

Research paper

Mucoadhesive properties of various pectins on gastrointestinal mucosa: An *in vitro* evaluation using texture analyzer

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Abstract

Mucoadhesive performance of various pectins with different degrees of esterification and molecular weights was examined with porcine gastrointestinal (GI) mucosa, i.e. buccal, stomach, small intestine and large intestine, using a texture analyzer equipped with mucoadhesive platform. The instrumental parameters and test conditions such as pre-hydration time of pectin disc, contact time, contact force, test speed of probe withdrawal, GI tissue and test medium were also studied. Two parameters derived from texture analysis, namely maximum detachment force (F_{\max}) and work of adhesion (W_{ad}), were used as parameters for comparison of mucoadhesive performance. The results indicated that degree of hydration of pectin disc affected the mucoadhesive properties. The mucoadhesion of pectin increased with the increased contact time and contact force, but not by the increased probe withdrawal speed. Tissue from different parts of GI tract and test medium also influenced the mucoadhesion. Pectins showed a stronger mucoadhesion on large intestinal mucosa than on small intestinal mucosa. The mucoadhesive properties of pectins on gastric mucosa depended on pH of the medium; a higher F_{\max} and W_{ad} in a pH 4.8 medium than a pH 1.2 medium was revealed. Additionally, pectin showed a significantly higher mucoadhesion than carbomer934P in most of the GI mucosa tested. The results also demonstrated that the mucoadhesive performance of pectins largely depended on their characteristics, i.e. higher degree of esterification and molecular weight gave a stronger mucoadhesion. These findings suggest that pectin can be used as a mucoadhesive carrier for GI-mucoadhesive drug delivery systems.

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1. Introduction

Mucoadhesive polymers are used to immobilize a drug delivery device on a specific site for targeted release and optimal drug delivery due to intimacy and duration of contact. Mucoadhesive polymers have been developed for buccal, nasal, ocular, vaginal and oral applications. So far, a considerable number of studies focusing on the mucoadhesive

properties of wide range of polymeric materials, particularly hydrophilic polymers containing numerous hydrogen bond (H-bond) forming groups, have been performed [1]. It has been proposed that the interaction between the mucus and mucoadhesive polymers is a result of physical entanglement and secondary bonding, mainly H-bonding and van der Waals attraction. These forces are related to the chemical structure of the polymers [2]. The types of surface chemical groups of mucoadhesive polymers that contribute to mucoadhesion include hydroxyl, carboxyl, amine and amide groups in the structure [3]. Peppas and Buri [4] suggested that polymer characteristics which are necessary for mucoadhesion are (i) strong H-bonding

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groups, (ii) strong anionic charges, (iii) high molecular weight, (iv) sufficient chain flexibility, and (v) surface energy properties favoring spreading onto mucus.

The present work concerns the pectin, anionic polysaccharide, which is rich in carboxylic groups and possible to interact with functional groups in mucus layer. Up until now, pectin has been commercially used as a food additive, a thickening agent and a gelling agent. Pectin has very complex structure which contains α -D-galacturonic acid with 1–4 linkage [5]. The galacturonic acid of the backbone is partially methyl-esterified which classifies pectin type. The degree of esterification (DE) less than 50% is low DE pectin while DE more than 50% is high DE pectin.

Several reports have demonstrated the mucoadhesive properties of pectin. Smart et al. [6] reported that pectin gave fair adhesiveness with mucus gel using Wilhemy plate method. On the contrary, Lehr et al. [7] found that pectin (with no identified source) showed no adhesion compared to polycarbophil or chitosan. With this test, thin films containing 1 mg/cm² of polymers were hydrated in the saline medium for 5 min, then tested with the pig small intestinal mucosa under very slight pressure (~ 10 mN), and kept in this position for 1 min. The hydration time of 5 min may be too long and, then, the thin films could be dissolved before testing, resulting in loss of mucoadhesive properties [6]. In fact, there are many factors affecting the mucoadhesive properties of polymers such as degree of hydration [4], ionic strength of medium [8], and their molecular structure feature [9–11]. Additionally, the physical properties, e.g. solution, gel-forming, and swelling properties, of pectin are different, depending on the types or characteristics of pectin. This information should be mentioned in the literature and should not be disregarded.

Schmidgall and Hensel [12] reported that rhamnogalacturonans with a low DE and linear oligogalacturonides derived from pectin showed a significant bioadhesion against colonic mucus membranes whereas high DE pectins and neutral polysaccharides were ineffective. Liu et al. [13] reported that pectin with higher net electrical charges showed a higher mucoadhesion with porcine colonic tissues than the less charged ones. The high DE pectin formed gel networks with endogenous mucin lining on the surface of mucosal tissue whereas low DE pectin was able to penetrate deeply toward the colonic intestinal wall, but did not adhere strongly on the tissues surface. However, the mucoadhesive properties of pectin (i.e. only two different DEs) have been studied in only the colonic tissues. To date, there is no comparable information of such properties in other gastrointestinal (GI) tissues.

