FORMULATION AND EVALUATION OF NASAL IN-SITU GEL OF BUPROPION HYDROCHLORIDE

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ABSTRACT
Bupropion Hydrochloride is widely used antidepressant in a world community of which 10% of its population ageing from 20 to 29 suffer from depression. Bupropion hydrochloride is available in oral dosage form. As drug administered through oral route undergoes first pass metabolism, only 5-20% of bupropion hydrochloride is bioavailable. The present study’s objective was to fulfil the need to overcome deficiency of bioavailability and other problems associated with administration with oral route. Accordingly the study commenced with the aim to formulate and evaluate nasal in-situ gel of bupropion hydrochloride, study completed with accessing the safety and stability of formulation. For the said purpose excipients like Poloxamer 407, Carbopol 940, and Methyl paraben were used for their thermosensitive, mocoadhesive and antimicrobial property respectively. The experimental method started with preliminary studies, based on result of same, $3^2$ factorial design was employed. Nine batches were formulated and were subjected for evaluation such as Appearance, pH, Drug Content, Gelation Time, Gelation Temperature, Gel Melting Temperature, Gel Strength, Mucoadhesion, Viscosity, Ex vivo Permeability & Histopathology. On basis of the results obtained F8 formulation was found to be good in terms of gelation temperature, gelation time, drug content, mucoadhesion, gel strength, rheological studies. Ex-vivo studies revealed the sustain effect of gel and finally safety parameter was confirmed by the histopathological studies. The formulation was found to be stable by accessing its stability over 6 months.

KEYWORDS: Bupropion Hydrochloride, in-situ gel, bioavailability, poloxamer 407, carbopol 940. Intranasal delivery.
INTRODUCTION
Depression is a significant contributor to the global burden of disease and affects people in all communities across the world. Today, depression is estimated to affect 350 million people.[1]
Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Moreover, depression often comes with symptoms of anxiety. These problems can become chronic or recurrent and lead to substantial impairments in an individual’s ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide. Almost 1 million lives are lost yearly due to suicide, which translates to 3000 suicide deaths every day. For every person who completes a suicide, 20 or more may attempt to end his or her life (WHO, 2012).[1]

Bupropion hydrochloride is a drug primarily used as an antidepressant. Bupropion selectively inhibits the neuronal reuptake of dopamine, norepinephrine and serotonin. The increase in norepinephrine may attenuate nicotine withdrawal symptoms and the increase in dopamine at neuronal sites may reduce nicotine cravings and the urge to smoke.

Bupropion Hydrochloride is available in oral dosage form, due to its extensive first pass Metabolism. Alternative route of drug administration was critically important, alternative routes such as parenteral could have been used but it has its own disadvantages, hence nasal route was selected. Various dosage form are available for nasal route viz. nasal spray, nasal drop, nasal gel, nasal powder, liposomes, nanoparticles, intranasal microsphere & intranasal micro emulsion. Based on need of safety, improved patient compliance, high residence time, reduction in frequency of drug administration, nasal gel was found to be ideal dosage form, that will overcome problem of low retention of nasal spray & nasal drop, bypass the irritation potential of nasal powder.

Nasal gels are high-viscosity thickened solutions or suspensions. The advantages of a nasal gel includes the reduction of post-nasal drip due to high viscosity, reduction of taste impact due to reduced swallowing, reduction of anterior leakage of the formulation, reduction of irritation by using soothing/emollient excipients and target to mucosa for better absorption. Poloxamer 407 is thermo-sensitive gelling agent, used for the formulation. In association with former gelling agent carbopol 940 is used to impart mucoadhesive property, the gel so formed is thermo-sensitive in nature i.e. at 32°-34° C it get transformed in gel from solution. This is because at low temperature in aqueous solution, a hydration layer surrounds PF-127
molecule. However, when the temperature is increased, the hydrophilic chain of the copolymer becomes desolvated and it results in the breakage of the hydrogen bonding that had been established between the solvent and these chains. This phenomena favours hydrophobic interaction among the polyoxypropylene domain and leads to gel formation.\(^2\)

**Experimental**

**Material**

Bupropion Hydrochloride kindly supplied as gift sample from Glenmark Pharmaceuticals, Mumbai. Carbopol 940 and Methyl Paraben received from Research Lab Fine Chem., Mumbai & Poloxamer 407 was kindly obtained from Glenmark Pharmaceuticals, Nashik.

