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Formulation and *in vitro* evaluation of xanthan gum-based bilayered mucoadhesive buccal patches of zolmitriptan

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ABSTRACT

A novel bilayered mucoadhesive buccal patch of zolmitriptan was prepared using xanthan gum (XG) as mucoadhesive polymer. Hydroxypropyl methylcellulose E-15 was used as film-former and polyvinyl alcohol (PVA) was incorporated, to increase the tensile strength of the patches. To study the effect of independent variables *viz.* concentrations of XG and PVA, on various dependent variables like *in vitro* drug release, *ex vivo* mucoadhesive strength and swelling index, 3^2 factorial design was employed. *In vitro* drug release studies of optimized formulation showed initially, rapid drug release; 43.15% within 15 min, followed by sustained release profile over 5 h. Incorporation of 4% dimethyl sulfoxide enhanced drug permeability by 3.29 folds, transported 29.10% of drug after 5 h and showed no buccal mucosal damage after histopathological studies. In conclusion, XG can be used as a potential drug release modifier and mucoadhesive polymer for successful formulation of zolmitriptan buccal patches.

1. Introduction

Buccal mucosa is the most attractive alternative site of systemic drug administration amongst the various transmucosal sites available, because it has expanse of relatively immobile smooth muscle and abundant vascularization (Sudhakar, Kuotsu, & Bandyopadhyay, 2006). Buccal cavity provides easy accessibility for self-medication and hence it is well accepted by patients (Abruzzo, Bigucci, Cerchiara, Cruciani, Vitali, & Luppi, 2012; Ratha Adhikari, Nayak, Nayak, & Mohanty, 2010). Moreover it is a potential site for controlled drug delivery of therapeutic agents because of low enzymatic activity compared to the gastro-intestinal tract (Junginger, Hoogstraate, & Coos Verhoef, 1999; Salamat-Miller, Chittchang, & Johnston, 2005). As buccal mucosa is highly permeable and usually rich in blood supply, it allows rapid uptake of drug in systemic circulation, leading to rapid onset of action (Consuelo, Falson, Guy, & Jacques, 2007) and in most cases avoids degradation by first-pass hepatic metabolism hence leading to high bioavailability (Satheesh Madhav, Shakya, Shakya, & Singh, 2009). Furthermore, buccal mucosa has several advantages like, it shows short recovery times after stress or damage, is tolerant to potential allergens (Scholz, Wolff, Schumacher, & Giannola, 2008; Shojaei, 1998) and provides intimate contact between a dosage form and the absorbing tissue (Bruschi & Freitas, 2005). It has versatility in designing unidirectional or multidirectional release system for both, local or systemic action (Vasantha, Puratchikody, Mathew, & Balraman, 2011). Various buccal adhesive delivery devices have been developed such as, buccal tablet, buccal films, buccal wafers, buccal gels and ointments (Hearden et al., 2012). But among various buccal dosage forms, buccal patches provide more flexibility, as they can be very easily administered and removed from the application site, terminating the input of drug whenever desired (Ratha Adhikari et al., 2010). The patches also have improved patient compliance due to their small size and reduced thickness (Morales & Mc Conville, 2011) and obviate the need for water. But one particular problem associated with buccal drug delivery system is the short residence time at the site of application. Use of bioadhesive polymers may overcome this problem (Perioli et al., 2004). Bioadhesive polymers are generally classified as natural and synthetic polymers (Salamat-Miller et al., 2005). But natural polysaccharides are generally preferred over the synthetic polymers, because they are non-toxic, less expensive and freely available (Bhardwaj, Kanwar, Lal, & Gupta, 2000).

In this study xanthan gum (XG), a high molecular weight extracellular heteropolysaccharide, produced by fermentation with the Gram-negative bacterium *Xanthamonas campestris*, (Talukdar & Kinget, 1995) was used for modifying drug release properties and mucoadhesivity. It is a heteropolysaccharide and the primary structure of XG consists of repeated pentasaccharide units formed by two glucose units, two mannose units, and one glucuronic acid unit, in the molar ratio 2.8:2.0:2.0. Its main chain consists of β-D-glucose units, which are linked at the 1 and 4 positions. Presence of glucuronic acid in side chains gives this polymer a negative charge. XG is a high molecular weight naturally produced cellulose

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derivative and its molecular weight ranges from 2×10^6 to 20×10^6 Da (Fukuda, Peppas, & McGinity, 2006; Garcia-Ochoa, Santos, Casa, & Gomez, 2000). Chemically XG is a polysaccharide which has polyanionic properties due to carboxylic groups and has very good bioadhesive strength (Kaur, Singh, & Tiwary, 2010). As it has excellent rheological properties, XG is used in many applications, mainly in food industry as thickening, suspending and stabilizing agent (Psomas, Liakopoulo-Kyriakides, & Kyriakidis, 2007). XG has also been used as binder (Sinha & Kumria, 2002) and mucoadhesive controlled-release excipient for buccal drug delivery. Furthermore, XG is generally regarded as non-toxic and non-irritant at the levels employed as pharmaceutical excipient (Rowe, Shesky, & Owen, 2006).

Zolmitriptan (ZMT) is a selective agonist of serotonin type 1B and 1D receptors, used in the treatment of migraine. ZMT undergoes extensively first pass metabolism, with oral bioavailability only 40% and has very short $t_{1/2}$ *viz.* 3 h. This makes oral route unsatisfactory. Presently ZMT is available in the form of conventional oral tablet (2.5 mg, 5 mg) mouth dissolving tablet (2.5 mg), and nasal spray (5 mg) in the market (Moffat, Osselton, & Widdop, 2004). But nasal route also has its own limitations such as rapid mucociliary clearance and low permeability of the nasal mucosa to the drugs (Alhalaweh, Andersson, & Velaga, 2009).

