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Gellan–thioglycolic acid conjugate: Synthesis, characterization and evaluation as mucoadhesive polymer

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ABSTRACT

Gellan–thioglycollic acid conjugate was synthesized with the objective to improve its mucoadhesive properties. Synthesis of conjugate was confirmed by –SH stretch in the Fourier-transform infrared spectra at 2571 cm^{-1}. It was found to contain 13.92 mM of thiol groups/g of the conjugate. Thiolation of gellan gum was found to slightly increase its degree of crystallinity and decrease its sensitivity to Ca^{2+}-induced gelation. On screening of gellan–thioglycollic acid conjugate for *ex-vivo* ocular tolerance using hen's egg chorio-allantoic membrane test and for biocompatibility by resazurin assay on Vero-cells, it was found to be non-irritant and biocompatible. Metronidazole gels formulated using gellan thioglycollic acid conjugate as bioadhesive agent showed 1.82-fold higher mucoadhesive strength than the gels formulated using gellan gum. Further, the metronidazole gels containing gellan and gellan–thioglycollic conjugate released the drug following first-order and Higuchi's square-root release kinetics. In conclusion, gellan–thioglycollic acid conjugate is a promising bioadhesive excipient.

1. Introduction

Natural gums and mucilages contain large number of hydroxyl, carboxyl and amino functional groups which because of their hydrogen-bond forming ability impart mucoadhesive characteristics on the natural polymers. Their mucoadhesive characteristic can be further improved by conjugation with covalent bond forming thiol-functional bearing compounds (Bernkop-Schnurch, Scholler, & Bieble, 2000). Thiolated polymers apart from improving the mucoadhesive properties have also been reported to improve cohesive properties (Bernkop-Schnurch et al., 2000), impart enzyme inhibitory capabilities (Bernkop-Schnurch & Thaler, 2000) and permeation enhancing effect on the polymer (Clausen & Bernkop-Schnurch, 2000, 2001). Gellan gum is an anionic exopolysaccharide secreted by *Pseudomonas elodea*. Gellan gum undergoes ionic gelation in the presence of cations. It has been used extensively as *in situ* gelling polymer in ophthalmic formulations (Rupenthal Ilva, Green, & Alany, 2011). During earlier study cysteine was conjugated to gellan gum and on the basis of rheological oscillatory measurements it was reported that gellan-cysteine conjugate improves the *in situ* gelling properties (Krauland, Leitner, & Bernkop-Schnurch, 2003).

In the present study thiol-functionalization of gellan gum was achieved by synthesizing gellan–thioglycolic acid conjugate (GTC). The synthesized GTC was characterized by differential scanning calorimetery (DSC), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The number of thiol group substituents was determined by Ellman's method. GTC was screened for *ex vivo* ocular tolerance by hen's egg test on chorio-allantoic membrane (HET-CAM) and for biocompatibility by resazurin assay on Vero cell lines. Thiolated gellan gum was explored for pharmaceutical applications as mucoadhesive agent by formulating bucoadhesive gels using metronidazole as a model drug. The gels were prepared using Carbopol-974P as a gelling agent, and GG or GTC as the bioadhesive agent. The formulated gels were characterized mechanically by texture profile analysis for hardness, cohesiveness and adhesiveness. Mucoadhesive characteristics of formulated gels were determined by modified physical balance method using chicken ileum as the model membrane while the *in vitro* release study was conducted using dialysis membrane.

2. Materials and methods

2.1. Materials

Gellan gum (C.P. Kelcogel, UK, Gelrite®) was gifted by Burzin & Leons, Argenturon (Mumbai, India), Metronidazole was obtained

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as gift sample from GMH Lab Pvt. Ltd. (Baddi, India). Thioglycolic acid (99% AR) and hydrochloric acid were purchased from SD Fine-Chem. Ltd. (Mumbai, India). Ellman's reagent [5,5-dithiobis (2-nitrobenzoic acid)] (DTNB) was purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). Fresh isolated chicken ileum was procured from the local butcher shop (Hisar, India). Ten days old fertilized hen's eggs were procured as gift samples from Indovax Pvt. Ltd. (Hisar, India). Metronidazole gel (Metrogyl®, Lekar Pharmaceuticals, Ankleshwar, India) was purchased from the local pharmacy store.

