desired vehicles [29, 30].

- Chemical form: The chemical form in which a drug is presented at the nasal mucosa can be important in determining its absorption. For example, conversion of a drug into a salt or ester form can alter its absorption [30].

- Partition coefficient and pKa: There is a quantitative relationship existed between the partition coefficient and nasal absorption constant. As per the pH partition theory, unionized species are absorbed better compared with ionized species and same holds true in the case of nasal absorption [30].

2.2.4.2 Factors Related to Formulation:

- Drug concentration, dose and dose volume: Drug concentration, dose and dose volume of administration are three interrelated parameters that impact the performance of the nasal delivery system. Nasal absorption of L-Tyrosine was shown to increase with drug concentration in nasal perfusion experiments. However, in another study, aminopyrine was found to absorb at a constant rate as a function of concentration [31]. In general, higher nasal absorption or therapeutic effect was observed with increasing dose. It is important to note how the dose is varied. If the drug is increasing by increasing formulation volume, there may be a limit as to what extent nasal absorption can be increased. The nostrils can retain only a limited volume,
beyond which a formulation will drain out of the nasal cavity. The ideal dose volume range is 0.05-0.20 ml with an upper limit of 0.25 ml [31-33].

- **Formulation pH:** The pH of the formulation can affect a drug’s permeation. The pH of the nasal formulation is important for the following reasons:
  - To avoid irritation of the nasal mucosa.
  - To allow the drug to be available in unionized form for absorption.
  - To prevent the growth of pathogenic bacteria in the nasal passage.
  - To maintain functionality of excipients such as preservatives and
  - To sustain normal physiological ciliary movement.

Lysozymes are found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection. It is therefore advisable to keep the formulation at a pH of 4.5 to 6.5 keeping in mind the physicochemical properties of the drug as drugs are absorbed in the unionized form and also to avoid nasal irritation [33-35].

- **Buffer capacity:** Nasal formulations are generally administered in small volumes ranging from 25 to 200 μl with 100 μl being the most common dose volume. Hence, nasal secretions may alter the pH of the administered dose. This can affect the concentration of un-ionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH *insitu* [36].

- **Osmolarity:** Drug absorption can be affected by tonicity of the formulation. Shrinkage of the epithelial cells has been observed in the presence of hypertonic solutions [36].

- **Solubilizers:** Aqueous solubility of a drug is always a limitation for nasal drug delivery in solution. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol, (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol (saturated polyglycolyzed C8-C10 glycerides) can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as HP-β-Cyclodextrins that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers. In such cases, their impact on nasal Irritancy should be considered [37].
• Preservatives: Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. Preservatives are based in small quantities and are not likely to affect drug absorption [36].

• Antioxidants: Depending upon the stability profile of a given drug in the formulation chosen, it may be necessary to use antioxidants to prevent drug degradation. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxytoluene and Tocopherol [37].

• Humectants: Many allergic and chronic diseases are often connected with crusts and drying of mucous membranes. Adequate intranasal moisture is essential for preventing dehydration. Therefore, humectants can be added especially in gel-based nasal products [38].

• Absorption Enhancers: When it becomes difficult for a nasal product to achieve its required absorption profile, the use of absorption enhancers is recommended [39].

2.2.4.3 Physiological factors:

• Effect of deposition on absorption: Deposition of the formulation in the anterior portion of the nose provides a longer nasal residence time. The anterior portion of the nose is an area of low permeability while posterior portion of the nose, where the drug permeability is generally higher provides shorter residence time.

• Nasal blood flow: Nasal mucosal membrane is having very rich vasculature. The blood flow and therefore drug absorption will depend upon the vasoconstriction and vasodilation of blood vessels.

• Effect of enzymatic activity: Several enzymes are present in nasal cavity which might affect stability of drug including protease and amino peptidase. The level of amino peptidase is much lower than that in gastrointestinal tract.

• Effect of pathological conditions: Intranasal pathologies such as allergic rhinitis, infections may affect nasal absorption. Nasal pathology can alter mucosal pH and thus affect absorption of drug. During common cold, the efficiency of an intranasal medication is often compromised [39].
2.2.5 Models for nose to brain transfer of drug: Different in vitro and in vivo models are described in literature to study brain uptake of drug through nasal mucosa.

