



American Journal of
Drug Discovery
and Development

ISSN 2150-427X



Academic
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Formulation and *In vitro/In vivo* Evaluation of Buccoadhesive Discs for Controlled Release of Calcium Channel Antagonist

^{1,2}Mohamed Haider, ²Magdy Mohamed and ²Muaadh Ali

¹Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, University of Sharjah, Sharjah, 27272, UAE

²Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Corresponding Author: Mohamed Haider, Department of Pharmaceutics and Pharmaceutical Technology, College of Pharmacy, University of Sharjah, Sharjah, 27272, UAE Tel: +97165057414

ABSTRACT

Diltiazem hydrochloride (DTZ HCl) buccoadhesive discs were developed to control the drug release and enhance its bioavailability by evading the first pass metabolism. Buccoadhesive discs were prepared by direct compression of mucoadhesive polymers, namely; polyvinylpyrrolidone (PVP K-30), Sodium Carboxymethyl Cellulose (SCMC) and Carbopol 934 (CP934) in combination with Eudragit L100-55, or Sodium Alginate (SA), as matrix polymers to control the drug release. *In vitro* characterization showed that drug release, swelling capacity, surface pH and mucoadhesion depended on the type of polymers used and their ratios. *In vivo* testing in human volunteers showed that most of the formulae had suitable mucoadhesion and controlled drug release, however those containing SA and SCMC mixtures showed optimum drug release and greater mucoadhesion values. Relative bioavailability of a selected DTZ HCl buccoadhesive formulation (SA/SCMC, 3:1) was determined and compared with that of a commercial sustained release oral tablet (Altiazem[®] SR) as a reference in rabbits. The percentage relative bioavailability of DTZ HCl from the selected buccoadhesive disc was found to be 197.7%. The estimated t_{max} and $AUC_{0-\infty}$ values were also significantly higher after buccal administration ($p < 0.05$). The proposed buccoadhesive formulation of DTZ HCl could be an alternative for the currently available oral therapy.

Key words: Buccoadhesive disc, diltiazem hydrochloride, buccal delivery, controlled release, relative bioavailability

INTRODUCTION

The Diltiazem hydrochloride (DTZ HCl) is a benzothiazepine calcium channel antagonist of a molecular weight 450.98 which has been shown to lead to an effective and well-tolerated treatment of stable, unstable and variant angina pectoris as well as mild to moderate systemic hypertension. It has also shown remarkable efficacy in terminating supraventricular tachycardia and in controlling the ventricular response to a trial fibrillation/flutter (Chaffman and Brogden, 1985; Elliott and Ram, 2011). Following oral administration, DTZ HCl suffers from extensive presystemic metabolism and the absolute bioavailability is approximately 40%, with a large inter-individual variation that is mainly due to first pass effect (Hermann and Morselli, 1985). The DTZ HCl is mainly metabolized by CYP3A4 enzyme which is mainly located in the liver but also found in the

intestine (Watkins *et al.*, 1987). Furthermore, DTZ HCl is rapidly eliminated from the body with a short elimination half-life ranging from 3-6 h (Chaffman and Brogden, 1985; Hermann and Morselli, 1985). Many attempts were made to develop sustained release preparations of DTZ HCl with extended clinical effects and a reduced dosing frequency (Chaffman and Brogden, 1985; Hendriks *et al.*, 1998). Delayed-release tablets using hydroxyethylcellulose as a gel-forming matrix (Matsuo *et al.*, 1996) 6-casein-chitosan microspheres prepared by an aqueous coacervation technique (Bayomi *et al.*, 1998), extended release matrix tablets using counter polymer in Polyethylene Oxides (Peo)/Polyethylene Glycol (PEG) (Kojima *et al.*, 2008), controlled release guar gum matrix tablets (Al-Saidan *et al.*, 2005), oral floating matrix tablet (Gambhire *et al.*, 2007; Anwar *et al.*, 2011), diffusion-controlled transdermal delivery system (Limpongsa and Umprayn, 2008) are examples of controlled-release forms of this drug which have been reported in different studies with claims to maintain effective plasma concentrations. However, due to the extensive first pass metabolism, oral formulation of DTZ HCl requires the administration of large doses of the drug to reach effective sustained therapeutic levels in the plasma which could result in serious toxic manifestations in case of system failure.

The interest in alternative routes of drug administration occurs from their ability to enhance the bioavailability of certain drugs by passing the liver and hence escaping first-pass metabolism (Shojaei, 1998; Limpongsa and Umprayn, 2008; Behra *et al.*, 2012). Many studies have demonstrated the feasibility of buccal delivery for low molecular weight drugs such as acyclovir (Rossi *et al.*, 2003) and propranolol (Manganaro and Wertz, 1996) as well as large molecular weight molecules such as dextrans (Hoogstraate *et al.*, 1996) and insulin (Morishita *et al.*, 2001). Drug delivery via the buccal route using mucoadhesive dosage forms offers such a benefit since drug molecules permeate the oral mucosa and directly diffuse into the systemic circulation where the non-keratinized mucosa and dense capillary vessel network in the buccal cavity allow for a rapid onset of action and better control of plasma levels with minimum fluctuations (Shojaei, 1998; Nafee *et al.*, 2003a; Salamat-Miller *et al.*, 2005; Sudhakar *et al.*, 2006; Behra *et al.*, 2012). In addition, buccal drug delivery is an effective and safe alternative route of administration for patients who are unable to intake drugs orally where drug absorption can be rapidly terminated in cases of toxicity by removal of the dosage form from the buccal cavity. Accordingly, different buccoadhesive dosage forms including adhesive tablets (Choi *et al.*, 2000; Shanker *et al.*, 2009), gels (Ishida *et al.*, 1983) and patches (Nafee *et al.*, 2003b; Patel *et al.*, 2006) were formulated using different mucoadhesive polymers such as, polyvinylpyrrolidone, sodium carboxymethyl cellulose and carboxyvinyl polymer for buccal delivery.

The selection of suitable polymers and optimization of the formulation from both the bioadhesion and controlled drug release aspects remain an important goal and challenge for development of an efficient buccal dosage form (Hassan *et al.*, 2009). An ideal buccoadhesive drug delivery system should possess good bioadhesive properties as these dosage forms should adhere to the mucosa to prolong the residence time of the dosage form and secure localized drug release for a required period of time. In addition, the delivery system should release the drug in a controlled and predictable manner, to achieve and maintain efficient plasma drug levels for a defined period of time for the desired therapeutic response (Shojaei, 1998). A polymer that has good mucoadhesive property may not necessarily have good controlled drug release behavior and vice versa (Munasur *et al.*, 2006). Therefore, a combination of a mucoadhesive polymer and a matrix or scaffolding polymer is essential to optimize both the localization and drug release kinetics, respectively, from buccoadhesive drug delivery systems.

The use of different types of Eudragits for controlled drug delivery has been well established (Moustafine *et al.*, 2005). Eudragit L100-55 is a particularly attractive matrix or scaffold for controlled release, due to its high chemical stability, good compactability and dissolution at pH above 5.5, equivalent to the range of salivary pH (5.5-7) (Hassan *et al.*, 2009; Al-Taani and Tashtoush, 2003; Ceballos *et al.*, 2005; Moustafine and Bobyleva, 2006). Sodium Alginate (SA) is a hydrophilic polymer that has been extensively used in fabrication of scaffolds for controlled drug delivery as well. It is easily and rapidly hydrated with water leading to considerable swelling of the polymer matrix and allowing the drug to diffuse at a controlled rate (Patel *et al.*, 2007).

The geometric shape of the drug delivery system plays a major role in stability, ease of manufacture and drug release (Yehia *et al.*, 2008). Previously, the formulation and release of DTZ HCl is reported from buccal film using several hydrophilic and hydrophobic film forming polymers either alone or in combination with bioadhesive polymers (Mohamed *et al.*, 2011). However, buccal discs offer advantages over adhesive films in terms of stability and easier manufacture on a large scale (Yehia *et al.*, 2008).

