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Formulation and Statistical Optimization of Fast Dissolving Buccoadhesive Films of an Antihypertensive Drug using Factorial Design

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ABSTRACT

The purpose of this investigation was to develop fast dissolving muco-adhesive buccal films of candesartan cilexetil by solvent casting technique to deliver candesartan into blood via buccal mucosa and to show immediate action using jack fruit gum as novel muco-adhesive polymer. A 2^2 factorial design was considered in optimizing the formulation by taking Jack Fruit Gum (JFG), muco-adhesive polymer and Hydroxy Propyl Methyl Cellulose (HPMC), film forming polymer as two independent variables at two levels (high and low). The response factors considered were tensile strength, bioadhesion force and drug release. Differential Scanning Calorimetry (DSC) analysis revealed no interaction between drug and polymers. Ex vivo diffusion studies were carried out using Franz diffusion cell, while bioadhesive properties were evaluated using porcine buccal mucosa as model tissue. Results revealed that bilayer film containing 0.1% (w/v) JFG and 0.6% (w/v) HPMC in the drug layer and 1% (w/v) Ethyl Cellulose (EC) in backing layer demonstrated diffusion of 94.12% through the porcine buccal mucosa. Thus, this study suggests that Jack fruit gum can act as a potential mucoadhesive polymer for buccal delivery of antihypertensive drug candesartan cilexetil.

Keywords: Interaction plots, Buccal film, Quality by design, Candesartan cilexetil, Jackfruit gum

INTRODUCTION

Fast dissolving delivery systems are gaining much more importance now a day as they offer immediate relief from serious conditions [1]. Buccal route is preferred mostly for the drugs which have poor solubility, dissolution and bioavailability and for the drugs which show high hepatic first pass metabolism. Buccal route is the most convenient route as it is noninvasive and more patient compliance. This is because the buccal mucosa is highly vascularized and the drugs are directly absorbed into blood stream and shows immediate action. Moreover this route can be used for both local and systemic effects [2-4]. As the drug directly reaches the blood, the dose can be minimized. Several buccal adhesive delivery devices have been developed such as tablet, wafers, gels and films. Overall, a muco-adhesive buccal film offers several benefits due to its small size, thickness and improved patient compliance compared to tablets and gels. Buccal films offer more surface area and offers rapid disintegration and rapid absorption [5,6]. The muco-adhesive buccal films adhere to the buccal mucosa and then the films are disintegrated after hydrating in saliva and release the drug. As the film adhered to buccal mucosa the released drug has more chances to get firstly absorb into the blood stream through mucosal layer.

Candesartan is an angiotensin receptor blocker which was commonly used to treat hypertension. It is a Biopharmaceutical Classification System (BCS) Class II drug with 15% oral bioavailability due to its poor solubility [7]. So in the present study an attempt was made to formulate candesartan fast dissolving buccal films by solvent casting technique to improve its bioavailability and to have fastest onset of action. There are several methods to formulate buccal films, solvent casting technique is one of the most common and simplest methods.

Natural polysaccharides have been widely used as bio-adhesive polymers because of their biocompatibility and biodegradability properties. In this study Jack Fruit Gum (JFG), a polysaccharide extracted from the pulp of *Artocarpus heterophyllus* Lam., family Moraceae was used for muco-adhesion. An attempt has been made in the present investigation to utilize JFG, which is abundantly available and a cheap source of polysaccharide in formation of a buccal film of candesartan cilexetil. Thus, the aim of this work was to develop and characterize a fast dissolving muco-adhesive buccal film of candesartan cilexetil using natural polysaccharide JFG.

MATERIALS AND METHODS

Materials

Candesartan cilexetil was obtained as a gift sample from Natco Pharma Ltd. (Hyderabad). Hydroxy Propyl Methyl Cellulose (HPMC) purchased from Noveon Inc. All other chemicals and solvents were of analytical grade.

Experimental design

A 2^2 randomized full factorial design was used for optimization of buccal films. In this model two factors were evaluated, each at two levels (high and low levels). The concentrations of muco-adhesive polymer (JFG) and film forming polymer (HPMC) were selected as independent variables. Tensile strength (Y1), bio-adhesion force (Y2) and % drug release at 40 min (Y3) were selected as response factors. The selected factor levels are summarized in Table 1.

Table 1: Translation of coded levels in actual units

Factor	Coded levels and their actual values	
	Low level (-1)	High level (+1)
Film forming polymer HPMC (%w/v) (A)	0.2	0.6
Mucoadhesive polymer JFG (%w/v) (B)	0.1	0.5

Compatibility of drug and excipients

FTIR analysis

The binary mixtures of drug and various excipients like jackfruit gum, HPMC E 50, used in formulations were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu-FTIR-460 Plus) for determination of interactions. Drug was mixed with excipient in 1:1 ratio and samples were stored for 30 days at 40 ± 2°/75 ± 5% RH. FT-IR spectra of these samples were then obtained after 30 days.

DSC analysis

The compatibility of drug with excipients was also analyzed using Differential Scanning Calorimetry by placing the sample in a DSC crucible, sealed and then analyzed.

Preparation of muco-adhesive buccal films

Muco-adhesive buccal films were prepared by solvent casting method. JFG and HPMC were soaked in 15 ml water for 4 h. and then candesartan cilexetil was dispersed in it. To this aspartame and propylene glycol were added and stirred on magnetic stirrer for 30 min and then sonicated for 30 min. The solution was poured in petri plate of size 7.7 cm in diameter and was dried in vacuum oven at 50°C for 24 h. The backing layer was prepared by ethanolic solution of Ethyl Cellulose (EC) (1%, w/v). The homogenous solution was poured on the dried medicated film. It was dried in vacuum oven at 50°C for 5 h. The dried bilayer films were cut into square pieces of sides 1 cm containing 8 mg of drug per patch**.** Table 2 shows the composition of formulated buccal films.

