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New insights into the mucoadhesion of pectins by AFM roughness parameters in combination with SPR

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ABSTRACT

The object of this study was to assess the mucoadhesion of the three main commercially available types of pectin by atomic force microscopy (AFM) and surface Plasmon resonance (SPR). Polyacrylic acid and polyvinyl pyrrolidone were used as positive and negative control, respectively.

Image analysis of the AFM scans revealed a significant change of roughness parameters when low-ester pectin was introduced to mica supported bovine submaxillary mucin, indicating a high mucoadhesion for this type of pectin. Only minor changes were observed with high-ester and amidated pectin. The same ranking order of adhesion affinity was confirmed by SPR.

In conclusion, a high specific mucin interaction of pectin with a high charge density was demonstrated directly on a molecular scale without interference from the viscoelastic properties or the intra-molecular interactions between the polymer chains themselves, using two independent methods.

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1. Introduction

Mucoadhesive drug formulations are generally considered advantageous, both to prolong the treatment time and the effect of locally acting drugs, but also as a strategy for increasing the bioavailability of elsehow poorly absorbed drugs, for example proteins and other peptides. In the last case, especially the buccal cavity has emerged as a promising alternative to peroral systemic administration, due to a lower enzymatic activity, a better accessibility and its robustness (Bruschi and de Freitas, 2005). Another advantage of the buccal cavity in the context of exploitation of mucoadhesion is the lower amount of fluid present as compared to for example the gastrointestinal tract. In fact, already in 2005, Rossi et al. published a paper suggesting that buccal drug delivery was a challenge already won. However, even under conditions when the amount of water is rather low, the hydration of a mucoadhesive formulation will increase with time, which will eventually lead to reduced adhesive properties (Surapaneni et al., 2006). When developing a formulation intended to stay on the mucosa for prolonged periods of time, it is therefore of importance to have knowledge about the mucoadhesive properties of polymers in an aqueous environment.

Many different polymers have been synthesized and tested with regard to mucoadhesion (Grabovac et al., 2005). Pectins are cheap, safe and abundant polymers that have been used extensively by the pharmaceutical and food industries (Bengmark, 1998). Commercial pectins consist of a galacturonic acid backbone (Fig. 1). The acid groups can be esterified (methoxylated) or amidated, as indicated by R in the figure. Based on the relative amount of ester groups, pectins are classified into high- and low-ester pectin (HM and LM pectin, respectively). Additionally, LM pectin can be amidated (NH pectin). Due to the manufacturing process, the average molecular weight (Mw) will typically vary between the types. Both the Mw and chemical structure are expected to influence on the mucoadhesion. The mucoadhesive properties of pectins have been investigated by many groups in the past. However, controversial results were reported, and still there is no consensus as to which type of pectin is optimal in view of mucoadhesiveness. LM pectin was reported to be more mucoadhesive than HM pectin for solutions (Schmidgall and Hensel, 2002) and gels (Liu et al., 2005), visualized on porcine colonic tissue and demonstrated by rheological synergism. In contrast, Thirawong et al. found, using a texture analyzer, that HM pectin discs were more mucoadhesive towards a porcine GI mucosa. Amidation had a positive effect on the mucoadhesion of LM pectin (Thirawong et al., 2007). Later they confirmed these findings by rheological synergism studies of pectin solutions and commercially available mucin (Thirawong et al., 2008).
One reason for these conflicting results may be the use of different formulations and methods to test mucoadhesion. Unfortunately, no standardized method exists, and the testing was done in many different ways, ranging from un-physiological in vitro situations to in vivo testing in animals and humans. Moreover, mucoadhesive interactions have been tested from nanoscale to bulk-level. Advantages and disadvantages exist for all methods. Generally speaking, there seems to be a conflict between gaining information about the important factors and mechanisms involved, and at the same time simulating the relevant in vivo conditions.

Atomic Force Microscopy (AFM) is an in vitro method that explores the topology and, occasionally, elasticity of a sample in a non-invasive way. A minute tip on a cantilever serves as a nanoscopic finger that probes a sample by scanning it for surface modulations. Essentially, an AFM generates an image that is a 3D map of constant interaction potential between the tip and the surface. The tip-sample interaction is, in a sense, translated into small changes in the bending of the cantilever as it touches or comes close to the sample. The reflection from a laser beam that is incident on the cantilever face can be read on a detector, electronically assembling the image proper from the cantilever deflection. Depending on the AFM settings and the sample details, the resolution for wet samples may go down to the Ångström range, and the images are then depicted on the cm-scale, i.e. in a magnification of ∼10⁴.