The aim of the present study was to develop and characterize a novel buccoadhesive controlled release discs for buccal drug delivery of DTZ HCl. The effect of the type and concentration of bioadhesive polymer and the nature of the matrix polymer on physicochemical properties, drug release rate, release mechanism and mucoadhesive strength were studied. In addition, *in vivo* bioavailability and buccoadhesion studies were carried out in rabbits and healthy human volunteers, respectively.

MATERIALS AND METHODS

Materials: Diltiazem hydrochloride (DTZ HCl) and Altiazem® SR (internal standard) were kindly provided by the Egyptian International Pharmaceutical Company EIPCO (10th of Ramdan, Egypt). Eudragit L100-55 was obtained from Rohm Pharma (Darmstadt, Germany). Sodium Alginate (SA), polyvinylpyrrolidone (PVP K-30), Carbopol 934 (CP934), sodium carboxymethyl cellulose (SCMC, low viscosity), D-mannitol, magnesium stearate and moxifloxacin hydrochloride were purchased from Sigma-Aldrich (St. Louis, USA). The HPLC grade of potassium dihydrogen phosphate, ortho phosphoric acid, acetonitrile and methanol were acquired from Merck KGaA, (Darmstadt, Germany). All other chemicals used in the study were of analytical grade and were used as received.

Preparation of buccoadhesive discs: All formulae comprised DTZ HCl (30 mg), 10 mg of mannitol as diluent, 1.5 mg magnesium stearate as lubricant and mixtures of matrix polymers Eudragit L100-55 or SA with bioadhesive polymers (PVP K-30, SCMC or CP934) in different w/w ratios (Table 1). The discs were prepared by a direct compression method using IR hydraulic Press (Model P16, Beckman, UK) equipped with 13 mm flat punches at a compression force of 5,000 kg for 15 sec (El-Samaligy *et al.*, 2004; Perioli *et al.*, 2004). The final expected total weight of the discs was 220 mg.

Physicochemical characterization of the buccoadhesive discs

Determination of weight variation, thickness and hardness: Weight variation was carried out using 5 discs from each formula. Discs were individually weighed using an electronic balance (model AC210 S, Sartorius, Germany), the mean weight of discs was calculated and the weight variation was determined. The thickness and diameter of the prepared discs were determined using

Table 1: Composition of DTZ HCl buccoadhesive discs

| Formula code | Ratio* | Eudragit L100-55 (mg) | SA (mg) | PVP K30 (mg) | SCMC (mg) | Carbopol 934 (mg) |
|--------------|--------|-----------------------|---------|--------------|-----------|-------------------|
| F1 | 3:1 | 135 | - | 45 | - | - |
| F2 | 2:2 | 90 | - | 90 | - | - |
| F3 | 1:3 | 45 | - | 135 | - | - |
| F4 | 3:1 | 135 | - | - | 45 | - |
| F5 | 2:2 | 90 | - | - | 90 | - |
| F6 | 1:3 | 45 | - | - | 135 | - |
| F7 | 3:1 | 135 | - | - | - | 45 |
| F8 | 2:2 | 90 | - | - | - | 90 |
| F9 | 1:3 | 45 | - | - | - | 135 |
| F10 | 3:1 | - | 135 | 45 | - | - |
| F11 | 2:2 | - | 90 | 90 | - | - |
| F12 | 1:3 | - | 45 | 135 | - | - |
| F13 | 3:1 | - | 135 | - | 45 | - |
| F14 | 2:2 | - | 90 | - | 90 | - |
| F15 | 1:3 | - | 45 | - | 135 | - |
| F16 | 3:1 | - | 135 | - | - | 45 |
| F17 | 2:2 | - | 90 | - | - | 90 |
| F18 | 1:3 | - | 45 | - | - | 135 |

*Eudragit L100-55 or SA to mucoadhesive polymer ratio

an electronic micrometer (model G, Peacock, Japan) and the mean thickness and diameter were calculated. The hardness of five discs from each formula was determined individually using hardness tester (model PTB311, Pharmatest, Germany) and average values were calculated for each formula.

Determination of content uniformity: Drug content uniformity of the buccoadhesive discs was determined by individually crushing five discs from each formula and dissolving the resulting fine particles in phosphate buffer (pH 6.8). The solution was filtered through 0.45 µm filter (Millipore Co., Bedford, MA, USA) and properly diluted with the same buffer. The absorbance was measured spectrophotometrically at the predetermined λ_{max} of DTZ HCl of 237 nm using UV/VIS spectrophotometer, UV-1601 PC (Shimadzu, Kyoto Japan) using solutions of plain discs as blanks.

Surface pH measurement: The surface pH of the discs was determined in order to investigate the possibility of any irritation in the oral cavity. Discs were allowed to swell in contact with 5 mL of distilled water for 2 h in Petri dishes, then the surface pH of each disc was determined using pH meter specially designed to measure the pH of small volumes of liquids (Jenway 3505, Essex, UK) (Bottenberg *et al.*, 1991; Charde *et al.*, 2008). The average pH of three readings was determined.

Determination of equilibrium swelling ratio: The swelling index (%) was experimentally determined using the equation (Desai and Kumar, 2004):

$$\text{Swelling index (\%)} = \frac{W_s - W_d}{W_d} \times 100$$

where, W_s is the weight of swollen disc and W_d is the weight of dry disc. Freshly prepared discs were weighed individually (W_d) and transferred to Petri dishes containing 25 mL of distilled water. Each

disc was completely soaked in water for 1 h at room temperature then removed. The excess water on the surface was carefully removed using filter paper and swollen discs were weighed (W_s). The weights of the discs were determined with a precision micro-balance and the average values of three measurements were taken for each sample for calculation of swelling index (%).

Release of DTZ HCl from buccoadhesive discs: The drug release studies were carried out using modified USP dissolution apparatus, type II (Type PTW, Pharma Test, Germany) equipped with paddles which was operated at the speed of 50 rpm. The release medium consisted of 250 mL of phosphate buffer solution of pH 6.8 and the release was performed at $37 \pm 0.5^\circ\text{C}$. The disc was attached from one side to a 3 cm diameter glass disc with instant cyanoacrylate adhesive, in order to follow the pharmacopeial requirements of paddle over disc. The glass disc was then placed at the bottom of the dissolution vessel with the disc facing the solution side, thereby allowing drug release only from the upper side of the buccal disc. The amount of drug released was measured at the pre-determined time intervals (0.25, 0.5, 1, 2, 3, 4, 5, 6 and 8 h) by withdrawing aliquots of 5 mL which were replaced immediately with fresh medium. The samples were filtered through 0.45 μm filter (Millipore Co., Bedford, MA, USA) then analyzed by UV spectrophotometry at 237 nm after proper dilution. The release studies were conducted in triplicates and the mean cumulative percentage drug released was plotted versus time. The absorbance of the polymeric additives was negligible and did not interfere with λ_{max} of the drug.

Kinetic analysis of the release data: In order to gain insight into the drug release mechanism from the prepared buccoadhesive discs, the release data was fitted according to zero order, first order and Higuchi equations. The following linear regression equation were employed for zero order kinetics:

$$F_t = Kt$$

where, F_t is the fraction of drug released in time t and K represents the apparent release rate constant or zero order release constant. First order kinetics was determined according to the equation:

$$\ln(1-F) = -Kt$$

where, F is the fraction of drug released in time t and K represents the first order release constant. Drug release following Higuchi model was determined using the equation:

$$F = Kt^{1/2}$$

where, F is the fraction of drug released in time t and K represents the Higuchi dissolution constant.

