Carbohydrate Polymers 90 (2012) 1543–1549

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis, characterization and evaluation of thiolated tamarind seed polysaccharide as a mucoadhesive polymer

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ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised form 5 July 2012
Accepted 7 July 2012
Available online 16 July 2012

Keywords:
Tamarind seed polysaccharide
Thiolated tamarind seed polysaccharide
Metronidazole
Mucoadhesion

ABSTRACT

In the present study, thiol-functionalization of tamarind seed polysaccharide was carried out by esterification with thioglycolic acid. Thiol-functionalization was confirmed by —SH stretch in Fourier-transformed infra-red spectra at 2586 cm^{-1}. It was found to possess 104.5 mM of thiol groups per gram. The results of differential scanning calorimetry and X-ray diffraction study indicate increase in crystallinity. Polymer compacts of thiolated tamarind seed polysaccharide required 6.85-fold greater force to detach from the mucin coated membrane than that of tamarind seed polysaccharide. Comparative evaluation of Carbopol-based metronidazole gels containing thiolated tamarind seed polysaccharide with gels containing tamarind seed polysaccharide for mucoadhesive strength using chicken ileum by modified balance method revealed higher mucoadhesion of gels containing thiolated tamarind seed polysaccharide. Further, the gels containing tamarind seed polysaccharide and thiolated tamarind seed polysaccharide released the drug by Fickian-diffusion following the first-order and Higuchi's-square root release kinetics, respectively.

1. Introduction

Natural polysaccharides are recently getting consideration as biopolymers as they are economical, easily available, non toxic, readily modified, biodegradable and biocompatible. They find extensive applications in pharmaceutical and food industry because of their diversity in structure and properties (Manjanna, Kumar, & Shivakumar, 2010). Tamarind seed polysaccharide (TSP) is one such polysaccharide which has been explored widely as a bioadhesive polymer. It is a mucilaginous polysaccharide derived from the seed kernels of "*Tamarindus indica*" Linn. (family – Fabaceae), a common and an important tree grown in South East Asia. TSP is a galactoxyloglucan derivative comprising of backbone chain of (1→4)β-D-glucans substituted with a side chain of α-D-xylopyranose linked (1→6) to glucose residues. The glucose, xylose and galactose units are present in the ratio of 2.8:2.25:1. It is rich in a high molecular weight polysaccharide (~65–72%) with the molecular weight of 720–880 kDa (Freitas et al., 2005; Kumar & Bhattacharya, 2008). TSP is used as stabilizer, thickener, gelling agent, and binder in food and pharmaceutical industries. It swells in water and form the mucilaginous solution after heating up. It forms a gel which is mainly used as a thickening and stabilizing agent in the food industry (Zhang et al., 2008).

Mucoadhesion of natural polymers by derivatisation with reagents bearing thiol functional groups have been used to improve the mucoadhesive and cohesive properties of the polymers (Bernkop-Schnürch, 2005). Apart from mucoadhesion thiomers have been found to enhance the oral permeation of protein and peptide drugs (Bernkop-Schnurch, Kast, & Guggi, 2003), inhibit efflux proteins (Werle & Hoffer, 2006), enzymes (Bernkop-Schnurch, Walker, & Zarti, 2001) and exhibit *in situ* gelling properties (Krauland, Leitner, & Bernkop-Schnurch, 2003).

In the present study an attempt was made to improve the mucoadhesive properties of tamarind seed polysaccharide by thiol-functionalization. Thiol group estimation was done by Ellman's reagent assay. Synthesized thiolated conjugate was further characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Mucoadhesive characteristics of TSP were comparatively evaluated with thiolated-TSP by conducting tensile tests employing texture analyser. Thiolated TSP was further explored as mucoadhesive agent by formulating Carbopol based gels of metronidazole. The gels were characterized mechanically by texture analyser and for mucoadhesive strength using chicken-ileum by modified physical balance method. *In vitro* release of drug from gels was studied using dialysis sac method.

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http://dx.doi.org/10.1016/j.carbpol.2012.07.028

2. Experimental

2.1. Materials

Tamarind kernel powder (TKP) and metronidazole were obtained as gift samples from Hindustan Gums and Chemicals Pvt. Ltd. (Bhiwani, India) and Ranbaxy Research Laboratories (Gurgaon, India), respectively. Thioglycolic acid (99% AR), triethanolamine, Carbopol 974P, Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and L-cysteine were procured from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). Commercial formulation of metronidazole gel (Metrogyl®, Lekar Pharmaceuticals, Ankleshwar, India) and fresh isolated chicken ileum were procured from the local market (Hisar, India).