Table 2: Composition of various buccal film formulations

Ingredients	F1	F2	F3	F4
Candesartan cilexetil	8 mg	8 mg	8 mg	8 mg
HPMC	0.2% w/v	0.2% w/v	0.6% w/v	0.6% w/v
Jack fruit gum	0.1% w/v	0.5% w/v	0.1% w/v	0.5% w/v
Propylene glycol	1.5% w/v	1.5% w/v	1.5% w/v	1.5% w/v
Aspartame	0.125% w/v	0.125% w/v	0.125% w/v	0.125% w/v
Water	Q.S	Q.S	Q.S	Q.S

Ethyl cellulose (1%, w/v) Backing layer on F1–F4 formulations

Characterization of buccal films

Thickness and weight

Screw gauge was used to measure the thickness of films. Three films, each of 1 cm^2 surface area were randomly selected and weighed. Then the average weight of the film was calculated.

Measurement of surface pH

Surface pH of film was determined to check whether the film causes irritation to the mucosa. The surface pH of randomly selected 3 films were measured using pH meter (Equip-Tronics, EQ-614, India) by placing the probe in close contact with the wetted film surface [8].

Folding endurance

Number of times a film can be folded at the same place without breaking or cracking gives the value of folding endurance. This was determined by repeatedly folding the films at the same place until they broke or were folded for 300 times whichever is less [9].

***Ex vivo* mucoadhesion study**

Mucoadhesive strength of all fabricated buccal patches was measured *ex vivo* (n=3) on a modified physical balance using the method described by Gupta et al. [10]. A piece of porcine buccal mucosa was tied to the open mouth of a glass vial filled completely with isotonic phosphate buffer, pH 6.8. The glass vial was tightly fitted in the center of a beaker filled with isotonic phosphate buffer (pH 6.8, temperature, 37 ± 1°C). The patches were stuck to the lower side of the rubber stopper with glue. The mass (in gram) required to detach the patches from the mucosal surface gave the measure of mucoadhesive strength (shear stress).

The following parameters were calculated from mucoadhesive strength:

$$\text{Force of adhesion (N)} = \frac{\text{Mucoadhesive strength}}{1000} \times 9.81$$

$$\text{Bond strength } (Nm^{-2}) = \frac{\text{Force of adhesion}}{\text{Surface area}}$$

In vitro release of candesartan cilexetil from buccal film

The commercially available dialysis membrane (obtained from Sigma Chemicals) was employed for the study, and the *in vitro* drug release study was carried out using a Franz diffusion cell [11]. The effective diffusion area was 1.8 cm^2. The receptor compartment (40 ml) was filled with Phosphate Buffer Saline (PBS), pH 6.8. The patches were fitted between the donor and receptor compartments of the diffusion cell. The drug release was performed at 37 ± 0.5°C, at a stirring speed of 50 rpm using a magnetic stirrer. Five milliliters of the sample from receptor medium was withdrawn at regular intervals and replaced immediately with an equal volume of phosphate buffer saline, pH 6.8. The amount of candesartan released into the receptor medium was quantified by using UV–visible spectrophotometer at 238 nm against a blank.

Ex vivo permeation studies

Ex vivo permeation study of film was carried out on Franz diffusion cells of diffusional diameter 1.76 cm and volume of 7 ml which were placed on six station magnetic stirring unit (Whirlmatic, Spectralab, India) using porcine buccal mucosa. The diffusion was carried out with Phosphate buffer pH 6.8 as receptor media maintained at 37 ± 5°C and was continuously stirred at 300 rpm with the help of a tiny teflon coated needle shaped magnetic. The diffusion was carried out for 30 min. Samples were withdrawn at predetermined time intervals of 5, 10, 15, 20, 30 min. At each time 0.5 ml samples were withdrawn and replaced with fresh phosphate buffer pH 6.8. These aliquots after centrifugation were diluted appropriately and analyzed using UV spectrophotometer (1800, Shimadzu, Japan) at 238 nm [12,13].

Stability studies

Films of formulae F3 were wrapped in a butter paper followed by aluminum foil and kept in an aluminum pouch which was heat sealed at the end and Stored at 30°C and 60% relative humidity. The films were evaluated periodically for percent drug content and time to dissolve the film. Stability studies were carried out for a period of 3 mon.

RESULTS AND DISCUSSION

Experimental trials were performed for all 4 possible combinations by 2^2 randomized full factorial design by using Design Expert software. Mathematical relationships generated for the studied response variables are expressed as Equations 3-5. The formulation layout for the factorial design batches F1–F4 is shown in Table 3.