2.2.5.1 Ex vivo Models used to study brain uptake through nasal mucosa: There is difficulty in obtaining human nasal tissue specimens because of ethical issues. Thus excised nasal mucosa is used for study of nasal transport and metabolism in various animal species. Most studies were performed with epithelia of excised skin from rabbits, bovine, sheep, and dog’s tissues. Compared to in vivo or cell culture methods, the availability of animal tissues for experimental use is better with respect to ex vivo techniques. Because not only the ethical issues of using great numbers of animals in experimentations are avoided, but also nasal epithelium is very readily available from local slaughterhouses. This means that no animal is sacrificed only to obtain these tissues. Ex vivo models are very suitable for studying nasal toxicity of drugs, formulations, and excipients [39, 40].

2.2.5.2 Cell lines cultures: The RPMI 2650 nasal epithelial cell line obtained for study of tumors in animals. This cell line mainly used for the study of nasal metabolism because it grow into multilayer and not form monolayer. That’s why it is used rarely for study of nasal transport [40].

2.2.5.3 In situ nasal perfusion model: The method simply involves circulating a buffered solution containing the drug under investigation through the rat or rabbit nasal mucosa and back into the test solution. The amount of drug absorbed is estimated by measuring the concentration of drug remaining at specified time intervals. The consideration of other potential sources of drug loss other than absorption (e.g., physical, chemical, and enzymatic degradation) is very important when using this method to investigate nasal drug absorption. A number of drugs, including digoxin, midazolam, sumatriptan, and tyrosine-linked drug molecules, have been studied using the rat in using in situ perfusion model [41-43].

2.2.5.4 In vivo Model: In vivo models are the best models available because of the combination of all biological aspects in the same model, such as physiological barriers, transporters, and metabolic pathways. However, these models are expensive, time-consuming, requires the mastering of animal-based assays. There are two types of animal models which can be used for nasal absorption studies: the whole animal model and isolated organ perfusion model. The animal models using rat, rabbit, dog, sheep, and monkey have been reported for assessment of
nasal absorption studies. Different parameters that can be used after nasal administration of formulation to ensure brain uptake are as follows:

a. Monitoring CNS effect: Different pharmacodynamic studies can be used to check effect of drug on brain with respect to control animals. A few studies included control animals which received drug through alternative route [40].

b. Measuring drug concentration in CNS: Many scientists used this parameter as indicator for brain uptake studies. Generally rat is most widely used animal model for such studies. Liquid Scintillation (LS) & High performance liquid chromatography (HPLC) are the two selected methods for detection and quantification of drug in brain. The detection of metabolites can be done also with HPLC analysis to check the drug concentration, but it is time consuming because of the necessity for more complicated sample work up before analysis & larger tissue sample is required in this case. Mouse olfactory bulbs were too small in size for HPLC detection. Drug concentration was determined in brain and plasma by either HPLC or LS and then Brain/Plasma ratio was measured as indicative parameter. Ideally these studies should include frequent and early samples from both plasma and brain after intra nasal and intravenous administration to ensure calculation of AUC. Brain uptake index was determined by taking ratio of $\text{AUC}_{\text{brain}}/\text{AUC}_{\text{plasma}}$ [44-45].

c. Visualizing Drug Transfer: This is non invasive technique which can be easily applied on animals. In this technique radiolabelled formulation was administered in nasal cavity and distribution of drug in body was visualized by gamma scintigraphy image. This technique gives clear and true idea about biodistribution of drug in animal model. The main limitation of this technique is requirement of expensive equipments [44-46].

2.2.6 Nasal Dosage Forms:
Several nasal formulations have been used for brain targeting. The formulations include liquid, semisolid and solid dosage forms. Liquid dosage forms include Nasal drops, nasal sprays, emulsion and microemulsions. Semisolid dosage forms include ointments, nasal gel and insitu gel while solid dosage forms include nasal powder, liposomes, and nanoparticles. Microemulsions has been recently explored as an alternative drug delivery system through nasal route to demonstrate a possible alternative to IV administration and a promising
approach for rapid delivery of CNS medications. Since microemulsion is optical isotropic, thermodynamically stable system and imparts relatively more lipophilicity to the formulation, poorly water soluble drugs and drugs, prone to hydrolysis can be successfully formulated and administered by microemulsion [47-50].
2.3 MICROEMULSION

The term “microemulsion” was first used by Schulman et al. in 1959 to describe a multiphase system consisting of water, oil, surfactant and alcohol, which forms a transparent solution. There has been much discussion about the word “microemulsion” to describe such systems. Although not systematically used today, some prefer the names “micellar emulsion” or “swollen micelles”.

Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a cosurfactant. In contrast to ordinary emulsions, microemulsions form by simple mixing of the components and do not require the high shear conditions generally required in the formation of ordinary emulsions.

The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible light, microemulsions are transparent. The microemulsion is formed readily and generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase [47-49].

