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Thiolated xyloglucan: Synthesis, characterization and evaluation as mucoadhesive *in situ* gelling agent

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ABSTRACT

The objective of present study was to enhance bioadhesive potential of xyloglucan by thiolation. Thiolation of xyloglucan was achieved with esterification with thioglycolic acid. Thiolated xyloglucan was characterized by NMR, DSC, and XRD analysis. Thiolated xyloglucan was determined to possess 4 mmol of thiol groups/g of polymer by Ellman's method. Comparative evaluation of mucoadhesive property of ondansetron containing *in situ* gel system of xyloglucan and thiolated xyloglucan using sheep nasal mucosa revealed higher *ex vivo* bioadhesion time of thiolated xyloglucan as compared to xyloglucan. Improved mucoadhesive property of thiolated xyloglucan over the xyloglucan can be attributed to the formation of disulfide bond between mucus and thiolated xyloglucan. *Ex vivo* permeation study conducted using sheep nasal showed improved drug permeation in formulation based on thiolated xyloglucan. In conclusion, thiolation of xyloglucan improves its bioadhesion and drug permeation without affecting the resultant gel properties.

1. Introduction

Since the concept of bioadhesion has been pioneered, numerous attempts were undertaken in order to enhance bioadhesivity of polymers. Thiolated polymers so-called thiomers have proven to be a promising new class of polymeric excipients in drug delivery. In contrast to traditionally used mucoadhesive polymers, which adhere to the mucus by non-covalent bonds, such as hydrogen bonds and ionic interactions (Peppas & Mikos, 1990), thiomers are capable of forming covalent bonds leading to improved mucoadhesive properties. The underlying mechanism is based on thiol/disulfide exchange reactions and on an oxidation process between the reactive thiol groups of the mucoadhesive polymer and cysteine-rich subdomains of the mucin glycoproteins (Gum et al., 1992). The formed disulfide bonds are representatives of the bridging structure most commonly encountered in biological systems, providing covalent adhesion of thiomers to the mucus layer.

Thiomers are synthesized by immobilizing thiol moieties on well established hydrophilic polymers such as poly-(acrylates) (Bernkop-Schnurch & Steininger, 2001), chitosan or alginate (Bernkop-Schnurch, Kast, & Richter, 2001). Apart from their strongly improved mucoadhesive properties, thiomers have also permeation enhancing, and enzyme inhibitory properties (Bernkop-Schnurch, 2000), which render them useful excipients particularly for the noninvasive application of peptide.

Natural polysaccharides and its derivatives are widely used in pharmaceutical and food industry as biodegradable and biocompatible polymer for a large number of applications including binding, thickening, emulsifying, gelling agent, *etc.*

Xyloglucans (XG) are the main glycans that interlace cellulose microfibrils in most flowering plants (Carpita & Gibeaut, 1993; Fry, 1989; McNeil, Darvill, & Fry, 1984). Besides being a structural component of primary cell walls, they play important roles in the control of cell expansion and as a reserve of carbon in the seeds of many dicotyledons (Buckeridge, Santos, & Tine, 2003). Seed xyloglucans have cellulose-like (1 → 4)-linked β-D-glucan main-chain, substituted at O-6 by single-unit β-D-xylopyranose (Xylp) side-chains. Some of them are further substituted at O-2 by β-D-galactopyranose (Galp). These seed xyloglucans have a large number of commercial and industrial applications, especially those obtained from seeds of *Tamarindus indica* (Buckeridge et al., 2003; Picout, Ross-Murphy, Errington, & Harding, 2003; Rao & Srivastava, 1973). Xyloglucan are widely used as common additives for food and cosmetic, where they act as thickeners and stabilizing agents (Maeda, Yamashita, & Morita, 2007; Yamatoya & Shirakawa, 2003).

The present study was designed with the objective to improve the mucoadhesive properties of xyloglucan by synthesizing thiolated xyloglucan (TXG). The numbers of thiol groups/g of thiolated xyloglucan were determined by Ellman's method. Thiolated xyloglucan was characterized by differential scanning calorimetry (DSC), X-ray diffraction analysis (XRD), and nuclear magnetic resonance (NMR). Thiolated xyloglucan was further explored

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for mucoadhesive applications by developing *in situ* gel system employing ondansetron as model drug. Mucoadhesive properties of xyloglucan and thiolated xyloglucan *in situ* gel were comparatively evaluated using *ex vivo* bioadhesion study employing freshly excised nasal mucosa. Further, the *in situ* gelling system was evaluated for gelation ability, viscosity, gel strength, texture analysis. Mucosal toxicity studies were carried to get safety profile of polymer. Formulation also subjected to *in vitro* and *in vivo* biodegradation.

2. Experimental

2.1. Materials

Xyloglucan (45% galactose removal ratio) was gifted by DSP Gokyo Food and Chemical Co. Ltd. (Fukusima, Japan). Ondansetron has obtained as gift sample from IPCA Laboratory (India Rep Office, Andheri, India). Thioglycolic acid (Merck Specialities Private Ltd.). L-Cysteine and Ellman's reagent (5,5-dithiobis (2-nitrobenzoic acid) (DTNB)) were procured from Hi-Media Laboratories Pvt Ltd. (Mumbai, India). All other chemicals used were of analytical grade.

