Original Article

Formulation and optimization of mucoadhesive buccal patches of losartan potassium by using response surface methodology

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Abstract

Background: This study was undertaken with an aim to systematically design a model of factors that would yield an optimized sustained release dosage form of an anti-hypertensive agent, losartan potassium, using response surface methodology (RSM) by employing 3^2 full factorial design.

Materials and Methods: Mucoadhesive buccal patches were prepared using different grades of hydroxypropyl methylcellulose (HPMC) (K4M and K100M) and polyvinylpyrrolidone-K30 by solvent casting method. The amount of the release retardant polymers – HPMC K4M (X_1) and HPMC K100M (X_2) was taken as an independent variable. The dependent variables were the burst release in 30 min (Y_1), cumulative percentage release of drug after 8 h (Y_2) and swelling index (Y_3) of the patches. *In vitro* release and swelling studies were carried out and the data were fitted to kinetic equations.

Results: The physicochemical, bioadhesive, and swelling properties of patches were found to vary significantly depending on the viscosity of the polymers and their combination. Patches showed an initial burst release preceding a more gradual sustained release phase following a nonfickian diffusion process.

Discussion: The results indicate that suitable bioadhesive buccal patches with desired permeability could be prepared, facilitated with the RSM.

Key Words: 3^2 factorial design, losartan potassium, mucoadhesive, response surface methodology, sustained release

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INTRODUCTION

The recent years have seen a major shift in the choice of route for delivery of therapeutic agents. Extensive research efforts have been focused on placing a drug delivery system in a particular region of the body for maximizing biological drug availability and minimizing dose-dependent side effects.[1] Peroral administration of drugs, the preferred route of drug administration, has several disadvantages, such as hepatic first pass metabolism, longer onset of action and enzymatic degradation of drugs within the gastrointestinal (GI) tract. Buccal delivery of drugs provides an attractive alternative to the peroral administration of drugs,

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particularly in overcoming deficiencies associated with the latter mode of administration. Various studies have been carried out to formulate a wide range of mucoadhesive buccal drug delivery devices, including tablets, films, patches, disks, ointments, and gels. Among these formulations, buccal patches are preferred owing to their good flexibility compared with tablets and more accurate dosing of the drug in comparison with gels and ointments.[2,3] Moreover, since mucoadhesion implies attachment to the buccal mucosa, patches can be formulated to exhibit a systemic or local action. Due to the versatility of the manufacturing processes, the release can be oriented either toward the oral cavity or the buccal mucosa; in the latter case, it can exhibit the advantage of avoiding the first pass effect by directing absorption through the venous system that drains from the cheek.[4]

Losartan potassium (LP) is a potent, highly specific angiotensin II type 1 receptor antagonist with anti-hypertensive activity. The drug is orally administered as 25 mg tablets once or twice daily with total daily doses ranging from 25 to 100 mg. Following oral administration, it is readily absorbed from the GI tract with an oral bioavailability of about 33% and a plasma elimination half-life ranging from 1.5 to 2.5 h. Administration of LP in a controlled release dosage form with dual release characteristics that is, burst release followed by an extended release, would be more desirable as these characteristics would allow a rapid onset, followed by protracted anti-hypertensive effects by maintaining the plasma concentrations of the drug well above the therapeutic concentration.[5,6]

Response surface methodology (RSM) is one of the widely used methods in the development and optimization of drug delivery systems. Based on the principles of design of experiments, the methodology encompasses the use of various types of experimental designs, generation of polynomial mathematical equations, and mapping of the response over the experimental domain to ascertain the optimum formulation(s). The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms. Central composite design, three-level factorial design, and Box–Behnken design are the different types of RSM designs available for statistical optimization of the formulations.[6,7]

The current study aimed at developing and optimizing a mucoadhesive bilayered buccal patch of LP. The bilayered design of the patch was selected to obtain unidirectional release of the drug. Because of the properties such as hydrophobicity, low water permeability, drug impermeability, and moderate flexibility, ethyl cellulose (EC) was used as a backing layer polymer. Computer-aided optimization technique, that is, three-level factorial design was employed. The independent variables for the present study were: Amount of release retardant polymers – hydroxypropyl methylcellulose (HPMC) K4M (X_1) and HPMC K100M (X_2). The dependent variables studied were the burst release in 30 min (Y_1), cumulative percentage release of drug after 8 h (Y2) and the swelling index (Y3).

MATERIALS AND METHODS

Materials

LP was provided as a gift sample by BHC Labs (Baddi, India). HPMC K4M, HPMC K100M, polyvinylpyrrolidone (PVP)-K30 and EC were purchased from Central Drug House (Delhi, India). All other chemicals used were of reagent grade. Fresh goat buccal mucosa was obtained from a local slaughterhouse and was used within 2 h of slaughter.

Methods

Preparation of mucoadhesive bilayered buccal patches

Backing layer

For preparing the backing layer EC (5% w/v) was dissolved in a mixture of acetone and isopropyl alcohol (65:35). 2%v/v dibutyl phthalate was added as the plasticizer. The plasticized EC solution was poured into a petriplate of 7.5 cm internal diameter on a level surface and allowed to air dry at controlled rate by covering the petriplate with a funnel.[8]

Mucoadhesive layer containing drug

Mucoadhesive layer was prepared by the solvent casting technique, using LP, plasticizer, and other film forming as well as release retarding polymers. The experiment was designed using a 3^2 full factorial design (Design Expert, Version 8.0.4.1, Stat-Ease Inc., Minneapolis, MN, USA). Different concentrations of polymer solutions were mixed in specified ratios as shown in Table 1. The hydrophilic polymers HPMC K4M, HPMC K100M and PVP-K30 were dissolved separately in ethanol (95%) and then incorporated into one. This polymeric dispersion was then stirred on a magnetic stirrer (Remi Equipments Ltd., India) for a period of 1 h to get a homogenous clear solution, followed by sonication for 15 min. Propylene glycol (PG) was added as a plasticizer and stirring was continued for another 30 min. To this mixture, a drug solution corresponding to 300 mg was added, mixed thoroughly with continued stirring and kept aside for few hours until all the entrapped air had escaped. This solution was then poured over the preformed backing

Table 1: The 3^2 full factorial design of composition of patches containing LP

Batch code	LP (mg)	HPMC K4M (X_1) (mg)	HPMC K100M (X_2) (mg)	PVP-K30 (mg)	EC (mg)	PG (ml)
LP_1	300	−1	−1	250	500	0.5
LP_2	300	0	−1	250	500	0.5
LP_3	300	+1	−1	250	500	0.5
LP_4	300	−1	0	250	500	0.5
LP_5	300	0	0	250	500	0.5
LP_6	300	+1	0	250	500	0.5
LP_7	300	−1	+1	250	500	0.5
LP_8	300	0	+1	250	500	0.5
LP_9	300	+1	+1	250	500	0.5

