

Development and Statistical Optimization of Buccoadhesive Films of Amiloride Hydrochloride: *In-vitro* and *Ex-vivo* Evaluation

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ABSTRACT

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The aim of present investigation was to develop an optimized buccoadhesive film of Amiloride hydrochloride (AMHCl), a BCS class III drug, to provide unidirectional sustained drug delivery to the buccal mucosa that has potential to enhance the bioavailability. The films were prepared using HPMC K4M as film former, carbopol 934P as buccoadhesive polymer and dimethyl sulfoxide as penetration enhancer, by solvent casting technique. The films were characterized for various pharmacotechnical parameters and 2^3 full factorial design was employed to study the effect of independent variables. The design was validated by extra design checkpoint formulation (F9). The responses of design were analyzed using Design Expert 8.0.2 and the analytical tools of software were used to draw Pareto charts. On the basis of software analysis, formulation F4 with desirability factor of 0.698 was selected as optimized formulation and was evaluated for independent parameters. Optimized formulation showed 8.3 hr *ex-vivo* residence time, good permeation (41.52%) through goat buccal mucosa and 85.15% drug release after 8hr. The release kinetics of optimized formulation best fitted the Higuchi model. Histopathological studies revealed no buccal mucosal damage. Hence F4 formulation can be concluded as promising drug delivery system to enhance the permeability limited absorption of AMHCl.

Keywords: Amiloride hydrochloride, 2^3 full factorial design, buccoadhesive film, optimization, pareto charts, response surface plots

INTRODUCTION

The oral drug delivery is considered to be the most preferred route by majority of the patients amongst the various available routes of drug delivery. However, oral administration of drugs has certain disadvantages such as hepatic first pass metabolism and enzymatic degradation within the GI tract that prohibits oral administration of various classes of drugs¹. Now-a-days buccal route is available as an alternate for oral delivery of drugs due to its attractive advantages; viz. larger surface area for drug application and good accessibility². Buccal mucosa is relatively permeable with a rich blood supply. Furthermore buccal delivery avoids first pass effect and provides facile removal of dosage form in case of need. Recently developed mucoadhesive buccal delivery systems such as creams³, adhesive tablets⁴, gels⁵, patches⁶, and films⁷. Tablets, films and patches appear to be the most preferred formulations. However, buccal films are preferable over adhesive tablets in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed off and removed by the continuous salivary secretion. Mucoadhesive films appear attractive because they can readily adhere to buccal mucosa;

they are retained for longer period of time and can be removed at any time during the treatment⁸.

Most of the research for drugs with high degree of lipophilicity such as fentanyl⁹ (logP 2.98), lidocaine¹⁰ (logP 1.62), omeprazole¹¹ (logP 2.20), propranolol¹² (logP 3.60) etc. have been reported for buccoadhesive delivery. Therefore, buccoadhesive films of amiloride hydrochloride, a BCS class-III drug were addressed in current investigations, to obtain unidirectional release of the drug, greater surface area of contact, and administer the bitter drug without taste masking^[13]. Because of the properties such as hydrophobicity, low water permeability, drug impermeability, and moderate flexibility, ethyl cellulose was used as a backing layer polymer to prevent drug loss¹⁴. Amiloride hydrochloride (AMHCl); 3,5 diamino-N-(aminoiminomethyl)-6-chloropyrazine carboxamide is a potassium sparing diuretic and antihypertensive agent that acts as Na⁺ channel blocker present at the luminal site¹⁵. It is a BCS class III drug i.e. high solubility and low permeability (log P value -0.76). The drug is incompletely (15 to 20%) absorbed from gastrointestinal tract¹⁶ and consequently results in low oral bioavailability (27%) due to its very low permeability through biological membrane, hence there is need to develop a suitable formulation of AMHCl to improve its bioavailability. AMHCl also has an unpleasant taste and buccoadhesive film is an appropriate approach to administer the bitter drugs without taste masking.

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Commercially only tablet formulations of AMHCl are available in the market while the literature reports available on AMHCl are nano-liposomal dry powder inhaler of AMHCl¹⁷ and liposomal formulation of AMHCl¹⁸ that has limitations like easy washout of the formulation from nose leads to invariable dose administration and thus low bioavailability, while collapsibility and instability of the liposomal formulation respectively also results similar problems. The concept of administration of AMHCl via buccal route by formulating the buccoadhesive film has not been fully explored so far as per best of our knowledge, hence to overcome the above mentioned problems, buccoadhesive films of AMHCl was developed and optimized using 2³ full factorial design aiming to enhance the permeability and consequently bioavailability and do deliver the drug in controlled manner.

MATERIAL AND METHODS

MATERIALS

AMHCl received as gift sample from Panacea biotech Ltd., Chandigarh, India; Ethyl cellulose received from Hercules Aqualon 100 USA; Propylene glycol received from Sigma Aldrich Chemie GmbH, Netherlands; Dimethyl sulfoxide received from s.d fine-chemicals Ltd, Mumbai, India, Dibutyl phthalate received from Qualigen chemicals, Mumbai, India; Hydroxypropyl methyl cellulose and Carbopol 934P received from central drug house, New Delhi, India. All the ingredients were of pure analytical grade.

