Formulation and In-vitro Evaluation of Mucoadhesive Buccal Patches of Cyproheptadine Hydrochloride

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ABSTRACT

The goal of the present investigation was to design and evaluate mucoadhesive buccal patches of Cyproheptadine Hydrochloride (CPH) which is a sedating antihistamine with antimuscarinic, serotonin-antagonist, and calcium-channel blocking action. Buccal films were made with Hydroxy propylcellulose (HPC EF) and Hydroxy Propyl Methyl Cellulose (HPMC E15) as mucoadhesive polymers. Permeation of CPH was calculated ex vivo using porcine buccal membrane. 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 F8 of HPMC E15 was found to give the better results and release of drug from the film followed Higuchi and Korsmeyer and Peppas models.

Keywords: Cyproheptadine Hydrochloride, ex vivo permeation, mucoadhesive, buccal patch, mechanical properties, diffusion.

INTRODUCTION

Recent years have seen an increasing interest in the development of 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 (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 and hence poor oral bioavailability (Rathbone et al., 1994). Conceivably buccal delivery systems provide easy administration, thereby increasing patient compliance. Cyproheptadine hydrochloride (CPH) is 4-(SH-dibeno [a,d] cyclopehten-5-ylidene)-1-methyl pipridine hydrochloride, potent antihistaminic, antimuscarine and antiserotonin drug and shows sedative with calcium-channel blocking along with stimulate appetite (Lefkowith et al., 1999) and weight gain activity in children and adults and sometimes used in adjunct therapy in children who are taking human growth hormone (Joanna et al., 2004).
Cyproheptadine is highly lipophilic drug, so its HCl salt is used which is slightly soluble in aqueous medium and the solubility decreases in acidic medium (stomach fluid) due to counter ion effect. Moreover it undergoes first-pass metabolism, so its bioavailability may be improved when delivered through buccal route and its dose is low i.e., 4-20mg/day, hence it can be conveniently loaded into a patch. The polymers selected for the formulation is hydroxyl propyl methylcellulose (HPMC E 15) and hydroxyl propyl cellulose EF (HPC EF). The polymers are water soluble and soluble in organic solvents like mixture of alcohol and dichloromethane or methanol and dichloromethane (Vamshi et al., 2007).

**EXPERIMENTAL**

Cyproheptadine hydrochloride was obtained as a gift sample from Vasudha Pharma Chem Ltd, Hyd, A.P, India. Hydroxyl Propyl Methylcellulose (HPMC E 15) and hydroxyl propyl cellulose EF (HPC EF) were 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 methanol (70:30) was placed in the receptor compartment. The donor compartment contained a mixture of 5 ml of PBS pH (6.6) and methanol (95:5) in which 4 mg of Cyproheptadine 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 (Elanco, India) at 285 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 CPH 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 procedure is repeated for HPC EF with out addition of plasticizer (Table 1).

![Table 1 Formulation Ingredients of Cyproheptadine hydrochloride Buccal Patches.](attachment:image)

**Characterization 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.

**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).
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 (hr)</th>
<th>Surface pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>F1</td>
<td>124±3.46</td>
<td>242±4.86</td>
<td>4.4±2.66</td>
<td>128±7.22</td>
<td>125.2±7.89</td>
<td>4±0.1</td>
<td>4.4±0.86</td>
<td>6.7±0.02</td>
</tr>
<tr>
<td>02</td>
<td>F2</td>
<td>132±2.68</td>
<td>284±6.22</td>
<td>7.6±5.32</td>
<td>96±12.4</td>
<td>116.2±4.72</td>
<td>3.9±0.3</td>
<td>3.5±0.64</td>
<td>6.6±0.01</td>
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<tr>
<td>03</td>
<td>F3</td>
<td>146±3.22</td>
<td>326±4.22</td>
<td>9.2±2.88</td>
<td>82±4.4</td>
<td>98.5±6.34</td>
<td>3.9±0.02</td>
<td>4.5±0.88</td>
<td>6.6±0.02</td>
</tr>
<tr>
<td>04</td>
<td>F4</td>
<td>166±3.88</td>
<td>384±3.45</td>
<td>13.4±5.86</td>
<td>64±7.4</td>
<td>82.4±6.86</td>
<td>4±0.2</td>
<td>4.4±0.66</td>
<td>6.8±0.03</td>
</tr>
<tr>
<td>05</td>
<td>F5</td>
<td>178±2.56</td>
<td>423±4.24</td>
<td>19.8±2.14</td>
<td>42±6.24</td>
<td>76.1±6.48</td>
<td>3.9±0.4</td>
<td>4.2±0.26</td>
<td>6.8±0.03</td>
</tr>
<tr>
<td>06</td>
<td>F6</td>
<td>112±2.52</td>
<td>396±2.28</td>
<td>6.2±3.44</td>
<td>152±6.22</td>
<td>Eroded</td>
<td>3.9±0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>07</td>
<td>F7</td>
<td>119±3.02</td>
<td>449±6.85</td>
<td>9.7±3.56</td>
<td>110±8.3</td>
<td>Eroded</td>
<td>4±0.01</td>
<td>4.2±0.26</td>
<td>6.6±0.01</td>
</tr>
<tr>
<td>08</td>
<td>F8</td>
<td>126±3.42</td>
<td>490±3.21</td>
<td>12.4±2.88</td>
<td>87±7.65</td>
<td>109.1±7.94</td>
<td>3.9±0.3</td>
<td>4.2±1.24</td>
<td>6.6±0.01</td>
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<tr>
<td>09</td>
<td>F9</td>
<td>144±2.44</td>
<td>523±2.45</td>
<td>14.6±2.74</td>
<td>76±8.56</td>
<td>115.6±9.65</td>
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<td>6.6±0.01</td>
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<tr>
<td>10</td>
<td>F10</td>
<td>163±3.24</td>
<td>574±6.65</td>
<td>17.1±2.43</td>
<td>65±4.64</td>
<td>167.0±10.46</td>
<td>4±0.2</td>
<td>4.1±0.98</td>
<td>6.8±0.04</td>
</tr>
</tbody>
</table>

