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Rheological synergy in aqueous mixtures of pectin and mucin

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ABSTRACT

The interaction between pectin and mucin was investigated by comparing the viscoelastic properties of mucin–pectin mixtures, using a small-strain oscillatory rheology, against those of the pure components. All pectins showed rheological synergism when mixed with mucin, as evidenced by an increase in dynamic moduli and a decrease in loss tangent. The interaction between pectin and mucin related to the physical entanglement of gel network and depended on pectin type, pectin concentration, mucin concentration and dispersion medium. Pectin with higher degree of esterification (DE) showed a greater interaction than that with lower DE. Amidated low DE pectin in deionized water showed the strongest interaction with mucin due to the presence of amide and carboxylic acid groups in its structure facilitating H-bond with mucin. Increasing of mucin concentration affected the mucoadhesive interaction of pectin. The results suggested that the study of rheological synergy can be used to indicate the mucoadhesive interaction of the materials to be used in mucoadhesive drug delivery systems.

1. Introduction

The concept of mucoadhesion has received considerable interest in formulation science in recent years, as a platform for controlled delivery. The attention has been focused on the potential of a mucoadhesive dosage form to prolong the residence time within a specified region of the body, and create an intimate contact with the absorbing membrane (Gu, Robinson, & Leung, 1988). This would decrease the diffusion path of the drug and could protect it from enzymatic activity or luminal degradation.

The mucoadhesive phenomenon is a complex process and numerous theories, which were developed to understand and explain the adhesive performance of adhesives, have been adapted to gain an understanding of bio/mucoadhesion. Six theories being proposed to explain the mucoadhesion phenomena are wetting, adsorption, diffusion, electronic, mechanical and fracture theories (Smart, 2005). 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 (Hassan & Gallo, 1990; Mortazavi, Carpenter, & Smart, 1992; Rossi, Ferrari, Bonferoni, & Caramella, 2001) and attenuated total reflection Fourier transform infrared spectroscopy (Jabbari, Wisniewski, & Peppas, 1993). It is generally accepted that chain interlocking (physical entanglement), conformational change and chemical interactions (like hydrogen and van der Waals bonds), which occurred between a mucoadhesive polymer and mucin (or mucus), are likely to produce changes in the rheological behavior of the two macromolecular species.

The interaction at the functional group level often resulted in the formation if mixtures capable of exhibiting rheological synergy (Caramella, Rossi, & Bonferoni, 1999), that is, to demonstrate gel-like properties when mixed, greatly in excess of when the mucin (or mucus) and polymer dispersions were examined separately. Several authors suggested that rheological synergism between polymer and mucin (or mucus) can be used as an *in vitro* parameter to determine the mucoadhesive properties of a material (e.g. Bonacucina, Martelli, & Palmieri, 2004; Caramella, Bonferoni, Rossi, & Ferrari, 1994; Caramella et al., 1999; Hassan & Gallo, 1990; Hägerström, Paulsson, & Edsman, 2000; Mortazavi et al., 1992; Rossi et al., 2001), and provides an acceptable model representative of the *in vivo* behavior of a mucoadhesive polymer.

The main components of the mucus layer include water (up to 95% by weight), mucin (generally 0.5–5% by weight), inorganic salts (about 1% by weight), carbohydrates and lipids. Mucin is chiefly responsible for the characteristic viscoelastic and gel forming properties of the mucus, essential to its protective role (Marriott & Gregory, 1990). Mucin is a block copolymer with branched and un-branched blocks. Both blocks contain protein backbone chains and the branched blocks contain many highly branched oligosaccharide chains. The oligosaccharide side chains have sugar residues such as galactose, fucose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid. At $pH > 3$, both

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sialic acid and sulfated sugars are fully ionized and this confers a net negative charge to the molecule (Marriott & Gregory, 1990). The dried mucin could be dispersed in aqueous medium because it contains numerous hydrogen bonding groups, e.g. the hydroxyl groups in the branched sugar chains, the amide groups in the backbone chains, and some carboxylic or sulfate groups in the terminal segments of branch chains (Peppas & Huang, 2004). In addition, these functional groups are responsible for the interactions with mucoadhesive polymers. Mucoadhesive polymers are generally hydrophilic molecules that contain numerous hydrogen bond forming groups and a number of charged and neutral polymers has been classified as mucoadhesive, since they are known to bind very strongly to mucin or mucus via non-covalent bonds (Smart, 2005).

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, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007). The mucoadhesive properties of pectin are presumably due to the formation, of secondary chemical bonds such as hydrogen bonds between hydrogen bonding groups (e.g. carboxyl groups) of pectin and functional groups of mucin glycoprotein in mucus (Thirawong et al., 2007). Clearly, this will depend on the chemistry of specific pectin and the environmental pH.

Pectin is a naturally occurring water-soluble polysaccharide which is 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 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; Sriamornsak, 2003).