Low (−1)=150; Medium (0)=250; High (+1)=350; X_1, X_2=Independent variables. EC: Ethyl cellulose, PG: Propylene glycol, LP: Losartan potassium

layer of EC and allowed to dry overnight, undisturbed at room temperature. The petriplate was covered with an inverted funnel to allow controlled evaporation of the solvent. After careful examination, the dried patches were removed, checked for any imperfections or air bubbles and cut into 25 mm diameter patches. The patches were packed in aluminum foil and stored in a glass container at room temperature till further use.[5,9]

Optimization of formulation

A 3^2 randomized full factorial design was employed in this study. Two factors, each at three levels, were evaluated and experimental trials were performed on all nine possible combinations [Table 1]. The amount of HPMC K4M (X_1) and the amount of HPMC K100M (X_2) were selected as independent variables. The burst release in 30 min (Y_1), *in vitro* cumulative percentage release of drug after 8 h (Y_2) and the swelling index (Y_3) were selected as dependent variables. Regression polynomials for the individual dependent variables were calculated with the help of Design Expert 8.0.4.1 software and applied to approximate the response surface and contour plots. A statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1.X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \text{(i)}$$

Where Y is the dependent variable, β_0 is the arithmetic mean response of the nine runs, and β_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing 1 factor at a time from its low to high value. The interaction terms ($X_1.X_2$) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The polynomial equations can be used to draw conclusions after considering the magnitude of the coefficient and the mathematical sign it carries (i.e., positive or negative).[7,10,11]

Characterization of prepared mucoadhesive patches

Weight and thickness of the patch

The average weight of 10 samples of each formulation was determined by weighing individually on a Digital Balance (Adventurer AX 523, Ohaus Corp. USA,). 10 samples of each formulation were taken, and the thickness was measured using micrometer screw gauge (MMO-25DS, Mitutoyo, Japan) at three different locations, and the mean thicknesses were calculated.[12]

Folding endurance

The folding endurance was determined by repeatedly folding one patch at the same place till it broke or folded up to 250 times without breaking. The number of times the film could be folded at the same place without breaking gives the value of the folding endurance.[13]

Surface pH determination

Patches (without backing layer) were left to swell for 3 h on agar plate prepared by dissolving 2% (m/v) agar in simulated human saliva (SHS; NaCl [0.126 g], KCl [0.964 g], KSCN [0.189 g], KH_2PO_4 [0.655 g], and urea [0.200 g] in 1 L of distilled water)[14] of pH 6.8 under stirring and then pouring the solution into a Petri dish until gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch.[15]

Drug content uniformity

Patches of 25 mm diameter designed to contain 55 mg of LP were dissolved by homogenization in a mixture of 5 ml ethyl alcohol and 2 ml of dichloromethane for 5 h with occasional shaking and diluted to 50 ml with distilled water. After filtration to remove insoluble residue, 1 ml of the filtrate was diluted to 10 ml with SHS of pH 6.8. The absorbance was measured at 205 nm using an ultraviolet (UV) spectrophotometer (UV - shimadzu 1601). The experiments were carried out in triplicate for the patches of all formulations.[16]

Percent moisture absorption

The buccal patches were weighed accurately and placed in the desiccators containing 100 ml of saturated solution of aluminum chloride, maintaining 76% and 86% relative humidity. After 3 days, the patches were taken out and weighed.

Percent moisture loss

The buccal patches were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After 3 days, the patches were taken out and weighed.[1] The percentage moisture absorption and moisture loss were calculated using the formula:

$$SI\ (\%) = \frac{W2\text{-}W1}{W1} \times 100 \quad \text{(ii)}$$

$$\text{Moisture loss}\,(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad \text{(iii)}$$

Drug release from backing layer

For determination of drug release from the backing layer, Franz diffusion cell was used. A bilayered buccal patch was placed between donor and receptor compartment. The complete unit was maintained at 37°C, donor compartment (3 ml) was filled with SHS pH 6.8 and receptor compartment (21 ml) contained phosphate buffer pH 7.4 with synchronous stirring. At predetermined interval 2 ml sample was removed from a donor compartment and analyzed at 205 nm by UV spectrophotometric analysis to check release of drug from the backing layer of the patch.[11]

Tensile strength measurement

Dried patch samples were cut into uniform strips (2.5 cm × 5 cm). Two pieces of cardboard (1 cm × 2.5 cm) were attached to the upper and the lower end of the patch using cyanoacrylate resin adhesive. Attaching the patch to the cardboard facilitates clamping it to the jaws of the modified device used for tensile strength (TS) measurement, thus preventing pressure on the patches and slipping prior to or during application. The modified device contains a rectangular frame with two jaws made up of aluminum.[17] One jaw is stationary in the front and the other one is movable and can be pulled by loading weights on the pan attached with string to the movable part. The patch on the cardboard was clamped between the two jaws of the device positioned at a distance of 3 cm. The weights were gradually added to the pan till the patch was broken. The weight necessary to break the patch was noted as breaking force and the simultaneous distance traveled by the pointer on the graph paper indicated the elongation at break (E/B).[18,19] TS and percent elongation can be obtained by following formula:

$$\text{Tensile strength}\,(\text{g/cm}^2) = \frac{\text{Force at break}}{\text{Cross-sectional area of sample}} \times 100 \quad \text{(iv)}$$

$$\%\text{Elongation at break} = \frac{\text{Increase in length}}{\text{Original length}} \times 100 \quad \text{(v)}$$

Ex vivo bioadhesive strength

Freshly excised buccal mucosa of an adult goat was used as a model membrane for the measurement of bioadhesive strength. Fresh goat buccal mucosa was obtained from a local slaughterhouse and used within 2 h 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 isotonic phosphate buffer pH 6.8 at 37°C. Bioadhesive strength of patch ($n = 3$) was measured on a modified two-arm physical balance.[17] The pan at the left arm of the balance was detached and to the lever of the left arm, was hung a vertical thread, which had a rubber stopper tied to its end, hanging downward. The patch to be tested was adhered to the downward facing side of the rubber stopper. Goat buccal mucosa was tied onto the open mouth of a glass vial filled with isotonic phosphate buffer. The vial was fitted in the center of a glass beaker filled with SHS (pH 6.8, 37°C ± 1°C). The apparatus was set such that the vial (mucosal membrane tied on it, facing upward) lies exactly below the rubber stopper (patch adhered onto it, facing downward). The rubber stopper was lowered so as to make the patch come in contact with the membrane. After facilitating the contact between the two, weight was put on the right limb of balance and increased gradually until the patch got detached from the buccal mucosa. The weight (gram force) required to detach the patch from the mucosal surface gave the measure of detachment stress, calculated by:

$$\text{Detachment stress}\ (\text{dyne/cm}^2) = \frac{(\text{w.g})}{\text{A}} \quad \text{(vi)}$$

where w is the weight required for the detachment of patch, g is the acceleration due to gravity considered as 980 cm/s, and A is the area of the mucosal surface exposed (cm^2).[17,20]

