



Mucoadhesion of pectin as evidence by wettability and chain interpenetration

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ABSTRACT

Different types of pectin were characterized for gastrointestinal (GI) mucoadhesion. The mechanisms of mucoadhesion of these pectins were investigated by measurements of surface tension, contact angle, and FTIR studies. The surface tension of different GI fluids was relatively the same and found to decrease after addition of mucin. The contact angle of tested fluids on pectin surfaces behaved as time-dependent and the values at 30 s were used for comparison. The type of pectin, addition of mucin and pectin surfaces influenced the contact angle of GI fluids. The thermodynamic work of adhesion ($W_{ad/therm}$) and spreading coefficient were calculated. The positive values of $W_{ad/therm}$ indicated that the pectin surfaces could be wet spontaneously by adhesional process. The pectin with higher molecular weight and higher degree of substitution showed a lower $W_{ad/therm}$, resulting from the low wettability. The spreading coefficient of tested fluids containing mucin on pectin surface was negative, suggesting that no spreading could be occurred spontaneously. ATR-FTIR studies revealed the changes resulting from interpenetration of pectin–mucin chains at film interface and the formation of hydrogen bonds. The results obtained from this study demonstrated that the wetting behavior and work of adhesion could be used to explain the mechanism of mucoadhesion of various pectins in GI conditions, and that a chain interdiffusion occurred at the interface of pectin film and mucin solution which supported the diffusion theory of mucoadhesion.

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1. Introduction

Pectin is a water-soluble polysaccharide found in the cell wall of most plants. Though it is a heterogeneous polysaccharide, pectin contains linear chains of (1–4)-linked α -D-galacturonic acid residues. The linear structure of pectin is partly interrupted by (1,2)-linked side-chains consisting of L-rhamnose residues and some others neutral sugars (Rolin, 1993). The galacturonic acids have carboxyl groups, some of which are naturally presented as methyl esters and others which are reacted with ammonia to produce carboxamide groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are an important means to classify pectin. The DE less than 50% is so-called low methoxy pectin while DE more than 50% is so-called high methoxy pectin (Rolin, 1993). Pectin is regarded as safe for human consumption and has been used successfully for many years in food and pharmaceutical industries. As it is rich of carboxylic groups and possible to interact with functional groups in mucus layer, it has been used as a mucoadhesive polymer for controlled drug

delivery (e.g., Liu, Fishman, Hicks, & Kende, 2005; Schmidgall & Hensel, 2002; Thirawong, Kennedy, & Sriamornsak, 2008; Thirawong, Nunthanid, Puttipipatkachorn, & Sriamornsak, 2007). Liu et al. (2005) reported that pectin with higher net electrical charges showed a higher mucoadhesion with porcine colonic tissues than the less charged ones. Recently, Thirawong et al. (2007, 2008) reported the mucoadhesive performance of various pectins on the gastrointestinal tract, investigating by texture analysis and viscometric study.

The mucoadhesive process involved in the formation of bioadhesive bonds has been described in three steps: (i) wetting and swelling of polymer to permit intimate contact with biological tissue, (ii) interpenetration of bioadhesive polymer chain and entanglement of polymer and mucin chains and (iii) formation of weak chemical bonds between entangles chains (Duchene, Touchard, & Peppas, 1988). From this process, the wetting and swelling behavior of dosage form using mucoadhesive polymers has a great impact on their adhesive properties (Mortazavi & Smart, 1993). The strong adhesion of many hydrophilic polymers to the mucosa is based on their capability of water uptake, where absorption, swelling and capillary effects lead to a water flux from the underlying tissue to the polymer (Duchene & Ponchel, 1992). A sufficient amount of water appears necessary to properly hydrate and expand the mucoadhesive network which exhibits available adhesive sites for bond formation and creates pores for diffusion

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of polymer chains to mobilize the interpenetration (Leung & Robinson, 1990).

The wetting theory, which is one of general theories of adhesion, has been adapted for the investigation of mucoadhesion (Smart, 2005). It involves the ability of a liquid to spread spontaneously onto a surface as a prerequisite for the development of adhesion (Adamson, 1990). In the case of tissue or polymer surfaces relevant for mucoadhesion, experimental difficulties are met, since both mucoadhesive polymer and mucus are hydrogels. Several techniques have been used for contact angle measurement (Andrade, King, Greggonis, & Coleman, 1979; Bodde, De Vries, & Junginger, 1990; Lehr, Bouwstra, Bodde, & Junginger, 1992). Andrade et al. (1979) measured contact angle of hydrogels being completely immersed and equilibrated with water. Lehr et al. (1992) used the captive bubble technique which was adapted from Andrade et al. (1979) to measure contact angle of polymers. The contact angle measured on the air bubbles are considered as water contact angle at a hydrogel–air interface while the contact angle measured on the *n*-octane droplets are considered as octane contact angle at a hydrogel–water interface. Therefore, the solid surface energy for hydrophilic surface could be calculated (Lehr et al., 1992). Li, Bhatt, and Johnson (1998) measured contact angle of water on the surface of a silicone-based mucoadhesive patch contained carbomer974P using an ophthalmic shadow scope. The corresponding work of adhesion between the water and the mucoadhesive patch was then estimated. The thermodynamic work of adhesion ($W_{ad/therm}$) represents the energy required to separate an interface between two substrates, e.g., mucus and polymer surface. To calculate $W_{ad/therm}$ for a mucus–surface interface, the surface tension of the mucus, the surface tension of the surface on which the mucus is resting, and the interfacial tension between the two need to be measured. However, if mucus behaves enough like a liquid to accept the Young's equation, the $W_{ad/therm}$ for a particular mucus interface can be calculated, in which the surface tension of the mucus (or mucin-contained liquid) and its contact angle on the surface need to be measured. Albers, Tomkiewicz, May, Ramirez, and Rubin (1996) measured the surface tension of liquid-like mucus using ring distraction technique, and the contact angle of mucus on glass slide and Teflon, thereafter the $W_{ad/therm}$ was calculated.

According to the diffusion theory of mucoadhesion, the interpenetration and entanglement of polymer chains are responsible for mucoadhesion. The diffusion theory has been supported by experimental studies using rheological technique (e.g., Mortazavi, Carpenter, & Smart, 1992; Thirawong et al., 2008) and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (e.g., Jabbari, Wisniewski, & Peppas, 1993). The spectroscopic analysis, particularly ATR-FTIR spectroscopy, has been applied successfully to study the chain interpenetration at the bioadhesive interface occurring between mucoadhesive polymer and mucin (Degim & Kellaway, 1998; Jabbari et al., 1993; Saiano, Pitarresi, Cavallaro, Licciardi, & Giammona, 2002).

