Drug Development and Industrial Pharmacy, 33:417–425, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600920630

informa
healthcare

In Vitro Measurements of Mucoadhesive Properties of Six Types of Pectin

Ellen Hagesaether and Sverre Arne Sande
Department of Pharmacy, School of Pharmacy, University of Oslo, Blindern, Oslo, Norway

ABSTRACT The objective of this study was to measure and compare the specific- and general mucin interaction of six pectin types from three manufacturers, differing mainly in the degree of methoxylation and degree of amidation. Mucoadhesive properties were measured using a texture analyzer. It was found that an intermediate degree of methoxylation (35 and 36%) improved the specific mucin interaction. Amidation did not increase mucin interaction. Samples from different manufacturers did not alter these conclusions. This study indicates that the general classification of pectin as a poor mucoadhesive, without differentiating between the amount and type of substituents, probably is an oversimplification.

KEYWORDS Mucoadhesion, Pectin, Texture analyzer, Degree of methoxylation, Amidation

INTRODUCTION

The concept of mucoadhesion is appealing, since the interaction of the drug formulation with the mucous membranes of eye, buccal membrane or small intestine will allow increased residence time by avoiding the bodily mechanisms for flushing foreign substances away from the membrane. This could allow drug release over sustained periods, reducing the need for readministration and/or reducing the amount of drug needed. The theories that describe the mechanism of mucoadhesion are well known (Dodou et al., 2005).

Many polymers are known to possess mucoadhesive properties, e.g. cellulose derivates, acrylates, chitosan, and chitosan derivates (Dodou et al., 2005) . Pectin, however, is listed in standard textbooks and review articles as a polymer exhibiting poor mucoadhesive properties (Duchêne et al., 1988). This is supported by several studies comparing pectin with other polymers in vitro. Smart et al. (1984) measured a low force required to detach a pectin-coated glass plate from an isolated mucous gel. It has been found that few pectin-coated particles remained on rat jejunum or stomach after washing (Ranga Rao & Buri, 1989). Lehr et al. (1992) measured the force of detachment as zero for swollen pectin films on porcine intestinal mucosa and Baloglu et al. (2003) found poor values for pectin tablets on cow vaginal mucosa. More recently, however, positive values have also been reported (Miyazaki et al., 2000),

Address correspondence to Ellen Hagesaether, Department of Pharmacy, School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway; E-mail: ellen.hagesather@farmasi.uio.no

concluded that an increase in the pectin content in mixed tablets of pectin and hydroxypropylmethylcellulose led to greater adhesive properties measured as tensile strength on a sublingual membrane. The pectin types used in these studies have generally not been well classified. There are exceptions, however (Nafee et al., 2004) tested tablets of pectin with high degree of methoxylation (DM). They found low tensile strength values when detaching the tablet from a buccal mucosal surface. (Schmidgall and Hensel, 2002) found in an in vitro system based on porcine colonic tissue that highly esterified pectins were not mucoadhesive, but that pectin-like rhamnogalacturonan with a low degree of esterification showed promising results.

Characterization of pectin by our group has shown that different qualities differ to a large extent in their pharmaceutical properties; for example they respond differently to different granulation liquids during manufacturing of pellets (Tho et al., 2001), and show different permeation of drugs through free films made of pectin and chitosan (Hiorth et al., 2003). This knowledge in combination with the contradictory results found in the literature regarding the mucoadhesive properties of pectin, led to this study where the aim was to compare mucoadhesion of different types of purified and characterized pectins in an environment simulating the small intestine. The pectin types chosen for study differ i.a. in functional groups, molecular weight and viscosity. All of these properties are known to affect the mucoadhesion (Dodou et al., 2005). Pectin types were obtained from several manufacturers in order to investigate if changing manufacturer would influence the mucoadhesive properties. As a reference for mucoadhesiveness, alginate was chosen. Alginate has a chemical structure similar to pectin, but is neither methoxylated nor amidated. Alginate is generally recognized as a substance with excellent mucoadhesive properties (Duchêne et al., 1988).

MATERIALS AND METHODS

Materials

Mucin from porcine stomach, type II, batch 034K0628 was purchased from Sigma-Aldrich, Schnelldorf, Germany, and used as received.

Sodium alginate from brown algae, low viscosity, batch 084K0005, M/G ratio of 1.56, degree of polymerization of 60–400 or a molecular weight range of 12,000–80,000 (information provided by the manufacturer), was purchased from Sigma-Aldrich and used as received.

All the pectin types were derived from citrus and kindly provided by the manufacturers. A fractional factorial design involving six pectin types, listed in Table 1, was chosen. The factors varied among the pectin types were; DM (three levels; high, low, and medium), degree of amidation (DA) (two levels; amidated or nonamidated) and manufacturer (three levels). Due to the manufacturing process, the molecular weight was correlated with the DM. All other chemicals used were of analytical grade.

