FABAD J. Pharm. Sci., 42, 3, 179-190, 2017

RESEARCH ARTICLE

Formulation and Evaluation of Baclofen Mucoadhesive Buccal Films

Muaadh A. Mohamed Ali[*](MUAADH), Ahmed Mohammed Sabati [**](SABATI), Bassam Abduh Ali [°],[***](BASSAM),

Formulation and Evaluation of Baclofen Mucoadhesive Buccal Films

SUMMARY

Aim of the present work was to formulate and design mucoadhesive buccal films of baclofen (muscle relaxant) using different hydrophilic polymers and to evaluate them. The baclofen was incorporated into film that was composed of film forming and bioadhesive polymers. Hydroxypropyl methyl cellulose (HPMC) was used as film forming agent. Both Carbopol 940 (CP940) and polyvinylpyrrolidone (PVP) were used as bioadhesive polymers. Propylene glycol (PG) was used as plasticizer. Films were fabricated by solvent casting technique and then they were subjected to various evaluation parameters. Drug-excipients compatibility test was studied and no possible drug-excipients interaction. After formulation, it was observed that the formulated buccal films had appeared with satisfactory physicochemical characters except formula B3. Low values of standard deviation in thickness, weight measurement and drug content data reflected no significant difference within the batch. The in vitro mucoadhesive strength values were satisfactory for maintaining them in oral cavity except for B1 and B7 that showed the lowest values. The data clearly showed that percentage release of baclofen after 8 hours using phosphate buffer (pH 6.8). By studying and analyzing the in-vitro data pharmacokinetically for the tested formulations, it was observed that the mechanism of drug release from most films found to be non-Fickian diffusion. According to the in vivo residence time study, it was detected that formulas B4 and B6 had the highest mucoadhesion time with 4.77 and 5.07 hours, respectively. Finally, it was concluded that formulation B4 exhibited the most satisfactory results representing good physicochemical properties, in vitro profiles, and suitable mucoadhesion strength.

Key Words: *Baclofen, transmucosal buccal, in-vitro release, mucoadhesive force, residence time, solvent casting technique*

Baclofen Mukoadhezif Bukkal Film Geliştirme ve Formülasyonu

ÖZET

Bu çalışmanın amacı, farklı hidrofilik polimerler kullanarak, baclofen (kas gevşetici) mukoadhezif bukkal film geliştirmektir. Baclofen biyoadhezif polimerler ile birleştirilerek film oluşturuldu. Hidroksipropil metil selüloz, filmleştirme ajanı olarak kullanıldı. Carbopol 940 ve polivinilprolidon biyoadhezif polimerler olarak kullanıldı. Propilen glikol plastikleştirici olarak kullanıldı. Filmler, solvent casting (çözücü döküm) tekniği ile oluşturuldu ve çeşitli değerlendirilen parametreler anlatıldı. İlaç – yardımcı madde geçimlilik testleri yapıldı ve herhangi bir ilaç-yardımcı madde etkileşimi saptanmadı. Formülasyonlardan B3 hariç, üretilen tüm formülasyonların fizikokimyasal karakterizasyonlarının uygun olduğu gözlendi. Kalınlık, ağırlık ve etkin madde miktarlarında düşük varyasyon katsayısı belirlendi, seriler arası fark olmadığı saptandı. En düşük değerleri gösteren B1 ve B7 formülasyonları hariç oral kavitedeki mukoadhezif güç değerleri yüksek olarak belirlendi. pH 6.8 fosfat tamponu kullanılarak yapılan ölçümlerde 8 saate kadar baclofen salımı belirlendi. İn vitro datalar farmakokinetik olarak incelendi ve ilaç salımının non-fick difüzyona uyduğu belirlendi. İn vivo alıkonulma zamanı çalışmalarına göre, B4 ve B6 formülasyonlarının mukozada kalma zamanlarının en yüksek olduğu (4.77 ve 5.07) saptandı. Sonuç olarak, en iyi fizikokimyasal özellik göstermesi, in vitro profili ve uygun bir mukoadhezif güç göstermesinden ötürü en iyi formülasyon olarak B4 belirlendi.

Anahtar Kelimeler: *Baclofen, transmukozal bukkal, in-vitro salım, mukoadhezif güç, alıkonulma zamanı, çözücü dökme yöntemi*

Received: 30.04.2017
Revised: 16.05.2017
Accepted: 25.05.2017

[*] Department of Pharmaceutics, Faculty of Pharmacy, University of Science and Technology, Taiz Branch, Taiz, Yemen,
[**] Department of Pharmaceutics, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen,
[***] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Science and Technology, Taiz, Yemen.

[°] Corresponding Author;
Telephone:+967777443417
Fax number:+9671290722
E-mail address: bassam00001@yahoo.com

INTRODUCTION

Among the large numbers of different routes of drug delivery, oral route is perhaps the most satisfactory route to the patients and the physicians. Buccal drug delivery using mucoadhesive polymers has been began since the early 1980s and until now. The buccal cavity is the most convenient and easily accessible site for the delivery of certain drugs for both local and systemic delivery as it is considered to be retentive dosage forms (Mahanthesha, 2013). Oral controlled release drug delivery has recently been of increasing interest to achieve improved therapeutic benefits, such as ease of administration, excellent accessibility, patient compliance and flexibility in formulation. Furthermore, oral transmucosal drug delivery provides direct access to the systemic circulation through the jugular vein bypassing the first pass hepatic metabolism and avoids pre-systemic elimination in the GI tract leading to high bioavailability with a smaller dosage and less frequent administration and decreased toxicity. The membranes that line the oral cavity are readily accessible and exhibit fast cellular recovery following local damage. The oral transmucosal absorption is generally rapid because of the rich vascular supply to the mucosa and the lack of a stratum corneum that considered the major barrier to absorption across the skin. Additionally, oral mucosal drug delivery is non-invasive and highly acceptable by patients to offers an alternative route when per-oral administration is not advised or impractical (Marina, 2010). These factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery. The sites of drug administration in the oral cavity include the floor of the mouth (sublingual), the mucosal tissues in the cheeks (buccal), palatal and the gums (gingival). The buccal and sublingual tissues are the primary focus for drug delivery via the oral mucosa because they are more permeable than the tissues in other regions of the mouth. Unlike the sublingual mucosa, the buccal mucosa offers many advantages because of its smooth and relatively immobile surface and its suitability for the administration of retentive dosage forms which is well accepted by patients. In particular, the sublingual route is generally employed for the delivery of drugs characterized by a high permeability across the mucosa and used in the treatment of acute disorders, whereas the buccal route is generally used in the treatment of chronic disorders when a prolonged release of the active substance is required. The buccal route is particularly an attractive non-invasive route for the treatment of either local or systemic therapies overcoming the drawbacks of conventional administration routes (Sangeetha, 2010). Many mucoadhesive dosage forms are suggested for buccal delivery, including adhesive tablets, gels, ointments, discs, patches, and films. Bioadhesion may be defined as the state in which two materials, at least one of which is biological in nature, are held together for extended period of time. When the attachment is to a mucous membrane, the phenomenon is referred as mucoadhesion. The development of oral mucoadhesive delivery systems was always of great interest as delivery systems capable to offer various advantages. Buccal drug delivery system should be flexible and possess good mucoadhesive properties to retained in the oral cavity for the desired duration. In addition, it should release the drug in a predictable manner to elicit the required therapeutic response (Bukka, 2012).