In this study, the effect of different gastrointestinal (GI) fluids on the wetting properties of various types of pectin was investigated by contact angle measurement between pectin surface and GI fluid, and surface tension measurement. The effects of type of pectin surface (i.e., compacted disc or film) and the addition of mucin on the contact angle were also investigated. The $W_{ad/therm}$ and spreading coefficient (S_s) were calculated using GI fluids containing mucin. The ATR-FTIR spectroscopic investigation of diffusion of water and chain interpenetration at a mucoadhesive interface consisting of pectin and mucin in various media was also studied. The results make it possible to draw conclusions about the mechanism of mucoadhesion of various pectins at different conditions of GI tract.

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 (Da)
<i>High methoxy pectin</i>			
CU201	70	0	200,000
CU501	56	0	180,000
<i>Low methoxy pectin</i>			
CU020	29	20	150,000
CU701	38	0	80,000

Note: The DE, DA and molecular weight are specified and reported by the manufacturer.

2. Materials and methods

2.1. Materials

Four commercial pectins with different DEs and molecular weights (MWs) (see Table 1) were kindly provided by Herbstreith & Fox KG (Germany). Partially purified powder of mucin from porcine stomach, type III, with bound sialic acid of 0.5–1.5%, was purchased from Sigma Chemical Co., Ltd., USA. All other chemicals were analytical grade and used as received without further purification.

2.2. Preparation of pectin samples

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 over silica gel at ambient temperature (i.e., at 25 °C) until used.

Pectin films were prepared by casting pectin solution (2% or 4% w/w in deionized water) in glass plate and dried at 40 ± 5 °C for 10 h. Films were cut into pieces (20×20 or 10×50 mm²) prior to the test and kept in desiccator over silica gel at ambient temperature (i.e., at 25 °C) until used. The thickness of film was measured and averaged from 16 points using a digital thickness tester (model MiniTest 600B, ElektroPhysic, Germany). Film thickness was around 30–50 µm.

2.3. Morphological studies

The surface morphology of pectin discs and films were examined under a scanning electron microscope (SEM) (model Max-im-2000, CamScan Analytical, Cambridge, UK) equipped with back-scattered electron detector at an accelerating voltage of 5 keV.

2.4. Wetting studies

2.4.1. Determination of surface tension

Media used in this study were distilled water (DW), normal saline solution (0.9% w/v sodium chloride, NSS), simulated gastric fluid USP without pepsin (SGF, pH 1.2), simulated saliva fluid (SSF, pH 6.75) and simulated intestinal fluid (SIF, pH 6.8). The mucin was also added to all media to make 1 mg/mL concentration. Surface tension of each medium was measured using the du Noüy interfacial tensiometer, ring method (model K8, Krüss, Germany). Calibration was performed prior to use the instrument and then verified using distilled water. The media were maintained at 25.0 ± 0.5 °C during the test. Five repeated measurements were made on separate aliquots of each medium. The averaged values were reported and used for calculation.

2.4.2. Determination of contact angle

Water contact angles (θ) were measured with droplets of each medium on the polymer surface, using the contact angle goniometer (model G1, Krüss, Germany) at $25 \pm 2^\circ\text{C}$. Five microliters of each medium were gently dropped on the surface of pectin discs or films using a microsyringe. All the droplets were released from 1 cm above the surface to minimize the inconsistency between each measurement. The angle between the tangent line and the polymer surface from goniometric scale, after 30 s and 2 min of the release of each droplet onto the surface was measured. Several droplets (i.e., 6–8 droplets) were measured for each polymer sample on both left and right sides. The averaged values for the contact angles were then used for all subsequent calculation.

2.5. ATR-FTIR analysis

2.5.1. Analysis method

An infrared spectrometer with an attenuated total reflectance (ATR) accessory was used for the interdiffusion studies. A thin film of pectin was carefully overlain, in direct contact, on the ZnSe crystal mounted on a Nicolet 4700 FTIR spectrometer (Thermo Electron Corporation, USA). The 500- μL mucin (5% w/v) in different media (i.e., DI water, SSF, SGF and SIF) was then placed onto the upper surface of pectin film. The top of ZnSe crystal was covered with plastic sheet to prevent solvent evaporation. The measurement range was 4000–400 cm^{-1} and the spectra were collected at every 30 s, with four averaged scans and a resolution of 4 cm^{-1} . The spectrophotometer was linked to a personal computer equipped with Nicolet Omnic software allowing the continuous automated collection and subsequent manipulation of spectra.

2.5.2. Calculation of diffusion coefficient

A diffusion model using Fick's second law that satisfies both initial and subsequent boundary conditions (Jabbari et al., 1993) has been employed to compare the experimental results of the evolution with time and other infrared bands at the polymer/mucin interface. The diffusion can be defined by the following equation:

$$\frac{C}{C_0} = \frac{A}{A_0} = 1 - \frac{4}{\pi} \cdot \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp \left[\frac{-D(2n+1)^2 \pi^2 t}{4h^2} \right] \quad (1)$$

where C is the water concentration at the interface at time t ; C_0 is the solubility of the water in the film; D is the water diffusion coefficient; h is the film thickness; n is an integer. Concentration terms can be replaced with experimental absorbances, i.e., $C/C_0 = A/A_0$; where A is the area under the water peak curve and A_0 is the area under the water peak curve corresponding to film saturation with the water (Degim & Kellaway, 1998). Diffusion coefficients were calculated by using a non-linear least square curve fitting software (Shattuck, 2007), in order to fit the experimental data to Eq. (1).

2.6. 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 ($p < .05$) 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. Surface tension of GI fluids

Table 2 shows the apparent surface tension of DW, NSS, SSF, SGF and SIF with and without mucin. Different GI fluids demonstrated

Table 2

Apparent surface tension of different media at $25 \pm 0.5^\circ\text{C}$ ($n = 5$)

Medium	Surface tension (mN/m)	
	Without mucin	With 1 mg/mL mucin
DW	72.9 ± 0.6	62.4 ± 0.5
NSS	74.8 ± 0.2	52.8 ± 0.5
SSF, pH 6.75	74.5 ± 0.4	51.8 ± 0.3
SGF, pH 1.2	74.4 ± 0.3	60.0 ± 0.2
SIF, pH 6.8	73.6 ± 0.4	54.6 ± 0.5

