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Research Article

Formulation and Evaluation of Bioadhesive Buccal Drug Delivery of Tizanidine Hydrochloride Tablets

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Abstract. The study aim was concerned with formulation and evaluation of bioadhesive buccal drug delivery of tizanidine hydrochloride tablets, which is extensively metabolized by liver. The tablets were prepared by direct compression using bioadhesive polymers such as hydroxylpropyl methylcellulose K4M, sodium carboxymethyl cellulose alone, and a combination of these two polymers. In order to improve the permeation of drug, different permeation enhancers like beta-cyclodextrin (β-CD), hydroxylpropyl beta-cyclodextrin (HP-β-CD), and sodium deoxycholate (SDC) were added to the formulations. The β-CD and HP-β-CD were taken in 1:1 molar ratio to drug in formulations. Bioadhesion strength, *ex vivo* residence time, swelling, and *in vitro* dissolution studies and *ex vivo* permeation studies were performed. *In vitro* release of optimized bioadhesive buccal tablet was found to be non-Fickian. SDC was taken in 1%, 2%, and 3% *w*/*w* of the total tablet weight. Stability studies in natural saliva indicated that optimized formulation has good stability in human saliva. *In vivo* mucoadhesive behavior of optimized formulation was performed in five healthy male human volunteers and subjective parameters were evaluated.

KEY WORDS: bioadhesive buccal tablets; *in vitro* evaluation; *in vivo* mucoadhesive behavior; permeation enhancers; stability studies in natural saliva; tizanidine hydrochloride.

INTRODUCTION

Bioadhesive buccal delivery of drugs is one of the alternatives to the oral route of drug administration, particularly to those drugs that undergo first-pass effect. The stratified squamous epithelium supported by a connective tissue lamina propria, which is present in buccal mucosa (1), was targeted as a site for drug delivery several years ago. Problems accompanied with oral route of administration such as extensive metabolism by liver, drug degradation in gastrointestinal tract due to harsh environment, and invasiveness of parenteral administration can be solved by administering the drug through the buccal route (2,3). The buccal route appears to offer a number of advantages, like good accessibility, robustness of the epithelium, usage of the dosage form in accordance with need, and comparatively less susceptibility to enzymatic activity. Hence, adhesive mucosal dosage forms were prepared for oral delivery, in the form of adhesive tablets (4,5), adhesive gels (6,7), and adhesive patches (8).

The permeation of hydrophilic drug through membrane is one of the major limiting factors for the development of bioadhesive buccal delivery devices. The epithelium that lines the buccal mucosa is a main barrier for the absorption of drugs (9). In order to improve buccal absorption, several approaches have been introduced. Increased permeation of the drug through the buccal membrane and prevention of the drug degradation by enzymes was achieved by changing the physicochemical properties of the drug (10). Alternatively, improving the bioadhesion and release characteristics of buccal delivery devices increases the amount of drug available for absorption (11). The incorporation of absorption enhancers to the buccal formulation is one interesting approach. Substances that facilitate the permeation through buccal mucosa are referred as permeation enhancers (12). Different types of potential permeation enhancers have been studied for buccal route to increase the penetration of drugs (13,14).

The complexation of steroidal hormones with cyclodextrins was not effective in increasing the permeation through buccal route, whereas condensation products of cyclodextrin with propylene oxide or epichlorohydrins were able to form complexes with estradiol, testosterone, and progesterone, thereby enhancing absorption through the buccal membrane in humans (15).

The delivery of hydrophilic macromolecular drugs via buccal membrane was made possible by incorporation of absorption or permeation enhancers, which could reduce barrier properties of the buccal epithelium (13–20).

Tizanidine hydrochloride (TZD HCL) is an imidazoline derivative, which acts as agonist on centrally located α_2

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receptors and this leads to myotonolytic effects on skeletal muscle (21–24). It is structurally and pharmacologically similar to clonidine and other α_2-adrenergic agonists (23,24). The correct mechanism of tizanidine in decreasing muscle tone and frequency of spasm is not clearly understood (24).

About 53% to 66% of the dose administered is being absorbed through the gastrointestinal tract after oral administration and the peak plasma concentration is reached within 1 to 2 h. Bioavailability of tizanidine is about 34% to 40% and half-life is 2.5 h. The drug is widely distributed throughout the body and 30% of drug binds to plasma proteins. It undergoes rapid and extensive first-pass metabolism in the liver (approximately 95% of a dose), leading to the oxidation of the imidazoline moiety, aromatic system, and the sulfur atom. This leads to lower bioavailability of tizanidine (25). In order to overcome such extensive first-pass metabolism, the drug is selected as suitable candidate for bioadhesive buccal drug delivery.

The aim of the present study was to develop a new bioadhesive sustained-release tablets for buccal drug delivery of tizanidine hydrochloride.