Ex vivo bioadhesion time

The *ex vivo* bioadhesion time was ascertained ($n = 3$) after application of the patches onto freshly cut goat buccal mucosa. The fresh goat buccal mucosa was fixed in the inner side of the beaker, above 2.5 cm from the bottom, with cyanoacrylate glue. One side of each patch was wetted with one drop of isotonic phosphate buffer pH 6.8 and pasted to the goat buccal mucosa by applying a light force with a fingertip for 30 s. The beaker was filled with 500 ml of SHS pH 6.8 and was kept at 37°C ± 1. After 2 min, a 50 rpm stirring rate was applied to simulate the buccal cavity environment, and patch adhesion was monitored up to 6 h. The time required for the patch to detach from the sheep buccal mucosa was recorded as the mucoadhesion time.[20]

Swelling study

The degree of swelling of bioadhesive polymer is an important factor affecting adhesion. The swelling rate of the mucoadhesive patch was evaluated by placing the patches in SHS solution pH 6.8 at 37°C ± 1. Three patches of each formulation were cut and weighed, and the average weight was calculated (W1). The patches were placed in SHS solution and were removed at time intervals of 30 min (up to 3 h), excess water on the surface was carefully absorbed using filter paper,

and swollen patches were reweighed. The average weight (W2) was calculated, and the swelling index was calculated by the formula:[16]

$$SI\ (\%) = \frac{W2\text{-}W1}{W1} \times 100 \quad \text{(vii)}$$

In vitro drug release study

The drug release study from the patches was carried out using a USP 23 Type-2 rotating paddle dissolution test apparatus (Electrolab). 250 ml of SHS solution (pH 6.8) at 37°C ± 5°C was used as the dissolution medium with a stirring rate of 50 rpm. A patch of 2.5 cm diameter was fixed onto a glass disc with the help of cyanoacrylate adhesive. The disc was put at the bottom of the dissolution vessel such that the patch remained on the upper side of the disc. Samples (5 ml) were withdrawn at a predetermined time interval of 30 min and replaced with an equal volume of dissolution medium. The samples were filtered through a 0.45 mm filter and appropriately diluted with SHS solution (pH 6.8) and assayed spectrophotometrically at 205 nm. The experiment was performed in triplicate and average values were reported.[3,5]

Kinetic modeling of dissolution data

Because qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. In this regard, the use of *in vitro* drug dissolution data to predict *in vivo* bio-performance can be considered as the rational development of controlled release formulations. In order to determine the drug release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were fitted into various model dependent methods such as zero order, first order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas model. Model dependent methods are based on different mathematical functions, which describe the dissolution profile.[21] Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. The preference of a certain release mechanism was based on the correlation coefficient (r) for the parameters studied, where the highest correlation coefficient is preferred for the selection of the mechanism of release. The release data of LP from different buccal patches prepared was fitted to following mathematical models like:

$$Q_t = Q_0 + K_0 t: \text{Zero order model} \quad \text{(viii)}$$

$$\log C = \log CK^t/2.303: \text{First order model} \quad \text{(ix)}$$

$$f_t = Q = K_H \times t^{1/2}: \text{Higuchi model} \quad \text{(x)}$$

$$W_0^{1/3} - W_t^{1/3} = \kappa t: \text{Hixson–Crowell model} \quad \text{(xi)}$$

$$M_t/M_\infty = Kt^n: \text{Korsmeyer–Peppas model} \quad \text{(xii)}$$

Where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most of the times, $Q_0 = 0$), K_0 is the zero order release constant expressed in units of concentration/time, C_0 is the initial concentration of drug, K is the first order rate constant, K_H is the Higuchi dissolution constant, W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and κ (kappa) is a constant incorporating the surface volume relation, $M_t/M\infty$ is a fraction of drug released at time t, K is the release rate constant and n is the release exponent.

The interpretation of diffusional release mechanisms from different polymeric films as depicted by Korsmeyer–Peppas model is as follows.[22-24]

Differential scanning calorimetry studies

Differential scanning calorimetry (DSC) studies were performed using (DSC-60, Shimadzu, Japan) and carried out under the following conditions: Sample weight (3–5 mg), scanning speed (5°C/min), and temperature range (50–300°C). Thermal analysis data were obtained using a TA 501 PC system with Shimadzu software programs (Shimadzu Corp. Japan). The pure drug, physical mixture and the optimized formulation were subjected to the study.

X-Ray diffraction studies

The crystalline state of different samples was evaluated with X-ray powder diffraction. The diffraction patterns were obtained at room temperature using a Philips Analytical X-ray BV (PW1710) diffractometer with cobalt as an anode material and graphite monochromator, operated at a voltage of 30 kV.

RESULTS AND DISCUSSION

In the present investigation, buccal patches of LP were prepared with different polymer combinations of HPMC K4M, HPMC K100M and PVP-K30 using solvent casting technique. A total of nine formulations were prepared in triplicate using a 3^2 factorial design. PG was used as the plasticizer. One of the major aims of the investigation was to study the effect of different grades of HPMC as well as their combination on various characteristics of the patch. The main characteristics under study were burst release at 30 min, cumulative percent drug release at 8 h, and swelling index of the patches. Impermeable backing layer is an essential part of the buccal mucoadhesive patch to obtain unidirectional drug flow. Backing layer

prevents the loss of drug at the required site and also minimizes the exposure of other tissues to the drug by preventing bidirectional flow. Therefore, in the present investigation, backing layer of EC was used.[3]

Weight and thickness of the patch

Physicochemical characteristics of the formulated patches are shown in [Table 2]. The prepared patches were smooth, colorless with good flexibility and showed no visible imperfection. Based on the quantities of the polymers, HPMC K4M and HPMC K100M, the thickness, as well as the weight of different patches were found to be varying. The patch thickness was observed to be in the range of 0.52 ± 0.06 mm to 0.85 ± 0.05 mm and weight was found to be in the range of 150 ± 1 mg to 194 ± 2 mg. It was observed that as the percent of the polymers increased, thickness and weight also increased, as more amount of polymer resulted in the thickness as well as the weight of patches.

Folding endurance

All the patches had the satisfactory folding endurance of >250. The range of folding endurance study ensured flexibility of these formulated buccal patches.