Several techniques for *in vitro* determination of mucoadhesion have been reported in the literature. Most *in vitro* methods for screening of the mucoadhesion are based on a measurement of either tensile or shear strengths [14]. Methods using tensile strength usually

examine the force necessary to separate two surfaces after mucoadhesive bonding has been established [15]. The employed instruments are modified balances or tensile testers. The Du Noüy tensiometer has also been modified to evaluate a relative adhesion capacity of polymers in powder form [16]. Methods based on measurement of shear strength determine the force that causes the adhesive polymer slide on mucus layer in the direction parallel to their plane of contact, for example, the Wilhemy plate method reported by Smart et al. [6]. This technique measures mucoadhesion by recording a maximum force from microbalance at the moment a polymer coated glass plate is detached from mucus gel. The samples used in this technique need to be coated on glass plate and their mucoadhesive performance is influenced by plate width, penetration depth of glass plate into mucus and rate of extraction out of mucus [17].

Recently, tensile test using texture analyzer has been reported for studying the mechanical characteristics of mucoadhesiveness of polymers and dosage forms [18,19]. In general, the mucoadhesion using this technique was evaluated through the measure of maximum force required to separate the polymer or dosage form from surface of substrate after contact at specified time and force, and the work of adhesion calculated. Several surface substrates such as porcine stomach tissue, chicken pouch tissue [20], bovine sublingual mucosa [21,22], bovine duodenal mucosa [22], mucin disc [23], and mucin gel [24] have been used as a model substrate using texture analyzer. The validation of the test using texture analyzer has been performed under simulated gastric condition using pig gastric mucosa [18] or simulated buccal conditions using chicken pouch tissues [20], in order to elucidate test conditions and instrumental parameters influencing the mucoadhesive test results. Tobyn et al. [18] found that contact time and force between pig gastric mucosa and sample, removal test speed of the probe, and pre-hydration time of polymer samples significantly affected the result obtained. However, from the literature, the test parameters' validation under simulated small intestinal or colon conditions has not yet been reported. Moreover, the test parameters, e.g. contact time, contact force, test speed, and test environment (i.e. pH, ionic strength), for texture analysis are varied among the published reports. Thus, comparing the mucoadhesive properties of polymers from differential test parameters would be complicated.

The objective of this study was, then, to validate the instrumental variables and test conditions when testing with texture analyzer under simulated GI conditions using porcine GI mucosa (i.e. buccal, stomach, small intestinal and large intestinal mucosa). The instrumental variables and test conditions studied are pre-hydration time, contact time, contact force, probe speed, GI mucosa, and test medium. Mucoadhesive performance of various pectins with different degrees of esterification and molecular weights under different GI conditions was also studied and discussed.

2. Materials and methods

2.1. Materials

Four pectins with different DEs (see Table 1) were kindly provided by Herbstreith & Fox KG (Germany). Polyacrylic acid crosslinked polymer (carbomer934P) manufactured by from Corel Pharma-Chem (India) was used. Chitosan (molecular weight of 100 kDa) with degree of acetylation of 95% was supplied from Seafresh Chitosan (Lab) Co., Ltd., Thailand. All other chemicals were of analytical grade and used as received without further purification.

2.2. Preparation of sample discs

Discs of 200-mg pectin sample were prepared by direct compression using a single punch hydraulic press (Model 15011, Specac, USA) with 9.53-mm diameter flat-faced tooling. The discs were compressed at the pressure of 2 tons for 20 s and kept in desiccator until used.

2.3. Preparation of GI tissues

GI tissues from different parts of porcine GI tract (i.e. buccal, stomach, small intestinal and large intestinal tissues) were obtained from animals immediately after slaughter at local slaughterhouse (Nakhon Pathom, Thailand). The tissues were washed with deionized water to remove non-digested food from lumen then placed in normal saline solution at 4 °C and used within 6 h. The underlying connective tissues were subsequently removed to isolate the mucosal membrane.

2.4. Study on test conditions of mucoadhesive test

Mucoadhesion testing of the sample discs was carried out using a texture analyzer (TA.XT plus, Stable Micro Systems, UK) with 50 N load cell equipped with mucoadhesive holder. A disc was attached to the cylindrical probe (10 mm in diameter) by double-sided adhesive tape. The tissue (about 20 × 20 mm) was equilibrated for 15 min at 37.0 ± 0.5 °C before placing onto the holder stage of mucoadhesive holder and maintained at 37 °C during the test in 200 mL of the medium. Fig. 1 demonstrates the scheme showing the process of the mucoadhesive test. The probe

with the disc attached was immersed in the test medium for a specified time prior to the test, the hydrated disc was then moved downward to contact with soaked tissue at a specified force and maintained until specified time, in steps A and B, respectively. The probe was subsequently withdrawn at a specified test speed in step C. By using the texture analyzer, the maximum force required to separate the probe from the tissue (i.e. maximum detachment force; F_{\max}) could be detected directly from Texture Expert 32 software and the total amount of forces involved in the probe withdrawal from the tissue (work of adhesion; W_{ad}) was then calculated from the area under the force versus distance curve (Fig. 2). These parameters were used to compare the different test conditions or formulations.