relatively the same surface tension, ranging from 72.9 to 74.8 mN/m. The surface tension of all fluids significantly decreased with addition of mucin (1 mg/mL). This indicates that the addition of mucin could reduce the surface tension of all fluids. A possible explanation is that mucin, having the hydrophilic and hydrophobic segments, acts as a surfactant which results in a reduction of the surface tension between water and air. Mikos and Peppas (1989) reported that the surface tension, using a pendent drop method, of buffered solution pH 7 was decreased from 69.7 to 44.8 mN/m after the addition of 1% w/v of porcine stomach mucin. The increase in mucin concentration from 1% to 30% w/v in buffered solution pH 7 did not affect the measured surface tension (Mikos & Peppas, 1989). A slight decrease in the liquid surface tension after addition of 0.5% w/v of bovine submaxillary mucin to water was reported (Bazskin, Proust, Monsenego, & Boissonnade, 1990). Lehr et al. (1992) also confirmed that the surface tension of the test media differed from that of pure water by less than 5%. In fact, the surface tension of human GI fluids was lowered due to the presence of surface active agents such as bile salt and mucin which could reduce the polar components in the liquid (Lehr, Bodde, Bouwstra, & Junginger, 1993). A surface tension in the range of 35–45 mN/m has been reported for samples of human gastric fluid in several works (e.g., Efentakis & Dressman, 1998; Vissink, De Jong, Busscher, Arends, & s-Gravenmade, 1986). In contrast, for human mixed saliva, a value of 53.1 ± 2.7 mN/m has been reported (Vissink et al., 1986) which is considerably higher than that for GI fluids. Therefore, the mucoadhesion to buccal mucosa may be stronger as compared to mucosa surfaces of the stomach and lower digestive tract.

3.2. Contact angle of GI fluids on pectin surfaces

The affinity of a liquid for a surface can be found using techniques such as contact angle goniometry to measure the contact angle of the liquid on the surface. When a liquid rests on the surface of a solid, in most instances the liquid will not completely wet the solid and will form a drop on the surface with a discrete contact angle (θ). For a liquid, whose contact angle on a given solid is higher than zero, Eq. (2) can be modified by a means of Young's equation (Adamson, 1990).

$$\gamma_s - \gamma_{SL} = \gamma_L \cdot \cos \theta \quad (2)$$

where γ_s is the surface energy of the solid phase in contact with air, γ_L is the surface tension (energy) of the liquid phase in contact with air, and γ_{SL} is the interfacial tension between the solid and liquid.

3.2.1. Effect of contact time on contact angle

The measured contact angle in this study is the water contact angle of medium on polymer surface. As shown in Fig. 1, the measured contact angle of medium (i.e., DW, NSS) on pectin disc was significantly decreased with the increased contact time from 30 to 120 s. This is probably because the wetting process is time-dependent, particularly for hydrophilic materials such as pectin. The dependence of the contact angle on the time implied a strong interaction between the medium and pectin surfaces or substrate.

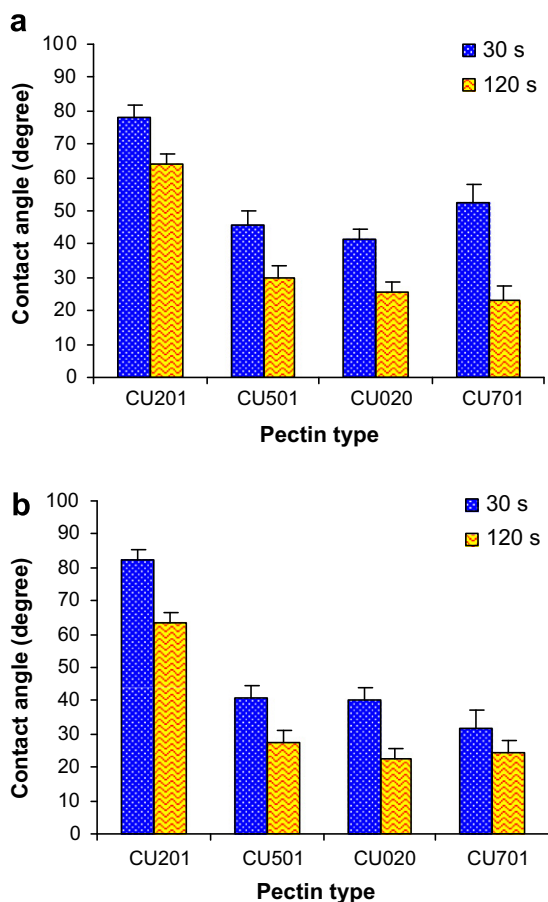


Fig. 1. Water contact angle of (a) distilled water and (b) NSS on pectin discs at 30 and 120 s ($n = 6-8$).

It also related to the pore on surface of pectin discs (discuss later in Section 3.2.2). Luner and Kamp (2001) also found that the contact angle of tested media (e.g., SGF) on poly(methyl methacrylate) surfaces behaved as time-dependent in the first several minutes. Contact angles for medium containing mucin on pectin disc showed a significant time-dependency (data not shown). Vissink et al. (1986) also reported the time-dependency of human saliva and saliva substitutes containing pig gastric mucin. They suggested that the components (i.e., mainly mucin) are very likely to adsorb to the solid-liquid and the liquid-air interface, resulting in a decrease of the contact angle. Due to the roughness of surface, resulting from the gel formation with time-dependence, the contact angle measurement was more complicated. Thus, the contact angle for all further studies was determined at 30 s after dropping of each medium on the pectin surfaces.

3.2.2. Effect of pectin type, medium, mucin and pectin surface on contact angle

The reduction of surface energy of either liquid or substrate decreased the tendency of adhesion occurrence. The reduction of contact angle, however, indicated increasing adhesion tendency. Fig. 2 shows the water contact angle values of each liquid medium on pectin surfaces. For pectin discs (Fig. 2a and c), the highest contact angle was found in high DE pectin (i.e., CU201) for all media, while that of the other three types of pectin was lower. This was due to the CU201 pectin is high molecular weight and contains high amount of methoxy groups, which is a hydrophobic part of the molecule, resulting in lower wettability with water. This is in good agreement with the report by Bodde et al. (1990) in which the

water contact angle of copolymer film prepared from acrylic acid increased with increasing butyl acrylate content, since the surface polarity of the copolymer film was decreased.

The contact angle of SGF (both with and without mucin) on the CU020 disc and that of SSF and SGF with mucin on CU701 disc could not be measured within 30 s because the medium penetrated into pectin discs rapidly and resulted in the wet surface. This result might relate to the gel formation of pectin in acidic environment. The result also corresponded to the higher water uptake with the less erosion of CU020 pectin in acidic medium than in water, as previously reported (Sriamornsak, Thirawong, Weerapol, Nunthanid, & Sungthongjeen, 2007). Addition of mucin in the medium could reduce the contact angle of all media on the discs of low DE pectin (Fig. 2c). The possible explanation is that the surface tension of these media decreased after addition of mucin (Mikos & Peppas, 1989), which facilitated the penetration of media through the hydrophilic surfaces of the pectin discs, e.g., CU020 and CU701.

