Biomedical Materials

PAPER

Synthesis of thiolated arabinoxylan and its application as sustained release mucoadhesive film former

RECEIVED
19 September 2017

REVISED
24 November 2017

ACCEPTED FOR PUBLICATION
27 November 2017

PUBLISHED
8 February 2018

Muhammad Zaman[1,2], **Muhammad Hanif**[1], **Kishwar Sultana**[2] **and Atta-Ur-Rehman**[2]

[1] Department of Pharmacy, Bahauddin Zakariya University, Multan 60800, Pakistan
[2] Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan

E-mail: Muhammadhanif14@yahoo.com

Keywords: arabinoxylan, thiolation, safety analysis, mucoadhesive films

Abstract

The present work aimed to synthesize thiolated arabinoxylan (TAX), and to evaluate its mucoadhesive potential. Synthesis of TAX was accomplished by esterification of arabinoxylan (AX) with thioglycolic acid (TGA). The appearance of a characteristic peak at 2516 cm^{-1} in the FTIR spectrum of TAX, and presence of 6.01 $\pm$ 1.03 m moles of thiol per gram of the polymer confirmed successful thiolation of AX. The incorporation of the thiol group considerably promoted mucoadhesive strength of the polymer—viz. 3.99-fold. Moreover, *in vivo* safety analysis in albino rats revealed TAX to be safe in the concentration range of 750–1000 mg kg^{-1} body weight. Synthesized TAX was utilized to prepare Tizanidine HCl (TZN HCl) loaded sustained release (SR) mucoadhesive buccal films using a solvent casting technique. Results proved that the prepared films were of uniform thickness, good mechanical strength (with folding endurance >300), acceptable moisture contents (5%–7%) and surface pH (6.23 $\pm$ 0.81 to 6.43 $\pm$ 0.49) compatible to that of the buccal cavity. Presence of greater that 90% of drug contents indicated the excellent drug loading ability of the prepared films. Results of *in vitro* dissolution studies and *ex vivo* permeation studies conducted respectively by USP dissolution apparatus II and Franz diffusion cell indicated that sustained effect of TAX was achieved for 8 h. These results have conclusively proven that TAX has the potential to improve the bioavailability of TZN HCl due to enhanced mucoadhesion in buccal cavity, hence signifying its suitability as a mucoadhesive buccal film former.

1. Introduction

Ispaghula (*Plantago ovata*) is important for its seeds, as seed powder or seed husk; and the mucilage obtained from seeds or husks has found numerous applications in the pharmaceutical industry [10–12]. The material has an appreciable potential to be used as a matrix for slow release of drugs, and is used in sustained release formulations [14, 15]. When the seed husk is treated with hot caustic soda solution, and subsequently neutralized, a gel type material known as arabinoxylan (AX) is obtained. AX is a hemicellulose, in which arabinose side chains are attached to a linear backbone of β-(1 $\rightarrow$ 4)-linked D-xylopyranosyl units. Ispaghula husk is the major source of AX, which is usually 45%–60% of the total weight of the husk. Extraction of the AX is usually carried out by alkali or hot water extraction techniques. AX offers great potential for use as a pharmaceutical excipient. It is non-irritant and non-toxic. AX has also shown potential for use as polymer to develop controlled release drug delivery matrices [16, 17], as well as mucoadhesive buccal films [9]. AX is a mucoadhesive polymer, and its strength of mucoadhesion can be enhanced by converting it into a thiolated form. Thiolated polymers are designed to enhance mucoadhesion and other additive properties. This modification is carried by introducing thiol groups in the backbones of non-thiolated polymeric chains, by replacement reactions or simple oxidation reactions [1, 2]. The introduction of a thiol moiety therefore enhances the mucoadhesive strength and mucoadhesion retention time of the thiolated polymer 2–140-fold. Currently, thiomers have comprehensive application as an auspicious pharmaceutical excipient in the assessment stage of pharmaceutical technology [3, 4]. They are extensively used in various

mucoadhesive dosage forms, especially in controlled release drug delivery systems [5, 6]. Moreover, they have potential for transmucosal [7], gastrointestinal, buccal, oral, nasal and ophthalmic delivery of drugs [8, 9]. Buccal films are very thin films, having size and shape similar to that of a postage stamp, which are simply placed on the tongue, where they readily become hydrated by saliva, and release drugs. Drugs can be absorbed directly from the highly vascularized buccal mucosa, entering the systemic circulation by escaping first-pass effects caused by hepatic metabolism. This helps to improve the bioavailability of drugs that undergo extensive first-pass effects [18]. Tizanidine HCl (TZN HCl), an α_2-adrenergic receptor agonist with myospasmolytic action, is used for the treatment of back pain, both alone and in combination with NSAIDs. It has a very short half-life of about 2.5 h, and undergoes extensive first-pass hepatic effects, which respectively cause increase in dose frequency and reduction in its bioavailability, and hence negatively impact patient compliance [20]. These characteristics make TZN HCl a potential candidate for delivery by sustained release mucoadhesive buccal film, to overcome these issues.

The purpose of the current study was to extract AX from ispaghula husk, and convert it into a novel mucoadhesive polymer: thiolated arabinoxylan (TAX). Its physicochemical characterization and evaluation of its safety profile in albino rats to assess whether it is safe or not for *in vivo* application were then undertaken, prior to its application as a sustained release mucoadhesive film former.

2. Materials and methods

2.1. Materials

TZN HCl was gifted by Pharmedic Laboratories Lahore Pakistan, and ispaghula husk was obtained from the local market of Lahore, Pakistan. TGA (Barcelona Spain), Ellman's reagent and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) (Sigma-Aldrich Gmbh Chemie, Germany) and distilled water were taken from the research laboratories of Bahauddin Zakariya University Multan, Multan, Pakistan. Hydroxypropyle methylcellulose (HPMC) K15M. Hydrochloric Acid (HCl), Acetic Acid, Sodium Hydroxide (NaOH), Sodium Chloride (NaCl) and Glycerol were purchased from Merck Darmstadt, Germany. All the chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of AX

AX was extracted from *Plantago Ovata* (ispaghula husk) by using the method described earlier by Saghir *et al* [21]. Briefly, 100 g of ispaghula husk was soaked in distilled water, and the pH of the mixture increased to 12 by adding 2.5% aqueous solution of NaOH. Muslin cloth was used to filter the gel twice from the mixture, leaving behind husk as a residue. Concentrated acetic acid was added to reduce the pH to 3. This change in pH resulted in the coagulation of the gel. Coagulated gel was repeatedly washed with distilled water, to neutralize the pH. The gel was then allowed to settle, decanted, and finally dried in an oven at 40 °C (figure 1).

2.2.2. Thiolation of AX

The method described by Anitha *et al* [22] was used for thiolation of AX. A 2 gm quantity of AX was added to sufficient distilled water to attain a final concentration of 2% w/v, and stirred using a magnetic stirrer at room temperature for 4 h to ensure complete hydration. EDAC having concentration of 0.125 mmol was added as a reagent, followed by addition of TGA in the ratio of 1:1 to AX. The pH of this mixture was adjusted to 5 using 1.0 M NaOH, and reaction was controlled by keeping the mixture in darkness for 4 h, under constant stirring, at room temperature. After that, TAX was isolated from the mixture by repeatedly subjecting it to the process of dialysis: once against 5 mM HCl in tubing of cellulose membrane having a molecular weight of 12 kDa for 3 days; twice against the same medium but with the addition of 1% NaCl; and finally, against 5 mM HCl for 2 days, to maintain the pH of the medium at 4 (figure 2). Control samples were prepared by the same method, but without adding TGA. Dried polymer was obtained by lyophilizing at −47 °C and 0.013 mbar pressure. The lyophilized product was kept safe in a desiccator for further analysis [22].

2.2.3. Percent yield of extraction method

Percent yield of AX obtained, with respect to the total mass of Ispaghula husk used, was calculated using the following equation (1):

$$\%\text{Yield} = \left[\frac{a}{b} \times 100\right], \quad (1)$$

where a is the mass of AX and b is the mass of Ispaghula husk.

2.2.4. Micromeritic studies of TAX

Micromeritics including bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose were studied, to observe the flow properties of TAX.

