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Delivery of Piroxicam With A Mucoadhesive Buccal Tablet: *In Vitro, Ex Vivo* and *In Vivo* Evaluation

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Research Article

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ABSTRACT

Piroxicam (PX) is one of the most potent non-steroidal, anti-inflammatory agents which also exhibit anti-pyretic activity in various types of non-rheumatic pains. Although the drug is well absorbed following oral administration, gastric irritation is still the most serious adverse effect. Thus there is a need for an alternative drug delivery system with better GI tolerability. Buccal administration of drugs provides a convenient route of administration for both systemic and and local actions and bypasses first-pass effects and avoids GI side effects. Therefore the aim of this study was to develop buccal tablets of piroxicam by using hydroxypropyl methylcellulose and chitosan as mucoadhesive agents. Tablets were prepared with direct compression method and evaluated for physical properties. *In vitro* dissolution studies showed that the release rate of PX from the formulations affected by type and ratio of polymers. The release mechanism of PX from buccal tablets follows diffusive mechanism with first order and Higuchi release kinetics. *In vivo* studies of optimum buccal tablet formulation carried out on human healthy volunteers showed that the relative bioavailability of PX was 67.52 ± 21.47%. These results demonstrate that buccal tablet formulation of PX seems to be an alternative drug delivery for patients especially suffering from GI disturbances

INTRODUCTION

Piroxicam (PX) is an oxicam derivative with potent non-steroidal anti-inflammatory activity. It is used in various acute and chronic musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis and in acute gout, dysmenorrhea and sometimes for pain associated with inflammation [1]. According to the Biopharmaceutical drug classification system (BCS), PX is a class 2 drug with low solubility and high permeability. Its pharmacokinetic pattern is characterized by slow and gradual absorption via the oral route [2-4].

This undesired property, may also increase the amount of gastrointestinal (GI) damage, due to long contact of drug with the mucous of GI system [5]. Thus there is a need for an alternative drug delivery system of PX with better gastro intestinal tolerability [6]. Buccal administration of drugs is a valid alternative to the perioral one since drugs directly diffuse into the systemic circulation. In particular, it is advantageous for those drugs that encounter degradation in the gastrointestinal tract or severe hepatic first-pass metabolism and require the administration of large doses to reach effective therapeutic levels in the target site.

This drug delivery route is also an alternative for drugs that have severe side effects on GI system such as PX [7,8].

By contrast, some drawbacks must be taken into account when a dosage form is proposed for buccal administration. Among

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these is the need for the dosage form to maintain its position for many hours against Buccal motion and salivary flow, the latter also being responsible for dissolving a possible relevant part of the drug, thus reducing the mucosal absorption [9].

Therefore, the first step in the development of a Buccal dosage form is the selection of an appropriate adhesive. A number of bio adhesive polymers have been investigated for buccal purposes, mainly carbopol, cellulose derivatives such as hydroxypropyl methylcellulose (HPMC) and chitosan [10].

Chitosan is the N-deacetylated product of chitin, a polysaccharide very abundant in nature. Chitosan is gaining increasing importance in the pharmaceutical field due to its favorable properties such as biocompatibility, non-toxicity, and biodegradability. It has been show that this polymer has good mucoadhesiveness and a significant enhancing effect on the permeation of drugs across the Buccal mucosa [11,12]. HPMC, a semisynthetic ether derivative of cellulose, has been used widely since the 1960's and it is non-toxic, readily compressible, and able to accommodate high levels of drug loading. It swells in contact with a liquid medium forming a gel that controls drug release and reduces irritation in the mouth [13,14]. The aim of this study was to develop buccal tablets of PX using Mucoadhesive polymers, namely HPMC and chitosan. Direct compression method was chosen to prepare matrix tablets. The employment of direct compression appears to be the most efficient and interesting approach economic and process development points of view. The straightforward manufacturing processes, scale-up and cost effectiveness are among the various advantages of direct compression [14]. *In vitro* and *ex vivo* tests were made on developed buccal tablet formulations. Optimum formulation was chosen for *in vivo* studies to carry on human healthy volunteers.

MATERIALS AND METHOD

The following reagents were used: Piroxicam (R & G Chemicals, England) was a generous gift from Kimetsan Chemical Company (Turkey), HPMC (4000 cPs-Sigma, USA) and chitosan (low density, Fluka, Switzerland), spray dried lactose (Meggle, Germany). All other chemicals and solvents were of analytical reagent grade.

EXPERIMENTAL

Preparation of Bio Adhesive Tablets

The tablets were prepared by direct compression of the drug with Mucoadhesive polymers. HPMC, chitosan and their mixtures with different ratios (9-39%) were used as Mucoadhesive. Magnesium stearate was used as lubricant. The powders were mixed and tablets, 8 mm diameter, containing 20 mg Piroxicam were prepared by using a hydraulic tablet press (Ayasli ucler, Ankara, Turkey). Compositions of buccal adhesive tablet formulations are given in **Table 1.**

Table 1. The composition of PX buccal tablets (mg).

