ORIGINAL ARTICLE

Formulation, Optimization, and Evaluation of Bioadhesive Xanthan Gum Based Buccal Patch for Sustained Delivery of Ropinirole Hydrochloride

Kalyani Kayande, Namrata Patil, Supriya Nikam

Department of Pharmaceutics, Sinhgad Institute of Pharmacy, Narhe, Pune, Maharashtra, India

Abstract

Introduction: Parkinson's disease is a neurological disorder in which there is a gradual loss of brain cells that make and store dopamine. Ropinirole hydrochloride (ROPH) is an anti-Parkinson's drug which undergoes extensively first-pass metabolism, with oral bioavailability 45%. **Aim:** The study aimed to formulation, optimization, and evaluation of ROPH buccal patch using xanthan gum (XG). **Materials and Methods:** Solvent casting method was used to prepare mucoadhesive buccal patch ROPH using XG as a mucoadhesive polymer, polyvinylpyrrolidone K90 as a film former and polycarbophil and hydroxypropyl methylcellulose K4M as a release retardant. **Results and Discussion:** The dissolution studies showed sustained release of drug about 97.86% for 8 h following Korsmeyer-Peppas model (r^2=0.989, n = 0.199). The optimization of all prepared batches was carried out by 3^2 factorial designs, the optimized batch F3 showed acceptable physicochemical properties and having swelling index 286.10%, mucoadhesive strength 26.90 g, tensile strength 0.04±0.01 N/mm^2, and *in vitro* drug release 97.86%. E*x vivo* permeability was carried out using sheep buccal mucosa and it was found to be increased by five folds than that of formulation without penetration enhancer. After histopathological evaluation cellular membrane was found to be intact and did not show any signs of necrosis. **Conclusion:** Thus, an attempt to formulate a stable mucoadhesive buccal patch was made. The *in vitro* studies have shown that this is a potential drug delivery system for ROPH with good stability and release profile.

Key words: Mucoadhesive, Xanthan gum, Ropinirole hydrochloride, 3^2 factorial design, *In vitro, Ex vivo*

INTRODUCTION

In mucoadhesive buccal drug delivery system when the mucoadhesive formulation gets in contact with the mucosal surface, due to hydration of polymer with mucin molecules they retained at the site for specific a period and release the drug in a controlled manner.[1] Due to the abundant blood vessels and smooth muscles, this route is more preferred.[2] It avoids the first-pass metabolism by direct entry of drug in systemic circulation.[3] It is painless, easily assessable; no need of water, the dangers of the formulation can be avoided by splitting or removal of formulation from the site of application.[4] Due to the low enzymatic activity, it is a potential site for controlled delivery of drugs as compared to the gastro intestinal mucosa.[5] It is for both local and systemic delivery of drug, it may be bi- directional or the release of drug can be modified by providing backing membrane for unidirectional release of the drug, to avoid dissolving of the drug in saliva and swallowing.[6] Various buccoadhesive formulations are available such as buccal tablets, buccal ointments, pastes, gels, nanoparticulates, and buccal patches or films. The buccal patches are more acceptable by the patient due to the more flexibility, easy accessibility, small size, and thickness.[7] The mucoadhesive polymer increases the residence time of the buccal patch. In general, the biodegradable, nontoxic, easily available, and cost-effective nature make the natural polymers more acceptable.[8]

Address for correspondence:
Kalyani Kayande, Department of Pharmaceutics, Sinhgad Institute of Pharmacy, Narhe, Pune - 411 041, Maharashtra, India. Mobile: +91-8149422492.
E-mail: kalyanipranav08@gmail.com

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In Parkinson's disease, there is loss of brain cells that make and store dopamine. Ropinirole hydrochloride (ROPH) undergoes extensive first-pass metabolism and has 45% oral bioavailability with very short $t_{1/2}$ (5 h). The dose of ROPH is 2–24 mg/day and the molecular weight is 296.836, this makes oral route unsatisfactory.[9] The conventional oral tablets of ROPH (0.25–1 mg) and extended release tablets (2–4 mg) are available in the market. ROPH has satisfied all the criteria required for the buccal patch system. Xanthan gum (XG) is used as a mucoadhesive polymer, obtained by fermentation process from *Xanthomonas campestris*, which is a high molecular weight extracellular heteropolysaccharide and shows all principle properties for mucoadhesion.[9]

The aim of the present work was to develop, optimize, and evaluate the mucoadhesive buccal patch of ROPH using XG as mucoadhesive polymer, which gives a sustained release of the drug for the treatment of Parkinson's disease.

MATERIALS AND METHODS

ROPH was obtained as a gift sample from Glenmark Pharmaceuticals Pvt. Ltd. (Mumbai). Hydroxypropyl methylcellulose (HPMC) K4M was purchased from Loba Chemie Pvt. Ltd. (Mumbai). XG of food-grade, propylene glycol (PG), polyvinylpyrrolidone (PVP) K90, dimethyl sulfoxide (DMSO), polyethylene glycol, tween 80, and polycarbophil were obtained commercially from Research-Lab Fine Chem (Mumbai).

