RESEARCH ARTICLE

Synthesis of Thiolated Cashew Gum and Its Evaluation as an Improved Mucoadhesive Agent in Drug Delivery

Pranab K. Bandyopadhyay*, Amit K. Nayak

Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj, Odisha, India

Received: 09th January, 2023; Revised: 06h February, 2023; Accepted: 07th March, 2023; Available Online: 25th March, 2023

ABSTRACT

The objectives of present research work were to extract cashew exudate gum (CG), perform thiolation of extracted CG, and evaluate the synthesized thiolated cashew exudate gum (TCG) as a mucoadhesive agent in designing metronidazole mucoadhesive gels and metronidazole mucoadhesive buccal discs. The CG was thiolated or thiol-modified via esterification utilizing thioglycolic acid and hydrochloric acid. Metronidazole mucoadhesive gels and metronidazole mucoadhesive buccal discs made of unmodified CG and TCG (as mucoadhesive agent) were formulated and evaluated to reveal their bio-mucoadhesive potentials in drug delivery. The yield of thiolated product (TCG) was 56.24%, and the thiol-group content in TCG was found to be 9.06 mM of thiol group/g of CG. FTIR analysis indicated the thiolation of CG in the synthesized TCG. Both types of formulations (mucoadhesive gels and buccal discs) made of TCG exhibited excellent improved *ex-vivo* bio-mucoadhesion and consistent pattern of metronidazole releasing over a prolonged time. The synthesized TCG can be utilized as an improved mucoadhesive material in designing bio-mucoadhesive systems for drug delivery.

Keywords: Buccal disc, Cashew gum, Drug delivery, Mucoadhesive, Plant gum.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.10

How to cite this article: Bandyopadhyay PK, Nayak AK. Synthesis of Thiolated Cashew Gum and Its Evaluation as an Improved Mucoadhesive Agent in Drug Delivery. International Journal of Drug Delivery Technology. 2023;13(1):66-74.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Plant-derived gums are natural biopolysaccharides, which are widely utilized in several industrial applications and numerous use in drug delivery.[1,2] Amongst all the plant-derived gums are biodegradable in nature. Besides the biodegradability issue, plant gums are well known for their low production cost, easy availability of raw materials from plant-based resources, and nontoxicity.[3] Thus, considering the benefits mentioned above, plant-derived gums are preferred as improved biodegradable and biocompatible biopolymeric excipients over synthetic agents for use in many pharmaceutical applications.

During the last few decades, a number of plant exudates-derived gums have been explored as natural biopolymers and exploited as pharmaceutical additives in the preparation of formulations of divergent types of liquid, semi-solid, and solid dosage forms.[1,3,4] Important and widely used plant exudates-derived gums as pharmaceutical excipients are cashew gum (CG),[5] moringa gum,[6] karaya gum,[7] etc. Amongst these, CG is a well-known exudates-derived gum produced from the gum exudates of the bark of a cashew plant (*Anancardium occidentale* L., which belongs to the family: Anacardiaceae).[8] In general, the gum exudation from the cashew tree was done by wounding the tree bark during pruning.[9,10] CG is an aqueous soluble branched heteropolysaccharide type gum of acidic nature. The reported molecular structure is reported to have a similar structure as of Arabic gum.[8] The polysaccharide chain of CG comprises an Arabin galactomannan structure with different side chains like galacturonic acid, glucuronic acid, and anacardic acid residues. CG also possesses some important biological characteristics, like antimicrobial activity.[9,10] The contents and ratio of monosaccharides in the CG are reported as variable depending on the cashew exudates-raw material sources, age of the cashew tree, process, and time of exudation. The chemical composition of CG has been reported that, on hydrolysis, CG produces glucose, galactose, rhamnose, mannose, and arabinose.[11] CG has already been utilized as a drug delivery excipient in many dosage forms for the delivery of drugs, like different types of tablets, multiple-unit systems (microparticles, nanoparticles, and small beads), gels, and pastes.[5,9,10] CG has already established the potential of applications as an emulsifier, thickener, gel-forming agent, matrix-forming agent, sustained release agent, etc.[8] It has also been reported as natural mucoadhesive agent in different kinds of bio-mucoadhesive dosage forms.[12]

The term 'Mucoadhesion' is defined as the adhesion of any system to the mucus-lining epithelial surface.[13]

**Author for Correspondence: pranab_btpuri@yahoo.com*

In general, mucoadhesion is attained via using different kinds of mucoadhesive polymeric candidates.[14] When mucoadhesive polymeric candidates are incorporated as mucoadhesive excipients in drug delivery systems, these can exert prolongation of adhesive contact onto the mucosal surface to increase the residence time for dosage forms. The mucoadhesive property of natural polysaccharide gums is due to the formation of non-covalent bonds, namely hydrogen bonding, ionic interactions, and Van der's Wall force, which are weak bonds.[15] For this reason, natural gums-based mucoadhesive systems fail to provide mucoadhesive contact and residence time over a prolonged period.[16,17] To improve the mucoadhesive of different natural gums, chemical modification via thiolation (*i.e*., introducing thiol group to the gum molecules) is being carried out and reported.[17] Thiol groups are able to form stronger covalent bonds (di-sulfide linkage) when coming in contact with glycoproteins of the mucus-lining.[16] The configuration of stronger covalent bonds in-between thiolated gums as mucoadhesive polymeric agent and mucus glycoproteins assurance the enhancement of bio-mucoadhesivity, which obviously facilitates excellent mucoadhesive contact and residence time over a prolonged period.[6] This also facilitates the localization of mucoadhesive dosage forms at the targeted area.

