2 BACKGROUND OF THE PROBLEM SELECTED FOR THE RESEARCH

2.1 Introduction

Along with the acceleration of population aging, there is the increase in the complexity on neurological disorders and remains a question mark and challenge to the pharma firms, to treat cerebrovascular diseases effectively. The blood brain barrier (BBB) represents an intractable obstacle for a large number of drugs and is the major tailback in drug delivery to the brain. Despite good progress in neurosciences and a corresponding high interest in brain delivery technologies, very few drugs have been marketed for the treatment of CNS disorders.

In the case of Neurological disorders special attention is to be given for development of dosage form which will be provided with minimum side effects. Population appraisal reveals the estimate that, 12% of the general population lives with migraine each year while its lifetime prevalence is estimated to be greater than 30%. Historically, migraine has been considered an episodic pain syndrome. The pathophysiologic origins of migraine are thought to reside in the brain. People with migraine are considered to have a genetically hyperexcitable nervous system with an enduring propensity to generate the clinical event of migraine when exposed to various changes in the internal and/or external environment [1, 2].

2.2 Migraine

Migraine is a chronic neurological disease characterized by recurrent moderate to severe headaches which is often associated with a number of autonomic nervous system symptoms. It is also termed as a multifactorial primary headache disorder, characterized by unilateral, intense and pulsatile headaches leading to cause of medical disability. Since migraine is a common illness, it creates an enormous health burden on patient and society. 80% to 89% of the economic burden is due to migraine which is conscientious for affecting work and productivity losses. Migraine is more common in women than men and is associated with the side effects like nausea, anorexia, vomiting, photophobia, and phonophobia [3].
2.2.1 Migraine Pathophysiology [4 - 6]

Clinical manifestations

Migraine is characterized by episodic pain, often throbbing and frequently unilateral and may sometimes be severe. Migraines are associated with nausea, vomiting, sensitivity to light, sound, movement and when treated, the attacks typically last for 4 to 72 h. The migraine is basically identified by the three diagnostic criterias classified as follows

**Migraine without aura**

- At least 5 attacks fulfilling B – D
- Headache attacks, lasting 4 – 72 h (untreated or unsuccessfully treated)
  
  Headache has at least two of the following characteristics
  
  1. Unilateral localization
  2. Pulsating quality
  3. Moderate to severe intensity
  4. Aggravation by walking stairs or similar routine physical activity
- During headache at least one of the following
  
  1. Nausea and/or vomiting
  2. Photophobia and phonophobia
- At least one of the following
  
  1. History, physical- and neurological examinations do not suggest association with head trauma, vascular or non-vascular disorders, use of or withdrawal from noxious substances, non-cephalic infections, metabolic disorders or disorder or cranial or facial structures
  2. History and/or physical- and/or neurological examinations do suggest such disorder, but it is ruled out by appropriate investigations
  3. Such disorder is present, but migraine attacks do not occur for the first time in close temporal relation with the disorder

**Migraine with aura**

- At least 2 attacks fulfilling B
- Migraine aura fulfills criteria for typical aura, hemiplegic aura, or basilar-type aura
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- Not attributed to another disorder

**Typical aura**
- Fully reversible visual, sensory or speech symptoms (Individually or in combination).
  - Visual symptoms including positive features (e.g. Flicking of lights, spots, lines) or negative features (e.g. Loss of vision), or unilateral sensory symptoms including positive features (e.g. Visual loss, pins and needles) or negative features (e.g. Numbness), or any combination
- At least one of the following
  a. At least one symptom develops gradually over a minimum of 5 min or different symptoms occur in succession or both
  b. Each symptom lasts for at least 5 min and for no longer than 60 min
- Headache that meets criteria for migraine without aura begins during the aura or follows aura within 60 min.

2.2.2 Theory and mechanism involved in migraine

2.2.2.1 Theories of migraine

**Vascular theory**

Scientifically, Harold Wolff [7] was the first researcher to measure the diameter of the extracranial arteries in patients suffering migraine attacks and found them to be dilated. Patients treated with vasoconstrictors were relieved from the pain and decreased the arterial dilation. It has been hypothesized that pain and other associated symptoms are caused due to the increased vascular pulsation which activate stretch receptors that in turn increase the activity of neuropeptide containing perivascular nerves [8].

**Neurological theory**

This theory suggests that migraine arises due to abnormal neuronal firing and neurotransmitter release in brain neurons. Theory primarily focuses on certain symptoms, such as premonitory symptoms occurring prior to an attack (prodrome), which are difficult to explain based on the vascular hypothesis. The theory reveals from the
facts that external factors, such as stress and hunger can precipitate migraine attacks to pathologies which basically is responsible begin and develop migraines. Cortical spreading depression, an expanding depolarization of cortical neurons suggested to underlie the aura or prodrome associated with initiation of migraine attack. During spreading depression, cortical function is disrupted subsequent to neuronal depolarization and increased extracellular potassium. These cortical changes are thought to be the cause of the transient sensory or motor impairments that frequently precede the painful period of a migraine attack [4, 9].

**Neurogenic theory**

Neurogenic theory suggests that migraine could be produced by electrically stimulating brain stem structures. Study is based on the stimulation studies which relate the acquaintance between the trigeminal nerve and the cranial vasculature. Trigeminovascular axons from blood vessels of the pia mater and dura mater release vasoactive peptides producing a sterile inflammatory reaction with pain. Neurogenic theory is an attempt to reunite the vascular changes in the neuronal dysfunction that may occur in migraine headache and proposes that migraine pain is associated with inflammation and dilation of the meninges, particularly the dura membrane surrounding the brain. Neurogenic dural inflammation is thought to result from the actions of inflammatory neuropeptides released from the primary sensory nerve terminals innervating the dural blood vessels [10]

**2.2.2.2 Mechanism involved**

Mechanism of migraine is still not fully understood, it is thought that migraine is a disorder of three major key factors, the cranial blood vessels, the trigeminal innervation of the vessels and the reflex connection of the trigeminal system in the cranial parasympathetic outflow. The substance of the brain is largely insensate indicating the pain generation by large cranial vessels, proximal intracranial vessels or by the dura mater (Martin et al., 1993; Feindel et al., 1960). Alterations in the activity of 5-hydroxytryptamine (5-HT)-containing neurons in the raphe nuclei and/or norepinephrine-containing pathways originating from the locus coeruleus lead to depolarization of
trigeminoperivascular sensory afferents and release of vasoactive neuropeptides resulting in vasodilation of pial and dural arteries (and arteriovenous shunts) and exacerbate nociceptive transmission leading to so-called sterile neurogenic inflammation.

As illustrated in the Figure 2.1 it can be observed that unidentified trigger factors for migraine activate and depolarize perivascular trigeminal axons, which release vasoactive neuropeptides to promote sterile neurogenic inflammation. Orthodromic and antidromic conduction along trigeminovascular fibers spreads the inflammatory response to neighboring tissues and transmits nociceptive information toward the trigeminal nucleus caudalis and higher nociceptive brain centers. Inhibitory 5-HT_1D receptors on perivascular fibers block the release of vasoactive neuropeptides and impulse conduction in trigeminovascular neurons. At the level of the brain stem, the increased trigeminal input causes reflex activation of the seventh cranial nerve, which innervates predominantly extracranial blood vessels, resulting in the amplification of the trigeminovascular activation by provoking strong cranial vessel dilation.

