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Original Article

DESIGN, DEVELOPMENT, AND CHARACTERIZATION OF LORATADINE SUSTAINED RELEASED BUCCAL FILM: *IN VITRO* AND *IN VIVO* STUDY IN BEAGLE DOGS

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ABSTRACT

Objective: The aim of this work was to develop and evaluate bucco-adhesive films of Loratadine (LTD) for sustained release use.

Methods: Design of twelve different sustained released buccal film formulas using Carbopol, pectin, sodium alginate, glycerol, carboxymethyl cellulose (CMC), hydroxypropyl cellulose (HPC), Gelatin, Hydroxyethyl cellulose (HEC), and Hydroxypropyl methylcellulose (HPMC) as mucoadhesive polymers. Films were evaluated for physicochemical properties, thickness, swelling, moisture content, drug content, *in vitro* dissolution. The pharmacokinetic parameters of optimal formula were evaluated in beagle dogs.

Results: The selected film formula (F6) showed accepted content and muco-adhesion properties. The *in vitro* release study showed prolonged release of drug from films over 10 h in optimal formulation. The bioavailability studies performed using beagle dogs model showed that there was 113.45% increase in the AUC_{0-24} of selected film compared with oral market tablets.

Conclusion: Bucco-adhesive films is a promising dosage form for improving the bioavailability of loratadine.

Keywords: Buccal drug delivery, Mucoadhesive polymers, Loratadine, Films, Bioavailability

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INTRODUCTION

Oral drug administration is the ideal and most used route due to its convenient characteristic and as a painless route [1]. But it has many limitations such as the first-pass effect, gastrointestinal enzymatic degeneration, in addition to slow onset of action [1]. Therefore, thinking of other alternative routes becomes important [2]. The mucus membrane of the mouth has been identified as a potential site for the absorption of drugs [2]. The mucosal lining of the buccal region offers an attractive drug-delivery route to enhance both systemic and local therapy [3]. In contrast to oral drug delivery, the buccal delivery system has distinctive advantages as for its higher permeability resulting in fast onset of action and also improving the bioavailability by bypassing the gastrointestinal tract (GIT) and avoiding the first-pass effect, enzymatic/acidic hydrolysis, and the food reaction [4, 5]. Therefore, increasing the therapeutic efficacy of the drugs [6]. In addition to, its capability to control the release of the drug for a long time with a slow and controlled manner [6]. Since the medication amount in buccal formulations is often smaller than in tablets and capsules, toxicity and other side effects are likely to be significantly decreased [7, 8]. Recently, numerous types of muco-adhesive dosage forms were developed [8, 9]. Muco-adhesive films were confirmed to enhance drug absorption over other buccal forms [10]. The ideal buccal films must have excellent muco-adhesive strength, suitable elasticity, softness, in addition to its ability to resist rupture owing to oral activity stress [11-13]. In order to establish strong adhesive contact with the mucosa, mucoadhesive polymers are used as excipients [12, 13]. First-generation mucoadhesive polymers, also known as conventional film-forming materials, include sodium alginate, Hydroxypropyl methylcellulose (HPMC), Pullulan, Methylcellulose, Polymethacrylate derivatives, Polyacrylate, and Chitosan [14]. In recent years, a new generation of muco-adhesive polymers known as thiomers or thiolated polymers has been developed [14]. It was observed that these newer materials were less susceptible to mucus turnover as they form covalent thiol bonds and so mimic the natural adhesion mechanism of secreted mucus glycoproteins, Poly (acrylic acid)-cysteine, Chitosan-thioglycolic acid, and Chitosan-cysteine were examples for these polymers [15]. The choice of proper mucoadhesive polymers is a main step in developing an effective mucoadhesive medication delivery system. These polymers must be stable, nonirritant, adhere rapidly, non-toxic, inert, compatible with the drugs, and cost-effective [16]. Plasticizers are one of the necessary components for oral films that they enhance flexibility and decrease brittleness by lowering the polymer's glass transition temperature; plasticizer enhances strip characteristics intensely [17, 18]. The plasticizer used should lend permanent flexibility to the film formulation, and this depends on both the volatile nature of the plasticizer in addition to the type of interaction of the plasticizer with the polymer. Moreover, a penetration enhancer is also used in buccal film formulation to enhance the release of the medication by allowing it to permeate the live tissue more easily [19, 20]. Buccal films can easily be scaled up due to the adaptableness and probability nature of the film and easy manufacturing procedures like hot melting extrusion (HME) and solving casting techniques [21, 22]. Moreover, developments in 3D printing technologies will also service to accommodate acceptable dosages of drugs in buccal films [23, 24]. Loratadine (LTD) (fig. 1) is one of the drugs that can be formulated as buccal film, LTD is one of newer generation of antihistaminic drugs that have less sedating effect [25]. It is acting as a selective drug for peripheral H1-receptor in the nose and conjunctivae, intended to relieve the symptoms of rhinorrhea, nasal congestion, sneezing, and also itching. Although it showed rapid absorption after oral administration, its absorption is limited and highly variable. LTD showed low oral bioavailability (less than 40%) because of its extensive first-pass metabolism [26]. It is a weakly ionizable basic drug with pH-dependent solubility that rapidly decreases as pH rises [27]. Kumria *et al.* designed a successful sustained release buccal films of LTD to provide prolonged protection against allergic rhinitis by using a solvent-casting method with HPMC (E5 and K100 blend) and also Eudragit® NE 30D as a retardant [28-30]. Moreover, other studies that involve LTD as a buccal dosage form, included a transfer-osomal gel formulation study that definite that inter-individual variability in absorption parameters was pointedly reduced when the buccal gel was used compared to LTD oral tablets [31, 32]. The study aimed to design a bucco adhesive film of LTD for

sustained release behavior. Different polymers in different ratios were used for preparing twelve buco adhesive film of LTD. Films also were characterized for drug content, muco-adhesive properties and *in vitro* drug release. Finally, *in vivo* pharmacokinetics behavior were tested for the selected optimized film in dogs' model based on cross-over design in comparison to market tablets as a reference.

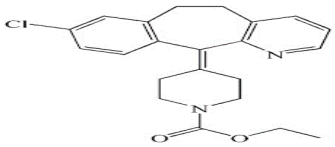

Fig. 1: Chemical structure of loratadine (LTD)

MATERIALS AND METHODS

Materials

LTD was a kind gift from SPIMACO for Pharmaceutical Industries, Sodium alginate (SA). Hydroxy propyl methyl cellulose (HPMC), Hydroxy propyl cellulose (HPC), Carboxymethyl Cellulose Sodium Salt (Na CMC) High Viscosity by Loba Chemie, UK, Chitosan cysteine (CS), Carbopol (Cp) (El Kahera Pharmaceuticals, Cairo, Egypt). Hydroxy ethyl cellulose (HEC), pectin (Brunsbüttel, Germany). Gelatin (gelatin powder for bacteriology by loba chemie, India. Sodium hydroxide and ethanol were HPLC grade. Loratadine tablets were supplied from SEDCO Pharmaceuticals (Cairo, Egypt). All other chemicals were of analytical grade and were used as get. Purified water was used from an ultra-pure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

Methods

Preparation of LTD buccal films

The casting technique was used to prepare LTD buccal films as it is a simple and low-cost preparation method and can easily be implemented at lab scale [33]. Using different concentrations and combinations of HPMC, HEC, HPC, CMC, Pectin, Gelatin, Na alginate, Cp, CS and glycerol as a plasticizer, all polymers were dissolved in definite volumes of distilled water and stirred till dissolved. Vary amounts as shown in table 1 were blended and stirred at 60 °C for one hour with the addition of glycerol [33]. Measured weights of LTD was dissolved in 1 ml ethanol and added to the mixture at room temperature, covered and stirred till a completely homogenous clear solution was obtained. The solution poured into the mold and release control layer were mixed separately according to table and poured above the medication layer. Molds were then covered with the Para film and be reserved in a desiccator at room temperature for further investigation.