Surface pH determination

Acidic or alkaline pH may cause irritation to the buccal mucosa and influence the degree of hydration of polymers. The surface pH of the patches ranged between 5.97 ± 0.15 and 7.02 ± 0.11. The results were found to be close to neutral in all the formulations, and this means that they have less potential to irritate the buccal mucosa.

Drug content uniformity

The drug content (%) in all formulations varied between the ranges of 89.70 ± 0.36% to 99.19 ± 0.21%. This indicates that the drug dispersed uniformly throughout the polymeric patches.

Percent moisture absorption

Moisture interaction studies are necessary to find out the physical stability of the film at high humid conditions and integrity of the film at dry conditions. The percent moisture absorption study was done over a period of 3 days and the results were found to be varied between 2.58% ±0.08% and 5.92% ±1.17%. The moisture absorption was found to increase with an increase in the viscosity of the polymer (HPMC K4M, HPMC K100M) as well as with the polymer concentration. The low moisture content in the formulation is highly appreciable to protect from microbial contaminations and bulkiness of the patches. Moreover, low moisture content in formulations helps them to remain stable from being a completely dried and brittle film.

Percent moisture loss

The results of percent moisture loss (PML) varied between 1.03% ±0.95% and 2.22% ±0.41% as shown in Table 2 and it can be observed that as the viscosity of

Table 2: Different parameters of prepared buccal patches

(a) Physicochemical parameters

Batch code	Weight (mg)†	Thickness (mm)†	Folding endurance†	Surface pH*	Drug content (%)*	PMA (%)*	PML (%)*
LP_1	150±1	0.52±0.06	>250	6.37±0.13	89.70±0.36	2.58±0.08	2.22±0.41
LP_2	161±1	0.65±0.03	>250	5.97±0.15	94.85±0.15	2.72±0.35	2.17±0.05
LP_3	172±3	0.68±0.02	>250	6.39±0.19	97.16±0.57	3.28±0.73	2.03±0.62
LP_4	161±2	0.59±0.05	>250	6.84±0.17	95.47±0.82	3.75±1.16	1.94±0.57
LP_5	172±2	0.67±0.01	>250	6.53±0.15	99.19±0.21	4.19±0.52	1.15±0.83
LP_6	183±1	0.75±0.06	>250	6.70±0.08	96.34±0.14	4.69±0.83	1.47±0.71
LP_7	172±3	0.70±0.04	>250	7.02±0.11	95.73±0.51	5.11±1.04	1.32±0.25
LP_8	183±2	0.79±0.03	>250	6.24±0.09	95.82±0.13	5.65±0.39	1.14±0.64
LP_9	194±2	0.85±0.05	>250	6.60±0.14	96.24±0.11	5.92±1.17	1.03±0.95

(b) Mechanical, bioadhesive and swelling parameters

Batch code	Mechanical properties		Bioadhesive properties		SI* (%)
	TS* (kg/mm²)	E/B* (%)	Detachment stress* (dyne/cm²×10³)	Residence time* (min)	
LP_1	0.163±0.08	38.96±1.31	2.106±0.547	145±2	16.49±0.05
LP_2	0.192±0.05	35.72±1.64	2.548±0.386	168±1	20.58±0.14
LP_3	0.215±0.02	31.49±2.85	3.038±0.294	185±1	22.64±0.62
LP_4	0.228±0.04	29.51±1.16	3.284±0.596	216±2	24.21±1.35
LP_5	0.261±0.08	27.43±2.15	3.936±1.025	247±2	25.46±0.73
LP_6	0.372±0.06	25.72±2.61	4.346±0.629	269±3	30.94±0.46
LP_7	0.394±0.03	22.53±1.49	4.966±1.029	293±1	31.96±1.05
LP_8	0.417±0.05	20.52±1.14	5.230±1.038	319±2	33.59±0.58
LP_9	0.469±0.02	16.96±2.73	5.942±0.359	347±1	34.49±0.35

†Values represented as mean±SD (n=10), *Values represented as mean±SD (n=3), TS: Tensile strength, E/B: Elongation at break, SI: Swelling index at 180 min, PMA: Percentage moisture absorption, PML: Percentage moisture loss, SD: Standard deviation, LP: Losartan potassium

the polymer increased, its moisture retention capacity increased thereby resulting in a gradual decrease of PML.

Drug release from backing layer

In an attempt to evaluate the performance of backing layer in avoiding release of LP, a study was conducted using Franz diffusion cell. Results of the study revealed that no drug was released in 150 min in the donor compartment of the diffusion cell. This indicated that EC membrane was impermeable to LS and the swelling of the mucoadhesive layer did not change the integrity of backing layer. Hence, the patch was found to be efficient for unidirectional release of LS through the buccal mucosa.

Optimization of formulation

A 3^2 randomized full factorial design as the RSM requires nine experiments. The responses observed for nine formulations prepared simultaneously fitted to first order, second order and quadratic models using Design Expert 8.0.4.1 software and the summary of analysis of variance (ANOVA) for selected factorial model are given in Table 3.

Table 3: Summary of ANOVA for selected factorial model

Source	Sum of square	df	Mean square	*F*	*P* value (*P* > F)
Response I					
Model	269.92	5	53.98	366.91	0.0002
X_1 - HPMC K4M	20.31	1	20.31	138.01	0.0013
X_2 - HPMC K100M	244.62	1	244.62	1662.60	<0.0001
X_1X_2	2.14	1	2.14	14.53	0.0318
X_1^2	0.023	1	0.023	0.16	0.7171
X_2^2	2.83	1	2.83	19.23	0.0220
Residual	0.44	3	0.15		
Cor total	270.36	8			
Response II					
Model	169.63	5	33.93	27.88	0.0102
X_1 - HPMC K4M	26.81	1	26.81	22.03	0.0183
X_2 - HPMC K100M	138.50	1	138.50	113.80	0.0018
X_1X_2	3.84	1	3.84	3.15	0.1739
X_1^2	0.46	1	0.46	0.37	0.5837
X_2^2	0.033	1	0.033	0.027	0.8806
Residual	3.65	3	1.22		
Cor total	173.28	8			
Response III					
Model	310.66	2	155.33	102.89	<0.0001
X_1 - HPMC K4M	39.58	1	39.58	26.22	0.0022
X_2 - HPMC K100M	271.08	1	271.08	179.57	<0.0001
Residual	9.06	6	1.51		
Corrected total	319.72	8			

Response I: Burst release in 30 min, Response II: Cumulative percent drug release in 8 h, Response III: Swelling index. HPMC: Hydroxypropyl methylcellulose, ANOVA: Analysis of variance

