Original Article

Assessment of Mucoadhesion Potential of Thiolated Pectin Extracted from *Citrus limon*

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ABSTRACT

Aim: This study aimed to extract pectin from *Citrus limon*, synthesize thiolated pectin (TFP), and assess its mucoadhesion capability utilizing *in-vitro* and *ex-vivo* methodologies. **Materials and Methods:** Wilhelmy`s approach, the falling sphere method, the modified physical balancing method, and others were used to measure the mucoadhesion force of pectin and TFP and compared with chitosan (CS) and sodium carboxymethylcellulose (SCMC). **Results:** FTIR spectra containing carboxyl and hydroxyl groups confirmed that pectin formed a hydrogen connection with the mucosa. The outcome demonstrates that, under the experimental setup utilized in this study, pectin's mucoadhesive qualities were equivalent to CS but less so than SCMC. The ability of the bulk of these polymers to adhere to one another is dependent on non-covalent chemical connections such as hydrogen bonds, ionic contacts, and van der Waals forces, amongst other types of interactions. Since these interactions are not as strong as covalent connections, localization of dosage forms cannot be guaranteed. To confirm the enhanced mucoadhesion potential of pectin through the thiolation procedure, pectin was thiolated and TFP was assessed for mucoadhesion potential using the same methodologies. **Conclusion:** In nutshell, pectin has good mucoadhesion properties, but to use pectin in mucoadhesion drug delivery systems, it is necessary to increase pectin's mucoadhesion potential through appropriate methods like thiolation, etc.

Keywords: Mucoadhesion, Pectin, Thiolation, Chitosan, Sodium Carboxymethylcellulose.

INTRODUCTION

The process of attaching two surfaces is called adhesion. Mucoadhesion is the word used to describe adherence to the mucosal membrane.[1] Since the 1980s, when the idea of mucoadhesion was first proposed, numerous initiatives have been undertaken to enhance the adhesive characteristics of polymers.[2-6] Weak bioadhesion is exhibited by mucoadhesive polymers because they are based on the development of non-covalent connections.[7-13] These types of bonding include hydrogen bonds, van der Waal forces, and ionic interactions.

Thiolated polymers also referred to as thiomers, are a novel class of polymeric matrices that imitate the way released mucus glycoproteins adhere to mucus layers naturally by covalently joining them via disulfide bonds.[14] The mucoadhesive polymer and the mucus layer are connected by disulfide bonds created when the thiol side chains of thiolated polymers contact the cysteine-rich subdomains of the mucus glycoprotein.[15,16] These polymers have improved mucoadhesive properties because the drug is localized at the disease site. They also have a longer residence time on mucosal tissue for delivery systems and high cohesive properties that prevent the failure of adhesive bonding. Many polymers have successfully undergone thiolation processes, including chitosan (CS), poly(acrylic acid), alginate, and polycarbophil.[17,18]

One of the most popularly growing tree fruits worldwide is the lemon (*Citrus limon* from the *Rutaceae* family). In terms of both economic worth and overall production, citrus fruits lead the pack. Peels (rind skin) and pulp make up the two sections of citrus fruits. The pulp, which is the fruit's

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edible portion, may be easily distinguished from the peels, which are a good source of pectin.[19] Natural polysaccharides and their derivatives are extensively used as biodegradable and biocompatible polymers in the pharmaceutical and food industries. A low-cost, secure polymer obtained from the citrus peel or apple pomace, pectin is a heterogenous polysaccharide. It contains linear chains of (1-4) connected β-d-galacturonic acid residues that are infrequently interrupted by rhamnogalacturonic acid residues, as well as α-l-rhamnopyranose via -1,2 linkage. Backbone galacturonic acid has some methyl esterification.[20] Due to its advantageous gelling qualities, pectin is frequently utilized in food technology.

A variety of pharmacological and biological uses benefit from pectin's biocompatibility, biodegradability, and lack of toxicity.[21] In pharmaceutical science, it is also employed and researched as a carrier and coating substance. It can be used as a medication delivery device for the best drug distribution and target release due to its bioadhesive characteristics toward gastrointestinal tissues.[18,22] The goal of the current work is to enhance pectin's mucoadhesive function by the creation of pectin thioglycolic acid conjugate. Using freshly excised intestinal mucosa, an *ex vivo* bioadhesion study was used to compare the mucoadhesive characteristics of pectin and TFP.

