INTRODUCTION

The buccal mucosa, along with other mucosal tissues, has been investigated as a potential site for controlled delivery of macromolecular therapeutic agents, such as peptides, proteins and polysaccharides because of its accessibility and low enzymatic activity compared to the gastro-intestinal tract. Another interesting advantage is its tolerance (in comparison with the nasal mucosa and skin) to potential sensitizers.

The potential of the buccal mucosa as an alternative site for the delivery of drugs into the systemic circulation has recently received much attention. There are many reasons why the buccal mucosa might be an attractive site for the delivery of therapeutic agents into the systemic circulation. Due to the direct drainage of blood from the buccal epithelium into the internal jugular vein, the first-pass metabolism in the liver and intestine may be avoided. This first-pass effect is a major reason for the poor bioavailability of some compounds when administered orally. Additionally, the mucosa lining the oral cavity is easily accessible, which ensures that a dosage form can be applied to the required site and can be removed easily in case of emergency.

The oral cavity is a moist environment; the membranes that line the oral cavity are covered with mucus which is derived mainly from minor salivary glands and are constantly bathed in saliva, an aqueous substance rich in inorganic salts, proteins and bacteria. Saliva has a variety of functions and is continuously secreted into, distributed around and removed from the oral cavity.

Based on the current understanding of biochemical and physiological aspects of absorption and metabolism of biotechnologically-produced drugs, they cannot be delivered effectively through the conventional oral route. Because after oral administration many drugs are subjected to presystemic clearance extensive in liver, which often leads to a lack of significant correlation between membrane permeability, absorption, and bioavailability. Difficulties associated with parenteral delivery and poor oral availability provided the impetus for exploring alternative routes for the delivery of such drugs.

Nebivolol is a third-generation β1-selective β-blocker used in the treatment of hypertension, it works by relaxing blood vessels and slowing heart rate to improve blood flow and decrease blood pressure. Nebivolol on oral administration undergoes extensive metabolism in the liver resulting in to a very poor (approximately 10-12%) bioavailability. Oral administration of nebivolol can also cause gastrointestinal disturbance and abdominal or stomach pain etc. In order to improve the bioavailability, efficacy and to minimize the side effects associated with oral administration, mucoadhesive buccal films of nebivolol using hydroxy propyl methyl cellulose and methyl cellulose were prepared by solvent casting technique. The films of nebivolol using hydroxy propyl methyl cellulose and methyl cellulose were smooth, elegant and uniform in thickness and weight. Among the two polymers used hydroxy propyl methyl cellulose showed an increased in-vitro residence time due to mucoadhesion nature of the hydroxyl propyl methyl cellulose. Drug content uniformity study showed uniform dispersion of the drug throughout the film in the range of 96.208±1.0705 to 98.887±0.2558 %.

In-vitro drug release study showed that more than 90% of drug was released at the end of 8 hr. The release profile of all the formulations was subjected to various kinetic equations and the results suggested that the drug was released by diffusion mechanism following super case-II transport.

Keywords: Nebivolol, Buccal films, Mucoadhesion, In vitro drug release and Diffusion.
procured from SD Fine Chem., Mumbai and all other chemicals used were of analytical grade.

**Preparation of Nebivolol Buccal Films**

Nebivolol mucoadhesive buccal films were prepared using hydroxy propyl methyl cellulose (HPMC) and methyl cellulose (MC) by solvent casting technique. Accurately weighed quantity of hydroxy propyl methyl cellulose was soaked ethanol (chloroform and ethanol in the ratio of 75:25 % v/v for methyl cellulose) for 24 hrs, the calculated amount of nebivolol was dissolved in the polymeric solution and propylene glycol was added gradually with continuous stirring. 5 ml resultant mixture was poured into each fabricated glass ring placed on aluminum foil in a petri dish, and then the petri dish was kept aside for drying at room temperature for 24 hours. The dried polymeric films were cut into circular films of 10 mm diameter for further evaluation.

**Evaluation of Nebivolol Mucoadhesive Buccal Films**

**Weight Uniformity**

A film of 10mm diameter was weighed using Shimadzu digital balance and the average weight was calculated (n=3).

**Thickness Uniformity**

The thickness of the film was measured using screw gauge with a least count of 0.01mm at three different spots of the film and the average thickness was calculated.

**Folding Endurance**

The flexibility of film can be measured quantitatively in terms of folding endurance. The folding endurance of the film was determined by repeatedly folding a small strip of the films at the same place till it broke. The number of times films could be folded at the same place without breaking indicated the value of folding endurance and the procedure was repeated for three times.