Previous studies have shown the rheological interaction between pectin and mucin by large-strain measurement using a simple Brookfield viscometer (Thirawong, Kennedy, & Sriamornsak, 2008). The objective of this study was, therefore, to look more closely into the interactions between pectin and mucin using dynamic oscillatory rheology. The pectin–mucin interactions in various media were examined. The effect of pectin type, pectin concentration (i.e. 1% and 2% w/w) and mucin concentration (i.e. 2.5%, 5.0% and 7.5% w/w) on oscillatory dynamic moduli were also investigated.

2. Experimental details

2.1. Materials

Four commercial pectins with different DEs and molecular weights (MWs) (see Table 1) were kindly provided by Herbstreith & Fox KG (Germany). Polyacrylic acid cross-linked polymer (carbomer934P) manufactured by Corel Pharma-Chem (India) was used. Partially purified powder of mucin from porcine stomach, type III, with bound sialic acid of 0.5–1.5% (Sigma Chemical Co., Ltd., USA), was selected for use to limit the variability in the rheological measurement owing to variability in native mucus samples. All other chemicals were analytical grade and used as received without further purification. Deionized (DI) water was prepared by reverse osmosis throughout all experiments.

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 (Dalton)
High methoxy pectin			
CU201	70	0	200,000
CU501	56	0	180,000
Low methoxy pectin			
CU020	29	20	150,000
CU701	38	0	80,000

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

2.2. Preparation of samples

Mucin dispersions and mucoadhesive polymer solutions were prepared in different media, i.e. DI water, simulated gastric fluid USP without pepsin (SGF, pH 1.2), and simulated intestinal fluid (SIF, pH 6.8). Dried mucin was hydrated with each medium by gentle stirring for 3 h at room temperature to yield a dispersion of 10% w/w. Six-gram aliquots of mucin dispersion (10% w/w) in DI water, SGF or SIF were mixed well with 6 g of each polymer in the corresponding media to give the concentration of 1.0% and 2.0% w/w for pectin. The final concentration of mucin was 5% w/w. All systems were equilibrated at 37.0 ± 0.1 °C for 1 h prior to analysis.

The samples of 1% or 2% w/w pure pectin and 5% w/w mucin were also measured in order to compare the increment of rheological parameters. The samples for studying the effect of mucin concentration on pectin solution (low DE pectin, CU701) in SIF were prepared by mixing the stock solutions of pectin (2.0% w/w) and mucin (15% w/w) to make the final concentration of 1% w/w pectin and 2.5%, 5.0% or 7.5% w/w mucin. In each case, the rheological experiments were performed once on each solution. At least three replicates of samples were measured.

2.3. Rheological characterization

The oscillatory shear experiments were conducted in the Paar Physica MCR 301 rheometer (Anton Paar GmbH, Austria) using either a 50-mm stainless-steel parallel plate with a 0.05-mm gap (CP50-1) or a concentric cylinder (DG26.7) measuring system. An initial strain amplitude sweep measurement was made to determine the shear independent plateau of the linear viscoelastic region (LVR). Subsequent frequency sweeps were carried out at strain values in the shear independent plateau over the frequency range from 100 to 0.1 Hz. These frequency sweeps were used to determine the storage or elastic modulus (G') and loss or viscous modulus (G''). Samples were individually loaded on the lower plate or the measuring geometry and allowed to stand at 37 °C for 5 min prior to the test. All samples were prepared in three replicates; each of them was analyzed once. The effect of pectin type, pectin concentration (i.e. 1% and 2% w/w), mucin concentration (i.e. 2.5%, 5.0% and 7.5% w/w), and dispersion medium (i.e. DI water, SGF and SIF) on oscillatory dynamic moduli were investigated.

2.4. Calculation of rheological synergism

The rheological synergism parameters were measured as a function of the dynamic moduli. The dynamic moduli (G' and G'') were measured as a function of frequency. The G' is the energy stored and recovered per cycle of deformation and reflects the solid-like component of a viscoelastic material. In contrast, G'' is the energy lost per cycle and reflects the liquid-like component. The viscoelastic properties of the samples were described using the loss tangent ($\tan\delta$), which is an indicator of the overall viscoelasticity of the sample being a measure of the energy loss to the energy stored per cycle (G''/G'). $\tan\delta < 1$ indicates a solid or gel-like component or elastic behavior, whereas $\tan\delta > 1$ indicates a liquid or viscous-like component (Rossi, Bonferoni, Fermi, Bertoni, & Caramella, 1996; Shah & Donovan, 2007). When tan δ became smaller, the elasticity of the material increased and the viscous behavior reduced. The rheological synergism or interaction between the polymer and mucin ($\Delta G'$ and $\Delta G''$), which is the difference between the actual viscoelastic values of the mixture of polymer and mucin (G'_{mix} and G''_{mix}) and the theoretical values defined as the sum of the viscoelastic components of polymer (G'_{p} and G''_{p}) and mucin (G'_{m} and G''_{m}), was calculated as follow:

$$\Delta G' = G'_{\mathrm{mix}} - (G'_{\mathrm{p}} + G'_{\mathrm{m}}) \tag{1}$$

$$\Delta G'' = G''_{\mathrm{mix}} - (G''_{\mathrm{p}} + G''_{\mathrm{m}}) \tag{2}$$

This is similar to the viscosity components of bioadhesion (Thirawong et al., 2008). The dynamic moduli determined at the intermediate frequency of 1.15 Hz were used in the calculation.