MATERIALS AND METHODS

Materials

Tizanidine hydrochloride is a gift sample from Vilin Biomed Ltd. (Rurki, India). Hydroxylpropyl methylcellulose K4M, sodium carboxymethyl cellulose (NaCMC), and ethyl cellulose are gift samples from Zydus Cadila (Ahmedabad, India). Beta-cyclodextrin (β-CD) and hydroxylpropyl beta-cyclodextrin (HP-β-CD) are provided by Dr. Reddy's laboratories (Hyderabad, India). Sodium deoxycholate (SDC) was purchased from Moly chem (Mumbai, India).

Methods

Solubility Studies

The solubility of TZD HCL in phosphate buffer solution of pH 6.6 was determined by phase equilibrium method. An excess amount of drug was taken into 50-ml conical flasks containing 20 ml of pH 6.6 phosphate buffers. These flasks were closed with aluminum foil and constantly agitated at room temperature for 24 h using rotary shaker (Remi Instruments, Mumbai, India). After 24 h, the solution was filtered through a 0.2-μm Whatman filter paper. The amount of drug solubilized was then estimated by measuring the absorbance at 319 nm using a UV spectrophotometer (Systronic Pc-Based Double-Beam Spectrophotometer 2202, Ahmedabad, India) (26). The studies were repeated in triplicate (n=3), and mean was calculated.

Ex Vivo Permeation of Drug Solution

Ex vivo permeation study of TZD HCL through the porcine buccal mucosa was performed using dissolution cell and membrane assembly (27), at 37±0.2°C and 50 rpm. The temperature and revolutions per minute were maintained by using magnetic stirrer (Remi, 2MLH, Mumbai, India). Porcine buccal mucosa was procured from a local slaughterhouse and used within 1 h of slaughter. The tissue was stored in Krebs buffer at 4°C upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and tied to the one side of open tube and this side of the tube (donor chamber) was brought in contact with the surface of the 50 ml pH 6.6 buffer solution (28) which was taken in 100-ml glass beaker (receiver chamber). After the buccal membrane was equilibrated for 30 min with buffer solution between both the chambers, the receiver chamber was filled with fresh buffer solution (pH 6.6), and the donor chamber was charged with 5 ml (1 mg/ml) of drug solution. Aliquots of 5 ml were collected at predetermined time intervals up to 6 h and the amount of drug permeated through the buccal mucosa was then determined by measuring the absorbance at 319 nm using a UV spectrophotometer. The medium was replaced with equivalent volume (5 ml) of buffer, which was prewarmed at 37°C (29). After performing the experiment in triplicate (n=3), mean values were calculated. The cumulative amount of the permeated drug was plotted against time. The flux (J) and the permeability coefficient (P) were calculated by using the following Eqs. 1 and 2:

$$J = dQ/dtA \tag{1}$$

$$P = (dQ/dt)/\Delta CA \tag{2}$$

Where J is flux (mg h^{-1} cm^{-2}); P is permeability coefficient (cm h^{-1}); dQ/dt is the slope obtained from the steady-state portion of the curve; ΔC is the concentration difference across the mucosa and A is the area of diffusion (cm^2).

Preparation of Double-Layered Buccal Tablets

The formulations were prepared as shown in Table I. Each tablet contains 4.56 mg of TZD HCl, which is equivalent to 4 mg of tizanidine base. Before direct compression, all the ingredients were screened through sieve no. 100 and then thoroughly blended in glass mortar with pestle. Blending was carried out separately for core (polymer and drug) and backing layer. The powder of backing layer was compressed using 8.0-mm flat-faced beveled-edge punch and dies set, on 16-stage rotary tablet compress machine (Cadmach, Ahmedabad, India) and blended powder of core layer was added on previously obtained backing layer and compressed again (30). In case of formulations F8 and F9 complexing with beta-cyclodextrin (β-CD) and hydroxylpropyl beta-cyclodextrin (HP-β-CD), respectively, drug to cyclodextrins were taken in 1:1 molar ratio. The amount of cyclodextrins required for single dose was shown in Table I. Complexes were prepared as follows; first, cyclodextrins were taken in glass mortar and little amount of water was added to make a slurry; later, the powder was added to the slurry by continuous trituration with pestle and this process is continued up to 30 min. This slurry containing drug and cyclodextrin was dried at 60°C for 15 min. After drying, the complex was passed through the sieve no. 100. The residual moisture content was found to be not more than 1.03% *w/w* 15 min at 60°C in Sartorius IR Balance moisture analyzer.

Thickness

The thicknesses of buccal tablets were determined using digital micrometer (Digital Caliper, Aerospace, India). Ten

Table I. Composition of Double-Layer Buccal Tablets of Tizanidine Hydrochloride

Ingredients (milligram per tablet)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
TZD HCL	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56	4.56
HPMCK4M	95	–	80	65	50	35	20	20	20	20	20	20
SCMC	–	95	15	30	45	60	75	75	75	75	75	75
β-CD	–	–	–	–	–	–	–	17.81	–	–	–	–
HP-β-CD	–	–	–	–	–	–	–	–	27.36	–	–	–
SDC	–	–	–	–	–	–	–	–	–	1.04	2.09	3.12
Pearlitol	4	4	4	4	4	4	4	4	4	4	4	4
Aspartame	1	1	1	1	1	1	1	1	1	1	1	1
Aerosil	1	1		1	1	1	1	1	1	1	1	1
Backing Layer (EC)	50	50	50	50	50	50	50	50	50	50	50	50

TZD HCL tizanidine hydrochloride, *SCMC* sodium carboxymethyl cellulose, *SDC* sodium deoxycholate, *EC* ethyl cellulose

individual tablets from each batch were used and the average thickness was calculated.

