11.0 FORMULATION AND DEVELOPMENT OF ETHOSOMAL IN-SITU NASAL GEL OF ZOLMITRIPTAN

11.1 Background of the investigation

Migraine is a common illness which imposes an enormous health burden on both patient and society leading to work and productivity losses causing economic burden [1]. Various therapeutic agents have been recognized and documented for the treatment of migraine. Introduction of triptans for the treatment of migraine has been a revolution with some limitations. Most triptans with short half-life durations like Rizatriptan (2.4 h), Eletriptan (5 h), Naratriptan (5.5 h), Sumitriptan (2 h), Almotriptan (3 h) and Zolmitriptan (2.5 h) [2] lowers the desired therapeutic action leading to frequent dosing to get the desired effect. Even though the patient acceptability of zolmitriptan is reliable, its use is limited due to its frequent dosing and associated side effects. Hence there is a need to develop a novel dosage form for brain targeted sustained and continuous delivery of zolmitriptan which would subsequently reduce associated side effects.

Zolmitriptan (ZMT) chemically ((S)-4-[[3-[2-dimethylamino] ethyl]-1Hindol-5yl]methyl]-2-oxazolidinone) is a 5-HT \textsubscript{1B/1D} receptor partial agonist which has been approved for use in the acute treatment of migraine [3]. ZMT has a better efficacy and tolerability profile at doses of 2.5–10 mg, undergoes first-pass metabolism and has poor bioavailability. Its C\textsubscript{max} is reported to be 2.7 to 9.9 ng/ml and plasma half-life is 1–2 h suggesting frequent dosing [4].

Presently, ZMT formulations available in market include tablet, nasal spray and orodispersible tablet dosage forms [5-7]. But the use of these dosage forms is limited due to the vomiting and gastric problems of the patients suffering from migraine [8]. To overcome these limitations, there is need to develop a nano formulation for targeting brain and simultaneously provide sustained release of drug. One such approach is nano ethosomal drug delivery system where it is hypothesized that drug can bypass blood brain barrier via olfactory lobes pathway [9]. Further, thermoreversible gel formulation of drug loaded ethosomes could provide a final dosage form for sustained release intranasal delivery of ZMT. However, intranasal drug delivery system must be precisely formulated...
to provide desirable release of drug across nasal mucosa and longer residence time in nasal cavity.

Herein, we have streamed our research work to formulate and optimize ZMT ethosomes by using $3^2$ factorial design. Optimized ethosomes were evaluated and further incorporated into thermosensitive gel form using carbopol 934 and HPMC K 100 as mucoadhesive agent and poloxamer 407 as and thermoreversible agent.

**11.2 Materials and Methods**

**11.2.1 Materials**

ZMT was obtained as a gift sample from Cipla ltd., Mumbai, India and Poloxamer 407 was obtained as a free sample from Shreya life sciences Pvt. Ltd., Aurangabad. Other chemicals like Carbopol 934, HPMC K100, Soya lecithin (30%) and membrane (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.

**11.3 Methodology**

**11.3.1 Preparation of ethosomes**

On the basis of preliminary investigations, a three level factorial design was used for formulations of ethosomes [10]. Concentration of Soya lecithin and ethanol were considered as independent factors whereas percent entrapment efficiency (EE) and vesicle size (VS) as dependent variables (Table 11.1). The polynomial equation generated was used to estimate the response shown by general equation,