**Method**

**Preparation of Gel\(^4\)**

The cold method has been chosen for the preparation of gels (Schmolka 1972). First of all the required amount of carbopol 940 was dissolved in distilled water followed by addition of drug and methyl paraben. Then the required amount of poloxamer 407 was slowly added to formulation kept at 50°C.

**Preliminary Batches**

Introductory preliminary batches were prepared by using Bupropion hydrochloride as the active ingredient.

**Table 1: Different composition of trial batches.**

<table>
<thead>
<tr>
<th>Batch no</th>
<th>Poloxamer 407 (%w/v)</th>
<th>Carbopol 940 (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Evaluation of Preliminary Batches**

**Gelation Temperature\(^5\)**

The Tsol–gel of the formulation was determined by test tube inversion method. Bupropion Hydrochloride in situ gel (2 ml) was transferred to a test tube and sealed with paraffin. This test tube was placed in the constant temperature water bath. The temperature of water bath was increased in the increments of 2°C and left to equilibrate at each new temperature.
However, in the region of Tsol–gel temperature was raised slowly in the increments of 0.5°C. The formulation was examined for gelation which was said to have occurred when the meniscus would no longer move upon tilting through 90°. Measurements were done in triplicate.

Experimental Design

Based on the results obtained for introductory preliminary batches experimental runs (Batches) for optimization using $3^2$ factorial design were designed by considering two independent variables.

I) $X_1 = \text{Poloxamer 407 Conc.}(\%\text{w/v})$

II) $X_2 = \text{Carbopol 940 Conc.}(\%\text{w/v})$.

Table 2: Actual and coded values for in situ gel Composition of gels of different batches.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Name</th>
<th>Units</th>
<th>Type</th>
<th>Low actual</th>
<th>High actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>Poloxamer 407</td>
<td>%w/v</td>
<td>Categoric</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>$X_2$</td>
<td>Carbopol 940</td>
<td>%w/v</td>
<td>Categoric</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Table 3: Composition of different batches.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion Hydrochloride</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Poloxamer 407</td>
<td>17%</td>
<td>18%</td>
<td>19%</td>
<td>17%</td>
<td>18%</td>
<td>19%</td>
<td>17%</td>
<td>18%</td>
<td>19%</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.2%</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
<td>q.s to 100ml</td>
</tr>
</tbody>
</table>

Preparation of gel using $3^2$ factorial design

The gel was prepared as per the method described above.

Evaluation of Gels

The prepared gels have been evaluated for

1. **Appearance**

The appearance of the gels was examined for clarity. The clarity of various formulations was evaluated by visual inspection under black and white background.
2. pH\textsuperscript{[3]}

The pH of each formulation was examined by using digital pH meter. The pH meter was first calibrated using buffer solutions of pH 4 and pH 7. Then gels was taken in a beaker and their pH was measured.

3. Drug Content Determination\textsuperscript{[16]}

In this study, each formulation (1 ml) was taken in a 100-ml volumetric flask diluted with D.W. up to the mark. After suitable dilutions the amount of drug was measured in the formulation by using ultraviolet spectroscopy.

4. Gelation Time\textsuperscript{[7]}

The T_{sol-gel} of the formulation was determined by test tube inversion method. Bupropion Hydrochloride in situ gel (2 ml) was transferred to a test tube and sealed with paraffin. This test tube was placed in the constant temperature water bath at 35 ± 1\textdegree C. The sample was examined for gelation. Measurement were done in triplicate.

5. Gelation Temperature and Gel Melting Temperature\textsuperscript{[4]}

Gelation temperature was determined as stated earlier. The obtained temperature is said to be T1. After attaining the temperature T1, further heating of gel causes liquefaction of gel and form viscous liquid and it starts flowing, this temperature is noted as T2 i.e. gel melting temperature.

6. Gel strength\textsuperscript{[5]}

The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature was measured by measuring the force required for the depression of gel at 35\textdegree C temperature. A sample of 50g of nasal gel was put in 100ml graduated cylinder and gelled in thermostatically controlled water bath at 37\textdegree C. A weight of 35g was placed over the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was measured by time in seconds required by the weight to penetrate 5 cm into the nasal gel.

6. Mucoadhesion\textsuperscript{[6]}

The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. The mucoadhesive force expressed as the detachment stress in dynes/cm² was determined from the minimal weight
that detached the mucosal tissue from surface of each formulation. Tied up below the height adjustable pan. While another slide with section of mucosa was fixed in inverted position to the underside of the same pan. Both the slides with mucosal section were fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2 min to ensure intimate contact between gel and both mucosal section. Then weight was kept increasing in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment
Mucoadhesive Strength (dynes/cm²) = mg/A,
Where,
m = weight required for detachment in gram,
g= Acceleration due to gravity (980cm/s²),
A = Area of mucosa exposed. The nasal mucosa was changed for each measurement.