Weight Variation Test

Weight variation test was performed for ten tablets from each batch using an electronic balance (Shimadju, Aux*220, Japan) and average values were calculated.

Hardness

Hardness test was conducted for three tablets from each batch using Monsanto hardness tester and average values were calculated

Assay

Five tablets were selected at random and were powdered in a mortar; and amount of powder equivalent to single dose was dissolved in 0.1 N HCL (31) by sonication for 30 min and filtered through Whatman filter (0.2 μm) paper. The drug content was analyzed spectrophotometrically at 319 nm using a UV spectrophotometer. Each measurement was carried out in triplicate and the average drug content was calculated.

Disintegration Test

The test was performed for buccal tablets without backing material. Form each batch, six randomly selected tablets were placed in US Pharmacopeia (USP) disintegration apparatus baskets (Electrolab ED-2L) and the process of disintegration was carried out for 4 h. Later, the baskets were lifted from the fluid and observed for complete disintegration of tablets (32).

Measurement of Bioadhesion Strength

Bioadhesive strength (BS) of the disks was measured on a modified physical balance (33). The apparatus consists of a modified double-beam physical balance in which the right pan was replaced with a lighter pan and the left pan was replaced with a glass slide (4-cm length and 2.5-cm width) which was suspended by means of Teflon rings and copper wire. The setup was balanced in such a way that it consists of 5 g of removable weights on right pan and equivalent amount of clay on other side. The height of the total setup was adjusted so as to accommodate a glass container of 6.6-cm height. In order to find the bioadhesion strength, first, buccal tablet (n= 3) was stuck to the glass slide with cyanoacrylate adhesive and balance was set in to weighing mode with the help of a knob that is situated at the base of the balance. Now, 5 g of weight on the right pan is removed. This lowered the glass slide along with the tablet over the membrane with a weight of 5.0 g. The entire setup was kept undisturbed for 5 min. Then, the weights on the right-hand side were slowly added in increments of 0.1 g until the tablet just separated from the membrane surface. The excess weight on the right pan, i.e., total weight minus 5 g, was taken as a measure of the bioadhesive strength.

Determination of the Ex Vivo Residence Time

The *ex vivo* residence (ER) time was found using a locally modified USP disintegration apparatus, which was applied by Nakamura *et al.* (34). The disintegration medium was composed of 800-ml pH 6.6 phosphate buffer maintained at 37°C. The porcine buccal tissue was tied to the surface of a glass slab, vertically attached to the apparatus. The buccal tablet was hydrated from one surface using 0.5-ml of pH 6.6 phosphate buffer and then the hydrated surface was brought in contact with the mucosal membrane. The glass slide was vertically fixed to the apparatus and allowed to run in such way that the tablet completely immersed in the buffer solution at the lowest point and was out at the highest point. The time taken for complete erosion or dislodgment of the tablet from the mucosal surface was noted. The experiments were performed in triplicate (n=3) and mean of triplicate was determined.

Swelling Studies of Buccal Tablets

Buccal tablets were weighed individually; initial weight was considered as W_1 and placed separately in Petri dishes (outside dimensions: 100-mm diameter×15-mm height; inside dimensions 88 mm diameter×12-mm height) containing 15 ml of phosphate buffer (pH 6.6) solution in such a way that the side of tablet which attaches to the buccal membrane was positioned to the bottom of the Petri dishes with the backing

membrane being viewable from the top. Tablets were soaked in such a way that the core tablet completely immersed in the buffer solution. At regular intervals (0.5, 1, 2, 3, 4, 5, and 6 h), the buccal tablets were removed from the Petri dishes using coverslips and excess surface water was removed carefully using the Whatman filter paper. The swollen tablets were then reweighed (W_2) (35,36). This experiment was performed in triplicate. The degree of swelling (water uptake) was calculated according to the following Eq. 3:

$$\text{Degree of swelling} = W_2 - W_1/W_1 \quad (3)$$

Surface pH Study

The bioadhesive buccal tablets ($n=3$) were made in contact with 1 ml of distilled water and allowed to swell for 2 h at room. The pH was measured by bringing the pH meter electrode (Cyber Ph-14l, Cyberlab, India) in contact with the surface of the tablet and allowing it to equilibrate for 1 min (37).