Form. Code	PX	Chitosan (Low density)	HPMC (4000 cPs)	Lactose (Spray dried)	Mg Stearate
F1	20	39		40	1
F2	20	-	39	40	1
F3	20	26	13	40	1
F4	20	13	26	40	1
F5	20	19.5	19.5	40	1
F6	20	30	9	40	1
F7	20	9	30	40	1
F8	20	21	18	40	1
F9	20	18	21	40	1
F10	20	10	-	69	1
F11	20	20	-	59	1
F12	20	-	10	69	1
F13	20	-	20	59	1
F14	20	-	-	79	1

Evaluation of Tablets

Drug content uniformity was determined by dissolving the crushed tablets in methanol and filtered through 0.45 µm PTFE filter. It was made necessary dilutions and analyzed in HPLC (Agilent 1100, Santa Clara, USA) with a UV detector set at 360 nm. Weight variation test is done by weighing 20 tablets individually; calculating the average weight and comparing the individual tablet weight to the average.

The thickness of the tablets was measured with a micrometer (Mauf, Poland) placed perpendicular to the diameter. The strength of tablet is expressed as tensile strength (N: Newton). The tablet crushing load, which is the force required to break a tablet into halves by compression. It was measured using a tablet hardness tester (Pharma Test PTB301, Hamburg, Germany). Friability test is performed to assess the effect of friction and shocks, which may often cause tablet to chip, cap or break. Friabilator (Pharma Test PTFRA, Hamburg, Germany) was used for the purpose. Pre-weighed sample of tablets was placed in the

friabilator, which was then operated 100 revolutions. Tablets were dusted and reweighed. Compressed tablets should not lose more than 1% of their weight.

Differential Scanning Calorimetry (DSC)

DSC thermo grams of the pure drug and mixture with polymers were recorded on a Schimadzu DSC-60, Japan. The instrument was calibrated with indium and zinc prior to analyzing the samples under nitrogen. All accurately weighed samples (5-6 mg) were placed into sealed aluminum pans and scanned at the heating rate of 5°C min^{-1} over the temperature range of 30-475°C.

***In Vitro* Dissolution Studies**

Dissolution studies were conducted in a USP Apparatus II (paddle method, Sotax AT7, Switzerland) with three replicates at 37 ± 0.5°C. Phosphate buffer (pH 7.4) was used as dissolution medium (500 ml) and the paddle rotation speed was kept at 50 rpm [14]. At predetermined time intervals 5 ml of dissolution sample was withdrawn and replaced with an equal volume of fresh medium. After filtration and dilution by a suitable mobile phase, the samples were analyzed by HPLC of which details given below.

Drug Release Kinetics

The *in vitro* dissolution dates of all formulations were subjected to goodness of fit test by linear regression analysis according to first order, zero order, Higuchi equation and Korsemeyer-Peppas model.

***In vitro* Permeation Studies**

Permeation studies carried out with unjacketed modified horizontal diffusion cells with a diffusional surface area of 1 cm^2 and 32 ml of receptor cell volume, placed in heating stirring module, through cellulose membrane (Typical molecular weight cut-off, 14 000). 2 ml of phosphate buffered saline was added on buccal tablet placed in donor phase. The receptor compartments were filled with phosphate buffered saline pH 7.4. The receptor phase was maintained at 37 ± 0.5°C. Aliquots of 500 µl were withdrawn periodically and replaced with the same volume of receptor fluid for 4 h. The samples were analyzed with UV spectrophotometer at the wavelength of 360 nm.

The cumulative amount of PX permeated per unit area was plotted against time, and the slope of the linear portion of the plot was used as steady state flux (J_{ss}). The permeability coefficient (K_P) was calculated with Eq. (1), in which C_V is the total donor concentration of the formulation

$$K_P = Jss / Cv \quad (1)$$

***Ex Vivo* Determination of Bio adhesion**

Instron 4411 Model Texture Analyzer (England) were used to determine the bio adhesion of tablets. Cattle buccal mucosa was obtained at the slaughterhouse. The freshly excised buccal mucosa transported to laboratory conditions in Krebs Henseleit solution at 4°C. Cyanoacrylate adhesive was used to fix the tablet and buccal mucosa. The tablet surface was wetted with 50 µl of artificial saliva. The tablet and mucosa were brought in contact, and kept in this condition for 10 min. Then, the tensile test was performed at a constant extension rate of 5 mm min^{-1}. The peak detachment force (F) and the work of adhesion (W, area under the force/distance curve) were recorded.

***In Vivo* Studies**

The *in vivo* study was carried out on eight healthy Caucasian volunteers. The study protocol was approved by the Ethical Committee of Gulhane Military Medical Academy (Ankara, Turkey) and each volunteer signed an informed form of consent before starting the trial. All the volunteers were active, ambulatory adults with no negative past medical history and had not taken any medication at least 7 days before starting the trial. They were not in the habit of smoking or drinking alcoholic beverages.

Dosage Schedule and Blood Sampling

The dosage forms, developed buccal tablet and commercially available conventional oral tablet (Oksikam, Sanofi Aventis), containing 20 mg of PX were administered in a single-dose, randomized, open, three-way crossover study. The volunteers were served a standardized breakfast. Two hours later, the oral tablet was taken with 200 ml water, while buccal tablet was applied to the cheek after drinking 200 ml water. Blood samples were taken either vena basilica or vena metacarpals at predetermined time points (before drug administration and after drug administration 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 360, 420, 480 minutes and 24 hours). Sera was separated and frozen prior to assay for PX by HPLC. All volunteers fasted during 8 hours. A 15-day washout period was left between dosing days.

