10 FORMULATION AND DEVELOPMENT EVALUATION, OPTIMIZATION AND PERMEATION STUDIES OF ETHOSOMAL GEL OF ELETRIPTAN HYDROBROMIDE

10.1 Background of the designed investigation

Migraine is a disease characterized by severe throbbing pain over one or both halves of the scalp followed by nausea, vomiting, photophobia and/or phonophobia. Presently available treatment of for migraine mainly includes triptans like Sumitriptan, Eletriptan, Zolmitriptan, Naratriptan, etc which acts by blocking serotonin receptors in blood vessels in the brain to decrease recurring pain [1,2].

Eletriptan hydrobromide (EH) which is chemically \(3-\{(R)-1\text{-methyl}-2\text{-pyrrolidinyl}\}\) methyl\(\text{-}5\{2\text{-}(\text{phenylsulfonyl})\text{-ethyl}\}\) indole hydrobromide acts as a potent antimigraine agent via its selective partial agonistic action at the 5-HT1B receptor [3, 4, 5]. The drug has an oral bioavailability of approximately 50 percent with an elimination half-life ranging from about 4 to 7 hours, metabolized mainly by cytochrome P450 (CYP3A4) hepatic enzyme system [6]. During migraine attack absorption of EH may be delayed by almost 50%, which leads to decreased bioavailability. Less bioavailability of EH may also be due to excessive first pass metabolism and poor aqueous solubility leading to increase in dosing frequency to achieve desired therapeutic index. Increased dosing of the available formulation of EH is associated with side effects like nausea, feelings of tingling/numbness, weakness, tiredness, drowsiness, or dizziness may occur by taking the drug orally. It is also reported that EH causes dose dependent increase in serotonin leading to very serious condition called serotonin syndrome/toxicity [7-9].

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 like EH. Moreover, EH owing to its lipophilic nature could easily penetrate through brain tissues [6]. 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. Present investigation was focused to develop novel ethosomal formulation for active targeting of EH through the olfactory pathway to brain tissues. Although very limited
research is done on novel formulations of EH 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 [10]. 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 [11].

Literature reveals that thermoreversible gel preparations of Sumatriptan [12] and Domperidone [13] have been developed and found to have increased permeation rate with prolonged nasal residence time and thereby improved nasal absorption [14]. Hence, intranasal ethosomal drug delivery system having advantages like biodegradability [15], targeting ability [16], biocompatibility [17], improved permeation profile [18] better stability than liposomes [19] and ease of usage could serve as a potential carrier for EH. Herein, we have streamed our work to formulate and characterize ethosomes using advanced analytical techniques like TEM, Malvern zetasizer etc. Thermoreversible gel was further formulated to incorporate EH loaded ethosomes into a finished dosage form. Gel was finally characterized for mucoadhesive strength, viscosity, in-vitro and ex-vivo drug release profile.
10.2 Materials and Methods

10.2.1 Materials

EH and Poloxamer 407 was obtained as a free sample from Zydus Cadila Ltd., India and Shreya life sciences Pvt. Ltd., Aurangabad, India respectively. Other chemicals like Carbopol 934P, cellophane membrane (12,000–14,000 M.W) and Soya lecithin (30%) were purchased from Hi-Media Lab Pvt. Ltd., Mumbai, India. All other reagents used were of analytical grade.

10.3 Methodology

10.3.1 Experimental design for formulation of ethosomes

A $3^2$ factorial design consisting of two independent variables at three levels (high, normal and low) depending on preliminary studies was used for formulation of ethosomes [20]. The independent variables selected for this study were $X_1$: Concentration of Soya lecithin and $X_2$: Concentration of ethanol. The response of dependent variables $Y_{EE}$: Percent entrapment efficiency and $Y_{VS}$: Vesicle size (nm) on independent variables is listed in table 10.1. Statistical model including polynomial terms was used to estimate the response shown by general binomial equation as given below