Purification of Pectin

In order to minimize expected batch to batch variations, the pectin types were purified. A 1.5 wt. %

TABLE 1 **Degree and Type of Substitution, Galacturonic Acid Content and Manufacturer of the Pectin Types Investigated (Information Provided by the Manufacturer)**

	Pectin classic		Genu® pectin		GRINDSTED® pectin	
	CU 902	CU 701	X-920-02	X-917-02	LA 410	RS 400
Batch	00406002	00501087	BA-2005-28	4450099	4010175812	0412744
DM	Low (4%)	Medium (35%)	Medium (36%)	Medium (31%)	Medium (27%)	High (70%)
DA	No (0%)	No (0%)	No (0%)	Yes (19%)	Yes (21%)	No (0%)
Total substitution	4%	35%	36%	50%	48%	70%
Galacturonic acid content	86%	89%	~90% (not analyzed)	~90% (not analyzed)	~80–95% (not analyzed)	~80–95 % (not analyzed)
Manufacturer	Herbstreith & Fox KG, Germany	Herbstreith & Fox KG, Germany	CPKelco, Denmark	CPKelco, Denmark	Dansico, Denmark	Dansico, Czech Republic

pectin solution in purified water was centrifuged for 2×3 hr at 3600 rpm, and the supernatant was dialyzed against purified water. Water was changed once a day for 7 days. The molecular weight cut off for the Spectra/Por® dialysis membrane was 8000. For the Pectin Classic DM 4% the pH was adjusted to pH = 7.0 with 1.0 M NaOH before centrifugation due to solubility problems at low pH. The remains after dialyzing were freeze dried and stored at low temperature.

Characterization

Viscosity

The specific viscosity η_{sp} was measured using a Micro-Ostwald capillary viscometer (type 516 10, Schott, Germany) at 20 ± 0.1°C. The concentrations used were 0.0125–0.250 wt. %. The intrinsic viscosity [η] was obtained from a plot of the reduced viscosity η_{red}, versus C (C is the concentration and $\eta_{red} = \eta_{sp}/C$). The plot should be linear as a positive deviation from linearity indicates polymer aggregation. In order to suppress aggregation, purified pectin was dissolved in 1.0 wt. % sodium hexamethaphosphate, pH 4.50 ± 0.05 (Christensen, 1954). The Huggins' constant k', was determined from the equation

$$\eta_{sp}/C = [\eta] + k'[\eta]^2 C.$$

Turbidity

The wavelength dependency (380–580 nm) of the transmittance at 25.0°C was measured with a Helios Gamma (Thermo Spectronic, Cambridge, UK) spectrophotometer for purified samples of 0.5 wt. % and 1.0 wt. % Pectin Classic DM 35% and Genu® pectin DM 36%, respectively, all in phosphate buffer having pH 6.8. The turbidity, τ, was calculated using the equation $\tau = (-1/L)\ln(I_t/I_0)$, where L is the light path length in the cell (0.95 cm), I_t is the transmitted light intensity, and I_0 is the incident light intensity.

To relate the wavelength dependency of the turbidity to the conformation of the polymer aggregates in the samples, it has been suggested to fit the data to the following equation: $\tau \sim \lambda^{-4+\beta}$. For particles much larger than the wavelength, the value of β is one for rods and two for spheres and coils. This way of interpreting turbidity data has previously been used by (Erbil & Saraç, 2002; Hiorth et al., 2005), but is still not properly tested and is probably not valid for all systems.

Mucoadhesion Test

Mucoadhesive properties of the polymers were evaluated by optimization of several tensile tests described in the literature (Rossi et al., 1996; Sandri et al., 2005) . The experimental conditions were chosen to optimize the limit of detection and precision. 250 μL of a 3.0 wt % solution of polymer or a 3.0 wt % dispersion of mucin, both in phosphate buffer pH 6.8, were evenly spread on a filter paper with an inert backing layer (Watman® Benchkote) of 2.5 × 2.5 cm. The samples were allowed to rest for 45 min to assure proper interaction between the polymer and the filter paper. The mucin sample was attached with double sided adhesive tape to the lower, stationary part of a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, England), and the polymer sample was attached to the upper, movable part (Fig. 1). The upper, movable part was lowered until it reached contact with the polymer sample, and a preload of 200 g was applied for 100 s, after which the upper part was raised with a speed of 0.01 mm/s. Displacement and force of detachment were recorded. To adjust for nonspecific interactions, the polymer samples and a mucin sample were also tested against pure buffer samples.

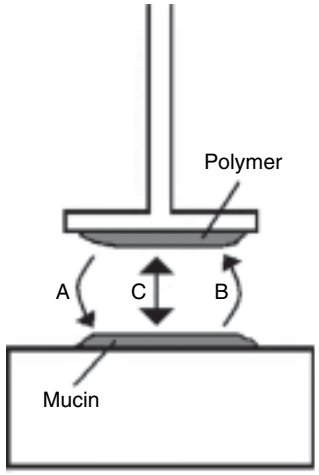

FIGURE 1 **Set up for the Mucoadhesion Measurements. The Arrows Indicted With Capital Letters Represent Relevant Forces in Action. A = Unspecific Adhesion of Polymer; B = Unspecific Adhesion of Mucin; C = Specific Interaction Between the Polymer and mucin.**

In order to test the effect of a substance known to break hydrogen bonds, urea was added to a sample consisting of 3.0 wt. % Genu® pectin DM 36 % in buffer and one sample of 3.0 wt. % mucin. The final urea concentration was 8 mol/kg solvent.