Physicochemical characteristic

Weight variation test

The test was carried out by taking ten films from each formula, and each film was weight separately by sensitive four digits' electronic balance. The average weight and standard deviation were calculated.

Average weight = Total weights of all films/number of films …… (1)

Thickness measurement

The thicknesses of three films from each formulation were measured on an individual basis with a micrometer; the average thickness of every sample was obtained as well as the standard deviation [34].

Uniformity of drug content test

Three separate buccal films from each formula were dissolved in 100 ml phosphate buffer pH 6.8 and stirred for one hour, five ml samples were taken and the drug content was assessed using UV spectrophotometer at 269 nm after proper dilution [35].

Swelling index measurement

The test was conducted by keeping three films from each formulation after weight on stainless steel sieving mesh pre-weighed. The mesh containing the film was submerged in a beaker containing 100 ml of phosphate buffer pH 6.8 and maintained in an oven at 37±2 °C. At regular one-hour time intervals, the mesh was removed, dried with tissue, and reweighed. An increase in weight was determined every hour until a constant weight was obtained [36].

The Swelling index was calculated as:

$$\%\square=\square_{\square}-\square_{0}/\square_{0}\times100 \ldots (2)$$

Whereas:

Wo = original weight or preweight of the film and

Wt = weight of the film at time t.

Moisture content percentage

It was calculated by weighing the three LTD buccal films from each formula, which then placed in a desiccator containing silica for 24 h and reweighed again [37]. The percentage moisture content was calculated:

% Moisture content= (initial weight-final weight/final weight) * 100 …. (3)

Moisture uptake measurement

Three films from each formulation were exposed to more than 80% relative humidity by placing them in a potassium chloride-saturated filled desiccator. The solution was prepared by dissolving 37.4g of KCl in 100 ml of distilled water, and the film was reweighed again after each hour until it gained a constant weight [38]. The %Moisture uptake was calculated:

% Moisture uptake = (final weight-initial weight/initial weight) *100 ….. (4)

Measurement of film's surface pH

The films were left in 10 ml of distilled water for one hour at room temperature, allowed to swell, and pH was determined by bringing the electrode in contact with the surface of the film for one minute [39].

Flexibility of LTD buccal film formulations

Three films from each LTD buccal film formula were selected randomly. The film was folded at the same position until cracking happened. The value of the film's folding endurance was denoted by the number of film folds before cracking occur. The mean folding endurance±SD was deliberated [40].

The *in vitro* residence time

The *in vitro* residence time was identified using disintegration apparatus at pH 6.8 phosphate buffer disintegration medium (600 ml, maintained at 37±0.5 °C). On the surface of a glass piece, the parts of rat abdominal mucosa (3 cm^2) were stuck and then the slab was vertically fixed to the apparatus. The formulation was hydrated using phosphate buffer and the hydrated surface was carried in contact with the membrane. The glass slab was then fixed to the apparatus and permitted to move up then down. The time required for whole erosion and/or detachment of the film from the surface was verified (n=3) [40].

Water vapor transmission through films

Sample films from all prepared formulations were tested for water vapor penetration. Three grams of anhydrous calcium chloride were reserved in an empty five ml test tube, and at the top of the test tubes blank films were fixed. The test tubes were weighed and they were retained in desiccators enclosing a saturated solution of potasium chloride to maintain RH at 75±5%. The desiccator was well closed. The test tubes were weighed every day for ten days. The water vapor permeation rate (V) was expressed by the following equation [41]

$$V = M * T/A \ldots. (5)$$

Where M: mass of water vapor transmitted, T: thickness of the film, A: surface area of the film

In vitro release study of LTD from buccal films

The *in vitro* release studies for LTD bucco-adhesive films were done in phosphate buffer (pH 6.8, 250 ml) solution at 37 °C using a modified dissolution apparatus. The modified apparatus was a 500 ml beaker with a bottom magnetic stirrer (adjusted at 50 rpm) and an electro-thermal hot plate was used for keeping the temperature at 37 °C. The film was located in a basket cavity and kept in a dissolution medium. One milliliter samples were taken at pre-determined time intervals (0.5,1,2,3,4,5,6,7,8 and 24 h) and replaced with an equal quantity of temperature equilibrated dissolution medium. Then the absorbance of each sample was measured by UV/Vis spectrophotometer at the determined wavelength 269 nm after a tenfold dilution of each of the withdrawn sample [42].

In vivo pharmacokinetic study of the selected LTD buccal film in beagle dogs

The *in vivo* pharmacokinetic study was accompanied in agreement with the ethical guidelines for laboratory animals use and was permitted by the Institutional Animal Ethics Committee, Qassim University (number 22/13/02). All procedures and care of the "beagle dogs" were in accordance with institutional guidelines in research. Six male beagle dogs weighing 12.5–14 kg were used and divided into two groups randomly (fig. 4), and the study was carried out in a crossover design in two phases with a washout period of one week to eliminate the effect of the previous drug treatment before the administration of the next drug dose. No food was allowed during the experiment up to 12 h. Water was available ad libitum thru the study period. During the first phase, films were located in the buccal membrane with the help of a clip for the first group, while the second group received orally one tablet of marketed product "10 mg" and vice versa in the second phase.

Blood sampling

Five milliliters of blood samples were withdrawn into a heparinized blood collection tube via a needle at zero, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h postdose. The plasma samples were got by centrifuging the blood samples at 4000 rpm for 15 min and were stored at −20 °C until further analysis. The plasma concentration was assayed using HPLC method using a fluorescence detector after proper validation of the method [43].

Chromatographic system and conditions

LTD concentrations in plasma samples were determined by a reported HPLC method after reviewing of its selectivity, accuracy and linearity as will be mentioned later [44]. A simple, rapid, and accurate high-performance liquid chromatographic method using fluorescence detection was used for the determination of LTD concentration in withdrawn plasma samples. Liquid–liquid extraction of LTD and diazepam (as internal standard) from plasma samples was completed with *n*-butyl alcohol and *n*-hexane mixture (2:98, v/v) in alkaline pH (8.2) followed by reverse extraction into diluted perchloric acid (0.1M). Chromatography was conducting using a C_{18} column (250 × 4.6 mm, 5 µm) under isocratic elution with acetonitrile: sodium dihydrogen phosphate: triethylamine in a ratio 43:57:0.02, v/v, and pH 2.4. Analysis process was run at a flow rate of 1.0 ml/min at temperature 25 °C. LTD was detected by a fluorescence detector set at 291 nm excitation wavelength and 480 nm emission wavelength. All data were collecting and treated using Lynx TMV 4.1 software (Waters Corp., Milford, MA, USA). The method was validated for selectivity, precision, accuracy, linearity, and carry over, extraction recovery and stability briefly before the beginning of this study [44].

Selectivity of the method

Selectivity was studied by comparing chromatograms of blank plasma obtained from beagle dogs with those of corresponding standard plasma samples spiked with LTD, IS, and plasma sample after buccal application of selected LTD-F6 formula.