Table 3: factorial design with corresponding 4 formulations

Formulation	Variable levels in coded form		Bioadhesion force (N)±S.D	Tensile strength (N/m^2) ± S.D	Drug release (%) after 20 min ± S.D
	HPMC	JFG			
F1	-1	-1	0.15± 0.03	83.33± 0.14	84.23± 0.5
F2	-1	+1	0.28± 0.02	155.56± 0.08	78.29 ± 0.6
F3	+1	-1	0.20± 0.03	111.11± 0.04	95.48± 0.4
F4	+1	+1	0.19± 0.01	105.56± 0.11	86.86± 0.8

Compatibility of drug and excipients

FTIR analysis

FTIR analysis of pure drug and drug and excipients were shown in Figures 1a-1c. The FTIR studies do not reveal any additional peak for the drug, indicated that the drug did not interact with excipients used in the films. The pure drug candesartan cilexetil showed characteristic absorption at 2941 cm^{-1}, 1752 cm^{-1}, 1714 cm^{-1} and 1613 cm^{-1}. This absorption peak at 2941 cm^{-1} was due to stretching of C-H bond, the peaks at 1752 cm^{-1} and 1714 cm^{-1} were due to two C-O bonds (carbonyl group) and peak at 1614 cm^{-1} was due to C-N bond. Figure 1a shows IR scan of pure drug and Figure 1b and 1c shows IR scan of pure drug with the excipients, so it was confirmed that there were no drug-excipient interactions.

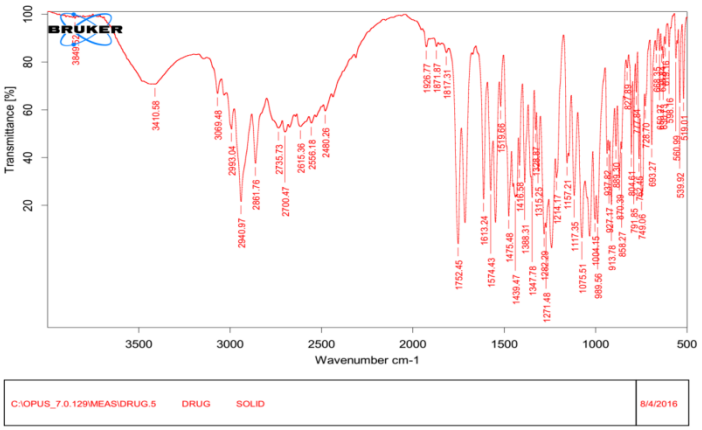

Figure 1a: FTIR of candesartan pure drug

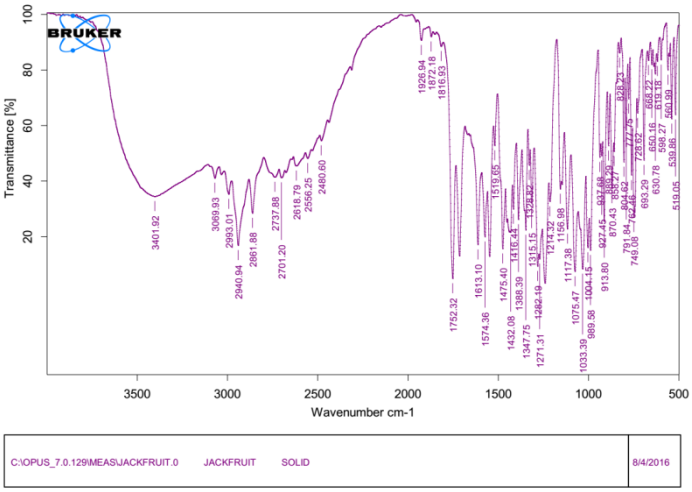

Figure 1b: FTIR of candesartan and jackfruit gum

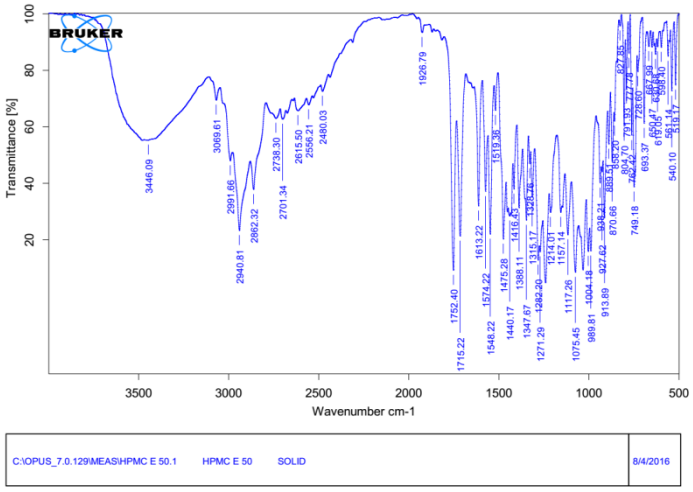

Figure 1c: FTIR of Candesartan and HPMC E 50

DSC analysis

The DSC of pure drug has a peak at 175°C and the drug with all the excipients have also shown the peaks in between 170-175°C. Thus, it confirms that the excipients used were compatible with the drug and can be used for further formulations. The DSC peaks were shown in Figures 2a-2c.