2.2. Synthesis of thiolated xyloglucan

The thiolated xyloglucan was synthesized by the esterification of xyloglucan with thioglycolic acid in the presence of hydrochloric acid. The reaction was carried out with 2 moles of thioglycolic acid for every 1 mole of hydroxyl group in xyloglucan. Xyloglucan (1 g) was dissolved in sufficient amount of hot water and was added with 0.223 mL of 80% thioglycolic acid and 1 mL of 7 N HCl. These were allowed to react for 150 min at 80 °C. The reaction mixture was poured in 500 mL of methanol. Precipitates of thiolated xyloglucan so obtained were washed twice with methanol and dried at room temperature.

2.3. Characterization of thiolated xyloglucan

2.3.1. Determination of thiol content

The degree of thiol group substitution was determined by quantifying the amount of thiol group on thiolated xyloglucan and xyloglucan by Ellman's method (Bernkop-Schnürch, Hornof, & Zoidl, 2003). An accurately weighed 50 mg of thiolated xyloglucan or xyloglucan was dissolved in 25 mL of distilled water. An aliquot of 2.5 mL of the polymer solution diluted with 2.5 mL of 0.5 M phosphate buffer (pH 8.0) was allowed to react with 5 mL of Ellman's reagent (DTNB, 0.03%, w/v in 0.5 M phosphate buffer pH 8.0) for 2 h at room temperature, followed by measurement of absorbance of the reaction mixture at 450 nm. The numbers of thiol groups in the polymer were calculated using the standard curve obtained by reacting xyloglucan solution containing varying amount of L-cysteine with Ell-man's reagent.

2.3.2. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance spectroscopy of the xyloglucan and thiolated xyloglucan was carried out by using NMR spectrometer (Bruker Avance III, 400 MHz). The xyloglucan and thiolated xyloglucan in powder form were scanned from 1 to 10 ppm range under the following measurement conditions: Magnet 9.4 T superconducting Magnet; Probe-BBO 400 MHz, with Z-gradient, 2H lock; for observation of nuclei like 1H, 13C, 31P, 15N, *etc.* with 1H decoupling. Any of these nuclei can be fully automatically selected and optimally tuned and matched.

2.3.3. Scanning electron microscopy (SEM)

The surface morphology of polymer was examined by scanning electron microscopy. A small amount of powder was spread on an aluminum stub, which was placed after gold sputtering in SEM chamber (JSM 6390®, USA). Photographs were taken at acceleration voltages of 20 kV electron beam.

2.3.4. Zeta potential measurement

Xyloglucan and thiolated xyloglucan were separately dispersed in distilled water to get stock solution of 1% (w/w). This dispersion was filled in zeta cell and zeta potential was determined using Zeta Sizer (Nano ZS 90, Malvern Instruments, UK) with the help of software.

2.3.5. Differential scanning calorimetry (DSC)

DSC thermograms of xyloglucan and thiolated xyloglucan were recorded using a differential scanning calorimeter (DSC, METTLER, TOLEDO, Switzerland). About 5 mg of sample was crimped in a standard aluminum pan and heated in a temperature range of 40–350 °C at a heating rate of 10 °C per min in nitrogen atmosphere.

2.3.6. X-ray diffraction (XRD) analysis

The X-ray diffractometry was carried out to investigate crystallinity of the xyloglucan and thiolated xyloglucan. Study was carried out using an X-ray diffractometer (Bruker AXS D8 Advance). The xyloglucan and thiolated xyloglucan in powder form were scanned from 3° to 80° diffraction angle (2θ) range under the following measurement conditions: source, nickel filtered Cu Kα radiation; voltage 40 kV; current 35 mA; step time 31.2 s; temperature range −170 °C to +450 °C.

2.3.7. In vitro degradation

The degradation performance of thiolated xyloglucan was studied in various simulated fluids like simulated body fluid (SBF), simulated lungs fluid (SLF) and simulated nasal fluids. Films of thiolated xyloglucan (0.005–1 mm thickness) obtained by casting method were placed in 10 mL each of simulated fluid which content in small vials. Then the vials were incubated in shaking incubator at 100 rpm at 37 °C for 1 h. The films were withdrawn at intervals of 10, 20 and 30 min, washed with distilled water, dried and subjected to degradation characteristic such as swelling degree and weight loss (Shi, Zhu, & Chen, 2010; Suggs, Krishan, Garcia, Peter, & Anderson, 1998).

2.3.7.1. Swelling degree. The swelling degree (SD) was characterized at 37 °C. The experiments were carried out by measuring the weight gain as a function of immersion time in 20 mL solution. The swelling degree was calculated according to equation given below.

$$\text{SD} \quad (\%) = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} \times 100 \tag{1}$$

where Wt is the wet weight and after degrading a predetermined time; Wo is the original weight of the sample.

2.3.7.2. % Weight loss. The weight loss was calculated by comparing the dry weight (Wd) of the remained sample after degradation for a predetermined time with the original dry weight (W0) of the sample as the equation. At pre-determined intervals of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 60 min; samples were taken out, purged with distilled water and subsequently dried until absolute desiccation, then weighted.

$$\text{Weightloss} \quad (\%) = \frac{\text{W0} - \text{Wd}}{\text{W0}} \times 100 \tag{2}$$

2.3.8. In vivo biodegradation study

The animal experiment was carried out in compliance with the protocol of the institutional animal ethical committee (Registration No. 651/02/C/CPCSEA under CPCSEA, India). For the study of *in vivo* study 3 male wistar rats (Avg wt. 200–300 g) are selected to

monitor the *in vivo* degradation. Anesthesia was induced by intraperitoneal injection of ketamine HCl (85 mg/kg body weight). An incision (2.5 cm) was inflicted laterally about the mid portion of the back. Subcutaneous pockets were formed around each incision, free film was inserted, and the wounds were closed by intermittent nylon sutures, 0.5 cm apart for 3 individual male wistar rats. Films were explanted at 10, 20, and 30 min for analysis (Tracy et al., 1999).