2.2. Synthesis of gellan–thioglycolic acid conjugate (GTC)

Synthesis of GTC was carried out by reacting gellan gum with thioglycolic acid in the presence of hydrochloric acid (Kaur, Yadav, Ahuja, & Dilbaghi, 2012). Gellan gum (1 g) was dissolved in 50 ml water aided by stirring and heating. Thioglycolic acid (0.3 ml) and hydrochloric acid (0.3 ml, 7 N) was added to the above solution. This mixture was allowed to react for 3 h at 80 °C under reflux conditions. The reaction mixture was precipitated with methanol (100 ml) and further washing was done with methanol. The resulting precipitate was kept at −80 °C for 4 h, and further dried by lyophilization using lyophilizer (Freeze dryer, Alpha 2-4 LD Plus, Martin Christ, Germany) for 24 h at −90 °C, at 0.0010 mbar.

2.3. Characterization of GTC

2.3.1. Determination of thiol group contents

The contents of thiol groups in GTC were determined by Ellman's method as reported earlier, with slight modification (Sharma & Ahuja, 2011). A 0.5% (w/v) solution of gellan gum (as control) and GTC in aqueous sodium hydroxide (0.5%, w/v) was prepared, and further diluted with phosphate buffer (5 M, pH 8.0) to a concentration of 0.15% (w/v). An aliquot of 5 ml of the above solution was allowed to react with 5 ml of Ellman's reagent (0.3% w/v) for 2 h at room temperature, followed by measurement of absorbance of the reaction mixtures at 450 nm. The number of thiol group substituents per gram of GTC were determined using a calibration curve prepared by reacting standard solutions of thioglycolic acid with Ellman's reagent as detailed above.

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

The spectra of gellan gum and gellan–thioglycolic acid conjugate were recorded on a Fourier-transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan). The data was recorded in the frequency region of 4500–400 cm^{-1}. Sample pellets were prepared with KBr.

2.3.3. Differential scanning calorimetery (DSC)

Thermal characteristics of gellan gum and gellan–thioglycolic acid conjugate were studied employing differential scanning calorimeter (Q 10 TA systems, USA) with nitrogen purge of 50 ml/min. The thermal curves were recorded over a temperature range of 40–250 °C at a heating rate of 10 °C per min employing cell constant calibration method. The calibration of the instrument was done with Indium, having a melting point of 158.26 °C and heat of fusion of 28.80 J/g.

2.3.4. X-ray diffraction (XRD)

The X-ray diffraction patterns were recorded using X-ray diffractometer (Table top XRD, Miniflex 2, Rigaku, Japan). The goniometer was a Miniflex 2 goniometer. The data were collected in the continuous scan mode using a step size of 0.02° (2θ). The scan range was 0–80°. Further parameters of the diffractometer were: Ni filtered Cu-Kα radiation; voltage 30 kV; tube current 15 mA; scan speed 0.05 min^{-1}.

2.3.5. Scanning electron microscopy (SEM)

The photomicrographs of gellan gum and gellan–thioglycolic acid conjugate were obtained using scanning electron microscope (SEMTRAC mini, Microtac, Inc.). The samples were mounted on stub containing double sided adhesive carbon tape. The electron micrographs were taken at an accelerating voltage of 20 kV.

2.3.6. Effect of cations on gelling behaviour of gellan–thioglycolic acid conjugate

Effect of calcium ions on gelling behaviour of gellan and gellan–thioglycolic acid conjugate was investigated employing partial ternary phase diagrams. Briefly, solution of gellan (0.1–1%, w/v) in water and gellan–thioglycolic acid conjugate (0.5–3.5%, w/v) in sodium hydroxide (0.25 N) were prepared. To the above prepared polymer solutions varying concentration of calcium chloride solutions were added and left overnight. The test tubes were observed for their consistency by tilting them at 90° and classified as solutions, viscous solution or gels on the basis of their visual appearance (Rupenthal Ilva et al., 2011).

2.3.7. Ex-vivo ocular tolerance (HET-CAM) study

Gellan gum and gellan-thioglycolic acid conjugate were evaluated for ocular tolerance by estimating their potential irritation in the chorioallantoic membrane (CAM) of hen's egg. The extent of potential irritation caused by the polymer (GG/GTC) in the chorioallantoic membrane of egg was the basis of the study (Luepke, 1985). The potential irritancy was estimated by observing the changes (haemorrhage, vasoconstriction and coagulation) occurring in the membrane within 5 min of application of the formulations (Kaur, Ahuja, Kumar, & Dilbaghi, 2012). HET-CAM study was done on 10 days old fertilized and incubated hen's egg. After 10 days incubation at 37 ± 0.5 °C, the shell was removed. The formulations were added (in triplicate) to the membrane and left in contact with CAM for 5 min. The CAM was examined for irritation effects and potential irritation (PI) was calculated by recording the onset time for each irritation effect.

$$\mathrm{PI}(\%) = \frac{(301-h)\times 5}{300} + \frac{(301-v)\times 7}{300} + \frac{(301-c)\times 9}{300} \quad (1)$$

where, h = onset time (s) for haemorrhage, v = onset time (s) for vasoconstriction, c = onset time (s) for coagulation. The range of PI score were classified as 0–0.9: non-irritant; 1–4.9: slight irritant; 5–8.9: moderate irritant; 9–21: severe irritant.