The procedure was repeated nine times for each measurement, with the exception of the measurements involving Genu® pectin DM 36% (without added urea) and amidated GRINDSTED® pectin, for which the intermediate precision was tested by preparing new solutions and repeating the measurements on different days. Measurements outside the quartiles ±1.5 times the interquartile range of the repeated measurements were excluded as outliers (Q1 – 1.5*IQR; Q3 + 1.5*IQR).

Interpretation of Mucoadhesion Data

A representative graph obtained from a Texture Analyzer is shown in (Hägerström et al., 2004). The maximum force, F_{max}, and area under the force versus time curve, AUC, from 0 force to 30 sec to ensure that all the graphs had levelled off, were obtained. AUC (g.s) is used to estimate the total work of detachment, and F_{max} (g) is used to estimate the maximum force of detachment. As illustrated in Fig. 1, three forces may be regarded as significant for the measured results. A is the unspecific adhesion of the polymer, B is the unspecific adhesion of mucin and C is the specific interaction between the polymer and mucin. Testing of polymer versus buffer allowed evaluation of force A (AUC_{buffer} and $F_{max\ buffer}$), while testing of mucin versus buffer allowed evaluation of force B (AUC_{mucin} and $F_{max\ mucin}$). By deducting these values from the measured AUC and F_{max} obtained when testing polymer versus mucin, the specific interaction between mucin and polymer could be found. As the values for mucin's unspecific adhesion are supposed to be independent of polymer type it could be ignored in the comparison between the polymers.

In ranging the polymers, data may be interpreted at two levels. The first level is the total mucoadhesion represented by AUC and F_{max}. The second level is the specific mucin interaction, represented by ΔAUC and ΔF_{max} ($AUC - AUC_{buffer}$ and $F_{max} - F_{max\ buffer}$, respectively), or the normalized parameters $\Delta AUC/AUC_{buffer}$ and $\Delta F_{max}/F_{max\ buffer}$. The normalized parameters indicate the relative increase and are used to compare samples of different cohesive properties and viscosity (Ferrari et al., 1997).

Standard errors for differences and quotients were calculated using error propagation theory.

RESULTS AND DISCUSSION

Characterization

Viscosity

The pectin types were characterized by capillary viscometry, which provides valuable information about the polymers. Most substantial are the intrinsic viscosity and the Huggins constant of the systems, which are summarized in Table 2.

There are large differences between the pectin types with respect to the maximum nonaggregated concentration (C without significant nonlinear deviation). Most of the qualities allow concentrations up to 0.20–0.25 wt. %. For Pectin Classic DM 35% and Genu® pectin DM 36%, however, the curves deviate from linearity at concentrations of only 0.064 wt. % and 0.038 wt. %, respectively, thereby indicating a very high tendency for self aggregation. The self aggregation of Pectin Classic DM 35% has been investigated (Kjøniksen et al., 2003; Kjøniksen et al., 2005), and mainly explained by extensive hydrogen bonding between the different polymer chains. The limited concentration range investigated for these two polymers renders the values obtained for the intrinsic viscosity and Huggins constant somewhat uncertain.

The intrinsic viscosity can be related to the volume an individual polymer chain occupies in the solution, and through the Mark-Houwink equation indicates

TABLE 2 **Intrinsic Viscosity and Huggins Constant for the Investigated Pectin Types**

	Pectin classic, DM 4%	Pectin classic, DM 35%	Genu® pectin, DM 36%	Genu® pectin, DM 31%, amidated	GRINDSTED®, DM 27%, amidated	GRINDSTED®, DM 70%
[η]	0.8	3.8	3.5	3.9	4.3	5.1
k'	0.85	0.23	0.44	0.42	0.63	0.76

the molecular weight (Christensen, 1954; Anger & Berth, 1986). The intrinsic viscosity decreases with a decreasing DM, indicating a corresponding decrease in the molecular weight. This is reasonable; since it is known that the manufacturing process used to manufacture low DM pectin from high DM pectin also invokes hydrolysis of the pectin backbone.

In relating the intrinsic viscosity to molecular weight, the Huggins constant should also be taken into account. The Huggins constant indicates the thermodynamic conditions of the polymer in the system; a value of 0.5 representing theta-conditions. The general picture is a correlation between degree of substitution and the Huggins constant. This is reasonable as increased hydrophilicity should lead to better thermodynamic conditions (lower Huggins constant). The exception is Pectin Classic DM 4% whose high Huggins constant indicate poor thermodynamic conditions. The difference in molecular weight between Pectin Classic DM 4% and the other qualities is consequently not as profound as the large difference in intrinsic viscosity would indicate.

The molecular weight for Pectin Classic DM 35% has previously been calculated to about 50,000, using a similar method (Hiorth et al., 2003).