This justifies a need to develop other effective alternatives and one of them is mucosal drug delivery systems, which are considered as potential sites for drug administration, allowing the drug to directly enter systemic circulation and bypass first-pass metabolism. The concept of administration of ZMT *via* buccal route by formulating the mucoadhesive bilayered buccal patches using XG has not been explored so far. Thus an attempt has been made to utilize XG, which is a cheap source of polysaccharide and abundantly available natural polymer, for formulating ZMT mucoadhesive bilayered patches.

The aim of this work was to develop and evaluate ZMT mucoadhesive bilayered patch that will demonstrate initially fast release and later prolonged release of the drug satisfying the need for an anti-migraine effect, using natural polysaccharide XG as mucoadhesive polymer and drug release modifier.

2. Materials and methods

2.1. Materials

ZMT was obtained as a gift sample from Cipla Ltd. (Mumbai). 3 M Scotchpak ™ 9732 (polyester and ethylene vinyl acetate laminate) was obtained as a gift sample from 3 M Corporation (USA). Hydroxypropyl methylcellulose (HPMC) E-15 LV Premium was purchased from Loba Chemie Pvt. Ltd. (Mumbai). Xanthan gum (XG) of food grade, propylene glycol (PG), polyvinyl alcohol (PVA), sorbitol and dimethyl sulfoxide (DMSO) were obtained commercially from Research-Lab Fine Chem (Mumbai). Acetonitrile, triethylamine and ammonium dihydrogen orthophosphate of HPLC grade were purchased from Research-Lab Fine Chem (Mumbai). Oleic acid was obtained from Burgoyne Urbidges and Co. (Mumbai). All other chemicals and solvents were of analytical grade.

2.2. Preparation of mucoadhesive bilayered buccal patches

Mucoadhesive bilayered buccal patches of ZMT were prepared by solvent casting method as described by Morales and Mc Conville (2011). ZMT was dissolved in distilled water and XG was then added in drug solution. This solution was heated till the gum dissolved. The hydrophilic polymer PVA was then added to this solution and kept aside for 20 min for swelling of the polymer. Further HPMC E-15 was added and this solution was kept in refrigerator for the purpose of cooling (since HPMC dissolves in cold water) till a clear solution was obtained (required 30 min). Later PG was added as a plasticizer and stirred with constant mechanical stirrer for 10–15 min. This solution was then sonicated for 30 min for complete removal of air bubbles. The resultant clear solution was then poured on 3 M Scotchpak™ 9732 foil, placed in glass Petri plate of size 7 cm in diameter and allowed to dry in a hot air oven, maintained at 50 °C for 5 h. The dried bilayered patch was then cut into circular pieces having diameter of 2 cm and area as 3.14 cm^2, containing 2.5 mg of drug per patch. Composition of ZMT mucoadhesive buccal patches is shown in Table 1.

2.3. Full factorial experimental design

A 3^2 randomized full factorial design was used for optimization of ZMT buccal patches. The design was also applied to study the effect of concentration of XG and PVA on physico-chemical characteristics of patches. The amount (%) of mucoadhesive polymer XG (X_1) and the amount (%) of film former, PVA (X_2) were selected as independent variables, in this study. These two factors were evaluated, each at three levels. The actual units of higher, middle and lower levels of factor X_1 were 0.1%, 0.2% and 0.3%, and for factor X_2 were 0.5%, 0.75% and 1%. The coding was +1, 0 and −1, respectively for higher, middle and lower levels of each factor. The dependent or response variables included swelling index (Y_1), % drug release in 15 min (Y_2), % drug release at 5 h (Y_3) and mucoadhesive strength (Y_4).

2.4. Characterization of buccal patches

2.4.1. Weight and thickness of patches

Three patches were selected randomly from each formulation of diameter 2 cm and were weighed individually on an analytical balance (Contech CB-50). Average weight was calculated along with standard deviation. The thickness of patches was assessed using a vernier caliper (Mitutoya, Japan) with a least count of 0.01 mm.

2.4.2. Surface pH measurement

Surface pH of patch was determined to check whether the film causes irritation to the buccal mucosa. The surface pH study was carried out by selecting 3 patches randomly and pH measurement was done using pH meter (Equip-Tronics, EQ-610, India). Patch was placed in a Petri dish containing 0.5 ml of distilled water and left for 1 h. After complete swelling, pH probe was placed in close contact with the wetted patch surface and pH was recorded for each patch (Koland, Charyulu, & Prabhu, 2010).

2.4.3. Drug content uniformity

The patches were tested for drug content uniformity by UV spectrophotometric method. Patches of 20 mm diameter were cut from three different places from the casted patches. Each patch was placed in 100 ml volumetric flask and dissolved in PBS pH 6.8; 2 ml was taken and diluted with the same up to 10 ml, to make final concentration of 5 μg/ml. The absorbance of the solution was measured at 222.5 nm using UV/visible spectrophotometer. The percentage drug content was determined using the standard graph and the same procedure was repeated for three patches of each formulation (Raghavendra Rao & Munde, 2011).

2.4.4. Folding endurance

Folding endurance of patches was determined manually by repeatedly folding the patch at the same place until it ruptures. The number of folding required to break or crack a patch was taken as the folding endurance. The experiments were performed in

Table 1
Composition of various mucoadhesive buccal patches.

Name of excipients	Different batches of ZMT formulation and concentration (% w/v)								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
Drug per patch (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
HPMC E-15	3	3	3	3	3	3	3	3	3
XG	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
PVA	0.5	0.75	1	0.5	0.75	1	0.5	0.75	1
PG[a]	40	40	40	40	40	40	40	40	40
3 M Scotchpak™ 9732 foil	Backing layer on A1–A9 formulations								

[a] Amount of PG in terms of % w/w of total dry polymer.

triplicate, and average values were reported (Sahni, Raj, Ahmad, & Khar, 2008).