Preparation of mucoadhesive buccal patches

Mucoadhesive buccal patches of ROPH were prepared by the solvent casting method. XG was dissolved in hot distilled water, hydrophilic polymer PVP K90 was added to this solution and kept aside for 30 min for swelling and dissolution of the polymer. HPMC K4M was added and kept in the refrigerator for 30 min. PG was added and stirred for 15 min. This solution was sonicated for 30 min for the removal of air bubbles. The resultant clear solution was poured on ethyl cellulose backing membrane placed in a glass Petri plate of size 9 cm in diameter and allowed to dry in a hot air oven maintained at 50°C for 3–4 h. The dried bilayered patch was then cut into 4 cm^2 containing 2.0 mg drug per patch. The composition of ROPH mucoadhesive buccal patches is shown in Table 1.

Full factorial experimental design

A 3^2 randomized full factorial design was used for optimization of ROPH buccal patches. It was applied to study the effect of concentration of XG and PVP K90 on the physicochemical characteristics of patches. The amount (%) of mucoadhesive polymer XG (X_1) and the amount (%) of film former, PVP K90 (X_2) were selected as independent variables and evaluated each at three levels. The actual units of higher, middle, and lower levels of factor X_1 were 0.3%, 0.2%, and 0.1%, and for factor, X_2 were 4%, 3%, and 2%. The coding was +1, 0, and −1, respectively, for higher, middle, and lower levels of each factor. The response variables included swelling index (R_1), mucoadhesive strength (R_2), and % drug release after 8 h (R_3).

Weight and thickness of patches

The uniformity of weight for patches was performed in triplicate using an analytical balance (Contech CB-50). The average weight was calculated along with standard deviation. The thickness of the patches was checked using a Vernier caliper (Mitutoya, Japan) with a least count of 0.01 mm.

Surface pH measurement

pH was measured using pH meter (Equip-Tronics, EQ-610, India). Buccal patches were kept to swell for 1 h in a Petri dish containing phosphate buffer (pH 6.8). After 1 h, pH probe was placed in close contact with the wetted patch surface and pH was recorded for each patch. The experiment was performed in triplicate and the average was taken.[10]

Drug content uniformity

The patches of 4 cm^2 were cut from three different places of the casted patches. Each patch was placed in a 100 ml volumetric

Table 1: Composition of ROPH mucoadhesive buccal patches

Ingredients	A1	A2	A3	A4	A5	A6	A7	A8	A9
ROPH (mg)	40.5	40.5	40.5	40.5	40.5	40.5	40.5	40.5	40.5
XG (% w/v)	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
PVP K90 (% w/v)	2.0	2.0	2.0	3.0	3.0	3.0	4.0	4.0	4.0
HPMC K4M (% w/v)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Polycarbophil (% w/v)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PG*	40	40	40	40	40	40	40	40	40
Water up to (ml)	20	20	20	20	20	20	20	20	20

*Amount of PG in terms of % w/w of total polymer weight. PG: Propylene glycol, PVP: Polyvinylpyrrolidone, HPMC: Hydroxypropyl methylcellulose, XG: Xanthan gum, ROPH: Ropinirole hydrochloride

flask and dissolved in phosphate-buffered saline (PBS) (pH 6.8). From this 2 ml was taken and diluted with the PBS pH 6.8 up to 10 ml, to make a final concentration of 4 µg /ml. The absorbance of the solution was measured at 249 nm using a UV-visible spectrophotometer. The procedure was repeated for three patches of each formulation batch and the % drug content was determined using the standard graph.[11]

Folding endurance

The number of folding required to break or crack a patch is called as folding endurance. It was determined manually by repeatedly folding the patch at the same place until it breaks. The experiments were performed in triplicate, and average values were reported.[12]

Tensile strength

The tensile strength of the patch was checked by Universal Tensile Strength Testing Machine (TexturePro CT V1.3 Build 15) equipped with a trigger force set at 0.07 N. The film of 400 mm^2 was randomly selected and ASTM D-882 method was used to perform the test. The test speed was constant (0.5 mm/s) until the film ruptured. The force when the patch broke was recorded (N).[13] The tensile strength at break value was calculated as:

$$\text{tensile strength}\left(\frac{\text{N}}{\text{mm2}}\right)=\left(\frac{\text{Force}}{\text{Area}}\right) \quad (1)$$