Turbidity

The values of β found from turbidimetric measurements of Pectin Classic DM 35% and Genu® pectin DM 36% are below one for all samples. This indicates that the aggregates probably will have an extended conformation. A possible explanation could be the existence of hairy regions within the pectin molecule, which may induce sterical hinderences towards aggregation in the interior parts of the molecule and thereby favoring aggregation at the ends of the molecules.

Mucoadhesion

From the results summarized in Table 3, the values of ΔAUC, Δ F_{max} and normalized parameters were calculated and presented in Figs. 2 and 3. There were no significant differences observed between comparable qualities from different manufacturers. It is also worth noting that for all measured and calculated values alginate displays intermediate values. Depending on the circumstances different qualities of pectin will consequently be expected to perform both better and worse than alginate.

TABLE 3 Total Work and Maximum Force of Detachment From the Displacement Measurements (Mean ± SD)

	Pectin classic		Genu® pectin		GRINDSTED® pectin		
	DM 4%	DM 35%	DM 36%	DM 31%, amidated	DM 27%, amidated	DM 70%	Alginic acid
AUC (g.sec)	1662 ± 161 (n = 9)	2101 ± 136 (n = 7)	2286 ± 354 (n = 21)	2329 ± 414 (n = 9)	2627 ± 236 (n = 11)	2505 ± 306 (n = 8)	1900 ±189 (n = 9)
AUC_{buffer} (g.sec)	1010 ± 53 (n = 7)	1176 ± 133 (n = 9)	1164 ± 135 (n = 21)	1890 ± 252 (n = 9)	1887 ± 212 (n = 8)	2327 ± 358 (n = 8)	1300 ± 42 (n = 6)
F_{max} (g)	184 ± 27 (n = 9)	266 ± 12 (n = 7)	283 ± 58 (n = 21)	290 ± 63 (n = 9)	337 ± 14 (n = 11)	311 ± 35 (n = 8)	213 ± 11 (n = 9)
$F_{max\ buffer}$ (g)	98 ± 11 (n = 7)	121 ± 23 (n = 9)	123 ± 26 (n = 21)	252 ± 49 (n = 9)	261 ± 26 (n = 8)	278 ± 42 (n = 8)	33 ± 7 (n = 6)