2.4.5. Tensile strength

Tensile strength of the patches was checked by Universal Tensile Strength Testing Machine (LS5, Lloyd Instruments Limited, UK) equipped with a 500 N load cell. Test was conducted under normal laboratory conditions. The film of 400 mm^2 was randomly selected and ASTM D-882 method was used to perform the test. The lower clamp was held stationary and the patch was pulled apart by the upper clamp at a speed of 50 mm/min. The force of the patch at the point when the patch broke was recorded (Avchat, Gujar, & Wagh, 2013). Nexygen Plus3 software was used for data collection and performance of calculations. The experiment was performed in triplicate, and average values were reported. The tensile strength at break value was calculated using formula:

$$\text{tensile strength}\,(\text{N/mm}^2) = \frac{\text{force at break}\,(\text{N})}{\text{initial cross sectional area}\,(\text{mm}^2)} \quad (1)$$

2.4.6. Ex vivo mucoadhesion time

The *ex vivo* mucoadhesion time was performed after application of the patches on freshly cut sheep buccal mucosa. Within 15 min of slaughter, buccal mucosa was isolated and immediately immersed in an ice-cold phosphate buffer saline (PBS) pH 6.8. It was kept immersed for 10 min, with aeration to maintain viability. The sheep buccal tissues were then fixed on the internal side of a beaker with cyanoacrylate glue. Each patch was divided in portions of 3.14 cm^2 and cut; a side of each patch was wetted with 50 μl of PBS pH 6.8 and was pasted to the sheep buccal tissue by applying a light force with the finger tip for 30 s. 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 for 300 min. Time required for the patch to detach from the sheep buccal mucosa or its complete erosion from the mucosa was recorded as the mucoadhesion time (Patel, Prajapati, & Patel, 2007a).

2.4.7. Swelling studies

The swelling index of buccoadhesive patch was evaluated by placing the patches in PBS pH 6.8 at 37 ± 0.5 °C (Kaur & Kaur, 2012). Three patches of each batch were cut and weighed, and the average initial weight was calculated as W_1. The patches were placed in PBS and were removed at time intervals of 5, 10, 15, 20, 25, 30 min till there was maximum increase in weight of the patches; excess water on the surface was carefully absorbed using filter paper, and swollen patches were reweighed. The experiment was carried out in triplicate. The average weight W_2 was calculated, and the swelling index was calculated by the formula:

$$\%\,\text{swelling index} = 100 \times \frac{(W_2 - W_1)}{W_1} \quad (2)$$

2.4.8. In vitro drug release studies

USP dissolution apparatus type II was used to study drug release from patch formulation 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 slide in such a way that 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. Samples (5 ml) were withdrawn at suitable time intervals and replaced with fresh dissolution medium. The amount of ZMT was determined by UV spectrophotometer at 222.5 nm (Jasco) with the help of standard curve of drug (range 1–5 μg/ml and $y = 0.0099x$; $r^2 = 0.9993$ in PBS pH 6.8). The test was performed on three patches of same formulation (Shidhaye, Saindane, Sutar, & Kadam, 2008). All formulations were subjected to various mathematical kinetic models like Zero-order, First-order, Higuchi and Korsmeyer-Peppas, to understand the release patterns and establish the kind of mechanism followed by ZMT release from patch matrix. The model with the highest correlation coefficient was considered to be the best fitting one (Ratha Adhikari et al., 2010).

2.4.9. In vitro mucoadhesive strength

The mucoadhesive strength of the mucoadhesive buccal patches was determined at room temperature using the two-arm balance method reported by Parodi, Russo, Caviglioli, Cafaggi, and Bigbardi (1996); Emami, Varshosaz, and Saljoughian (2008) with minor modifications. Fresh sheep buccal mucosa was obtained from a local slaughterhouse and used for the study within 2 h of slaughter. The mucosal membrane was separated by removing underlying fat and loose tissues, and 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 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, *i.e.* total weight minus 5 g is force required to separate the patch from mucosa. The weight, in grams, required to detach the patch from the mucosal surface provided the measure of mucoadhesive strength (Abu-Huwaij, Asaf, Salem, & Sallam, 2007). The experiments were performed in triplicate, and average values were reported.

2.4.10. Ex vivo drug permeation studies

2.4.10.1. Isolation of buccal mucosa and pre-experimental setup. The optimized formulation showing the desired release in 15 min and after 5 h, best mucoadhesive strength, optimum swelling index,

and better *in vitro* residence time was subjected to permeation studies through the sheep buccal mucosa in order to find out the extent of drug permeability in terms of permeation flux and permeation coefficient. ZMT being hydrophilic drug has low permeability through the buccal mucosa and hence different penetration enhancers like DMSO (Aungst & Rogers, 1989), sorbitol and oleic acid (Rathi, Dhamecha, Patel, Saifee, & Dehghan, 2011) were incorporated to increase its permeability. Fresh sheep buccal mucosa obtained within 15 min of slaughter, from a local slaughterhouse was immediately separated from underlying fat and loose tissues, surgically. The isolated mucosal membrane of thickness 2 mm was washed with water and then with PBS pH 6.8. For maintaining the viability of buccal mucosa, it was immediately immersed in ice-cold PBS pH 6.8 for 15 min and bubbled. The extent and rate of mucosal permeation of ZMT across sheep buccal mucosa were carried out using Franz diffusion cell. The buccal mucosa with 3.14 cm^2 as an effective area was then mounted on diffusion cell, with mucosal surface facing donor compartment and serosal side facing receptor compartment. Both the compartments were filled with PBS pH 6.8 and bubbled. After the temperature reached 37 °C (required 10 min), permeability studies were started (Lalla, Gurnaney, & Narayan, 2002; Pund, Rasve, & Borade, 2013).