Ex vivo mucoadhesion time

The freshly cut sheep buccal mucosa was isolated within 15 min of slaughter and immediately kept in an ice cold PBS (pH 6.8) to maintain aeration viability. The sheep buccal tissues were then fixed on the internal side of a beaker with cyanoacrylate glue. Each patch was divided in to portions of 4 cm^2, one side of which was wetted with 50 µl of PBS pH 6.8 and was pasted to the sheep buccal tissue by applying a light force. The beaker was filled with 200 ml of the PBS pH 6.8, was kept at 37±0.5°C, and was aerated. After 2 min, a 50 rpm stirring rate was applied to simulate the buccal cavity environment and the patch adhesion was monitored. The time at which the patch gets detached from the sheep buccal mucosa or complete erosion of patch from the mucosa was recorded as the mucoadhesion time.[14]

Swelling studies

The swelling index of mucoadhesive patches was carried out by placing the patch in PBS pH 6.8 at 37 ± 0.5°C. Three patches from each batch were cut and weighed, the average initial weight was calculated (W_1). The patches were placed in PBS and were removed at time intervals of 5, 10, 15, 20, 25, and 30 min till there was a maximum increase in weight of the patches; excess water present on the surface of the patch was removed, and swollen patches were reweighed (W2).[15] The swelling index was calculated as:

$$\%Swelling=\frac{(W2-W1)}{W1}\times 100 \quad (2)$$

In vitro drug release studies

USP dissolution apparatus type II was used to study drug release from the ROPH patches under sink conditions at 37°C ± 0.5°C and 50 rpm. A single patch was placed in 500 ml dissolution media containing PBS pH 6.8. A patch was applied on glass a slide in such a way that the mucoadhesive layer of the patch was in contact with dissolution media and non- adhesive backing layer was fixed on the slide with the help of two sided adhesive tape. 5 ml sample was withdrawn at suitable time intervals and replaced with a fresh dissolution medium.[16] The amount of ROPH was determined by a UV spectrophotometer at 249 nm (Jasco) with the help of a standard curve of a drug (range 0–30 ug/ml and y =0.0327x+0.0108; r^2= 0.996 in PBS pH 6.8). The test was performed on the three patches of the same formulation. All formulations were subjected to various mathematical kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas to understand the release patterns and establish the kind of mechanism followed by ROPH release from patch matrix. The model with the highest correlation coefficient was considered the best fitting one.

In vitro mucoadhesive strength

The mucoadhesive strength of the buccal patches was determined at room temperature using the two-arm balance method with minor modifications. Sheep buccal mucosa was obtained from the local slaughterhouse and used within 2 h for this study. The mucosal membrane was separated by removing underlying fat and loose tissues, and a thickness of 2 mm was obtained. The membrane was then washed with distilled water and then with PBS pH 6.8 at 37°C. The buccal mucosa was cut into pieces and again washed with PBS pH 6.8. A piece of buccal mucosa was then fixed to the bottom of a smaller beaker with the help of cyanoacrylate glue. Two pans of the balance were balanced with a 5 g weight on the right-hand side pan. The buccal patch was then stuck to the lower side of left-hand side pan with help of two way adhesive tape and then was brought in contact with the mucosa placed on small beaker by removing 5 g weight from the right pan of the balance. The balance was kept in this position for 5 min and then water was added slowly at 100 drops/min to the right-hand side pan until the patch detached from the mucosal surface. The excess weight on the pan, that is, total weight minus 5 g is the force required to separate the patch from the mucosa. The mucoadhesive strength is the weight, in grams, required to detach the patch from the mucosal surface. The experiments were performed in triplicate, and average values were reported.[17]

Ex vivo drug permeation studies

ROPH being a hydrophilic drug has low permeability through the buccal mucosa and hence different penetration enhancers such as DMSO, PEG 400m and tween 80 were incorporated to increase its permeability. Fresh sheep buccal mucosa obtained within 15 min of slaughter was immediately separated from the underlying fat and loose tissues. The isolated mucosal membrane of thickness 2 mm was washed with water and then with PBS pH 6.8. The viability of buccal mucosa was maintained by immediately immersing in ice cold PBS pH 6.8 for 15 min. The extent and rate of mucosal permeation of ROPH across sheep buccal mucosa were carried out using Franz diffusion cell. The buccal mucosa with 4 cm^2 area was then mounted on diffusion cell, with the mucosal surface facing a donor compartment and serosal side facing receptor compartment. Both the compartments were filled with PBS pH 6.8 and bubbled. After the temperature reached at 37±1°C (required 10 min), permeability studies were started.[13] Receptor compartment was now replaced with fresh 15.5 ml of pre warmed (37°C) degassed PBS pH 6.8. A 4 cm^2 patch of each formulation under study was placed on the pre incubated buccal mucosa and the side exposed to donor compartment was of ethyl cellulose as backing membrane. Similarly, the donor compartment was also replaced with fresh 1 ml of PBS pH 6.8. The cell contents were stirred (50 rpm) using magnetic bead at 37±1°C to simulate the buccal cavity environment. Aliquots of 1 ml withdrawn at regular intervals, (every 1 h) for 8 h were filtered and analyzed for ROPH content. To maintain the constant volume in receptor chamber, same volume of PBS pH 6.8 was replenished in the receptor chamber after every sampling.[18]