Baclofen is a centrally acting muscle relaxant that has absorption window in upper G.I. tract and due to this, available dosage forms display low bioavailability. Oral baclofen has a number of significant pharmacokinetic limitations as it is only absorbed in the upper small intestine by saturable active transport mechanisms. In addition, baclofen has a short half-life of 3 to 4 hours and it is rapidly cleared from the blood. It is difficult to formulate into sustained release products because on arrival to colon (or even before) its absorption is low or nonexistent. The dose of baclofen must be frequent daily four times but the multiple administration produces high incidence of adverse effect, moreover conventional dosage form leads to fluctuation in plasma drug level. Therefore efforts will make to increase the residence of baclofen at or above the absorption window by preparing mucoadhesive film for buccal retention considering fact that it is stable in oral mucosal condition (Gavaskar, 2010).

The aim of this study was to formulate and evaluate mucoadhesive buccal films of baclofen to prolong the residence time providing sustained action, which ensure satisfactory drug release to a mucosa and to avoid loss of drug resulting from wash out with saliva. The new buccal mucoadhesive films were prepared using several mucoadhesive polymers, as hydroxypropylmethyl cellulose (HPMC), polyvinyl pyrrolidone (PVP) and Carbopol 940 (CP940). Effect of polymer type, proportion and combination were studied on drug release rate, release mechanism and mucoadhesion time to optimize the prepared formulations.

MATERIALS AND METHODS

Materials

Baclofen was kindly supplied as a gift from Alfath Group (Pharo Pharma, Egypt); Hydroxypropyl methyl cellulose (HPMC) and propylene glycol (PG)

were supplied as a gift from Yemeni-Egyption company, Sana'a, Yemen. Polyvinylpyrrolidone (PVP) was supplied kindly by Pharmacare, Sana'a, Yemen. Carbopol 940 (CP940) was purchased from Loba Chemie (Mumbai, India). Ethyl alcohol (70%) was purchased from Al-Shamel Chemicals Co. Sana'a, Yemen. All other chemicals were of analytical grade and provided by UST laboratories. Water used in this study was distilled.

Compatibility study of Baclofen with polymers

In this study, the drug-excipients physical mixtures were prepared on 1:1 ratio, weighed accurately and then they were gently mixed in a mortar (Magdy, 2011). So each method was applied on pure samples of drug and polymers individually and drug-polymer mixture separately. Then freshly prepared samples of pure drug, pure polymer and drug-polymer physical mixture were analyzed and evaluated as follows:

Fourier Transform Infrared Analysis (FTIR) Studies

FTIR is a procedure to identify the drug and it also to detect the compatibility of baclofen with used polymers. FTIR data of dried samples of pure baclofen, polymers and physical mixtures of them were recorded in FTIR spectrophotometer using the potassium bromide disc method. Each sample was pulverized and intimately mixed with dried IR grade KBr powder at a weight ratio of 1:100, and then pressed using a hydrostatic press at a pressure of 10 tons for 5min at room temperature. The result disc was placed in the sample holder and the spectra were recorded over the wave number range 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The location of peak of pure baclofen and pure polymer were analyzed and compared with spectra of their physical mixture.

Ultraviolet Spectrophotometry (UV) Studies

Baclofen, pure polymers and drug-polymer solutions were prepared in phosphate buffer solution pH 6.8. The prepared solutions were filtered and then subjected to UV scanning by means of spectrophotometer at wavelength range of 200 nm to 400 nm. In the same way, the UV spectra of pure baclofen and pure polymer were recorded and then compared with those UV spectra of their solutions.

Preparation of Baclofen Mucoadhesive Buccal Films

Seven baclofen buccal films composed of different ratios and combinations of different polymers were formulated. Baclofen buccal films were prepared (Hanif, 2015) by solvent casting technique, using combination of three polymers that were HPMC, CP940 and PVP as mentioned on Table (1). Specific weighed amount of the polymer(s) was dissolved in ethanol 70% homogenously. Weighed baclofen was dissolved in distilled water. The solutions of both baclofen and polymers then were mixed together with slow stirring to get a semisolid viscous solution. Propylene glycol (as plasticizer) was added to the final mixture. The mixture was placed on ultrasonic to remove air bubbles and then poured into 6.2 cm diameter glass petri dish and allowed to dry over night at room temperature till a flexible film was formed. The dried film was carefully removed from the petri dish and stored in a desiccator until another use. Dosage units were made by cutting film into parts of 3.7 cm^2 surface area such that one film contained 21 mg baclofen, packed in aluminum foil, and stored in glass containers at room temperature to maintain their integrity and elasticity.

Table 1. Formulation composition of Baclofen mucoadhesive buccal films

Formulation code	HPMC (mg)	CP940 (mg)	PVP (mg)	Ethanol 70% (ml)	PG (ml)	D.W (ml)
B1	150	50	0	6	0.5	3.5
B2	150	0	50	6	0.5	3.5
B3	150	40	10	6	0.5	3.5
B4	150	10	40	6	0.5	3.5
B5	150	30	20	6	0.5	3.5
B6	150	20	30	6	0.5	3.5
B7	150	25	25	6	0.5	3.5

Evaluation of Baclofen Mucoadhesive Buccal Films

Visual Examination of the Prepared Mucoadhesive Buccal Films

The physical appearance is an important factor specially for patient acceptance and product stability. The formulated dried buccal films were carefully removed from petri dish, checked and examined visually and formulated films with any imperfection, air bubble, cracks or heterogeneous surface were excluded from further studies (Bhatia, 2012).