For further investigation of the release mechanism, the release data was also fitted to Korsmeyer-Peppas equation which is frequently used to describe the drug release behavior from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved. The Korsmeyer-Peppas equation (Korsmeyer *et al.*, 1983):

$$M_t/M_\infty = k t^n$$

where, k is a kinetic constant featuring both structural and geometric characteristics of the matrix tablets, n represents the diffusional exponent that depends on the release mechanism and M_t/M_∞ is the fraction of drug dissolved at time t . When determining the n exponent, only the portions of the release profile where $M_t/M_\infty \leq 0.6$ were employed to provide the accurate values.

***In vitro* mucoadhesion measurements:** The adhesion of the prepared discs to mucosal membrane was determined using inverted surface of chicken pouch (stripped off its contents and surface fats) as the model mucosal tissue (Wong *et al.*, 1999a; El-Samaligy *et al.*, 2004). The chicken pouches were kept frozen at -20°C in a phosphate buffer saline solution pH 6.75 and only thawed to room temperature before use.

The mucoadhesion strength was checked using a modified balance method (Ali *et al.*, 2002; Kerec *et al.*, 2002). Briefly, a balance was taken and its left pan was replaced with a weight to the bottom of which a buccoadhesive disc 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, $37^\circ\text{C} \pm 0.5^\circ\text{C}$) with a level slightly above the membrane. The weight which was attached to the buccal disc, 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 and the weight (in grams) needed for the separation of the two surfaces was recorded as the mucoadhesive strength. The experiments were performed in triplicate and average values were reported.

***In vivo* buccoadhesion performance of plain discs:** Plain discs were tested in four healthy volunteers (25-40 years) following the regulations approved by the Institutional *in vivo* Ethics Committee (Cairo University, Cairo, Egypt). Each volunteer was provided with 3 discs from each formula. After wiping off the excessive saliva, each disc was applied to the gingival mucosa above the canine tooth by pressing for 30 sec onto the mucosa and left for a period of 8 h (Yehia *et al.*, 2009). The volunteers were asked to record the residence time; which is the time required for complete erosion or detachment of the disc from the buccal mucus membrane and also to monitor for fragment loss, salivary level variation, bad taste, swelling, irritation or pain.

***Ex vivo* permeation studies:** The *ex vivo* drug permeation study of DTZ HCl through chicken pouch membrane was performed using the method described by Tayel *et al.* (2010). The apparatus used to test the permeation consisted of a glass tube (1.3 cm diameter) opened from both ends. Each disc was pressed on the mucosal side of chicken pouch for 30 sec and the loaded membrane was stretched over the open end of a glass tube, made water tight by rubber band forming a donor chamber. In order to simulate the conditions inside the buccal cavity, 2 mL phosphate buffer pH 6.8 were transferred to the donor chamber. The tube was attached to the shaft of the USP dissolution apparatus and immersed in USP dissolution apparatus flask containing 250 mL of phosphate buffer (pH 7.4) such that the membrane was just below the surface of the recipient solution. The permeation study was completed at $37 \pm 0.5^\circ\text{C}$ and steady rotation speed of 50 rpm for 8 h. Samples of five milliliters were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 8 h and were immediately compensated for by an 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 in milligrams 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 equation:

$$J_{ss} = (dQ/dt)/A$$

where, J_{ss} 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 using the equation:

$$P = J_{ss}/C_d$$

where, P is the permeability coefficient and C_d is the concentration of the drug in the donor chamber (De Caro *et al.*, 2008). The experiments were performed in triplicates and mean values \pm SD were calculated to compute the flux and permeability coefficient.

***In vivo* studies in rabbits**

Study design: The *in vivo* studies were carried out to compare the pharmacokinetics of DTZ HCl from F13 buccoadhesive disc formulation containing 30 mg DTZ HCl (group A) to an oral commercial sustained release tablet (Altiazem[®] SR) containing the same dose as a reference (group B) in white New Zealand rabbits with mean weight 1.79 ± 0.24 kg using a non-blind, two-group, randomized, parallel design. Four rabbits were randomly assigned to each of the two treatment groups of equal size ($n = 4$).

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 (Cairo University, Cairo, Egypt), under the supervision of a registered veterinarian.

Animals were fasted overnight and kept in individual cages before the study day. On the day of the study, animals were lightly anesthetized by an intramuscular injection of a 1:5 mixture of xylazine (1.9 mg kg^{-1}) and ketamine (9.3 mg kg^{-1}) before the assigned treatment was given (Alur *et al.*, 1999). The light plane of anesthesia was maintained by an intramuscular injection of one-third of the initial dose of xylazine and ketamine mixture as needed. In group A, upon the induction of anesthesia, a drop of water was placed on the surface of the buccoadhesive disc then the disc was applied to the oral cavity, on the buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed against the mucosa for about 30 sec to ensure adhesion. Oral Altiazem[®] SR tablets were administered to test animals by carefully placing each as far back in the oral cavity as possible to prevent the animal from spitting it out. Blood samples (2 mL) were withdrawn from the ear vein of rabbits using a 23 G needle at 0.5, 1, 2, 3, 5, 7 and 10 h post dosing and collected in heparinized tubes. Blood samples were centrifuged at $3000 \times g$ for 10 min to separate the plasma. The clear supernatant plasma layer was collected in labeled tubes and stored immediately at -20°C until analysis.

Blood sample analysis: The quantitative determination of DTZ Hcl was performed by High-Performance Liquid Chromatography (HPLC, Shimadzu LC-20A, Shimadzu, Japan) using of a Shimadzu LC-20A pump, SIL-20 autosampler, an SPD 20A UV/VIS detector and a μ Bondapak C-18 column ($250 \times 4.6 \text{ mm ID}$; particle size $5 \mu\text{m}$) (Waters, USA). The mobile phase consisted of a mixture of potassium dihydrogen orthophosphate buffer (0.05 M, pH 4.6), acetonitrile and anhydrous ethanol at 70:25:5 v/v. The final pH was adjusted to 4.6 using 85% orthophosphoric acid

(British Pharmacopoeia Commission, 2009). The mobile phase was filtered through a 0.45 μm membrane filter and was then degassed by ultrasonication. Analysis was carried out at a flow rate of 1.3 mL min^{-1} using a detection wavelength of 260 nm. Peak areas were calculated with Shimadzu LC Solution software.

Frozen plasma samples were thawed at ambient temperature ($25\pm 2^\circ\text{C}$) for at least 60 min, followed by adding 100 μL of moxifloxacin hydrochloride as internal standard (IS) ($100 \mu\text{g mL}^{-1}$ in methanol) and 4 mL of diethyl ether to 1 mL thawed plasma sample. The mixture was then vortexed for 2 min by using a vortex mixer and centrifuged at 3000 rpm for 10 min. After centrifugation the upper organic layer was separated and the solvent was evaporated in vacuum oven to dryness. The residue was reconstituted with 400 μL of mobile phase and 20 μL were injected into column.

Chromatograms obtained showed no interfering with determination of the DTZ HCl and IS peaks which were well resolved (data not shown). The retention times were approximately 4.3 and 7.4 min for the drug and IS, respectively. The calibration curve for DTZ HCl was constructed from measurements of five concentrations in the range of 10-200 ng mL^{-1} in spiked plasma. Calibration curve for DTZ HCl was linear and the relative coefficient of correlation (r^2) was 0.997. Precision and accuracy were evaluated by spiking blank plasma with DTZ HCl at three concentration levels: 50, 100 and 200 ng mL^{-1} . The coefficients of variation (CVs) for the intra-day precision were: 3.47% at 50 ng mL^{-1} , 0.92% at 100 ng mL^{-1} and 4.42% at 200 ng mL^{-1} . The CVs for day-to-day precision were: 3.37% at 50 ng mL^{-1} , 5.73% at 100 ng mL^{-1} and 8.59% at 200 ng mL^{-1} . The relative error, determined by comparing the measured concentrations to the expected concentrations, was less than 10%. The absolute recovery of DTZ HCl at 50, 100 and 200 ng mL^{-1} 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^{-1} .