In Vitro Drug Release of Buccal Tablets

The USP XXIII rotating paddle method was used to study the drug release from the buccal tablets. The dissolution medium consisted of 200 ml of phosphate buffer pH 6.6. The experiment was performed at 37±0.2°C, with a rotation speed of 50 rpm. The backing layer of buccal tablet was attached to the glass slide with instant adhesive (cyanoacrylate adhesive). The slide was placed at the bottom of the dissolution vessel. Samples (5 ml) were withdrawn at predetermined time intervals and equivalent amount was replaced with fresh medium. The samples were filtered through Whatman filter (0.2 μm) paper and analyzed after appropriate dilution by UV spectrophotometer at 319 nm.

Ex Vivo Permeation of Buccal Tablets

Ex vivo permeation study of TZD HCL tablets through the porcine buccal mucosa was performed using dissolution cell and membrane assembly (27), the temperature was maintained by using a water bath and a thermometer assembled to it. Simulated buccal movements were maintained by using magnetic stirrer (Remi, 2MLH, Mumbai, India). Porcine buccal mucosa was procured from a local slaughterhouse and used within 1 h of slaughter. The tissue was stored in Krebs buffer at 4°C upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and tied to the one side of open tube and this side of the tube (donor chamber) was brought in contact with the surface of the 50-ml pH 6.6 buffer solution (28) which was taken in a 100-ml glass beaker (receiver chamber). After the buccal membrane was equilibrated for 30 min with buffer solution between both the chambers, the receiver chamber was filled with fresh buffer solution (pH 6.6), and the buccal tablet was carefully placed in the donor chamber using a forceps in such a way that core tablet can abreast to the buccal membrane in which 1 ml of buffer solution (pH 6.6) was added (38). Samples (5 ml) were collected at predetermined time intervals and filtered through a 0.2-μ filter, and the amount of drug permeated through the buccal mucosa was then determined by measuring the absorbance at 319 nm using a UV spectrophotometer. The medium in the receiver chamber was replaced with an equivalent volume of buffer (5 ml), which was prewarmed at 37°C. The experiments were performed in triplicate ($n=3$) and mean value was used to calculate the flux and permeability coefficient. The enhancement ratio was determined by dividing the cumulative amount of permeated TZD HCL in the presence of SDC at the end of 6 h $(Q)_{6\ h}$(enh) by the amount of TZD HCL alone $Q_{6\ h}$(control), Eq. 4;

$$\text{Enhancement ratio} = (Q)_{6h}(\text{enh})/(Q)_{6h}(\text{control}) \quad (4)$$

Stability of Buccal Tablets

Stability studies of buccal tablets were performed for optimized formulation (F12) in normal human saliva. The saliva was collected from humans (aged 22–26) and filtered through Whatman (0.2 μm) filter paper. Buccal tablets were placed in separate Petri dishes containing 5 ml of human saliva and placed in a temperature-controlled oven (Bio-Technics, India) for 6 h at 37±0.2°C. At regular time intervals (0, 2, 4, and 6 h), the buccal tablets were examined for change in color, surface area, and integrity (39). The experiments were repeated in triplicate ($n=3$) in a similar manner.

In Vivo Mucoadhesive Performance of Buccal Tablets

This study was conducted after obtaining permission from the institutional human ethical committee and then informed consent was obtained from all the volunteers before conducting the study. This study was conducted as per the guidelines prescribed by the committee under the supervision of the principal investigator. *In vivo* studies were conducted by applying tablets ($n=3$) on five healthy volunteers' (aged 22–26 years) gums to obtain the residence time, the subjective parameters, and loss of the fragment, and the possible production of irritation or pain. Volunteers were given optimized buccal tablets (F12) along with an instruction sheet and were asked to press the buccal tablet against the buccal mucosa for about 1 min. For the purpose of the photography proof, in one volunteer, buccal tablet was applied to the inner side of the lower lip and photographs were taken immediately after application and after 2, 4, and 6 h. *In vivo* behavior of the bioadhesive buccal tablet was shown in Fig. 1.Volunteers were then asked to record the time of application and time of dislodgment of tablet. After completion of the study, a questionnaire was given to volunteers to collect information regarding the parameters such as irritancy, comfort, taste, dry mouth, salivation, dislodgment of the system during the study, and heaviness of the system at the place of application. Though food intake is avoided from 0.5 h prior to the beginning of the study to the end of the study, water intake is permitted after the initial 0.5 h (40).

RESULTS

Solubility of the drug in the pH 6.6 phosphate buffer was found to be 12.85 mg ml^{-1}. The flux and permeability coefficient of drug solution was found to be 0.0775 mg h^{-1} cm^{-2} and 0.0180 cm h^{-1}, respectively. The values of weight variation and

Fig. 1. *In vivo* mucoadhesive behavior of optimized formulation (F12)

friability were found to be within the limits of conventional oral tablets stated in the Indian Pharmacopoeia (1996). In addition to these evaluation tests, disintegration, thicknesses, hardness, and percentage of drug content tests were conducted, and the results were shown in Table II.

Measurement of Bioadhesive Strength and *Ex Vivo* Residence Time

These evaluation tests were conducted for all the formulations and there was a gradual increase in both the bioadhesive strength and the *ex vivo* residence time form F3 to F7. The maximum BS (21.152 g) and ER (8 h) were found in the formulation F7. F1 was found to have low BS and ER of 4.012 g and 2 h, respectively. Formulations from F10 to F12 have shown similar results as that of the F7. The BS, ER, and surface pH values were shown in Table III.

