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Viscometric study of pectin–mucin interaction and its mucoadhesive bond strength

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Abstract

The rheological characteristics of aqueous dispersion of four types of pectin and their mixtures with mucin were investigated using a simple viscometric method with a Brookfield viscometer, and were compared to the known mucoadhesive chitosans and Carbomer934P. The viscometric method introduced the parameter, $\eta_{enhance}$ or viscosity enhancement, which is an empirical determinant of the absolute force of bioadhesion. The higher the molecular weight, the greater the $\eta_{enhance}$ indicating the rheological synergism. The force of bioadhesion was also found to be dependent on molecular weight of pectin, its initial viscosity and environmental pH. These results are in agreement with an already reported *in-vitro* test on gastrointestinal mucosa. This corroborates the use of pectin as a mucoadhesive polymer for gastrointestinal mucoadhesive drug delivery system.

Keywords: Pectin; Mucin; Mucoadhesion; Mucoadhesive properties; Viscometry; Viscosity enhancement; Viscosity synergism; Force of bioadhesion

1. Introduction

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 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).

Due to its biocompatibility, biodegradability and non-toxicity, pectin represents an attractive biopolymer for a variety of pharmaceutical and biomedical applications. Pectin has shown promise in engineering drug carriers for oral drug delivery (e.g. Charlton, Davis, & Illum, 2007; Liu et al., 2004; Sriamornsak, Sungthogjeen, & Puttipipatkhachorn, 2007; Sriamornsak, Thirawong, & Puttipipatkhachorn, 2005; Sriamornsak, Thirawong, Weerapol, Nunthanid, & Sungthongjeen, in press). The uses of pectin in biomedical applications include tissue engineering, e.g. improvement of cell adhesion and proliferation of osteoblasts, as demonstrated by *in vitro* cell culture (Liu et al., 2004). Chemically, the structure of pectin has many hydrogen bond forming groups such as hydroxyl, carboxyl and amide groups. This may allow the interaction between pectin and biological mucus, e.g. gastrointestinal mucus. However, only a few reports have investigated the mucoadhesive properties of pectin. Smart, Kellaway, and Worthington (1984) reported a fair adhesiveness of pectin

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when tested with mucus gel using a Wilhemy plate method. Recently, pectin has been demonstrated to possess mucoadhesive properties on to different gastrointestinal mucosa (Liu, Fishman, Hicks, & Kende, 2005; Schmidgall & Hensel, 2002; Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, in press). 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., in press). Clearly, this will depend on the chemistry of the specific pectin and the environmental pH.

The main components of the mucus layer include water (up to 95% by weight), mucin (generally no more than 5% by weight), inorganic salts (about 1% by weight), carbohydrates and lipids. Mucin represents more than 80% of organic components of mucus and controls the gel-like structure (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 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).

It is generally accepted that chain interlocking, conformational changes and chemical interactions which occur between a mucoadhesive polymer and mucin (or mucus) are likely to produce changes in the rheological behavior of the two macromolecular species. In this context, the viscosity of a molecular dispersion of a completely hydrated polymer and mucin may be considered as a reflection of the strength of the mucoadhesive joint. Some researchers observed a synergistic increase in viscosity, when a putative mucoadhesive polymer and mucin were mixed together. Viscosity synergism has been proposed as an *in-vitro* parameter to measure the mucoadhesive properties of various polymers, including chitosan and polyacrylic acid (Hassan & Gallo, 1990; Rossi, Ferrari, Bonferoni, & Caramella, 2001), and in those studies it was concluded that a greater viscosity synergism was indicative of a stronger polymer–mucin interaction.

Determination of mucoadhesive bond strength is also important in the development of mucoadhesive drug delivery systems as it can quantitatively compare different bioadhesive materials and allow for quality control testing. A simple procedure to assess the absolute force of bioadhesion through monitoring viscometric changes of mucin–polymer mixtures was proposed by Hassan and Gallo (1990). Viscosity components of bioadhesion (η_b) were calculated from Eq. (1):

$$\eta_t = \eta_m + \eta_p + \eta_b \quad (1)$$

where η_t is the viscosity of the system, η_m and η_p are the individual viscosities of mucin and polymer, respectively. Consequently, the force of mucoadhesion (F) represents the additional intermolecular frictional force per unit area and can be determined by Eq. (2):

$$F = \eta_b \sigma \quad (2)$$

where σ is the shear rate (s^{-1}). The η_b is based on experimentally measured values at the same concentration, temperature, time and shear rate. These two parameters (η_b and F) give a direct estimate of the polymer–mucin interactions occurring in mucoadhesion (Hassan & Gallo, 1990).

The aim of this study was to investigate the pectin–mucin interactions in various media through viscosity measurements performed on pectins alone and on their mixtures with mucin. This would allow us to demonstrate differences in response due to differences between pectins and to assess the extent to which the pectin–mucin interactions were affected by different media. Chitosan and carbomer934P, well-known mucoadhesive polymers (Rossi et al., 2001), were employed in this study to serve as comparative standards for cationic and anionic mucoadhesive polymers, respectively.

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. Polyacrylic acid cross-linked polymer (carbomer934P) manufactured by Corel Pharma-Chem (India) was used. Chitosans (MW of 40,000 and 100,000 Da) with degree of acetylation of 85% and 95%, respectively, were purchased from Seafresh Chitosan (Lab) Co., Ltd. (Thailand) and were referred as LMW and MMW chitosans, respectively. All other chemicals were analytical grade and used as received without further purification. Deionized (DI) water was prepared by reverse osmosis (Purelab RO100, USF Ionpure, Germany) throughout all experiments.

2.2. Moisture content analysis

The moisture content of all pectins (about 2 g) was measured by heating at 105 °C using a moisture analyzer (model MA 40, Sartorious AG, Germany). The measurements were performed in triplicate; the means and standard deviation are presented.