2.3.1 Structure:-

Microemulsion is dynamic systems in which the interface is continuously and spontaneously fluctuating. The interface is stabilized by an appropriate use of surfactants and/or cosurfactants. The mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon their proportions. The flexibility of the surfactant film is an important factor in this regard. Besides microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases, and lamellar structures depending on the ratio of the components. The internal structure of a microemulsion vehicle is very important for the diffusivity of the phases, and thereby also for the diffusion of a drug in the respective phases. Researchers have been trying constantly to understand the complicated phase behaviour and the various microstructures encountered in the microemulsion systems [48-50]. Fig. 4 represents structure of o/w microemulsion.
Three types of microemulsions are most likely to be formed depending on the composition (Fig 5):

1. Oil in water microemulsions wherein oil droplets are dispersed in the continuous aqueous phase

2. Water in oil microemulsions wherein water droplets are dispersed in the continuous oil phase

3. Bi-continuous microemulsions wherein microdomains of oil and water are interdispersed within the system
A well-known classification of microemulsions is that of Winsor who identified four general types of phase equilibrium [51]:

Type– I The surfactant is preferentially soluble in water and oil-in-water (O/W) microemulsions form (Winsor I). The surfactant-rich water phase coexists with the oil phase where surfactant is only present as monomers at small concentration.

Type– II The surfactant is mainly in the oil phase and water-in-oil (W/O) microemulsions form. The surfactant-rich oil phase coexists with the surfactant-poor aqueous phase (Winsor II).

Type – III A three-phase system where a surfactant-rich middle-phase coexists with both excess water and oil surfactant-poor phases (Winsor III or middle-phase microemulsion).

Type – IV A single-phase (isotropic) micellar solution, that forms upon addition of a sufficient quantity of amphiphile (surfactant plus alcohol).

Depending on surfactant type and sample environment, types I, II, III or IV form preferentially, the dominant type being related to the molecular arrangement at the interface.
2.3.2. Advantages of microemulsion based systems [52]

1. Microemulsions are thermodynamically stable system.

2. Microemulsions act as super solvents of drug. They can solubilize hydrophilic and lipophilic drugs including drugs that are relatively insoluble in both aqueous and hydrophobic solvents. This is due to existence of microdomains of different polarity within the same single-phase solution.

3. The dispersed phase, lipophilic or hydrophilic (oil-in-water, O/W, or water-in-oil, W/O microemulsions) can behave as a potential reservoir of lipophilic or hydrophilic drugs, respectively.

4. They can be sterilized by filtration.

5. Microemulsions are easy to prepare and require no significant energy contribution during preparation.

6. Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.

7. The use of microemulsion as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

8. Microemulsion increases the rate of absorption due to smaller globule size and eliminates variability in absorption.

9. Microemulsion improves bioavailability of hydrophobic drugs by increasing area per volume ratio required for efficient mass transfer and by improving permeation due to presence of surfactant.

2.3.3. Disadvantages of microemulsion based systems [52]

1. Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
2. The surfactant must be nontoxic for using pharmaceutical applications.

3. Microemulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon microemulsion delivery to patients.

2.3.4. Conditions necessary to produce microemulsions

The three important conditions required for producing microemulsion are as follows:

- **Ultra low interfacial tension at o/w interface:** The ultra-low interfacial tension at oil/water is a prime requirement to produce microemulsions. Emulsifiers or surfactants must be carefully chosen so that an ultra low interfacial tension (<10^{-3} \text{ mN/m}) can be obtained. Very low interfacial tension that leads to spontaneous emulsification of oil in water or water in oil. Interfacial tension is affected by the presence of a co-surfactant, as well as electrolyte and/or temperature, pressure, and oil chain length [53].

- **Sufficient high concentration of surfactant:** The concentration of emulsifiers or surfactants must be high enough to provide the number of surfactant molecules needed to stabilize the microdoplets produced by an ultra-low interfacial tension. Because microemulsions are in the range of 100-1000 Å droplet diameter will create 10^6 \text{ cm}^2 of total interfacial area per millimeter of the microemulsion. Therefore, the large concentration (10-40%) of surfactant is required to stabilize the newly created interface of microemulsion droplets. By proper adjustment of hydrophilic and hydrophobic groups of the surfactant, the surfactant molecules can preferentially partition into the interface and minimize their concentration in the bulk oil and water phases [51].