2.4. Preparation of in situ gel system

Drug loaded *in situ* gelling system was prepared by slowly adding a weighed amount (2.5%, w/w) of each of the polymer xyloglucan (XG) and thiolated xyloglucan (TXG) into cold water. The mixture was slowly homogenized by using magnetic stirrer (Remi Instruments, India). About 2.0 g of ondansetron was dissolved in resulting solution. Appropriate quantity of benzalkonium chloride was added as a preservative. The pH of formulation was adjusted between 4.5 and 5.5. The formulations were filled in glass vials (capacity 10-mL), capped with rubber closures and sealed with aluminum caps. Formulations were stored at 4–8 °C until use.

2.5. Evaluation of prepared in situ gels

2.5.1. Gelation studies

Gelation is the process, by which the liquid phase makes a transition to gel. In brief, a 10-mL transparent vial containing a magnetic bar and each formulation were placed in a temperature water bath. The gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked by visual inspection and graded as indicated in Table 1. Each preparation was tested thrice to control the repeatability of the measurement (Balasubramaniam, Kant, & Pandit, 2003).

2.5.2. Viscosity measurements

Viscosities of formulations before and after gelation were measured by using Brookfield DV-E viscometer using spindle number-3 at 100 rpm shear rate. The viscosity was recorded at increasing temperature in range of 20–30 °C (Edsman, Carlfors, & Petersson, 1998).

2.5.3. Gel strength determination

It is expressed in terms of time (in seconds) required by a 35 g piston for penetration of 5 cm distance, through the 50 g gel formulation. Test was performed using 'gel strength apparatus' modified at laboratory. XG and TXG solutions (50 g) were placed in a 100 mL measuring cylinders and gelation was induced by means of temperature. The piston (weight: 35 g) was then placed onto the gel. The gel strength was measured as the time (seconds) required moving the piston 5 cm down through the gel (Mahajan, Shah, & Surana, 2011).

2.5.4. Ex vivo bioadhesive strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method by Murthy, Majithiya, and Ghosh (2006). In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. At the time of testing, a section of nasal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The surface area of each exposed mucosal membrane was 4.2 cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe. Probe with mucosal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a slight force was applied for 2 min to ensure intimate contact between the tissue and the sample. The mucoadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation.

$$\text{Detachment stress} = \frac{m \times g}{A} \quad (3)$$

where m is the weight added to the balance in grams, g is the acceleration due to gravity taken as 980 cm/s^2 and A is the surface area of sheep nasal mucosa.

2.5.5. Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2 Texture Analyzer in TPA mode, as previously described (Jones, Lawlor, & Woolfson, 2002). Formulation (35 g) was transferred into 50-mL bottles, taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (35 mm diameter) was forced down into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). At least five replicate analyses of each sample were performed at temperatures of 25 °C and 35 °C. From the resulting force–time plots, the hardness (the force required to attain a given deformation), cohesiveness (the work required to deform the hydrogel in the down movement of the probe) and adhesiveness (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived.

2.5.6. Ex vivo permeation studies

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue sample was sandwiched in Franz diffusion cells (capacity 16 mL) displaying a permeation area of 3.14 cm^2. Phosphate buffer (pH 6.6) was added to the acceptor chamber. The temperature was maintained at 37 °C. After a pre-incubation time of 20 min, formulation was placed in the donor chamber. At predetermined time points, 1-mL samples was withdrawn from the acceptor compartment, replacing the sampled volume with phosphate buffer after each sampling, for a period of 5 h. The samples withdrawn were filtered and used for analysis. Blank samples (without drug) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV–visible spectrophotometer at 310 nm.

Permeability coefficient (P) was calculated by the following formula:

$$P = \frac{dQ/dt}{C_0 \times A} \quad (4)$$

where dQ/dt is the flux or permeability rate (mg/h); C_0 is the initial concentration in donor compartment; and A is the effective surface area of nasal mucosa (Mahajan & Gattani, 2010).

Table 1
Evaluation parameters of the *in situ* gel.

Sr. no.	Formulation	Degree of gelation	Viscosity study (cp)		Gel strength (s)	Mucoadhesive force (dyne/cm^2)	Zeta potential (mV)
			Solution	Gel			
1	XG	++	51 ± 0.73	512 ± 0.51	14 ± 1.96	4500.13 ± 91.19	21.7 ± 1.67
2	TXG	++	95 ± 1.21	1430 ± 1.25	38 ± 1.78	6500.41 ± 56.15	22.2 ± 2.64

2.5.7. Mucosal toxicity studies

Being sensitive than other mucosa nasal mucosa was used to study mucosal toxicity. Mucosa incubated in phosphate buffer solution (pH 6.6) after collection it was compared with tissue incubated in the diffusion chamber with gel formulation (TXG). Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 μm) were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope, to detect any damage to the tissue during *ex vivo* permeation study (Murthy et al., 2006).