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)	Moisture content[a](%)
High methoxy pectin				
CU201	70	0	200,000	10.11 ± 0.30
CU501	56	0	180,000	9.95 ± 0.12
Low methoxy pectin				
CU701	38	0	80,000	9.11 ± 0.26
CU020	29	20	150,000	11.96 ± 0.05

Note: The %DE, %DA and molecular weight are specified and reported by the manufacturer.
[a] The moisture content of triplicate samples was measured.

2.3. Preparation of samples

Pectin was dispersed in DI water with gentle stirring at room temperature. Stock solutions (1.25% w/w) of all types of pectin were prepared. To study the effect of ionic strength, the necessary amount of 1.0 M sodium chloride (NaCl) and DI water were added to 40 g of stock solution to make 50 g of 1.0% w/w pectin in 0.05, 0.10, 0.15 and 0.20 M NaCl. Triplicate solutions were prepared.

To study the influence of various concentrations of glucose added, stock solutions (2.0% w/w) of all types of pectin and 69% w/w glucose were prepared in DI water. The glucose solution and DI water was added into 15 g of 2% w/w pectin to make 30 g of 1% w/w pectin in 11.5, 23.0, 28.75 or 34.5% w/w glucose solutions. Triplicate solutions were prepared.

To study the polymer–mucin interaction, mucin solutions were prepared in different media, i.e. simulated gastric fluid USP without enzyme (SGF), simulated intestinal fluid pH 6.8 without enzyme (SIF) and DI water. Different concentrations of stock solutions were prepared, i.e. 2.0% w/w for pectin, 1.4% w/w for chitosan or 0.8% w/w for carbomer934P. 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 SGF, SIF or DI water were mixed well with 6 g of each polymer in the corresponding media to give the concentration of 1.0% w/w for pectin, 0.7% w/w for chitosan and 0.4% w/w for carbomer934P. The final concentration of mucin was 5% w/w. All systems were kept at 37.0 ± 0.1 °C for 1 h to equilibrate prior to analysis. In each case the viscometric experiments were performed once on each solution.

The combination of low methoxy pectin (CU701) and various concentrations of mucin in SIF was studied. The stock solutions of pectin (2.0% w/w) and mucin (15% w/w) were mixed together to make the final concentration of 1% w/w pectin and 2.5, 5.0 or 7.5% w/w mucin. The final mixtures of pectin and mucin were allowed to stand at 37.0 ± 0.1 °C for 1 h prior to analysis. At least three replicates of samples were measured.

2.4. Viscosity measurements

The rheological properties and flow behavior of all formulations were measured using a Brookfield Model DV-III programmable viscometer (Brookfield Engineering Laboratories, Inc., USA) with SC4-18/13R spindle and small sample adaptor at 37.0 ± 0.1 °C. Samples of each formulation were added to the chamber of the viscometer and allowed to equilibrate for at least 2 min prior to test. The measurements were made within the shear rate. The flow behavior index or non-Newtonian index (n) and consistency index (K_c) can be derived from the power law expressed in Eq. (3) (Walter, Rao, Cooley, & Sherman, 1985). The solutions were tested in triplicate at shear rates up to about 25 s^{-1}.

$$\tau = K_c \gamma^n \tag{3}$$

where τ is the shear stress and γ is the shear rate. For comparison purposes, the apparent viscosity at a shear rate of 3.96 s^{-1} was taken from the data directly.

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 ($p < .05$) 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. Moisture content

The moisture contents of the pectin powders, measured at 105 °C, are shown in Table 1 and range from 9% to 12%. These figures are typical of commercial polysaccharide powders. This result suggested that all pectins contained one H_2O molecule/monosaccharide (Sriamornsak, 2002). The preparation of pectin solutions in all other experiments was performed by making a correction for the moisture content.

3.2. Effect of ionic strength and glucose concentration on apparent viscosity of pectin solutions

The viscosities of 1% w/w pectin in various ionic strengths (0.05–0.20 M NaCl) are shown in Fig. 1. The

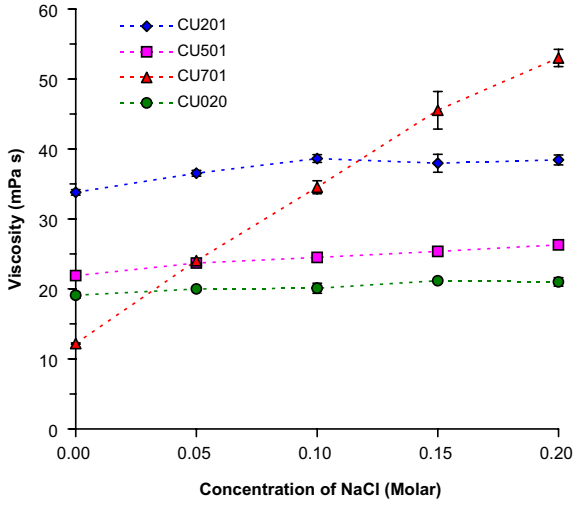

Fig. 1. Apparent viscosity (at 3.96 s^{-1}) of different pectins (1% w/w) at different ionic strengths ($n = 3$, SD shown). Lines are draw as a guide for the eye.

viscosity of the high methoxy (CU201 and CU501) and low methoxy amidated (CU020) pectins were relatively insensitive to increasing ionic strength. However, the viscosity of the CU701 was significantly increased by the ionic strength. Because CU701 has the lowest DE, it is expected that it will possess the highest charge density and it is likely that this will limit chain entanglements and lead to a low viscosity in water. As the ionic strength is increased, the polyanionic charges are likely to be suppressed, and this lead to the significant increase in viscosity shown in Fig. 1. It is apparent that CU701 had the highest viscosity at a near-physiological ionic strength of 0.15 M.