2.3.8. Cytotoxicity screening

Cytotoxicity of GG and GTC was screened employing Vero cell lines using resazurin assay method. Briefly, Vero cells were placed in 96-well plates in a density of 1×10^5 cells in DMEM (Dulbecco's modified eagle media) culture media having 5% FBS (foetal bovine serum) and incubated for 24 h at 37 °C in 5% CO_2 humidified incubator (Iqbal et al., 2012). Proliferation of cells occurred after 24 h, which were then viewed under microscope. Fifty microliters of samples of GTC and GG (0.05%, w/v) were added to the above cells and incubated for another 24 h at 37 °C and 5% CO_2 incubation. After 24 h, 20 μl of resazurin (1 mg/ml, in DMEM) was then added to each well and were incubated for 4 h at 37 °C and 5% CO_2 humidified incubator. After 4 h the plate was observed in ELISA spectrophotometer at 573 nm. Cytotoxicity (%) was calculated as follows

$$\%\mathrm{Cytotoxicity} = \frac{\mathrm{Abs_u} - \mathrm{Abs_t}}{\mathrm{Abs_u}} \times 100. \quad (2)$$

where $\mathrm{Abs_u}$ is the absorbance of cells not treated with any polymer, $\mathrm{Abs_t}$ is the absorbance of cells treated with GG or GTC.

2.4. Evaluation of gellan–thioglycolic acid conjugate as mucoadhesive polymer

2.4.1. Formulation of gels

Gellan–thioglycolic acid conjugate was comparatively evaluated with gellan gum as bioadhesive agent in Carbopol-based gels of metronidazole. Gels were prepared by dispersing Carbopol 974 P at a concentration of (1.5%, w/v) into an aqueous solution containing metronidazole (1%, w/v) and gellan–thioglycolic acid conjugate or gellan gum (1%, w/v). It was allowed to hydrate overnight followed by addition of triethanolamine (0.5 v/v) to form gels.

2.4.2. Physicochemical characterization of gel

2.4.2.1. Determination of viscosity. Viscosity of gel formulations was determined by Brookfield viscometer (Brookfield DV-E Viscometer) at various speeds using spindle number 6.

2.4.2.2. Mechanical characterization of gels. Mechanical properties of gels such as hardness, cohesiveness and adhesiveness were analysed using texture analyser (TA-XT21, Instron Testing Machine) equipped with a 5 kg load cell. An aluminum cylindrical probe (P/25) of diameter 25 mm was depressed twice into the gel sample to a 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.

2.4.2.3. Evaluation of mucoadhesive strength of gels. Mucoadhesive strength of gels was comparatively evaluated using modified physical balance method (Bansal et al., 2009). The modified physical balance apparatus consisted of a tared two-pan 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 pan of the balance. Fresh chicken intestinal membrane was glued to the lower and upper glass plates using cyanoacrylate adhesive. An accurately weighed 1 g of the gel formulation was sandwiched between the chicken intestinal tissues, which was then compressed by applying a preload force (or contact pressure) of 60 g for 5 min (preload time). After the preload time, a gradually increasing weight was applied on the second pan 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.

2.4.2.4. In vitro drug release. *In vitro* release of metronidazole from various gel formulations was comparatively evaluated by dialysis sac method (Bansal et al., 2009). One gram of the gel formulation was placed in a dialysis sac (cut off 10 kDa), which was then tied to the paddle of USP type II dissolution apparatus (TDT-08L, Electrolab,

