International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 1, 2023

Original Article

THIOLATED MORINGA EXUDATE GUM AS IMPROVED BIO-MUCOADHESIVE AGENT IN THE FORMULATIONS OF DENTAL PASTE AND GEL

PRANAB KUMAR BANDYOPADHYAY*, AMIT KUMAR NAYAK*

Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Mayurbhanj 757086, Odisha, India
Email: pranab_btpuri@yahoo.com

Received: 19 Oct 2022, Revised and Accepted: 18 Nov 2022

ABSTRACT

Objective: The objectives of the current study were to synthesize thiolated moringa exudate gum (TMEG) via thiolation of moringa exudate gum (MEG) and to evaluate TMEG as an improved bio-mucoadhesive agent in semi-solid formulations like dental paste and gel. MEG-and TMEG-based mucoadhesive dental pastes of aceclofenac and mucoadhesive gels of metronidazole were prepared and changes in bio-mucoadhesion capability were studied.

Methods: In the current study, extracted MEG was modified to synthesize TMEG via thiolation (by utilizing thioglycolic acid in an acidic milieu) to study improvement in bio-mucoadhesive capability. MEG-and TMEG-based mucoadhesive dental pastes of 1% w/w aceclofenac and mucoadhesive gels of 1% w/w metronidazole were prepared with MEG and TMEG (as mucoadhesive excipient) in order to evaluate a comparative view of improvement in bio-mucoadhesion.

Results: The yield percentage of TMEG was found to be 44.62% and the thiol group in TMEG was found 4.17 mmol of thiol group/g of MEG. FTIR analysis results indicated the thiolation of MEG in the synthesized TMEG. Both types of semi-solid formulations (mucoadhesive dental pastes of 1% w/w aceclofenac and mucoadhesive gels of 1% w/w metronidazole) prepared using TMEG as mucoadhesive excipient exhibited excellent improved *ex vivo* bio-mucoadhesion and a sustained pattern of drug-releasing over a prolonged period.

Conclusion: The synthesized TMEG can be used as an improved mucoadhesive agent in the designing of bio-mucoadhesive semi-solid formulations for prolonged drug delivery.

Keywords: Mucoadhesive polymer, Plant polysaccharide, Moringa exudate gum, Drug delivery, Dental paste, Mucoadhesive gel

DOI: https://dx.doi.org/10.22159/ijap.2023v15i1.46647. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Natural polysaccharides obtained from various sources are being used as natural mucoadhesive agents [1-3]. At present times, these polysaccharides have multiple uses in various industrial fields, like in the processed food, biomedical, pharmaceutical, water purification, engineering, and electronics industries [4-7]. These natural polysaccharides are biodegradable and biocompatible. Along with this, they are very easily available and economical. In the pharmaceutical industry, they are accepted as an important group of materials for multiple pharmaceutical uses [5, 6]. These polymers are modified physically or chemically to improve their utility in pharmaceuticals as well as in other existing and emerging fields [8-10]. Numerous techniques are being explored to exploit their application potentiality with high-end accuracy and specification. Many researchers approached techniques like thiolation, carboxymethylation, grafting, cross-linking, *etc*., to alter or improve the usefulness of natural polysaccharides [8, 10, 11]. The thiolation technique among these techniques is very important to improve the mucoadhesive potential of these natural polysaccharides [12, 13]. Many approaches were there to impart and improve mucoadhesive by forming hydrogen bonds, van der Waals force, ionic bonds, *etc* [14, 15]. However, these bonds are weak and provide insufficient bonding strength, thereby decreasing the residence time of materials in the desired site(s). However, modification of polysaccharides by thiolation technique makes the derivatives a stronger mucoadhesive agent by forming covalent bonds [13]. Thiolated polysaccharides are also called thiomers, which have active thiol groups in the molecules [12]. These thiol groups form disulfide bonds with cysteine present in the glycoprotein of the mucus membrane [16]. Disulfide bond formation enhances the bio-adhesion capability of the modified polysaccharide [12, 13]. Many polysaccharides have been successfully modified to thiomers by various investigators to improve mucoadhesivity. Psyllium husk [17], pectin [18], alginate [19], tamarind seed polysaccharides [15], gellan gum [20], xanthan gum [21], Karaya gum [22], *etc*., showed significant improvement in bio-mucoadhesive after derivatization.

An exudate from the bark of *Moringa oleifera* Lam. (family: Moringaceae) tree, called moringa exudate gum (MEG), contains huge amounts of polysaccharide [23, 24]. Acid hydrolysis of MEG produces residues like L-galactose, L-arabinose, L-rhamnose and L-gluconic acid. MEG has been used as a disintegrant, binding agent, and drug release retardant in several pharmaceutical formulations [23]. MEG possesses a lot of medicinal applications in the treatment of dysentery, intestinal cancer, and asthma [25, 26]. In this study, extracted MEG was modified to form thiolated moringa exudate gum (TMEG) in order to study improvement in bio-mucoadhesive capability. Two semi-solid formulations were prepared with MEG and TMEG in order to evaluate a comparative view of improvement in bio-mucoadhesion. MEG-and TMEG-based bio-mucoadhesive dental pastes of aceclofenac and bio-mucoadhesive gels of metronidazole were prepared and changes in bio-adhesion capability were studied. The uses of TMEG as a bio-mucoadhesive agent in mucoadhesive dental pastes and gels have not been reported till date. Therefore, the current research is novel of its kind.

MATERIALS AND METHODS

Materials

MEG was extracted from the crude bark exudate-materials of *Moringa oleifera* Lam. trees present in Jharpokharia area, district-Mayurbhanj, Odisha, India (Located at 22.17 °N 86.63 °E with an average elevation of 130 m or 430 ft) in the month of June, 2022 and taxonomical identification was carried out by the Department of Pharmacognosy, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia, Odisha India. The voucher specimen number of the sample is SIPS/COG/HERB/2201. Sample has been preserved in the laboratory for future reference. Both natural exudates and exudate materials were produced by stabbing the *Moringa oleifera* Lam. trees were collected from multiple trees. The exudate was purified, extracted, and dried. This extracted product (MEG) was used in this study.

Aceclofenac (BS Traders Pvt. Ltd., India), metronidazole (BS Traders Pvt. Ltd., India), thioglycolic acid (99%, Hi-Media Laboratories Pvt. Ltd.,

India), lactose (SD Fine Chemicals Ltd., India), calcium carbonate (Loba Chemie Pvt. Ltd., India), sodium lauryl sulfate (SD Fine Chemicals, India), Carbopol 940 (Loba Chemie Pvt. Ltd., India), potassium dihydrogen orthophosphate (SD Fine Chemicals, India), sodium hydroxide (SD Fine Chemicals, India), Ellman's reagent [DTNB; 5,5′-dithiobis (2-nitrobenzoic acid)] (Hi-Media Laboratories Pvt. Ltd., India), L-cysteine (Merck Specialties Pvt. Ltd., India), PEG 4000 (Loba Chemie Pvt. Ltd., India), glycerine (Loba Chemie Pvt. Ltd., India), methylparaben (SD Fine Chemicals, India), camphor (Qualigens Fine Chemicals, India), and hydrochloric acid (SD Fine Chemicals Ltd., India) were used. All other chemicals, reagents, and solvents were of analytical grade.