The contact angle between pectin film and medium, however, could be determined for all pectin types and media (Fig. 2b and d). This might be due to the difference in the texture of pectin film and disc. The film showed the continuous surface which was different from the disc that showing pore or crack, as shown in Fig. 3. Most of the pectin films showed a higher water contact angle than pectin discs, except for CU201. The difference in contact angle between pectin discs and films might be caused by their surface characteristics. The disc surfaces were rough and contained many gaps between particles, which facilitated the penetration of the wetting medium and disallowed the observation of a stable drop. Therefore, the lower contact angles for the rougher surface of pectin discs were expected. This effect contrasted to the surface of pectin films which was smooth and homogenous (Fig. 3). Tröger, Lunkwitz, and Bürger (1997) also demonstrated that the surface porosity affected the measured contact angle which could be corrected by a calculation from their proposed model. Media containing mucin did not show a significant difference in the contact angle of various pectin films except for CU701 which showed the lowest contact angle (Fig. 2d). The rank order of contact angle values between pectin films and medium is CU201 > CU501 \approx CU020 > CU701, which is similar to the rank order of MW and DE.

Fig. 4 shows the relationship between the water contact angle on pectin surfaces and the MW or degree of substitution (DE + DA) of pectins in different media. There was a good relationship between the water contact angle on pectin films and the MW of pectin, particularly in distilled water (Fig. 4a). A better relationship with degree of substitution of pectin (both DE and DA) was observed (Fig. 4b); the r^2 was higher than those with MW. The higher the degree of substitution, the lower the number of carboxyl groups. This indicated that the wettability on pectin films depended on the hydrophobicity of the pectin molecules which resulted from the number of methoxy and amide groups; the wettability decreased with the increase of the DE and DA of pectin.

In case of pectin discs, a modulate relationship between the contact angle and the MW was observed, only when in contact to NSS and SGF as its r^2 was about 0.54 and 0.50, respectively (data not shown). No relationship between water contact angle and degree of substitution ($r^2 < .17$), except for SGF ($r^2 = .84$), was observed. This was probably due to the fact that the porosity and gaps between particles on surface influenced the measured contact angle, resulting in the difference in contact angle between the medium and surface.

3.3. Thermodynamic work of adhesion ($W_{ad/therm}$) and spreading coefficient (S_c)

In general, wetting is a displacement of one element from a surface by another; usually displacement of air from a solid surface by

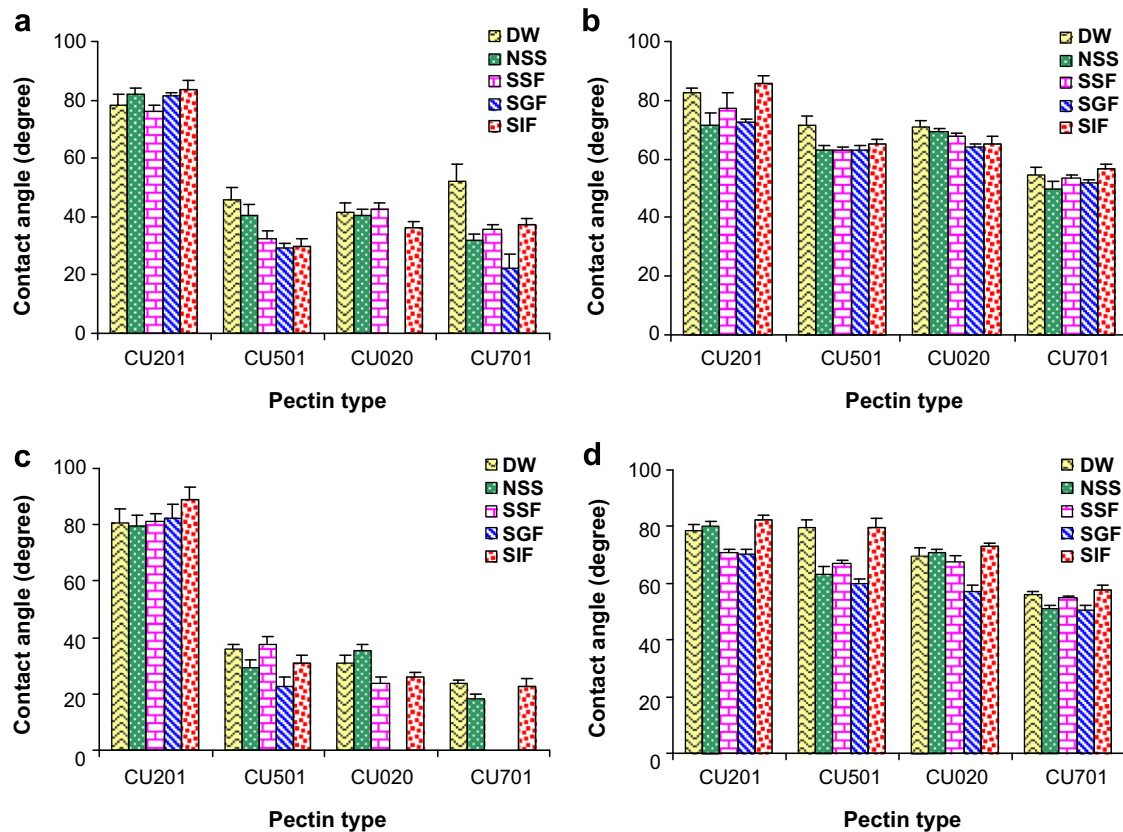


Fig. 2. Contact angle of different media without mucin on (a) pectin discs and (b) pectin films, and those with mucin on (c) pectin discs and (d) pectin films, at 30 s ($n = 6-8$).

water or aqueous solution. Three types of wetting phenomena have been accepted. These are adhesional, spreading and immersional wetting. Only adhesional and spreading wetting have importance in mucoadhesion processes. Spreading is dependent on a so-called spreading coefficient (Adamson, 1990). The droplet adhesion is mainly influenced by the ability of the liquid to penetrate into the porous solid. A liquid not originally in contact with the substrate adheres to it. The driving force of adhesional wetting is known as a work of adhesion, i.e., $W_{ad/therm}$ in this study. The $W_{ad/therm}$ is expressed in the following equation:

$$W_{ad/therm} = \gamma_L + \gamma_S - \gamma_{SL} \quad (3)$$

Combining Eqs. (2) and (3) results in the $W_{ad/therm}$ between the liquid and the mucoadhesive substrate as shown in the following equation:

$$W_{ad/therm} = \gamma_L(\cos\theta + 1) \quad (4)$$

Work of adhesion is a characteristic of two surfaces, each with unique surface tension that shares an interface. It is the work done to overcome the interfacial tension, resulting in a separation of two substances (Albers et al., 1996). The $W_{ad/therm}$ is the work required to separate the test medium from pectin surfaces. The positive value of $W_{ad/therm}$ indicated that the wetting between pectin and medium is a spontaneous process (Luner & Kamp, 2001). It is worth to note that the $W_{ad/therm}$ calculated here is different from the work of adhesion (W_{ad}) calculated from texture analysis (Thirawong et al., 2007).