Buccal mucosa sensitivity test

The final optimized formulation with 5% DMSO was subjected for a buccal mucosa sensitivity test to determine the pathological changes occurring in the cell morphology and tissue organization after the application of the buccal patch. After completion of the diffusion experiment for 8 h, buccal mucosa was collected and repeatedly washed with PBS pH 6.8. Small portion of the mucosa was fixed in 10% buffered formalin solution and dehydrated. Sections were taken by microtome at 4 µm perpendicular to the epithelial surface, stained with hematoxylin eosin (HE) and examined under a digital microscope (Motic) to evaluate any histological changes in the epithelium and the adjacent connective tissue. Control buccal mucosa was also treated and examined similarly.[19]

Accelerated stability studies

The optimized formulation was subjected to aggravated conditions of temperature and relative humidity by wrapping it in aluminum foil and packaging it in a glass container. The buccal patches were kept in a stability chamber at 40±0.5°C temperature and 75±5% RH for 3 months. The formulation was tested for changes in the appearance, mucoadhesive strength, drug content, release behavior and, surface pH.[20]

RESULTS AND DISCUSSION

Formulation optimization using full factorial experimental design

Design Expert 11.0 software was used for studying the effect of independent variables on responses. The experimental design layout developed for nine possible combinations of ROPH buccal patch formulations [Table 2]. Various models such as linear, 2FI, Quadratic and Cubic, were fitted to the data and the model which fit best was suggested by software and was tested for analysis of variance (ANOVA). Regression polynomials were calculated for the individual dependent variables and then contour plots and 3D surface graphs were obtained for each individual dependent variable. Mathematical models were generated for each individual dependent variable or response (R) and expressed as equations 3 to 5. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1, X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity.

Weight and thickness of patches

The average thickness of all the mucoadhesive patches ranged from 0.10 to 0.15 mm. The weight of patches was measured with a digital balance ($n = 3$) and the average weight of all the patches varies from 67.76 ± 0.50 to 85.70 ± 0.50 [Table 3]. Thus, there was a proportional gain in the weight of patches with an increase in the thickness of patches.

Surface pH measurement

Surface pH of all the patches was found to be in the range of 6.8–7.5 [Table 3]. Hence, no mucosal irritation was expected from these prepared patches.

Drug content uniformity and folding endurance

Drug content uniformity of formulation A-1 to A-9 varied from 1.98 ± 0.01 to 2.00 ± 0.01, respectively [Table 3], which was within the desirable range. Folding endurance was determined manually, which ranged from 324 ± 2.50 to 364 ± 1.52 for batch A1-A9, respectively. The folding endurance was found to be increased with increasing concentration of PVP K90 and XG. This confirms that the films were not brittle.

Table 2: Experimental design layout of ROPH buccal patch formulations

Run	Formulation code (FC)	Factor X1 (XG)	Factor X2(PVP K90)	Response 1 (R1)	Response 2 (R2)	Response 3 (R3)
Coded levels of variables				Swelling Index (%)	Mucoadhesive Strength (g)	% Drug release at 8 h
1	A1	−1	−1	278	25.0	98.41
2	A2	−1	0	282	25.3	96.85
3	A3	−1	1	294	26.2	96.69
4	A4	0	−1	286	26.7	96.44
5	A5	0	0	298	27.5	95.75
6	A6	0	1	305	28.4	95.09
7	A7	1	−1	292	28.9	94.67
8	A8	1	0	315	29.6	93.56
9	A9	1	1	324	30.1	91.65

Table 3: Result of different buccal patches containing ROPH

FC	Thickness* (mm)	Weight uniformity * (mg)	Surface pH*	Folding endurance*	Drug content uniformity* (mg)	*Ex vivo* mucoadhesion time (min)
A1	0.10±0.010	67.76±0.50	7.2±0.10	324±2.50	1.98±0.01	288
A2	0.10±0.005	71.69±0.60	7.1±0.10	344±2.00	2.00±0.01	286
A3	0.11±0.010	72.95±0.96	7.0±0.08	360±1.52	1.98±0.01	285
A4	0.12±0.005	75.03±0.95	6.9±0.10	331±1.00	1.99±0.01	294
A5	0.13±0.010	76.11±0.20	6.8±0.05	346±1.52	1.99±0.00	293
A6	0.13±0.010	77.54±0.79	6.9±0.10	363±1.15	1.99±0.01	290
A7	0.14±0.010	79.93±0.51	6.8±0.09	341±2.08	1.99±0.01	298
A8	0.15±0.010	83.14±0.65	6.8±0.05	348±1.00	1.98±0.01	296
A9	0.15±0.010	85.70±0.50	6.8±0.05	364±1.52	1.98±0.01	293

*All values are mean ± SD, *n*=3

Ex vivo mucoadhesion time

Mucoadhesion time of patches containing a higher proportion of XG was found to be more (A7, A8, and A9) than other batches of ROPH buccal patch [Table 3]. It was found that the mucoadhesion time varies with change in concentration of polymer and there exists a direct relationship between the swelling index and mucoadhesion time of the buccal patch.