Methodology

Purification, isolation, and extraction of MEG

In brief, 500 g of dried exudate material from the bark of *Moringa oleifera* Lam. trees was soaked in 1 L of warm distilled water (45 °C) for 12 h with occasional stirring by a glass rod. The formed slurry was filtered with muslin cloth. Filtrate was mixed with an equal volume of acetone and the precipitate was separated and washed with acetone multiple times. Precipitate was dried in tray drier at 50 °C. Dried MEG was grounded with a mortar and pestle. Grinded powder was passed through a sieve (mesh size 80) and stored in an air-tight container inside a desiccator.

Thiolation of MEG

MEG was thiol-esterified by reacting it with thioglycolic acid in a strong acidic environment [15]. In brief, 12 ml of 80% thioglycolic acid was added to 50 ml of 2% w/v aqueous solution of MEG. Then, 4 ml of 7 N hydrochloric acid was added to it. The mixture was kept for 2.5 h at 80 °C to allow the completion of the esterification reaction. This mixture was then mixed with acetone to separate TMEG precipitate and it was dried in a tray drier at 50 °C. Dried TMEG was grounded with a mortar and pestle. TMEG powder was passed through a sieve (mesh size 80) and stored in an air-tight container inside a desiccator.

Estimation of thiol group content

Ellman's method was used to estimate the thiol group content in TMEG [15]. In brief, 50 mg sample of TMEG was dissolved separately in 25 ml of 1 N sodium hydroxide solution (aqueous) and mixed with 25 ml of 0.5 M phosphate buffer (pH 8.0). Then, 5 ml of Ellman's reagent (DTNB, 0.03% w/v) was added to this reaction mixture and kept for 2 h at room temperature. Thiol group content was calculated by measuring absorbance at 450 nm. A calibration curve was used in which L-cysteine solution was used.

Fourier transform-infra-red (FTIR) spectroscopy

The samples of MEG and TMEG were grinded with a definite quantity of KBr and small pellets were prepared by compression in a hydraulic press with 75 kg/cm^2 pressure for 30 sec. These pellets were scanned by a FTIR spectrophotometer (Perkin-Elmer Spectrum RX I, USA) in a scan range of 4000-400 cm^{-1} and analyzed.

Preparation of aceclofenac dental pastes using MEG and TMEG

Dental pastes containing aceclofenac (1% w/w) were prepared using MEG and TMEG (as mucoadhesive agents) by simple trituration method. Glycerin (28 g), methylparaben (0.50 g), MEG (2.50 g), sodium laurylsulfate (1.50 g) and half the quantity of distilled water were mixed together and then, triturated in a mortar and pestle to blend it, uniformly. Aceclofenac powder (1% w/w) was dispersed in the remaining half portion of water and it was added to the blended mixture in the pestle and mortar. This prepared mixture was triturated for 30 min. Calcium carbonate powder (41.50 g) was added gradually to this mixture with the help of a sieve (No. 75) and trituration continued during addition. Finally, camphor (1.5 g) was added to it and blended by trituration until a smooth, uniform paste was prepared. Dental pastes containing aceclofenac (1% w/w) were also prepared by the same procedure using 2.50 g of TMEG in place of MEG. The formulas of different dental pastes containing aceclofenac prepared using MEG and TMEG are given in table 1.

Table 1: Formula of different aceclofenac dental pastes prepared using MEG and TMEG

Codes	DP-MEG	DP-TMEG
Calcium carbonate (g)	41.50	42.00
Glycerine (g)	28.00	28.00
MEG (g)	2.50	-
TMEG (g)	-	2.50
Methyl paraben (g)	0.50	0.50
Camphor (g)	1.50	1.50
Sodium lauryl sulfate (g)	1.50	1.50
Aceclofenac (% w/w)	1.00	1.00
Water qs to 100 g	qs	qs

Evaluation of aceclofenac dental pastes

Determination of aceclofenac content

Dental pastes (5 mg) were separately taken and dissolved in 100 ml of distilled water by stirring it for 30 min with the help of a magnetic stirrer (Remi Motors, India). Rotational speed and temperature were maintained at 400 rpm and 40 °C, respectively. The solutions were separately filtered through a Whatman filter paper. Absorbances of the filtrates were separately measured in a UV-VIS spectrophotometer (Shimadzu, Japan) at the wavelength (λ_{max}) of 274.5 nm against the blank sample [29].

Determination of pH

Dental pastes (1 mg) were separately taken in glass beaker. Distilled water (100 ml) was added to both samples and dispersed uniformly with the help of a magnetic stirrer (Remi Motors, India) maintaining a rotational speed of 400 rpm. The pH of the solutions was separately measured within 5 min of preparation of dispersion [29].

Determination of viscosity

Dental pastes (1 mg) were separately taken in a glass beaker. Distilled water (100 ml) was added to both samples and dispersed uniformly with the help of a magnetic stirrer (Remi Motors, India) maintaining a rotational speed of 400 rpm. A cone and plate type viscometer (Brookfield, Middle-boro, MA) was used to measure the viscosity of prepared dental pastes at a spindle speed of 100 rpm and room temperature. Viscosity calculations were done in Rheocalc V2.6 software [29].

Determination of tube spreadability and tube extrudability

Dental pastes (1 mg) were separately taken on a square-shaped 100 cm^2 glass plate. Another plate of the same size and shape was placed over it. A weight of 2 kg was applied above the glass plate for 30 min. The diameter of the dental paste was measured in cm [30].

A clean collapsible lacquered aluminum tube with a tip opening of 5 mm was filled with dental pastes containing aceclofenac (1% w/w). The tube was pressed with fingertip and extrudability was determined by measuring the percentage of quantity, which had come out through 5 mm opening immediately after pressing with the fingertip [30].