- **Sufficiently low fluidity and low surface viscosity of the interfacial film:** The third major consideration in formulating microemulsion is the flexibility or fluidity of the interface to promote the formation of microemulsion. Therefore short chain alcohols are often added along with surfactant [54].
2.3.5. Composition of Microemulsions:-

1. Oil
2. Surfactant and co-surfactant
3. Water

Lipophilic drugs are preferably solubilized in o/w microemulsions. The main criterion for selecting the oil phase is that the drug should have high solubility in it. This will minimize the volume of the formulation to deliver the therapeutic dose of the drug in an encapsulated form.

The oil component influences curvature by its ability to penetrate and hence swell the tail group region of the surfactant monolayer. Short chain oils penetrate the tail group region to a greater extent than long chain alkanes, and hence swell this region to a greater extent, resulting in increased negative curvature (and reduced effective HLB). Saturated (for example, lauric, myristic and capric acid) and unsaturated fatty acids (for example, oleic acid, linoleic acid and linolenic acid) have penetration enhancing property of their own. Fatty acid esters such as ethyl or methyl esters of lauric, myristic and oleic acid have also been employed as the oil phase.

The surfactant chosen must be able to lower the interfacial tension to a very small value which facilitates dispersion process during the preparation of the microemulsion and provide a flexible film that can readily deform around the droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region. It is generally accepted that low HLB surfactants are favoured for the formulation of w/o microemulsion, where as surfactants with high HLB (>12) are preferred for the formation of o/w microemulsion. Generally large amount of surfactant is required for stabilization of large interfacial area produced by nanoglobules [54].

In most cases, single-chain surfactants alone are unable to reduce the o/w interfacial tension sufficiently to produce a microemulsion. The presence of cosurfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition. If a single surfactant film is desired, the lipophilic chains of the surfactant should be sufficiently short, or contain fluidising groups (e.g. unsaturated bonds). Thus generally short to medium chain length alcohols (C3-C8) are commonly added as
Surfactants and cosurfactant decreases the interfacial tension and their molecular structures also affect the curvature of the interface. The hydrocarbon chain are rather closely packed, they repel one another sideways and as a result have a tendency to curve the interface around the water side. The counterions of the ionic headgroups of ionic surfactant and bulky polar groups of non ionic surfactants repel one another side ways and thus tend to curve interface around oil side. Thus generally higher concentration of salts or cosurfactants generally produces water in oil microemulsion while lesser concentration of salts and higher concentration of surfactant produce oil in water microemulsion [56].

A large number of oils and surfactants are available which can be used as components of microemulsion systems but their toxicity, irritation potential and unclear mechanism of action generally limit their use. One must choose materials that are biocompatible, non-toxic, clinically acceptable, and use emulsifiers in an appropriate concentration range that will result in mild and non-aggressive microemulsions. The emphasis is, therefore, on the use of generally regarded as safe (GRAS) excipients.

**2.3.6 Method of preparation:** Microemulsions can be prepared by two methods viz. phase titration method and Phase inversion method. Generally microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component Fig. 6. The region can be separated into w/o or o/w microemulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the metastable systems are not included.
Phase inversion of microemulsions occurs upon addition of excess of the dispersed phase or in response to temperature. During phase inversion drastic physical changes occur including changes in particle size that can affect drug release both \textit{in vivo} and \textit{in vitro}. These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, this can be achieved by changing the temperature of the system, forcing a transition from an o/w microemulsion at low temperatures to a w/o microemulsion at higher temperatures (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. This method is referred to as phase inversion temperature (PIT) method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone [54].
2.3.7 Microemulsions Characterization: In contrast to the ease of preparation of microemulsion, it is difficult to characterize microstructure of microemulsion. However, the knowledge is essential for their successful scaling up for commercial production and exploitation. Microemulsion science has relied heavily on characterization techniques, both from the perspective of fundamental elucidation of phase and nanostructural behavior. Phase behavior studies are most essential for the study and selection of surfactant system. Phase behavior can be studied by using pseudoternary diagrams that combine more than one component in the vertices of the ternary diagram. Phase diagram provide information on the boundaries of the different phases as a function of composition variables and temperatures, and, more important, structural organization can be also inferred. Phase behaviour studies also allow comparison of the efficiency of different surfactants for a given application [56].