Film Thickness

The thickness of the formulated buccal films was determined by means of digital micrometer (Sterling

Manufacturing Company, India). The thickness of six films was measured and the mean ± SD values were calculated of all formulations (Khanna, 1996; Cilurzo, 2008).

Weight Uniformity

The variations in weight may be due to change in density of different combination of polymers as a result of difference in molecular weight and proportion of the polymer used in the films. Films (3.7 cm^2) were cut and the weights of three films were weighed individually (Khanna, 1996; Cilurzo, 2008) using digital electronic balance (Kern-ABS, UK) and the average weights ± SD were calculated.

Folding Endurance

Folding endurance is a good test to examine the physical and mechanical properties of the films. Three films of each formulation of size (1×2 cm) were cut by using sharp blade. Folding endurance of the buccal films was determined by repeatedly folding one film at the same place till it broke or folded up to 200 times manually, which is considered satisfactory to reveal good film properties. The number of times of film could be folded at the same place without breaking gave the value of the folding endurance for film. This test was done on all the films for three times (SNR, 2010).

Determination of Drug Content

Uniformity of drug content was determined by dissolving the baclofen buccal film by homogenization in 250 ml of phosphate buffer solution pH 6.8 for 2 hours with occasional shaking on a magnetic stirrer. Aliquot 1 ml was withdrawn and diluted with the same solution up to suitable volume and the resulting solution was filtered through Whatman filter paper. The amount of baclofen was determined spectrophotometrically at λ_{max} 268 nm. The average of drug content of three films was taken as final reading (Magdy, 2011; Saxena,2011).

Surface pH Measurement

The films were allowed to swell by keeping it in contact with 5 ml of distilled water (pH 6.5 ± 0.05) for 2 hours at room temperature and pH was noted by bringing glass electrode of pH meter in contact with the microenvironment of the swollen films and allowing it to equilibrate for 1 minute. The average pH ± SD of three determinations was reported (Dixit, 2009).

Swelling Studies

The swelling rate of mucoadhesive film was evaluated by placing the film in phosphate buffer solution pH 6.8 at 37 ± 1°C. Buccal films (n=3) were weighed individually (W_1) and placed separately in petri dishes containing 25 mL of phosphate buffer (pH 6.8) solution. The dishes were stored at room temperature. Then, films were removed and excess surface water was removed carefully using the filter paper after specified time intervals of 5 minutes and 15 minutes. The swollen films were then again weighed (W_2) and swelling index (SI) was calculated (Desai, 2004; Yehia, 2009) using the equation (1):

$$SI = [(W_2 - W_1) \div W_1] \times 100 \qquad (1)$$

Where, W_1 = Initial weight of the patch W_2 =Final weight of the patch.

Determination of *In Vitro* Mucoadhesion Strength

The mucoadhesion strength was evaluated using a modified balance method (Kerec, 2002; Ali, 2002). The cleaned chicken pouch membrane was used as model mucosa for these studies (Wong, 1999; El-Samaligy, 2004). The chicken pouches were kept frozen at –20°C in a phosphate buffer saline solution (pH 6.8), and only thawed to room temperature before use. Briefly, a balance was taken and its left pan was replaced with a weight to the bottom of which a buccal film was attached. Both sides were then balanced with weight. A piece of chicken pouch membrane was fixed to a rubber cork, which was already attached to the bottom of the beaker containing phosphate buffer (pH 6.8, 37°C) with a level slightly above the membrane. The weight, which was attached to the buccal film, was brought into contact with the membrane, kept undisturbed for two minutes and then the pan was raised. Weights were continuously added on the right side pan in small increments. The weight of water, in grams, required to detach the film from the mucosal surface gave the measure of bioadhesive strength. The experiments were performed in triplicate, and average values were reported. From the mucoadhesive strength, force of mucoadhesion was calculated as in equation (2):

Force of mucoadhesion (N) = (Mucoadhesive strength /1000) x 9.81 (2)

The means of all data were presented with their standard deviations (mean ± SD). The calculated values of mucoadhesive strength were analyzed statistically using the SPSS program. A statistically significant difference was considered when $P < 0.05$.

***In-Vitro* Drug Release Studies**

The USP dissolution tester XXIII-B with rotating paddle was used to study the drug release from the prepared buccal films. The dissolution medium consisted of 250 ml of phosphate buffer solution of pH 6.8 that simulated to saliva. The release was performed at 37±0.5°C with a rotation speed of 50 rpm. The one

side of the buccal film (3.7 cm^2 surface area) was attached to a 5 cm diameter glass disk with instant adhesive. The film with glass disk was allocated to the bottom of the dissolution vessel so that the buccal film faced upright thereby allowing drug release only from the upper side of the film. Samples of 5 ml were withdrawn at pre-determined time intervals and replaced with fresh medium. The samples were filtered through Whatman filter paper and analyzed to determine the baclofen amount after appropriate dilution by UV spectrophotometry at λ_{max} 268 nm. The release studies were conducted in triplicates and the mean values were plotted versus time. The absorbance of the polymeric additives was negligible and did not interfere with λ_{max} of the drug (Consuelo, 2007).

Kinetic Analysis of *In Vitro* Release Data for Baclofen Mucoadhesive Buccal Films

Data of *in-vitro* release were fit into different equations and kinetic models to explain the release kinetics of baclofen from the buccal films. The kinetic models used were a zero-order, first order, Higuchi model and Peppas equation. For all the buccal formulations, the obtained results from the buccal film formulations were plotted in various kinetic models and results were interpreted. To investigate more precisely the effect of the polymeric blend on the release of baclofen, the results were fit and analyzed according to the well-known semi-empirical Peppas equation (3):

$$M_t / M_\infty = kt^n \qquad (3)$$

Where M_t / M_∞ is fractional release of the drug, '*t*' denotes the release time, '*k*' represents a constant, incorporating structural and geometrical characteristics of the drug/polymer system (device) and '*n*' is the diffusional exponent and characterizes the type of release mechanism during the dissolution process. The values of *n* were estimated by linear regression of log M_t / M_∞ vs. log (t) of different formulations. It is

important to note that for determination of the exponent *n*, only the initial portion of the release curve ($M_t / M_\infty \leq 0.7$) must be used. For non-Fickian release, the value of *n* falls between 0.45 and 0.89; while in case of Fickian diffusion, *n*= 0.45; for zero-order release (case II transport), *n*=0.89 and for supercase II transport, *n* >0.89 (Peppas, 1985; Dash, 2010).