METHODS

Experimental design

A 2³ randomized full factorial design was used in this study. Three factors were evaluated, each at two levels and

experimental trials were performed on all eight possible combinations (Table 1). The amount of HPMC K4M as film former (X₁), the amount of carbopol 934P as buccoadhesive polymer (X₂) and concentration of DMSO as penetration enhancer (X₃) were selected as independent variables. The percent cumulative drug release (% CDR) at 8th hr, *ex-vivo* residence time and cumulative % permeation at 8th hr respectively were selected as dependent variables. Regression polynomials for the individual dependant variables were calculated with the help of Design Expert 8.0.2 software (Stat-Ease, Inc, USA) and applied to approximate the response surface and contour plots. The general model as shown below was generated-

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + \dots + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 + \dots + B_{123}X_1X_2X_3 + \dots \text{eq. (1)}$$

B₁ is estimated coefficient for the factor X₁, similarly B₂ and B₃ are estimated coefficients for the factor X₂ and X₃ respectively. The main effects (X₁, X₂ and X₃) represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when three factors are simultaneously changed.

Preparation of buccoadhesive films

Backing layer: For preparation of backing layer a glass petri dish of 9 cm diameter was used as a casting surface. Backing membrane of ethyl cellulose was fabricated by slowly pouring a solution containing 500 mg of ethyl cellulose and 2% dibutyl phthalate in 10 ml ethanol to the glass petri dish and air drying for 1 hr.

Buccoadhesive layer containing drug: 3% w/v HPMC K4M was dissolved in 10 ml of ethanol and water (3:2) under constant stirring till a clear solution was obtained. To this 1%

Table 1: 2³ Full Factorial Design of Buccoadhesive Films of AMHCl and the Response Parameters (n=3)

Frmulation Code	Drug (mg)	Independent Variable #			Response Parameter		
		HPMC K4M % w/v (X ₁)	Carbopol 934 % w/v (X ₂)	DMSO % v/v (X ₃)	% CDR at 8 th hr	<i>Ex-vivo</i> residence time (hrs)	Cumulative % Permeation at 8 th hr
F1	5	-1	-1	-1	90.42 ± 0.86	6.5 ± 0.12	34.26 ± 1.20
F2	5	-1	-1	+1	84.60 ± 1.74	7.1 ± 0.17	37.12 ± 0.58
F3	5	-1	+1	-1	87.23 ± 0.78	8.2 ± 0.12	40.31 ± 0.83
F4	5	-1	+1	+1	85.15 ± 1.47	8.3 ± 0.16	41.52 ± 0.97
F5	5	+1	-1	-1	76.52 ± 1.36	7.9 ± 0.12	34.16 ± 0.48
F6	5	+1	-1	+1	73.84 ± 0.94	8.0 ± 0.08	38.54 ± 0.71
F7	5	+1	+1	-1	75.20 ± 0.82	9.1 ± 0.14	41.36 ± 0.82
F8	5	+1	+1	+1	69.18 ± 1.36	9.5 ± 0.17	43.41 ± 0.76
F9 *	5	0	0	0	76.45 ± 1.56	8.0 ± 0.96	37.32 ± 1.42

#Actual values: X₁, +1=600 mg, -1=300mg 0=450mg; X₂, +1=100mg, -1=50mg 0=75mg; X₃, +1=0.6ml, -1=0.3ml, 0=0.45ml

*extra design check point formulation

w/v neutralized carbopol 934P (0.5g f carbopol 934P was neutralized by approximately 0.2g of sodium hydroxide) and 5% v/v propylene glycol was added by stirring using magnetic stirrer. Then sufficient amount of AMHCl was added with stirring so as to have 5mg of drug per 2 cm diameter of film. The mixture was stored at low temperature in order to remove air bubbles. The resultant clear solution was then poured on preformed backing layer of ethyl cellulose and allowed to dry undisturbed for 4 h at 60°C in the oven to ensure complete removal of solvent. The dried film was cut into discs of 2 cm diameter and packed in aluminum foil and stored in desiccators.

PHARMACOTECHNICAL CHARACTERISTICS OF BUCCOADHESIVE FILMS

Film thickness, weight and content uniformity

The thickness of F1-F8 films was measured using screw gauge (Mitutoyo corporation, Kawasaki, Japan) and the weight of films was determined using electronic balance (Sansui, Japan). For content uniformity, the film was dissolved in 100 mL isotonic phosphate buffer pH 6.8 ± 0.2, filtered through 0.4µ nylon disc filter and resultant solution was analyzed by UV spectrophotometer (Pharmaspec 1700, Shimadzu, Kyoto, Japan) at 362 nm. The experiment was performed in triplicate.

Surface pH

The micro environmental pH of F1-F8 formulations was measured so as to predict its effect on buccal mucosa. The formulations were first wetted by adding 1 ml distilled water to its surface. The surface pH was then recorded by bringing a glass electrode near the surface of the formulation and allowing it to equilibrate for 1 min. The average pH ± SD was determined for all formulations.

Swelling index

Buccoadhesive films were weighed individually (designated as W_1) and placed separately in 2% agar gel plates, incubated at 37 ± 1°C and examined for any physical changes. At regular 1-hour time intervals until 3 hours, films were removed from the gel plates and excess surface water was removed carefully using the filter paper. The swollen films were then reweighed (W_2) and the swelling index (SI) was calculated using the following formula:

$$SI = \frac{W_2 - W_1}{W_1} \times 100 \quad \dots \text{eq. 2}$$

The experiment was performed in triplicate and average ± SD values were recorded.

Folding endurance

Folding endurance of the films was determined by repeatedly folding and unfolding the film at the same place till it broke or

for 300 times, which is considered to be a satisfactory value to reveal good folding endurance properties. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

Ex-vivo buccoadhesive Strength

The *ex-vivo* buccoadhesive strength of the prepared films was measured from modified physical balance using the fresh goat's buccal mucosa which was cut into pieces and washed with phosphate buffer pH 6.8. A piece of buccal mucosa was tied in the open mouth of a glass vial, filled with phosphate buffer pH 6.8. This glass vial was tightly fitted into a glass beaker filled with phosphate buffer pH 6.8, 37°C ± 1°C so it just touched the buccal surface. The film was stuck to the lower side of a rubber stopper with cyanoacrylate adhesive. Two pans of the balance were balanced with a 5g weight on the right-hand side pan. The 5g weight was then removed from the left hand side pan, which lowered the pan along with the film over the mucosa. The balance was kept in this position for 5 minutes of contact time. The water was added slowly at 100 drops per min to the right-hand side pan until the film detached from the mucosal surface. The weight in grams required to detach the film from the mucosal surface provided the measure of mucoadhesive strength. The experiments were performed in triplicate and mean ± SD values were reported.