HPLC Assay of Sera Samples

The sample preparation: 50 µL meloxicam (internal standard) at concentration of 8000 ng/mL were added to 950 µL of the sera sample in a conical test tube and vortexes for one minute. Then 500 µL 0.1 M o-phosphoric acid and 1500 µL mixtures of acetonitrile and methanol (4:1 v/v) were added. After vortexing, the mixture was centrifuged at 4400 rpm for 30 min. The upper layer was taken and evaporated to dryness under a stream of nitrogen, then reconstituted with 475 µL of mobile phase.

The amount of PX in the samples was determined with a validated HPLC method. The chromatographic separation was performed using an isocratic elution. The mobile phase components were acetonitrile: methanol: 0.04 M KH_2PO_4 (40:10:50, v/v) and delivered at a flow rate of 1 ml min^{-1}. The separation was carried out at 15^0C, on a reversed phase ACE C18 column (150 x 4.6 mm, 5 µm particle size). The DAD detector was set at 360 nm and injection volume was 50 µL. The validation parameters of the method were given in **Table 2.**

Table 2. Validation parameters of PX in the HPLC analysis.

Linearity range	10-2500 ng/mL
Correlation coefficient	0.999
Detection limit	2.5 ng/mL
Quantification limit	7.5 ng/mL
Intra-day precision (RSD% for 25 ng/ml))	1.495
Inter-day precision (RSD% for 25 ng/ml)	2.115

Pharmacokinetic Data Analysis

Pharmacokinetic parameters, including the area under the curve (AUC) of the serum concentration curve and mean PX concentration after each administration were obtained directly from the serum PX concentrations. The AUCs for each administration were calculated by the linear trapezoidal rule. The relative bioavailability of PX after buccal administration were calculated according to the following Equation (2)

$$F_R = [(AUC_{buccal\ tablet} \times D_{commercial\ oral\ tablet.}) / (AUC_{commercial\ oral\ tablet} \times D_{buccal\ tablet})]\ 100\% \quad (2)$$

Where F_R is the relative bioavailability and D is the administered dose.

Statistical Analysis

Statistical analysis was performed with (repeated measures) variance analysis (SPSS 15.0 for Windows software). Tukey-Kramer's adjustment, which controls the experiment wise error rate at $\alpha = 0.05$ level, was used to determine significance among all possible pairs of formulations and interactions. At $p \leq 0.05$, data were considered to be significant.

RESULTS AND DISCUSSIONS

During the last two decades, Tran's epithelial routes have been extensively explored by pharmaceutical researchers as alternative routes of delivery. Drug application to mucosal membranes is often chosen to reach the site of action with direct systemic absorption and to lessen side-effects especially in the GI system [15,16].

Evaluation of Physical Properties of Buccal Tablets

The comparison of physical properties of the buccal tablets is shown in **Table 3.** Drug uniformity results were found to be good among different batches of tablets, and the percentage of drug content was more than 98%. The results also showed acceptable and homogenous distribution of drug in tablets.

Table 3. Physical properties of the buccal tablets of PX.

Code	CU (%) (n=10)	WV (mg) (n=20)	T (mm) (n=10)	F (%) (n=20)	H (Newton) (n=10)
F1	101.03 ± 1.21	98.13 ± 1.91	1.53 ± 0.06	0.48	40.08 ± 0.27
F2	98.64 ± 0.58	97.63 ± 2.46	1.67 ± 0.04	1.03	30.27 ± 1.53
F3	102.52 ± 0.59	100.26 ± 0.26	1.59 ± 0.02	0.53	42.39 ± 2.03
F4	99.25 ± 0.72	101.43 ± 1.38	1.63 ± 0.03	0.86	35.11 ± 0.19
F5	101.43 ± 0.98	100.59 ± 0.58	1.66 ± 0.03	0.68	41.44 ± 1.53
F6	102.87 ± 0.17	99.59 ± 0.41	1.52 ± 0.05	1.06	43.04 ± 0.56
F7	98.66 ± 0.58	97.55 ± 2.51	1.58 ± 0.04	2.27	31.48 ± 2,51
F8	106.89 ± 1.36	101.25 ± 1.23	1.59 ± 0.01	0.71	39.93 ± 1.22
F9	104.12 ± 1.03	100.76 ± 0.75	1.54 ± 0.05	0.87	33.77 ± 0.58
F10	102.86 ± 0.29	100.25 ± 0.25	1.52 ± 0.06	1.04	35.84 ± 0.81
F11	102.36 ± 0.87	100.71 ± 0.70	1.64 ± 0.03	0.68	39.37 ± 2.52
F12	101.22 ± 2.03	100.29 ± 0.29	1.54 ± 0.09	1.16	38.25 ± 2.77
F13	112.26 ± 1.54	100.72 ± 0.71	1.55 ± 0.01	0.92	29.61 ± 0.07
F14	103.83 ± 1.26	100.68 ± 0.68	1.63 ± 0.07	0.89	35.74 ± 3.51

Note: CU: Content Uniformity, WV: Weight Variation, T: Thickness, F: Friability, H: Hardness

The weight and thickness of the formulations ranged from 97.55 to 101.41 mg and from 1.52 to 1.67 mm, respectively. All tablets prepared in this study meet the USP requirements for weight variation of all formulae was less than 2% (USP 31). In all the

formulations, the hardness test indicated good mechanical strength, whereas friability is less than 1% (except F6, F7, F10 and F12), which indicated that the tablets had a good mechanical resistance and the friability is within the compendial limits (USP 31). The high friability index of above formulations may be explained with manufacturing process of tablets. Direct compression process of tablets sometimes can cause high friability index due to lack of enough binding effect among powder particles.