Evaluation of *In Vivo* Mucoadhesion Time of the Mucoadhesive Buccal Films in Human Volunteers

Four healthy male adult volunteers, aged between 22 and 28 years, participated in the study. The study followed the rules approved by the ethical committee (MECA NO.: (2017/06)). Prior to the test, the volunteers were educated with the procedure and purpose of test. They were asked to rinse their mouth with distilled water before a piece of the drug free film was placed on their buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed onto the mucosa for about 30 seconds till the film adhered to the buccal mucosa (Magdy, 2011; Prakasami, 2014). Food and water were not allowed for first 2 hours after application of films. The volunteers were asked to record the residence time of the film on buccal mucosa in the oral cavity (time of complete erosion or detachment of the film from the buccal mucus membrane) and to monitor for fragment loss, irritation, bad taste, swelling, dry mouth or increase in salivary flow.

RESULTS & DISCUSSION

Compatibility study of Baclofen with polymers

Fourier Transform Infrared Analysis (FTIR) Studies

The compatibility of baclofen with the selected polymers was confirmed by FTIR Spectroscopy. Samples of pure baclofen with or without the selected polymers were scanned in the region of a wave number range of 4000 cm^{-1} to 400 cm^{-1}. The scans were examined for presence of finger print of drug, shifting, appearing and masking of drug peaks by presence of polymer in the formulation. The FTIR spectra of pure baclofen and physical mixture of different polymers were observed on Figure (1). The FTIR spectrum of baclofen exhibited the characteristic absorption peaks at 3300 cm^{-1} ($N\text{-}H_2$ stretching), 2984.542 cm^{-1} (aromatic C-H stretching), broad peak at 2598.642 cm^{-1} and extend up to 3100 cm-1(O-H of alcohol and carboxylic acid stretching), 2155.047 cm^{-1} (alkynyl C≡C stretching), 1922.494 cm^{-1} (carboxylic acid C=O steretching), 1626.875 cm^{-1}(alkenyl C=C stretching) , 1531.447 cm^{-1} (aromatic C=C bending) and the peaks that were appeared at region less than 1500cm-1 considered as fingerprint for baclofen. 1401.322 cm^{-1} (O-H bending), 1245.755 cm^{-1} (C-O stretching), 834.388 cm^{-1} (C-Cl stretching) and 700-800 cm^{-1} (benzene). These data are in good agreement with that reported in literature for baclofen (Kulkarni, 2011; Shaikh, 2013).

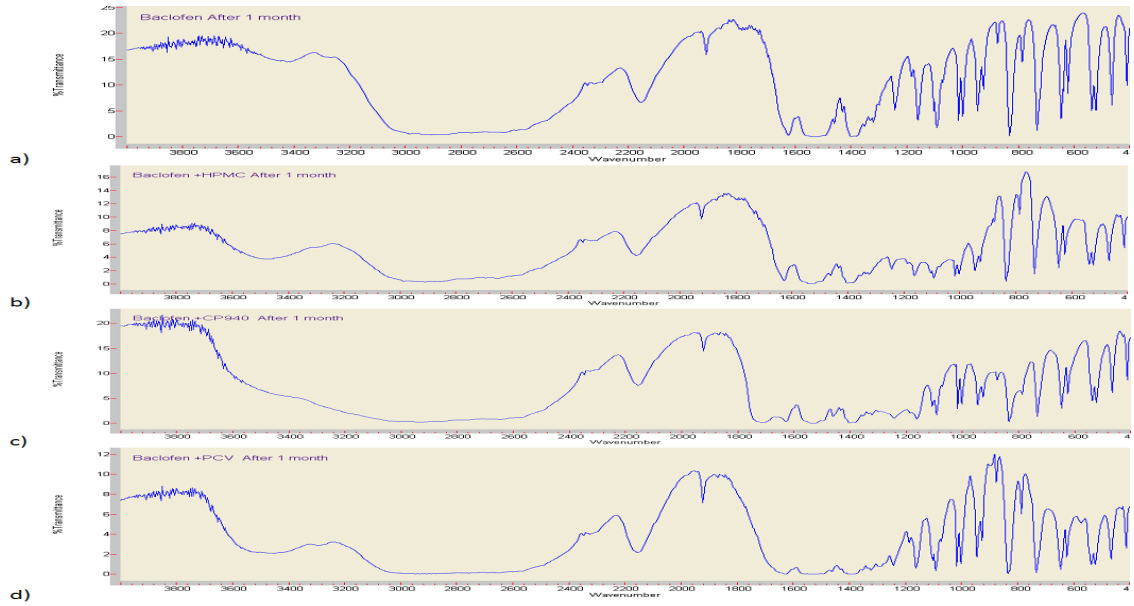

Figure 1. FTIR Spectra for Baclofen **(a),** Baclofen-HPMC **(b),** Baclofen-CP940 **(c)** and Baclofen-PVP **(d)** physical mixtures.

Figure(1) represents comparative FTIR spectra of pure baclofen and its freshly prepared physical mixtures with different polymers selected for the study. The IR spectra of these mixtures showed the absorption bands, indicating the presence of drug and polymer. It was observed that all bands of baclofen were maintained at the same positions in FT-IR spectra of baclofen blends with HPMC, CP940 and PVP which means that no possible interaction between baclofen and studied polymers. Since the main peaks of drug-polymer mixture were approximately the same as peaks of pure drug. Thus FTIR analysis results proposed that the baclofen and polymers used were compatible.

UV- Spectrophotometric Scanning Studies

The UV-spectrophotometric scanning of pure baclofen showed one triple peak of maximum at 268 nm. The UV scanning of pure baclofen and baclofen-excipient mixtures showed the main characteristic peak at λ_{max} 268 nm for most polymers were shown in Figures (2). According to these data, neither peak shift nor peak intensity changes were recorded, indicating absence of interaction between baclofen and these excipients.