Two types of pectin (i.e. CU201 and CU701) were selected as representatives of high and low DE pectins, respectively, to validate the instrumental parameters and test conditions (i.e. pre-hydration time of the sample disc in test medium, contact force between the sample disc and tissue, contact time of the sample disc to the tissue, and test speed of the probe removal from the tissue). Five pre-hydration times (0.5, 2, 5, 10 and 20 min) were studied at the contact force of 0.05 N, contact time of 60 s and probe speed of 0.5 mm/s. The effect of contact force (i.e. 0.05, 0.1, 0.2 and 0.5 N) and contact time (i.e. 10, 30, 60, 180 and 600 s) was investigated using pre-hydration time of 5 min and probe speed of 0.5 mm/s. Four probe withdrawal speeds (0.1, 0.3, 0.5 and 1.0 mm/s) were further studied using pre-hydration time of 5 min, contact force of 0.05 N and contact time of 60 min. The probe without disc was also tested to check the uniformity of the GI tissue. In order to confirm reproducibility and validity of obtained data, 6 to 10 measurements were performed on the fresh tissue samples for each condition.

2.5. Study on the effect of GI tissue and test medium on mucoadhesion of pectin

The effect of GI tissue and test medium was also investigated using different parts of porcine GI tract with their relevant medium, i.e. buccal mucosa with simulated saliva fluid pH 6.75 (SSF), gastric mucosa with simulated gastric fluid USP without pepsin (SGF) or citric-phosphate buffer, pH 4.8 (representing the fasted or fed state, respectively), small intestinal mucosa (duodenum part) and large intestinal mucosa with simulated intestinal fluid pH 6.8 (SIF).

Table 1
Designation and properties of pectin examined in the study

Pectin type and designation	Degree of esterification (% DE)	Degree of amidation (% DA)	Molecular weight (kDa)
<i>High methoxy pectin</i>			
CU201	70	0	200
CU501	56	0	180
<i>Low methoxy pectin</i>			
CU701	38	0	80
CU020	29	20	150

Note: This information is specified and reported by the manufacturer.

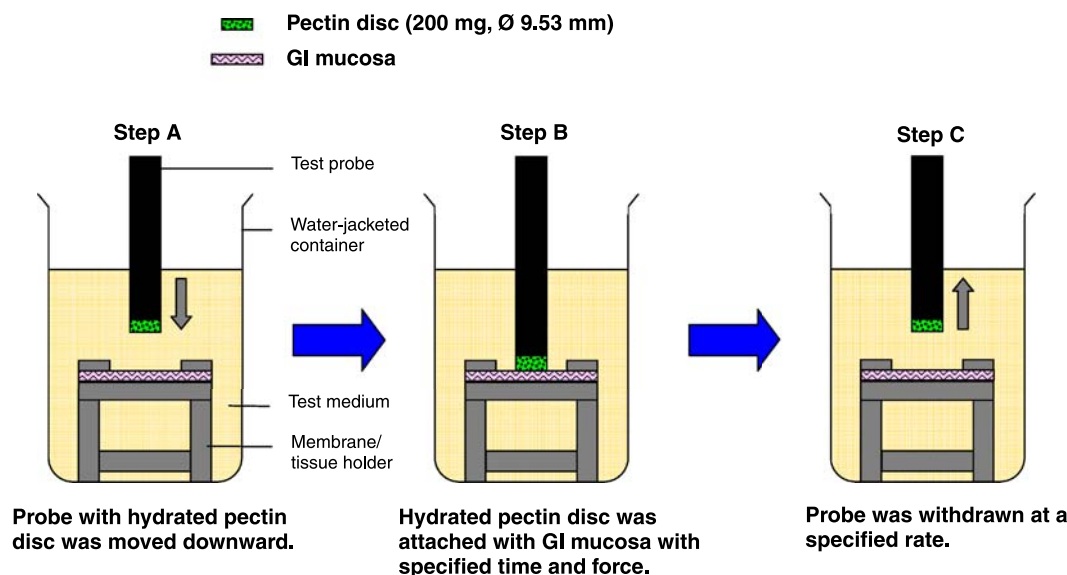


Fig. 1. Process of mucoadhesive test on gastrointestinal mucosa by texture analyzer with mucoadhesive holder.

The tests were employed using a condition chosen from the results of previous Section 2.4, that is, a pre-hydration time of 5 min, contact force of 0.05 N, contact time of 60 s and probe speed of 0.5 mm/s.

2.6. Study on pectin type on mucoadhesive properties

Four types of pectin, namely CU201, CU501, CU020 and CU701, were used to compare the mucoadhesive performance of low and high DE pectins. The test conditions were pre-hydration time of 5 min, contact force of 0.05 N, contact time of 60 s and a probe speed of 0.5 mm/s. The common mucoadhesive polymers, e.g. carbomer934P and chitosan, were used as controls.

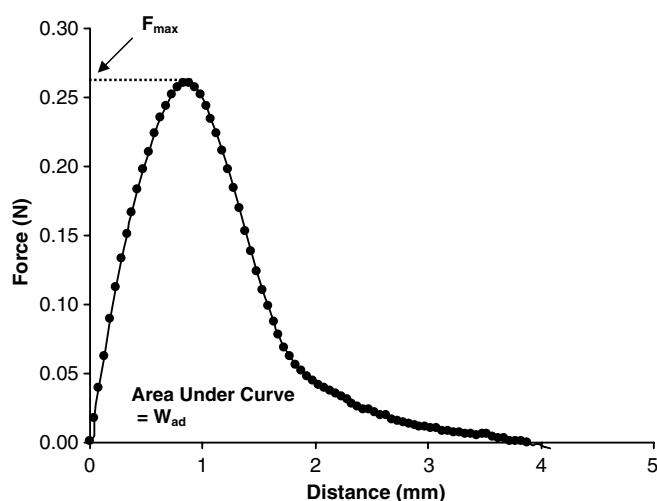


Fig. 2. A typical plot of force versus distance data for pectin disc (CU020 tested with small intestinal mucosa) from the mucoadhesive test using texture analyzer. The height of the peak is the maximum force required to separate the probe from the tissue (i.e. maximum detachment force; F_{\max}) and the total amount of forces involved in the probe withdrawal from the tissue (work of adhesion; W_{ad}) is calculated from the area under the force versus distance curve.

