**In Vitro and in Vivo Evaluation of Buccal Bioadhesive Films Containing Salbutamol Sulphate**

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The aim of present study was to prepare and evaluate buccal bioadhesive films of salbutamol sulphate (SS) for the treatment of asthma. The films were designed to release the drug for a prolonged period of time so as to reduce the frequency of administration of the available conventional dosage forms of SS. The different proportions of sodium carboxymethylcellulose (SCMC) and Carbopol 940P (CP 940P) were used for the preparation of films. Carbopol was used to incorporate the desired bioadhesiveness in the films. The films were prepared by solvent casting method and evaluated for bioadhesion, in vitro drug release and anti asthmatic effect (bronchoprotection) in histamine induced bronchospasm of guinea pigs. In vitro drug release from the film was determined using a modified Franz diffusion cell while bioadhesiveness was evaluated with a modified two-arm balance using guinea pig buccal mucosa as a model tissue. Films containing SCMC : CP 940P ratio of 76 : 24 was found to be the best with moderate swelling along with favorable bioadhesion force and in vitro drug release. The drug release mechanism was found to follow non-Fickian diffusion as release mechanism. The prolonged in vivo effect (bronchoprotection) obtained from the buccal bioadhesive film of SS administered via buccal route may improve the treatment of asthmatic disorders by reducing the frequency of administration which is associated with the tolerance effect of SS. Additionally for the clinical benefit, it is also expected to reduce the major adverse effects of SS such as tachycardia and arrhythmias via buccal absorption.

Key words salbutamol sulphate; buccal bioadhesive film; Carbopol 940P; in vivo effect

Salbutamol sulphate (SS) is a short-acting β2-adrenergic agonist that has been widely used in the treatment of asthmatic disorders and chronic obstructive lung diseases.1—3) When given orally, its systemic bioavailability is only 50% as it is subjected to first pass metabolism in liver and extensive presystemic metabolism mainly due to sulfation in the duodenal mucosa.4) The elimination half-life of SS is 2.7 to 5.5 h. Also the repeated administration of SS leads to tolerance to its bronchodilator effect.4,5) These attributes of SS have been focused to utilize the potential of buccal bioadhesive film of SS prepared using a combination of SCMC and Carbopol 940P (CP 940P) which would prolong and improve the anti asthmatic effect of SS.

**Experimental**

**Materials** Salbutamol sulphate was obtained as gift sample from Glaxo SmithKline Pharmaceuticals Ltd., Mumbai, India. Sodium carboxymethylcellulose (SCMC), hydroxypropylmethylcellulose, chitosan and xanthan gum have been described for the formulation of bioadhesive systems but none of these polymer possess all the characteristics of an ideal polymer (nontoxic, nonirritant, strong non covalent adhesion, sustained release, stable and cheap) for a bioadhesive drug delivery system.8) Carbopol is excellent bioadhesives but with potential mucosal irritating character.9) Irritant properties of Carbopol can be reduced by combining it with other non-irritant bioadhesive polymers like SCMC. Although various in vitro investigations were carried out for the buccal formulations of SS, but none of these studies have been focused to utilize the in vivo potential of buccal formulations of SS.12,13)

Therefore, the present study was aimed to design and evaluate (including in vivo potential) buccal bioadhesive film of SS prepared using a combination of SCMC and Carbopol 940P (CP 940P) which would prolong and improve the anti asthmatic effect of SS.

**Preparation of the Buccal Bioadhesive Films** The weighed amount of CP 940P was added to one-third portion of the required doubled distilled water (DDW) and kept undisturbed until a clear solution was formed. Then it was stirred for 1 h. SS was dissolved in a minimum volume of DDW and added to SCMC contained in a dry beaker. The remaining two-third portion of DDW was added to the above mixture with stirring to form a homogenous dispersion. The CP 940P solution and required volume of PEG 400 (Qualigens Fine Chemicals, Mumbai, India), histamine (Sigma-Aldrich Chemicals Private Limited, Bangalore, India) and urethane (Merek Chemicals Private Limited, Mumbai, India) were used. All other chemicals were of analytical grade.