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2.$$  

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels are specified in the original units for each factor. Whereas the equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded
equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 11.1: Observed responses from $3^2$ factorial design of ZMT loaded ethosomal formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Independent variables</th>
<th>Dependent variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$: Concentration of Soya lecithin</td>
<td>$X_2$: Concentration of ethanol</td>
</tr>
<tr>
<td></td>
<td>EE: Entrapment efficiency (%)</td>
<td>VS: Vesicle size (nm)</td>
</tr>
<tr>
<td>ZE1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>ZE2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>ZE3</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>ZE4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>ZE5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*ZE6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ZE7</td>
<td>1</td>
<td>-1</td>
</tr>
<tr>
<td>ZE8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ZE9</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Indicates optimized formulation, $X_1$: Concentration of Soya lecithin, $X_2$: Concentration of ethanol; EE: Percent entrapment efficiency; VS: Vesicle size (nm)
Ethosomes were formulated by ethanol injection method [11] by using Mixture A and B. Mixture A consisting of propylene glycol, ethanol and soya lecithin was kept stirring (IKA India Private Limited, India) at 30°C, speed 700 rpm for 30 mins. Mixture B consisting of dissolved ZMT in distilled water was injected slowly into mixture A using syringe at the flow rate of 200µL/min to make up the volume up to 50 ml and stirred for additional 30 mins. Prepared ethosomes were then subjected to thermostatically maintained (4°C) probe sonication (Rivotek Ultrasonic sonicator, Mumbai, India) for 45 minutes for 3 cycles of 15 mins each (15 sec on/off cycle). Formulations were then stored in refrigerator for further characterization.

11.3.2 Evaluation of ethosomes

ZMT loaded ethosomal nano-dispersion was subjected to centrifugation at 50,000 rpm for 1hr at 4°C using micro-ultracentrifuge (Thermo scientific Sorvall MX 150 Micro-Ultracentriguge, India) to estimate drug entrapment. Supernatant liquid was analysed for free ZMT at UV wavelength of 281 nm (Shimadzu 1800, Japan) and calculated by using formula

\[
\text{percent entrapment efficiency} = \frac{\text{amount of drug encapsulated}}{\text{total amount of drug used in formulation}} \times 100
\]

Vesicle size, polydispersibility index (PDI) and zeta potential of ethosomes after appropriate dilutions were analyzed by Malvern Zeta Sizer (Nano ZS, Malvern Instruments Ltd, UK). Ethosomes were initially examined for its shape under optical trianocular microscope coupled with camera (Metzer M, Optical Instruments Pvt. Ltd, India) at magnification of 10x and 40x. Optimized formulation was then finally analysed by transmission electron microscopy (TEM) (Tecnai G2 Spirit Bio Twin; FEI, Czech republic) coupled with camera. Samples of ethosomes formulation (10µl) were retained onto copper grids until drying and then stained using 2% w/v aqueous uranyl acetate and scanned to obtain images.
11.4 Determination of gelation temperature

Poloxamer 407 in the concentration range of 13-20 % w/v was dissolved in water and studied for its gelation temperature by visual inspection method [12]. Beaker containing 10 ml of polaxomer 407 solution in water was kept on digital stirrer where the temperature was increased with an increment of 1°C/min and the temperature at which bead stops moving due to formation of gel was recorded as gelation temperature.

11.5 Formulation of in situ nasal gel of ZMT loaded ethosomes

Optimized concentration of polaxomer 407 was used for formulation of intranasal gels (table 11.2) using carbopol 934 and HPMC K100 as mucoadhesive agent. Different concentration of Carbopol 934 and HPMC K100 was slowly added to ZMT loaded ethosomal dispersion in water followed by addition of Poloxamer 407 solution with continuous stirring. All formulated gels were stored for further evaluation.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (% w/v)</th>
<th>PoloxamerP 407 (% w/v)</th>
<th>Carbopol 934 (% w/v)</th>
<th>HPMC K100</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZS</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>ZPG</td>
<td>2</td>
<td>18</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>ZG2</td>
<td>2</td>
<td>18</td>
<td>0.2</td>
<td>--</td>
</tr>
<tr>
<td>ZG3</td>
<td>2</td>
<td>18</td>
<td>0.3</td>
<td>--</td>
</tr>
<tr>
<td>ZG4</td>
<td>2</td>
<td>18</td>
<td>0.4</td>
<td>--</td>
</tr>
<tr>
<td>ZG5</td>
<td>2</td>
<td>18</td>
<td>--</td>
<td>0.5</td>
</tr>
<tr>
<td>ZG6</td>
<td>2</td>
<td>18</td>
<td>--</td>
<td>1</td>
</tr>
<tr>
<td>ZG7</td>
<td>2</td>
<td>18</td>
<td>--</td>
<td>1.5</td>
</tr>
</tbody>
</table>
11.5.1 Evaluation of in-situ gel formulations

