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Simple Controlled Release Delivery System for an Anti-hypertensive Drug via Buccal Route

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MMG and MME designed the study, wrote the protocol and managed the analysis of the study. Authors MME and SG managed the literature searches and revision of the paper. Author WMK carried out the experimental work, performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Objective: This study aims to develop controlled release buccal tablets of losartan potassium based on bioadhesion using direct compression technique.
Materials and Methods: The bioadhesive buccal tablets of losartan potassium were prepared after preliminary drug-excipients compatibility studies and micromeretics study for powder blends. The tablets were prepared by direct compression utilizing carbopol 934LR as a primary bioadhesive polymer either with or without chitosan or hydroxypropyl methylcellulose E15LV as secondary polymers. Other excipients included PVP K30 as a binder, magnesium stearate as a lubricant and mannitol as a diluent. The tablets were evaluated for weight variation, thickness and diameter, hardness, friability, drug content, surface pH, *Ex-vivo* residence time and bioadhesion force, *In-vitro* swelling and drug release study. The analysis of the release profiles in the light of distinct kinetic models (zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer–Peppas) was carried out.
Results and Discussion: The formula containing 40% w/w bioadhesive polymers of carbopol 934LR and chitosan (1:2) was selected as the optimum one based on a ranking

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methodology and then, it was subjected to *Ex-vivo* permeation and physical stability study in human saliva. Swelling index was 78.32±1.84% after 7h and tablets showed a neutral surface pH. *Ex-vivo* residence time was long enough for more than 10h. *Ex-vivo* bioadhesion force was 0.38±0.01N. Drug release was 57.64±3.43% after 8h following zero order kinetics with a steady state permeation flux of 0.959mg/cm^{2}h. Tablets were physically stable in human saliva.
Conclusion: These formulae improved, controlled and prolonged the release of losartan potassium from a buccal bioadhesive system for at least 8h in a simple way which can achieve a high patient compliance.

Keywords: Buccal; controlled release; losartan potassium; carbopol 934LR; chitosan.

1. INTRODUCTION

Controlled release dosage forms have high popularity and medical value. Buccal drug delivery systems offer a direct path to the blood circulation through the internal jugular vein escaping from the hepatic first pass effect leading to a higher bioavailability of drugs [1,2]. They are also cheap, easy to be produced with high patient compliance, self administrable and the patient can even terminate the drug delivery process at any time. Combining drug release control and bioadhesion techniques can result in a highly performing and interesting drug delivery system from pharmaceutical and medical point of view.

Hypertension is a well established independent risk factor for cardiovascular diseases and stroke. In developing countries, heart diseases and stroke resulting from hypertension are the first and third causes of morbidity and mortality [3]. High blood pressure or hypertension kills around 1.5 million people yearly in South-East Asia which makes it the most important risk factor for non communicable diseases such as heart attack and stroke, according to the World Health Organization [4]. Many conventional oral formulations for hypertension offer convenience and ease of use but produce unreliable blood levels and inconsistent response [5].

Losartan potassium is an angiotensin II receptor blocker for the treatment of hypertension [3]. It is freely soluble in water (Class-III BCS) with a partition coefficient log P (octanol/water) 4.01. It can be easily absorbed after oral administration but it suffers from an extensive pre-systemic metabolism in liver and only 33% from the oral dose reaches the systemic circulation and 14% is converted to an active metabolite [6]. Peak plasma concentrations occur after 1h of administration.

The aim of this work is to develop a simple convenient controlled release bioadhesive buccal delivery system for losartan potassium that has a long duration of action up to 12h and allows the drug to avoid hepatic first pass metabolism, can be administered less frequently, gives better therapeutic response and improves patient compliance.

2. MATERIALS AND METHODS

2.1 Materials

Losartan potassium was kindly received as a gift sample from The Egyptian International Pharmaceutical Industries Co. (EIPICO), Tenth of Ramadan city, Egypt. Carbopol 934LR and HPMC E15LV were purchased from Alpha Chemika, Mumbai, India. Chitosan (Low

molecular weight) and magnesium stearate were purchased from Oxford Laboratory, Mumbai, India. Poly vinyl pyrrolidone K30 was purchased from Winlab, Leicestershire, U.K. Mannitol was purchased from Loba Chemie Pvt. Ltd., India. All other chemicals used in this study were of an analytical grade.

2.2 Preformulation Studies

2.2.1 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) study using Shimadzu 435 U-O4 IR spectrometer (Japan) was performed. Samples of losartan potassium, carbopol 934 LR, chitosan, HPMC E15LV separately and physical mixtures of each substance with losartan potassium were analyzed. Discs of potassium bromide (KBr) were prepared by grinding each sample with KBr using a mortar and pestle, compressing the sample in a KBr die using a pressure of 8 tons in an IR press [7]. The scanning range was 4000-400cm^{-1}.

2.2.2 Thermal analysis

Differential scanning calorimetry (DSC) of losartan potassium, carbopol 934 LR, chitosan, HPMC E15LV separately and physical mixtures of each substance with losartan potassium was performed. It was carried out on accurately weighed samples (7.5mg) in closed aluminum pans using Shimadzu DSC-50 (Japan). The heating rate was 10°C/min and nitrogen flow rate was 40 ml/min from 25°C to 300°C [8].

2.2.3 Micromeritics study

All the ingredients were allowed to pass through sieve number 60, USA standard test sieve, ASTME-11, Gilson company (USA). Each ingredient was weighed for each formula and the ingredients were then mixed together using a porcelain mortar and pestle. The powder blends were finally evaluated by determining the following parameters: Angle of repose, Hausner ratio and Carr's index.

2.2.3.1 Angle of repose

It was determined using the fixed funnel method. The powder mixture was poured through a funnel which was raised vertically at a fixed height (h) equal to 1 cm in all experiments [9]. Radius of the powder pile (r) was measured. Angle of repose (Ɵ) was calculated according to the following equation:

$$\text{Tan } Ɵ = 1 / r \text{ ----------} \quad (1)$$

Where "Ɵ" is the angle of repose, "1" is the height of the funnel (cm) and "r" is the radius of powder pile.

2.2.3.2 Bulk and tapped densities

Apparent bulk density (D_b) was measured by pouring a known weight (M) equal to 5g of the powder mixture into a graduated measuring cylinder. Bulk volume (V_b) was determined.

$$D_b = M / V_b \text{ ----------} \quad (2)$$

Where "M" is the weight of powder (5g) and "V_b" is the bulk volume of powder.

The measuring cylinder containing the known weight of powder blend (M) was tapped for a fixed number of times (10 times) by raising it to a height of 30cm for all formulae. The minimum volume (V_t) obtained in the cylinder was recorded [10]. Apparent tapped density (D_t) was calculated according to the following equation:

$$D_t = M / V_t \text{ ----------} \quad (3)$$

Where "M" is the weight of powder (5g) and "V_t" is the volume after tapping for 10 times (tapped volume of powder).