Spreading coefficient (S_c) is the negative free energy associated with spreading liquid over solid surface (Luner & Kamp, 2001). It is given as:

$$S_c = \gamma_L(\cos\theta - 1) \quad (6)$$

The S_c should be positive for the liquid to spread spontaneously over the solid.

Due to the requirement of the solid surfaces which should be ideally smooth, homogenous and non-deformation when using the Young's equation (Adamson, 1990), only the $W_{ad/therm}$ and S_c of pectin films were calculated (Table 3). The S_c for all fluids on different pectin films were negative (-67.8 to -19.6), indicating that there are no spreading of the fluid on the pectin film surfaces. The S_c values were in the same range for all the studied GI fluids and were found to decrease after addition of mucin. However, different pectins demonstrated the difference in the S_c values, i.e., CU201 showed the most negative values while CU701 showed the least negative values. These values were consistent with the $W_{ad/therm}$ values, i.e., CU201 showed the lowest while CU701 showed the highest. This implied that CU201 showed the weakest bioadhesion while CU701 had the strongest bioadhesion. It is possibly due to the difference in the MW and the number of methoxy groups in pectin structure. A higher MW and the presence of hydrophobic moieties in pectin structure may result in the lower S_c and $W_{ad/therm}$.

Fig. 5 shows the relationship between the $W_{ad/therm}$ of pectin films and the MW or degree of substitution of pectins in different media. The $W_{ad/therm}$ of pectin films in contact with each medium has a good relationship to both the MW and the degree of substitution as the r^2 ranged from .64 to .93. A higher MW or degree of substitution of pectin showed a lower in the $W_{ad/therm}$, resulting from its higher contact angle. These results, however, contrasted to the results from the mucoadhesion test by texture analysis conducting on hydrated samples in excess medium (Thirawong et al., 2007), in which the higher MW and a higher degree of substitution of pectin demonstrated a stronger mucoadhesion. The work of adhesion (W_{ad}), calcu-

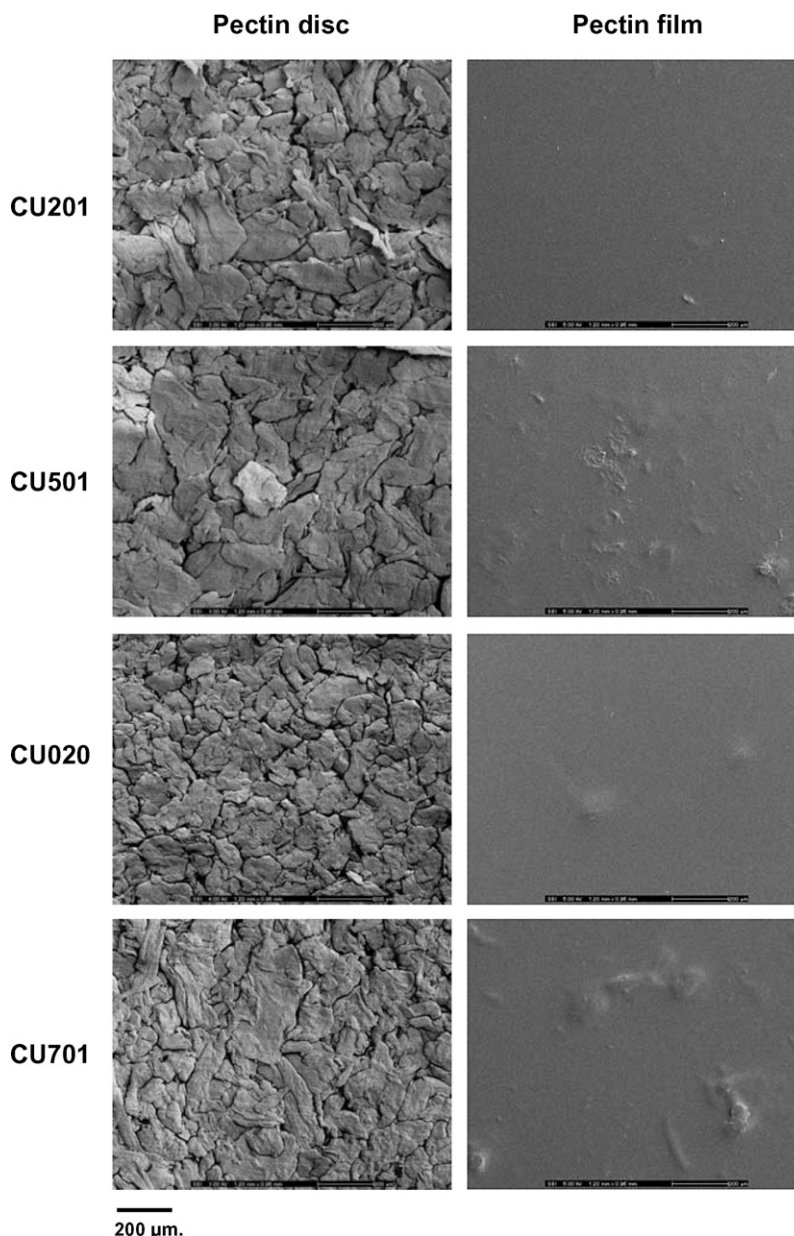


Fig. 3. SEM images of the surface of pectin discs (left column) and films (right column).

lated from texture analysis that corresponded to the fracture theory. This differs a little from the wetting theory in that it relates the adhesive strength to the forces required for the detachment of the two involved surfaces (e.g., mucosa and pectin surfaces) after adhesion. The failure of the adhesive bond normally occurs at the weakest component, which is typically a cohesive failure within one of the adhering surfaces (Adamson, 1990). Therefore, in this study, it is likely that the hydrophilicity of molecules is necessary for the wettability of pectin in medium and plays an important role on the mucoadhesion of pectin.

In case of the lack of spreading, liquid formed droplets over the substrate with a finite contact angle. To spread, the liquid drop has to get energy from the outside of the system. This may be an advantage for the buccal bioadhesive drug delivery where the dosage forms can be applied with external force/pressure. In this study, the positive values of the $W_{ad/therm}$ demonstrated that the wetting between pectin and medium could be occurred spontane-

ously by adhesional wetting. The wetting and spreading processes may play an important role in the molecular interaction between pectin surfaces and substrates involving in mucoadhesion (e.g., buccal or GI mucosa). The impact of the thermodynamic balance of surface free energy on the molecular interaction between two hydrogel surfaces was also proposed by Lehr et al. (1992). They believed that mucoadhesion involved a two-step process; the adsorptive contact governing primarily by surface-energy effects and spreading processes and the interdiffusion of polymer chains across the interface enhancing bond formation. Moreover, the electrostatic interactions between pectin and mucin could influence the $W_{ad/therm}$. Therefore, the mucoadhesion of the pectin-based formulations can not be predicted by surface properties alone.