The model *F* value of 366.91 imply that the model is significant. There was only a 0.02% chance that such a large "model *F* value" could occur due to noise. Values of " *P* (probality) > F" <0.05 indicate model terms are significant. In this case X_1, X_2, $X_1 \times {}_2$, X_2^2 were significant model terms. Values >0.1 indicate the model terms are not significant [Table 3]. The final model in terms of coded factors for burst release of drug in 30 min was as follows:

$$\text{Burst release} = +15.71 - 1.84\,X_1 - 6.39\,X_2 + 0.73\,X_1X_2 - 0.11\,X_1^2 + 1.19\,X_2^2 \quad \text{(xiii)}$$

A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between the factor and the response. Coefficients with higher order terms or more than one factor term in the regression equation represent quadratic relationships or interaction terms, respectively. It also shows that the relationship between responses and factors is not always linear. Used at different levels in a formulation or when more than one factors are changed simultaneously, a factor can produce different degree of response. Two-dimensional contour plots and three-dimensional response surface plots are presented in Figures 1–3, which are very useful to study the interaction effects of the factors on the responses. These types of plots show the effects of two factors on the response at a time. The contour plot and response surface plot [Figure 1a] revealed that a corresponding decrease in the burst release of drug takes place with an increase in the concentration of HPMC K100M. Water-soluble drugs are released primarily by diffusion of dissolved drug molecules across the HPMC gel layer, while poorly soluble drugs are primarily released by erosion mechanism. The formulations with lower level of polymers exhibited higher burst release, which can be ascribed to dissolution of the drug present initially at the surface of the polymeric patch as it imbibes water and starts swelling.[6] The plots also revealed that both the HPMC K4M and HPMC K100M had a negative effect on the burst release, and the effect of concentration of HPMC K100M was more than that of HPMC K4M.

As evident from Table 3, The model *F* value of 27.88 implied the model was significant. There was only a 1.02% chance that a "model *F* value" this large could occur due to noise. Values of "*P* > F" <0.05 indicated that X_1, X_2 were significant model terms. The final model for *in vitro* cumulative percent drug release in 8 h was as follows:

$$\text{Drug release} = +89.26 - 2.11\,X_1 - 4.80\,X_2 + 0.98\,X_1X_2 + 0.48\,X_1^2 - 0.13\,X_2^2 \quad \text{(xiv)}$$

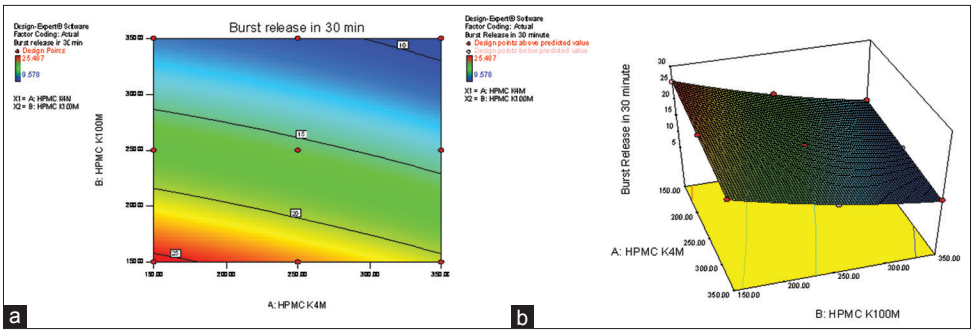

Figure 1: Effect of hydroxypropyl methylcellulose K4M (X_1) and hydroxypropyl methylcellulose K100M (X_2) on (a) response Y_1 (burst release in 30 min), (b) response Y_2 (cumulative % drug released in 8 h)

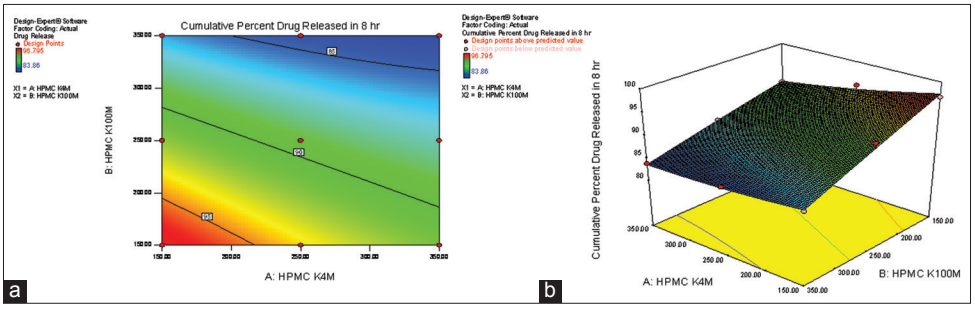

Figure 2: Swelling behavior of buccal patches (n = 3) (a) 1–5 and (b) 6–9, in simulated human saliva (pH 6.8)

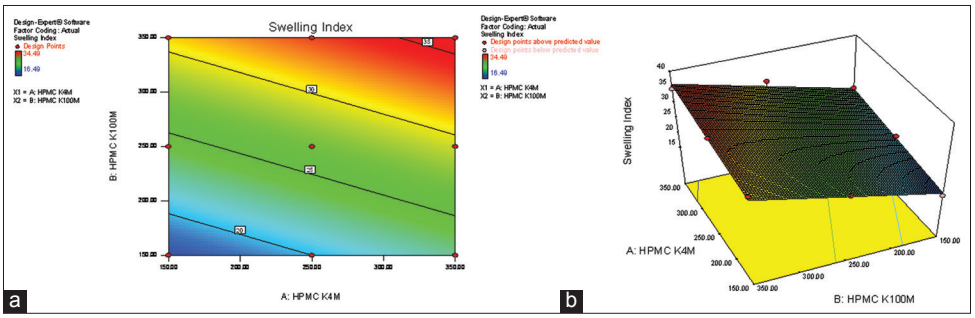

Figure 3: Drug release profiles of buccal patches; n = 3 (a) 1–5 and (b) 6–9, containing losartan potassium

The "Pred. R^2" of 0.8084 was found to be in reasonable agreement with the "Adj. R^2" of 0.9438. The "Adeq precision" measures the signal to noise ratio. A ratio >4 is desirable and the ratio of 20.470 indicated an adequate signal.

The contour plot and response surface plot [Figure 1b] revealed that about 97% drug was released after 8 h when both the HPMC K4M and HPMC K100M were at the lowest level, and the decrease in % drug release was polymer concentration dependent. Furthermore, the HPMC K100M resulted in greater reduction in % release as compared to the HPMC K15M thus showing a dominant effect over the latter. This also indicated a slight nonlinear trend between the factors X_1 and X_2.