This setup can allow mucoadhesion to be directly tested on a molecular scale by measuring the force–distance curve between a mucus surface and a polymer microsphere that is attached to an AFM cantilever as a colloidal tip. This approach was successfully used by Cleary et al. to study the molecular interaction between glass beads coated with a co-polymer and bovine submaxillary mucin on a planar carrier, in aqueous surroundings under different pH and salt concentrations (Cleary et al., 2004). Later Catron et al. (2006) did a similar study, but used a sharp AFM tip that was covalently functionalized with polymer molecules to test their affinity for adhering to a mucous interface. However, in Cleary et al.’s self-assessment of their method they point to its complexity regarding functionalization of both the colloidal probe and the planar surface, as well as the effect on reproducibility. This is also indicated by Li et al. (2010) who mostly focused on achieving a smooth and uniform polymer coating of the AFM tip.

The alternative is to focus on topological images that refrain from detailed force measurements, but give an overview of the coverage of a surface with mucin, and on its modification upon interaction with another polymer (Dedinaite et al., 2005). Scans of polymers premixed with mucin have also been used (Deacon et al., 2000). Even this may be a challenge, as getting good and informative pictures of soft matter, especially of swelling polymers in an aqueous environment, is difficult, and there is a risk that the assessment of the pictures will become somewhat subjective unless a detailed method for the image analysis can be defined.

In this paper we report on a study about the mucoadhesion of pectins, performed by AFM in an aqueous environment cell. We propose to compare and quantify the different interaction behavior found by use of statistical roughness parameters that can be extracted by image analysis from the topographical pictures taken by AFM. Bovine submaxillary mucin (BSM), earlier used to simulate the buccal mucosa, was deposited onto mica and then scanned in air and immersed in water. The pre-coated mica surface was then incubated with different pectin polymers that were introduced into the bulk of the water cell. After washing to remove excessive polymer, AFM images were taken under full hydration. The images obtained were assessed by extracting surface roughness parameters. Their changes depending on the sample details were taken as quantifiers of mucosal adhesion of the polymers under regard. The three main commercially available types of pectin (LM, NH and HM pectin, Table 1) were tested by this method and the outcome was compared to the one obtained for polyacrylic acid (PAA) as a positive control and to the one for polyvinyl pyrrolidone (PVP) as a negative control. PAA and PVP are known to possess high and low mucoadhesion, respectively, e.g. (Thongborisute and Takeuchi, 2008). The results for pectin were validated by an independent method based on surface plasmon resonance (SPR). In short, the change of refractive index upon adhesion of polymer to a test chip is optically explored by the shift of the Plasmon resonance angle in total reflection conditions, which is converted to SPR response measured in resonance units (RU). The SPR response correlates linearly with mass bound to the sensorchip plus the refractive index of the bulk medium (Mol and Fischer, 2010). The test chip consisted of an Au surface that was pre-coated with mucin and mounted into the flow cell of a Biacore instrument. Upon injection of the different polymers into the flow cell, their mucoadhesion was directly monitored. If the polymer permanently binds to the mucinated surface, the refractive index along the sensor chip surface is modified, leading to a quantitative SPR signal in so called response units (RU, in effect proportional to the interfacial refractive index).

2. Materials and methods

2.1. Materials

Mucin from bovine submaxillary glands (BSM), type I–S, batch 068K7001, was purchased from Sigma–Aldrich and used as received.

5 different test polymers were used, all dissolved in pure water. The LM, NH and HM pectin were derived from citrus and kindly provided by the manufacturer (CP Kelco, Denmark) and used as received. Details are listed in Table 1. The pectins differed in the degree of and functionalization (methoxylation and amidation) of the acid groups, as well as Mw. Polyacrylic acid (PAA; Carbopol 980, batch 80035089) and polyvinyl pyrrolidone (PVP; Povidone 25, batch 81430488) were used as positive and negative control, respectively. They were both of Ph. Eur. quality and purchased from Caelo, Hilden, Germany. Both were used as received.