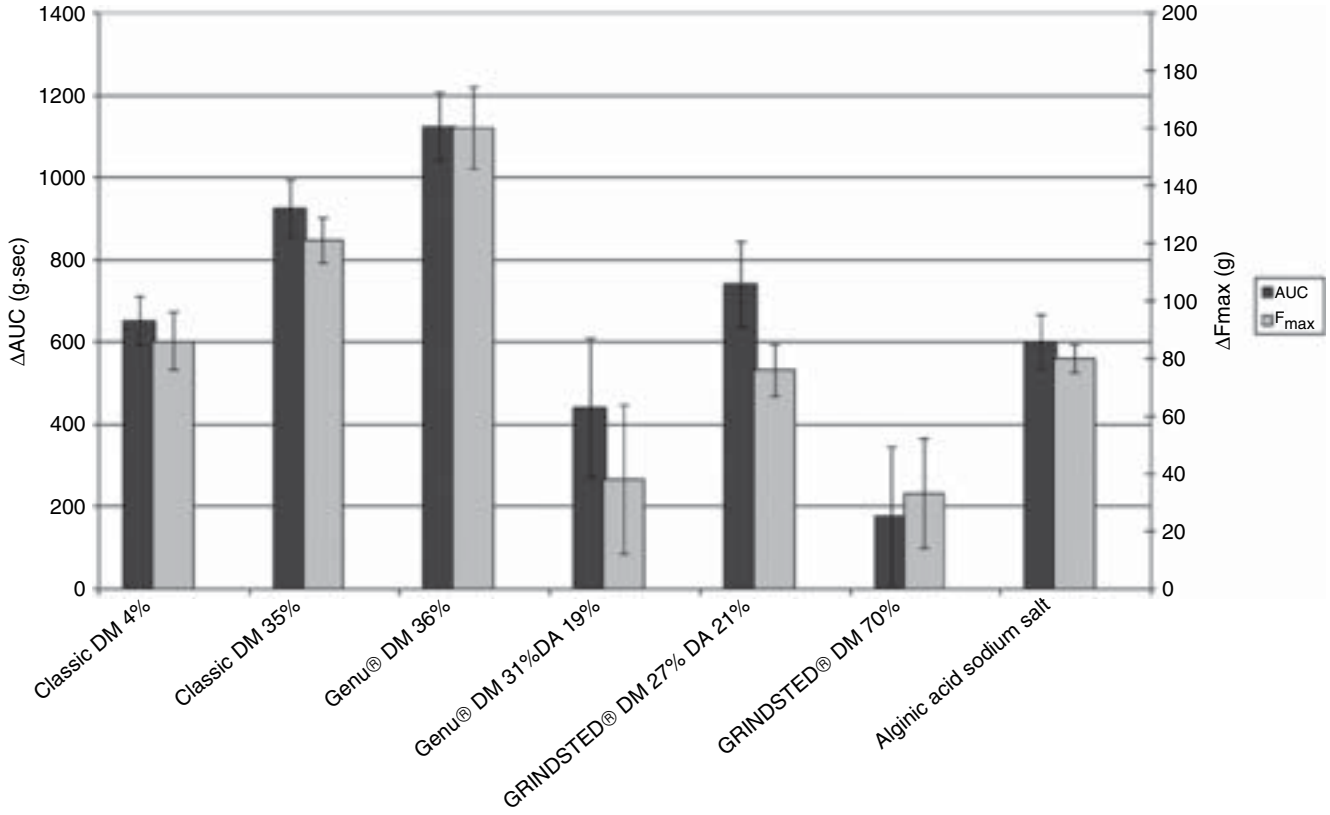

FIGURE 2 **The Calculated Specific Mucin Interaction (Total Work and Maximum Force of Detachment) for the Investigated Polymers.**

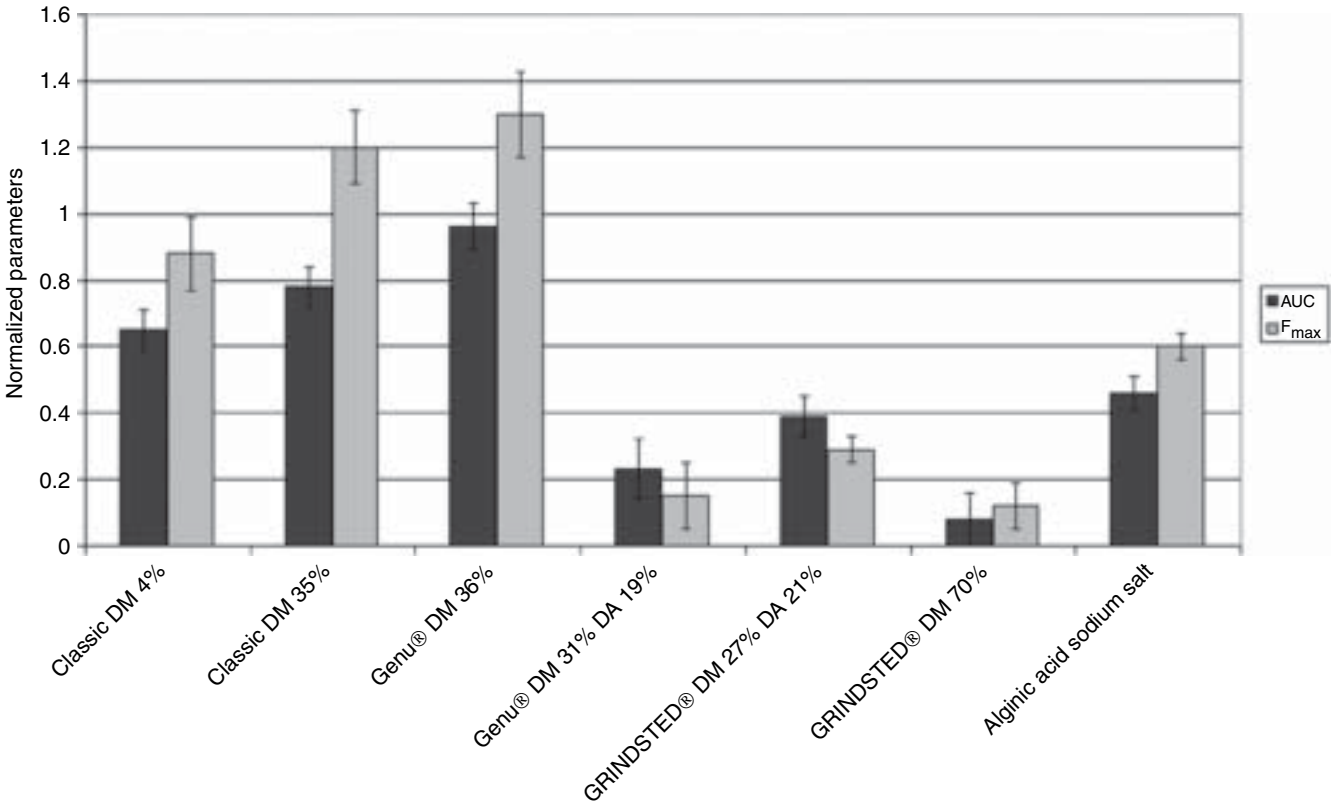

FIGURE 3 **The Calculated Values of the Normalized Total Work and Maximum Force of Detachment Parameters for the Investigated Polymers.**

The values of AUC, ΔAUC and $\Delta AUC/AUC_{buffer}$ correlate well with the values of F_{max}, ΔF_{max} and $\Delta F_{max}/F_{max\ buffer}$, respectively. It is therefore not necessary to discuss changes in the AUC values separately from the changes in the F_{max} values, as both parameters can equally be used for assessing mucoadhesion and mucin interaction in this study.

Ranging of the polymers with respect to ΔAUC and ΔF_{max}, correlate well with the order obtained by evaluating the normalized parameters. The only exception is amidated GRINDSTED® pectin, which has moderate values for ΔAUC and ΔF_{max}, but because of high values of unspecific adhesion (AUC_{buffer} and $F_{max\ buffer}$) the values for the normalized parameters are low.