Differential Scanning Calorimetry (DSC)

Polymers used in formulations can exhibit an interaction with active substance. DSC studies can give information if there are any interactions between these chemicals. DSC studies showed that there were no interactions between PX and the polymers. **Figure 1** shows the DSC thermo grams of pure HPMC, chitosan and PX, and compares them with the scan of a compressed tablet containing all these materials.

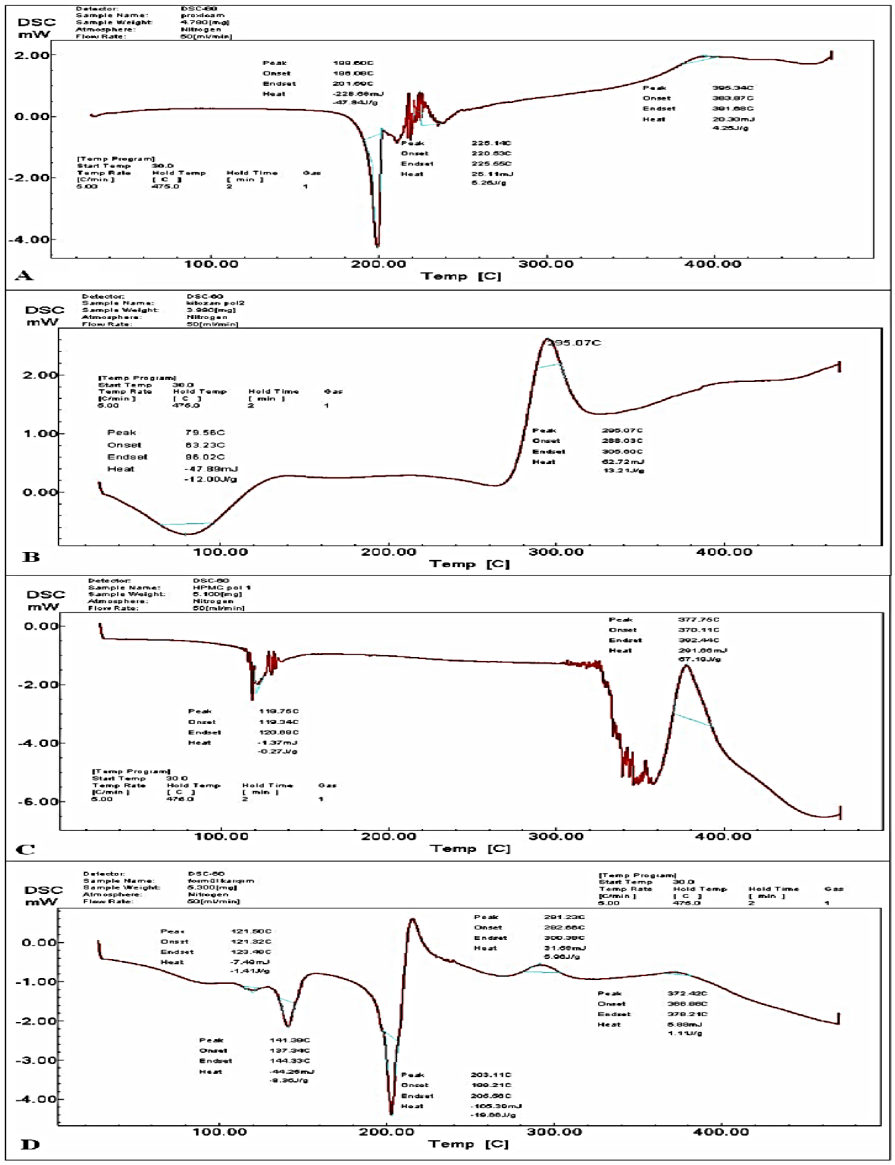

Figure 1. DSC curves A: pure piroxicam, B: pure chitosan, C: pure HPMC, D: F5 formulation.

It was observed no shift in peak position, or appearance of new or disappearance of existing peaks, and this shows us that the ingredients were stable and there was no interaction with each other physically or chemically as a result of compacting stress.

***In Vitro* Dissolution Studies**

Solubility plays an important role in the dissolution of a drug substance from a solid dosage form. For water insoluble drugs, difficulties are usually encountered in selecting a dissolution medium of acceptable volume and a composition as well as a good discriminating power [17]. Previous studies showed that 500 ml phosphate buffer (pH 7.4) provides sink condition for dissolution of PX from tablet dosage forms [18].

Drug dissolutions profiles of formulations are shown in **Figure 2.** The release rate of PX from the formulations affected by type and ratio of polymers **(Table 4).** The release of PX in 5 h study (dissolution time period) was incomplete for the formulations which contain at least 30% HPMC (F2 and F7). All other formulations released almost all drug during the dissolution period. High

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concentration of HPMC can cause thick coat of polymer around the drug. This gel layer increases the diffusional path length of the drug. Its viscous nature also affects the diffusion coefficient of the drug. Because the diffusional release of a drug primarily may be controlled by the gel thickness (diffusion layer), increasing the polymer level tends to decrease drug release [19,20].