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**Thickness Testing** The thickness of ten randomly selected films from every formulation batch was determined using a standard screw gauge.14)
Table 1. Formulas for Buccal Bioadhesive Films of SS

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Amount of SS (mg/cm² of the film)</th>
<th>Polymer concentration (% v/v of gel)</th>
<th>Ratio of SCMC to CP 940P concentration (% v/v of gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSB1</td>
<td>4.2</td>
<td>3</td>
<td>76:24</td>
</tr>
<tr>
<td>SSB2</td>
<td>4.2</td>
<td>3</td>
<td>83:17</td>
</tr>
<tr>
<td>SSB3</td>
<td>4.2</td>
<td>3</td>
<td>90:10</td>
</tr>
</tbody>
</table>

Weight Uniformity The weight of each of ten randomly selected films from every formulation batch was determined by using an electronic balance (Adair Dutt & Co., Kolkata, India).

Folding Endurance Folding endurance was determined by repeatedly folding the ten films at the same place till it broke or folded up to 300 times.

Drug Content Uniformity Uniformity of drug content was determined according to the following procedure. Ten randomly selected films of each formulation batch were weighed accurately and dissolved in 10 ml of phosphate buffer (pH 6.8). Half millilitre of this SS solution was transferred into a 100 ml volumetric flask containing 20 ml of phosphate buffer (pH 6.8), and stirred continuously for 1 h on a magnetic stirrer. The volume was made up to 100 ml with phosphate buffer (pH 6.8) and the absorbances were measured in UV/Vis spectrophotometer (ppm, Tokyo, Japan). Concentrations of SS were calculated from a standard calibration curve of SS in phosphate buffer (pH 6.8) without interferences of excipients.

Microenvironment pH The microenvironment pH of the prepared buccal bioadhesive SS films was determined to evaluate the possible irritation effects on the mucosa. The films were left to swell in 5 ml of distilled water (pH 6.8) in small beakers, and the pH was measured after 8 h by placing the electrode in contact with the microenvironment of the swollen films. The average pH of five determinations was reported.

Swelling Studies of Buccal Bioadhesive Films of SS The swelling index of the prepared buccal bioadhesive SS films was determined by weighing films and recording their weights before placing them separately in weighed beakers. The initial weights of the films were recorded (W₀). Fifteen millilitres of phosphate buffer (pH 6.8) was added to each beaker and then placed in an incubator at 37±0.5 °C. At time intervals of 1, 2, 4, 6 and 8 h, excess water was carefully removed, and the swollen films were weighed (Wₙ).15) Time intervals of swelling index studies (up to 8 h) were kept similar to the time intervals of in vitro release for their comparisons. The experiment was repeated three times. The swelling index was determined from the formula:

\[ \text{swelling index} = \left( \frac{Wₙ - W₀}{W₀} \right) \]

Mechanical Characterization of the Films Mechanical parameters, tensile strength and elongation at break were calculated from the load time profiles of the films using instron® tensile tester. Upper and lower grips of the sample with a gauge length of 5 cm×1 cm, were attached to the crosshead and the base plate respectively in such a way that the former was located exactly 5 cm above the latter. The crosshead was moved upwards at a speed of 1 cm/s. The force and elongation were measured when the film broke.13) Results were reported as the mean (±S.D.) of five replicates.

The following equations were used:

\[ \text{tensile strength (kg mm}^{-2}) = \frac{\text{force at break (kg)}}{\text{initial cross-sectional area of sample (mm}^2)} \]

\[ \text{elongation at break (% mm}^{-2}) = \frac{\text{increase in length (mm)×100}}{\text{original length (mm)×cross sectional area (mm}^2)} \]