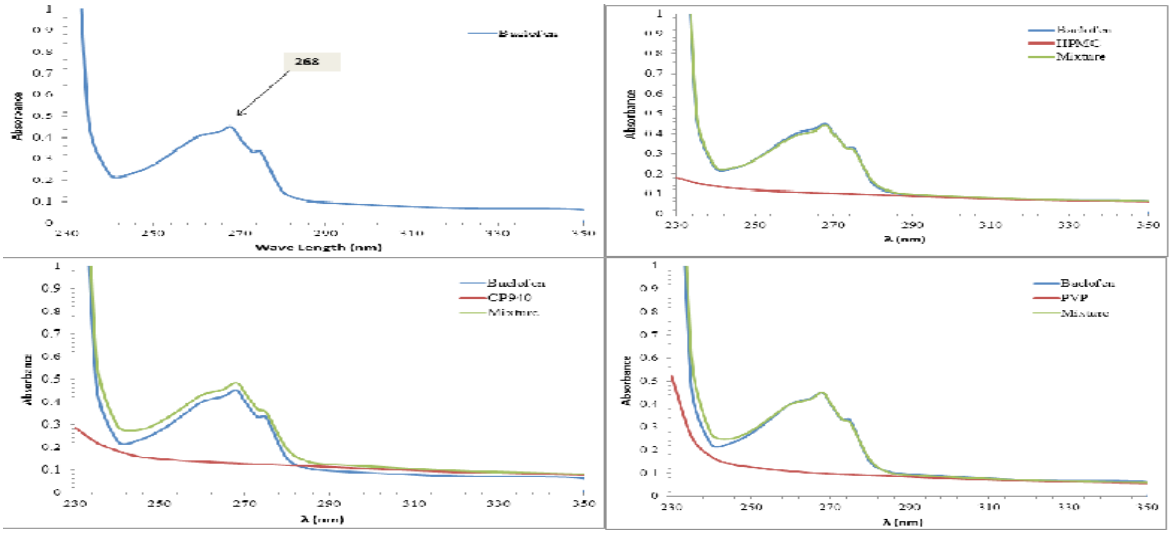

Figure 2. UV- absorbance spectrum of baclofen with different polymers.

Visual Examination of the Formulated Mucoadhesive Buccal Films:

All the prepared formulations were elegant in appearance, showed no visible cracks and showing good flexibility except (B3) that was excluded from further studies due to the cracks and fragmentations appeared.

Film Thickness

Average and standard deviation of all readings were calculated and recorded in Table (2). Thickness was found to be in the range of 0.567±0.186 mm to 1.467±0.288 mm. From the results obtained it was confirmed that all the films were ideal and uniform (in each formula less than 5%, n=6) and did not have any significant differences in the thickness at different points (Magdy, 2011; AB, 2013). B2 batch showed the minimum thickness while B4 batch showed the maximum.

Weight Uniformity

A large variation in weight signifies the inefficiency of the method applied and high chances are there for non-uniformity in drug content (AB, 2013). From the results shown in Table (2), it was observed that all the buccal films were uniform in weight and there was no significant difference in the weight of the individual formulations from the average value, the variations were all within normal limits and the weight variation was less than 5% (Zamani, 2016). Weight uniformity was found to be in range of 38.00±3.61 mg to 62.67±2.52 mg for formula B1 and B7 respectively.

Folding Endurance

The flexibility of the polymeric thin films which is required for their easy handling can be measured with respect to its folding endurance. The folding endurance is determined by folding the film repeatedly at 180° angle of the plane at the same place until it breaks. All the films resisted breakage upon folding them for more than 300 times at same place and did not show any cracks even after folding for more than 300 times. Hence it was taken as the end point. The folding endurance of the film was optimum and therefore the films exhibited excellent physical (Mukherjee, 2013) and mechanical properties.

Determination of Drug Content

Drug content of formulations was determined using UV-Spectrophotometer and results showed that the drug was uniformly distributed throughout the films and standard deviation of all the films was very less and within the limits (Saini, 2011) as recorded in Table (2). Average drug content was found between 91.70 ± 0.30 % (B1) and 102.23 ± 0.68 % (B7) of added amount of baclofen per film. On this basis, the results of content uniformity revealed that baclofen was distributed evenly throughout the films regardless of the polymer type and ratios.

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

Formulation code	Film thickness (mm) ± SD	Film weight (mg) ± SD	Folding endurance	Drug content % ± SD
B1	0.967±0.137	38.00±3.61	> 300	91.70±0.30
B2	0.567±0.186	48.33±1.53	> 300	99.03±0.80
B4	1.467±0.288	58.33±3.06	> 300	101.07±0.40
B5	1.133±0.216	55.00±2.00	> 300	97.37±0.81
B6	1.383±0.214	55.67±1.53	> 300	99.23±0.49
B7	0.883±0.223	62.67±2.52	> 300	102.23±0.68

Surface pH Measurement

Surface pH of films was determined by using pH meter and recorded in Table (3). Surface pH values were ranged from 6.74 ± 0.09 to 7.77 ± 0.04. The surface pH of all prepared films was within satisfactory limit of 7.0 ± 1.5 and hence no mucosal irritation was expected and ultimately achieved patient compliance (Lia, 2016). These results suggested that the polymeric blend identified was suitable for oral application owing to the acceptable pH measurements.

Swelling Study

In many cases the degree and rate of swelling play a key role in controlling the release of the drug. Hence, these parameters can be considered as the indicator for bioadhesive or mucoadhesive potential and drug release profiles (Roy, 2010; Kumria, 2014). Swelling studies of prepared films were performed using pH 6.8 phosphate buffer for 5 and 15 minutes and the results are showed in Table (3). In general, the uptake of water into the film, producing an increase in weight and swelling ratio was dependent on the content of each polymer in the polymer blend films. Maximum swelling (after 15 minutes) was observed in buccal mucoadhesive film B6 (316.97 %) while formulation B5 showed minimum swelling (59.17%). The buccal films were not dissolved nor eroded, indicating that the cohesiveness of the polymers is sufficient to guarantee the stability of the system and therefore, they were accepted for further studies.