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

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 methanol. Methanol 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 285 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 methanol (95.5), at 37±0.5°C and 25 rpm. Samples were collected at different time intervals and analyzed by using UV-Vis spectrophotometer at 285 nm. 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 polymers used for the formulation of mucoadhesive patches are hydrophilic polymers. 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. 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:

\[
\% \text{ Moisture absorbed} = \frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}
\]

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 formulations. The following equations were used to calculate the mechanical properties of the films.

Tensile strength (kg.mm⁻²) = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample (mm}² \text{)}}

Elongation at break (%mm⁻²) = \frac{\text{Increase in length (mm) \times 100}}{\text{Original length \times Cross sectional area (mm}² \text{)}}.

The results of the experiment are presented in Table 2.

In vitro Bioadhesive Strength

The bioadhesive strength of the Buccal patches was determined using an ultra test (Mecmesin, west Sussex UK) equipped with a 5-kg load cell. The fresh porcine buccal mucosa obtained from slaughterhouse was stored in simulated saliva solution (2.38 g Na₂HPO₄, 0.19 g KH₂PO₄ and 8.00 g NaCl in 1000 ml of distilled water at pH 6.75). The porcine buccal mucosa...
was secured tightly to a circular stainless steel adapter of a diameter 2.2 cm provided with the equipment. This was fixed to advanced force gauze. The buccal patch to be tested was placed over another cylindrical stainless steel adaptor of similar diameter and mounted on the platform of motorized test stand. Buccal patch with a backing membrane was adhered on to it using a solution of cyanoacrylate adhesive. All measurements were conducted at room temperature. During Measurement 100µl of 1% mucin solution of crude mucin procured from sigma chemicals was used to moisten the porcine buccal membrane. The upper support was lowered at a speed of 0.5 mm/s until contact was made with the tissue at the predetermined force of 0.5 N for a contact time of 180 sec. At the end of the contact time upper support was withdrawn at a speed of 0.5mm/s to detach the membrane from the patch. Data collection and calculations were performed using the data plot software package of the instrument. Two parameters, namely the work of adhesion and peak detachment force were used to study the buccal adhesiveness of patches (Vamshi et al., 2007). The work of adhesion was determined from the area under force distance curve while the peak detachment force required detaching from tissue.

**Ex vivo Permeation of Cyproheptadine hydrochloride Patches through Porcine Buccal Membrane.**

*Ex vivo* permeation of CPH 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 & methanol. 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 CPH in the samples was analyzed by UV-Vis Spectrophotometer at the wavelength of 285 nm. All the experiments were performed in triplicates (Figure 4).

**FTIR studies**

The physical mixtures of drug and HPC EF, drug and HPMC E 15 were prepared. The IR spectra for the pure drug and physical mixtures were obtained by using an FTIR spectrometer (PERKIN ELMER FT-IR Spectrometer, Spectrum Two, Singapore).