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

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 10.1: Observed responses from $3^2$ factorial design of EH 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>E1</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>E2</td>
<td>-1</td>
<td>0</td>
</tr>
<tr>
<td>E3</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>E4</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>E5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E6*</td>
<td>0</td>
<td>+1</td>
</tr>
<tr>
<td>E7</td>
<td>+1</td>
<td>-1</td>
</tr>
<tr>
<td>E8</td>
<td>+1</td>
<td>0</td>
</tr>
<tr>
<td>E9</td>
<td>+1</td>
<td>+1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coded values</th>
<th>Actual values</th>
</tr>
</thead>
<tbody>
<tr>
<td>X$_1$ (% w/v)</td>
<td>X$_2$ (% v/v)</td>
</tr>
<tr>
<td>-1</td>
<td>2.5</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>+1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Indicates optimized formulation, $X_1$: Concentration of Soya lecithin, $X_2$: Concentration of ethanol; $Y_{EE}$: Percent entrapment efficiency; $Y_{VS}$: Vesicle size (nm)

10.3.2 Preparation of ethosomes

Ethosomes were formulated using $3^2$ factorial design (table 10.1) by ethanol injection-sonication method [21]. Initially, EH (1% w/v) was dissolved in ethanol and
added to the mixture containing soya lecithin and propylene glycol (13% v/v). The mixture was uniformly stirred using magnetic stirrer (IKA India Private Limited, India) at 30°C, speed 700 rpm for 30 mins. During stirring, double-distilled water was added to the solution in stream line using syringe at the flow rate of 200 µL/min to make up the volume up to 50 ml. Mixture was then subjected to probe sonication (Rivotek Ultrasonic sonicator, Mumbai, India) for total of 45 mins for 3 cycles of 15 mins each (15 sec on/off cycle). Formulation was then stored in refrigerator for further characterization.

10.4 Evaluation of ethosomes

10.4.1 Entrapment efficiency

Drug entrapment efficiency of ethosomes was determined by centrifugation method. EH loaded ethosomal nanodispersion was subjected to centrifugation at 50,000 rpm for 1hr at 4°C using micro-ultracentrifuge (Thermo scientific Sorvall MX 150 Micro-Ultracentrifuge, India). Supernatant liquid was collected and analysed for free EH by UV spectrophotometry (Shimadzu 1800, Japan) at 272 nm. The percent entrapment efficiency was calculated by using given formula,

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

10.4.2 Vesicle size and zeta potential analysis

Ethosomal samples were diluted appropriately with deionized water and analyzed by Malvern Zeta Sizer (Nano ZS, Malvern Instruments Ltd, UK) to determine vesicle size, polydispersibility index (PDI) and zeta potential at room temperature.

10.4.3 Morphological studies

Formation of vesicles was initially confirmed by examining ethosomes under optical trianocular microscope coupled with camera (Metzer M, Optical Instruments Pvt. Ltd, India) at magnification of 10x and 40x. Transmission electron microscopy (TEM) (Tecnai G2 Spirit Bio Twin; FEI, Czech republic) coupled with camera was used to
visualize nanostructure of ethosomes. 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.

10.4.4 Determination of gelation temperature

Gelation temperature was determined by visual inspection method [22] to optimize the concentration of poloxamer 407 (13–20 % w/v) for preparation of thermoreversible gel. Beaker containing 10 ml aqueous solution of polaxomer 407 was kept on digital stirrer with thermostat facility. Beaker was heated at constant heating rate of 1°C/min with stirring and the temperature at which the magnetic bead stopped moving due to gelation was measured as gelation temperature.

10.4.5 Formulation of in situ nasal gel of ethosomal EH

Intranasal gels were prepared by cold technique [23] where EH loaded ethosomes were dispersed in water to get 2 % w/v drug content (table 10.2). To the above mixture, Carbopol 934P was slowly added followed by addition of Poloxamer 407 with continuous stirring. Triethanolamine (1-2 drops) were finally added to form a gel and kept in cool condition (4–8°C) for further characterization.

| Table 10.2: Composition of in-situ gel formulations |
|---------------------------------|------|-----|-----|
| **Formulation code** | **Drug (% w/v)** | **Poloxamer 407 (% w/v)** | **Carbopol 934 (% w/v)** |
| EG1 | 2 | 18 | 0.1 |
| EG2 | 2 | 18 | 0.2 |
| EG3 | 2 | 18 | 0.3 |
| EG4 | 2 | 18 | 0.4 |
| EG5 | 2 | 18 | 0.5 |
10.4.6 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 272 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 [24]. 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. 100 mg of gel was placed in between two mucosal tissues and held in contact for 2 min. dummy granules were then added slowly into the other pan till the tissues get separate. The nasal mucosal tissue was changed for each measurement. The mucoadhesive strength of gel formulation was determined from minimal weight that detach the mucosal tissues from the surface of each formulation and was expressed as the detachment stress in dyne/cm² as mentioned below,

\[ \text{Mucoadhesive strength (dyne/cm²) } = \frac{m \cdot g}{A} \cdot 100 \]

