8 FORMULATION AND DEVELOPMENT OF THERMOREVERSIBLE IN-SITU NASAL GEL OF NARATRIPTAN HYDROCHLORIDE

8.1 Background of the investigation designed

Intractable migraine presents a significant treatment challenge to both patient and physician. Migraine is a chronic neurological disease characterized by recurrent moderate to severe headaches which often is associated with a number of autonomic nervous system symptoms. Typically the headache affects one half of the head, is pulsating in nature, and lasts from 2 to 72 h. Associated symptoms include nausea, vomiting and sensitivity to light, sound or smell and pain which may worsen by physical activity [1].

Oral route is considered to be the most convenient and cheap route of drug administration but owning to its inefficiencies such as poor bioavailability [2], first pass metabolism [3], drug solubility [4] and absorption [5] issues, there is an obvious need for alternative but novel systems for drug delivery [6]. Parenteral routes of administration like subcutaneous route is another alternative but the dislike of injection make this dosage form less acceptable for the patient [7]. The percutaneous route is also used for controlled delivery of drugs which bypasses the first pass metabolism, but has limitations for permeability of the skin to many drugs [8]. As an preferred alternative to this, transmucosal routes including the nasal, buccal, pulmonary, rectal and vaginal routes can be used to overcome above mentioned issues wherein, intranasal (nasal cavity) route of drug provides a number of distinctive benefits, such as, low proteolytic activity, prevention of harsh environmental conditions, hepatic first pass metabolism, and potential direct delivery to the brain could serve the advantage of ease of access, improving bioavailability [9], good permeability mainly for lipophilic and low molecular weight drugs, [10], bypass first pass metabolism [11], ease of self-administration, ease of handling and can even be administered to patients in vomiting and unconscious state [12]. Nasal gels have also been engaged to enhance drug delivery efficiency by reducing swallowing and anterior leakage of formulation. Moreover, recent advancement has
revealed that the nasal mucosa can act as the site for directly delivering the therapeutics to the CNS through the olfactory lobes, which helps to circumvent the BBB [13].

Naratriptan Hydrochloride (NH) is a selective 5-HT$_1$ agonist developed for the acute treatment of migraine which acts by stimulating constriction of dilated cranial arteries and by inhibiting the release of neurogenic inflammatory mediators [14]. Naratriptan is available only in oral form with the recommended dose of 2.5 mg as its oral bioavailability of 50-60% [15] and exhibits a six fold higher affinity for the human recombinant 5-HT$_1$B receptor than sumatriptan [16].

The physiological characteristics of the nasal mucosa and nasal mucociliary clearance are the two main considerations in designing nasal formulations. To overcome these constrains, the approach was to develop a mucoadhesive thermosensitive nasal formulation capable of undergoing sol–gel transition at the nasal temperature. Nasal drug delivery is mainly to improve drug absorption rate and bioavailability by using permeation enhancer by prolonging the drug residence time at the nasal absorption site through biodegradable thermosensitive polymers. The thermoreversible gel formulation helps to prolong the drug contact time and releases the drug in a controlled manner, which results in improved local and systemic bioavailability, reduced dose requirements, improved patient safety and acceptability [17]. Consequently, thermoreversible system helps the drug to be manufactured as liquid dosage form which will perform phase transitions at physiological nasal temperature [18]. Hence, at in-situ nasal physiological conditions bioadhesive thermoreversible gels could be very useful for efficient delivery of drugs for targeting brain through olfactory lobe. In recent decade different formulations of Sumatriptan [19], Rizatriptan benzoate [20], Zolmitriptan [21], has been studied to provide the drug via intranasal route in the form of thermoreversible gel, whereby the drug was released in sustained form by adhering the gel to the local tissues and releasing the drug to the brain through the olfactory lobes. From the Pharmacokinetic standpoint intranasal administration circumvents first pass metabolism and drug absorption is rapid due to the existence of a rich vascular system and highly permeable structures within the permeable membrane [22].

Hence, the objective of present investigation was to develop thermoreversible gel formulations of NH for intranasal delivery using thermoreversible polymer poloxamer...
407 and mucoadhesive polymer Carbopol 934P, which would enhance nasal residence time and absorption of drug across nasal-mucosal membrane.

**8.2 Materials and Methods**

**8.2.1 Materials**

NH was provided as a gift sample from Orchid Chemicals and Pharmaceuticals Ltd. Chennai, India. Poloxamer 407 was obtained as a free sample from Shreya life sciences Pvt. Ltd., Aurangabad, India. Carbopol 934P and cellophane membrane (12,000–14,000 M.W) were purchased from Hi-Media Lab Pvt. Ltd., Mumbai, India. All other reagents used were of analytical grade.