7. Viscosity

The viscosity of prepared gel formulations was measured by using Brook-field DV-II pro plus viscometer (Brookfield Engineering Labs. Inc.,) Viscosity measurements were made at variable temperature and variable shear rate. For temperature dependency study, formulation was subjected to constant shear rate at different temperature from 26 to 40°C. During this testing the temperature was raised gradually by 1°C and viscosity of the sample was measured after attaining the set temperature. Measurements were done in triplicate. Steady shear sweep test was carried out by measuring the viscosity at constant temperature of 25°C and 34°C but varying the rotation speed of spindle from 10 to 100.

8. Ex vivo Diffusion Study

Ex vivo drug diffused study was performed for the optimized formulation, marketed formulation and controlled formulation by using 24ml Franz diffusion cells containing phosphate buffer saline (PBS) pH 6.4. The sheep nasal mucosa was used for the study. 1ml of sample was placed in donor compartment & diffusion study was conducted for 7 hrs. at 34 ± 1°C. 1ml sample was withdrawn at 1/2 hr. for an hour and then every 1 hr. & the same quantity of phosphate buffer saline (PBS) pH 6.4 was added to it.

9. Histopathological Study

Histopathological evaluation of tissue after collection was compared with tissue incubated in the diffusion chamber with gel formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained
with haematoxylin and eosin (HE). Sections were examined under amotic microscope, to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study.

10. Stability Study\textsuperscript{[10]}

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with the time under the influence of a variety of environmental factors. Such as temperature, humidity, light and to establish a re-test period for the drug substance or a shelf life for the drug product & recommended storage condition. Drug and their formulations are exposed to variable storage condition throughout their shelf life, during storage, shipment & handling. In addition to this diversity of conditions with respect to temperature and humidity, in various countries, also propels to investigate the stability of drugs and their formulations under the influence of various storage conditions. Stability assessment started with studies on the substance to determine degradation products & degradation pathway. On the ICH, Harmonized Tripartite Guidelines on stability testing of New Drug substance & product, fundamental recommendations are summarized.

In order to determine stability of gels, the samples were kept in air tight glass vials packed by aluminium foil. Samples were also stored at 5°C ± 3°C for 6 months. These samples were also evaluated for drug content, gelation temperature and physical characteristics.

11. Best Fitting Model

Best fitting model was determined. The first order equation describes the release from the systems where dissolution rate is dependent on the concentration of the dissolving species. Higuchi equation describes the release from system where solid drug is dispersed in insoluble matrix and the rate of drug release is related to the rate of diffusion. The Korsmeyer-Peppas equation is used to analyse the release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena could be involve.

12. Flux\textsuperscript{[9]}

Flux is the amount of permeant crossing a membrane per unit area into the circulating system per unit time. It is given in units of mass/area/ time. It is calculated to determine the saturation of nasal mucosa initially further as and when the consecutive reading are taken.
RESULTS AND DISCUSSION

Evaluation of Preliminary Trial Batches

a) Gelation Temperature

![Gelation Temperature](image)

**Figure 1**: Comparison of gelation temperature of preliminary batches.

Preparation of Gel

Gel was prepared as per method given above depending on the data obtained from preliminary studies.

Evaluation of Gels

The different batches of in situ gel prepared by using $3^2$ factorial design were evaluated for following parameters.

