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DESIGN, DEVELOPMENT AND EVALUATION OF MUCOADHESIVE PATCHES OF LOSARTAN FOR BUCCAL DELIVERY

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ABSTRACT

The buccal region offers an attractive route of administration for systemic drug delivery. Losartan is an angiotensin II receptor antagonist readily absorbed from the GIT, following oral administration. It has low bioavailability as it undergoes extensive first pass metabolism and low elimination half life. The present study was aimed at studying controlled release behavior of the drug using hydrophilic and hydrophobic polymers. The mucoadhesive polymers used in the formulation were ethylcellulose (hydrophobic), hydroxypropyl methyl cellulose and polyvinyl pyrrolidone (hydrophilic). These polymers were used to evaluate the effect of hydrophilic and hydrophobic polymers on the release pattern of the drug. Various mucoadhesive buccal films were prepared by employing EC alone, HPMC alone, PVP alone and in combination of all polymers in different ratios by solvent casting method using ethanol, and water as solvents, propylene glycol as plasticizer. The prepared mucoadhesive buccal films were evaluated for their physicochemical parameters such as weight uniformity, thickness uniformity, folding endurance, drug content, surface pH, swelling index, *in vitro* release studies, *ex vivo* mucoadhesion time, *ex vivo* permeation studies and stability studies. Patches exhibited controlled release for a period of 10 hrs. The mechanism of drug release was found to be Fickian diffusion and followed the zero-order kinetics. The mucoadhesive buccal patches of Ethylcellulose-Hyroxypropyl methyl cellulose in ratio of 1:4 (EH4) was concluded to be optimized. The optimized patches showed comparable swelling index, significantly higher mucoadhesive strength, higher *in-vitro* drug release, more mucoadhesion time and more cumulative percentage of drug permeated than mucoadhesive buccal patches of ethylcellulose-polyvinyl pyrrolidone in ratio of 1:4 (EP4). From the above results, it can be concluded that blends of hydrophobic and hydrophilic polymers is better than single polymer

to obtain sustained drug release and can be used to formulate mucoadhesive buccal patches of losartan to bypass first pass metabolism and hence bioavailability of losartan.

KEYWORDS: Losartan, mucoadhesive, buccal delivery, hydrophilic and hydrophobic polymers.

1. INTRODUCTION

Buccal delivery of drugs provides an attractive alternative to other conventional methods of systemic drug administration, since buccal mucosa is relatively permeable with rich blood supply and acts as an excellent site for the absorption of drugs.[1,2,3] The administration of drugs via buccal route facilitates a direct entry of drug molecules into the systemic circulation, avoiding the first-pass metabolism and drug degradation in the harsh gastrointestinal environment, which are often associated with oral administration.[4,5,6] The buccal cavity is easily accessible for self medication, and hence it is safe and well accepted by patients. Buccal drug delivery offers a safer method of drug delivery, since drug action can be promptly terminated in case of toxicity by removing the dosage from the buccal cavity. The term bioadhesion is typically used to describe the adhesion between polymer either synthetic or natural to soft tissue. In instances when bond is formed between mucus membrane and polymer, the term mucoadhesion is used.[7,8] Mucus membrane is one, in which the goblet cells are present for the secretion of mucus, which is composed of glycoprotein mucin[9]. Buccal mucosa presents a relatively smooth and immobile surface for the placement of mucoadhesive dosage form. Mucoadhesive buccal patches may be preferred over adhesive tablets in terms of flexibility and comfort and also they do not get easily washed away or removed by saliva as may in case of oral gels[10,11,12,13].

Losartan, an angiotensin II receptor antagonist is used in the management of high blood pressure (hypertension). Losartan is rapidly absorbed following an oral dose but undergoes extensive first pass metabolism, resulting in only 25-35% oral bioavailability. The half-life of losartan is 2 hours.[14,15] Hence, the aim was to prepare mucoadhesive buccal patches of losartan to ensure satisfactory drug release in oral cavity with the use of blends of hydrophilic and hydrophobic polymers and thereby to avoid first pass metabolism and prolong duration of action. The influence of variables such as concentration of the hydrophilic and hydrophobic polymers on *in-vitro* release profiles of patches prepared by solvent casting method was determined.

2. MATERIAL AND METHOD

2.1 Material

Losartan was obtained as a gift sample from Jackson Laboratory Pvt. Ltd., Amritsar, India. Ethylcellulose, Hydroxypropyl methyl cellulose (HPMC $K_{15}M$), Polyvinyl pyrrolidone (PVP $K_{30)}$ were purchased from S.D. Fine chemicals Ltd., Mumbai, India. Other chemicals used were of analytical grade.

2.2 METHODS

2.2.1 Formulation of Losartan Loaded Mucoadhesive Buccal Patches

Mucoadhesive patches were prepared by solvent casting method.

2.2.1.1 Formulation of Ethylcellulose-HPMC mucoadhesive buccal patches

Ethylcellulose solution was prepared in 50 ml of ethanol and HPMC solution was prepared in 50 ml of distilled water. Losartan was accurately weighed in quantity such that 2 cm^2 patches contained 50 mg drug and then dissolved in above prepared ethylcellulose solution. Ethylcellulose solution containing drug was added to HPMC solution with constant stirring on a magnetic stirrer. Propylene glycol 1% w/v was added as plasticizer.

2.2.1.2 Formulation of Ethylcellulose-PVP mucoadhesive buccal patches

Ethylcellulose solution was prepared in 50 ml of ethanol and PVP solution was prepared in 50 ml of distilled water. Losartan was accurately weighed in quantity such that 2 cm^2 patches contained 50 mg drug and then dissolved in above prepared ethylcellulose solution. Ethylcellulose solution containing drug was added to PVP solution with constant stirring on a magnetic stirrer. Propylene glycol 1% w/v was added as plasticizer. The above prepared solutions of ethylcellulose-HPMC and ethylcellulose-PVP were then homogenized for 2 hrs and casted on a specially fabricated Teflon coated petridish by placing on a leveled surface. Inverted funnel was kept over the petridish to avoid sudden evaporation. Patches were then allowed to dry at room temperature for 2 hrs and further dried in a hot air oven at 40^o C for 48 hrs. The dried patches were carefully examined for imperfections or entrapped air bubbles and cut into 2 cm^2 patches (equivalent to 50 mg of drug). Then the patches were packed in an aluminium foil and stored in an air tight glass container to maintain their integrity and elasticity.

2.2.2 Physicochemical characterization of formulated buccal patches

A. Weight uniformity and Thickness

The assessment of weight and patch thickness was done in 3 different randomly selected patches from each formulation. Patches were directly weighed on a digital balance and patch thickness was measured at 5 different randomly selected spots on patches using a screw gauge. [16]

B. Folding endurance

Folding endurance of patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 200 times without breaking. [17]

C. Drug content uniformity

Drug content uniformity was determined by dissolving the patch in 100 ml of an isotonic phosphate buffer (pH 6.8) for 8 h by homogenization under occasional shaking. Then 5 ml solution was taken and diluted with isotonic phosphate buffer pH 6.8 up to 20 ml, and the resulting solution was filtered through a 0.45 µm Whatman filter paper. The drug content was then determined after proper dilution at spectrophotometer. The experiments were carried out in triplicate. [18]

D. Surface pH Determination

A combined glass electrode was used for this purpose. The patches were allowed to swell by keeping them in contact with 1 ml of distilled water (pH 6.8±0.1) for 2 hrs at room temperature, and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min. The surface pH of the patches was determined in order to investigate the possibility of any side effects in the oral cavity. As acidic or alkaline pH is bound to cause irritation to the buccal mucosa, hence attempt was made to keep the surface pH of the patches close to the neutral pH [19].