2.2.3.3 Hausner ratio

Hausner ratio is an indirect measure for flowability of the powders. It is the ratio between tapped density and bulk density [11].

$$\text{Hausner ratio} = D_t / D_b \text{ ----------} \quad (4)$$

2.2.3.4 Carr's index (compressibility %)

It is a simple way for the measurement of powder flowability. This percentage is indirectly related to the relative flow rate, cohesiveness, and particle size of the powder [12]. It can be calculated from the equation:

$$\text{Compressibility \%} = (D_t - D_b) / D_t \times 100 \text{ ----------} \quad (5)$$

2.3 Preparation of Bioadhesive Buccal Tablets

Bioadhesive buccal tablets of losartan potassium were prepared by direct compression using flat 8mm punches tablet compress machine, CPMD-10, Chamunda Pharma machinery Pvt. Ltd., Ahmedabad (India). First, ingredients were screened through sieve number 60 then, thoroughly mixed geometrically (except magnesium stearate) in a porcelain mortar with a pestle for 15min. Magnesium stearate was added later and mixed for additional 5min. The powder mixture was finally compressed. The composition of the bioadhesive buccal tablets formulae is mentioned in Table 1.

Table 1. Composition of losartan potassium bioadhesive buccal tablets

Formula code	Carbopol 934LR(mg)	Chitosan(mg)	HPMC E15LV(mg)	Mannitol(mg)
F1	10	-	-	107
F2	20	-	-	97
F3	10	50	-	57
F4	20	40	-	57
F5	10	-	50	57
F6	20	-	40	57

**Each tablet contains 25mg losartan potassium, 6mg pvp k30, 2mg magnesium stearate*

2.4 Evaluation of Prepared Bioadhesive Buccal Tablets

2.4.1 Weight variation

Twenty tablets were randomly selected from each batch and the average weight was determined. Then, each tablet was weighed and the weight of each tablet was compared with the average weight [13]. The mean±SD was calculated.

2.4.2 Thickness and diameter

Thickness and diameter of tablets was measured using digital Mitutoyo caliper (Japan) and expressed in mm [13]. The test was performed using ten tablets from each batch and the mean±SD was calculated.

2.4.3 Hardness

Hardness of the tablets was measured using Campbell tablet hardness tester (India) [14] and expressed in kg/cm^2. This test was performed using ten tablets from each batch and the mean±SD was calculated.

2.4.4 Friability

Friability test for the tablets was performed using Roche Friabilator. Pre-weighed sample of 10 tablets was placed in the friabilator and the tablets were subjected to 25r/min for 4min. Tablets were then dedusted using a brush and reweighed [15]. The % friability (F) was calculated according to the following equation:

$$F = [(W_{initial} - W_{final})/ (W_{initial})] \times 100 \text{ ----------} \quad (6)$$

"F" represents the percentage weight loss; "$W_{initial}$" and "W_{final}" are the initial and final weights, respectively.

2.4.5 Drug content

Three tablets from each batch were randomly selected and separately ground in a porcelain mortar using a pestle [16]. Each one was then dissolved in phosphate buffer pH 6.8 and filtered through a whatmann filter paper .The filtrate was diluted with the buffer solution and the drug content was analyzed spectrophotometrically at λ_{max} =253nm using Hitachi U-2900, UV spectrophotometer (Japan) and a standard curve of the drug (y=0.0274x+0.037; r^2=0.999 in phosphate buffer pH 6.8 and range 5-30µg/ml). A test on a placebo tablet from each formula was carried out to eliminate any possible interference from other ingredients of the tablets. The mean±SD was calculated.

2.4.6 Surface pH

Surface pH was measured to show if tablets can be irritant to the buccal mucosa. Three tablets from each batch were randomly selected and each one was soaked in 1ml of distilled water in separate Petri plates for 2h [17]. The surface pH was determined by bringing the combined glass electrode of a pH-meter, Jenway (UK) in contact with the surface of the tablets and permitting it to equilibrate for 1 min. The mean±SD was calculated.

2.4.7 *In-vitro* swelling study

Three buccal tablets from each batch were weighed separately (W_1) and each tablet was placed over the gel surface of 2% w/v agar Petri plates with the core of the tablet facing the surface of the gel and incubated at 37±1°C [18,19]. At predetermined time intervals up to 7 h, each tablet was removed out of the Petri plate and excess surface water was carefully removed using a filter paper. Each tablet was reweighed (W_2) at the previously specified

time intervals. Swelling index (SI) after predetermined time intervals was calculated according to the following equation:

$$\% \text{ Swelling index} = [(W_2 - W_1)/ (W_1)] \times 100 \quad \text{---------- (7)}$$

"W_2" is final weight of each tablet after a certain time t and "W_1" is initial weight of each tablet.

2.4.8 *Ex-vivo* residence time

Tablets residence time was determined using a modified disintegration apparatus, Erweka Apparatebau GmbH (Germany) [20]. The medium used was 800 ml phosphate buffer pH 6.8 at 37°C. A piece of sheep buccal mucosa from a local slaughterhouse was glued to a glass slide using an instant adhesive. The slide was vertically fixed to the disintegration apparatus. Each tablet was wetted from one surface by 0.5ml of phosphate buffer pH 6.8. The wet surface was left in contact with the mucosal membrane for 5min. The glass slide was allowed to move downwards and upwards keeping the tablet completely immersed in the buffer solution [21]. The time till complete dissolving or detachment of each tablet from the mucosal membrane surface was recorded. This experiment was carried out in a triplicate (n=3) for each batch and the mean±SD was calculated.

2.4.9 *Ex-vivo* bioadhesion force

Bioadhesion force was measured using a modified physical balance [22]. It is a normal double beam physical balance in which a glass beaker substituted the right pan and a glass slide fixed to the base of a glass beaker substituted the left pan .The mucous membrane was glued to the base of an inverted glass beaker. The modified balance was set up so that any increase in the weight of the right side will pull the tablet adhering to the mucous membrane upwards till detachment. Each tablet was wetted from one surface with 0.5 ml phosphate buffer pH 6.8, and then allowed to be in contact with the mucosal surface for 5 min. Distilled water was added from a burette inside the right side glass beaker until the tablet was detached from the membrane. The volume of the distilled water consumed was recorded and it represents the mucoadhesive strength in grams. Bioadhesion force (N) can be calculated according to the following equation:

$$\text{Bioadhesion force (N)} = \text{weight of distilled water (g)} \times G/1000 \quad \text{---------- (8)}$$

"G" is the acceleration due to gravity and it equals 9.81m/s^2. This experiment was performed in a triplicate for each batch (n=3) and the mean±SD was calculated.