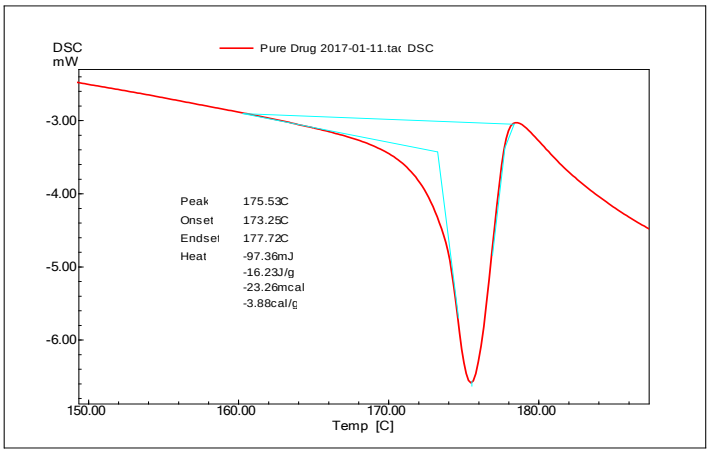

Figure 2a: DSC of pure drug candesartan

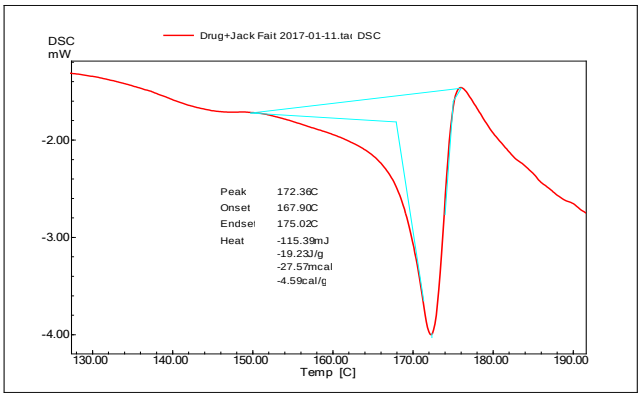

Figure 2b: DSC of pure drug candesartan + jack fruit gum

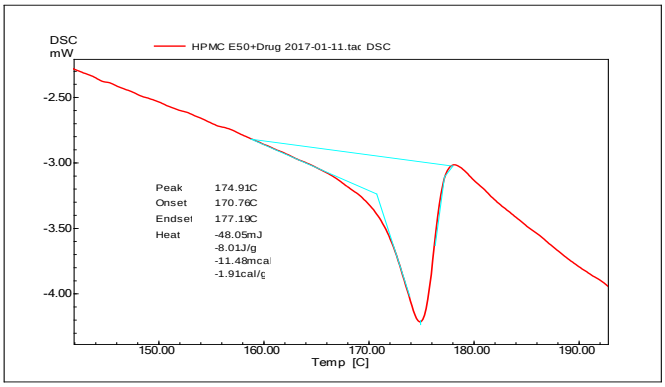

Figure 2c: DSC of pure drug candesartan + HPMC E 50

Characterization of buccal films

Physico-chemical characteristics of the bilayer films were shown in Table 4.

Table 4: Physicochemical characteristics and *Ex vivo* permeation data of the film

Formulation	Thickness (mm) ± S.D	Weight uniformity (g) ± S.D	Surface pH ± S.D	Folding endurance ±S.D	Permeation studies (%) ±S.D
F1	0.21 ± 0.063	0.49 ± 0.006	6.82 ± 0.54	185 ± 1.02	83.15 ± 0.2
F2	0.23 ± 0.076	0.58 ± 0.005	6.65 ± 0.48	190 ± 1.24	79.32 ± 0.6
F3	0.25 ± 0.053	0.59 ± 0.005	6.7 ± 0.60	179 ± 1.13	94.12 ± 0.1
F4	0.27 ± 0.067	0.69 ± 0.006	6.92 ± 0.52	199 ± 1.31	85.09 ± 0.4

Thickness and weight

The average thickness of all prepared buccal films ranged from 0.21-0.27 mm. Weight variation values (g) of film (1 cm^2) for formulations F1-F4 were found to be between 0.4 and 0.7 g. As the thickness of the films increases, proportional gain in weight of films was observed. This depicts uniform film casting.

Measurement surface pH

Surface pH for formulation F1–F4 was found to range from 6.65-6.92 which were in the range of salivary pH (6.5-7.2). Thus, no mucosal irritation was expected.

Folding endurance

As the film forming polymer concentration increases there observed an increase in folding endurance. Folding endurance values for films indicates high mechanical strength of these films. This is highly desirable because it would not allow easy dislocation of the films from the site of application or breaking of film during administration.

Effect of formulation variables on *in vitro* bioadhesion force

This is an important property as it ensures delivery of drug at the site of administration. The bioadhesion force was found to increase with increase in concentration of jack fruit gum and decrease in concentration of HPMC. The ANOVA for the response bioadhesion force was given in the Table 5. The polynomial equation for R1 (Bioadhesion force) in terms of coded and actual factors were given in Equations 1 and 2.

From the equations and the plots obtained it was clear that HPMC and HPMC-JFG combination has negative effect and JFG has positive effect on bioadhesion force. Bioadhesion Force of buccal films increases with increase in jack fruit gum concentration and decreases with HPMC. The half normal, interaction, contour, 3D surface and predicted vs actual plots for the response factor bioadhesion force were given in Figures 3a-3e.

Table 5: ANOVA for response bioadhesion force (Response 1)

Summary output	
Regression statistics	
Multiple R	0.670402
R Square	0.449438
Adjusted R square	-0.65169
Standard error	0.07
Observations	4

ANOVA						
	df	**SS**	**MS**	**F**	**Significance F**	
Regression	2	0.004	0.002	0.408163	0.741999	
Residual	1	0.0049	0.0049			
Total	3	0.0089				
	Coefficients	**Standard error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	0.18	0.09424	1.910009	0.307052	-1.01744	1.377438
HPMC	-0.05	0.175	-0.28571	0.822829	-2.27359	2.173586
JFG	0.15	0.175	0.857143	0.548875	-2.07359	2.373586

Final equation in terms of coded factors: R1=+0.21 -0.010* A +0.030* B -0.035* AB (1)

Final equation in terms of actual factors: Bioadhesion force=+0.075000+0.21250* HPMC+0.50000* JFG-0.87500* HPMC* JFG (2)