Simple physicochemical characterization involves measurement of clarity, % Transmittance and viscosity. Clarity and % Transmittance may provide idea about the globule size and differentiate them from macroemulsion. Electrical conductivity remains a simple and inexpensive technique for microemulsion characterization. It primarily reveals whether an aqueous or oil phase or both phases are continuous. The conductivity measurement technique has been used to determine the type of microemulsion, and to estimate phase boundaries resulting from changes in composition or temperature. Conductivity greater than 0.01 mScm$^{-1}$ indicates the presence of water in continuous phase observed in bicontinuous or solution type of microemulsion. Simple dye test or dilution tests can also be used to predict type of microemulsion. The rheological properties of microemulsions depend on the type, shape and number density of aggregates present, as well as the interactions between these aggregates. Hence, microstructural changes such as sphere-rod or discontinuous to bicontinuous transitions are reflected in microemulsion rheology. Viscosities measurements may provide indication regarding the presence of rod like or warm like reverse micells. The formation and the properties of microemulsion can be studied by measuring the interfacial tension. Ultra low values of interfacial tension are correlated with phase behavior, particularly the existence of surfactant phase or middle-phase microemulsions in equilibrium with aqueous and oil phases [55].
More complex characterization techniques employed to reveal the detailed physicochemical properties of microemulsion region. Transmission (TEM) and scanning (SEM) techniques have been used to study internal and surface mesophase nanostructure. In case of microemulsion generally cryo-TEM and and freeze-fracture (FFEM) techniques are used. More recently, these studies have extended to studying compositions of increasing complexity and to elucidate the substructure of the polar and nonpolar compartments within the system. Small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and static as well as dynamic light scattering are widely applied techniques in the study of microemulsions. These methods are very valuable for obtaining quantitative information’s on the size, shape and dynamics of the components [57].

Dynamic light scattering (DLS), also known as photon correlation spectroscopy, can be used to analyze microemulsion droplet size via determination of hydrodynamic radius which can be extracted from measurements of the diffusion constants of diluted dispersed phase (droplets) undergoing Brownian motion. The parameter calculated is defined as the translational diffusion coefficient. The particle size is then calculated using the Stokes- Einstein equation. The technique employs relatively simple equipment and involves very short experimental times. The major drawback of this technique is the dilution of the sample required for the reduction of interparticle interaction.

Application of SAXS in determining shape and size of microemulsion droplets relies on the difference in the ability of oil and water phases to scatter x-rays. NMR has been used in the characterization of microemulsions to study diffusion properties of components and relaxation behavior [55].
2.3.8 Applications of Microemulsions in pharmacy:

To attain the highest pharmacological effects with least side effects of drugs, drugs should be delivered to target sites without significant distribution to non-target areas. Microemulsion systems have emerged as novel vehicles for drug delivery which allow sustained or controlled release for transdermal, topical, oral, nasal, intravenous, ocular, and parenteral and other administration routes of drugs. Microemulsion drug delivery is a practical delivery platform for improving target specificity, therapeutic activity, and reducing toxicity of drugs.

- **Microemulsions for oral Delivery:** Oral delivery offers the opportunity to deliver peptide and protein drugs. Usually when peptides and proteins are delivered orally, they are degraded in the GI and are not therapeutically active. Delivery of these molecules using microemulsions, though, increases their bioavailability. Currently, an oral cyclosporine formulation called Neoral® is on the market. A soft capsule is administered which contains an oil solution of drug and surfactant, which is converted to an o/w microemulsion when it comes in contact with the aqueous stomach environment. Microemulsions have given this drug more rapid and reproducible absorption with less inter- and intra-patient variability [52].

- **Microemulsions for transdermal and topical Delivery:** Microemulsions have the ability to enhance transdermal drug delivery. Using a transdermal route instead of oral eliminates systemic side effects, avoids first pass metabolism, and maintains plasma drug levels for a longer period of time. Microemulsions favor drug partitioning into skin by modifying the thermodynamic activity of the drug. The volatile water component can evaporate over time leaving an oil/surfactant solubilizing layer that may irritate the skin [53].

- **Microemulsions for ocular Delivery:** Ocular delivery of microemulsions shows great promise as well. Many properties inherent to microemulsions, such as low viscosity, transparency, and thermodynamic stability, prove to be very advantageous when it comes to this type of dosage form. Similarly to other routes of administration, microemulsions increase the water solubility of certain drugs and enhance absorption into the eye. This could ultimately lead to decreased number of applications [52].

- **Microemulsions for parenteral Delivery:** Microemulsions possess low viscosity, and because of this characteristic, make them ideal for injections and intravenous use.
• Microemulsions for cosmetics: The transparent nature of microemulsions makes them ideal for cosmetics. They possess low viscosity and are easily absorbed by the skin. Because the systems contain an oil phase and a water phase, water-soluble and oil soluble components can be added together in the same formulation easily. Microemulsions can be applied in this industry as cleaners, hair products, perfumes, gels, and skin care products, to name only a few [52].