2.4.10 *In-vitro* drug release study

The drug release study of the tablets was performed using USP dissolution apparatus type II, Erweka Apparatebau GmbH, DT 600 (Germany). The dissolution medium was composed of 500ml phosphate buffer pH 6.8. The drug release study was carried out under sink conditions at 37±0.5°C and a paddle rotation speed 50r/min. The tablet was glued to a glass slide using a cyanoacrylate instant adhesive permitting the drug to be released from one side only. The slide was fixed under the paddle near the bottom of dissolution cup. Samples (5ml) were withdrawn at predetermined time intervals then, replaced by the same volume of fresh medium to maintain the sink conditions [23]. The samples were filtered using a whatmann filter paper and analyzed spectrophotometrically as previously mentioned. This

experiment was performed in a triplicate for each batch (n=3) and the mean±SD was calculated.

2.4.11 Drug release kinetics study

The *In-vitro* drug release profile of various formulae was subjected to regression analysis and kinetics study by five models which are zero order, first order, Higuchi square root kinetics, Hixson-Crowell and Korsmeyer-Peppas to determine the order of the drug release rate and the mechanism by which losartan potassium was released from polymeric matrices [24]. The kinetic parameters for zero order model was calculated by plotting the time in hours versus the percent of losartan potassium released, for first order by plotting the time in hours versus log percent retained of losartan potassium, for Higuchi's diffusion model by plotting the square root of time versus the percent of losartan potassium released, for Hixson-Crowell cube root model by plotting time in hours versus the cube root of the initial concentration minus the cube root of percent retained of losartan potassium and by plotting log time in hours versus log cumulative percent drug release of losartan potassium for Korsmeyer-Peppas model. The regression coefficient (r) for each kinetics model was obtained and for Korsmeyer-Peppas model the drug release exponent (n) was obtained to determine the release mechanism of losartan potassium using the portion of the release curve where cumulative percent drug release was less than 60% [25].

2.4.12 Selection of optimum formula

Different formulae were ranked according to the data obtained from the *In-vitro* swelling study, surface pH, *Ex-vivo* residence time, *Ex-vivo* mucoadhesion, *In-vitro* drug release and kinetics study. Further analysis based on parameters with high priority was performed to select the optimum formula which was subjected to *ex-vivo* permeation and physical stability study in human saliva.

2.4.13 *Ex-vivo* permeation study

Permeation study through sheep buccal mucosa was performed using a Franz diffusion cell, Hanson (USA) at 37±0.5°C and a rotation speed 50r/min using a magnetic stirrer [18,19]. Sheep buccal mucosa was obtained from a local slaughterhouse. Epithelial membrane was surgically separated from underlying connective tissues then; it was clamped between the donor and receptor chambers of the diffusion cell. The buccal membrane used had a thickness of 1.5mm and a surface area of diffusion 113mm^2. It was allowed to equilibrate in phosphate buffer pH 6.8 between the chambers for 30 min. The receptor chamber was filled with a volume of 25 ml phosphate buffer pH 7.4 [26] which mimic the pH of the blood stream [27]. The buccal tablet was placed inside the donor chamber and 1 ml phosphate buffer pH 6.8 was added [28]. Samples (0.5ml) were withdrawn from the receptor chamber at predetermined time intervals and replaced by the same volume of the fresh medium. The samples were then diluted with phosphate buffer pH 7.4 up to 10ml in a volumetric flask then filtered through a whatmann filter paper. The amount of drug permeated (Q) through the buccal mucosa was determined using a UV spectrophotometer as mentioned before. This study was performed in a triplicate (n=3) and the mean±SD was calculated. Other permeation parameters such as steady state flux (J_{ss}), permeability coefficient (P) and diffusion coefficient (D) were calculated according to the following equations:

$$J_{ss} = 1/A(dQ/dt)_{ss} = PC_o = DKC_o/h \quad \text{----------} \quad (9)$$

Where:

"J_{ss}" is the permeation rate constant, the flux, at steady state (mg/cm^{2}h) which obtained from the slope of the regression line divided by the area of diffusion
"A" is the effective diffusion surface area of the buccal membrane (1.13cm^2)
$(dQ/dt)_{ss}$ is the amount of the drug passing through the membrane per unit time (the slope) at steady state (mg/h)
"P" is the permeability coefficient (cm/h)
"C_o" is the drug concentration in the donor phase, (mg/ml)
"D" is the diffusion coefficient of the drug (cm^2/h)
"K" is the partition coefficient of the drug between the buccal membrane and the delivery system
"h" is the effective path length (0.15cm)

$$D = h^2/6L \text{ ----------} \quad (10)$$

Where:

"h" is the effective path length (0.15cm).
"L" is the lag time (h), which was estimated by back extrapolation of the linear portion of the line.

2.4.14 Physical stability of buccal tablets

This study was performed in normal human saliva which was collected from 26 years old healthy volunteer and filtered through a whatmann filter paper. Each tablet was immersed in a separate Petri plate which contains a volume of 5ml normal human saliva up to 6h and removed out at predetermined time intervals. Each buccal tablet was observed for any change in color and collapse of the tablet [29,30]. Thickness and diameter were measured. The study was carried out using 6 tablets.

2.5 Statistical Analysis

All figures were produced using *MS Excel® 2010* software and all data in figures and tables are expressed in mean±SD which were calculated using this software. Figures contain error bars which were all produced using the same previously mentioned software.

3. RESULTS AND DISCUSSION

3.1 Preformulation Studies

3.1.1 Fourier transform infrared spectroscopy

Losartan potassium is 2-butyl-4-chloro-L-[[20-(lH-tetrazol-5-yl)[l,l0-biphenyl]-4-yl]methyl]-lH-imidazole-5-methanol. It has a broad absorption band in the IR spectrum at 3200cm^{-1} and twin bands at 996 and 1010cm^{-1} due to tetrazole ring and a sharp band at 1460cm^{-1} due to imidazole ring. Further bands due to O-H and C-O stretch at 933 and 1074cm^{-1}, respectively. N-H stretch gives a band at 2956cm^{-1} and C=N stretch gives another one at 1426cm^{-1}. One band due to C-N stretch of tertiary amine at1260cm^{-1}. One sharp band due to aryl chloride at 1114cm^{-1}. These are the characteristics of losartan potassium [31].

FT-IR spectra of losartan potassium, each polymer and a physical mixture of losartan potassium with each polymer are shown in Fig. 1.

The spectrum for losartan potassium alone showed the previously mentioned characteristic bands at 3197.98, 997.20, 1008.77, 1458.18, 933.55, 1074.35, 2956.87 and 1423.47cm^{-1}, respectively.

The spectrum for the physical mixture of losartan potassium and carbopol 934LR retained all the characteristic bands of the molecular structure of losartan potassium at 3199.91, 997.20, 1008.77, 1452.40, 933.55, 1074.35, 2956.87 and 1421.54cm^{-1}. The spectrum for the physical mixture of losartan potassium and chitosan also retained the same characteristic bands of losartan potassium at 3203.76, 997.20, 1008.77, 1460.11, 933.55, 1074.35, 2956.87 and 1421.54cm^{-1}. The spectrum for the physical mixture of losartan potassium and HPMC E15LV retained most characteristic bands of losartan potassium at 1008.77, 1458.18, 933.55, 1080.14, 2956.87 and 1425.40cm^{-1}.