In some reported literature, a few natural gums, namely tamarind seed polysaccharides,[15] xanthan gum,[17,18] karaya gum,[19] moringa gum,[6] etc., have chemically been modified via thiolation for the improvement of their mucoadhesivity to be exploited as mucoadhesive agents in the outline design of various types of biomucoadhesive dosage forms. However, the attempt for thiolation of CG is still not reported for use as an improved mucoadhesive agent in designing of biomucoadhesive dosage forms. In the present research, thiolation of CG via the incorporation of thiol (-SH) groups within the CG molecular structure was considered to enhance the biomucoadhesion property of unmodified CG, and the synthesized thiolated-CG (TCG) was evaluated as an improved mucoadhesive agent in two different kinds of drug delivery systems (one semi-solid and one solid formulation) - metronidazole mucoadhesive gels as well as metronidazole mucoadhesive buccal discs.

MATERIALS AND METHODOLOGY

Materials

In this work, CG was obtained from the raw exudates-materials of the barks of a fully grown cashew plant (*A. occidentale* L.) collected from the Jharpokharia area in the district of Mayurbhanj, Odisha, India in the summer of July 2020. The gum exudation from the cashew tree was done by wounding the tree bark during pruning. Thioglycolic acid (99% AR, Hi-Media Laboratories Pvt. Ltd., India), Ellman's reagent, which is also called DTNB (Hi-Media Laboratories Pvt. Ltd., India), superior grade hydrochloric acid from SD. Fine Chemicals Limited., L-cysteine, triethanolamine from Merck Specialties Pvt. Ltd., India, Lactose (SD Fine Chemicals Ltd., India., India), PEG-4000andCarbopol 940, both from LobaChemie Pvt. Ltd., etc., were obtained from the Hi-Media Laboratories Pvt. Ltd., India. Metronidazole was employed as the model drug and was purchased from BS Traders, India. The remaining substances which were used were analytical-grade chemicals, reagents, and solvents.

CG Extraction of from Raw Exudates-materials from the Barks of Cashew Plant

CG was extracted carefully from raw exudates-materials of the barks of the cashew plant.[5] The crude exudates-materials were dried overnight at a temperature of 45 ± 2°C in a tray drier to make materials brittle. The dried crude cashew exudates-materials were powdered by milling. The milled powders of crude exudates-materials were sieved, and 500 g of sieved material was solubilized in 1 L distilled water. The solution of crude cashew exudates-materials was then boiled in a water bath for one hour while being stirred occasionally. The prepared solution was gradually cooled down to room temperature and subsequently, it was allowed to cool in a refrigerator for overnight to settle the proteins, other undissolved matters, etc. The supernatant solution was decanted and, subsequently, was concentrated to 1/3rd of the preliminary volume at 45 ± 2°C using a temperature control water bath for 1-hour with moderate intermittent stirring. The warm solution was then cooled at normal room temperature, and the solution of concentrated raw cashew exudates-materials was mixed with twice the volume of acetone with stirring using a glass rod continuously. The formed precipitate was collected via a filtration process and, subsequently, repeatedly washed using acetone. The washed precipitate material (in wet condition) was separated and, subsequently, was slowly dried overnight at a temperature of 45 ± 2°C in a tray drier. Precipitated material, after complete drying, was powdered and sieved through an 80 mesh size screen. Finally, for the further experiment, the powdered CG was stored in the air-tight container inside a desiccator.

Synthesis of Thiolated-CG (TCG)

In brief, extracted CG was thiolated or thiol-modified via the esterification reaction utilizing thioglycolic acid (as a thiolating agent) and hydrochloric acid.[6] In 50 mL distilled water, 12 g CG was dissolved to prepare an aqueous solution of extracted CG. To the aqueous solution of CG, 7.2 mL of 99% thioglycolic acid and 4 mL of 7N of HCl were added for the esterification reaction. After that, the mixture of CG (12 g), 99% thioglycolic acid (7.2 mL), and 7 N HCl (4 mL) was allowed for reaction at 80°C temperature for a duration of three hours. The above reaction mixture was cooled and, subsequently, was poured into acetone (1-L) in a separate beaker. A creamy-white precipitate of TCG was formed and washed thrice with the volume of acetone to exterminate the unreacted thioglycolic acid. The obtained material was dried in a tray drier at a temperature of 45 ± 2°C for 12 hours. The dried TCG powder was ground with a mortar and pestle. The fine powder was passed via a sieve of mesh size 80 and then, stored in a container inside a moisture-free desiccator.

Characterization of TCG

Thiol Group Contents Estimation in TCG

Thiol group contents in TCG, which was freshly synthesized, were estimated by quantification of the thiol-group by Ellman's method.[20] Briefly, 0.1% w/v solution of TCG was prepared in a 5 M phosphate buffer of pH 8. This aqueous solution in buffer was then allowed for reaction with 5 ml 0.03% w/v solution of Ellman's reagent in the same phosphate buffer of pH 8 at normal room temperature for two hours. Substitution of thiol groups in the synthesized TCG was estimated by determining the absorbance of this mixture at a wave length of 450 nm. The amount of thiol group substituted (Thiol group/g) in TCG was estimated using the calibration curve prepared by the reaction of a standard preparation of L-cystine with Ellman's reagent.

Fourier Transform Infrared (FTIR) Spectroscopy

The samples of extracted CG (obtained from the crude exudates-materials of cashew tree barks) and synthesized TCG (by thiolation reaction) were analyzed by FTIR spectrophotometer. For FTIR spectroscopy analysis, samples were analyzed using potassium bromide pellets (containing samples) made of IR-grade potassium bromide. The analysis was done in 4000–400 cm^{-1}scan range

Manufacturing of Metronidazole Gels Made of CG and TCG by Aqueous Dispersion Method

To evaluate the mucoadhesive of unmodified CG and newly synthesized TCG, gels containing metronidazole as a model drug were manufactured, where Carbopol 940 was used as a gel-producing agent. These metronidazole gels were produced by the aqueous dispersion method. Unmodified CG and synthesized TCG were employed as mucoadhesive agents in these gel formulations. Briefly, 1% w/v solution of Carbopol 940 and 0.5% w/v solution of metronidazole were nicely mixed with aqueous solutions containing unmodified CG or synthesized TCG (0.5% w/v) separately. Both the mixtures were hydrated for 12 hours, followed by the incorporation of glycerin (0.5% w/v) for formation of gels. The formulation chart for manufacturing metronidazole gels is given in Table 1.