• Microemulsions for Nasal Delivery: Microemulsion has been recently explored as an alternative drug delivery system through nasal route to demonstrate a possible alternative route to intravenous administration. Intranasal administration confers a simple, practical, cost effective, convenient and noninvasive route of administration for rapid drug delivery to the brain. It is a promising approach for rapid onset delivery of CNS medicament from ancient times. Recently various scientists have explored intranasal microemulsion as alternative delivery system for treatment of various central nervous disorders. Microemulsion improves absorption of drug through nasal mucosa due to its lipophilic nature and smaller globule size, which allows the reduction of dose and systemic side effects and may also, be effective to achieve faster onset of action [54].
2.3.9 Optimization of Microemulsions: Optimization techniques for the dosage forms such as tablets, capsules and injectables have been extensively studied, while few optimization techniques such as titration techniques and pseudoternary phase diagrams have been reported for optimization of microemulsions. Pseudoternary phase a diagram is usually used to comprehensive study the microemulsion region and its phase behavior, although construction of phase diagram is expensive and time consuming process [57].

Experimental design and statistical analysis have been widely used to develop formulation as well as in process optimization and validation. The major advantage of experimental design for development of pharmaceutical products is that it allows all potential factors to be evaluated simultaneously and systematically. Using experimental design, one can evaluate the effect of each formulation factor on each response and possibly the effects of interaction between factors and, therefore, to identify the critical parameters based on statistical analysis. Once identified, the optimal formulation could be defined by using a proper experimental design to optimize the levels of all critical factors. The levels of experimental design could not be chosen arbitrarily, where the composition is a factor of interest, because the sum of all the fractions of components equal to unity. Scientist have used various mixture design experiments for optimization of microemulsion composition including Simplex design, D-optimal design, central composite design, Box- Benkhan design. The mixture design techniques are effective in reducing the numerous test runs in laboratories and generate response surfaces to predict values [58-59].

In simple mixture experiments for microemulsion optimization the total concentration of water, surfactant and oil phase in the formulation was kept constant generally to unity while the ratio of the three was varied according to the specific design.

The simplex design for a three-component system is represented by an equilateral triangle in two-dimensional space. In simplex centroid design Ten batches were prepared as followed: three vertexes (A, B, C), three one third points between vertices (AB, BC, AC), other three at two third points between vertices (AB, BC, AC), and the center point (ABC) (Fig 7).
All responses (properties) of interest would be measured for each mixture in the design and modeled as a function of the components. The optimization can be done by plotting response in form of graphs or my developing mathematical equation. Typically, polynomial functions are used for modeling, but other functional forms can also be used. For three components, the linear polynomial model for a response $y$ is

$$Y = B_0 + B_1 X_1 + B_2 X_2 + B_3 X_3 + B_{12} X_1 X_2 + B_{23} X_2 X_3 + B_{13} X_1 X_3 + B_{123} X_1 X_2 X_3$$

Where $Y$ is the dependent variable, $B_0$ is the arithmetic mean response of the nine runs; $B_i$ is the regression coefficient for the factor $X_i$, $X_1$, $X_2$, $X_3$ stand for the main effects, $X_1 X_2$, $X_2 X_3$, $X_1 X_3$, $X_1 X_2 X_3$ are the interaction terms between the main effects and shows how the response changes when two factors are changed simultaneously; $X_{12}$, $X_{23}$, $X_{13}$, $X_{123}$ are the quadratic terms included to investigate non linearity[58-60].

Scientists have focused their research toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as epilepsy, migraine, emesis, depression in last few decades.
2.4 EPILEPSY

Epilepsy is a brain disorder in which a person has repeated seizures (convulsions) over time. Epilepsy is one of the most common neurological disorders affecting about 65 million people globally. It affects 1% of the population by age 20 and 3% of the population by age 75. It is more common in males than females with the overall difference being small. Most of those with the disease (80%) are in the developing world. Around 14 people per 1,000 people are expected to suffer from epilepsy in countries like India with higher estimates in children and young adults, and in rural areas [61].

Seizures are episodes of disturbed brain activity that cause changes in attention or behavior. Epilepsy seizures usually begin between age of 5 and 20, but they can happen at any age. There may be a family history of seizures or epilepsy. Epilepsy may develop because of an abnormality in brain wiring, an imbalance of nerve signaling chemicals called neurotransmitters, or due to combination of these factors. Two common forms of epilepsy are reported - Grandmal (tonic-clonic seizures) and Petitmal (absence seizures). Seizures are divided into two major categories - focal seizures and generalized seizures. However, there are many different types of seizures in each of these categories. But the underlying neuronal abnormality in epilepsy is poorly understood [62].