Table 3. Surface pH and swelling indices of Baclofen mucoadhesive buccal films

Formulation code	pH ± SD	Swelling % after 5 min ± SD	Swelling % after 15 min ± SD
B1	7.14 ± 0.11	103.47±7.69	124.21±11.34
B2	7.42 ± 0.08	93.64±15.03	109.37±11.51
B4	7.00 ± 0.10	56.60±2.31	65.51±1.52
B5	6.74 ± 0.09	52.16±10.56	59.17±7.19
B6	7.54 ± 0.08	168.63±8.40	316.97±5.74
B7	7.77 ± 0.04	134.83±9.01	204.7±4.13

In vitro Mucoadhesion Strength Measurements

Mucoadhesion is a very important aspect for maintaining high drug levels at the site of administration and prevents expulsion of formulation. The mucoadhesive properties of the films were successfully tested using the modified physical balance system. In this study, the mucoadhesive strength was determined by measurement of the weight required for the detachment. Mucoadhesion strength and mucoadhesion force of the prepared baclofen films on chicken pouch mucosa as a function of polymers type and concentration have been shown in Table (4). The mean mucoadhesive strength was found to be in the descending order of B6> B4> B2> B5> B1> B7 (71.60> 66.00> 43.80> 36.40> 29.10> 19.20 grams). However, differences do exist due to change in the polymer type or composition of the film. The mucoadhesion properties increased with increasing content of the bioadhesive polymers for all the polymers studied. The increase in mucoadhesivity may be due to the formation of a strong gel that penetrates deeply into the mucin molecules (Park, 1987). In literature, it is reported that polymers with hydrophilic groups, such as carboxyl and hydroxyl groups, bind strongly to the oligosaccharide chains of the mucous layer (Kim, 2007). For buccal films prepared with F6 (HPMC 15%, CP940 2% and PVP 3%) had the highest mucoadhesive strength (71.60 ± 4.722) whereas B7 (HPMC 15%, CP940 2.5% and PVP2.5%) showed the lowest in vitro mucoadhesive strength (19.20 ± 2.387) on chicken pouch mucosa. These values showed that the mucoadhesion was satisfactory for buccal films to be attached on the mucous membrane (Korsmeyer, 1983). The calculated values of mucoadhesion strength (mean ± SD) failed to demonstrate statistical significance (p>0.05).

Table 4. *In vitro* mucoadhesion measurements of Baclofen mucoadhesive buccal films

Formula code	Formulation composition	Mucoadhesion strength (g) ± SD	Force of mucoadhesion (N) ± SD
B1	(15%HPMC,5%CP940)	29.10 ±2.408	0.29 ±0.024
B2	(15%HPMC,5%PVP)	43.80 ±1.643	0.43 ±0.016
B4	(15%HPMC,1%CP940,4% PVP)	66.00 ±3.674	0.65 ±0.036
B5	(15%HPMC,3%CP940,2%PVP)	36.40 ±3.050	0.36 ±0.030
B6	(15%HPMC,2%CP940,3% PVP)	71.60 ±4.722	0.70 ±0.046
B7	(15%HPMC,2.5%CP940,2.5% PVP)	19.20 ±2.387	0.19 ±0.023

In-Vitro Drug Release Studies of the Baclofen Mucoadhesive Buccal Films

The data obtained from *in-vitro* drug release study performed up to 8 hours gives a clear indication that prepared films showed necessary controlled release profile. When the films were subjected to dissolution test, rate of permeation of the dissolution medium into the films determined the drug release rate (Sekhar, 2008; Magdy, 2011). The *in vitro* release profiles of baclofen from different mucoadhesive films are shown in Figure (3). The graph was plotted between cumulative percentage drug released and time. It is apparent from the plots that the drug release could be sustained and was varied with respect to the proportion of polymers. Release of baclofen after eight minutes from different mucoadhesive buccal films was on the following descending order: B5 > B4 > B2 > B1 > B7 > B6 by the percentages (97.31 > 95.09 > 93.12 > 90.11 > 85.82 > 67.34 %/w) respectively. It was observed that the difference between the first four formulations was very low and the release of them was on the range (90.11% - 97.31%) whereas the rest formulations (B7 and B6) gave release far from the former 85.82% and 67.34% respectively. *The* $T_{50\%}$ values of *in vitro* release study were in increasing order: B5< B2 <B4 <B1=B7< B6 (1.30< 1.37< 1.40< 1.60< 2.6 hours)

as shown in Table (5). In general, a formulation with an appropriate controlled release profile with at least 80% drug release over an 8h period was desired for the purpose of this study for buccal delivery (Perumal, 2008; Cynthia, 2011). This means that all formulations were ideal for optimizing the purpose of this study except B6 that was 67% only that indicated very slow release. *In-vitro* drug release studies showed that release rate of drug increased when using the HPMC with pure PVP or pure CP940 whereas when using the combination PVP and CP940 there was decrease in the release and this depended on the ratio of these two polymers as the ratio increase the release was decreased. Maximum *in-vitro* release was found to be 97 % over a period of 8 h in batch B5 while minimum *in-vitro* release was found to be 65 % in batch B6. These results were further supported by swelling studies results, where highest swelling was shown by B6, so resulting in slower drug release.

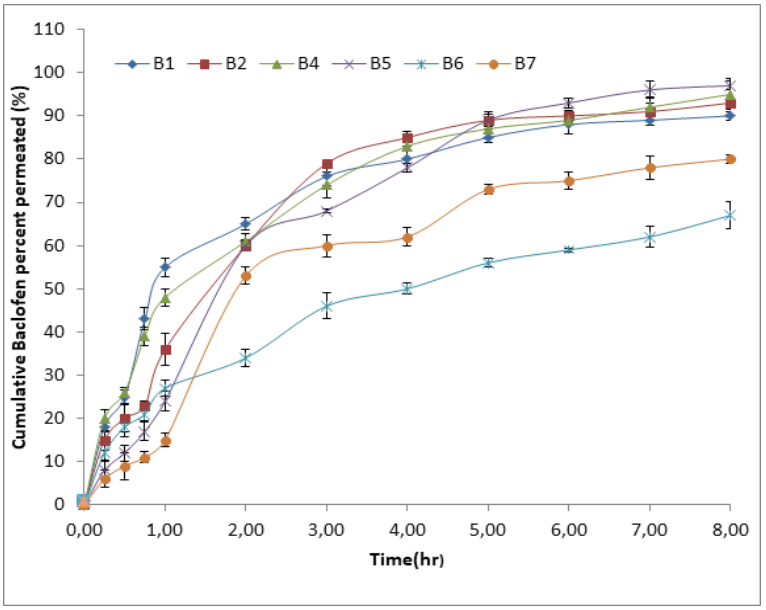

Figure 3. Release profiles of Baclofen from different mucoadhesive buccal films

Kinetic Analysis of *In Vitro* Release Data for Baclofen Mucoadhesive Buccal Films

The obtained values of *k* (kinetic constant), *n* (diffusional exponent) and r^2 (correlation coefficient) of the *in vitro* release data of baclofen from mucoadhesive buccal films are presented in Table (5). In general, the values of the determination coefficient obtained after fitting the data to the Peppas equation were high. The '*n*' values can be used to characterize diffusion release mechanism. The '*n*' value for the most formulations B1, B2, B4, B5 and B7 were between 0.45 to 0.89, which indicates both drug diffusion in the hydrated matrix and the polymer relaxation called anomalous (non-fickian) diffusion. From the '*n*' value, it can be concluded that drug release mechanism from film is diffusion with swelling of polymer. For B6, value of *n* was found to be 0.281 indicating Fickian release kinetics.