MATERIALS AND METHODS

Pectin was purchased from Sigma Aldrich, St. Louis, MO, USA. CS and sodium carboxymethylcellulose (SCMC) was bought from Yarrow Chemicals Ltd, Mumbai, India. The gum was produced from fresh citrus fruits that were acquired at the neighborhood market. The remaining solvents and chemicals used were of analytical grade.

Method of Isolation and Extraction

The methodology described by Alfa Ezhil Rose *et al.* for pectin extraction was used.[23] Distilled water (150 ml) was mixed with 5 g of powdered lemon peel. To keep the pH constant (1.5), around 0.8 ml of 70% HNO_3 was added. The aforementioned solution was agitated for 60 min at 80°C to generate an acid extract, which was then filtered three times before being collected separately. This was allowed to coagulate for three hours at 4°C using an equal volume of 99.1% ethanol. Centrifugation was used to separate the insoluble fraction, which was subsequently cleaned with 55% and 75% ethanol, respectively.

Characterization of Pectin[24]

The extracted pectin was characterized for the following parameters.

Moisture Content

One gram of the material was weighed in desiccators, dried in an oven set to 100°C for four hours, and then chilled over silica gel. To achieve agreement with the Fischer method, the observed percent moisture is multiplied by one percent.

Equivalent Weight

The amount of esterification and anhydrouronic acid concentration is determined using equivalent weight. Samples equivalent to 0.5 g were taken in glass contained and 5 ml of ethanol was added and mixed. Then, 100 ml of distilled water and 1 g of salt chloride were also added to improve the terminal point. The last step was adding 6 drops of phenol red and titrating it against 0.1 N sodium hydroxide. Purple was used to signify the titration point. This neutralized solution was kept to measure the amount of methyl.

$$\text{Equivalent weight} = \frac{\text{Weight of sample} \times 1000}{\text{ml of alkali} \times \text{normality of alkali}}$$

Methoxyl Content

By saponifying the pectin and titrating the released carboxyl groups, the methyl concentration was ascertained. After determining the neutral solution's equivalent weight, sodium hydroxide (0.25 N) was added to 25 ml. The mixture was briskly stirred, then left to stand for 30 min at 25°C. After waiting for 30 min, 25 ml of 0.25 N HCl was added, and it was titrated using the equivalent weight titration method against 0.1 N sodium hydroxide to the same endpoint as before.

$$\text{Methoxyl content \%} = \frac{\text{ml of alkali} \times \text{Normality of alkali} \times 3.1}{\text{Weight of sample}}$$

Estimation of Anhydrouronic acid

By using equivalent weight and the methoxyl content value, it is important to know the anhydrouronic acid concentration to assess the purity and level of esterification. The total AUA of pectin was calculated using the following formula.

$$\text{\% of AUA} = \frac{176 \times 0.1z \times 100w \times 1000}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

Where AUA's molecular weight unit is 176 g, z is the corresponding weight in ml of sodium hydroxide, y is the methoxyl content in ml of sodium hydroxide, and w is the sample's weight.

Determination of degree of esterification (DE)

Based on methoxyl and AUA levels, the DE of pectin was assessed and determined using the formula;[25]

$$\% \text{ DE} = \frac{176 - \% \text{ Methoxyl content}}{31 - \% \text{ AUA}} \times 100$$

Where AUA=Anhydrouronic acid content

Flow Properties

The flow characteristics of extracted pectin were assessed for the angle of repose, bulk density, tapped density, Hausner's ratio, and compressibility index.

Synthesis of TFP[26,27]

The isolated pectin was esterified using thioglycolic acid in the presence of HCl to obtain TFP. Six grams of pectin were mixed with 50 ml of hot distilled water. Thioglycolic acid (3.6 ml) was combined with 2 ml of HCl (7N), and the mixture was then left to react for around two and a half hours at 80°C. In order to create the final precipitate, the reaction mixture was transferred into 0.5 liters of methanol and then discarded. After two or three washing in methanol, this was allowed to dry and then placed in a desiccator for storage. TFP was produced in an environment where there was no oxygen (carried out under argon).