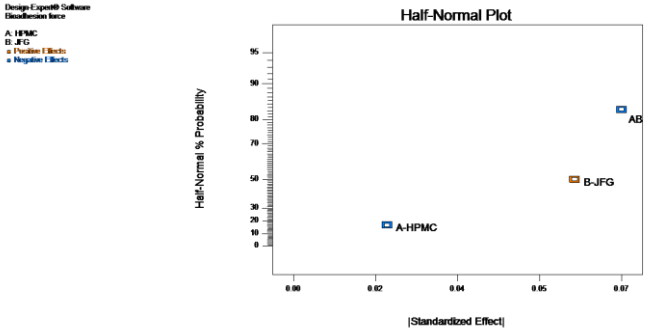

Figure 3a: Half normal plot for bioadhesion force as response

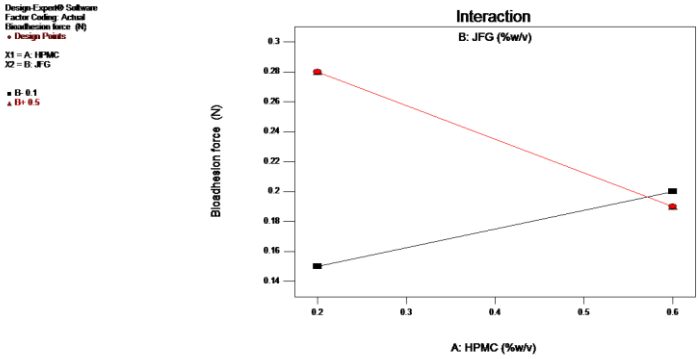

Figure 3b: Interaction plot for biaoadhesion force as response

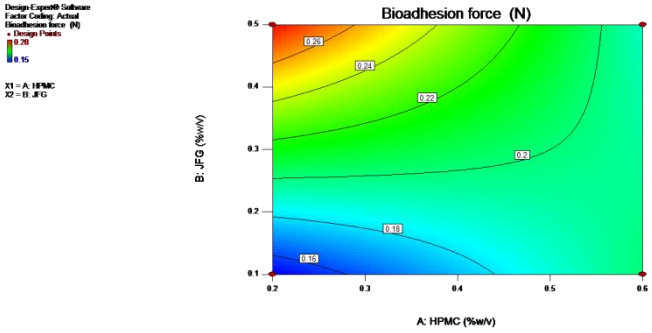

Figure 3c: Contour plot for biaoadhesion force as response

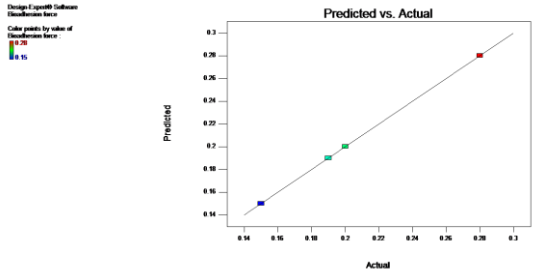

Figure 3d: Predicted vs actual graph for biaoadhesion force as response

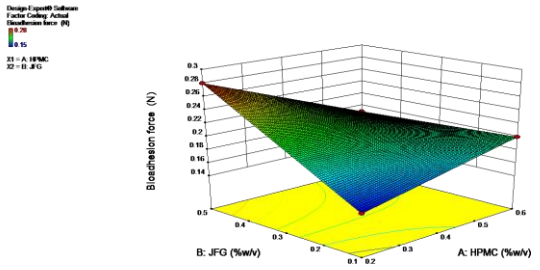

Figure 3e: 3D surface plot for biaoadhesion force as response

Effect of formulation variables on tensile strength

The observation of the data for all formulations for tensile strength (Table 6) depicts that films have sufficient strength to withstand wear and tear occurring during administration and transportation. The ANOVA for the response tensile strength was given in the Table 6. The polynomial equation for R2 (tensile strength) in terms of coded and actual factors were given in Equations 3 and 4. From the equations and the plots, it was clear that HPMC and HPMC-JFG combination has negative effect and JFG has positive effect on tensile Strength. Tensile strength of buccal films increases with increase in jack fruit gum concentration and decreases with HPMC. The half normal, interaction, contour, 3D surface and predicted vs actual plots for the response factor tensile strength were given in Figures 4a-4e.

Table 6: ANOVA for response tensile strength (Response 2)

Summary output	
Regression Statistics	
Multiple R	0.670454
R Square	0.449508
Adjusted R Square	-0.65148
Standard Error	38.89
Observations	4

ANOVA						
	df	**SS**	**MS**	**F**	**Significance F**	
Regression	2	1234.988	617.4939	0.408279	0.741951	
Residual	1	1512.432	1512.432			
Total	3	2747.42				
	Coefficients	**Standard error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	99.995	52.35726	1.909859	0.307072	-565.267	765.2571
HPMC	-27.775	97.225	-0.28568	0.822851	-1263.14	1207.586
JFG	83.35	97.225	0.85729	0.548821	-1152.01	1318.711

Final equation in terms of coded factors: R2=+113.89-5.55* A+16.67* B-19.45* AB …(3)

Final equation in terms of actual factors: Tensile strength=+41.66000+118.06250* HPMC+277.80000*JFG-486.12500*HPMC *JFG …(4)