As seen on the Figure 2.1 orthodromic conduction transmits this nociceptive impulse to the central nervous system, where it may induce associated symptoms accompanying migraine. Simultaneously, antidromic conduction along the trigeminovascular fibers spreads the depolarization to the neighboring tissues. In addition, reflex activation of intrinsic cholinergic neurons from the facial nerve which innervates predominantly extracranial blood vessels amplifies trigeminovascular depolarization and hence the sterile neurogenic inflammation [4, 11].
2.2.3 Pharmacodynamic characteristics of second-generation 5-HT_{1B}/1D agonists

At least seven different 5-HT receptor subtypes (5-HT_1 through 5-HT_7) have been now identified. Furthermore, the 5-HT_1 receptor family comprises five different receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}) that couple to a G-protein to inhibit cyclic AMP formation. Immuno histological data suggest that 5-HT_{1B} receptors in the human trigemino vascular system predominate mainly in meningeal arteries, while 5-HT_{1D} receptors are found on trigeminal nerve endings. Stimulation of 5-HT_{1B} receptors results in selective vasoconstriction of cranial vessels, whereas activation of 5-HT_{1D} receptors directly inhibits the release of proinflammatory neuropeptides. Functional activity studies indicate that all 5-HT_1 agonists possess high affinity and relative specificity for 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} subtype receptors while having no appreciable affinity for 5-HT_2, 5-HT_3, or non-serotonergic receptors [12, 13].
2.2.4 Overview of second-generation 5HT\textsubscript{1B}/5HT\textsubscript{1D} agonists

Considering the efficacy, pharmacokinetic (low bioavailability, plasma half-life, and low liposolubility) and pharmacodynamic shortcomings of available triptans have prompted the pharmaceutical industry to develop more potent and selective 5-HT\textsubscript{1B}/1D agonists. The triptans have been developed considering the drawbacks of the sumatriptan. Some of the products are present in the market for oral (conventional tablets, orodispersible tablets) and nasal delivery (nasal spray) [14, 15]. An overview of the pharmacokinetic properties of the second-generation 5-HT1B/1D agonists is given in Table 2.1 [1].

Inhibition of neurogenic inflammation and restoration of central inhibition provide a rationale for using 5-HT\textsubscript{1B}/1D agonists in the acute treatment of migraine. The clinical efficacy of eletriptan is one of the highest among the oral second-generation 5-HT\textsubscript{1B}/1D agonists; however, its downside might be its slightly higher incidence of treatment-related adverse events [16]. Although data for frovatriptan are scanty, the drug appears to be one of the most promising agents as its long half-life translates to the lowest rate of headache recurrence reported for second-generation 5-HT\textsubscript{1B}/1D agonists. Furthermore, it has been shown to have a low incidence of adverse events [17].

Naratriptan offers a slightly higher efficacy profile compared to sumatriptan. Furthermore, the headache recurrence rate following naratriptan is among the lowest reported with the newer second-generation 5-HT\textsubscript{1B}/1D agonists. The wafer formulation of rizatriptan offers a convenient benefit for the treatment of migraine headache [18]. Rizatriptan has a faster onset of action than oral sumatriptan and makes more patients headache free with significantly less migraine-associated symptoms [19]. Zolmitriptan is one of the best-documented second generation 5-HT\textsubscript{1B}/1D agonists [12]. The drug is rapidly effective and well tolerated, making a good alternative to sumatriptan.

Various aspects of economy and comparing clinical efficacy are needed to elaborate statistically to determine the place of each of these second-generation 5-HT\textsubscript{1B}/1D agonists in the treatment of acute migraine. Although differences among second-generation 5-HT\textsubscript{1B}/1D agonists appear to be small, clinical response and tolerability vary considerably among individual patients. Furthermore, up to 25% of patients do not respond to any of these agents. Whether this is due to pharmacodynamic reasons or an
aberrant form of migraine remains unsolved but would justify development of other antimigraine agents [20-22].

Table 2.1: Pharmacokinetic Properties of Oral Sumatriptan and Second-Generation 5-HT1B/1D Agonists [12]

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Sumatriptan (100mg)</th>
<th>Almotriptan (12.5mg)</th>
<th>Eletriptan (80mg)</th>
<th>Frovatriptan (2.5mg)</th>
<th>Naratriptan (2.5mg)</th>
<th>Rizatriptan (10mg)</th>
<th>Zolmitriptan (2.5mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>54.0-78.4</td>
<td>49.5</td>
<td>107-190</td>
<td>4.2 - 7.0</td>
<td>7.8- 14.4</td>
<td>20</td>
<td>1.3- 4.7</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.5 - 2.3</td>
<td>1.4- 3.8</td>
<td>1.0 – 1.5</td>
<td>2 – 4</td>
<td>0.8 - 4.1</td>
<td>1- 3</td>
<td>0.5 – 0.6</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>14</td>
<td>70-80</td>
<td>50</td>
<td>24-30</td>
<td>63-74</td>
<td>40-45</td>
<td>40-49</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>2</td>
<td>3.0-3.7</td>
<td>3.6-6.9</td>
<td>25</td>
<td>4.5-6.6</td>
<td>1.8-3</td>
<td>1.5-3.6</td>
</tr>
<tr>
<td>Protein binding (%)</td>
<td>14-21</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>28-31</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>Major metabolic enzyme</td>
<td>MAO-A</td>
<td>CYP 3A, MAO-A</td>
<td>CYP 3A4</td>
<td>NR</td>
<td>CYP 450</td>
<td>MAO-A</td>
<td>CYP 1A2 MAO-A</td>
</tr>
<tr>
<td>Volume of distribution(L/Kg)</td>
<td>2.4-3.3</td>
<td>2.5</td>
<td>2.4</td>
<td>3- 4</td>
<td>2.4-2.9</td>
<td>1.3-2.5</td>
<td>7.0-8.3</td>
</tr>
<tr>
<td>Clearance (ml/min/kg)</td>
<td>3.5-3.9</td>
<td>8.6</td>
<td>6.6</td>
<td>1.9-3.1</td>
<td>2.7-3.8</td>
<td>3.2-5.3</td>
<td>2.0-3.1</td>
</tr>
<tr>
<td>Liposolubility</td>
<td>Low</td>
<td>NR</td>
<td>High</td>
<td>NR</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

Considering the urgency of the treatment the formulation is required to be developed to release in the localized area with quick therapeutic effects. It has been reported that most of the formulations available are present in the tablet dosage form for oral drug delivery. Available drug formulations with given route of administration have certain limitations as delayed release, first pass metabolism, poor bioavailability, side effects like nausea, vomiting and anorexia. So study is urged to develop a formulation that could be given by the proper route of administration whereby it avoids the limitations occurred by oral route.
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2.3 Intranasal Drug Delivery

Intranasal route offers a practical, palatable and non-invasive delivery of therapeutic agents directly to the brain and spinal cord bypassing the blood-brain barrier (BBB) eliminating the need for systemic administration and its related potential side effects. Intranasal delivery can be advantageous for delivery of much central nervous system (CNS) therapeutics, including those that can cross the BBB upon systemic administration. Technology allows feasibility for the drugs that do not cross the BBB but are intended to be delivered to the central nervous system within minutes and most importantly does not necessarily require any chemical modification to therapeutic agents. Possible mechanisms for intranasal therapeutics to reach the brain from the nasal mucosa is to transport the drug by diffusion within perineuronal channels, perivascular spaces, or lymphatic channels directly connected to brain tissue or cerebrospinal fluid (CSF) [23]. Intranasally administered therapeutics reach the CNS via the olfactory and trigeminal neural pathways. A wide variety of therapeutics, including both small molecules and macromolecules with different carrier transport pathways can be targeted to the olfactory system and connected memory. Both the olfactory and trigeminal nerves innervate the nasal cavity, providing a direct connection with the CNS. Direct delivery of therapeutics from the nose to the brain was initially attributed to the olfactory pathway [24].

The nasal mucosa due its wide rich epithelial vasculature offers numerous benefits as a target tissue for drug delivery, with rapid onset of drug action, plasma drug profiles resembling intra venous infusions, potential for central nervous system delivery, and bypass of first-pass metabolism [25].