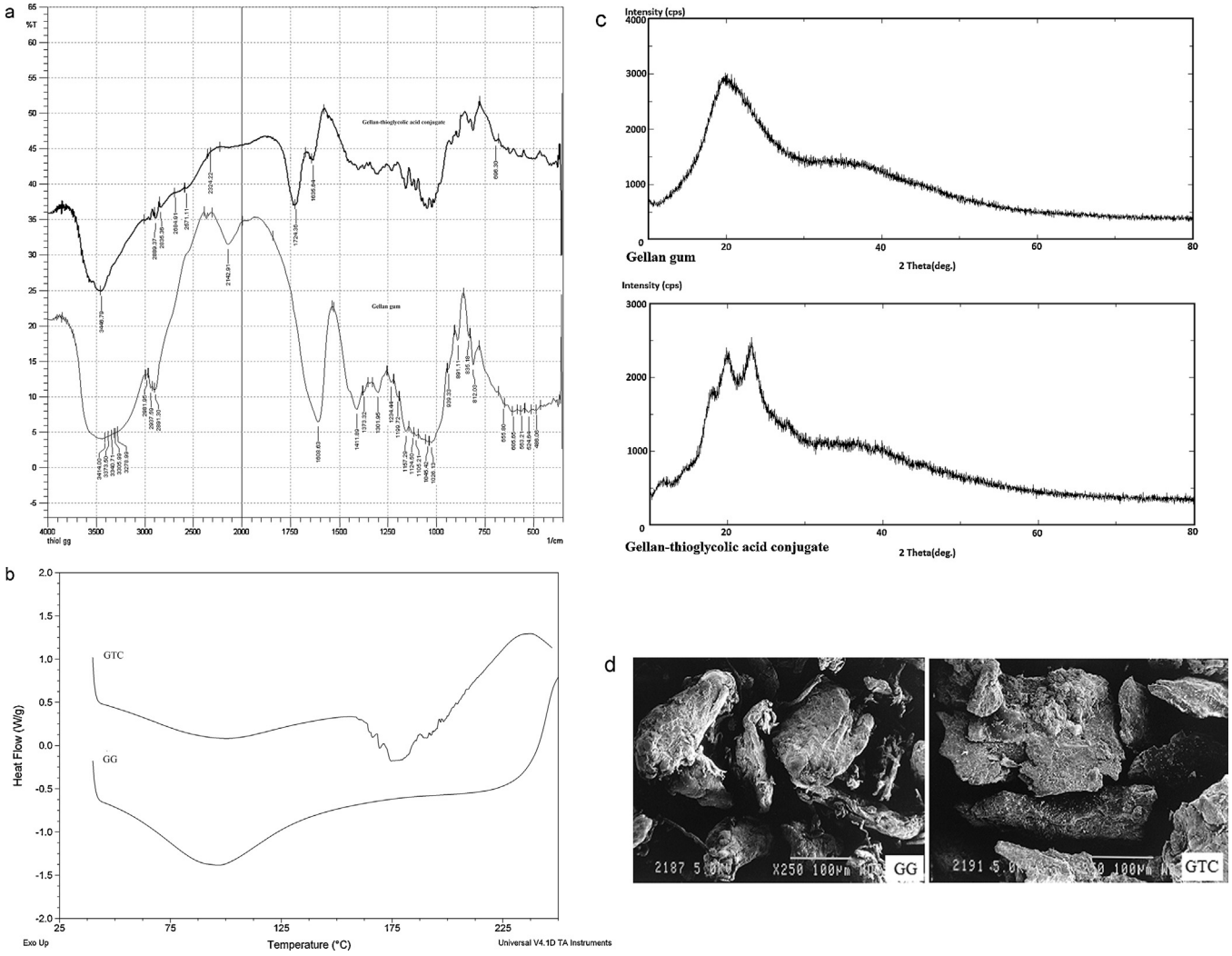


Fig. 1. (a) Fourier transform infra-red spectroscopy. (b) Differential scanning calorimetery. (c) X-ray diffractograms. (d) Scanning electron micrograph of GG and GTC.

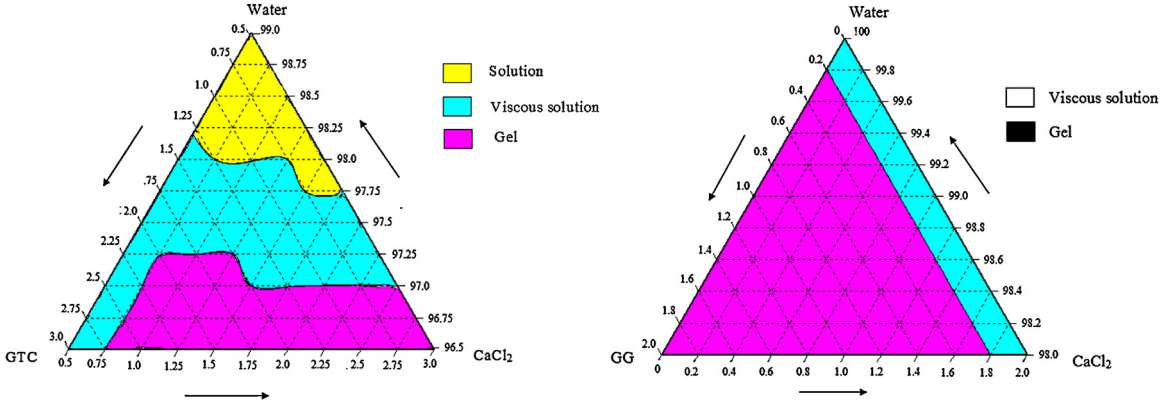


Fig. 2. Partial ternary phase diagrams of GG and GTC.