Specific Mucin Interaction

The measurements of mucin and buffer alone gave an AUC_{mucin} value of 572 g.sec (SD = 82, $n = 15$) and a $F_{max\ mucin}$ value of 68 g (SD = 14, $n = 15$). Deducting these values from the estimated ΔAUC and ΔF_{max} yields the polymer's specific interaction with mucin (C in Fig. 1). Only Pectin Classic DM 35% and Genu® pectin DM 36% display values significantly different from 0 (353 ± 70 g.s and 53 ± 9 g for Pectin Classic DM 35% and 550 ± 85 g.s and 92 ± 14 g for Genu® pectin DM 36%). It is, therefore, reasonable to conclude that only Pectin Classic DM 35% and Genu® pectin DM 36% showed specific interaction with mucin in these experiments.

The first step in mucoadhesion that has to take place prior to the polymer engaging in bonding with the mucin, is wetting of the two surfaces and interpenetration of the polymers (Saiano et al., 2002). Hydrophilic polymers are therefore generally considered more mucoadhesive than more hydrophobic polymers (Garcia & Ghaly, 2001). The polymers` contact angles with water have been found to correlate well with the mucoadhesion (Li et al., 1998). It has also been found that polymers are more mucoadhesive if the polymer and mucin can obtain intimate surface contact (Hägerström et al., 2003). Consequently hydrophobicity and contraction of pectin types (reflected in the Huggins constant) can hinder mucoadhesion. This can partly explain the poor mucin interaction of Pectin Classic DM 4% and GRINDSTED® pectin DM 70% and the high mucin interaction of Pectin Classic DM 35%.

Our results indicate that mucin interaction of pectin is increased upon lowering the level of substitution, up to a certain level (pectin with a very low level of substitution is known to have poor solubility (Tho et al., 2003), as is also reflected in the Huggins constant of Pectin Classic DM 4%). This indicates that hydrogen bonding between the free carboxyl groups of pectin and mucin is the dominating mechanism. The strength of this force is illustrated by the fact that they manage to overcome the electrostatic repulsion of the charged carboxylic acid groups of both pectin and mucin, which are both almost fully dissociated at pH 6.8. The importance of hydrogen bonding in mucoadhesion has been highlighted several times, and the presence of –COOH groups and –OH groups are generally regarded as beneficial with respect to mucoadhesion (Saiano et al., 2002; Mortazavi, 2003).

It is also possible that Pectin Classic DM 35% and Genu® pectin DM 36% have some, up till now unknown, features that make them particularly favorable for hydrogen bonding. The results from capillary viscometry could indicate this, as the polymer chains start to aggregate at extremely low concentrations. To investigate the effect of hydrogen bonds further, urea, a well-known hydrogen bond breaking agent, was added to the pectin and mucin samples used in the Genu® pectin DM 36% measurements, and the measurements were repeated. The results are summarized in Table 4.

TABLE 4 **Comparison of Total Work and Maximum Force of Detachment for Genu® Pectin DM 36% With and Without Urea (Mean ± SD)**

Force	Without urea	With added urea	Change
AUC (g.s)	2286 ± 354 (n = 21)	1179 ± 71 (n = 9)	–1107 (– 48%)
AUC_{buffer} (g.s)	1164 ± 135 (n = 21)	1042 ± 130 (n = 9)	–122 (–10 %)
AUC_{mucin} (g.s)	572 ± 82 (n = 15)	290 ± 97 (n = 6)	–282 (–51 %)
F_{max} (g)	283 ± 58 (n = 21)	149 ± 19 (n = 9)	–134 (–53 %)
$F_{max\ buffer}$ (g)	123 ± 26 (n = 21)	124 ± 21 (n = 9)	–1 (–1 %)
$F_{max\ mucin}$ (g)	68 ± 14 (n = 15)	42 ± 20 (n = 6)	–26 (–38 %)

As can be seen from Table 4, both the cohesiveness of the mucin sample and pectin sample decrease upon adding urea, but the most substantial decrease is seen in the specific interaction between Genu® pectin DM 36% and mucin, which is no longer significant. This finding supports the theory that hydrogen bonding is the dominant mechanism in the current system.

Pectins with a high DM generally have a higher molecular weight as seen from the intrinsic viscosity results. Up to a certain level, a high molecular weight is known to enhance mucoadhesion due to a more pronounced interpenetration with the mucin (Batchelor et al., 2004; Bromberg et al., 2004). In our study, the molecular weight represented by the intrinsic viscosity was not found to be an important factor for mucin interaction. Interpenetration might, however, be important if the pronounced self aggregation observed for Pectin Classic DM 35% and Genu® pectin DM 36% yields aggregates of elongated shapes that go way beyond the range of molecular weight of the pectin types, leading to the observed strong mucin interaction. The turbidity data indicate that this could be a possible explanation.