The viscosities of all types of pectin were increased as the concentration of glucose was increased; the CU701 (low DE) pectin was most affected (Fig. 2). This is because the addition of glucose reduced the concentration of free water in the system, with a consequent decrease in the effectiveness of pectin–solvent interactions that normally compete with pectin–pectin interactions. Therefore the increased glucose concentration promoted self-association of the pectin chains (Evageliou, Richardson, & Morris, 2000). It is possible that the shorter chain length of the CU701 (Table 1 shows that CU701 has the lowest molecular weight of all the pectins studies) favored the self-association process.

3.3. Effect of medium and mucin on apparent viscosity and flow behavior of pectin solutions

Fig. 3 shows the apparent viscosity of pectin (1% w/w) in various media (i.e. SGF, SIF and DI water) at 37 °C. The rank order of the apparent viscosity of pectin, in all media, is CU201 > CU501 > CU020 > CU701. The viscosity of the non-amidated pectins correlated to the DE, i.e. CU201 (70% DE) > CU501 (56% DE) > CU701 (38% DE) as shown in Table 1. As the DE increases, it is likely

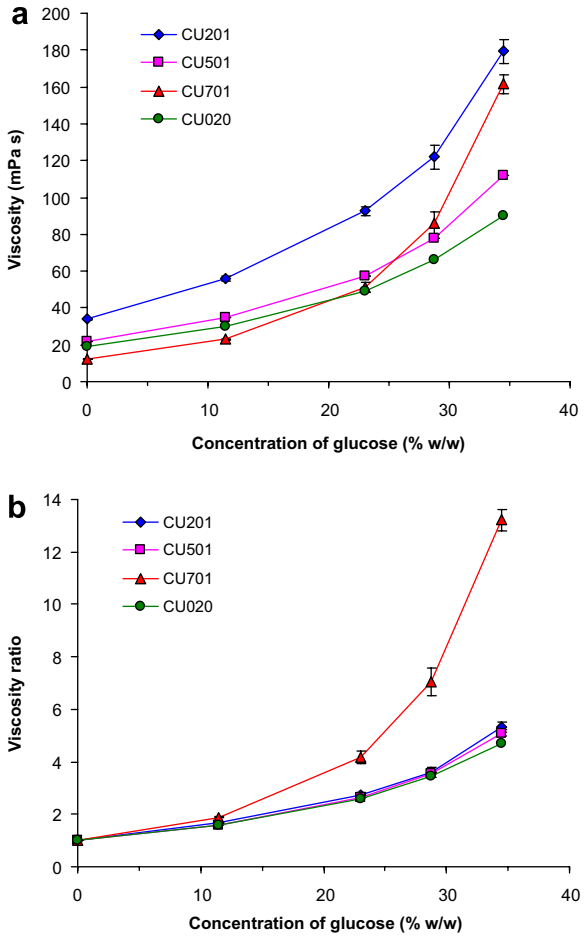

Fig. 2. Effect of the concentration of glucose added in pectin solution on (a) apparent viscosity and (b) viscosity ratio of different pectins (1% w/w). Viscosity ratio is the viscosity of the mixture of pectin and glucose divided by the viscosity of the pectin alone (i.e. 0% glucose).

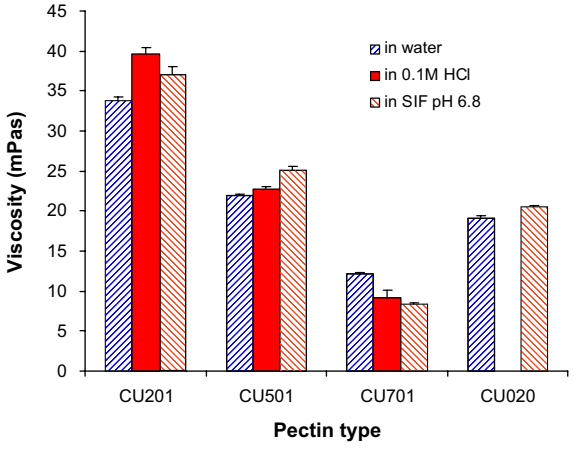

Fig. 3. The effect of pectin type on the apparent viscosity of pectin (1% w/w) in various media at 37 °C.

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. Although the amidated low methoxy pectin

(CU020) had a lower DE than CU701, the amide groups would be likely to contribute to pectin chain associations through hydrogen bonding (Alonso-Mougán, Meijide, Jover, Rodríguez-Núñez, & Vázquez-Tato, 2002), and this lead to CU020 showing a higher viscosity than CU701. In addition, the viscosity of the pectin solutions is correlated to their nominal MW as shown in Table 1. It is generally accepted that the chain length (i.e. MW) and viscosity are intimately related (Morris, Cutler, Ross-Murphy, & Rees, 1981).

The viscosity of the pectins in each medium was statistically different and the viscosity in the acidic SGF (pH 1.2) was higher than in SIF (pH 6.8). The carboxyl groups in pectin were primarily uncharged in a low pH environment (pK_a of pectin is about 3–4) (Rolin, 1993) and able to form hydrogen bonds resulting in labile intermolecular cross-links. As the pH is increased, molecular ionization increases and this resulted in a loss of the hydrogen bonded network (Riley et al., 2001).

The plots of apparent viscosity of polymer, pectin or mucin alone or of a combination of polymer and mucin versus shear rate, in various media, are shown in Fig. 4. All the combination systems of pectin (or other polymers) and mucin also showed pseudoplastic behavior. Some data points, at higher shear rate, of the combination systems could not be measured. Under conditions of increased shear, the polymer chains become progressively disentangled and the hydrogen bonds may be broken resulting in a reduction in the polymer dimensions and the release of any entrapped solvent (Aulton, Twitchell, & Hogan, 1997), and thus a reduction in viscosity, as shown in Fig. 4, because their torque reached 100%, thus higher than the capacity of the viscometer.