a) Evaluation parameter for different batches

**Table 4**: Evaluation parameters for different batches (F1 - F9).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>pH</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>96.09</td>
<td>97.09</td>
<td>101.2</td>
<td>94.49</td>
<td>99.89</td>
<td>101.6</td>
<td>98.69</td>
<td>99.69</td>
<td>94.89</td>
</tr>
<tr>
<td>Gelation Time (minute)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Gelation Temperature $^0\text{C}$</td>
<td>38.7 ± 0.5</td>
<td>38.3 ± 0.5</td>
<td>37.8 ± 0.5</td>
<td>36.26 ± 0.5</td>
<td>35.7 ± 0.5</td>
<td>34.8 ± 0.5</td>
<td>32.9 ± 0.5</td>
<td>32.1 ± 0.5</td>
<td>31 ± 0.5</td>
</tr>
<tr>
<td>Gel melting temperature $^0\text{C}$</td>
<td>53.2 ± 0.5</td>
<td>54.2 ± 0.5</td>
<td>54.2 ± 0.5</td>
<td>54.1 ± 0.5</td>
<td>54.5 ± 0.5</td>
<td>54.3 ± 0.5</td>
<td>54.5 ± 0.5</td>
<td>54.2 ± 0.5</td>
<td>54.3 ± 0.5</td>
</tr>
<tr>
<td>Gel Strength (sec)</td>
<td>20</td>
<td>25</td>
<td>29</td>
<td>21</td>
<td>27</td>
<td>32</td>
<td>22</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Mucoadhesion Dynes/cm²</td>
<td>980</td>
<td>2082</td>
<td>3062</td>
<td>1102</td>
<td>2205</td>
<td>3185</td>
<td>1225</td>
<td>2327</td>
<td>3430</td>
</tr>
<tr>
<td>n=3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. pH
pH of all formulation was found to be in desired pH limit of nasal cavity.

2. Percent Drug Content
The above data shows that all the formulations are having the percent drug content in the range of 94-102% which is satisfactory.

3. Gelation Temperature
The above data shows wide range of gelation temperature for different formulations. It indicated that as the concentration of polymer increases the gelation temperature of the formulation decreases. Formulations F6 and formulation F8 are having the gelation temperature in the desired range of 32-34 °C.

4. Mucoadhesion Strength
Use of polymers with strong bioadhesive capacities can significantly limit the total clearance of the formulation from the nasal cavity. An optimal system for nasal drug delivery would therefore be fluid enough for easy administration yet would not undergo rapid initial clearance, and would have sufficient interaction with the mucosal surface to continue to limit clearance for extended time periods. Residence time of any formulation in nasal cavity depends on the mucoadhesive strength of polymers. The results obtained clearly indicate, as the concentration of carbopol 940 increases, the mucoadhesive strength of the gel increases. So, it can retain on the nasal surface for longer period of time. Formulation F6, F8 and F9 have sufficient mucoadhesive strength.

5. Gel Strength
The gel strength is an indication for the viscosity of the nasal gel at physiological temperature. The data obtained indicate, as the polymer concentration increases the gel strength of the formulation also increases. The formulation F9 has the highest gel strength. Rheological analysis (F1 - F9).
From the above graph, it is clear that as the temperature increases, the viscosity of the formulation increases. At the gelation temperature there is a sudden increase in the viscosity indicating the conversion of sol to gel. For formulation F8 and F6 it is found between 32-34°C.

Figure 2: Comparison of viscosity change with temperature for different formulations.

Figure 3: Comparison of viscosity change with change in Shear Rate (At 25°C) for different formulations.
All the formulations showed pseudo plastic rheological flow after studying at various temperatures, as evidenced by shear thinning and an increase in the shear stress with increased angular velocity. It was found that the rheological parameter was directly dependent on the polymer concentration of the formulations. At 25°C all the formulations were having low viscosity and at 34°C formulations F6, F8 and F9 have shown high viscosity. This indicates the conversion of these formulations from sol to gel. It was also observed that the viscosity of all the formulations were decreasing with increase in shear rate.

6. Ex vivo Diffusion Study

Table 5: Comparison of ex vivo permeability of different formulations.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>39.64</td>
<td>30.11</td>
<td>31.30</td>
<td>27.14</td>
<td>29.52</td>
<td>23.57</td>
<td>21.19</td>
<td>16.42</td>
<td>17.02</td>
</tr>
<tr>
<td>1</td>
<td>57.09</td>
<td>51.61</td>
<td>49.47</td>
<td>51.02</td>
<td>47.33</td>
<td>44.35</td>
<td>42.69</td>
<td>42.92</td>
<td>43.04</td>
</tr>
<tr>
<td>2</td>
<td>80.42</td>
<td>74.35</td>
<td>70.30</td>
<td>66.61</td>
<td>62.21</td>
<td>52.33</td>
<td>65.78</td>
<td>57.69</td>
<td>58.28</td>
</tr>
<tr>
<td>3</td>
<td>93.04</td>
<td>87.21</td>
<td>88.28</td>
<td>76.14</td>
<td>74.83</td>
<td>74.11</td>
<td>79.83</td>
<td>71.02</td>
<td>71.73</td>
</tr>
<tr>
<td>4</td>
<td>101.26</td>
<td>101.5</td>
<td>99.47</td>
<td>88.88</td>
<td>86.97</td>
<td>83.35</td>
<td>90.78</td>
<td>80.42</td>
<td>81.14</td>
</tr>
<tr>
<td>5</td>
<td>101.26</td>
<td>101.5</td>
<td>99.47</td>
<td>88.88</td>
<td>86.97</td>
<td>83.35</td>
<td>90.78</td>
<td>80.42</td>
<td>81.14</td>
</tr>
<tr>
<td>6</td>
<td>101.26</td>
<td>101.5</td>
<td>99.47</td>
<td>88.88</td>
<td>86.97</td>
<td>83.35</td>
<td>90.78</td>
<td>80.42</td>
<td>81.14</td>
</tr>
<tr>
<td>7</td>
<td>101.26</td>
<td>101.5</td>
<td>99.47</td>
<td>88.88</td>
<td>86.97</td>
<td>83.35</td>
<td>90.78</td>
<td>80.42</td>
<td>81.14</td>
</tr>
</tbody>
</table>