2.4.10.2. Zolmitriptan permeation study. Receptor compartment was now replaced with fresh 15.5 ml of prewarmed (37 °C) gassed PBS pH 6.8. A 3.14 cm^2 patch of each formulation under study was placed on the preincubated buccal mucosa and the side exposed to donor compartment was of 3 M Scotchpak™ 9732 foil 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 (temperature was controlled using constant circulation water bath), to simulate the buccal cavity environment. Aliquots of 1 ml withdrawn at regular intervals, (every 1 h) for 5 h were filtered and stored at −20 °C, until analyzed for ZMT content. In order to maintain the constant volume in receptor chamber, same volume of PBS pH 6.8 was replenished in the receptor chamber after every sampling (Shidhaye et al., 2008). The amount of drug permeated was quantified using HPLC method of analysis with the help of standard curve of drug ($y = 165{,}175x$; $r^2 = 0.9987$, range 5–13 ppm) and mobile phase as 0.01% triethylamine: acetonitrile: 0.02 M ammonium dihydrogen phosphate; 28.2: 25: 46.8 (pH-3.2) at a flow rate of 1 ml/min. HPLC system (Jasco; 2000 series) was equipped with a reverse phase C18 column (Kromasil 100-5C18; 250 × 4.6 mm, 5 μm in particle size), a PU-2080 Plus isocratic pump, a 20 μl injection loop and a UV-2075 Plus absorbance detector (λ = 222 nm). Computerized data acquisition and treatment were performed with the Borwin Chromatography Software (Danavena, Bhoomaiah, Phani, & Balmurali, 2011). The graph of cumulative amount of ZMT (μg) permeated per unit of mucosal surface area (cm^2) against time (h) was plotted. Permeation flux (J, μg cm^{-2} h^{-1}), was calculated from this graph as the amount of ZMT passing across 1 cm^2 of the permeation membrane per unit time. The slope of the linear portion of the curve was considered as permeability flux. Permeability coefficient (P, cm h^{-1}) was calculated as permeability flux divided by initial total donor chamber concentration (2.5 mg/ml). Enhancement ratio indicates ratio of permeability coefficient with enhancer to permeability coefficient without enhancer (Lalla et al., 2002; Pund et al., 2013).

2.4.11. Buccal mucosa sensitivity test

The final optimized formulation was subjected for buccal mucosa sensitivity test to check if any irritation has occurred after patch application. After completion of the diffusion experiment, 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 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 (Yedurkar, Dhiman, Petkar, & Sawant, 2013).

3. Results and discussion

3.1. Full factorial experimental design

Design Expert 8.0 software was used for studying effect of independent variables on responses. Experimental design layout developed for 9 possible combinations of ZMT buccal patch formulations is shown in Table 2. Various models such as Linear, 2FI, Quadratic and Cubic, were fitted to the data and the model which fit well 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 Eqs. (3)–(6). 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_1X_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.

3.2. Characterization of buccal patches

Physico-chemical characteristics of the bilayered patches are shown in Tables 2 and 3.

3.2.1. Weight and thickness of patches

The average weight and thickness of all the patches is given in Table 3. Weight variation values (mg) of different ZMT patches were found to be in the range of 64–80 mg. The average thickness of all the mucoadhesive patches ranged from 0.14 to 0.17 mm. Thus there was proportional gain in weight of patches with that of increase in the thickness of patches. The thickness and weight uniformity values were uniform for the patches within the respective group of formulation type. This shows that the patches cast were uniform.

3.2.2. Surface pH measurement

Surface pH of patch was determined to check whether the patch causes irritation to the mucosa. The pH of all the patches was found to be in the range of that of normal pH of saliva 6.8–7.5 (Table 3). Hence no mucosal irritation was expected from these prepared patches.

3.2.3. Drug content uniformity

The percentage drug content was determined by UV spectrophotometer at 222.2 nm (Jasco V-630, Japan) method using the standard calibration curve and the same procedure was repeated for three patches of each formulation. Results are shown in Table 3. As the drug content values of same formulation did not show a significant difference, it can be concluded that the drug was uniformly dispersed in buccal patches.

3.2.4. Folding endurance

The number of folding required to break or crack a patch was taken as the folding endurance. The folding endurance was found to be increased with an increasing concentration of PVA and XG. All the patches showed good value of folding endurance (more than 200 was considered to be good value) and the formulations A3, A5 and A7–A9 showed folding endurance values more than 300

Table 2
Experimental design layout of ZMT buccal patch formulations.

Run	FC	Factor X_1 (XG) Coded levels of variables	Factor X_2 (PVA)	Response 1 (Y_1) Swelling index (%)	Response 2 (Y_2) % Drug release in 15 min	Response 3 (Y_3) % Drug release at 5 h	Response 4 (Y_4) Mucoadhesive strength (g)
1	A1	−1	−1	270.48	44.95	95.86	18.00
2	A2	−1	0	229.2	49.32	97.11	14.15
3	A3	−1	1	168.79	51.12	99.26	12.13
4	A4	0	−1	310.78	39.30	92.13	24.34
5	A5	0	0	251.34	41.87	93.31	21.13
6	A6	0	1	174.32	43.15	95.09	20.78
7	A7	1	−1	363.60	36.22	86.55	33.89
8	A8	1	0	283.30	37.50	87.54	33.12
9	A9	1	1	194.61	37.95	89.02	32.98

(Table 3). This confirms that there will be no breakage of patch during administration.

3.2.5. Tensile strength

The tensile strength of all formulations is given in Table 3. It was found that tensile strength increased with an increasing amount of PVA and increasing amount of XG. The values showed that the mechanical strength of patches was enough to bear stress during transport and administration of patches.

3.2.6. Ex vivo mucoadhesion time

Ex vivo mucoadhesion time of the formulations (Table 3) was found to be dependent on swelling index. *Ex vivo* mucoadhesion time was directly proportional to the swelling index; it was observed highest for formulation showing highest swelling index. The *ex vivo* mucoadhesion time of patches (A7, A8, A9) containing highest amount of XG was found to be more than formulations (A1, A2, and A3) containing lowest amount of XG.