3. Results and discussion

3.1. Synthesis of thiolated xyloglucan

The covalent attachment of xyloglucan to thioglycolic acid was achieved by ester bonds formation between hydroxyl group of beta-galactane moieties of xyloglucan and carboxyl group of thioglycolic acid (Fig. 1).

After complete drying, product appeared as off-white odorless powder, soluble in water. The average yield of this synthesis amounted to 48% of the utilized amount of xyloglucan. Thioglycolic acid is soluble in water and methanol. Precipitation with methanol from an aqueous solution and subsequent washing by keeping the precipitate over night was found to be sufficient purification method for thiolated xyloglucan. Thiolated xyloglucan was found to contain 4 mmol of thiol groups/g as determined by quantifying the amount of thiol group by Ellman's method.

3.2. Nuclear magnetic resonance (NMR)

In order to explore possible modification of xyloglucan, we compared the ^{1}H NMR spectra of xyloglucan and thiolated xyloglucan (Fig. 2). As illustrated in Fig. 2, the majority of XG and TXG chemical shifts were between 1 and 8 ppm. NMR spectra of xyloglucan showed singlet at 2.1 (—CH_2) and multiplets at 3.91 (—O—CH_2), 4.50 (—O—CH_2—OH), (—O—CH—), 5.1 (—O—) and multiplets at 3.42, 3.76, due to —CH (OH). NMR spectra of thiolated xyloglucan showed singlets similar to xyloglucan but additional singlets at 3.27 due to (SH). This result confirms thiolation of xyloglucan (Silverstein, Webster, & Kiemle, 2005).

3.3. Scanning electron microscopy (SEM)

Scanning electron microscopic images show surface morphology of xyloglucan and thiolated xyloglucan. The shape of xyloglucan and thiolated xyloglucan was found to be round (Fig. 3). Both xyloglucan and thiolated xyloglucan had smooth surfaces without crack, hole and fracture.

3.4. Zeta potential measurements

Xyloglucan and thiolated xyloglucan had zeta potential values -21.7 ± 1.67 mV and -22.2 ± 2.64 mV respectively indicating anionic nature in distilled water (Table 1). The result showed that zeta potential was negative and it was shifted to higher negative value after thiolation. This might owe presence of large number of —OH groups as anionic structures.

3.5. Differential scanning calorimetry (DSC)

Fig. 4 shows the DSC thermo grams of xyloglucan and thiolated xyloglucan. The DSC curve of xyloglucan shows one sharp endotherm at 139.44 °C with heat of fusion of −100.72 J/g and one sharp exotherm at 332.33 °C with heat of fusion 81.23 J/g. DSC thermo gram of thiolated xyloglucan shows an endotherm at 119.75 °C with heat of fusion of −419.73 J/g. Thus, a decrease in the endothermic transition temperature and heat of fusion of xyloglucan was observed on thiolation of xyloglucan.

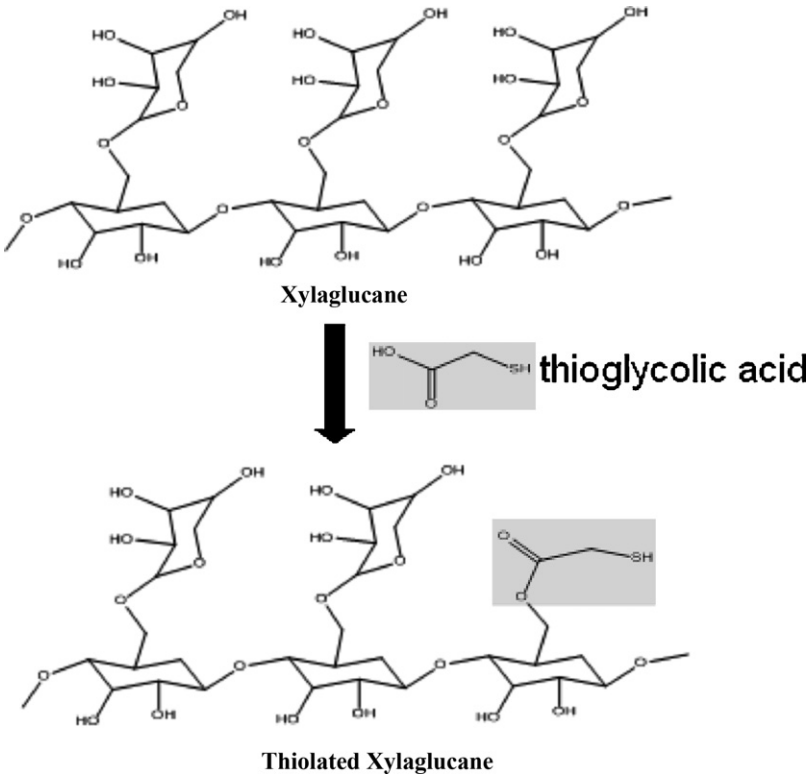

Fig. 1. Synthesis of thiolated xyloglucan.