% swelling index

The degree of swelling of bio-adhesive polymer is an important factor affecting bioadhesion. All the patches showed a maximum increase in swelling after 1 h. Figure 1 shows the comparative swelling index of different formulations of ROPH buccal patches.

In vitro drug release studies

In vitro drug release profiles are shown in Figure 2. An immediate drug release followed by gradual release was successfully observed for all XG patches.

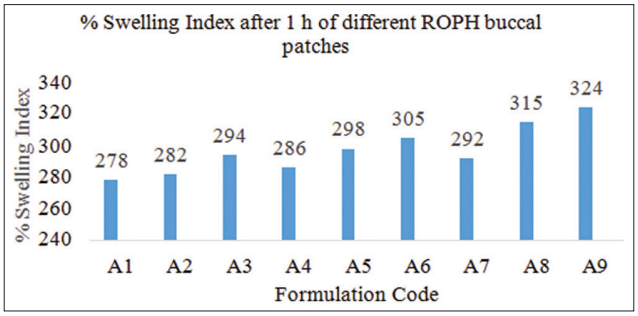

Figure 1: Bar graph showing % swelling index of ROPH buccal patches after 1 h

In Vitro mucoadhesive strength

The weight required to detach the patch from the mucosal surface provided the measure of mucoadhesive strength which is showed in Table 4.

Effect of formulation variables on swelling index

Swelling property is an important parameter, which governs the consistent and prolonged release of drug and effective

Table 4: *Ex vivo* mucoadhesion characteristics of ROPH buccal patches

Formulation code	A1	A2	A3	A4	A5	A6	A7	A8	A9
Mucoadhesive strength* (g)	25.00± 0.80	25.30±0.60	26.20±0.20	26.70±0.05	27.50±0.05	28.40±0.30	28.90±0.10	29.60±0.10	30.10±0.10

*All values are mean±SD, *n*=3

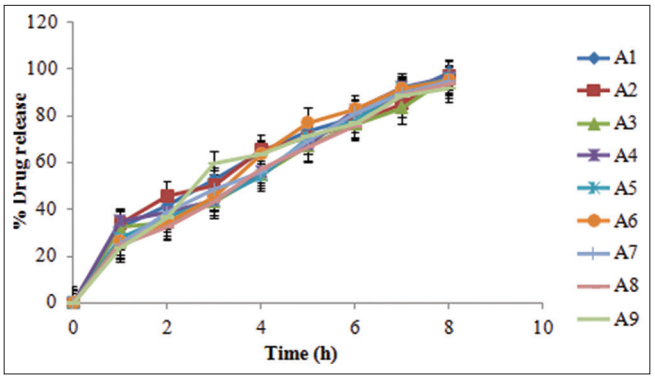

Figure 2: Comparative *in vitro* drug dissolution profiles of ROPH buccal patches

mucoadhesion.[21] Due to the presence of soluble excipients, PVP K90, HPMC K4M, and XG, swelling of patches was started within 3.4 min. All the patches showed a maximum increase in swelling after 1 h. Swelling index of all the formulations is shown in Table 2.

Formulation A9 containing the highest concentration of XG (0.3%) and the lowest concentration of PVP K90 (4%), showed highest value of swelling index.

On the application of factorial design, the quadratic model was suggested by software and found to be significant with model F value of 22.11, $P < 0.0001$ and $R^2 = 0.973$, which implied that the model was significant. There was only 1.42% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" <0.05 for each term was obtained which indicated that model term was significant. In this case, X_1, X_2, X_1^2, and X_2^2 were significant model terms. The model for response R_1 (Swelling index) is:

$$R_1 = 297.56 + 12.83X_1 + 11.17X_2 + 4.00X_1X_2 - 1.17X_1^2 - 1.83X_2^2 \quad (3)$$

The given Eq. 3 indicates that both X_1 (concentration of XG) and X_2 (concentration of PVP K90) have a positive effect on the percent swelling index. It means that the increase in concentration of XG increases the extent of swelling of the patches. PVP K90 shows good swelling index values, greater hydration rates, which would permit faster and ready disentanglement of individual chains, thus increasing the porosity of the film and gives good release.[14] The swelling index of patches increases with an increase in the concentration of XG and PVP K90 because XG is also hydrophilic in nature which swells in water. The combined effect of factor X_1 (XG) and factor X_2 (PVP K90) can be further interpreted with the help of the contour plot and response surface plot [Figure 3]. The effect of PVP 90 concentration on response was found to be more significant than that of XG concentration. The combination of PVP K90 with XG led to increases in swelling index.