Pharmacokinetic analysis: Pharmacokinetic characteristics from plasma data following administration of the buccoadhesive disc and reference formulation, Altiazem[®] RS, were estimated for each rabbit by using a computer program, WinNonlin[®] (version 1.5, Scientific consulting, Inc., Cary, NC, USA). Noncompartmental analysis was used. C_{max} (ng mL^{-1}) and t_{max} (h) were the observed maximal drug concentration and its time, respectively. The area under the curve, $\text{AUC}_{(0-t)}$ ($\mu\text{g h mL}^{-1}$), was calculated using the trapezoidal rule from zero time to the last time of blood sample. The area under the curve from zero to infinity, $\text{AUC}_{(0-\infty)}$ (ng h mL^{-1}), was calculated as:

$$\text{AUC}_{(0-\infty)} = \text{AUC}_{(0-t)} + C_t/k$$

where, C_t is the last measured concentration at the time t and k is the terminal elimination rate constant estimated by log-linear regression analysis on data visually assessed to be a terminal log-linear phase. MRT, the mean residence time was calculated as:

$$\text{AUMC}_{(0-\infty)}/\text{AUC}_{(0-\infty)}$$

where, AUMC is the area under the first moment versus-time curve from $t = 0$ to infinity. The relative bioavailability (f_{rel}) of DTZ HCL from the buccoadhesive disc in comparison to the reference commercial tablet was calculated as:

$$\text{AUC}_{\text{F13}}/\text{AUC}_{\text{Altiazem}} \times 100$$

Statistical analysis: Statistical comparisons between two groups were made using the two-tailed, unpaired Student's t-test. A p-value of less than 0.05 was considered statistically significant. The one-way analysis of variance (ANOVA) F-test for testing the equality of several means followed by Tukey's post t-test analysis was used for multiple comparisons. For the pharmacokinetic parameters C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and t_{max} , statistical analyses were performed using a two-sample t test assuming unequal variances wherein $p < 0.05$ were considered statistically significant. Statistical inferences were based on untransformed values for the C_{max} and AUC parameters and observed values for t_{max} . Statistical calculations were carried out using the software SPSS 14.0 (SPSS Inc., Chicago, USA) The significant level was set at $\alpha = 0.05$ for all statistical tests.

RESULTS AND DISCUSSION

Physicochemical characterization of buccoadhesive discs: The physical properties of the prepared buccoadhesive controlled-release DTZ HCl discs are presented in Table 2. The determination of the mean weight of five discs from each formula showed that all the discs were within the weight range of 216.0-222.4 mg. The polymer composition and ratio did not have a sizeable effect on the weight of the prepared discs and weight variation remained within acceptable limits as shown by the low SD values indicating good weight uniformity.

The average thickness of all prepared buccal discs ranged from 1.212±0.004-1.390±0.014 mm which shows the uniformity of the compression process used to prepare the discs and excludes variations in drug release due to changes in diameter or force of compression. Similarly, the hardness of the prepared discs showed acceptable values from 3-10 kg cm⁻². The obtained hardness values confirm the excellent compactability properties of the used polymers which allowed direct compression even in the absence of other excipients. Disc formulae F7-F9 containing different

Table 2: Physicochemical properties of buccoadhesive disc

| Formula code | Thickness (mm) | Weight (mg) | Hardness (kg cm ⁻²) | pH | Drug content (%) | Swelling index (%) | |
|--------------|----------------|-------------|---------------------------------|-----------|------------------|--------------------|--------------|
| | | | | | | After 15 min | After 60 min |
| F1 | 1.366±0.005 | 217.6±0.55 | 6.3±0.3 | 5.14±0.16 | 100.57±0.42 | 8.58±1.30 | 25.28±1.20 |
| F2 | 1.364±0.005 | 219.8±0.84 | 5.3±0.3 | 5.53±0.08 | 101.94±0.48 | 12.97±3.12 | 43.622±1.44 |
| F3 | 1.372±0.011 | 220.6±1.34 | 4.5±0.5 | 5.36±0.11 | 102.17±0.36 | -2.89±4.09 | Eroded |
| F4 | 1.308±0.011 | 216.0±0.71 | 7.3±0.3 | 5.72±0.05 | 100.39±0.55 | 81.45±7.34 | 122.73±7.61 |
| F5 | 1.280±0.010 | 216.8±0.84 | 7.2±0.3 | 6.29±0.05 | 100.48±0.44 | 129.65±4.78 | 192.17±9.13 |
| F6 | 1.266±0.015 | 218.2±1.10 | 7.2±0.3 | 6.61±0.06 | 99.01±0.41 | 151.25±6.66 | 280.43±18.60 |
| F7 | 1.320±0.032 | 217.4±1.82 | 9.5±0.5 | 4.38±0.09 | 97.54±0.42 | 57.76±2.91 | 116.44±3.23 |
| F8 | 1.286±0.011 | 216.8±1.79 | 9.8±0.3 | 4.58±0.15 | 96.03±0.71 | 72.95±3.54 | 123.64±6.43 |
| F9 | 1.240±0.016 | 217.8±1.10 | 10.0±0.5 | 4.60±0.07 | 95.85±0.28 | 102.48±1.59 | 152.36±5.58 |
| F10 | 1.330±0.007 | 220.6±0.89 | 3.0±0.5 | 7.11±0.11 | 99.93±0.71 | 181.34±12.18 | 403.66±11.61 |
| F11 | 1.361±0.008 | 222.4±0.89 | 3.3±0.3 | 6.94±0.06 | 100.20±0.21 | 179.67±10.92 | Eroded |
| F12 | 1.390±0.014 | 221.4±0.55 | 3.5±0.5 | 6.67±0.08 | 100.20±0.40 | 88.48±7.38 | Eroded |
| F13 | 1.276±0.005 | 217.4±0.89 | 4.8±0.3 | 7.00±0.15 | 99.74±0.48 | 182.21±3.65 | 389.17±16.62 |
| F14 | 1.244±0.005 | 217.4±1.14 | 5.0±0.5 | 6.99±0.08 | 100.16±0.35 | 180.47±4.16 | 382.06±7.21 |
| F15 | 1.244±0.005 | 219.6±0.55 | 5.8±0.3 | 7.12±0.08 | 99.65±0.42 | 194.31±12.61 | 361.21±9.66 |
| F16 | 1.248±0.004 | 219.6±0.55 | 6.2±0.8 | 6.01±0.13 | 98.05±0.50 | 151.83±3.55 | 359.82±21.31 |
| F17 | 1.224±0.005 | 220.4±0.55 | 8.2±0.3 | 6.06±0.63 | 97.22±0.28 | 150.80±0.16 | 317.85±1.35 |
| F18 | 1.212±0.004 | 217.4±0.55 | 8.8±0.3 | 5.37±0.07 | 96.03±0.42 | 125.74±2.82 | 267.4±13.96 |

combination of Eudragit L100-55 and CP934 showed significantly higher hardness values than other formulae which may be due to the higher bulk densities of both polymers compared to other polymers used.

The determination of mean (%) drug content showed that all prepared discs are in compliance to the pharmacopoeial limits where the average percentage drug content lied within the range of 85-115% of the claimed dose and the standard deviation was less than 0.71%.