ASSESSMENT OF RESPONSE PARAMETERS

Ex-vivo residence time

The *ex-vivo* residence time was studied (n = 3) after application of film on freshly cut goat buccal mucosa. The fresh goat buccal mucosa was fixed in the inner side of a beaker about 2.5 cm from the bottom. One side of each film was wetted with 1 drop of phosphate buffer pH 6.8 and pasted to the buccal mucosa by applying a light force with a fingertip for 30 seconds. The beaker was filled with 500 ml of phosphate buffer pH 6.8 and was kept at 37°C ± 1°C. After 2 minutes, a 50-rpm stirring rate was applied to simulate the buccal cavity environment, and film adhesion was monitored for 8 hr. The time required for the film to detach from the goat buccal mucosa was recorded as the residence time.

In-vitro drug release

The USP XXIII dissolution apparatus (paddle over disc) was used to study *in-vitro* drug release from buccoadhesive films. 250 ml of phosphate buffer pH 6.8 was used as dissolution medium at 37.0 ± 0.5°C and a rotation speed of 50 rpm was maintained. One side of the buccal film was attached to the glass disk and kept at the bottom of dissolution vessel in inverted position. Aliquots of 5 ml sample were withdrawn at each half an hour intervals and replaced with fresh medium each time to maintain the sink conditions. The samples were filtered through 0.45µm nylon mesh filter paper and were

analyzed at 362nm by UV spectrophotometer. The model dependent parameters were calculated using PCP-Disso-Ver.2.0 software, Pune, India.

***In-vitro* buccal permeation**

The *in-vitro* buccal permeation study of AMHCl through goat buccal mucosa was performed using franz diffusion cell. A specimen of fresh goat buccal mucosa was mounted between the donor and receptor compartments. The film was placed on the mucosa, and the compartments were clamped together. The donor compartment was filled with 1 ml of phosphate buffer pH 6.8. The receptor compartment was filled with isotonic phosphate buffer pH 7.4 maintained at $37.0 \pm 0.2^\circ\text{C}$ and hydrodynamics in the receptor compartment were maintained by stirring magnetically at 50 rpm. Aliquots of 1ml sample were withdrawn at predetermined time intervals and analyzed by UV spectrophotometer at 362nm.

STATISTICAL ANALYSIS OF RESPONSES BY DESIGN EXPERT SOFTWARE

Design Expert 8.0.2 software (Stat-Ease, Inc, USA) was used for the analysis of effect of each variable on the designated response. Pareto charts were made for the analysis of each response coefficient for its statistical significance. Quantitative and qualitative contribution of each variable on each of the response was analyzed. The significant response polynomial equations generated by design expert were used to validate the statistical design^{19, 20}. Response surface plots were generated to visualize the simultaneous effect of each variable on each response parameter. Possible interactions between X1X2, X2X3 and X1X3 were also studied and analyzed.

Validation of experimental design

The polynomial equations were utilized for validation of the experimental design^{19, 20}. An extra checkpoint formulation F9 was prepared with the predicted value of 76.45% for *in-vitro* drug release (%CDR at 8th hr), 37.32% cumulative permeability at 8th hr and 8.0 hr *ex-vivo* residence time. Experimental values were determined by formulating and evaluating F9 and close resemblance between predicted and experimental values indicated validity of the generated model. Finally an optimized formulation was selected on the basis of higher *in-vitro* drug release after 8 hr (%CDR), higher *ex-vivo* residence time, and higher cumulative % permeability at 8th hr with good desirability factor using software analysis.

DIFFERENTIAL SCANNING CALORIMETRY ANALYSIS

Differential scanning calorimetry (DSC) analysis was performed for detecting drug-polymer interaction. DSC

thermograms were recorded on a differential scanning calorimeter equipped with liquid nitrogen sub ambient accessory (Perkin-Elmer). The instrument was operated under nitrogen pure gas at a rate of 20 ml min^{-1} . Approximately 3 to 4 mg of each sample of pure drug, polymers, physical mixture and film were hermetically sealed in a flat-bottomed aluminium pan and heated over a temperature range of 20 to 250°C at a linear heating rate of 10°C/min .

HISTOPATHOLOGICAL STUDY

Histopathological evaluation of goat buccal mucosa tissue (control) incubated in phosphate buffer saline solution pH 6.8 was compared with that treated with buccal film for 8 hr. The tissue was properly washed twice using normal saline solution to remove the adhered tissues and protein. The tissue was fixed with 10% formalin, routinely processed and set in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin. Examine the transverse sections of treated goat buccal mucosa under light microscope to detect any cellular damage to buccal mucosa tissue.

RESULTS AND DISCUSSION

The buccoadhesive films of AMHCl were successfully prepared using HPMC K4M as film former, carbopol 934P as buccoadhesive polymer, propylene glycol as plasticizer and DMSO as permeation enhancer as per experimental design (Table 1). Films consisting of a drug loaded buccoadhesive layer composed of HPMC K4M and carbopol 934P (hydrophilic); and a drug free non adhesive protective layer (hydrophobic); made up of ethyl cellulose. It is important to mention that a perfect binding between buccoadhesive layer and protective backing layers was achieved. All the prepared buccoadhesive films (F1-F8) were characterized for physical characteristics and various pharmacotechnical parameters; and are shown to be uniform in appearance, transparent, flexible and having a smooth surface without entrapped air spaces.