Linearity and lower limit of quantification (LLOQ)

Calibration curves were prepared by making serial dilution of the working stock and assaying standard plasma samples at six concentrations of LTD ranging 200-2000 ng/ml. The validation of LLOQ was conducted in at least six different batches of blank plasma. It was validated using an LLOQ sample for which an acceptable accuracy (RE) within 20% and a precision (R. SD) below 20% were obtained [45].

Precision and accuracy

For determining the intra-day accuracy and precision, a replicate analysis of plasma samples of LTD was performed on the same day, the run consisted of a calibration curve and six replicates of each low, mid, and high-concentration quality control samples. The inter-day accuracy and precision were assessed by analysis of three batches on different days. The precision was expressed as the relative standard deviation (R. SD) and the accuracy as the relative deviation (RD).

Stability of the method

The effect of freeze and thaw cycles on the LTD and IS stability in plasma was determined by analyzing LTD and IS concentrations (low, mid and high concentration) in plasma samples which meeting four freeze-thaw cycles. After completion of every cycle, the samples were analyzed and the experimental concentrations were compared with the nominal values. The accuracy values of three concentrations in four freeze-thaw cycles were calculated. In order to estimate the stability of LTD and IS in the prepared sample, three QC samples at low, mid and high concentration were kept at sample temperature for about 6 h. Then, the samples were analyzed and the concentrations obtained were compared with the nominal values [45].

Calibration curve of LTD in plasma

Standard calibration curve of LTD in plasma was constructed using drug-free beagle dog plasma (200 µl) spiked with 500 ng/ml of internal standard (IS) and standard LTD solutions to obtain the concentration range of 100-2500 ng/ml. The spiked plasma was then subjected to the same extraction procedure as the samples. Unknown sample concentrations of LTD were calculated from this calibration curve, which was obtained by plotting the final concentration versus the peak area ratios of varying amounts of LTD [46].

Plasma sample preparation

Aliquot of plasma samples collected from different dogs were subjected to protein precipitation as follows. 0.5µg/20 µl of Diazepam as internal standard (IS) were spiked into 200 µl of plasma samples and transferred to Eppendorf tube and the mixture was vortexed for 10 sec. Then, 800 µl of acetonitrile were added and the mixture was vortexed for 1 min followed by centrifugation at 20,000 rpm for 15 min at 10 °C. The supernatant was transferred into a clean glass tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 100 µl of water: acetonitrile mixture (50:50, v/v), vortexed for 1 min, centrifuged at 3000rpm for 5 min, transferred into a plastic autosampler vial with pre-slit septum (Waters, USA) where 1 µl was injected into the HPLC [46].

Pharmacokinetic analysis

Plasma concentration of LTD was presented as the mean±S. E. Pharmacokinetic parameters were estimated using model-independent methods. The elimination rate constant (K) was estimated from linear regression analysis of the terminal portion of the log-linear blood concentration–time profile of a drug. The elimination half-life (t1/2) was calculated from the terminal elimination rate constant using the formula t1/2 = 0.693/K. The maximum peak drug concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were derived directly from the individual blood levels. The areas under each drug concentration-time curve (AUC, µg ml^{-1} h) were calculated by the linear trapezoidal rule using stripe computer program. The relative bioavailability (F_{rel}) was calculated using the following equation:

$$\text{Frel.} = \frac{\frac{[AUC]□6}{\text{dose}}}{\frac{[AUC]\text{commercial tablet}}{\text{dose}}} * 100 \ldots\ldots (6)$$

Statistical analysis

Difference between the means of each pharmacokinetic parameter of the batach and commercial tablets was analyzed using ANOVA of unpaired data (Graph Pad Instat 3.0 software). Differences between means were considered statistically non-significant if the p value was>0.05.

RESULTS AND DISCUSSION

In the presented study, LTD buccal films were prepared using solvent casting method, which could be described as a simple and low-cost technique. The ideal mucosal adhesive buccal film should be flexible, soft, compact, mechanically strong, and have adequate mucosal adhesive strength [30]. The prepared films were visually inspected and found to be elegant in appearance, flexible, homogeneous and most of them are easily removed from the mold. The study involved the use of the following polymers gelatin, Pectin, NaCMC, CS, HPMC, CP, HPC, and SA. The experiment involved the using of different polymers in different concentrations for achieving sustained behavior of drug release. Physicochemical evaluation of the buccal films, *in vitro* releases parameters, and *in vivo* performance in dogs were studied.

Table 1: Ratios of the compositions of LTD buccal films formulations

Code	First layer components ratio						Sustained layer components ratio		
	P	Cp	ALG	Gly	HEC	Gel	HPMC	HPC	CMC
F1	1	-	1	1		1	1	1	-
F2	2	-	2	1		1	1	-	1
F3	1	-	2	1		1	-	1	1
F4	2		2	1		1	1	1	1
F5		1		1	1	1	1	1	-
F6		2		1	2	1	1	-	1
F7		1		1	2	1	-	1	1
F8		2		1	2	1	1	1	1
F9				1		1	1	1	-
F10				1		1	1	-	1
F11				1		1	-	1	1
F12				1		1	1	1	1

P: Pectin, Cp: Carpobol, ALG: Alginate, Gly: Glycerin, HEC: Hydroxy ethyl cellulose, Gel: Gelatin, HPMC: Hydroxy propyl methyl cellulose, HPC: Hydroxy propyl cellulose, CMC: Na Carboxy methylcellulose

Table 2: Weight variation of formulated loratadine sustained released buccal film F1-F12

LTD-BF	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Wt (mg)	0.103	0.107	0.107	0.109	0.108	0.111	0.110	0.104	0.105	0.104	0.114	0.06
SD	0.005	0.007	0.010	0.008	0.006	0.017	0.015	0.001	0.025	0.015	0.021	0.025
T(mm)	0.12	0.13	0.14	0.13	0.13	0.14	0.15	0.15	0.16	0.16	0.16	0.18
SD	0.001	0.002	0.001	0.002	0.001	0.003	0.003	0.002	0.003	0.002	0.001	0.002
Moist%	0.15	0.56	0.18	2.4	2.3	2.3	2.2	2.1	2.4	2.2	2.0	2.2
SD	0.05	0.001	0.021	0.021	0.011	0.031	0.021	0.041	0.001	0.041	0.021	0.011
%cont.	84.5	80.16	92.3	97.34	98.11	98.36	97.35	97.91	96.87	95.66	96.99	96.54
SD	3.64	2.32	3.26	2.74	2.72	2.60	2.51	1.67	2.20	3.02	1.80	1.20
Most 1h	1.12	1.32	1.22	2.13	2.23	2.49	2.51	3.92	3.71	3.52	2.87	2.99
SD	0.021	0.029	0.023	0.025	0.025	0.071	0.088	0.03	0.02	0.04	0.09	0.10
Most 2h	3.54	2.90	3.72	4.84	5.09	5.19	5.39	5.02	4.88	4.76	4.87	3.98
SD	0.92	1.02	1.01	0.93	0.98	0.88	0.98	0.85	0.95	0.92	1.01	1.02
Res. T.	2.23	2.13	2.23	2.21	2.63	2.22	2.25	2.24	2.20	2.43	2.21	2.33
SD	0.56	0.16	0.36	0.16	0.45	0.45	0.51	0.50	0.36	0.34	0.55	0.11
Fold.	96	99	101	161	166	162	131	145	154	147	155	158
SD	13	16	12	12	18	10	14	12	13	16	12	14

BF: Buccal Film, F: Formula, SD: standard deviation, W: weight, T: thickness, Moist%: Moisture content percentage. Res. T.: *In vitro* residence time. %cont.: Percentage drug content. Fold: Folding endurances. Results presented as mean±SD

Characterization of LTD bucco-adhesive films

Weight variation test

The average weights of all buccal films, as well as the standard deviation of the weight measurements for each formulation, have been calculated. As shown in table 2, the results of the average weight in grams of all formulations ranged between 0.103±0.005 and 0.114±0.021 mg for F1, and F11, respectively. The recorded average weight shows the appropriateness of prepared film for buccal use for adults that studies showed the adults could withhold medicated batches up to 0.4 mg [47].