2.3.1 Advantages with improved patient compliance and limitations of intranasal delivery (Sam et al. 1995) [26, 27]

- Highly vascularized mucosa and large surface area favors rapid absorption and onset of pharmacologic action
- Trained person not required
- Needle-free (painless) and non-invasive delivery
- User-friendly, self-medication possible
Liposome based drug delivery system for brain targeting through intranasal route

- Good penetration for lipophilic and low molecular weight drugs
- Bypass BBB, allowing direct delivery of drug to CNS system via olfactory region
- Useful for local and systemic delivery
- Less chemical and enzymatic degradation
- Low dose required
- Avoids first pass metabolism
- Lower side effects
- High bioavailability

Limitations of intranasal delivery

Limitation Factors
- Pathologic conditions such as cold or allergies are responsible for reduced nasal absorption which might be responsible for altered nasal bioavailability
- Constituents added in dosage forms may sometimes, cause irritation and irreversible damage of the cilia layer of nasal mucosa.
- Muco-ciliary clearance influence permeability of drug
- High concentration of absorption enhancers disrupt and dissolves nasal membrane
- Acts as a barrier for high molecular weight compounds

2.3.2 Anatomy of nasal cavity

As the nasal route plays an important role in local systemic drug delivery, vaccines and access to the central nervous system, create the challenge and interest in the Pharma industry. The anatomy and the physiology of the nasal cavity is important factor to be considered in designing the dosage form. Anatomically, the nasal cavity can be divided into three functional regions as illustrated in the (Figure 2.2).
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Figure 2.2: Anatomy of nasal cavity showing the relation with pathway for olfactory lobe.

- Nostrils dividing into vestibular region having an area of 10-20 cm². It is covered with stratified, keratinised and squamous epithelium.
- Respiratory region having an area of about 130 cm² and occupying the major nasal cavity and consists of three turbinates namely inferior, middle and superior.
- Olfactory region has an area of about 10 - 20 cm² located in the roof of the nasal cavity and on the upper part of the nasal septum containing the receptors for the smell sensation. Local delivery of drugs in the nasal cavity can be used to treat allergies, congestion and infection [28].

2.3.3 Mechanism of drug absorption through nose

The mechanisms by which intranasally delivered substances enter the CNS have not been fully elucidated, but it is thought to involve a combination of neuronal, vascular and lymphatic transport methods. Mechanism is basically reliant on the region where the delivered agent is placed within the nasal cavity and the physicochemical properties of the therapeutic being administered.
The initial step in the absorption of drug from the nasal cavity is passage through the mucous membrane. Small unchanged particles with low molecular weight easily get access through the mucosal layer whereas large or charged particles may find it more difficult to cross. Subsequent to a drug’s passage through the mucus membrane, several mechanisms exist for drug absorption through the mucosa, include transcellular or simple diffusion across the membrane, paracellular transport via movement between cell and transcytosis by vesicle carriers.

Currently, numerous small molecule drugs and vaccines are formulated for intranasal use. Recent advances in intranasal delivery include drug atomizers (ViaNase™) to saturate the nasal cavity, gels that aid binding to the nasal epithelium, and nano-sized vectors for targeting and added protection of the therapeutic agent [24]. The nasal route clearly provides a much safer route than direct surgical administration.

Drugs and biomolecules administered intranasally have two main mechanisms that utilize these neuronal connections to the brain:

- The first mechanism involves an aqueous route (paracellular) of transport, which involves the transport of water soluble compounds. This route is considered to be slow and passive, linking an inverse log-log correlation between intranasal absorption and the molecular weight of water-soluble compounds observing poor bio-availability for drugs with high molecular weight (greater than 1000 Daltons).

- The second mechanism involves transport through a lipoidal route (transcellular process), responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Passive transcellular diffusion occurs “through cells” and primarily applies to small, lipophilic molecules that are taken up by the nerve terminals of olfactory neurons. Drugs are also carried to the cell membranes through the active transport route by transporting through the opening of tight junctions by carrier mediated process [29, 30].

Figure 3.1 demonstrates the transport of the drug molecule through the olfactory epithelium into the central nervous sytem. The respiratory regions of the nasal cavity have a variety of mechanisms that prevent xenobiotics access to the blood and CNS, such as mucus-producing goblet cells and ciliated epithelial cells. If overcome, there are pathways along the trigeminal and olfactory nerves which serve as the main sites of...
intransal transport of molecules to the brain. The olfactory bulbs are reached through the olfactory receptor neurons (ORNs). Perivascular transport is thought to occur through perivascular spaces. The ORNs are surrounded by olfactory unsheathing cells along their axons, and it is thought that xenobiotics are directly shuttled to the brain and CSF via transport through the perineuronal spaces. [31]

Figure 2.3: Transport of xenobiotics through the olfactory epithelium into the CNS.

As stated above, vascular and lymphatic systems are thought to also play a large role in nose-to-brain transport. Since, the olfactory and respiratory mucosa is highly vascularized, small molecular weight drugs are able to be absorbed into the systemic circulation. Perivascular spaces inside the brain act as a lymphatic system, allowing neuron-derived substances, CSF and substances within CSF to be drained from interstitial fluid. Acting in reverse, arterial pulsations and bulk flow mechanisms provide the driving force by which substances can be transported throughout the parenchyma, giving this distribution system the name “perivascular pump”. In other words, perivascular spaces are able to act as a sort of delivery path, allowing the spread of intranasally delivered therapeutics throughout the brain via the “perivascular pump”. This mechanism works along with the perineuronal channels that surround the ORNs to allow molecules to move from the nasal mucosal surface into deep regions of the brain in a relatively short time (<1 hour). Recent work also suggested the importance of the rostral migratory stream
(RMS) in the retrograde flow of intranasally applied therapeutics from the olfactory bulb to the rest of the brain. [32]

2.3.4 Barriers for absorption through nasal drug delivery

Barriers which mainly affect the nasal drug delivery are discussed below.

Low bioavailability

- Bioavailability of polar drugs is mainly low (about 10% for low molecular weight drugs and 1% for peptides such as calcitonin and insulin).
- Polar drugs have limited nasal absorption with a large molecular weight.
- Polar drugs with a molecular weight below 1000 Da will pass the membrane by the latter routes.
- Nasal absorption of polar drugs is enhanced by coadministration of absorption-enhancing agents.
- Hydriphillic lipophilic balance [33].

Enzymatic barrier

- When peptides and proteins cross the nasal mucosa, there is the possibility of an enzymatic degradation of the molecule in the lumen of the nasal cavity or during passage through the epithelial barrier, which can limit the bioavailability of the drug.
- These two sites contain exopeptidases (mono and diamino peptidases), cleave peptides at their N and C termini, and endopeptidases (serine and cysteine), which can attack internal peptide bonds. The enzymes present in nasal mucosa provides a pseudo-first-pass effect which acts as an enzymatic barrier to protect the lower respiratory airways from toxic agents. In addition, there are various barriers in the nasal membrane for protection from the microorganisms, allergens and irritating substances from the environment that must be overcome by drugs before they can be absorbed into the systemic circulation. The use of enzyme inhibitors, cosolvents, and prodrugs may be the approaches to overcome this barrier [34].
Mucociliary clearance

It is an essential factor which involves the combined action of the mucus layer and cilia is called mucociliary clearance, which plays the role in removing the entrapped particles in the mucus layer. Mucus traps the particles of dust, bacteria and drug substances and is transported towards the nasopharynx at a speed of 5 - 8 mm/min where it is swallowed. The normal mucociliary transit time in humans has been reported to be 13 to 15 minutes. It has also been shown that for liquid and powder formulations which are not bioadhesive, the half-life for clearance is of the order of 15 – 30 min [35].

Protective barriers

Mucin, the protein in the mucus, has the potential to bind to solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes such as pH, temperature etc. The nasal membrane is a physical barrier and the mucociliary clearance is a temporal barrier to drug absorption across the nasal epithelium [33].

2.3.5 Factors influencing nasal drug absorption

Various factors affect the systemic bioavailability of drugs that are administered through the nasal route. Those are physiochemical properties of the drugs, the anatomical and physiological properties of the nasal cavity and the type and characteristics of selected nasal drugs delivery system.