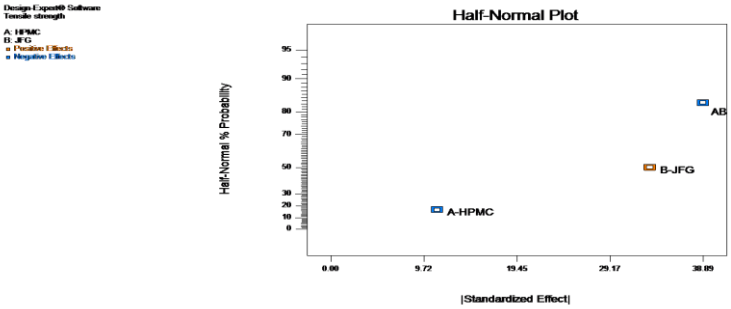

Figure 4a: Half normal plot for tensile strength as response

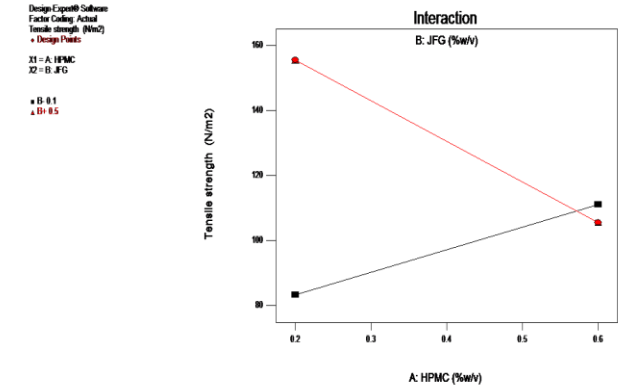

Figure 4b: Interaction plot for tensile strength as response

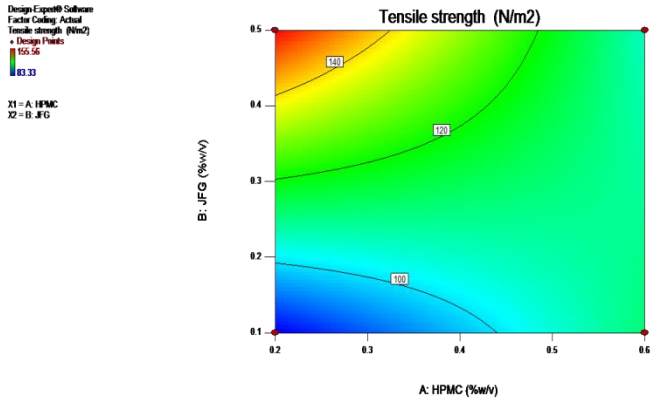

Figure 4c: Contour plot for tensile strength as response

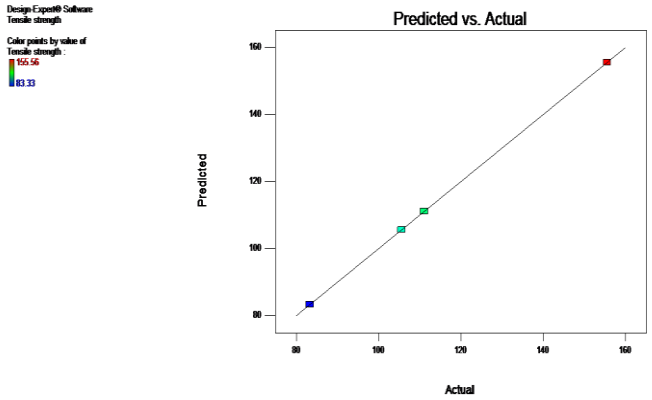

Figure 4d: Predicted vs Actual graph for tensile strength as response

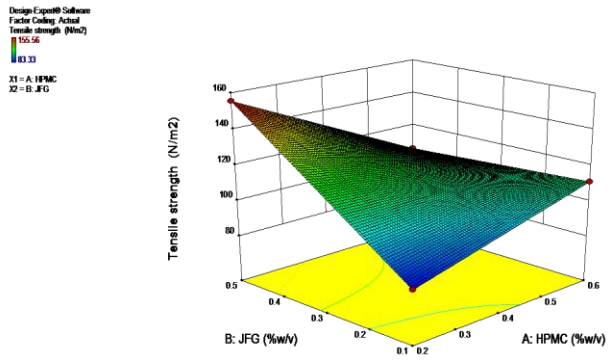

Figure 4e: 3D surface plot for tensile strength as response

Effect of formulation variables on *in vitro* release of candesartan cilexetil from buccal film

The ANOVA for the response drug release was given in the Table 7. The polynomial equation for R3 (drug release) in terms of coded and actual factors were given in Equations 5 and 6. From the equations and the plots, it was clear that JFG and HPMC-JFG combination has negative effect and HPMC has positive effect on bioadhesion force. The cumulative percentage release increased with increase in concentration of HPMC. The half normal, interaction, contour, 3D surface and predicted vs actual plots for all the three response factors were given in Figures 5a-5e.

Table 7: ANOVA for response drug release (Response 3)

Summary output	
Regression statistics	
Multiple R	0.994115
R Square	0.988264
Adjusted R Square	0.964793
Standard error	1.34
Observations	4

ANOVA						
	df	**SS**	**MS**	**F**	**Significance F**	
Regression	2	151.2065	75.60325	42.10473	0.108332	
Residual	1	1.7956	1.7956			
Total	3	153.0021				
	Coefficients	**Standard error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	81.765	1.80403	45.32352	0.014044	58.84262	104.6874
X Variable 1	24.775	3.35	7.395522	0.085563	-17.7908	67.34079
X Variable 2	-18.2	3.35	-5.43284	0.115883	-60.7658	24.36579

Final equation in terms of coded factors: R3=+86.22+4.95*A-3.64*B-0.67*AB …(5)

Final equation in terms of actual factors: Drug release=+79.75500+29.80000* HPMC-11.50000* JFG-16.75000*HPMC *JFG … (6)