2.7. Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively.

3. Results and discussion

3.1. Effect of pre-hydration time of pectin disc on mucoadhesive properties

Sample discs of high (CU201) or low (CU701) DE pectin were immersed in test medium for 0.5, 2, 5, 10 or 20 min prior to attachment to a duodenal part of small intestinal mucosa. The pectin disc was pulled out at a speed of 0.5 mm/s after attachment to the mucosa at a contact force of 0.05 N and contact time of 60 s. Fig. 3 shows the F_{\max} and W_{ad} of pre-hydrated discs after attachment to duodenal mucosa. The results showed that the F_{\max} of both pectins did not change significantly when the disc was hydrated up to 20 min whereas the W_{ad} slightly decreased from the first 2 min then maintained until 20 min. These findings indicate that the optimum degree of hydration and swelling of pectin disc could influence the mucoadhesive properties. This is in agreement with Ponchel et al. [25] who reported that, for their test system with bovine sublingual mucosa, there was an optimum pre-hydration time of around 10 min before the tablet showed maximum adhesive characteristics. Leung and Robinson [8] suggested that a sufficient amount of water is necessary to properly hydrate and expand the mucoadhesive network to expose available adhesive sites for bond formation, create pores

or channels for diffusion of polymer chains and mobilize the polymer chains for interpenetration. However, if an excessive quantity of water is available, the hydrated polymers start to form gels and eventually a slippery mucilage, resulting in all adhesive properties being lost since the polymers dissolve in the available water [6].

Additionally, high DE pectin showed a higher W_{ad} than low DE pectin while the F_{max} was not significantly different. It is possible that high molecular weight of the high DE pectin could be entangled more than low DE pectin with low molecular weight [26]. The expansion of the entangled chain of high DE pectin, during the withdrawal of pectin disc from mucosa, was probably longer than that of low DE pectin, resulting in high area under force versus distance curve. In order to investigate the effect of other instrumental parameters and test conditions, the pre-hydration time of pectin disc of 5 min was selected, based on this finding.

3.2. Effect of contact time and contact force on mucoadhesive properties

Fig. 4 shows the effect of contact time between the sample disc and GI mucosa on the F_{max} and W_{ad} of pectin discs

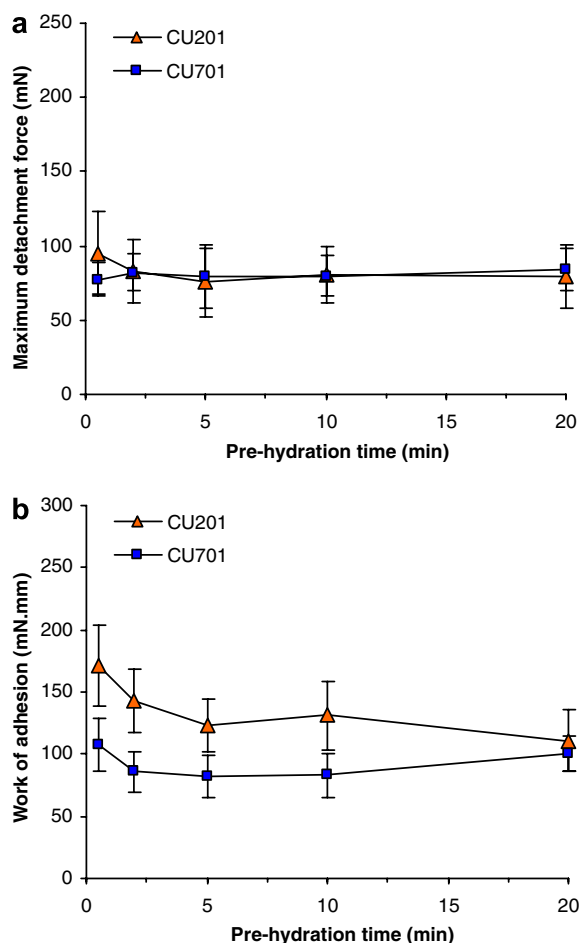


Fig. 3. Effect of pre-hydration time of pectin discs (CU201 and CU701) on (a) maximum detachment force, and (b) work of adhesion, against porcine small intestinal mucosa ($n = 6–10$).

