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Buccal Mucoadhesive Films Containing Antihypertensive Drug: *In vitro*/*in vivo* Evaluation

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ABSTRACT

Mucoadhesive drug delivery systems for diltiazem hydrochloride in the form of buccal films were developed and characterized for improving bioavailability. Several hydrophilic and hydrophobic film forming polymers either alone or in combination with bioadhesive polymers were used for film fabrication. The bioadhesive polymers studied were sodium carboxymethyl cellulose (SCMC), hydroxypropyl cellulose (HPC). Prepared films were evaluated for various physicochemical characteristics such as weight variation, thickness, drug content uniformity, folding endurance, surface pH, and in vitro drug release. The in vitro mucoadhesive strength and permeation studies were performed using chicken pouch mucosa. Further, in vivo testing of mucoadhesion time and acceptability were performed in human subjects. Results indicated that drug release, swelling index and mucoadhesion performance were found to depend upon polymer type and proportion. The majority of the developed formulations presented suitable adhesion and the mechanism of drug release was found to be non-Fickian diffusion. Good correlation was observed between in vitro drug release and in vitro drug permeation with correlation coefficient ranged between of 0.945 to 0.980. In addition, from healthy human volunteers, bioadhesive behavior were found to be satisfactory. Drug bioavailability of a selected diltiazem hydrochloride adhesive buccal film, F26 (1% HPC and 2%SCMC) was determent and compared with that of a commercial sustained release oral tablet (Altiazem® RS) as a reference formulation. The obtained C_{max} and $AUC_{0-\infty}$ values were higher for buccal administration than oral administration and the difference was statistically significant ($p < 0.05$). The percentage relative bioavailability of diltiazem hydrochloride from the selected buccal mucoadhesive film in rabbits was found to be 165.2%.

Keywords: Mucoadhesive films; Diltiazem hydrochloride; Buccal delivery; chicken pouch membrane; Relative bioavailability.

INTRODUCTION

Although the oral administration of drugs has been the preferred route of administration for the patients and clinicians, certain disadvantages such as hepatic first pass metabolism, gastric irritation, and enzymatic degradation within the gastrointestinal tract have been identified [1]. The buccal route has been advocated as an alternative route of administration for drugs which undergo extensive hepatic first pass metabolism or which are susceptible to degradation and presystemic metabolism in the gastrointestinal tract [1, 2]. This route is well vascularized with venous blood draining the buccal mucosa reaching the heart directly via the internal jugular vein. Moreover, buccal delivery for the transmucosal absorption of drugs into the system circulation provides a number of advantages such rapid onset of action, sustained delivery, high permeability, high blood flow, and is easily accessible for both application and removal of a drug delivery device [2, 3].

Recently, various mucoadhesive mucosal dosage forms have been developed, which included adhesive tablets [4, 5], gels [6], ointments [7], and more recently films [8, 9]. Adhesive buccal film may be preferred over adhesive tablet in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which is easily washed away and removed by saliva. Moreover, buccal films also ensure more accurate dosing of drugs when compared to gels and ointments [10].

Diltiazem hydrochloride (DH), a benzothiazepine calcium channel antagonist agent has been widely used in the treatment of stable, variant and unstable angina pectoris, mild to moderate systemic hypertension and many other cardiovascular disorders, with a generally favorable adverse effect profile. Diltiazem hydrochloride is subjected to an extensive and highly variable hepatic first pass metabolism by CYP3A4 followed by an oral administration and the absolute bioavailability is approximately 40%, with a large inter individual variation. The interindividual variation may be explained by a variable first pass effect [11-13]. The short half-life value of diltiazem hydrochloride (3-5 hours), low molecular weight, optimum log partition coefficient (2.79) [14], and its extensive and highly variable first pass metabolism following oral administration make it a suitable candidate for administration by the buccal route to avoid the hepatic first pass metabolism.

The aim of this study was, therefore, to formulate and evaluate buccal mucoadhesive films for improving bioavailability of diltiazem hydrochloride. The new buccal mucoadhesive films were prepared using several film-forming polymers, as sodium alginate (SALG), hydroxypropylmethyl cellulose (HPMC), polyvinylalcohol (PVA), Eudragit NE30D and Eudragit L100 . Among various possible bioadhesive polymers, sodium carboxymethyl cellulose (SCMC) and hydroxypropyl cellulose (HPC) were selected in this study. In order to prepare films having the appropriate characteristics, film-forming polymers were initially used alone and successively in combination with bioadhesive polymers. Effect of polymer type, proportion and combination were studied on drug release rate; release mechanism, mucoadhesive strength, adhesion time and drug permeation to assess the suitability of the prepared formulations. *In vivo* bioavailability and acceptability studies were carried out in rabbits and healthy human volunteers, respectively.

EXPERIMENTAL SECTION

2.1. Materials

Diltiazem hydrochloride (DH), Hydroxypropyl methyl cellulose (HPMC), Hydroxypropyl cellulose (HPC, low viscosity) and moxifloxacin hydrochloride (internal standard) were kindly supplied by the Egyptian International Pharmaceutical Company (EIPICO, Egypt); Eudragit NE 30D and Eudragit L100 were from Rohm Pharma (Darmstadt, Germany); sodium alginate (SALG) and Polyvinyl alcohol (PVA, Hot water soluble) were from Loba Chemie (Mumbai, India); sodium carboxymethyl cellulose (SCMC, low viscosity), propylene glycol, sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate were from El-Nasr Pharmaceutical Chemicals Co., (Cairo, Egypt); diethyl ether (Norway); potassium dihydrogen phosphate, HPLC Grade (Merck, Germany); ortho phosphoric acid, HPLC Grade (Merck, Germany); acetonitrile and methanol were HPLC grade (Merck, Germany). All other chemicals were of analytical grade, and water used in this assay was doubly distilled.

2.2. Preparation of Diltiazem hydrochloride films:

Solvent casting method [15, 16] was used to prepare buccal mucoadhesive films of DH using several hydrophilic and hydrophobic film forming polymers either alone or in combination with bioadhesive polymers. SALG, HPMC, and PVA are hydrophilic, film-forming polymers and Eudragit NE 30D and Eudragit L100 are hydrophobic, film-forming polymers. The bioadhesive polymers studied were sodium carboxymethyl cellulose (SCMC) and Hydroxypropyl cellulose (HPC) and they were used to prepare buccal films with tow different concentrations (1 and 2%). Propylene glycol was used as a plasticizer. The composition of the assayed formulations is given in Table 1.

2.2.1. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing HPMC as a film-forming polymer:

The required amount of DH was dissolved in the required amount of distilled water containing 50% (w/w) propylene glycol with constant stirring. Subsequently, the weighed quantity of the HPMC (2%, w/v), was mixed with the bioadhesive polymer. The mixture then was gradually added to the solution with constant stirring. Once it was fully hydrated and gel consistency was obtained, the medicated gel was left overnight at room temperature to ensure clear, bubble-free gel. The gel was cast into glass petri dish (7 cm diameter, 10 mm depth) and allowed to dry at 40^{o}C in an oven until a flexible film was formed. The dried film was carefully removed from petri dish, checked for possible imperfections or air bubbles.

Dosage units were made by cutting film discs of 13 mm diameter such that one film contained 30 mg DH, packed in aluminium foil, and stored in glass containers at room temperature.

Films of DH without bioadhesive polymers were prepared and the preparation method was the same as described above.

2.2.2. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing SALG as a film-forming polymer:

The required amount of DH was dissolved in the distilled water containing 50% propylene glycol with constant stirring. Subsequently, the weighed quantity of the SALG (2%, w/v), was mixed with the bioadhesive polymer. The steps that followed were the same as the methods previously described in Section 2.2.1.

Films of DH without bioadhesive polymers were prepared and the preparation method was the same as described above.

Table 1: The composition of the diltiazem hydrochloride buccal mucoadhesive films

Code	Film-forming polymer	Bioadhesive polymer		Code	Film-forming polymer	Bioadhesive polymer	
		SCMC % (w/v)	HPC % (w/v)			SCMC % (w/v)	HPC % (w/v)
F1	**SALG**	-	-	**F16**	**Eudragit NE 30 D**	-	-
F2	**SALG**	1%	-	**F17**	**Eudragit NE 30 D**	1%	-
F3	**SALG**	2%	-	**F18**	**Eudragit NE 30 D**	2%	-
F4	**SALG**	-	1%	**F19**	**Eudragit NE 30 D**	-	1%
F5	**SALG**	-	2%	**F20**	**Eudragit NE 30 D**	-	2%
F6	**HPMC**	-	-	**F21**	**Eudragit L-100**	-	-
F7	**HPMC**	1%	-	**F22**	**Eudragit L-100**	1%	-
F8	**HPMC**	2%	-	**F23**	**Eudragit L-100**	2%	-
F9	**HPMC**	-	1%	**F24**	**Eudragit L-100**	-	1%
F10	**HPMC**	-	2%	**F25**	**Eudragit L-100**	-	2%
F11	**PVA**	-	-	**F26**	**-**	2%	1%
F12	**PVA**	1%	-	**F27**	**-**	1%	2%
F13	**PVA**	2%	-	**F28**	**-**	2%	2%
F14	**PVA**	-	1%				
F15	**PVA**	-	2%				

2.2.3. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing PVA as film-forming polymer:

PVA (5%, w/v) was dissolved in 2/3 the quantity of hot distilled water (temperature between 80-100°) with stirring. DH and propylene glycol were added to the cooled PVA solution with constant stirring. Then, the bioadhesive polymer was added with continuous stirring and the final volume was adjusted with water.