***In vitro* drug release test**

Dialysis membrane (having molecular cut-off of 10 KDa) and a glass-made permeation cell (having both the ends open; height 10 cm,

outer diameter 3.7 cm, and inner diameter 3.1 cm) were used in the *in vitro* drug release test [29, 30]. Before use, the dialysis membrane was soaked with distilled water for a period of 24 h and then, it was attached to the end part of a glass-made permeation cell using strong glue. Dental pastes containing aceclofenac (1% w/w) were separately taken and placed in the permeation cell and were immersed below the surface of the medium contained in the receptor compartment. To be used as a receptor compartment, 100 ml phosphate buffer (pH 6.8) was taken in a beaker was employed in this test and the receptor medium was agitated by a magnetic stirrer (Remi Motors, India) at 37±0.5 °C. Samples (5 ml) from the receptor medium were collected at customary time interval and immediately replaced with equal volume of phosphate buffer. The collected samples were filtered through a Whatman filter paper. Absorbances of the filtrates were measured in a UV-VIS spectrophotometer (Shimadzu, Japan) at the wavelength (λ_{max}) of 274.5 nm against the blank sample.

Evaluation of ex vivo bio-mucoadhsivity

Buccal mucosa of goat was used for evaluation of *ex vivo* bio-mucoadhsivity of dental pastes containing aceclofenac by modified physical balance method [29]. It was collected from a local slaughterhouse within 1h of the slaughter of the animal and dipped into phosphate buffer (pH 6.8) after initial cleaning and immediately brought into the laboratory. The buccal mucosal layer was separated from collected buccal mucosa with the help of a sharp scalpel and immersed into phosphate buffer (pH 6.8) and incubated for 1 min at a temperature of 37±0.5 °C. All fat layers and undesirable tissues were removed before the separation of mucosal layers. The membrane was finally rinsed multiple times in phosphate buffer of pH 6.8 and used.

A modified physical balance was used to study the mucoadhesion capability of the prepared aceclofenac dental pastes. Processed goat buccal mucosal membrane was attached to the base below the left side of the balance pan with the help of glue. Another piece of processed goat buccal mucosal membrane was attached to the bottom of the pan with the help of glue. Aceclofenac dental pastes (1 g) were separately placed in between two membranes and pressed with fingertip for 5 min with moderate pressure in order to set the dental paste in between the membranes. Weight required in g to detach two membranes was noted and shear stress was determined [31].

$$\text{Force of adhesion (N)} = \text{MS} \times 9.81/1000$$

$$\text{Bonding strength (M/m2)} = \text{FA/SA}$$

MS = Mucoadhesive strength

FA = Force of adhesion

SA = Surface area of mucosal surface

Preparation of metronidazole gels using MEG and TMEG

In brief, 0.5% w/v aqueous solution of MEG was prepared separately and mixed with 1% w/v solution of metronidazole powder. This mixture solution was then mixed with a 1% w/v aqueous solution of Carbopol 974 P. The newly formed final mixture solution was allowed to stand for 12 h to allow sufficient hydration. The solution was then mixed with 1% w/v glycerin and mixed gently to make MEG-containing metronidazole gel. TMEG containing metronidazole gel was also prepared by the same procedure using a 0.5% w/v aqueous solution of TMEG in place of MEG. The formulas of different metronidazole gels prepared using MEG and TMEG are given in table 2.

Table 2: Formula of different metronidazole gels prepared using MEG and TMEG

Codes	G-MEG	G-TMEG
Carbopol 940 (% w/v)	1.00	1.00
Glycerine (% w/v)	0.50	0.50
MEG (% w/v)	0.50	-
TMEG (% w/v)	-	0.50
Metronidazole (% w/v)	0.50	0.50

Evaluation of metronidazole gels using MEG and TMEG

Determination of metronidazole content

Metronidazole gels (5 mg) were separately taken and dissolved in 100 ml of distilled water by stirring it for 30 min with the help of a magnetic stirrer (Remi Motors, India). Rotational speed and temperature were maintained at 400 rpm and 40 °C, respectively. The solutions were separately filtered through a Whatman filter paper. Absorbances of the filtrates were separately measured in a UV-VIS spectrophotometer (Shimadzu, Japan) at the wavelength (λ_{max}) of 320 nm against the blank sample [15].

Determination of pH

Metronidazole gels (1 g) were separately taken in a glass beaker and 100 ml solutions were separately prepared using distilled water. Determination of pH was done in the similar way as described in the previous section (where pH determination of pastes was stated).

Determination of viscosity

Metronidazole gels (1 g) were separately taken in a glass beaker and 100 ml solutions were separately prepared using distilled water. Determination of viscosity was done in the similar way as described in the previous section (where viscosity determination of pastes was stated) at a spindle speed of 100 rpm and room temperature using a cone and plate type viscometer (Brookfield, Middle-boro, MA) [30].

***In vitro* drug release test**

Metronidazole gels (1 g) prepared using MEG and TMEG were separately taken and placed inside a dialysis bag (having a molecular cut-off of 10 KDa) [15]. The dialysis bag (containing 1 g metronidazole gel) was attached to the paddle of a USP type II dissolution apparatus. The apparatus was filled with phosphate buffer (pH 6.8). The paddle was dipped in the phosphate buffer release medium and rotated at a speed of 50 rpm at 37 °C. Samples (5 ml) from the release medium were collected at the customary time interval and immediately replaced with an equal volume of phosphate buffer. The collected samples were filtered through a Whatman filter paper. Absorbances of the filtrates were measured in a UV-VIS spectrophotometer (Shimadzu, Japan) at the wavelength (λ_{max}) of 274.5 nm against the blank sample.

Evaluation of ex vivo bio-mucoadhsivity

Buccal mucosa of goat was used for evaluation of *ex vivo* bio-mucoadhsivity of metronidazole gels using by modified physical balance method [29]. The procedure was similar as described in the previous section (where an evaluation of *ex vivo* bio-mucoadhsivity of pastes was stated).

Statistical analysis

Statistical analysis of all data was completed using MedCalc software.

RESULTS AND DISCUSSION

Extraction of MEG and thiolation of MEG to produce TMEG

MEG is a plant exudate-derived polysaccharide posing an arabinogalactan structure reported to contain L-galactose, L-arabinose, L-rhamnose and L-gluconic acid residues [32]. The yield

of extracted MEG from the bark exudate-material of *Moringa oleifera* Lam. trees was found 18.54%. The extracted MEG was found as creamy in color and soluble in water (both hot and cold). The yield of the thiolated product-TMEG was found, 44.62%. The obtained TMEG was creamy-white colored and it was found soluble in water (both hot and cold). In the present investigation, the thiolation reaction among the hydroxyl (-OH) groups of MEG (from the bark exudate-material of *Moringa oleifera* Lam. trees) and carboxyl (-COOH) group of thioglycolic acid (used as thiolating agent) was performed to synthesize the thiolated product (i.e., TMEG). This reaction occurred in the acidic environment as 7 N hydrochloric acid was used in the thiolation reaction. A schematic representation of the thiolation of MEG using thioglycolic acid to synthesize the thiolated product (i.e., TMEG) is presented in fig. 1.