The rheograms for these solutions can be well described by the power law, as shown in Eq. (3). The flow behavior index (n) was calculated from the plots of shear stress versus shear rate. The consistency index (K_c) was then estimated at a shear rate of 1 s^{-1}. The exponent 'n' from the power law model is an indication of departure from Newtonian behavior. For pseudoplastic fluids, $0 < n < 1$, and for dilatant fluids, $n > 1$. As n approaches 1, flow becomes less shear dependent, and $n = 1$ for Newtonian flow. Dilute polysaccharide solutions are generally Newtonain but at moderate concentration, the curves may show dilatant or pseudoplastic flow (Walter et al., 1985). The flow behavior and consistency index of polymer alone and its combination with mucin, in various media, are shown in Table 2. It can be seen that flow behavior index of pectin and combination systems were not much different and corresponded to a shear-thinning flow behavior (pseudoplastic fluid). The shear-thinning behavior of pectin solutions (or combinations of pectin and mucin) can be rationalized in terms of polymer entanglements, where (under shear) the rate of disentanglement exceeds the rate at which the new entanglements form and this leads to a reduction in the cross-link density and, in consequence, the viscosity decreased, as was reported by Manoj, Watson, Hibberd, Fillery-Travis, and Robins (1998) who analyzed the flow behavior of hydroxyethylcellulose solutions. On the other hand, flow behavior index of chitosan and carbomer934P were increased when mixed with mucin indicating more Newtonian behavior. The consistency index (K_c), an indicator of the viscous nature of the solution at low shear rate (i.e. at 1 s^{-1}), was observed to increase after mixing with mucin. It is thought that the combination of polymer and mucin induced overlap of the polymer chains, increased competition for 'free' water leading to entanglement of the polymer (and mucin) chains and possibly promoted interactions between the polymer and mucin molecules by hydrogen bonding (Aulton et al., 1997).

3.4. Effect of mucin on viscosity enhancement (synergism)

Table 3 shows the expected viscosity (η_{exp}), the observed viscosity (η_{obs}), viscosity enhancement ($\eta_{enhance}$) and relative viscosity enhancement (η_{rel}) of the combination systems of polymer and mucin as expressed in Eqs. (4)–(6).

$$\eta_{exp} = \eta_p + \eta_m \quad (4)$$

$$\eta_{enhance} = \eta_{obs} - \eta_{exp} \quad (5)$$

$$\eta_{rel} = \eta_{obs}/\eta_{exp} \quad (6)$$

The η_p and η_m are the viscosity of the polymer and mucin alone, respectively. In all cases, the observed viscosity (η_{obs}) was higher than the expected viscosity (η_{exp}) and the viscosity enhancement ($\eta_{enhance}$) was calculated as shown in Eq. (5). The $\eta_{enhance}$ is equivalent to the viscosity component of bioadhesion (η_b) referred to in Eq. (1).

Fig. 5 shows the viscosity of the polymer, mucin and their viscosity enhancement, $\eta_{enhance}$, after mixing in different hydrating media. It is apparent that the $\eta_{enhance}$ of systems containing pectin was higher than those containing chitosans or carbomer934P. This suggested that the pectins are able to interact more strongly with mucin as compared to other polymers. In general, this is in good agreement with previous reports of the mucoadhesive properties of these types of polymers (Mortazavi & Smart, 1994), where the polymers that have been reported to be capable of interacting with mucus in their fully hydrated state and in the presence of an excess amount of water induce the greatest viscosity synergism. In contrast, chitosan (in SGF) or carbomer934P (in DI water) may lose some of their mucoadhesive strength upon over-hydration and produce only limited gel strengthening when mixed with the mucin. Rossi et al. (2001) suggested that two different types of rheological interaction could be found between chitosan and mucin in both DI water and 0.1 M HCl, depending on chitosan concentration and chitosan:mucin weight ratio: one was characterized by a minimum in viscosity and occurred at higher chitosan:mucin weight ratio, the other one produced a positive rheological synergism and was observed in the presence of an excess of mucin. The other possible explanation is the difference in the initial concentration of the polymers used in the study, and this was unavoidable due to the