The steps that followed were the same as previous. Films of DH without bioadhesive polymers were prepared and the preparation method was the same as described above.

2.2.4. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing HPC and SCMC:

The required amount of DH was dissolved in the distilled water containing 50% propylene glycol with constant stirring. Subsequently, the weighed quantity of the HPC was mixed with the weighed quantity of SCMC. The mixture was gradually added to the solution with constant stirring. The steps that followed were the same as previous.

2.2.5. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing Eudragit NE 30 D as film-forming polymer:

The required amount of DH was dissolved in the required amount of distilled water containing 50% propylene glycol with constant stirring. Subsequently, the weighed quantity of the bioadhesive polymer was gradually added to the solution with constant stirring. Then, Eudragit NE 30 D (20%, v/v) was successively added to the mixture. The steps that followed were the same as previous. Films of DH without bioadhesive polymers were prepared and the preparation method was the same as described above.

2.2.6. Preparation of Mucoadhesive Diltiazem hydrochloride Films containing Eudragit L-100 as film-forming polymer:

The required amount of DH was dissolved in 2/3 the quantity of phosphate buffer 6.8 containing 50% propylene glycol with stirring. Subsequently, the weighed quantity of the bioadhesive polymer was gradually added to the solution with constant stirring. Eudragit L-100 (4%, w/v) was dispersed in the 1/3 the quantity of phosphate buffer 6.8 with stirring. Then, Eudragit L-100

dispersion was successively added to the mixture under constant stirring to obtain homogeneous dispersions.

The steps that followed were the same as previous. Films of DH without bioadhesive polymers were also prepared.

2.3. Evaluation of Diltiazem Hydrochloride films:

2.3.1. Film thickness

The thickness of the prepared films was determined by means of micrometer. The thickness of four films was measured and the average thickness was determined.

2.3.2. Weight Uniformity

For evaluation of film weight three films of every formulation were taken and weighed individually on a digital balance (Sartorius GmbH, Gottingen, Germany). The average weights were calculated.

2.3.3. Folding Endurance

Three films of each formulation of size (1 × 2 cm) were cut. Folding endurance of the buccal films were determined by repeatedly folding one film at the same place till it broke or folded up to mpre than 200 times at the same place without breaking which gave the value of folding endurance of film [17].

2.3.4. Surface pH

The method adopted by Bottenberg et al [18] was used to determine the surface pH of the tablet. A combined glass electrode was used for this purpose. The films were allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.5 ± 0.05) for 2 hours at room temperature and pH was noted by bringing glass electrode of pH meter (Jenway 3505, Essex, UK) in contact with the microenvironment of the swollen films and allowing it to equilibrate for 1 minute. The average pH of three determinations was reported.

2.3.5. Drug content

The diltiazem hydrochloride buccal film unit of each formulation was dissolved in 250 ml of phosphate buffer (pH 6.8), then stirred and filtered. The amount of diltiazem hydrochloride was determined spectrophotometrically at λ_{max} 237 nm [19]. The average of drug contents of three films was taken as final reading. Concentrations of DH were calculated from a standard calibration curve of DH in phosphate buffer (pH 6.8).

2.3.6. Swelling Study

Buccal films (n=3) were weighed individually (W1) and placed separately in petri dishes containing 5 mL of phosphate buffer (pH 6.8) solution. The dishes were stored at room temperature. Then, films were removed and excess surface water was removed carefully using the filter paper after specified time intervals. The swollen films were then again weighed (W2) and swelling index (SI) was calculated using the following formula (Eq. 1) [20, 21]:

$$SI\ (\%) = \frac{(W2 - W1)\ x}{W1} 100\ \% \qquad (1)$$

2.3.7. *In Vitro* Drug Release

The US Pharmacopeia XXIII rotating paddle method was used to study the drug release from the designed buccal mucoadhesive films. The dissolution medium consisted of 250 ml of phosphate

buffer solution of pH 6.8. The release was performed at 37±0.5^{o}C with a rotation speed 50 rpm. The one side of the buccal film was attached to a 3 cm diameter glass disk with instant adhesive (cyanoacrylate adhesive). The film with glass disk was placed at the bottom of the dissolution vessel so that the film dosage form faced upright thereby allowing drug release only from the upper side of the film [9]. Samples of 5ml were withdrawn at pre-determined time intervals and replaced with fresh medium. The samples were filtered through 0.45-µm filter (Millipore Co., Bedford, MA, USA) and analyzed after appropriate dilution by UV spectrophotometry (Jenway 6715, Essex, UK) at λ_{max} 237 nm. The release studies were conducted in triplicates and the mean values were plotted versus time.

2.3.8. *In vitro* mucoadhesion study

The mucoadhesion strength was checked using a modified balance method [22, 23]. The chicken pouch membrane (removed of its contents and surface fats) was used as model mucosa for these studies [24, 25]. The chicken pouches were kept frozen at –20°C in a phosphate buffer saline solution (pH 6.8), and only thawed to room temperature before use. Briefly, a balance was taken and its left pan was replaced with a weight to the bottom of which a buccal film was attached. Both sides were then balanced with weight. A piece of chicken pouch membrane was fixed to a rubber cork, which was already attached to the bottom of the beaker containing phosphate buffer (pH 6.8, 37^{o}C) with a level slightly above the membrane. The weight, which was attached to the buccal film, was brought into contact with the membrane, kept undisturbed for two minutes and then the pan was raised. Weights were continuously added on the right side pan in small increments. The weight of water, in grams, required to detach the film from the mucosal surface gave the measure of bioadhesive strength. The experiments were performed in triplicate, and average values were reported. From the bioadhesive strength, force of adhesion was calculated (Eq. 2) [23],

Force of adhesion (N) = (Bioadhesive strength /1000) x 9.81 (2)

2.3.9. *In vivo* Residence Time Measurement Using Human Volunteers

Four healthy male adult volunteers, aged between 27 and 40 years, participated in the study. The study followed the rules approved by the ethical committee. Prior to the test, the volunteers were educated with the procedure and purpose of test. They were asked to rinse their mouth with distilled water before a piece of the drug free film was placed on their buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed onto the mucosa for about 30 s till the film adhered to the buccal mucosa [21]. The volunteers were asked to record the residence time of the film on buccal mucosa in the oral cavity (time of complete erosion or detachment of the film from the buccal mucus membrane) and to monitor for irritation, bad taste, swelling, dry mouth or increase in salivary flow. Repetition of application of the mucoadhesive films using the same human volunteer was allowed after a five-day rest period.

2.3.10. *In vitro* transmucosal permeation study

Formulations which possessed the best results were exposed to permeation testing of the drug through chicken pouch membrane [24, 26] using the method described in Tayl, et al [26]. The apparatus used to test the permeation consisted of a glass tube (1.3 cm diameter) opened from both ends. Each film was pressed on the mucosal side of chicken pouch for 30 s and the loaded membrane was stretched over an open end of the glass tube and made water tight by rubber band forming donor chamber. Two milliliters phosphate buffer pH 6.8 was transferred to the donor chamber to simulate the conditions inside the buccal cavity. The tube was attached to the shaft of the USP dissolution apparatus. The tube was then immersed in 250 ml of phosphate buffer pH 7.4 contained in the USP dissolution apparatus flask so that the membrane was just below the

surface of the recipient solution. The temperature was maintained at 37±0.5°C, and the apparatus was run at 50 rpm for 8 h. Samples of five milliliters were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 8 hr, and were compensated for by equal volume of fresh buffer. The concentrations of the samples were calculated from the absorbance measured at λ_{max} 237 nm.