Characterization of TFP

Thiol conjugation was confirmed by characterizing TFP in the following methods.

FTIR

The interaction of electromagnetic radiation with rotational or vibrational resonances inside a molecular structure is investigated using infrared spectroscopy. Using the KBr disc approach, the polymer's FTIR spectra were captured. The reference (sigma standard) and the measured scan were then compared.[27,28]

XRD analysis

TFP's crystallinity was investigated utilizing XRD analysis using Inxitu Benchtop XRD. The subject material is ground into a fine powder and homogenized before the examination, and the bulk composition is determined based on an average. The following settings were used to scan polymers in their ambient state between 5 and 80 diffraction angles: Voltage: 35 kV, current: 30 mA, and 5 scans per minute.[29,30]

Assessment of mucoadhesion potential of pectin and TFP

Through *in vitro* and *ex vivo* procedures, the mucoadhesive properties of the extracted pectin and TFP were compared with those of CS and SCMC. The mucous was purchased from a local market, for *ex vivo* methods after an animal was sacrificed, and kept there until it was removed for the experiment using Tyrode's solution.

Mucoadhesion studies via rotating cylinder

At a compression force of 5 tonnes, 200 mg of mucoadhesive polymers were pressed for thirty seconds using a die with a diameter of 13 mm on an infrared hydraulic press (Perkin Elmer, England). The discs that were manufactured had a diameter of 13 mm and an average thickness of 1.26 mm. By following the steps outlined below, the adhesion time of the produced discs to the stomach mucosa was determined using the rotating cylinder method. Before the mucosa was stretched onto a cylinder made of stainless steel, discs were connected to newly excised areas of it (approximately 12 cm^2 in total) (diameter 2.5 cm, height 3.7 cm; apparatus I-Basket, USP). After being completely submerged in 100 ml of simulated stomach fluid at a temperature of 37.2 degrees Celsius, the cylinder was then moved into the dissolution device (USP Type-I). The cylinder was spun at a rate of 125 revolutions per minute (RPM). Every thirty minutes, the test system was subjected to a visual inspection, and the resulting changes were meticulously documented. This process continued until all of the discs had either dissolved or been detached from the mucosa. At a minimum of five different times, this test was performed on each polymer disc.[31]

Shear stress measurement[32]

Two 10 ×10 × 0.5 centimeter-thick rectangular glass plates were used to make up the device, similar to one described in the literature.[18] A table now has one of the glass plates permanently adhered to its surface. The second glass plate, which is suspended from a pan by a wire and pulley and is located above the first glass plate, is in the uppermost position. It will take more force to separate the upper plate if any mucoadhesive material is sandwiched between the plates. Pectin and TFP are manufactured in a range of concentrations, such as 0.5% and 1.5% w/v. A 100 g weight was placed on the upper plate to disperse the pectin and TFP solution between the two plates after it had been deposited in a predetermined volume between the plates. The weight that had been left on the upper plate was removed after five minutes. The weights on the pan steadily increased until the upper plate separated from the bottom plate. The test was once more carried out by extending the solution's contact time with the plates (up to 30 min). This weight placement has been recorded. Similar to

how it was done the first time, the test is carried out again with the following solutions using 0.5 and 1.5% w/v of CS and SCMC.

Wilhelmy's Method[33]

The mucous solution is contained in a beaker that serves as the equipment, as described in the literature.[18] A thread and two pulleys were used to attach a rectangular glass plate (2 × 5 cm) to a weight tray. On a suitable stand, the assembly was fixed for the experimental configuration. The counterweight tray and the glass plate weigh the same amount, maintaining an equal opposing force.

Pectin (1% w/v) and TFP were applied to the glass plate, which was then dried for three hours at 60°C. The mucous solution kept in the beaker was then allowed to contact the covered glass plate. The weight tray had a glass plate attached to it, and the weight on the tray was regulated to keep the glass plate still. The glass plate is left submerged in the mucous solution for the allotted amount of time. The weight on the weight tray was gradually increased after the predetermined amount of time until the glass plate was pulled free from the solution. It was observed how many grams were needed to separate the glass plate. Using a freshly coated plate and changing the glass plate's contact time with the mucous solution, the same operation was carried out once more. For the remaining mucoadhesive materials, CS and SCMC, this experiment was run.