2.2. Isolation of tamarind seed polysaccharide (TSP)

TKP was used as a precursor to isolate TSP by the method as described earlier (Rao, Ghosh, & Krishna, 1946). In brief, a dispersion of TKP (10%, w/v) was prepared in cold distilled water to make slurry, which was then poured into 400 ml of boiling distilled water with stirring and boiled for further 20 min. The resulting dispersion was kept overnight to allow settling of fibres and proteins, followed by centrifugation at 6000 rpm (C24, Remi Instruments Ltd., Mumbai, India) for 20 min. The supernatant so obtained was collected and poured into twice the volume of absolute ethanol with continuous stirring. The precipitated TSP was pressed and then dried at 50–55 °C.

2.3. Synthesis of thiolated TSP

Thiolation of isolated TSP was done by esterification of TSP with thioglycolic acid in the presence of hydrochloric acid (Sharma & Ahuja, 2011). TSP (6 g) was dissolved in 50 ml of distilled water. To the above solution 3.6 ml of thioglycolic acid and 2 ml of 7 N HCl was added. The components were allowed to react at 80 °C for 180 min. Thiolated TSP was precipitated by pouring the above reaction mixture in 500 ml of methanol. The creamy white precipitate so obtained was washed thrice with methanol and was freezed at −80 °C for 4 h followed by lyophilization in laboratory model freeze dryer (Alpha 2-4 LD Plus, Martin Christ, Germany) for 24 h at −90 °C, at 0.0010 mbar.

2.4. Characterization of thiolated TSP

2.4.1. Determination of thiol group contents

The number of thiol groups substitution on the thiolated TSP were determined by quantifying the amount of thiol groups on thiolated TSP and TSP (control) by Ellman's method as described earlier (Hornof, Kast, & Bernkop-Schnürch, 2003). Briefly, aqueous solution (0.1%, w/v) of thiolated TSP or TSP (control) in phosphate buffer (pH 8.0, 5 M) was incubated with Ellman's reagent (0.03%, w/v) in phosphate buffer (pH 8.0, 5 M) for 2 h at 25 °C, followed by measurement of absorbance of the reaction mixture at 450 nm. The number of thiol groups/g of the polymer were determined using a calibration curve prepared by reacting standard solutions of L-cysteine with Ellman's reagent as explained above.

2.4.2. Fourier transform infra-red spectroscopy (FT-IR)

TSP and thiolated TSP samples were subjected to FT-IR spectroscopy in a Fourier-transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan) in a range of 4500–500 cm^{-1} as KBr pellets.

2.4.3. Differential scanning calorimetry (DSC)

The thermal behaviour of TSP and thiolated TSP was studied by using a differential scanning calorimeter (Q10 TA systems, USA) under nitrogen purge of 50 ml/min. The sample placed in the standard aluminium pan was heated over a temperature range of 40–250 °C employing a heating rate of 10 °C per min. The DSC instrument was calibrated with indium, having melting point of 158.26 °C and a heat of fusion of 28.89 J/g (at 10 °C min^{-1}).

2.4.4. X-ray diffraction analysis (XRD)

The powder X-ray diffractograms of TSP and thiolated TSP were recorded employing X-ray diffractometer (Miniflex 2, Rigaku, Japan). The main characteristics and setting parameters of the diffractometer were: Nickel filtered Cu Kα radiation; voltage 30 kV; tube current 15 mA; scan speed 0.05 min^{-1}; angular range 10–80° (2θ).

2.4.5. Scanning electron microscopy (SEM)

The electron photomicrographs of TSP and thiolated TSP were recorded using scanning electron microscope (SEMtrac mini, Microtrac, Inc., USA). The samples were mounted on stub containing double adhesive carbon tape and recorded at an accelerating voltage of 20 kV.

2.4.6. Evaluation of mucoadhesive potential of TSP and thiolated TSP

Polymer compacts of TSP and thiolated TSP powders (200 mg) were prepared by direct compression method employing a single punch IR hydraulic press (KP, 795, Kimaya Engineers, Thane, India) using 13 mm diameter die at a pressure of 10 tons for 60 s and kept in desiccator until used. A comparative evaluation of mucoadhesive potential of TSP and thiolated TSP was done by carrying out tensile tests on their polymer compacts employing texture analyser (TAX_2, Stable Microsystem, UK). The analyzer was equipped with a 5 kg load cell. The polymer compacts were attached to the upper probe, while a cellophane membrane hydrated with mucin dispersion (0.3%, w/v) was attached to the lower probe as the model membrane (Martinac, Voinovich, Perissutti, & Franceschinis, 2005). During the measurement the probe was lowered at a rate of 0.1 mm/s until a contact with the model membrane at a constant force of 0.25 N was obtained. The force was maintained for 5 min and the upper probe was moved upwards at a rate of 0.1 mm/s. The force required to detach the polymer compact from membrane was considered as index of mucoadhesive potential.

