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

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Received August 11, 2009; accepted December 18, 2009; published online December 24, 2009

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 β_2-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 adminstration of SS leads to tolerance to its bronchodilator effect.[4,5] These attributes of SS makes it a suitable candidate for controlled buccal delivery. Recent years have seen an increasing interest in the development of novel buccal bioadhesive dosage forms including bioadhesive tablets, gels and films.[6] However, buccal bioadhesive films are preferable over buccal bioadhesive tablets in terms of flexibility and comfort.[7] These buccal bioadhesive films are useful for both for systemic delivery of drugs, as well as for local targeting of drugs to a particular region in the body.[6,8] A wide range of polymers of synthetic, semi synthetic and natural origin like Carbopol, Polycarbophil, 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] Carbopols are excellent bioadhesives but with potential mucosal irritating character.[9] Irritant properties of Carbopols can be reduced by combining it with other non-irritant bioadhesive polymers like SCMC.

To control the delivery rate as well as to improve the bioavailability attempts have been made to deliver SS through transdermal, buccal and nasal route.[10—13]

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.

Experimental

Materials Salbutamol sulphate was obtained as gift sample from Glaxo SmithKline Pharmaceuticals Ltd., Mumbai, India. Sodium carboxymethylcellulose (S.D. Fine Chemicals, Mumbai, India), Carbopol 940P (Loba Chemicals Private Limited, Mumbai, India), poly ethylene glycol 400 (PEG 400) (Qualigens Fine Chemicals, Mumbai, India), histamine (Sigma-Aldrich Chemicals Private Limited, Bangalore, India) and urethane (Merck Chemicals Private Limited, Mumbai, India) were used. All other chemicals were of analytical grade.

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 homogeneous dispersion. The CP 940P solution and required volume of PEG 400 were added to the dispersion of SCMC and stirred for 6 h (Table 1). The gel thus obtained was kept overnight undisturbed under refrigeration to ensure the formation of clear, bubble-free gel which was finally poured on a borosilicate glass mould (10 cm×10 cm×1.5 cm), allowed to settle and dried under convective flow of hot air at a temperature of 40—45 °C for 48—72 h till a flexible film was formed. After drying, the films were cut into smaller pieces of 1 cm×1 cm sizes, wrapped in aluminum foil and stored in glass containers were preconditioned at room temperature and relative humidity of 60%.

Thickness Testing The thickness of ten randomly selected films from every formulation batch was determined using a standard screw gauge.[14]

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Table 1. Formulas for Buccal Bioadhesive Films of SS

Batch code	Amount of SS (mg/cm^2 of the film)	Polymer concentration (% v/v of gel)	Ratio of SCMC to CP 940P	PEG 400 concentration (% v/v of gel)
SSB1	4.2	3	76 : 24	1.5
SSB2	4.2	3	83 : 17	1.5
SSB3	4.2	3	90 : 10	1.5

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).[14)]

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.[14)]

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 milliliter 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 at 275 nm (Jasco 7800, UV/Vis Spectrophotometer, 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 (W_1). Fifteen milliliters 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 (W_2).[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:

$$\text{swelling index} = \frac{(W_2 - W_1)}{W_1} \quad (1)$$

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.[16)] Results were reported as the mean (±S.D.) of five replicates.

The following equations were used:

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

$$\text{elongation at break (\% mm}^{-2}) = \frac{\text{increase in length (mm)} \times 100}{\text{original length (mm)} \times \text{cross sectional area (mm}^2)} \quad (3)$$

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.[7,17)] 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 10 l 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.[7)] 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)]

$$M_t/M_\infty = K_t^n \quad (4)$$

where M_t/M_∞ is the fractional drug released at time t, k is a kinetic constant incorporating structural and geometrical characteristics of the drug/polymer system (device), 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 h 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 anesthesia. A maintenance dose (50—100 mg) of anesthesia was given as required. After being satisfied with the level of anesthesia, tracheal cannulation was done to keep the respiratory tract patent. The respiratory excursions were recorded by securing the guinea pig skin over xiphisternum 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-

Table 2. Physicochemical Characteristics of the Prepared Buccal Bioadhesive Films of SS

Batch code	Thickness (mm) (mean±S.D.[a])	Weight (mg) (mean±S.D.[a])	Drug content (mg) (mean±S.D.[a])	Folding endurance pH (mean±S.D.[a])	Microenvironment (mean±S.D.[b])
SSB1	0.70±0.04	68±1.45	4.12±0.08	>300	5.8±0.50
SSB2	0.65±0.19	65±3.68	4.13±0.10	>300	5.7±0.65
SSB3	0.65±0.09	66±2.35	4.09±0.15	>300	5.5±0.73

a) $n=10$; S.D.: standard deviation for ten determinations. *b*) $n=5$; S.D.: standard deviation for five determinations.

Table 3. Swelling Index of Buccal Bioadhesive Films of SS at Different Time Intervals

Time (h)	Swelling index (mean±S.D.[a])		
	SSB1	SSB2	SSB3
1	0.950±0.160	2.170±0.080	3.310±0.100
2	1.400±0.200	2.900±0.100	4.180±0.160
4	1.990±0.090	4.200±0.190	5.890±0.300
6	2.930±0.160	5.120±0.040	6.900±0.180
8	3.630±0.200	6.000±0.160	7.960±0.090

a) $n=3$; S.D.: standard deviation for three determinations.