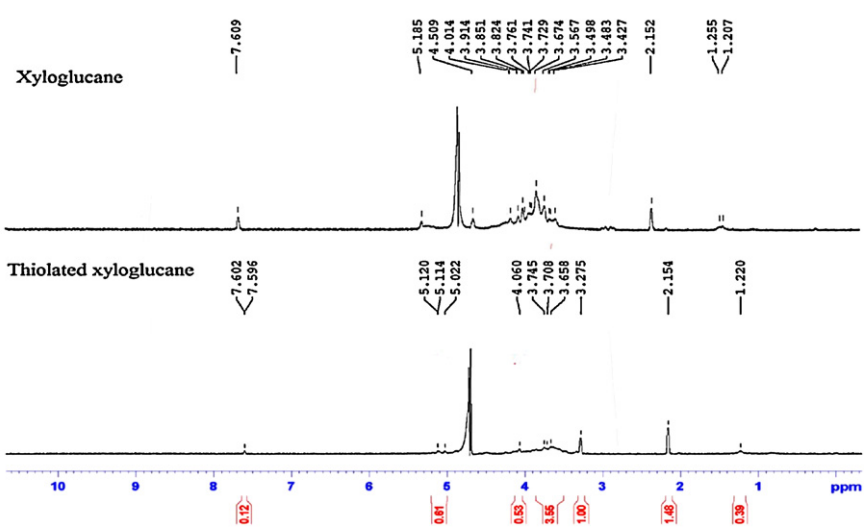

Fig. 2. NMR spectra of xyloglucan and thiolated xyloglucan.

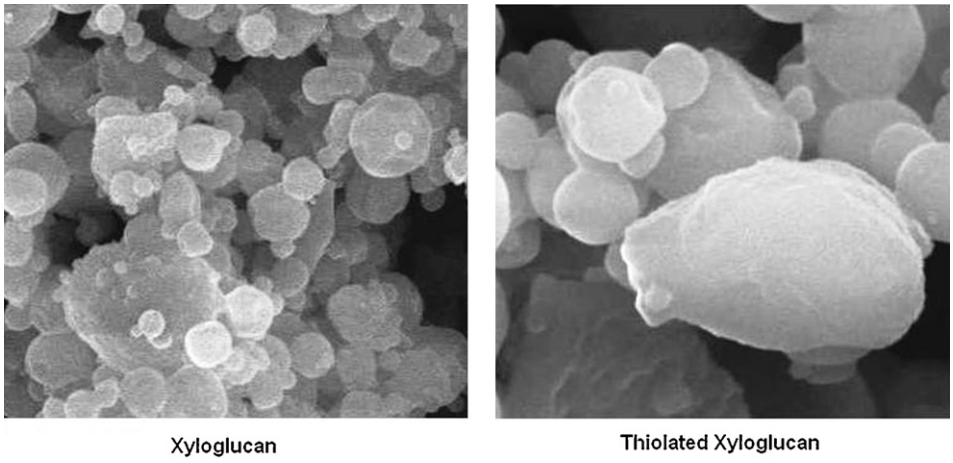

Fig. 3. Scanning electron microscopic images of xyloglucan and thiolated xyloglucan.

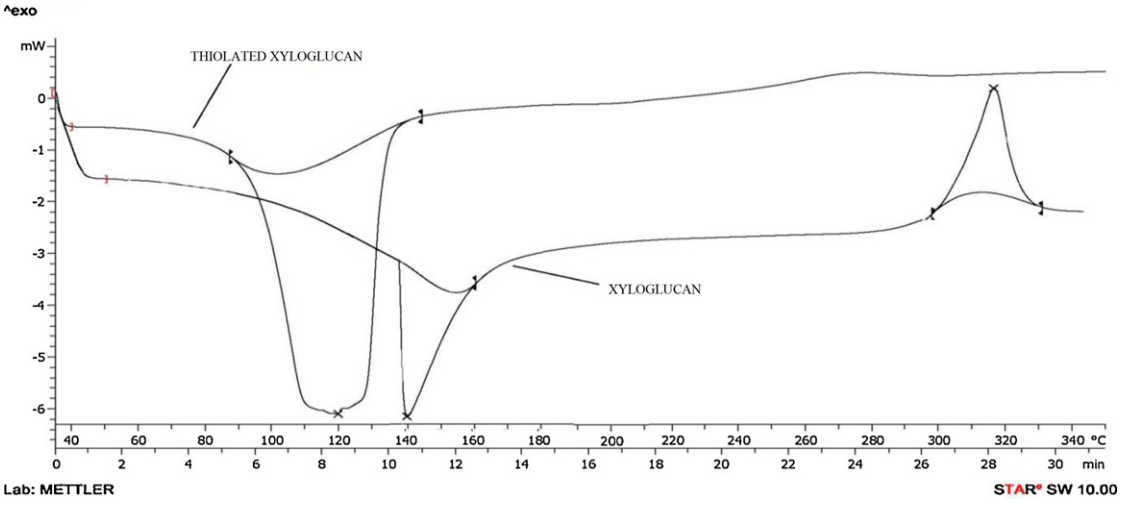

Fig. 4. DSC thermograms of xyloglucan and thiolated xyloglucan.