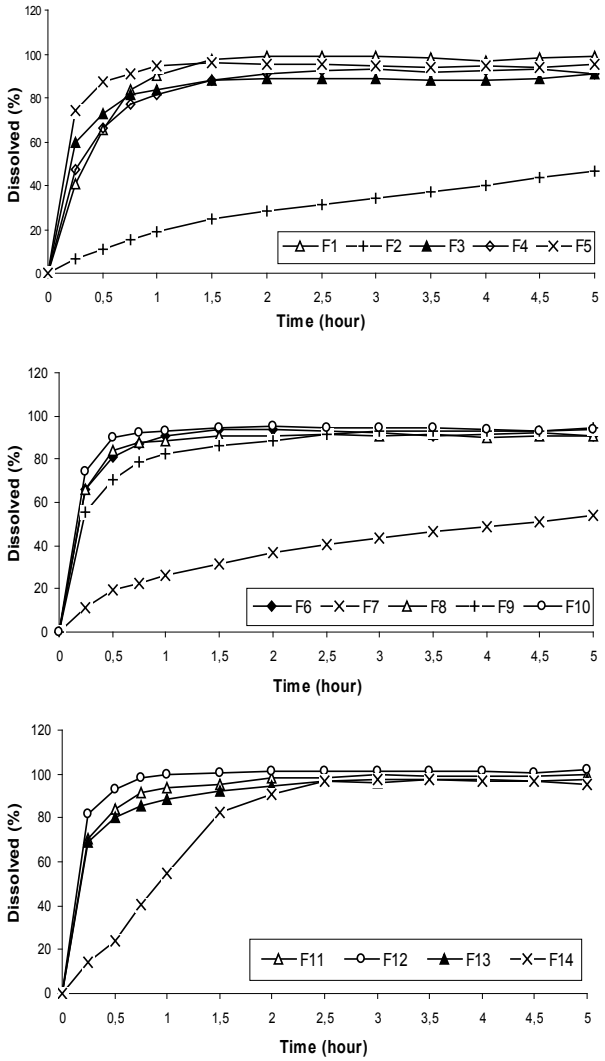

Figure 2. Dissolution profiles of buccal tablet formulations.

Formulations which contain chitosan at a ratio of 10, 20 and 39 gave dissolution rate 93.68 ± 6.69, 99.58 ± 5.52, 98.89 ± 5.55 respectively at the end of 5 hours **(Table 4).** These results show that PX dissolves completely even at the highest concentration of chitosan. Chitosan is a polymer material which is already known its properties of dissolution rate enhancer of drugs that are poorly soluble in water such as PX [21]. This result is also consistent with previous studies demonstrated that prednisolone when ground and mixed with chitosan achieved an enhancement of its dissolution

Table 4. Dissolution of PX from buccal tablets at the end of the 5 hours and polymers ratios used in the formulations.

Formulation Code	Dissolved %	Chitosan/HPMC ratio
F1	98.89 ± 5.55	39/0
F2	46.41 ± 3.71	0/39
F3	90.92 ± 11.11	2/1
F4	91.04 ± 10.95	1/2
F5	94.94 ± 6.01	1/1
F6	90.99 ± 5.80	10/3
F7	53.98 ± 2.90	3/10
F8	90.54 ± 7.15	7/6
F9	94.22 ± 5.40	6/7
F10	93.68 ± 6.69	10/0
F11	99.58 ± 5.62	20/0
F12	102.31 ± 5.53	0/10
F13	97.17 ± 6.11	0/20
F14	94.96 ± 4.93	0/0

Properties [22]. A similar result was also observed upon loading dexamethasone into spray-dried chitosan microparticles [23]. When HPMC and chitosan were used together in the formulation, the release profile of PX changed according to the ratios of these polymers. It was observed no significant release profile throughout the dissolution profile between at 1:2, 2:1, 6:7 and 7:6 ratios of HPMC/chitosan **(Figure 2 and Table 4).** The similar dissolution profiles of the formulations in the case of increasing or decreasing of HPMC or chitosan ratio up to 13 percent (F3-F8, F3-F6, F3-F4 and F8-F9, **Figure 2**) may explained with different mechanism of action of chitosan and HPMC on the release of PX. The expected results such as dissolution profile sometimes cannot be observed in the case of combination of different polymers in a formulation. But the use of these two polymers in equal ratio (1:1 or 19.5%) exhibited the fastest drug release ($p>0.05$). This can be explained with that the use of these polymers at above mentioned ratio can be associated to a higher degree of matrix hydration and a greater contribution of the matrix relaxation/erosion process to the predominant release mechanism. The hydration process of swellable hydrophilic matrices, as a function of time of exposure to an aqueous environment, involves progressively swelling, swelling/erosion and disentanglement/dissolution. Swelling and hydration of the polymer matrix occurs at a rate that is mainly a function of matrix composition and dissolution medium penetration into the matrix [24].

Drug Release Kinetics

Drug release kinetics parameters were given in **Table 5.** It was found that first order kinetics was predominant for F1, F4, F5, F9, F10, F11 and F13 formulations. Porous matrice structure especially formed by the presence of chitosan in the formulation may have an effect on predominant observation of this kinetic model. As clearly indicated in **Table 5** the formulations (except F2) did not follow a zero-order release pattern probably due to the fast initial dissolution of the drug from the superficial layers of the tablet. Increasing the amount of HPMC in the formulation (F2 formulation) resulted in a reduction in the drug release rate **(Figure 2)** and a linearization of the release curve, leading to a shift from anomalous type of release towards a swelling-controlled mechanism.