2.5. 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. Small deformation rheology

Freshly prepared pectin–mucin mixtures were subjected to an initial amplitude sweep to determine the LVR where the values of the moduli are independent of the applied deformation. Subsequent frequency sweeps were carried out at strain values within the LVR in the frequency range of 0.1–100 Hz. Figs. 1–3 show the rheological profiles of various types of pectin (2% w/w), mucin (5% w/w) and their mixtures in DI water, SGF and SIF, respectively. The G' and G'' of the mucin demonstrated frequency dependence with a cross-over at about 2 Hz in DI water. This indicated that mucin behaved as an entangled polymer system. This is similar to that reported for homogenized mucus gel by Madsen, Eberth, and Smart (1998). All pure pectin solutions (2% w/w) in different media did not give a strong gel as the G'' was greater than the G' (i.e. viscous behavior became dominant over elastic behavior), which resulted in a higher $\tan\delta$ (Table 2). When the mucin (5% w/w) was added, the mixture showed more elastic behavior than the individual polymer ($\tan\delta$ close to 1.0 and dynamic moduli increased), indicating a stronger gel in the structure. The higher G'' than G' and a substantial decline at low frequencies indicated that the physical entanglement of pectin and mucin may occur (Clark & Ross-Murphy, 1987; Ross-Murphy & McEvoy, 1986).

The magnitude of G', G'' and the shape of the profiles directly related to the gel strength of the sample (Madsen et al., 1998). In a strong cross-linked gel, the G' would be much larger than G'' and not influenced by the frequency of oscillation or the experimental time, whilst a physical entangled gel network would have G'' over G' at some points in the frequency range with a substantial decline in G' at low frequency (Clark & Ross-Murphy, 1987; Ross-Murphy & McEvoy, 1986). This is because polymer chains in physical entangled system needed time to untangle at low frequencies. In contrast, there was insufficient time for any network rearrangement within the period of one oscillation at high frequency, resulting in an elastic deformation. Hence, all pectin–mucin mixtures in all media (Figs. 1–3) behaved like an entangled system which was weaker in gel strength than the cross-linked gel.

3.2. Effect of mucin on rheological enhancement (synergism)

As seen from Figs. 1–3, there is an increase in the viscoelastic moduli of pectin–mucin compared to the pectin and mucin solutions alone, showing rheological synergism. The viscoelastic parameters ($\Delta G'$ and $\Delta G''$) of the combination systems in different media are shown in Figs. 4 and 5. This is similar to that found by the viscosity measurement using Brookfield viscometer (Thirawong et al., 2008). The rank order of $\Delta G'$ and $\Delta G''$ of the combinations in DI water was CU020 > CU201 > CU501 > CU701. The highest $\Delta G'$ and $\Delta G''$ of CU020 might be due to the presence of amide groups, together with carboxyl groups, in its structure. Thus, the H-bond formation may be stronger than other types of pectin, particularly in water. Pectin with higher DE showed a greater interaction than that with low DE. As the DE increases, it is likely that the charge density will decrease and the extent of chain entanglement (and probably inter-chain and intra-chain associations between the methyl groups) is likely to increase (Thirawong et al., 2008). Most pectin showed stronger interaction with mucin in DI water than in SIF and SGF. Similar effects were observed for viscosity enhancement. This is probably due to the effect of pH and ionic strength of the medium that influenced the association of pectin molecules.

The carboxyl groups of pectin and the sialic acid of mucin were uncharged in SGF and able to form H-bonding or other non-electrostatic interactions. The pH of pectin in DI water was around 3.2–4.3 which is close to its pK_{a} (about 3–4). It implied that pectin was in unionized form and, probably, could interact with mucin via H-bonding. This result corresponded with the studies of rheological behavior of carbomer934P and mucus glycoproteins, having the optimum pH for gel strengthening around its pK_{a}. It is suggested that a certain number of unionized carboxyl groups within the polymer chains is required for optimal interaction with the mucin (Mortazavi et al., 1992). As the pH environment increased (i.e. in SIF), the carboxyl groups and sialic acid were both negatively charge. Therefore, the H-bonds could be replaced by electrostatic interaction, resulting in a less of the H-bonded network in gel structure. The electrostatic interaction between polymer and mucin with the same charge is generally repulsive, therefore, reduced chain entanglements are expected. Low DE pectin with the greatest number of carboxyl groups would have the highest negative charge density in SIF, which decreased the chain entanglement of pectin and mucin. Both CU701 and CU020 could not be dispersed homogeneously in SGF because of the formation of insoluble gel so that the viscosity and moduli could not be measured.