2.5. Formulation of TSP and thiolated TSP gels

Mucoadhesive property of TSP and thiolated TSP were evaluated by formulating gels using metronidazole as the model drug. Carbopol 974P was used as the gelling agent, and TSP and thiolated TSP were employed as mucoadhesive agents. Briefly, Carbopol 974P (1.5%, w/v) was dispersed into aqueous solution of metronidazole (1%, w/v) containing TSP or thiolated TSP (1%, w/v) and allowed to hydrate overnight followed by addition of triethanolamine to form gels.

2.6. Physicochemical characterization of metronidazole loaded gel

2.6.1. Determination of viscosity

Viscosity of Carbopol-based metronidazole gels containing TSP (CTSP) and thiolated TSP (CTTSP) was measured using Brookfield viscometer (Brookfield DV-E Viscometer) at various speeds using spindle 6 at 25 °C.

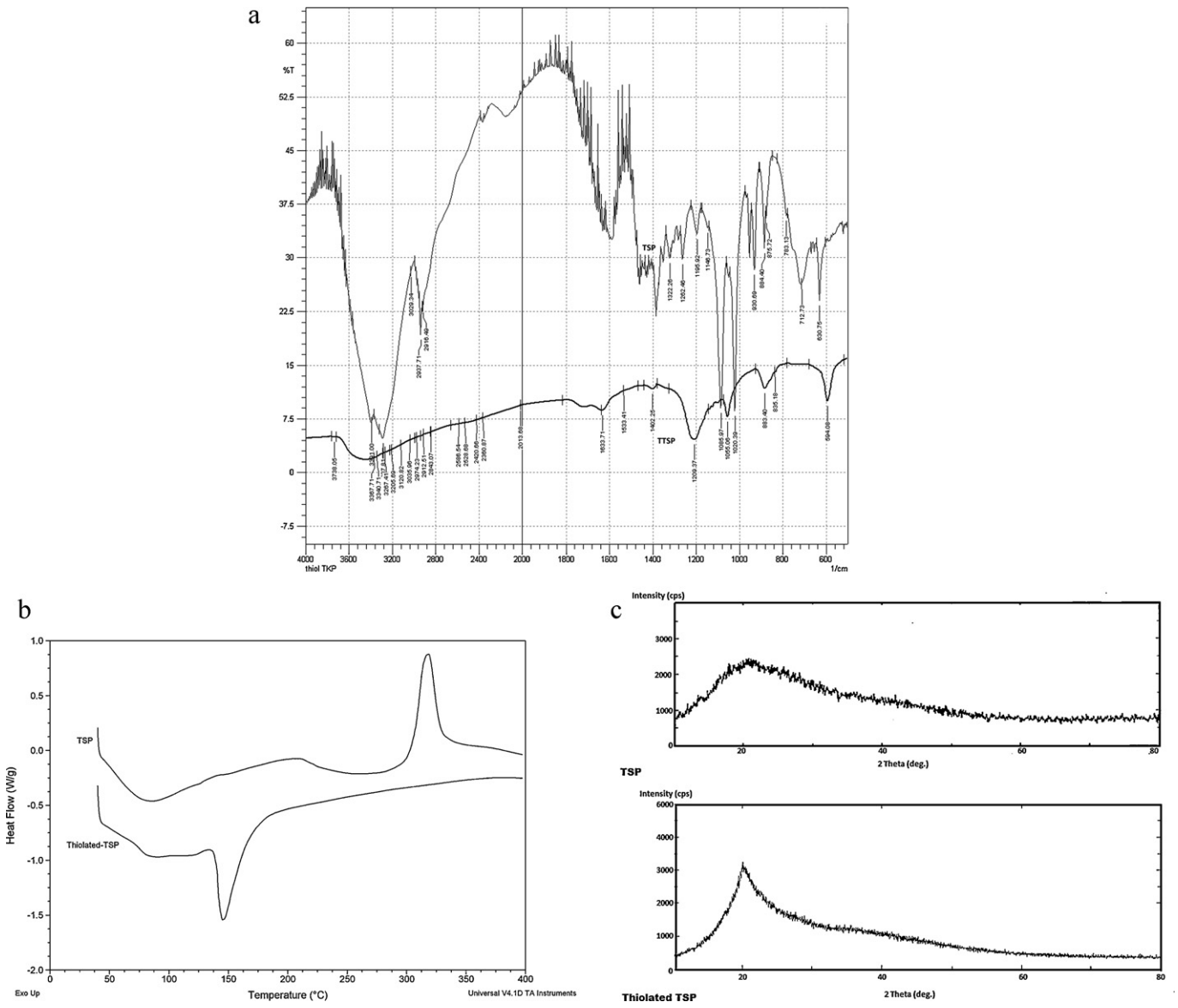

Fig. 1. The FT-IR spectra (a), DSC curve (b), X-ray diffractogram (c) of TSP and thiolated TSP.