Swelling Studies of Buccal Tablets

From F3 to F7, maximum swelling was found at 4 h; from these, F7 was found to have high 770.67° of swelling at 4 h. Nearly same degree of swelling was observed for F10, F11, and F12 as that of the formulation F7, whereas maximum swelling was found at 3 h for F1 and F9. The comparison of degree of swelling of all formulations was shown in Fig. 2.

Table II. Physicochemical Properties of Double-Layer Buccal Tablets of Tizanidine Hydrochloride

Form code	Weight variation	*F* (%)	DT (h)	Thickness (mm)	Hardness (kg/cm^2)	Percent drug content
F1	202.71±2.85	0.47	0.83±0.26	2.70±0.08	3.8±0.3	99.43±0.53
F2	203.36±3.70	0.23	2.50±0.63	2.68±0.09	5.0±0.5	99.35±0.38
F3	201.17±3.68	0.11	0.92±0.38	2.72±0.06	4.3±0.1	99.32±0.41
F4	202.10±4.07	0.11	1.25±0.52	2.69±0.08	4.3±0.3	99.42±0.05
F5	202.11±4.04	0.05	1.33±0.61	2.71±0.06	4.7±0.8	99.22±0.29
F6	203.12±4.25	0.02	2.92±0.38	2.69±0.08	4.7±0.8	99.36±0.27
F7	202.77±4.25	0.04	3.92±0.49	2.70±0.07	5.0±0.5	99.41±0.50
F8	222.07±2.18	0.04	4.83±0.26	3.13±0.02	8.0±0.5	99.70±0.34
F9	233.18±1.40	0.04	0.83±0.26	3.43±0.03	5.2±0.3	99.27±0.34
F10	204.76±1.68	0.49	3.92±0.38	2.74±0.03	5.0±0.5	99.37±0.34
F11	204.66±1.82	0.23	3.92±0.58	2.75±0.02	5.0±0.5	99.29±0.26
F12	205.06±1.60	0.03	3.92±0.38	2.75±0.03	5.0±0.5	99.45±0.36

F friability, *DT* is disintegration time

Table III. Results of Bioadhesion Strength, *Ex Vivo* Residence Time Surface pH of All Formulations (n=3)

Form code	Bioadhesion strength (g)	ER (h)	Surface pH
F1	4.013±0.002	2.17±0.29	5.97±0.07
F2	16.503±0.003	5.50±0.50	6.42±0.37
F3	6.012±0.001	2.50±0.50	6.53±0.32
F4	6.674±0.001	2.67±0.29	6.78±0.19
F5	7.637±0.001	3.50±0.50	6.71±0.17
F6	15.852±0.001	4.00±0.50	6.89±0.17
F7	21.153±0.002	6.67±0.76	6.94±0.12
F8	16.066±0.001	4.50±0.50	6.68±0.26
F9	17.004±0.004	1.83±0.29	6.79±0.16
F10	21.159±0.001	6.83±1.04	6.93±0.03
F11	21.161±0.001	7.17±0.76	6.88±0.11
F12	21.160±0.001	7.17±1.04	6.94±0.10

ER ex vivo residence time

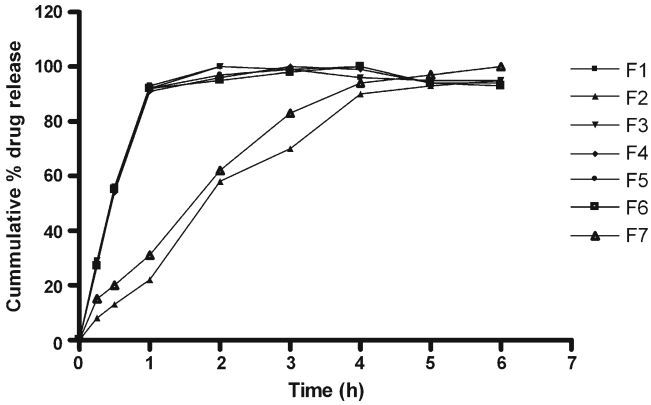

Fig. 3. Plot of cumulative percentage drug release *vs* time for F1–F7 formulations

In Vitro Drug Release from Buccal Tablets

F1 and F3 were found to release 99.90±0.36% and 99.97±0.18% of the drug just within 2 h whereas F2 released 94.96±0.07% of the drug in 6 h. The formulation F7 was found to release 99.85±0.00% of the drug in 6 h. F9 released about 99.85±0.14% drug in 3 h. Formulations F8 and F10 through F12 released the drug similar to that of F7. The comparison of cumulative drug release of all formulations was shown in Figs. 3 and 4.