3.2.7. Effect of formulation variables on swelling index

Swelling property becomes an important parameter to be studied as appropriate swelling behavior of a buccal adhesive system governs the uniform and prolonged release of drug and effective mucoadhesion (Peppas & Buri, 1985). Swelling of patches was started within 5 min due to presence of soluble excipients, PVA, HPMC E-15 and XG. All the patches showed maximum increase in swelling after 1 h. Swelling index of all the formulations is shown in Table 2. Formulation A7 containing highest concentration of XG (0.3%) and lowest concentration of PVA (0.5%), showed highest value of swelling index. On applying factorial design, the quadratic model was suggested by software and found to be significant with model F value of 1747.68, p value <0.0001 and R^2 value of 0.9997 which implied that model was significant. And there was only a 0.01% chance that a "Model *F*-Value" this large could occur due to noise. Values of "Prob > *F*" less than 0.05 for each term was obtained which indicated that every model term was significant. In this case X_1, X_2, X_1^2, X_2^2 were significant model terms. The model for response Y_1 (Swelling index) is as follows:

$$Y_1 = +250.49 + 28.84X_1 - 67.86X_2 - 16.82X_1X_2 + 6.18X_1^2 - 7.52X_2^2 \quad (3)$$

The above given Eq. (3) indicates that X_1 (concentration of XG) has positive and that X_2 (concentration of PVA) has a negative effect on percent swelling index. That is increase in XG amount led to increase in the extent of swelling of the patches. Similar conclusion was derived by Kaur et al. (2010). As XG is hydrophilic anionic polymer, it has much higher affinity for water and when the film is placed in an aqueous medium, a gel is formed due to uncoiling of the structure of XG molecules and formation of hydrogen bonds with water molecules. This leads to an increase in the diameter of the film progressively and a distinct gel-sol boundary develops. This is how the rate of swelling is increased (Talukdar & Kinget, 1995). But the swelling index of patches decreased with an increase in concentration of PVA. The combined effect of factor X_1 (XG) and factor X_2 (PVA) can be further interpreted with the help of contour plot and response surface plot (Fig. 1). The effect of PVA concentration on response was found to be more significant than that of XG concentration. The combination of PVA with XG led to decrease in swelling index of XG. That is significant decrease in swelling index was obtained at same level of XG with increase in PVA concentration.

3.2.8. Effect of formulation variables on % drug release in 15 min and at 5 h

As ZMT is an anti-migraine candidate, an immediate drug release followed by gradual release of drug is necessary to satisfy anti-migraine effect. Therefore % drug release in 15 min forms an important parameter to be studied and hence was selected as dependent variable for the purpose of optimization. An immediate drug release followed by gradual release was successfully observed for all xanthan gum patches (Table 2).

The coefficient of regression value was found to be highest for Korsmeyer-Peppas model by all formulations (0.956–0.983) and hence the release mechanism was found to follow

Table 3
Physicochemical evaluation parameters of ZMT buccal patches.

FC	Weight uniformity[a] (mg)	Thickness[a] (mm)	Surface pH[a]	Drug content uniformity[a] (mg)	Folding endurance[a]	Tensile strength[a] (N/mm^2)	*Ex vivo* mucoadhesion time (min)
A1	64 ± 0.057	0.14 ± 0.04	7.39 ± 0.01	2.49 ± 0.01	280 ± 2.51	9.12 ± 0.03	276
A2	67 ± 0.050	0.14 ± 0.03	7.22 ± 0.01	2.50 ± 0.02	292 ± 1.00	11.13 ± 0.04	269
A3	68 ± 0.036	0.15 ± 0.03	7.33 ± 0.02	2.49 ± 0.03	305 ± 1.52	13.11 ± 0.01	266
A4	72 ± 0.050	0.15 ± 0.02	7.02 ± 0.05	2.48 ± 0.01	294 ± 1.52	10.12 ± 0.05	289
A5	74 ± 0.050	0.15 ± 0.01	7.12 ± 0.02	2.50 ± 0.01	310 ± 1.52	11.89 ± 0.03	287
A6	76 ± 0.032	0.15 ± 0.03	7.13 ± 0.01	2.51 ± 0.01	322 ± 1.52	13.14 ± 0.01	286
A7	78 ± 0.047	0.16 ± 0.03	6.83 ± 0.03	2.48 ± 0.03	308 ± 1.52	11.13 ± 0.02	295
A8	79 ± 0.037	0.16 ± 0.02	6.93 ± 0.03	2.49 ± 0.01	321 ± 2.08	13.16 ± 0.03	290
A9	80 ± 0.040	0.17 ± 0.02	6.85 ± 0.02	2.50 ± 0.02	338 ± 2.51	15.95 ± 0.03	287

[a] All values are mean ± SD, $n = 3$.