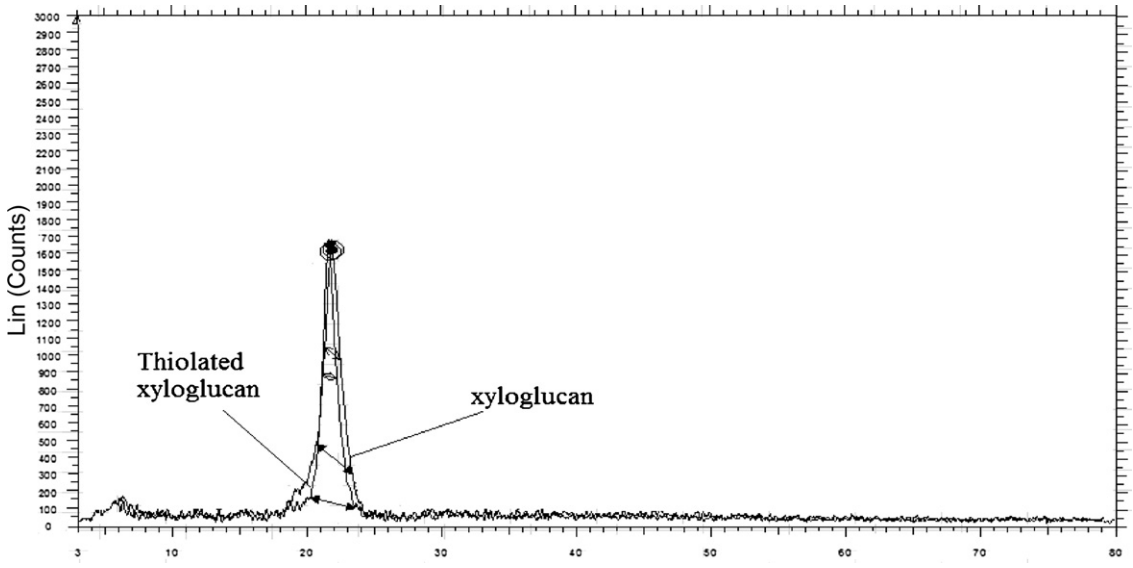

Fig. 5. XRD spectra of xyloglucan and thiolated xyloglucan.

3.6. X-ray diffractometry (XRD)

Fig. 5 displays the X-ray diffraction spectra of xyloglucan and thiolated xyloglucan. X-ray diffractogram of xyloglucan is typical of amorphous material with characteristic peaks appearing at 20.15° (2θ) while the diffractogram of thiolated xyloglucan shows characteristic peak at 20.3° (2θ). The peak intensity of thiolated xyloglucan is slightly greater than the xyloglucan. No marked changes are observed on thiolation.

3.7. In vitro degradation

Fig. 6A shows the swelling ability of films in different simulated fluids within 35 min. The swelling degree was larger in SBF than that in SNF/SLF. The swelling behavior of films was similar in SNF and SLF. The weight losses of films in SBF, SLF and SNF at 10, 20, 30 and 35 min were shown in Fig. 6B reflects that for samples with same polymer content, the weight loss speed in SBF was slower than SLF and SNF. The result can be explained by that rapid weight loss was mainly caused by the breaking down of xyloglucan molecules.

3.8. In vivo degradation

In the *in vivo* study the rate of degradation was rapid maintaining 30% weight at the end of 20 min. The films showed complete degradation by the end of 30 min. Complete films could not be recovered at the end of 40 min, due to foreign body response. As a result of the *in vivo* implantation the typical response results in the accumulation of cells such as macrophages around the foreign body. Free radicals, acidic products or enzymes produced by these cells during the foreign body response may accelerate degradation.

3.9. Characterization of in situ gel system

3.9.1. Gelation studies

The thermal gelation characteristic of xyloglucan solution was studied by measuring gel formation temperature (GFT), was in the range of 25–30 °C. Gelation characteristics was assessed on ordinal scale ranging between – (no gelation), ++ (immediate gelation remains for few hours), +++ (immediate gelation remains for longer duration). Both xyloglucan and thiolated xyloglucan showed immediate gelation remains for few hours (Table 1). Gelation characteristic of xyloglucan does not affected on thiolation. Rapid gelation favors the inter diffusion process between the polymer and mucus layer providing stronger adhesion.

3.9.2. Viscosity measurement

Apparent viscosity values were measured for liquid formulation and gel using Brookfield viscometer DVE with spindle no. 3 at 100 rpm. The results showed (Table 1) marked increase in viscosity for both polymers after sol to gel transition. TXG based gel is more viscous than XG based gel.

3.9.3. Gel strength measurement

In the development of an *in situ* gelling system, the gel strength is important in finding the condition, which allows easy administration as liquid and enhance residence time at administration site. Optimal *in situ* gel must have suitable gel strength so as to be administered easily and can be retained at mucosal surface for longer time

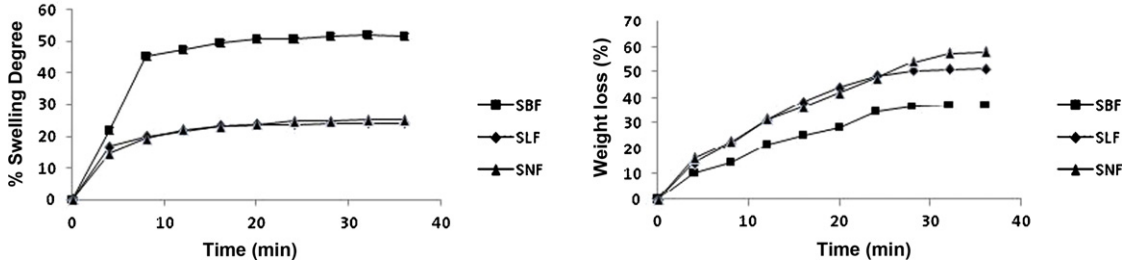

Fig. 6. *In vitro* degradation. (A) Swelling index of polymer. (B) Percent weight loss of polymer in SBF, SLF, SNF.

after administration. In thiolated xyloglucan gels marked increase in gel strength was observed. The gel strength values between 25 and 50 s were considered sufficient. The gel strength less than 25 s may not retain its integrity and may erode rapidly while gels having strength greater than 50 s are too stiff and may cause discomfort to the mucosal surfaces. The TXG showed the gel strength values in the range 35–40 s (Table 1) which are acceptable (Yong, Choi, & Rhee, 2001).