2.2.4.1. Bulk density (ρ_b)

Bulk density was calculated using method I of the US Pharmacopeia, 2006. A measured amount of powder was added to a graduated cylinder of 100 cm^3 capacity. Without tapping the cylinder, the material was flattened, and apparent volume (V_b) was noted from the adjacent graduated unit. Equation (2) was used to calculate the bulk density (ρ_b) and measured in cm^3:

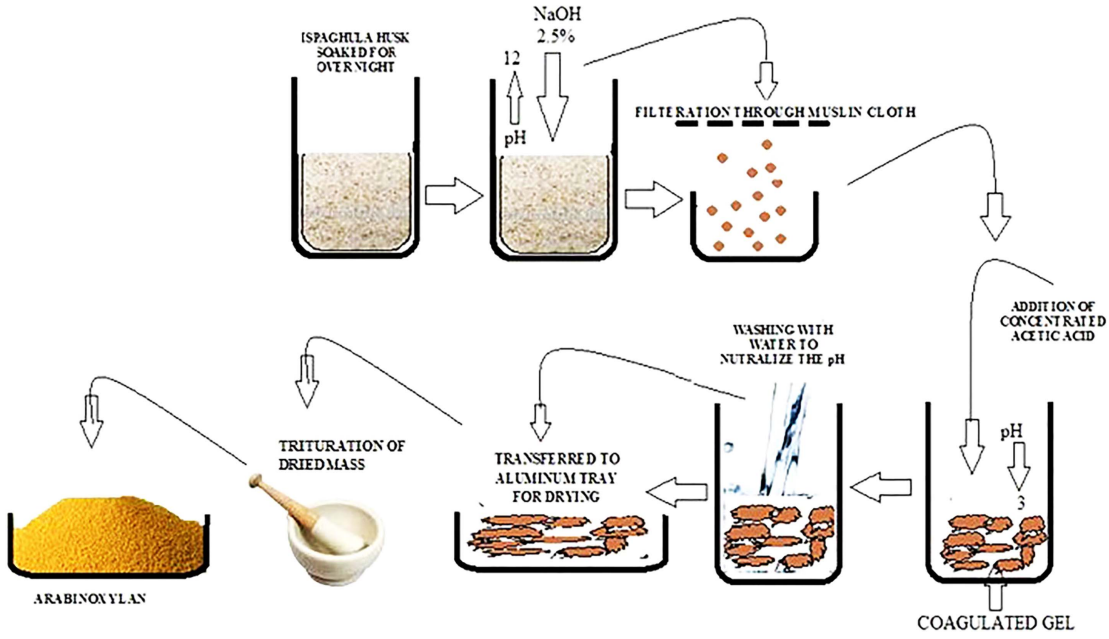

Figure 1. Pictorial illustration of alkali extraction of AX from Ispaghula husk.

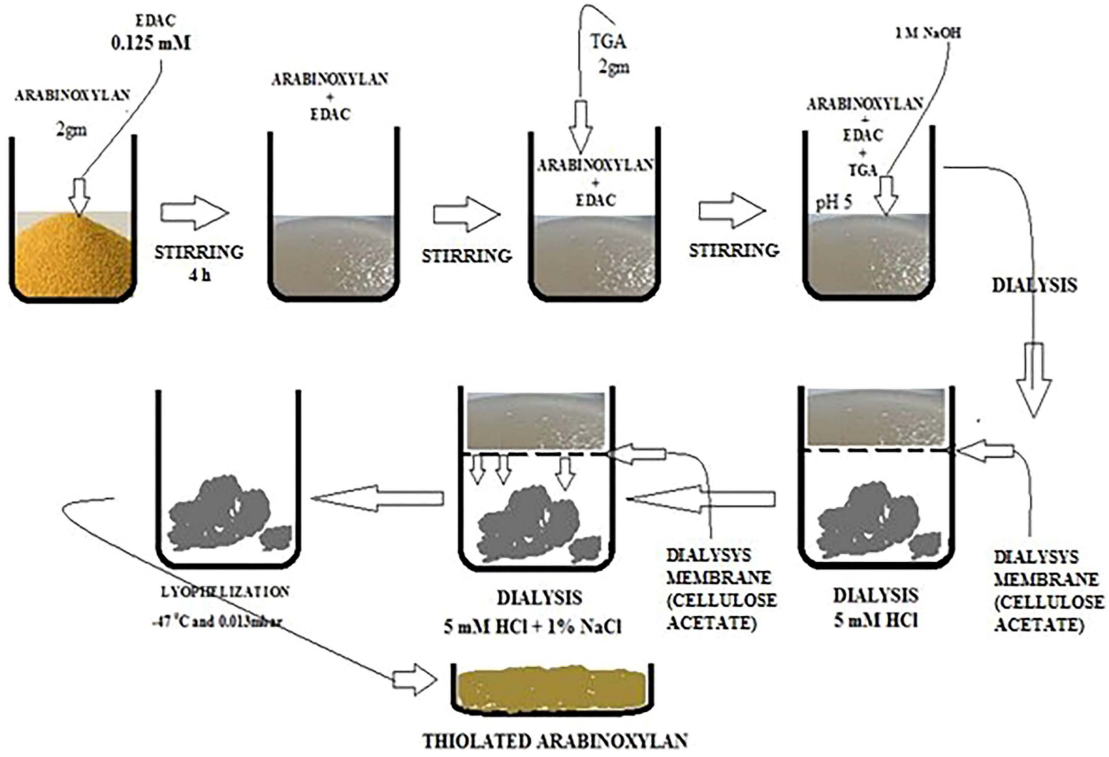

Figure 2. Schematic diagram illustrating the process of thiol modification of AX to synthesize TAX.

$$\rho_b = M/V_b, \quad (2)$$

where M is mass of the material and V_b its volume.

2.2.4.2. Tapped density (ρ_t)

The powder was shifted to a graduated glass cylinder having filling capacity of 100 cm^3. The apparent volume of the material without compressing (V_b) was noted. 100 taps were performed, volume (V_t) was noted again, and subsequent cycles of hundred taps were repeated until a minor difference of 0.2% between successive cycles was achieved. Equation (3) was used to compute the tapped density (ρ_t) in cm^3:

Table 1. Flow properties and corresponding values of angle of repose, C.I. and Hausner ratio.

Flow properties	Angle of repose	C.I	Hausner ratio
Excellent	25–30	⩽10	1.00–1.11
Good	31–35	11–15	1.12–1.18
Fair	36–40	16–20	1.19–1.25
Passable	41–45	21–25	1.26–1.34
Poor	46–55	26–31	1.35–1.45
Very poor	56–65	32–37	1.46–1.59
Very, very poor	>66	>38	>1.60

$$\rho_t = M/V_t, \tag{3}$$

where M is mass of the material and V_t its tapped volume.

2.2.4.3. Compressibility index (C.I. %)

This is an easy and commonly used method to assess the flow properties of materials. The compressibility index of the powder was determined using equation (4):

$$\text{C.I.}(\%) = \frac{100(V_b - V_t)}{V_b}, \tag{4}$$

where V_b is initial volume and V_t tapped volume.

2.2.4.4. Hausner's ratio

It is another vital parameter to explain the flow properties of powders [23]. Equation (5) was used to calculate the Hausner's ratio. This parameter has no units, as it is the ratio between two values of the same kind:

$$\text{Hausner's ratio} = V_b/V_t, \tag{5}$$

where V_b is initial volume and V_t tapped volume.

2.2.4.5. Angle of repose

A fixed funnel and cone method was used to measure the angle of repose [24]. A glass petri dish was placed on a flat surface, and a glass funnel fixed over it with the help of a tripod stand, in such a way that the distance between its tip and a paper surface was 20 mm. Very carefully, the powder was transferred onto the flat surface through the funnel to form a heap. The height (h) and diameter (d) of the heap were measured, and the angle of repose calculated using equation (6):

$$\tan \varnothing = \frac{2h}{d}, \tag{6}$$

where h is the height of the powder cone and d its mean diameter [25]. The flow properties of the material were categorized by comparing the obtained results with table 1.

2.2.5. Determination of thiol contents of TAX

Thiol content per gm of the polymer was determined by the quantification of thiol substituents using the Elman's reagent method [26, 27]. Dispersions of AX and TAX having 0.5% w/v concentration were prepared,and diluted with 0.5 M phosphate buffer of pH 8.0 to the final concentration of 0.15% w/v. A 5 ml quantity of the polymer solution was reacted with equal volume of 0.3% w/v solution of Ellman's reagent for 2 h at room temperature. The absorbance of the sample was then checked at 450 nm, using a double beam UV−visible spectrophotometer (PG Instruments T80, UK). The numbers of thiol constituents per gram of TAX were determined using a calibration curve prepared by reacting standard solutions of TGA with Ellman's reagent (0.3% w/v).

2.2.5.1. Fourier transform infrared spectroscopic (FTIR) analysis

FTIR is considered to be a useful technique to observe any structural modification of polymers. A comparison between unmodified (AX) and modified (TAX) polymers was made by comparing their graphs, obtained by scanning the samples in the range of 500 cm^{-1} to 4000 cm^{-1} using FTIR (Agilent Carry 360 FTIR, United States).

2.2.5.2. Thermal analysis

Thermal behavior can be changed with structural modification of the polymer. In order to find out the variation in the thermal behavior of AX after introduction of the thiol moiety into its polymeric backbone, differential scanning calorimetry (DSC) was performed (DSC-60A Thermal analyzer, Shimadzu Japan). An accurately weighed amount (7 mg) of the sample was sealed in an aluminum pan, and heated at the rate of 10 °C min^{-1} from 35 °C to 800 °C, under continuous flow of nitrogen at the rate of 25 ml min^{-1} [28].

2.2.5.3. X-ray diffraction (XRD) studies

The XRD is an important method to observe any change in the structure of polymeric particles upon chemical modification. Samples of AX and TAX were taken and analyzed with an x-ray diffractometer (JDX-3532 JEOL Japan) under constant experimental conditions: tube voltage 45 kV; tube current 40 mA; and scanning angle (2θ) 5–50° [28].

2.2.5.4. Surface morphological studies

Scanning electron microscopy (SEM) (JSM-6490A, Tokyo Japan) was performed to observe the change in surface morphology of polymeric particles after thiolation.