2.4.1 Pathophysiology of Epilepsy: In epileptic seizures, due to structural or functional problems within the brain, a group of neurons begin firing in an abnormal, excessive and synchronized manner. This results in a wave of depolarization known as a paroxysmal depolarizing shift. Normally, after an excitatory neuron fires it becomes more resistant to firing for a period of time. This is due in part from the effect of inhibitory neurons, electrical changes within the excitatory neuron, and the negative effects of adenosine. In epilepsy the resistance of excitatory neurons to fire during this period is decreased. Another mechanism of epilepsy may be the up-regulation of excitatory circuits or down-regulation of inhibitory circuits following an injury to the brain. These secondary epilepsies occur through processes known as epileptogenesis. Failure of the blood–brain barrier may also be a causal mechanism as it would allow substances in the blood to enter the brain. Focal seizures begin in one hemisphere
of the brain while generalized seizures begin in both hemispheres. Some types of seizures may change brain structure, while others appear to have little effect [63].

Epilepsy may occur as a result of a number of other conditions including: tumors, strokes, head trauma, previous infections of the central nervous system, genetic abnormalities, and as a result of brain damage around the time of birth. Between 6 and 20% of epilepsy is believed to be due to head trauma [64].

Other risks include Alzheimer's disease, multiple sclerosis, tuberous sclerosis, and autoimmune encephalitis. Malnutrition is a risk factor seen mostly in the developing world, although it is unclear however if it is a direct cause or an association [65].
2.4.2 Treatment of epilepsy: Normal treatment includes use of antiepileptic drugs, surgery and ketogenic diet. There are a number of medications available. Epilepsy surgery may be an option for people with focal seizures that remain a problem despite other treatments. These other treatments include at least a trial of two or three medications. The goal of surgery is total control of seizures and this may be achieved in 60–70% of cases. Common procedures include: cutting out the hippocampus via an anterior temporal lobe resection, removal of tumors, and removing parts of the neocortex [66-67].

A ketogenic diet (high-fat, low-carbohydrate and adequate protein) appears to decrease the number of seizures by half in about 30–40% of children. About 10% manage to stay on the diet for a few years, 30% had constipation, and other adverse effects were common. Less radical diets were easier to tolerate and may be effective. It is unclear why this diet works. Exercise has been proposed as possibly useful for preventing seizures with some data to support this claim.

It has been reported that various vitamins including vitamin B6 plays important role in treatment of epilepsy. Pyridoxal phosphate is the active form of pyridoxine (vitamin B6). In the synthesis of GABA, pyridoxal phosphate binds to glutamic acid decarboxylase (GAD) and catalyzes the decarboxylation of glutamate to form GABA. GABA is a very important inhibitory neurotransmitter (GABA) as discussed earlier, and when it is not present, the neurons continue to fire, and this leads to epileptic seizures. Thus inclusion of vitamins is also recommended during treatment of epilepsy [67].

Approximately 20–30% of patients with epilepsy have seizures that are refractory to treatment with the currently available antiepileptic drugs (AEDs) and patients will experience breakthrough seizures. The choice of anticonvulsant is based on seizure type, epilepsy syndrome, other medications used, other health problems, and the person's age and lifestyle. Patients with epilepsy usually need to take drugs continuously for many years. Although the majority of seizures will be self-limiting, seizures that persist for more than 5 min require prompt intervention. In addition, there are circumstances where chronic administration of medications is required but due to lack of oral access and incompatibility with intravenous formulations of medications, alternative routes of drug dosing are required. Therefore, the treatment of epilepsy requires administration of medications for both acute and chronic treatment using
multiple types of formulations. Long established antiepileptic drugs include phenytoin, carbamazepine, sodium valproate, Phenobarbital, benzodiazepines including lorazepam and midazolam etc. Phenytoin, carbamazepine and valproate appear to be equally effect in both focal and generalized seizures [68].
2.4.3 Rational for selection of Carbamazepine and Phenytoin

Carbamazepine and Phenytoin are most widely used drugs in the treatment of epilepsy. Currently the available marketed formulations for these drugs are in the form of oral or injectable preparations. Table 1 enlisted the marketed preparations for carbamazepine while Table 2 describes some of the available marketed formulations for phenytoin. Oral preparations include tablet, capsules, suspensions but these preparations include various side effects like delayed onset of action, various systemic side effects and loss of drug. While injectable preparations shows poor patient compliance and requires qualified medical practitioner for administration [63].