Evaluation of Metronidazole Gels Made of CG and TCG

Viscosity Measurement

The viscosities of formulated metronidazole containing unmodified CG and synthesized TCG were determined by Brookfield viscometer with spindle 6 at different rotational speeds at normal room temperature.

Ex-vivo Biomucoadhesivity Determination

The *ex-vivo* biomucoadhesivity of metronidazole gels made of unmodified CG and TCG was assessed through modified physical balance with a 2-pan balance and 2-glass plates. One side of the lower plate was permanently attached to the basement, and the upper pan was fixed to one arm of modified balance using cyanoacrylate adhesive.[21] The natural biological membrane which was used in this study of biomucoadhesivity evaluation was an excised and fresh mucosal membrane of the goat intestine, which was fixed with cyanoacrylate adhesive to the bottom of the upper pan and on the upper surface of the lower pan, 1-g of metronidazole gel was placed for biomucoadhesivity evaluation. The upper pan was positioned over the lower pan with the application of initial force by the tip of finger for 5 minutes. A gradual increase in weighing mass was loaded on the second pan of the modified balance till the detachment of plates. The applied weight (in g) needed to detach the lower plate from the upper plate was recorded as mucoadhesive strength of the prepared gels. The strength of mucoadhesion (g), adhesion force (N) as well as strength of bonding (N/m^2) were easily calculated employing the following formula:[22]

Mucoadhesive strength = The mass (in g) needed for the detachment of the lower plate from the upper plate.

$$\text{Adhesion force (N)} = \frac{\text{Strength of mucoadhesion} \times 9.81}{1000}$$

$$\text{Strength of bonding (N/m}^2\text{)} = \frac{\text{Adhesion force}}{\text{Mucosal membrane surface area}}$$

In-vitro Drug Release Study

Briefly, accurately weighed metronidazole gels (1-g) made of unmodified CG and TCG were individually placed within the dialysis sac (having 10 kDa molecular weight cut-off), and then, the dialysis sac containing the gel sample was fixed to the paddle of a dissolution apparatus (Campbel Electronics). The phosphate buffer of pH 6.8 was used as the drug release media, and the paddle of the dissolution apparatus was submerged in it. The entire drug release system was controlled, temperature controlled at 37 ± 0.5°C, and a 50 rpm paddle speed was applied. Aliquots were sampled from the drug release medium contained in the dissolution apparatus at different time points. The same volume (*i.e.*, 5 mL) of freshly prepared release medium was immediately replaced at the same time-point of sample removal. The metronidazole contents in the sampled aliquots for different metronidazole gels were assessed by UV-vis spectrophotometer (Shimadzu, Japan) at a wavelength of 320 nm.

Manufacturing of Metronidazole Buccal Discs Made of Extracted CG and Synthesized TCG

In brief, powdered 100 mg CG (extracted) or TCG (synthesized), 100 mg metronidazole, 50 mg lactose, and 25 mg PEG 4000 were thoroughly blended and then compressed for 1-minute at 75 kg/cm^2 by IR hydraulic press to produce 13 mm discs. The formulation chart for manufacturing metronidazole buccal discs is given in Table 2.

Table 1: Formulation chart for the manufacturing of these metronidazole gels made of CG and TCG

Code	*CG (% w/v)*	*TCG (% w/v)*	*Carbopol 940 (% w/v)*	*Glycerin (% w/v)*	*Metronidazole (% w/v)*
G-CG	0.5	-	1	0.5	0.5
G-TCG	-	0.5	1	0.5	0.5

Table 2: Formulation chart for the manufacturing of metronidazole buccal discs made of extracted CG and synthesized TCG

Code	*CG (mg)*	*TCG (mg)*	*Lactose (mg)*	*PEG 4000 (mg)*	*Metronidazole (mg)*
G-CG	75	-	75	25	10
G-TCG	-	75	75	25	10

Evaluation of Metronidazole Buccal Discs Made of Extracted CG and Synthesized TCG

Determination of Weight Uniformity

The weight uniformity of prepared metronidazole buccal discs made of unmodified CG and synthesized TCG was determined by weighing 20 buccal discs, individually, from each formulation by an electronic analytical balance. The average weights, as well as standard deviation, were determined. The variation of weight (%) was estimated by using this equation.[23]

Variation of weight (%)=Standard deviation/Mean weight X 100

Thickness Uniformity Determination

The thicknesses of 6 metronidazole buccal discs made of unmodified CG and TCG were measured, in which a digital slide caliper was used. The mean thickness was calculated.

Content Uniformity Determination

Uniformity of the drug content of unmodified CG and synthesized TCG-based metronidazole buccal discs was determined by estimating metronidazole contents in 6 buccal discs of each formulation. The sampled buccal discs were crushed and powdered by pressing with pastel in a clean mortar. The crushed and powdered sample accurately weighed 100 mg was dissolved in a phosphate buffer of pH 6.8 followed by filtration using No. 40 Whatman® filter paper. The metronidazole concentration in the filtered liquid were appropriately diluted by freshly prepared phosphate buffer (pH 6.8) determined, and absorbance was estimated at a wavelength of 320 nm using a UV-vis spectrophotometer (Shimadzu, Japan).

Measurement of Friability

The friability of metronidazole buccal discs made of unmodified CG and synthesized TCG was determined by a friability tester USP 23 (Electro Lab, India). For each formulation of buccal discs, six discs were weighed by an electronic analytical balance and placed in the friability for evaluation. These sampled discs were then rotated for a duration of 4 minutes at a rotational speed of 25 rpm. The discs were reweighted accurately after careful dedusting. The friability (%) values of these metronidazole buccal discs made of unmodified CG and TCG were calculated using the following formula:[6]

$$\%\text{Friability} = (I–F)X100/I$$

where I = Initial weight of the disc, F =Finalweight of the disc after friability.