2.2.3 Measurement of mucoadhesive strength

Preparation of buccal mucosa

Fresh goat buccal mucosa was obtained from a local slaughter house and used within 2 hrs of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer pH 6.8 as moistening fluid. A modified balance method was used for determining the *ex-vivo* mucoadhesive strength. Goat buccal mucosa was fixed on the plane surface of glass slide attached (with adhesive tape) to bottom of smaller beaker, kept inverted in 500 ml beaker

attached to the bigger beaker. Phosphate buffer pH 6.8 was added to the beaker up to the upper surface inverted beaker with buccal mucosa. The buccal patch of 4cm^2 was stuck to the lower side of the upper clamp with cyanoacrylate adhesive. The exposed patch surface was moistened with phosphate buffer pH 6.8 and left for 30 sec for initial hydration and swelling. Then the platform was slowly raised until the patch surface came in contact with mucosa. Two sides of the balance were made equal before study by keeping a weight on the right hand pan. A weight of 5 g was removed from the right hand pan, which lowered the pan along with the patch over the mucosa. The balance was kept in this position for 5 minutes contact time. Then weights were slowly added to the right hand pan until the patch detached from the mucosal surface. This detachment force gave the mucoadhesive strength of the buccal patches in grams. Force of adhesion was calculated from the mucoadhesive strength. [20]

Force of adhesion (N) = Mucoadhesive strength/1000*9.81

2.2.4 *In vitro* Swelling Studies: The degree of swelling of mucoadhesive polymer is important factor affecting adhesion. Upon application of the mucoadhesive material to a tissue, a process of swelling may occur. Buccal patch was weighed (W_1), placed in phosphate buffer solution pH 6.8 at 37±0.5^{o}C. After regular time intervals (upto 10 h), the patches were removed from the petri dish and excess surface moisture was removed carefully using the filter paper. The swollen mucoadhesive patches were then reweighed (W_2) and the swelling index was calculated. [21]

$$\text{Swelling Index} = \frac{W_2 - W_1}{W_1}$$

2.2.5 *In vitro* release studies

The release of Losartan from the prepared mucoadhesive buccal patches of 4 cm^2 was carried out in phosphate buffer pH 6.8 at 37 ± 0.5^{0}C. Each mucoadhesive patch was adhered to the side wall of a vessel (100 ml beaker) using cyanoacrylate. Adequate sink conditions were provided by placing 50 ml of phosphate buffer pH 6.8 in each vessel. Each covered vessel was fitted with a magnetic stirrer rotating at a rate of approximately 150 rpm. After time intervals each of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 hours, 3 ml sample was withdrawn, filtered through a whattman filter paper and assayed spectrophotometrically. Immediately after each sample withdrawal, a similar volume of phosphate buffer pH 6.8 was added to the release medium to maintain the volume in the vessel constant [22].

2.2.6 Release kinetics

To study the release kinetics, data obtained from *in-vitro* dissolution study was fitted in various kinetic models: zero order as cumulative percent of drug released *vs.* time, first order as log cumulative percentage of drug remaining *vs.* time and Higuchi's model as cumulative percent drug released *vs.* square root of time, Hixon crowel describes the release from systems when there is a change in a surface area and diameter of particles. To determine the mechanism of drug release, the data was fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released *vs.* log time and the exponent n was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is fickian; if 0.5<n <1.0, then it is anomalous transport. If n is 1.0, it is case II transport and if n>1.0, then it is super case II transport. [23]

2.2.7 Selection of formulations

Selection of formulations was done on the basis of results obtained from swelling studies, mucoadhesive strength and *in vitro* release studies. After the selection of batches further studies (*ex vivo* mucoadhesion time and *ex vivo* permeation studies) were performed.

2.2.8 *Ex vivo* mucoadhesion time

Preparation of simulated saliva

According to IP, Sodium chloride 4.5g, potassium chloride 0.3g, sodium sulphate 0.3g, ammonium acetate 0.4g, urea 0.2g, lactic acid 3g and distilled water up to 1000ml, adjusting pH of solution to 6.8 by 1 M sodium hydroxide solution.

The residence time for the formulation, that is, the time taken for the patch to detach or erode completely from the mucosa was measured *ex vivo*, by application of the patch on freshly excised goat buccal mucosa. The goat buccal mucosa was cut to an appropriate size of a 9 cm^2 square and fixed on the internal side of a beaker with cyanoacrylate glue. The patch of 4cm^2 was first wetted with simulated saliva fluid and attached to the goat buccal tissue by applying light pressure with a finger tip for 20 seconds. The beaker was filled with 200 ml simulated saliva fluid and kept at 37^0C on a magnetic stirrer. After two minutes, a 50 rpm stirring rate was applied to simulate the buccal cavity environment, and during the test, the time taken for the patch to completely erode or detach from the mucosa was observed as the *ex vivo* mucoadhesion time. [24]

2.2.9 *Ex vivo* Permeation studies

Permeation studies were carried out, to evaluate the permeability of drug across the buccal mucosal membrane, by using glass surface Franz diffusion cell.

Goat buccal mucosa was obtained from local slaughter house and used within 2 hrs of slaughter. The tissue was stored in phosphate buffer pH 6.8 solution upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and clamped in between donor and receiver chambers of the diffusion cells for permeation studies. Receptor compartment contained 21 ml of pH 6.8 phosphate buffer while donor compartment was filled with 3 ml simulated saliva of pH 6.8. The patch was placed on the mucosal surface in donor compartment and 2 ml aliquots were removed at time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 hours from the receptor compartment while the solution was being stirred continuously using magnetic stirrer, replacing it with fresh 2 ml medium each time. The experiment was carried out at 37 ± 0.5^0C. Samples were analysed spectrophotometrically as for dissolution samples (Supriya *et al*., 2008). The drug permeated per cm^2 of patch was calculated and plotted against time and the flux was calculated as drug permeated per cm^2 per hr. [25] The steady state was determined from the slope of the linear portion of a cumulative amount of drug permeated *vs*. time plot. The lag time was determined by extrapolating the linear portion of the cumulative amount permeated *vs*. time curve.

2.2.10 Permeation kinetics

To study the permeation kinetics, data obtained from permeation studies were fitted in various kinetic models: zero order as cumulative percent of drug released *vs*. time, first order as log cumulative percentage of drug remaining *vs*. time and Higuchi's model as cumulative percent drug released *vs*. square root of time. To determine the mechanism of drug permeate, the data were fitted into Korsmeyer and Peppas equation as log cumulative percentage of drug released *vs*. log time, and the exponent n was calculated from slope of the straight line. For slab matrix, if exponent is 0.5, then diffusion mechanism is fickian; if 0.5< n< 1.0, mechanism is non-fickian, n=1 to case II (relaxation) transport, and n> 1 to super case II transport.

2.2.11 Stability studies

2.2.11.1 Stability studies in human saliva

The stability study of patches was performed in natural human saliva. Samples of human saliva were collected from 10 humans (ages 18-40 years) and filtered. The patches were placed in separate petridishes containing 5 ml of human saliva and put in temperature-

controlled oven at $37^0C \pm 0.2^0C$ for 10 hrs. At regular time intervals, patches were examined for changes in colour, shape, collapse and physical stability.