Milli-Q water from a QTIM000EX obtained from Millipore A/S, with a 0.22 μm filter unit on the outlet was used as solvent. All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Atomic force microscopy (AFM)

AFM was performed with a PicoScan – AFM (PicoSPM from Molecular Imaging Inc. (now Agilent Technologies Inc.)) using a scanner that has a maximal range of 30 μm × 30 μm. Sam-

![Fig. 1. A schematic illustration showing a part of the galacturonic backbone of pectin, as well as the substitution at the carboxylic acid.](image-url)
samples were deposited onto mica (G250-1 from Plano GmbH, Germany). AFM imaging was done in air in the contact mode (cantilevers by NanosensorsTM, type PPP-NCL-50, polymer to be studied for its mucoadhesive properties. Coated samples that were incubated in aqueous solutions of a test BSM. Mucoadhesion was then later explored by doing AFM on pre-

---

### Table 1

<table>
<thead>
<tr>
<th>Brand</th>
<th>Low-ester (LM) pectin</th>
<th>Amidated (NH) pectin</th>
<th>High-ester (HM) pectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>LM-12 CG-Z</td>
<td>LM-102 AS-Z</td>
<td>Type B Rapid Set-Z</td>
</tr>
<tr>
<td>Degree of methoxylation</td>
<td>33%</td>
<td>30%</td>
<td>70%</td>
</tr>
<tr>
<td>Intrinsic viscosity, $[\eta]$ (dL/g)</td>
<td>3.35</td>
<td>4.31</td>
<td>5.36</td>
</tr>
<tr>
<td>Huggins' constant, $k'$</td>
<td>0.38</td>
<td>0.37</td>
<td>0.44</td>
</tr>
</tbody>
</table>

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2.2.2. Surface plasmon resonance (SPR)

SPR was performed in a Biacore 3000 equipped with BIAevaluation software (GE Healthcare, Uppsala, Sweden) using a bare gold sensorchip (type SCB AU-S, Xantec Bioanalytics GmbH, Düsseldorf, Germany). The microfluidic flow system of the Biacore instrument forms 4 flow cells on the sensorchip that can be addressed individually. The samples were filtered through a 0.45 μm pore filter (Minisart, 16555 80412103) before injection to remove any dust particles or other big sized objects that might interfere with the free flow in the microchannel lines. All liquids used for SPR were degassed before use in order to avoid the development of gas bubbles that can interfere with the flow and detection.

At first a baseline with pure degassed water was established over a course of 3 min. Then the chip was conditioned with BSM dissolved in pure water (0.10 wt.%). Five injections of BSM were applied sequentially, followed by water washing, to condition each of the sub-cells separately, covering the detection surface of the Au chip with a layer of BSM. The final outcome for each of the flow cells was then found in RU units for each of the flow channels on the chip. Finally, test polymer solutions were applied, each in at least 3 subsequent injections to individual BSM coated channels and the specific SPR signals were recorded. LM, NH and HM pectin, as well as the negative control PVP (all provided as 0.10 wt.% in water solution) were tested for binding. All experiments were performed at 25 °C; the flow rate was 15 μL/min, and the injection time for BSM and the test polymers was 1 min. After each injection, the cell was flushed with water for 4 min.

3. Results and discussion

3.1. AFM

Representative images from the 3 essential preparative steps are shown in Fig. 2. First, the mica carrier was exposed to the BSM solution. After evaporation of the solvent, it was found fully covered with BSM (Fig. 2a)). The lighter areas indicate more concentrated and bulky BSM, while the black areas represent a thinner layer of BSM. The covering is hence not uniform. The BSM samples were rehydrated and then imaged in the wet state in a water cell (Fig. 2b)). The BSM probably swells and hence changes conformation when rehydrated. This gives rise to a different roughness variation, as can be seen by a lot of small blobs and a few big ones (lighter areas) sitting on a background of apparently more homogenous topology. Then, the BSM coating in the water cell was incubated with test polymer by injection of the selected polymer type and, after incubation, the excess polymer was removed by washing. A scan obtained from PAA as test polymer is shown in Fig. 2c). In this case the surface topology appears smoother, indicating that PAA interacted with the BSM surface. Blobs can still be identified. Although, in general, the individual scans reveal an uneven surface containing different features, all samples were considered as macroscopically homogeneous on average. Therefore all scans from the accidentally chosen spots in a sample are represen-
The images show that BSM and test polymer are deposited onto the carrier sheet. Still, distinct conclusions regarding quantification of adhesion of test polymer to the initial BSM coating can hardly be drawn from the shear appearances of the samples. Only a statistical analysis and subsequent comparison among samples of different preparations does allow a quantification of the mucoadhesive potential of the diverse test polymers.

The selected quantities (see Eqs. (1.1) and (1.2)) from the statistical image analysis are presented graphically in Fig. 3a and b. They compile the results obtained by AFM under hydrated conditions. The initial BSM values (light bars) are an average from all samples and are used as start values which are compared to the properties after application of test polymer (dark bars; averaged as indicated in the figure caption).