Determination of surface pH: The resemblance of surface pH of the discs to physiological pH is essential to avoid any potential irritation to the buccal mucosa after continuous application of mucoadhesive discs. All formulae, except F7, F8 and F9, had shown a surface pH values around the physiological pH (Table 2). These results reveal that the designed formulations provide an acceptable pH in the range of salivary pH (5.5-7.0) suggesting no risk of mucosal damage or irritation upon application. The lower pH values obtained from formulations containing different combination of Eudragit L100-55 and CP934 (F7-F9) might be due to their high content of carboxyl groups which may cause slight irritation to the mucus membrane when applied (Al-Taani and Tashtoush, 2003; Munasur *et al.*, 2006).

Swelling studies: Swelling behavior of a buccoadhesive system is critical for effective adhesion and uniform and prolonged release of the drug (Desai and Kumar, 2004). Swelling index for each formula was calculated with respect to time and the values obtained at 15 and 60 min are shown in Table 2. The results suggest that both the type of matrix polymer and type and concentration of the incorporated mucoadhesive polymers may have a substantial role in achieving the desired bioadhesion and drug release profiles. In general, discs containing Eudragit L100-55 showed less swelling than those containing SA at the same ratio and type of bioadhesive polymer used. These results can be attributed to the hydrophobic nature of Eudragit L100-55 especially when compared with SA, a hydrophilic polymer, that can retain large amounts of water is expected to have a higher rate and extent of swelling. Those results were further confirmed by the increase in the observed swelling index % associated with the increase in the amount of SA in the formulation.

Although, all mucoadhesive polymers used in the study are hydrophilic and can retain large amounts of water (Perioli *et al.*, 2004), discs containing SCMC showed significant superior concentration-dependent swelling properties when combined with either Eudragit L100-55 or SA, followed by those containing similar amounts of PVP K-30 then CP934. The results of the swelling study also showed that formulations F3, F11 and F12 were not able to preserve their integrity and disintegrated after 1 h probably due to an increase in surface wettability and water penetration within the matrix that can be attributed to the presence of PVP especially at higher concentrations (Nafee *et al.*, 2003b).

Drug release from buccoadhesive disc: A perfect controlled-release drug delivery system would release part of the drug load immediately after administration to rapidly achieve the therapeutic level then subsequently sustain this drug level for an extended period of time (Remunan-Lopez *et al.*, 1998). A release of 20-50% of the drug after 3 h ensures that there is no dose dumping from the dosage form. In addition, formulations with an appropriate controlled-release profile of at least 80% drug release over a period of 8 h were desired for the purpose of this study.

The cumulative percentage drug release profiles of DTZ HCl from buccoadhesive discs containing PVP K-30, SCMC and CP934 Fig. 1a-c, respectively showed that the drug release

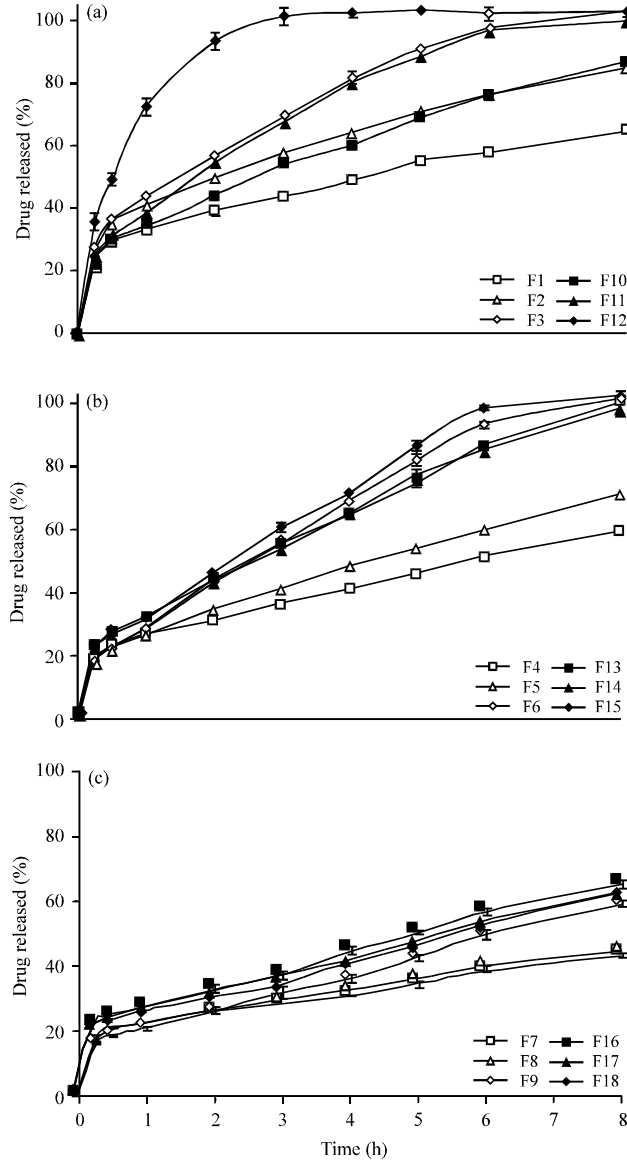


Fig. 1(a-c): Release profile of DTZ HCl from buccal discs containing, (a) PVP K-30, (b) SCMC and (c) CP 934P as a mucoadhesive polymer. Points represent the mean \pm standard deviation for $n = 3$

was influenced by the type of matrix and bioadhesive polymer used and their ratio in each formula. In all discs, a burst release was observed within the first hour, followed by a slow gradual increase in the cumulative percentage of drug released up to 8 h. The release of the drug was significantly faster from discs containing SA than from those containing Eudragit L100-55 using the same type and ratio of the bioadhesive polymer which may be due to the differences in the hydration, gelling and erosion behavior of both matrix polymers and is in agreement with the swelling data where discs containing Eudragit L100-55 had smaller swelling indexes compared to those containing SA. In theory, the higher the uptake of water by the polymer, the more the amount of drug diffused out from the polymer matrix (Remunan-Lopez *et al.*, 1998). Accordingly, SA, being a hydrophilic

polymer, has the ability to hydrate more readily than Eudragit L100-55, retain water in its structure and spontaneously form a gel which is swellable, erodible and with faster drug release (Moustafine and Bobyleva, 2006).

The addition of increasing amounts of bioadhesive polymers to Eudragit L100-55 containing discs significantly increased the release rate of the drug. As shown in Fig. 1a-c, DTZ HCl release rates from discs containing Eudragit L100-55 were improved by decreasing the matrix to mucoadhesive polymer ratio, although, the release rates from those disc formulae were still lower than those from discs containing similar mucoadhesive polymer to SA ratios. This could be explained again by the ability of hydrophilic polymers to absorb water, increase swelling and gel expansion leading to an increase in the release of the drug. Moreover, the hydrophilic mucoadhesive polymers would dissolve, creating more pores and channels for the drug to diffuse out of the disc (Wong *et al.*, 1999a; Shidhaye *et al.*, 2010). Therefore, those results suggest that the rate of penetration of the release medium into the discs and hence the rate of release of the dissolved drug, are a function of the amount of the hydrophilic polymer dispersed throughout the matrix.

The time required for 50% ($T_{50\%}$) of DTZ HCl to be released from different mucoadhesive discs is shown in Fig. 2, as another measure of the controlled release of the drug from the prepared mucoadhesive discs. Formulae showing small $T_{50\%}$ values would be not suitable for sustained drug delivery of DTZ HCl as the drug should be slowly released from the buccal discs over a prolonged time period to enhance the complete absorption of the drug from the oral mucosa and reduce the washing out effect of human saliva.

