Research Paper

Formulation and In vitro Evaluation of Mucoadhesive Buccal Patches of Ondansetron Hydrochloride

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ABSTRACT: The goal of the present investigation was to design and evaluate mucoadhesive buccal patches of Ondansetron Hydrochloride (OND) which is used for nausea and vomiting associated with cancer chemotherapy and radiotherapy. Permeation of OND was calculated ex vivo using porcine buccal membrane. Buccal films were developed by solvent-casting technique using Hydroxy Propyl Methyl Cellulose (HPMC E15) as mucoadhesive polymer. The patches were evaluated for weight variation, thickness variation, surface pH, moisture absorption, in vitro residence time, mechanical properties, in vitro release, ex vivo permeation studies and drug content uniformity. The formulation F3 was found to give the better results and obeys first order kinetics.

KEYWORDS: Mucoadhesive; Buccal patch; ondansetron hydrochloride; mechanical properties; in vitro studies; ex vivo studies

Introduction
Recent years have seen an increasing interest in the development of novel mucoadhesive buccal dosage forms. These are useful for the systemic delivery of drug as well as for local targeting of drug to a particular region of the body (T. Nagai et al., 1993, Khar et al., 2002). Buccal delivery for the transmucosal absorption of the drug into the systemic circulation offers number of advantages for those drugs that suffer from first pass metabolism in the liver, poor oral bioavailability (Rathbone et al., 1994). Conceivably buccal delivery systems provide easy administration, thereby increasing patient compliance (Hoogstraate et al., 1998, Chowdary et al., 2002).

Ondansetron is a serotonin 5-HT3 receptor antagonist used mainly as an antiemetic to treat nausea and vomiting following chemotherapy (Steven et al., 2002, Blake et al., 1993). Ondansetron hydrochloride was selected as the model drug for the investigation because it has got certain characteristics that a drug should possess to get absorbed through buccal route viz., biphasic solubility and low molecular weight (365.9 g/mol) (Harris et al., 1992, Hans et al., 1999). Moreover it undergoes first-pass metabolism, so its bioavailability may be improved when delivered through buccal route. Patients may have frequent vomiting following chemotherapy and in such cases they may not swallow a tablet to prevent vomiting (Junginger et al., 1999). So there is a need to develop a buccal patch of Ondansetron Hydrochloride, which increases patient compliance. Its bioavailability when administered by oral route is only 50% to 60% and its dose is low i.e., 4-8mg, hence it can be conveniently loaded onto a patch.

The polymer selected for the formulation is hydroxyl propyl methylcellulose (HPMC E 15). The polymer is water soluble and soluble in organic solvents like mixture of alcohol and dichloromethane or methanol and dichloromethane (Vamshi et al., 2007).

Materials and Methods

Materials
Ondansetron Hydrochloride was obtained as a gift sample from Natco Pharma, Hyd, A.P, India. Hydroxy Propyl Methylcellulose (HPMC E 15) was procured from Loba Chemicals Pvt Ltd., India. All other reagents used were of analytical grade. The films were prepared by solvent casting method.

Tissue Isolation
Buccal tissue was taken from pigs at a slaughter-house. It was collected within 10 minutes after slaughter of the pig and tissue was kept in Krebs buffer solution. It was transported immediately to the laboratory and was mounted within 2 hours of isolation of buccal tissue. The
tissue was rinsed thoroughly using phosphate buffer saline to remove any adherent material. The buccal membrane from the tissue was isolated using surgical procedure. Buccal membrane was isolated and buccal epithelium was carefully separated from the underlying connective tissue. Sufficient care was taken to prevent any damage to the buccal epithelium.

**Ex vivo permeation studies through porcine buccal mucosa**

The buccal epithelium was carefully mounted in between the two compartments of a Franz diffusion cell with an internal diameter (ID) of 2.4 cm (4.52 cm² area) and with a receptor compartment volume of 24 ml. 24 ml of mixture of phosphate buffer solution (PBS) pH (7.4) and ethanol (60:40) was placed in the receptor compartment. The donor compartment contained a mixture of 5 ml of PBS pH (6.6) and ethanol (60:40) in which 5 mg of Ondansetron Hydrochloride was dissolved. The donor compartment also contained phenol red at a concentration of 20 µg/ml. This is because phenol red acts as a marker compound and is not expected to permeate through the porcine buccal membrane. Absence of phenol red in the receiver compartment indicates the intactness of the buccal membrane. The entire setup was placed over magnetic stirrer and temperature was maintained at about 37°C. The samples were collected at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 hr and stored under refrigerated conditions till the analysis was carried out by using UV-Visible spectrophotometer (Elico, India) at 310 nm. All the experiments were performed in triplicates (Vamshi et al., 2007, Luana et al., 2004).