Thus, it was concluded that there is no interaction took place between losartan potassium and different polymers within the formulae.

3.1.2 Thermal analysis

Losartan potassium exists in at least two polymorph forms. Polymorph Form I, characterized by DSC endotherm at 230-250°C, converts, while heated, into polymorph Form II, characterized by endotherm melting point at 276°C [32].

DSC scans of losartan potassium, each polymer and a physical mixture of losartan potassium with each polymer are shown in Fig. 2.

DSC thermogram of losartan potassium showed a sharp characteristic melting endotherm at 271.28°C. The thermograms for carbopol 934LR, chitosan and HPMC E15LV did not show any sharp characteristic endothermic peaks within the temperature range of this study. The thermogram for the physical mixtures retained the sharp characteristic endothermic peak of losartan potassium at 264.49°C, 265.08°C and 269.43°C, respectively.

Hence, DSC thermograms of the physical mixture of the drug and each polymer showed the characteristic endothermic peak of losartan potassium with nearly the same intensity and sharpness and minor shifts for the peak. Thus, it was confirmed that drug and polymers are compatible with no doubt of interactions to take place within the formulae.

3.1.3 Micromeritics study

3.1.3.1 Angle of repose (Ɵ)

The angle of repose is an important parameter which indicates the flow ability of the powders. The powders with values less than 20° exhibit excellent flow ability; between 20° and 30° have good flow ability; between 30° and 34°show passable flow ability; while above 34° have very poor flow ability [33]. USP specifications are slightly different from the previous publication and these values are: 25-30° for excellent flow, 31-35° for good flow, 36-40° for fair flow, 41-45° for passable flow, 46-55° for poor flow and more than 55° shows very poor flow properties for the powders.

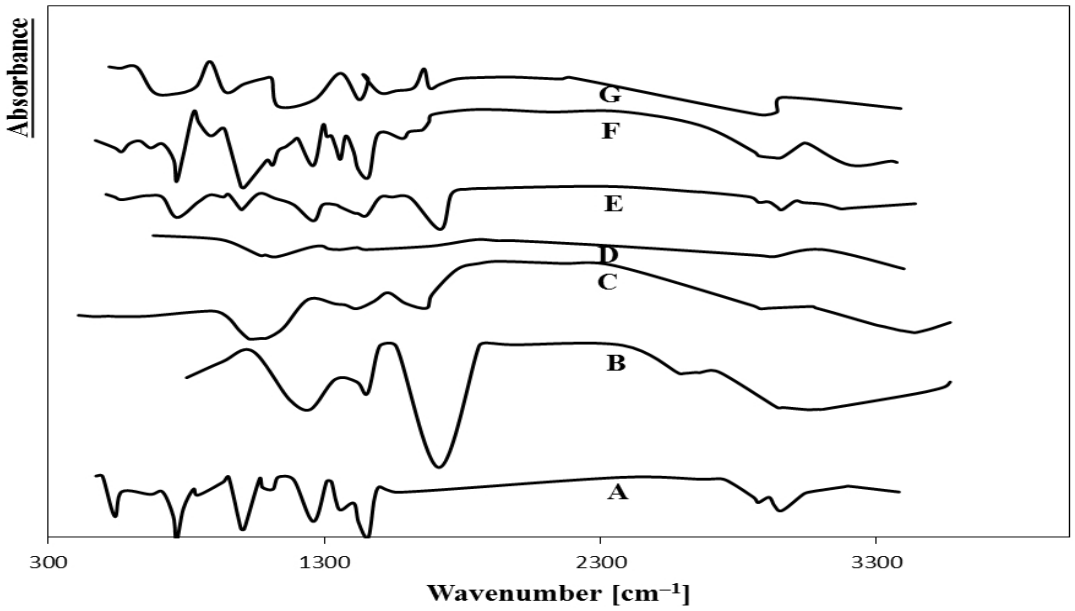

Fig. 1. FT-IR spectra of (A) losartan potassium, (B) carbopol 934LR, (C) chitosan, (D) HPMC E15LV, (E) physical mixture of losartan potassium and carbopol 934LR, (F) physical mixture of losartan potassium and chitosan, (G) physical mixture of losartan potassium and HPMC E15LV

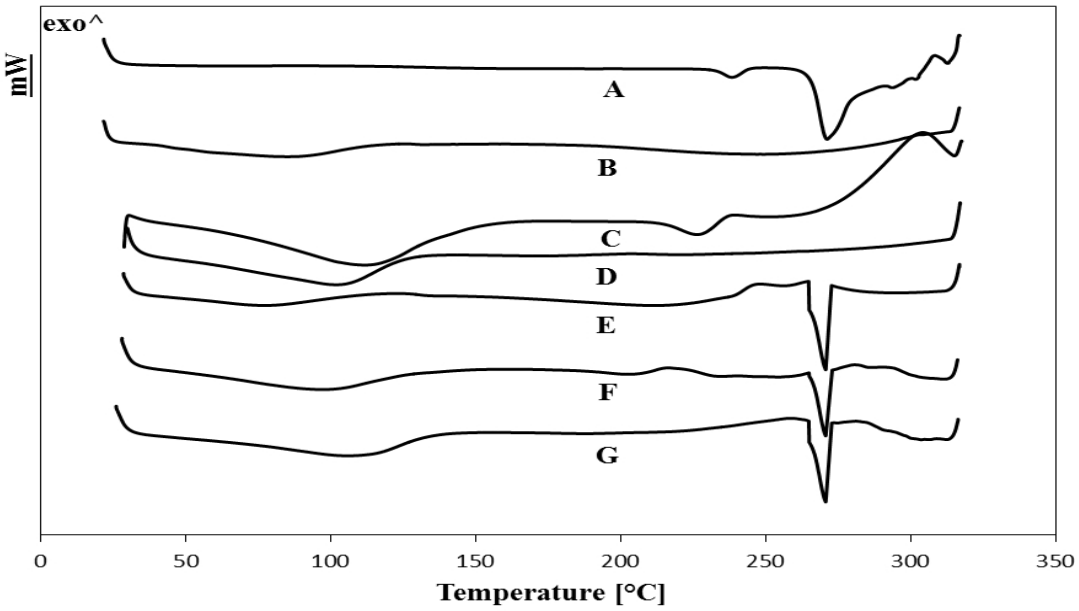

Fig. 2. DSC scans of (A) losartan potassium, (B) carbopol 934LR, (C) chitosan, (D) HPMC E15LV, (E) physical mixture of losartan potassium and carbopol 934LR, (F) physical mixture of losartan potassium and chitosan, (G) physical mixture of losartan potassium and HPMC E15LV

Different formulae powder blends showed an angle of repose in the range of 25.13±0.231° to 29.50±1.50°, as shown in Table 2. These results indicate that all formulae had excellent or at least good flow ability. The best flow ability was exhibited by the powder blends which were containing secondary polymers chitosan or HPMC E15LV (F3 and F5) may be due to that the presence of these polymers within the formula reduced the cohesive forces between carbopol 934LR particles which are very hygroscopic in nature and these cohesive forces affected the flow ability of the batches containing carbopol 934LR only (F1 and F2). Formulae F1 and F2 were also containing a higher quantity of mannitol which may adversely affected the flow properties. However, it was concluded that the powder blends of all formulae were free flowing and suitable for direct compression.