The % cumulative amount of permeated drug per square centimeter was plotted versus time (h) and steady-state flux was measured from the slope of the linear portion of the plot using the following equation (Eq. 3):

$$\text{Flux} = Jss = (dQ/dt)/A, \quad (3)$$

where *Jss* is the steady-state flux; dQ/dt is the permeation rate; A is the active diffusion area (1.33 cm^2). The permeability coefficient *P* was calculated as follows (Eq. 4):

$$P = Jss/Cd, \quad (4)$$

where *P* is the permeability coefficient and Cd is the donor drug concentration [27]. The experiments were performed in triplicate (n = 3) and mean value was used to calculate the flux and permeability coefficient.

2.3.11. Determination of bioavailability

2.3.11.1. Administration and blood collection

The potential of the fabricated buccal mucoadhesive films to deliver diltiazem hydrochloride to the systemic circulation in a sustained fashion was evaluated by conducting the following study. New Zealand white rabbits with mean weight of 1.79± 0.24 kg were selected. The animals were housed individually for at least 1 week prior to experimentation and allowed food and water ad libitum. The study was conducted as per guidelines prescribed by Institutional Animal Ethics Committee, under the supervision of registered veterinarian.

Animals were fasted for overnight and stored in individual cages before the experiment was carried out. Animals were lightly anesthetized by an i.m. injection of a 1:5 mixture of xylazine (1.9 mg/kg) and ketamine (9.3 mg/kg) [28]. The light plane of anesthesia was maintained by an i.m. injection of one-third of the initial dose of xylazine and ketamine mixture as needed. The animals were divided into tow equal groups each having four rabbits. The animals of first group were dosed with 30 mg of oral commercial sustained release tablet (Altiazem® SR) as a reference formulation, while second group animals received 30 mg of the tested diltiazem hydrochloride buccal mucoadhesive film (F26). Upon the induction of anesthesia, mucoadhesive film was applied to oral cavity, on the buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed against the mucosa.

Blood samples (2 mL) were withdrawn from the ear vein of rabbits using a 23 G needle. Samples were withdrawn at 0.5, 1.0, 2.0, 3.0, 5.0, 7.0 and 10.0 h post dosing and collected in heparinized tubes. Blood samples were centrifuged at 3000 x g for 10 min to separate the plasma. The clear supernatant serum layer was collected in labeled tubes and stored immediately at -20 °C until analysis could be performed.

2.3.11.2. Samples analysis

The quantitative determination of diltiazem hydrochloride was performed by high-performance liquid chromatography (HPLC, Shimadzu LC-20A, Shimadzu, Japan) using of a Shimadzu LC-20A pump, SIL-20 A autosampler, a SPD 20A UV/VIS detector and a µBondapak C-18 column

(250 mm x 4.6 mm ID; particle size 5 µm) (Waters, USA). The mobile phase consisted of a mixture of potassium dihydrogen orthophosphate buffer (0.05 M, pH 4.6) and acetonitrile (75:25 v/v). The final pH was adjusted to 4.6 using 85% orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and was then degassed by ultrasonication. Analysis was run at a flow rate of 1.3 ml/min and the detection wavelength was 260 nm.

Frozen serum samples were thawed at ambient temperature (25±2 °C) for at least 60 min, followed by adding 100 µl of moxifloxacin hydrochloride as internal standard (IS) (100 µg/ml in methanol) and 4 ml of diethyl ether to 1 ml thawed plasma sample. The mixture was then mixed for 2 min by using a vortex mixer and centrifuged at 3000 rpm for 10 min by centrifuge machine. After centrifugation the upper organic layer was separated and then solvent was evaporated in vacuum oven to dryness. The residue was reconstituted with 400 µl of mobile phase and 20 µl injected into column.

Chromatograms obtained showed no interfering with determination of diltiazem hydrochloride and the diltiazem hydrochloride and IS peaks were well resolved (data not shown). The retention times were approximately 4.5 min for diltiazem hydrochloride and 8.4 min for IS. The calibration curve for diltiazem hydrochloride was constructed from measurements of five concentrations in the range of 10 to 200 ng/mL in spiked plasma. Calibration curve for diltiazem hydrochloride was linear, and the relative coefficient of correlation (r^2) was 0.997. Precision and accuracy were evaluated by spiking blank plasma with diltiazem hydrochloride at three concentration levels: 50, 100 and 200 ng/mL. The coefficients of variation (CVs) for the intra-day precision were: 3.47% at 50 ng/mL, 0.92% at 100 ng/mL and 4.42% at 200 ng/mL. The CVs for day-to-day precision were: 3.37% at 50 ng/mL, 5.73% at 100 ng/mL and 8.59% at 200 ng/mL. The relative error, determined by comparing the measured concentrations to the expected concentrations, was less than 10%. The absolute recovery of diltiazem hydrochloride at 50, 100 and 200 ng/mL was 91.3, 102.58 and 96.58%, respectively. Thus, the overall recovery was> 91%. The limit of detection was estimated to be 5 ng/mL.

2.3.11.3. Pharmacokinetic Analysis

The maximum plasma concentration (C_{max}) and the time required to reach C_{max} (T_{max}) were directly read from the arithmetic plot of time vs plasma concentration of diltiazem hydrochloride. The area under the plasma concentration vs time curve ($AUC_{0-\infty}$) was determined by means of trapezoidal rule. The relative bioavailability of diltiazem hydrochloride from tested buccal mucoadhesive film in comparison to reference formulation (Altiazem® RS, oral tablets) was calculated by dividing its $AUC_{0-\infty}$ with that of Altiazem® SR.

2.4 Statistics

All data was expressed as the mean value ±S.D. Statistical analysis was performed using the one-way analysis of variance (ANOVA) test. Differences were considered to be significant at a level of $p < 0.05$.

RESULTS AND DISCUSSION

All the prepared polymeric films except F14, F15, F19 and F20 were elegant in appearance, homogeneous, thin, flexible, possesses a smooth surface and no spot or stain was found on the films. Films prepared using PVA and HPC (F14 and 15) were not homogeneous and showed a rough surface. Non-homogeneous surface of films was posing the problems, such as unequal distribution of drug in film. Thus, F14 and 15 were excluded from further studies. In addition, polymeric films prepared using Eudragit NE 30D and HPC (F19 and 20) were attached more

strongly to the bottom of casting surface, hard to peel after dry, brittle in nature and showed visible cracks and breaks. They were also excluded from further studies.

Table 2. Thickness, weight, drug content and folding endurance of diltiazem hydrochloride buccal mucoadhesive films

	Thickness (mm) ± SD	Film wieght (mg) ± SD	Drug content (%) ± SD	Folding endurance
F1	0.376 ± 0.014	78.75 ± 3.59	103.21 ± 0.26	> 200
F2	0.420 ± 0.010	111.50 ± 3.70	102.14 ± 0.28	> 200
F3	0.760 ± 0.021	178.75 ± 2.75	101.09 ± 0.89	> 200
F4	0.385 ± 0.010	100.50 ± 2.65	100.99 ± 0.91	> 200
F5	0.518 ± 0.005	139.50 ± 3.11	100.99 ± 0.91	> 200
F6	0.333 ± 0.029	81.50 ± 1.29	98.76 ± 0.57	> 200
F7	0.420 ± 0.022	109.00 ± 1.83	98.72 ± 0.29	> 200
F8	0.619 ± 0.022	174.75 ± 3.50	99.70 ± 0.34	> 200
F9	0.535 ± 0.033	131.50 ± 1.91	101.34 ± 0.77	> 200
F10	0.795 ± 0.030	182.50 ± 1.29	102.03 ± 0.40	> 200
F11	0.790 ± 0.010	187.50 ± 1.29	97.46 ± 0.72	> 200
F12	0.780 ± 0.035	185.25 ± 1.71	96.38 ± 0.79	> 200
F13	0.820 ± 0.010	223.00 ± 1.15	98.25 ± 0.49	> 200
F14	-	-	-	-
F15	-	-	-	-
F16	0.540 ± 0.010	145.75 ± 0.96	94.80 ± 1.08	> 200
F17	0.775 ± 0.029	206.33 ± 3.06	96.70 ± 0.94	> 200
F18	0.835 ± 0.017	218.67 ± 1.53	96.93 ± 1.02	> 200
F19	-	-	-	-
F20	-	-	-	-
F21	0.610 ± 0.014	161.50 ± 1.73	92.70 ± 0.77	> 200
F22	0.755 ± 0.019	196.00 ± 3.37	93.09 ± 0.55	> 200
F23	0.846 ± 0.024	211.75 ± 2.63	94.13 ± 0.82	> 200
F24	0.715 ± 0.013	182.75 ± 1.50	94.14 ± 0.81	> 200
F25	0.795 ± 0.017	201.25 ± 0.96	96.62 ± 0.92	> 200
F26	0.435 ± 0.022	115.00 ± 4.08	99.34 ± 0.85	> 200
F27	0.458 ± 0.026	114.25 ± 2.06	99.95 ± 0.70	> 200
F28	0.665 ± 0.030	179.50 ± 2.38	100.17 ± 0.58	> 200

The important physicochemical parameters of the fabricated buccal mucoadhesive films of diltiazem hydrochloride are presented in Table 2 and 3. The film thicknesses were observed to be in the range of 0.33±0.03 mm to 0.85±0.02 mm.