PHARMACOTECHNICAL CHARACTERISTICS OF THE FILMS

The pharmacotechnical characteristics data of the films are shown in (Table 2). Based on the quantities of the HPMC K4M and Carbopol 934P, the thickness of different formulations was found to vary from $1.0 \pm 0.05 \text{ mm}$ to $1.09 \pm 0.05 \text{ mm}$. The weight of the films found to vary from $22.0 \pm 0.16 \text{ mg/cm}^2$ to $47.5 \pm 0.05 \text{ mg/cm}^2$. It can be concluded that as the concentrations of HPMC K4M and carbopol 934P increases, both film thickness and weight also increases. All the films displayed more than 90% drug content that confirming the uniformity of drug content. The surface pH found to ranged from 5.1 ± 0.05 to 6.8 ± 2.02 . The obtained

Table 2: Pharmacotechnical evaluation of buccoadhesive films (F₁-F₈)

Formulation Code	Thickness* (mm) ± SD	Film weight* (mg/cm ²) ± SD	% Drug content* ± SD	Surface pH* ± SD	Swelling index* (%) ± SD	Folding endurance* ± SD	Ex-vivo buccoadhesive strength* (gm) ± SD
F1	1.00 ± 0.05	22.0 ± 0.16	92.17 ± 0.06	5.2 ± 0.03	23.8 ± 2.5	314 ± 9.5	32 ± 2.04
F2	1.03 ± 0.01	26.4 ± 0.36	91.82 ± 0.02	5.1 ± 0.05	21.9 ± 2.6	312 ± 7.5	35 ± 1.94
F3	1.09 ± 0.05	28.0 ± 0.13	93.42 ± 0.05	6.2 ± 0.45	26.5 ± 2.8	343 ± 9.0	40 ± 0.96
F4	1.12 ± 0.01	32.0 ± 0.12	93.92 ± 0.03	6.3 ± 1.02	28.6 ± 2.8	340 ± 5.5	41 ± 1.54
F5	1.15 ± 0.05	37.2 ± 0.12	93.39 ± 0.04	6.2 ± 2.35	23.4 ± 5.7	326 ± 7.0	39 ± 1.06
F6	1.18 ± 0.05	42.3 ± 0.36	89.81 ± 0.03	6.5 ± 0.2	24.6 ± 2.8	324 ± 6.0	40 ± 1.36
F7	1.24 ± 0.05	43.0 ± 0.1	92.67 ± 0.05	6.3 ± 1.26	26.0 ± 2.8	355 ± 2.5	42 ± 1.82
F8	1.28 ± 0.05	47.5 ± 0.05	93.60 ± 0.04	6.8 ± 2.02	27.7 ± 5	358 ± 7.5	44 ± 1.34
F9 #	1.16 ± 0.24	35.4 ± 0.68	93.28 ± 0.06	6.5 ± 1.68	25.8 ± 2.36	308 ± 4.5	35 ± 1.64

*each value in the table is the mean ± SD of three estimations (n=3), #extra design check point formulation

values were close to buccal mucosa pH6.4 hence no mucosal irritation and allergic response can be expected due to formulation.

Swelling study

The swelling study was done to calculate degree of swelling of buccal films (F1-F8) in simulated saliva solution. The films started to swell within 5 min due to presence of swellable HPMC K4M and carbopol 934P, and maximum degree of swelling was observed after 30 min. Percent swelling index was calculated as per equation 2, films containing high level of carbopol 934P (F3, F4, F7, F8) exhibited higher degree of swelling as compared to films containing low level of carbopol 934P (F1, F2, F5, F6). This is due to the concentration based swelling behavior of carbopol 934P, more the carbopol 934P available for swelling, more will be the swelling index which is beneficial for buccoadhesion^[21]. Swelling phenomenon of the polymers makes strong secondary hydrogen bonding with buccal mucosa and thus results in mucoadhesion. Swelling results in the formation of a thick swollen mass which provide unidirectional release of drug in sustained manner.

Folding endurance

All the developed formulations were flexible and displayed good folding endurance ranging from 278.0 ± 7.5 to 358 ± 9.5 due to presence of propylene glycol as plasticizing agent. Carbopol 934P generally is known to increase the softness and flexibility which could be related to its highly crosslinked conformation and configuration¹⁰.

Ex-vivo buccoadhesive strength

The results of *ex-vivo* buccoadhesive strength for AMHCl buccal films are shown in Table 2. The formulations (F1 to F8) exhibited buccoadhesion strength of 32 ± 2.04g to 44 ± 1.34 g,

and the cut off value for buccoadhesion of a dosage form is 33g^[22]. Thus F1 was rejected and among the rest of the formulations containing high level of carbopol 934P (F3, F4, F7, F8) exhibited higher buccoadhesive strength than F2, F5, F6 formulation which may be due to surface adhesion phenomenon as well as due to formation of secondary hydrogen bonds with mucosa as a result of rapid swelling of carbopol 934P²³. Buccoadhesion is also regulated by the addition of HPMC K4M. It has synergistic effect on buccoadhesive strength over carbopol 934P, correspondingly F7 and F8 displayed highest buccoadhesive strength. This may be attributed to the hydrosolubility of HPMC K4M that despite its moderate swelling properties promoted liquid entry and entrapment in the polymer network and thus increased mucoadhesion²⁴.