Bioadhesive Force The force required to detach the bioadhesive films from the mucosal surface was applied as a measure of the bioadhesive performance. The method of Singh et al. was used for measuring the bioadhesion strength of the films.5)7) The instrument is broadly composed of a modified two arm physical balance in which the right pan had been replaced by a formulation holding glass plate (10×5 cm) and counter balanced by a water collecting pan suspended to the left arm. The pan received a siphon tube from a 101 bottle, which was kept at a high place in such a way that water head in the bottle always remains above the water collecting pan. The siphon tube bears a flow regulating device. Nylon thread was used to suspend both the glass plate and the pan. An acrylate tissue mounting stage (1.8 cm×1.8 cm×8 cm) was attached to the center of a glass beaker (16 cm diameter and 18 cm height). Glass beaker was filled with phosphate buffer (pH 6.8) to simulate in vivo saliva conditions. A magnetic stirrer provided with temperature control was used to maintain the temperature of phosphate buffer (pH 6.8) in glass dish at 37±0.5 °C. A piece of guinea pig buccal mucosa, 3 cm long, was tightly secured on the upper surface of the acrylate tissue mounting stage with thread. Films were fixed on the centre of the formulation holding glass plate with an adhesive (Fevi Quick®). The exposed film surface was moistened with phosphate buffer (pH 6.8) and left for 30 s for initial hydration and swelling. Then glass plate (with the film) was kept on the mucosal tissue secured on the tissue mounting stage in such a way that films completely remained in contact with mucosa. The whole assembly was kept undisturbed for 3 min (preload time) to establish the adhesion between the film and mucosal tissue. The glass plate (weight 50 g) itself acted as a preload. After the preload time, water collecting pan was suspended to the left arm and water was added in it, by the siphon tube, at a constant rate of 200 drops/min until detachment of the film from mucosal surface took place. A support was kept under the water collecting pan to hold it at the time of detachment. Weight of water collected in the pan at the time of detachment was measured. The experiment was performed in triplicate.

In Vitro Drug Release Studies SS released from the prepared buccal bioadhesive films of SS was determined by introducing single film in modified Franz diffusion cell {(external diameter: 3.0 cm, internal diameter: 2.8 cm, total height of the apparatus: 8.0 cm, height of the receptor compartment: 5.0 cm) with a hat shaped stainless steel wire mesh basket for placing the films (2.6 cm diameter and 1 cm height)} having 30 ml of phosphate buffer (pH 6.8) in receptor compartment. The receptor compartment maintained at 37±0.5 °C was continuously stirred at 100 rpm. Samples were withdrawn at predetermined time intervals of over 8 h and replaced with equal volumes of the dissolution medium equilibrated at the same temperature. Drug concentration of the withdrawn samples was analyzed after filtration (0.45 µm Millipore filter) by UV/Vis Spectrophotometer at 275 nm (Jasco 7800, UV/Vis Spectrophotometer, Tokyo, Japan). All experiments were carried out in triplicate.

Drug Release Kinetics To examine the release mechanism of SS from the prepared buccal bioadhesive films, the results were analyzed according to the following equation.18,19)

\[ M/M₀ = Kₙ^n \]

where \( M/M₀ \) is the fractional drug released at time \( t \), \( k \) is a kinetic constant incorporating structural and geometrical characteristics of the drug/polymer system (source), and \( n \) is the diffusional exponent that characterizes the mechanism of drug release. For non-Fickian release, the \( n \) value falls between 0.5 and 1.0 (0.5≤n<1.0), whereas in the case of Fickian diffusion, \( n<0.45 \); for zero-order release (case II transport), \( n=1 \), and for super case II transport, \( n>1 \).20,21)

In Vivo Efficacy of Buccal Bioadhesive Film of SS Animals: Guinea pigs (weight of 200—300 g) (Institute of Medical Sciences, Banaras Hindu University, India) were used to study the in vivo effect (bronchoprotection on the histamine induced bronchospasm) of buccal bioadhesive films of SS. The animals were kept (housed) at 12:12 light to dark cycle to maintain their biological rhythm. Before experimentation they were fed with standard diet with water ad libitum. All experimental procedures were reviewed and approved by the animals and ethics review committee of Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, India.

Surgical Techniques and Recordings: Guinea pigs were anaesthetized with urethane (dose of 1.3—1.5 g/kg using 0.5 g/ml stock solution) intraperitoneally sufficient to achieve anaesthesia. A maintenance dose (50—100 mg) of anaesthesia was given as required. After being satisfied with the level of anaesthesia, tracheal cannulation was done to keep the respiratory tract patent. The respiratory excursions were recorded by securing the guinea pig skin over xiphistemum and connecting it to a force displacement transducer via a thread. The percent respiratory rate was computed by counting the number of deflections recorded on a chart recorder (Bio Devices, Ambala, India).

Bronchoprotection Effect on the Histamine Induced Bronchospasm: Guinea pigs were divided into four groups for this study (n=6). The modified method of Mohammed et al. was used to study the bronchoprotection effect of buccal bioadhesive films of SS on the histamine induced bron-
The microenvironment pH of different batches showed the acidic nature of CP 940P.23) Folding endurance was found to be more than 300 for each case, indicative of reasonable flexibility of the films.