3.9.4. *Ex vivo* bioadhesion studies

The *ex vivo* bioadhesion was studied by the method reported by Murthy et al. using drug loaded *in situ* gelling system based on xyloglucan and thiolated xyloglucan. The mucoadhesion force is an important parameter in studying bioadhesion. The mucoadhesion force for XG and TXG was 4500.13 ± 91.17 dynes/cm^2 and 6500.41 ± 56.15 dynes/cm^2 respectively (Table 1). Thiolated xyloglucan showed almost 1.5-folds improved mucoadhesive property as compared to xyloglucan. The mucoadhesive property of xyloglucan can be explained by the fact that secondary hydroxyl (—OH) groups are principle source of mucoadhesion. The improved mucoadhesive property of thiolated xyloglucan can be attributed to the formation of disulfide bond between the —SH groups of thiolated polymers and mucus *via* thiol/disulfide exchange reactions. Moreover polymers with charge density can serve as good mucoadhesive agents. It has also been reported that anionic polymers are more effective bioadhesive than cationic or non ionic polymers. (A zeta potential study revealed anionic nature of xyloglucan and thiolated xyloglucan.)

3.9.5. Texture analysis

In designing gel type formulation, particularly in respect to prolonged retention time at the site of administration for gels destined for mucosal delivery, a balance between gel adhesiveness and gel cohesiveness should be maintained. Texture analysis could provide a reliable overview of these properties. Texture profile analysis spectra of *in situ* gel obtained by plotting load (N) *vs.* time (s) gives the hardness 0.20 ± 0.02 N, cohesiveness 2.34 ± 0.21 N*S and adhesiveness −0.001 J. Gel hardness expresses the applicability of gels to site of application or adhesiveness which can be an indicator for the retention time on the site of application.

3.9.6. *Ex vivo* permeation studies

Fig. 7 compares *ex vivo* permeation of drug across nasal mucosa from in stiu gelling system based on xyloglucan and thiolated xyloglucan. The percent drug permeated after 270 min was found to be 95.52% from XG formulations and 96.23% from TXG formulations. The permeability coefficient values (P) were found to be 0.04572 cm^2/h and 0.0562 cm^2/h. The P values determined in thiolated xyloglucan were significantly higher than non thiolated

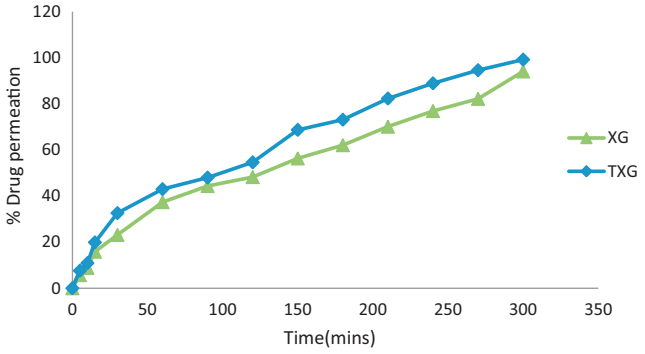

Fig. 7. *Ex vivo* drug permeation across nasal mucosa from xyloglucan and thiolated xyloglucan *in situ* gelling system.

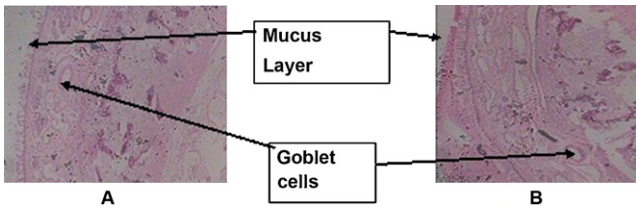

Fig. 8. Photomicrograph of sheep nasal mucosa used in mucosal toxicity. (A) Formulation treated mucosa. (B) Untreated mucosa.

polymer. As a plausible hypothesis, this effect might be ascribed to thiol groups causing stronger adhesion of thiomer to mucosal surface.

3.9.7. Mucosal toxicity

Photomicrographs of sheep nasal mucosa after the permeation studies were observed for histopathological changes in comparison with the normal (untreated) mucosa (Fig. 8). The section of mucosa treated with formulation showed very slight degeneration of nasal epithelium along with no erosion. There was no sign of remarkable destructive effect of formulations on the treated nasal mucosa.

4. Conclusion

Xyloglucan has been proved in many articles as a promising *in situ* gelling agent for the mucosal route of administration. In this study, thiolation of xyloglucan was carried out to enhance bioadhesive property of xyloglucan. Thiolation of xyloglucan did not alter its gelling ability. Moreover thiolated xyloglucan has permeation enhancing effect without causing mucosal toxicity. The *in situ* gel system based on thiolated xyloglucan exhibits better texture and idea gelling properties along with good *in vitro in vivo* degradation. The present study gives an extensive characterization of a thiolated xyloglucan and should therefore facilitate the development of new drug delivery systems providing a greatly prolonged residence time on various mucosal tissues like nasal, rectal, ocular, vaginal, *etc.*

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References

Balasubramaniam, J., Kant, S., & Pandit, J. K. (2003). In vitro and *in vivo* evaluation of the Gelrite® gellan gum based ocular delivery system for indomethacin. *Acta Pharmaceutica*, *53*, 251–261.