Table 3. pH and swelling index of diltiazem hydrochloride buccal mucoadhesive films

	pH ± SD	Swelling Index (%)± SD		
		After 5 min	After 15 min	After 30 min
F1	7.53 ± 0.07	176.99 ± 7.74	251.99 ± 7.13	319.78 ± 8.07
F2	7.19 ± 0.03	190.99 ± 8.68	291.39 ± 19	370.62 ± 4.4
F3	7.42 ± 0.07	208.72 ± 9.72	341.9 ± 9.5	447.28 ± 11
F4	7.13 ± 0.09	178.48 ± 8.95	276.58 ± 4.48	316.46 ± 5.37
F5	7.39 ± 0.08	167.50 ± 5.74	220.23 ± 7.89	241.54 ± 11.2
F6	5.64 ± 0.05	Eroded	--	--
F7	6.93 ± 0.12	137.74 ± 2.30	240 ± 8.12	Eroded
F8	6.77 ± 0.09	176.14 ± 5.95	377.78 ± 5.24	443.06 ± 7.2
F9	6.65 ± 0.05	129.37 ± 4.89	Eroded	--
F10	6.47 ± 0.08	107.58 ± 6.10	Eroded	--
F11	5.56 ± 0.06	37.56 ± 8.38	60.23 ± 10.1	83.04 ± 6.31
F12	5.86 ± 0.03	48.90 ± 8.34	72.69 ± 9.3	96.02 ± 8.46
F13	6.46 ± 0.11	60.81 ± 8.82	89.48 ± 4.83	134.67 ± 10.7
F14	-	-	-	-
F15	-	-	-	-
F16	5.49 ± 0.08	0.00 ± 0.00	0.31 ± 0.29	1.04 ± 0.76
F17	5.74 ± 0.08	33.53 ± 3.62	50.39 ± 5.97	56.54 ± 4.75
F18	6.06 ± 0.06	58.24 ± 1.13	95.16 ± 7.48	112.49 ± 7.96
F19	-	-	-	-
F20	-	-	-	-
F21	5.53 ± 0.02	8.82 ± 0.76	16.13 ± 1.55	14.27 ± 1.4
F22	5.57 ± 0.02	60.21 ± 6.58	87.94 ± 2.06	91.06 ± 2.64
F23	5.59 ± 0.02	148.46 ± 11.14	239.5 ± 17	380.21 ± 12.1
F24	5.58 ± 0.04	33.87 ± 0.25	54.56 ± 1.07	38.7 ± 1.69
F25	5.57 ± 0.03	33.64 ± 3.96	39.72 ± 2.29	35.11 ± 2.09
F26	6.74 ± 0.02	195.16 ± 5.93	305.18 ± 4.73	350.21 ± 5.54
F27	6.51 ± 0.06	154.90 ± 6.93	213.99 ± 7.3	235.41 ± 7.1
F28	6.74 ± 0.04	115.14 ± 9.27	160.1 ± 10.9	226.77 ± 8.07

Drug loaded films (1 × 2 cm) were tested for uniformity of weight. The films were found to be uniform. The average weight of the film was found to be in the range of 78.75±3.59 mg to 223±1.15 mg (Table 2).

Average drug content was found between 92.7±0.77 % (F21) and 103.21±0.0.26 % (F1) of added amount of diltiazem hydrochloride per film (1 × 2 cm) (Table 2). Low SD in thickness, weight measurement and drug content data reflected no significant difference within the batch.
All the films resisted breakage upon folding them for more than 200 times at same place and did not show any cracks even after folding them for more than 200 times. Therefore the films exhibited good physical and mechanical properties.

3.1. Surface pH

Attempts were made to keep the surface pH as close to buccal/ salivary pH as possible. The surface pH of all films was within satisfactory limit of 7.0±1.5 [29] and hence no mucosal irritation was expected and ultimately achieved patient compliance (Table 3). These results suggested that the polymeric blend identified was suitable for oral application owing to the acceptable pH measurements.

3.2. Swelling study

Appropriate swelling behavior of a buccal adhesive system is an essential property for uniform and prolonged release of drug and effective mucoadhesion [21, 29]. The percentage of swelling of diltiazem hydrochloride mucoadhesive films was monitored during 30 min in phosphate buffer solution (pH 6.8) and data are shown in Table 3. Results indicated that the prepared buccal films containing hydrophilic film forming polymers (SALG, HPMC and PVA) had higher swelling index compared to films prepared using hydrophobic film forming polymers (Eudragits). The addition of hydrophilic bioadhesive polymers increased surface wettability and, consequently, water penetration within the matrix [17]. It was observed that SCMC imparted continuous increase in swelling with time and SCMC containing films showed higher percent swelling than HPC containing films at the same concentration due to presence of more hydroxyl group in the SCMC molecules which hold more amount of water in their network [30]. Increasing HPC content from 1 to 2% w/v was found to reduce the extent of swelling of the films may be due to the rapid dissolution and erosion in the swelling medium resulting in decreasing its percentage of swelling.

The highest percentage swelling after 30 min was obtained for F3 (447.28%) owing to its higher hydrophilic nature as a result of the presence of SALG and high concentration of SCMC. On the other hand, the lowest percentage swelling after 30 min was obtained for F16 (1.04%) owing to the hydrophobic nature of Eudragit NE 30D. The swelling capacity of diltiazem hydrochloride films containing Eudragits was low because of the hydrophobic effect exerted by Eudragits contents in film and had the lowest swelling index among the prepared films. Similar results were obtained by Patel et al, who showed that Eudragit L-100 films had weak swelling property [17].

All films prepared using of HPMC except F8 did not preserve their integrity throughout the experiment and showed fragmentation within 30 min after that maximum hydration was reached. These may pose the problems, such as unexpected burst release of drug and short residence time on the buccal mucosa. Furthermore, in the eroded films, the water soluble hydrophilic additives dissolve rapidly introducing porosity. The void volume is thus expected to be occupied by the external solvent diffusing into the film and thereby accelerating the dissolution of the gel [21, 30]. One of the major requirements in developing buccal film system is the maintenance of the morphology of the film, i. e., the film should not be dissolved for a certain period of time. Thus, F6, F7, F9 and F10 were excluded from further studies. The other diltiazem hydrochloride films were not dissolved nor eroded, indicating that the cohesiveness of the polymers is sufficient to guarantee the stability of the system and therefore, they were accepted for further studies.

3.3. *In vitro* Mucoadhesion study

Bioadhesion is a very important aspect for maintaining high drug levels at the site of administration and prevents expulsion of formulation [31]. Bioadhesion strength and bioadhesion force of the prepared diltiazem hydrochloride films on chicken pouch mucosa as a function of SCMC and HPC concentration have been shown in Table 4. The use of chicken pouch as a model mucosa has been reported by Mumtaz and Ch'ng (1995) and was chosen for the present study [32]. It was observed that films formulated using hydrophilic film forming polymers, especially, SALG and HPMC showed higher bioadhesive strength values than films prepared using hydrophobic film forming polymers (Eudragits). Choi et al. (1998) suggested that polymers with hydrophilic groups, such as carboxyl and hydroxyl groups, bind strongly to the oligosaccharide chains of the mucous layer [33].

For buccal films prepared with only film forming polymers, films containing only SALG (F1) had the highest bioadhesive strength (36.24 ± 4.86). Furthermore, SALG films showed higher bioadhesive strength values than other films having similar compositions of bioadhesive polymer. SALG is one of the polysaccharides that possess a mucoadhesive property because it contains numerous hydrogen bond forming groups, i.e., carboxyl and hydroxyl groups [34]. It has been proposed that the interaction between the mucus and hydrophilic polymers occurs by physical entanglement and chemical interactions, such as hydrogen bonding [34].

Film containing only Eudragit L100 (F21) showed the lowest mucoadhesive strength (8.92 ± 4.16). Whereas, no bioadhesion detected with films containing only Eudragit NE 30D (F16) which indicated that Eudragits has weak or no bioadhesive properties (Table 4). The addition of hydrophilic polymers into Eudragit based films was found to improve the bioadhesiveness of the films. This finding was in agreement with findings reported in the literature [17]. Increasing SCMC content from 1% to 2% led to an increase in the bioadhesion strength of PVA, Eudragit NE 30D as well as Eudragit L100 films. The opposite is true for SALG films, when SCMC content increased from 1% to 2% led to a slight decrease in the bioadhesion strength from 71.14±6.49 to 67.59±7.41 gr. Explanation for this might be possibility of decreased mucoadhesion due to the higher degree of swelling (Table 3). Since excessive hydration can result in a reduction of interaction between mucoadhesive polymers and mucin, making more difficult and less efficacious the mucoadhesion process [35, 36].