Effect of formulation variables on mucoadhesive strength

Bioadhesion/mucoadhesion may be defined as the adhesion between a polymer and a biological membrane, for example, mucus. The strength of bioadhesion was affected by diverse factors such as the molecular weight of polymers, contact time with mucus, swelling rate of the polymer, and natural membrane used in the study.[22] Thus, formulation A7–A9 showed the highest bioadhesion due to their highest swelling index, ensuring adhesion of patch at the site of administration.

On applying factorial design, the quadratic model was suggested by software and found to be significant with model F value of 118.53, $P < 0.0001$ and $R^2 = 0.9950$, which implied that model was significant. In addition, there was only a 0.12% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" <0.05 for each term were obtained which indicated that every model term was significant. In this case, X_1, X_2, X_1^2, and X_2^2 were significant model terms.

$$R_2 = +27.48 + 2.02X_1 + 0.6833X_2 - 0.0167X_1^2 + 0.0833X_2^2 \quad (4)$$

The Eq. 4 indicates that X_1 (concentration of XG) has a positive and that X_2 (concentration of PVP K90) also has a positive effect on mucoadhesive strength. That is an increase in XG amount led to an increase in the mucoadhesive strength. XG being anionic polyelectrolyte containing glucuronic acid and pyruvate in its side chain thus showed excellent mucoadhesive characteristics.[23] XG showed strong mucoadhesive characteristics due to physical entanglements and secondary interactions (hydrogen bonds) between the free COO^- groups of XG and mucin glycol-proteins.[24]

The combined effect of factor X_1 (XG) and factor X_2 (PVP K90) can be further interpreted with the help of the contour plot and response surface plot [Figure 4]. The effect of XG concentration on response was found to be more significant than that of PVP K90 concentration. Significant decrease in the % drug release from the formulation at 8 h was seen after increasing the concentration of XG and PVP K90.

Effect of formulation variable on *In vitro* drug release

ANOVA for % Drug release (R_3) at 8 h is shown in Table 2. On applying factorial design, the quadratic model was suggested

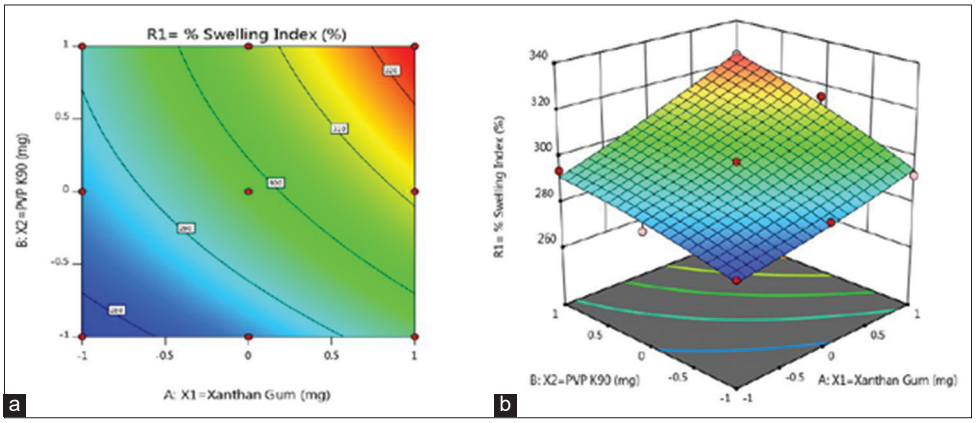

Figure 3: (a) Two dimensional contour plot (b) Three dimensional response surface plots for response R_1 (Swelling index)

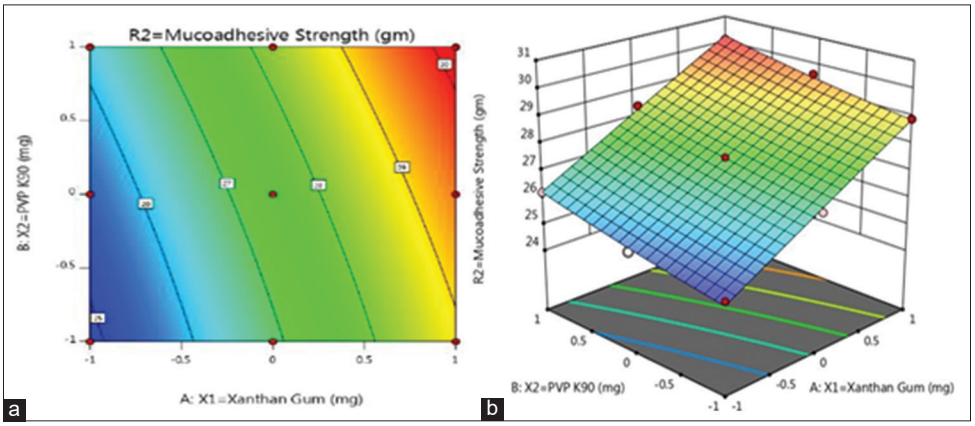

Figure 4: (a) Two dimensional contour plot; (b) Three dimensional response surface plots for response R_2 (Mucoadhesive strength)