2.2.11.2 Stability studies at room temperature and incubator temperature: Stability of the product may be defined as the capability of a particular to remain with the physical, chemical, therapeutic and toxicological specifications. The patches were stored in aluminium foil at room temperature 25 ± 2^0C and $60 \pm 5\%$ RH and in incubator 40 ± 2^0C and $75 \pm 5\%$ RH for 2 months (according to ICH guidelines). Parameters include mucoadhesive strength and drug release were evaluated at predetermined time intervals i.e. 30 and 60 days. [26]

2.2.12 Comparison of optimized formulations of EC-HPMC and EC-PVP mucoadhesive buccal patches: On the basis of results obtained from swelling studies, mucoadhesive strength, *in-vitro* release studies, *ex-vivo* mucoadhesion time and *ex-vivo* permeation studies, comparison of the optimized formulations of EC-HPMC and EC-PVP was done.

2.2.13 Statistical Analysis

Graph pad prism 5 was used for statistical analysis. All studies were done in triplicates unless specified and data represent the mean ± SD. The statistical analysis was performed using student's t-test. A difference below the probability level was considered statistical significant.

3. RESULT AND DISCUSSION

3.1 Formulation of Losartan Loaded Mucoadhesive Buccal Patches

Seventeen formulations with different concentrations of polymers, each containing 50 mg of drug and propylene glycol (1%w/v) as plasticizer were prepared by solvent casting method and were evaluated.

Table 1: Composition of formulated mucoadhesive buccal patches.

S. No	Formulation Code	Polymer Ratio	Ethyl cellulose (mg)	Hydroxypropyl methylcellulose (HPMC $K_{15}M$) (mg)	Polyvinyl pyrrolidone (PVP K_{30}) (mg)	Losartan (mg)
1.	PE	-	600	-	-	50
2.	PH	-	-	600	-	50
3.	PP	-	-	-	600	50
4.	EH1	1:1	300	300	-	50
5.	EH2	1:2	200	400	-	50
6.	EH3	1:3	150	450	-	50
7.	EH4	1:4	120	480	-	50
8.	EH5	2:1	400	200	-	50
9.	EH6	3:1	450	150	-	50

10.	EH7	4:1	480	120	-	50
11.	EP1	1:1	300	-	300	50
12.	EP2	1:2	200	-	400	50
13.	EP3	1:3	150	-	450	50
14.	EP4	1:4	120	-	480	50
15.	EP5	2:1	400	-	200	50
16.	EP6	3:1	450	-	150	50
17.	EP7	4:1	480	-	120	50

Concentration of propylene glycol (1%w/v) was kept constant in all the formulations.

3.2 Physicochemical characterization of formulated mucoadhesive buccal patches

The prepared mucoadhesive buccal patches were evaluated for various physicochemical parameters and the results are depicted in Table 2.

3.2.1 Weight uniformity and Thickness

Weight of all formulations ranged between 124.8±0.33 mg to 137.3±0.82 mg. This shows the uniformity in weight of all formulations.

From the results, it is evident that the thickness of the patches varied from 0.33±0.14 mm to 0.52±0.63 mm. This shows the uniformity in thickness.

3.2.2 Folding endurance

It is clear from results that the folding endurance increased with increase in HPMC and PVP concentration up to 209±10 and 206±8 no. of folds respectively. Folding endurance test results indicated that the patches would maintain the integrity with buccal mucosa when applied.

3.2.3 Drug content uniformity

Good uniformity in drug content was found among different formulations of mucoadhesive buccal patches and the percentage of drug content varied from 96.21 ± 0.36% to 99.83 ± 0.44%.

Table 2: Physicochemical parameters of the formulated mucoadhesive buccal patches.

Formulation Code	Weight Uniformity (mg)	Thickness (mm)	Drug Content (%)	Folding Endurance (no. of folds)	Surface pH
PE	124.8 ± 0.33	0.39 ± 0.15	97.63 ± 0.43	199 ± 8	4.6 ± 0.08
PH	129.5 ± 0.62	0.48 ± 0.25	96.21 ± 0.36	205 ± 11	4.16 ± 0.05
PP	131.4 ± 0.48	0.35 ± 0.18	97.55 ± 0.24	201 ± 9	4.34 ± 0.11
EH1	136.6 ± 0.82	0.41 ± 0.36	98.76 ± 0.26	199 ± 7	6.8 ± 0.06
EH2	130.3 ± 0.42	0.34 ± 0.14	97.09 ± 0.62	202 ± 10	6.7 ± 0.11

EH3	134.5 ± 0.94	0.49 ± 0.49	98.36 ± 0.67	207 ± 9	6.9 ± 0.04
EH4	128.2 ± 0.54	0.38 ± 0.12	99.83 ± 0.44	209 ± 10	7.0 ± 0.05
EH5	125.6 ± 0.31	0.46 ± 0.85	97.70 ± 0.85	193 ± 5	6.6 ± 0.03
EH6	126.7 ± 0.94	0.40 ± 0.45	96.82 ± 0.15	195 ± 7	6.9 ± 0.06
EH7	129.1 ± 0.82	0.35 ± 0.76	98.63 ± 0.11	197 ± 12	7.0 ± 0.09
EP1	125.9 ± 0.84	0.34 ± 0.49	97.32 ± 0.41	194 ± 7	6.7 ± 0.11
EP2	127.9 ± 0.46	0.39 ± 0.14	98.26 ± 0.43	196 ± 15	6.9 ± 0.08
EP3	131.6 ± 0.34	0.52 ± 0.63	98.31 ± 0.54	200 ± 8	6.8 ± 0.09
EP4	126.3 ± 0.88	0.35 ± 0.44	99.25 ± 0.39	206 ± 8	7.1 ± 0.04
EP5	129.4 ± 0.96	0.40 ± 0.21	96.22 ± 0.43	190 ± 10	6.7 ± 0.05
EP6	137.3 ± 0.82	0.38 ± 0.53	97.63 ± 0.26	192 ± 8	7.0 ± 0.11
EP7	133.8 ± 0.34	0.36 ± 0.38	98.00 ± 0.65	193 ± 11	7.1 ± 0.08

3.2.4 Surface pH Determination

The surface pH of the buccal patches was determined to optimize both drug release and mucoadhesion. The surface pH of the formulation of formulations EH1-EH7 (EC- HPMC) and EP1-EP7 (EC- PVP) was found to be within 6.6±0.03 to 7.1±0.08 units. Therefore, these formulations should not cause any irritation to the buccal mucosa. The pH of the mucosa is reported to be 6.8[27]. Therefore the formulations meant for delivering the drug directly to the buccal mucosa must have almost similar pH to avoid any harmful effect of the dosage form.

The pH of the formulation containing EC, HPMC and PVP alone was found to be 4.6, 4.16 and 4.34 respectively which is acidic in nature. Therefore the patches comprising of EC, HPMC and PVP alone although were mucoadhesive, cannot be used for formulation of mucoadhesive patches since these can cause damage to buccal mucosa due to their acidic pH.

3.3 Measurement of mucoadhesive strength

The mucoadhesive patches formulated using ethyl cellulose alone depicted very less mucoadhesive strength while formulation PH containing HPMC alone showed the maximum mucoadhesive strength. Results indicated that the force required to detach the patches from the mucosal surface increased with the increase in HPMC and PVP concentration in formulations EH1-EH4 (EC-HPMC) and formulations EP1-EP4 (EC-PVP).This behaviour could possibly be attributed to the amount and nature of the polymer particles. The HPMC and PVP particles were finer and higher in quantity and so provided greater surface area for contact with the mucus membrane. As a result, mucoadhesion was enhanced since the patch contained higher amount of HPMC and PVP. Mucoadhesive interaction may result from hydrogen bonding or other types of bonding made possible by the hydrophilic nature of HPMC and PVP.