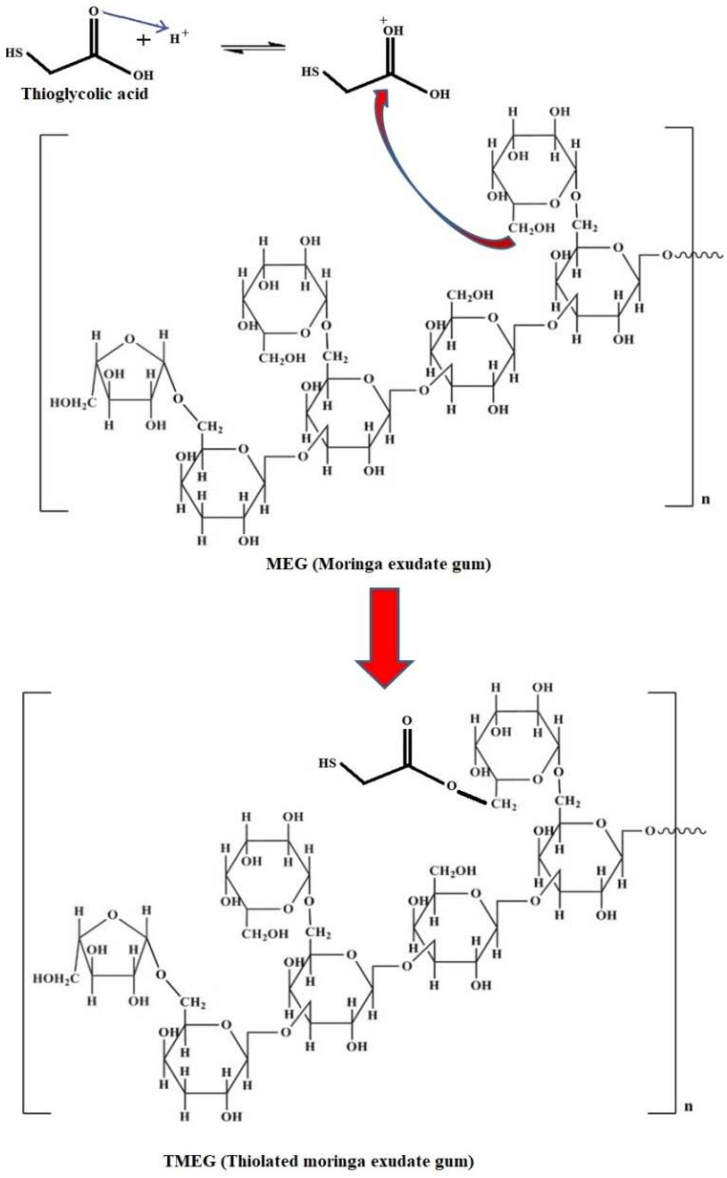

Fig. 1: Schematic representation of thiolation of MEG using thioglycolic acid to synthesize the thiolated product (i.e., TMEG)

Thiol group contents in TMEG

By Ellman's method, the thiol-group content was estimated in the synthesized thiolated product (i.e., TMEG). The content of the thiol group in TMEG was found to be 4.17 mmol of thiol group/g of MEG as estimated.

FTIR analysis

The FTIR spectrum of MEG (extracted from the bark exudate-material of *Moringa oleifera* Lam. trees and unmodified) and TMEG (synthesized and thiol-modified) is presented in fig. 2. The FTIR spectra of MEG (fig. 2a) presented characteristic peaks at 3423.97 cm^{-1} for–OH stretching vibration, at 2936.88 $cm^{-1}$for–CH stretching vibration of alkane, at 1623.49 cm^{-1} for-COOH stretching vibration of glucoronic acid and at 1073.65 cm^{-1} for C-O stretching of primary alcohol. In the FTIR spectrum of TMEG (fig. 2b), different characteristic peaks of the extracted MEG (unmodified) were observed to be present in a very minute/without any significant shifting or alteration of peaks. However, a weak shoulder at 2558.64 cm^{-1} for-SH stretching of the thiol group was identified in the FTIR spectrum of TMEG, which was not observed in the FTIR spectrum of unmodified MEG. This occurrence suggested the successful thiolation of MEG using thioglycolic acid. In recent studies, it has also been reported that the characteristic peaks representing the thiol group in polysaccharides is feebly detectable in the FTIR spectroscopy analysis [32, 33].

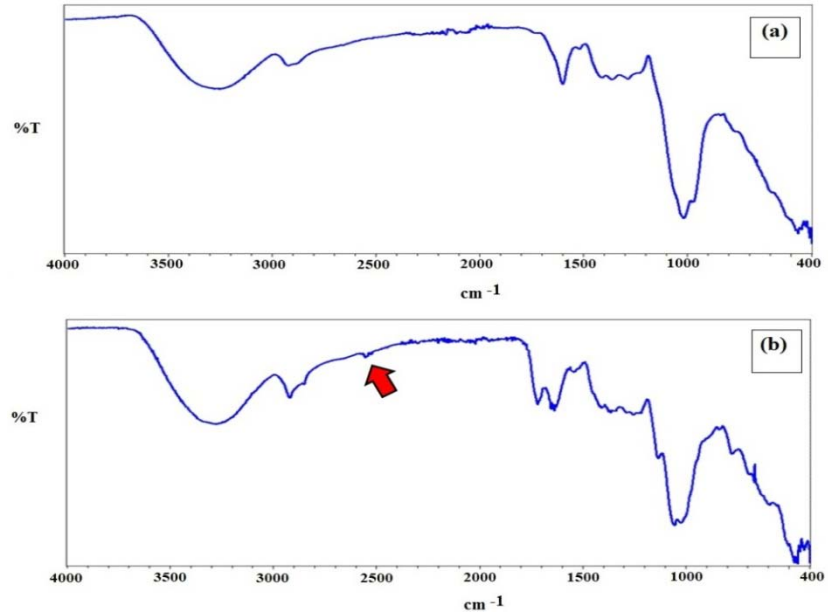

Fig. 2: The FTIR spectrum of: (a) MEG (extracted from the bark exudate-material of *Moringa oleifera* Lam. trees and unmodified) and (b) TMEG (synthesized and thiol-modified)

Preparation of aceclofenac dental pastes using MEG and TMEG

For the evaluation of the bio-mucoadhesivity potential of TMEG, aceclofenac dental pastes were prepared using the conventional triturating method, where 1% w/w aceclofenac was incorporated as a model drug (table 1). In these aceclofenac dental pastes (DP-MEG and DP-TMEG), calcium carbonate was incorporated as an abrasive agent, whereas unmodified MEG and synthesized TMEG were incorporated as mucoadhesive excipients. In addition, glycerin as cosolvent and humectant, sodium laurylsulphate as the surfactant, methylparaben as a preservative, and camphor as a flavoring agent were incorporated. Both the prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were white-colored and sticky in nature.