3.4. ATR-FTIR characterization

Fig. 6a shows the FTIR of various types of pectin film, in the frequency region from 4000 to 500 cm^{-1} . The broad band in the range

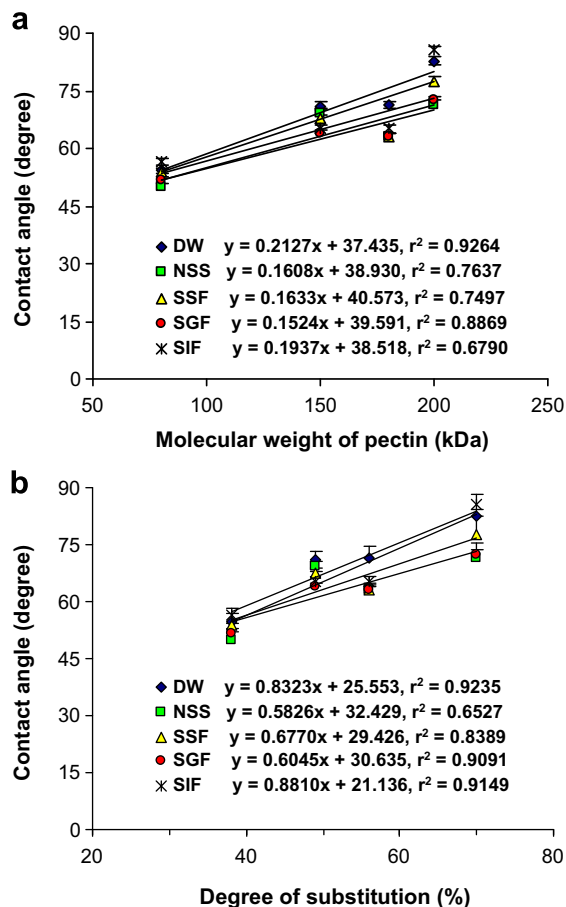


Fig. 4. Relationship between the water contact angle and (a) MW and (b) degree of substitution (DE + DA) of pectin in different media without mucin. The correlation coefficient (r^2) from linear regression analysis in each medium is shown.

of 3500–3000 cm^{-1} was due to OH stretching extensively involved in H-bonding. The characteristic absorption band was observed at 1736 cm^{-1} assigned to C=O stretching of methyl ester (COOR) group and a peak around 1600 cm^{-1} assigned to C=O stretching of COO[−] group (Séné, McCann, Wilson, & Grinter, 1994). The amidated low DE pectin (CU020) has three bands at 1736 cm^{-1} (C=O vibration), 1670 cm^{-1} (amide I) and 1588 cm^{-1} (amide II) (Sinitsya, Copikova, Prutyantov, Skoblya, & Machovic, 2000). The absorption bands around 1200–900 cm^{-1} called carbohydrate region (McCann, Hammouri, Wilson, Belton, & Roberts, 1992). All pectins showed a rich in uronic acid because of the high intensity peaks

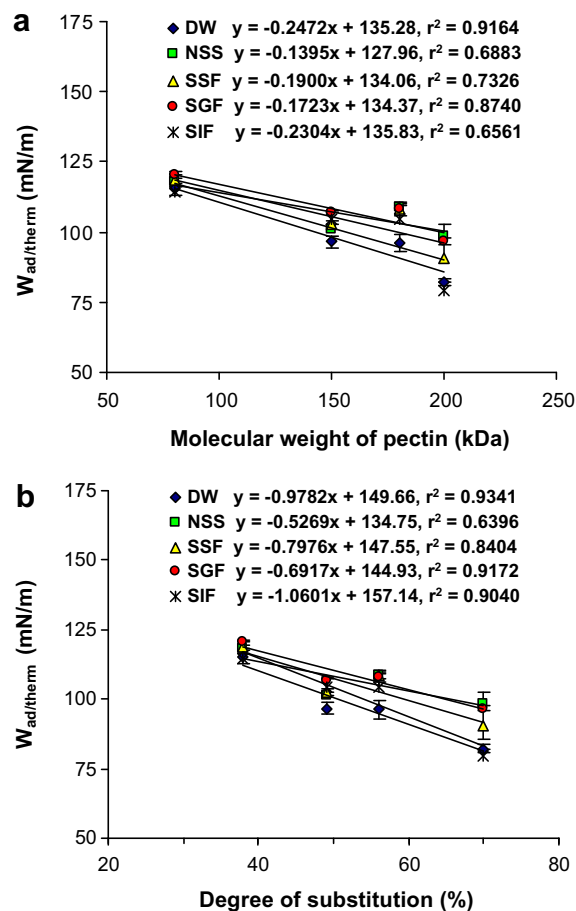


Fig. 5. Relationship between the $W_{ad/therm}$ of pectin films and (a) MW and (b) degree of substitution (DE + DA) of pectin in different media without mucin. The correlation coefficient (r^2) from linear regression analysis in each medium is shown.

around 1100 and 1018 cm^{-1} (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998). Fig. 6b demonstrates the FTIR spectra of a porcine gastric mucin powder and mucin dispersion (5% w/w, in DI water). Mucin is attributable to amino acids or to oligosaccharide groups. IR spectrum of mucin, in powder form, was different from those in aqueous solution, due to the absence of water. The primary amine deformation (expected at 1600 cm^{-1}) is masked by a stronger amide I (C=O stretch, 1654 cm^{-1}) which is unaffected by pH change. A peak around 1553 cm^{-1} was assigned to anti-symmetric stretch of carboxylate anion which is enhanced by increasing pH (Patel et al., 2003). Spectrum of mucin in DI water showed

Table 3

Thermodynamics work of adhesion ($W_{ad/therm}$) and spreading coefficient (S_c) of various liquid media with and without mucin on pectin film

Medium		Thermodynamic work of adhesion (mN/m)				Spreading coefficient (mN/m)			
		CU201	CU501	CU020	CU701	CU201	CU501	CU020	CU701
DW	Without mucin	82.2	96.3	96.6	115.2	−63.6	−49.5	−49.2	−30.6
	With mucin	74.5	73.9	84.2	97.5	−50.3	−50.9	−40.6	−27.3
NSS	Without mucin	98.5	108.8	101.2	118.2	−51.1	−40.8	−48.4	−26.7
	With mucin	61.8	76.6	70.2	86.0	−43.8	−29.0	−35.4	−19.6
SSF	Without mucin	90.6	108.3	102.8	118.6	−58.4	−40.7	−46.2	−30.4
	With mucin	68.9	72.0	71.5	81.7	−34.7	−31.6	−32.1	−21.9
SGF	Without mucin	96.8	108.2	107.0	120.4	−52.0	−40.6	−41.8	−28.4
	With mucin	79.9	90.0	92.6	98.0	−40.1	−30.0	−27.4	−22.0
SIF	Without mucin	79.4	104.5	104.6	114.3	−67.8	−42.7	−42.6	−32.9
	With mucin	61.7	64.5	70.6	83.7	−47.5	−44.7	−38.6	−25.5