The *in vitro* bioadhesive strength exhibited by diltiazem hydrochloride films was satisfactory for maintaining them in oral cavity except for F16 and F17. This aspect was further confirmed by measurement of bioadhesive time. F16 which containing Eudragit NE 30D alone and F17 containing Eudragit NE 30D with 1% SCMC possessed the lowest bioadhesive strength values, less than 8 gr. Therefore, F16 and F17 have been excluded from further studies.

3.4. *In vitro* Release study

The *in vitro* release profiles of diltiazem hydrochloride from different mucoadhesive films containing 1% SCMC, 2% SCMC, 1% HPC and 2% HPC are shown in Figures 1, 2, 3 and 4, respectively. The time for 50% of diltiazem hydrochloride to be released from the different mucoadhesive films is presented in Table 5. It can be seen that increasing the concentration of SCMC from 1% to 2% in films containing hydrophilic film forming polymer comparatively reduced the drug release, whereas the opposite is true for films containing hydrophobic film forming polymers. This finding was also supported by the results of swelling study (Table 3), where the highest swelling index was also exhibited by films containing hydrophilic film forming polymer with 2%SCMC. Although the marked increase in surface area during swelling

can promote drug release but the increase in diffusion path length of the drug may paradoxically delay the release [30].

Table 4: *In vitro* and *In vivo* bioadhesion measurments of diltiazem hydrochloride mucoadhesive films

	***In vitro* mucoadhesion measurements**		***In vivo* bioadhesion time (hr) ± SD**
	Bioadhesion strength (gm) ± SD	**Force of biodhesion (N) ± SD**	
F1	36.24 ± 4.86	0.356 ± 0.048	3.00 ± 0.35
F2	71.14 ± 6.49	0.698 ± 0.064	5.04 ± 0.30
F3	67.59 ± 7.41	0.663 ± 0.073	4.88 ± 0.18
F4	48.78 ± 9.01	0.478 ± 0.088	3.50 ± 0.35
F5	54.12 ± 9.41	0.531 ± 0.092	3.21± 0.06
F8	47.77 ± 9.68	0.469 ± 0.095	3.38 ± 0.53
F11	10.41 ± 5.20	0.102 ± 0.051	0.63 ± 0.18
F12	19.35 ± 6.10	0.190 ± 0.060	1.13 ± 0.18
F13	26.20 ± 4.13	0.257 ± 0.041	1.45 ± 0.07
F16	-	-	-
F17	3.66 ± 0.51	0.036 ± 0.005	-
F18	8.11 ± 1.89	0.080 ± 0.019	1.08 ± 0.11
F21	8.92 ± 4.16	0.087 ± 0.041	0.79 ± 0.06
F22	20.33 ± 2.96	0.199 ± 0.029	2.00 ± 0.07
F23	29.16 ± 7.81	0.286 ± 0.077	2.63 ± 0.18
F24	17.09 ± 3.07	0.168 ± 0.030	1.08 ± 0.11
F25	21.92 ± 3.43	0.215 ± 0.034	2.17 ± 0.23
F26	50.26 ± 5.35	0.493 ± 0.052	5.38 ± 0.18
F27	54.13 ± 7.13	0.531 ± 0.070	3.25 ± 0.35
F28	70.02 ± 4.89	0.687 ± 0.048	4.25 ± 0.35

DH release was slower form films containing SCMC than films containing HPC. This could have been due to the higher swelling profile and slower erosion rate of SCMC based films, which created a thick gel barrier, resulting in an increase in diffusional path length of drug and the consequent reduction of drug release [15, 21]. These results were consistent with the literature, in which many authors have generally observed that increasing the amount of hydrophilic polymer in the films produces a water-swollen gel-like state that can substantially reduce the permeation of the dissolution medium into the films and thus retard the drug release [37, 38].

It was obvious that the slowest release was obtained from films containing Eudragit polymers. This could be attributed to the high hydrophobic properties, and the consequent lower dissolution and slower erosion of Eudragit films, which prevented free and deep water penetration into the film [39]. The addition of hydrophilic bioadhesive polymers to the Eudragits films improved the bioadhesion as well as the penetration and release rates of diltiazem hydrochloride, as shown in Figures 1-4.

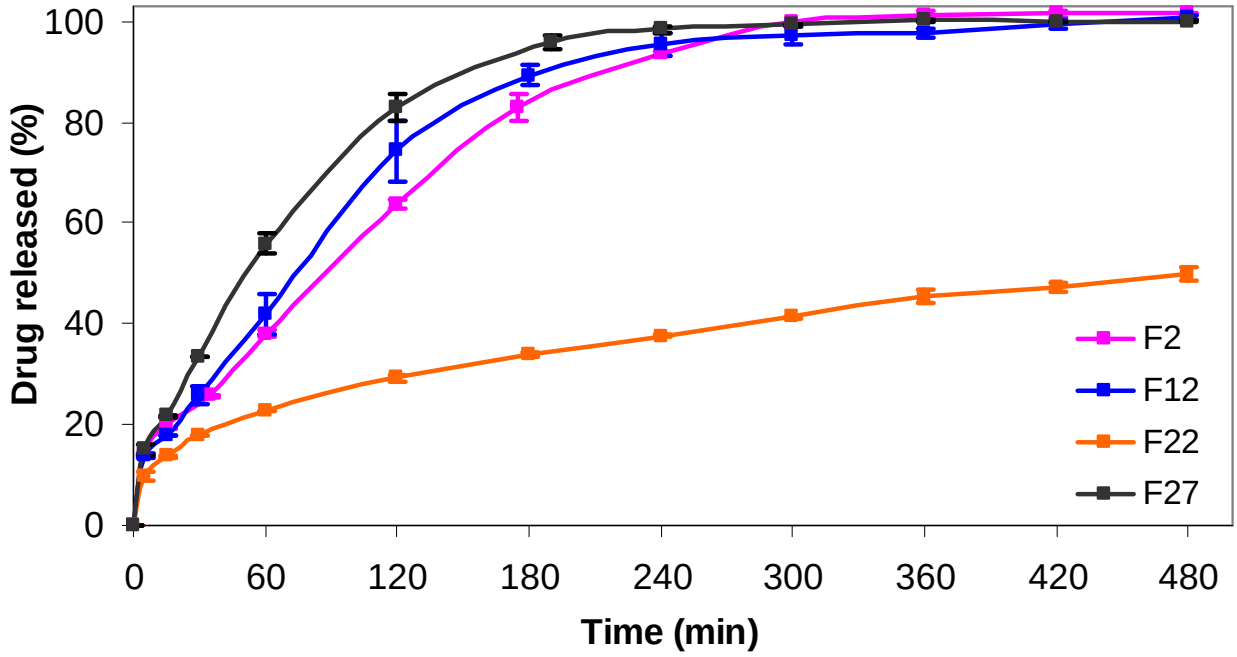

Figure 1. Release profile of diltiazem hydrochloride from buccal mucoadhesive films containing 1% SCMC as bioadhesive polymer