Evaluation of aceclofenac dental pastes

Aceclofenac content

The prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac presented aceclofenac content of 96.08±4.16% and 97.34±3.24%, respectively (table 3). The obtained result of aceclofenac content (within>95%) in both the medicated dental pastes indicated uniform mixing and distribution of aceclofenac with other excipients [29, 30].

Table 3: Aceclofenac content (%), pH, viscosity (cps), tube spreadability (cm) and tube extrudability (%) of aceclofenac (1% w/w) dental pastes containing MEG and TMEG (DP-MEG and DP-TMEG)

Results	DP-MEG	DP-TMEG
Aceclofenac content (%)[a]	96.08±4.16	97.34±3.24
pH	6.50	6.30
Viscosity (cps)	63240.38	58774.55
Tube spreadability (cm)[a]	6.33±0.40	6.40±0.32
Tube extrudability (%)[a]	87.02±2.43	91.42±4.75

Data are expressed as [a]mean±SD, n = 3

pH

The pH of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were 6.50 and 6.30, respectively (table 3). Both the pH of were very close to the standard range of oral pH and therefore, the result demonstrated that the prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac should not exert any irritation at the oral mucosal site after application [30].

Viscosity

Viscosities of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were 63240.38 cps and 58774.55 cps, respectively (table 3). From the obtained results, it was obvious that the dental paste containing TMEG (i.e., DP-TMEG) showed less viscosity than that of MEG.

Tube spreadability and tube extrudability

The tube spreadability of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were found at 6.33±0.40 cm and 6.40±0.32 cm, respectively (table 3). The tube spreadability of DP-TMEG dental paste was found to be higher than that of DP-TMEG dental paste. The higher tube spreadability by DP-TMEG dental paste could be due to less viscosity, which might facilitate spreading swiftly [29].

The tube extrudability of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were found 87.02±2.43% and 91.42±4.75%, respectively (table 3). The tube extrudability of DP-TMEG dental paste was found higher than that of DP-TMEG dental paste. The higher tube spreadability by DP-TMEG dental paste could be due to less viscosity, which might facilitate to extrude from the tube easily. The high quality of tube extrudability of pastes is advantageous for easy removal from the collapsible tubes during dispensing and, therefore, improves patient compliance [29].

***In vitro* drug release test**

The *in vitro* drug release studies of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac were studied in

phosphate buffer (pH 6.8). The comparative *in vitro* aceclofenac release from both these dental pastes is presented in fig. 3. The DP-TMEG dental pastes containing TMEG demonstrated a comparatively sustained pattern of aceclofenac releasing than that of DP-MEG dental paste containing MEG. Both the pastes were found to be sustained over a longer time period. The *in vitro* aceclofenac release result of DP-MEG and DP-TMEG dental pastes was not consistent with the viscosity result (as the DP-MEG dental pastes showed comparatively higher viscosity than that of DP-TMEG dental pastes). The comparatively sustained aceclofenac releasing from DP-TMEG dental pastes could be on account of *in situ* cross-linking, which might be contributed by the di-sulfide linkage of TMEG.

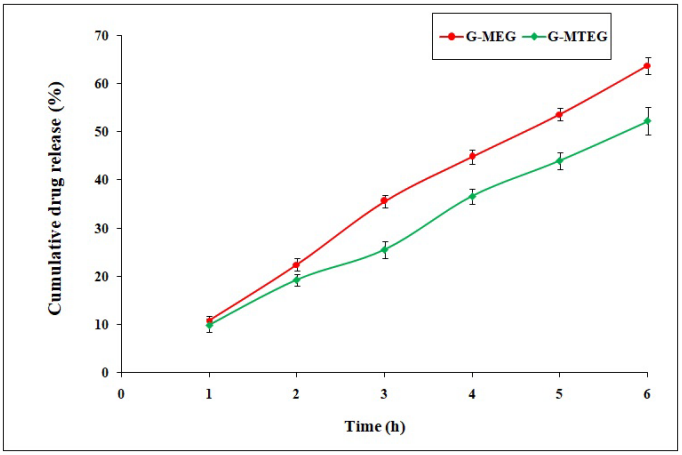

Fig. 3: Comparative *in vitro* aceclofenac release from 1% w/w aceclofenac dental pastes containing MEG and TMEG (DP-MEG and DP-TMEG) in phosphate buffer, pH 6.8 [Data are expressed as mean±SD, n = 3]

The model fitting results for *in vitro* aceclofenac release from 1% w/w aceclofenac dental pastes containing MEG and TMEG (DP-MEG and DP-TMEG) in phosphate buffer, pH 6.8 is presented in table 4. The DP-MEG and DP-TMEG dental pastes followed both the zero-order model ($Q = k_0 t + Q_0$; R^2 = 0.9947 and 0.9962, respectively) and the Korsmeyer–Peppas model ($Q = k_{KP} t^n$; R^2 = 0.9968 and 0.9967, respectively) as the best-fit model (table 4). Q and Q_0 symbolize the aceclofenac released at the time-point, t and 0, respectively; k_0, and k_{KP} symbolize rate constants in zero-order and Korsmeyer-Peppas models, respectively. Besides these, n symbolizes the release exponent. The release exponent values (n) of DP-MEG and DP-TMEG dental pastes were measured and these were 0.9890 and 0.9265, respectively, suggesting that the *in vitro* release of aceclofenac from these dental pastes followed the mechanism of case-II transport (n value ≥ 1). It suggested the polymeric chain relaxation-dependent drug releasing [30].