(CU201 and CU701). The results showed that the F_{max} and W_{ad} of both pectins were significantly increased with the increased contact time. This is consistent with those obtained by Tobyn et al. [18] and Wong et al. [20] in which different types of polymers (e.g. carbomer, polycarbophil, hydroxypropylmethyl cellulose, sodium carboxymethylcellulose) and model mucosa were used. In this case, the increase of F_{max} and W_{ad} is most likely because the degree of hydration and swelling was sufficient to expand the mucoadhesive network. Increasing contact time may provide interdiffusion and chain entanglement between pectin and mucin chain in mucus membrane. This is in agreement with Leung and Robinson [8] who demonstrated that mucoadhesion of carbomer was a time-dependent process supporting the proposed interpenetration as being a time-dependent process. An increase in contact resulted in an increase in formation of secondary bonds and diffusional path or depth of interpenetration between two macromolecules. Increasing contact time between the mucoadhesive polymer and the mucus layer could, therefore, increase the mucoadhesive strength [8]. However, Shojaei et al. [11] reported that increasing contact time

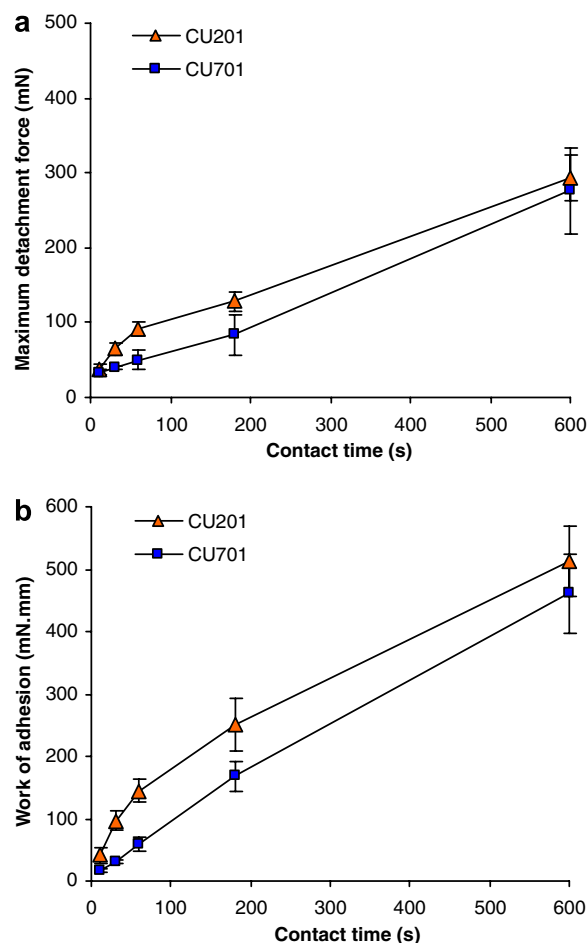


Fig. 4. Effect of contact time between the pectin discs (CU201 and CU701) and small intestinal mucosa on (a) maximum detachment force, and (b) work of adhesion, against porcine small intestinal mucosa ($n = 6–10$).

between copolymer (acrylic acid and 2-ethylhexyl acrylate) film and buccal tissue yielded a linear increase in mucoadhesive forces for up to 60 s, further increase in contact time (120–300 s) led to a plateau. They explained that cohesive energy of the copolymers may decrease substantially after the first minute of contact leading to physical deformation of the polymeric film due to water sorption. Furthermore, it was, again, found that high DE pectin showed a higher W_{ad} than low DE pectin, at all contact times studied.

Fig. 5 shows the effect of contact force applied between the sample disc and GI mucosa on the F_{max} and W_{ad} of pectin discs (CU201 and CU701). The F_{max} and W_{ad} tended to increase with the increased contact force. These results agree with the study by Tobyn et al. [18]. Nevertheless, Wong et al. [20] observed that no significant increase in the W_{ad} was seen at a contact force above 0.5 N, due to a maximum intimate contact. They suggested that too high contact force may not be advantageous but may damage the mucosa without achieving better contact. However, the contact time was shown to be a critical factor in affecting the mucoadhesion results. It may be thought that an initial stage of mucoadhesion process begins with the

establishment of an intimate contact between the polymer and mucosal surface followed by interpenetration of polymers to form secondary chemical bonds [2]. Hence, contact time is important to allow sufficient hydration, swelling, interpenetration and bond formation for mucoadhesion. Based on the physiological condition in GI tract after oral administration, mucoadhesive samples could not be forced to attach directly to the mucosa. As such, contact force employed for studying the effect of other test conditions was kept at the lowest force, i.e. 0.05 N, with a contact time of 60 s.

3.3. Effect of probe withdrawal speed from GI mucosa on mucoadhesive properties

Fig. 6 shows the effect of test speed of the probe removal from the tissue on the F_{max} and W_{ad} of pectin discs (CU201 and CU701). The results showed that there was no significant change in both F_{max} and W_{ad} at each increment of probe speed. These results differed from those of the previous studies [11,20] in which probe speed appeared to influence the mucoadhesive properties of polymer. This might be due to the difference in test conditions employed. Both of those previous experiments used non-hydrated samples before attachment to model mucosa. Wong et al. [20] conducted the experiments using carbomer and methylcellulose buccal tablets without pre-hydration time, and a chicken pouch tissue which was wetted with 200 μ L of SSF. They observed that low probe speeds such as 0.1 and 0.3 mm/s produced larger variation as compared to higher probe speed, e.g. 0.5 and 1.0 mm/s. Shojaei et al. [11] tested the non-hydrated copolymer film with porcine buccal tissue which was soaked with simulated gingival fluid periodically during the test. Due to the viscoelastic nature of the mucoadhesive bond, increasing the rate of stress producing from the crosshead removal speed resulted in less time for bond deformation and, therefore, the tensile strengths, which represent the mucoadhesive properties, were increased [11].