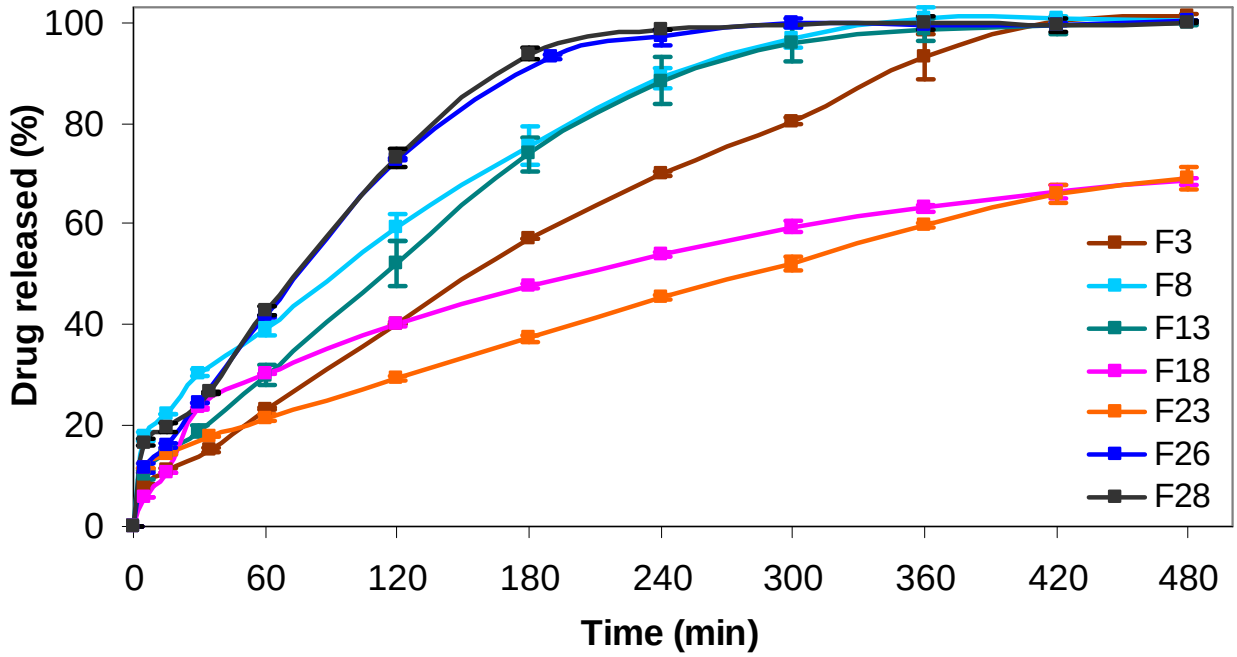

Figure 2. Release profile of diltiazem hydrochloride from buccal mucoadhesive films containing 2% SCMC as bioadhesive polymer.

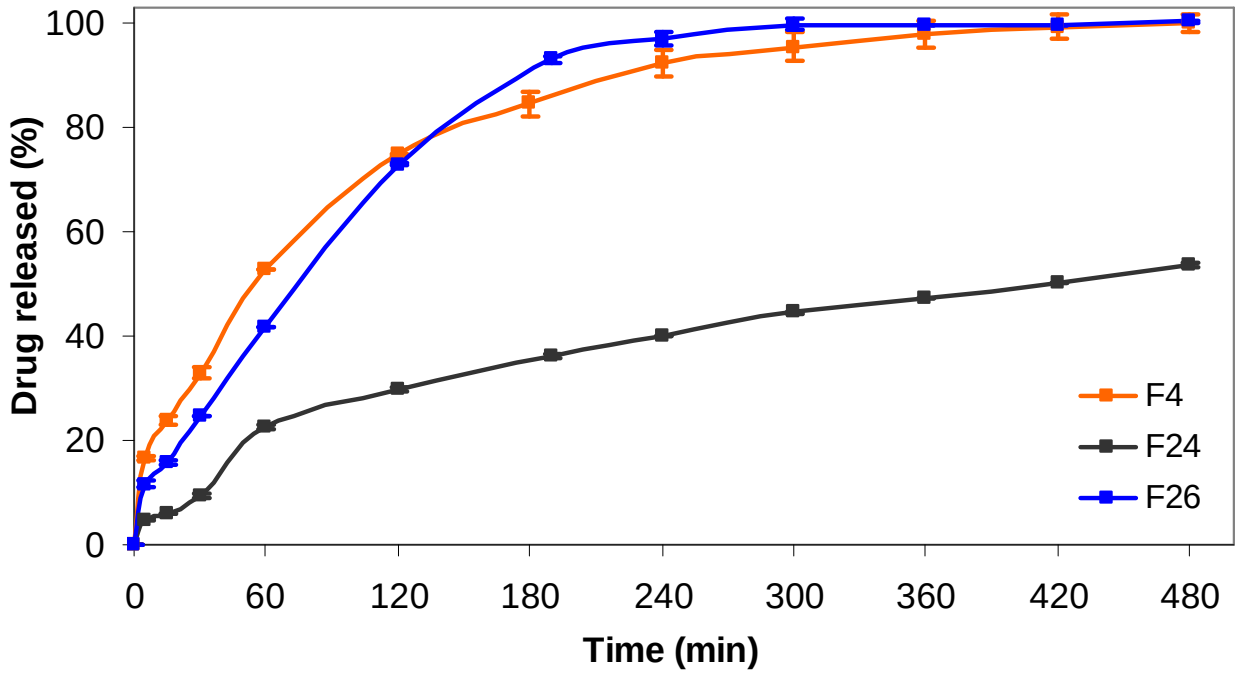

Figure 3. Release profile of diltiazem hydrochloride from buccal mucoadhesive films containing 1% HPC as bioadhesive polymer.

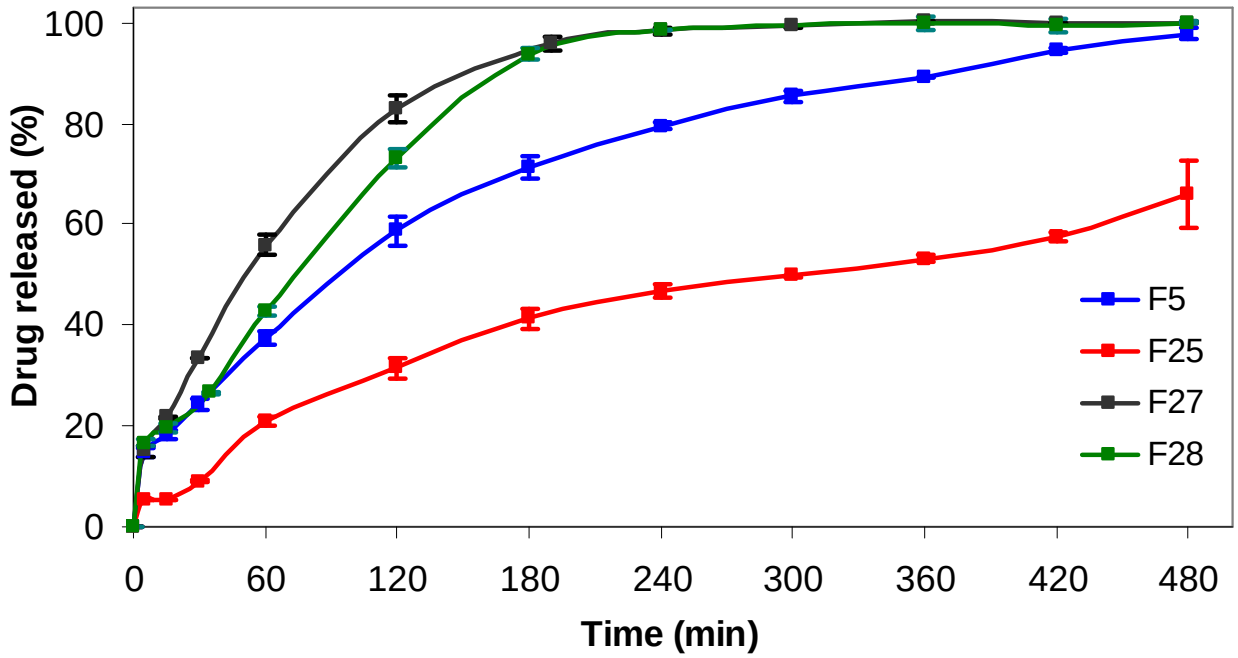

Figure 4. Release profile of diltiazem hydrochloride from buccal mucoadhesive films containing 2% HPC as bioadhesive polymer.

Buccal film containing only drug and Eudragit L-100 (F21) showed the minimum *in vitro* drug release, only 50.31 % drug release was achieved in 8 hours with a $T_{50\%}$ of 480 min. The drug release rate appeared to increase with an increasing amount of the hydrophilic polymers. As when the concentration of SCMC increased from 1% (F22) to 2% (F23) w/v the drug release increased from 50.42 to 69.11 % in 8 hours and $T_{50\%}$ significantly decreased from 480 to 278.27 min (p <0.05). When the concentration of HPC increased from 1% (F24) to 2% (F25) w/v the

drug release increased from 53.78 to 66.18 % in 8 hours and $T_{50\%}$ significantly decreased from 420 to 301.21 min ($p < 0.05$), as shown in Table 5. F18 containing Eudragit NE 30D and 2%SCMC (F21) showed 68.46 % drug release in 8 hours with a $T_{50\%}$ of 203.10 min. It was clear that, the drug release from the Eudragit films could be significantly modified by addition of the hydrophilic polymers. This observation was in good agreement with the results obtained by Bodmeier and Paeratakul [40]. The increase in rate of drug release could be explained by the ability of the SCMC and HPC to absorb water due to their hydrophilicity, thereby promoting the dissolution, and hence the release, of the highly water-soluble drug. Moreover, the hydrophilic polymers would leach out and, hence, create more pores and channels for the drug to diffuse out of the films [40].