Table 4: The model-fitting results for *in vitro* aceclofenac release from % w/w aceclofenac dental pastes containing MEG and TMEG (DP-MEG and DP-TMEG)

Code	Zero-order model	First-order model	Higuchi model	Korsmeyer-Peppas model	Release exponent (n)
DP-MEG	0.9947	0.9069	0.7226	0.9962	0.9890
DP-TMEG	0.9968	0.9399	0.7343	0.9967	0.9265

Evaluation of *ex vivo* bio-mucoadhsivity

The *ex vivo* bio-mucoadhesion of prepared DP-MEG and DP-TMEG dental pastes containing 1% w/w aceclofenac (which were prepared using MEG and TMEG as mucoadhesive agents, respectively) onto the excised goat buccal mucosal membrane was tested via modified physical balance method. The *ex vivo* bio-mucoadhesion parameters (namely mucoadhesive strength, force of adhesion and bonding strength) of different dental pastes containing MEG and TMEG are presented in table 5. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of DP-MEG dental paste were found as 116.38±8.54 g, 1.14 x 10^{-4}N, and 40.20 x 10^{-2}N/m^2, respectively. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of DP-TMEG dental paste were found as 144.84±9.39 g, 1.42 x 10^{-4}N, and 50.03 x 10^{-2}N/m^2, respectively. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of DP-TMEG dental paste were relatively higher than those of DP-MEG dental paste. The bio-mucoadhesive potential of MEG (extracted and unmodified), when employed as mucoadhesive agent in the DP-MEG dental paste can be explicated by the fact of developing different kinds of noncovalent bonds (such as ionic bonds, hydrogen bonds, Van der Walls force, *etc.*) among-OH groups occurred in the MEG molecular structure and glycoproteins occurred in the mucus (as buccal mucosal membrane was used as mucosal surface in the *ex vivo* bio-mucoadhesion testing). However, noncovalent bonds are acknowledged for their weaker bio-mucoadhesion. On the contrary, the improved bio-mucoadhesivity of the DP-TMEG dental paste (prepared using TMEG as a mucoadhesive agent) can be explicated by the capability of developing stronger covalent bonds (owing to the formation of di-sulfide linkage) by thiol groups present in the TMEG (thiolated product) in contact with the glycoproteins occurred in the mucus. Therefore, TMEG can be utilized as an improved bio-mucoadhesive agent in dental paste-based systems for localized drug delivery.

Table 5: *Ex vivo* bio-mucoadhesivity results (mucoadhesive strength, force of adhesion and bonding strength) of aceclofenac (1% w/w) dental pastes containing MEG and TMEG (DP-MEG and DP-TMEG)

Code	Mucoadhesive strength (g)[a]	Force of adhesion (N)	Bonding strength (N/m^2)
DP-MEG	116.38±8.54	1.14 x 10^{-4}	40.20 x 10^{-2}
DP-TMEG	144.84±9.39	1.42 x 10^{-4}	50.03 x 10^{-2}

Data are expressed as [a]mean±SD, n = 3

Preparation of metronidazole gels using MEG and TMEG

For the evaluation of the bio-mucoadhesive potential of TMEG, mucoadhesive gels of metronidazole were prepared, where 1% w/w metronidazole was incorporated as a model drug. In these metronidazole gels (G-MEG and G-TMEG), 0.5% w/v Carbopol 940 was incorporated as a gel-producing excipient, whereas 0.5% w/v unmodified MEG and synthesized TMEG were incorporated as mucoadhesive excipients. In addition, 0.5% w/v glycerin was incorporated as a plasticizer. Both the prepared G-MEG and G-TMEG metronidazole gels were white-colored and sticky in nature.

Evaluation of metronidazole gels using MEG and TMEG

Metronidazole content

The prepared G-MEG gel and G-TMEG gel containing 1% w/w metronidazole presented metronidazole content of 95.86±3.70% and 96.35±4.22%, respectively (table 6). The obtained result of metronidazole content (within>95%) in both the medicated gels indicated uniform mixing and distribution of metronidazole with other excipients.

pH

The pHs of prepared G-MEG gel and G-TMEG gel containing 1% w/w metronidazole were 6.40 and 6.30, respectively (table 6). Both the pH was very close to the standard range of oral pH (pH 6.8) and therefore, the result demonstrated that the prepared G-MEG gel and G-TMEG gel should not exert any irritation at the oral mucosal site after application [15].

Viscosity

The viscosities of prepared G-MEG gel and G-TMEG gel containing 1% w/w metronidazole were 726.52 cps and 274.38 cps, respectively (table 6). From the obtained results, it was obvious that the gel containing TMEG (i.e., G-TMEG) showed less viscosity than that of MEG.

Table 6: Metronidazole content (%), pH and viscosity (cps) of 1% w/ w metronidazole gels containing MEG and TMEG (G-MEG and G-TMEG)

Results	G-MEG	G-TMEG
Metronidazole content (%)[a]	95.86±3.70	96.35±4.22
pH	6.40	6.30
Viscosity (cps)	726.52	274.38

Data are expressed as [a]mean±SD, n = 3

***In vitro* drug release test**

The *in vitro* metronidazole released from G-MEG and G-TMEG gels containing 1% w/w metronidazole was tested in phosphate buffer (pH 6.8). The comparative *in vitro* metronidazole released from both these gels is presented in fig. 4. The G-MEG gel containing MEG demonstrated almost the entire metronidazole release within 6 h. On the other hand, the G-TMEG gel containing TMEG demonstrated a comparatively sustained pattern of metronidazole release than that of G-MEG gel containing MEG. The comparatively sustained metronidazole release from G-TMEG gel could be on account of *in situ* cross-linking, which might be contributed by di-sulfide linkage of TMEG.

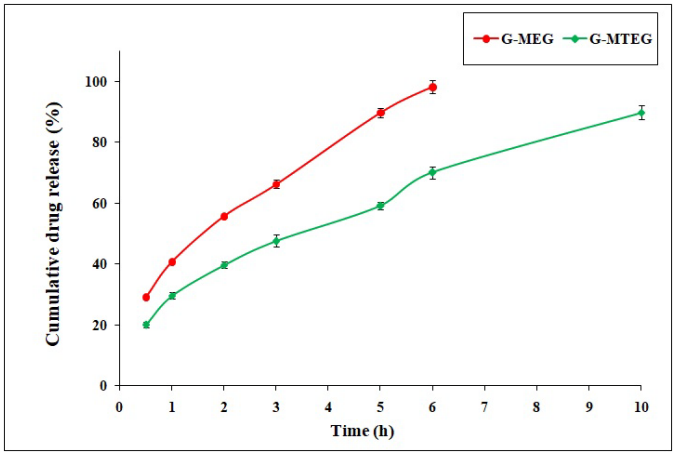

Fig. 4: Comparative *in vitro* metronidazole release from 1% w/w metronidazole gels containing MEG and TMEG (G-MEG and G-TMEG) in phosphate buffer, pH 6.8 [Data are expressed as mean±SD, n = 3]

The model fitting results for *in vitro* metronidazole release from 1% w/w metronidazole gels containing MEG and TMEG (G-MEG and G-TMEG) in phosphate buffer, pH 6.8 is presented in table 7. The G-MEG and G-TMEG gels followed both the Higuchi model ($Q = k_H t^{1/2}$; R^2 = 0.9967 and 0.9942, respectively) and Korsmeyer–Peppas model ($Q = k_{KP} t^n$; R^2 = 0.9976 and 0.9958, respectively) as best-fit model (table 7). Q and Q_0 symbolize the aceclofenac released at the time-point, t and 0, respectively; k_H, and k_{KP} symbolize rate constants in the Higuchi model and the Korsmeyer-Peppas model, respectively. Besides these, n symbolizes the release exponent. The release exponent values (n) of G-MEG and G-TMEG gels were measured and these were 0.4845 and 0.4875, respectively, suggesting demonstrated diffusion-dependent releasing.