The low values measured for GRINDSTED® pectin DM 70%, indicate that hydrophobic associations between the hydrophobic methyl groups of pectin and mucin seem to be a negligible mechanism. Introducing amide groups on pectin, does not increase the mucin interaction. In fact, total degree of substitution displays a higher correlation to mucin interaction than discriminating between methyl groups and amide groups, again pointing at hydrogen bonding involving free carboxyl groups of pectin as the dominating mechanism. This is not surprising taking into account the greater polarization of O–H bonds compared to N–H bonds.

General Mucin Interacion

The main emphasis in this discussion has been laid on the specific interactions between the different pectin types and the mucin. The in vivo relevance for these measurements compared to the values for mucoadhesion, represented by the total values of AUC and F_{max} is, however, unclear. A higher degree of substitution leads to higher values of AUC and F_{max} for pectin. The exception is amidated GRINDSTED® pectin (total substitution of 48%), which has the overall highest values. The explanation for the general correlation is that when no specific interaction between the mucin and polymer can be detected, the values of AUC_{buffer} and $F_{max\ buffer}$ will correlate with the values of AUC and F_{max} (Hägerström et al., 2004). The values of AUC_{buffer} and $F_{max\ buffer}$ are generally regarded to reflect the rheologic properties (viscosity) and the intrinsic cohesiveness of the samples (Ferrari et al., 1997; Hägerström et al., 2004). Pectin types with a high degree of substitution are more viscous. This is probably due to poorer solubility (leading to more hydrophobic interactions) when decreasing the amount of charged acid groups. It might also be due to higher molecular weight leading to entanglements within the pectin network, even though the higher molecular weight was not found to increase specific mucoadhesion by diffusing into the mucin network. For an in vivo situation, it is reasonable to expect that a high general adhesion of a formulation would be beneficial. Based on this presupposition the amidated GRINDSTED® pectin is the most promising candidate as it has the highest value of AUC and F_{max} (see Table 3), even though it was not possible to detect any specific interaction with mucin. Which of the measurements that are the most relevant in vivo, can only be investigated by performing in vivo measurements. The important issue is, however, that by varying the amount and type of the substituents, pectin may be tailored to display high values of either specific or general interactions.

CONCLUSION

In this study, DM and DA were indeed found to be important parameters for the specific and general interaction of pectin with mucin, while the observed effects are only insignificantly dependent on the manufacturer. The pectin types with a moderate DM (˜35%) displayed a particularly strong specific interaction with mucin. The two pectin samples stood out from the rest in their ability to aggregate, interpreted as an unusual high ability to engage in hydrogen bonding. Introducing amide groups on the pectin did not increase the interaction with mucin. In fact, total substitution of the carboxylic acid groups seemed to be a better indication of specific mucin interaction.

The pectin types with the highest degree of total substitution (amidated and DM 70%) showed the highest tendencies for general interaction. This could be relevant parameters for an in vivo situation, even

though the ability to engage in specific interactions with mucin was limited. This study indicates that the general classification of pectin as a poor mucoadhesive substance, without differentiating between the amount and type of substituents, probably is an oversimplification.

REFERENCES

Anger, H., & Berth, G. (1986). Gel permeation chromatography and the Mark-Houwink relation for pectins with different degrees of esterification. *Carbohydr. Polym.*, *6*, 193–202.

Baloglu, E., Özyazici, M., Hizarcioglu, S. Y., & Karavana, H. A. (2003). An in vitro investigation for vaginal bioadhesive formulations: bioadhesive properties and swelling states of polymer mixtures. *Farmaco*, *58*, 391–396.

Batchelor, H., Dettmar, P. W., Hampson, F. C., Jolliffe, I., & Craig, D. (2004). Microscopic techniques as potential tools to quantify the extent of bioadhesion of liquid systems. *Eur. J. Pharm. Sci.*, *22*, 341–346.

Bromberg, L., Temchenko, M., Alakhov, V., & Hatton, T. A. (2004). Bioadhesive properties and rheology of polyether-modified poly(acrylic acid) hydrogels. *Int. J. Pharm.*, *282*(1–2), 45–60.

Christensen, P. E. (1954). Methods of grading pectin in relation to the molecular weight (intrinsic viscosity) of pectin. *Food Res.*, *19*, 163–171.

Dodou, D., Breedveld, P., & Wieringa, P. A. (2005). Mucoadhesives in the gastrointestinal tract: revisiting the literature for novel applications. *Eur. J. Pharm. Biopharm.*, *60*, 1–16.

Duchêne, D., Touchard, F., & Peppas, N. A. (1988). Pharmaceutical and medical aspects of bioadhesive systems for drug administration. *Drug Dev. Ind. Pharm.*, *14*, 283–318.

Erbil, C., & Saraç, A. S. (2002). Description of the turbidity measurements near the phase transition temperature of poly(N-isopropyl acrylamide) copolymers: the effect of pH, concentration, hydrophilic and hydrophobic content on the turbidity. *Eur. Polym. J. 38*, 1305–1310.

Ferrari, F., Rossi, S., Martini, A., Muggetti, L., De Ponti, R., & Caramella, C. (1997). Technological induction of mucoadhesive properties on waxy starches by grinding. *Eur. J. Pharm. Sci.*, *5*(5), 277–285.