In general, a formulation with an appropriate controlled release profile with at least 80% drug release over an 8-h period was desired for the purpose of this study for buccal delivery. For Eudragits based films; it was evident that while drug release was controlled, only approximately 50.31-68.11% diltiazem hydrochloride was released from the film at the end of 8 h. On the other hand, the data clearly shows that percentage release of diltiazem hydrochloride was maximum (97.71% - 100.74%) for formulations containing hydrophilic film forming polymers. The release was completed after 8 h for most these films. In the case of films F4 ($T_{50\%}$=58.2 min) and F27 (T50%=53.5 min), diltiazem hydrochloride release is relatively fast. Perhaps, a slower rate could be more convenient for mucoadhesive films, which have to be attached to the mucosa for at least 4 h [41]. Thus, F4, F27 and Eudragit based films would not be considered appropriate for a controlled drug release profile. The other formulations were considered suitable for diltiazem hydrochoride release as more than 90% diltiazem hydrochloride was released from these films at the 8th hour of dissolution while still maintaining a controlled release profile throughout the study.

3.4.1. Kinetic Analysis of Diltiazem hydrochloride *In Vitro* Release Data

To investigate more precisely the effect of the polymeric blend on the release of diltiazem hydrochloride, the results were analyzed according to the well-known semi-empirical Peppas equation (Eq. 5) [42]:

$$Mt / M\infty = Kt^n \quad (5)$$

where Mt /M∞ is fractional release of the drug, ‘t’ denotes the release time, ‘K’ represents a constant, incorporating structural and geometrical characteristics of the drug/polymer system (device) and ‘n’ is the diffusional exponent and characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.45 and 0.89; while in case of Fickian diffusion, n= 0.45; for zero-order release (case II transport), n=0.89 and for supercase II transport, n >0.89[42]. The obtained values of K (kinetic constant), n (diffusional exponent) and r^2 (correlation coefficient) of the *in vitro* release data of diltiazem hydrochloride from mucoadhesive films are presented in Table 5. For most of the tested formulations, the values of n on fitting the simple power equation $Mt/M\infty = Kt^n$ were between 0.45 and 0.89 for the release of diltiazem hydrochloride from all the film formulations except for F21 and F22, indicating anomalous (non-Fickian) release kinetics, where drug release is controlled by combination of diffusion and polymer chain relaxation mechanisms [42, 43].

Table 5: Release Kinetics of the diltiazem hydrochloride from buccal mucoadhesive films, analyzed using the well-known Peppas equation $Mt/M\infty = Kt^n$: :

	K	n	Time for 50 % drug release (min)	r^2
F1	0.07147	0.4748	60.17	0.992
F2	0.03602	0.5929	84.51	0.968
F3	0.02398	0.5958	163.72	0.961
F4	0.06987	0.4842	58.23	0.980
F5	0.03763	0.5668	95.97	0.994
F8	0.06279	0.4619	89.29	0.990
F11	0.05436	0.4755	63.47	0.993
F12	0.02616	0.6892	72.31	0.992
F13	0.02199	0.6609	113.00	0.979
F18	0.02822	0.5410	203.10	0.972
F21	0.03026	0.4395	480.00	0.979
F22	0.04964	0.3727	480.00	0.999
F23	0.03501	0.4724	278.27	0.975
F24	0.01768	0.5663	420.00	0.972
F25	0.01441	0.6214	301.21	0.963
F26	0.03656	0.6046	75.64	0.971
F27	0.05488	0.5552	53.48	0.979
F28	0.02951	0.6606	72.50	0.978

3.5. *In Vivo* Bioadhesive Performance of Diltiazem hydrochloride Mucoadhesive Films:

The mean residence time values of various films on buccal mucosa are depicted in Table 4. The time required for the complete removal of the buccal film from the buccal mucosae varied with the composition of the film. The bioadhesive polymers predominately increased the *in vivo* residence time of mucoadhesive films. SCMC and HPC are hydrophilic polymers and may have more affinity towards mucin which comprises of 95% water [44]. This may be the reason for longer residence time of films containing bioadhesive polymers. All films eroded completely except PVA and Eudragit based films, which dislodged and detached from the buccal mucosa. These films remained intact without erosion.

The highest residence time was detected for F2, F3, F26 and F28 with adhesion time of 5.04, 4.88, 5.38 and 4.25 hrs, respectively. Films containing PVA, Eudragit NE 30D or Eudragit L-100 as film forming polymer showed the lowest adhesion time. For Eudragits based films this can be attributed to the hydrophobic nature and lower swelling indexes of Eudragit polymers caused a reduction of interaction between mucoadhesive polymers and mucin. On the other hand, for PVA based films, the excessive hydration and increased surface area of the PVA based films, permitting more water influx, results in a reduction of interaction between mucoadhesive polymers and mucin, and then faster dislocation from mucosal surface [35, 44].

Visual examination of the volunteer's mucosal tissue after the removal of the film revealed no signs of damage to the mucosa. Only PVA based films showed an excessive increase in diameter and surface area which considered undesired, since it might cause discomfort.

Buccal mucoadhesive films exhibited short adhesion time were considered unsuitable for prolonged intra-oral delivery of diltiazem hydrochloride and excluded from the permeability and bioavailbility studies. It was noted that the only F2, F3, F26 and F28 films exhibited a reasonable and satisfactory adhesion in the oral cavity for over 4 h. Therefore they were selected for *in vitro* permeability studies. They could be arranged according to their residence times as follows; F26> F2> F3 > F28.

3.6. Permeation of diltiazem hydrochloride through chicken buccal membrane:

In vitro permeation profiles of diltiazem hydrochloride from the four selected mucoadhesive films (F2, F3, F26 and F28) through the chicken pouch membrane are shown in Figure 5. The permeation parameters were calculated from the linear portion of the permeation graph. These parameters are listed in Table 6. The results indicated that diltiazem hydrochloride can permeate easily across the mucosal membrane. This was due to high aqueous and lipid solubilities of diltiazem hydrochloride. Good correlation was observed between *in vitro* drug release and *in vitro* drug permeation with correlation coefficient ranged between of 0.945 to 0.980. The % cumulative amount of diltiazem hydrochloride penetrated through the membrane was indicated that the penetration of drug through the chicken pouch epithelium was rapid up to the first 2 hours followed by a low penetration in the next 6 hours (Figure 5). % Cumulative amount of DH permeated in 8 hr was between 66.54 and 82.70 % and flux was calculated to be in the range 3.333 to 4.625 % h^{-1} cm^{-2}. These values are sufficiently high to ensure permeation through the buccal mucosa. Referring Figure 5 and Table 6, the fastest diltiazem hydrochloride penetration was observed for the film containing 1%HPC and 2%SCMC (F26) followed by F28, F2 and F3, respectively. Although from the comparison of profiles of the different films we observed that permeability behavior was not statistically different ($P > 0.05$).

F26 (1%HPC, 2%SCMC) film could be considered the most optimum buccal mucoadhesive film in the consideration of ease of preparation, excellent bioadhesion values and expected to present a better drug release under normal physiological conditions without the risk of mucosal irritation, convenient *in vivo* residence times (5.38 hr) and release rates as indicated by $t_{50\%}$ values. The penetration study revealed that the optimized film (F26) was more effective at supplying diltiazem hydrochloride to the oral mucosa than the other tested films. The flux, permeation coefficient, and cumulative drug permeated from formulation F7 were found to be 4.625 % h^{-1} cm^{-2}, 25.6961 ± 0.3323 x 10^{-6} cm h^{-1}, and 82.7 ± 1.61 %, respectively. F26 was thus selected for the bioavailability studies.