Physiochemical properties of drug

Molecular weight

A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 Daltons. Absorption decreases significantly if the molecular weight is greater than 1000 Daltons except with the use of absorption enhancers based on the reports by Fisher et al. and Yamamto et al. it can be concluded that the permeation of drugs less than 300 Da is not significantly influenced by the physicochemical properties of the drug like molecular weight, size, formulation pH, pKa.
Liposome based drug delivery system for brain targeting through intranasal route

of molecule, which will mostly permeate through aqueous channels of the biological membrane [36].

**Particle size**

It has been reported that particles greater than 10 μm in size are deposited in the nasal cavity. Particles that are 2 to 10 μm can be retained in the lungs, and particles of less than 1 μm are exhaled.

**Chemical form**

The chemical form of a drug is important in determining absorption. For example, conversion of the drug into a salt or ester form can alter its absorption (Huang et al.) for example in-situ absorption of carboxylic acid esters of L-tyrosine was significantly greater than that of unmodified L-Tyrosine [27].

**Polymorphism**

Polymorphism affects the rate of drug dissolution, solubility, and absorption through biological membrane [27].

**Solubility and dissolution rate**

Drug solubility and dissolution rates are important biopharmaceutical factors in determining nasal absorption from powders and suspensions. In the nasal cavity, the deposited particles need to be dissolved prior to absorption. Solubility of a drug or dosage form is the first prerequisite for absorption and bioavailability of dosage form. The fluid available for dissolution of drug particles in nasal cavity or mucosa is very less when compared to the gastrointestinal fluid [30].

**Lipophilic-hydrophilic balance**

Absorption of drug substance through biological membrane may be dependent on hydrophilic lipophilic balance of the compound. On increasing lipophilicity, the nasal absorption of the compound is supposed to be increased. Although, in one study it was found that lipophilic compounds alprenolol and propranolol were well absorbed from the
nasal mucosa, in comparison to the hydrophilic drug metoprolol. Lipophilic compounds tend to readily cross biological membranes via the transcellular route since they are able to partition into the lipid (bilayer) of the cell membrane and diffuse into and traverse the cell in the cell cytoplasm. Nasal absorption of steroids was directly correlated with lipophilicity of drug molecules and was found to be independent of pH [37].

**Partition Coefficient and pKa**

The pH partition theory states that non ionized species are absorbed well, when compared with ionized species, and hence it is the same in the case of nasal absorption. Jiang et al. conducted a study to determine the quantitative relationship between the physicochemical properties of drugs and their nasal absorption, using diltiazem hydrochloride and paracetamol as model drugs. The results showed that a quantitative relationship existed between the partition coefficient and the nasal absorption constant [27].

**pH and mucosal irritation**

Change in the pH can lead to irritation to nasal mucosa and can affect the drug’s permeation. To avoid nasal irritation, the pH of the nasal formulation should be adjusted to nasal pH (4.5 – 6.5). Nasal secretions contain lysozyme, which, at acidic pH, destroys certain bacteria. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection [35].

**Osmolarity**

Isotonic solutions are administered for shrinkage of the nasal epithelial mucosa, because of the effect of osmolarity on the absorption. This results in increased permeation of the compound because of structural changes. Isotonic solutions are also known to inhibit or cease ciliary activity [35].

**Buffer capacity**

Nasal formulations are administered in small volumes ranging from 25 to 200 µL. Therefore, nasal secretions may alter the pH of the administrated dose, which can affect
the concentration of nonionized drug available for absorption. Hence, an adequate formulation buffer capacity may be required to maintain the pH in-situ [38].

**Viscosity**

At the same time, highly viscous formulations interfere with the normal functions like ciliary beating or muco-ciliary clearance, and thus alter the permeability of drugs (Johal et al. 2014). A higher viscosity of the formulation increases contact time between the drug and the nasal mucosa, thereby increasing permeation time [35].

**Nasal physiology**

- **Nasal blood flow**

The nasal mucosal membrane is very rich in vasculature and plays an important role in thermal regulation and humidification of the inhaled air, and therefore the drug absorption will depend upon the vasoconstriction and vasodilatation of the blood vessels [35].

- **Effect of enzymatic activity**

Many enzymes might affect the stability of drugs which are present on the nasal mucosa. For example, proteins and peptides are subjected to degradation by proteases and aminopeptidases at the mucosal membrane [27].

- **Effect of pathological conditions**

Intranasal pathologies such as infections, nasal surgery, cold, and allergic rhinitis may affect the nasal muco-ciliary transport process and/or capacity for nasal absorption. Nasal pathology also alters mucosal pH, and thus affects the absorption of drugs [27].

**Nasal formulations**

The deposition area is mainly a function of the delivery system and delivery device. Different dosage forms and their application to deliver the drugs in nasal cavity are elaborated in brief:
**Nasal drops** are the simplest and most convenient delivery systems among all the formulations. The main disadvantage of this system is the lack of dose precision and mucociliary clearance.

- Both **solution** and **suspension** formulations can be formulated into **nasal sprays**. Due to the availability of metered dose pumps and actuators, a nasal spray can deliver an exact dose anywhere from 25 to 200 μL. The particle size and morphology (dispersions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly. Due to mucosal irritation by powders, solution and suspension sprays are preferred and claimed as safe dosage form.

- **Intranasal emulsions** and **nanoparticles** have not been studied as extensively as other liquid nasal delivery systems. One of the major disadvantages is poor patient acceptability. The physical stability of emulsion formulations and precise delivery are some of the main formulation issues.

- **Powder dosage form** provides the advantage of stability due to absence of preservative. However, the suitability of the powder formulation is dependent on the solubility, particle size, aerodynamic properties and nasal irritancy of the active drug and/or excipients.

- **Nasal gels** are thickened solutions or suspensions, of high-viscosity. The advantages of a nasal gel include the reduction of post-nasal dripping due to its high viscosity, reduction of the taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients, and target delivery to the mucosa for better absorption. Vitamin B$_{12}$ and apomorphine gel have been successfully employed to achieve desired therapeutic concentrations following nasal administration [30, 39, and 40].

**Drug concentration, required dose and dose volume**

Drug concentration, dose and volume of administration are three interrelated parameters that impact the performance of the nasal delivery system. *Ex-vivo* experiments in rats demonstrated the effect of drug concentration on nasal drug absorption. However, few experiments showed different effects of drug concentration on the absorption of drugs, for example the absorption of aminopyrine from rat nasal mucosa was constant as
a function of its concentration. Nasal absorption of salicylic acid was decreased with increasing concentration of administered drug and low absorption of high concentration of salicylic acid was lined with its nasal epithelial toxicity and nasal membrane resistance [27].

2.3.6 Strategies to improve absorption from nasal cavity

**Permeation enhancers**

A variety of permeation enhancers have been investigated to improve the nasal absorption, like fatty acids, bile salts, phospholipids, surfactants, cyclodextrin, etc., which act via different mechanisms such as inhibition of enzyme activity, reduction of mucus viscosity, decreasing mucociliary clearance, opening tight junctions, and solubilizing or stabilizing the drug [41].

**Prodrug approach**

Prodrugs are the inactive chemical moiety which becomes active at the target site. This approach is mainly used to improve the physicochemical properties such as taste, solubility, and stability of formulation. This approach includes derivatization of C and N termini, esters, and cyclic prodrugs [42].

**In-situ gel**

The conversion into gel by the influence of stimuli including temperature, pH, and ionic concentration is possible with substances like Carbopol, cellulose derivatives, lecithin, chitosan, etc. These formulations generally control the problems of administration [35].

**Nasal enzyme inhibitors**

Enzyme inhibitors like protease and peptidase are used as inhibitors for the formulation of peptide and protein molecules. Other examples are bile salts, amastatin, bestatin, boroleucine, fusidic acids, etc [27].
Structural modification

Structural modification without changing the pharmacologic activity, can also improve nasal absorption. Chemical modifications are mainly used to modify the physicochemical properties of the drug such that they lead to improved nasal absorption of drug [27].