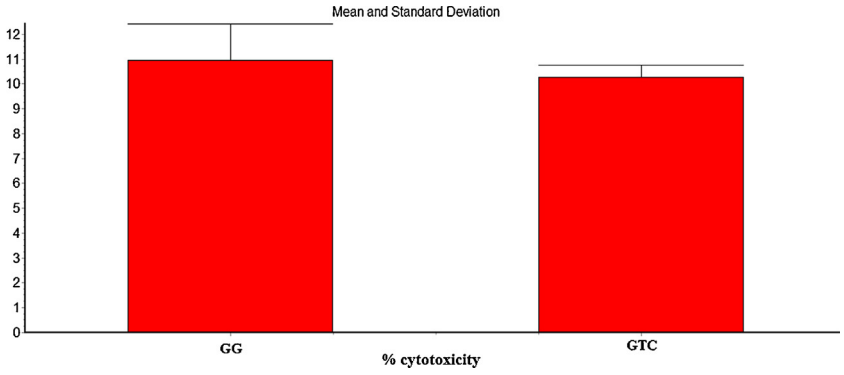


Fig. 3. Comparative cytotoxicity of GG and GTC against Vero cell lines.

India) and immersed in the media. The release media consisted of phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C and rotated at the speed of 100 rpm. A sample aliquot of 3 ml was withdrawn at various intervals and replaced with equal volumes of fresh media. The contents of metronidazole in the samples were analysed by measuring the UV absorbance at 320 nm.

3. Results and discussion

Synthesis of gellan–thioglycolic acid conjugate was achieved by esterification of the hydroxyl groups of gellan gum with carboxyl group of thioglycolic acid. The average yield of gellan–thioglycolic acid conjugate was found to be 64%. It was found that repeated washing of the product with methanol was sufficient to remove the unreacted thioglycolic acid from the product. The product was off-white in colour, insoluble in water but dissolves when made alkaline. Further, it was found to contain 13.39 ± 0.51 mM of thiol groups/g of the polymer as determined by Ellman's method.

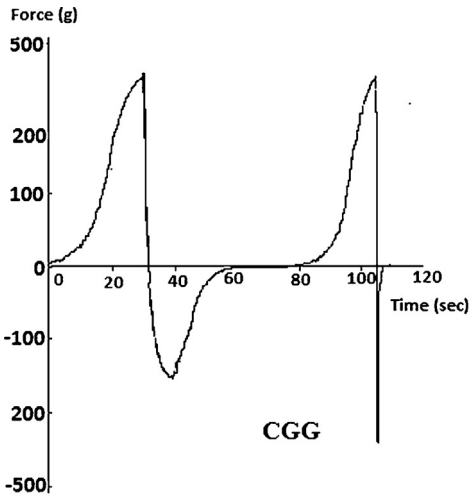
Fig. 1(a) depicts the IR spectra of gellan gum and gellan–thioglycolic acid conjugate. The spectra of gellan gum shows a broad absorption band comprising of overlapping contributions of –CH stretching of carboxylic acid group at 3373.50 cm^{-1} and –OH stretching of alcohols at 3414 cm^{-1}. The peaks appearing at 2981.30 cm^{-1} and 2937.59 cm^{-1} can be attributed to –CH stretching of alkanes, while the peaks at 1608 and 1411 cm^{-1} are due to assymteric and symmetric stretching of carboxylate. Presence of cyclic ether is confirmed by peaks at 1124.50 cm^{-1} and 1105.21 cm^{-1}. The spectra of gellan–thioglycolic acid conjugate shows a peak at 3448.79 cm^{-1} owing to –OH stretching, peak appearing at 1724.38 cm^{-1} can be ascribed to ester (–C=O) stretching. Gellan–thioglycolic acid conjugate shows a characteristic peak of thiol (–SH) group at 2571.11 cm^{-1}, which confirms the formation of gellan–thioglycolic acid conjugate.