Thickness measurement

The thickness of prepared LTD formulations was ranged between 0.12±0.002 and 0.18±0.005 for F1 and F12, respectively (table 2), which could be clarified based on the polymer content in each formula. The thickness of the formulations was suitable for adult use in the buccal cavity without irritation or bulkiness feel for the patients [48].

Drug content percentage

Table 2 shows that the average drug percentage ranged between 80.16±2.32% and 98.36±2.60% for F2 and F6, respectively. The results showed that the drug was uniformly dispersed in the matrix of the polymer, with an accepted loading and a significantly high loading of the drug was observed in all formulations. The drug percentage is accepted for some formulations, while is decreases for others, indicating the interaction of the drug with the polymer under the experiment conditions [49].

Surface pH measurement

Both acidic and alkaline pH may cause irritation to mucosa and may disturb the drug release and degree of polymer hydration. Hence, the surface pH of buccal films was determined to improve both drug release from batches and its muco-adhesion. Surface pH of all the buccal film formulation, have been measured. As shown in table 2, pH of all formulations ranged between 5.97-6.34 for F1 and F6, respectively. Films showed pH resemble to that of the buccal cavity which improves its compatibility with buccal mucosa without irritation or induction of inflammation. Previous studies reported that buccal dosage forms with pH less than 5 or more than 8.5 may lead to mucosal sensitization and increased possibility of buccal inflammation which decrease patient compliance [49]. The reported pH of LTD buccal batches was within accepted pH ranges which helps to maintain the batch for the treatment duration without patient discomfort feelings.

The moisture content %

The moisture content (%) percentage of the prepared LTD buccal films formulations ranged between 0.15±0.05 to 2.3±0.031 for F1 and F6 respectively. The difference between films in the moisture content might be due to the changes in the polymers content and concentrations. The optimal moisture content in the film is necessary to assure the ideal softness, flexibility, and stability [43]. The results showed the significant increase in film moisture content with Carpobol, HPMC, and EC. While pectin and alginate films showed lower moisture content. The lower moisture content in these formulations might be due to the lower ability of alginate and pectin to adsorb and keep moisture that increase the compactness of the film network. The high content of carboxylic acid groups in Cp, HPMC and EC helps in increasing its swelling ability. This could explain the high moisture content in formulations (F5-F12).

The percentage moisture uptake (%)

Moisture uptake is crucial in order to assess the physical stability of the films at high humidity, which is typical of the oral cavity. Table-2 showed that the % Moisture uptake after 1 hour was ranged between 1.12±0.021% and 3.92±0.03 for F1 and F8, respectively, and after 2 h was ranged between 2.90±1.02% and 5.39±0.98% for F2 and F7 respectively. Results showed the physical stability of batches in a humid environment. These results are parallel with the ones showed in the swelling study. These results in a good correlation with a study done by Pilicheva *et al.* who prepared multilayer buccal films of benzydamine, tolfenamic acid and betahistine [50].

Swelling measurement of LTD-buccal films

The swelling behaviors of LTD buccal films formulations are presented in fig. 2. Fig. showed that the swelling behavior of F1, F2, F3, and F4 shows 10.21±1.56% increase in size after 1 hour and reach maximum swelling index 40.23±2.34% after 3 h then a slight decrease in size was observed. F5, F6, F7, and F8 showed regular increase in size till 3 h followed by decrease in size at fourth hour except F6 which retain its size. F9-F12 showed rapid increase in a swelling index followed by minor decrease in the fourth hour The swelling performance of films is based on their structure and polymeric content. Polymeric hydrogels like CMC, HPMC, and HPC are three-dimensional cross-linked systems that have the capability to engross water and swell without losing their shape. Their swelling performance is exaggerated mainly by outer conditions (ie pH, T). Under the research condition the CMC, HPMC, and HPC did not swell to its peak value due to the partial swelling of these polymers at inspection pH [51]. Swelling behavior of F5 and F6 showed rapid swelling could be expressed based on the increase of HPMC and HPC content in the formula which helps in the rapid absorption of water and increase in size. F1, F2, F3, and F4 showed significant lower swelling under experiment conditions which could be interpreted based on the ALG and P properties which have much lower ability to adsorbe water. Erosion of polymers is responsible for loss of some of its weight after 4 h in some formulation.

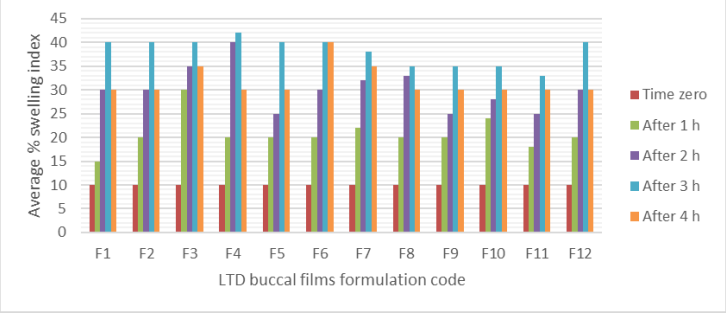

Fig. 2: Swelling behavior of LTD buccal films in phosphate buffer (pH 6.8). Error bars were omitted

Flexibility of LTD buccal films

Folding strength was measured physically by folding the film frequently at a same point till they cut. Table 2 showed the value of the film's folding endurance which is identified by the number of film folds before the film cracked. F1 and F5 displayed the minimum and maximum endurance, respectively (96±13 and 166±18). Significant increase in folding endurance for F4, F5, and F8, compared to the other films, which could be explained based on the presence of higher contents of methyl cellulose derivatives polymers in these formulations. Graphs also showed that CP increases the flexibility of formula while lower flexibility was observed for P and ALG films. Stiff films may section during storage and may miss part of drug dose. In addition, firm film has lower ability to be adjusted in the buccal cavity. Moreover, it may cause discomfort, irritation, sensation and loss of part of the film during application. Folding endurance results showed good elasticity of LTD buccal films.

***In vitro* residence time**

Significant differences were noticed in the residence time of different formulations. Previous studies by Jacob, S. and Nair, A. B. *et al.*, mentioned that, greater erosion rate was detected with the non-ionic polymers i.e. ALG and P [49]. It might be clarified based on particle swelling leading to the increase in the internal matrix swelling, which encourages disintegration and leakage of drug, leaving a highly porous matrix [30, 47]. The *in vitro* residence time of formulations was in order of F6>F5>F4>F10>F9>F8>F7>F12>F11>F1>F2>F3. The *in vitro* residence time of the films were ranged between 4.3±0.5 and 5.9±0.3 h for F3 and F6, respectively; this residency considered optimal and therefore, films revealed accepted swelling and drug release properties.