As shown in Table 3, The model F value of 102.89 impliy that the model is significant. There was only a 0.01% chance that such a large "model F value" could occur due to noise. The swelling index followed a linear model, and the values of "$P > F$" <0.05 indicated that X_1, X_2 were significant model terms. The final model for cumulative percent drug release in 8 h was as follows:

$$\text{Swelling index} = +26.71 + 2.57\ X_1 + 6.72\ X_2 \qquad \text{(xv)}$$

The "Pred R^2" of 0.9309 was found to be in reasonable agreement with the "Adj R^2" of 0.9622. It was evident from the contour plot and response surface plot [Figure 1c] that a gradual increase in swelling index occurred with an increase in polymer concentration. Moreover, the effect of HPMC K100M on swelling index was more prominent than that of HPMC K4M. The reason could be attributed to the increased viscosity of the polymer.

The optimum formulation was selected by applying constraints on the three dependent variables, that is, burst release in 30 min (15–24%), *in vitro* cumulative percent drug release in 8 h (85–95%) and swelling index (20–30%). Various response variables were adjusted and ccomprehensive evaluation of feasibility search alongwith exhaustive grid search was done. This led us to the formulation LP_5 (with polymer levels of HPMC K4M, 250 mg and HPMC K100M, 250 mg) that was found to fulfill the maximum requisite of an optimum formulation. On "trading off" various response variables and comprehensive evaluation of feasibility search and exhaustive grid search, the formulation composition with polymer levels of HPMC K4M, 250 mg and HPMC K100M, 250 mg (formulation LP_5) was found to fulfill the maximum requisite of an optimum formulation.

Tensile strength measurement

Tensile testing gives an indication of the strength and elasticity of the patch, reflected by the parameters TS and E/B. A soft and weak patch is characterized by low TS and high E/B, whereas a hard and brittle patch is defined by a moderate TS and low E/B. Moreover, a soft and tough patch is characterized by moderate TS and high E/B, whereas a hard and tough patch is characterized by a high TS and E/B. Hence, it is suggested that a suitable buccal patch should have a relatively moderate TS and E/B.[15] For buccal application, soft and tough inserts are preferred as these can withstand the continuous mechanical stress due to movement of jaws or in the buccal cavity. The TS of the patches varied between 0.163 ± 0.08 and 0.469 ± 0.02 kg/mm^2 and E/B was between 16.96 ± 2.73 and 38.96 ± 1.31% [Table 2]. The observed results revealed that TS increased with an increase in polymer concentration, moreover, polymer with high viscosity had prominent effect on TS. The result for E/B described an inverse relation with the polymer concentration. TS values indicate that there is no statistically significant difference between the next immediate formulations. However, statistically significant difference was observed in E/B values between the next immediate formulations at $P < 0.05$.

Ex vivo bioadhesive strength

Bioadhesion may be defined as the adhesion between a polymer and a biological membrane, e.g. mucus. The strength of bioadhesion is affected by various factors such as molecular weight of polymers, contact time with mucus, swelling rate of the polymer, and biological membrane used in the study. All patches showed appreciable bioadhesive detachment stress, which ranged between 2.106 ± 0.547 and 5.942 ± 0.359 dyne/cm^2 × 10^3 [Table 2] indicating a potential of sustaining the stay and enhancing contact with buccal mucosa. Various mechanisms have been proposed to explain the *in vitro* bioadhesion or mucoadhesion phenomena. These included electrical double layers, electrostatic attractions, hydrogen bonding, Van der Waals force, hydrophobic bonding, wetting, diffusion-interpenetration, physical entanglements, and surface-free energy.[25] Most of the hydrophilic polymers have the ability to absorb water and swell. This can increase the potential to adhere onto mucosal surfaces. This is the simplest mechanism of adhesion and has been defined as "adhesion by hydration." PVP has a high water solubility that critically limits its application as an effective mucoadhesive polymer, because after hydration, the formed gel starts to disintegrate due to dissolution. This leads to slippery mucilage and loss of the adhesive properties. On the other hand, HPMC is a nonionic polymer containing only hydroxyl groups, which can form weak hydrogen bonds with mucous layers. Furthermore, owing to its slow rate of hydration it can form a strong surface gel that efficiently adheres onto the mucosal surface and remains in contact for a longer time. For this reason, it can be characterized as one of the most effective mucoadhesive polymers.[17,26] Results of the bioadhesive detachment force suggested that PVP and HPMC came in unison to create a new matrix with an enhanced mucoadhesion accompanied by the individual properties of each polymer. Mixing with a second polymer can enhance the mucoadhesive properties of a novel polymer. It was also observed that as the concentration of HPMC increased in the patches, the mucoadhesive force also increased. Increasing the polymer amount may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting consequently in the augmentation of bioadhesive strength. Moreover, the effect of concentration of HPMC K100M was found to be more significant than that of HPMC K4M. This could be attributed to the high viscosity of HPMC K100M resulting in extensive interpenetration into the mucous layer and forming a stronger surface gel.

Ex vivo bioadhesion time

The *ex vivo* bioadhesion time (residence time) of the patches varied from 145 ± 2 to 347 ± 1 min [Table 2]. It was observed that a gradual increase in the residence time occurred with a concomitant increase in the polymer viscosity. The observation can be assigned to the inherent property of the polymer HPMC that although showing significantly higher swelling is less water affined and hence tends to retain its structure better.[27] In addition, increased viscosity led to formation of surface gel that maintained its structural integrity for a longer period of time, thereby resulting in increased residence time.

Swelling study

Swelling behavior was assessed by measuring equilibrium degree of swelling by the weight method. The swelling profile of all the formulations, as shown in Figure 2 revealed that the swelling index of the patches increased with an increase in the polymer concentration as well as with the HPMC viscosity. The finding was in agreement with some previously published data.[28] The swelling of the HPMC matrices can be mainly attributed to the disruption of hydrogen bonding among the polymeric chains. When water penetrates the solid HPMC, it inserts itself into the hydrogen bonds between adjacent polymer chains. As more water comes among the chains, the forces between the chains diminish. The macromolecular chains initially gain rotational freedom and begin to occupy more space and this is evidenced by polymer swelling. The penetrating water fills the voids between the polymer chains and diffuses into denser regions of the polymer, forcing additional chains apart.[29] As the viscosity increased (HPMC K100M > HPMC K4M), the hydrodynamic volume occupied by the hydrated polymer chains also increased, consequently resulting in greater swollen mass of the matrices.