Table 6. Permeability parameters of tested diltiazem hydrochloride buccal mucoadhesive films

	$(R^2)^a$	Flux % cm^{-2} h^{-1}	Permeability Coefficient (x 10^{-6})cm s^{-1}	R^2	% of Drug permeated at 480 min ± SD
F2	0.959	3.439	19.1039 ± 1.3730	0.999	70.21± 0.44
F3	0.980	3.333	18.5161 ± 0.8941	0.996	66.54 ± 1.32
F26	0.967	4.625	25.6961 ± 0.3323	0.993	82.70 ± 1.61
F28	0.945	3.919	21.7706 ± 0.3272	0.997	73.27 ± 1.46

[a] *In vitro release – in vitro permeability correlation.*

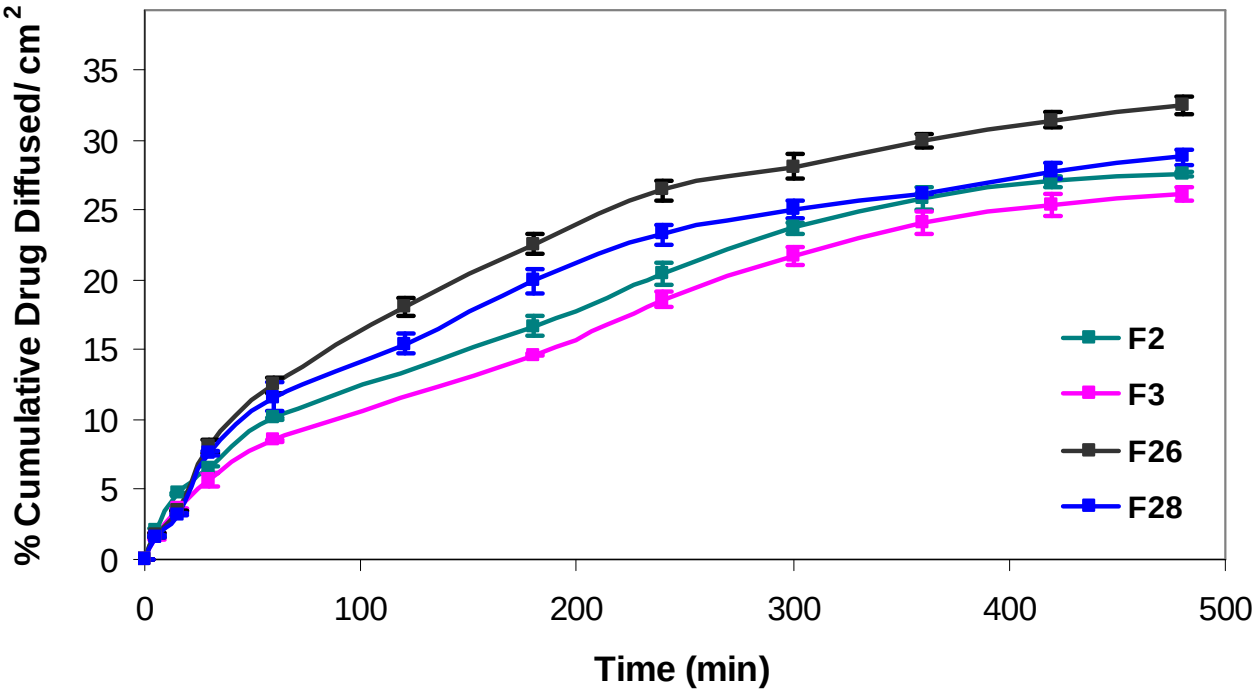

Figure 5. *In vitro* Permeation profile of diltiazem hydrochloride through Chicken Pouch mucosa, the values represented mean ± S.D (n=3).

3.7. Pharmacokinetic study

The mean plasma level profiles (mean ± SD) of diltiazem hydrochloride obtained following the application of adhesive buccal film (F26) containing 30 mg drug and from an oral administration of sustained release commercial tablet (Altiazem® RS) at the same dose to rabbits are compared in Figure 6. A summary of the pharmacokinetic parameters derived from the study data is listed in Table 6. Following oral administration of the reference product, the C_{max} was achieved after 2.0 h of oral dosing. Unlike that for the oral administration, after buccal administration of the mucoadhesive film (F26), C_{max} was achieved 3.0 h after dosing. The mucoadhesive formulation spent longer times to reach the maximum drug concentration in the systemic circulation. The mean value of C_{max}, $AUC_{0\text{-}10}$ and $AUC_{0\text{-}\infty}$, was significantly higher ($P < 0.05$) for drug administered from buccal mucoadhesive film (F26) than oral tablet demonstrating improved bioavailability of diltiazem hydrochloride from tested buccal formulation, but the mean value of MRT failed to demonstrate statistical significance ($p > 0.05$) even though it is higher for buccal film (Table 7).

The bioavailability of the selected mucoadhesive formulation (F26) containing 30 mg of diltiazem hydrochloride was determined and compared with the reference oral tablet (Altiazem® SR) containing the same amounts of diltiazem hydrochloride. The F26 showed relative bioavailability of 165.2 % with respect to Altiazem® SR. The enhancement of the relative bioavailability of diltiazem hydrochloride from buccal route is a direct result of the elimination of the hepatic first-pass metabolism on buccal delivery of the diltiazem hydrochloride. Moreover, the introduced mucoadhesive formulation offered a more sustained delivery profile than oral tablet with the absence of sharp peaks. Further clinical trials in humans of the introduced mucoadhesive preparations are also encouraged.

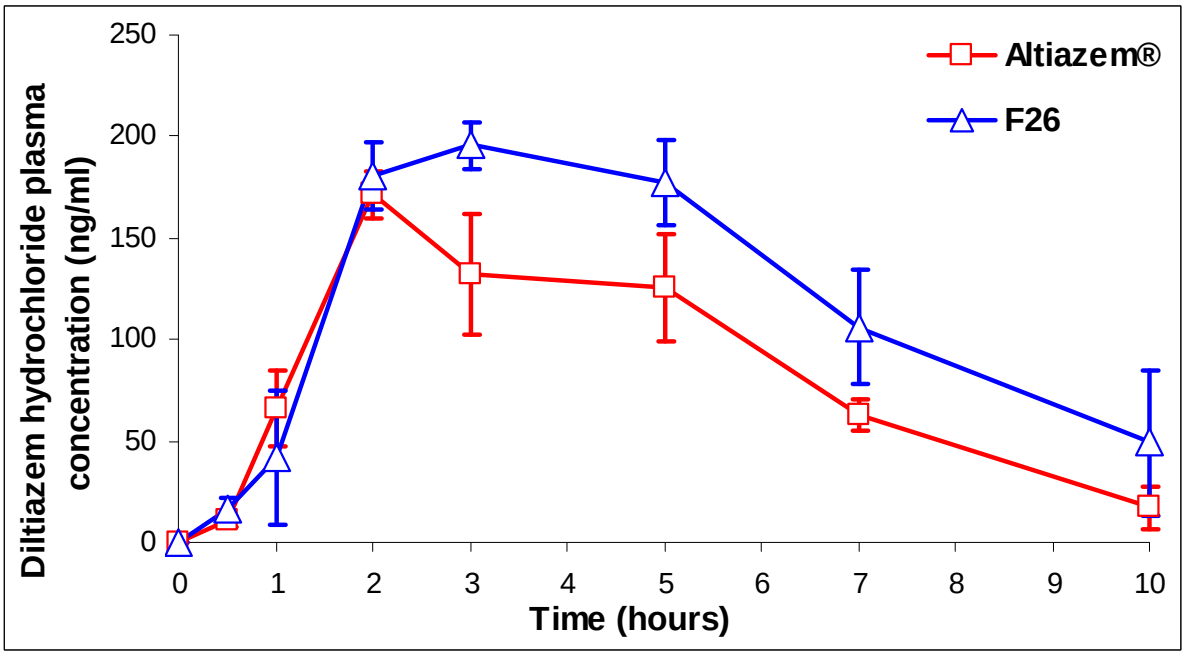

Figure 6. Mean plasma concentration profile of diltiazem hydrochloride following administration of single dose (30 mg) in rabbits by buccal (F26) and oral route (Altiazem®) (Mean ± SD of four independent determinations)

Table 7. Pharmacokinetic parameters of diltiazem hydrochloride after buccal and oral administration [a]

Parameters	Altiazem® SR oral tablet (Reference product)	Mucoadhesive buccal film (F26)
C_{max} (ng/ml)	171.32 ± 11.12	195.58 ± 11.65
T_{max} (hr)	2.00 ± 0.00	3.00 ± 0.00
AUC_{0-10} (ng.hr/ml)	859.24 ± 129.30	1206.27 ± 137.61
$AUC_{0-\infty}$ (ng.hr/ml)	925.06 ± 180.90	1527.98 ± 378.22
MRT (hr)	4.81 ± 0.57	6.84 ± 2.53
Relative bioavailability (%)	-----	165.2 %

[a] *Each value represents the mean ±SD. (n = 4).*

CONCLUSION

New buccal mucoadhesive film formulations containing diltiazem hydrochloride had been prepared with satisfactory physicochemical characterizations. The release patterns and bioadhesion properties can be controlled by changing the polymer type and concentration. The diltiazem hydrochloride administered to healthy rabbits via buccal route showed a significant improvement in bioavailability when compared to oral route. This increased bioavailability of diltiazem hydrochloride from designed formulations may also result in substantial dose reduction. The present study indicates a good potential of the prepared buccal mucoadhesive films containing diltiazem hydrochloride for systemic delivery with added advantages of circumventing the hepatic first pass metabolism and substantial dose reduction. This study confirmed the potential of the above buccal dosage forms as a promising candidate for buccal delivery of diltiazem hydrochloride.

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