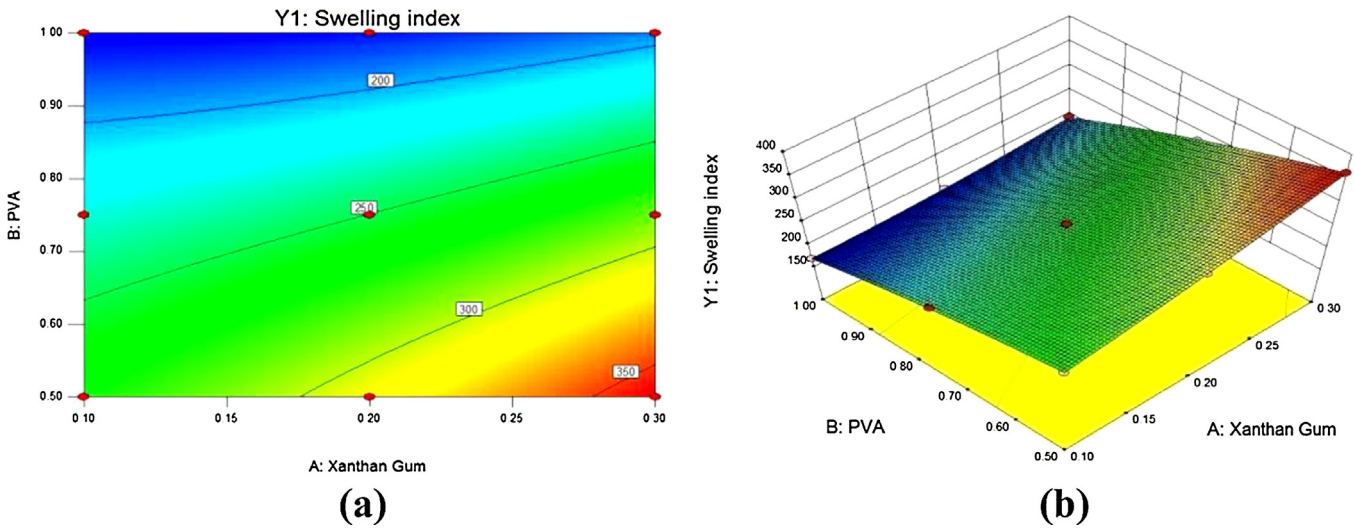

Fig. 1. Two dimensional contour plot (a), three dimensional (3D) response surface plots for response Y_1 (swelling index) (b).

Korsmeyer-Peppas model. The diffusion exponent n value was found to be less than 0.5 (0.23–0.28) for all the formulations, indicating quassi-Fickian diffusion of drug through the patches.

On applying factorial design, the quadratic model was suggested by software for response Y_2 and found to be significant with model F value of 456.94, p value <0.0002 and R^2 value of 0.9987 which implied that model was significant. And there was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 for each term was obtained which indicated that every model term was significant. In this case X_1, X_2, X_1^2, X_2^2 were significant model terms. The model for response Y_2 (% drug release in 15 min) is as follows:

$$Y_2 = +41.96 - 5.61X_1 + 1.97X_2 - 1.09X_1X_2 + 1.41X_1^2 - 0.77X_2^2 \quad (4)$$

In Eq. (4) – sign of X_1 indicates that factor X_1 (concentration of XG) has negative effect, and + sign of X_2 indicates that factor X_2 (concentration of PVA) has positive effect on response Y_2 (% drug release in 15 min). That is % drug release in 15 min decreases with increase in XG concentration and increases with increase in PVA concentration.

On applying factorial design, the quadratic model was suggested by software for response Y_3 and found to be significant with model F value of 5704.57, p value <0.0001 and R^2 value of 0.9999 which implied that model was significant. And there was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 for each term was obtained which indicated that every model term was significant. In this case X_1, X_2, X_1^2, X_2^2 were significant model terms. The model for response Y_3 (% drug release at 5 h) is as follows:

$$Y_3 = +93.29 - 4.85X_1 + 1.47X_2 - 0.23X_1X_2 - 0.95X_1^2 + 0.33X_2^2 \quad (5)$$

From Eq. (5) it is clear that the drug release rate appeared to increase with an increasing amount of factor X_2 (concentration of PVA) and decreased with an increasing amount of factor X_1 (concentration of XG). The combined effect of factor X_1 and factor X_2 can be further interpreted with the help of contour plot and 3D response surface plots (Fig. 2) showing the effect of concentration of XG and PVA on % drug release in 15 min and at 5 h.

The release of drug was found to be dependent on swelling index. Many authors state that, greater the swelling index of the polymer, slower is the release of the drug (El-Samaligy, Yahia, & Basalious, 2004; Guo, Skinner, Harcum, & Barnum, 1998; Patel, Prajapati, & Patel, 2007b). Hence the decrease in the drug release rate may be explained due to an extensive swelling property of XG. As the proportion of XG was increased in the formulation, erosion of the patches slowed down. So formulations containing lowest amount of XG (0.1%) got eroded first. This in turn affected drug release rate. The swelling index of patches decreased with an increasing concentration of PVA and thus enhanced the release rate of the drug. A3 formulation which contained lowest amount of XG (0.1%) and highest concentration of PVA (1%) showed highest drug release in 15 min. In this case the results indicated that XG concentration had more significant negative effect on drug release than the effect of PVA concentration. That is significant decrease in % drug release in 15 min and at 5 h was obtained at same level of PVA with increase in XG concentration.

3.2.9. *Effect of formulation variables on mucoadhesive strength*

Mucoadhesive strength is an important property to be determined because it ensures the attachment of dosage form and delivery of drug at the site of administration. The direct relationship between the swelling index and adhesion force has been described by many authors (Kaur & Kaur, 2012; Kaur et al., 2010; Patel et al., 2007b; Ratha Adhikari et al., 2010). Formulation A7–A9 therefore showed highest bioadhesion due to their highest swelling index, thus 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 777.47, p value <0.0001 and R^2 value of 0.9992 which implied that model was significant. And there was only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 for each term was obtained which indicated that every model term was significant. In this case X_1, X_2, X_1^2, X_2^2 were significant model terms. The model for response Y_4 (mucoadhesive strength) is as follows:

$$Y_4 = +21.49 + 9.29X_1 - 1.72X_2 + 1.24X_1X_2 + 1.96X_1^2 + 0.89X_2^2 \quad (6)$$

Eq. (6) shows that the bioadhesion force increased with an increase in concentration of XG, but decreased with an increase