The formulated gels were evaluated for their physicochemical properties viz - pH, clarity and drug content. Prior to use, pH meter 510 (Eutech Instruments Pvt. Ltd., Singapore) was calibrated by standard buffer solutions of pH 4 and pH 7 (Thermo Fisher scientific standard buffers). The clarity was checked against standard white and black background apparatus. The drug content of the gel was determined by spectroscopic method, where appropriate dilutions were made in the linear range and absorbance was measured at 281 nm against blank. The viscosity of the developed formulations was determined by Brookfield viscometer (DV3T Rheometer, USA) at 32 ± 2°C. The force required to detach the formulation from nasal mucosal tissue was recorded as the mucoadhesive potential of gel formulations [13]. Sheep nasal tissue was obtained from slaughter house and intact mucosal membrane was isolated within 1 h after killing the animal. The mucosa was separated from bone cartilage and tissues were cut into small portions. Two tissue portions 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 and dummy granules were then added slowly into the other pan till the tissues get separate. The mucoadhesive strength of gel formulation was determined from minimal weight that detach the mucosal tissues from the surface of each formulation and was calculated by the formula: (M x G / A) x 100, where M= weight required for detachment in grams, G = Acceleration due to gravity (980 cm/s²), A= area of mucosa exposed and expressed as the detachment stress in dyne/cm².

11.5.6 In-vitro drug release study and ex-vivo drug permeation study from ZMT loaded thermoreversible intranasal gel

Drug release and permeation studies from gel was determined by using Franz diffusion six cells system (Thermo Fischer scientific, Haake S5P Newington, USA) using
cellophane dialysis membrane (soaked in receptor medium for 2 hours) for in vitro drug release study [14, 15] (Molecular weight: 12000-14000 KDa).

For ex-vivo permeation studies, intact nasal mucosa membrane was identified and separated from sheep nasal cavity, cleaned and stored in buffer for 2 h prior to experimentation. Membranes were then fixed on Franz diffusion cells having effective permeation area of 2 x 2 cm². ZMT loaded ethosomal gel equivalent to 2.5 mg of ZMT was loaded into donor compartment whereas receptor compartment was filled with 12 ml of phosphate buffer pH 6.4 and maintained at fixed temperature (34 ± 1°C) and standard stirring speed. An aliquots 0.5 ml were withdrawn at predetermined intervals from receptor compartment and replaced with fresh buffer till 24 h. The samples were diluted appropriately and analyzed by UV spectrophotometer at 281 nm. The effective permeability coefficient (cm/s) across sheep nasal mucosa under steady state conditions was calculated according to the equation: \((\frac{dc}{dt})_{ss} \frac{V}{AC_D}\), where \((\frac{dc}{dt})_{ss} (\mu g \text{ mL}^{-1} \text{s}^{-1})\) change of concentration under steady-state; \(A (\text{cm}^2)\) is the permeation area; \(V (\text{mL})\) the volume of the receiver compartment; and \(C_D (\mu g \text{ mL}^{-1})\) is the initial donor concentration.