**RESULTS AND DISCUSSIONS**

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

The cumulative amount of CPH that had penetrated through the buccal epithelium was determined. 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 level of Phenol red, which was used as marker compound, was found in the receiver compartment. Hence it did not show any penetration whereas CPH could penetrate freely. This indicated that the membrane was intact. The result is shown in (Figure 1). The flux was calculated to be 15.906 µg/hr.cm².

![Fig 1. Ex-Vivo Permeation of Cyproheptadine Hydrochloride through Porcine Buccal Mucosa](image)

**Physicochemical Characteristics of the Patches**

Physicochemical characteristics of the patches are 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 and F6 to F10. In thickness variation test, 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. The surface pH of all formulations ranged from 6.6 to 6.8 and hence no mucosal irritation was expected. The results of content uniformity confirmed uniformity of drug content in the patch.

**In vitro Drug Release Studies**

Phosphate buffer pH 6.6 and methanol (95:5) was used as medium for the release studies to show the drug release profile of CPH patches containing different ratios of polymers 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.

When compare the drug release, less percentage was released from HPC EF than HPMC E 15 due to lower solubility of HPC EF. The patches (F6, F7) released the drug much faster than the other formulations. With F8, F9 and F10 also showed T₅₀ values of less than one hour. This is because the polymer HPMC E 15 used was a low viscosity polymer and unlike the other grades of polymers like HPMC K4M, K15 or K100M, HPMC E 15 dissolves much faster. Formulations with higher polymer content (F8, F9 and F10) have shown increased T₅₀ 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 (Figure 2 & 3).

![Figure 2](image1)

**Figure 2.** In vitro Drug Release Profile of Cyproheptadine Hydrochloride Buccal Patches (HPC EF)

![Figure 3](image2)

**Figure 3.** In vitro Drug Release Profile of Cyproheptadine Hydrochloride Buccal Patches (HPMC E 15)

Data of the in vitro release was fit in to different equations and kinetic models to explain the release kinetics of CPH from these buccal patches. The kinetic models used were a zero-order equation, first-order equation, Hixson-Crowell equation, Higuchi release and Korsmeyer and Peppas models (Table 3). In case of HPMC E 15 the best fit with the highest correlation value was shown by Higuchi and Korsmeyer and Peppas. In case of HPC EF all formulations follow zero order along with Higuchi and Peppas models.

**Moisture Absorption Studies**

Results of moisture absorption studies are presented in the Table 2. In case of HPC EF the percentage moisture absorbed ranged from about 76.18% to 125.22% w/w and from 109.17% to 167.02% w/w for HPMC E 15. The swelling was slower with HPC EF than HPMC E 15. The formulations F6 and F7 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.

**Mechanical Properties of Films**

The results of the mechanical properties of 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. Similar pattern was observed in formulations with both the polymers HPC EF and HPMC E 15. Tensile strength values indicate that there is no statistically significant difference between the next immediate formulations. (Vamshi et al., 2007).

**In vitro Bioadhesion Studies**

In vitro bioadhesion study was performed for formulation (F8). The peak detachment force and work of adhesion were found to be 0.860 ± 0.42 N and 0.542 ± 0.43 mJ respectively. With these above mentioned results it is concluded that the polymer possess reasonable bioadhesion in terms of peak detachment force and elongation at break values.

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

The results of drug permeation from buccal patches of CPH 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 F8 among the last three formulations and about 75.82% of CPH could permeate through the buccal membrane in 4 hrs (Figure 4).
FTIR studies

To know the interaction between the drug and polymers used in the preparation of patches IR spectroscopy was carried out for the test preparations. The IR spectra of the physical mixtures showed the same absorption bands as the pure drug, indicates the absence of interaction between Cyproheptadine, HPC EF and HPMC E 15 (Fig 5).

CONCLUSION

Good results were obtained both in vitro and Ex vivo conditions for prepared films. Cyproheptadine Hydrochloride could permeate through porcine buccal membrane as evidenced from the results of ex vivo drug permeation studies. Buccal patches can be formulated using HPC EF and HPMC E 15 which are soluble in both water as well as organic solvents. In vitro release studies demonstrate the suitability of developed formulations for the release of Cyproheptadine Hydrochloride. Satisfactory drug release rates and final percentage of drug release could be obtained from the selected formulations. Buccal patches were produced good mechanical properties measured in terms of tensile strength and elongation at break values. Drug release was slow for HPC EF patches due to lower solubility of the polymer when compare 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 Cyproheptadine Hydrochloride possess reasonable bioadhesion measured in terms of in vitro bioadhesion strength.

ACKNOWLEDGEMENT

The authors are thankful to University Grants Commission (UGC), New Delhi, India for providing fellowship and contingency to carryout the research.

REFERENCES