Falling sphere method[34]

The falling sphere method was carried out using a device described in the literature.[18] A clean burette was placed in a stainless steel stand with a 10% mucous solution. The selection and grouping of several mustard grains with comparable weights. The needed amount of mucoadhesive substance was put onto each set of mustards. When a grain of coated mustard was thrown into the burette, the amount of time it took the mustard to move from zero to the fifth mark was noted. The same technique was carried out again using mustards coated with various amounts of the same mucoadhesive substance. All of the mucoadhesive compounds were subjected to the entire set of experiments at various doses.

Detaching Force Measurement Apparatus[35,36]

The apparatus described in the earlier study was used to measure the detaching force.[18] The right arm of a physical balance was placed underneath a sufficient piece of stomach mucosa that had been cut off and stretched on a glass plate. A different glass plate was attached to the balance's modified right arm. Both of the glass plates were superimposed on one another by the assembly on the right arm.[37] The mucoadhesive tablets prepared with test polymers were applied to the gut that had been stretched. A drop of water is used to moisten the glass plate, which is suspended from the right arm and permitted to touch the tablet resting on the skin.[38,39] Before the tablet is placed, a beaker that is attached to the left arm is calibrated to counteract the equal weight of the right arm assembly. Water was slowly added to the conical flask, increasing the weight on the left arm. It is calculated how much water is needed to lift the glass plate and tablet out of the intestinal skin. Subtract the tablet's weight from the water poured into the left arm to determine the separation weight. The same technique was carried out again using fresh tablets made of the same mucoadhesive substance at contact durations of 5, 10, 15, and 30 min. For all mucoadhesive compounds, the entire experiment is conducted again.[40]

The following formula is used to get the force in Newton's;

$$F = \frac{0.00981}{2} \times W$$

Where W is the amount of water.

RESULTS AND DISCUSSION

The results of various physicochemical characteristics of extracted pectin were summarized in Table 1. A 78.9% yield for the pectin extraction was demonstrated (Figure 1). The percentage of moisture content of the pectin was confirmed to be satisfactory and within limits (below 2%). Nitric acid was used to extract pectin from powdered lemon peel, and the equivalent weight was discovered to be 125. A higher equivalent weight would result in more gel formation. Methoxyl concentration was discovered to be 18.6% in pectin. Methoxyl content plays a significant role in regulating pectins' ability to set quickly and form gels as well as their gel-forming capability. Accordingly, the extracted pectin's AUA level was discovered to be 141.08%. The AUA measures the extracted pectin's purity and shouldn't be less than 65%. As mentioned in Food Chemical Codex, 1996.[41] The DE can be expressed as a percentage of the total amount of esterified carboxyl groups or as a percentage of the total amount of methoxylated pectin. DE can be between 0 to 100%, and the observed value was 74%. High methoxyl pectins are those that have a DE greater than 50%, whereas low methoxyl pectins have a DE that is less than 50%. At a low pH (4.0) high methoxyl pectin gel and sucrose (55%), makes the polymer is less soluble.

Table 1: Extracted pectin characterization.	
Parameter	**Result**
Organoleptic characteristics	Light brown amorphous powder with no odor and slightly gummy taste.
Percentage Yield	78.9%
Moisture Content	0.165%
Angle of repose	9.09˚ (excellent)
Equivalent Weight	125
Methoxy Content	18.6%
Anhydrouronic acid Content	141.08%
Degree of Esterification	74%
Compressibility index	26.8 (poor)

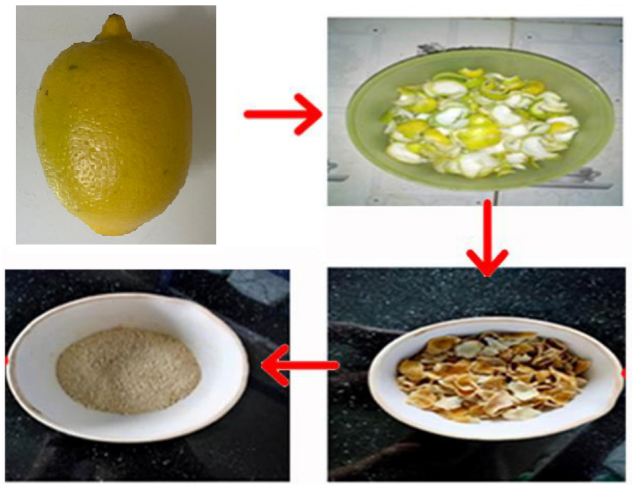

Figure 1: Process of extraction of pectin from lemon peel.