11.5.7 Histopathological evaluation of mucosal tissue

Treated and non-treated nasal mucosal membranes were stored in 10 ml of buffered formalin to fix the tissues for histopathological studies. Standard haemotoxylin and eosin staining method was used and sections were then examined under light microscope to detect any change in tissue structure during *ex-vivo* drug permeation studies [15].
11.8 Result and Discussion

11.8.1 Preparation of ethosomes

Three level factorial design clearly demonstrated that the independent variables i.e., concentration of soya lecithin (A) and concentration of ethanol (B) demonstrated significant effects on dependent variables i.e., percent entrapment efficiency (EE) and vesicle size (VS) (Table 11.1). The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Binomial equations generated from Design-Expert® Software (Stat-Ease Inc., Minneapolis) are

\[ EE = 61.64 + 13.48A + 4.64B - 2.29AB - 9.48A^2 + 0.038B^2 \]

where positive sign indicates that entrapment efficiency is directly proportional to both the independent variables A and B.

\[ VS = 239.37 + 51.17A - 51B - 4.42AB + 11.94A^2 + 4.44B^2 \]

where positive sign indicates that vesicle size is directly proportional to A and negative sign indicate inversely proportional relation with B.

Results of ANOVA (Table 11.3) for measured response confirm the significance of the model from F value and P value. Surface response curve and contour plots (Figure 11.1) demonstrate the effect of the A and B on EE and VS. The Model F-value of 575.64 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The Model F-value of 10.74 implies the model is significant. There is only a 3.94% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.
Result of the diagnostic reports to express the predicted vs. actual values are shown in Figure 11.2 and 11.3 whereas the residuals verses soyalecithin (%w/v)/ethanol for entrapment efficiency and vesicle size are expressed in the Figure 14.4.

### Table 11.3: Results of analysis of variance for measured response for EE and VS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Degree of freedom</th>
<th>Sum square value</th>
<th>Mean square value</th>
<th>f-Value</th>
<th>p-Value</th>
<th>R² Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>For Y&lt;sub&gt;EE&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>5</td>
<td>1419.67</td>
<td>283.93</td>
<td>575.64</td>
<td>0.0001</td>
<td>0.99</td>
</tr>
<tr>
<td>Residual</td>
<td>3</td>
<td>1.48</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>8</td>
<td>1421.15</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For Y&lt;sub&gt;VS&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>5</td>
<td>31715.54</td>
<td>6343.11</td>
<td>10.74</td>
<td>0.0394</td>
<td>0.95</td>
</tr>
<tr>
<td>Residual</td>
<td>3</td>
<td>1772.60</td>
<td>590.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>8</td>
<td>33488.14</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EE: Percent entrapment efficiency; VS: Vesicle size (nm).
CHAPTER 11

Formulation And Development Of Ethosomal In-Situ Nasal Gel Of Zolmitriptan

Liposome based drug delivery system for brain targeting through intranasal route

Design-Expert® Software
Factor Coding: Actual
Entrapment efficiency (%)
Design Points
68
32.1667
X1 = A: Soyalecithin
X2 = B: Ethanol
3.5 3.7 3.9 4.1 4.3 4.5
35 37 39 41 43 45
Entrapment efficiency (%)
A: Soyalecithin (% (w/v))
B: Ethanol (% (v/v))40
50 60

Design-Expert® Software
Factor Coding: Actual
Vesicle size (nm)
Design Points
346.66
171.667
X1 = A: Soyalecithin
X2 = B: Ethanol
3.5 3.7 3.9 4.1 4.3 4.5
35 37 39 41 43 45
Vesicle size (nm)
A: Soyalecithin (% (w/v))
B: Ethanol (% (v/v))200
250
300
350

I

II
Figure 11.1: Contour plot (I and II) and Response surface plot (III and IV) showing effect of independent variables A and B on percent entrapment efficiency and vesicle size (nm). A = Concentration of soya lecithin and B = Concentration of ethanol.
**Figure 11.2**: Normal plots of residuals and the predicted vs. actual for entrapment efficiency