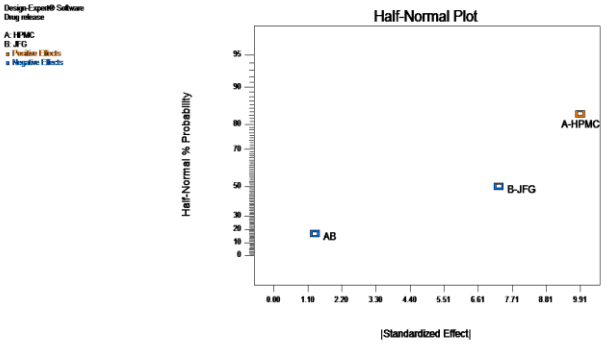

Figure 5a: Half normal plot for drug release as response

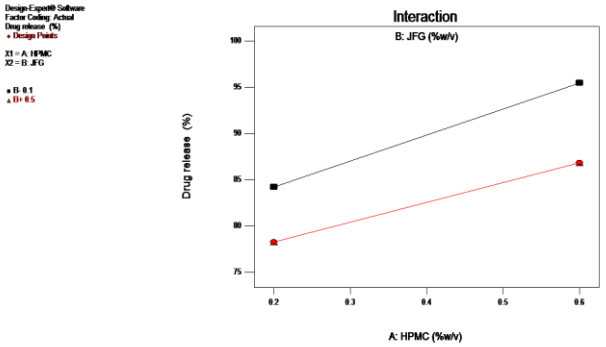

Figure 5b: Interaction plot for drug release as response

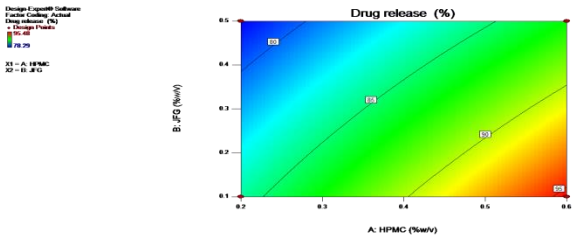

Figure 5c: Contour plot for drug release as response

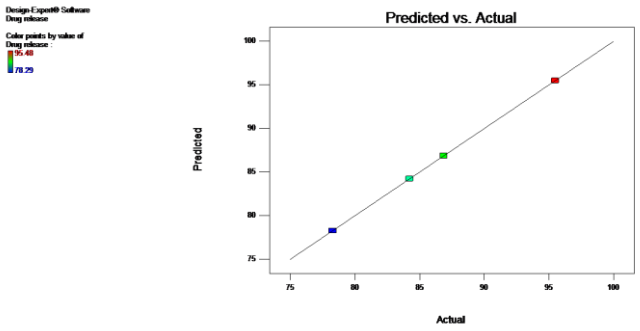

Figure 5d: Predicted vs actual graph for drug release as response

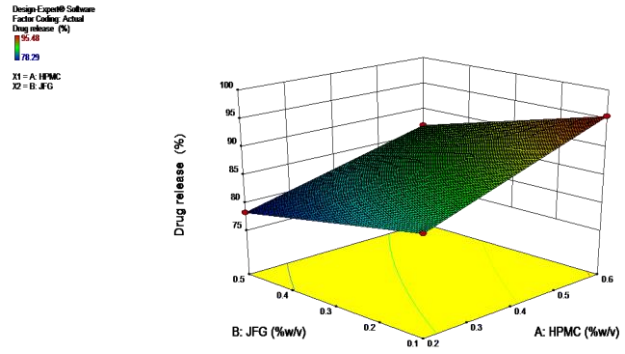

Figure 5e: 3D surface plot for drug release as response

Ex vivo permeation studies

The permeation profiles of candesartan across porcine buccal mucosa were shown in Table 4. Films containing higher percentage of HPMC provided greater amount of permeated drug than other formulations. Formulation F3 showed highest diffusion of around 94% at the end of 30 min. The tensile strength and bioadhesion force were also higher for F2 and F3 formulations compared to other formulations.

In vitro drug release studies

This observation can be correlated with *in vitro* drug release profiles, which influences drug availability at the absorption site. Though both F2 and F3 showed better tensile strength and bioadhesion force, F3 was selected as optimized formula due to more drug release than F2. The higher drug release and thus higher permeation of F3 may be due to presence of higher amount of water soluble film forming polymer HPMC. The formulation F4 also contained higher concentration of HPMC but the release was less because it also contained higher amount of water insoluble mucoadhesive polymer jack fruit gum which may retard the drug release from the formulation.

In vitro drug release data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetics and according to Higuchi and Peppas models to ascertain mechanism of drug release. From the results, which were shown in Table 8 it was evident that all the formulations displayed first order release kinetics. Higuchi and Peppas models reveals that drug release is by non-Fickian diffusion (n values from 0.55-0.88). The plots were shown in the Figures 6a-6d.