Swelling Index Studies The swelling state of the polymer (in the formulation) was reported to be crucial for its bioadhesive behaviour. Adhesion occurs shortly after the beginning of swelling but the bond formed between mucosal layer and polymer is not very strong. The adhesion will increase with the degree of hydration until a point where over hydration leads to an abrupt drop in adhesive strength due to disentanglement at the polymer/tissue interface.16)

The swelling profiles of different batches of the films are shown in Table 3. These profiles indicate the uptake of water into the film, producing an increase in weight. The swelling index of the prepared buccal bioadhesive films showed swelling rates in the order: SSB3 > SSB2 > SSB1, indicating that as the concentration of CP 940P was decreased, the swelling index increased. The maximum swelling was attained in 8 h for all the batches SSB1, SSB2 and SSB3.

Mechanical Properties and Bioadhesive Force Table 4 shows the mechanical properties of the prepared drug loaded films. The results show that decrease in CP 940P content reduced both the tensile strength and elongation break significantly, indicative of a weaker and less elastic, less flexible films. The films with high concentration of CP 940P resulting into the formation of hard and brittle films.

Bioadhesive force of the prepared films on guinea pigs buccal mucosa as a function of CP 940P and SCMC ratio have been shown in Table 4. Films containing 24% of CP 940P ratio possessed the highest bioadhesive force (51.76 g); decrease in CP 940P content resulted into a decrease in bioadhesion. The swelling index increased. The maximum swelling was attained in 8 h for all the batches SSB1, SSB2 and SSB3.

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In Vitro Drug Release Studies Figure 1 shows release profiles of the buccal bioadhesive films of SS. The rate and extent of drug release increased (from SSB1 to SSB3) as the concentration of CP 940P decreased in SCMC based films. Sustained release was observed in all the cases which may be attributed to the highly coiled network of CP 940P.22) The difference in cumulative percent release of all the formulations was found significant (p<0.05, one way ANOVA). SSB3 batch showed the highest cumulative percent release (99.20±0.23 after 8 h) which may be attributed to the higher swelling ability of SCMC (Table 3). Pronounced swelling along with erosion of SCMC matrix allowed the drug to diffuse at a faster rate. SSB1 batch showed cumulative percent release from CP 940P.
release of 52.90±0.83 after 8 h which is significantly (p<0.05, one way ANOVA) less than SSB3 batch. The presence of CP 940P in the ratio of 24% decreased the drug release from the SSB1 batch. Based on the slope and intercept values of in vitro release curves, cumulative percent releases were expected to reach 100% for batches SSB1, SSB2 and SSB3 at 15, 10, 8 h, respectively (Fig. 1).

**Drug Release Kinetics**  The values of n as estimated by linear regression of log $M_t/M_\infty$ vs. log(t) of different formulations are shown in Table 5. The n values were between 0.5 and 1.0 for the release of SS from all the film formulations, indicating non-Fickian release kinetics, which is indicative of drug release mechanisms involving a combination of both diffusion and chain relaxation mechanisms (Table 5). The differences in the swelling rate of the buccal film formulations did not change the drug release mechanisms.24–26)

The sustained release rate ($K=13.71$) among all the batches was shown by batch SSB1 containing 24% ratio of CP 940P (Table 5). This batch showed maximum bioadhesive force with moderate swelling rate (Tables 3, 4). The batches SSB2 and SSB3 showed faster release rate ($K=23.37$ and 26.22, respectively) with corresponding increase in swelling rate (Tables 3, 5). As the ratio of CP 940P was reduced the swelling of the buccal films increased (Tables 1, 3). The marked increase in surface area, due to swelling of these buccal films, resulted in a faster drug release rate.

It was concluded that buccal bioadhesive films containing 24% ratio of CP 940P (Batch SSB1) was characterized by moderate swelling rate, maximum bioadhesive force as well as slower rate of in-vitro drug release which are favorable for sustained release buccal film. However, batches SSB2 and SSB3 containing low concentration of CP 940P showed less bioadhesive force and more swelling rate which leads to higher rate of in-vitro drug release when compared with batch SSB1. Therefore, only batch SSB1 was selected for investigation of further in-vivo studies.