Extracted pectin was further used to synthesize TFP using thioglycolic acid and the process was illustrated in Figure 2.

Figures 3 and 4 display the FT-IR spectrum of pectin and TFP in the spectral region of 4000 to 500 cm^{-1}. The following was shown by the infrared measurements of the pectin: 1236 (C-O stretch from the carboxylic group), 2852 (corresponding to SH stretch and a weak band of mercaptans); 1726 (C=O stretch from the ester); 3305 (corresponding to OH stretch and a broad band of the carboxylic group); and 1095 (C-O stretch from ester as well as C–O stretch from primary alcohol). The infrared data from the TFP validated the -SH stretch at 2584, and the ester's C=O stretch was enhanced while the other groups remained unchanged.

Pectin's X-ray diffractogram has characteristic peaks at 19.56 and 24.68, which indicate that the substance is

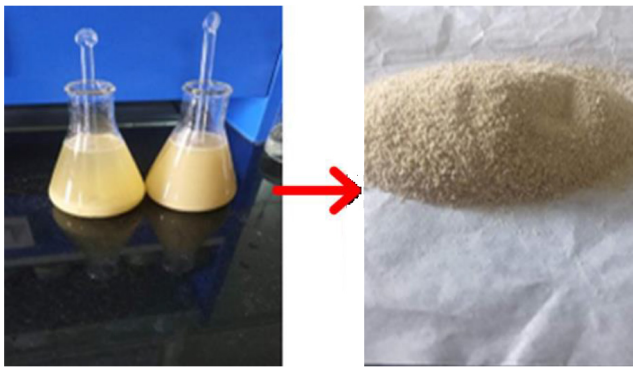

Figure 2: Preparation of TFP from pectin.

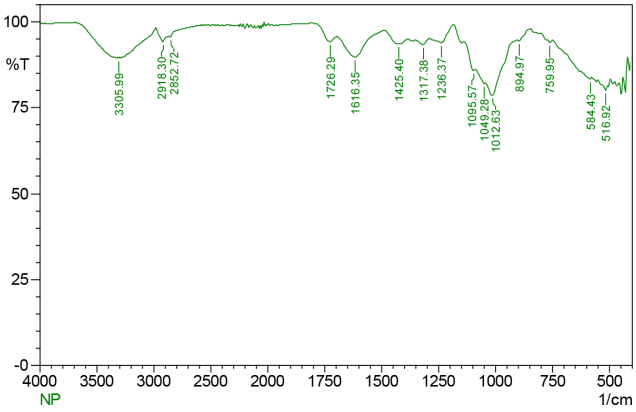

Figure 3: FTIR spectrum of pectin.

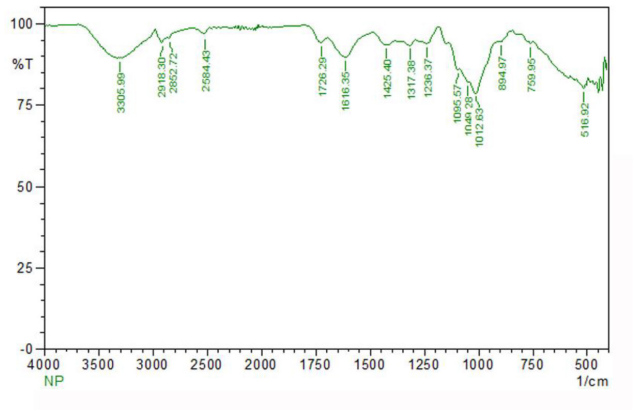

Figure 4: FTIR spectrum of TFP.

amorphous. TFP's diffractogram exhibits a recognizable peak at 15.21 and 22.52 nm. TFP has a slightly higher peak intensity than pectin [Figures 5 and 6]. The FTIR and XRD characterizations of pectin corroborated its thiolation.