**8.3 Methodology**

**8.3.1 Determination of gelation temperature**

Temperature at which the liquid (sol) phase converts to gel form is termed as gelation temperature. The sol–gel transition temperature of the prepared in-situ gel formulations was determined by visual inspection method [19]. Briefly, the solutions of poloxamer 407 in the concentrations (15–20 % w/v) were prepared by stirring (magnetic stirrer, IKA India Private Limited, India) in a transparent 10 ml glass bottle sealed with paraffin. The vial was heated at constant rate with an increment of 1°C and the temperature at which the magnetic bead stopped moving due to gelation was considered as gelation temperature. Gels which showed gelation temperature very close to nasal temperature (32-34°C) were selected for further evaluation. Effect of Carbopol 934P on phase transition temperature was evaluated by dispersing different concentration (0.1–0.5 % w/v) in optimized poloxamer 407 solutions.

**8.3.2 Formulation of in situ nasal gel of NH**

Poloxamer 407 gel was prepared by dissolving the optimized poloxamer 407 concentration in cold (4°C) water. The hazy solution formed was kept in refrigerator (2–4°C) overnight for complete dissolution resulting in a clear solution. The ‘cold’ method was adopted for the formulation of NH thermoreversible gels [23] where Carbopol 934P
(0.1 to 0.4 % w/v) concentrations was added slowly to the optimized poloxamer 407 solution containing drug with continuous stirring at 4°C. Formulated gels where then finally stored at 4°C for further evaluation.

Table 8.1: Composition of NH thermoreversible gel formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (% w/v)</th>
<th>Poloxamer 407 (% w/v)</th>
<th>Carbopol 934 (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>Pure drug solution (0.5%)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>NG</td>
<td>0.5</td>
<td>18</td>
<td>--</td>
</tr>
<tr>
<td>G1</td>
<td>0.5</td>
<td>18</td>
<td>0.1</td>
</tr>
<tr>
<td>G2</td>
<td>0.5</td>
<td>18</td>
<td>0.2</td>
</tr>
<tr>
<td>G3</td>
<td>0.5</td>
<td>18</td>
<td>0.3</td>
</tr>
<tr>
<td>G4</td>
<td>0.5</td>
<td>18</td>
<td>0.4</td>
</tr>
<tr>
<td>G5</td>
<td>0.5</td>
<td>18</td>
<td>0.5</td>
</tr>
</tbody>
</table>

8.3.4 Evaluation of gel

8.3.4.1 Physico-chemical properties of in-situ gel

The formulated gels were evaluated for pH, clarity, drug content, viscosity and gel strength. The pH of each formulation was determined by pH meter. Initially, the pH meter (Eutech Instruments Pvt. Ltd., Singapore) was calibrated using standard buffer solutions of pH 4 and pH 7 (Thermo Fisher scientific standard buffers). The clarity was checked against white and black background and was graded as turbid (+), clear (++) and very clear (+++). Drug content was determined spectrophometrically using UV spectrophometer (Shimadzu 1800, Japan) at 283 nm. Rheological studies were performed with a thermostatically controlled Brookfield viscometer (DV3T Rheometer, USA) fitted with suitable spindle at varying temperatures (20 - 40°C).

Gel strength was determined by placing a standard weight of 35 g onto 50 g of thermoreversible gel (placed in 100 ml beaker) maintained at gelation temperature using controlled water bath. The time in seconds by the weight to penetrate 5 cm deep into the container was recorded as gel strength [24].
The mucoadhesive potential was determined by measuring the force required to detach the formulation from nasal mucosal tissue. Two nasal mucosa tissue portions measuring approximately 20 x 20 mm² were tied to two different glass slides using thread. One glass slide was fixed on the underneath portion of a pan balance with two sided adhesive tape facing downside. The other slide was fixed on wooden board of balance in such a way that the tissue was just beneath and facing upper tissue. Gel (100 mg) was placed in between two mucosal tissues and held in contact for 2 min followed by adding dummy granules slowly into the other pan till the tissues get separate [20]. Minimal weight that detached the mucosal tissue from the surface of each formulation was recorded as mucoadhesive strength and expressed as the detachment stress in dyne/cm². It was calculated by the formula,

$$\text{Mucoadhesive strength} \left( \frac{\text{dyne}}{\text{cm}^2} \right) = \frac{m \times g}{A} \times 100$$

Where m = weight required for detachment in grams, g = acceleration due to gravity (980 cm/s²), and A= area of mucosa exposed.