**Figure 11.3**: Predicted vs. actual and normal plots of residuals for entrapment efficiency
CHAPeR 11

FORMULATION AND DEVELOPMENT OF ETHOSOMAL IN-SITU NASAL GEL OF ZOLMITRIPTAN

Figure 11.4: Diagnostic graphs for residuals verses soyalecithin (% w/v) /ethanol for entrapment efficiency and vesicle size.
The results showed that optimized formulation of ZMT loaded ethosomes demonstrated desirable properties i.e., more than 60 percent entrapment efficiency and vesicle size of less than 200 nm when compared with other formulations. Graphical optimization (Figure 11.5) highlights the working area to get the desired vesicle size and entrapment efficiency. Graph illustrates the optimum concentration of ethanol (39 - 42%) and soyalecithin (3.7 - 4%) to get the optimized results.
11.8.2 Evaluation of ethosomes

Optimized ethosomes (E6) showed 171.67 nm vesicle size, 66.33 % drug entrapment efficiency, 10 mV zeta potential (Figure 11.6), 0.201 polydispersibility index with spherical morphology.
Figure 11.6: Characterization of ZMT loaded ethosomes, A: Vesicle size (nm), B: Percent entrapment efficiency and C: Zeta Potential (mV). All results are expressed in mean ± Std. Dev (n=3).
Results revealed that vesicle size and entrapment efficiency of ethosomes was directly proportional to concentration of ethanol and inversely proportional to the concentration of soya lecithin used in the formulation. Ethanol is said to cause modifications in net surface charge of the system and subsequently causes some degree of stearic stabilization leading to decrease in mean vesicle size [16]. Soya lecithin is used as a coating lipid for formation of ethosomes which supports the statement that increase in vesicle size increases with increase in concentration of soya lecithin [17]. Polypropylene glycol is characterized by a high hydrophobicity and show good solvent capability for ZMT due to its related properties, such as polarity, partition coefficient, and ability to interpenetrate the lipids. It not only acts as humectant but also act as a penetration enhancer for delivery of drug through nasal mucosa [18]. Zeta potential, polydispersibility index of optimized formulation suggest desirable stability of the formulation.

Morphological examination by trianocular microscope reveals that spherical multilamellar vesicles which was further studied by TEM. TEM micrograph confirms drug loaded vesicles with smooth and spherical morphology (Figure 11.7a and 11.7b).

Figure 11.7a: Preliminary microscopic image of ZMT ethosomes at magnification of 10 x and 40x.
Figure 11.7b: Morphological images of ethosomes by TEM with varying sizes at 100, 200, and 500 nm.
Table 11.4: Results of gelation temperature

<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 41</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>29–30</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>26–27</td>
</tr>
</tbody>
</table>

11.9 Determination of gelation temperature

Results of gelation temperature (Table 11.4) indicates that poloxamer 407 showed optimized gelation temperature of 30-34°C (nasal temperature) [19] at the concentration of 18 %w/v. Poloxamer 407 is known to exhibit thermoreversible property due to its negative coefficient of solubility to block copolymer micelles. It is water soluble copolymer of ethylene oxide and propylene oxide and exhibits monomolecular micelles at low concentrations (upto 10%) and multimolecular aggregates at higher concentrations (above a critical gel concentration) leading to formation of gel [20]. The addition of poloxamer 407 might have interfered with soya lecithin molecules causing voids in the region of phospholipid of the membrane bilayer, leading to relaxation of bilayer membrane and thereby improved drug permeation.