3.1.3.2 Bulk and tapped densities

The flow ability of different formulae powder blends was investigated by measuring both the bulk and tapped density and then, the Hausner ratio and Carr's index were calculated. The values for the bulk densities were in the range of 0.311±0.002 to 0.359±0.003g/cm^3. While the values for the tapped densities ranged from 0.393±0.017 to 0.615±0.017g/cm^3 as illustrated in Table 2.

3.1.3.3 Hausner ratio

This ratio can give an indication about the flow properties. The values less than 1.25 show better flow ability than values more than 1.25 [33].

Hausner ratio values for the formulae powder blends ranged from 1.263±0.053 to 1.714±0.062, as illustrated in Table 2. So, it was concluded that the powder blends which were containing chitosan showed the best flow ability.

3.1.3.4 Carr's index (compressibility %)

This index is indirectly related to the flow ability as a compressible material will be less flow able. The value of this index can affect the flow properties of solid materials. Index values between 5 and 12 have excellent flow ability; between 12 and 16 have good flow ability; between 18 and 21 have fair passable flow ability; between 23 and 35 have poor flow ability; while the values between 33 and 38 exhibit very poor flow ability [33].

The values of different powder blends ranged from 20.751±3.242 to 41.615±2.152%, as illustrated in Table 2. These results suggest that all formulae powder blends had fair or slightly poor flow ability except for powder blends which were containing carbopol 934LR alone (F1 and F2) as they showed very poor flowability and a glidant should be included within the formulae powder blends to become suitable for direct compression process.

According to the micromeritics study, it was concluded that the best flow characters were exhibited by the powder blend which was containing carbopol 934LR and chitosan 1:2 (F4) and the worst flow ability was shown from powder blends which were containing carbopol 934LR alone (F1 and F2) due to its hygroscopic nature and may be due to containing larger quantity of mannitol than other formulae powder blends.

Table 2. Micromeritics study of different formulae powder blend

Formula code	Angle of repose (±SD)	Bulk density (g/cm^3±SD)	Tapped density (g/cm^3±SD)	Hausner ratio (±SD)	Carr's index (±SD)
F1	29.167±1.258	0.353±0.007	0.546±0.017	1.547±0.069	35.263±2.920
F2	29.500±1.500	0.359±0.003	0.615±0.007	1.714±0.062	41.615±2.152
F3	25.133±0.231	0.318±0.005	0.415±0.017	1.306±0.075	23.259±4.403
F4	26.833±0.115	0.311±0.002	0.393±0.017	1.263±0.053	20.751±3.242
F5	26.200±0.300	0.345±0.012	0.448±0.008	1.299±0.068	22.895±4.076
F6	28.233±0.208	0.355±0.004	0.492±0.014	1.388±0.049	27.880±2.605

3.2 Evaluation of Prepared Bioadhesive Buccal Tablets

In the present study, bioadhesive tablets containing 25mg losartan potassium were prepared as shown in Fig. 3 (A) in six formulae with two different amounts (10 and 20mg/tablet) of the mucoadhesive polymer carbopol 934LR as a primary polymer with and without secondary polymers chitosan and HPMC E15LV which were added in two different amounts (40 and 50 mg/tablet). Selection of ratios of carbopol was on the basis of the main objective of the study which is a controlled release system. So, carbopol was added as a sustained release agent in the range of 5-30% w/w in addition to its role as a bioadhesive polymer [34]. Chitosan and HPMC were added by the same two ratios for comparison based on the concentration needed for cellulose derivatives to act as extended release matrix formers which is in the range of 15-35% w/w [34]. PVP K30 was added to act as a binder in a fixed concentration 4% w/w. Magnesium stearate was added as a lubricant. Mannitol was used as a diluent due to its pleasant taste. All batches of the bioadhesive tablets were subjected to post-compression evaluation which is illustrated in Table 3.

All tablets had an average weight in the range of 152.05±2.08 to 156.65±1.42mg. Tablets of different formulae passed the weight variation test according to the acceptable weight variation range stated in USP (±7.5%) for tablets with an average weight in the range of 80-250mg. Thickness of the tablets ranged from 3.21±0.05 to 3.51±0.07mm with a diameter in the range of 8.03±0.01 to 8.04±0.01mm which indicates fairly uniform ones.

All tablets had hardness in the range of 6.06±0.94 to 6.82±0.56Kg/cm^2. Hardness varied among different formulae according to the type and concentration of bioadhesive polymers. The hardness variation was minimized by modifying the hydraulic pressure of the tablet compress machine prior to compaction of each formula powder blend.

The percent weight loss of the tablets due to friability was in the range of 0.25±0.06 to 0.47±0.08%. The friability values indicated that none of the formulae exceeded 0.47% which was found to be in agreement with the acceptable friability range for tablets (not more than 0.5-1%). The results of hardness and friability tests showed that losartan potassium bioadhesive tablets were mechanically tough enough and can withstand the pressure and rigors during transportation and handling.

The percent drug content of all tablets was found to be in the range of 98.50±0.56 to 99.33±0.29% which is acceptable according to USP (2007) specifications for losartan potassium tablets (98.5-101%).

3.3 Surface pH

Compatibility of the buccal tablets with mucous membrane is an important issue. An irritation or even a serious damage for mucosal surface will occur if the tablets exhibit an extreme surface pH (acidic or alkaline) during the duration of application which may trigger the patient to terminate the treatment before gaining the full therapeutic efficacy.

Therefore, it was necessary to measure changes of the surface pH which occur within the tablets after the drug release. Tablets should have a surface pH in the range of salivary pH which is 5.6–7.9 [35]. Surface pH of different tablets was in the range of 5.50±0.05 to 6.73±0.10 as illustrated in Table 3. The surface pH differed according to the concentration and nature of the bioadhesive polymers. Carbopol 934LR has an acidic nature as it is composed of several acrylic acid units. So, incorporation or increasing carbopol concentration can shift surface pH of tablets to the acidic side. Chitosan and HPMC show a slightly acidic pH in aqueous solutions. So, tablets which were containing chitosan (F3 and F4) or HPMC (F5 and F6) showed lower surface pH values than the tablets contained carbopol only (F1 and F2). However, all tablets had surface pH values within the salivary pH range. So, it was concluded that all tablets can be tolerated by patients and cause no local irritation or damage to the buccal mucosal surface.