### 8.3.5 In-vitro drug release study

Drug release from gel was determined by using six cell thermoregulated Franz diffusion cell system (Thermo Fischer scientific, Haake S5P Newington, USA). Artificial dialysis membranes (mol. Wt. 1200-1400 KDa) were soaked in receptor medium for 2h prior to use. Phosphate buffer saline (12 ml) pH 6.4 was added into the receptor chamber maintained at 34 ± 1°C. Gel equivalent to 2.5mg of drug was placed into donor compartment and the setup was kept on stirring. An aliquots of 1ml were withdrawn at predetermined time intervals from receptor compartment and replaced with fresh buffer till 12 h. The samples were diluted suitably and analyzed spectrophotometrically at 283 nm and the amount of drug released was determined using calibration curve. Release kinetics was studied by using software DD solver [25].

### 8.3.6 Ex-vivo drug permeation study

Nasal cavity of sheep was obtained from local slaughter house immediately after its sacrifice and transported to the laboratory by keeping it in saline phosphate buffer pH
6.4. The superior nasal membrane was identified and separated from nasal cavity and made free from adhered tissues. The study was conducted with six cell thermoregulated Franz diffusion cell system where tissue samples were fixed on Franz diffusion cells having effective permeation area of 2 x 2 cm$^2$. Phosphate buffer saline (12 ml) pH 6.4 was added to the acceptor chamber and agitated with magnetic stirrer at 34 ± 1°C. After pre-incubation time of 30 min, thermoreversible gel formulations were placed in the donor compartment. Sample aliquots (1 ml) were withdrawn at predetermined time interval up to 12 h and were replaced with same volume of phosphate buffer saline pH 6.4. The samples were filtered and analyzed by UV spectrophotometer at 283 nm. Permeability coefficient was drug formulations were calculated by using following formula,

$$Permeability\ coefficient = \frac{(dc/dt)_{ss}}{A} \times \frac{V}{C_D}$$

where $(dc/dt)_{ss}$ (µg mL$^{-1}$ s$^{-1}$) change of concentration under steady-state; A (cm$^2$) is the permeation area; V (ml) the volume of the receiver compartment; and $C_D$ (µg mL$^{-1}$) is the initial donor concentration.

### 8.3.7 Histopathological evaluation of mucosal tissue

Histopathological evaluation of tissue incubated in PBS (pH 6.4) was compared with tissue incubated in the diffusion chamber with gel formulation. Tissue was fixed in 10% buffered formalin (pH 7.2). Paraffin sections were cut on glass slides and stained with haemotoxylin and eosin. Sections were examined under light microscope by a pathologist who was blinded to the study, to detect any damage to tissue during ex-vivo drug permeation.

### 8.3.8 Stability studies of NH loaded thermoreversible in-situ gel

Stability studies of the optimized formulations were carried out at 40 ± 2°C, 75 ± 5% RH at an interval of one month for 3 consecutive months. The results were compared with respect to gelation temperature, pH, viscosity, drug content and drug release to indicate stability for optimized formulation [26].
8.4 Result and Discussion

8.4.1 Determination of gelation temperature

Phase transition temperature determination is a preliminary step in the formulation of thermoreversible gel. Gelation temperature of gel formulations is shown in table 8.2 which suggests that Polaxomer in the concentration of 18% w/v showed optimized results for phase transition at 32-33°C. As the concentration of poloxamer increased from 18 to 20 %, transition temperature decreased from 34 to 27 °C.

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Poloxamer 407 (% w/v)</th>
<th>Gelation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>No gelling till 42</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>No gelling till 40</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>Viscosity increased at 38</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>32–33</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>30–31</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>26–27</td>
</tr>
</tbody>
</table>

In general, thermoreversible gel should form gel in the temperature range of 30-34°C which is very close to nasal temperature. Thermoreversible intranasal gels are expected to turn to gel form at nasal temperature and exist in solution form when stored at room temperature. If the gelation temperature of a thermoreversible formulation is lower than 25°C, a gel may be formed at room temperature, leading to difficulty in manufacturing, handling, and administering whereas when the gelation temperature is higher than 34°C, solution form will not show phase transition at the nasal temperature resulting in the nasal clearance of the administered drugs at an early stage. Thermoreversible polymer poloxamer 407 is known to exhibit in situ gelling property due to their negative coefficient of solubility to form block copolymer micelles [27]. Increase in temperature is directly proportional to the number of micelles formation leading to construction of tightly packed micelles which subsequently forms gel. Effect of
concentration of carbopol 934p on gelation temperature on gelation was studied (figure 8.1) where results confirm that all formulations were converted to gel form in the range of 25-31°C. It was found that by addition of carbopol 934P from 0.1 to 0.3% gelation temperature was lowered from 31°C to 29°C. On further addition of carbopol (0.4% and more) the gelling temperature was reduced to 25.4°C. Considering the physiological temperature of the nose formulation G2, G3 and G4 were considered for further studies.