Mucoadhesion

Mucoadhesion can be defined as the state in which two materials are held together for a long period. Mucoadhesive polymers make intimate contact with the biological membrane, and after the establishment of contact, they penetrate into the tissue surface sometime acting as the penetration enhancers. Both natural as well as synthetic polymers have been reported as safe for intra nasal delivery [43].

2.4 Thermoreversible Gel Formulation

However, drug delivery via nasal mucosa is also associated with certain drawbacks including drug loss due to rapid mucociliary clearance resulting in a short residence time of the formulation, potential for ciliotoxicity, enzymatic degradation, and relatively smaller surface area of absorption. These limitations can be minimized by the use of mucoadhesive polymers. Recent developments in the field of polymer science and technology has led to the development of various stimuli sensitive hydrogels like pH, temperature and ion sensitive which are employed for nasal drug delivery. Attempts have been made to overcome these drawbacks and to increase the nasal bioavailability of drugs through use of bioadhesives, permeation enhancers, in-situ gelling systems, microspheres, and nanoparticles, among others. Mucoadhesive in-situ polymers tents to change the phase from liquid to gel form at the nasal temperature and tends to adhere to the mucosal surface area from where it release the drugs for maximum duration of time by retaining the drug at the local site hence improves the bioavailability and avoids the limitations occurred by nasal solutions [44].
2.4.2 Approaches of in-situ gel drug delivery

Different approaches and its mechanisms to trigger the in-situ gel formation of biomaterials are listed broadly as follows: [Physiological stimuli (e.g., temperature and pH), physical changes in biomaterials (e.g., solvent exchange and swelling), chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization)].

In-situ formation based on physiological stimuli

Temperature triggered system Temperature-sensitive hydrogels are probably the most commonly studied class of environment-sensitive polymer systems in drug delivery research. The use of biomaterial (natural as well as synthetic) which leads to the phase transition from sol to gel is triggered by increase in temperature. The important application of the system is that there is no external energy required to form the gelation which complements the ideal temperature whereby the phase transition would takes place at the ambient and the physiological temperature. A useful system should be tailorable to account for small differences in local temperature, such as might be encountered in appendages at the surface of skin or in the oral cavity. Examples of such systems are Nisopropylacrylamide, Pluronics and protein polymers (ProLastins) which undergo an irreversible sol gel transition.

pH triggered systems

The system is based on the formation of in-situ gel is induced by the change in the physiologic stimuli. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The polymers mostly used to achieve gelation at pH change are carbomers or its derivatives polyvinylacetal diethylaminoacetate and poly (ethylene glycol). Mixtures of poly methacrylic acid and Poly ethylene glycol have also been reported as a pH sensitive system to achieve gelation [27, 29, and 35].
2.4.3 *In-situ* formation based on physical mechanism [27, 45]

**Swelling**

*In-situ* formation may also occur by the material absorbing the water from surrounding environment and expand to desired space. One such substance is myverol 18-99 (glycerol mono-oleate), which is polar lipid that swells in water to form lyotropic liquid crystalline phase structures. It has some bioadhesive properties and can be degraded in vivo by enzymatic action.

**Diffusion**

This method involves the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix. Eg. Nmethyl pyrrolidone.

**In-situ formation based on chemical reactions**

Chemical reactions that results *in-situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.

**Ionic crosslinking**

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive crosslinking. While k-carrageenan forms rigid, brittle gels in reply of small amount of $K^+$, icarrageenan forms elastic gels mainly in the presence of $Ca^{2+}$. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes *in-situ* gelling in the presence of mono- and divalent cations, including $Ca^{2+}$, $Mg^{2+}$, $K^+$ and $Na^+$. Gelation of the low-methoxy pectins can be caused by divalent cations, especially $Ca^{2+}$. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. $Ca^{2+}$ due to the interaction with glucuronic acid block in alginate chains.
Enzymatic cross-linking

*In-situ* formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation.

Photo-polymerisation

Photo-polymerisation is basically used for *in-situ* formation of biomaterials. Electromagnetic radiations are used to form gel to a solution of monomers or reactive macromer by injecting the initiator into a tissues site. These systems can be designed readily to be degraded by chemical or enzymatic processes or can be designed for long term persistence *in-vivo*. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photo initiator. Photopolymerizable systems when introduced to the desired site via injection get photo cured *in-situ* with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature.

2.4.5 Formulation design

The design of *in-situ* gel formulation depends on the physicochemical properties of the drug molecule, the diseased condition for which treatment is required, the patient population and the marketing preference. Physicochemical factors include molecular weight, lipophilicity and molecular charge; an anatomical and physiological factor includes membrane transport, pH of tissue fluid, and mucocilliary clearance (as in case of nasal administrations). While the formulation factors include, clarity, pH, gelation temperature, viscosity, osmolarity and spreadability [46].
2.4.6 Evaluation and characterization of *in-situ* gelling system

*In-situ* gels are evaluated & characterized by the following parameters

**Clarity**

The clarity of formulated solution is determined by visual inspection under black & white background.

**Texture analysis**

Texture profile analyzer is used to analyze the consistency, firmness and cohesiveness of *in-situ* gel. Which are mainly indicated by measuring the gel strength & easiness in administration in vivo higher value of adhesiveness of gel are needed to maintain an intimate contact with mucus surface.

**pH of gel**

pH can be determined by taking the formulation in beaker and 1ml NaOH added drop wise with continuous stirring. pH is checked by using pH meter.

**Sol-Gel transition temperature and gelling time**

Phase transition is the process where the sol is changed to gel form by change in the temperature and the temperature the sol-gel transition occurs is termed as Phase transition temperature. It is being evaluated by two methods visual inspection method and by indicating the by movement of meniscus on tilting the tube. Thermoreversible polymers are used for *in-situ* gel forming systems. To evaluate the gelation temperature sol meniscus is first noted when kept in a sample tube at a specific temperature and then heated at a specified rate. In the visual inspection method the solution is heated with constant rate with an increment of 1°C on thermoregulated magnetic stirrer at constant speed, movement at which bead stops due to gelation is recorded as gelation temperature [47].

**Gel-Strength**

It is denoted by the time taken by standard weight to penetrate by 5cm dip into the gel. This parameter can also be evaluated using a rheometer. Depending on the
mechanism of the gelling of gelling agent used, a specified amount of gel is prepared in a beaker, from the sol form. This gel containing beaker is raised at a certain rate, so pushing a probe slowly through the gel. The changes in the load on the probe can be measured as a function of depth of immersion of the probe below the gel surface [48].

**Gelling capacity**

*In-situ* gel is mix with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25μl & normal volume of tear fluid in eye is 7μl) to find out gelling capacity of ophthalmic product. The gelation assessed visually by noting the time for & time taken for dissolution of the formed gel [49].

**Viscosity and rheology**

Rheological study reveals the viscosity properties of the sol and gel transmissions. The viscosity measured by using Brookfield viscometer, cone & plate viscometer or some other type of multipoint viscometers. *In-situ* gel formulation is placed in sample tube. The viscosity of these formulations should be such that no difficulties are envisaged during their administration by the patient during administration. Formulation should have viscosity 5-1000 mPas, before gelling & after ion gel activation by eye will have viscosity of from about 50-50,000 mPas [44].

**Isotonicity evaluation**

Isotonicity is important characteristics of ophthalmic preparation. Isotonicity is maintained to prevent tissue damage or irritation of eye. All ophthalmic preparation are subjected to isotonicity testing, science they exhibited good release characteristics & gelling capacity & the requisite velocity. Formulation mixed with few drops of blood & observed under microscope at 45x magnification & compared with standard marketed ophthalmic formulation [50].