As the concentration of pectin was increased to 2%, the dynamic moduli for both the pectin solution alone and the pectin–mucin mixture increased (2- to 11-fold) (Fig. 5). This is because the increased amount of mucoadhesive H-bond forming groups in the mixtures promoted a stronger bond formation between pectin and mucin. This is in a good agreement with the results obtained by Madsen et al. (1998) which the rheological response in the polymer–mucus gel was concentration dependent.

Another method to consider the magnitude of the rheological synergism is by a consideration of the relative rheological synergism (i.e. G'_{rel} and G''_{rel}) or the normalized interaction parameters (Rossi et al., 1995), which express the relative increment in viscoelasticity with regard to the polymer and mucin solutions alone and can be calculated from Eqs. (3) and (4), respectively,

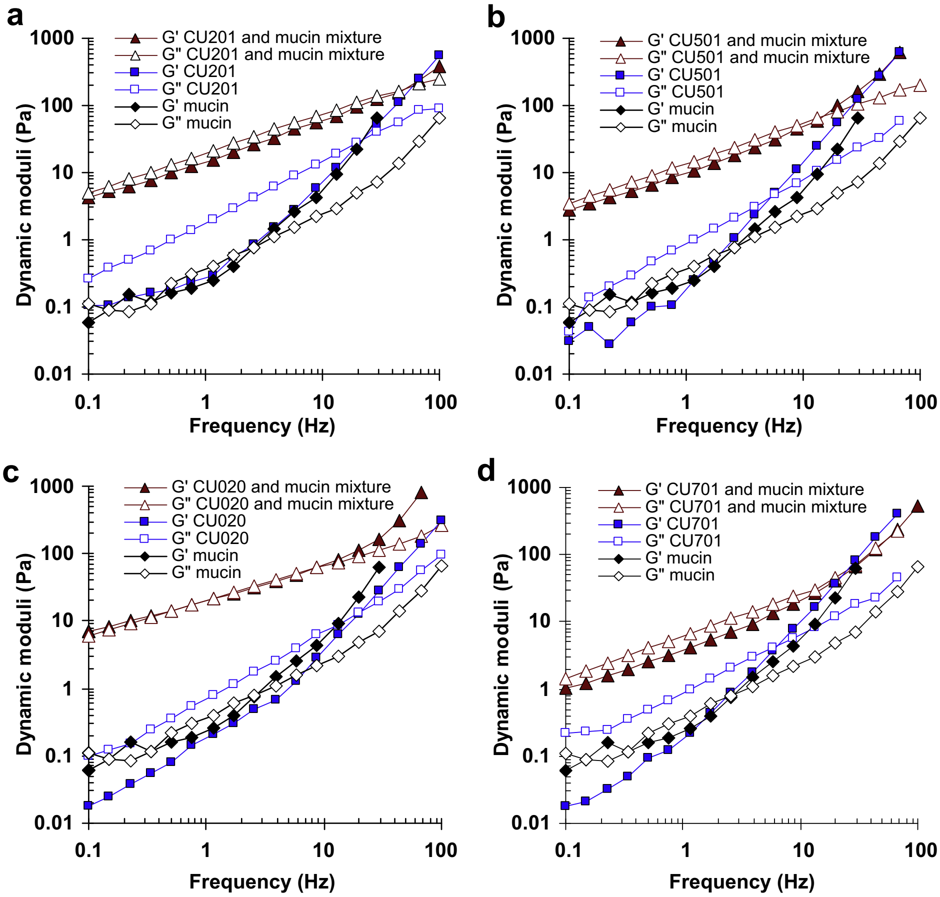

Fig. 1. The frequency dependence of the elastic modulus (G', filled symbol) and the viscous modulus (G'', opened symbol) for pectin (2% w/w) and its mixture with mucin (5% w/w) in DI water; (a) CU201, (b) CU501, (c) CU020 and (d) CU701. The dynamic moduli of mucin (5% w/w) are shown in all figures for comparison purpose.