11.10 Formulation and evaluation of in situ nasal gel of ethosomal ZMT

Finalized concentration of poloxamer (18 %) was used for formulation of in-situ nasal gel using carbopol 934 (0.2 to 0.5% w/v) and HPMC K100 (0.5 to 1.5% w/v) as mucoadhesive agents. The effect of mucoadhesive agents on gelation temperature of the formulations (Table 11.5) indicates the change in gelation temperature which is directly proportional to the concentration of mucoadhesive agents.
**Table 11.5: Effect of mucoadhesive agents on gelation temperature of the formulations**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Percent w/v of Poloxamer 407</th>
<th>Carboxymethylcellulose w/v</th>
<th>Carbopol 934 (w/v)</th>
<th>HPMC K100 (w/v)</th>
<th>Gelation temperature (°C) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZG1</td>
<td>18</td>
<td>0.1</td>
<td>—</td>
<td>—</td>
<td>34.6 ± 0.46</td>
</tr>
<tr>
<td>ZG2</td>
<td>18</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>33.7 ± 0.35</td>
</tr>
<tr>
<td>ZG3</td>
<td>18</td>
<td>0.3</td>
<td>—</td>
<td>—</td>
<td>31.33 ± 0.29</td>
</tr>
<tr>
<td>ZG4</td>
<td>18</td>
<td>0.4</td>
<td>—</td>
<td>—</td>
<td>28.3 ± 0.52</td>
</tr>
<tr>
<td>ZG5</td>
<td>18</td>
<td>—</td>
<td>0.5</td>
<td>1</td>
<td>31.43 ± 1.00</td>
</tr>
<tr>
<td>ZG6</td>
<td>18</td>
<td>—</td>
<td>1</td>
<td>0.5</td>
<td>30.07 ± 0.38</td>
</tr>
<tr>
<td>ZG7</td>
<td>18</td>
<td>—</td>
<td>1.5</td>
<td>1</td>
<td>27.6 ± 0.36</td>
</tr>
</tbody>
</table>

Effect of mixture of carbopol 934 and HPMC K100 with Poloxamer 407 (18% w/v) on gel strength, viscosity, gelling temperature and mucoadhesive strength (Figure 11.8) were the main determinant for finalizing the optimized concentration of carbopol 934 and HPMC K100. Optimized in situ gel formulations G3 and G6 were considered as optimized due to the desirable gel strength (25-50 seconds), high mucoadhesive strength and desirable gelation temperature (30-31°C). Gel strength above 50 seconds suggests that the formulated gels were very stiff and may cause nasal irritation which may subsequently lead to patient non-compliance to dosage [21]. Gelation temperature above 35°C can cause handling; administration problems leading to loss or drainage of drug from nasal cavity, whereas, gelation temperature less than 25°C could cause manufacturing and storage problems.

Physicochemical evaluation of gel formulation reveals that pH of all the optimized formulations was found close to 6.1 ± 0.2 which is desirable and acceptable for nasal mucosal permeation studies. The drug content of the gel formulations was determined spectrophotometrically at 281 nm and was found to be in the range of 98 to 99 %. Viscosity and mucoadhesive strength of gel formulations was found to be directly proportional to the concentration of Carbopol 934 and HPMC K100 used in formulation.
Carbopol 934 is chemically a polyacrylate polymer having abundant carboxylic groups which tends to form hydrogen bonding with nasal mucus membrane leading to increase in mucoadhesive strength [22]. Hydrophilic nature of HPMC may result in increase in the tortuosity or gel strength relating to formation of uneven and tough mess like gel. HPMC undergoes rapid hydration when exposed to aqueous medium leading to chain relaxation and thereby increase in viscous gelatinous layer [23]. Higher mucoadhesive strength and gelatin like structure might have prolonged drug retention in nasal cavity leading to subsequently increase in permeation across nasal mucosal tissue.

![A. Gel Strength](image-url)
Figure 11.8: Evaluation of formulations for A. Gel strength, B. Viscosity of formulations at sol state (8 ± 2°C) C. Mucoadhesive strength of gel formulations (dynes/cm²). All determinations are expressed in mean ± Std. Dev (n=3).
Table 11.6: Model fitting for optimized thermoreversible gel formulations (G3 and G6).