Where, \( m \) = weight required for detachment in grams, \( g \) = Acceleration due to gravity (980 cm/s²), \( A \) = area of mucosa exposed.
10.4.7 In-vitro drug release study from EH loaded thermoreversible intranasal gel

Drug release from gel was determined by using Franz diffusion six cells system (Thermo Fischer scientific, Haake S5P Newington, USA) using cellophane dialysis membrane [25] (Molecular weight: 12000-40000 KDa). Prior to experimentation, pieces of cellophane membrane were soaked in receptor medium for 2 hours. Membranes were then fixed on Franz diffusion cells having effective permeation area of 2 x 2 cm². EH loaded ethosomal gel equivalent to 10 mg of EH was loaded into donor compartment whereas receptor compartment was filled with 12 ml of phosphate buffer pH 6.4. The study was conducted at the temperature of 34 ± 1°C with standard stirring speed. An aliquots 0.5 ml were withdrawn after every hour from receptor compartment and replaced with fresh buffer till 8 hrs. The samples were diluted suitably and analyzed UV spectrophotometer at 272 nm.

10.4.8 Ex-vivo drug release study from EH loaded thermoreversible intranasal gel

Nasal cavity of sheep was obtained from local slaughter house immediately after its sacrifice and stored in saline phosphate buffer pH 6.4. Intact nasal mucosa membrane was identified and separated from nasal cavity, cleaned and stored in buffer. The study was conducted using six cells Franz diffusion system with thermostat facility. Tissue samples were fixed on Franz diffusion cells having effective permeation area of 2 x 2 cm². EH loaded ethosomal gel equivalent to 10 mg of EH was loaded into donor compartment whereas receptor compartment was filled with 12 ml of phosphate buffer pH 6.4. The study was conducted at the temperature of 34 ± 1°C with standard stirring speed. An aliquots 0.5 ml were withdrawn after every hour from receptor compartment and replaced with fresh buffer till 8 hrs. The samples were diluted suitably and analyzed by UV spectrophotometer at 272 nm [26]. The effective permeability coefficient (cm/s) across sheep nasal mucosa under steady state conditions was calculated according to the given equation.
\[ \text{Permeability coefficient} = (dc/dt)_{ss} \times \frac{V}{AC_D} \]

where \((dc/dt)_{ss}\) (\(\mu\)g 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 \(C_D\) (\(\mu\)g mL\(^{-1}\)) is the initial donor concentration.

### 10.4.9 Histopathological evaluation of mucosal tissue

Histopathological evaluation of tissue incubated in PBS (pH 6.4) was compared with post experiment tissue samples to determine the effect of formulation on nasal mucosa membrane. Both tissue samples were stored in buffered formalin to fix the tissue for histopathology studies. Standard haematoxylin 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 [27].
CHAPTER 10 Formulation and Evaluation of Ethosomal In-Situ Nasal Gel Of EH

10.5 Result and Discussion

10.5.1 Experimental design for formulation of ethosomes

In the present investigation, the effect of concentration of soya lecithin ($X_1$) and concentration of ethanol ($X_2$) on percent entrapment efficiency ($Y_{EE}$) and vesicle size ($Y_{VS}$) was studied by using $3^2$ factorial design are shown in table 10.1. Response variables ($Y_{EE}$ and $Y_{VS}$) showed clear dependence on independent variables ($X_1$ and $X_2$). The fitted equation relating the response $Y_{EE}$ and $Y_{VS}$ to the transformed factor are shown in following equations

$$Y_{EE} = 59.49 + 14.57 X_1 + 4.12 X_2 - 2.50 X_1X_2 - 8.43 X_1^2 - 0.78 X_2^2$$

$$Y_{VS} = 262.00 + 56.17 X_1 - 53.33 X_2$$

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Coefficient of regression parameters</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$b_{12}$</th>
<th>$b_{11}$</th>
<th>$b_{22}$</th>
<th>$R^2$ Value</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{EE}$</td>
<td>$b_0$</td>
<td>59.49</td>
<td>14.57</td>
<td>4.12</td>
<td>-2.50</td>
<td>-8.43</td>
<td>-0.78</td>
<td>0.9934</td>
<td>0.0018</td>
</tr>
<tr>
<td>$Y_{VS}$</td>
<td>$b_0$</td>
<td>262.00</td>
<td>56.17</td>
<td>-53.33</td>
<td>-</td>
<td>-</td>
<td>0.9573</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