![Figure 8.1: Study on effect of concentration of carbopol 934P on phase transition temperature.](image)

**8.5 Evaluation of thermoreversible gel of NH**

**8.5.1 Physicochemical properties**

The pH of all the formulations was found to be 6.0 ± 0.3 which is in the range of nasal pH (5.5 to 6.5) [28]. This confirms that all the formulations were compatible with nasal mucosa. All formulations were found to be clear except G3 and G4 which confirms that clarity is inversely proportional to the concentration of carbopol used in formulations.
Figure 8.2: Evaluation of NH in-situ gel. 3a. Viscosity of gel formulations, 3b. Gel strength (sec) and 3c. Mucoadhesive strength of gel formulations (dynes/cm²). All determinations are expressed in mean ± Std. Dev (n=3).
Drug content of all formulations was found in the range of 97 and 99% of the initially added drug quantity i.e. 0.5% w/v in all formulations. Viscosity was carried out at the temperature of 8 ± 2°C for all formulations to determine its consistency during storage time. It was noted that viscosity was directly proportional to the concentration of carbopol 934P (0.1% to 0.4%) as shown in Figure 8.2A. Gel strength is defined as a measure of the ability of a colloidal dispersion to develop and retain a gel form, based on its resistance to shear. Results of gel strength (Figure 8.2B) suggest that carbopol is mainly responsible for increasing the gel strength of formulations. Gel strength of less than 25 seconds suggests that the gel could not be retained in nasal cavity due to its solution nature, whereas gel strength in the range of 26-50 seconds are desirable which could be retained in nasal cavity due to its gel form. Gel strength above 50 seconds suggests undesirable stiffness of gel formulation which may lead to irritation and discomfort in drug delivery.

The mucoadhesive strength was determined on the basis of detachment stress, where the force required to detach two mucous membranes from each other was measured. It was indicated from observation (Figure 8.2C) that the concentration of Carbopol 934p in formulation was directly proportional to mucoadhesive strength of gel. Cross-linked polyacrylate nature of Carbopol 934p with abundant carboxylic groups tends to form hydrogen bonding with sugar residues in mucus membrane leading to strengthened network between polymer and mucus membrane. It was observed that drug retention and absorption across mucosal tissue was directly proportional to mucoadhesive strength [20].

8.5.6 In-vitro release study

The drug-release behaviors of NH solution, Poloxamer 407 drug solution and G2-G3 were firstly measured in-vitro via the dialysis membrane. As recorded from Figure 8.3, the release of NH solution was rapid and almost complete within 6 h, while the incorporation of drug into the Poloxamer 407 significantly retarded drug release to about 50% after 12 h, suggesting that poloxamer acts as retardant to sustain the drug release.

However the maximum amount of drug was released from G4 than that of G2 and G3 indicating the effect of carbopol on drug release. Consequently, the data obtained
from the \textit{in-vitro} release experiments (table 8.3) were kinetically analyzed by different mathematic models like Zero order, first order, Higuchi, Korsmeyer–Peppas and Hixson-Crowell representing goodness of fit in terms of $R^2$ values. From the Peppas exponential equation $\log \frac{M_t}{M} = \log k + n \log t$, where $\frac{M_t}{M}$ is the fraction of released drug at time $t$, $k$ is a release constant and is dependent on structural and geometric characteristics of the drug/polymer system, and $n$ is the release exponent and is indicative of the release mechanism. If $n < 0.45$, the drug is released from the polymer with a Fickian diffusion mechanism. If $0.45 < n < 0.89$, this indicates anomalous or non-Fickian release. If $n > 0.89$, the main mechanism is matrix erosion.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>PG</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Models</td>
<td>Parameters</td>
<td>$R^2$</td>
<td>Parameters</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Zero ($K_0$)</td>
<td>5.18</td>
<td>0.92</td>
<td>5.91</td>
<td>0.94</td>
</tr>
<tr>
<td>First ($K_1$)</td>
<td>0.07</td>
<td>0.98</td>
<td>0.09</td>
<td>0.99</td>
</tr>
<tr>
<td>Higuchi ($K_H$)</td>
<td>14.5</td>
<td>0.94</td>
<td>16.5</td>
<td>0.94</td>
</tr>
<tr>
<td>Korsmeyer-Peppas ($K_{KP}$)</td>
<td>10.26</td>
<td>0.98</td>
<td>11.11</td>
<td>0.99</td>
</tr>
<tr>
<td>n=0.69</td>
<td>n=0.71</td>
<td>n=0.69</td>
<td>n=0.74</td>
<td></td>
</tr>
<tr>
<td>Hixson-Crowell ($K_{KC}$)</td>
<td>0.02</td>
<td>0.99</td>
<td>0.03</td>
<td>0.98</td>
</tr>
</tbody>
</table>