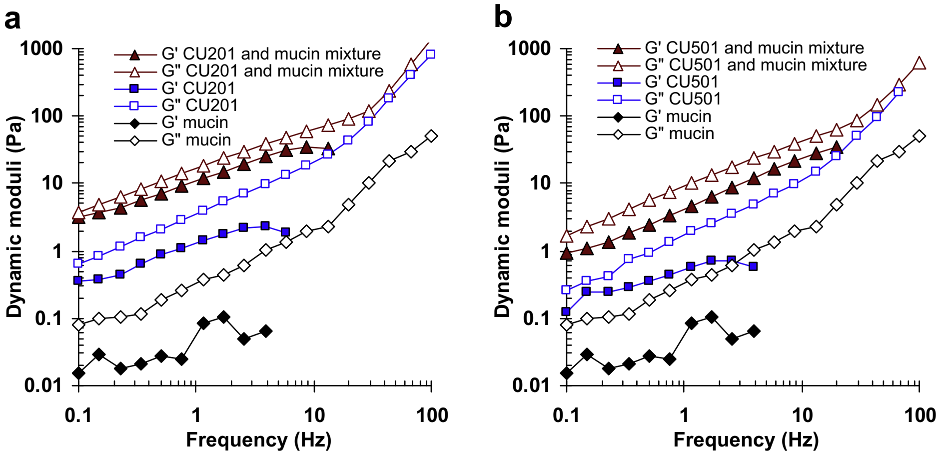

Fig. 2. The frequency dependence of the elastic modulus (G', filled symbol) and the viscous modulus (G'', opened symbol) for pectin (2% w/w) and its mixture with mucin (5% w/w) in SGF; (a) CU201 and (b) CU501. The dynamic moduli of mucin (5% w/w) are shown in all figures for comparison purpose.

$$G'_{\mathrm{rel}} = \frac{\Delta G'}{G'_{\mathrm{p}} + G'_{\mathrm{m}}} \tag{3}$$

$$G''_{\mathrm{rel}} = \frac{\Delta G''}{G''_{\mathrm{p}} + G''_{\mathrm{m}}}. \tag{4}$$

This is comparable to the rheological synergistic effect that expressed as a proportion of the unmixed materials viscosities (Thirawong et al., 2008). A relative rheological synergism equals to one means there is no interaction between polymer and mucin. A higher value of relative rheological synergism shows rheological synergism between the polymer and mucin and is indicative of potentially mucoadhesive associations between them. The calculated G'_{rel} and G''_{rel} of various pectins in different media are shown in Fig. 6. It was observed that the G'_{rel} and G''_{rel} of pectin showed the same trend as $\Delta G'$ and $\Delta G''$ parameters, i.e., most pectin showed stronger interaction with mucin in DI water than in SIF and SGF. The excep-

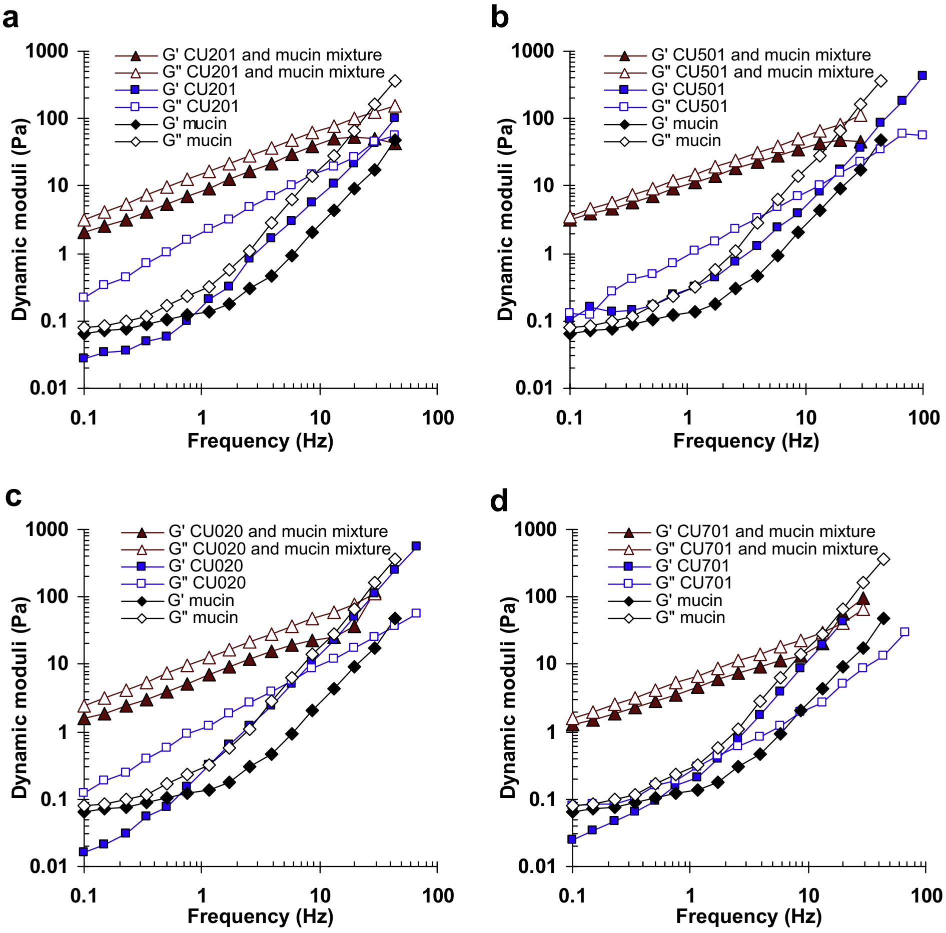

Fig. 3. The frequency dependence of the elastic modulus (G', filled symbol) and the viscous modulus (G'', opened symbol) for pectin (2% w/w) and its mixture with mucin (5% w/w) in SIF; (a) CU201, (b) CU501, (c) CU020 and (d) CU701. The dynamic moduli of mucin (5% w/w) are shown in all figures for comparison purpose.