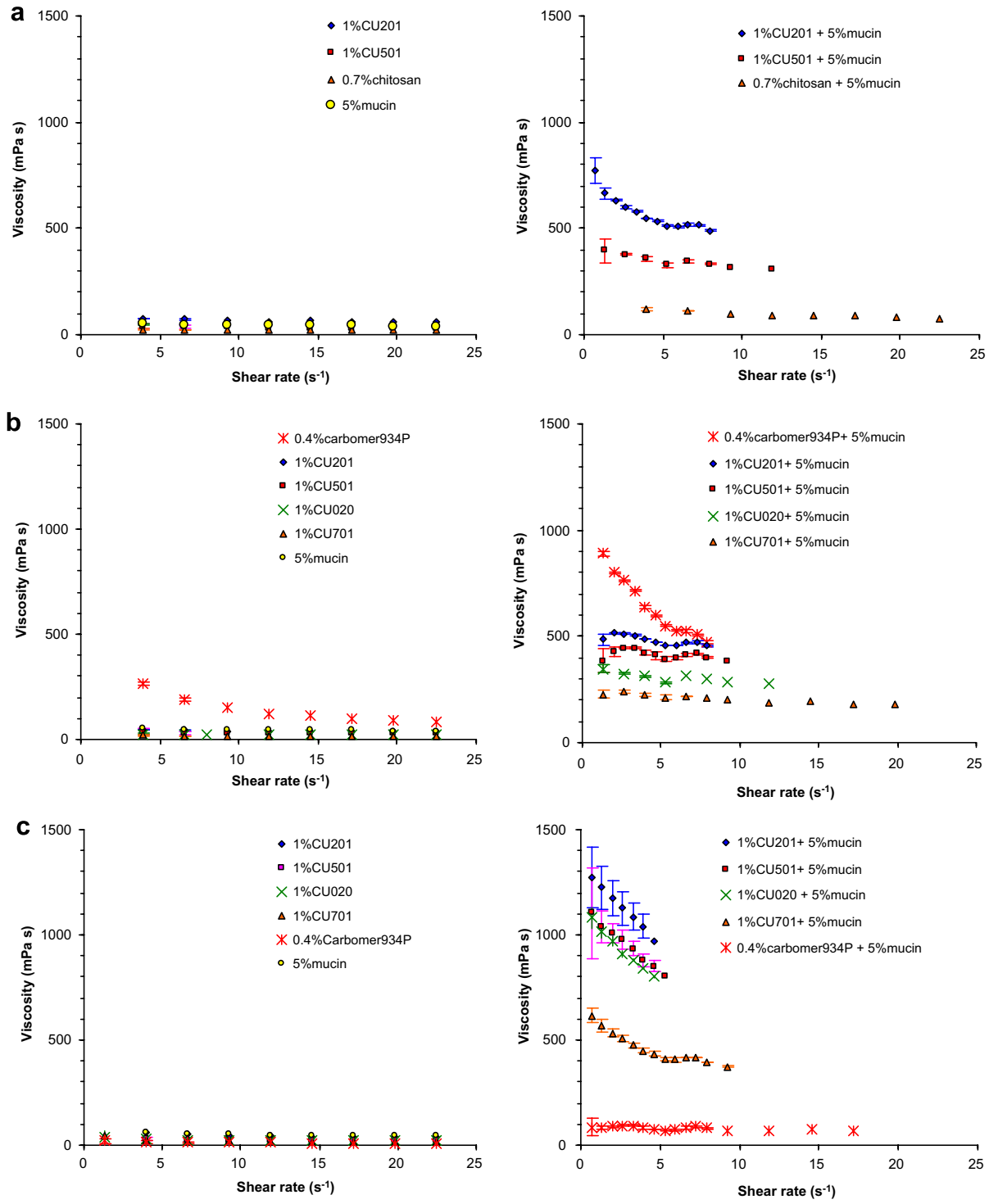

Fig. 4. Apparent viscosity of polymer alone (left) and combination of polymer and mucin (right) of different polymers at various shear rate in (a) SGF, (b) SIF and (c) deionized water.

torque limits of the viscometer. The $\eta_{enhance}$ of carbomer934P and mucin in SIF was significantly higher than in DI water. This is probably due to the interaction between carbomer and mucin by forming physical entanglements followed by hydrogen bonds with sugar residues on the oligosaccharide chains, resulting in the formation of a strengthened mucus gel network (Mortazavi, 1995).

The viscosity of combination systems in all media seemed to be dependent on the initial viscosity of pectin. For example, viscosity of pectin CU201 (1% w/w) was higher than pectin CU501 at the same concentration, hence the higher $\eta_{enhance}$ of combination systems was observed. The pectin CU201 demonstrated the highest $\eta_{enhance}$ in all media (Fig. 5) indicating the greatest synergism. This

Table 2
Flow behavior index (n) and consistency index (K_c) derived from the power law model of different polymers in SGF, SIF and deionized water

Polymer	Flow behavior index, n		Consistency index, K_c (mPa s)	
	Polymer	Polymer + mucin	Polymer	Polymer + mucin
In SGF				
5% Mucin	0.89	–	57.6 ± 2.1	–
1% Pectin CU201	0.88	0.83	89.9 ± 13.9	697.1 ± 40.6
1% Pectin CU501	0.87	0.85	46.4 ± 6.8	442.4 ± 31.2
1% Pectin CU701	ND	ND	ND	ND
1% Pectin CU020	ND	ND	ND	ND
0.7% LMW chitosan	0.60	0.88	18.3 ± 6.5	80.5 ± 10.2
0.7% MMW chitosan	0.69	0.83	48.2 ± 11.3	128.2 ± 20.5
0.4% Carbomer934P	ND	ND	ND	ND
In SIF				
5% Mucin	0.89	–	56.5 ± 1.6	–
1% Pectin CU201	0.90	0.96	51.8 ± 5.0	505.2 ± 84.8
1% Pectin CU501	0.89	0.97	42.3 ± 2.2	432.5 ± 91.3
1% Pectin CU701	0.76	0.88	25.4 ± 5.3	258.8 ± 33.4
1% Pectin CU020	0.94	0.91	28.1 ± 2.2	351.9 ± 58.5
0.7% LMW chitosan	ND	ND	ND	ND
0.7% MMW chitosan	ND	ND	ND	ND
0.4% Carbomer934P	0.36	0.63	615.3 ± 11.3	1047.0 ± 29.5
In deionized water				
5% Mucin	0.83	–	77.7 ± 16.7	–
1% Pectin CU201	0.87	0.90	57.6 ± 5.8	1240.7 ± 117.3
1% Pectin CU501	0.81	0.88	43.0 ± 9.2	1068.3 ± 131.8
1% Pectin CU701	0.71	0.80	40.4 ± 16.4	593.8 ± 34.3
1% Pectin CU020	0.83	0.85	36.2 ± 3.4	1041.9 ± 102.0
0.7% LMW chitosan	ND	ND	ND	ND
0.7% MMW chitosan	ND	ND	ND	ND
0.4% Carbomer934P	0.71	0.93	23.9 ± 8.2	90.2 ± 27.4