2.2.5.5. Swelling index (SI)

1 gm of TAX was taken in a graduated cylinder with a capacity of 50 cm^3 to observe the swelling power of the powder. Initial volume (V_i) of the powder was noted, and sufficient distilled water was added to make the volume up to 50 ml. The mixtures were allowed to stand overnight for maximum swelling, the final volume (V_f) noted, and SI calculated using equation (7):

$$\text{SI} = \frac{V_f}{V_i} \times 100, \tag{7}$$

where V_i is initial volume and V_f final volume.

2.2.5.6. pH of aqueous dispersions

The pH of AX and TAX was measured by preparing their 1% (w/v) aqueous dispersions in distilled water. The probe of a digital pH meter (bench top pH meter with 25CW microprocessor (BANTE instruments, China)) was dipped in the dispersion until a constant pH was observed.

2.2.5.7. ex vivo *mucoadhesive strength*

Reformed physical balance method was used to the determine the mucoadhesive strength of AX and TAX [29, 30]. The apparatus used consisted of a two pan physical balance. Three glass slides were taken in such a way that one of them was glued to the bottom of one of the pans, a second slide attached at the base beneath that pan, and a third attached to the other pan of the balance to tare the apparatus. Pieces of buccal mucosal membrane were attached separately to both slides (one glued to the pan and other glued to the base beneath that pan) and a circular disc prepared by compressing 100 mg of powder was inserted between the mucosal membranes attached with those slides. A pre-load force of 50 gm was applied for 5 min over the pan to stick the slides to each other. The physical balance was elevated by holding its lever, and weights were applied in a gradually increasing manner on the second pan, until the glass slides holding powder discs were isolated from each other. The weight (g) required for separation of slides was noted, and converted into the index of mucoadhesive strength. Force of detachment was measured in newtons (N).

2.2.5.8. in vivo *safety studies in albino rats*

All animal experiments were approved by the Instructional animal research ethic committee of the Faculty of Pharmacy, The University of Lahore, Lahore void ref no 'IAEC-2016-18'. Nine (9) female albino rats weighing 200–225 g were purchased from the animal house of The University of Lahore, Lahore, and kept for a week under the laboratory conditions (Temperature 25 °C) with plentiful water and food supply. Animals were divided into three groups ($n = 3$). Group 1 was untreated control, Group 2 was treated with AX, and Group 3 (Test Group) with TAX.

Three types of studies were performed, (1) skin irritation, (2) GIT disturbance and (3) effect on buccal mucosa.

- **Skin irritation:** Hairs were removed using hair-removing cream. A 1 ml quantity of gel of AX or TAX (3%) was applied on a circular area of around 1 cm^2 of the skin, while control animals were treated with water only. After 4, 8 and 24 h, the animals were observed for signs of skin inflammation (redness, swelling and erythema).
- **Gastrointestinal effects:** 5 ml of AX and TAX gel (3%) was administered by oral gavage. The animals were observed daily for a week to detect any sign of GIT discomfort. Every 24 h, stools were observed for consistency, size, shape, color and numbers of stool pellets.
- **Effect on buccal mucosa:** Animals were anesthetized using thiopental 80 mg kg^{-1} through intraperitoneal routes. 50 mg of powdered TAX and AX were placed in the buccal cavity, and pressed softly against the mucosal membrane with the help of a micro spatula to ensure firm attachment of the polymer to the buccal mucosa. After 24 h, rats were analyzed for any signs of infection or inflammation. In order to observe the effects on mucosal cells, the rats were sacrificed under anesthesia, buccal tissue was removed, fixed in 10% formalin, and processed for H and E staining and histopathological evaluation using optical microscopy.

Table 2. Composition of TZN HCl containing SR mucoadhesive buccal films.

Formulations	TARX(mg)	Glycerol (mg)
MBF1	200	30
MBF2	250	67
MBF3	300	45

• Constant concentrations of HPMC K15M and Polysucralose (2 ml of 2% solutions) were used in each formulation.

2.3. Preparation of mucoadhesive buccal films

SR mucoadhesive buccal films were fabricated using a solvent casting technique, in accordance with the formulation design described in table 2. For ease and accuracy of the preparation, w/v aqueous solutions (or dispersions) of all the required ingredients were prepared as follows: 3% solution of TZN HCl, 3% dispersion of TAX, 6% solution of glycerol, 2% solutions of HPMC K15 and of polysucralose. The required volume of polymer solution was taken in a beaker of 100 ml capacity under continuous stirring, and glycerol solution added to form mixture 1. Drug solution was taken in another beaker, and HPMC K 15 added to form mixture 2. Mixture 2 was poured into mixture 1, sweetener added along with a few drops of flavoring agent, and stirring continued until a homogeneous mixture was obtained. The final mixture was poured into a petri dish of approximately 24 cm^2 surface area. The petri dish was placed under an inverted funnel in a hot air oven at 40 °C for 24 h to dry the films. Dried films were removed from the petri dish using a sharp knife, packed in aluminum foil, and placed in the desiccator for future use (figure 3).

2.3.1. Evaluation of prepared films

Mucoadhesive buccal films containing TZN HCl were evaluated for thickness, folding endurance, moisture content, surface pH, and surface morphology using

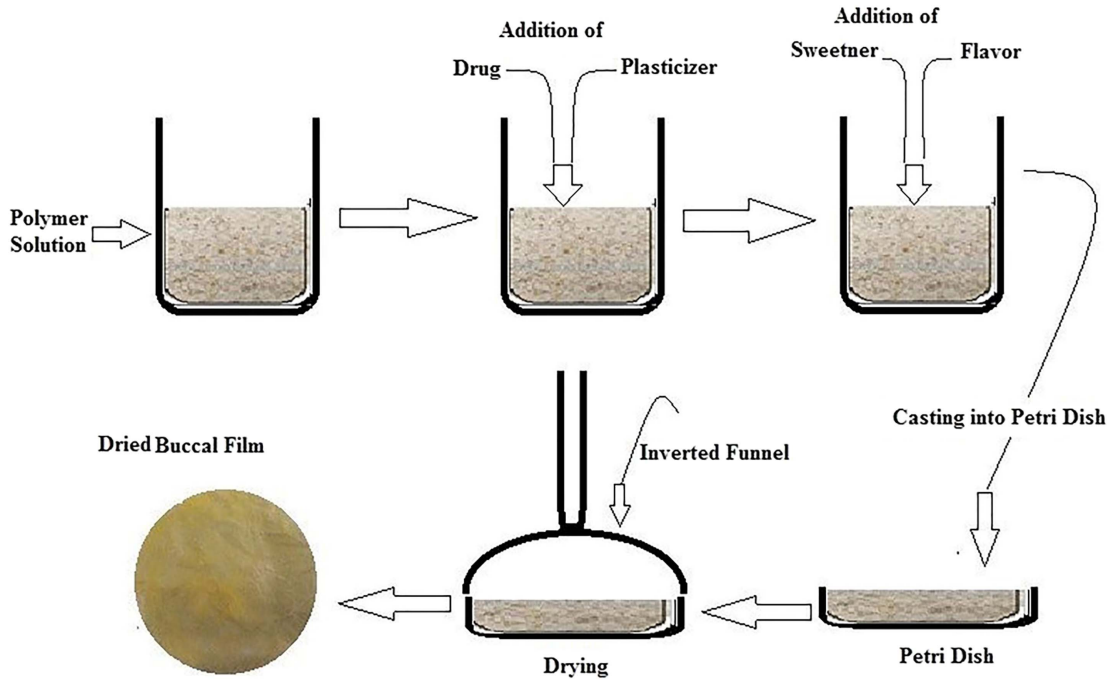

Figure 3. Diagram showing solvent casting process for the preparation of mucoadhesive buccal films of TZN HCl.

scanning electron microscopy. *Ex vivo* mucoadhesion strength, drug contents, *in vitro* drug release and *ex vivo* drug permeation were also assessed.

2.3.2. in vitro drug release of the films

in vitro drug release studies were carried out in USP II dissolution apparatus [31, 32]. Phosphate buffer (pH 6.8, 500 ml) was used as dissolution medium. Working conditions of the apparatus were set at 37 ± 0.5 °C temperature and 50 rpm paddle speed. A small strip containing unit dose of the drug was cut from each film, carefully placed on the surface of a glass slide with the help of a paper pin, and thes slide placed in the vessel. After each hour, 5 ml of aliquot was drawn, and the same volume of fresh medium was added in order to maintain the total dissolution medium volume at 500 ml. Studies were carried out for 8 h, and percentage drug release was calculated using spectrophotometry by analyzing the sample absorbance at 228 nm.