**Swelling studies**

Swelling studies are conducted with a cell, equipped with thermo jacket to maintain a constant temperature .The cell contains artificial tear fluid (composition –
0.67g sodium chloride, 0.20g Sodium bicarbonate, 0.008g calcium chloride.2H₂O & distilled water q.s to 100g). Swelling medium equilibrating at 37°C one milliliter of formulated solution is placed in dialysis bag & put into the swelling medium. At specific time interval the bag is removed from the medium & weight is recorded. The swelling of the polymer gel as a function of time is determined by using the following relationship [51].

\[ \% S_t = \frac{(W_t - W_0)}{W_0} \times 100 \]

Where, \( S_t \) = Swelling at time ‘t’. \( W_0 \) = Initial weight of gelling solution. \( W_t \)=Final weight of gel.

**Mucoadhesive strength**

Mucoadhesion testing was carried out using a texture analyzer (CT3, Brookfield, USA) with 50 N load cell equipped with mucoadhesive holder. Sheep nasal mucosa is utilized as the model membrane to determine mucoadhesive potential. The tissue (about 20×20 mm) is equilibrated for 15 min at 37.0 ± 0.5°C before placing onto the holder stage of mucoadhesive holder. The probe is then lowered at a rate of 0.5 mm/s until it is in contact with the membrane. A contact force of 1 N is maintained for 60s, and the probe is subsequently withdrawn at a rate of 0.5 mm/s to a distance of 15 mm. By using the texture analyzer, the maximum force required to separate the probe from the tissue (i.e., maximum detachment force in grams; \( F_{max} \)) could be detected directly from Texture Pro. CT V 1.3 Build 14 software.

In the second method two sheep nasal tissue portions approximately 20 x 20 mm² are tied to two different glass slides using thread. One glass slide is fixed on the underneath portion of a pan balance with two sided adhesive tape facing downside. The other slide is fixed on wooden board of balance in such a way that the tissue lies just beneath and facing upper tissue. Gel (100 mg) is placed in between two mucosal tissues and held in contact for 2 min and dummy granules are then added slowly into the other pan till the tissues get separate. The mucoadhesive strength of gel formulation is determined from minimal weight that detach the mucosal tissues from the surface of each formulation and is calculated by the formula: \((m \times g / A) \times 100\),
where \( m = \) weight required for detachment in grams, \( g = \) acceleration due to gravity (980 cm/s\(^2\)), \( A = \) area of mucosa exposed and expressed as the detachment stress in dyne/cm\(^2\) [46, 48].

**Statistical analysis**

Analysis of variance (ANOVA) is used in testing the difference between calculated parameters using SPSS statistical package. Statistical difference yielding \( P \leq 0.05 \) is considered. Duncan multiple comparison is applied when necessary to identify which of the individual formulations are significantly different.

**High performance liquid chromatography**

The HPLC system is used in reversed phase mode. Analysis is performed on a Nova pack C18 packed column (150 mm length X 3.9 mm i.d).

**Fourier transformer infra red (FTIR)**

The possibility of drug excipient interaction is investigated by FTIR studies. The FTIR graph of pure drug & combination of drug with excipient are recorded by using KBR pellets.

**Thermal analysis**

Thermo gravimetric analysis can be conducted for *in-situ* forming polymeric system to quantitative the percentage of water in hydrogel. Different scanning calorimetry is used to observe any changes in thermograms as compared with pure ingredients used thus indicating the interaction.

**In vitro drug release studies**

In vitro release study of *in-situ* gel solution is carried out by using Franz diffusion cell. The formulation is placed in donor compartment & freshly prepared simulated tear fluid in receptor compartment. Between receptor & donor compartment dialysis membrane is placed (0.22 \( \mu \)m pore size). The whole assembly is placed on
thermostatically controlled magnetic stirrer. The temperature of the medium is maintained at 37°C± 0.5°C. 1ml sample is withdrawn at predetermined time interval of 1h for 6h the sample volume of fresh medium is replaced. The withdrawn sample is diluted to 10ml in volumetric flask with respective solvent & analyzed by UV spectrophotometer at respective nm using reagent blank. The drug content calculated using an equation generated from standard calibration curve. The percentage cumulative drug release (% CDR) calculated. The obtained data is further subjected to curve fitting for drug release data. The best fit model and kinetic studies can be checked for Zero order, First order, Highuchi, Krosmeyers peppas & Hixon crowell, to study the fickian and non-fickian diffusion mechanism [48].

**In-vitro permeation studies**

*In-vitro (ex-vivo)* permeation studies are carried out on fresh nasal tissues. Nasal mucosal tissue is carefully removed from the nasal cavity of sheep, obtained from the local slaughter house. Tissue samples are inserted in Franz diffusion cells displaying a permeation area of 0.785 cm². Twenty milliliters of phosphate buffer saline (PBS) pH 6.4 at 34°C is contained in the receptor chamber. To ensure oxygenation and agitation, a mixture of 95% O₂ and 5% CO₂ is bubbled through the system. The temperature within the chambers is maintained at 34°C. After a pre-incubation time of 20 min, pure drug solution and formulation is placed in the donor chamber. At predetermined time points, samples are withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.4 after each sampling. The samples withdrawn are filtered and used for analysis. Blank samples can be run simultaneously throughout the experiment to check for any interference. The amount of permeated drug is determined using a suitable analytical technique [52].

**Antimicrobial activity:- Sterility testing**

Sterility testing is carried out as per the IP 1996. The formulation is incubating for not less than 14 days at 30 - 35°C in the fluid thioglycolate medium to find the growth of bacteria & at 20 -25°C in Soya bean casein digest medium to find the growth of fungi in formulation.
Accelerated stability studies

Formulation is replaced in amber colored vials & sealed with aluminum foil for the short term accelerated stability study at 40 ± 20°C & 75 ± 5% RH as per International Conference of Harmonization (ICH) State Guidelines. Sample is analyzed at every month for clarity, pH, gelling capacity, drug content, rheological evaluation & in vitro dissolution [53].

Histopathological studies

Two mucosa tissue pieces (2-3 cm²) were mounted on in vitro diffusion cells. One mucosa was used as control (0.6 mL water) and the other was processed as treated with 0.6 mL of optimized formulation (conditions similar to in vitro diffusion). The mucosa tissues were fixed in 10% neutral carbonate formalin (24 h), and the vertical sections were dehydrated using graded solutions of ethanol. The subdivided tissues were stained with hematoxylin and eosin. The sections under microscope were photographed at original magnification × 100. The microscopic observations indicate that the organogel has no significant effect on the microscopic structure of the mucosa. The surface epithelium lining and the granular cellular structure of the nasal mucosa were totally intact. No major changes in the ultrastructure of mucosa morphology could be seen and the epithelial cells appeared mostly unchanged [48].

2.5 Nanocarriers

Nanotechnology is a potential area of current research due to its widespread applications in treating many diseases with targeted therapy. Nanoscale formulations could be successfully manipulated for targeted therapy of antimigraine drugs. It is expected that on administration of nanocarriers like intranasal ethosomes should reach at the targeted receptors via nose to brain active targeting through olfactory lobes. Figure 4. illustrates various types of nanocarriers which includes Liposomes, Polymeric nanoparticles, Gold, Iron oxide and carbon which hold great promise for drug delivery applications in nanomedicine due to its beneficial properties, such as better encapsulation, bioavailability, control release, and lower toxic effect.
2.5.1 Liposomes

It is important to understand that intranasal administration delivers less than 1% of the total dose to the brain, with larger accumulation in the olfactory bulbs and along the trigeminal nerve path. Within the past decade, there has been a surge of research into nano-sized vectors to aid drug delivery into the CNS. As mentioned before, the extracellular perineuronal channels (that are the most likely path of brain entry following intranasal delivery) have a diameter around 10-15 nm, giving nanoparticles a size limit for rapid delivery to the brain Liposomes are one example of nanoparticles for drug delivery that have attracted significant attention because of their ability to increase delivery to the target site of drug action [55].

Liposomes are self-assembling spherical vesicles with a size ranging from 20 nm to 10 μm. Liposomes may exist as uni- and multilamellar vesicles. Unilamellar vesicles consist of a lipid bilayer separating the aqueous core from an outer aqueous environment, while multilamellar vesicles have multiple lipid bilayers separating the different aqueous environments. Liposomes are formed spontaneously when dry lipids is dispersed in an aqueous media. They have the ability to function as drug carriers for both hydrophilic,
lipophilic and amphiphilic drugs. Hydrophilic drugs can be encapsulated in to the aqueous core, while lipophilic and amphiphilic drugs can be incorporated within the lipophilic bilayer.