Regardless the type of bioadhesive polymers, discs containing Eudragit L100-55, showed a proportional significant decrease in $T_{50\%}$ with decreasing Eudragit to mucoadhesive polymer ratio. The same effect was also observed for discs containing SA, except for F16-F18 containing SA/CP934, although the $T_{50\%}$ values for those discs were significantly less than that of Eudragit L100-55 containing discs having similar amounts of the mucoadhesive polymer. As discussed earlier, this decrease in $T_{50\%}$ is probably due to the ability of SA to retain large amounts of water more than Eudragit L100-55 leading to higher rate and extent of swelling. The type of mucoadhesive polymer also affected the $T_{50\%}$ values where discs prepared using PVP K-30, regardless the type of matrix polymer, showed a significantly smaller $T_{50\%}$ values followed by discs containing SCMC then CP934. The addition of PVP has been known to disintegrate a disc very rapidly, resulting in fast dissolution of the drug (Lee and Chien, 1995) while the incorporation of SCMC has been previously reported to alter the structural properties of the disc matrix by increasing the disc porosity, thus allowing a rapid penetration of the dissolution medium into the disc which facilitate the drug release (Munasur *et al.*, 2006). On the other hand, Discs containing CP934 (Fig. 1c and 2) showed slow and gradual drug release throughout the experimental time without reaching 100% drug release. The drug release behavior from CP934 discs may be due to the presence of uncharged carboxyl group that forms hydrogen bonds which imbibe water and also holds water inside the mucoadhesive gel matrix producing a water-swollen gel-like state that substantially control and reduce the penetration of more liquids through the gelled network and retarding the drug diffusion (Varshosaz and Dehghan, 2002; Narendra *et al.*, 2005). In SA/CP934 containing discs, as the amount of CP934 increased, both the viscosity and strength of gel formed increased which decreased the water diffusion into the disc and thereby decreases the drug release rate and in turn increases the $T_{50\%}$. This behavior is in agreement with the results obtained during

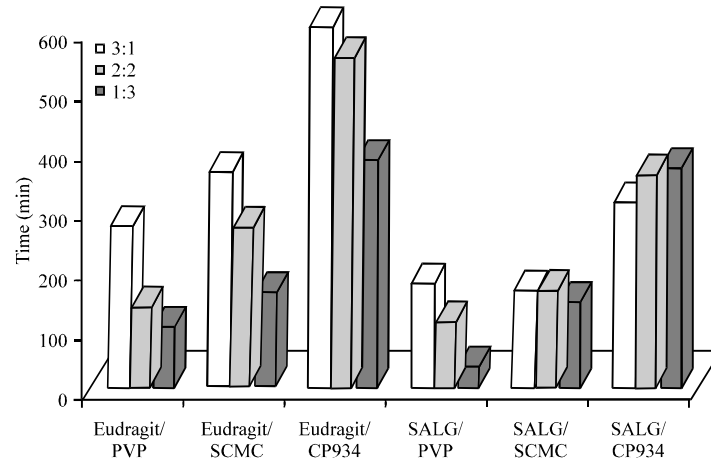


Fig. 2: Time for 50% drug release (min) of DTZ HCl from buccoadhesive discs. Bars represent the mean for n = 3 samples

the swelling study showing an increase in swelling index upon increasing the SA content and suggests that higher concentration of PVP K-30 is not suitable for the purpose of sustaining the release of DTZ HCL from the prepared buccoadhesive discs.

The observed effects of polymer composition and ratio on the cumulative release of DTZ HCl from the prepared discs correlates with previously reported release study of the same drug from buccal film (Mohamed *et al.*, 2011). However, the overall release from the buccal film was greater than that released from the discs. The difference in the rate of release can be attributed to the difference in the surface-to-volume ratio of the two geometries (Megeed *et al.*, 2004).

Kinetic analysis of the release data: Drug release from matrix/mucoadhesive polymers drug delivery systems is a complex process that may be purely diffusion or erosion controlled, or may exhibit a combination of these mechanisms (Remunan-Lopez *et al.*, 1998; Alur *et al.*, 1999). In order to determine the kinetics of drug release, the *in vitro* release data of DTZ HCl from buccoadhesive discs was analyzed according to zero order, first order, Higuchi model and Korsmeyer-Peppas equation (Table 3). Higuchi model is applicable when the release of drug is largely governed by diffusion through water-filled pores in the matrix. The preference of a certain mechanism was based on the determination of coefficient of correlation (r^2) for the parameters studied only for the early stages ($\leq 60\%$) of drug release, where the highest coefficient of correlation is preferred for the selection of the order of release.

Accordingly, coefficients of correlation (r^2) of each model were calculated by linear regression analysis and used to evaluate the accuracy of the fit. The *in vitro* release data is in favor of zero-order release kinetic except in case of F1, F2, F3 and F8, indicating that the drug release was nearly independent of its concentration which could be considered as an advantage for fabricated systems.

Interpretation of release exponent values (n) of the Korsmeyer-Peppas equation enlightens in understanding the release mechanism from the dosage form. For non-Fickian release, the value of n falls between 0.45 and 0.89 while for case II transport, n = 0.89 and for supercase II transport,

Table 3: Mathematic modeling and drug release kinetics from buccoadhesive discs

| Formula code | Correlation coefficient (r^2) | | | Release order | Parameters obtained from Korsmeyer-Peppas model | | | Cumulative drug released (%) (\pm SD) |
|--------------|-----------------------------------|-------------|---------------|---------------|---|--------|--------|--|
| | Zero order | First order | Higuchi model | | K | n | r^2 | |
| F1 | 0.9854 | 0.9493 | 0.9930 | Diffusion | 0.0643 | 0.3733 | 0.9916 | 64.90 \pm 2.09 |
| F2 | 0.9813 | 0.9360 | 0.9968 | Diffusion | 0.0808 | 0.3788 | 0.9985 | 85.03 \pm 0.26 |
| F3 | 0.9872 | 0.9270 | 0.9937 | Diffusion | 0.1063 | 0.3546 | 0.9817 | 102.57 \pm 0.79 |
| F4 | 0.9981 | 0.9707 | 0.9782 | Zero | 0.0501 | 0.3903 | 0.9672 | 59.28 \pm 0.94 |
| F5 | 0.9928 | 0.9440 | 0.9912 | Zero | 0.0369 | 0.4719 | 0.9911 | 70.57 \pm 0.56 |
| F6 | 0.9987 | 0.9441 | 0.9797 | Zero | 0.0311 | 0.5569 | 0.9898 | 101.08 \pm 1.21 |
| F7 | 0.9924 | 0.9672 | 0.9713 | Zero | 0.0915 | 0.2330 | 0.9373 | 43.23 \pm 0.46 |
| F8 | 0.9854 | 0.9473 | 0.9894 | Diffusion | 0.0556 | 0.3300 | 0.9732 | 44.62 \pm 0.39 |
| F9 | 0.9975 | 0.9764 | 0.9603 | Zero | 0.0245 | 0.5043 | 0.9699 | 59.10 \pm 1.11 |
| F10 | 0.9931 | 0.9505 | 0.9881 | Zero | 0.0526 | 0.4489 | 0.9917 | 86.68 \pm 1.69 |
| F11 | 0.9912 | 0.9602 | 0.9777 | Zero | 0.0606 | 0.4645 | 0.9867 | 99.82 \pm 2.16 |
| F12 | 0.9990 | 0.9834 | 0.9964 | Zero | 0.0882 | 0.5115 | 0.9963 | 100.91 \pm 2.51 |
| F13 | 0.9994 | 0.9667 | 0.9754 | Zero | 0.0367 | 0.5241 | 0.9925 | 99.97 \pm 1.12 |
| F14 | 0.9985 | 0.9699 | 0.9804 | Zero | 0.0350 | 0.5336 | 0.9840 | 97.88 \pm 2.36 |
| F15 | 0.9984 | 0.9755 | 0.9874 | Zero | 0.0295 | 0.5791 | 0.9956 | 101.68 \pm 1.64 |
| F16 | 0.9963 | 0.9737 | 0.9702 | Zero | 0.0273 | 0.5120 | 0.9875 | 65.15 \pm 1.32 |
| F17 | 0.9987 | 0.9816 | 0.9646 | Zero | 0.0256 | 0.5088 | 0.9864 | 60.89 \pm 1.14 |
| F18 | 0.9947 | 0.9876 | 0.9464 | Zero | 0.0187 | 0.5585 | 0.9771 | 60.67 \pm 0.27 |

$n > 0.89$. In case of $n \leq 0.45$, the release is governed by a pure Fickian diffusion mechanism that describes release of a drug from a matrix governed by diffusion (Yehia *et al.*, 2008). For most of the tested formulations, the values of n were between 0.45 and 0.89 except F1-F4, F7, F8 and F10. These values suggest that the release mechanism could be considered, in general, non-Fickian, although they were very close to the Fickian limit of $n = 0.45$, especially in the case of F4 and F10 (Table 3). This means that drug release is controlled both by drug diffusion process through the matrix and by polymeric chain relaxation time (Perioli *et al.*, 2008).