Table 2
tan δ at frequency of 1.15 Hz of pectin (2% w/w) and the mixture with mucin (5% w/w) in different media ($n = 3$)

Pectin type		DI water	SGF	SIF
CU201	Pectin alone	5.45 ± 0.66	3.05 ± 1.58	8.54 ± 0.85
	Mixture	1.36 ± 0.03	1.53 ± 0.03	1.76 ± 0.02
CU501	Pectin alone	4.56 ± 1.29	2.90 ± 0.55	3.44 ± 0.86
	Mixture	1.39 ± 0.07	2.24 ± 0.07	1.33 ± 0.01
CU020	Pectin alone	4.31 ± 1.41	N/A	3.88 ± 0.99
	Mixture	1.00 ± 0.02	N/A	1.82 ± 0.07
CU701	Pectin alone	4.89 ± 1.13	N/A	2.07 ± 0.95
	Mixture	1.61 ± 0.08	N/A	1.48 ± 0.12

tion is for the G'_{rel} of CU701 in SIF and G''_{rel} of CU201 in DI water, due to their initial G' and G'' value. The relative approach to synergism is the parameter to consider how many times stronger (or weaker) the polymer–mucin mixture is compared to the gel itself. The limitation of these parameters when the G'_{rel} and G''_{rel} approach infinity (if the moduli close to zero) or when the G'_{rel} and G''_{rel} are negative, was suggested (Hägerström & Edsman, 2003). As seen from Fig. 6, however, there is no problem with the limitation of negative values of synergism parameters of different pectins.

In SIF, the rheological synergism of combined systems containing pectin (as shown in Fig. 6) was higher than those containing carbomer934P (Thirawong, 2007), i.e. G'_{rel} and G''_{rel} of the mixtures containing carbomer934P are −3.4 ± 0.2 and 2.3 ± 0.2, respectively. Hägerström et al. (2000) also reported the negative interaction between carbomer and mucin (from porcine stomach, 4% w/w in water and simulated tear fluid) and a small positive interaction at the lowest concentration of carbomer (0.5% w/w). At the concentration of 0.5% w/w of carbomer934P in simulated tear fluid, the swollen gel particles were probably not in contact with each other due to the presence of ions. The addition of mucin resulted in a swelling of the gel particles forcing them into contact with each other and the entanglement or weak intermolecular forces between mucin and carbomer binding the gel particles together. Rossi et al. (1995) also suggested that the negative interaction of carbomer and mucin was caused by the presence of ions in the mucin, resulting in a shield of charged carboxylic groups and the carbomer adapted a less expanded structure, then changing the rheological properties. Riley et al. (2001), however, reported a large increase in synergistic interaction between mucus and carbomer934P (0.5% w/w).

Fig. 7 shows the relationship between the relative rheological synergism and the MW of pectin and Table 3 shows the r^2 of the plots between relative rheological synergism of pectin versus MW, %DE or degree of substitution of pectin in DI water and SIF. Due to only two types of pectin in SGF were measured, the r^2 in SGF could not be calculated by linear regression analysis. It is noted that no relationship between G'_{rel} and MW, DE or degree of substitution of pectin was observed due to a lower r^2 of the plots (0.02–0.14). However, it is observed that the G''_{rel} increased with the increased MW of pectin (except for amidated

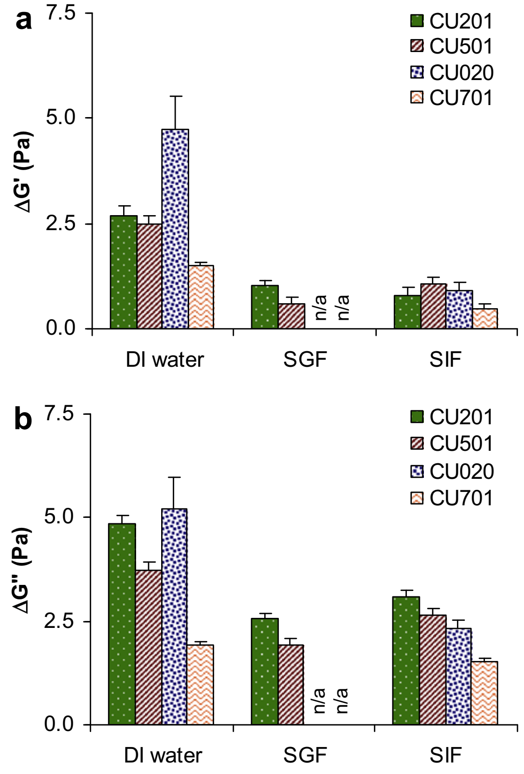

Fig. 4. Rheological synergism at a frequency of 1.15 Hz, expressed as (a) $\Delta G'$ and (b) $\Delta G''$ of pectin (1% w/w) and mucin (5% w/w) in different media ($n = 3$).

pectin). The G''_{rel} related well with the degree of substitution in both DI water and SIF as the r^2 of the plots showed the highest value.