2.3.3. ex vivo *permeation studies*

ex vivo permeation studies were performed by using a Franz diffusion cell to assess the permeation of TZN HCl across buccal mucosa [33]. Buccal mucosa of goat was used in the current study, obtained from a local slaughterhouse. Mucosa was washed with isotonic phosphate buffer, and then kept in normal saline to preserve it for further use [34]. The Franz diffusion cell consists of two compartments: one donor and one recipient compartment. The recipient compartment of the cell, having filling capacity of 13 ml, was filled with phosphate buffer medium (pH 6.8). A piece of the buccal mucosa equivalent in size to the opening of the diffusion cell receptor compartment was mounted over the receptor compartment. A strip of film containing unit dose of the drug was placed on this buccal mucosa. Next, the donor compartment was placed over it, and the two compartments clamped together. An LP needle was used to collect a sample of 3 ml after every 2 min until the 10th min of the study, and thereafter at 5 min intervals up to 30 min. Experimental conditions were set at 37°C ± 0.5 °C and 50 rpm speed of the shaker water bath. Each aliquot drawn was replaced with the same volume of fresh medium, to maintain constant volume. The amount of drug permeated was calculated using spectrophotometry by analyzing the sample absorbance at 228 nm.

2.3.4. Kinetic modeling of drug release and drug permeation data

Kinetic models including zero order ($Q_1 = Q_0 + K_o t$) and Korsmeyer−Peppas model ($M^t/M^0 = kKPtn$) were applied to measure the release kinetics and pattern of drug release [5] from the formulated buccal films.

2.3.5. Statistical analysis

The effect of increasing concentration of TAX and associated percentage of glycerol on cumulative amount of drug released and mucoadhesion strength was assessed by the application of two-way ANOVA followed by Tukey's multiple comparisons test. *P* values were calculated at 95% significance level.

3. Results and discussion

3.1. Percent yield of extraction method

39% of AX was successfully extracted from 100 gm of Ispaghula husk. The percent yield of the isolated AX was a bit low as compared to that reported earlier [21]. This may be due to two reasons: one, that filtration was done using muslin cloth instead of vacuum filtration with sintered glass crucible; two, due to repeated filtration, which was done to assure the absence of particulate material [9].

3.2. Micromeritic studies

AX was considered as a poorly flowing powder, as reported earlier by Zaman *et al* [9]. However, a significant change in the flow properties was observed when it was converted into TAX. Results of micromeritics showed TAX to have bulk density $0.619 \pm 0.031\ cm^3\ ml^{-1}$ and tapped density $0.692 \pm 0.001\ cm^3\ ml^{-1}$. The value of Hausner's ratio (1.117 ± 0.01) suggested that the flow of TAX was good to excellent, which was further confirmed by the value of angle of repose ($27.93° \pm 1.03$). Bahulkar *et al* have also performed micromeritics of thiolated karaya gum, and reported good flow properties of thiolated polymer [35].

3.3. Determination of Thiol contents

Thiol content of the polymer was determined by the Ellman's reagent method—using a spectrophotometer—to be 6.01 ± 1.03 mmol g^{-1} [36]. This thiol content was evidence of successful incorporation of the thiol moiety into the backbone of AX—converting it into TAX, with improved mucoadhesive properties.

3.3.1. FTIR studies

FTIR has proved to be an effective technique to observe and confirm whether the modification has accomplished successfully or not. In case of AX, a broad absorption band was noticed at 3321 cm^{-1}, which can be accredited to −OH stretching of alcohols. A peak came into sight at 2931 cm^{-1}, due to −CH stretching of alkanes. The peak at 1030 cm^{-1} was recognized as C–O stretching of ether. The other peaks observed at 895, 717, and 609 cm^{-1} were due to polymer backbone bending. The results were comparable to those reported by Iqbal and Saghir in their study for evaluation AXs as drug carriers, Bashir *et al* while performing physicochemical characterization and evaluation of suspending properties of AX, and Erum *et al*, who have done acute toxicity studies of AX [17, 21, 37, 38]. The IR spectra of TAX revealed all the important absorption bands, sufficient to prove the ester linkage between AX and TGA. The key functional groups appeared, including the C=O of ester and –SH stretching at 1710 cm^{-1} and 2516 cm^{-1} respectively. The C–O–C stretch appeared at 1031 cm^{-1}. The stretch appeared at 1031 cm^{-1}, while the peaks at 892, 771, and 643 cm^{-1} were attributed to polymer backbone bending. Other related stretching frequencies were also observed in the appropriate regions of the infrared spectra (figure 4).

3.3.2. Thermal analysis

DSC thermograms of AX and TAX were recorded to observe the change in their thermal behavior AX showed an endothermic peak at about 71.12 °C, with a heat of fusion of 180.39 J g^{-1} and a sharp peak at 305.72 °C with heat flow of 242.80 J g^{-1}. While, in case of TAX, endothermic peaks appeared at 61.72 °C and 202.01 °C with heat flow 28.10 J g^{-1} and 31.29 J g^{-1} respectively. Hence, the absence of sharp exothermic peak, appearance of additional endothermic peaks and decrease in transition temperature indicated the successful modification of AX (figure 5).

3.3.3. XRD studies

Figure 6 shows the x-ray diffraction spectra of AX and TAX in the range of 5°–50° (2θ). AX exhibited the characteristic of an amorphous material, as tno sharp peak was observed. However, diffractogram of TAX showed three distinguishing sharp peaks at 32°, 35° and 45° (2θ) that indicate slight proliferation in the crystallinity of AX on addition of the thiol group. Conversion of an amorphous powder into a crystalline phase was also noticed by Bahulkar *et al*, when they were working on thiolation of karaya gum in 2015 [35].

3.3.4. Surface morphological studies

Scanning electron microscopic observation of AX and TAX revealed that the particles were of irregular shape and variable size. However, TAX showed comparatively more irregularity, both in shape and surface morphology (figure 7).

3.3.5. Swelling index

The swelling properties of AX were observed; it was noted that the material had an appreciable water holding capacity, as its swelling index was found to be more than 50% [17]. Results of the study demonstrated that modification of AX into TAX enhanced the swelling strength of the powder, as the swelling index of the latter was calculated to be more than 80%. Adequate swelling power indicates the potential of the material to be used as a matrix forming agent, as well as a binding and disintegrating agent. Prüfert *et al* also pointed out an increase in the swelling power of polymers upon thiolation [39].

3.3.6. pH of aqueous dispersion

When isolating or synthesising a polymer with the aim to use it as excipient in buccal drug delivery, it is of great importance to evaluate its pH. Its compliance with the pH of buccal cavity is worthy of consideration because of its impact on patient compliance. pH of 1% aqueous dispersions of AX was 6.75 ± 0.17; that of TAX, 6.69 ± 0.13. These results prove that extracted

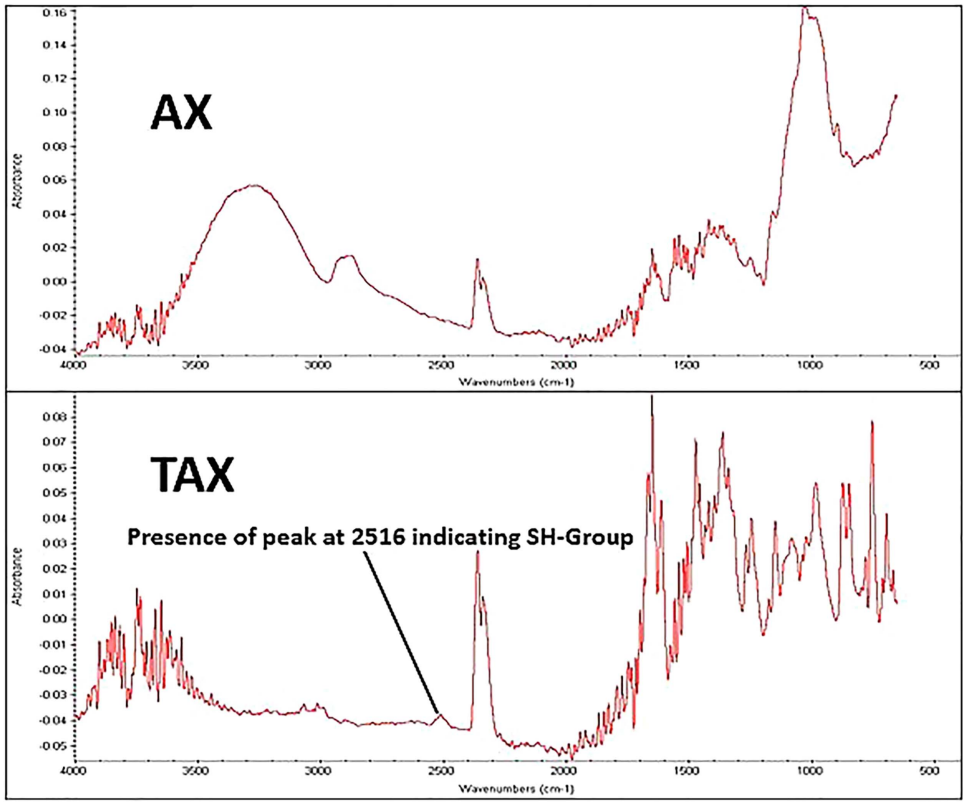

Figure 4. Showing the FTIR spectrum and DSC thermogram of AX and TAX. FTIR scan of TAX advocating a successful conversion of AX into TAX.