3.4 *In-vitro* Swelling Study

Swelling is an intrinsic property of most bioadhesive polymers may be due to that they are hydrophilic in nature and have gel forming ability. Swelling of the tablets can affect the drug release process, mucoadhesion strength, residence time and patient compliance.

Different formulae swelled by time when remained in contact with aqueous media Fig.3. due to absorption of moisture by different bioadhesive polymers as shown in Fig.4. Swelling index values of tablets after 7 h was in the range of 59.17±1.47 to 78.32±1.84%. Swelling index varied according to nature and concentration of bioadhesive polymers. Tablets which were containing chitosan (F3 and F4) showed higher swelling index values than other ones may be due to high gel forming capacity and insolubility of chitosan at pH>6.5. When carbopol concentration was increased, the tablets absorbed more moisture and the thickness of the gel layer formed on the surface of tablets increased. Swelling study showed the relative capacities of bioadhesive polymers for moisture absorption and whether the tablets maintained their integrity after swelling. According to the study, it was concluded that swelling index values of none of the tablets exceeded 78.32% after 7h and different tablets kept their integrity even after swelling. So, it was considered that all tablets had acceptable swelling index.

3.5 *Ex-vivo* Residence Time

Complete and effective drug delivery to the systemic circulation depends on the duration of bioadhesion of buccal tablets to the mucous membrane. So, it was necessary to determine the residence time of the delivery system inside the buccal cavity after its application and the bioadhesive force of different formulae tablets.

Different tablets showed long residence times suitable enough to deliver losartan potassium for at least 7.02±0.53h (F1) up to 10.5±0.50h (F4) as illustrated in Table 3. Residence time depended on nature and concentration of the bioadhesive polymers within the formula.

Carbopol 934LR had a strong bioadhesive property due to interpenetration of polymer chains into the mucus membrane which can be enhanced by increasing its concentration or addition of a secondary polymer such as chitosan and HPMC E15LV which showed good bioadhesive characters may be due to their ability to form a viscous gel layer at the surface of the tablets. Secondary polymers may also enhance water uptake ability of the matrix which promoted the interpenetration process.

3.6 *Ex-vivo* Bioadhesion Force

The force of bioadhesion of different formulae may affect the process of drug delivery and patient compliance. The drug delivery system should adhere with a sufficient strength and does not detach easily after its application due to normal physical movements of the oral cavity during talking or food mastication. However, very strong bioadhesion may cause a serious injury or damage to the mucous membrane.

Different batches showed bioadhesion force in the range of 0.15±0.02 to 0.38±0.01N as illustrated in Table 3. It depended on nature, concentration of bioadhesive polymers and gel layer viscosity. Carbopol 934LR showed a strong enough bioadhesion force may be due to formation of secondary bioadhesion bonds with mucin and interpenetration of polymer chains in the interfacial region. Bioadhesion force can be enhanced by increasing carbopol 934LR concentration or addition of a secondary bioadhsive polymer. Chitosan showed bioadhesive properties due to the ability of chitosan to interact with the mucous fluid which is rich in glycoproteins by combining amine groups with negatively charged groups on the tissue surface. HPMC E15LV showed a bioadhesive nature may be due to its ability to absorb water rapidly and form a viscous gel layer at the surface of tablets. Secondary polymers may also facilitate the interpenetration process or allow the tablets to form a highly viscous gel layer which promoted the force of bioadhesion.

3.7 *In-vitro* Drug Release Study

Drug release process in this study is critical since the main objective is to develop a controlled release drug delivery system. It is often performed to show the behavior of the delivery system in ideal situations, which could give some information about its *In-vivo* performance.

Different batches showed prolonged drug release by at least 57.64±3.43% (F4) after 8h except for batches which were containing HPMC E15LV (F5 and F6). They showed a cumulative drug release percentage of 33.55±1.11% and 24.26±1.95%, respectively after 8h. Drug release from different batches depended on nature and concentration of bioadhesive polymers. Batches which were containing secondary polymers and higher concentration of bioadhesive polymers (F3-F6) showed a more sustained release than other batches. The bioadhesive polymers swell when comes in contact with aqueous media due to water uptake and formation of a gel layer at the surface of the tablets which varied in terms of thickness and viscosity among different batches. Swelling index of different formulae increased as a result of increasing carbopol 934LR which exposed the drug to a longer path of diffusion and this inversely affected the drug release process. Although Chitosan based formulae (F3 and F4) showed a higher swelling index, they had a higher cumulative drug release percentage after 8h than HPMC E15LV based formulae which showed a very slow drug release and this may be due to that chitosan formed a less viscous gel layer than HPMC E15LV which facilitated the drug release from the system.

Table 3. Post-compression evaluation of different formulae

Formula code	Hardness (Kg/cm^2±SD)	Thickness (mm±SD)	Diameter (mm±SD)	Friability (%±SD)	Weight variation (mg±SD)	Drug content (%±SD)	Surface pH (±SD)	*Ex-vivo* residence time (h±SD)	*Ex-vivo* bioadhesion force (N±SD)
F1	6.12±0.74	3.21±0.05	8.03±0.01	0.47±0.08	152.25±2.46	98.50±0.56	6.73±0.10	7.02±0.53	0.15±0.02
F2	6.06±0.94	3.29±0.03	8.03±0.01	0.41±0.02	152.05±2.08	99.00±0.50	6.60±0.16	8.03 ±0.45	0.20±0.02
F3	6.19±0.65	3.24±0.04	8.04±0.01	0.34±0.02	154.73±2.01	98.77±0.31	6.12±0.07	9 ±0.50	0.29±0.01
F4	6.82±0.56	3.39±0.03	8.04±0.01	0.25±0.06	155.50±1.47	99.33±0.29	6.08±0.23	10.5±0.50	0.38±0.01
F5	6.08±0.61	3.51±0.07	8.04±0.01	0.41±0.05	154.90±1.67	98.60±0.36	6.07±0.04	8.15±0.35	0.22±0.01
F6	6.48±0.56	3.48±0.04	8.04±0.01	0.32±0.06	156.65±1.42	98.79±0.20	5.50±0.05	10±0.41	0.26±0.01