Liposomes were defined as an artificial microscopic vesicle consisting of a central aqueous compartment surrounded by one or more concentric phospholipid layers (lamellae) (Fig. 5). Furthermore, hydrophilic (in the aqueous cavity), hydrophobic (within lipidic membrane) and amphiphilic substances are able to be incorporated within these vesicles developing large potential applications. Numerous researchers have worked with these structures since Bangham’s discovery, making of liposomes the most popular nanocarrier systems [56].

The encapsulation will offer protection from plasma and tissue hydrolytic enzymes and allow the drug to reach its cellular destination. Uptake into cells is by endocytosis. One of the main benefits to liposomes is their ease of adding surface modifications for cell targeting (i.e. monoclonal antibodies), evasion from the immune system (i.e. polyethylene-glycol), and/or detection (i.e. fluorescent tags). A range of lipids can be used to generate cationic, neutral or anionic vectors. For example, a liposome having an overall cationic charge will greatly increase cell-binding as well as aid in condensation of DNA (forming lipoplexes). This may be of particular interest to intranasal delivery, as cationic liposomes are thought to bind sialic acid residues in the nasal cavity and increase residence time at the nasal mucosa [57-59].
CHAPTER 2

Background of the Problem Selected for the Research

Liposome based drug delivery system for brain targeting through intranasal route

Figure 2.5 Schematic drawing demonstrating the liposomes structure and lipophilic or hydrophilic drug entrapment models.

Types of Liposomes
Liposomes can be obtained in different sizes and layers (single bilayer or multiple layers) depending on the preparation methods chosen

2.5.1.1 Classification of liposomes
Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types,

(i) Conventional liposomes
(ii) pH-sensitive liposomes
(iii) Cationic liposomes
(iv) Immunoliposomes and
(v) Long-circulating liposomes.
Otherwise, vesicle size is a critical parameter in determining circulation half-life of liposomes, and both size and number of bilayers influence the extent of drug encapsulation within liposomes. Thus, liposomes were typically classified on the basis of their size and number of bilayers into (Fig. 2):

(i) Small unilamellar vesicles (SUV): 20–100 nm
(ii) Large unilamellar vesicles (LUV): > 100 nm
(iii) Giant unilamellar vesicles (GUV): > 1000 nm
(iv) Oligolamellar vesicle (OLV): 100–500 nm and
(v) Multilamellar vesicles (MLV): > 500 nm.

In contrast to conventional liposomes, ethosomes shows smaller vesicle size, higher entrapment efficiency as well as improved stability. It is expected that on administration of nanocarriers like intranasal ethosomes should reach at the targeted receptors via nose to brain active targeting through olfactory lobes. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin and mucous layer in terms of quantity and depth when compared to conventional liposomes that are known mainly to deliver drugs to the outer layers [60]. Furthermore, the ethosomal carrier can provide an effective intracellular delivery of hydrophilic, lipophilic or amphiphilic molecules [61]. Basically ethosomes exhibit lipid bilayers like liposomes however they differ from liposomes in terms of composition (high content of ethanol). Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs. Considering the aspect investigation is being focused to develop novel ethosomal formulation for active targeting of CNS drugs through the olfactory pathway to brain tissues. Although very limited research is done on novel formulations of CNS drugs to improve the targeting potential, most of the studies have shown that intranasal drug delivery system could act as the innovative formulation to improve the drug targeting to brain and thereby decrease in dosing frequency and associated side effects. To increase the rate of penetration and release in the intranasal region ethosomes act as better choice of vesicle when compared to other forms of liposomes [62]. Ethosomes have become an area of research interest, due to its potential enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. Ethosomes can act as a carrier for
large and diverse group of drugs with different physicochemical properties and found a number of applications in pharmaceutical, biotechnological and cosmetic fields.

### 2.5.2 Ethosomes

Ethosomes are basically termed as soft, malleable lipid vesicles composed mainly of phospholipids, alcohol (ethanol or isopropyl alcohol) in relatively high concentration (20-45%) and water. Ethosomes were first developed by Touitou et al., (1997), as additional novel lipid carriers composed of ethanol, phospholipids, and water. “Ethosomes are ethanolic liposomes” Ethosomes can be defined as noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin in terms of quantity and depth when compared to conventional liposomes that are known mainly to deliver drugs to the outer layers of skin. Furthermore, the ethosome carrier can provide an effective intracellular delivery of hydrophilic, lipophilic or amphiphilic molecules shown in figure 2.6. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization. Therefore, when it comes in contact with vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. The lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and improves the drug distribution ability in the stratum corneum lipids. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer periods of time [64].

![Figure 2.6: Proposed diagram of ethosomes.](image)

**Table 2.2: Different Additive Employed In Formulation of Ethosomes.**


<table>
<thead>
<tr>
<th>Class</th>
<th>Example</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phospholipid</strong></td>
<td>Soya phosphatidyl choline</td>
<td>Vesicles forming component</td>
</tr>
<tr>
<td></td>
<td>Egg phosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dipalmitylphosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distearilphosphatidyl choline</td>
<td></td>
</tr>
<tr>
<td><strong>Polyglycol</strong></td>
<td>Propylene glycol</td>
<td>As a skin penetration enhancer</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td>Ethanol</td>
<td>For providing the softness for vesicle membrane. As a penetration enhancer</td>
</tr>
<tr>
<td></td>
<td>Isopropyl alcohol</td>
<td></td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>Cholesterol</td>
<td>For providing the stability to vesicle membrane</td>
</tr>
<tr>
<td><strong>Dye</strong></td>
<td>Rhodamine-123</td>
<td>For characterization study</td>
</tr>
<tr>
<td></td>
<td>Rhodamine red</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluoresceine Isothiocyanate (FITC)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6- Carboxy fluorescence</td>
<td></td>
</tr>
<tr>
<td><strong>Vehicle</strong></td>
<td>Carbopol 934, HPMC</td>
<td>As a gel former</td>
</tr>
</tbody>
</table>

### 2.5.2.1 Composition of Ethosomes

Basically, ethosomes exhibit lipid bilayer like liposomes; however they differ from liposomes in terms of composition (high content of ethanol). The ethosomes are composed of hydroalcoholic or hydro/glycolic phospholipid in which the concentration of alcohol is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid, phosphatidylserine, Phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Some preferred phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1 percent can also be added to the preparation. Alcohol, such as ethanol and isopropyl alcohol; among glycols, propylene glycol and Transcutol are generally used which may range from 20 to 50% in the final product. In addition to non-ionic surfactants (PEG-alkyl ethers) and cationic lipids (cocoamide, POE
alkyl amines, dodecylamine, cetrimide etc) can be combined with the phospholipids in the preparations [65]. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%. Various additives (table 2.2) which are used for formulation of ethosomes are listed in the table.

2.5.2.2 Advantages of Ethosomal Drug delivery [66]

In comparison to other transdermal and dermal drug delivery systems,
- Ethosomal system is passive, noninvasive and is available for immediate commercialization.
- Provides the platform for the delivery of large and diverse group of drugs (peptides, protein molecules).
- Composition is safe and the components are approved for pharmaceutical and cosmetic use.
- Low risk profile: The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
- Patient compliance is considered as ethosomes are administrated in semisolid form (gel or cream).
- Acts as permeation enhancer for drug through skin for transdermal and dermal delivery.
- High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of ethosomes.
- Widely proved applications in Pharmaceutical, Veterinary, and Cosmetic fields.
- Simple method for drug delivery in comparison to iontophoresis and phosphophoresis and other complicated methods.