Water vapor permeation study

Water vapor permeation results showed that all LTD buccal films were having good permeability to vapor. Vp ranged between 5.60±0.21 and 11.01±0.18 for F2 and F10, respectively. Results shown that the vapor permeation is dependent on the polymer content of the formulation. Higher rate was observed with gelatin formulations which is attributed to the chemical nature of gelatin which is amine polysaccharides that form a thin hydrated gel that permits a higher rate of vapour permeation. The combination of gelatin with other polymers (HPMC, HPC, SA) showed a reduction in permeation due to the increase in gel strength. Thus lower permeation of Cp formulations may be due to the highly cross-linked structure, which produce viscous coherent matrix that hinders vapor permeation. Relevant results were reported by Gajdošová *et al.*, and Semalty *et al.* for ciclopirox olamine and enalapril maleate mucoadhesive buccal film [50, 47].

***In vitro* release study**

The cumulative release of LTD from various films is shown in fig. 3. Release data, ranged from 49.2±2.5% to 97±3.2% in 24 h. The dissolution profiles for formulations F1, F2 and F3 were showed slow and incomplete release, that after 24 h only 69±1.7%,59±3.9%, and 74±2.2%. from F1, F2, and F3 were rereleased respectively. The formulations F4, F5, and F6 showed higher release (fig. 3). The percentage release of LTD from the formulations was found to be

85.4±2.6% for F4, 86.8±2.5% for F5, and 97.23±2.6% for F6 after 24 h. In complete release of LTD after 24 h was observed for F7, F8, F9 that less than 74.5±2.7% 70.2±4.4%, and 71.1±6.2% was released, respectively. Rapid release of LTD was observed with F11, F12 that almost 90.2±2.5% and 91.0±1.1% of the drug was released, respectively, within the first 4 h, while lowest amount of the drug was released from F10, that only 45.5±2.9 % was released within 24 h. A sustained medication release profile that follows zero-order kinetics would be optimal, where the film releases a constant amount of drug as a function of time, resulting in uniform drug concentrations across [48]. The LTD release from HPMC films began slow, with only around 20% of the drug released after 1 hour, followed by slow release up to 6 h resulting in a sustained release profile of LTD. This is in agreement with the results obtained by a study done by Lim *et al.* who reported that the release of curcumin from HPMC-PVA film was sustained with roughly 20% of curcumin being released after 1h, which is comparable with our findings [47].

The release of the drug from the film takes more time when the content of the polymer in the sustained layer increases, as seen in fig. 3. LTD release appeared to be biphasic, as shown by a higher rate of drug release in the first 3 h (the amount of drug released was 20–70%), followed by slow rate for up to 8 h in F6. Study done by Nair *et al.* found that rizatriptan was released by biphasic pattern from buccal films [29] with an increased rate of drug release in the first 2h. In buccal route, this pattern of release is accepted, as the rapid release in the first phase ensures adequate drug availability on the mucosal surface for absorption, followed by slow release to prolong the drug release [49]. Based on release data, F6 was selected for further *in vivo* bioavailability study.

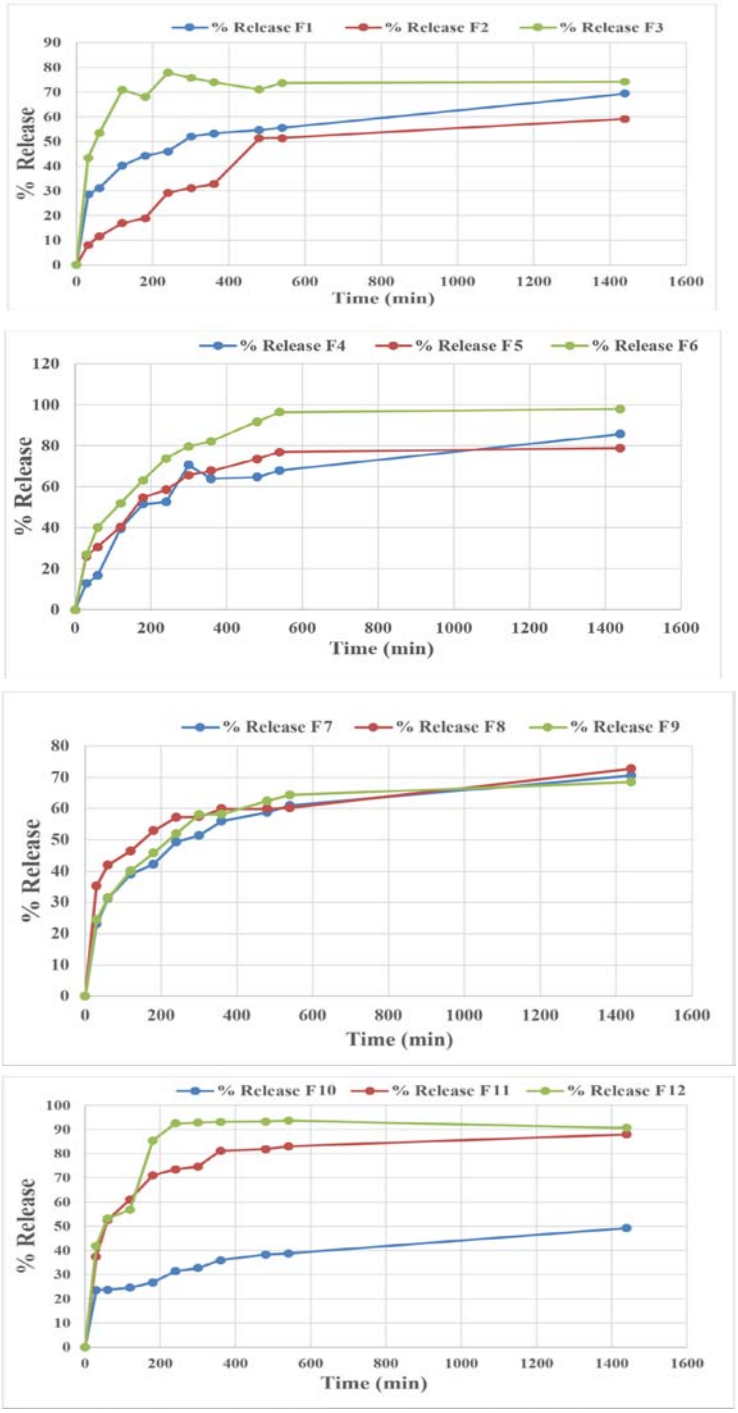

Fig. 3: Release profile of LTD from buccal films (F1-F12) in phosphate buffer (pH 6.8) buffer for 24 h

Table 3: Stability of LTD in dog plasma under indicated conditions

	Added (ng/ml)	Found (ng/ml)	Accuracy %	RSD%
Pretreatment for 6 h	50	49.75±0.89	99.5	3.26
	1000	978±24.36	97.8	4.59
	4000	3898.25±37.25	97.46	1.52
Freez-thow stability (36 h, n=3)	50	48.95±0.85	97.9	10.21
	1000	988±14.25	98.8	1.58
	4000	3847.54±102.32	96.19	3.31

Results presented as mean±SD, n= 3

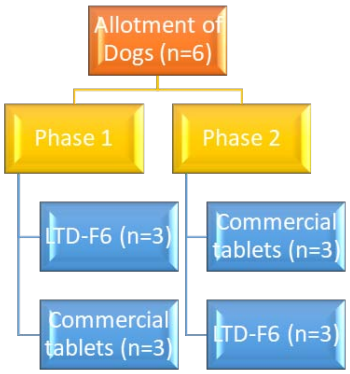

Fig. 4: *In vivo* study design shows treatment phases

Selectivity

No interference from endogenous substance was observed at the retention time of LTD and Diazepam (IS).