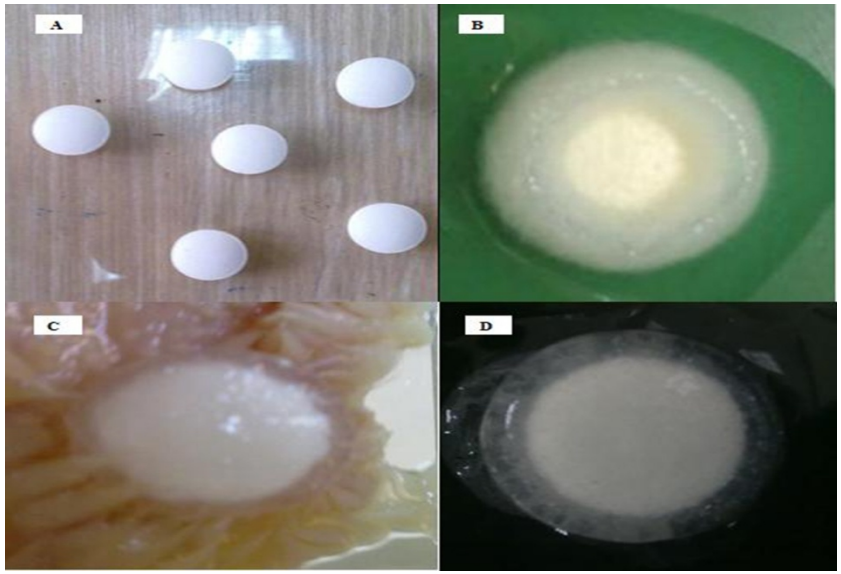

Fig. 3. Photographs of (A) Bioadhesive tablets after compression, (B) Chitosan based tablets swelling during surface pH study, (C) Carbopol 934LR based tablets swelling during residence time study, (D) Swollen HPMC E15LV based tablets at the end of the drug release study

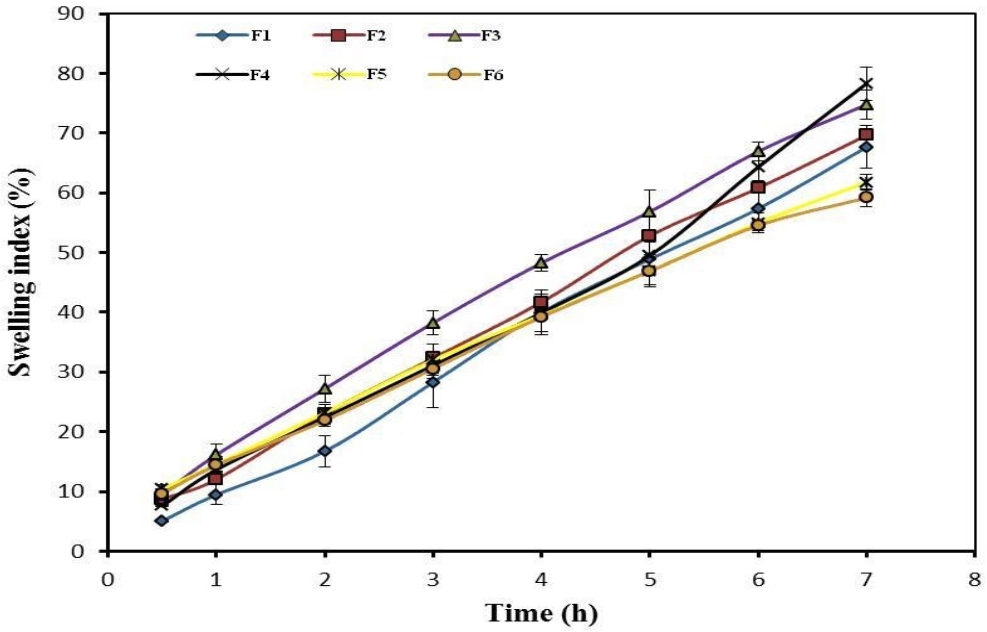

Fig. 4. Swelling index profile of different formulae

Formula which was containing carbopol 934LR and chitosan in the ratio of 1:2 (F4) showed an ideal drug release profile as shown in Fig.5. which was the most suitable one for a controlled release delivery system. It showed a cumulative drug release less than 20% after 2h and less than 60% after 8h. So, it was supposed that the drug release process may continue for at least 12h.

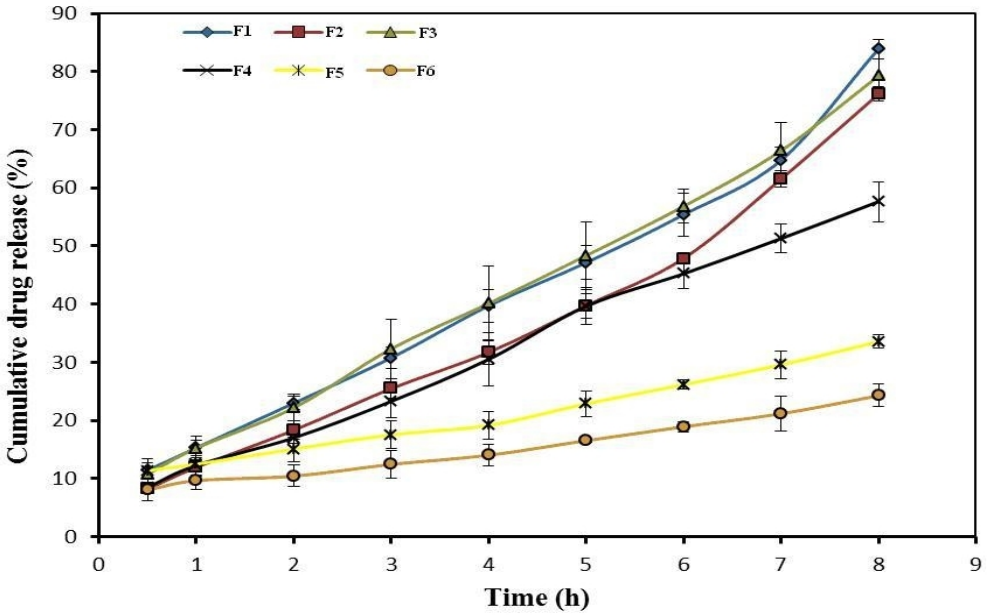

Fig. 5. *In-vitro* drug release profile of different formulae

3.8 Drug Release Kinetics Study

Kinetics study was carried out to determine the order of drug release process and the mechanism by which the drug was released from the delivery system. Different kinetic models were applied to analyze the results of *In-vitro* release study which are zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas model. According to the highest regression coefficient as illustrated in Table 4, it was concluded that all formulae released losartan potassium in a constant rate according to zero order kinetics model which may be an important feature for a sustained release delivery system. The most uniform drug release pattern was showed by the tablets which were containing chitosan as a secondary polymer (F3 and F4). Drug release mechanism from all formulae was by dissolution according to Hixson-Crowell kinetics model except formulae contained carbopol 934LR only (F1 and F2) and the formula contained carbopol 934LR and chitosan in the ratio of 1:5 (F3) which released the drug by diffusion mechanism according to Korsmeyer-Peppas model. Release exponent (n) of Korsmeyer-Peppas model indicated that the type of diffusion of losartan potassium from formulae (F1, F2 and F3) was non-fickian (anomalous) as $0.43<n<0.89$ [25]. The bioadhesive delivery system of losartan potassium showed a uniform, prolonged and constant rate drug release which may be an ideal property for a controlled release system.