The results obtained with in vitro permeability study clearly indicate the permeation of drug through nasal mucosa. It was observed that the formation of gel retards the extent of

Figure 4: Comparison of viscosity change with change in Shear Rate (At 34°C) for different formulations.
permeability. As the concentration of polymer in the formulation increases, the rate of permeation decreases. It was observed that the formulation F8 and F9 have shown maximum (about 100%) permeability in 7 hours as compared to the remaining formulations. This shows the possibility of extended release formulation of Bupropion Hydrochloride in sol-gel form. This delay in release was observed may be due to the more amount of gelling agent.

7. Best fitting model for in vitro drug diffusion study
The first order equation describes the release from the systems where dissolution rate is dependent on the concentration of the dissolving species. Higuchi equation describes the release from system where solid drug is dispersed in insoluble matrix and the rate of drug release is related to the rate of diffusion. The Korsmeyer-Peppas equation is used to analyse the release of pharmaceutical polymeric dosage forms when the release mechanism is not well known or when more than one type of release phenomena could be involved.

8. Flux
From the flux study it is concluded that initially when the mucous is not saturated high flux is observed as and on the further readings are noted due to saturation of the

9. Histopathological Study

Fig 5, 6: Control: Nothing abnormal architecture is normal. H&E 20X. Treated- normal lining epithelium no detected, Necrosis or infiltration of inflammatory Cells seen. H&E 20X.
The safety of intranasal formulations is of crucial importance for medications given by nasal route. The tissue damage by Bupropion Hydrochloride in situ gel was evaluated by paraffin section stained with haematoxylin and eosin. Histopathology was performed to observe the integrity of mucosa and irritation or toxicity caused to nasal mucosa by formulation, if any. As seen in figure no histological sections of treated and untreated nasal mucosa were found similar in tissue architecture after 7 h exposure. In normal nasal mucosa (untreated) (negative control), the structure of the mucosa was well preserved (Figure A). The surface pseudo epithelium displayed normal characteristics. After treatment with the Bupropion hydrochloride in situ gel, no marked alteration was observed as compared to negative control from the histological structure (Figure B). There was also no any evidence of haemorrhage, necrosis and ulceration in Bupropion hydrochloride treated and untreated nasal mucosa.

10. Optimization

Analysis of Data Using Design
ANOVA for Response Surface linear Model

Table 6: Model terms and p-value.

<table>
<thead>
<tr>
<th>Source</th>
<th>p-value Gelation temp</th>
<th>p-value Mucoadhesion</th>
<th>p-value 90% drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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<td>A-PoloxamerConc</td>
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<td>&lt;0.0001</td>
<td>0.1135</td>
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<tr>
<td>B-CarbopolConc</td>
<td>&lt; 0.0001</td>
<td>0.0004</td>
<td>0.0036</td>
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</table>

Fig 7, 8: Control: Nothing Abnormal Treated- Intact lining epithelium. H&E Detected architecture is normal. H&E 40X.
p-Values of less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. (in case of 90% drug release only B is significant)

1) Effect on Gelation Temperature

The graph reveals the contribution of poloxamer & carbopol to gelation temperature. As the signs of poloxamer and carbopol both are negative it is concluded that both have polymer inverse relation with the gelation temperature.