As the concentration of EC increased in formulations EH5-EH7 (EC-HPMC) and formulations EP5-EP7 (EC-PVP), the mucoadhesive strength was found to increase. This may be due to combination of hydrophilic and hydrophobic nature which gains the bond strength with mucosal surface (Figure 1 and Figure 2).

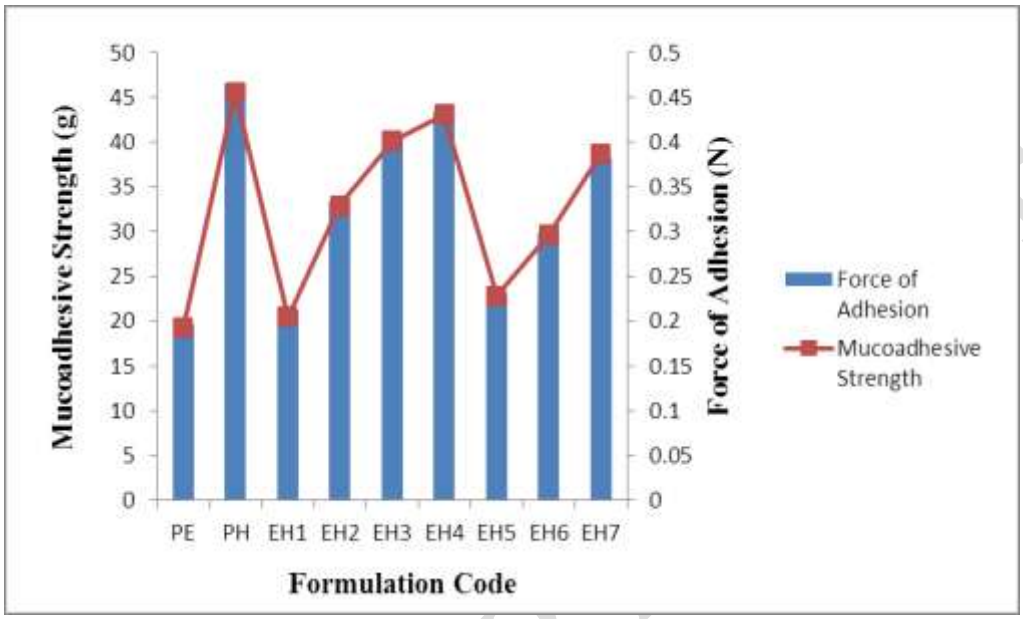

Figure 1: Mucoadhesive strength of buccal patches of EC-HPMC.

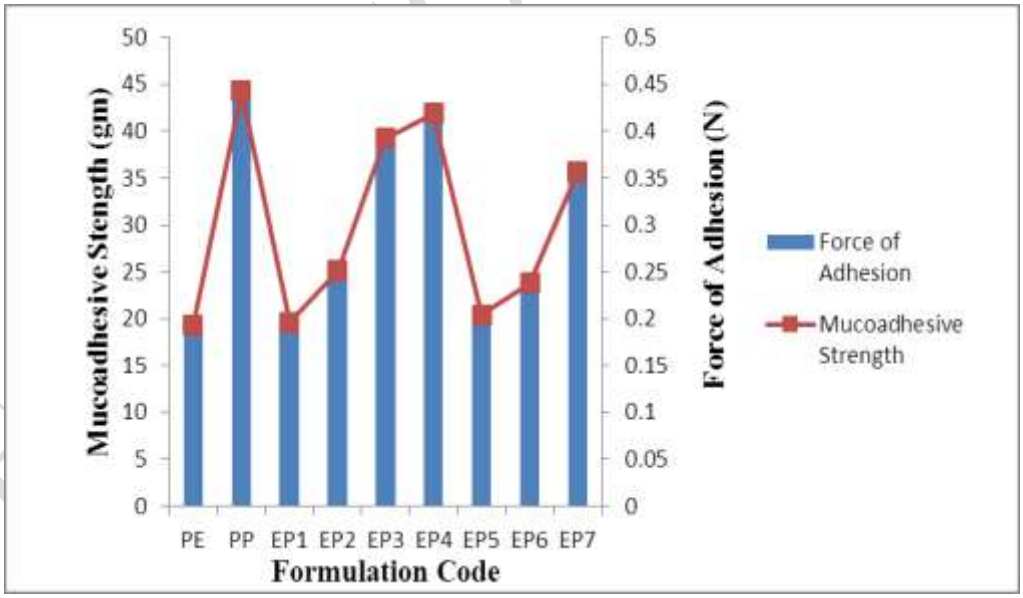

Figure 2: Mucoadhesive strength of buccal patches of EC-PVP.

3.4 *In vitro* Swelling Studies

Swelling index of all the investigational formulations i.e. formulations containing EC, HPMC, PVP, EC-HPMC patches (formulation EHI-EH7) and EC-PVP patches (formulation

EP1-EP7) was monitored for 10 hrs in phosphate buffer pH 6.8 (Figure 3, Figure 4 and Figure 5). Mucoadhesive buccal patches containing EC alone (formulation PE) showed least swelling index of 149.6 ± 0.58 % within 10 hrs as EC is water insoluble and less hydrophilic and therefore subject to lesser swelling upon hydration. Mucoadhesive buccal patches comprising of HPMC alone (formulation PH) and PVP alone (formulation PP) showed 274.46 ± 0.52 % and 270.34 ± 0.39 % swelling index respectively within 10 hrs due to their hydrophilic nature leading to greater swelling upon hydration. This can be explained on the basis that when the patch is placed in an aqueous medium, liquid penetrates into the patch and a gel is formed. Uptake of water results in relaxation of originally stretched, entangled or twisted polymer chain resulting in exposure of all polymer mucoadhesive sites for bonding to occur. As a result, the diameter of the patch increases progressively. Being hydrophilic in nature, HPMC and PVP after hydration and swelling, goes into solution and erodes.

The results of the swelling studies indicated that the rate of swelling was the function of HPMC and PVP concentration because an increase in concentration of these hydrophilic polymers showed increased swelling index. The highest swelling was seen for formulations EH4 and EP4. Both these formulations contain a high ratio of water soluble polymer HPMC and PVP respectively. It was observed that there was a proportionate increase in swelling of patch as the concentration of hydrophilic polymers increased. When the concentration of hydrophilic polymer is low then the swelling degree was also low (formulations EP1, EH1, EP5, EP6, EH5, EH6).