$Y_{EE}$: Percent entrapment efficiency; $Y_{VS}$: Vesicle size (nm)

The value of correlation coefficient (Table 10.3) indicates good fit. Results of ANOVA on measured responses were recorded in Table 10.4 and surface response curve and contour plots are shown in Figure 10.1. To demonstrate the effect of the $X_1$ and $X_2$, the response surface plots were generated for the dependent variables $Y_{EE}$ and $Y_{VS}$ using Design-Expert® Software (Stat-Ease Inc., Minneapolis). Figure 10.5 shows the working space for optimization to achieve the defined responses.

Result of the diagnostic reports to express the predicted vs. actual values (Figures 10.2 and 10.3) whereas the residuals verses soyalecithin (%w/v)/ethanol(%v/v) for entrapment efficiency and vesicle size are expressed in the Figure 10.4.
Table 10.4: Results of analysis of variance for measured response for $Y_{EE}$ and $Y_{VS}$

<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>
</tr>
</thead>
<tbody>
<tr>
<td>For $Y_{EE}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>5</td>
<td>1543.28</td>
<td>5308.66</td>
<td>90.21</td>
<td>0.0018</td>
</tr>
<tr>
<td>Residual</td>
<td>3</td>
<td>10.26</td>
<td>3.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>8</td>
<td>1553.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For $Y_{VS}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>2</td>
<td>35994.83</td>
<td>17997.42</td>
<td>67.19</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>1607.17</td>
<td>267.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>8</td>
<td>37602.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$Y_{EE}$: Percent entrapment efficiency; $Y_{VS}$: Vesicle size (nm).

In the Model F-value of 90.21 implies the model is significant. There is only a 0.17% 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, A$^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The Model F-value of 36.73 implies the model is significant. There is only a 0.68% 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. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.
The results showed that optimized formulation of EH loaded ethosomes demonstrated desirable properties i.e., more than 63 percent entrapment efficiency and vesicle size of less than 200 nm when compared with other formulations. In equation 5, $b_1$ and $b_2$ bearing positive sign indicates that percent entrapment efficiency is directly proportional to the independent variables ($X_1$ and $X_2$). In equation 6, $b_1$ bearing positive sign indicates that vesicle size is directly proportional to $X_1$, whereas negative sign of $b_2$ suggests that vesicle size is inversely proportional to $X_2$. The results obtained are elaborated in the following figures.

![Graph showing percent entrapment efficiency vs. concentration of soyalecithin and ethanol](image)
CHAPTER 10

Formulation and Evaluation of Ethosomal In-Situ Nasal Gel Of EH Liposome based drug delivery system for brain targeting through intranasal route

---

**B.**

Vesicle size (nm)

A. Concentration of ethanol (% v/v)

B. Concentration of soya lecithin (% w/v)

---

**C.**

Percent entrapment efficiency

Conc. of soya lecithin (% w/v)

Conc. of ethanol (% v/v)
Figure 10.1: Contour plot (A and B) and Response surface plot (C and D) showing effect of $X_1$ and $X_2$ on percent entrapment efficiency and vesicle size (nm). $X_1 = A$: Concentration of soya lecithin and $X_2 = B$: Concentration of ethanol.
Figure 10.2: Predicted vs. actual and normal plots of residuals for entrapment efficiency
Figure 10.3: Predicted vs. actual and normal plots of residuals for entrapment efficiency
Formulation and Evaluation of Ethosomal In-Situ Nasal Gel Of EH

Liposome based drug delivery system for brain targeting through intranasal route

Residuals vs. A:Soyalecithin (% w/v)

Residuals vs. B:Ethanol (% v/v)
Figure 10.4: Diagnostic graphs for residuals verses soyalecithin (% w/v) /ethanol for entrapment efficiency and vesicle size.
Figure 10.5: Overlay plot showing the working space for optimization of ethosomes.