**Assay of phenol red**

To 250 µl of sample solution, 250 µl of acetonitrile was added and vortexed to precipitate the proteins. To this 1 ml of 0.2 M NaOH was added, vortexed and to this 3.5 ml of distilled water was added to make the volume to 5 ml, vortexed, centrifuged and absorbance of supernatant was measured at 563 nm using UV-Vis Spectrophotometer.

**Method (solvent casting method)**

Weighed quantity of HPMC E15 was taken in a boiling tube. To this, 20 ml of solvent mixture of dichloromethane: methanol (1:1) was added and vortexed. Sufficient care was taken to prevent the formation of lumps. The boiling tube was set-aside for 6 hours to allow the polymer to swell. After swelling, measured quantity of propylene glycol was added to this mixture and vortexed. Finally weighed quantity of Ondansetron Hydrochloride was dissolved in 5 ml of solvent mixture, added to the polymer solution and mixed well. It was set-aside for some time to exclude any entrapped air and was then transferred into a previously cleaned anumbra petriplate. Drying of these patches for 8 hrs was carried out in oven placed over a flat surface.

The patches formed were removed carefully, placed in a vacuum oven and vacuum was applied to remove traces of solvent if any. They were stored in a desiccator till the evaluation tests were performed. The composition of the patches is given below. Formulated patches were then subjected to the weight variation test, thickness variation test and content uniformity test.

**Characterisation of Buccal Patches**

**Weight variation test**

Each formulation was prepared in triplicate and ten patches each equivalent to 15.0 mm were cut from each plate. Their weight was measured using Shimadzu digital balance. The mean ± SD values. (Table 2) were calculated for all the formulations.

**Thickness variation test**

The thickness of the patches was measured by digital srew guage (Digimatic outside micrometer, Mitutoyo, Japan). The mean ± SD values. (Table 2) were calculated for all the formulations.

**Table 1 Formulation Ingredients of Ondansetron Hydrochloride Buccal Patches.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Ondansetron Hydrochloride (mg)</th>
<th>HPMC E15 (mg)</th>
<th>Propylene glycol (µl)</th>
<th>DCM&amp; Methanol (1:1) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>132</td>
<td>2000</td>
<td>300</td>
<td>25</td>
</tr>
<tr>
<td>F2</td>
<td>132</td>
<td>2250</td>
<td>337</td>
<td>25</td>
</tr>
<tr>
<td>F3</td>
<td>132</td>
<td>2500</td>
<td>375</td>
<td>25</td>
</tr>
<tr>
<td>F4</td>
<td>132</td>
<td>2750</td>
<td>412</td>
<td>25</td>
</tr>
<tr>
<td>F5</td>
<td>132</td>
<td>3000</td>
<td>450</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 2 Evaluation of the patches.

<table>
<thead>
<tr>
<th>S.No</th>
<th>F.Code</th>
<th>Weight (mg)</th>
<th>Thickness (µm)</th>
<th>Tensile Strength (kg/mm²)</th>
<th>Elongation at break (%mm-2)</th>
<th>%Moisture Absorbed</th>
<th>Drug content (mg)</th>
<th>In-vitro residence time</th>
<th>Surface pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>F1</td>
<td>107±3.69</td>
<td>396±2.49</td>
<td>6.23±0.92</td>
<td>152±14.11</td>
<td>Eroded</td>
<td>4.87±0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>02</td>
<td>F2</td>
<td>119±3.98</td>
<td>449±7.67</td>
<td>9.79±2.17</td>
<td>110±12.39</td>
<td>Eroded</td>
<td>5±0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>03</td>
<td>F3</td>
<td>126±4.96</td>
<td>490±5.12</td>
<td>12.41±4.36</td>
<td>87±9.56</td>
<td>109.17±2.31</td>
<td>4.91±0.39</td>
<td>4.10±1.56</td>
<td>6.5±0.027</td>
</tr>
<tr>
<td>04</td>
<td>F4</td>
<td>144±5.64</td>
<td>523±2.36</td>
<td>14.62±1.75</td>
<td>76±8.12</td>
<td>115.61±7.29</td>
<td>5±0.2</td>
<td>4.05±2.24</td>
<td>6.6±0.017</td>
</tr>
<tr>
<td>05</td>
<td>F5</td>
<td>163±6.29</td>
<td>574±13.17</td>
<td>17.11±2.89</td>
<td>65±5.13</td>
<td>167.02±13.61</td>
<td>4.95±0.1</td>
<td>4.15±0.876</td>
<td>6.7±0.081</td>
</tr>
</tbody>
</table>