Mucoadhesion studies: The mucoadhesive strength (g) of the prepared discs was measured using chicken pouch as a model mucosa due its convenience and surface uniformity (Wong *et al.*, 1999b). The results show that the mucoadhesive strength exhibited by all formulations is satisfactory for maintaining them at the buccal site (Table 4). Nevertheless, the mucoadhesive characteristics were found to be affected by the type of matrix polymer used as well as the nature and concentration of the mucoadhesive polymers which on hydration, adhere to mucosal surface.

The bioadhesion strength and time were indirectly proportional to the matrix/mucoadhesive polymers ratio. When the concentration of the mucoadhesive polymer is high, the number of penetrating polymeric chains per unit volume of the mucus is high resulting in greater interaction and vice versa (Charde *et al.*, 2008). The maximum mucoadhesive strength was observed in formulations containing CP934 followed by those containing SCMC then PVP K-30. This may be due to the ability of CP934 to form secondary bioadhesion bonds with mucin and the interpenetration of the polymer chains in the interfacial region while the other polymers only undergo superficial mucoadhesion (Hassan *et al.*, 2009). In addition, the weaker mucoadhesion of

Table 4: Results of *in vitro* and *in vivo* bioadhesion of DTZ HCl buccoadhesive delivery systems

| Formula code | <i>In vitro</i> adhesion strength (g) (\pm SD) | <i>In vivo</i> bioadhesion performance | | |
|--------------|---|--|--------------------------------|------------|
| | | Adhesion time (h) (\pm SD) | Adhesive strength | Irritation |
| F1 | 6.88 \pm 2.03 | 0.63 \pm 0.18 | Slightly adhesive and slippery | None |
| F2 | 8.94 \pm 1.36 | 4.13 \pm 0.18 | Adhesive and slippery | None |
| F3 | 10.40 \pm 1.39 | 0.71 \pm 0.06 | Slightly adhesive | None |
| F4 | 27.10 \pm 3.68 | 4.38 \pm 0.18 | Adhesive | None |
| F5 | 33.82 \pm 3.35 | 7.08 \pm 0.12 | Adhesive | None |
| F6 | 39.06 \pm 3.82 | >8 | Adhesive | Mild |
| F7 | 29.67 \pm 4.83 | >8 | Adhesive | None |
| F8 | 41.17 \pm 2.88 | >8 | Very adhesive | Mild |
| F9 | 46.92 \pm 2.91 | >8 | Very adhesive | Moderate |
| F10 | 23.49 \pm 2.81 | 3.25 \pm 0.35 | Adhesive | None |
| F11 | 25.03 \pm 2.63 | 1.13 \pm 0.18 | Adhesive | None |
| F12 | 22.82 \pm 2.20 | 0.88 \pm 0.06 | Slightly adhesive | None |
| F13 | 40.11 \pm 1.38 | 8.00 \pm 0.35 | Adhesive | None |
| F14 | 46.57 \pm 2.64 | 7.04 \pm 0.29 | Adhesive | None |
| F15 | 44.29 \pm 3.87 | >8 | Adhesive | None |
| F16 | 61.68 \pm 3.10 | >8 | Very adhesive | Mild |
| F17 | 70.47 \pm 4.32 | >8 | Very adhesive | Mild |
| F18 | 74.98 \pm 3.13 | >8 | Very adhesive | Moderate |

discs containing PVP K-30 may be due to the high solubility of PVP in water that results in faster erosion and detachment from the membrane.

The results also showed that discs containing SA had significantly stronger adhesion values than those containing Eudragit L100-55. This superior mucoadhesion can be attributed to faster swelling and higher flexibility of polymeric chains of SA implying a better interaction with mucin (Davidovich-Pinhas and Bianco-Peled, 2010).

***In vivo* mucoadhesive studies of drug free buccal discs:** Since the drug used in the present study was an antihypertensive agent, placebo discs were used in the *in vivo* acceptability study on healthy human volunteers. The response of healthy human volunteers to each subjective parameter and their experiences with discs were documented. The results presented in Table 4 are in good correlation with those obtained from swelling and *in vitro* mucoadhesion studies where the longest adhesion times were observed with CP934 containing discs (>8 h) followed by that prepared using SCMC then PVP K-30. The short mucoadhesion time of PVP K-30 containing discs can be attributed to their faster erosion owing to the hydrophilic nature of the polymer and its disintegration at a very fast rate causing fragments to be dislodged from the disc and scattered throughout the buccal cavity, leading to taste alteration, salivation and feeling of discomfort reported by the volunteers. Therefore, the incorporation of PVP K-30 was found to have a negative effect on the mucoadhesion time which correlates with previous studies done by Shidhaye *et al.* (2008), who reported similar effect after using PVP K-30 in formulation of buccal patch of sumatriptan succinate. On the other hand, no disc fragmentation or bad taste were observed for discs containing SCMC and CP934 which successfully maintained their integrity and adhesion for a sufficient period of time before complete erosion.

However, signs of mild to moderate gum irritation have generally accompanied the use of discs containing CP934, except F7. This irritant effect is probably due to the slower hydration rate of those discs and the strongly formed mucin links (Siepmann and Peppas, 2001). In addition, most of the subjects reported some difficulty in removing the discs containing higher amounts of CP934 which sticks on the mucosa tenaciously after absorbing the saliva.

Discs containing SCMC showed good acceptability by the volunteers. There were no reported side effects such as taste alteration, heaviness, severe salivation, irritation, pain or discomfort on the gum, except for F6 where only mild irritation on the gum was reported. Therefore, DTZ HCl discs containing SCMC as a mucoadhesive polymer showed superior comfort when placed in the human buccal cavity and can be considered more suitable than those containing CP934 or PVP K-30 for buccal delivery of the drug.

Ex vivo Permeation of DTZ HCl buccoadhesive discs: Based on the collected data, the best formulations were selected on the basis of surface pH, *in vitro* release studies, *in vitro* and *in vivo* bioadhesion profile for further *ex vivo* permeation studies. Discs formulae that showed acidic surface pH values, less than 80% drug released after 8 h, loss of fragments in release media, in *in vivo* bioadhesion time less than 7 h, bad taste or irritation of the buccal mucosa were considered unacceptable and were excluded from further permeation and pharmacokinetic studies. Formulae F13, F14 and F15 containing SA and SCMC at different ratios showed suitable mucoadhesion properties, good acceptability by volunteers and no irritation during the period of study. They also showed $T_{50\%}$ in the range of 130-160 min and a percentage cumulative drug release of more than 90% after 8 h with zero-order release kinetics. In addition, their pH values were in the acceptable range (5.53-7.12). Therefore, they were chosen for *ex vivo* permeability and *in vivo* study.