Mathematical Model Fitting of *In Vitro* Drug Release

The *in vitro* percentage drug release of optimized formulation F12 was attempted to fit into mathematical models. The n and R^2 values for zero-order, first-order, Higuchi and Peppas, and Hixson Crowell models (41) were represented in Table IV. The Peppas model is widely used, when the release mechanism is not well known and when more than one type of release is involved (42). The semiempirical equation is shown as Eq. 5:

$$M_t/M_\infty = kt^n \tag{5}$$

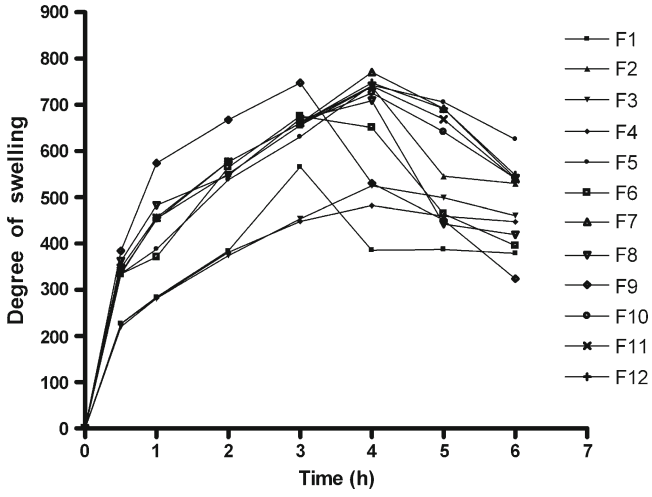

Fig. 2. Plot of degree of swelling *vs* time for all formulations

Where M_t/M_∞ is fraction of the drug released at time t; k represents a constant, incorporating structural and geometrical characteristics of the buccal devices; and n is the diffusion exponent, which characterizes the type of release mechanism during the dissolution process.

For non-Fickian release, the value of n falls between 0.5 and 1.0, while in case of Fickian diffusion, n=0.5; for zero-order release (case II transport), n=1; and for supercase II transport, n is greater than 1. Observation of all the R^2 values indicated the maximum for Higuchi, Peppas, and Hixson Crowell. The n value of formulation F12 was 0.686 and it also had the highest R^2 (0.9816).

Ex Vivo Permeation of Buccal Tablets

Based on the above results, formulations F7 to F12 were selected for the *ex vivo* permeation study. The flux, permeation coefficient, and cumulative drug permeated from formulation F7 were found to be 0.3342 mg h^{-1} cm^{-2}, 0.0844 cm h^{-1}, and 30.06%, respectively. No improvement in drug permeation was found in case of F8 and F10. There was improvement in cumulative percentage drug permeated for the formulations F9, F11, and F12. The values of flux, permeation coefficient, and cumulative drug permeated from optimized formulation (F12)

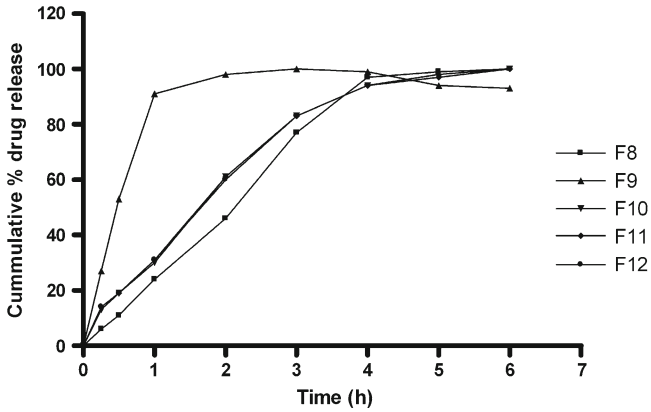

Fig. 4. Plot of cumulative percentage drug release *vs* time for F8–F12 formulations

Table IV. Estimated Values of n (Diffusional Exponent) and R^2 (Correlation Coefficient) for Optimized Formulation

Form code	Zero-order R^2	First-order R^2	Higuchi R^2	Peppas model		Hixson Crowell R^2
				n	R^2	
F12	0.9130	0.9148	0.9698	0.686	0.9816	0.9972

were found to be 0.8127 mg h^{-1} cm^{-2}, 0.2052 cm h^{-1}, and 62.73± 7.47%, respectively, with an enhancement factor of 1.9. The slope, flux, and permeability coefficient for formulations F7 to F12 were shown in Table V. Comparison of cumulative percentage drug permeated for all (F7 to F12) formulations was shown in Fig. 5.

Stability of Buccal Tablets

Based on the above result, stability studies were conducted only for optimized formulation F12. There was no change in the color and integrity of the tablets. The change in surface area (cm^2) at 0, 2, 4, and 6 h was found to be 0.5, 1.32, 2.00, and 2.50 cm^2, respectively.

In Vivo Mucoadhesive Behavior

This study was conducted for optimized formulation F12. In bioadhesive buccal drug delivery, comfort of system in oral cavity is given utmost importance. The result of five healthy human volunteers to each subjective parameter was calculated and shown in Table VI.