2.6.2. Mechanical characterization of gels

Prepared gels were analyzed for their different mechanical parameters such as hardness, cohesiveness and adhesiveness using texture analyser (TA-XT21, Stable Microsystem Surrey, UK). An analytical probe of diameter 3.5 cm was depressed twice into each sample to a definite depth of 15 mm at a defined rate of 0.5 mm/s, with a recovery period of 15 s between the end of first compression and beginning of second compression. The analysis was done using a 5 kg load cell.

2.6.3. Evaluation of mucoadhesive strength of gels

The mucoadhesive strength of gels was evaluated by modified physical balance method (Bansal et al., 2009). The modified physical balance apparatus comprised of a tared two-arm balance, one side of which contained two glass plates and the other side contained a container. One of the two glass plates (lower plate) was attached permanently to the base of the stage, and the other (upper plate) was glued to the base of one arm of the balance. The membrane used for mucoadhesive testing was fresh chicken intestinal membrane. Fresh chicken intestine was glued to the lower plate and another was glued to the upper plate by using cyanoacrylate adhesive. An accurately weighed 1 g of the gel was placed on the chicken intestine glued to the upper side of the lower plate. Then, the upper plate was placed over the lower plate and 60 g preload force (or contact pressure) was applied for 5 min (preload time). After removal of the preload force, a gradually increasing weight was applied on the second arm of the balance by controlled addition of water from the burette till the plates were detached from each other. The weight of the water required for the detachment of the glass plates was recorded and the mucoadhesive strength of the applied gels was calculated as force of detachment.

2.6.4. In vitro drug release

An accurately weighed 1 g gel was placed in a dialysis sac (cut off 10 kDa). The release rate of drug from the gels was determined using USP type II dissolution apparatus (TDT-08L, Electrolab, India) (Bansal et al., 2009). Dialysis sac was tied with paddle. The paddle was then immersed in the phosphate buffer (pH 6.8) maintained at $37 \pm 0.5\,^{\circ}C$ and was rotated at the speed of 100 rpm. Sample aliquots of 3 ml were withdrawn at regular intervals and media volume was maintained by adding equal volumes of fresh media.

The contents of metronidazole in the samples were determined spectrophotometrically by measuring the absorbance at 320 nm.

3. Results and discussion

Thiolation of tamarind seed polysaccharide was achieved by esterification of the hydroxyl groups of galactoxylan moieties of TSP with carboxyl groups of thioglycolic acid. The product was off-white in colour and soluble in water. The unreacted thioglycolic acid was removed by repeated washings with methanol. Thiolated TSP was found to contain 104.5 mM of thiol groups/g, as determined by the Ellman's method.

Fig. 1(a) shows the IR spectra of TSP and thiolated TSP. The spectra of TSP display a characteristic broad peak at 3391 cm^{-1} representing hydroxyl (—OH) groups of glucan backbone. Peaks at 2937.71 cm^{-1} and 2916.49 cm^{-1} can be attributed to C—H stretching of alkanes. Peak appearing at 1085.97 cm^{-1} is due to (C—O—C) stretching of cyclic ether. Cyclic C—H bending was confirmed by the peaks at 783 cm^{-1} and 712 cm^{-1}. The IR spectra of thiolated TSP shows the characteristic peak of thiol (—SH) group at 2586.54 cm^{-1} and hydroxyl group peaks at 3367.71 cm^{-1} and 3340.71 cm^{-1}. C—H stretching of alkanes was confirmed by peaks at 2974.23 cm^{-1} and 2912.51 cm^{-1}. Peaks at 1402.25 cm^{-1} and 1209.37 cm^{-1} can be attributed to C—O stretching of ester. Peak appearing at 549.08 cm^{-1} confirms the —OH bending of alcohols in thiolated TSP.

Fig. 1(b) compares the DSC thermograms of TSP and thiolated TSP. The thermal curve of TSP shows broad endotherm at 85.29 °C and 270.78 °C with heat of fusion of 203.8 J/g and 68.36 J/g respectively followed by an exotherm at 318.49 °C with heat flow of 84.43 J/g, while the thermal curve of thiolated TSP shows a broad endotherm at 81.78 °C with heat of fusion of 22.85 J/g followed by somewhat sharp endotherm at 145.38 °C with heat of fusion of 75.88 J/g. Thus the shift in the endothermic peaks and disappearance of exothermic peak in the thermal curve of thiolated TSP indicates the modification of TSP.

Fig. 1(c) exhibits the X-ray diffractograms of TSP and thiolated TSP. The diffraction curve of TSP is typical of amorphous material with no sharp peaks. The diffraction pattern of thiolated TSP is similar to TSP with no sharp peaks but with greater intensity, indicating increased degree of crystallinity on thiolation.

Fig. 2(a) and (b) shows the shape and surface of TSP and thiolated TSP as examined under scanning electron microscope. The shape of TSP and thiolated TSP was found to be polyhedral. A close examination of surface morphology reveals that thiolated TSP was rougher in comparison to TSP.