The results revealed that the optimized formulation had a value of $n = 0.69$, indicating that the major mechanism was diffusion-controlled drug release or Non-Fickian diffusion kinetics. The initial release more than 30% was observed and as the time proceeded carbopol retarded the release and also acted as permeation enhancer when compared with other formulations. The drug-release profile tend to follow the models, but it was identified better for first order ($R^2 = 0.99$) than others.
In-vitro release study

In-vitro release study from in situ nasal gel was carried out for 12 h using thermoregulated Franz diffusion cell at 34 ± 1°C. Cumulative drug release from all the formulations after 12 h was found to be more than 65%. The release data of gel formulation (table 8.3) were kinetically analyzed by different mathematic models like Zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell representing goodness of fit in terms of $R^2$ values. Since the calculated n values (table 8.3) obtained from Korsmeyer-peppas model were between 0.5 and 1, the NH release from gel formulations followed Non-Fickian release mechanism, suggesting that the release was controlled by the diffusion rate and the relaxation rate of carbopol 934 polymer matrix.
8.5.8 Ex-vivo drug permeation study

Ex-vivo drug permeation study reveals that cumulative amount of drug permeated was rapid for initial 2 h followed by slow release of around 66% in 12 h. Permeability coefficient \((10^{-5} \text{ cm/s} \text{ cm}^{-1})\) of NP, G2, G3 and G4 were found to be \(3.04 \pm 0.09\), \(3.47 \pm 0.05\), \(3.76 \pm 0.12\) and \(4.56 \pm 0.16\) respectively. Initial rapid release may be due to the exposure of fresh surface of drug present in gel form to the sheep mucosa and sustained drug release after 2 h may be due to the polymers like poloxamer 407 [27, 29]. Earlier studies have indicated that the poloxamer slightly decreases the rate of drug release due to enhanced micellar structure and gel network. However, comparing drug permeation from all gel formulations suggests that drug release is directly proportional to concentration of carbopol. This may be due to increase in the ionized carboxyl groups of carbopol 934p which has caused conformational changes in the polymer chain followed by swelling of polymer matrix leading to relaxation of gel network confirming that carbopol enhance rate of permeation [20].

![Figure 8.4: Ex-vivo permeation studies of gel formulations through sheep nasal mucosa. All determinations are expressed in mean ± Std. Dev (n=3)](image-url)
8.5.9 Histopathological evaluation of mucosal tissue

Histopathology of the nasal mucosal tissue (figure 8.5) indicated absence of toxicity. Both gel treated and untreated mucosal membranes showed similar microscopic tissue architecture with intact columnar structure of epithelial cells.

Figure 8.5: Histopathological evaluation of sections of sheep nasal mucosa. 6a. mucosal tissue incubated in PBS (treated) 6b. Mucosal tissue incubated in diffusion chamber with G4 formulation (untreated).

8.5.10 Stability studies of optimized NH loaded thermoreversible in-situ gel

Stability studies (ICH guideline Q1A (R2)) of optimized formulation were performed w.r.t. determining factors viz. gelation time, gel strength, mucoadhesive strength, viscosity, in-vitro diffusion studies and ex-vivo permeation studies. Sample analysis after 1, 2 and 3 month showed no significant change in all determining factors suggesting stability of gel formulations.
8.6 Conclusion

Present study represents formulation of thermosensitive intranasal gel for NH using poloxamer 407 and carbopol 934P. Formulation (G3) was found to be optimized due to its desirable gelation temperature (29°C), mucoadhesive (4213 dyne/cm²) and gel strength (47 sec). In-vitro release and ex-vivo permeation studies suggests that carbopol not only acts as mucoadhesive agent but also as an penetration enhancer where as poloxamer acts as thermoreversible polymer leading to sustained release of drug for longer time. In conclusion, intranasal gel of NH could be better alternative to existing conventional dosage form to improve drug bioavailability and patient compliance.
8.7 References