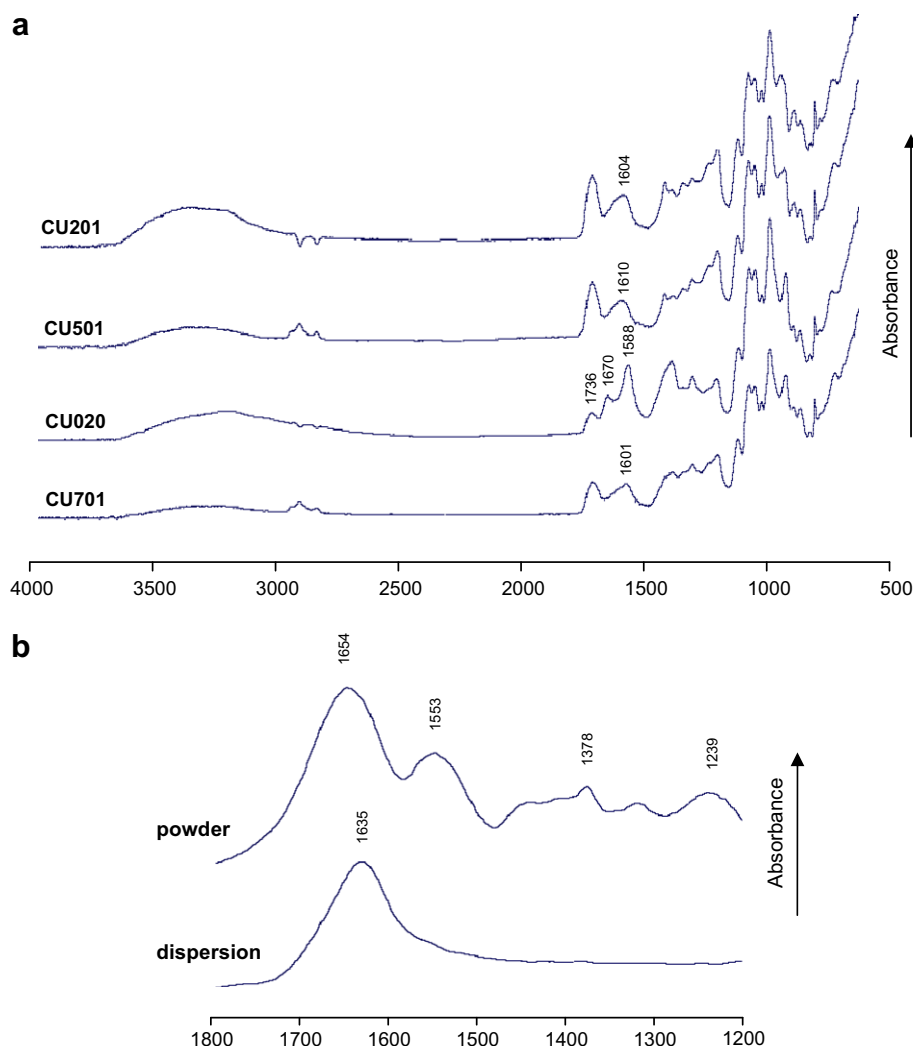


Fig. 6. FTIR spectra of (a) of different pectin films and (b) porcine gastric mucin powder and dispersion in DI water (5% w/w).

one strong peak around 1635 cm^{-1} . Saiano et al. (2002) reported a band at 1550 cm^{-1} assigned to $\text{C}=\text{O}$ stretching vibration of sialic acid of mucin. However, there was a very low intensity of this band in this study (Fig. 6b). This might be due to the different types of mucin used in the study; the mucin type I-S from bovine submaxillary glands in pH 7 buffer was used in the study of Saiano et al. (2002) while the type III porcine stomach mucin was used in this study.

Fig. 7 shows the time evolution of the spectra for CU201 film in contact with a mucin in DI water (5% w/w) from ATR-FTIR analysis. Other pectin films in contact with mucin in different media showed similar spectra characteristics (data not shown). The integrated area of OH stretching band at 3400 cm^{-1} was used to monitor the diffusion of water as an indirect measurement of any change resulting from interpenetration of polymer–mucin chains at the aqueous solution/polymer film interface (Saiano et al., 2002). As water diffused through the film, there would be a steady concentration build-up of the water at the crystal/polymer film interface. When the water wet the pectin film, the intensities of the ester band around 1736 cm^{-1} decreased and the intensities of the peaks around 3400 and 1640 cm^{-1} increased. The peak located at 1640 cm^{-1} was due to in-plane H–O–H bending vibration (Silverstein, Bassler, & Morrill, 1981). The characteristics of carbohydrate region (around $1200\text{--}900\text{ cm}^{-1}$) of pectin were also chan-

ged. Peaks at 1100 and 1074 cm^{-1} shifted of about 5 cm^{-1} and broad band spectra were found (Fig. 6b). The peak of mucin around 1550 cm^{-1} was not observed due to small intensity of the spectrum prior to the test. Saiano et al. (2002), however, reported that the mucin peak (around 1550 cm^{-1}) showed a slight increase with time after in contact with α,β -poly(*N*-hydroxyethyl)-DL-aspartamide (PHEA) and α,β -polyaspartylhydrazide films.

Fig. 8 shows the relative absorbance of water as a function of time for pectin film in contact with a mucin in various media. The integrated area under the band around 3400 cm^{-1} was used for comparison. The experimental data were fitted to the diffusion model in Eq. (1), using non-linear curve fitting software, and then the diffusion coefficients were calculated. The best fit with the experimental data gave a mean diffusion coefficient of water through pectin film (thickness of $60 \pm 3\text{ }\mu\text{m}$) as shown in Table 4. The diffusion coefficients of water from different media through pectin films were not much different, in the range from 1.03×10^{-6} to $1.32 \times 10^{-6}\text{ cm}^2/\text{s}$. Amidated low DE pectin (CU020) film showed the highest diffusion coefficient of water from both DI water and SIF, indicating that water could diffuse through CU020 film in a faster rate than other pectin films. This led to the interdiffusion of pectin and mucin chains. The possible explanation is that CU020 contains both amide and carboxyl groups in the structure, which can form stronger H-bond than