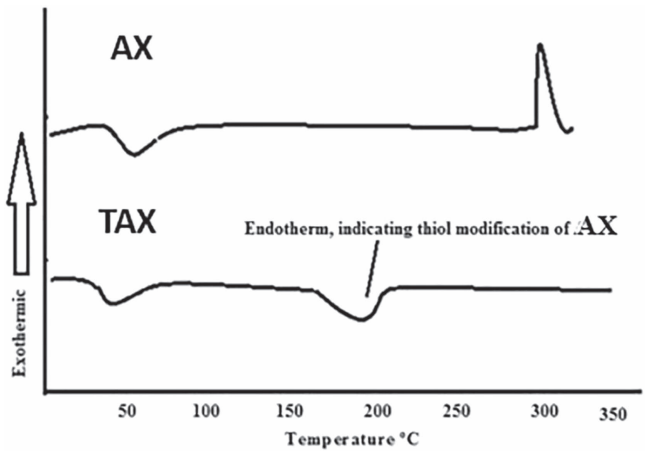

Figure 5. Thermal analysis describing the variation in DSC thermogram of TAX from AX provides evidence of successful thiol modification of AX.

as well as synthesized polymers would be compatible with buccal cavity pH (6.5–7.5). However, TAX showed a slightly lower value than that of AX, which may be due to some residues of the TGA components.

3.3.7. ex vivo mucoadhesive strength of AX and TAX

In order to evaluate the increase in mucoadhesive strength, a comparative analysis was done between AX and TAX. Results were clear evidence of improved mucoadhesion of the polymer upon its thiol modification. There was an augmentation of about 3.97 $\pm$ 0.97-fold in the mucoadhesion, as it had been increased from 2.32 $\pm$ 1.02 (AX) to 9.23 $\pm$ 0.91 (TAX). Reported results were in accordance with those reported by Gök *et al*, who described an enhancement in the mucoadhesion strength of the polymer upon thiolation [40]. Similarly, Kiani *et al* have also reported the improved mucoadhesion of thiolated carboxymethyl dextran, when they were evaluating its potential as a nano-carrier for colonic drug delivery [41].

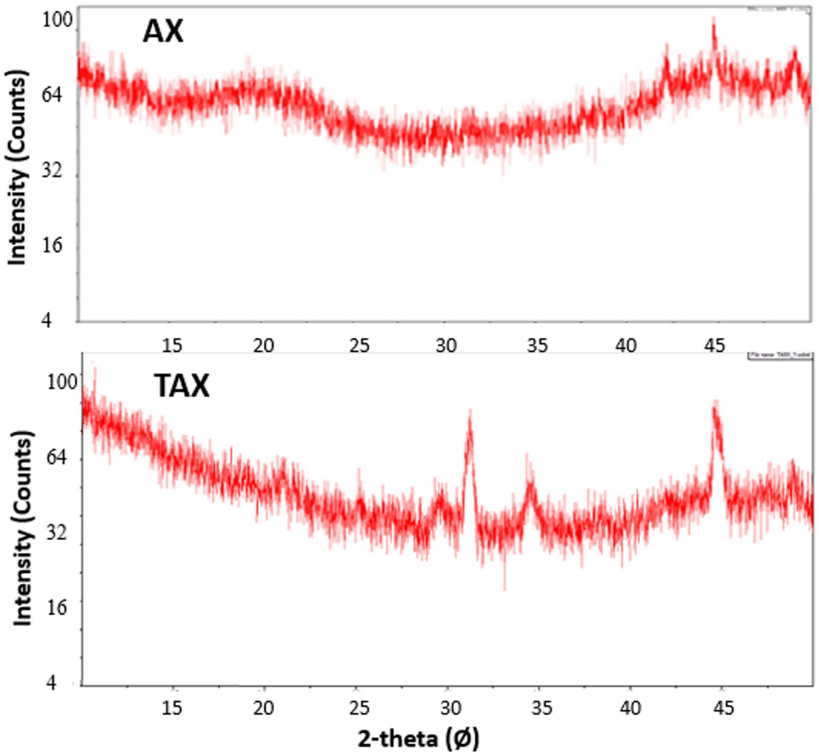

Figure 6. XRD of AX and TAX showing the change in AX structure from amorphous to a slightly crystalline TAX.

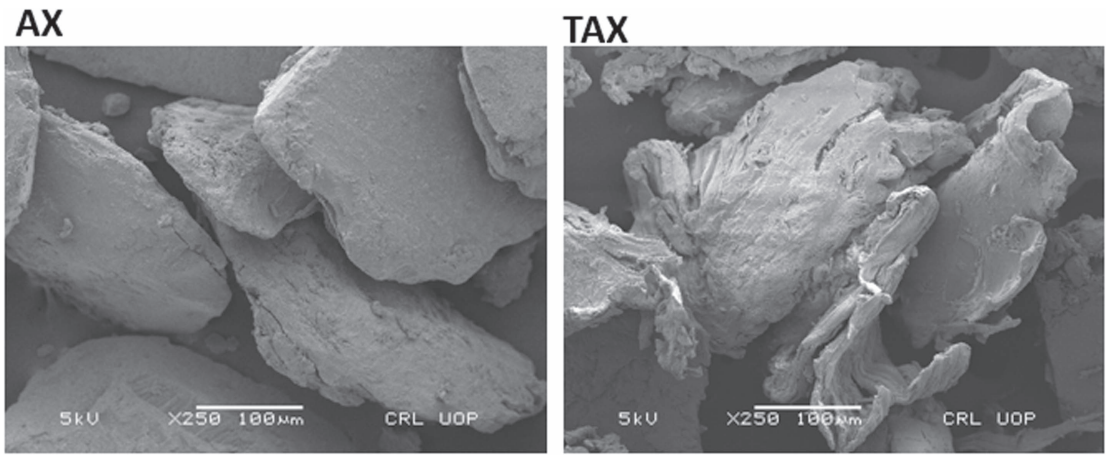

Figure 7. SEM images of AX and TAX illustrating the change in the appearance of particle in AX upon conversion into TAX.

3.3.8. in vivo safety studies in albino rats

Studies were conducted to evaluate that whether AX and TAX are safe to be used as effective carriers of drugs for *in vivo* administration. Both AX and TAX were applied to the skin of albino rats, and there were no any signs of redness or swelling erythema having appeared on the skin (figure 8). When the rats' skin was observed after 24 and 48 h, a slight constriction of the skin observed, which may be attributed to drying of the gel. However, after 15 days, normal hair growth was detected over the entire skin surfaces of the subject animals. Similarly, results of oral physiological compatibility studies proved that it is safe to administer the drug through the oral route, following administration of 5 ml of 3% AX gel and TAX gel separately to individual animals. After 24 h, no signs of any discomfort were observed and rats showed normal behavior. Number of pellets (stool) were counted, total weight was measured, shape, size and color of the stools were observed (table 3). Irregularly shaped, sized and a bit softer stools, having brownish color, were detected after 24 h in the AX treated rat, while in case of the TAX treated rat, a similar type of stool with comparable softness but with better symmetry in size and shape was obtained. The findings of these studies also supported its suitability for use as mucoadhesive buccal film former, as histopathological evaluation revealed that there were no any signs of damage to the