Table 4. Kinetics analysis of drug release from different formulae

Formula code	Zero order	First order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas	
	r^a	r^a	r^a	r^a	r^a	n^b
F1	0.9905	0.9227	0.9631	0.9537	0.9919	0.6435
F2	0.9908	0.9539	0.9595	0.9696	0.9940	0.7057
F3	0.9980	0.9835	0.9893	0.9929	0.9931	0.6761
F4	0.9979	0.9866	0.9749	0.9918	0.9894	0.7062
F5	0.9786	0.9733	0.9479	0.9753	0.9559	0.3849
F6	0.9837	0.9824	0.9741	0.9829	0.9530	0.3822

[a]Regression coefficient; [b]Release exponen

3.9 Selection of Optimum Formula

General descending ranking order of different formulae according to different parameters which are *In-vitro* swelling study, surface pH, *Ex-vivo* residence time, *Ex-vivo* mucoadhesion, *In-vitro* drug release and kinetics study as illustrated in Table 5. revealed that formulae containing chitosan as a secondary polymer (F3 and F4) are the optimum formulae. Since the major objective of the study is to control and prolong the delivery of losartan potassium using a mucoadhesive buccal system, *In-vitro* drug release, *Ex-vivo* residence time and *Ex-vivo* mucoadhesive strength were selected as high priority parameters among others. Formula containing carbopol 934LR and chitosan in the ratio of 1:2 (F4) showed superior results according to the high priority parameters over the other formula (F3). So, it was concluded that the optimum formula is F4.

3.10 *Ex-vivo* Permeation Study

This study was performed to ensure that losartan potassium was released from the optimum formula (F4) and then permeated through the buccal mucous membrane which can be considered as an indicator for *In-vivo* performance of the drug.

Table 5. Ranking order of different formulae according to different parameters

Formula code	Ranking order of different formulae								
	Swelling study	Surface pH	Residence time*	Mucoadhesive strength*	Drug release*	Kinetics study	Total	General final ranking	High priority ranking
F1	4	1	6	6	1	4	22	4	4
F2	3	2	5	5	3	3	21	3	4
F3	2	3	3	2	2	1	13	1	2
F4	1	4	1	1	4	2	13	1	1
F5	5	5	4	4	5	6	29	6	4
F6	6	6	2	3	6	5	28	5	3

* *High priority parameters*

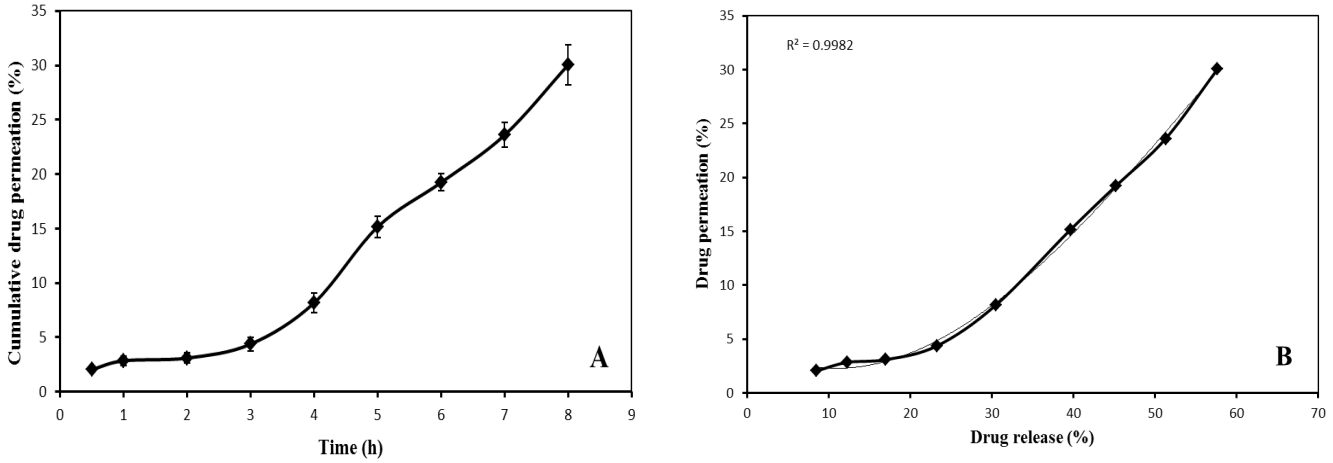

Fig. 6. (A) *Ex-vivo* permeation of losartan potassium from optimum formula F4 through sheep buccal mucosa. (B) Correlation between *In-vitro* drug release and *Ex-vivo* permeation of losartan potassium for optimum formula F4

Sheep buccal mucous membrane is a suitable membrane model for this study. It is cheap, easy to be obtained, easy to maintain and mimics human buccal mucous membrane in terms of keratinization as both are non-keratinized.

The study proved that losartan potassium was released from the buccal tablets and permeated slowly as shown in Fig.6 (A) may be due to that losartan potassium belongs to class III BCS drugs which have high aqueous solubility and low permeability. Losartan potassium showed a cumulative permeation of 30.05±1.87% through sheep buccal mucosa within 8h and the steady state permeation flux (J_{ss}) was 0.9599mg/cm^{2}h. Diffusion coefficient (D) of losartan potassium was 0.0019 cm^2/h and permeability coefficient (P) was 0.0259 cm/h.

Correlation between *In-vitro* drug release and *Ex-vivo* drug permeation showed that there was a good correlation with a regression coefficient (r) of 0.9991 as shown in Fig.6 (B). Sharp increase in permeation of losartan potassium after 30% drug release which was within 4h may be due to release of chitosan from polymer matrix and interaction with tight junction of the epithelial layer which facilitated the paracellular transport of the hydrophilic drug.

3.11 Physical Stability of Buccal Tablets

Stability study in normal human saliva was carried out to ensure that the tablets can withstand the surrounding environment of the oral cavity without collapse or any major changes in physical properties. The study showed that the tablets of optimum formula (F4) remained intact for up to 6h with no color changes. Thickness and diameter increased to 3.96±0.07 and 8.93±0.06mm, respectively after 6h due to absorption of moisture and swelling of the tablets. According to this study, the tablets can retain their physical characters during the period of application to the buccal mucous membrane which may ensure a consistent drug delivery and therapeutic response.

4. CONCLUSION

A simple controlled release delivery system for losartan potassium based on bioadhesion using a combination of bioadhesive polymers (40 % w/w) of carbopol 934LR and chitosan in the ratio of 1:2 can be an optimum way to administer such drugs. Pharmacokinetics study, *In-vivo* studies and controlled clinical studies are still required before the launch of the product into the market.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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