Fig. 3 compares mucoadhesive strength of polymer compacts of TSP and thiolated TSP, respectively. The maximum force of detachment of TSP and thiolated TSP discs from mucin coated model membrane was found to be 592.905 ± 161.48 mN and 4062.5 ± 845.15 mN, respectively. Thus, thiolated-TSP exhibits 6.85-fold greater mucoadhesive strength than TSP.

Greater mucoadhesive potential of thiolated TSP compared to TSP prompted us to explore it for mucoadhesive applications. The mucoadhesive properties of thiolated TSP were comparatively evaluated with TSP by formulating the gels employing metronidazole as the model drug. Since, TSP and TTSP gels at different concentrations due to their different swelling properties. Thus, to compare the mucoadhesive properties of TSP and TTSP, gels were formulated using Carbopol 974P as a gelling agent at concentration of 1.5% (w/v) while TSP and TTSP were used as bioadhesive agents at concentration of 1% (w/v).

Table 1 presents the results of mechanical characterization and the mucoadhesive strength of various batches of metronidazole gels. Mechanical properties of the gels were characterized in

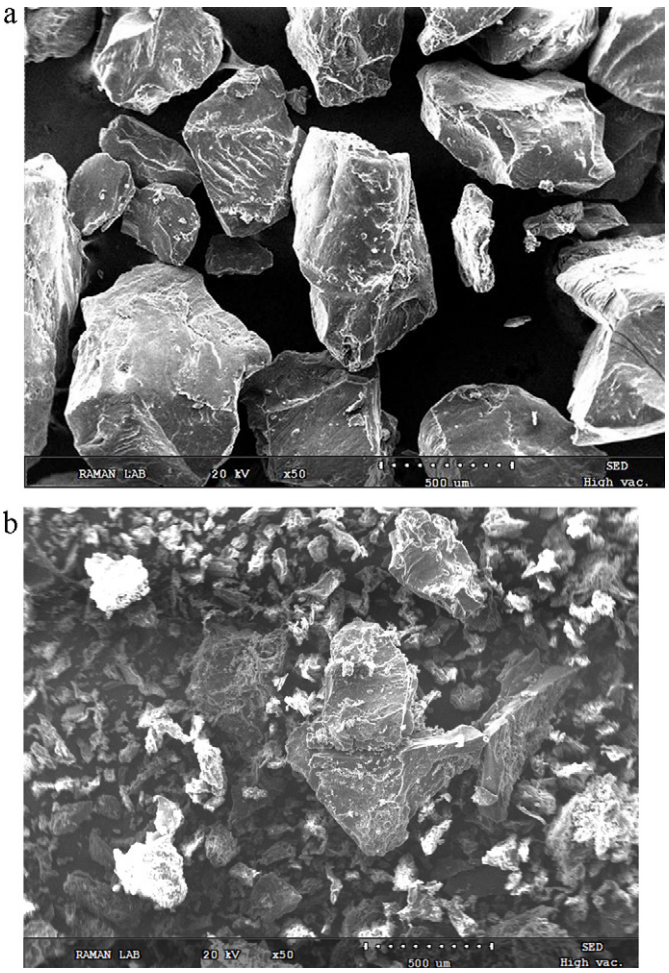

Fig. 2. Scanning electron micrographs of (a) TSP and (b) thiolated TSP.

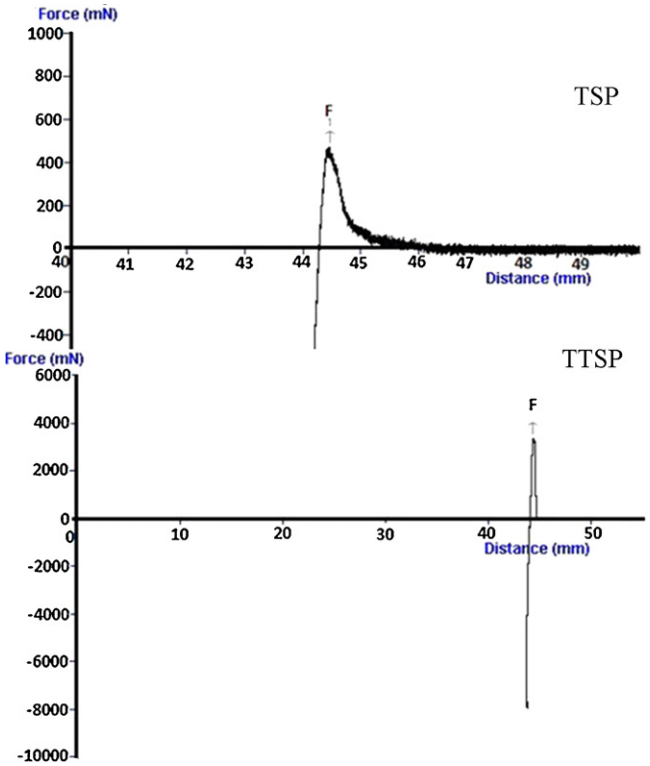

Fig. 3. Tensile test profiles of TSP and thiolated TSP polymer compacts.