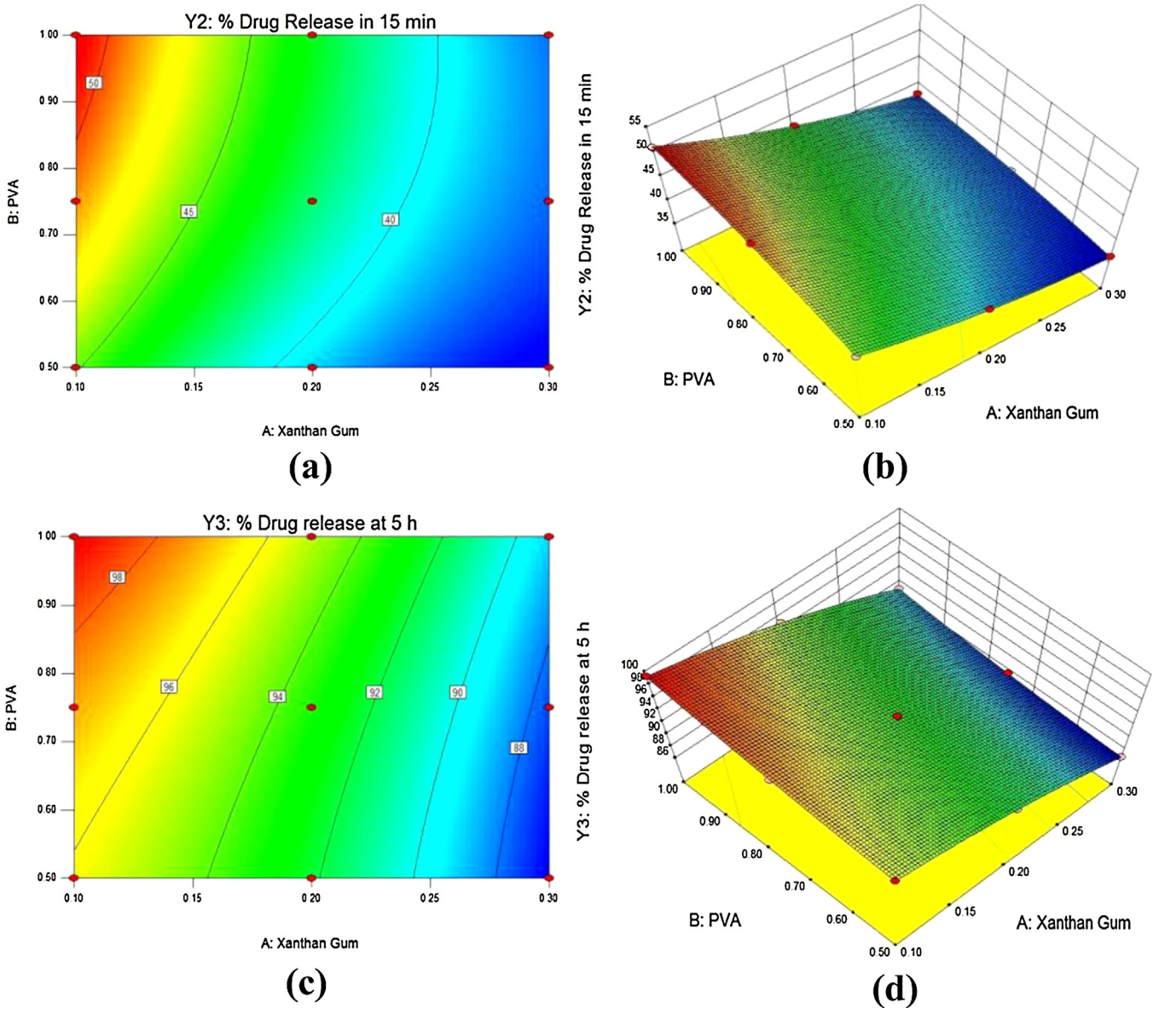

Fig. 2. Two dimensional contour plot for Y_2 (% drug release in 15 min) (a), three dimensional (3D) response surface plots for Y_2 (b), two dimensional contour plot for Y_3 (% drug release at 5 h) (c), three dimensional (3D) response surface plots for Y_3 (b).

in concentration of PVA. XG is an anionic polyelectrolyte containing glucuronic acid and pyruvate in its side chain (Richardson & Ross-Murphy, 1987). XG thus showed excellent mucoadhesive characteristics. The reason behind this strong mucoadhesivity is due to physical entanglements and secondary interactions (hydrogen bonds) between the free $-COO^-$ groups of XG and mucin glycoproteins which has already been explained by Andrews, Laverty, and Jones (2009). The combined effect of factor X_1 (XG) and factor X_2 (PVA) can be further interpreted with the help of contour plot and response surface plot (Fig. 3). The effect of XG concentration on response was found to be more significant than that of PVA concentration. The combination of XG with PVA led to increase in mucoadhesivity of PVA. That is significant increase in mucoadhesive strength was obtained at same level of PVA with increase in XG concentration.

3.2.10. Optimization of formulation

After application of 3^2 factorial experimental design, the process was optimized for the response variables Y_1–Y_4. If the swelling index of formulation is very large, this may cause patient discomfort on application of the patch, thus leading to patient incompliance. Also if mucoadhesive strength is very high then the patch removal from buccal mucosa becomes very difficult process and may damage buccal epithelium (Park, Song, Jee, Kim, & Kim, 2012). Hence the formulation showing optimum swelling index in range (150–200%), desired drug release in 15 min (more than 40%) and after 5 h (more than 90%) and sufficient mucoadhesive strength being targeted in range of 15–25 g, was to be selected as optimized formulation. Formulation A6 showed all these desirable characteristics with swelling index as 174.32%, 43.15% drug release in 15 min, 95.09% drug release after 5 h and sufficient mucoadhesive strength, *i.e.* 20.78 g. Thus formulation A6 containing 0.2% XG, 1% PVA and 3% HPMC E-15 was taken as optimized formulation and was subjected to permeation studies through the sheep buccal mucosa with and without penetration enhancers in order to find out the extent of drug permeability in terms of permeability flux and permeability coefficient.