F.Code: Formulation Code; All values indicate mean±Standard Deviation

Surface pH of Films

For determination of surface pH, three films of each formulation were allowed to swell for 2 hr on the surface of an agar plate. The surface pH was measured by using pH meter. Electrode was placed on the surface of the swollen patch allowing it to equilibrate for 1 min. A mean of three readings was recorded. (Table 2)

Assay of the patches

The formulated patches were assayed for drug content in each case. Three patches from each formulation were assayed for content of drug. Each formulation was casted in triplicate and one patch from each was taken and assayed for content of drug.

Procedure

Patches from each formulation were taken and each patch was cut into small pieces. They were then allowed to dissolve in water. Water was taken in conical flasks and placed on a rotary shaker overnight to aid dissolution. An aliquot of the solution was taken and centrifuged. Absorbance of the resulting supernatant solution was measured using UV-Vis spectrophotometer at a wavelength of 310 nm against water as blank. Results are presented in Table 2

In vitro Release Studies

Drug release from the bioadhesive buccal patch was studied by using dissolution apparatus (Elico). Patches of desired size were cut and since the patches were meant to release the drug from only one side, an impermeable backing membrane was placed on one side of the patch. The dissolution assembly was prepared by adhering the patch onto a glass slide using a solution of cyanoacrylate adhesive. It was then placed in dissolution apparatus. The dissolution test was performed using 500 ml PBS pH (6.6) and ethanol (60:40), at 37±0.5°C and 25 rpm. Samples were collected at different time intervals and analyzed using UV-Vis spectrophotometer. The release studies were performed in six replicates and mean values were taken (Vamshi et al., 2007, Mashru et al., 2005).

Moisture Absorption Studies

The polymer used for the formulation of mucoadhesive patches is hydrophilic polymer. The moisture absorption studies give an indication about the relative moisture absorption capacities of polymers and an idea whether the formulation maintains its integrity after absorption of moisture. 5% w/v agar in distilled water, in hot condition, was transferred into Petri plates and it was allowed to solidify. Six drug free patches of each formulation were selected and weighed.

Moisture Absorption Studies

They were placed in desiccator overnight prior to the study to remove moisture if any and laminated on one side with water impermeable backing membrane. They were placed on the surface of the agar and incubated at 37°C for one hour in incubator. The patches were removed and weighed again. The percentage of moisture absorbed can be calculated using the formula: % Moisture absorbed = Final weight – Initial weight/ Initial weight ×100. Results are presented in Table 2

Measurement of Mechanical Properties

Mechanical properties of the films (patches) were evaluated using a microprocessor based advanced force gauze equipped with a motorized test stand (Ultra Test, Mecmesin, West Sussex, UK), equipped with a 25 kg load cell. Film strip with the dimensions 60 x 10 mm and free from air bubbles or physical imperfections, were held between two clamps positioned at a distance of 3 cm. A cardboard was attached on the surface of the clamp to prevent film from being cut by the grooves of the clamp. During measurement, the strips were pulled by the top clamp at a rate of 2.0 mm/s to a distance till the film broke.
The force and elongation were measured when the films were broken. Results from film samples, which were broken at end and not between the clamps were not included in observations. Measurements were run in six replicates for each formulation. The following equations were used to calculate the mechanical properties of the films. Tensile strength (kg.mm⁻²) = Force at break (kg)/Initial cross sectional area of the sample(mm²) and Elongation at break(%)mm⁻² = [Increase in length (mm)]100/[Original length] [Cross sectional area (mm²)].