Table 1
Evaluation of mucoadhesive strength and mechanical parameters of gels.

Formulation	Mucoadhesive strength (N)	Hardness (g)	Adhesiveness (g s)	Cohesiveness
CTSP	0.3136 ± 0.0353	191.1	−2086	0.8123
CTTSP	0.5096 ± 0.0802	78.4	−562.5	0.8760
Metrogyl	0.3005 ± 0.0502	119.9	−1837	0.7916

terms of hardness, adhesiveness and cohesiveness. The hardness of the formulation, which was measured as the peak height of the force–time curve (Fig. 4) describes the resistance to compression which indicates the ease by which product can be removed from the container. The hardness of the gels were found to be in the order of CTSP > Metrogyl > CTTSP. The hardness of the formulation reflects their viscosity (Bansal et al., 2009). Adhesiveness of the formulation indicates the work required to overcome the forces of attraction between the sample surface and the surface of the probe, and is measured as area of the first negative peak of the force–time curve. The adhesiveness of the formulations was found in the order CTSP > Metrogyl > CTTSP. Cohesiveness of the formulation describes the structural reformation following compression. It is the ratio of the areas under the second positive peak to the first positive peak. The order of cohesiveness of the formulation was as follows CTTSP > CTSP > Metrogyl.

Formulations for the buccal delivery should possess low hardness but high cohesiveness. Low hardness of the gel formulation means minimum work would be required for removal of the gel from the container while higher adhesiveness and cohesiveness indicates retention of the formulation for prolonged duration and complete structural recovery of the formulation after application. Among all the formulations gels containing thiolated TSP (*i.e.*, CTTSP) possess least hardness and adhesiveness but highest cohesiveness. However, the adhesiveness (force of detachment between the steel probe and the gel surface) of the gel formulation cannot be regarded as true indicator of mucoadhesive potential. Therefore, to have the true indication of mucoadhesive property of the gels, the gels were tested with fresh chicken intestinal membrane employing modified physical balance method. Maximum mucoadhesive strength (the force required for the detachment of chicken intestinal membrane from the gel surface) was observed in case of CTTSP gel formulation.

Fig. 5 compares the *in vitro* release profile of metronidazole from the CTSP and CTTSP gels with marketed formulation of metronidazole. It can be observed from the release rate profile that the commercial gel formulation (Metrogyl®) provided the slowest release of metronidazole followed by CTTSP and the fastest release of drug occurred from CTSP. The release rate of drug from the gel formulation usually depends upon the diffusion of the drug through the viscous gel matrix.

Fig. 6 compares the viscosity of Carbopol-based metronidazole gels containing TSP (CTSP) and thiolated-TSP (CTTSP) with a commercial formulation of metronidazole gel, measured using Brookfield viscometer. It can be observed from the plot that the viscosity of the formulations follow the order CTSP > Metrogyl > CTTSP. The results of our release rate study are not consistent with their viscosity. The disagreements between the release rate of Carbopol-based formulations and the commercial formulation could be due to the different gelling agents used in the formulation. Even though CTSP had the viscosity greater than CTTSP, it provided faster release than the CTTSP. This could be due to interaction of metronidazole with thiolated-TSP or due to *in situ* cross linking exhibited by disulfide linkages of thiolated polymers. However, further studies are needed to comment more on this aspect.

To determine the release kinetics and mechanism of release, the release rate was fitted into various kinetic models. Table 2 presents