Table 7: The model-fitting results for *in vitro* metronidazole release from 1% w/w metronidazole gels containing MEG and TMEG (G-MEG and G-TMEG)

Code	Zero-order model	First-order model	Higuchi model	Korsmeyer-Peppas model	Release exponent (n)
G-MEG	0.9871	0.9219	0.9967	0.9976	0.4845
G-TMEG	0.9722	0.8591	0.9942	0.9958	0.4875

Evaluation of *ex vivo* bio-mucoadhsivity

The *ex vivo* bio-mucoadhesion of G-MEG gel and G-TMEG gel containing 1% w/w metronidazole (which were prepared using MEG and TMEG as mucoadhesive agents, respectively) onto the excised goat buccal mucosal membrane was tested via modified physical balance method. The *ex vivo* bio-mucoadhesion parameters (namely mucoadhesive strength, force of adhesion and bonding strength) of different metronidazole gels containing MEG and TMEG are presented in table 8. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of G-MEG metronidazole gel were found as 8.22±0.37 g, 8.06 x 10^{-2}N, and 283.94 N/m^2, respectively. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of G-TMEG metronidazole gel were found as 10.05±0.48 g, 9.86 x 10^{-2}N, and 347.15 N/m^2, respectively. The *ex vivo* mucoadhesive strength, force of adhesion and bonding strength values of G-TMEG metronidazole gel were relatively higher than those of G-MEG metronidazole gel. These results were also similar to the results of the mucoadhesive dental pastes of 1% w/w aceclofenac as presented earlier. Similarly, the improved bio-mucoadhesive by the G-TMEG metronidazole gel (prepared using TMEG as a mucoadhesive agent) can be explicated by the capability of developing stronger covalent bonds (owing to the formation of di-sulfide linkage) by thiol groups present in the TMEG (thiolated product) in contact with the glycoproteins occurred in the mucus (comparable to the mucoadhesive dental pastes of 1% w/w aceclofenac as presented earlier). Therefore, TMEG can be utilized as an improved bio-mucoadhesive agent in bio-mucoadhesive gel-based systems.

Table 8: *Ex vivo* bio-mucoadhesive results (mucoadhesive strength, force of adhesion and bonding strength) of 1% w/w metronidazole gels containing MEG and TMEG (G-MEG and G-TMEG

Code	Mucoadhesive strength (g)[a]	Force of adhesion (N)	Bonding strength (N/m^2)
G-MEG	8.22±0.37	8.06 x 10^{-2}	283.94
G-TMEG	10.05±0.48	9.86 x 10^{-2}	347.15

[a]mean±SD, n = 3

CONCLUSION

Natural polysaccharides are natural mucoadhesive agents due to the presence of numerous hydroxyl groups in molecules. MEG is also a natural polysaccharide and its mucoadhesive potential is improved by thiolation. The yield percentage of the thiolated product was 44.62%, which is a good production rate. Thiol group content was measured by Ellman's method and it was found that TMEG contains 4.17 mmol of the thiol group. Gel and paste were prepared separately by both MEG and TMEG to study its impact on drug release and bio-mucoadhesive in various formulations. Metronidazole gel prepared with TMEG shows 22% more mucoadhesive strength as well as force of adhesion. Metronidazole release from prepared gel fit into various kinetics models and this data suggests diffusion-dependent release. Aceclofenac paste prepared with MEG and TMEG showed a 24% increase in bio-mucoadhesion potential. Aceclofenac release study of the paste fit into various kinetic models suggesting a polymeric chain relaxation-dependent drug release. It can be concluded that the thiolation of MEG produces a product which can produce formulations which show improved bio-mucoadhesivity.

ACKNOWLEDGEMENT

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

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Prezotti FG, Siedle I, Boni FI, Chorilli M, Muller I, Cury BSF. Mucoadhesive films based on gellan gum/pectin blends as a potential platform for buccal drug delivery. Pharm Dev Technol. 2020 Feb;25(2):159-67. doi: 10.1080/10837450.2019.1682608, PMID 31623500.
2. Hasnain MS, Rishishwar P, Ali S. Floating-bioadhesive matrix tablets of hydralazine HCl made of cashew gum and HPMC K4M. Int J Pharm Pharm Sci. 2017 Jul;9(7):124-9. doi: 10.22159/ijpps.2017v9i7.18945.
3. Ratha Adhikari SN, Panda S. Development evaluation and characterization of buccal patches containing atenolol using hydrophilic polymers. Int J App Pharm. 2022 Mar-Apr;14(2):61-7. doi: 10.22159/ijap.2022v14i2.43589.
4. Lovegrove A, Edwards CH, De Noni I, Patel H, El SN, Grassby T. Role of polysaccharides in food, digestion, and health. Crit Rev Food Sci Nutr. 2017 Jan 22;57(2):237-53. doi: 10.1080/10408398.2014.939263, PMID 25921546.
5. Das S, Ghosh A, Das R, Nandi G, Ghosh LK. Development of quality control parameters for standardization of a novel mucilage obtained from okra (*Abelmoschus esculentus* (L.) Moench) fruit. Int J Curr Pharm Res. 2021 May;13(3):28-41.
6. Hasnain MS, Rishishwar P, Ali S. Use of cashew bark exudate gum in the preparation of 4 % lidocaine HCl topical gels. Int J Pharm Pharm Sci. 2017 Aug;9(8):146-50. doi: 10.22159/ijpps.2017v9i8.19815.
7. Nazarzadeh Zare E, Makvandi P, Tay FR. Recent progress in the industrial and biomedical applications of tragacanth gum: a review. Carbohydr Polym. 2019 May 15;212:450-67. doi: 10.1016/j.carbpol.2019.02.076, PMID 30832879.
8. Rani N, Dev D. Formulation and evaluation of fast disintegrating tablet of propranolol hydrochloride using modified tamarind seed gum as a natural superdisintegrant. Asian J Pharm Clin Res. 2022 Sep;15(9):185-92. doi: 10.22159/ajpcr.2022.v15i9.45284.
9. Sen SO, Nayak AK, Devbhuti P, Ghosh A. Modification of gums by periodate oxidation: A natural cross-linker. Int J Pharm Pharm Sci. 2019 Jul;11(7):1-6. doi: 10.22159/ijpps.2019v11i7.31058.
10. Kaur L, Gupta GD. Gum karaya-g-poly (acrylamide): microwave assisted synthesis, optimisation and characterisation. Int J App Pharm. 2020;12(5):143-52. doi: 10.22159/ijap.2020v12i5.37732.
11. Hasnain MS, Al-Muhanna MK, Anwar N, Siddique MUM, Ahsan MN, Ahmed SA. Drug delivery dosage forms made of tailor-made natural polysaccharides. In: Nayak AK, Hasnain MS, editors. Tailor-made polysaccharides in drug delivery. Academic Press, Elsevier Inc; 2022. p. 29-62.
12. Naveen NR, Gopinath C, Rao DS. A spotlight on thiolated natural polymers and their relevance in mucoadhesive drug delivery system. Future J Pharm Sci. 2018; Jun 4;4(1):47-52. doi: 10.1016/j.fjps.2017.08.002.
13. Bandyopadhyay PK, Hasnain MS, Nayak AK. Thiolated polysaccharides in drug delivery. In: Nayak AK, Hasnain MS, editors. Tailor-made polysaccharides in drug delivery. Academic Press, Elsevier Inc; 2022. p. 82-106.
14. Nayak AK, Pal D, Santra K. Development of pectinate-spatula mucilage mucoadhesive beads of metformin HCl by central composite design. Int J Biol Macromol. 2014 May;66:203-11. doi: 10.1016/j.ijbiomac.2014.02.023, PMID 24560618.