In the present study, the pre-hydrated pectin disc was tested on small intestinal mucosa soaked in 200 mL of test medium. With this test, pectin disc and GI mucosa were excessively hydrated, providing an openness of the interacting networks to facilitate interpenetration and bond formation between polymer chains. Thus, a probe withdrawal speed may not influence the mucoadhesive performance of pre-hydrated pectin disc.

3.4. Effect of different parts of GI tissue and test media on mucoadhesive properties

In order to evaluate the effect of tissue from different parts of GI tract and its relevant test medium on mucoadhesion of various types of pectin, buccal mucosa with SSF, stomach mucosa with SGF or citric-phosphate buffer, pH 4.8, small intestinal (duodenal section) and large intestinal mucosa with SIF were used. A pectin disc was hydrated in

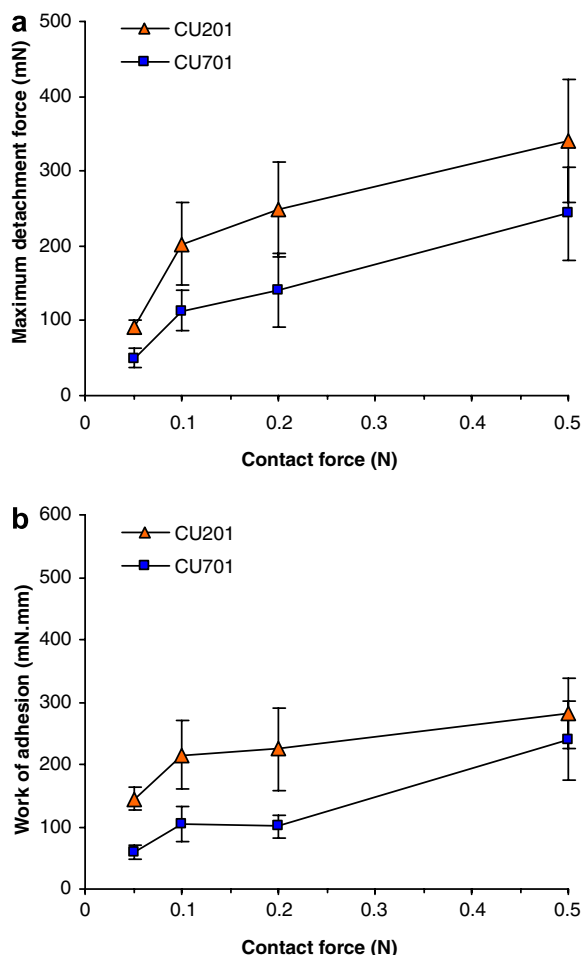


Fig. 5. Effect of contact force applied between the pectin discs (CU201 and CU701) and small intestinal mucosa on (a) maximum detachment force, and (b) work of adhesion, against porcine small intestinal mucosa ($n = 6-10$).

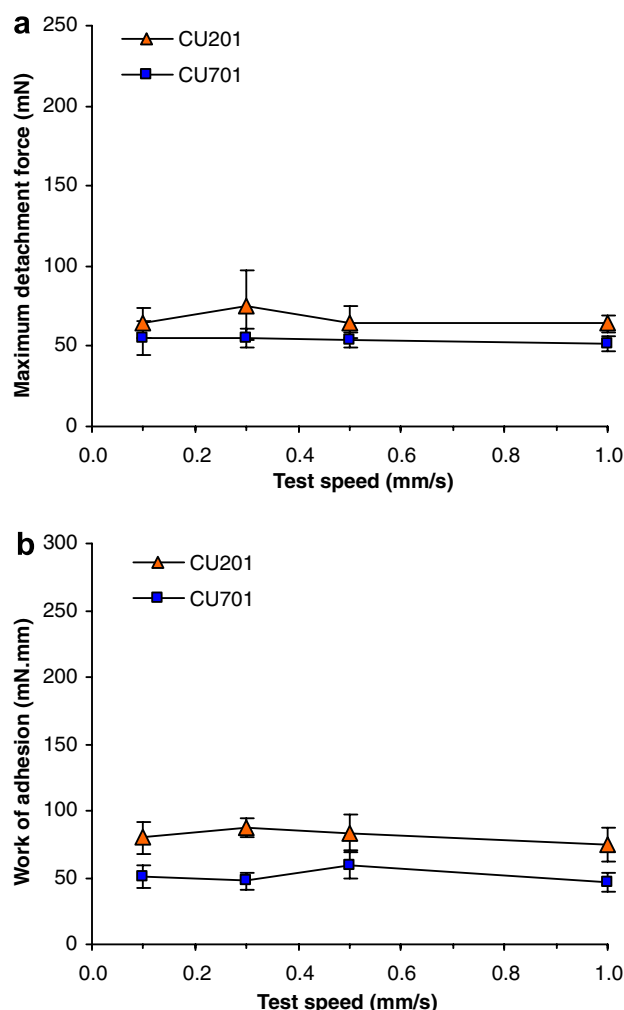


Fig. 6. Effect of probe withdrawal speed of pectin discs (CU201 and CU701) on (a) maximum detachment force, and (b) work of adhesion, against porcine small intestinal mucosa ($n = 6–10$).

each medium for 5 min prior to contact with GI mucosa at a force of 0.05 N for 60 s. Probe withdrawal speed was 0.5 mm/s.