Table (5): Kinetic analysis of baclofen released from different mucoadhesive buccal films.

Formula code	Correlation coefficient (r^2)			Korsmeyer-Peppas model			
	Zero order	1st order	Higuchi model	*K*	*N*	$t_{50\%}$* (hr)	r^2
B1	0.822	0.631	0.952	0.3232	0.474	1.60	0.958
B2	0.866	0.678	0.966	0.3645	0.580	1.37	0.970
B4	0.871	0.657	0.976	0.3505	0.559	1.40	0.981
B5	0.920	0.727	0.980	0.3952	0.612	1.30	0.982
B6	0.934	0.710	0.966	0.2479	0.281	2.60	0.968
B7	0.901	0.741	0.962	0.3252	0.509	1.60	0.966

*Time for 50% drug release (t_{50}% (hour))

***In Vivo* Mucoadhesive Performance of Baclofen Mucoadhesive Buccal Films in Healthy Human Volunteers:** (Ethical Committee : MECA NO.: (2017/06))

Another important factor in developing buccal film system is the maintenance of adhesive film on the mucous surface (Peh, 1999). *In vivo* mucoadhesion characteristics of the formulated buccal films, without drug, were evaluated in four healthy male human volunteers. The comfort, irritation, duration of adhesion and ease of removal of the films were also evaluated during the test on human volunteers. The residence time values of various films on buccal mucosa are depicted in Figure (4). It was observed that the tested mucoadhesive buccal films were readily retained on the buccal mucosa and the bioadhesive polymers predominately increased the *in vivo* residence time of mucoadhesive films. Buccal films exhibited short adhesion time were considered unsuitable for prolonged intra-oral delivery of baclofen and excluded from the permeability and bioavailability studies. It was noted that the only B2, B4 and B6 films exhibited a reasonable and satisfactory mucoadhesion in the oral cavity for over 3.5 hours (Puratchikody, 2011) whereas the other formulations were excluded due to the low values of residence times. They could be arranged according to their residence times as follows; B6> B4> B2. Visual examination of the mucosal tissue after the removal of the film revealed no signs of damage to the mucosa with either of the polymers. Volunteers reported no irritation during or after the study.

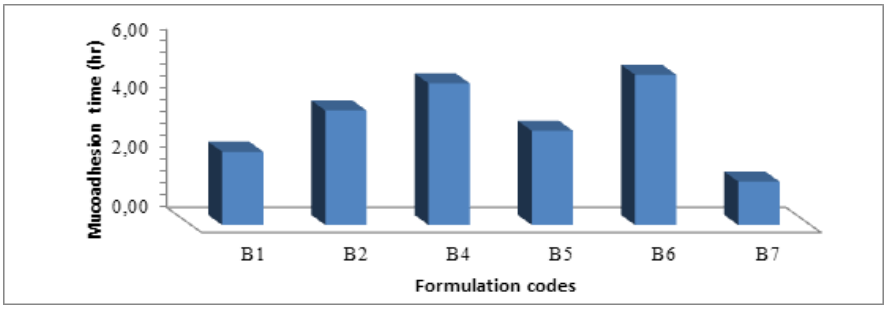

Figure 4. *In vivo* Mucoadhesion time of Baclofen mucoadhesive buccal films evaluated in healthy human volunteers.

CONCLUSION

From the present work, it was concluded that the formulated mucoadhesive buccal films containing baclofen had been prepared with satisfactory physicochemical properties except formula B3 that was excluded due to the misshaped appearance and presence of cracks in the film. Low values of standard deviation in thickness, weight measurement and drug content data reflected no significant difference within the batch. The surface pH of all films was within satisfactory limit of 7.0±1.5 and hence no mucosal irritation was expected and ultimately achieved patient compliance. The *in vitro* mucoadhesive strength values were satisfactory for maintaining them in oral cavity except for B1 and B7 which showed the lowest mucoadhesive strength values, less than 30 grams. The release patterns and mucoadhesion properties can be controlled by changing the polymer type and concentration. The data clearly showed that percentage release of baclofen was maximum (95.09% - 97.31%) for formulations B4 and B5. On the other hand, B6 showed the minimum *in vitro* drug release; only 67.34 % drug release was achieved in 8 hours. For most of the tested formulations, the mechanism of drug release was found to be non-Fickian diffusion. The highest *in vivo* residence time was detected for B4 and B6 with mucoadhesion time of 4.77 and 5.07 hrs, respectively. Films of B7 showed the lowest mucoadhesion time. Formulation B4 exhibited a good *in vitro* profiles, and suitable mucoadhesion strength and was therefore selected for other recommended studies including stability study and *in vivo* evaluation test.

REFERENCES

AB, Kumria R,H.S.(2013). In vitro techniques to evaluate buccal films. *J Control Release*,166,10–21.

Ali, J., R. Khar, A. Ahuja, and R.K.(2002). Buccoadhesive erodible disk for treatment of oro-dental infections: design and characterisation. *Int J Pharm*,238(1-2),93-103.

Bhatia, Monika Sachdeva and M.B.(2012). Formulation and evaluation of transdermal patch of pregabalin. *International Journal Pharmaceutical Sciences and Research*,3(2),569-575.

Brown, H.D.F.(2011). Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms. *Indian J Pharm Sci.*,73(3),338–353.

Bukka, Mukul Dwivedi, LVG Nargund and K. P.(2012). Formulation and Evaluation of Felodipine Buccal Films containing Polyethylene Oxide. *International Journal of Research in Pharmaceutical and Biomedical Sciences*,3(3),1153-1161.

Cilurzo, Cupone IE, Minghetti P, Selmin F, M.L.(2008). Fast dissolving films made of maltodextrins. Eur. *J. Pharm. Biopharm.*,70,895–900.

Consuelo, Falson F, Guy RH, J.Y.(2007). Ex vivo evaluation of bioadhesive films for buccal delivery of fentanyl. *J Control Release,*122(2),135–140.