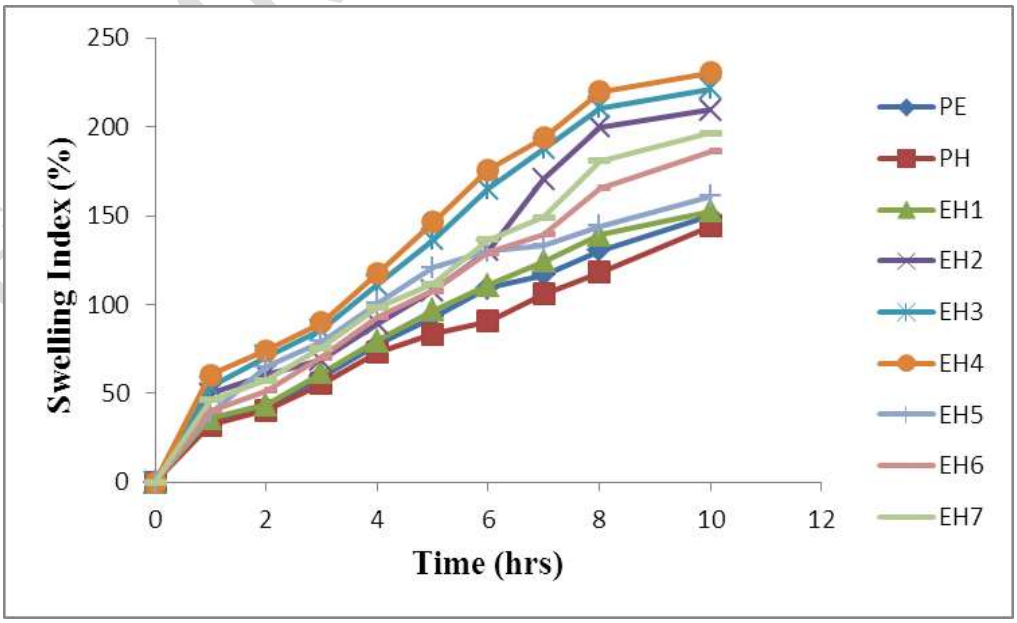

Figure 3: Swelling index of EC-HPMC mucoadhesive buccal patches.

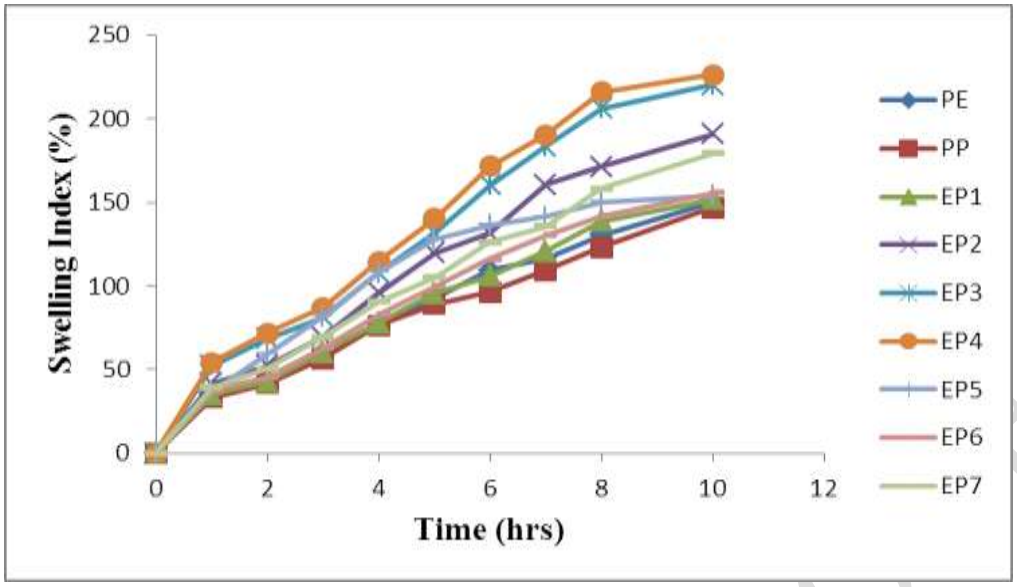

Figure 4: Swelling index of EC-PVP mucoadhesive buccal patches.

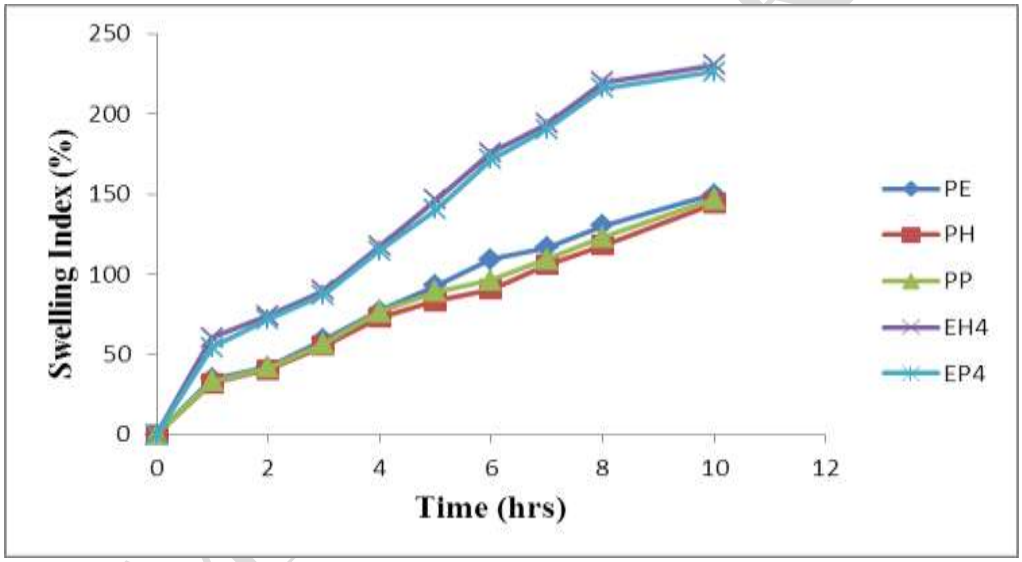

Figure 5: Comparison of swelling index of formulations EH4 and EP4 with formulations containing single polymers of mucoadhesive buccal patches.

3.5 *In vitro* release studies

In vitro release studies were conducted as per USP procedure using phosphate buffer pH 6.8 as dissolution medium. The *in vitro* profiles of losartan from various formulations are given in Figure 6, Figure 7 and Figure 8. Mucoadhesive patches formulated with ethyl cellulose (formulation PE), HPMC (formulation PH) and PVP (formulation PP) alone were found to be in acidic in nature as surface pH for formulation PE was 4.6 ± 0.08, for formulation PH was 4.16 ± 0.05 and for formulation PP was 4.34 ± 0.11, therefore the formulations were excluded from the *in vitro* studies. It was observed that with the increase in HPMC and PVP

concentration in the mucoadhesive buccal patches (formulations EHI-EH4 and EP1-EP4), the release of losartan was enhanced. The reason attributed to the above observation may be the hydrophilic nature of HPMC and PVP which can promote the entry of solution into the particles causing maximum swelling. This process greatly improves the solubility of drug and thus accelerates its dissolution. Hence formulations EH4 (EC- HPMC) and EP4 (EC- PVP) showed higher % of release compared to other formulations.