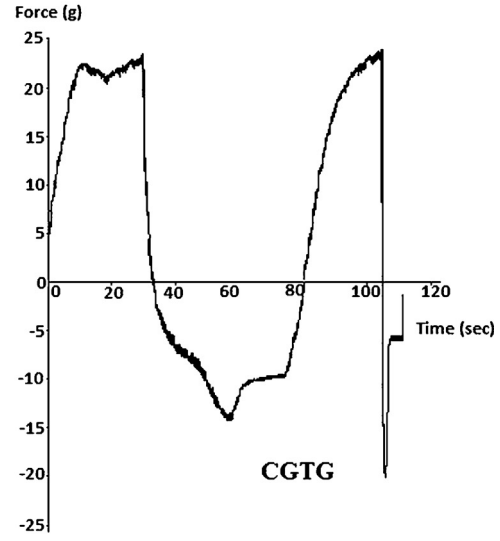
Fig. 1(b) represents the DSC thermograms of gellan gum and gellan–thioglycolic acid conjugate (GTC). The thermal curve of GG is characterized by a broad endotherm at 96.68 °C with heat of fusion of 275.0 J/g while the thermal curve of GTC shows broad endotherms at 97.54 °C and 179.74 °C with heat of fusion of 120.2 J/g and 15.19 J/g, respectively. Thus, the shift in endothermic peaks and appearance of one more endothermic peak in the thermogram of GTC indicate modification of GG.

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

Formulation	Mucoadhesive strength (N)	Hardness (g)	Adhesiveness (g sec)	Cohesiveness
CGG	0.310 ± 0.0282	262.7	−1921	0.669
CGTG	0.565 ± 0.0396	23.8	−424.2	0.6879
Metrogyl	0.3005 ± 0.052	119.9	−1837	0.7916

CGG: gel containing gellan, CGTG: gel containing gellan–thioglycolic acid conjugate.
* Values are mean ± SD(n = 3).





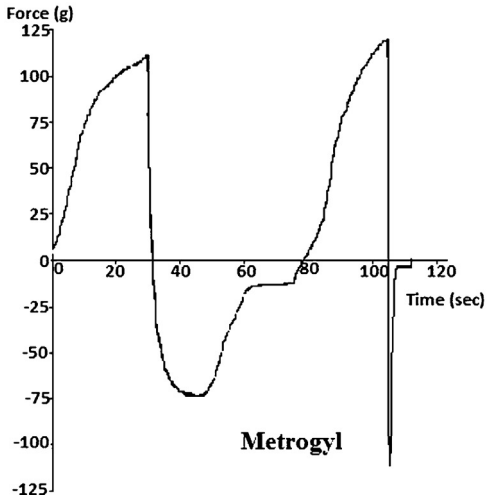


Fig. 4. Texture profile analysis of CGG, CGTG and Metrogyl.

Fig. 1(c) displays the X-ray diffractograms of gellan gum and gellan–thioglycolic acid conjugate. The thermal curve of gellan gum is typical of amorphous material with no sharp peaks, whereas the diffraction pattern of gellan–thioglycolic acid conjugate displays somewhat sharp peaks, indicating slight increase in degree of crystallinity on thiolation.

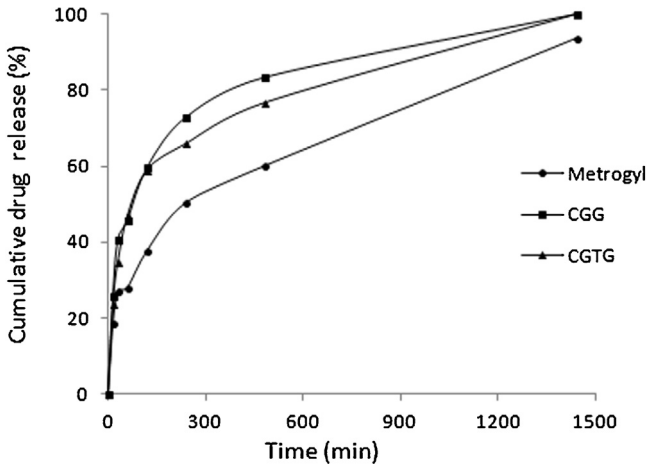


Fig. 5. *In vitro* release profile of metronidazole from CGG, CGTG and Metrogyl.

Fig. 1(d) exhibits the scanning electron micrographs showing shape of gellan gum and gellan–thioglycolic acid conjugate. It can be observed from the figure that both gellan gum and gellan–thioglycolic acid conjugate particles are polyhedral in shape with conjugate particles somewhat rougher.