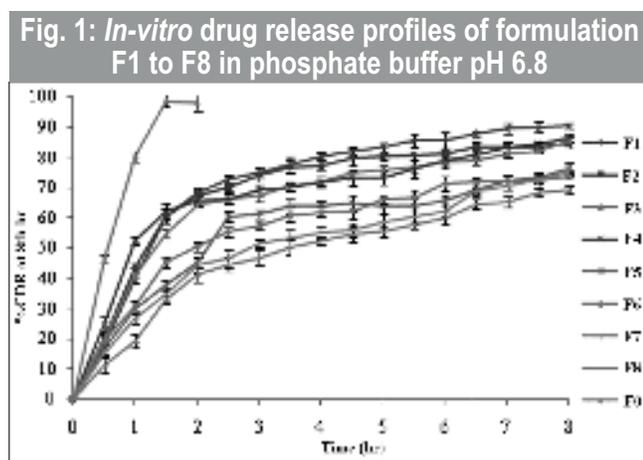
ASSESSMENT OF RESPONSE PARAMETERS

Ex-vivo residence time

The *ex-vivo* residence time of the formulations ranged between 6.5 ± 0.12 to 9.5 ± 0.17 hr. As seen from Table 1, the films containing high level of carbopol 934P (F3, F4, F7, F8) showed higher residence time of 8.2 to 9.5 hr as films containing low level of carbopol 934P (F1, F2, F5, F6) that show residence time of 6.5 to 8.0 hr. This may due to surface adhesion phenomenon as well as due to formation of secondary hydrogen bonds with goat buccal mucosa as a result of rapid swelling of carbopol 934P²³. Among F3, F4, F7, F8 formulations, F7 and F8 show higher residence time than F3 and F4 due to presence of HPMC K4M at high level. Hence it can be concluded that *ex-vivo* residence time increased with increase in the HPMC concentration in the formulation. The addition of DMSO did not affect the *ex vivo* residence time appreciably. The effect of levels of polymers has been statistically discussed in the later sections.

In-vitro drug release

The comparative *in-vitro* drug release profiles of F1-F8 films are shown in Figure 1. Films containing low level of HPMC K4M (F1, F2, F3, F4) displayed higher *in-vitro* drug release ($85.15 \pm 1.47\%$ to $90.42 \pm 0.86\%$) than formulations containing high level of HPMC K4M (F5, F6, F7, F8) that displayed only $69.18 \pm 1.36\%$ to $76.52 \pm 1.36\%$ drug release after 8 hr which may due to increased viscosity offered by the gelling of the hydrophilic HPMC K4M polymer. The increased viscosity of formulation resulted in a corresponding decrease in the drug release. A similar observation has been obtained by Dortune²⁵ where a decrease in atenolol release was obtained on increasing the concentration of HPMC and carbopol 934P.



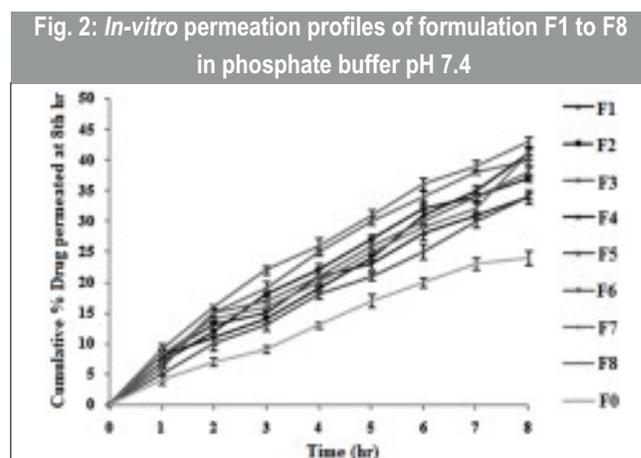
Though highest % CDR of $90.42 \pm 0.86\%$ at 8th hr was recorded for F1, the formulation was rejected based on poor *ex-vivo* residence time, thus F3 was considered as second best formulation in terms of %CDR (87.23%) and least by F8 ($69.18 \pm 1.36\%$) which is showing an inverse relation between concentration of HPMC K4M and *in-vitro* drug release (Table 1). In formulations F1, F2, F3, F4 drug release decrease with increasing the concentration of carbopol 934P. Since carbopol934P is insoluble in simulated saliva and swelling behavior of carbopol934P is attributed to unchanged COOH group that get hydrated by forming hydrogen bonds on imbibing with water and therefore extending polymer chain²⁶. It was observed that films containing combination of high levels of both carbopol 934P and HPMC K4M exhibited delayed drug release indicating better matrix characteristics. Strong matrix integrity inhibits the entry of dissolution media and delays the dissolution of drug. To investigate the release kinetics of drug release from buccal films, the release data was subjected to fit various kinetics models (such as zero order, first order, higuchi, peppas or hixon crowell's model) by using software PCP disso ver. 2.0 Pune, India and value of r^2 , n and k were determined. All buccal films showed 'n' values

in the range of 0.51 to 0.76 indicating that the drug release followed non-fickian diffusion and the best fit model was observed to be higuchi model ($r^2=0.9945$) implying that the films can provide sustained drug release up to 8 hr.

