Development And Characterization Of Buccal Film: Tacrolimus As A Model Drug

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ABSTRACT

Buccal drug delivery has become a relevant route of administration. It has got various advantages, masking the first pass metabolism being the primary one, thus ensuring increased drug bioavailability at the site of action. Tacrolimus, an immunosuppressant, calcineurin inhibitor is the drug of choice. Oral tablet suffers from high first pass metabolism and topical formulations such as ointments readily washout from the site of application. Tacrolimus formulated as mucoadhesive buccal film provided prolonged effect than topical formulations. Films (F1 & F2) were prepared by solvent casting technique. Chitosan being the best mucoadhesive polymer with wound healing properties increased the retention time of the film at the wound site. HPMC ensured good film property. Propylene glycol was used as plasticizer and citric acid as salivary stimulator and cross linker. The compatibility of drug in the formulation was confirmed by FTIR. Formulated films were subjected to various evaluation parameters, and all the physical parameters were within the acceptable limits. In vitro release was found to be better for F1. Ex vivo studies of F1 using goat mucous showed a permeation of 76.5%.

Key words: Tacrolimus–film–Chitosan–HPMC–Propylene glycol

1 INTRODUCTION

Buccal drug delivery has got various advantages, masking the first pass metabolism being the primary one, thus ensuring increased drug bioavailability at the site of action [1]. Current [2] topical formulations include ointments which has the major drawback of washout. So it necessitates for a dosage form which is more mucoadhesive and has increased retention time. Chitosan, the best mucoadhesive polymer with wound healing property was used along with HPMC to provide good film forming property [3]. Tacrolimus, is used as a model drug for the preparation of buccal film.

2 MATERIALS AND METHODS

2.1 Materials

Tacrolimus was obtained from Yarrow Chem Products, Mumbai. Pre

2.2 formulation studies [4]

It determined the physicochemical parameters of the drug to ensure its safety and effectiveness (physical appearance, solubility and pH).

2.3 Drug-Excipient compatibility studies [5]

FTIR Spectroscopy of pure drug (Tacrolimus) and the polymers (Chitosan and HPMC) were carried out to determine their interactions.

2.4 Construction of Calibration [6]

2.4.1 Preparation of standard stock solution

2.4.1.1 Stock A100mg Tacrolimus was accurately weighed and dissolved in 50ml acetonitrile.

2.4.1.2 Stock B0.5ml of stock A and 0.5 ml of concentrated H\textsubscript{2}SO\textsubscript{4} taken in a 10ml standard flask was made up to the volume using distilled water.

2.4.2 Determination of λ\textsubscript{max}

Stock solution was scanned from 200 - 400nm by UV spectrophotometer.
2.4.3 Calibration of Tacrolimus [7]

From the above stock solution 1ml, 2ml, 3ml, 4ml, 5ml and 6ml were pipetted out and made up to 10ml by using distilled water in standard flasks to produce 10, 20, 30, 40, 50 and 60 μg/ml respectively. The absorbance was measured at 297nm in a UV spectrophotometer using distilled water as blank. The data’s were tabulated. The concentration was plotted against absorbance.

2.5 Development of tacrolimus film formulation [8]

Chitosan and HPMC were dissolved in 1% dil.acetic acid and distilled water respectively and these two solutions were mixed together. Citric acid dissolved in distilled water and propylene glycol was added to the above solution mixture. To this the required quantity of Tacrolimus was added by dissolving the drug in a small quantity of ethanol. The mixture was kept under magnetic stirrer for 5 minutes. Then it was casted to the petridish and kept for drying at 60°C for 48 hours.

3 CHARACTERIZATION OF TACROLIMUS FILM [9]

3.1 Weight variation
Films were taken and the weight was checked on digital weighing balance.

3.2 Film thickness
The thickness of each film was measured using a micrometer screw gauge and the average was determined.

3.3 Tackiness
Films were taken and pressed against the finger tip and the results were recorded.

3.4 Folding endurance [10]

It determines the brittleness of the film. The films were randomly selected and repeatedly folded at the same place until it broke and the values were recorded.