Fig. 2 represents the partial ternary phase diagrams showing effect of Ca^{2+} ions on gelling behaviour of gellan gum and gellan–thioglycolic acid conjugate. Gellan gum is anionic polysaccharide which gels in the presence of cations. Ion-promoted gelation of gellan gum follows two steps, in the first step the random coil chains of gellan gum form double helices followed by complexation of double helices with cations at junctional zones leading to their aggregation (Tang, Lelievre, Tung, & Zeng, 1994). The phase diagram of gellan gum shows that gellan gum forms gel at a concentration >0.2% (w/v) in the presence of Ca^{2+} ions. Thiolation of GG results in modification of its gelling behaviour in the presence of ions. It can be observed that the aqueous solution of GTC forms a clear solution at a concentration <1.25% (w/v) in the presence of smaller concentration of Ca^{2+} ions (0.75%, w/v), but on increasing the concentration of Ca^{2+} ions, the solution becomes viscous, Further it was observed that GTC forms gel at concentration >2.0% (w/v) in the presence of Ca^{2+} ions at concentration >0.75% (w/v). It can be inferred from the result that thiolation of GG with thioglycolic acid, diminishes the interaction between the Ca^{2+} ions

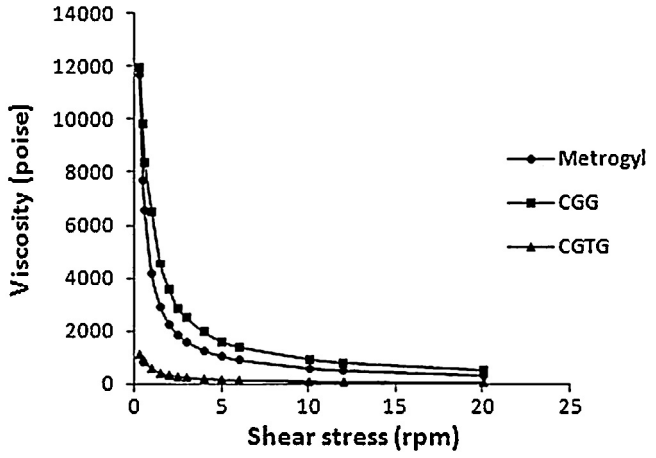


Fig. 6. Viscosity of CGG, CGTG and Metrogyl.

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
CGG	0.5972	0.9061	0.846	0.9444	0.2858
CGTG	0.6438	0.8438	0.8736	0.9832	0.3004
Metrogyl	0.8245	0.987	0.9723	0.977	0.332

CGG: gel containing gellan, CGTG: gel containing gellan–thioglycolic acid conjugate.

and the backbone chains of GG, as a result higher amount of Ca^{2+} is required for gelation of GTC.

Gellan gum has been earlier evaluated as a *in situ* gelling polymer for ocular delivery of indomethacin, timolol-maleate, gatifloxacin etc (Rupenthal Ilva et al., 2011). Thus to check whether thiolation affects the ocular irritation potential of GG, the GTC was comparatively evaluated with GG for *ex vivo* ocular tolerance employing HET-CAM assay. HET-CAM assay is used as an alternative to Draize rabbit eye test for evaluating the potential ocular irritation of compounds of formulations to be instilled in the eye. HET-CAM is a simple, rapid & sensitive test cost effective compared to the Draize rabbit's eye test. In the present study, GG and GTC was used in the concentration of 0.05%, (w/v) and no signs of irritation i.e. haemorrhage, vasoconstriction and coagulation were observed on the CAM uptill 5 min after application of the solution. This indicates that thiolation does not affect its ocular irritation potential.

GG is a biocompatible polymer. During earlier studies, it was found to possess excellent biocompatibility (Cencetti, Belline, Longinotti, Martinelli, & Martricardi, 2011). To check whether conjugation with thioglycolic acid affects its biocompatibility GG and GTC were screened for *in vitro* cytotoxicity against Vero cell lines employing resazurin assay method. Resazurin is reduced enzymatically by mitochondrial enzymes to resorufin, which is excreted outside the cells. The reduction of resazurin to resorufin reflects the cell viability (Czekanska, 2011). The results (Fig. 3) of resazurin assay reveal cell viability of 89.1% and 89.7% in the presence of GG and GTC after 24 h respectively, indicating that these polymers are not harmful to the cells.

Because of the difference in gelling behaviour of gellan and gellan–thioglycolic acid conjugate it was not possible to formulate gels employing equal concentration of gellan and gellan–thioglycolic acid conjugate as gelling agent. Thus, bioadhesive application of gellan–thioglycolic acid conjugate was comparatively evaluated with gellan gum by formulating Carbopol-based gels using metronidazole as the model drug. The bioadhesive gels were formulated employing Carbopol 974 P as a gelling agent at a concentration of 1.5% (w/v) while gellan or gellan–thioglycolic acid conjugate were used as bioadhesive agents at a concentration of 1% (w/v).