In-vitro buccal permeation

AMHCl being hydrophilic with log P value of -0.76 exhibits low permeability through buccal mucosa and there is a need to enhance its buccal permeation with help of permeation enhancer²⁷ that causes perturbation and dissolution of paracellular fluid, enhancing its paracellular transport²⁸. Based on this fact, different concentrations of DMSO were tried to improve the permeation of AMHCl through buccal mucosa. The results suggested that on increasing the concentration of DMSO up to 6%, permeability of drug increased. As a preliminary investigation F0, film F1 without permeation enhancer (acted as a reference formulation for displaying the influence of permeation enhancer, F0) was prepared as it had low levels of rate controlling polymers based on the assumption that low levels of rate controlling polymers will result in higher drug release facilitating concentration gradient directed permeation across the mucosa as compared to rest of the formulations. *In-vitro* buccal permeation of F0 resulted in % cumulative drug permeation of $24.36 \pm 4.87\%$ at 8th hr (data not shown). Hence it was thought worthwhile to improve its permeation. DMSO has been reported as effective permeation enhancer for enhancing transbuccal diffusion of small and macromolecules like sumatriptan succinate²⁹ and insulin³⁰ respectively. In the present study incorporation of DMSO showed almost two times significant permeation enhancement ($p < 0.05$) across goat buccal mucosa, probably by opening mucosal non-selective porous pathway.

In the experimental design, formulations F2, F4, F6 and F8 containing high level of DMSO showed higher permeation of AMHCl than formulations F1, F3, F5 and F7 which is highlighting the significance of level of DMSO (Figure 2). Amongst all the films containing high levels of DMSO, the descending order for permeability coefficient was



F8>F4>F6>F2 and it can be concluded that proper formulation optimization is essential.

STATISTICAL ANALYSIS OF RESPONSES BY DESIGN EXPERT SOFTWARE

Based on the results obtained for *ex-vivo* residence time, % CDR at 8th hr and cumulative % drug permeation at 8th hr, the response polynomial coefficients were determined in order to evaluate each response. Each response coefficient was studied for its statistical significance by Pareto charts as shown in (Figure 3). Pareto charts establish 't' value of effect that is studied by two limit lines namely the Bonferroni limit line (t value of effect=3.752) and t limit line (t value of effect=2.345). Coefficients with t value of effect above the Bonferroni line are designated as certainly significant coefficients with t value of effect between Bonferroni line and t limit line are termed as coefficients likely to be significant, while t value of effect below the t limit line is statistically insignificant coefficient and should be removed from the analysis. Thus non-significant response coefficients were deleted and the following significant polynomial response equation(s) for *ex-vivo* residence time, %CDR at 8th hr and cumulative % drug permeation at 8th hr were generated^{19,20}.

$$Ex\text{-}vivo\text{ residence time} = 8.08 + 0.55X_1 + [0.70 \times (X_2)] + [0.15 \times (X_3)] + [0.10 \times (X_1X_2X_3)] \dots\dots\dots eq. (3)$$

$$\% \text{ CDR at } 8^{th} \text{ hr} = 79.88 - 6.63X_1 + [-0.88 \times (X_2)] + [-2.13 \times (X_3)]$$

$$+ [-0.88 \times (X_1X_2X_3)] \dots\dots\dots eq. (4)$$

$$\text{Cumulative \% drug permeated at } 8^{th} \text{ hr} = 38.55 - 0.55X_1 + [1.30 \times (X_3)] \dots\dots\dots eq. (5)$$

Validation of experimental design

These equations were utilized for validation of the experimental design. An extra design checkpoint formulation (F9) was prepared and the predicted value(s) for *ex-vivo* residence time, % CDR at 8th hr and cumulative % permeation at 8th hr were generated²⁰. Experimental values were determined by formulating and evaluating F9, and close resemblance between predicted and experimental values indicated validity of the generated model (Table 1 & 3).

Interaction studies and response surface plots

The possible interactions between X₁X₂, X₂X₃, and X₁X₃ for each response were also investigated (Figure 4). Graphically the interactions are visualized by lack of parallelism in the lines but in this case, parallel lines obtained the response parameter *ex-vivo* residence time suggesting lack of interaction between the dependent variables for this particular response. However Cumulative % drug permeated at 8th hr was affected by each interaction term(s), whereas % CDR at 8th hr was predominantly affected by X₁ and X₂. This in turn indicates that the experimental design has maximum efficiency in estimating the main effects. Figure 4 shows the

Table 3: Evaluation of Extra Design Checkpoint Formulation F9 and Optimized Formulation F4				
Response parameter	Formulation code	Predicted value	Experimental value	% RSD
% CDR at 8 th hr	F9	79.88	76.45 ± 1.56	1.75
	F4	84.89	85.15 ± 1.47	1.23
Cumulative % permeation at 8 th hr	F9	38.55	37.32 ± 1.42	0.58
	F4	41.01	41.52 ± 0.97	1.68
Ex-vivo residence time (hr)	F9	6.08	6.00 ± 0.96	0.98
	F4	6.3	6.3 ± 0.16	1.88