Linearity and LLOQ

Fig. 6 shows that the standard calibration curves for LTD were linear over the concentration range of 100-2500 ng/ml (R2>0.99). The lower limit of quantification for LTD was 100 ng/ml with RE within±20% and R. SD lower than 20%.

Precision and accuracy

The intra-day precision for low, middle and high QC levels of LTD were 5.68%, 5.53% and 2.04%, and that of inter-day analysis were 5.39%, 7.49% and 10.05%. The accuracy of low, middle and high QC levels of LTD were 99.48%, 103.10% and 97.59%.

Stability

The stock solution of LTD in plasma was found to be stable at sample room 7 °C for 6 h, at freeze and thaw stability (table 3). The results from all stability tests demonstrated good stability of LTD overall steps of the determination.

***In vivo* performance of LTD in selected formula**

Pharmacokinetic study of the optimized mucoadhesive buccal film of LTD compared with marketed LTD tablets were investigated in beagle dogs on two phases (fig. 4). The mean concentration-time profiles for LTD batch and commercial product are shown in fig. 7 and table 4. Results revealed that, after buccal adhesion of buccal batch F6 and oral use of market product to beagle dogs, drug appeared in plasma after a 0.53±0.12 h, 0.51±0.1 h, and 0.70±0.24h, respectively. Mean peak drug concentration of F6 C_{max} (2.009±0.15 µg/ml) was higher than that of market product (1.376±0.42µg/ml). The mean time to reach the peak concentration (t_{max}) was non statistically significant difference (P>0.05). Moreover, there was a significant difference (P>0.05) in the terminal elimination rate constant among the two product. The AUC_{0-24} value was 54.09±12.46, and 64.72±11.92 (µg. h. ml^{-1}) for market product and F6, respectively, suggest that buccal batch of LTD showed higher rate and extent of drug absorption than market oral tablets. The relative bioavailability of F6 was 113.45% higher than market tablets. The higher bioavailability of mucoadhesive batch may be interpreted on the base of (i) The rapid diffusion of outermost drug particles that inter to blood flow directly without metabolism helps to appear of the drug in the circulation faster than oral tablets (ii) In addition the polymer content in batch system; forming a swollen gel with longer diffusion path that could substantially reduce the release rate of LTD from the batch.

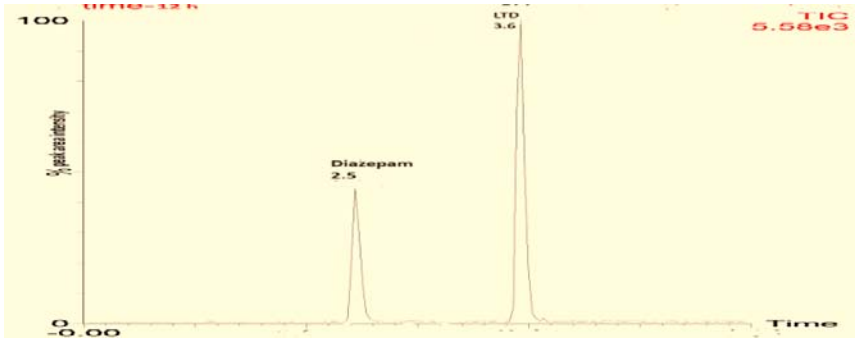

Fig. 5: HPLC chromatograms of mean residence time (MRT) of LTD and diazepam (IS)

Table 4: Measured pharmacokinetics of LTD following buccal attachment of film and oral administration of marketed tablets in beagle dogs

Parameters	LTD market tablets	LTD batch (F6)
AUC_{0-24h} (µg. h. ml^{-1})	54.09±12.46	64.72±11.92
$AUC_{0-\alpha}$ (µg. h. ml-1)	59.31±9.45	67.69±11.90
C_{max} (µg ml^{-1})	2.009±0.51	1.376±0.42
T_{max} (h)	3.03±0.16	2.68±0.21
F		113.45

Results presented as mean±SE

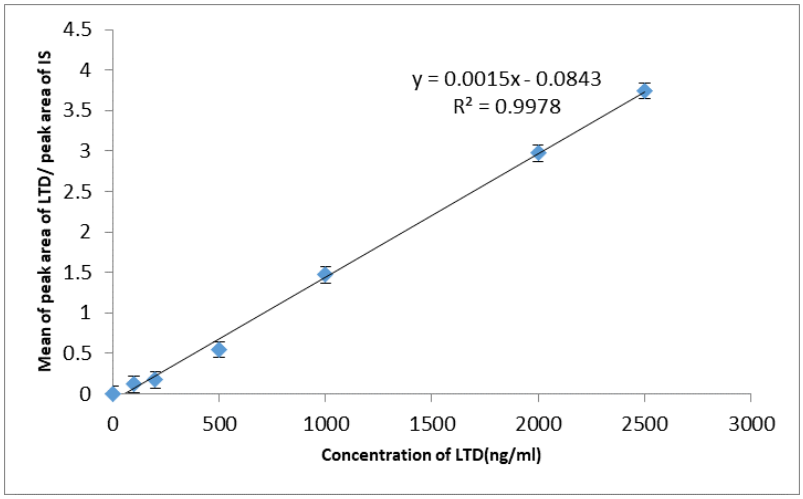

Fig. 6: HPLC standard calibration curve of LTD in dog plasma

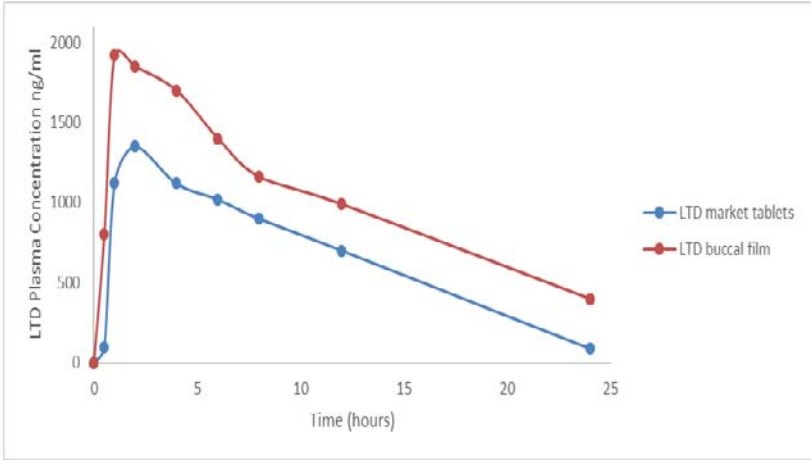

Fig. 7: LTD plasma concentration-time profiles in dogs after administration of buccal film (F6) and market tablets. Error bars were omitted

In vitro/in vivo correlation

In an attempted to find the type of relationship between LTD plasma concentration and concentration of the drug released from selected batch (F6) in the dissolution medium. Plasma concentrations of a drug were plotted against the concentration of drug released *in vitro* at the same time of 2, 4, 6, 8 h (fig. 8). The results revealed linear correlation coefficient R2 of 0.9977 was obtained. Consequently; plasma drug concentration can be anticipated from *in vitro* release studies under the adopted experimental conditions without the need of performing *in vivo* studies using the following equation:

$$y = 0.9137x-0.0504 \quad \ldots\ldots\ldots (7)$$

Where x is the concentration of drug released *in vitro* (µg/ml) and y is the *in vivo* plasma concentration (µg/ml).