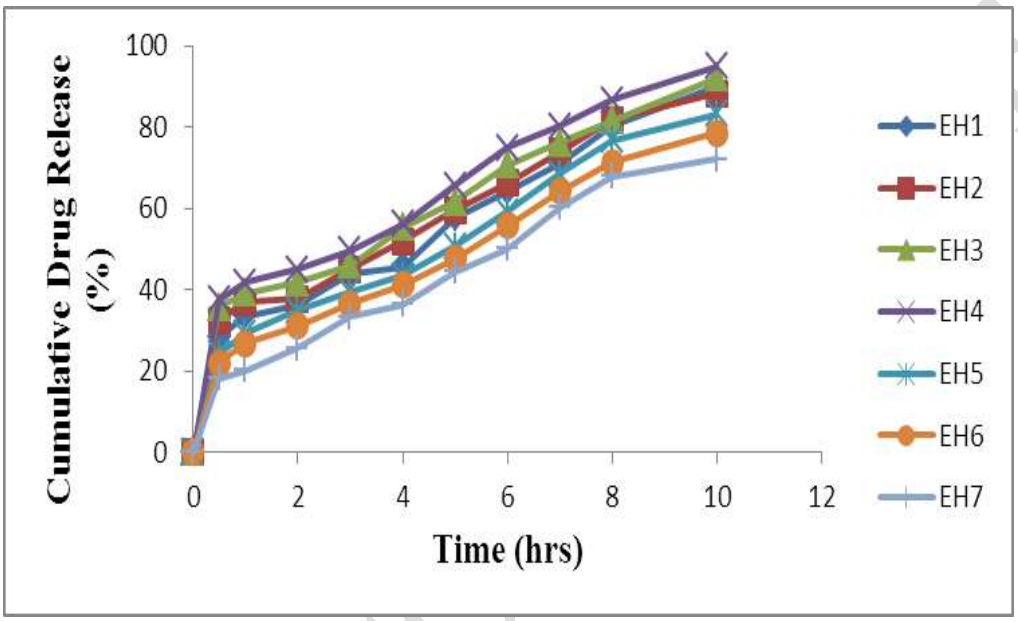

Figure 6: *In vitro* release profile of losartan from EC-HPMC mucoadhesive buccal patches.

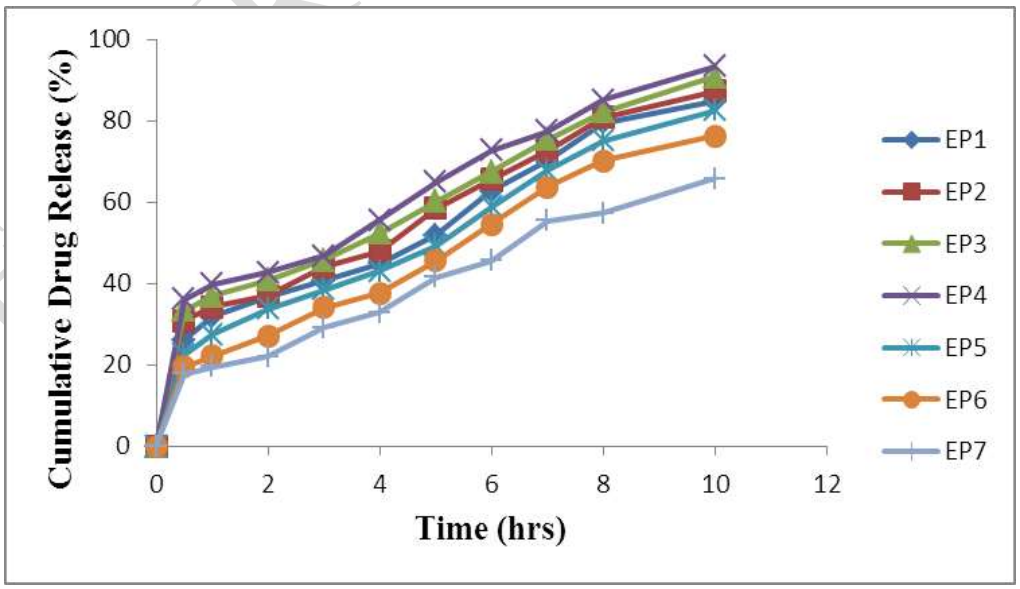

Figure 7: *In vitro* release profile of losartan from EC-PVP mucoadhesive buccal patches.

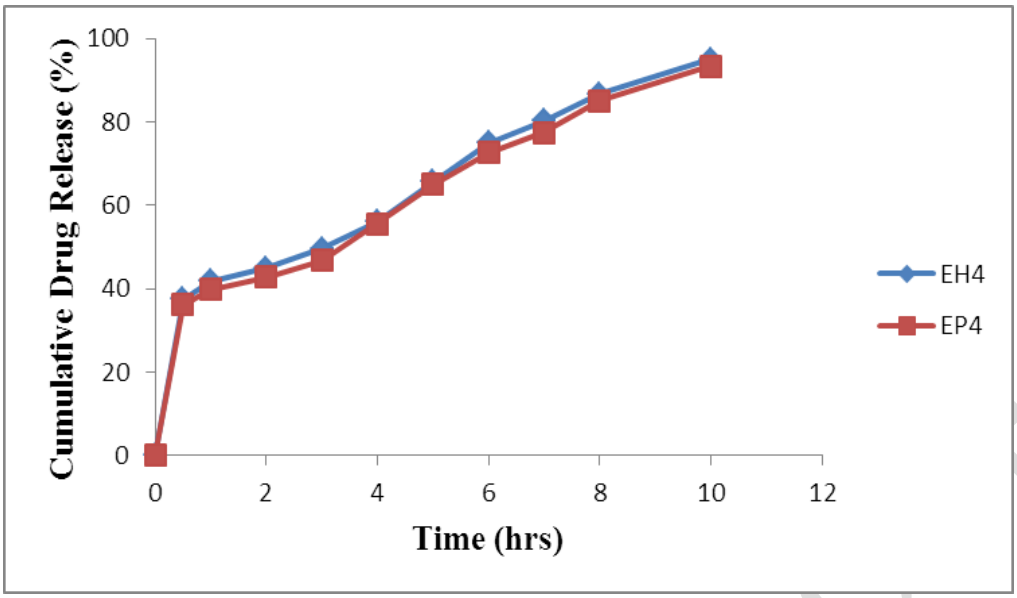

Figure 8: Comparison of *in vitro* release profile of losartan from optimized formulations EH4 and EP4 of mucoadhesive buccal patches.

3.6 Release kinetics: Kinetic analysis of the *in vitro* release data of losartan from mucoadhesive buccal patches is shown in table 3 and 4. The value of n in all the formulations was close to 0.5 suggesting that losartan was released from the mucoadhesive patch by Fickian diffusion. The formulations were best fitted to Zero order kinetics which indicated drug release by diffusion in controlled way.

Table 3: Release kinetic parameters of EC-HPMC mucoadhesive buccal patches.

S. No	Formulation code	Zero order r^2	First order r^2	Higuchi model r^2	Hixon Crowell r^2	Korsmeyer and Peppas (n)	Release order and Main Transport Mechanism
1.	EH1	0.985	0.975	0.943	0.972	0.382	Zero, Fickian
2.	EH2	0.984	0.980	0.944	0.827	0.348	Zero, Fickian
3.	EH3	0.987	0.977	0.939	0.974	0.325	Zero, Fickian
4.	EH4	0.987	0.973	0.946	0.983	0.318	Zero, Fickian
5.	EH5	0.986	0.869	0.944	0.948	0.419	Zero, Fickian
6.	EH6	0.986	0.983	0.949	0.943	0.431	Zero, Fickian
7.	EH7	0.984	0.980	0.951	0.965	0.497	Zero, Fickian

Table 4: Release kinetic parameters of EC-PVP mucoadhesive buccal patches.