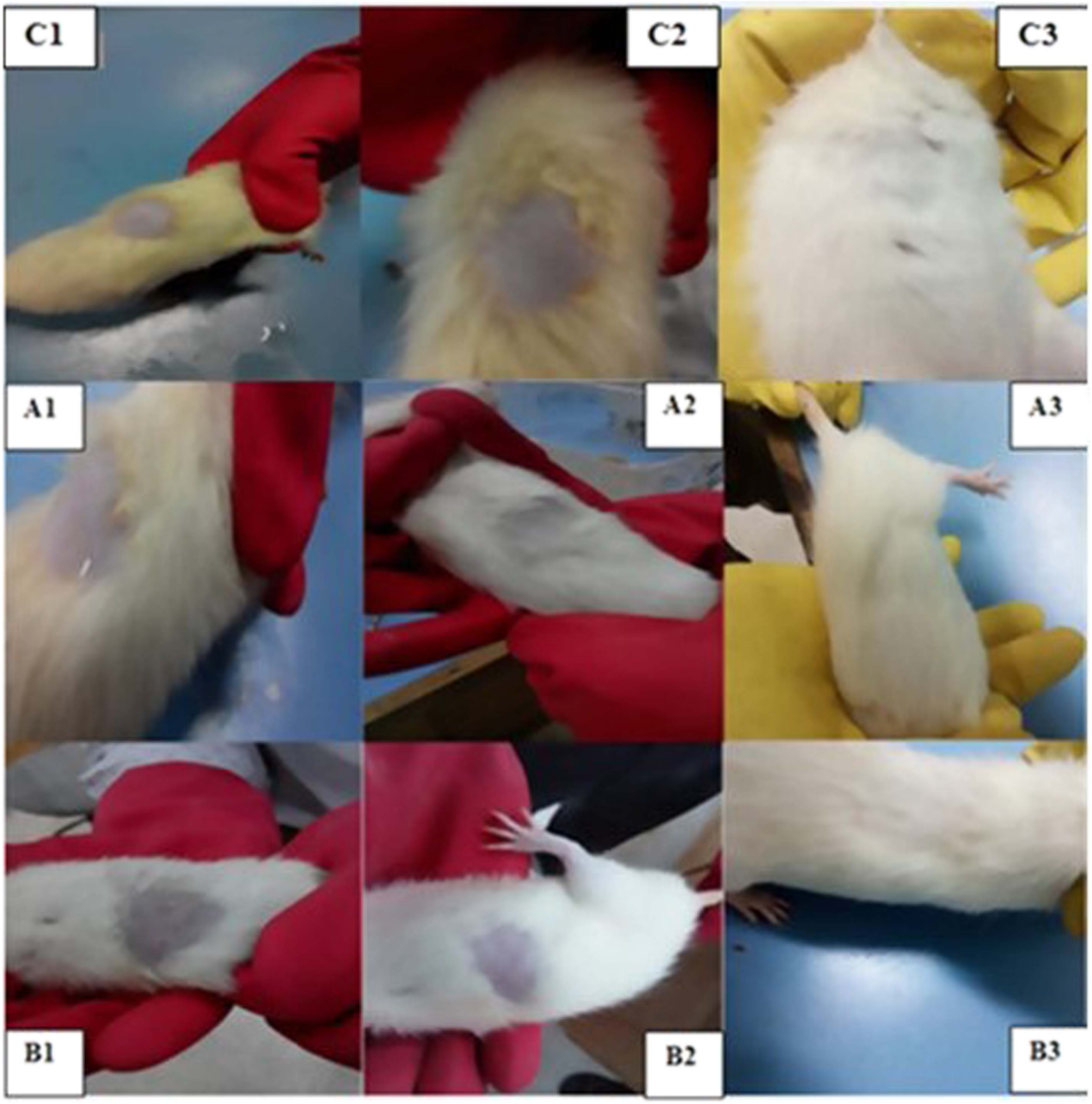

Figure 8. Skin compatibility studies (Reading from top to bottom) Control (C1, C2 and C3) AX treated (A1, A2 and A3) and TAX treated (B1, B2 and B3). C1, A1 and B1 (First day when the AX and TAX gel was applied). C2, A2 and B2 (Condition of the skin after 24 h) and C3, A3 and B3 (After 15 days of the treatment).

mucosal membrane, and buccal mucosal tissues showed normal cell morphology (figure 9). In the figure, it can be clearly seen that normal adipose tissues were present in all three groups, along with normally growing muscles. Capillaries and sebaceous glands can also be observed in their normal physiology. Moreover, necrosis, inflammation and infiltration of neutrophils and macrophages were absent. The concentrations of unmodified as well as modified polymers used in the current studies have conclusively indicated that the polymers are safe at concentration from about 750 mg kg^{-1} to 1000 mg kg^{-1} body weight [38].

3.4. Evaluation of mucoadhesive buccal films

Thickness of the film was 0.137 $\pm$ 0.081 mm, 0.149 $\pm$ 0.061 mm and 0.157 $\pm$ 0.063 mm for MBF1, MBF2 and MBF3 respectively, while surface pH was compatible to that of the buccal cavity, ranging from 6.23 $\pm$ 0.81 to 6.43 $\pm$ 0.49. Drug contents and moisture contents were greater than 90% and 5 to 7% respectively in all the formulations, while folding endurance was >300 in all the prepared films. SEM showing a uniform mixing of the drug in the polymeric meshwork, indicating the good drug-holding capability of TAX (figure 10). Mucoadhesion strength of the prepared films was found to increase along with the polymeric contents of the films, indicating that thiolated polymers are capable of enhancing the mucoadhesion strength of the material.

3.4.1. in vitro drug release studies

Glycerol is known to increase the plasticity and elasticity of films. Beside this, it is considered as an effective dissolution- and permeation-enhancing agent. The reason behind this enhanced dissolution is the hygroscopic nature of glycerol, which causes an increase in the water uptake by mucoadhesive film

Table 3. Effects of AX and TAX on gastrointestinal tract of albino rats.

	Control		AX Treated		TAX Treated	
Studied Parameters	After 24 h	After 48 h	After 24 h	After 48 h	After 24 h	After 48 h
No of pellets	33 ± 1.2	31 ± 0.9	21 ± 1.4	23 ± 1.1	27 ± 0.8	30 ± 0.8
Weight of the stool (g)	4.37 ± 2.1	4.08 ± 0.4	2.85 ± 0.3	2.93 ± 0.3	4.5 ± 0.1	4.6 ± 0.3
Shape of the stool	Regular, cylindrical	Regular, cylindrical	Irregular, compact	Regular, cylindrical	Regular, cylindrical	Regular, cylindrical

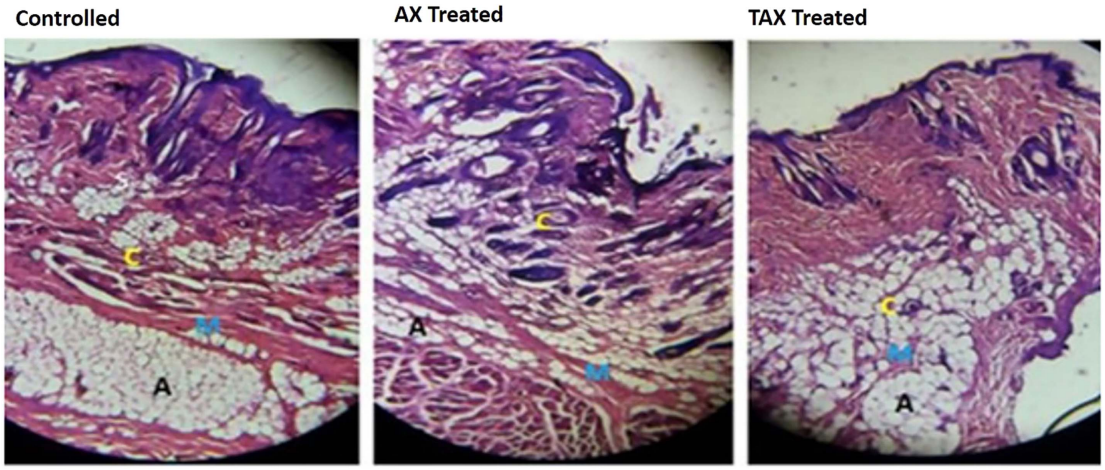

Figure 9. Histopathological slides of epithelial cells describing comparative analysis of Control, AX and TAX treated animal. Adipose tissue (A), muscles (M), mapillaries (C) and sebaceous glands (S) were present in their normal form.

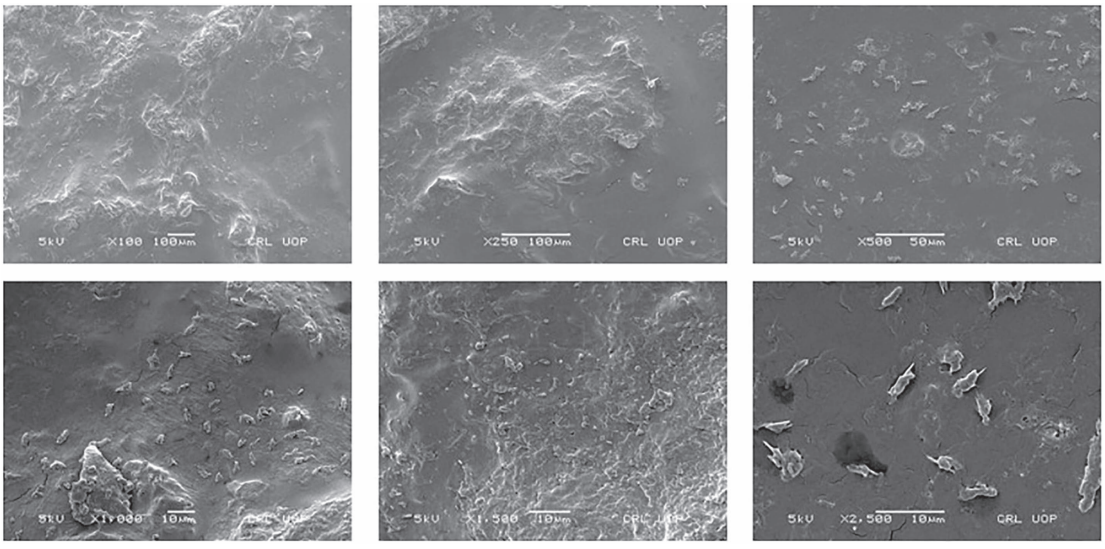

Figure 10. Scanning electron microscopic image of prepared mucoadhesive buccal films taken at different resolution powers (100x to 2500x), indicating uniform mixing of the drug in the meshwork of the polymer.

from the available liquid medium in the surroundings, and hence greater availability of the dissolving medium for drug dissolution. Moreover, glycerol may reduce the viscosity of polymeric gels, by forming swellable polymer (TAX) around the drug particles, causing greater release of the drug. This is a possible interpretation of the somewhat increased drug release from the formulations holding greater amounts of glycerol. As described earlier, the main barrier in the release of the drug through the gel layer is the thickness

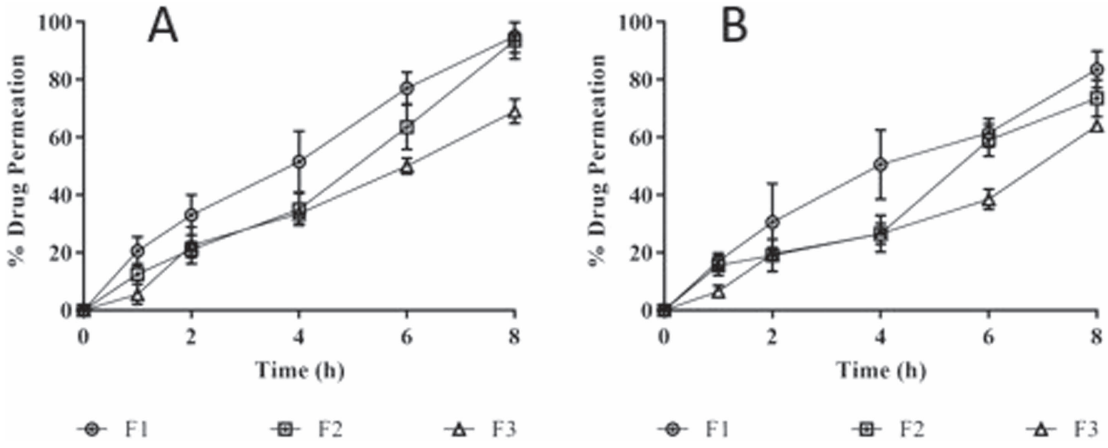

Figure 11. Graphical presentation of cumulative amount TZN HCl, released (A) and permeated (B) from mucoadhesive buccal films.