In vitro drug release study

The *in vitro* drug release profiles of LP from bioadhesive patches are shown in Figure 3. It could be concluded from the results that the patches containing lesser concentration of the polymer gave a faster release of the drug. The rate of drug release decreased substantially on increasing the viscosity of HPMC. An initial burst release was observed in all formulations, followed by a polymer controlled release of the drug. The difference in burst effect of the initial time was a result of difference in the viscosity of the polymers. As evident from Figure 3, polymeric system with low viscosity polymer (HPMC K4M) yielded a faster initial burst effect. Some of the previously published data also have reported that increased viscosity resulted in a corresponding decrease in the drug release and HPMC with higher viscosity (HPMC K100M) resulted in thicker gel layer formation.[30] The reason could be ascribed to dissolution of the drug present initially at the surface of the polymeric matrix as the patches imbibe water and start swelling. As dissolution progresses, the gradual swelling of the outer layer creates proportionately new areas for drug diffusion. Since the polymeric matrix is hydrophilic, the permeation of dissolution medium takes place in the matrix and initiates dissolution of drug from the inner layers. The dissolution rate is counter-balanced by gel formation of the matrix, which takes place simultaneously. The balance between the swelling and gelling characteristics of the matrix system is critical in maintaining the desired drug release rate.[6] It has been postulated that, increasing the molecular weight or viscosity of the polymer in a matrix formulations increases the gel layer viscosity and thus slows down drug dissolution. Furthermore, the greater the viscosity of the gel, the more resistant is the gel to dilution and erosion, thus controlling the drug dissolution. In general, the penetration rate of water in matrices containing hydrophilic polymer is determined by the equilibrium between promotive forces of admission of water and those that act against its admission, that is, the viscosity forces. In so far as the movement of drugs like LP through gelatinous layer is controlled by the diffusion, the process gets slower in more viscous medium that is, developed around the dosage form.[31] Moreover, polymer of higher viscosity induces greater chain entanglement than a polymer of low viscosity. Therefore, it is difficult for longer chains to dissolve because of the high energy required for pulling them off the matrix. Thus, higher viscosity polymers induce the formation of a thicker gel layer after hydration.[32]

It was also observed that the influence of HPMC K100M fraction on the amount of drug released, was higher at later stages (7 h, 8 h) than at the earlier stages of drug release (after 2 h and 4 h). The results could be attributed to the swelling and hydration ability of HPMC. Higher polymer grade as HPMC K100M required more time for water penetration, swelling and formation of gel layer, and hence its impact on drug release at the early stages of examination was smaller. HPMC K4M, as a lower viscosity grade polymer, required less time for water penetration, swelling, and gel formation so the influence of this polymer on the drug release was more evident at earlier stages. Moreover, the gel barrier of HPMC K4M having viscosity smaller than K100M, showed less impact on the amount of the drug released at the later stage.

Kinetic modeling of dissolution data

In order to determine the release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were subjected to kinetic treatment. Regression coefficient values obtained for each formulation were compared to understand the release kinetics [Table 4]. Comparison of R^2 values obtained by zero order, first order, and Higuchi kinetic equation revealed that *in vitro* drug release followed zero order kinetics as the R^2 values obtained by zero order kinetic equation were close to unity. For a polymeric film or patch, different values of "n" indicate different release pattern [Table 5]; n = 0.5 for Fickian (Case I) release, >0.5 but <0.89 for non-Fickian (anomalous) release, 0.89 for Case II (zero order) release and >0.89 for super Case II

Table 4: Kinetic modeling of drug release

Batch code	Zero order (R^2)	First order (R^2)	Higuchi's model (R^2)	Hixon-Crowell model (R^2)	Korsmeyer-Peppas model	
					R^2	n
LP_1	0.981±0.0051	0.922±0.039	0.997±0.0046	0.982±0.0046	0.995±0.0035	0.522±0.27
LP_2	0.986±0.0061	0.951±0.022	0.989±0.006	0.988±0.006	0.982±0.007	0.594±0.23
LP_3	0.996±0.0066	0.937±0.029	0.984±0.0044	0.977±0.007	0.982±0.0049	0.633±0.204
LP_4	0.998±0.006	0.897±0.052	0.976±0.0076	0.957±0.018	0.982±0.0045	0.708±0.162
LP_5	0.997±0.006	0.934±0.031	0.971±0.01	0.971±0.011	0.980±0.005	0.74±0.143
LP_6	0.993±0.0066	0.947±0.026	0.967±0.015	0.974±0.013	0.984±0.0078	0.81±0.105
LP_7	0.993±0.0042	0.954±0.021	0.970±0.012	0.977±0.009	0.990±0.004	0.832±0.091
LP_8	0.991±0.0051	0.960±0.016	0.970±0.011	0.980±0.007	0.990±0.0062	0.864±0.071
LP_9	0.991±0.0064	0.946±0.022	0.960±0.014	0.969±0.009	0.988±0.004	0.93±0.031

Selected release model-non-Fickian diffusion. R^2 is coefficient of determination, n is release exponent for Korsmeyer-Peppas model, values are as "mean±SD".
SD: Standard deviation, LP: Losartan potassium

Table 5: Interpretation of diffusional release mechanisms from polymeric films

Release exponent (n)	Drug transport mechanism
0.5	Fickian diffusion
$0.5< n < 0.89$	Non-Fickian transport
0.89	Case II transports
Higher than 0.89	Super Case II transport

type of release.[22] From Table 4, it is evident that all but one value of n fall between the range 0.5 and 0.89, which is a clear indication of anomalous or non-Fickian transport. Only formulation LP_5 demonstrated drug release mechanism by super Case II transport as observed from the n value (0.93). In swellable system, factors affecting the release kinetics are liquid diffusion rate and polymeric chain relaxation rate. When the liquid diffusion rate is slower than the relaxation rate of the polymeric chain, the diffusion is fickian; whereas when the relaxation process is very slow when compared to diffusion the Case II transport occurs. When liquid diffusion rate and polymer relaxation rate are of the same order of magnitude, anomalous or nonfickian diffusion is observed. On the basis of these considerations, it is clear that the drug release from our formulation was controlled by both phenomenon (i.e., diffusion as well as swelling), a case of non-Fickian transport. Transport from swellable systems may often lead to release under conditions that do not agree with Higuchi's or the Fickian behavior. Most transport processes in glassy polymers fall between two limiting cases; as such, they can be represented by a coupling of the Fickian and Case II transport mechanisms. Anomalous drug transport behavior is intermediate between Fickian and Case II; this is reflected by the fact that anomalous behavior is defined by values of n between 0.5 and 0.89.[33]

Statistical analysis (ANOVA) of the percent drug release (in 8 h) revealed that release of the drug from formulation LP_5 showed a statistically significant difference ($P < 0.05$) with respect to other formulation LP_1, LP_2 and LP_9. No statistically significant difference ($P < 0.05$) in percent drug release was observed between LP_3 and LP_5, LP_6 and LP_8, and LP_6 and LP_9.