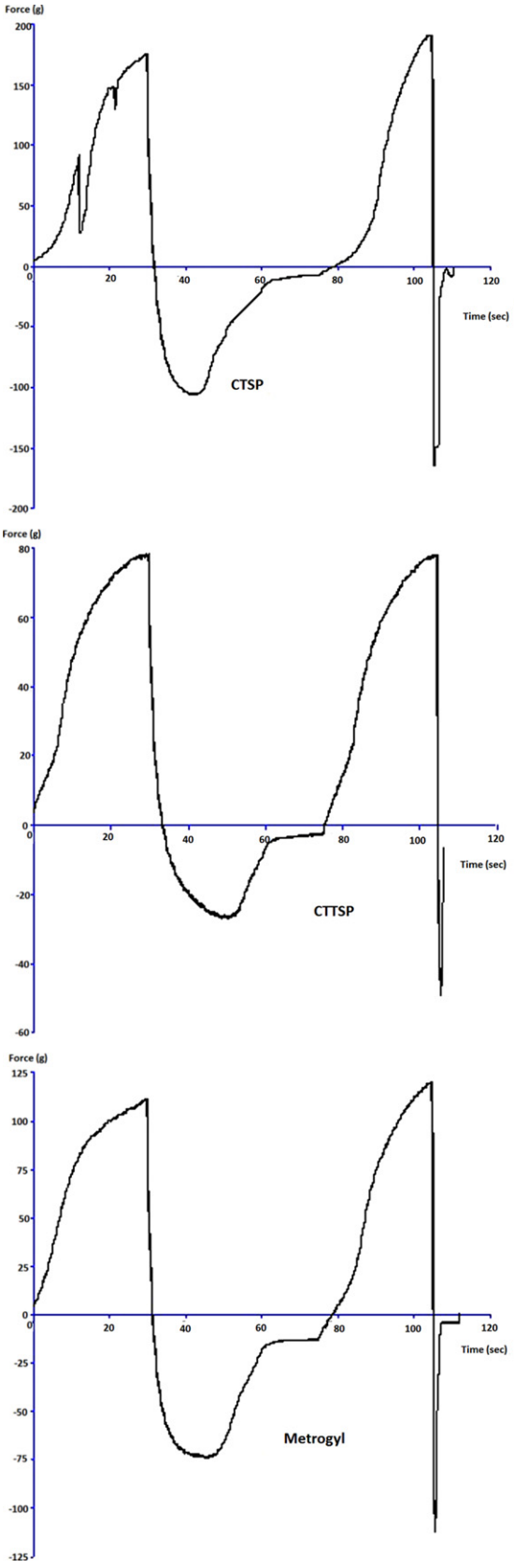

Fig. 4. Mechanical characterization of metronidazole gel formulations.

Table 2
Modelling and release kinetics of metronidazole from various gel formulations.

Formulation	Zero-order	First-order	Higuchi's square root	Korsemeyer–Peppas	
	R^2	R^2	R^2	R^2	n
CTSP	0.5693	0.9367	0.8167	0.9189	0.2432
CTTSP	0.6146	0.7881	0.8671	0.9451	0.3072
Metrogyl	0.8245	0.987	0.9723	0.977	0.332

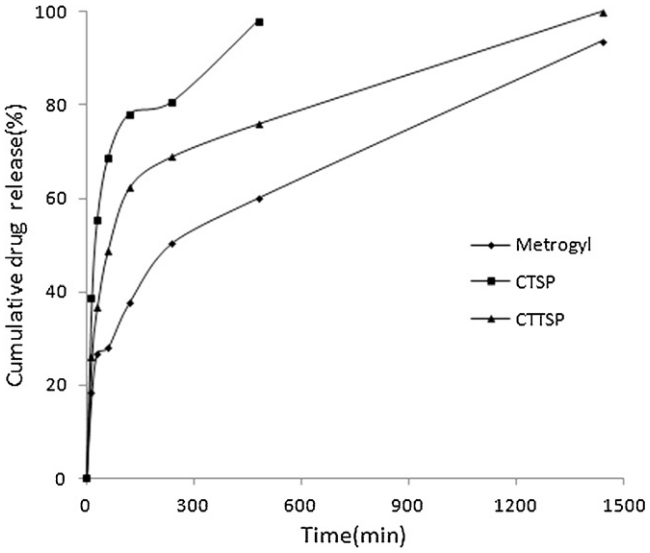

Fig. 5. *In vitro* release profile of metronidazole from various gel formulations.

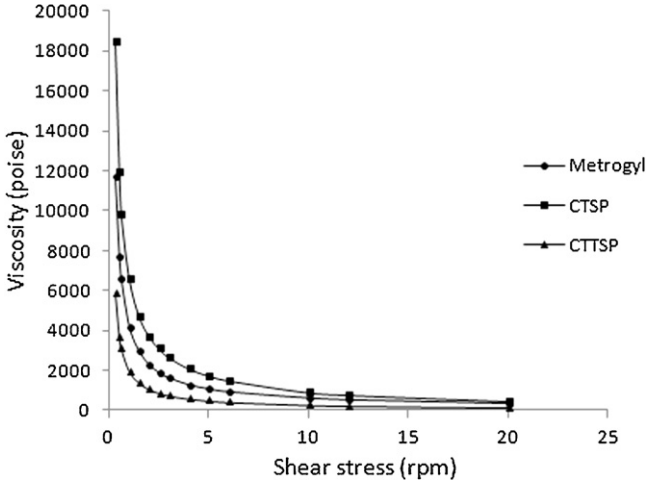

Fig. 6. Rheological profile of metronidazole gel formulations.

the results of modelling and release kinetics applied to various formulations of metronidazole gel. The marketed formulation and CTSP gel followed the 1st order release kinetics while the CTTSP formulation showed a best fit in Higuchi's square-root release kinetics. Further the value of '*n*', the release exponent of Korsemeyer and Peppas equation ($n < 0.5$) indicates that the mechanism of release of drug is diffusion (Costa & Loba, 2001; Jones, Woolfson, Brown, & O'Neill, 1997).