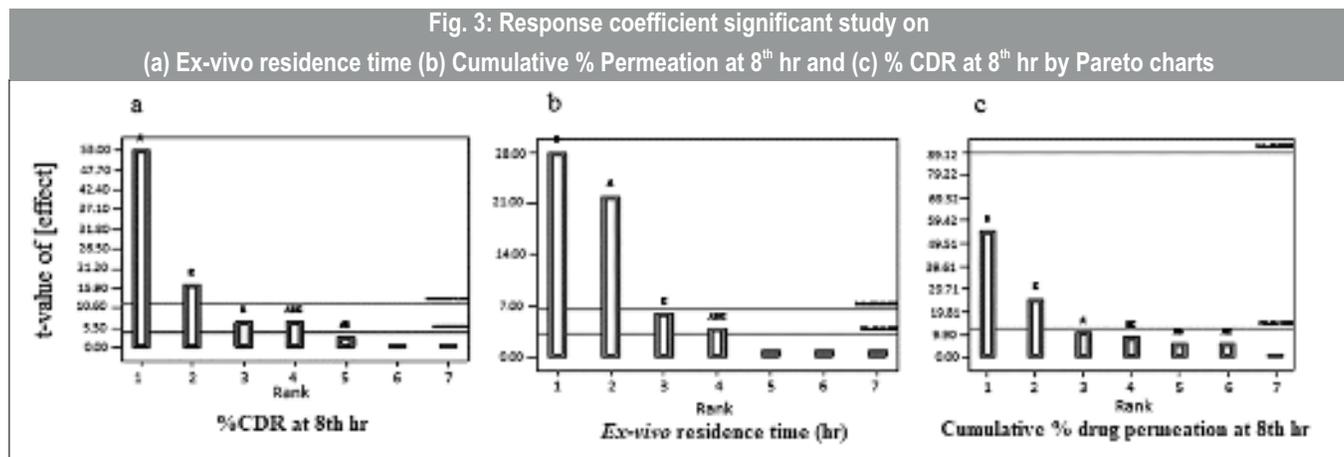
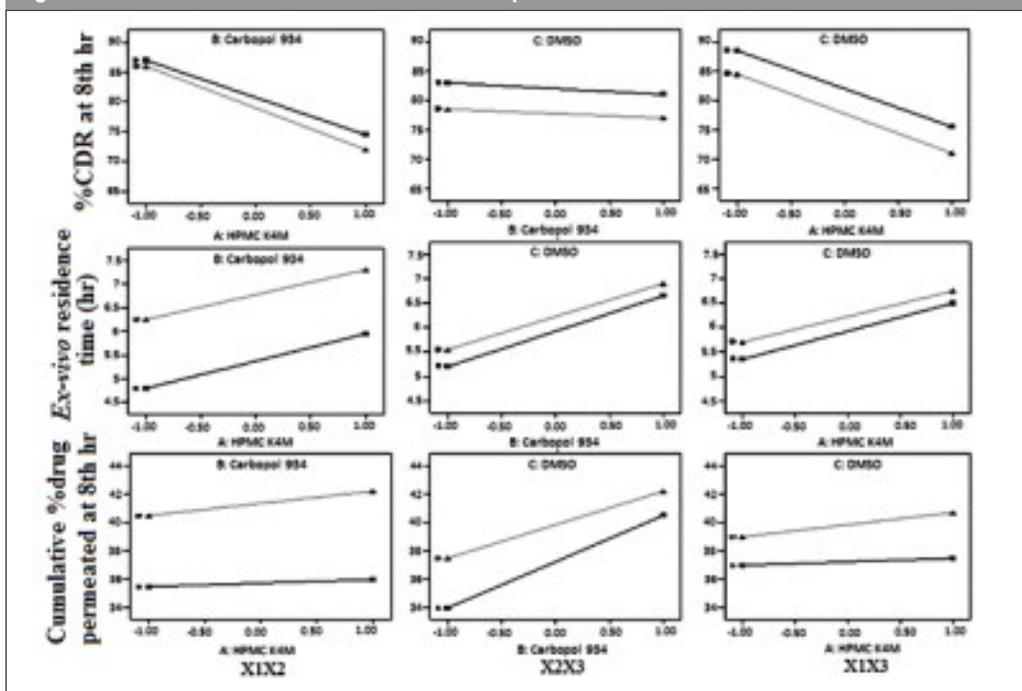


Fig. 4: Interaction studies between various independent variables of buccoadhesive formulations



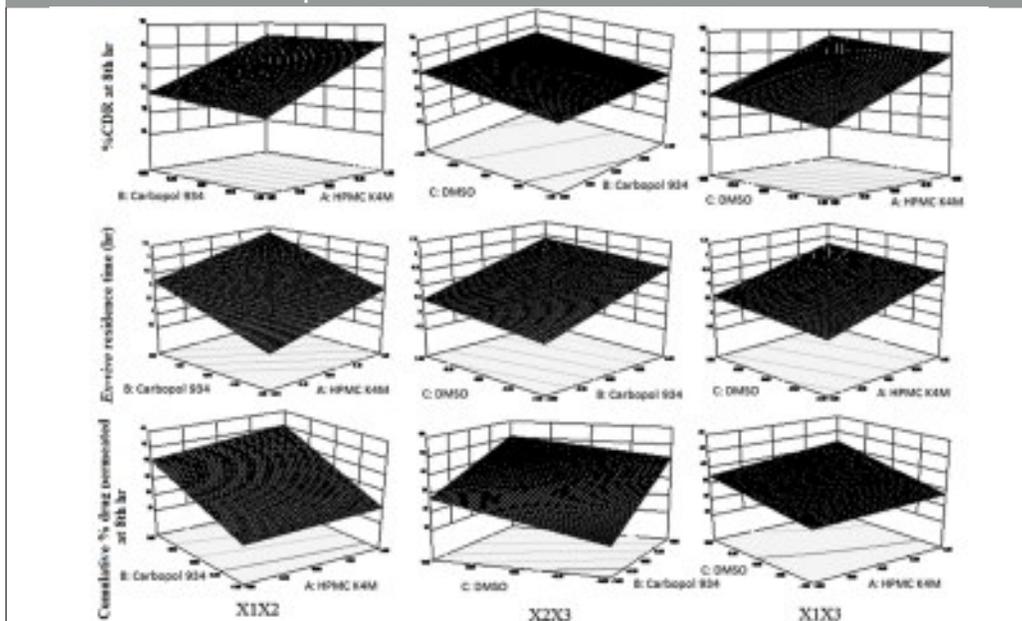
qualitative effect of the variables used and the quantitative effects can be estimated by response surface plots. The response surface plots (Figure 5) generated using polynomial equations represent quantitative simultaneous effect of any two variables on response parameter taking one variable at constant level. The results were similar to interaction studies but were quantifiable. However Design Expert software can analyze both qualitative and quantitative effects of variables

on the response parameters and hence can facilitate selection of optimized formulation.