Mucoadhesion Studies via Rotating Cylinder

Mucoadhesion potential was assessed for pectin, CS, and SCMC and compared with TFP. Table 2 shows the average adhesion time for all examined polymers.

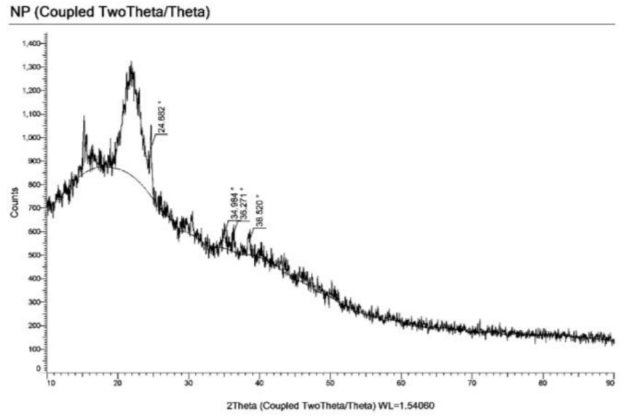

Figure 5: XRD diffractogram of pectin.

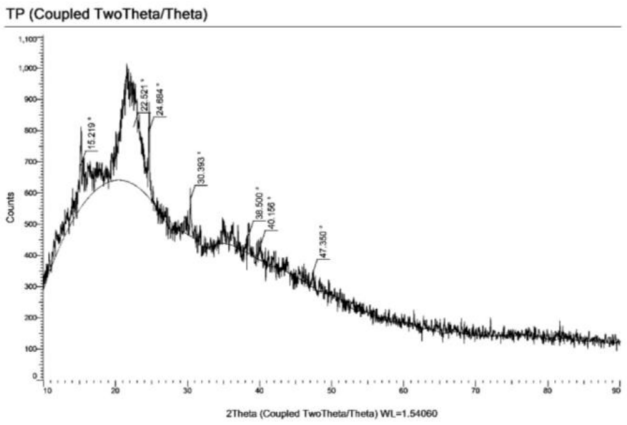

Figure 6: XRD diffractogram of TFP.

Table 2: Mucoadhesion of various polymers determined via rotating cylinder.

Sl. No	Mucoadhesive polymer	Mean mucoadhesion time (h)
1.	Pectin	2.3
2.	CS	3.5
3.	SCMC	5.8
4.	TFP	6.4

The mean period of adherence on the mucosa for CS was 3.5 hr, placing it second after SCMC in terms of mucoadhesive characteristics. The mean time of adhesion for extracted pectin is mild. With a mean of 6.4 hr, TFP has the longest mean mucoadhesion time. The rank order was found to be Pectin < CS < SCMC < TFP.

Shear Stress Measurement

A polymer concentration of 1.5% was used in the experiment, while the contact period was varied. It takes roughly three to four times more weight to separate glass plates when the contact time is increased (from

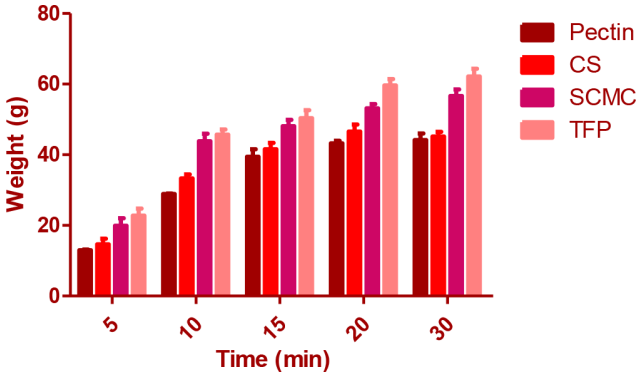

Figure 7: Report of shear stress measurement for 1.5% of the polymer at different contact times. CS, chitosan; SCMC, sodium carboxymethylcellulose; TFP, thiolated pectin.