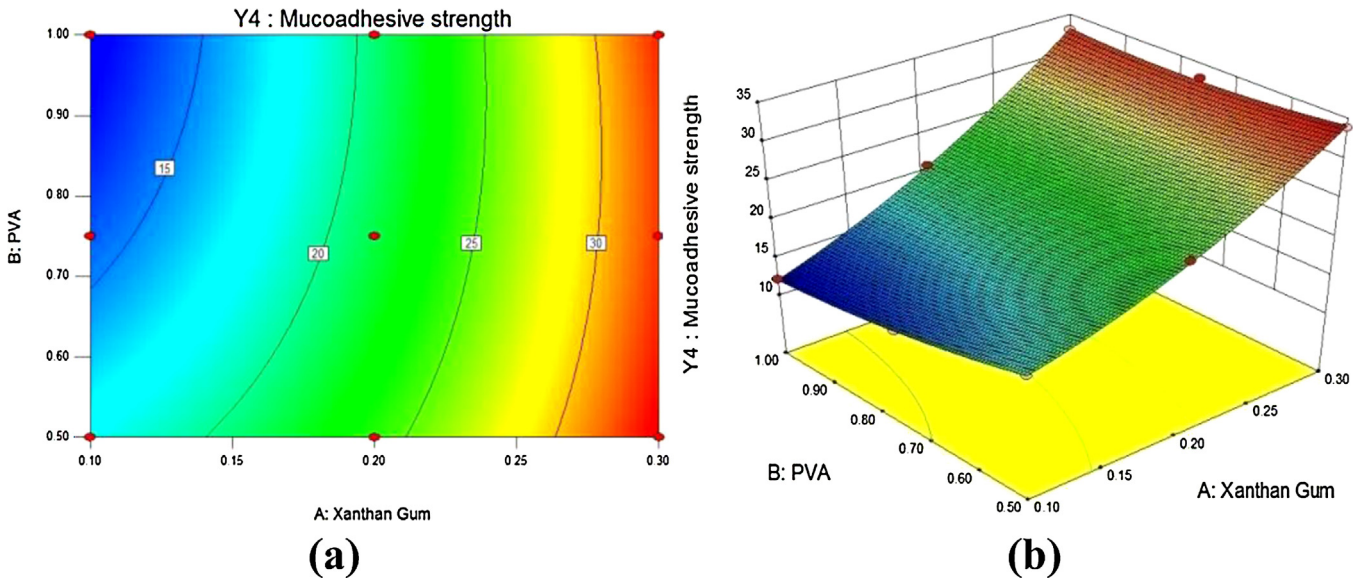

Fig. 3. Two dimensional contour plot (a), three dimensional (3D) response surface plots for Y_4 (mucoadhesive strength) (b).

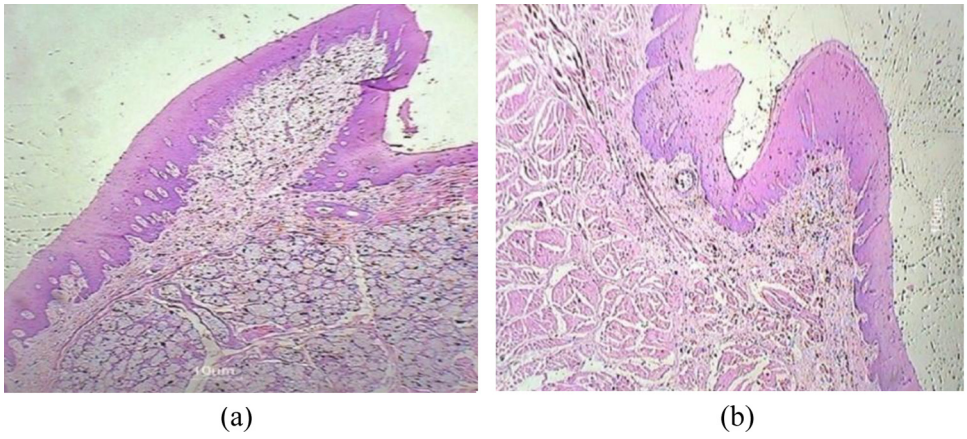

Fig. 4. Histopathological evaluation of sheep buccal mucosa, control (a), sample buccal mucosa (b).

3.2.11. *Ex vivo drug permeation studies*

The permeation characteristics of A6 formulation, containing different penetration enhancers and that of without penetration enhancer, are shown in Table 4.

Maximum increase in permeability was obtained from patch containing 4% DMSO. Formulation containing 4% DMSO, increased drug permeability by three folds and transported 29.10% of drug after 5 h as compared to that of formulation without any enhancer; which showed only 11.53% drug permeation after 5 h. While 4% sorbitol and 4% oleic acid transported 20.55% and 18.43% of drug after 5 h respectively. Shidhaye et al. (2008) also tried DMSO as penetration enhancer to enhance the penetration of Sumatriptan drug. They obtained 12% permeation of the drug through sheep buccal mucosa using 3% DMSO.

Table 4
Ex vivo permeation characteristics of A6 formulation with and without enhancers.

Formulation	P (cm h^{-1}) × 10^{-3}	(J, μg cm^{-2} h^{-1})	ER
Without penetration enhancer	2.51	6.28	–
DMSO (4%)	8.27	20.69	3.29
Sorbitol (4%)	4.90	12.25	1.95
Oleic acid (4%)	3.77	9.44	1.50

Hence A6 formulation containing 4% DMSO as penetration enhancer, 0.2% XG, 1% PVA and 3% HPMC was taken as final optimized batch.

3.2.12. *Buccal mucosa sensitivity test*

The final optimized formulation A6 containing 4% DMSO was subjected for buccal mucosa sensitivity test. The sections of control and sample mucosa (treated with final optimized formulation) observed under digital microscope (Motic, B1 Advanced series) are shown in Fig. 4. 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, formulation containing 4% DMSO is safe for buccal administration of ZMT through mucoadhesive patches.

4. Conclusion

The results have shown that XG has potential to modify drug release rate and shows good bioadhesion. In combination with 1% PVA and 3% HPMC E-15; 0.2% XG has shown promising fast initial drug release within 15 min (43.15%) followed by gradual release over 5 h (95.09%). Hence this natural polysaccharide which is economic and abundantly available can be used as a potential drug

release modifier and mucoadhesive polymer for successful formulation of ZMT bilayered mucoadhesive buccal patches, which may prevent hepatic metabolism to a large possible extent. But use of penetration enhancer is necessary to achieve maximum permeation of drug through buccal mucosa.

From the present study one can conclude that XG based mucoadhesive buccal patches of ZMT can be successfully prepared with considerable good stability and tolerability. But future *in vivo* studies is necessary to confirm permeation and retention of these patches.

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