The independent and response variables were related using polynomial equation with statistical analysis through Design-Expert® software. The values of the coefficients X1, X2 are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response. The larger coefficient means the independent variable has more potent influence on the response.
2) **Effect on Mucoadhesion**

![3D Response of poloxamer conc and carbopol conc on mucoadhesion.](image)

The graph reveals the contribution of poloxamer & carbopol to Mucoadhesion, the equations states that Poloxamer significantly contributes to Mucoadhesion. Carbopol also contributes but to lesser extent. As the signs of poloxamer and carbopol both are positive it is concluded that both have polymer direct relation with the Mucoadhesion.

3) **Effect on Drug Release**

![3D Response of poloxamer conc and carbopol conc on T90%](image)

3D Response of poloxamer conc and carbopol conc on T90%.
Final Equation in Terms of Actual Factors

Time for 90% drug release = 4.00000 + 0.33333*poloxamer + 0.83333*carbopol

The graph reveals the contribution of poloxamer & carbopol to drug release, the equations states that carbopol significantly contributes to extended release of drug, poloxamer also contributes but to lesser extent. As the signs of poloxamer and carbopol both are positive it is concluded that both have polymer direct relation with the drug release.

11. Stability Study

The gel was evaluated for gelation temperature. Drug content and physical characteristic after 6 months and the gel was found to be stable.

Summary

The aim of study was to achieve brain targeted drug delivery of Bupropion Hydrochloride for the patients suffering from depression.

Initially preformulation was carried out to evaluate the purity of drug and purity was confirmed which eventually revealed the suitability of ingredient to be used in formulation. For the same purpose analysis like FTIR, melting point, etc. were carried out Preliminary batches was formulated to find the suitable concentration of polymer for desired gelation temperature.

Initially the preliminary batches were formulated by using concentration 16, 17, 18, 18, 19 of poloxamer and 0.1, 0.2, 0.1, 0.2, 0.3 of carbopol this preliminary batch were subjected to gelation temperature measurement.

Based on the results obtained from preliminary batches $3^2$ factorial design was applied for the preparation of 9 batches of concentrations ranging from 17 to 19 and 0.1 to 0.3 of poloxamer and carbopol respectively. This 9 batches were then subjected to evaluation parameter such as appearance, pH, gelation time, gelation temperature, gel melting temperature, drug content uniformity and texture analysis like mucoadhesion, and gel strength.

The appearance was clear enough, pH was found to be desired limit, the gelation temperature varied with change in concentration of poloxamer and carbopol but F8’s gelation temperature was found to be satisfactory. Gelation time was found to be almost uniform for all the formulations. Further the gel melting temperature was also was found to be uniform.
The drug content varied from 94% to 102% which was satisfactory.

Texture analysis was carried out further which comprised of parameters like mucoadhesions and gel strength, the study of former parameter revealed that the poloxamer as well as carbopol was responsible for mucoadhesion except F1, F4 & F7 other all showed good mucoadhesive strength, the later parameter’s evaluation showed the same results.

As the formulation is temperature dependent two types of rheological studies were carried out, rheological analysis at different temperature and rheological analysis at different shear rate, the former studies showed that as the temperature increased simultaneously the viscosity also increased, but in case of changing shear rate analysis it showed pseudo plastic characteristic i.e. as the shear rate increased the viscosity decreased.

The formulation were also evaluated for drug release characteristics by performing ex-vivo diffusion studies which suggests that increase in carbopol concentration sustains the release of the formulation, the time for release increases in ascending order from F1 to F9. The data of ex vivo study was then subjected to flux calculation, from the study it was concluded that initially there is high level of flux and the further it decreases this is because primarily nasal mucosa has no saturation and after a time period it gets saturated.

Optimization was done using design expert, it was done on the basis of results obtained for gelation temperature, time required for 90% drug release, mucoadhesion and the optimized batch (F8) was used for the further evaluations. The optimized batch was evaluated for ex vivo diffusion study, histopathological evaluation, compatibility study.

Histopathological studies was carried out on F8 formulation, the studies didn’t disclosed any damage to nasal mucosa, from this it was concluded that formulation is safe. Stability studies were also performed 2 parameter were evaluated for this studies viz. gelation temperature, drug content, for all parameter were found to be satisfactory.

Hence from the overhead studies we may conclude the safety, acceptability, reliability of the formulation based on its specific gelation temperature, good mucoadhesion, decent ex vivo diffusion, safe histopathological results, considering all this parameter F8 formulation was found to be optimum and up to the objective. Therefore the formulation of nasal in-situ gel of Bupropion Hydrochloride was up to the objective.
REFERENCES
10. ICH harmonised tripartite guideline Stability testing of new drug substances and products Q1A (R2), 1-24.