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Parameters</th>
<th>G3</th>
<th>Parameters</th>
<th>G6</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zero (K₀)</strong></td>
<td>5.273</td>
<td>0.6798</td>
<td>5.037</td>
<td>0.9605</td>
<td></td>
</tr>
<tr>
<td><strong>First (K₁)</strong></td>
<td>0.116</td>
<td>0.9968</td>
<td>0.101</td>
<td>0.9946</td>
<td></td>
</tr>
<tr>
<td><strong>Higuchi (K_H)</strong></td>
<td>20.412</td>
<td>0.9903</td>
<td>19.206</td>
<td>0.9971</td>
<td></td>
</tr>
<tr>
<td><strong>Korsmeyer-peppas (K_KP)</strong></td>
<td>16.677, n=0.582</td>
<td>0.9857</td>
<td>13.345, n=0.648</td>
<td>0.9927</td>
<td></td>
</tr>
<tr>
<td><strong>Hixson-Crowell (K_KC)</strong></td>
<td>0.033</td>
<td>0.9989</td>
<td>0.029</td>
<td>0.9933</td>
<td></td>
</tr>
</tbody>
</table>

11.10.2 *In-vitro* drug release and *Ex-vivo* permeation studies drug release from ZMT loaded thermoreversible intranasal gel

The release data of gel formulation (Table 11.6) 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 R² values. Cumulative drug release of G3 and G6 (Figure 11.9) suggests that there was no significant difference in the drug release from gel formulation consisting of carbopol 934 and HPMC K100 after 24 h. However, drug release after 12 h was more in G3 than G6 suggesting that carbopol gel showed initial burst release which could be due to chemical nature of carbopol 934. Carbopol 934 is not only a mucoadhesive abut also acts as permeation enhancer, whereas HPMC K100 shows sustained release due to its complex mesh like structure [23].
Figure 11.9: *In-vitro* drug release studies of optimized gel formulations through cellophane dialysis membrane. All determinations are expressed in mean ± Std. Dev (n=3)

Permeation study (Figure 11.10) revealed that both in vitro drug release and ex-vivo permeation go hand in hand. Comparison of G3 and G6 with ZS and ZPG suggests that carbopol and HPMC plays a major role in mucoadhesion, permeation and release kinetics. Permeability co-efficient of gel formulations was found to be 5.92 and 5.9 µg/cm² min for G3 and G6 formulations.
Figure 11.10: Ex-vivo permeation studies of optimized gel formulations through sheep nasal mucosa (ZS: zolmitriptan solution, ZPG: zolmitriptan poloxamer 407 gel). All determinations are expressed in mean ± Std. Dev (n=3)
11.10.3 Histopathological evaluation of mucosal tissue

Histopathology studies (Figure 11.11) of treated and untreated sheep nasal mucosa suggest that there was negligible difference in cellular structure of samples. This suggests that formulated gels containing ethosomes, carbopol 934, HPMC K100 and poloxamer 407 are non-toxic in nature and could be used in treatment of migraine.

Figure 11.11: Histopathological evaluation of sections of sheep nasal mucosa. A: mucosal tissue incubated in PBS (untreated), B and C. mucosal tissue incubated in diffusion chamber (treated) of G3 and G6 formulations respectively.
11.11 Conclusion

Three factorial design was effectively used for optimization of ethosomal formulation where significant relation was observed between independent (Concentration of soya lecithin and ethanol) and dependent (percent entrapment efficiency and vesicle size) variables. Optimized ethosomes formulation (E5) showed 171.67 nm vesicle size and 66.33 percent drug entrapment efficiency. These ethosomes were formulated as a thermoreversible gel using thermoreversible polymer (poloxamer 407) and mucoadhesive polymers (carbopol 934 and HPMC K100). Optimized gel formulations G3 (containing carbopol 934) and G6 (containing HPMC K100) showed comparatively same release profile after 24 hours. Overall, ZMT loaded ethosomal intranasal gel could serve as a better alternative to existing dosage forms for effective treatment of recurrent migraine.
11.12 References