Table 5. Correlation coefficients and release exponent n according to different kinetic equations used for describing PX release behaviour from buccal tablet formulations.

Form. Code	Zero Order r^2	First Order r^2	Higuchi's Equation r^2	Korsemeyer-Peppas r^2 n	Form. Code
F1	0.453	0.996	0.864	0.729	0.238
F2	0.965	0.989	0.998	0.992	0.621
F3	0.505	0.869	0.858	0.786	0.114
F4	0.544	0.953	0.898	0.812	0.191
F5	0.303	0.932	0.858	0.614	0.061
F6	0.307	0.846	0.813	0.632	0.083
F7	0.943	0.977	0.993	0.988	0.496
F8	0.331	0.666	0.640	0.621	0.077
F9	0.652	0.857	0.791	0.882	0.155
F10	0.247	0.709	0.654	0.541	0.053
F11	0.530	0.956	0.777	0.811	0.097
F12	0.481	0.717	0.943	0.697	0.056
F13	0.847	0.972	0.897	0.896	0.105
F14	0.693	0.978	0.953	0.892	0.642

The relative good correlation with Higuchi kinetic model highlights the drug release mechanism as diffusion controlled maybe due to pore formation in tablets during the dissolution period. This mechanism was also supported with datas of Korsemeyer-Peppas kinetic model evaluation. All formulations 'n value, which describe the drug release mechanism, were between 0.053-0.642, indicating that the mechanism of the drug release was diffusion controlled.

In Vitro Permeation Studies

The release parameters of PX from buccal tablets are given in **Table 6.** The differences in the flux values of PX from the tablets could be attributed to the polymers used. The presence of HPMC in the formulation produced a swollen gel in contact with water. The viscosity of the gel slowed the diffusion of PX to the membrane interface. This could decrease the flux of PX from the tablets formulated with increasing concentration of HPMC (F2-F4, F6-F9). The use of HPMC and chitosan with equal amount (F5-19.5%) gave the maximum steady state flux value of 0.201 ± 0.011 mg/cm^2 hour compared to other formulations ($p<0.05$). Due to the PX low aqueous solubility and small volume (2 ml) of donor chamber, only a limited amount of drug can dissolve inside the hydrated polymeric matrices. Incorporation of chitosan in formulation makes PX dissolve easily in a hydrated polymeric environment, resulting in a higher diffusional driving force and faster drug release [25,26].

Ex Vivo Bioadhesion Studies

It has been proposed that mucoadhesion occurs in three stages. The first stage involves the formation of an intimate contact between the mucoadhesive and the mucus.

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Table 6. Drug release parameters (By using cellulose membrane at the end of A 4-h period) of the formulations (n=3).

Form. Code	Released%	Jss (μg/cm².h)	P (cm / h)
F1	2.91 ± 0.02	177.29 ± 30.63	17.73 ± 3.64
F2	2.75 ± 0.27	129.71 ± 11.19	12.97 ± 1.81
F3	2.54 ± 0.01	135.12 ± 2.55	13.51 ± 0.47
F4	2.68 ± 0.40	128.93 ± 6.07	12.89 ± 0.71
F5	3.34 ± 0.19	201.47 ± 11.18	20.15 ± 1.63
F6	2.32 ± 0.05	80.86 ± 8.91	8.09 ± 0.66
F7	2.59 ± 0.11	112.58 ± 10.44	11.26 ± 1.19
F8	3.08 ± 0.77	89.64 ± 17.37	8.97 ± 1.86
F9	2.66 ± 0.05	87.32 ± 7.03	8.58 ± 0.71
F10	3.06 ± 0.30	122.29 ± 21.11	12.23 ± 2.26
F11	2.51 ± 0.23	143.92 ± 26.78	14.39 ± 2.76
F12	2.84 ± 0.18	112.89 ± 6.23	11.29 ± 0.59
F13	2.98 ± 0.51	127.74 ± 12.71	12.78 ± 1.33
F14	2.39 ± 0.24	81.24 ± 2.32	8.16 ± 0.39

Note: J_{ss}: Steady state flux P: Permeability coefficient.

Secondly, the mucoadhesive macromolecules swell and interpenetrate with the mucus macromolecules, becoming physically entangled. Thirdly, these molecules interact with each other via secondary, non-covalent bonds such as hydrogen bonds [27,28].

Results of the mucoadhesion study were reported in **Table 7.** As is known, both work of adhesion and detachment force can be used to assess mucoadhesion, although some authors claim that the former is the best parameter [29,30].

Table 7. Bioadhesive parameters of buccal tablets.