The results of comparison of formulated metronidazole gels with commercial formulation Metrogyl® (Lekar Pharmaceutical Pvt. Ltd.) for their mechanical characteristics and the mucoadhesive strength are presented in Table 1. The gels were characterized mechanically for their hardness, adhesiveness and cohesiveness.

The peak height of the force–time curve (Fig. 4) gives the hardness of the formulation. It describes the resistance to compression indicating the ease by which product can be removed from the container (Bansal et al., 2009). The gel formulations containing gellan gum (CGG) showed maximum hardness followed by Metrogyl®, while gel containing thioglycolic acid conjugate (CGTC) showed the least hardness. The area of the first negative peak of the force–time curve measures the adhesiveness of the formulation. It indicates the work required to overcome the forces of attraction between the gel surface and the surface of the probe. The adhesiveness of the formulations was found to be consistent with the results of hardness of gels i.e. CGG being more adhesive than Metrogyl® followed by CGTC. The ratio of the areas under the second positive peak to the first positive peak measures the cohesiveness of the formulation. It describes the structural recovery of the formulation following compression. The cohesiveness of the gel formulation was found to follow the order-Metrogyl > CGTC > CGG.

Gel formulations designed for efficient buccal delivery should have low hardness but high cohesiveness; so that minimum work would be required for its removal from the container and it attains complete structural recovery after application. The higher adhesiveness of the formulation is desired for prolonged retention in the buccal cavity. Gel formulations containing gellan–thioglycolic acid conjugate (CGTG) showed less hardness and adhesiveness but more cohesiveness than the gellan gum containing formulation (CGG). However, the adhesiveness (force of detachment between the aluminium probe and the gel surface) of the gel formulation cannot be considered as true indicator of mucoadhesive potential. Thus, the gels were further tested with fresh chicken intestinal membrane using modified physical balance method, to observe their real mucoadhesive potential. The results of the study revealed that the force required for the detachment of chicken intestinal membrane from gel containing gellan–thioglycolic acid conjugate (CGTG) was maximum, followed by gels containing gellan (CGG) and Metrogyl®. Further, the CGTG showed a 1.82-fold higher mucoadhesive potential than CGG.

Fig. 5 displays the *in vitro* release profile of metronidazole from various gel formulations. It can be inferred from the plot that the commercial gel formulation (Metrogyl®) provided the slowest release of metronidazole, while the CGTG showed slightly lesser release than CGG. 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 gel formulations based on Carbopol with commercial formulation, measured using Brookfield viscometer. The viscosity of the formulations follow the order CGG > Metrogyl > CGTG. The results of release rate study cannot be explained on the basis of 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 the viscosity of CGG is greater than CGTG, it provided slightly faster release of drug. This could be due to interaction of metronidazole with gellan–thioglycolic acid conjugate or due to *in situ* cross linking exhibited by disulfide linkages of thiolated polymers. However, further investigations are required to comment more on this aspect.

The release rate data of metronidazole-loaded gels and marketed formulation were fitted into various kinetic models to estimate their release kinetics and mechanism of release (Table 2). The results of model fitting revealed that the gel formulation containing GG (i.e, CGG) and marketed formulation (Metrogyl®) released the drug by first-order kinetics while gel formulations containing GTC (i.e, CGTG) released the drug by Higuchi's square root kinetics. Further, the value of 'n', ($n < 0.5$) the release exponent of Korsemeyer and Peppas equation indicates that the mechanism of release of metronidazole from gel formulations is diffusion through the matrix (Costa & Loba, 2001).

4. Conclusion

Conjugation of thiol groups on gellan gum was achieved by its esterification with thioglycolic acid. Characterization of gellan–thioglycolic acid conjugate (GTC) revealed decrease in the sensitivity of gellan gum (GG) to cation-promoted gelation, and improvement in mucoadhesive properties on thiolation. Further, it was observed that thiolation does not affect the non-irritant nature and biocompatibility of the GG. The bioadhesive applications of GTC were comparatively evaluated with GG by formulating Carbopol-based metronidazole gels. On the basis of the results of this study, it can be concluded that thiol conjugation of GG affects its gelling behaviour but improves the bioadhesive properties without affecting its biocompatibility. Thus, GTC possesses excellent potential for use as bioadhesive excipient in pharmaceutical formulation.

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