Differential scanning calorimetry studies

DSC monitors heat effects associated with phase transitions and chemical reactions as a function of temperature. In a DSC the difference in heat flow to the sample and a reference at the same temperature, is recorded as a function of temperature. The DSC thermograms of pure LP, physical mixture and optimized buccal patch are shown in Figure 4. The DSC thermogram of LP features a single sharp melting endotherm, having a peak temperature of 264.82°C, with two minor endotherms at 208.64°C and 71.03°C. Onset and endset temperatures for the major endotherm were 255.86°C and 279.61°C respectively. Similar results were reported in some earlier works.[34] The thermograms of the physical mixtures of LP with other excipients (1:1) showed the existence of the drug exothermic peak which indicated the absence of interaction between LP and other excipients. In the optimized formulation, endothermic peak of drug was well preserved with slight changes in terms of broadening or shifting towards the lower temperature. It has been reported that the quantity of material used, especially in drug–excipients mixtures, affects the peak shape and enthalpy. Thus, these minor changes in the melting endotherm of drug could be due to the mixing of drug and excipient, which lowers the purity of each component in the mixture and may not necessarily indicate potential incompatibility. Thus, it could be concluded that LP was compatible with all the excipients used in the formulation.

XRD studies

XRD studies are carried out in order to investigate the state of drug whether amorphous or crystalline as such in pure form and its polymer blends. The presence of sharp peaks generally indicate the crystalline nature

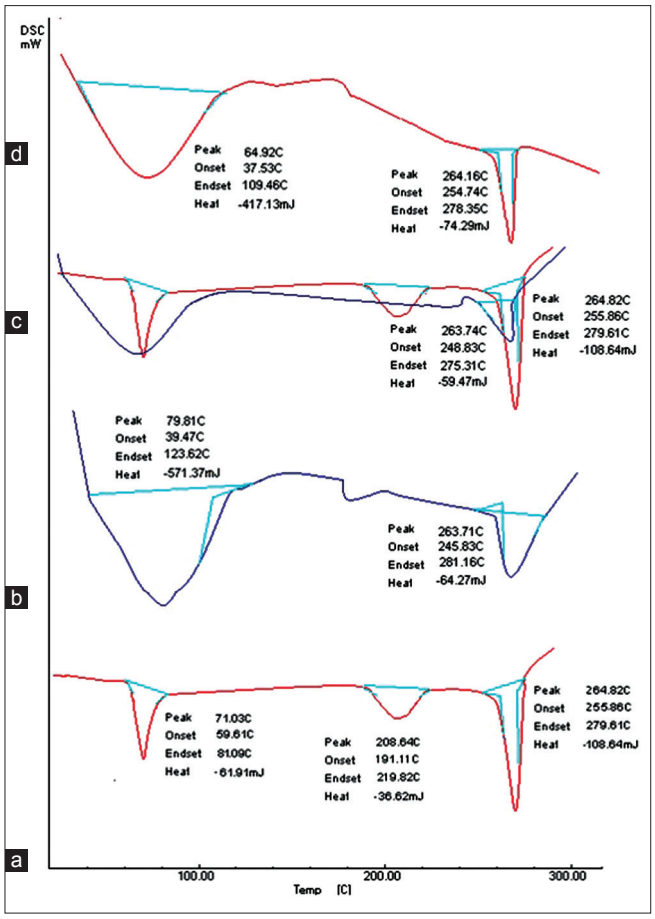

Figure 4: Differential scanning calorimetry thermograms of (a) pure losartan potassium, (b) physical mixture, (c) overlay of physical mixture and losartan potassium plain and (d) optimized buccal patch losartan potassium 5

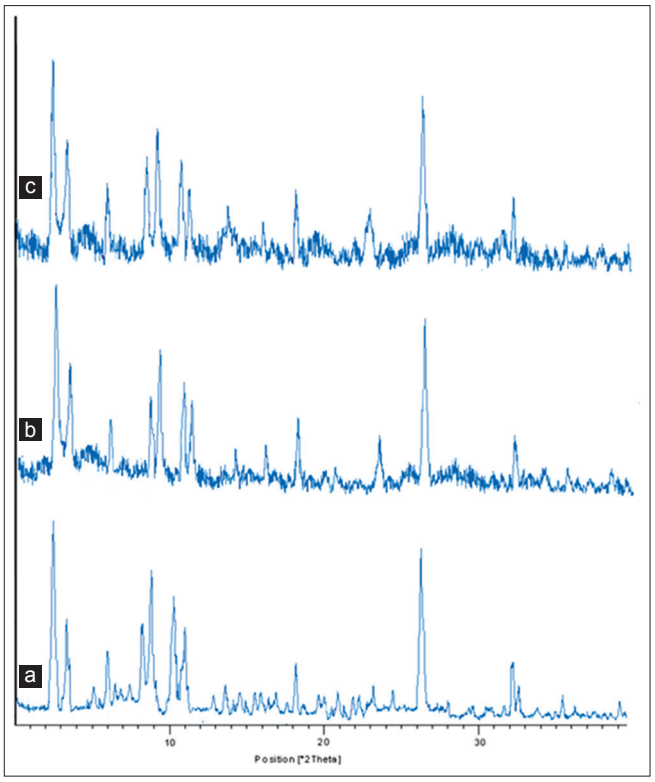

Figure 5: X-ray diffractometry spectra of (a) pure losartan potassium, (b) physical mixture and (c) optimized buccal patch losartan potassium 5

which are absent in case of amorphous drugs. The X-ray diffractogram of pure LP, physical mixture and optimized product are shown in Figure 5. The pure LP exhibited the diffraction peaks in the range of 4–35° 2θ, similar observations being reported in some earlier works.[34]. The X-ray diffractogram of LP confirms its crystalline nature, as evidenced from the appearance of number of sharp and intense peak. The diffraction pattern of drug polymer physical mixture showed a decline in the intensity of peaks, indicating a slight tendency toward amorphous nature. However, the diffraction pattern of optimized product represented complete appearance of sharp peaks, which suggest that the drug is still in its crystalline nature and there is no inhibitory effect of selected polymers on the crystallization of drug.

CONCLUSION

Novel mucoadhesive buccal patches of LP with unidirectional drug delivery were formulated with the intention of obtaining better therapeutic efficiency by controlling drug release, thereby improving patient compliance and increasing bioavailability with decreased dosing and fewer side effects. The patches were formulated with HPMC K4M, HPMC K100M and PVP-K30 and optimized using a two factor, three-level 3^2 full factorial design. The quantitative effect of these factors at various levels on the burst release etc., could be predicted by using polynomial equations. The RSM studied for the various responses helped in understanding the interaction effects between the combination and ratio of the different grades of HPMC. The *in vitro* studies have shown that this is a potential drug delivery system for LP with considerably good characteristics and release profile. Further work is suggested to support its efficacy claims by long-term pharmacokinetic and pharmacodynamic studies in human subjects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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