**Swelling Index**

A buccal film of 10 mm diameter was weighed on a pre-weighed cover slip, the initial weight of the film was recorded (W₀) and then it was kept in a petri dish containing 5 ml of phosphate buffer pH 6.8. The cover slip was removed at time interval of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hr, and excess of water was carefully removed and swollen film was re-weighed (Wₜ). The percentage swelling (%S) was calculated by following formula:

\[
\%S = \frac{Wₜ - W₀}{W₀} \times 100
\]

The mean %S was calculated (n=3).

**Surface pH**

The film was allowed to come in contact with 1ml of phosphate buffer pH 6.8 for 1-3 min. The surface pH was measured using pen pH meter (n=3).

**Drug Content Uniformity**

This study was carried out to know the complete and uniform dispersion of the drug throughout the film. The film of 10 mm diameter was dissolved in methanol and the absorbance of the solution (after suitable dilution) was measured at 282 nm using UV/visible spectrophotometer (Shimadzu UV-1700). The percentage drug content was calculated with the help of calibration curve (n=3).

**In-vitro Drug Release**

The drug release from buccal film was studied by standard cylindrical tube method using sigma dialysis membrane. The membrane was tied to one end of open cylinder and is acted as donor compartment, the buccal film was placed inside this compartment and it was in contact with the receptor compartment containing 100 ml of phosphate buffer pH 6.8. The diffusion medium was stirred continuously using magnetic stirrer and the temperature was maintained at 37±0.5ºC. 5 ml sample was withdrawn from the receptor compartment at periodic intervals and the same was replaced by equal volume of fresh buffer solution. The samples were analyzed for drug content spectrophotometrically at 282 nm. The amount of drug released was calculated with the help of standard calibration curve and cumulative percentage drug release was calculated. In-vitro release data were subjected to release kinetic equations and were plotted for various graphs.

**Ex vivo Mucoadhesive Strength**

Sheep buccal mucosa was obtained from a local slaughterhouse; the mucosal membrane was separated by removing the underlining fat and loose tissues. The membrane was washed with distilled water and subsequently with isotonic phosphate buffer (IPB) solution of 6.8 pH at 37°C. The bioadhesive strength of the film was measured on modified physical balance.

**In vitro Residence Time**

The in-vitro residence time was determined using a locally modified USP disintegration test apparatus. A segment of sheep buccal mucosa of 3 cm long was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive films were hydrated from one surface using 15 µl IPB and then 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 in 800 ml IPB maintained at 37°C, so that the film was completely immersed in the buffer solution at the lowest point.
Fig. 1: Mucoadhesive strength of patches for batch BFN1-BFN7

Fig. 2: In vitro residence time profile for BFN1-BFN7

Fig. 3: In vitro drug release from nebivolol buccal films prepared using HPMC 2% (BFN1), 4% (BFN2), 6% (BFN3), 8% (BFN4) and MC 2% (BFN5), 3% (BFN6) and 4% (BFN7).

Table 1: Formulation of Nebivolol Buccal films

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation Code</th>
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<tbody>
<tr>
<td></td>
<td>BFN1</td>
</tr>
<tr>
<td>HPMC (%w/v)</td>
<td>2</td>
</tr>
<tr>
<td>MC (%w/v)</td>
<td>-</td>
</tr>
<tr>
<td>Propylene Glycol* (%w/w)</td>
<td>30</td>
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</table>

* Percentage of polymer weight Each patch contains 5 mg of nebivolol
Table 2: Evaluation of nebivolol buccal films for thickness, weight variation, folding endurance, swelling index, surface pH, mucoadhesive strength, *in vitro* residence time and percent drug content uniformity.