Ex vivo permeation studies were carried out on the selected buccoadhesive discs formulae to assess the ability and possible mechanisms of drug transport across the buccal epithelium in order to get some insight into the absorption kinetics and bioavailability of drug molecules (Nair *et al.*, 2012). The permeation parameters of DTZ HCl through chicken pouch membrane for tested formulations are shown in Table 5. The results indicated that the drug can permeate easily through the chicken pouch membrane and hence could possibly permeate through the human buccal membrane. The F13 which contains SA and SCMC in ratio of 3:1 showed the highest permeation parameters with steady state flux (J_{ss}) equal to $360.8 \pm 18.0 \mu\text{g cm}^{-2} \text{h}^{-1}$, followed by F14 and F15 with J_{ss} equal to 319.3 ± 17.7 and $331.2 \pm 20.9 \mu\text{g cm}^{-2} \text{h}^{-1}$, respectively. There was no significant difference between F13, F14 and F15 for the flux or permeability coefficient ($p > 0.05$).

Accordingly, F13 was then selected as optimized disc for the bioavailability studies in the consideration of ease of preparation, optimum drug release, superior *ex vivo* permeation profile, excellent bioadhesion values and expected to present a better drug release under normal physiological conditions without the risk of mucosal irritation.

Table 5: Permeability parameters of tested DTZ HCl buccoadhesive discs

| Formula code | Flux (J_{ss}) ^a $\mu\text{g cm}^{-2} \text{h}^{-1}$ | Permeability coefficient (P) ^a ($\times 10^{-6} \text{cm s}^{-1}$) |
|--------------|--|---|
| F13 | 360.8±18.0 | 7.16±0.36 |
| F14 | 319.3±17.7 | 6.34±0.35 |
| F15 | 331.2±20.9 | 6.57±0.41 |

^aEach value represents the mean±SD

Pharmacokinetic study: The selection of an animal model for the *in vivo* studies should primarily consider the resemblance of the animal mucosa to the human oral mucosa in terms of its anatomical composition and enzymatic activity which represent the physical and metabolic barriers of the oral mucosa, respectively. Rabbits are generally considered as a suitable animal model for the testing of buccal drug delivery due to the existence of a non-keratinized buccal mucosa similar to humans (Shojaei, 1998; Martin *et al.*, 2003) and therefore they were selected as the most appropriate animal model to conduct the bioavailability studies following the application of buccoadhesive formulation (F13). The mean plasma level profile (mean±SD) of DTZ HCl obtained following the application of F13 buccoadhesive discs containing 30 mg of the drug was compared to that obtained after oral administration of sustained release commercial tablet (Altiazem® SR) at the same dose (Fig. 3). A summary of the pharmacokinetic parameters derived from the study data is listed in Table 6.

The absorption from sustained release commercial tablets was faster, reaching peak plasma concentration in 2 h, whereas, following administration of buccoadhesive disc F13, the mean T_{max} was 5 h post-dosing. The mean value of C_{max} was also higher for drug administration from buccoadhesive discs than from oral tablet, though this difference was not statistically significant ($p>0.05$). The mean value of $AUC_{0-\infty}$ was higher for drug administered from buccoadhesive discs than from oral tablet and the difference was found to be statistically significant ($p<0.05$) demonstrating improved bioavailability of DTZ HCl from buccoadhesive F13 formulation. F13 also offered a more sustained delivery profile of the drug with the absence of sharp peaks and maintained elevated plasma concentrations during the entire application period. The mean

Table 6: Pharmacokinetic parameters of DTZ HCl following administration of 30 mg in Altiazem® SR oral tablet and F13 buccal disc in rabbits^a

| Parameter | Oral tablet | Buccoadhesive disc (F13) |
|---|-------------|--------------------------|
| C_{max} (ng mL ⁻¹) | 171.3±9.6 | 186.7±15.7 |
| T_{max} (h) | 2 | 5 |
| MRT (h) | 4.81±0.49 | 8.73±2.82 |
| AUC_{0-10} (ng h mL ⁻¹) | 859.2±111.9 | 1276.1±135.3 |
| $AUC_{0-\infty}$ (ng h mL ⁻¹) | 925.1±156.7 | 1828.8±366.9 |
| Relative bioavailability (%) | - | 197.7 |

^aEach value represents the mean±SD. (n = 4)

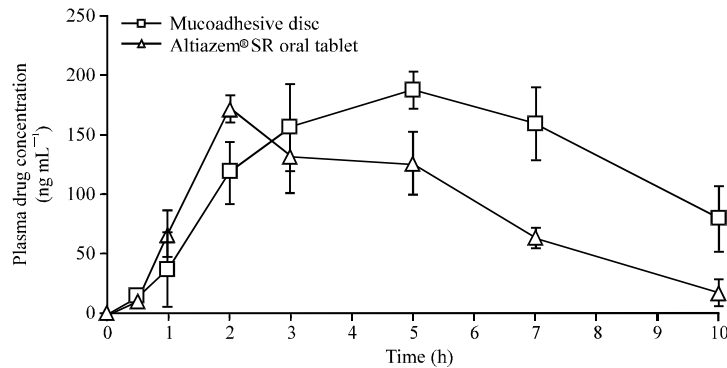


Fig. 3: Plasma concentration following administration of 30 mg DTZ HCl in buccoadhesive discs F3 or Altiazem® SR oral tablets in rabbits. Points represent the mean±standard deviation for n = 4

residence times (MRT) following oral ingestion of Altiazem® SR tablets and F13 disc were 4.8 ± 0.5 h and 8.7 ± 2.8 h ($p < 0.05$), respectively which is another indication on the *in vivo* performance of the buccal buccoadhesive disc in providing a sustained drug delivery.

Bioavailability of DTZ HCl following buccal administration was found to be 197.7% relative to oral sustained release tablets. Thus, the buccoadhesive delivery system designed in the present study, was found to provide prolonged steady-state concentration of the drug with minimal fluctuations and improved bioavailability. This higher relative bioavailability of DTZ HCl can be attributed to reduced presystemic metabolism of the drug when administered via buccal route.

CONCLUSION

A bioadhesive buccal drug delivery system for DTZ HCl was developed as an alternative to avoid first-pass effect associated with oral administration, provide extended release and improve drug bioavailability. New buccoadhesive formulae for controlled release of DTZ HCl were successfully developed in which release patterns and bioadhesion properties can be controlled by changing the type of matrix and mucoadhesive polymers and their ratios. The overall studies indicated that the combination of SA and SCMC showed satisfactory release profile and mucoadhesive properties. They were convenient to apply and remove from the buccal mucosa and did not appear to damage the underlying tissue. Thus, they could be useful for buccal administration of diltiazem hydrochloride. Formulation F13 with SA: SCMC in 3:1 ratio exhibited a good balance between *in vitro* drug release, *in vitro* drug permeation and bioadhesion strength and was therefore selected as the optimized formulation for *in vivo* evaluation. The DTZ HCl administered to rabbits via buccal route showed a significant improvement of bioavailability compared to that achieved by oral administration of Altiazem® SR tablet. In addition, bioadhesive buccal disc showed sustained plasma level profile with the absence of sharp peaks, demonstrating that a sustained delivery of DTZ HCl was achieved by the buccal route. This increase in bioavailability of the drug from designed formulations may also result in substantial reduction of the administered dose.

ACKNOWLEDGMENTS

This study has benefited from the financial support of Ministry of Higher Education and Scientific Research of Yemen. The authors are thankful to the Center of Applied Research and Advanced Studies, Faculty of Pharmacy, Cairo University, Egypt for developing the HPLC method. The authors wish to express their appreciation to Egyptian International Pharmaceutical Company (EIPICO), Egypt; for providing diltiazem hydrochloride, moxifloxacin hydrochloride and Altiazem® SR oral tablets as gift samples. We would also like to thank Rhom Pharma for their generous gift of Eudragit L100-55 through Hinrichs Trading Company, Cairo.

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