Note: ND, not determined.

possibly resulted from its higher MW. Additionally, the viscosity of pectin seemed to relate to the number of methoxyl and carboxyl groups in its molecule. The carboxyl groups of pectin and the sialic acid of mucin (pK_a 2.6) were uncharged in SGF indicating the interaction might be hydrogen bonding or other non-electrostatic interaction. In a higher pH environment, the carboxyl group and sialic acid were both negatively charged. The electrostatic interaction between polymer and mucin with the same charge is generally repulsive; therefore reduced chain entanglements are expected. Low methoxy pectin CU701 with highest carboxyl groups has highest negative charge density in SIF, decreasing the chain entanglement with mucin. This result was in contrast to high methoxy pectin. The rank order of $\eta_{enhance}$ of the pectin–mucin combinations is CU201 > CU501 > CU020 > CU701, which is the same rank order as the viscosity of the pectins themselves.

A second way to consider the magnitude of the rheological synergism is by a consideration of the relative viscosity enhancement (η_{rel}), as shown in Eq. (6). This would allow the viscosity enhancement effect to be expressed as a proportion of the unmixed materials viscosities. A $\eta_{rel} = 1$ means there is no interaction between polymer and mucin. A higher value of η_{rel} shows rheological synergism between the polymer and mucin and is indicative of potentially mucoadhesive associations between them. The calculated η_{rel} is also shown in Table 3. It is found that pectin showed the higher η_{rel}, compared to chitosan and carbomer934P. The η_{rel} of combinations of mucin and pectin in different media is also shown in Fig. 6. The η_{rel} was higher in DI water, than in SGF and SIF. It is observed that the η_{rel} increased with the MW of pectin. The increase of η_{rel} is somewhat limited as the plateau was seen.

3.5. Effect of mucin concentration

The η_{obs} and $\eta_{enhance}$ of combination systems of 1% w/w low methoxy pectin (CU701) and various concentrations of mucin, in SIF, are shown in Table 5. An increase in mucin concentration (up to 7.5% w/w) produced a statistically significant increase in the viscosity parameters, including the η_{rel} (Fig. 7). These results indicate 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 equal to 1:10) where chitosan hydrochloride solutions (1.5–4%) were studied (Rossi, Ferrari, Bonferoni, & Caramella, 2000). In this case, it is probable that the increase of the sialic acid groups of mucin while the number of mucoadhesive binding sites on pectin chain was unchanged. There are possibly excess mucoadhesive binding sites (on pectin) when lower concentration of mucin was used. It is thought that mucoadhesive binding sites on high methoxy pectin chain are denser than

Table 3
Expected viscosity (η_{exp}) and observed viscosity (η_{obs}) of the combination system between pectin and mucin in various media at 37 °C ($n = 3$)

Sample	Viscosity (mPa s)		
	SGF[e]	SIF[f]	Deionized water
1% CU 201 and 5% mucin			
η_{exp}[a]	90.5	70.8	75.7
η_{obs}[b]	469.5 ± 11.2	441.0 ± 4.0	1023.8 ± 77.7
$\eta_{enhance}$[c]	379.0	370.2	948.1
η_{rel}[d]	5.2	6.2	13.5
1% CU 501 and 5% mucin			
η_{exp}	63.8	63.3	62.1
η_{obs}	293.1 ± 8.9	373.7 ± 16.6	819. 8 ± 63.4
$\eta_{enhance}$	228.8	310.4	757.7
η_{rel}	4.6	5.9	13.2
1% CU 701 and 5% mucin			
η_{exp}		45.4	52.6
η_{obs}	ND	168.4 ± 3.4	336.6 ± 14.3
$\eta_{enhance}$		122.9	283.9
η^{rel}		3.7	6.4
1% CU 020 and 5% mucin			
η_{exp}		57.9	59.0
η_{obs}	ND	269.7 ± 7.6	754.2 ± 48.2
$\eta_{enhance}$		211.7	695.1
η_{rel}		4.7	12.8
0.7% LMW chitosan and 5% mucin			
η_{exp}	40.4		
η_{obs}	48.8 ± 0.5	ND	ND
$\eta_{enhance}$	8.4		
η_{rel}	1.2		
0.7% MMW chitosan and 5% mucin			
η_{exp}	50.2		
η_{obs}	61.0 ± 1.2	ND	ND
$\eta_{enhance}$	10.9		
η_{rel}	1.2		
0.4% Carbomer934P and 5% mucin			
η_{exp}		70.3	47.1
η_{obs}	ND	359.9 ± 5.1	65.9 ± 2.4
$\eta_{enhance}$		289.6	18.8
η_{rel}		5.1	1.4

Note: ND, not determined.
[a] Expected viscosity (η_{exp}) is the viscosity of polymer plus viscosity of mucin (5% w/w), the estimated percentage of relative standard deviation in the worse case is 5–10.
[b] Observed viscosity (η_{obs}) is the viscosity of the combination system measured via Brookfield viscometer.
[c] Viscosity enhancement ($\eta_{enhance}$) = $\eta_{obs} - \eta_{exp}$.
[d] Relative viscosity enhancement (η_{rel}) = η_{obs}/η_{exp}.
[e] SGF = simulated gastric fluid, pH 1.2, without enzyme.
[f] SIF = simulated intestinal fluid, pH 6.8, without enzyme.

those on low methoxy pectin chain due to the higher viscosity when mixed with mucin (data not shown). Therefore we would expect that as mucin was increased the interaction would increase to a limiting plateau.