Selection of optimized formulation

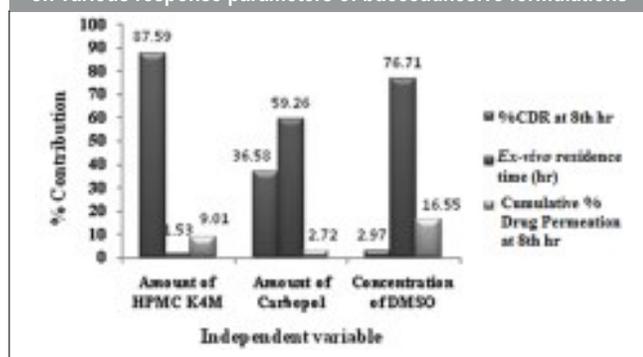
The qualitative and quantitative influence of independent variables on *ex-vivo* residence time, % permeability and % CDR were clearly interpreted from (Figure 6) by Design Expert that is an equally advantageous tool for selection of

Fig. 5: Response surface plots showing influence of independent variables on response parameter of buccoadhesive formulations



optimized formulation. The tool offers the possibility to vary each variable simultaneously and presents possible optimum selections with their respective desirability value^[30]. According to our criteria of higher %CDR at 8th hr, higher residence time and higher cumulative % drug permeated after 8 hr, F4 was selected as optimized formulation (desirability factor of 0.698). Consequently, the coded optimized level for the amount of HPMC K4M, concentration of carbopol 934P and volume of DMSO for F4 were identified as -1, +1 and +1 respectively. These coded optimized values can be converted to actual optimized values by using principles of transformation by use of the following equation^{20,31}.

Fig. 6: Percentage contribution and effect of independent variables on various response parameters of buccoadhesive formulations



$$\text{Coded value } x = \frac{\text{Actual value (X')} - \text{Average of the actual level}}{\frac{1}{2} \text{ difference between actual levels}}$$

The release of AMHCl from F4 best followed Higuchi model ($r^2=0.9829$) and the possible mechanism for drug release might be diffusion of drug from the swollen matrix of HPMC K4M and carbopol 934P.

DIFFERENTIAL SCANNING CALORIMETRY ANALYSIS

Differential scanning calorimetry (DSC) analysis was performed for detecting drug-polymer interaction. DSC thermogram showed sharp endothermic peaks at 65 °C and 75 °C; and a broad endothermic peak at 300°C corresponding to the melting point of HPMC K4M, Carbopol 934P and amiloride hydrochloride respectively (Figure 7). DSC of additive concentrate F4 also displayed endothermic peaks at 65°C, 75°C and 300°C indicated the presence of HPMC K4M, carbopol 934P and amiloride hydrochloride respectively. No other peak was appeared in the DSC of additive concentrate confirmed that there is no interaction between drug and polymers.

HISTOPATHOLOGICAL STUDY

The goat buccal mucosa specimen at the end of permeation study of optimized formulation F4 was subjected to

Fig. 7: DSC analysis of (a) Carbopol 934P (b) HPMC K4M (c) drug (d) physical mixture and (e) Formulation F4.t

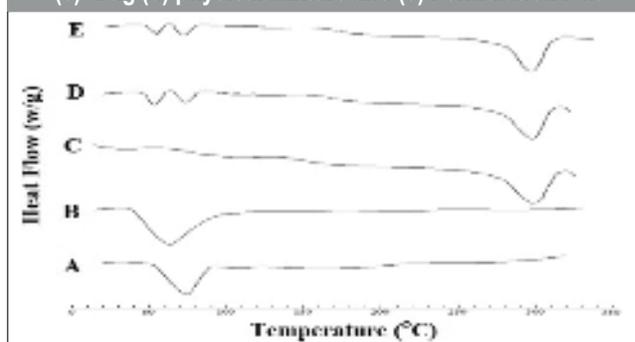
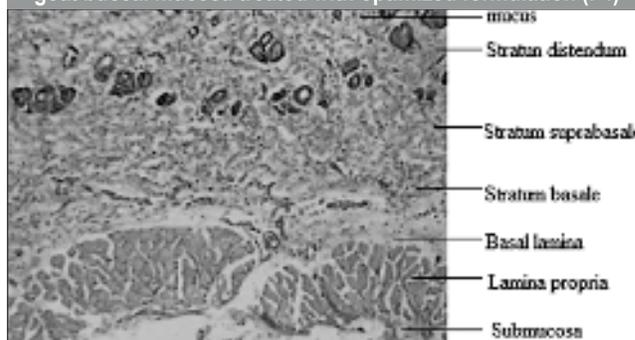


Fig. 8: Histopathological evaluation of transverse section of goat buccal mucosa treated with optimized formulation (F4)



histopathological evaluation. The microscopic observation of the transverse section showed no damage to the buccal mucosa at cellular level (Figure 8). All the layers mucus, stratum distendum, stratum basale, basal lamina and submucosa were found to be intact establishing the non-toxicity of the optimized film.

CONCLUSION

An optimized buccoadhesive film of amiloride hydrochloride was developed that has the potential to enhance the permeability limited bioavailability and to provide a unidirectional sustained drug delivery through the buccal mucosa. The developed buccoadhesive film exhibited sufficient pharmacotechnical properties and buccoadhesive character and was sustained the drug release of highly water soluble drug for 8 hr without causing any damage to the buccal mucosa. However the *in-vivo* performance of the developed formulation should be investigated that will decide its appropriateness in the clinical practice.

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DECLARATIONS OF INTEREST

The authors report no declarations of interest.

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