Determination of Ex-vivo Biomucoadhsivity

The *ex-vivo* biomucoadhesivity of metronidazole buccal discs made of unmodified CG and TCGwas evaluated via the determination of biomucoadhesion time employing excised and fresh mucosal membrane of the goat buccal mucosal membrane, which was collected from a local slaughtering house within 1hour of the slaughter of the animal. The collected goat buccal mucosal membranes were cleaned via the removal of underlying fatty materials and, subsequently, ringed thoroughly by using simulated saliva fluid of pH 6.8. The goat buccal mucosal membrane was then attached to the paddle of the dissolution apparatus. One buccal disc was sampled and attached onto the internal part of the buccal mucosal membrane employing a minimum pressure for approximately 30 seconds. The *ex-vivo* biomucoadhesion time was determined by estimating the time required for the separation of buccal discs from the membrane interface.

In-vitro Evaluation of Drug Release

In-vitro release of metronidazole from buccal discs made of unmodified CG and synthesized TCG was performed through dissolution apparatus (USP type-II, Campbel Electronics, India). Simulated saliva fluid of pH 6.8 was used as a drug release medium for the *in-vitro* drug release study. Briefly, 250 mL of simulated saliva fluid of pH 6.8 was taken in the dissolution vessel, which was maintained at 37 ± 0.5°C with 50 rpm of paddle speed. One buccal disc was glued with the cyanoacrylate glue in the inner part of the dissolution vessel. Aliquots were sampled from drug release medium contained in the dissolution apparatus at different time points and the same volume (*i.e*., 5 mL) of freshly prepared release medium was immediately replaced at the same time-point of sample removal. The metronidazole contents in the sampled aliquots for different metronidazole gels were measured with the help of a UV-vis spectrophotometer (Shimadzu) at a of wavelength of 320 nm.

STATISTICAL METHOD OF ANALYSIS

Experimental data were expressed as mean ± SD.Microsoft Excel 2002 was used to perform the basic statistical analysis.

RESULTS AND DISCUSSION

Extraction of CG and Thiolation of CG

CG is reported as a plant-derived heterogeneous polysaccharide posing Arabin galactomannan structure with different side chains like galacturonic acid, glucuronic acid, and anacardic acid residues.[5,8] The yield of extracted CG was found 20.07%. This result was almost comparable with the previous research, where it was reported as 19.22%.[5] In this study, the chemical esterification in between-OH groups present in the Arabin galactomannan structure of extracted CG and -COOH groups present in thioglycolic acid occurred to produce the thiolated product (*i.e.,* TCG) under the acidic milieu (as 7 N HCl was used in the synthesis). A schematic representation of thiolation of CG using thioglycolic acid to produce TCG is presented in Figure 1. The yield of TCG was found 56.24%. The synthesized

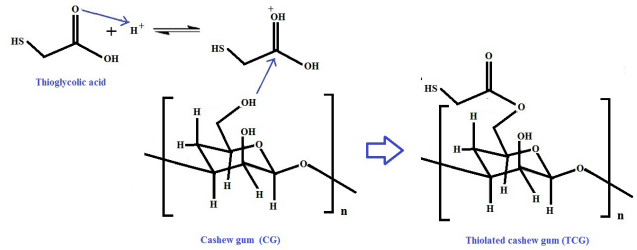

Figure 1: Schematic representation of thiolation of CG using thioglycolic acid to produce TCG.

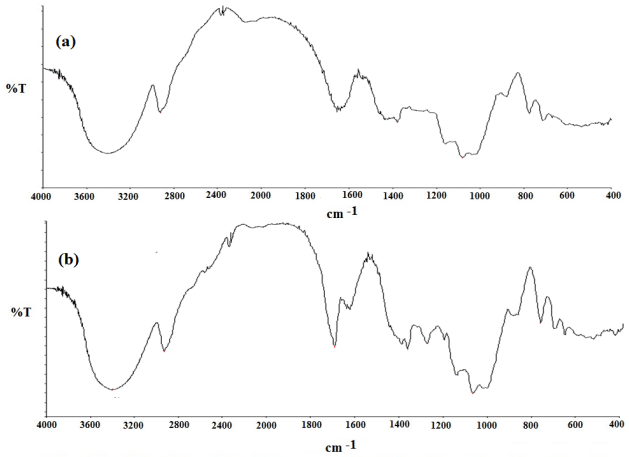

Figure 2: FTIR spectrum of (a) unmodified CG and (b) thiol-modified CG (TCG).

thiolated CG was creamy-white in color and soluble in both hot as well as cold water.

Characterization of TCG

Estimation of Thiol Group

Using Ellman's method, the thiol-group concentration in synthetic TCG was 9.06 mM of thiol group/g of CG.

FTIR Analysis

The FTIR spectrum of extracted CG (unmodified) and synthesized thiol-modified CG (*i.e.,* TCG) is presented in Figure 2. The FTIR spectra of CG (Figure 2a) displayed characteristic broad peaks at 3413.83 cm^{-1} because of–OH vibration of stretching and at 2926.52 cm^{-1} owing to–CH vibration of stretching. One absorption peak was observed at 1654.43 cm^{-1} due to the scissor vibration of O-H in bonded water molecules in the unmodified CG. Along with this, peaks at 1376.69 cm^{-1} for symmetrical deformation of -CH_2 groups, at 1150.72 cm^{-1} for glycosidic bonds (C-O-C) present in polysaccharide structure, and stretching vibration for –CO at 1081.14 cm^{-1}. FTIR spectrum of TCG (Figure 2b), several typical peaks of the extracted CG (unmodified) were noticed to be present with very minute/without any significant shifting/ alteration. Particularly, typical peaks at 3402.14 cm^{-1} for –OH vibration of stretching and at 2927.64 cm^{-1} for –CH vibration of stretching, at 1376.96 cm^{-1} for symmetrical deformation of -CH_2 groups,at 1152.44 cm^{-1} for glycosidic bond (C-O-C) of polysaccharide structure, and at 1081.05 cm^{-1} for –CO vibration of stretching. However, a weak shoulder at 2562.35 cm^{-1} due to -SH or thiol-group stretching of was detected in the FTIR spectrum of extracted TCG. However, it was not visible in the extracted CG. This observation indicated the successful thiolation of CG by thioglycolic acid. In addition, more intense typical peak at 1708.06 cm^{-1} was visible for extracted TCG (for O-H scissor vibration of bonded water molecules). In recent studies, it has also been reported that the characteristic peaks representingthiol-group in polysaccharides is feebly detectable in the FTIR spectroscopy analysis.[6,24]