Table 8: Release kinetics of various formulations

Formulation	Zero order plot R2	First order R2	Higuchi plot R2	Peppas plot R2	Peppas plot n value
F1	0.967	0.991	0.938	0.965	0.62
F2	0.974	0.994	0.933	0.972	0.55
F3	0.936	0.976	0.953	0.955	0.88
F4	0.963	0.989	0.943	0.969	0.71

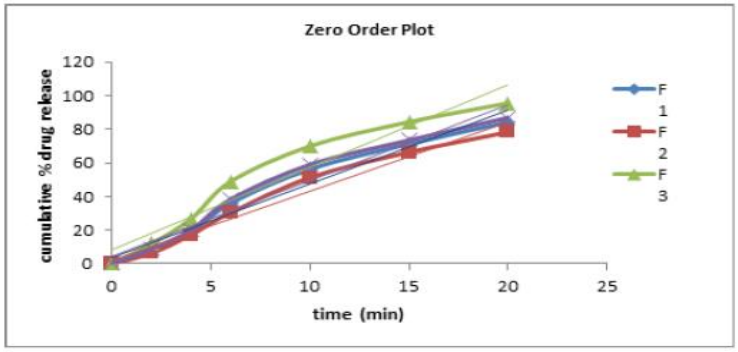

Figure 6a: *In vitro* release of candesartan cilexetil from buccal film (Zero order plot)

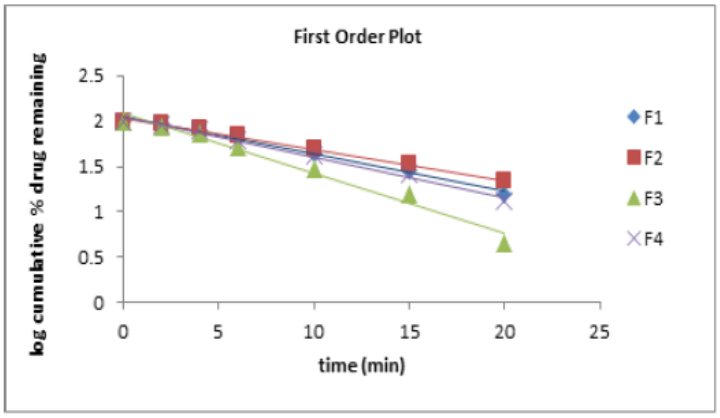

Figure 6b: *In vitro* release of candesartan cilexetil from buccal film (First order plot)

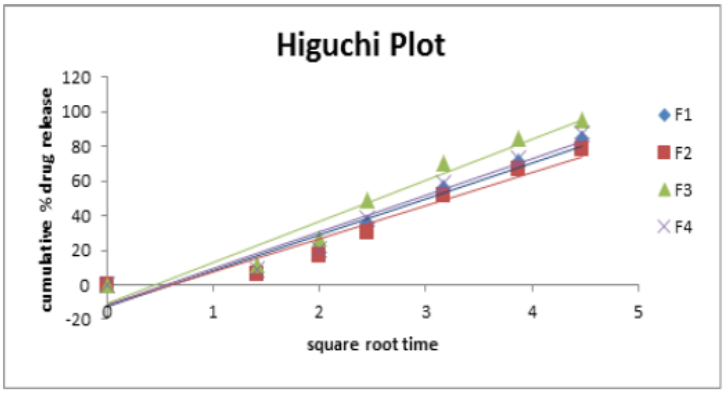

Figure 6c: *In vitro* release of candesartan cilexetil from buccal film (Higuchi Plot)

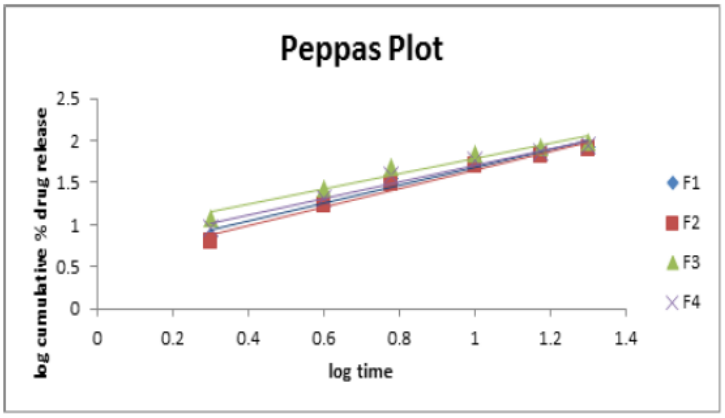

Figure 6d: *In vitro* release of candesartan cilexetil from buccal film (Peppas Plot)

Optimization

The computer optimization technique by the desirability approach was used to produce the optimum formulation. The process was optimized for the response variables R1-R3. The optimized formula was arrived by setting maximum percentage drug release at 20 min with a better bioadhesion force and tensile strength. Formulation F3 was found to be optimized formulation.

Stability studies

The films did not show any statistically significant change in appearance, % drug content, and disintegration time on storage. The % drug content and disintegration responses were same as that of the responses before the storage. This indicated that F3film was stable after storage.

CONCLUSION

In the present study, fast dissolving mucoadhesive buccal film of candesartan cilexetil were prepared using jack fruit gum and hydroxyl propyl methyl cellulose, which released the drug within 20 min, which would prevent first-pass metabolism to a large possible extent. ¿Bilayer films were prepared by 2^2 level factorial design and effect of formulation variables on drug release, bioadhesion force and tensile strength were analyzed by applying the computer optimization technique. Based on the results for dependent variables, formulation F3 was found to be optimal formulation.

Thus, an attempt of formulating a stable fast dissolving mucoadhesive buccal film of candesartan for treatment of hypertension using novel polysaccharide polymer jack fruit gum was made by optimization technique. Jack fruit gum showed good bioadhesion along with film forming polymer HPMC. Thus, cheap and abundantly available natural polysaccharide JFG could be a promising vehicle for systemic delivery of drugs like candesartan cilexetil through buccal route. The *in vitro* studies have shown that this is a potential drug delivery system for candesartan with considerable release profile. But, *in vivo* studies in future would be needed to confirm these results.

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