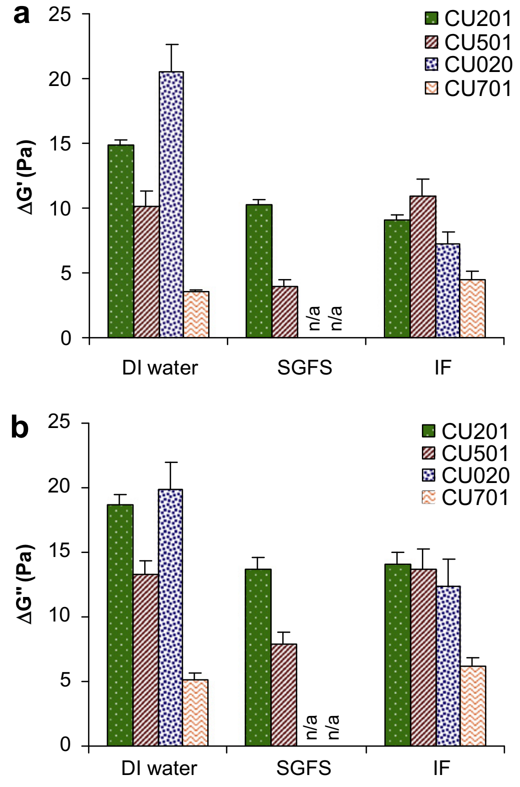

Fig. 5. Rheological synergism at a frequency of 1.15 Hz, expressed as (a) $\Delta G'$ and (b) $\Delta G''$ of pectin (2% w/w) and mucin (5% w/w) in different media ($n = 3$).

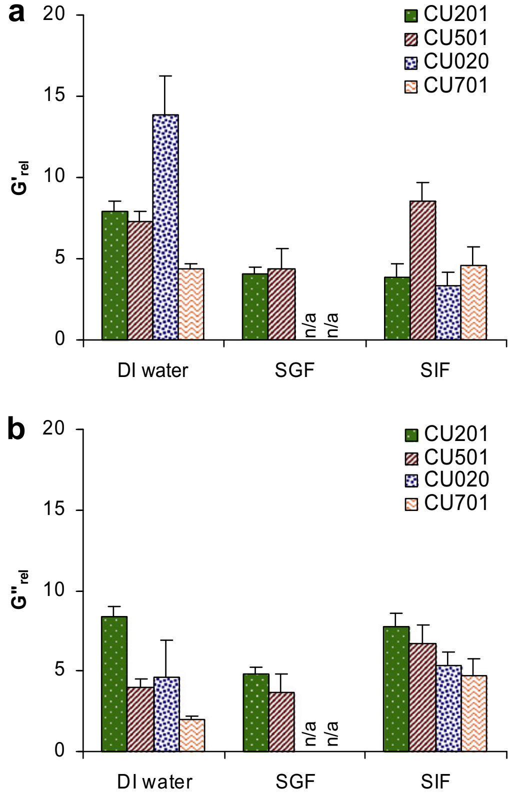

Fig. 6. Relative rheological synergism, expressed as (a) relative storage modulus (G'_{rel}) and (b) relative loss modulus (G''_{rel}) of mixtures of mucin (5% w/w) and pectin (1% w/w), chitosan (0.7% w/w) or carbomer934P (0.4% w/w) in different media ($n = 3$).

3.3. Effect of mucin concentration

Fig. 8 shows the rheological synergism ($\Delta G'$ and $\Delta G''$) of the combination systems of various types of pectin (1% w/w) and different mucin concentrations in SIF. An increase in mucin concentration (up to 7.5% w/w) produced a statistically significant increase in the rheological parameters. The rheological synergism was increased with the increased mucin concentration. The results indicated that there is a maximum stoichiometry of the interaction product which has not been reached even at the pectin:mucin weight ratio of 1:7.5. Such a ratio is comparable to that observed in a previous work (occurred at a weight ratio equals to 1:10) where chitosan hydrochloride solutions (1.5–4% w/w) were studied (Rossi, Ferrari, Bonferoni, & Caramella, 2000). This is similar to the relative viscosity that reported earlier (Thirawong et al., 2008), indicating that the interaction between pectin and mucin not only depend on the pectin concentration but also the mucin concentration. It is probably that sialic acid groups of mucin in the combination systems was increased with the increased mucin concentration while the number of mucoadhesive binding sites on pectin chain was unchanged. Excess mucoadhesive binding sites of pectin may be presented when lower concentration of mucin was used. Fuongfuchat et al. (Fuongfuchat, Jamieson, Blackwell, & Gerken, 1996) demonstrated higher moduli and viscosities of the mixture of concentrated mucin and alginate, compared to those of diluted mucin. It was probably due to a high number of binding sites of mucin, leading to gel formation.