Manufacturing of Metronidazole Gels Made of CG and TCG

To evaluate the biomucoadhesivity of TCG, mucoadhesive gels were formulated, where metronidazole (1% w/w) was used as a model drug. In these gels (G-CG and G-TCG), 0.5% w/v Carbopol 940 was used as a gel-forming agent, whereas 0.5% w/v solution of bothunmodified CG or synthesized TCG were employed as mucoadhesive agents in these gel formulations. In addition, glycerin (0.5% w/v) was used to form gels.

Evaluation of Metronidazole Gels Made of CG and TCG

Viscosity

Viscosities of all these metronidazole gels made of unmodified CG and TCG weredetermined by Brookfield viscometer. The comparative viscosity results of metronidazole gels are presented in Figure 3. It was observed that the viscosity of G-CG metronidazole gel made of unmodified CG as mucoadhesive agent was higher than that of G-TCGmetronidazole gel made of TCG.

Ex-vivo Biomucoadhsivity

The *ex-vivo* biomucoadhesion of prepared metronidazole gels made of unmodified CG and TCG (G-CG and G-TCG) onto the excised goat buccal mucosa was evaluated by employing modified physical balance. The *ex-vivo* biomucoadhesion results which include bonding strength, a force of adhesion, and strength of mucoadhesion of metronidazole gels made of unmodified CG and TCG (G-CG and G-TCG) are presented in Table 3. The measured *ex-vivo* bonding strength, force

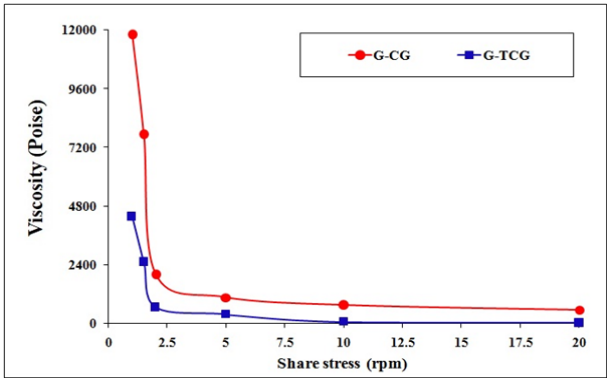

Figure 3: The comparative viscosity results of metronidazole gels made of unmodified CG and TCG(G-CG and G-TCG).

Table 3: *Ex-vivo* mucoadhesivity results (bonding strength, force of adhesion, and strength of mucoadhesion) of metronidazole gels made of unmodified CG and TCG (G-CG and G-TCG)

Code	*Strength of mucoadhesion (g)[a]*	*Force of adhesion (N)*	*Bonding strength (N/m^2)*
G-CG	9.25 ± 0.11	9.07 x 10^{-2}	319.51
G-TCG	10.18 ± 0.17	9.99 x 10^{-2}	351.65

of adhesion, and strength of mucoadhesion of G-TCG metronidazole gel made of TCG (used as mucoadhesive agent) were comparatively higher than those of G-CG metronidazole gel made of unmodified CG (used as mucoadhesive agent). The biomucoadhesivity potential of unmodified CG, when used as mucoadhesive agent in the formula of G-CG metronidazole gel, can be explained by the hydroxyl groups (present in the CG molecules) developing weak bonds like ionic interactions, Van der's Wall force and hydrogen bonds with the mucus glycoproteins. These non-covalent bonds are well-known for their weaker adhesion. In contrast, the enhanced biomucoadhesivity by the G-TCG metronidazole gels made of TCG can be explained by the fact of ability of thiol groups (present in the synthesized TCG) to develop more stronger covalent bonds (due to the formation of disulfide linkage) in contact with the mucosal glycoproteins. Therefore, TCG can be used as an improved mucoadhesive agent in biomucoadhesive gel-based systems for drug delivery.

In-vitro Release of Drugs

The *in-vitro* metronidazole released from mucoadhesive gels made of unmodified CG and TCG(G-CG and G-TCG) was evaluated in a phosphate buffer of pH 6.8. In Figure 4, the *in-vitro* metronidazole releasing comparison between unmodified CG and TCG (G-CG and G-TCG) mucoadhesive gels is presented. The G-CG mucoadhesive gel made of unmodified CG demonstrated almost total metronidazole release within 6 hours of sin drug release study. In contrast, the G-TCGmucoadhesive gel made of TCG demonstrated a slower and more sustained pattern of metronidazole releasing than that of G-CGmucoadhesive gel made of unmodified CG. The viscosity result was inconsistent with the *in-vitro* metronidazole release result (The G-CG metronidazole gel showed comparatively higher viscosity than that of G-TCG metronidazole gel). The comparatively slower-sustained metronidazole release from G-TCG metronidazole gel made of TCG could be due to the possible interaction of metronidazole with TCG. In addition, it might be caused owing to *in-situ* cross-linking, which the disulfide linkage of TCG might contribute.