Form. Code	Detachment Force (N)	Stretching at the detachment point (mm)	Work of Adhesion (N.mm)
F1	6.80 ± 0.26	0.80 ± 0.10	2.73 ± 0.43
F2	2.53 ± 0.15	2.57 ± 0.06	3.25 ± 0.22
F3	3.70 ± 0.17	1.03 ± 0.25	1.90 ± 0.38
F4	2.77 ± 0.06	1.10 ± 0.10	1.52 ± 0.14
F5	0.33 ± 0.06	6.97 ± 0.06	1.16 ± 0.19
F6	7.20 ± 0.17	0.53 ± 0.12	1.93 ± 0.46
F7	1.77 ± 0.21	5.43 ± 0.25	4.82 ± 0.80
F8	5.60 ± 0.26	0.87 ± 0.12	2.44 ± 0.45
F9	1.93 ± 0.12	3.57 ± 0.15	3.44 ± 0.14
F10	0.97 ± 0.15	5.33 ± 0.35	2.59 ± 0.21
F11	1.33 ± 0.15	4.60 ± 0.26	3.06 ± 0.19
F12	2.80 ± 0.17	7.23 ± 0.25	10.12 ± 0.39
F13	2.63 ± 0.12	1.06 ± 0.21	1.39 ± 0.16
F14	1.67 ± 0.15	3.60 ± 0.17	3.01 ± 0.23

The bio adhesive forces of PX buccal bio adhesive tablets were not dependent upon the shape of tablet, since it was fixed in the flat form, 8 mm in diameter. Furthermore, the bio adhesive forces of the PX tablets were hardly affected by the swelling degree of the tablet components, the bio adhesive polymers, since both were very water soluble or swellable.

The bio adhesive forces of the PX buccal adhesive tablets were chiefly affected by the nature of the bioadhesive polymers, since they were different in nature. HPMC could bind very weakly with the neutral cellulose groups. The comparatively weak bio adhesion force of the non-ionic polymer HPMC (F2, F4, F7 and F11 formulations) may attributed to the absence of a proton-donating carboxyl group which reduces its ability for the formation hydrogen bonds [31]. Chitosan, compared to HPMC, strongly binds due to its positive charges at neutral pH that enable an ionic interaction with the negative charges of salicylic acid residues of the mucus. Formulations F1 and F6 contain high amount of chitosan exhibited high detachment force 6.80 ± 0.26 and 7.20 ± 0.17 respectively compared to other formulations ($p<0.05$). HPMC could be used to control the bioadhesive force of chitosan due to its weak bioadhesive force [21,32].

The comparison of all formulations showed us that there was no a good correlation between polymer ratios and detachment force for formulations containing HPMC-chitosan (F4-F5, F1-F6, F2-F7, F8-F9). This can be attributed different mucoadhesive characteristic of two polymers can affect detachment force value in an unpredictable way. Incorporporation of HPMC into formulations containing chitosan can change the mucoadhesive characteristic of it and it is hard to settle a correlation value to decide which combination will give the desired mucoadhesive property. So a comprehensive study design was to be done to explain in which extent the incorporation of different mucoadhesive polymers in a formulation can affect its detachment force and stretching at the detachment point of the formulation.

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But to decide for choosing optimum formulation, it must be taken into consideration that excess bioadhesive force can damage to the mucous membrane in case of detachment from application site. So F5 formulation containing equal amounts of chitosan and HPMC (F5-19.5%) exhibits desired bioadhesive property according to its minimum detachment force and maximum stretching at the detachment point [32].

In Vivo Experiments

The principal mechanism of buccal absorption is passive diffusion. Two main pathways seem to be implicated in passive diffusion across mucosae: intracellular (or trans cellular) and intercellular (or Para cellular). Within the intercellular spaces there are two ways: one, hydrophobic, goes through the lipid domains; the other hydrophilic, relates to the aqueous channels associated with the polar head groups of lipids and proteins. The intrinsic physicochemical properties of the drug, such as solubility, partitioning, stability, crystallinity, thermodynamic activity, molecular size, pKa and half-life are also limiting factors of drug absorption through buccal mucosae [15].

According to the results of *in vitro* and *ex vivo* experiments in terms of high dissolution and permeability rate, enough and desired mucoadhesion at the buccal site, F5 formulation was chosen as an optimum one for *in vivo* studies. The tablets applied on buccal mucosa remained attached for 5 h without any discomfort **(Figure 3).** No irritation or pain on the mucosa was reported.

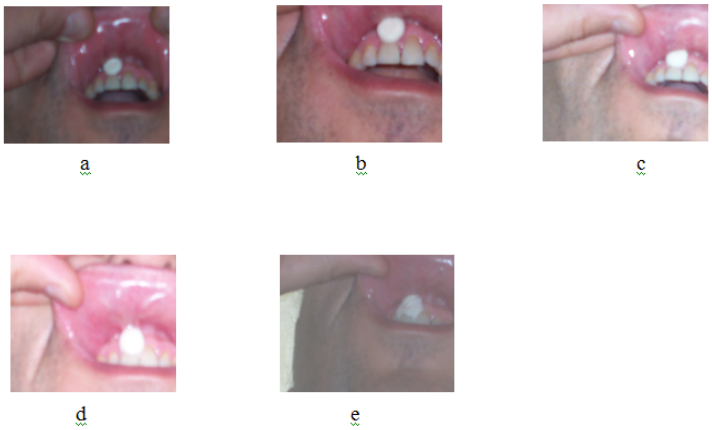

Figure 3. Images of the buccal tablets in the buccal region after application. A) After 1 hour B) After 2 hours C) After 3 hours D) After 4 hours E) After 5 hours.

The pharmacokinetic parameters of PX were determined after the administration of buccal adhesive tablet and oral administration of commercially available tablet formulation in human healthy volunteers. The results are shown in **Table 8.**

Table 8. Pharmacokinetic parameters of oral (commercial tablet formulation) and developed optimum buccal tablet formulation (F5).