<table>
<thead>
<tr>
<th>F. Code</th>
<th>Weight (mg) ±SD</th>
<th>Thickness (mm) ±SD</th>
<th>Folding Endurance ±SD</th>
<th>Swelling Index (%) ±SD</th>
<th>Surface pH ±SD</th>
<th>Mucoadhesive strength (gm)</th>
<th><em>In Vitro</em> Residence time (Hrs) ±SD</th>
<th>Drug Content Uniformity (%) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFN</td>
<td>6.333± 0.577</td>
<td>0.072± 0.0098</td>
<td>412±2.516</td>
<td>35.18 ± 2.188</td>
<td>6.82±0.105</td>
<td>3.100±0.1</td>
<td>2.10±0.050</td>
<td>97.176± 1.122</td>
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<tr>
<td>BFN1</td>
<td>10.666± 1.527</td>
<td>0.10± 0.0152</td>
<td>367±2.516</td>
<td>40.956 ± 0.871</td>
<td>6.78±0.155</td>
<td>3.766±0.115</td>
<td>2.27±0.025</td>
<td>98.661± 0.127</td>
</tr>
<tr>
<td>BFN2</td>
<td>14.000± 1</td>
<td>0.156± 0.0115</td>
<td>338±3.605</td>
<td>45.964 ± 2.406</td>
<td>6.94±0.227</td>
<td>4.333±0.115</td>
<td>3.23±0.036</td>
<td>96.393± 1.465</td>
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<tr>
<td>BFN3</td>
<td>16.333± 1.154</td>
<td>0.223± 0.0152</td>
<td>298±3.605</td>
<td>66.359 ± 1.547</td>
<td>6.60±0.438</td>
<td>4.766±0.057</td>
<td>3.9±0.309</td>
<td>98.057± 0.204</td>
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<tr>
<td>BFN4</td>
<td>6.333± 0.577</td>
<td>0.076± 0.0057</td>
<td>394±4.582</td>
<td>25.291 ± 1.866</td>
<td>6.67±0.375</td>
<td>4.300±0.1</td>
<td>4.12±0.502</td>
<td>98.887± 0.255</td>
</tr>
<tr>
<td>BFN5</td>
<td>6.666± 0.577</td>
<td>0.103± 0.0057</td>
<td>377±2.516</td>
<td>30.764 ± 0.941</td>
<td>6.22±0.420</td>
<td>5.633±0.057</td>
<td>5.35±0.133</td>
<td>96.208± 1.070</td>
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<tr>
<td>BFN6</td>
<td>10.333± 0.577</td>
<td>0.13± 0.0152</td>
<td>359±5.033</td>
<td>35.616 ± 2.808</td>
<td>6.56±0.526</td>
<td>6.260±0.115</td>
<td>5.52±0.072</td>
<td>97.494± 0.656</td>
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CONCLUSION

In the present research work, nebivolol mucoadhesive buccal film were prepared using varying concentration of HPMC and MC by solvent casting technique with an objective of improved bioavailability.

All the formulations possessed the good mucoadhesion, and they were free from irritation and released the drug completely by diffusion mechanism following super case –II transport.

Acknowledgement

Authors are thankful to Ajanta Pharma Ltd., Mumbai for providing gift samples of nebivolol. Authors are also thankful to the Principal of HKES's college of Pharmacy, Gulbarga for providing lab facilities to carryout the research work.

REFERENCES


Table 3: Drug release kinetics of nebivolol buccal films

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Regression coefficient (R²) values</th>
<th>Peppas Slope Values (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order</td>
<td>First order</td>
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<tr>
<td>BFN₁</td>
<td>0.995</td>
<td>0.854</td>
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<tr>
<td>BFN₂</td>
<td>0.991</td>
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<tr>
<td>BFN₃</td>
<td>0.988</td>
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<tr>
<td>BFN₄</td>
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<tr>
<td>BFN₅</td>
<td>0.993</td>
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</tr>
<tr>
<td>BFN₆</td>
<td>0.990</td>
<td>0.961</td>
</tr>
<tr>
<td>BFN₇</td>
<td>0.989</td>
<td>0.962</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

In the present research work, mucoadhesive buccal films of nebivolol (Table 1) were prepared using HPMC and MC by solvent casting technique to increase the efficacy of drug by improving its bioavailability. The prepared films were evaluated for various parameters.

The thickness of the film prepared measured in the range of 0.072 to 0.23 mm, the results (Table 2) suggested that the films were thin enough and they did not cause any inconvenience after their application into the buccal cavity. The surface pH of the film was in the range of 6.22 to 6.94, the pH of films was nearer to the salivary pH, hence any irritation was not observed to the mucus membrane of the buccal cavity.

The films were also evaluated for folding endurance and mucoadhesive strength (Fig 1), the higher values of these parameters indicated that the films were flexible enough and they were not detached easily. These helped in retaining the films for longer period of time at the site of application and it was well supported by longer in-vitro residence time values (Fig 2). The buccal films were also evaluated for drug content uniformity test; the results showed that the drug was uniformly dispersed in the range of 96.208 to 98.887 %.

Finally, the films were evaluated for drug release kinetics for a period of 8 hours, the release profiles were subjected to various kinetic equations like Higuchi diffusion equation ($Q = Kt^{1/2}$) and Peppas exponential equation ($Q = Kt^n$) to ascertain the drug release mechanism (Table 3). In both the cases, the plots (Fig. 3-6) were found to be fairly linear and the linearity was well supported by higher regression coefficient values ($r^2$ values were nearer to one) and slope values of the Peppas equation are more than one ($>1$) in all the cases which suggested that the drug was released by diffusion mechanism following super case-II transport.