Bernkop-Schnurch, A. (2000). Mucoadhesive polymers. In S. Dumitriu (Ed.), *Polymeric biomaterials* (pp. 147–165). New York: Marcel Dekker.

Bernkop-Schnürch, A., Hornof, M., & Zoidl, T. (2003). Thiolated polymers–thiomers: Synthesis and *in vitro* evaluation of chitosan–2-iminothiolane conjugates. *International Journal of Pharmaceutics*, *260*, 229–237.

Bernkop-Schnurch, A., Kast, C. E., & Richter, M. F. (2001). Improvements in the mucoadhesive properties of alginate by the covalent attachment of cysteine. *Journal of Control Release*, *71*, 277–285.

Bernkop-Schnurch, A., & Steininger, S. (2001). Synthesis and characterization of mucoadhesive thiolated polymers. *International Journal of Pharmaceutics*, *194*, 239–247.

Buckeridge, M. S., Santos, H. P., & Tine, M. A. S. (2003). Mobilisation of storage cell wall polysaccharides in seeds. *Plant Physiology and Biochemistry*, *8*(1–2), 141–156.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, *3*(1), 1–30.

Edsman, K., Carlfors, J., & Petersson, R. (1998). Rheological evaluation of poloxamer as an *in situ* gel for ophthalmic use. *European Journal of Pharmaceutical Sciences*, *6*, 105–112.

Fry, S. C. (1989). The structure and functions of xyloglucan. *Journal of Experimental Botany*, *40*(211), 1–11.

Gum, J. R., Hicks, J. W., Jr., Toribara, N. W., Rothe, E.-M., Lagace, R. E., & Kim, Y. S. (1992). The human MUC2 intestinal mucin has cysteine-rich subdomains located both upstream and downstream of its central repetitive region. *Journal of Biological Chemistry*, *267*, 21375–21383.

Jones, D. S., Lawlor, M. S., & Woolfson, A. D. (2002). Examination of the flow rheological and textural properties of polymer gels composed of poly (methyl vinyl ether-comaleic anhydride) and poly (vinylpyrrolidone): Rheological and mathematical interpretation of textural parameters. *Journal of Pharmaceutical Sciences*, *91*, 2090–2101.

Maeda, T., Yamashita, H., & Morita, N. (2007). Application of xyloglucan to improve the gluten membrane on bread making. *Carbohydrate Polymers*, *68*, 658–664.

Mahajan, H. S., & Gattani, S. G. (2010). Nasal administration of ondansetron using a novel microspheres delivery system. Part II: *Ex vivo* and *in vivo* studies. *Pharmaceutical Development and Technology*, *15*(6), 653–657.

Mahajan, H. S., Shah, S. K., & Surana, S. J. (2011). Nasal in situ gel containing hydroxy propyl b-cyclodextrin inclusion complex of artemether: Development and in vitro evaluation. *Journal of Inclusion Phenomenon Macrocyclic Chemistry*, *70*, 49–58.

McNeil, M., Darvill, A. G., & Fry, S. C. (1984). Structure and function of the primary cell walls of plants. *Annual Review Biochemistry*, *53*, 625–663.

Murthy, R. S. R., Majithiya, R. J., & Ghosh, P. K. (2006). Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. *AAPS PharmSciTech*, *7*, 1–7.

Peppas, N. A., & Mikos, A. G. (1990). Kinetics of mucus–polymer interactions. In R. Gurny, & H. E. Junginger (Eds.), *Bioadhesion—Possibilities and future trends*. Germany: Wissenschaftliche Verlags GmbH Stuttgart.

Picout, D. R., Ross-Murphy, S., Errington, N., & Harding, S. E. (2003). Pressure cell assisted solubilization of xyloglucans: Tamarind seed polysaccharide and detarium gum. *Biomacromolecules*, *4*, 799–807.

Rao, P. S., & Srivastava, H. C. (1973). *Industrial gums: Polysaccharides and their derivatives*. Diego: Academic Press. (pp. 369–411).

Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2005). Spectrophotometric identification of organic compounds. *John Wiley and Sons*, 150–151.

Shi, R., Zhu, A., & Chen, D. (2010). In vitro degradation of starch/PVA films and biocompatibility evaluation. *Journal of Applied Polymer Sciences*, *115*, 346–357.

Suggs, L. J., Krishan, R. S., Garcia, C. A., Peter, S. J., & Anderson, J. M. (1998). In vitro and *in vivo* degradation of poly (propylene fumarate-co-ethylene glycol) hydrogel. *Journal of Biomaterial Research*, *42*, 312–320.

Tracy, M. A., Ward, K. I., Firouzabadien, L., Wang, Y., Dong, N., Qwa, R., et al. (1999). Factors affecting the degradation rate of poly(Lactide-co-glycolate) microspheres *in vivo* and *in vitro*. *Biomaterials*, *20*, 1057–1062.

Yamatoya, K., & Shirakawa, M. (2003). Xyloglucan: Structure, rheological properties, biological functions and enzymatic modification. *Current Trends in Polymer Science*, *8*, 27–72.

Yong, C. S., Choi, J. S., & Rhee, J. D. (2001). Effect of sodium chloride on the gelation, gel strength and bioadhesive force of poloxamer gels. *International Journal of Pharmaceutics*, *275*, 195–205.