**In Vivo Efficacy of Buccal Bioadhesive Film of SS**  In vivo bronchoprotection effect of buccal bioadhesive film of SS was carried out with an animal model—histamine induced bronchospasm (percent respiration rate) in guinea pigs.27) The in vivo effect of SS solution and buccal bioadhesive film of SS are presented in Table 6. Histamine exposure increased the normal percent respiration rate in guinea pigs after the saline administration up to 4 h (Table 6). SS solution significantly (p<0.05, one way ANOVA) reduced the histamine induced percent respiration rate up to 1.5 h when compared to saline control (Table 6). Buccal bioadhesive film of SS significantly (p<0.05, one way ANOVA) reduced the histamine induced percent respiration rate up to 4 h compared to saline control (Table 6). Compared with SS solution, buccal bioadhesive film of SS showed prolonged reduction (from 1.5 to 4 h) of histamine induced percent respiration rate (Table 6). It has reduced nearer to the values of normal percent respiration rate without histamine exposure (Table 6). These results showed the bronchoprotection effect buccal bioadhesive film of SS up to 4 h.

**Conclusions**

All the prepared SS buccal bioadhesive films gave a reasonable bioadhesive force, which is important for prolonging the adhesion of the film with the buccal mucosa, thus improving the overall therapy of asthma. Decrease in CP 940P concentration resulted in increasing the swelling index. Bioadhesive force decreased with the low ratio of CP 940P. Also, decrease in CP 940P content reduced both the tensile strength and elongation break. The prepared buccal bioadhesive films of SS provided a controlled and prolonged in vitro release of SS. Bronchoprotection of buccal bioadhesive films of SS was prolonged up to 4 h. This would be important for better patient compliance because of the decrease in the frequency of administration. Additionally, it may avoid the tolerance formation of SS.

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**Table 5. Linear Correlation Coefficient (r), Determination Coefficients ($r^2$), Kinetic Release Constants (K), and Diffusion Exponents (n) after Fitting the Release Data of SS to the Simple Power Law (log $M_t/M_\infty$ vs. log t)**

<table>
<thead>
<tr>
<th>Batch code</th>
<th>$r^2$</th>
<th>K</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSB1</td>
<td>0.992</td>
<td>13.71</td>
<td>0.6913</td>
</tr>
<tr>
<td>SSB2</td>
<td>0.996</td>
<td>23.37</td>
<td>0.5948</td>
</tr>
<tr>
<td>SSB3</td>
<td>0.998</td>
<td>26.22</td>
<td>0.6812</td>
</tr>
</tbody>
</table>

*a* $n$ is the diffusion release exponent, indicative of the release mechanism; $n=0.5$ in case of the diffusion mechanism, $n=1$ for zero-order release, and for super case II transport, $n>1$. *n* lies between 0.5 and 1.0 (0.5 < $n$ < 1) for non-Fickian (anomalous) release and $n<0.45$ for Fickian release mechanism.

**Table 6. Effect of Buccal Bioadhesive Film of SS (Batch SSB1) on the Histamine Induced Bronchospasm (Percent Respiration Rate)**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>S.S solution (mean±S.D.)*</th>
<th>Batch SSB1 (mean±S.D.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96±4.7</td>
<td>188±4.7</td>
</tr>
<tr>
<td>1.5</td>
<td>92±3.8</td>
<td>190±2.9</td>
</tr>
<tr>
<td>2</td>
<td>97±2.9</td>
<td>191±3.3</td>
</tr>
<tr>
<td>2.5</td>
<td>99±3.3</td>
<td>192±4.7</td>
</tr>
<tr>
<td>3</td>
<td>101±3.9</td>
<td>196±4.8</td>
</tr>
<tr>
<td>3.5</td>
<td>100±4.8</td>
<td>193±1.1</td>
</tr>
<tr>
<td>4</td>
<td>98±3.7</td>
<td>197±3.7</td>
</tr>
</tbody>
</table>

*a* $n=6$. S.D.: standard deviation for six determinations. *p* < 0.05 compared with saline control group using Dunnett’s test, following significant one way ANOVA.
Present study showed the in vivo efficacy of buccal bioadhesive films of SS on the histamine induced bronchospasm. In future, pharmacokinetics studies will be carried out to assess the efficacy of buccal bioadhesive films of SS. The SS concentrations in the biological samples released from the buccal films of SS will be studied in healthy human volunteers.\(^{28}\)

References