The results of the experiment are presented in Table 2

**Measurement of in vitro Residence Time**

The in vitro residence time was determined using USP disintegration apparatus. The disintegration medium was 800 ml of PBS (pH 6.6) maintained at 37±2°C. The segments of porcine buccal mucosa, each of 3 cm length, were glued to the surface of a glass slab, which was then vertically attached to the apparatus. Three mucoadhesive films of each formulation were hydrated on one surface using PBS (pH 6.6) and the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down. The film was completely immersed in the buffer solution at the lowest point and was out at the highest point (Monasemalty et al., 2008, Buket et al., 1996). The time required for complete erosion or detachment of the film from the mucosal surface was recorded as given in Table 2

**Ex vivo Permeation of Ondansetron Hydrochloride Patches through Porcine Buccal Membrane.**

Ex vivo permeation of OND from buccal patches through porcine buccal membrane was studied. Porcine buccal mucosa was obtained and buccal membrane was isolated. The membrane was mounted over a Franz diffusion cell and a buccal patch was placed over the membrane. A dialysis membrane was placed over the membrane so as to secure the patch tightly from getting dislodged from the membrane (the buccal patch was sandwiched between the buccal mucosa and the dialysis membrane). The two compartments of diffusion cell were filled with PBS & ethanol. The setup was placed over a magnetic stirrer with temperature maintained at 37°C. Samples were withdrawn and replenished immediately from the receiver compartment at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 hr. They were stored under refrigerated conditions till the analysis was carried out. The content of OND in the samples was analyzed by UV-Vis Spectrophotometer at the wavelength of 310 nm. All the experiments were performed in triplicates. (Figure 3)

**Results and Discussions**

**Drug Penetration Studies through the Porcine Buccal Membrane**

The cumulative amount of Ondansetron Hydrochloride (OND) that had penetrated through the buccal epithelium. This model, which was aimed at simulation in vitro drug penetration, was found to be useful. The tissue could be isolated successfully because no detectable levels of Phenol red, which was used as marker compound, was found in the receiver compartment. Hence it did not show any penetration whereas Ondansetron Hydrochloride could penetrate freely. This indicated that the membrane was intact. The results is shown in (Figure 1). The flux was calculated to be 23.601 µg/hr.cm².
Formulation of Buccal Patches of Ondansetron Hydrochloride

**Optimizing the polymer content**

Patches were formulated with HPMC E 15. Polymer content of less than 2.00g was insufficient to retain the drug and it was precipitated out as particles on the patch. The experiment was initiated by taking 1.25g of polymer and as the polymer concentration increased the patch could accommodate more amount of Ondansetron Hydrochloride. Precipitation of the drug was predominant with 1.25g of polymer and as the polymer concentration was increased the precipitation decreased. No precipitation was observed with 2.00g and above of the polymer.

**Optimizing the plasticizer content**

Plasticizer concentration of 5% v/w of film former was insufficient to form films. Plasticizer concentration at 5-10% v/w yielded more flexible films. Further increasing the concentration of plasticizer above 25% v/w resulted in enormous increase in the drying time. Higher plasticizer concentrations resulted in poor bioadhesion of films.

**Optimizing the solvent volume**

Solvent volume of 8-14 ml resulted in viscous solution; hence complete transfer of the solution could not be ensured. Solvent volume of 16-25 ml was sufficient to solubilize the drug and cast the films. Increasing the solvent volume above 25 ml resulted in the formation of patches with entrapped air bubbles. Further increasing the solvent volume resulted in the formation of bilayered patches, indicating that drying of the patches might have taken place at two stages. The films used in the study were prepared by taking 25 ml of the solvent volume. The results of weight variation test for various buccal patches (formulations) were shown in (Table 2). Results of weight variation test indicated uniformity in weight of the patches, as evidenced by SD values and the weight of patches increased from F1 to F5.

In thickness variation test (Table 2), the thickness was found to be uniform. The thickness increased with increase in polymer concentration and a direct relation existed between the thickness and weight of the patches. Results of thickness variation test indicated uniformity in thickness of the patches, as evidenced by SD values.

Considering the fact that acidic or alkaline pH may cause irritation to the buccal mucosa and influence the degree of hydration of polymers. The surface pH of the buccal films was determined to optimize both drug permeation and mucoadhesion. The surface pH of all the films was within the range of salivary pH. (Table 2). No difference was found in surface pH of different films.