Table 4. Kinetic modeling of data obtained from *in vitro* drug release and *ex vivo* drug permeation studies of TZN HCl containing mucoadhesive buccal films.

Kinetic models		*in vitro* drug release studies		
		MBF1	MBF2	MBF3
Zero order	R^2	0.9748	0.9804	0.9883
	k^o	12.517	10.938	8.566
Korsmeyer–Peppas model	R^2	0.9970	0.9891	0.9885
	k_{KP}	18.711	7.635	9.040
	n	0.779	1.194	0.971
Kinetic models		*ex vivo* drug permeation studies		
Zero order	R^2	0.9596	0.9599	0.9665
Korsmeyer–Peppas model	k^o	10.884	9.103	7.393
	R^2	0.9941	0.9600	0.9680
	k_{KP}	17.617	8.515	6.427
	n	0.735	1.036	1.076

of that layer formed by the swellable polymers [42]. The same mechanism was followed by TAX to retard the release of TZN HCl from mucoadhesive buccal films. The real pathway followed by the drug to move across the gel layer is the liquid medium, captured in the gel pores; therefore, the factors that influence diffusivity of the drug in pure liquid phases can control penetration within gels. Thus, the thickness of the gel layer might be the deciding factor which determines the release of drug from films. Further, polymeric contents are responsible for the thickness of the gel layer. Therefore, by controlling the concentration of the polymer, thickness can be controlled, and hence the release of the drug. Findings of the current studies were in agreement with the stated claim, as the formulations with lower amounts of TAX released greater amounts of TZN HCl, and vice versa (figure 11) [24, 43].

3.4.2. ex vivo permeation of the drug

Permeation of buccal mucosa may be a limiting factor for various drugs delivered through this route. To overcome this problem, permeation-enhancing agents, which are capable of reducing permeation hurdle of the mucosal membrane, could be used. A variety of drugs can be delivered through this route by using a reversible method of reducing the barrier potential of mucosal tissues. Such permeation enhancers are used to change this hindering potential by increasing cell membrane fluidity, extracting intercellular lipids, interacting with epithelial protein domains or altering mucus structure or rheology, and hence increasing the permeability of the membrane to the drug [44]. Glycerol has the ability to penetrate into the membrane [45] and maintain the penetrability characteristics related with a hydrated membrane, even in circumstances of low water activities, which would otherwise dehydrate the membrane and restrict its penetrability. 83.5 ± 3.2%, 73.4 ± 4.21% and 60 ± 5.31% of the drug was found to be permeated across the goat buccal mucosa from the selected formulations MBF1, MBF2 and MBF3 respectively (figure 11). Here, it can be observed that, as the amount of the polymer was increased, the percentage of the permeated drug was decreased. However, from these results, the conclusion can be drawn that a controlled release formulation with suitable membrane permeating ability can be developed by adjusting appropriate concentrations of the film former and plasticizer.

3.4.3. Kinetic modeling

Zero order and Korsmeyer–Peppas models were applied to find out the mechanism and pattern of drug release from the prepared films. Zero order is the prediction of whether the release of the drug depends upon its initial concentration in the drug reservoir or not. On the other hand, a power law indicates how the drug will seep out from the film. Will it diffuse out following fickian or non-fickian diffusion patterns, or

Table 5. Statistical analysis of release, permeation data of drug and mucoadhesion strength of TZN HCl containing mucoadhesive buccal films.

Tukey's multiple comparisons test	Mean diff.	Significant	Summary	*P* value
% Drug release				
MBF1 versus MBF2	8.583	Yes	**	0.0023
MBF1 versus MBF3	16.08	Yes	****	<0.0001
MBF1 versus MBF3	7.5	Yes	**	0.0069
% Drug permeation				
MBF1 versus MBF2	8.25	Yes	**	0.0065
MBF1 versus MBF3	14.67	Yes	****	<0.0001
MBF2 versus MBF3	6.417	Yes	*	0.0341
Mucoadhesion test				
MBF1 versus MBF2	−0.85	Yes	***	0.0002
MBF1 versus MBF3	−1.198	Yes	****	<0.0001
MBF2 versus MBF3	−0.3475	Yes	*	0.0180

release by erosion of the dosage form? Application of kinetic modelling has resulted in the finding that release as well as permeation of the drug was dominated by the Korsmeyer−Peppas model (table 4). Higher values (0.9748 to 0.9883) of correlation coefficient (R^2) for zero order kinetics proved controlled release of TZN HCl from the film, independently of its initial concentration. Similarly, permeation was also controlled and independent from initial concentration, as R^2 for zero order was higher, ranging from 0.9596 to 0.9665. This the confirms the suitability of TAX along with glycerol for the formulation of mucoadhesive buccal films of TZN HCl. The best fit model for all the formulation was the Korsmeyer −Peppas model, indicating that both release and permeation of the drug followed diffusion from the gel layer of the swellable polymer around the drug particles. Values of *n* were taken to indicate that diffusion was non-fickian and followed super case II anomalous transport (table 4) for all formulations except permeation of MBF1, which followed a non-fickian diffusion pattern.

3.4.4. Statistical analysis

Two-way ANOVA followed by Tukey's multiple comparisons test suggested a significant variation of studied parameters of the different formulations. All three formulations were compared with each other, and it was observed that both polymer and plasticizer have pronounced effects on drug release, as well as on the mucoadhesion strength of the films. Increased concentration of the polymer showed negative impact on the release of the drug, but plasticizer may have a positive effect. The formulations with comparatively greater amounts of polymer released smaller quantities of the drug, and vice versa. *P* values in the range of <0.0001 to 0.0069 confirmed significant variability in the release of the drug upon variation in the concentrations of polymer and plasticizer. The influence of TAX and glycerol on drug permeation was analogous to those on release of the drug from formulated mucoadhesive films. Intra-formulation variations of permeability were significant, with *p* values less than 0.05 (<0.0001 to 0.0341). Similarly, when mucoadhesion strength was investigated statistically, it was observed that constructive response resulted upon increasing concentrations of both polymer and plasticizer. The formulations having larger concentrations showed greater mucoadhesion strength; the results of comparative analysis were significant with *p* values ranging from <0.0001 to 0.0180 (table 5).

4. Conclusion

The objectives of the study have been productively achieved, as AX was successfully extracted, converted into TAX and applied as an SR mucoadhesive film former. Safety analysis in albino rats has proven that it is safe for *in vivo* studies as well. TAX has shown reliable compatibility with TZN HCl, as well as capacity for loading sufficient amounts of the drug. It has clearly displayed its effectiveness, and may enhance patient compliance because of reduced number of unit doses of TZN HCl due to its sustained release from the film, which will increase its plasma concentration and effective half-life. The study reveals a promising scope in diverse areas. From the pharmaco-therapeutic perspective, it will increase bioavailability of TZN HCl, which otherwise might deteriorate due to extensive first-pass effect. This is due to direct absorption of drug into blood circulation from the buccal route, bypassing the hepatic first-pass effect. In a nutshell, TAX is found to be a useful mucoadhesive film former for transmucosal delivery of drugs in a sustained manner.

Acknowledgments

Author is thankful to Higher Education Commission (HEC) of Pakistan for its finiancial support to complete the project successfully.

Conflict of interest

The authors have nothing to disclose.