3.6. Force of bioadhesion

A bioadhesive force is required between the drug device and the mucosal surface to successfully retain the device and retard the natural clearance processes. The force of adhesion was calculated from the empirical equation expressed in Eq. (2). The single shear rate of 3.96 s^{-1} was selected to compare adhesion force between polymer and mucin because the viscosity at this shear rate able to be determined in all samples. As shown in Table 4, all types of pectin showed higher bioadhesion force with mucin in DI water than that in SIF and SGF. The force of bioadhesion seems to depend on the molecular weight of pectins as well as their initial viscosity and the environmental pH. At lower pH (i.e. in SGF), the adhesion force of pectin with mucin was low, compared to that at higher pH (i.e. in SIF). Moreover, the MMW chitosan gave a higher adhesive force with mucin, in SGF, than the LMW chitosan. Force of adhesion of carbomer934P with mucin in SIF was markedly higher than in DI water, resulting from their initial viscosities in each media.

Table 4
Apparent viscosity, viscosity enhancement at shear rate of 3.96 s^{-1} and force of bioadhesion between polymer and mucin in various media ($n = 3$)

Sample	Apparent viscosity of polymer at a shear rate of 3.96 s^{-1} (mPa s)	Viscosity enhancement (mPa s)	Force of bioadhesion (mPa)
In SGF			
5% Mucin	51.0 ± 3.0	–	–
1% Pectin CU201	75.0 ± 10.4	422.9 ± 9.5	1674.7 ± 37.6
1% Pectin CU501	41.0 ± 5.2	264.9 ± 17.6	1049.0 ± 69.7
1% Pectin CU701	ND	ND	ND
1% Pectin CU020	ND	ND	ND
0.7% LMW chitosan	11.0 ± 3.6	71.7 ± 1.5	283.9 ± 5.9
0.7% MMW chitosan	17.7 ± 7.4	107.0 ± 12.1	423.7 ± 47.9
0.4% Carbomer934P	ND	ND	ND
In SIF			
5% Mucin	49.3 ± 2.3	–	–
1% Pectin CU201	46.3 ± 4.2	390.6 ± 4.0	1546.8 ± 15.8
1% Pectin CU501	36.1 ± 1.6	337.5 ± 5.2	1336.5 ± 20.6
1% Pectin CU701	20.0 ± 1.7	155.7 ± 10.5	616.6 ± 41.6
1% Pectin CU020	25.3 ± 1.5	238.6 ± 6.0	944.8 ± 23.8
0.7% LMW chitosan	ND	ND	ND
0.7% MMW chitosan	ND	ND	ND
0.4% Carbomer934P	266.2 ± 6.7	324.4 ± 6.2	1284.6 ± 24.6
In deionized water			
5% Mucin	62.7 ± 5.7	–	–
1% Pectin CU201	49.7 ± 1.5	1039.5 ± 57.1	4116.4 ± 226.1
1% Pectin CU5 01	31.0 ± 5.0	784.1 ± 28.8	3105.0± 114.0
1% Pectin CU701	25.7 ± 1.5	361.5 ± 2.6	1431.5 ± 10.3
1% Pectin CU020	28.0 ± 6.1	749.8 ± 18.1	2969.2 ± 71.7
0.7% LMW chitosan	ND	ND	ND
0.7% MMW chitosan	ND	ND	ND
0.4% Carbomer934P	16.0 ± 5.2	5.0 ± 8.7	19.8 ± 34.4

Note: ND, not determined.

The strong interaction or high force of adhesion of pectins (especially high methoxy pectin) in all media tested with mucin may be due to their high molecular weight. The low bioadhesion of low methoxy amidated pectin (i.e. CU020) is clearly due to its low molecular weight. It is thought that higher interaction represent higher bond strength (Hassan & Gallo, 1990). Thus, the interfacial bond