10.6 Characterization of vesicular system

Drug loaded ethosomes were characterized for vesicle size, percent entrapment efficiency, zeta potential, polydispersity index and morphological characteristics.
Figure 10.6: Characterization of eletriptan 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).
Ethosomes vesicle size (Figure 10.2A) suggests that formulation E6 prepared with 40% v/v of ethanol and 3% of soya lecithin showed acceptable vesicle size with 191 ± 6.5 nm. Vesicle size of ethosomes was directly proportional to concentration of ethanol and inversely proportional to the concentration of soya lecithin used in the formulation. Percent drug entrapment efficiency of the EH ethosomal formulation is presented in Figure 10.2B. Optimized ethosomal formulation (E6) showed entrapment efficiency of 63.33 ± 2.31%. Entrapment efficiency was directly proportional to the concentration of ethanol as ethanol help to improve solubility of EH. The zeta potential of optimized formulations was observed in the range of -9 ± 2 to -21 ± 2 (Figure 10.2C) whereas the particle polydispersibility index was observed in the range of 0.28 ± 0.012 to 0.48 ± 0.034. The optimized formulation E6 has the Zeta potential of -20 ± 3 and PDI of 0.281±0.012 suggesting desirable stability of 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 [28]. 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 [29]. Polypropylene glycol is characterized by a high hydrophobicity and show good solvent capability for EH 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 ethosomal delivery through nasal mucosa [30].

Preliminary morphological examination by trianocular microscope coupled with camera suggested that spherical multilamellar vesicles have been formulated which was further studied by TEM. TEM micrographs clearly depict drug loaded vesicles with predominantly spherical morphology and smooth surface (Figure 10.3).
Figure 10.7: A. Preliminary microscopic image of eletriptan ethosomes at magnification of 40x and B. Morphological study of ethosomes by TEM.
10.7 Determination of gelation temperature

Nasal \textit{in-situ} gel will gel are expected to increase the residence time of the dosage form at the local site without drainage or loss of drug content. As the temperature of the nasal cavity is 34°C [31], present investigation was aimed at preparing thermoreversible liquid formulations using poloxamer 407 that may be transformed into gel below 34°C. Lower gelation temperature (27°C) of gels may lead to difficulty in formulation and its intranasal administration. If the gelation temperature is higher than 36°C, formulation will exist in a liquid form in nasal cavity resulting in drainage of dosage form [32]. In the present investigation, gelation temperature of formulation with different concentration of poloxamer were studied and results are represented in table 10.5. It clearly indicates that poloxamer 407 showed optimized gelation temperature of 33-34°C at the concentration of 18 %w/v.

Table 10.5: 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>30–31</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>26–27</td>
</tr>
</tbody>
</table>

Poloxamer 407 is known to exhibit thermoreversible property due to their negative coefficient of solubility to block copolymer micelles. It is water soluble copolymer of ethylene oxide and propylene oxide. Poloxamer 407 exhibits monomolecular micelles at low concentrations (upto 10%) and multimolecular aggregates at higher concentrations result (above a critical gel concentration) leading to formation of gel [33]. 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.

10.8 Formulation and evaluation of in situ nasal gel of ethosomal EH

Optimized concentration of poloxamer 407 (18% w/v) was used for formulation of thermoreversible intranasal gel. Carbopol 934P in varying concentrations (0.1 to 0.5% w/v) was used as a gelling agent as well as mucoadhesive polymer. Effect of mixture of carbopol 934P and Poloxamer 407 (18% w/v) on gelling temperature, viscosity and mucoadhesive strength is as shown in Figure 10.4. It was found that by addition of carbopol 934P in the concentration range from 0.1 to 0.5%, (table 10.2) causes lowering of gelation temperature from 35°C to 25°C. As the physiological temperature of the nasal mucosa is in the range of 30-34°C formulation G1 and G5 were not considered for further evaluation. G1 formulation having higher gelation temperature 35 ± 0.5°C can cause handling, administration problems leading to loss or drainage of drug from nasal cavity, whereas, G5 which is showing lower gelation temperature of 25 ± 0.5°C could cause manufacturing and storage problems.
**CHAPTER 10**  
*Formulation and Evaluation of Ethosomal In-Situ Nasal Gel of EH*

**A** Gelation Temperature

<table>
<thead>
<tr>
<th>Formulations</th>
<th>EG1</th>
<th>EG2</th>
<th>EG3</th>
<th>EG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature [°C]</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

**B** Viscosity

<table>
<thead>
<tr>
<th>Formulations</th>
<th>EG1</th>
<th>EG2</th>
<th>EG3</th>
<th>EG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity (cps)</td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>400</td>
</tr>
</tbody>
</table>