S. No	Formulation code	Zero order r^2	First order r^2	Higuchi model r^2	Hixon Crowell r^2	Korsmeyer and Peppas (n)	Release order and Main Transport Mechanism
1.	EP1	0.980	0.971	0.937	0.968	0.396	Zero, Fickian
2.	EP2	0.986	0.965	0.942	0.977	0.369	Zero, Fickian
3.	EP3	0.985	0.919	0.947	0.889	0.342	Zero, Fickian
4.	EP4	0.987	0.955	0.946	0.942	0.329	Zero, Fickian

5.	EP5	0.986	0.941	0.950	0.867	0.440	Zero, Fickian
6.	EP6	0.985	0.982	0.947	0.971	0.488	Zero, Fickian
7.	EP7	0.985	0.872	0.949	0.945	0.466	Zero, Fickian

3.7 Selection of formulations

Selection of formulations was done on the basis of results obtained from swelling studies, mucoadhesive strength and *in vitro* release studies. Formulations EH3, EH4 (EC-HPMC) and EP3, EP4 (EC-PVP) were selected because they had shown comparable swelling index, significantly higher mucoadhesive strength (40.89 ± 0.29g, 43.46 ± 0.11g and 39.73 ± 0.54g, 42.09 ± 0.39g respectively) and drug release 92.02 ± 0.39% and 94.93 ± 0.80%; 90.73 ± 0.68% and 93.28 ± 0.49% up to 10 hrs than rest of the formulations. Further studies were performed on the selected formulations (EH3, EH4; EP3, EP4).

3.8 *Ex vivo* mucoadhesion time

This study was performed on mucoadhesive buccal patches of EC-HPMC (formulations EH3, EH4) and EC-PVP (formulations EP3, EP4). The results are given in Table 5.

Table 5: *Ex vivo* mucoadhesion time of mucoadhesive buccal patches.

Mucoadhesive Buccal Patch	Formulation Code	*Ex vivo* mucoadhesive time (h)
EC-HPMC	EH3	11.4 ± 0.20
	EH4	11.9 ± 0.34
EC-PVP	EP3	10.2 ± 0.26
	EP4	11.3 ± 0.12

3.9 *Ex vivo* Permeation studies

Permeation studies were carried out on mucoadhesive buccal patches of EC-HPMC (formulations EH3, EH4) and EC-PVP (formulations EP3, EP4). The cumulative percentage of drug permeated was 92.19 ± 0.71% for formulation EH4 and 88.29 ± 0.78% for formulation EP4 maximum in 10 hrs (Figure 9). The EH3, EP3 and EP4 formulations showed significantly less cumulative percentage of drug permeated with lower flux and significantly higher lag time when compared with EH4 (EC-HPMC 1:4) formulation which explained that EH3, EP3 and EP4 formulations showed less permeation as more percentage of drug was retained in skin and took more time to permeate through the skin (Table 6).

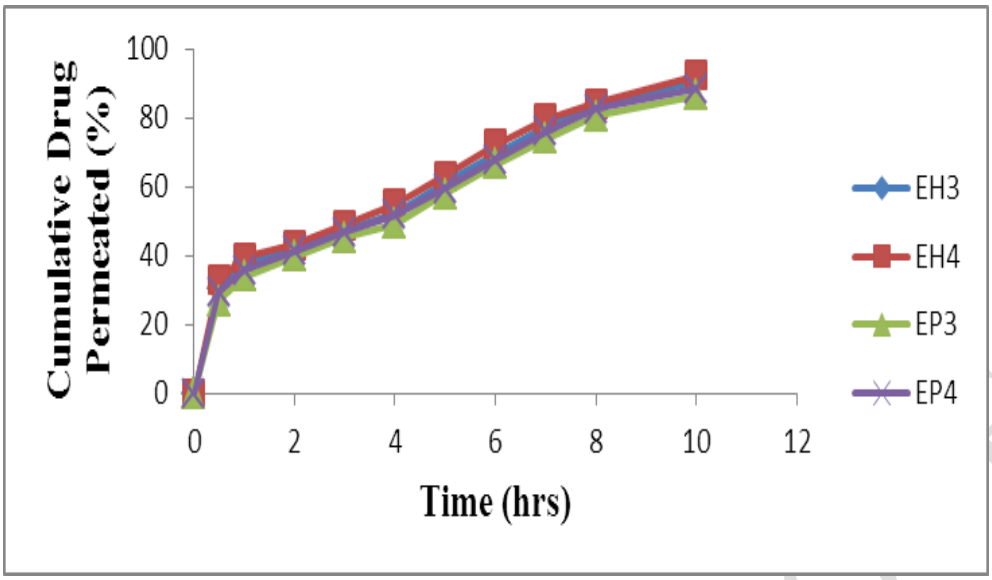

Figure 9: Drug permeation profile of formulations EH3, EH4 and EP3, EP4 of mucoadhesive buccal patches.

Table 6: Permeation parameters of formulations EH3, EH4 and EP3, EP4 of mucoadhesive buccal patches.

Formulation code	Flux (mg/cm^2/hr)	Lag time (hr)
EH3	0.622 ± 0.06	0.84 ± 0.01
EH4	0.651 ± 0.01	0.71 ± 0.03
EP3	0.518 ± 0.04	0.98 ± 0.05
EP4	0.546 ± 0.03	0.92 ± 0.01

3.10 Comparison of *in vitro* release & *ex vivo* permeation studies

When *in vitro* release studies were compared with *ex vivo* permeation studies, formulations EH3, EH4 of EC-HPMC and EP3, EP4 of EC-PVP mucoadhesive buccal patches were correlated. They revealed that the formulation which showed high drug release also showed high permeation of drug through skin.

3.11 Permeation kinetics

The formulations EH4, EH3 (EC-HPMC) and EP3, EP4 (EC-PVP) were best fitted to zero order kinetics which indicated drug permeation by diffusion in controlled way. To analyze the permeation mechanism of drug from mucoadhesive buccal patches, the data was fit to Korsmeyer-Peppas model. The 'n' value obtained was close to 0.5 suggesting that losartan was permeated from patches through aqueous channels of polymer by Fickian diffusion release model. From the results, formulations EH4 and EP4 were selected for stability studies (Table 7).

Table 7: Kinetic parameters of Permeation of EC-HPMC and EC-PVP mucoadhesive buccal patches.

S. No	Formulation code	Zero order r^2	First order r^2	Higuchi model r^2	Hixon Crowell r^2	Korsmeyer -Peppas (n)	Release order and Main Transport Mechanism
1.	EH3	0.985	0.952	0.956	0.968	0.368	Zero, Fickian
2.	EH4	0.987	0.979	0.963	0.952	0.353	Zero, Fickian
3.	EP3	0.983	0.963	0.959	0.979	0.396	Zero, Fickian
4.	EP4	0.986	0.981	0.962	0.975	0.376	Zero, Fickian

3.12 Stability studies

3.12.1 Stability studies in human saliva

The stability study of the optimized patches EH4 (EC-HPMC) and EP4 (EC-PVP) was done in natural human saliva for 10 hrs. The patches did not exhibit any significant changes in their color, shape and satisfactory physical stability.

3.12.2 Stability studies at room and incubator temperature

Stability studies of the formulated EC-HPMC and EC-PVP mucoadhesive buccal patches were carried out by storing the formulation EH4 (EC-HPMC) and EP4 (EC-PVP) at room temperature (25 ± 2^0C and 60 ± 5% RH) and incubator temperature (40 ± 2^0C and 75 ± 5% RH) for two months (acc. to ICH guidelines). Parameters namely mucoadhesive strength and drug release were carried out. The results revealed no changes in the physical appearance of the formulation after two months study. Drug release profile and mucoadhesive strength of formulation EH4 and EP4 stored at room temperature (25 ± 2^0C) and incubator temperature (40 ± 2^0C) after one and two months shown in Figure 10, Figure 11 and Table 8 respectively.