Dash, Padala Narasimha Murthy and P.C.(2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica ñ Drug Research,*67(3),217-223.

Desai, K.G. and T.M.K.(2004). Preparation and evaluation of a novel buccal adhesive system. *AAPS PharmSciTech,*5(3), Article 35.

Dixit, P.S. (2009). Oral strip technology: overview and future potential. *J. Controlled Release,*139,94–107.

El-Samaligy, M.S., S.A. Yahia, and E.B.B.(2004). Formulation and evaluation of diclofenac sodium buccoadhesive discs. *Int J Pharm,*286(1-2),27-39.

Gavaskar, E.Venkateswarlu, D.Kumaraswamy, D. Dooda, M.N.(2010). Formulation and evaluation of mucoadhesive tablets of baclofen. *International Journal Of Pharmacy&Technology,*2(2),396-409.

Hanif, Muhammad Zaman and V.C.(2015). Polymers used in buccal film: a review. *Designed Monomers and Polymers,*18(2),105-111.

Kerec, M., M. Bogataj, B. Mugerle, M. Gasperlin, and A.M.(2002). Mucoadhesion on pig vesical mucosa: influence of polycarbophil/calcium interactions. *Int J Pharm*,241(1),135-143.

Khanna , Agarwal S, A.A.(1996). Preparation and evaluation of bioerodible buccal tablets containing clotrimazole. *Int. J. Pharm.*,138,67–73.

Kim, Ahn JS, Choi HK, Choi YJ, C.CS.(2007). A Novel Mucoadhesive Polymer Film Composed of Carbopol, Poloxamer and Hydroxypropylmethylcellulose. *Arch Pharm Res.*,30,381–386.

Korsmeyer, Gunny R, P.NA.(1983). Mechanism of solute release from porous hydrophilic polymers. *Int J Pharmaceutics*,15,25-35.

Kulkarni et al.(2011). Fabrication and evaluation of biliayer matrix tablets of baclofen using ethyl cellulose. *International Journal of Advances in Pharmaceutical Research*,2(5),193–198.

Kumria, Nair AB, G.G.(2014). Buccal films of prednisolone with enhanced bioavailability. *Drug Deliv.*,23,471–478.

Lia, Zhao-Ming Yeb, J-B. W.(2016). Mucoadhesive buccal films of tramadol for effective pain management. *Rev Bras Anestesiol.*,10,8-16.

Magdy, Mohamed, Mohamed Haider, M.A.M.A. (2011). Buccal Mucoadhesive Films Containing Antihypertensive Drug:In vitro/in vivo Evaluation. *Journal of Chemical and Pharmaceutical Research*, 3(6),665-686.

Mahanthesha, Nagaraja.T.S and Bharathi D.R. (2013). Design and evaluation of timolol maleate buccal patches using tween as permeation enhancer. *International Journal of Universal Pharmacy and Bio Sciences,* 2,12-20.

Marina, R.N. Charyulu and P. P. (2010). Mucoadhesive films of Losartan Potassium for Buccal delivery: Design and Characterization. *Indian J.Pharm. Educ. Res.*, 44(4), 31-43.

Mukherjee and B.S.(2013). Design and characterization of double layered mucoadhesive system containing bisphosphonate derivative. *ISRN Pharm*,13,1–10.

Park, R.JR.(1987). Mechanisms of bioadhesion of polyacrylic acid hydrogels. *Pharm Res.*,4,457-464.

Peh, K.K. and C.F.W.(1999). Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. *J Pharm Pharm Sci.*,2(2),53-61.

Peppas, N.A.(1985). Analysis of Fickian and non-Fickian drug release from polymers. *Pharm Acta Helv.*,60(4),110-111.

Perumal, V.A., D. Lutchman, I. Mackraj, and T. Govender, Formulation of monolayered films with drug and polymers of opposing solubilities. *Int J Pharm*, 2008. 358(1-2),184-191.

Prakasami and R.B.(2014). Evaluation of cellulose polymers for buccal film formulation of rasagiline. *Asian J Pharm Clin Res,*7(3),83-87.

Puratchikody, Viswanadhan V. P.(2011). Development and characterization of mucoadhesive patches of salbutamol sulfate for unidirectional buccal drug delivery. *Acta Pharm.*,61(5),157–170.

Roy and P.B.(2010). Bioadhesive polymeric platforms for transmucosal drug delivery systems – a review. *Trop J Pharm Res.*,9,91–104.

Saini , Arun Nanda , Monika Hooda, K.(2011). Fast dissolving films: Innovative drug delivery system. *Pharmacologyonline*,2,919-928.

Sangeetha, D. Nagasamy Venkatesh, PN Krishan and R. S. (2010). Mucosa as a route for systemic drug delivery. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,*1(3),178.

Saxena, Gulab Tewari, S.A.S.(2011). Formulation and evaluation of mucoadhesive buccal patch of acyclovir utilizing inclusion phenomenon. *Brazilian Journal of Pharmaceutical Sciences*,47(4),54-62.

Sekhar, K.C., K.V. Naidu, Y.V. Vishnu, R. Gannu, V. Kishan, and Y.M.R.(2008). Transbuccal delivery of chlorpheniramine maleate from mucoadhesive buccal patches. *Drug Deliv.*,15(3),185-191.

Shaikh, MA. Shende and AM.S.(2013). Formulation Development and Evaluation of Gastro Retentive Mucoadhesive Tablets Using Synthetic Polymers. *International Journal of Research in Pharmaceutical and Biomedical Sciences,*4(4),1264-1271.

SNR, Nayak BS, Nayak AK, M.B.(2010). Formulation and evaluation of buccal patches for delivery of atenolol. *AAPS PharmSciTech.,*11,1038–1044.

Wong, C.F., K.H. Yuen, and K.K.P.(1999). An in-vitro method for buccal adhesion studies: importance of instrument variables. *Int J Pharm,*180(1),47-57.

Yehia, S.A., O.N. El-Gazayerly, and E.B.B.(2009). Fluconazole mucoadhesive buccal films: in vitro/in vivo performance. *Curr Drug Deliv,*6(1),17-27.

Zamani, Muhammad Hanifi and A.A.Q.(2016). Effect of polymer and plasticizer on thin polymeric buccal films of meloxicam designed by using central composite table design. *Acta Poloniae Pharmaceutica ñ Drug Research,*73(5),1351-1360.