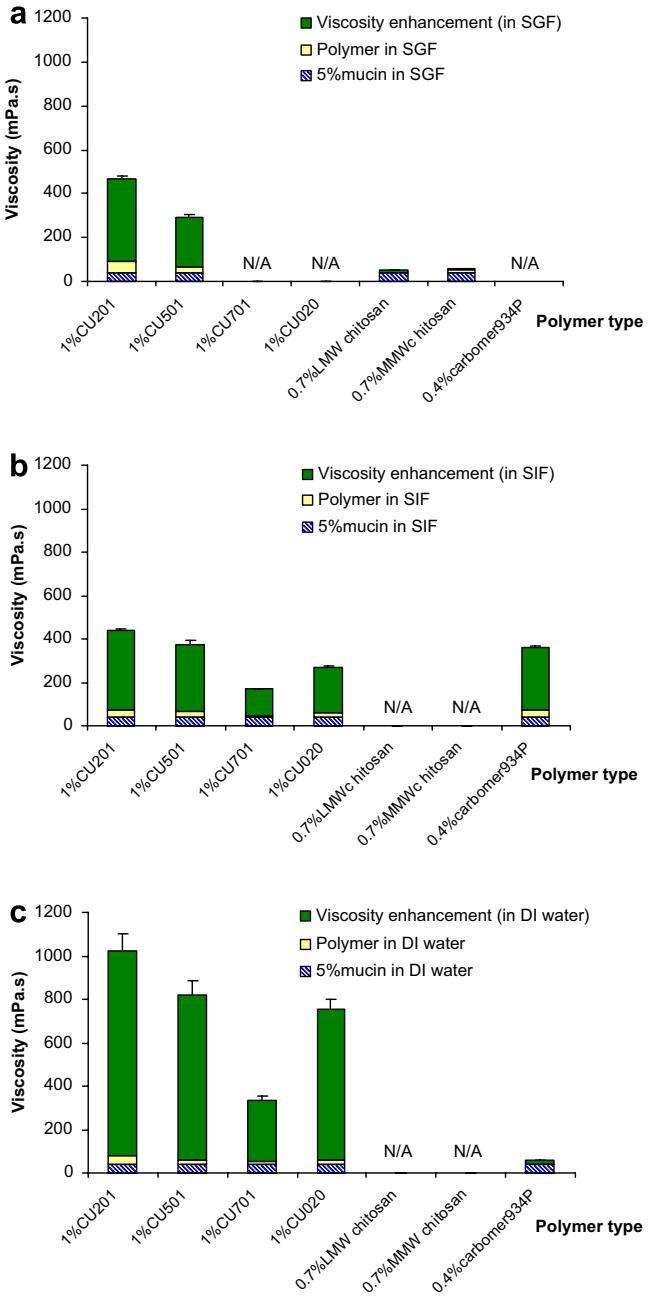

Fig. 5. Viscosity of various polymers and their viscosity enhancement after mixing with mucin in (a) SGF, (b) SIF, and (c) deionized water. The total height of the combined bar graph shows the observed viscosity of the combination system. The average and standard deviation of three replicates measured at a shear rate of 3.96 s^{-1} are shown (N/A = not applicable).

formation between high methoxy pectin and mucin is stronger than for those of low methoxy pectin. Both LMW and MMW chitosans showed a lower interaction with mucin, in SGF. This is probably due to their MW and association ability of sialic acid of mucin in SGF. The higher MW of pectin or chitosan may increase the probability for polymer–mucin interfacial interactions (Hassan & Gallo, 1990). As mucin (pK_a 2.6) is unionized in SGF (pH 1.2), therefore, the force of interaction is not solely due to ionic attractions.

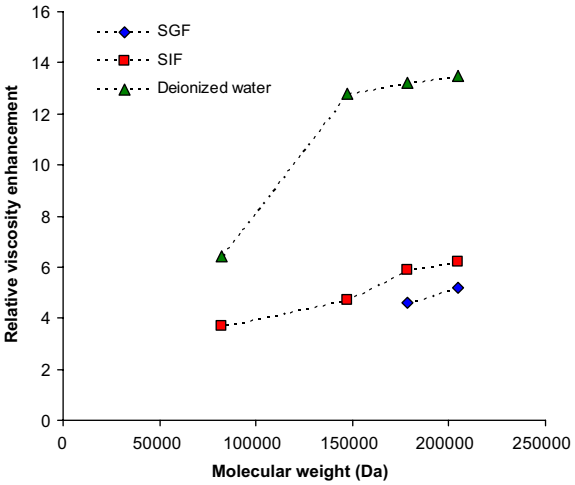

Fig. 6. Relative viscosity enhancements of combination systems in different media, measured by Brookfield viscometer at 37 °C. The lines are drawn as aids for the eye.

Table 5
Apparent viscosity and viscosity enhancement at shear rate of 3.96 s^{-1} of the combinations of pectin CU701 (1% w/w) and various concentrations of mucin in SIF, pH 6.8 ($n = 3$)

Sample	Apparent viscosity at a shear rate of 3.96 s^{-1} (mPa s)	Viscosity enhancement (mPa s)	Force of bioadhesion (mPa)
2.5%mucin + 1%CU701	43.3 ± 1.6	23.8 ± 1.6	94.2 ± 6.3
5.0%mucin + 1%CU701	225.0 ± 9.0	155.7 ± 10.5	616.6 ± 41.6
7.5%mucin + 1%CU701	782.0 ± 67.9	644.6 ± 67.9	2552.6 ± 268.9

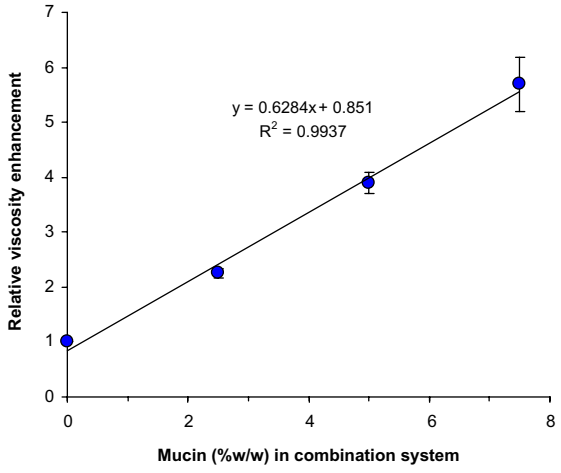

Fig. 7. Effect of concentration of mucin in the combination of 1% pectin CU701 and mucin on the relative viscosity enhancement value in SIF.

Hydrogen bonds as well as other bonds, such as polar, non-polar or physical bonds, may also exist.

4. Conclusion

In this study, viscosities of four types of commercial pectin (CU201, CU501, CU701 and CU020), poly(acrylic acid)

cross-linked polymer (carbomer934P) and chitosans with low and medium molecular weights were measured by rotational viscometry with a Brookfield viscometer. Only the viscosity of low methoxy pectin (CU701) was increased by the increased ionic strength. However, the viscosity of all pectins increased when glucose was added. The combination systems of pectin and mucin in SGF, SIF and DI water showed a higher viscosity than polymer alone indicating a synergistic interaction. High methoxy pectin showed a greater interaction that lead to a higher force of bioadhesion with mucin, compared to low methoxy pectin. These results are in agreement with an already established *in-vitro* test on gastrointestinal mucosa using texture analyzer (Thirawong et al., in press). This corroborates the use of pectin as a mucoadhesive polymer for gastrointestinal mucoadhesive drug delivery system. It was concluded that the use of pectin will advance the study of the rheological synergism of natural polymer, and will allow its relevance to the mucoadhesion process to be evaluated further. Moreover, it is reasonable to expect that the binding forces, e.g. hydrogen bonding, electrostatic forces may influence the mucoadhesion process of pectin polysaccharide, which can be evaluated by other methods, for example, NMR and AFM. These issues will be discussed in future publications.

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