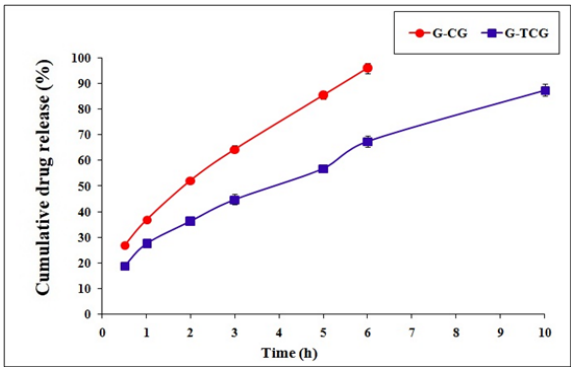

Figure 4: Comparative *in-vitro* release of drug from metronidazole gels made of unmodified CG and TCG(G-CG and G-TCG) in phosphate buffer of pH 6.8 [Data are expressed as mean ± SD, n = 3].

Table 4: The model-fitting results for *in-vitro* release of drug from metronidazole gels made of unmodified CG and synthesized TCG (G-CG and G-TCG)

Code	*Zero-order kinetics model*	*First-order kinetics model*	*Model of Higuchi*	*Model of Korsmeyer-Peppas*	*Exponent of release (n)*
G-CG	0.9896	0.9252	0.9957	0.9981	0.5091
G-TCG	0.9784	0.8728	0.9920	0.9953	0.5033

The model fitting results for *in-vitro* drug release of metronidazole from these gels are presented in Table 4. The *in-vitro* metronidazole releasing from both metronidazole gels (G-CG and G-TCG) followed the Korsmeyer-Peppas model (R^2 = 0.9981) G-CG and R^2 = 0.9953 for G-TCG) as it also fits best to this model. However, the Higuchi model (R^2 = 0.9957 for G-CG and R^2 = 0.9920 for G-TCG) also fits best to the Korsmeyer-Peppas model in both the cases of metronidazole gels (G-CG and G-TCG). Further, the release exponent values(n) of the Korsmeyer-Peppas equation ($n \leq 0.5$) were computed. For the G-CG mucoadhesive gel made of CG, the value of n was 0.5091, while it was 0.5033 for the G-TCG mucoadhesive gel made of TCG. Thus, the results demonstrated almost diffusion-dependent releasing by these metronidazole mucoadhesive gels made of unmodified CG and TCG (G-CG and G-TCG) in a phosphate buffer of pH 6.8.

Manufacturing of Metronidazole Buccal Discs Made of CG and TCG

In the current research, metronidazole buccal discs of 13 mm diameter were manufactured via a compression press process (75 kg/cm^2 for 1-minute). In the formula of metronidazole buccal discs, 75 mg lactose and 25 mg PEG 4000 were contained as diluent and plasticizer, respectively. Here, unmodified CG and TCG were also employed as mucoadhesive agents, which were incorporated in the formula of buccal discs individually. Metronidazole (100 mg) was used as a model drug in these formulations of buccal discs.

Evaluation of Metronidazole Buccal Discs Made of CG and TCG

Weight Uniformity

The average weight (mg) and weight variation (%) of metronidazole buccal discs metronidazole buccal discs made of unmodified CG and TCG (D-CG and D-TCG) are tabulated in Table 5. The average weight of D-CG metronidazole buccal discs made of unmodified CG (220.86 ± 7.44 mg) was approximately comparable to that of D-TCG metronidazole buccal discs made of TCG (212.64 ± 6.09 mg). The weight variation (%) values of both the metronidazole buccal discs

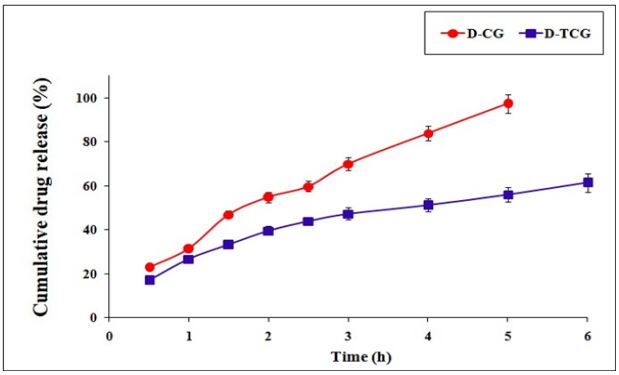

Figure 5: Comparative *in-vitro* release of metronidazole from prepared buccal discs made of CG and TCG (D-CG and D-TCG) in simulated saliva fluid, pH 6.8 [Data are expressed as mean ± SD; n = 3].

(D-CG and D-TCG) were less than 5%, indicating that powdered unmodified CG or TCG, lactose, PEG 4000, and metronidazole were thoroughly blended during manufacturing.

Thickness Uniformity

By digital slide calipers, the thicknesses of both the metronidazole buccal discs were determined. The thickness of D-CG metronidazole buccal discs made of unmodified CG (1.13 ± 0.06 mm) was found to be approximately comparable to that of D-TCG metronidazole buccal discs made of TCG (1.19 ± 0.07 mm) (Table 5). The results indicated that the application of identical force of compression (75 kg/cm^2) for a period of 1-minute by means of hydraulic press was used to manufacture 13 mm diameter buccal discs.

Content Uniformity

The drug content of D-CG metronidazole buccal discs made of unmodified CG (97.47 ± 3.18%) was found to be approximately comparable to that of D-TCG metronidazole buccal discs made of TCG (98.26 ± 4.05%) (Table 5). Therefore, the obtained results indicated the presence of contained metronidazole, uniformly, and within thecompendial quality limit in both the buccal discs made of unmodified CG and TCG (D-CG and D-TCG).

Friability

The friability (%) values of both the metronidazole buccal discs made of unmodified CG and TCG (D-CG and D-TCG) were less than 1% w/w (Table 5), which conformed the compendial friability specifications for tablet. The estimation of friability is usually used to assess the resistance of the solid dosage forms to abrasion.