4. Conclusion

Thiol functionalization of tamarind seed polysaccharide was accomplished by esterification of its hydroxyl groups with thioglycolic acid, which was confirmed by FT-IR analysis. Further characterization revealed increase in crystallinity, surface roughness and improvement in mucoadhesive properties of TSP on thiolation. To explore its mucoadhesive applications, Carbopol-based metronidazole gels were formulated. These gels were compared with the marketed formulation on different aspects such as mechanical characterization, viscosity, mucoadhesive strength and *in vitro* drug release. The promising mucoadhesive properties of thiolated TSP warrant its further exploration in formulation of different pharmaceutical dosage forms.

Acknowledgements

The authors are thankful to Hindustan Gums and Chemicals for gift sample of tamarind kernel powder, to Dept. of Applied Physics, Guru Jambheshwar University of Science and Technology, Hisar and Coordinator, UGCSAP, Dept. of Pharm. Sciences and Drug Research, Punjabi University, Patiala for providing facilities for SEM and texture profile analysis, respectively. Authors also want to thank Dept. of Technical Education, Haryana State Government for granting Sir C.V. Raman fellowship to H. Kaur.

References

Bansal, K., Rawat, M. K., Jain, A., Rajput, A., Chaturvedi, T. P., & Singh, S. (2009). Development of satranidazole mucoadhesive gel for the treatment of periodontitis. *AAPS PharmSciTech*, *10*(3), 716–723.

Bernkop-Schnürch, A. (2005). Thiomers: A new generation of mucoadhesive polymers. *Advanced Drug Delivery Reviews*, *57*, 1569–1582.

Bernkop-Schnurch, A., Kast, C. E., & Guggi, D. (2003). Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: Thiomer/GSH systems. *Journal of Controlled Release*, *93*(2), 95–103.

Bernkop-Schnurch, A., Walker, G., & Zarti, H. (2001). Thiolation of polycarbophil enhances its inhibition of intestinal brush border membrane bound aminopeptidase N. *Journal of Pharmaceutical Sciences*, *90*(11), 1907–1914.

Costa, P., & Loba, J. M. S. (2001). Modelling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences*, *13*, 123–133.

Freitas, R. A., Martin, S., Santos, G. L., Valenga, F., Buckeridge, M. S., Reicher, F., et al. (2005). Physico-chemical properties of seed xyloglucans from different sources. *Carbohydrate Polymers*, *60*, 507–514.

Hornof, M. D., Kast, C. E., & Bernkop-Schnürch, A. (2003). *In vitro* evaluation of the viscoelastic properties of chitosan–thioglycolic acid conjugates. *European Journal of Pharmaceutics and Biopharmaceutics*, *55*(2), 185–190.

Jones, S. D., Woolfson, D. A., Brown, F. A., & O'Neill, J. M. (1997). Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: *In vitro* release kinetics, syringeability, mechanical and mucoadhesive properties. *Journal of Controlled Release*, *49*, 71–79.

Krauland, A. H., Leitner, V. M., & Bernkop-Schnurch, A. (2003). Improvement in the in situ gelling properties of deacetylaed gellan gum by immobilization of thiol groups. *Journal of Pharmaceutical Sciences*, *92*, 1234–1241.

Kumar, C. S., & Bhattacharya, S. (2008). Tamarind seed: Properties, processing and utilization. *Critical Reviews in Food Science and Nutrition*, *48*, 1–20.

Manjanna, K. M., Kumar, T. M., & Shivakumar, B. (2010). Natural polysaccharide hydrogels as novel excipients for modified drug delivery systems: A review. *International Journal of ChemTech Research*, *2*(1), 509–525.

Martinac, A., Filipović-Grčić, J., Voinovich, D., Perissutti, B., & Franceschinis, E. (2005). Development and bioadhesive properties of chitosan–ethylcellulose microspheres for nasal delivery. *International Journal of Pharmaceutics*, *291*, 69–77.

Rao, P. S., Ghosh, T. P., & Krishna, S. (1946). Extraction and purification of tamarind seed polysaccharide. *Journal of Scientific & Industrial Research*, *4*, 705.

Sharma, R., & Ahuja, M. (2011). Thiolated pectin: Synthesis, characterization and evaluation as a mucoadhesive polymer. *Carbohydrate Polymers*, *85*, 658–663.

Werle, M., & Hoffer, M. (2006). Glutathione and thiolated chitosan inhibit multidrug resistance P-glycoprotein activity in excised small intestine. *Journal of Controlled Release*, *111*(1–2), 41–46.

Zhang, X., Zhang, H., Wu, Z., Wang, Z., Niu, H., & Li, C. (2008). Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*, 526–534.