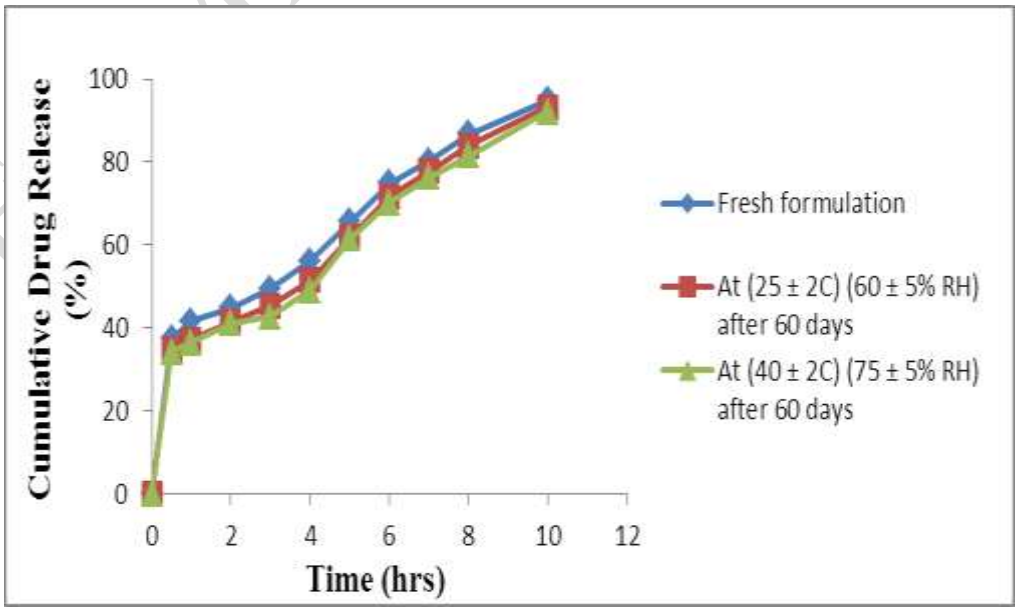

Figure 10: *In vitro* release studies of optimized formulation EH4 after 60 days.

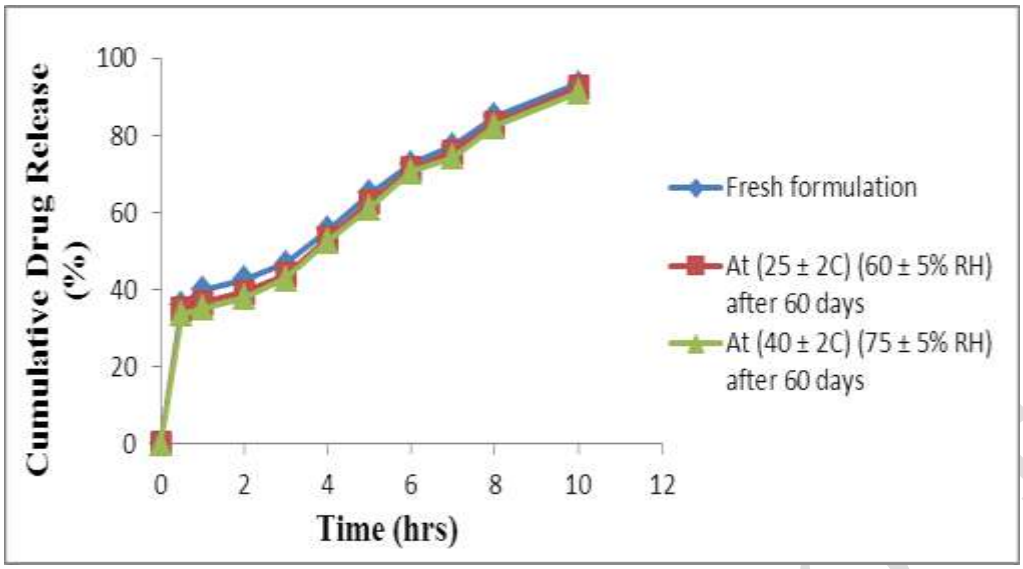

Figure 11: *In vitro* release studies of optimized formulation EP4 after 60 days.

Table 8: Mucoadhesive strength of optimized formulation EH4 and EP4 after 30 days and 60 days storage.

Formulation code	At room temp. (25 ± 2^{0}C) (60 ± 5% RH)		At incubator temp. (40 ± 2^{0}C) (75 ± 5% RH)	
	After 30 days	After 60 days	After 30 days	After 60 days
EH4	42.1 ± 0.3	41.7 ± 0.1	41.1 ± 0.2	40.5 ± 0.3
EP4	41.4 ± 0.02	41.2 ± 0.4	40.7 ± 0.05	40.2 ± 0.06

Analysis of mucoadhesive strength and percentage drug release after two months of storage at room temperature and incubator temperature showed that in formulation EH4 and EP4 there was no significant change. Hence these formulations are stable at all the temperatures.

After optimizing all the parameters, the formulations EH4 and EP4 were found to be best on the basis of mucoadhesive strength, degree of swelling, release profile, permeation studies and stability analysis. It was concluded that formulation EH4 (EC-HPMC 1:4) and EP4 (EC-PVP 1:4) were capable of controlling the rate of drug release.

3.13 Comparison of optimized formulations of EC-HPMC and EC-PVP mucoadhesive buccal patches

On the basis of results obtained from swelling studies, mucoadhesive strength, *in-vitro* release studies, *ex-vivo* mucoadhesion time, *in-vitro* permeation studies EC-HPMC mucoadhesive buccal patches showed comparable swelling index, significantly higher mucoadhesive strength i.e. 43.46 ± 0.11 g, higher *in-vitro* drug release i.e. 94.93 ±0.80%, higher mucoadhesion time i.e. 11.9 ± 0.34 h and more cumulative percentage of drug permeated i.e. 92.19 ± 0.50% than EC-PVP mucoadhesive buccal patches.

4. CONCLUSION

In this study, different mucoadhesive patches of drug, losartan were successfully prepared by solvent casting method for buccal delivery. The mucoadhesive buccal patches of EC-HPMC and EC-PVP were displaying comparable swelling index, sufficient mucoadhesive strength and *in vitro* drug release. The mucoadhesive buccal patch EH4 (EC-HPMC 1:4) showed comparable swelling index, significantly higher mucoadhesive strength, higher *in-vitro* drug release, more mucoadhesion time and more cumulative percentage of drug permeated than EP4 (EC-PVP 1:4) mucoadhesive buccal patches. Mucoadhesive buccal patches containing single polymers showed less mucoadhesive strength and less swelling than that of the EC-HPMC and EC-PVP mucoadhesive buccal patches. From the above results, it can be concluded that blends of hydrophobic (EC) and hydrophilic (HPMC and PVP) polymers is better than single polymer to obtain sustained drug release and can be used to formulate mucoadhesive buccal patches of losartan. The present investigation conclusively demonstrated that the loading of losartan into EC-HPMC mucoadhesive buccal patches leads to prolongation of drug release, enhanced retention time of the drug over the buccal mucosa thereby minimizing the limitations of conventional drug delivery. As the buccal patches deliver the drug through mesenteric circulation, first pass metabolism is bypassed thereby improving the therapeutic efficacy of drug. The data obtained in the study clearly indicated the potential of mucoadhesive buccal patches as a promising candidate for the controlled delivery of losartan.

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