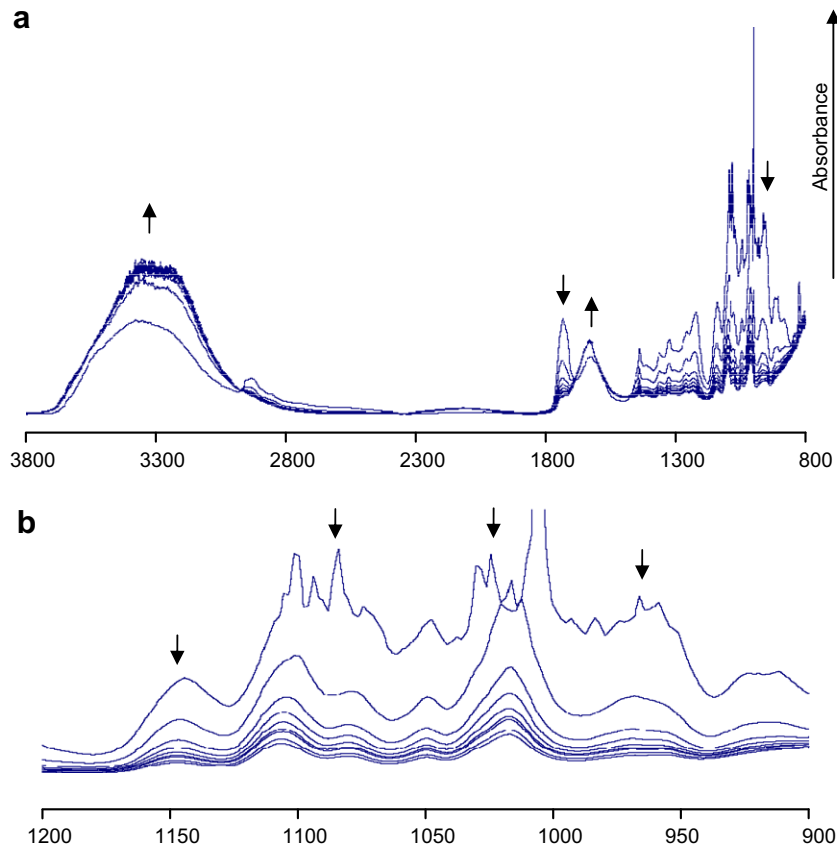


Fig. 7. Time evolution of the spectrum of CU201 film in contact with mucin in DI water (5% w/w) in the range of (a) 3800–800 cm⁻¹ and (b) 1200–900 cm⁻¹.

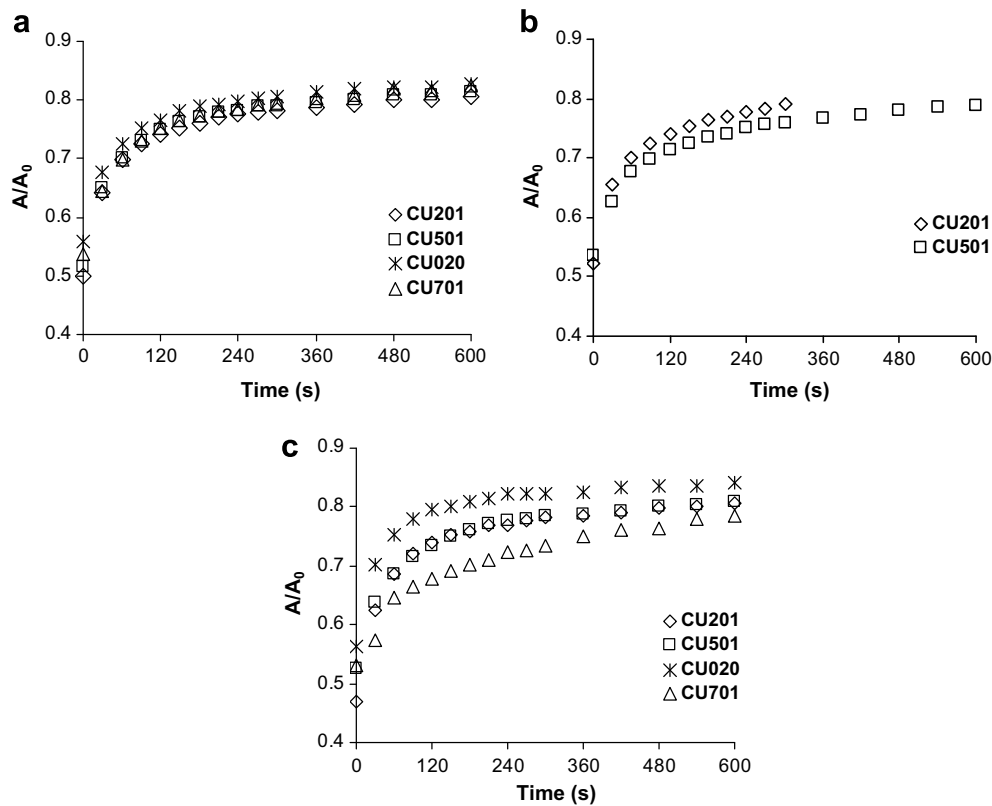


Fig. 8. Relative absorbance of water as a function of time for pectin film in contact with a mucin (5% w/w) in (a) DI water, (b) SGF and (c) SIF.

Table 4Diffusion coefficient (cm^2/s) of water through different pectin films

Pectin	DI water ($\times 10^{-6}$)	SGF ($\times 10^{-6}$)	SIF ($\times 10^{-6}$)
CU201	1.19	1.07	1.17
CU501	1.21	1.11	1.18
CU020	1.26	N/A	1.32
CU701	1.20	N/A	1.03

Note: N/A = not applicable. The film thickness was $60 \pm 3 \mu\text{m}$.

other types of pectin. The diffusion coefficient of water from SGF through high DE pectin film was lower than those from DI water and SIF. This might be due to carboxylate anion was converted to carboxylic acid with SGF, resulting in a lowered diffusion of water through the film.

4. Conclusions

The initial step in mucoadhesion is the wetting process which permits an intimate contact with mucin in mucus layer. Following this phase, the polymer hydrates by extraction of water from the glycoprotein gel resulting in the opportunity for interfacial diffusional mixing of polymer and glycoprotein chains.

The wetting properties of mucoadhesive pectin could be determined by the contact angle of medium on pectin surfaces. The roughness and porous structure of pectin discs gave a lower contact angle than pectin films. The high DE pectin showed a greater water contact angle but lower $W_{\text{ad/therm}}$ with medium than low DE pectin, indicated the low wettability of high DE pectin. The water contact angle of pectin showed a good relationship to their molecular weight and degree of substitution. The wetting studies demonstrated that all types of pectin could be wet spontaneously by adhesional process. The hydrophilicity or hydrophobicity of the molecules also played an important role for thermodynamics of wetting, which could be used to support the explanation of mucoadhesive properties of pectin.

The ATR-FTIR analysis of pectin film in contact with mucin revealed the changes resulting from interpenetration of polymer–mucin chains at film interface and the formation of H-bonding. The diffusion coefficient of water through pectin film was calculated and a slight difference was found when tested in different media. The results from ATR-FTIR studies demonstrated that a chain interdiffusion occurred at the interface of pectin film and mucin solution which supported the diffusion theory of mucoadhesion.

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