4. Conclusion

The associative interaction between pectin and mucin can be evaluated by comparing the viscoelastic properties of mucin–pectin

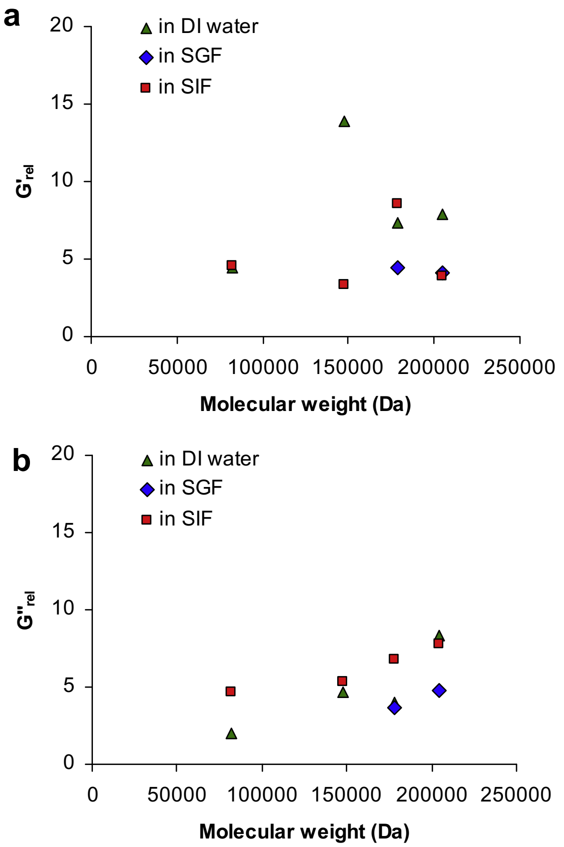

Fig. 7. Plots of the relationship between MW of pectin and rheological synergism, expressed as (a) G'_{rel}, and (b) G''_{rel} of the combination systems containing pectin (1% w/w) and mucin (5% w/w) in different media.

Table 3
Correlation coefficient (r^2) of the plots between relative rheological synergism versus MW, DE or degree of substitution of pectin, from linear regression analysis, in DI water and SIF

Relationship	DI water	SIF
G'_{rel} VS		
MW	0.1267	0.0407
DE	0.1460	0.0893
Degree of substitution	0.0247	0.0014
G''_{rel} VS		
MW	0.7015	0.8566
DE	0.4961	0.8428
Degree of substitution	0.8909	0.9605

mixtures against those of the pure components. Pectin has a significant interaction with mucin, as evidenced by an increase in dynamic moduli and a decrease in loss tangent. High DE pectin showed a greater interaction than low DE pectin. Amidated low DE pectin in DI water showed the strongest interaction with mucin due to the presence of amide and carboxylic acid groups in the structure. Increasing of mucin concentration affected the mucoadhesive interaction of pectin. The results provided useful information on the structural properties of pectin and mucin and the structural changes in the pectin network induced when mixing pectin with mucin. It adds to the perception that molecular interpenetration may be an important factor in mucoadhesion by strengthening the mucin (or mucus) in the mucoadhesive–mucosal interfacial layer.

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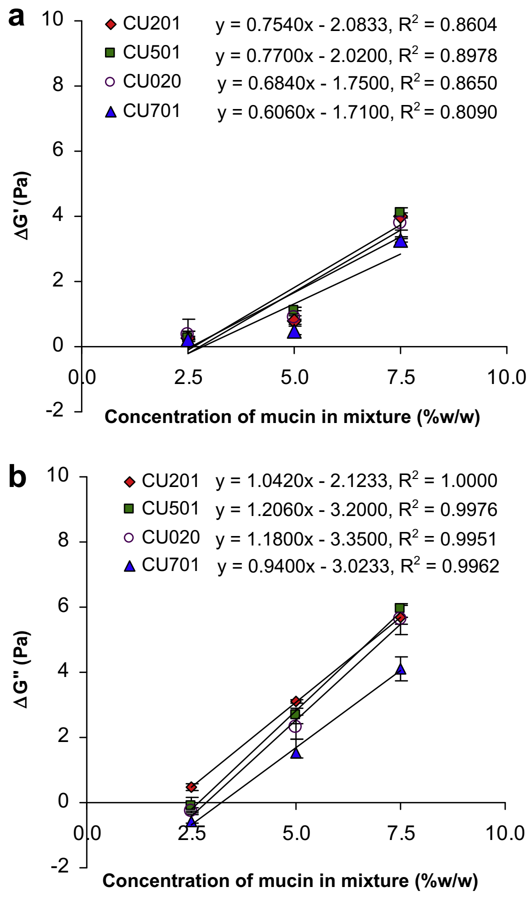

Fig. 8. Effect of mucin concentration in the combination of pectin (1% w/w) and mucin on the synergism of dynamic moduli, in SIF, at frequency of 1.15 Hz; (a) $\Delta G'$ and (b) $\Delta G''$ ($n = 3$). The correlation coefficient from linear regression analysis is shown.

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