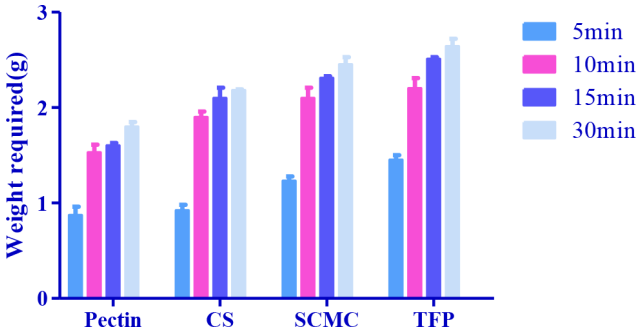

Figure 8: Mucoadhesion strength is characterized by Wilhelmy's method. CS, chitosan; SCMC, sodium carboxymethylcellulose; TFP, thiolated pectin.

five to thirty minutes). When measuring shear stress, a material's mucoadhesiveness is shown by how much weight is needed to separate the upper glass plate from the bottom plate. Figure 7 shows the various weights needed for each material at its particular period. Pectin has demonstrated mucoadhesive strength that is comparable to CS but weaker than SCMC nearly at all contact times. TFP showed the highest shear stress measurement compared to the other polymers, as was to be expected.

During the period when the mucoadhesive substance is in contact with the glass plate that contains the mucous solution, a binding force is formed between the mucous and the mucoadhesive substance. Keeping the glass plate in touch with the mucus solution for longer periods results in the formation of a stronger connection, which requires a greater amount of force to be severed (Figure 8).

Different mucoadhesive polymer combinations were used to cover the mustard. Due to the mucus solution's high stickiness, traveling more than 50 marks required greater time as concentration increased. In Table 3, the reports were displayed.

Table 3: Report of falling sphere method at different concentrations.

	Time required (minutes) to travel at different concentration		
	Conc (%)	**0.5%**	**1%**	**3%**
Polymer	Pectin	8.8±1.5	9.2±0.8	9.3±1.2
	CS	7.5±0.5	7.6±0.8	7.9±0.9
	SCMC	9.3±1.8	9.6±2.0	10.5±2.2
	TFP	10.5±2.0	10.8±2.5	11.2±3.2

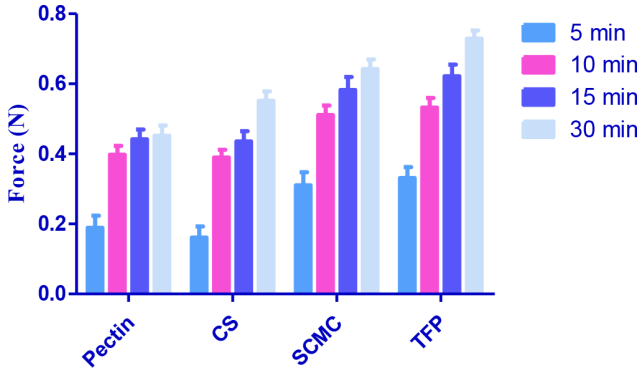

Figure 9: Detaching force measurement at different contact times. CS, chitosan; SCMC, sodium carboxymethylcellulose; TFP, thiolated pectin.

Figure 9 shows the calculated mucoadhesive strength in Newton's. For all polymers, the detachment force increased as the contact time increased. Comparing SCMC to the other polymers, the detachment force was highest in SCMC.

CONCLUSION

Citrus limon pectin was successfully extracted, and its various physicochemical characteristics were assessed. Pectin was tested for mucoadhesion, and the results indicated that, under the experimental setup utilized in this work, its mucoadhesive strength was comparable to CS but lower than SCMC. Pectin has good mucoadhesion properties, however, to use pectin in mucoadhesion drug delivery systems, it is necessary to increase pectin's mucoadhesion potential by appropriate methods like thiolation, etc. TFP was consequently created utilizing thioglycolic acid. FTIR and XRD were used to further confirm thiolation. TFP's mucoadhesion capability was better than that of other polymers. To remark further in this regard, additional *in vivo* research is required.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

ABBREVIATIONS

AUA: Anhydrouronic acid; **CS:** Chitosan; **DE:** Degree of esterification; **FTIR:** Fourier transform infrared spectroscopy; **SCMC:** Sodium carboxymethylcellulose; **TFP:** Thiolated pectin; **XRD:** X-ray diffraction.

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