The F_{\max} and W_{ad} of various pectins tested on different GI mucosa are shown in Fig. 7. The common mucoadhesive polymers, carbomer934P and chitosan, were used for comparison, as they showed good mucoadhesive properties in many reports, e.g. [7,10,27]. However, the test on chitosan discs was limited as they, unfortunately, disintegrated within 30 s after immersing in all media. The chitosan films, on the other hand, could be tested and reported [7].

Among various types of GI tissue, all pectins showed the strongest F_{\max} and W_{ad} on the large intestinal tissues (discuss later). The F_{\max} of all pectin discs on the buccal, stomach (both in the pH 1.2 and 4.8 media), and small intestinal mucosa were not significantly different, ranging from 55 to 85 mN. The W_{ad} of pectins on the gastric mucosa at pH 1.2 (fasted state) showed lower value than those on the gastric mucosa at pH 4.8 (fed state), buccal mucosa, small intestinal mucosa and large intestinal mucosa. This may be due to the difference in anatomy and characteristics of mucosa in

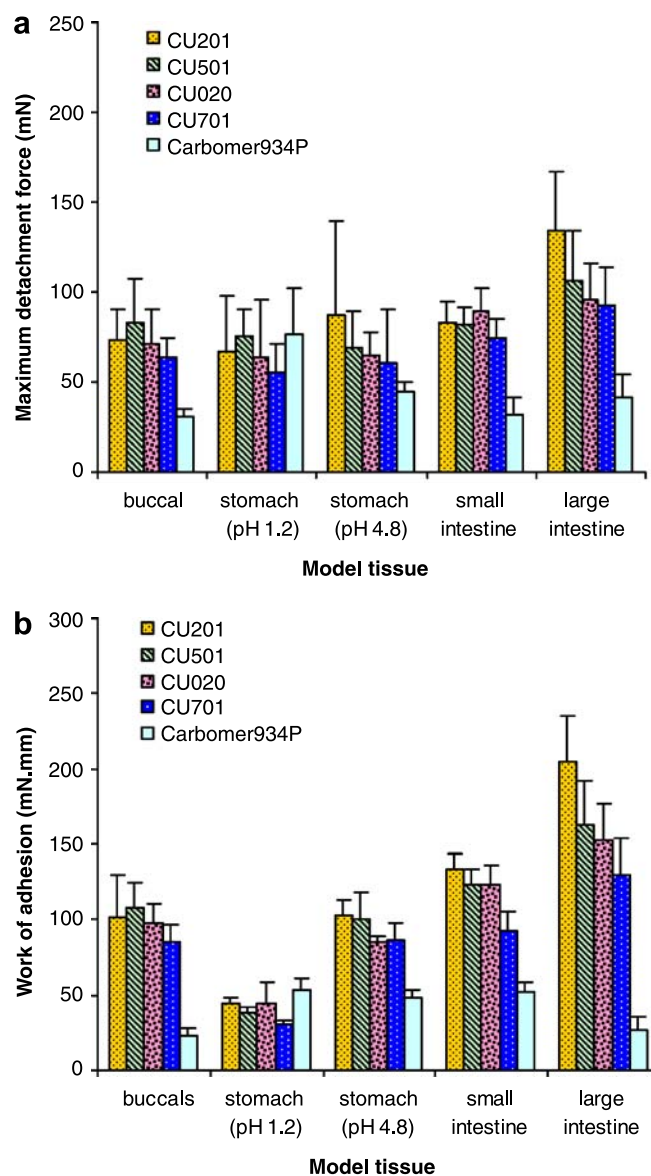


Fig. 7. Effect of GI mucosa and test medium on (a) maximum detachment force, (b) work of adhesion, of various types of pectin ($n = 6–10$).

different regions of GI tract [28]. The content of mucin, a major component in GI mucosa, was different in different parts of GI tract. In the stomach and intestines, the mucin released by goblet cells forms parts of mucus blanket covering the epithelia [28], resulting in viscous gel depending on the pH of the environmental medium. Buccal tissues do not have mucus layer, however, the mucin could be found in the saliva. This would attribute to the difference in bioadhesive properties of different GI mucosa. Accili et al. [22] reported that mucoadhesive properties of carbomer974 depended on the mucosa characteristics. The high mucoadhesion of carbomer974 in the sublingual and esophageal mucosa took place on the basis of their low amount of sialic acids (residues found in mucin) in these regions, thus of water bound, which reduces the gelation rate of carbomer.

Although, the medium used for testing of small and large intestinal mucosa was the same (i.e. SIF), large intestinal mucosa showed a stronger mucoadhesion than small intestinal mucosa. This is probably due to the fact that there is a difference in the functional histology of epithelia of small and large intestinal mucosa [28]. The absence of villi in large intestine, at the tissue level, may benefit the mucoadhesion as the attachment between the sample disc and mucosa or epithelia can occur easily. Moreover, in the cellular level, the ratio of goblet cells in large intestine is higher than in other parts of GI tract resulting in higher mucin level, and thus the mucoadhesion onto the large intestinal mucosa is higher. Mucoadhesive properties of pectins onto gastric mucosa depended on the environmental pH; a better mucoadhesion at pH 4.8 than at pH 1.2. This is probably due to the fact that pectin (pK_a of 3–4) is rapidly converted from carboxylate anions (pectin salt) to free carboxyl groups or unionized forms (pectinic acid), as the concentration of hydrogen ions increases at pH 1–2, which has the ability to swell less on hydration being virtually insoluble [26]. At higher pH, pectin can be ionized, swelled and form hydrogel [26], which contribute to the interdiffusion and the formation of interchain bridges between the polymer and biological substrate [15]. Furthermore, the sialic acid and sulphate residues in mucin glycoprotein of mucus were fully ionized at the pH more than 2.6; this confers a net negative charge to the molecule [15]. These findings demonstrated that mucosa from different parts of GI tract and test medium largely influenced the mucoadhesive performance of pectin.