2.5.2.3 Limitations of ethosomes [67]

- Poor yield.
- Loss of product during transfer form organic to water media.
2.5.2.4 Method for Preparing Ethosomes

Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, do not require any sophisticated equipment and are easy to scale up at industrial level.

1. Cold Method

This is the most common method utilized for the preparation of ethosomal formulation [68]. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. The mixture is heated to 300°C in a water bath. The water heated to 300°C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

2. Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 400°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400°C. Once both mixtures reach 400°C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method [69].

3. Injection Method

Ethosomes were prepared using different concentrations of lecithin, ethanol, isopropyl alcohol, propylene glycol. Phospholipids and drug was dissolved in ethanol and propylene glycol. The mixture was heated to 30°C in water bath. In this solution distilled water was added slowly in a fine stream with a constant mixing at 700 rpm in a closed vessel. The temperature was maintained at 30°C during the experiment. The mixing was continued for 5 minutes. The preparation was stored at 4°C. Ethosome prepared by the above procedure were subjected to sonication at 4°C using probe sonicator in 3 cycles of 5 minutes with 5 minutes pause between the cycles [70].
4. Mechanical dispersion method

Soya phosphatidylcholine is dissolved in a mixture of chloroform: methanol in round bottom flask (RBF). The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form a thin lipid film on wall of the RBF. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the RBF at suitable temperature [71].

![Proposed mechanism for skin delivery of ethosomal systems.](image)

**Figure 2.7:** Proposed mechanism for skin delivery of ethosomal systems.

The drug absorption probably occurs in following two phases
2.5.5.5 Mechanism of permeation [72]

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the ‘ethanol effect’ whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the ‘ethosome effect’, which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in Figure 2.7.

1. **Ethanol effect**

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. **Ethosomes effect**

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

2.5.5.6 Stability of ethosomes

Ethosomes offer better stability as compared to conventional liposomes [73]. In case of liposomes, upon storage they tend to fuse and grow into larger vesicles and this fusion and breakage of liposome vesicles on storage pose an important problem of drug leakage from the vesicles. The absence of electrostatic repulsion is likely to account for the tendency of neutral liposomes to aggregate, whereas in ethosomes, ethanol causes a modification of the net charge of the system (impart negative charge to the system) and confers it some degree of steric stabilization leading to increased stability of vesicles against agglomeration and drug leakage from vesicles. Increasing the concentration of
ethanol from 20 to 45% increases the entrapment efficiency owing to an increase in the fluidity of the membranes. However, a further increase in the ethanol concentration (>45%) destabilizes the vesicles and probably makes the vesicle membrane more leaky, thus leading to a decrease in entrapment efficiency.

2.5.5.7 Characterization and evaluation of Ethosomes

Size Analysis

Several techniques are available for assessing size and size distribution of ethosomes which include microscopy techniques, size-exclusion chromatography (SEC), and static or dynamic light scattering. Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS).

A recently developed microscopic technique known as atomic force microscopy (AFM) has been utilized to study ethosome morphology, size and stability. Ethosomes can be analyzed without manipulations by AFM, scanning probe microscopes with dimensional resolution (0.1nm). AFM analysis is rapid, powerful and relatively non-invasive technique. It can provide information on morphology, size, as well as on the possible aggregation processes of ethosomes during their storage [74].

HPLC along with SEC can be used to separate and quantify ethosomes population under physiological conditions. This mechanism leads to separation based on large particles elution before smaller particles. Conventional SEC is frequently used for liposomes separation from unencapsulated materials as a final purification step, but the use of HPLC-SEC for analysis offers increased resolution of liposome populations and reduced sample size and enhances reproducibility [75].

Transmission electron microscopy and Scanning electron microscopy provided the morphology and detail size characterization [76]. Photon correlation spectroscopy (PCS), is broadly used in ethosomes size distribution analysis. Basically PCS measures the time-dependant fluctuations of light scattered from particles with brownian motion, which results from collisions between suspended particles and solvent molecules [77].
CHAPTER 2

Background of the Problem Selected for the Research

Measurement of particle size distribution could also be achieved using density gradient stabilized sedimentation whereby particles that are lower in density than the fluid in which they are suspended can be accurately analysed.

**Zeta potential**

The zeta potential of a particle is the overall charge that a particle acquires in a particular medium. It is a physical property which is exhibited by any particle in suspension. To measure the zeta potential, a laser is used to provide a light source illuminating particles within the samples. The incident laser beam passes through the centre of the sample cell and the scattered light at an angle of about 13° is detected. When an electric field is applied to the cell, any particles moving through the measurement volume will lead to fluctuation of the detected light with a frequency proportional to the particle speed. This information is passed to a digital signal processor, then to a computer and hence potential zeta is calculated. Particles suspension with zeta potentials > +30 mV or < −30 mV are normally considered stable [78]

**Drug entrapment Efficiency**

Drug entrapment efficiency denotes the separation between the free drug and the encapsulated drug by using high speed centrifuge. The ultracentrifugation technique [79] was reported as a simple and fast method for the separation of drug-loaded liposomes from their medium. Drug loaded ethosomal nano-dispersion is subjected to centrifugation at 50,000 rpm for 50 minutes at 4°C using micro-ultracentrifuge (Thermo scientific Sorvall MX 150 Micro-Ultracentriguge, India) to estimate drug entrapment. Supernatant liquid was analysed for free drug UV, HPLC or LCMS and calculated by formula; Percent entrapment efficiency = (amount of drug encapsulated / total amount of drug used in formulation) x 100.

**Transition temperature**

The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC). The Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland) is used. The transition
temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute, within a temperature range from 20°C–300°C [74].

**Drug content**

Method is developed to access the drug content present in the formulation. Drug content of the ethosomes can be determined by using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

**Skin permeation studies**

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM).

**Drug Uptake Studies**

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 μL RPMI medium was added. Cells were incubated with 100 μL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

**Skin Permeation Studies**

The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using CLSM. In another method skin of the test animal is separated from the underlying connective tissues. The excised skin is placed on the aluminium foil, by gently teasing the dermal side of the skin for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume is 1.0 cm² and 10 mL, respectively. The temperature is maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 mL of pH 6.5). Excised skin is placed in between the donor and the receptor compartment. Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1, 2, 4, 8, 12, 16, 20, and 24 h time intervals and analyzed by HPLC assay [80].
Stability studies

A stable pharmaceutical dosage form maintains its physical integrity and does not adversely influence the chemical integrity of the active ingredient during its life. The successful introduction of dosage forms depends upon a well-defined stability study. In designing a stability study, physical, chemical and microbial parameters must be considered and evaluated. This wisdom is also required for the liposome dosage form. A stability study must include a section for product characterization and another section concerning the product stability during storage. The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM. Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier [81].

2.5.5.8 Therapeutic Applications of Ethosomes [62, 63]:

- Transdermal delivery of hormones
- Delivery of anti-parkinsonism agent
- Transcellular delivery
- Topical delivery of DNA
- Delivery of anti-arthritis drug, delivery of antibiotics
- In the treatment herpetic infection
- Delivery of anti-viral and antifungal agents
- Delivery of problematic drug molecules
- Used for treatment of chemotherapy induced nausea and vomiting
- Delivery of NSAIDs agents
- Treatment of acne and the dermatological diseases
- Delivery of anti-hypertensive agent
- Used to treat Alzheimer’s disease
- Pilosebaceous targeting
- Delivery of anti hyperlipidemic agent
- Used in angina pectoris
Liposome based drug delivery system for brain targeting through intranasal route

- used as bronchodilator
- Herbal ethosomes

Hence considering the applicability, patient compliance, biodegradability, stability, targeting ability, biocompatibility, improved permeation profile, better stability than liposomes, and ease of usage ethosomes could serve as potential carrier for antimigraine drugs. Literature reveals that thermoreversible gel preparations have been developed and found to have increased permeation rate with prolonged nasal residence time and thereby improved nasal absorption. Considering the applications and novelty in dosage form study was focused to develop drug loaded ethosomal insitu thermoreversible gel for brain targeting through intranasal route.
2.6 References