Form	C_{max} (ng/ml)	T_{max} (hour)	$AUC_{0\text{-}24\ h}$ (ng/ml. hour)	F_{rel} (%)
Oral tablet	2076.52 ± 395.09	4.13 ± 0.88	34416.07 ± 5978.37	-
Buccal tablet	1395.22 ± 431.15	5.63 ± 0.52	23236.52 ± 7334.59	67.52 ± 21.47
F_{rel}: Relative bioavailability (relative to oral tablet administration)				

Mean sera concentrations of PX are depicted in **Figure 4.** It was shown that PX was more rapidly absorbed from the oral tablets than from buccal ones. The peak level was reached 4.13 ± 0.88 h after administration of oral tablet compared to 5.63 ± 0.52 h after the buccal one (**Table 8**, p>0.05). The Tmax value of oral application is consistent with previous studies reveal that it takes more than 2 h to reach the maximum concentration [33]. The rapid absorption of PX after oral application compared to buccal one can be explained with rapid dissolution of PX in the gastro intestinal tract. For poorly soluble, highly permeable (class II drugs like PX), the rate of oral absorption is often controlled by the dissolution rate in the GI tract [34].

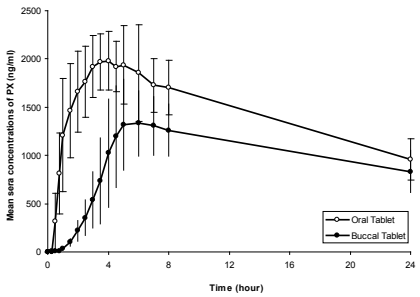

Figure 4. Serum concentration profiles of PX (n=8) after application of oral tablet (commercial) and buccal tablet (optimum formulation, F5).

The $AUC_{0\text{-}24h}$ and C_{max} values of oral tablet were significantly higher than that of the buccal tablet formulation. Relative bioavailability value of the buccal tablet was 67.52 ± 21.47. This comparable value of buccal tablet with oral one can be attributed

to formulation composition. Especially chitosan polymer used in oral mucosal formulations exhibited better drug release and effective *in vivo* absorption profile.

It was found that chitosan might be increasing the thermodynamic activity of the penetrant which results in enhanced penetration. Research on the permeability of oral mucosa indicated that the majority of compounds pass through the Para cellular pathway. However, the major intercellular barrier in oral epithelium consists of organized lipid lamellae in the intercellular regions of the superficial layers of the epithelium. As chitosan has been shown to be capable of disrupting lipid micelles in the intestine, the permeabilizing effect can be attributed to its interference with the lipid organization in the buccal epithelium [12,26]. Also chitosans are suggested to enhance absorption of drugs through mucoadhesion by binding strongly to negatively-charged biological surfaces such as mucous membranes and improved aqueous solubility of a drug [35,36].

On the other hand low bioavailability of PX after buccal application can be explained with several factors. PX is an ionizable water insoluble drug at a physiological pH. Specifically, PX can be ionized as a zwitterion with two pKa values (pKa_1 = 1.86 and pKa_2 = 5.46). A zwitterion drug possesses a large intramolecular multipole moment due to its multiplicity of oppositely charged groups. Consequently, most of these drugs have low solubility in polar and nonpolar media, as well as a low lipophilicity [37]. As a result, since PX is a lipophilic drug, it will readily diffuse across the buccal mucosa but may have problems of dissolution in the saliva, which, in turn, will limit its absorption [38].

On the contrary, the slower, progressive absorption pattern may contribute to the reduction of adverse effects by preventing abrupt rises in plasma levels of piroxicam. In a manner similar to slow release pharmaceutical formulations [33]. All these parameters showed us that permeation of PX from buccal mucosa was low and time to reach the maximum concentration in the sera was longer compared to commercial conventional tablet formulation. This shows us one of the major disadvantages associated with buccal drug delivery is the low flux which results in low drug bioavailability. Various compounds have been investigated for their use as buccal penetration enhancers in order to increase the flux of drugs through the mucosa. Since the buccal epithelium is similar in structure to other stratified epithelia of the body, enhancers used to improve drug permeation in other absorptive mucosae have been shown to work in improving buccal drug penetration [39]. For insance, hydroxypropyl-β-cyclodextrin was used to enhance permeation of PX from buccal mucosa. It was found that adding hydroxypropyl-β-cyclodextrin to the formulation increase the solubility of PX and also release rate [25]. From this point of view, it seems a logical approach to add penetration and solubility enhancer to the formulation to obtain fast pharmacological response and equal and comparable bioavailability values versus conventional oral tablet.

CONCLUSION

Chitosan can be evaluated as an ideal polymer for buccal tablet formulations because its mucoadhesive and solubility enhancer property for poorly soluble active ingredients such as PX. Buccal tablet formulation contains equal amount of chitosan and HPMC (19.5%) seems to be an alternative drug delivery of PX especially for patients suffering from GI disturbances.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Ali Rıza Kepsutlu: Carried out the preparation of buccal tablets, *in vitro-ex vivo* permeation studies and drafted the manuscript. Cetin TAS: participated in analysis of the drug and participated in drafting the manuscript. Ayhan Savaser: carried out the evaluation of buccal tablets and dissolution studies. Yalcin Ozkan: carried out DSC analysis Tamer BAYKARA: Supervisor. All authors read and approved the final manuscript.

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