ORCID iDs

Muhammad Zaman https://orcid.org/0000-0003-1768-2979

References

[1] Suchaoin W *et al* 2016 Mucoadhesive polymers: synthesis and *in vitro* characterization of thiolated poly (vinyl alcohol) *Int. J. Pharmaceutics* **503** 141–9
[2] Cheewatanakornkool K, Niratisai S and Sriamornsak P 2016 Bioadhesiveness of thiolated pectin for buccal delivery of carbenoxolone sodium *Asian J. Pharm. Sci.* **11** 124–5
[3] Hanif M, Zaman M and Qureshi S 2015 Thiomers: a blessing to evaluating era of pharmaceuticals *Int. J. Polym. Sci.* **2015** 146329
[4] Morishita M, Khafagy E-S, Shofner J P and Peppas N A 2016 Oral delivery systems *Biodrug Delivery Systems: Fundamentals, Applications and Clinical Development* (Boca Raton, FL: CRC Press) ch 11 p 172
[5] Hanif M, Zaman M and Chaurasiya V 2015 Polymers used in buccal film: a review *Des. Monomers Polym.* **18** 105–11
[6] Yandrapu S K, Kanujia P, Chalasani K B, Mangamoori L, Kolapalli R V and Chauhan A 2013 Development and optimization of thiolated dendrimer as a viable mucoadhesive excipient for the controlled drug delivery: an acyclovir model formulation *Nanomedicine: Nanotech. Bio. Med.* **9** 514–22
[7] Roy S, Pal K, Anis A, Pramanik K and Prabhakar B 2009 Polymers in mucoadhesive drug-delivery systems: a brief note *Des. Monomers Polym.* **12** 483–95
[8] Shahnaz G, Perera G, Sakloetsakun D, Rahmat D and Bernkop-Schnürch A 2010 Synthesis, characterization, mucoadhesion and biocompatibility of thiolated carboxymethyl dextran–cysteine conjugate *J. Controlled Release* **144** 32–8
[9] Zaman M, Hanif M and Khan M A 2018 Arabinoxylan based mucoadhesive oral films of tizanidine hcl designed and optimized by using central composite rotatable design *Polym.-Plast. Technol. Eng.* **57** 471–83
[10] Madgulkar A R, Rao M R and Warrier D 2015 Characterization of Psyllium (Plantago ovata) Polysaccharide and its uses *Polysaccharides* (Springer) pp 871–90
[11] Deokar G, Kshirsagar S, Deore P A and Kakulte H 2016 Pharmaceutical benefits of Plantago ovate (Isabgol seed): a review *Pharm. Biol. Evaluations* **3** 32–41
[12] Shirsand S, Suresh S, Para M, Swamy P and Kumar D N 2009 Plantago ovata mucilage in the design of fast disintegrating tablets *Indian J. Pharm. Sci.* **71** 41–5
[14] Erum A, Bashir S, Saghir S and Sarfraz R M 2015 Arabinoxylan from Plantago ovate (Husk) a novel binder and superdisintegrant *Dhaka University J. Pharm. Sciences* **13** 133–41
[15] Lee C J, Nah C S, Teng C S, Jun W W and Saravanan M 2015 Spray dried calcium gelled arabinoxylan microspheres: a novel carrier for extended drug delivery *Chem. Pap.* **69** 1325–30
[16] Bashir S, Erum A, Saghir S, Tulain U R and Rashid A 2014 Physicochemical characterization and evaluation of suspending properties of arabinoxylan from Ispaghula (Plantago ovata) husk *Pak. J. Pharm. Sci.* **27** 1761–6
[17] Iqbal M S, Akbar J, Hussain M A, Saghir S and Sher M 2011 Evaluation of hot-water extracted arabinoxylans from ispaghula seeds as drug carriers *Carbohydrate Polym.* **83** 1218–25
[18] Mandeep K, Rana A and Nimrata S 2013 Fast dissolving films: an innovative drug delivery system *Int. J. Pharm. Res. Allied Sci.* **2** 14–24
[19] Saini P, Kumar A, Sharma P and Visht S 2012 Fast disintegrating oral films: a recent trend of drug delivery *Int. J. Drug Dev. & Res.* **4** 80–94
[20] Jain N K, Kulkarni S K and Singh A 2002 Modulation of NSAID-induced antinociceptive and anti-inflammatory effects by α 2-adrenoceptor agonists with gastroprotective effects *Life Sciences* **70** 2857–69
[21] Saghir S, Iqbal M S, Hussain M A, Koschella A and Heinze T 2008 Structure characterization and carboxymethylation of arabinoxylan isolated from Ispaghula (Plantago ovata) seed husk *Carbohydrate Polym.* **74** 309–17
[22] Anitha A, Deepa N, Chennazhi K, Nair S, Tamura H and Jayakumar R 2011 Development of mucoadhesive thiolated chitosan nanoparticles for biomedical applications *Carbohydrate Polym.* **83** 66–73
[23] Zaman M *et al* 2013 Formulation and *in vitro* evaluation of sustained release matrix tablets of cellulose based hydrophilic and hydrophobic polymers loaded with loxoprofen sodium *Indo American J. Pharma. Res.* **3** 7389–98
[24] Zaman M *et al* 2015 Fabrication and analysis of hydroxypropylmethyl cellulose and pectin-based controlled release matrix tablets loaded with loxoprofen sodium *Adv. Polym. Technol.* **34** 1–7
[25] Geldart D, Abdullah E, Hassanpour A, Nwoke L and Wouters I 2006 Characterization of powder flowability using measurement of angle of repose *China Particuology* **4** 104–7
[26] Sharma R and Ahuja M 2011 Thiolated pectin: synthesis, characterization and evaluation as a mucoadhesive polymer *Carbohydrate Polym.* **85** 658–63
[27] Bhatia M, Ahuja M and Mehta H 2015 Thiol derivatization of Xanthan gum and its evaluation as a mucoadhesive polymer *Carbohydrate Polym.* **131** 119–24
[28] Mukhija U, Soni N, Chawla A and Bhatt D 2012 Physical properties and dissolution behaviour of Meloxicam/Poloxamer solid dispersions prepared by hot melt method and microwave assisted method *Int. J. Res. Pharmacy Sci.* **2** 64–74
[29] Saxena A, Tewari G and Saraf S A 2011 Formulation and evaluation of mucoadhesive buccal patch of acyclovir utilizing inclusion phenomenon *Braz. J. Pharm. Sci.* **47** 887–97
[30] Bhatia M and Ahuja M 2013 Thiol modification of psyllium husk mucilage and evaluation of its mucoadhesive applications *ScientificWorld J.* **2013** 1–7
[31] Tomar A, Sharma K, Chauhan N S, Mittal A and Bajaj U 2012 Formulation and evaluation of fast dissolving oral film of dicyclomine as potential route of buccal delivery *Int. J. Drug Dev. Res.* **4** 408–17
[32] Prasanthi N, Krishna C S, Gupta M E, Manikiran S and Rao N R 2011 Design and development of sublingual fast dissolving films for an antiasthmatic drug *Der Pharmacia Lettre* **3** 382–95
[33] Singh T P, Singh R K, Shah J N and Mehta T A 2014 Mucoadhesive bilayer buccal patches of Verapamil hydrochloride: formulation development and characterization *Int. J. Pharm. Pharm. Sci.* **6** 234–341
[34] Sharma N, Ojha H, Pathak D P, Goel R and Sharma R K *Ex vivo* complexation, skin permeation, interaction and cytodermal toxicity studies of p-tertbutylcalix [4] arene nanoemulsion for radiation decontamination *Life Sci.* 2017 **168** 65–76
[35] Bahulkar S S, Munot N M and Surwase S S Synthesis, characterization of thiolated karaya gum and evaluation of effect of pH on its mucoadhesive and sustained release properties *Carbohydrate Polym.* 2015 **130** 183–90
[36] Hanif M and Zaman M 2017 Thiolation of arabinoxylan and its application in the fabrication of controlled release mucoadhesive oral films *DARU J. Pharm. Sci.* **25** 6

[37] Bashir S, Erum A, Saghir S, Tulain U R and Rashid A 2014 Physicochemical characterization and evaluation of suspending properties of arabinoxylan from Ispaghula (Plantago ovata) husk *Pak. J. Pharm. Sci.* **27** 1761–6
[38] Erum A *et al* 2015 Acute toxicity studies of a novel excipient arabinoxylan isolated from Ispaghula (Plantago ovata) husk *Drug Chem. Toxicology* **38** 300–5
[39] Prüfert F, Bonengel S, Menzel C and Bernkop-Schnürch A 2017 Enhancing the efficiency of thiomers: utilizing a highly mucoadhesive polymer as backbone for thiolation and preactivation *Eur. J. Pharm. Sci.* **96** 309–15
[40] Gök M K *et al* 2017 The effects of the thiolation with thioglycolic acid and l-cysteine on the mucoadhesion properties of the starch-graft-poly (acrylic acid) *Carbohydrate Polym.* **163** 129–36
[41] Kiani M, Tekie F S M, Dinarvand M, Soleimani M, Dinarvand R and Atyabi F 2016 Thiolated carboxymethyl dextran as a nanocarrier for colon delivery of hSET1 antisense: *in vitro* stability and efficiency study *Mater. Sci. Eng.* C **62** 771–8
[42] Das A, Gupta B and Nath B 2012 Mucoadhesive polymeric hydrogels for nasal delivery of penciclovir *J. App. Pharm. Sci.* **2** 158
[43] Bansal S, Bansal M and Garg G 2013 Formulation and evaluation of fast dissolving film of an antihypertensive drug *Int. J. Pharm. Chem. Biol. Sci.* **3** 1097–108
[44] Velmurugan S and Dodla S 2013 Buccal penetration enhancers-an overview *Asian J. Pharm. Clinical Res.* **6** 39–47
[45] Singla R and Lee C H 2003 Effects of glycerol on the *in vitro* percutaneous absorption of all-trans retinoic acid *Pharm. Dev. Tech.* **8** 229–37