Table 4. Mechanical Properties and Bioadhesive Force of the Prepared Buccal Bioadhesive Films of SS

Batch code	Tensile strength ($kg\,mm^{-2}$) (mean±S.D.[a])	Elongation at break ($\%\,mm^{-2}$) (mean±S.D.[a])	Bioadhesive force (g) (mean±S.D.[b])
SSB1	9.48±0.24	36.25±1.70	51.76±0.47
SSB2	4.63±0.11	20.21±1.12	48.79±0.62
SSB3	2.33±0.25	9.88±1.15	47.20±0.95

a) $n=5$; S.D.: standard deviation for five determinations. *b*) $n=3$; S.D.: standard deviation for three determinations.

chospasm.[22] One group ($n=6$) of guinea pigs were treated with saline aerosol using an electronic nebulizer (Nuneb Basic®, MRK healthcare, Mumbai, India) and another two groups were treated with two test compounds (SS solution (1 mg/ml) equivalent to the dose of buccal film (batch SSB1) as aerosol and buccal film batch SSB1 *via* buccal route for buccal absorption). Approximately 34% of SS was released up to 4 h during the *in vitro* release of buccal bioadhesive film of SS (batch SSB1) from its total drug content (4.2 mg) which was equivalent to 1.42 mg of SS. Therefore, similar dose equivalent to 1.42 mg of SS was used in the control aerosol treatment. Thereafter, each guinea pig from all the three groups was placed in a histamine chamber and challenged with a histamine aerosol generated from a 1000 μg/ml solution of histamine, given intratracheally using an electronic nebulizer (Nuneb Basic®, MRK healthcare, Mumbai, India) at different time intervals (1, 1.5, 2, 2.5, 3, 3.5, 4 h later to treatment) and recordings of percent respiration rate were made for a minute. The experiment was repeated three times using another two guinea pigs from all the three groups. Normal percent respiration rate also recorded up to 4 h using one more group ($n=6$) of guinea pigs as a control without histamine exposure.

Statistical Analysis The results obtained with *in vitro* studies were subjected to statistical analysis with one way ANOVA. *In vivo* data were also analyzed with one way ANOVA followed by Dunnett's test (saline control and test compounds). Differences are considered significant at a level of $p<0.05$.

Results and Discussion

Physicochemical Characteristics of SS Buccal Bioadhesive Films The buccal bioadhesive films containing SS were successfully prepared by solvent casting technique. Physical characteristics of the films are summarized in the Table 2. The films from all the batches were translucent and flexible with the thickness ranging from 0.65 to 0.70 mm. Thickness of batch SSB1 was slightly different from the other batches. The higher CP 940P ratio in the buccal batch SSB1 showed a thickness variation when compared with the thicknesses of batches prepared with lower ratios of CP 940P (Table 2). The mass of films ranged from 65 to 68 mg. Drug content uniformity of films ranged from 4.09 to 4.13 mg. Low S.D. in thickness, weight measurement and drug content data reflected no significant difference within the batch.

The microenvironment pH of different batches showed the acidic nature of CP 940P.[23] Folding endurance was found to 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 shows 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. But no statistically significant difference was found in bioadhesive force between SSB1, SSB2 and SSB3 ($p>0.05$, one way ANOVA).

***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 case which may be attributed to the highly coiled network of CP 940P.[12] 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

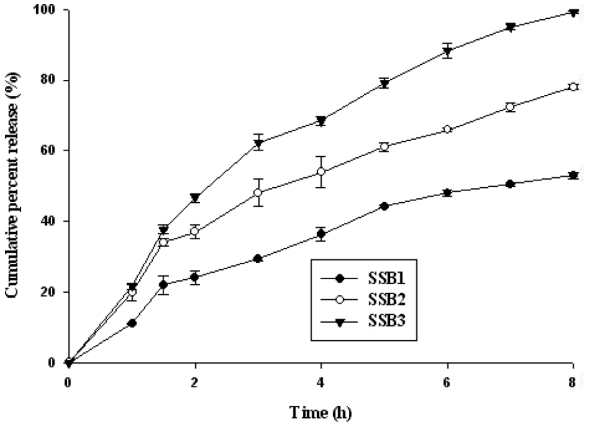

Fig. 1. Release Profiles of SS from Different Buccal Bioadhesive Films in Phosphate Buffer (pH 6.8) ($n=3$)

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 adminstration 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.

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.* $\log t$)

Batch code	r^2	K	$n^{a)}$
SSB1	0.992	13.71	0.6913
SSB2	0.996	23.37	0.5948
SSB3	0.998	26.22	0.6812

a) n=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)

Time (h)	Normal percent respiration rate without histamine exposure (mean±S.D.[a)])	Saline control (mean±S.D.[a)])	SS solution (mean±S.D.[a)])	Batch SSB1 (mean±S.D.[a)])
		Percent respiration rate after 60 s of histamine exposure		
1	96±4.7	188±4.7	88±5.5*	81±3.1*
1.5	92±3.8	190±2.9	127±3.4*	89±2.8*
2	97±2.9	191±3.3	193±4.6	91±4.7*
2.5	99±3.3	192±4.7	199±4.1	83±4.5*
3	101±3.9	196±4.8	192±3.2	79±4.4*
3.5	100±4.8	193±1.1	191±2.6	91±4.6*
4	98±3.7	197±3.7	193±3.4	94±2.2*

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.

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 adminstration. Additionally, it may avoid the tolerance formation of SS.

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)]

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