DISCUSSION

The solubility of drug was conducted in pH 6.6 phosphate buffer because it is the average pH of the oral cavity. F1 (HPMC K4M alone) and formulations containing high concentration of the HPMC K4M (F3, F4, and F5) have shown low disintegration time (DT) and ER due to rapid disintegration. F2 (NaCMC alone) and formulations F6 to F12 containing high concentration of NaCMC have shown higher DT and ER; this might be due to slow disintegration and slow water uptake by the formulations, whereas formulation F9 (contains HP-β-CD) showed lower DT and ER; this might be due to its high solubility (HP-β-CD) and overhydration of HP-β-CD that weakens the formed bonds (38).

In 1986, Longer and Robinson (43) defined the term bioadhesion as the attachment of a synthetic or natural macromolecule to mucus and/or an epithelial surface. In general, mucoadhesion/bioadhesion may be defined as the adhesion between a bioadhesive polymer and mucus. Mucoadhesion is considered to occur in four major stages: wetting, interpenetration, adsorption, and formation of secondary chemical bonds between mucus membrane and polymer. The strength of mucoadhesion is affected by different factors like molecular weight of the polymer, contact time with membrane, degree of swelling of the polymer, and the type of biological membranes used in the study (44). The adhesion will increase with the degree of hydration until a point where overhydration leads to a sudden decline in BS, which might be due to the disentanglement at the polymer/tissue interface. The degree of swelling was increased with the increase in the concentration of NaCMC; this led the formulations F2 and F5–F12 to have higher BS and F9 were found to have low BS; this might be due to overhydration of the HP-β-CD. NaCMC is the polyanionic polymer containing carboxylic groups, which form hydrogen bonds with the tissue. Rapid rate of hydration of NaCMC led to higher degree of swelling in a short period of time, which improved entanglement of polymer chains with the mucus. This hypothesis was confirmed with that previously reported by Lerhr *et al.* (45). All these factors have contributed to the higher BS of F12.

The surface pH of the buccal tablets was determined in order to investigate the possibility of any irritation effects *in vivo*, as acidic or alkaline pH may cause irritation to the buccal mucosa. Surface pH of the optimized formulation F12 was found to be 6.85 (near to neutral pH). It was inferred that neutral pH of the formulation does not cause any irritation to the mucosa.

Appropriate swelling property of a buccal adhesive device is required for uniform and prolonged release of drug

Table V. The Slope, Flux, and Permeability Coefficient of F7 to F12 Formulations

Form code	Slope	J (mg h^{-1} cm^{-2})	P (cm h^{-1})
F7	0.1679	0.3342	0.0844
F8	0.1974	0.3929	0.0992
F9	0.371	0.7385	0.1865
F10	0.2035	0.4051	0.1023
F11	0.2531	0.5038	0.1272
F12	0.4083	0.8127	0.2052

J flux, P permeability coefficient

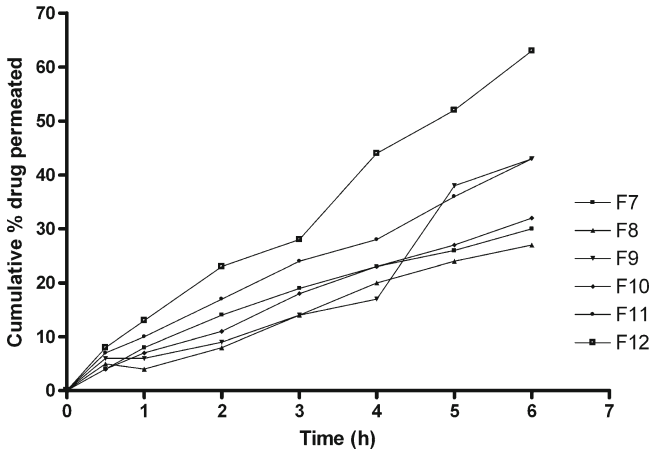

Fig. 5. Plot of cumulative percentage drug permeated *vs* time for formulations F7 to F12

Table VI. Response of Healthy Human Male Volunteers to Various Subjective Parameters (n=3)

SI no.	Criteria	Volunteer's response (%)
1	Irritation	
	None	100
	Slight	–
	Moderate	–
	Severe	–
2	Taste	
	Normal	80
	Slightly	20
	Very unpleasant	–
	Pleasant	–
	Very pleasant	–
3	Comfort	
	Very comfortable	–
	Comfortable	80
	Slightly uncomfortable	20
	Moderately uncomfortable	–
	Severely uncomfortable	–
4	Dryness of mouth	
	None	80
	Slight	20
	Moderate	–
	Severe	–
5	Salivary secretion	
	None	20
	Slight	60
	Moderate	20
	Severe	–
6	Heaviness at the place of attachment	
	None	90
	Slight	10
	Moderate	–
	Severe	–
7	Dislodgement of the system during study	
	No	100
	Yes	–

with proper mucoadhesion (46).The degree of swelling indicated that the rate of swelling is directly proportional to NaCMC content and inversely proportional to HPMC K4M content. The high amount of water intake by NaCMC at a faster rate might have resulted in the higher rate and extent of swelling (F7, F10, F11, and F12). Some buccal tablet formulations (F1, F9, and F3 to F5) did not preserve their integrity throughout the experimentation and were disintegrated with in 2 h. The highest loss was observed for the buccal tablets (F1 and F3) containing a high concentration of HPMC K4M as mucoadhesive polymer. Some of the buccal tablet formulations (F1, F3, and F9) were not successfully recovered and handled from the buffer solution. After reaching the maximum degree of swelling (3 to 4 h), buccal tablets (F2, F6, F8, and F9) did not maintain their integrity.