15. Kaur H, Yadav S, Ahuja M, Dilbaghi N. Synthesis, characterization and evaluation of thiolated tamarind seed polysaccharide as a mucoadhesive polymer. Carbohydr Polym. 2012 Nov 6;90(4):1543-9. doi: 10.1016/j.carbpol.2012.07.028.
16. Grewal P, Mundlia J, Ahuja M. Thiol modified Moringa gum–a potential bioadhesive polymer. Carbohydr Polym. 2019 Apr 1;209:400-8. doi: 10.1016/j.carbpol.2018.12.100, PMID 30732824.
17. Bhatia M, Ahuja M. Thiol modification of psyllium husk mucilage and evaluation of its mucoadhesive applications. Scientific World Journal. 2013 Nov 20;2013:284182. doi: 10.1155/2013/284182, PMID 24348147.
18. Hintzen F, Hauptstein S, Perera G, Bernkop Schnürch A. Synthesis and *in vitro* characterization of entirely S-protected thiolated pectin for drug delivery. Eur J Pharm Biopharm. 2013 Nov;85:1266-73. doi: 10.1016/j.ejpb.2013.09.017, PMID 24095893.
19. Khalid S, Abbas G, Hanif M, Shah S, Shah SNH, Jalil A. Thiolated sodium alginate conjugates for mucoadhesive and controlled release behavior of metformin microspheres. Int J Biol Macromol. 2020 Dec 1;164:2691-700. doi: 10.1016/j.ijbiomac.2020.08.116, PMID 32810533.
20. Jalil A, Asim MH, Le NN, Laffleur F, Matuszczak B, Tribus M. S-protected gellan gum: a decisive approach towards mucoadhesive antimicrobial vaginal films. Int J Biol Macromol. 2019 Jun 1;130:148-57. doi: 10.1016/j.ijbiomac.2019.02.092, PMID 30779984.
21. Bhatia M, Ahuja M, Mehta H. Thiol derivatization of xanthan gum and its evaluation as a mucoadhesive polymer. Carbohydr Polym. 2015 Oct 20;131:119-24. doi: 10.1016/j.carbpol.2015.05.049, PMID 26256167.
22. Bahulkar SS, Munot NM, Surwase SS. Synthesis, characterization of thiolated karaya gum and evaluation of effect of pH on its mucoadhesive and sustained release properties. Carbohydr Polym. 2015 Oct 5;130:183-90. doi: 10.1016/j.carbpol.2015.04.064, PMID 26076615.
23. Badwaik HR, Hoque AA, Kumari L, Sakure K, Baghel M, Giri TK. Moringa gum and its modified form as a potential green polymer used in biomedical field. Carbohydr Polym. 2020 Dec 1;249:116893. doi: 10.1016/j.carbpol.2020.116893, PMID 32933701.
24. Gupta S, Kachhwaha S, Kothari SL, Bohra MK, Jain R. Surface morphology and physicochemical characterization of thermostable moringa gum: a potential pharmaceutical excipient. ACS Omega. 2020 Nov 4;5(45):29189-98. doi: 10.1021/acsomega.0c03966, PMID 33225150.
25. Sharma K, Kumar M, Waghmare R, Suhag R, Gupta OP, Lorenzo JM. Moringa (*Moringa oleifera* Lam.) polysaccharides: extraction, characterization, bioactivities, and industrial application. Int J Biol Macromol. 2022 Jun 1;209(A):763-78. doi: 10.1016/j.ijbiomac.2022.04.047, PMID 35421412.
26. Nayak AK, Hasnain MS, Dhara AK, Pal D. Plant polysaccharides in pharmaceutical applications. In: Pal D, Nayak AK, editors. Bioactive natural products for pharmaceutical applications, advanced structured materials. Vol. 140. Berlin: Springer; 2021. p. 93-125. doi: 10.1007/978-3-030-54027-2_3.
27. Gupta S, Jain R, Kachhwaha S, Kothari SL. Nutritional and medicinal applications of Moringa oleifera lam.-review of current status and future possibilities. J Herb Med. 2018 Mar;11:1-11. doi: 10.1016/j.hermed.2017.07.003.
28. Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or pharmacological potential of *Moringa oleifera* lam. Nutrients. 2018 Mar 12;10(3):343. doi: 10.3390/nu10030343, PMID 29534518.
29. Hasnain MS, Rishishwar P, Rishishwar S, Ali S, Nayak AK. Extraction and characterization of cashew tree (*Anacardium occidentale*) gum; use in aceclofenac dental pastes. Int J Biol Macromol. 2018 Sep;116:1074-81. doi: 10.1016/j.ijbiomac.2018.05.133, PMID 29791876.
30. Hasnain MS, Rishishwar P, Ali S, Nayak AK. Preparation and evaluation of aceclofenac dental pastes using Dillenia fruit gum for periodontitis treatment. SN Appl Sci. 2020 Feb 17;2(3):1-8. doi: 10.1007/s42452-020-2240-3.
31. 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 May 29;10(3):716-23. doi: 10.1208/s12249-009-9260-z, PMID 19479385.
32. Grewal P, Mundlia J, Ahuja M. Thiol modified Moringa gum–a potential bioadhesive polymer. Carbohydr Polym. 2019 Apr 1;209:400-8. doi: 10.1016/j.carbpol.2018.12.100, PMID 30732824.
33. 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 Biol Macromol. 2018 Jul 15;114:270-82. doi: 10.1016/j.ijbiomac.2018.03.133, PMID 29578009.