Table 5: The results of weight uniformity (average weight and weight variation), thickness uniformity, content uniformity, and friability of metronidazole buccal discs made of unmodified CG and TCG (D-CG and D-TCG)

Code	*Average weight (mg)*[a]	*Weight variation (%)*	*Thickness (mm)*[b]	*Drug content (%)*[b]	*Friability (%)*[b]
D-CG	220.86 ± 7.44	3.37	1.13 ± 0.06	97.47 ± 3.18	0.79 ± 0.05
D-TCG	212.64 ± 6.09	2.86	1.19 ± 0.07	98.26 ± 4.05	0.90 ± 0.05

[a]Mean ± S.D., n = 20
[b]Mean ± S.D., n = 6

Ex-vivo Biomucoadhsivity

The *ex-vivo* biomucoadhesivity of metronidazole buccal discs made of unmodified CG and TCG(D-CG and D-TG) was assessed by measuring their biomucoadhesion time using goat buccal mucosal membrane. The *ex-vivo* biomucoadhesion time of D-TCG metronidazole buccal discs made of TCG (17.75 h) was comparatively higher (almost 1.85-fold) than that of D-CG metronidazole buccal discs made of unmodified CG (9.4 h).In the same way, improved *ex-vivo* biomucoadhesivity of G-TCG metronidazole gels made of TCG was observed as compared to that of G-CG metronidazole gels made of unmodified CG. The improved *ex-vivo* biomucoadhesivity of formulations containing thiol-modified CG (*i.e.,* TCG) can be accredited by the aptitude of thiol groups present in the structure of synthesized TCG to form stronger covalent bonds in contact with glycoproteins of mucous because of the formation of disulfide linkage. Therefore, synthesized TCG can be employed as an improved mucoadhesive agent in buccomucoadhesive drug delivery system.

In-vitro Release of Drug

The *in-vitro* release of metronidazole from buccal discs made of unmodified CG and TCG (D-CG and D-TCG) was evaluated in the simulated fluid of pH 6.8. The comparative *in-vitro* release of metronidazole from mucoadhesive buccal discs are presented in Figure 5. The D-CG mucoadhesive buccal discs made of unmodified CG released almost total metronidazole within 5 hours. On the contrary, D-TCG mucoadhesive buccal discs made of TCG showed a consistent pattern of metronidazole release over a period of 6 hours (61.44 ± 4.30%). The slower metronidazole released by D-TCG mucoadhesive buccal discs made of TCG can be attributed to a stronger covalent bond formation between glycoprotein in the mucous membrane and the thiol-group present in TCG (which was incorporated within the formula of D-TCG metronidazole buccal discs). This phenomenon also might be attributed to *in-situ* cross-linking contributed by the di-sulfide linkage of TCG.

The model fitting results for *in-vitro* metronidazole release from these metronidazole buccal discs in the simulated fluid of pH 6.8 is presented in Table 6. *In-vitro* metronidazole releasing from D-CG metronidazole buccal discs made of unmodified CG followed the model of Korsmeyer-Peppas ($R^2 = 0.9916$) as the

Table 6: The model-fitting results for ***in vitro*** release of drug from these buccal discs prepared with unmodified CG and TCG(D-CG and D-TCG)

Code	*Zero-order kinetics model*	*First-orderkinetics model*	*Model of Higuchi*	*Model of Korsmeyer-Peppas*	*Exponent release (n)*
D-CG	0.9846	0.8947	0.9301	0.9916	0.6425
D-TCG	0.9096	0.7845	0.9721	0.9807	0.4988

best-fit model, while the zero order kinetic model ($R^2 = 0.9846$) was also found closer to fit Korsmeyer-Peppas model. On the other hand, *in-vitro* metronidazole releasing from D-TCG metronidazole buccal discs made of TCG followed the model of Korsmeyer-Peppas ($R^2 = 0.9807$) as the best-fit model, while Higuchi model ($R^2 = 0.9721$) was also found closer to fit Korsmeyer-Peppas model. Further, the release exponent values(n) of the Korsmeyer-Peppas equation ($n \leq 0.43$) were computed for D-CG mucoadhesive buccal discs made of unmodified CG, the value of n was 0.6425, while it was 0.4988 for D-TCG mucoadhesive buccal discs made of TCG. Thus, the results demonstrated an anomalous transport mechanism based on diffusion as well as polymeric relaxation-dependent releasing by D-CG mucoadhesive buccal discs made of unmodified CG in the phosphate buffer of pH 6.8. In contrast, D-TCG mucoadhesive buccal discs made of TCG demonstrated almost diffusion-dependent releasing mechanism.

CONCLUSION

In the present work, thiolation of CG was carried out via the incorporation of thiol (-SH) group within the CG molecular structure (yield, 56.24%) to enhance the biomucoadhesion property of unmodified CG. The thiol-group content in synthesized TCG was found 9.06 mM of thiol group/g of CG, which was determined by Ellman's method. FTIR analysis indicated the thiolation of CG in the synthesized TCG. The TCG was evaluated as a mucoadhesion-enhancing agent in two different kinds of delivery of a drug (one semi-solid and one solid formulation) - metronidazole mucoadhesive gels as well as metronidazole mucoadhesive buccal discs. Both the formulations (mucoadhesive gels and buccal discs) exhibited excellent improved *ex-vivo* biomucoadhesion, when TCG was used as mucoadhesive agent. The *in-vitro* metronidazole release from both mucoadhesive gels and buccal discs demonstrated a slower and more sustained pattern of metronidazole release when TCG was used in their respective formula. The overall results revealed that TCG can be utilized as an improved mucoadhesion-enhancing agent in designing biomucoadhesive formulations for drug delivery.

ACKNOWLEDGEMENT

Both authors are grateful to the GB of Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj-757086, Odisha, India, for providing the necessary research facility.