In the case of carbomer934P, it showed a higher W_{ad} onto the stomach (both at pH 1.2 and 4.8) and small intestinal mucosa than those onto the large intestinal and buccal mucosa. Carbomer934P showed a significantly higher mucoadhesion than pectin, only on the stomach mucosa at pH 1.2. Carbomer has been claimed to be a potential mucoadhesive polymer, because of a lot of carboxylic groups (56–58%, calculated on a dry basis) that could interact with the functional groups of mucus [25,27].

Gu et al. [29] proposed that the mucoadhesion of carbomer at high pH medium (e.g. pH 6.2) could be due to dissociation of the carboxyl groups of carbomer and electrostatic repulsions between the negatively charged carboxyl groups causing the uncoiling and expansion of the molecule. This resulted in swelling and gel formation, thus making the polymer more susceptible to mechanical chain entanglement and secondary interactions with the mucus glycoprotein [29]. Water movement from mucus gel to dry polymer compacts (e.g. carbomer934 discs) also contributed to the dehydration of mucus layer which increased the adhesive and cohesive properties and caused the strengthening of the mucoadhesive joint [30]. However, excess polymer hydration led to a reduction in the strength of polymer–mucosa bond since the density of the functional groups promoting the adhesion decreases [3] or the polymer hydrates, gels and eventually forms

slippery mucilage [30]. Smart [10] noted that the adhesive failure of carbomer934P discs resulted from cohesive failure of the formed gel layer. Therefore, the force and work required to separate from mucosa were decreased. Similar results have been reported by Tobyn et al. [18] that mucoadhesion of carbomer934P tablets was largely decreased during the first 5 min of pre-hydration time due to the decreased mucoadhesive bond strength. In this study, the same conditions with pectin discs were used, i.e. the carbomer934P discs were pre-hydrated in medium for 5 min before testing with soaked GI tissue. It is assumed that the dry carbomer disc would absorb excess water to form a mucilage gel surface, thus weakening the mucoadhesive bonding.

3.5. Effect of pectin types on mucoadhesive properties

Pectins with different DEs and molecular weights (see Table 1) were selected to assess the mucoadhesive performance. The results showed that mucoadhesive performance of low DE pectin (CU701) was significantly lower than that of high DE pectin (CU201, CU501), when tested in all GI tissues and test media. The presence of amide groups in the structure (i.e. CU020) enhanced the mucoadhesion of low DE pectin as its W_{ad} value came close to that of high DE pectins, except for the stomach tissue at pH 4.8.

This result, somewhat, differed from those reported earlier [12,13], in which low DE pectin demonstrated a stronger interaction with porcine colonic tissues than high DE pectin. Liu et al. [13] suggested that the higher mucoadhesion of low DE pectin was possibly due to its higher net negative charges than those of high DE pectin, when both pectins were similar in the ratio of molecular weight to molecular size. However, in this study, it is likely that the molecular size of pectin played an important role on the mucoadhesion of pectin, and probably showed a stronger influence than the number of H-bond forming groups (i.e. $-COOH$) represented by a lower DE.

The mucoadhesive performance of high DE pectin (CU201) containing low amount of H-bond forming groups was mainly influenced by its high molecular weight which facilitates coil entanglement [14]. The mucoadhesion of low DE pectin (CU701), however, could be explained by a large amount of H-bond forming groups (about 64%), which promote secondary chemical bond formation in mucoadhesion process. The results showed that a higher degree of esterification and molecular weight of pectin demonstrated a stronger mucoadhesion. In case of amidated pectin (CU020), some carboxylic groups in the structure of low DE pectin were substituted with amide groups, resulting in strong H-bond forming groups to strengthen the mucoadhesive bonding. The rank order of mucoadhesive performance of examined pectins on to the GI mucosa appeared to be similar to the rank order of their degree of esterification and molecular weight (i.e. CU201 > CU501 > CU020 > CU701).

4. Conclusions

A texture analysis method, similar to other mechanical methods, is a convenient way of comparing new mucoadhesive polymers. The measurement of mucoadhesion could be influenced by the instrumental parameters and test conditions such as pre-hydration time of pectin disc, contact time, contact force, test speed of probe withdrawal, GI tissue and test medium. Therefore, a test system should be adequately assessed, as presented in this study, to optimize the conditions for conducting the measurements. Under the experimental conditions selected, the mucoadhesive performance of pectin onto different GI mucosa could be compared. It was found that the molecular weight and degree of esterification of pectin influenced its mucoadhesive performance.

The results suggest that pectin appears to be a potential mucoadhesive polymer for GI-mucoadhesive drug delivery system. We are continuing the experiments with these pectins, in particular to investigate its mucoadhesive properties in both *ex vivo* and *in vivo* tests. These issues will be discussed in future publications.

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