An ideal sustain-release system should be able to release the drug immediately to attain the therapeutic level at a faster rate and maintain this drug level for a prolonged period of time. The buccal tablets (F1, F9, and F3 to F5) released the drug in 3 h, for the same reason as was explained for the swelling of buccal tablets. The extensive swelling of formulation F2 creates a thick gel barrier, which retards and increases the path length for the diffusion of the drug molecules from buccal tablets; this might be the reason for lower cumulative percentage of drug release (94.96). In order to overcome this problem, a combination of the HPMC K4M and NaCMC (F3 to F7) was used. At 19% and 71% concentrations of HPMC K4M and NaCMC, respectively, F7 released 99.64% of the drug in 6 h. So this formulation F7 was selected to study the effect of the permeation enhancers. Hence, formulations F8 to F12 have the same concentration of polymer as that of F7. Due to high solubility of HP-β-CD, F9 released the total drug in 3 h. The remaining formulations, i.e., F8 and F10–F12 released the drug similar to that of F7. The β-CD (F8) has higher compressibility than HP-β-CD (F9); this led to increased hardness (8 kg cm^{-2}). This might be the reason for retarded release for 6 h when compared with F9.

The n and R^2 values of Peppas model indicated that the release of TZD HCL was found to be non-Fickian diffusion. Hixson Crowell model shows (R^2=0.9972) that the formulation mechanism of release depends on the thickness and diameter.

The *ex vivo* permeation study was conducted by taking the 50 ml of 6.6 pH phosphate buffer in receiver chamber in order to maintain sink conditions. There was no effect of β-CD in formulation F8. Addition of HP-β-CD (F9) increased

the cumulative percentage of drug permeated; the reasons might be due to forming complex with the individual molecules which improves the diffusible form of the drug species at the tablet–buccal membrane interface and due to increasing the solubility of TZD HCL. These results were similar to those produced by Mira and Mario (38). The HP-β-CD also has the ability to remove cholesterol and phospholipids (especially phosphatidyl choline and sphingomyelin) from the outer layer of the membrane, thus increasing the permeability of hydrophilic molecules. The HP-β-CD was reported to dissolve the membrane components without penetrating into the membrane. Therefore, the effects were mild and reversible (47). All these effects might contribute to enhanced permeation of the drug. From the result, it was inferred that 1% of SDC (F10) had no effect on permeation of the drug and 2% of SDC (F11) extracted only mucosal lipid from the intercellular spaces. Thus, this enhances the diffusivity of the drug via the paracellular (passing between the cells) or polar route. Higher concentration of SDC (F12), i.e., 3%, can extract lipids from the cell membranes, along with the extraction of mucosal lipid from the intercellular spaces by the formation of micelles. This resulted in enhancing passive diffusivity of the drug via transcellular (crossing the cell membranes and entering the cell) and paracellular routes (20). It was mentioned that SDC can also cause the uncoiling and extension of the protein helices, which leads to opening of the polar pathways for diffusion (48). All these effects might contribute to enhancing the permeation of the drug.

From the stability studies, it was known that optimized formulation F12 had stability in human saliva, which if failed might have led to color change. It was reported that color of the omeprazole changed to yellow when it was placed in human saliva (49). Physical properties of the TZD HCL buccal tablets such as thickness and diameter slightly changed owing to swelling of the system in human saliva. Buccal tablets have maintained their integrity in the natural human saliva throughout the experiment, exhibiting sufficient strength of the system.

From the human volunteer studies of optimized formula (F12), it was observed that slightly bitter taste was found at 4 h, which might be due to higher swelling of the mucoadhesive polymers. The excess swelling was responsible for the increased thickness of the buccal tablet and this led to improved radial release of TZD HCL, which was negligible during initial hours. This radial release increased the amount of the drug into mouth and was responsible for slightly bitter taste.

CONCLUSION

Development of bioadhesive buccal drug delivery of tizanidine hydrochloride tablets was one of the alternative routes of administration to avoid first-pass effect and provide prolonged release. In addition, these formulations reduce the need of frequent administration and enhance patient compliance. A combination of sodium carboxymethyl cellulose and hydroxypropyl methylcellulose K4M results in sustained buccal drug delivery. The *in vitro* drug release was found to be non-Fickian. The results strongly suggest that increase in the permeation was due to the effect of sodium deoxycholate on paracellular and transcellular pathways. From healthy human volunteers, subjective parameters and mucoadhesive behavior were found to be satisfactory.

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