REFERENCES

1. Dehghani Soltani M, Meftahizadeh H, Barani M, Rahdar A, Hosseinikhah SM, Hatami M, Ghorbanpour M. Guar (*Cyamopsis tetragonoloba* L.) plant gum: From biological applications to advanced nanomedicine. Int J BiolMacromol. 2021;193(Pt B):1972-1985.
2. Nayak AK, Hasnain MS, Dhara AK, Pal D. Plantpolysaccharidesin pharmaceutical applications.In: PalD, Nayak AK (eds) *Bioactive Natural Products for Pharmaceutical Applications*, Advanced StructuredMaterials,vol140.Springer,Cham,2021, 93-125.
3. Nayak AK, Hasnain MS. Plant polysaccharides in drug delivery applications, In Nayak AK, Hasnain MS,(eds), *Plant Polysaccharides-Based Multiple-Unit Systems for Oral Drug Delivery*, Springer, Singapore,2019, 19-23.
4. Nayak AK, Hasnain MS, Pal K, Banerjee I, Pal D. Gum-based hydrogels in drug delivery. In: Pal K, Banerjee I, SarkarP, KimD, DengW-P, DubeyNK, Majumder K (eds.,), *Biopolymer-Based Formulations*, *Biomedical and FoodApplications*, ElsevierInc., UnitedStates,2020, 605-645.
5. Hasnain MS, Rishishwar P, Rishishwar S, Ali S, Nayak AK. Extraction and characterization of cashew tree (*Anacardiumoccidentale*) gum; use in aceclofenacdental pastes. Int J BiolMacromol. 2018; 116:1074-1081.
6. Grewal P, Mundlia J, Ahuja M. Thiol modified Moringa gum - A potential bioadhesive polymer. CarbohydrPolym. 2019; 209:400-408.
7. Alange VV, Birajdar RP, Kulkarni RV. Functionally modified polyacrylamide-graft-gum karaya pH-sensitive spray dried microspheres for colon targeting of an anti-cancer drug. Int J Biol Macromol. 2017; 102:829-839.
8. Nayak AK, Ansari MT, Sami F, Bera H, Hasnain MS. Cashew gum in drug delivery applications. In:Hasnain MS, Nayak AK, (eds.,), *Natural Polysaccharides in Drug Delivery and Biomedical Applications*, Academic Press, Elsevier Inc., United States,2019:pp. 263-283.
9. Torquato D, Ferreira M, Sá G, Brito ES, Pinto GA, Azevedo E. Evaluation of antimicrobial activity of cashew tree gum. *World J Microbiol Biotechnol. 2004;20*;505-507.
10. Campos DA, Ribeiro AC, Costa EM, Fernandes JC, Tavaria FK, Araruna FB, Eiras C, Eaton P, Leite JR, Manuela Pintado M. Study of antimicrobial activity and atomic force microscopy imaging of the action mechanism of cashew tree gum. CarbohydrPolym. 2012;90(1):270-274.
11. Silva TM, Santiago PO, Purcena LL, Fernandes KF. Study of the cashew gum polysaccharide for the horseradish peroxidase immobilization—Structural characteristics, stability, and recovery. *Mater SciEng: C, 2010;30*:526-530.
12. Magalhães APSPA, Toma HK, do Carmo FA, Mansur CRE. Development of purified cashew gum mucoadhesive buccal tablets containing nystatin for treatment of oral candidiasis. Drug Dev Ind Pharm. 2021;47(5):825-837.
13. Shaikh R, Raj Singh TR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioall Sci. 2011;3(1):89-100.
14. Khutoryanskiy VV. Advances in mucoadhesion and mucoadhesive polymers. MacromolBiosci. 2011 Jun 14;11(6):748-764.
15. Kaur H, Yadav S, Ahuja M, Dilbaghi N. Synthesis, characterization and evaluation of thiolated tamarind seed polysaccharide as a mucoadhesive polymer. Carbohydr Poly. 2012;90(4):1543-1549.
16. Bernkop-Schnürch A. Thiomers: a new generation of mucoadhesive polymers. Adv Drug Deliv Rev. 2005;57(11):1569-1582.
17. Bandyopadhyay PK, Hasnain MS, Nayak AK. Thiolated polysaccharides in drug delivery, In NayakAK, Hasnain MS,(Editors), *Tailor-MadePolysaccharidesin DrugDelivery*, Academic Press, ElsevierInc., UnitedStates,2022, pp. 82-106.
18. Bhatia M, Ahuja M, Mehta H. Thiol derivatization of xanthan gum and its evaluation as a mucoadhesive polymer. CarbohydrPolym. 2015; 131:119-24.
19. Bahulkar SS, Munot NM, Surwase SS. Synthesis, characterization

of thiolated karaya gum, and evaluation of the effect of pH on its mucoadhesive and sustained release properties. CarbohydrPolym. 2015; 130:183-190.
20. Hornof MD, Kast CE, Bernkop-Schnürch A. *In vitro* evaluation of the viscoelastic properties of chitosan-thioglycolic acid conjugates. Eur JPharmBiopharm. 2003;55(2):185-190.
21. Bansal K, Rawat MK, Jain A, Rajput A, Chaturvedi TP, Singh S. Development of satranidazole mucoadhesive gel for the treatment of periodontitis. AAPS PharmSciTech. 2009;10(3):716-23.
22. Hasnain MS, Rishishwar P, Ali S, Nayak AK.Preparation and evaluation of aceclofenac dental past esusingdilleniafruitgum for periodontitis treatment.SNApplSci2020;2(3):1-8.
23. NayakAK,HasnainMS,MalakarJ. Development and optimization of hydroxyapatite-ofloxacinimplants for possible bone-implantable delivery in osteomyelitis treatment. Curr Drug Deliv.2013; 10:241-250.
24. Medeiros Borsagli FGL, Carvalho IC, Mansur HS. Amino acid-grafted and N-acylated chitosan thiomers: Construction of 3D bio-scaffolds for potential cartilage repair applications. Int J BiolMacromol. 2018; 114:270-282.