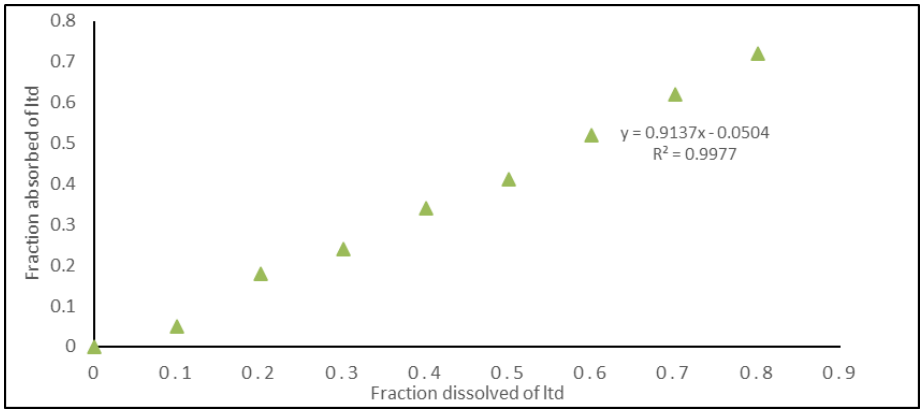

Fig. 8: Relationship between the LTD fraction dissolved *in vitro* and the LTD fraction absorbed *in vivo* for buccal film F6

CONCLUSION

Improving the bioavailability and prolonging the antihistaminic effect of LTD was succeeded by loading the drug in a muco-adhesive buccal film. Different polymers in different ratios were verified for achieving the optimized formula. F6 composed of carbopol, HEC, Gelatin, and sustained layer formed from HPMC, HPC, and CMC was found to be the ideal formula based on content, muco-adhesion, flexibility, *in vitro* residency and release parameters. Optimum LTD buccal film showed a marked improvement (113.54%) in the bioavailability of LTD compared to market tablets. Simplicity of use, ease of administration, absence of irritation in addition to the high bioavailability and longer duration, make LTD buccal films promising dosage form.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

REFRENCES

1. Gilhotra RM, Ikram M, Srivastava S, Gilhotra N. A clinical perspective on mucoadhesive buccal drug delivery systems. J Biomed Res. 2014;28(2):81-97. doi: 10.7555/JBR.27.20120136, PMID 24683406.
2. Pathan SA, Iqbal Z, Sahani JK, Talegaonkar S, Khar RK, Ahmad FJ. Buccoadhesive drug delivery systems-extensive review on recent patents. Recent Pat Drug Deliv Formul. 2008;2(2):177-88. doi: 10.2174/187221108784534135, PMID 19075906.
3. Ruiz Rubio L, Alonso ML, Perez Alvarez L, Alonso RM, Vilas JL, Khutoryanskiy VV. Formulation of Carbopol®/poly(2-ethyl-2-oxazoline) s mucoadhesive tablets for buccal delivery of hydrocortisone. Polymers. 2018;10(2):175. doi: 10.3390/polym10020175, PMID 30966211.
4. Zhang H, Zhang J, Streisand JB. Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. Clin Pharmacokinet. 2002;41(9):661-80. doi: 10.2165/00003088-200241090-00003, PMID 12126458.
5. Abruzzo A, Bigucci F, Cerchiara T, Cruciani F, Vitali B, Luppi B. Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr Polym. 2012;87(1):581-8. doi: 10.1016/j.carbpol.2011.08.024, PMID 34663007.
6. Shaikh R, Raj Singh TR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci. 2011;3(1):89-100. doi: 10.4103/0975-7406.76478, PMID 21430958.
7. Puratchikody A, Prasanth VV, Mathew ST, Ashok KB. Buccal drug delivery: past, present and future-a review. Int J Drug Deliv. 2011;3:171-84.
8. Kianfar F, Antonijevic MD, Chowdhry BZ, Boateng JS. Formulation development of a carrageenan-based delivery system for buccal drug delivery using ibuprofen as a model drug. J Biomater Nanobiotechnol. 2011;02(5):582-95. doi: 10.4236/jbnb.2011.225070.
9. Chinna Reddy P, Chaitanya KS, Madhusudan Rao Y. A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. Daru. 2011;19(6):385-403. PMID 23008684.
10. Kraisit P, Limmatvapirat S, Nunthanid J, Sriamornsak P, Luangtana Anan M. Preparation and characterization of hydroxypropyl methylcellulose/polycarbophil mucoadhesive blend films using a mixture design approach. Chem Pharm Bull (Tokyo). 2017;65(3):284-94. doi: 10.1248/cpb.c16-00849, PMID 27980251.
11. Abruzzo A, Bigucci F, Cerchiara T, Cruciani F, Vitali B, Luppi B. Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr Polym. 2012;87(1):581-8. doi: 10.1016/j.carbpol.2011.08.024, PMID 34663007.
12. Montero-Padilla S, Velaga S, Morales JO. Buccal dosage forms: general considerations for pediatric patients. AAPS PharmSciTech. 2017;18(2):273-82. doi: 10.1208/s12249-016-0567-2, PMID 27301872.
13. Jacob S, Nair AB, Patel V, Shah J. 3D printing technologies: recent development and emerging applications in various drug delivery systems. AAPS PharmSciTech. 2020;21(6):220. doi: 10.1208/s12249-020-01771-4, PMID 32748243.
14. Mythri G, Kavitha K, Kumar MR, Singh SJ. Novel mucoadhesive polymers–a review. J Appl Pharm Sci. 2011;01(08):37-42.
15. Ponchel G. Specific and non-specific bioadhesive particulate systems for oral delivery to the gastrointestinal tract. Adv Drug Deliv Rev. 1998;34(2-3):191-219. doi: 10.1016/S0169-409X(98)00040-4.
16. Shinde P, Salunkhe V, Magdum C. Buccal film: an innovative dosage form designed to improve patient compliance. Int J of Pharmaceutical and Chemical Science. 2012;1(4):1262-78.
17. Arunachalam A, Karthikeyan M, Konam K. Fast dissolving drug delivery: a review. J Glob Trends Pharm Sci. 2010;1(1):92-110.
18. Mishra A, Pathak AK. Plasticizers: a vital excipient in novel pharmaceutical formulations. Curr Res Pharm Sci. 2017;7(1, May):1-10. doi: 10.24092/CRPS.2017.070101.
19. Banyal M, Joshi S. Transdermal patch: an innovative technique for transdermal drug delivery system. IJPPR. 2021;20(3):109-31.
20. Burad S, Markad K, Kulkarni N, Dhole S. Assessment and outcome on preparations, characterization of topical targeted nano sponge based drug delivery: critical review. Asian J Pharm Clin Res. 2023;16(5):19-26.
21. Park CR, Munday DL. Evaluation of selected polysaccharide excipients in Buc-coadhesive tablets for sustained release of nicotine drug development and Indus BF Pharmacy. 2004;30(6):609-17. PMID 15285334.
22. Nair AB, Kumria R, Harsha S, Attimarad M, Al-Dhubiab BE, Alhaider IA. *In vitro* techniques to evaluate buccal films. J Control Release. 2013;166(1):10-21. doi: 10.1016/j.jconrel.2012.11.019, PMID 23219961.
23. Mahajan A, Chhabra N, Aggarwal G. Formulation and characterization of fast dissolving buccal films: a review. Scholars Res Libr. 2011;3:152-65.
24. Chakraborty P, Dey S, Parcha V, Bhattacharya SS, Ghosh A. Design expert supported mathematical optimization and predictability study of buccoadhesive pharmaceutical wafers of loratadine. BioMed Res Int. 2013;2013:197398. doi: 10.1155/2013/197398, PMID 23781498.
25. Arya A, Sharma V, Pathak K. Pharmaceutical evaluation and dynamic vapor Sorption studies of fast dissolving intraoral films of loratadine. Pharm Dev Technol. 2013;18(6):1329-38. doi: 10.3109/10837450.2012.685659, PMID 22663089.
26. El-Hammadi M, Awad N. Investigating the use of liquisolid compacts technique to minimize the influence of ph variations on loratadine release. AAPS PharmSciTech. 2012;13(1):53-8. doi: 10.1208/s12249-011-9719-6, PMID 22101967.
27. Kumria R, Nair AB, Al-Dhubiab BE. Loratidine buccal films for allergic rhinitis: development and evaluation. Drug Dev Ind Pharm. 2014;40(5):625-31. doi: 10.3109/03639045.2014.884125, PMID 24506459.
28. Elkomy MH, El Menshawe SF, Abou-Taleb HA, Elkarmalawy MH. Loratadine bioavailability via buccal transferosomal gel: formulation, statistical optimization, *in vitro*/*in vivo* characterization, and pharmacokinetics in human volunteers. Drug Deliv. 2017;24(1):781-91. doi: 10.1080/10717544.2017.1321061, PMID 28480758.
29. Pa B, Virsen Tg, Najmuddin M. Formulation and *in vitro* evaluation of buccal tablets of loratadine for effective treatment of allergy; 2011.
30. Mura P, Corti G, Cirri M, Maestrelli F, Mennini N, Bragagni M. Development of mucoadhesive films for buccal administration of flufenamic acid: effect of cyclodextrin complexation. J Pharm Sci. 2010;99(7):3019-29. doi: 10.1002/jps.22068, PMID 20127823.
31. Rajaram DM, Laxman SD. Buccal mucoadhesive films: a review. Syst Rev Pharm. 2016;8(1):31-8. doi: 10.5530/srp.2017.1.7.
32. Kaur A, Kaur G. Mucoadhesive buccal patches based on interpolymer complexes of chitosan-pectin for delivery of carvedilol. Saudi Pharm J. 2012;20(1):21-7. doi: 10.1016/j.jsps.2011.04.005, PMID 23960773.

33. Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Patel V, Sreeharsha N. Development of mucoadhesive buccal film for rizatriptan: *in vitro* and *in vivo* evaluation. Pharmaceutics. 2021;13(5):728. doi: 10.3390/pharmaceutics13050728, PMID 34063402.
34. Buanz ABM, Belaunde CC, Soutari N, Tuleu C, Gul MO, Gaisford S. Ink-jet printing versus solvent casting to prepare oral films: effect on mechanical properties and physical stability. Int J Pharm. 2015;494(2):611-8. doi: 10.1016/j.ijpharm.2014.12.032, PMID 25526674.
35. Khanna R. Preparation and evaluation of bioerodible buccal tablets containing clotrimazole. International Journal of Pharmaceutics. 1996;138(1):67-73. doi: 10.1016/0378-5173(96)04531-0.
36. Yang TZ, Wang XT, Yan XY, Zhang Q. Phospholipid deformable vesicles for buccal delivery of insulin. Chem Pharm Bull (Tokyo). 2002;50(6):749-53. doi: 10.1248/cpb.50.749, PMID 12045327.
37. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccoadhesive films of atenolol. Indian J Pharm Sci. 2008;70(2):175-9. doi: 10.4103/0250-474X.41451, PMID 20046708.
38. Khurana R, Ahuja A, Khar R. Development and evaluation of mucoadhesive nitrate. Indian J Pharm Sci. 2000;62:447-51.
39. Verma N, Ghosh AK, Chattopadhyay P. Preparation and *in vitro* assessment of mucoadhesive buccal patches containing carvedilol. Int J Pharm Pharm Sci. 2011;3(3):218-20.
40. Desu P, Sahu M. Formulation and evaluation of fast dissolving film of zolmitriptan. Int Res J Pharm. 2012;3(1):373-6.
41. Shridhar I, Joshi A. Formulation and characterization of buccal patch of Ondanserton hydrochloride. Int J Pharm Res Dev. 2013;5(8):84-94.
42. El Sharawy AM, Shukr MH, Elshafeey AH. Formulation and optimization of duloxetine hydrochloride buccal films: *in vitro* and *in vivo* evaluation. Drug Deliv. 2017;24(1):1762-9. doi: 10.1080/10717544.2017.1402216, PMID 29172829.
43. Yehia SA, El-Gazayerly ON, Basalious EB. Fluconazole mucoadhesive buccal films: *in vitro*/*in vivo* performance. Curr Drug Deliv. 2009;6(1):17-27. doi: 10.2174/156720109787048195, PMID 19418952.
44. Amini Hossein, Ahmadiani Abolhassan. Rapid determination of loratadine in small volume plasma samples by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2004;809(2):227-30. doi: 10.1016/j.jchromb.2004.06.022, PMID 15315769.
45. Koland M, Charyulu R, Prabhu P. Muco-adhesive films of losartan potassium for buccal delivery: design and characterization. Indian J Pharm Educ. 2010;44:315-23.
46. Patel VM, Prajapati BG, Patel JK, Patel MM. Physicochemical characterization and evaluation of buccal adhesive patches containing propranolol hydrochloride. Curr Drug Deliv. 2006;3(3):325-31. doi: 10.2174/156720106777731082, PMID 16848734.
47. Semalty A, Semalty M, Nautiyal U. Formulation and evaluation of mucoadhesive buccal films of enalapril maleate. Indian J Pharm Sci. 2010;72(5):571-5. doi: 10.4103/0250-474X.78522, PMID 21694987.
48. Kumria R, Nair AB, Goomber G, Gupta S. Buccal films of prednisolone with enhanced bioavailability. Drug Deliv. 2016;23(2):471-8. doi: 10.3109/10717544.2014.920058, PMID 24892626.
49. Jacob S, Nair AB, Boddu SHS, Gorain B, Sreeharsha N, Shah J. An updated overview of the emerging role of patch and film-based buccal delivery systems. Pharmaceutics. 2021;13(8):1206. doi: 10.3390/pharmaceutics13081206, PMID 34452167.
50. Pilicheva B, Uzunova Y, Marudova M. Polyelectrolyte multilayer films as a potential buccal platform for drug delivery. Polymers (Basel). 2022;14(4):14(4):734. doi: 10.3390/polym14040734, PMID 35215645.
51. Gajdosova M, Vetchy D, Muselik J, Gajdziok J, Jurica J, Vetcha M. Bilayer mucoadhesive buccal films with prolonged release of ciclopirox olamine for the treatment of oral candidiasis: *in vitro* development, ex vivo permeation testing, pharmacokinetic and efficacy study in rabbits. Int J Pharm. 2021;592:120086. doi: 10.1016/j.ijpharm.2020.120086, PMID 33188896.