3.5 Percent elongation
The initial lengths of the films were measured. It was then stretched to a lesser extent and the final length was noted.

% Elongation = (L-Lo) × 100/Lo

L = final length, Lo = Initial length

3.6 Film softening upon storage
Films were stored in desiccators for 48 hours and softening was determined.

3.7 Percentage moisture loss [11]

It is performed to ensure the physical stability of the films. Films were weighed and kept in desiccators containing fused anhydrous calcium chloride for 3 days. It is then weighed again and the percentage moisture loss was calculated.

% moisture loss = (initial weight – final weight) / initial weight × 100

3.8 Transparency

Transparency of the film was determined using UV Spectrophotometer. Films were taken and placed in spectrophotometer cell and analyzed at 600nm. Transparency was calculated as:

Transparency = [log T600] / B C

T600 - Transmittance at 600nm
B - Thickness of the film
C - Concentration

3.9 Surface pH

pH of the film should be neutral; otherwise it may cause irritation when it is too acidic or basic. The surface pH was determined using a combined pH electrode. The film was made slightly wet using water and the electrode was brought in contact with the film. pH reading from the digital meter was taken.

3.10 Drug content [12]

For this test, a film was taken and placed in a beaker containing 5ml phosphate buffer (pH 6.8) and 5ml alcohol. Medium was stirred for proper dissolution on orbital shaker for 4 hours. Later the content was filtered using whatman filter paper and the filtrate sample was analyzed by UV spectrophotometer at 297 nm.

3.11 Invitro disintegration studies [12]

Disintegrating time is defined as the time (seconds) at which a film breaks when brought in contact with water or saliva. Film was immersed in a beaker containing 25ml phosphate buffer 6.8. It was swirled at every 10sec. The time at which the film started to break was noted. By this method, cumulative percentage of drug retained was calculated.

3.12 Invitro dissolution studies [12]

In vitro drug dissolution was performed using USP paddle type apparatus. The studies were carried out at 37°C with stirring speed of 100 rpm in 900 ml phosphate buffer 6.8. 5 ml of samples were withdrawn at predetermined time intervals of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 hrs and replaced with the same volume of buffer. The samples were collected and the concentration was determined at 297nm using UV spectrophotometer.
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3.13 Stability studies [13]
Stability studies of the formulation were performed to check whether the product is stable or not. For this, film was wrapped in a butter paper, and then with aluminum foil wrapped over it and packed in aluminum for two months at room temperature, refrigerator temperature and incubator and determined its physical and chemical stability.

3.14 Ex vivo permeation study [14]
Ex vivo permeation study of Tacrolimus buccal film was carried out using fresh goat buccal mucosa. Goat buccal mucosa was tied to one end of an open ended cylinder, which acts as a donor compartment. The F1 formulation of buccal film was placed in such a way that it should be in contact with the mucus membrane. The receptor compartment was filled with phosphate buffer pH 6.8. The assembly was maintained at 37°C and stirred magnetically. Samples were withdrawn at predetermined time intervals and analyzed by UV spectrophotometer at 297nm.

4 RESULT AND DISCUSSION

4.1 Physical appearance
Tacrolimus was found to be a white amorphous powder with non hygroscopic nature.

4.2 Melting point
Melting point was found to be between 113-1150C.

4.3 Solubility Studies
It was found that Tacrolimus is insoluble in water. It was soluble in ethanol, acetone, chloroform, acetonitrile and other organic solvents.

4.4 pH determination
pH of Tacrolimus was found to be 6.61.

4.5 Determination of $\lambda_{\text{max}}$
The stock solution was scanned between the range of 200-400nm by UV spectrophotometer. From the graph, it was concluded that the $\lambda_{\text{max}}$ of Tacrolimus is 297nm.

4.6 FT – IR study
The obtained spectrum showed that there was no major shifting in the frequencies which confirmed that the drug was compatible with all excipients used in the formulation.