Table 1 Marketed preparation of Carbamazepine

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Brand Name</th>
<th>Strength</th>
<th>Name of company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Tablet</td>
<td>Zen</td>
<td>100 mg</td>
<td>Intas Laboratories</td>
</tr>
<tr>
<td></td>
<td>Mazetol</td>
<td>100 mg</td>
<td>Piramal Health care</td>
</tr>
<tr>
<td></td>
<td>Zeptol</td>
<td>100 mg</td>
<td>Sun Pharmaceuticals Ltd</td>
</tr>
<tr>
<td></td>
<td>Tegrital</td>
<td>100 mg</td>
<td>Novartis Healthcare</td>
</tr>
<tr>
<td>Chewable Tablet</td>
<td>Zeptol child</td>
<td>100 mg</td>
<td>Sun Pharmaceuticals Ltd</td>
</tr>
<tr>
<td>Sustained Release Tablet</td>
<td>Mazetol SR</td>
<td>100, 400 mg</td>
<td>Abbott Healthcare</td>
</tr>
<tr>
<td></td>
<td>Tegrital CR</td>
<td>100, 400 mg</td>
<td>Novartis Healthcare</td>
</tr>
<tr>
<td>Oral suspension/ syrup</td>
<td>Tegrital</td>
<td>100 mg/5 ml</td>
<td>Novartis Healthcare</td>
</tr>
<tr>
<td></td>
<td>Zen</td>
<td>100 mg/5 ml</td>
<td>Intas Laboratories</td>
</tr>
<tr>
<td></td>
<td>Mazetol</td>
<td>100 mg/5 ml</td>
<td>Abbott Healthcare</td>
</tr>
</tbody>
</table>
### Table 2 Marketed preparations of Phenytoin

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Brand Name</th>
<th>Strength</th>
<th>Name of company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>Epicent</td>
<td>100 mg</td>
<td>Crescent Pharmaceuticals ltd.</td>
</tr>
<tr>
<td></td>
<td>Eptoin</td>
<td>50 mg, 100 mg</td>
<td>Abbott Healthcare</td>
</tr>
<tr>
<td></td>
<td>Gentoin</td>
<td>100 mg</td>
<td>Gentech Healthcare ltd</td>
</tr>
<tr>
<td>Sustained Release Tablet</td>
<td>Fentoin ER</td>
<td>100 mg</td>
<td>Sunpharma ltd</td>
</tr>
<tr>
<td>Capsule</td>
<td>Dilantin</td>
<td>25 mg, 100 mg</td>
<td>Pfizer</td>
</tr>
<tr>
<td>Oral Suspension</td>
<td>Eptoin</td>
<td>30 mg/5 ml</td>
<td>Abbott Healthcare</td>
</tr>
<tr>
<td>Injections</td>
<td>Dilantin</td>
<td>25 mg/ml</td>
<td>Pfizer</td>
</tr>
<tr>
<td></td>
<td>Eptoin AMP</td>
<td>50 mg/ml</td>
<td>Abbott Healthcare</td>
</tr>
</tbody>
</table>

- BCS class-II drugs (having poor solubility and good permeability) making them suitable candidate for preparation of o/w microemulsion.
- Small molecular weight (Less than 300 Da) suitable for nasal delivery.
- High lipophilicity (Log P greater than 2) making them suitable candidate for preparation of microemulsion.
2.5 Challenges in preparation of intranasal microemulsion for Brain targeting:

• Nasal cavity is having very smaller instillation volume and thus one has to select excipients which allow maximum incorporation of selected drugs in the microemulsion. This can be achieved by screening of components for solubility studies.

• Optimization of composition is very important which can provide satisfactory results with minimal use of surfactant and cosurfactant. Microemulsion formulation are generally prepared and optimized by pseudoternary phase diagrams. However, mixture experiments like simplex centroid design provides an alternative tool to more effectively determine the feasible region of microemulsion formulations. These methods also provide for the development of the optimal formulations within the region of feasible formulations identified.

• Microemulsion intended for intranasal administration must be carefully prepared and evaluated as toxicity of the components used for preparation of microemulsion may lead to disruption of nasal epithelium which may allow entry of hazardous agents directly in to systemic circulation and brain. Scientists have used different In Vitro and In vivo nasal toxicity study using rat as an animal model to ensure safety of nasal formulation.

• It is very important to ensure faster and higher uptake of drug in to brain rather than systemic circulation from prepared microemulsion in comparison to available dosage formulation. This can be ensured by pharmacodynamic, In Vivo brain uptake study and gamma scientigraphy study.

So in the present study the carbamazepine/phenytoin loaded intranasal microemulsion was prepared and evaluated for physicochemical parameters and pharmacodynamic parameters. Brain uptake study for carbamazepine/phenytoin from optimized microemulsion was performed in rats.