*Liposome based drug delivery system for brain targeting through intranasal route*
10.8.1 Evaluation of in-situ gel formulations

Results of physicochemical evaluation of gel formulation are as shown in Figure 10.4. The pH of all the formulations was found to be 6.1 ± 0.2 which is desirable and acceptable for nasal mucosal permeation studies. The drug content of the gel formulations was determined by UV spectrophotometer after suitable dilutions at 272 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 used in formulation. It could be due to the cross-linked nature of carbopol 934P, chemically a polyacrylate polymer having abundant carboxylic groups which tends to form hydrogen bonding with sugar residues in oligosaccharide chains present in mucus membrane. These chemical interaction leads to an association between polymer and mucus membrane leading to increase in mucoadhesive strength [34]. Higher
mucoadhesive strength could prolong retention and subsequently increase the absorption across nasal mucosal tissue.

10.8.2 In-vitro drug release and Ex-vivo permeation studies drug release from EH loaded thermoreversible intranasal gel

Cumulative drug release from the EH loaded thermoreversible gel formulations were found to be in the range of 80 ± 1.7 to 89.33 ± 3.05 percent after 24 hrs (Figure 10.5), where G4 showed best release profile when compared to other formulations.

![Graph showing drug release over time](image)

Figure 10.9: *In-vitro* drug release studies of gel formulations through cellophane dialysis membrane. All determinations are expressed in mean ± Std. Dev (n=3)
Figure 10.10: Ex-vivo permeation studies of gel formulations through sheep nasal mucosa. All determinations are expressed in mean ± Std. Dev (n=3)

Ex-vivo drug permeation study of EH loaded ethosomal gels (G2, G3 and G4) was carried out on freshly excised sheep nasal tissue using Franz diffusion cell system. The sinus anatomy in sheep is closely comparable to humans [35] suggesting reasonable co-relation between in-vitro and in-vivo studies. Results of ex-vivo permeability studies (Figure 10.6) suggest that the permeation kinetics was best in formulation G4 when compared to other formulation. Permeability co-efficient for gel formulations was calculated and found to be 9.32 ± 0.18, 10.04 ± 0.18 and 10.53 ± 0.35 (10^-5 cm/s) for G2, G3 and G4 formulations respectively. Earlier studies have indicated that the poloxamer 407 slightly decreases the rate of drug release due to enhanced micellar structure and gel network. Drug permeation through nasal mucosa was found to be directly proportional to the concentration of carbopol 934P. This may be due to increase in the ionized carboxyl groups which might have caused conformational changes and swelling in the polymer chain and gel network to become relaxed, resulting in initial delay in drug permeation for 3 hours.
10.8.3 Histopathological evaluation of mucosal tissue

Histopathology of the nasal mucosal tissue (Figure 10.7) clearly indicated integrity of mucosa and absence of any irritation or toxicity. Both gel treated and untreated mucosal membranes showed similar microscopic tissue architecture. Absence of damage to mucus secreting glands, cell necrosis and epithelium confirmed that these ethosomal thermoreversible gels were safe to nasal mucosa and could be administered by intranasal route to treat migraine.

Figure 10.11: Histopathological evaluation of sections of sheep nasal mucosa. A. mucosal tissue incubated in PBS (treated), B. mucosal tissue incubated in diffusion chamber with G4 formulation (untreated).
10.9 Conclusion

In the present investigation, EH loaded ethosomal thermoreversible gel with favorable physico-chemical property for intranasal delivery to brain was developed. Ethosomes were formulated by using $3^2$ factorial design which reveals that concentration of soya lecithin and ethanol are the main determinants responsible for optimization of formulation. Optimized ethosomal formulation E6 having the concentration of soyalecithin (3 %w/v) and ethanol (40 %v/v) showed desirable entrapment efficiency (63.33%) and vesicle size around 191 nm. Thermoreversible gels of EH loaded ethosomes were formulated by using poloxamer 407 (18% w/v) and carbopol 934P (0.4% w/v) as thermoreversible and mucoadhesive polymers. These thermoreversible gels showed suitable gelation temperature, viscosity, mucoadhesive strength and release kinetics. This route provides needle free, non-invasive method of targeting the brain by passing the blood brain barrier and avoiding hepatic first pass metabolism in delivering the drug to the brain as a safe and effective drug delivery system for treatment of migraine.
10.10 References