Table 1. Formulation of Tacrolimus buccal film

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td>10 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>Chitosan</td>
<td>100 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>HPMC</td>
<td>200 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>Citric acid</td>
<td>250 mg</td>
<td>250 mg</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>0.6 ml</td>
<td>0.6 ml</td>
</tr>
<tr>
<td>1% acetic acid</td>
<td>20 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>Purified water</td>
<td>30 ml</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Summary of evaluated parameters for F1 & F2 buccal films

<table>
<thead>
<tr>
<th>Formulation</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Film forming Capacity</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Peel ability</td>
<td>Very Good</td>
<td>Good</td>
</tr>
<tr>
<td>Tackiness</td>
<td>Tacky</td>
<td>Tacky</td>
</tr>
<tr>
<td>Weight Variation (mg)</td>
<td>15±2.12</td>
<td>10±1.40</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>0.09±0.002</td>
<td>0.086±0.002</td>
</tr>
<tr>
<td>Surface pH</td>
<td>6.20</td>
<td>4.50</td>
</tr>
<tr>
<td>Folding endurance</td>
<td>630±0.03</td>
<td>550±0.03</td>
</tr>
<tr>
<td>Percentage moisture loss(%)</td>
<td>4.29±0.21</td>
<td>3.15±0.08</td>
</tr>
<tr>
<td>Film softening</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Percent elongation (%)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Disintegration time (min)</td>
<td>30-45</td>
<td>15-20</td>
</tr>
<tr>
<td>Transparency</td>
<td>-8.2</td>
<td>-5.7</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>70.93</td>
<td>67.38</td>
</tr>
</tbody>
</table>
4.7 Development and Evaluation of Tacrolimus films

**Invitro dissolution studies:** The dissolution profile of tacrolimus in different formulations is shown in the figure (fig 5). F1 formulation showed greater release than F2. From the kinetics data it was concluded that F1 follows zero order (concentration independent) drug release. From the Kosmeyer Peppas Model, the mechanism of drug release from the F1 formulation was confirmed to be Supercase II ie, diffusion + erosion.

**Exvivo permeation study:** Exvivo permeation study was performed on F1 since it gave maximum drug release compared to F2 formulation. Goat oral mucosa was used to study the drug permeation since the goat oral mucosa resembles the oral mucosa of humans. Permeation studies of formulation F1 showed permeation of 76.52% at the end of 6 hours. These result showed that the drug is getting absorbed through the oral mucosa.

Stability studies: The formulation F1 and F2 were subjected to short term stability studies and were analyzed. The drug content and disintegration time were evaluated and there were no significant changes.

**CONCLUSION**

Tacrolimus is used as a model drug, which is an immunosuppressant, calcineurin inhibitor which undergoes first pass metabolism, and it’s bioavailability is very low (11.2-19.1%). The relevance of our study was to develop mucoadhesive Tacrolimus buccal films so that the first pass metabolism in the liver was avoided, and thus dose could be reduced. The currently available topical formulations suffered from salivary washout. Thus the development of mucoadhesive buccal films provided the advantage of advanced retention time, prolonged release of the drug and better bioavailability at the site of action. Mucoadhesive tacrolimus films were prepared successfully using chitosan and HPMC as polymers by solvent casting method. The developed formulation showed satisfactory results for peel ability, tackiness, weight variation, surface pH, folding endurance, and percentage moisture loss, film softening upon storage, percent elongation, disintegration time, transparency and drug content. Films showed disintegration time of 30-45 mins, formulation F1 showed better release and followed zero order kinetics. Kos-
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meyer Peppas Model showed that the mechanism of drug release was Supercase II (erosion + diffusion). Ex vivo permeation study showed a release of 76.5%.

6 ACKNOWLEDGMENT
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7 AUTHOR’S CONTRIBUTION
Ashwathy P, Anjitha Anil, Arjun R, Ragavi R designed and performed the project under the guidance, supervision and support of Ms. Saritha A Surendran.

8 CONFLICT OF INTEREST
All authors have no conflict of interest to report.

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