by software and found to be significant. The Model F-value of 24.78 implies that the model is significant. There is only a 1.21% chance that F-value this large could occur due to noise. Values of "Prob > F" <0.0500 indicate model terms are significant. In this case, X_1, and X_2 are significant model terms, $P < 0.0001$ and $R^2 = 0.976$, which implied that the model was significant. Values of "Prob > F" <0.05 for each term were obtained which indicated that every model term was significant. In this case, X_1, X_2, X_1^2, and X_2^2 were significant model terms.

The model for response R_3 (% drug release at 8 h) is as follows:

$$R_3 = +95.69 - 2.01X_1 - 1.01X_2 - 0.3250X_1X_2 - 0.4550X_1^2 + 0.45X_2^2 \quad (5)$$

From Eq.5, it is clear that the drug release rate appeared to negative sign of X_1 in above equation indicates that the drug release rate decreased with an increasing concentration of XG. The release of a hydrophilic drug like ROPH from hydrophilic matrices, for example, XG, proceeds through the viscous gel layer (boundary layer control) which is formed surrounding the film on contact with the medium. As the thickness of the gel increases, the diffusion path length increases, this, in turn, causes a decrease in drug release rate from the matrices.[25] The combined effect of factors X_1 and X_2 can be further interpreted with the help of contour plot and 3D response surface plots [Figure 5] showing the effect of concentration of XG and PVP K90 on % drug release at 8 h. Furthermore, from Eq.5 and surface plots, the results indicated that XG concentration had more significant negative effect on response than the positive effect of PVP K90 concentration. That is significant decrease in % drug release at 8 h was obtained at increasing concentration of PVA with increase in XG concentration.

Validation of model

Reliability of the developed model evaluated experimentally by determining the responses for the optimized trial along with several random trails covering the entire range of experimental domain. The predicted and experimental values of all response variables and % prediction error were calculated by formula,

$$\%\text{prediction error} = \frac{\text{Experimental valuef}}{\text{experimental d}} \times 100 \quad (6)$$

$$\text{Experimental value}$$

As % prediction error was found to be <4% in all cases, model suggested by software was found to be valid.[26] Based on desirability, F3 selected as the optimized batch [Table 5].

Ex vivo drug permeation studies

The permeation profile of F3 formulation, containing different penetration enhancers and that of without penetration enhancer, across sheep buccal mucosa are shown

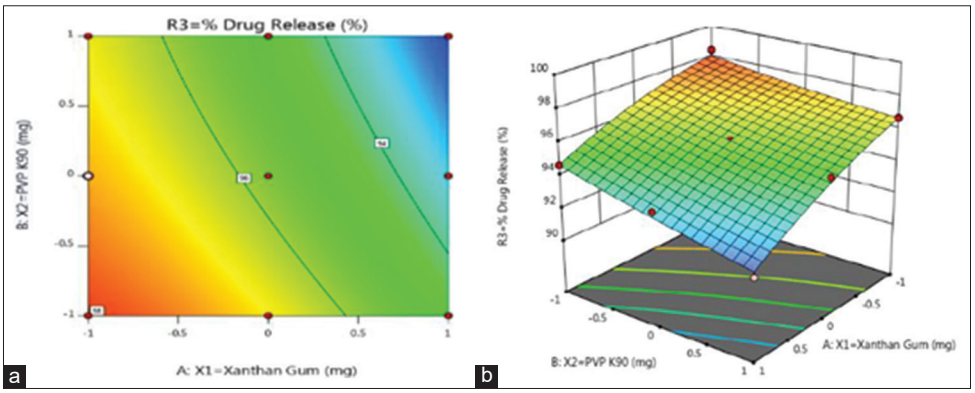

Figure 5: (a) Two dimensional contour plot (b) Three dimensional (3D) response surface plots for R_3 (Drug release)

Table 5: Predicted and experimental values of response variables and % prediction error