**In vitro Drug Release Studies**

Phosphate buffer pH 6.6 and ethanol (60:40) was used as medium for the release studies to show the drug release profile of Ondansetron Hydrochloride patches containing different ratios of polymer to drug. It is apparent from the plots that the drug release was governed by polymer content. An increase in the polymer content was associated with decrease in drug release rates. There appeared no significant difference in the final percentage of drug release.

The patch (F1) released the drug much faster than the other formulations. With F2 and F3 also showed T 50 values of less than one hour. There was a difference in the release rates in the first 1 hr, later the drug release was almost in a similar pattern (Luana Periolia et al., 2004). This is because the polymer HPMC E 15 used was a low viscosity polymer and unlike the other grades of polymer like HPMC K4M, K15 or K100M, HPMC E 15 dissolves much faster. Formulations with higher polymer content (F4 and F5) have shown increased T 50 values.

Increasing the amount of the polymer in the patches produced the water swollen gel like state that could substantially reduce the penetration of the dissolution medium into the patches and so the drug release was retarded.

To confirm the mechanism of drug release Higuchi’s plots were drawn for all the formulations. Figure 2 shows graphical representation of cumulative % drug release versus square root of time. The Higuchi’s plots were found to be linear with correlation coefficient values of 0.8879, 0.9026, 0.9068, 0.8941, 0.8788 for F1, F2, F3, F4, F5 respectively. It was concluded that the release of drug from the films followed the diffusion controlled mechanism in all the formulations. The plots of log cumulative % drug release versus time were found to be linear for the formulations. On the basis of plots it was concluded that the release of the drug from the films have obeyed first order kinetics. The correlation coefficient values were found to be -0.9805, -0.926, -0.9061, -0.8864, -0.8345 for F1, F2, F3, F4, F5 respectively.
Moisture Absorption Studies

Results of moisture absorption studies are presented in the Table 2. The percentage moisture absorbed ranged from about 109% to 167% w/w. The formulations F1 and F2 eroded during the test. Hence these may not be suitable for formulation of buccal patches as the structure of the patch might get deformed easily with the drug being released into the saliva, which is undesirable. No statistical difference was observed between the formulations F3 and F4. This indicates that the moisture absorption capacities of these formulations are same.

Mechanical Properties of Films

The results of the mechanical properties i.e., tensile strength and elongation at break are presented in (Table 2). Tensile strength increased with increase in the polymer content but elongation at break values decreased with the increase in polymer content. Tensile strength values indicate there is no statistical difference was observed in elongation at break values between the next immediate formulations (Vamshi et al., 2007).

In vitro residence time

In vitro residence time was determined for the formulations F3, F4, F5. Because the formulations F1, F2 were eroded in moisture absorption study. So they were not suitable as buccal patches (Mona Semalty et al., 2008). The in vitro residence time of the formulations was in order of F5 > F3 > F4 and they are represented in (Table 2).

Ex-vivo Permeation of Ondansetron Hydrochloride through Porcine Buccal Membrane from Buccal Patch

The results of drug permeation from buccal patches of Ondansetron Hydrochloride through the porcine buccal mucosa reveal that drug was released from the formulation and permeated through the porcine buccal membrane, hence they can possibly permeate through the human buccal membrane. The results indicated that the drug permeation was more in F3 among the last three formulations and about 74.4% of Ondansetron Hydrochloride could permeate through the buccal membrane in 4 hrs. (Figure 3)
Conclusion

Good results were obtained both in vitro and Ex vivo conditions for prepared films. Ondansetron Hydrochloride could permeate through porcine buccal membrane as evidenced from the results of ex vivo drug permeation studies. Buccal patches can be formulated using HPMC E 15 which is soluble in both water as well as organic solvents. In vitro release studies demonstrate the suitability of developed formulations for the release of Ondansetron Hydrochloride. Satisfactory drug release rates and final percentage of drug release could be obtained from the selected formulations. Buccal patches with good mechanical properties measured in terms of tensile strength and elongation at break values may be produced with HPMC E 15. Lower concentrations of HPMC may not be suitable for the development of buccal formulations, as they tend to lose their structure immediately and higher concentrations of HPMC may not release drug rapidly. Buccal patches developed for Ondansetron Hydrochloride possess reasonable bioadhesion measured in terms of in vitro residence time and elongation at break values.

References