Garcia, J., & Ghaly, E. S. (2001). Evaluation of bioadhesive glipizide spheres and compacts from spheres prepared by extruder/marumerizer technique. *Pharm. Dev. Technol.*, *6*(3), 407–417.

Hiorth, M., Kjøniksen, A.-L., Knudsen, K. D., Sande, S. A., & Nyström, B. (2005). Structural and dynamical properties of aqueous mixtures of pectin and chitosan. *Eur. Polym. J.*, *41*, 1718–1728.

Hiorth, M., Tho, I., & Sande, S. A. (2003). The formation and permeability of drugs across free pectin and chitosan films prepared by a spraying method. *Eur. J. Pharm. Biopharm.*, *56*(2), 175–181.

Hägerström, H., Bergström, C. A. S., & Edsman, K. (2004). The importance of gel properties for mucoadhesion measurements: a multivariate data analysis approach. *J. Pharm. Pharmacol.*, *56*, 161–168.

Hägerström, H., Edsman, K., & Strømme, M. (2003). Low-frequency dielectric spectroscopy as a tool for studying the compatibility between pharmaceutical gels and mucous tissue. *J. Pharm. Sci.*, *92*(2), 1869–1881.

Kjøniksen, A.-L., Hiorth, M., & Nyström, B. (2005). Association under shear flow in aqueous solutions of pectin. *Eur. Polym. J.*, *41*(4), 761–770.

Kjøniksen, A.-L., Hiorth, M., Roots, J., & Nyström, B. (2003). Shear-induced association and gelation of aqueous solutions of pectin. *J. Phys. Chem. B*, *107*, 6324–6328.

Lehr, C.-M., Bouwstra, J. A., Schacht, E. H., & Junginger, H. E. (1992). In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.*, *78*, 43–48.

Li, C., Bhatt, P. P., & Johnston, T. P. (1998). Evaluation of a mucoadhesive buccal patch for delivery of peptides: In vitro screening of bioadhesion. *Drug Dev. Ind. Pharm.*, *24*(10), 919–926.

Miyazaki, S., Kawasaki, N., Nakamura, T., Iwatsu, M., Hayashi, T., Hou, W.-M., & Attwood, D. (2000). Oral mucosal bioadhesive tablets of pectin and HPMC: in vitro and in vivo evaluation. *Int. J. Pharm.*, *204*, 127–132.

Mortazavi, S. A. (2003). Extended frequency sweep: A more realistic rheological approach to investigate the process of mucoadhesive polymer-mucus gel chain interpenetration. *Iranian Polym. J.*, *12*(5), 413–420.

Nafee, N. A., Ismail, F. A., Boraie, N. A., & Mortada, L. M. (2004). Mucoadhesive delivery systems. I. Evaluation of mucoadhesive polymers for buccal tablet formulation. *Drug Dev. Ind. Pharm.*, *30*(9), 985–993.

Ranga Rao, K. V., & Buri, P. (1989). A novel in situ method to test polymers and coated microparticles for bioadhesion. *Int. J. Pharm.*, *52*, 265–270.

Rossi, S., Bonferoni, M. C., Ferrari, F., Bertoni, M., & Caramella, C. (1996). Characterization of mucin interaction with three viscosity grades of sodium carboxymethylcellulose. Comparison between rheological and tensile testing. *Eur. J. Pharm. Sci.*, *4*(3), 189–196.

Saiano, F., Pitarresi, G., Cavallaro, G., Licciardi, M., & Giammona, G. (2002). Evaluation of mucoadhesive properties of α,β-poly(N-hydroxyethyl)-DL-aspartamide and α,β-poly(aspartylhydrazide) using ATR–FTIR spectroscopy. *Polym.*, *43*, 6281–6286.

Sandri, G., Rossi, S., Bonferoni, M. C., Ferrari, F., Zambito, Y., Di Colo, G., & Caramella, C. (2005). Buccal penetration enhancement properties of N-trimethyl chitosan: Influence of quarternization degree on absorption of a high molecular weight molecule. *Int. J. Pharm.*, *297*(1–2), 146–155.

Schmidgall, J., & Hensel, A. (2002). Bioadhesive properties of polygalacturonides against colonic epithelial membranes. *Int. J. Biol. Macromol.*, *30*, 217–225.

Smart, J. D., Kellaway, I. W., & Worthington, H. E. C. (1984). An in-vitro investigation of mucosa-adhesive materials for use in controlled drug delivery. *Eur. J. Pharm. Biopharm.*, *36*, 295–299.

Tho, I., Kleinebudde, P., & Sande, S. A. (2001). Extrusion/spheronization of pectin-based formulations. I. Screening of important factors. *AAPS Pharm. Sci. Tech.*, *2*(4).

Tho, I., Sande, S. A., & Kleinebudde, P. (2003). Disintegrating pellets from a water-insoluble pectin derivative produced by extrusion/spheronisation. *Eur. J. Pharm. Biopharm.*, *45*, 371–380