FC	Variables		Predicted Value			Experimental value			Predicted error		
	XG (X_1)	PVP K90 (X_2)	R1	R2	R3	R1	R2	R3	R1	R2	R3
F1	0.1	4	291.22	26.21	96.60	292.90	27.02	95.03	0.57	2.99	−1.65
F2	0.098	3.25	290.78	26.29	96.72	287.93	26.10	96.09	0.98	−0.72	−0.65
F3	0.2	2.0	284.55	26.87	96.81	286.10	26.90	97.86	0.54	0.11	1.08
F4	0.031	1.81	287.38	27.24	96.41	286.93	27.64	96.71	−0.15	1.44	0.31
F5	0.027	1.99	285.85	27.15	96.56	288.40	26.94	96.04	0.88	−0.77	−0.54
F6	0.2	3.0	297.55	29.55	95.69	298.08	30.06	94.90	0.17	1.6	−0.83

in Figure 6. Drug penetration was increased by 5% of drug after 8 h with patch containing 5% DMSO as compared to that of formulation without any penetration enhancer, while 5% Tween 80 and 5% PEG 400 transported about 2% and 3% of drug after 8 h, respectively. Hence, F3 formulation containing 5% DMSO as penetration enhancer, 0.2% XG, 2% PVP K90, 3% HPMC, was taken as final optimized batch.

Kinetics of drug release

The coefficient of regression value found to be highest for Korsmeyer-Peppas model (0.989). The diffusion exponent *n* value was found to be <0.5 (0.199), indicating Quasi-Fickian diffusion of drug through the patch.[14]

Tensile strength

Tensile strength for patch (F3) was found to be 0.04±0.01 N/mm^2, which indicates sufficient strength to withstand wear and tear occurring during administration and transportation.

Buccal mucosa sensitivity test

The optimized formulation was subjected to a buccal mucosa sensitivity test. The sections of control and sample mucosa (treated with final optimized formulation) observed under a digital microscope (Motic, B1 Advanced series) are shown in Figure 7. The histopathological evaluation of sections showed that cellular membrane was intact and there was no damage to the epithelial layer. Cell necrosis was not observed and hence it can be concluded that ROPH patch containing 5% DMSO is safe for buccal administration.

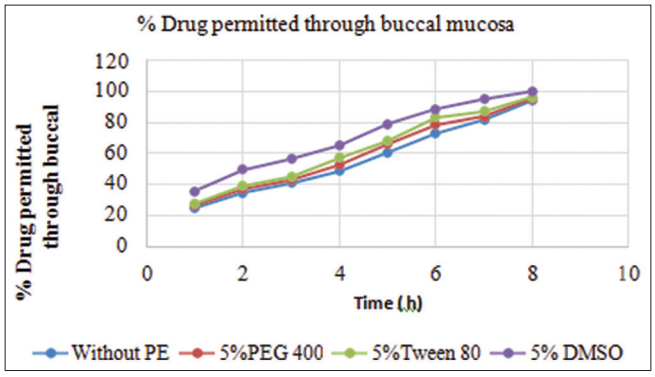

Figure 6: *Ex vivo* permeation of ROPH in the presence of different penetration enhancers

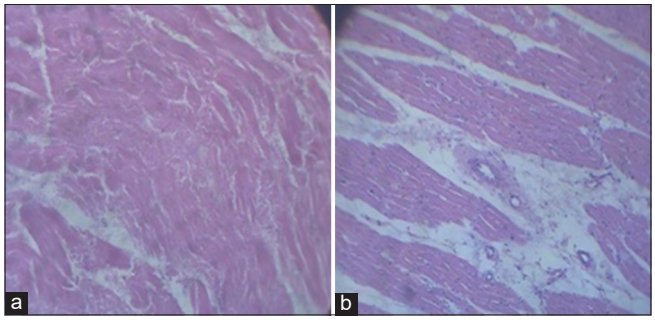

Figure 7: Histopathological evaluation of sections of sheep buccal mucosa (a) control (b) sample

Accelerated stability studies

ROPH buccal patch (F3) containing 5% DMSO showed no significant change in appearance, surface pH, mucoadhesive strength, drug content, and % drug release after 8 h during 3 months study performed at 40°±2°C and 75%±5 relative humidity. This indicates that the optimized formulation was stable.

CONCLUSION

In the present study, mucoadhesive buccal patch for ROPH was developed based on natural polymer XG and PVP K90 using solvent casting method. Formulation released the drug over a period of 8 h, which would prevent first pass metabolism. After application of 3^2 factorial design, it was found that the concentration of XG and PVP K90 had significant effect on dependent variables such as swelling index, % drug release, and mucoadhesive strength. Formulation F3 was found to be optimal formulation which showed b swelling index (286.10%), drug release (97.86%), and mucoadhesive strength (26.90 g). About 5% DMSO improved the drug permeability by 5%. Thus, an attempt to formulate a stable mucoadhesive buccal patch of ROPH for treatment of Parkinson's disease using XG was made. The *in vitro* studies have shown that this is a potential drug delivery system for ROPH with good stability and release profile. Further work is suggested to prove efficacy claims by pharmacokinetic and pharmacodynamic study in animals.

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ETHICAL APPROVAL

The article does not contain any study with human participants or animals performed by any of the authors.

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