The root mean square of the roughness average of BSM was not changed by PVP, serving as the negative control polymer (Fig. 3a)). On the other hand, the positive control polymer PAA reduced the variation of the average roughness drastically, as was also apparent from direct observations (Fig. 2c)). The pectins, at a 10 times lower concentration, displayed intermediate values. Among the pectins, only LM pectin inflicted changes that were statistically significant. The samples were more homogeneously flat over the whole scan area after application of PAA and LM pectin, indicating an interaction with BSM. In general, the peak–peak height values confirmed this picture (Fig. 3b)).

### 3.2. Surface plasmon resonance/Biacore

The Biacore method has previously been reviewed as an alternative in vitro method to detect the mucoadhesive properties of polymers, and the results obtained corresponded well with the results of other mucoadhesion tests (Takeuchi et al., 2005).

First the Au-surface was covered with BSM. BSM attached very effectively to the surface of gold as demonstrated by the increase in SPR response: the average change relative to the water baseline for the 4 flow cells after five injections was $633 \pm 48$ (SD) RU. Furthermore, the BSM was attached relatively stably to the Au surface as seen by the slow decrease of SPR response after the flow was changed to water. Next, test polymers were injected in the BSM coated flow cells and the change in SPR response for each individual channel with test polymer was recorded. As an example the result displayed by LM pectin is shown in Fig. 4, as well as an explanation for the $\Delta$RU values. The initial BSM baseline value was set to 0 RU. During the first injection, a large increase in SPR response is obtained. This signal is due to both the adhesion of test polymer (LM pectin) to the BSM matrix on the chip as well as the presence of the test polymer in the bulk solution.
water, the net SPR response that is significant for the adhesion is obtained (injection 1). Further injection (↑) resulted in additional adhesion with declining efficacy, until the surface is saturated. The total increase in net SPR response of all injections after flushing with water can be taken as an indicator of adhesion affinity. The results for all test polymers are compiled into Table 2.

The negative control, PVP, displayed a very low change of SPR response relative to the baseline. This was also the case for NH pectin. LM pectin displayed the largest increase in SPR response, indicating an accumulation of LM pectin at the BSM interface. The values of HM pectin were in between those of LM and NH pectin. The results from the Biacore method thus correlated well with the observations from AFM: among the pectins, mucoadhesion was highest for LM pectin. Unfortunately, the experiments with PAA using this set-up were unsuccessful due to clogging. A high mucoadhesion for PAA compared to PVP has previously been reported using SPR (Thongborisute and Takeuchi, 2008).

### Table 2

Increment in SPR response obtained from the Biacore measurements after injections of test polymers over a BSM covered Au-surface (the explanation for the values is given in Fig. 4).

<table>
<thead>
<tr>
<th>∆RU/polymer</th>
<th>LM pectin (∆RU)</th>
<th>NH pectin (∆RU)</th>
<th>HM pectin (∆RU)</th>
<th>PVP (∆RU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection 1</td>
<td>141.8</td>
<td>8.7</td>
<td>86.6</td>
<td>18.2</td>
</tr>
<tr>
<td>Injection 2</td>
<td>49.5</td>
<td>9.9</td>
<td>31.3</td>
<td>11.7</td>
</tr>
<tr>
<td>Injection 3</td>
<td>34.5</td>
<td>11.1</td>
<td>16.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Sum</td>
<td>225.8</td>
<td>29.7</td>
<td>134.4</td>
<td>40.8</td>
</tr>
</tbody>
</table>
or (Anger and Berth, 1986), the Mw can be estimated to vary from
in Table 1. Using the Mark–Houwinks relationship, \[ \eta = K \times M^a \]

The intrinsic viscosities for the different types of pectin can be seen
negative control, PVP (Povidon 25) has a Mw of about 15–30 kDa.
known to possess a very high Mw of a few million Da, while the
most types of surfaces, including those covered by mucus. For the
to a higher tack and hence a general tendency for adherence to
is important. In general, for highly soluble polymers like pectin, a
mimics the buccal mucosa and test polymer solutions of a low
achieved by studying the molecular interactions between a surface
ing water from the underlying mucosa, leading to adhesion by
may be reached. The other is related to the formulation extract-
formulating, liberating the polymer chains. In this way, the opti-
mum flexibility and minimal intra- and intermolecular bonding, i.e.
cast films by tensile tests (Hagesaether and Sande, 2007, 2008).
subtracted), which was demonstrated for 3.0 wt.% solutions and
casted films by tensile tests (Hagesaether and Sande, 2007, 2008).
Solutions of mucin, pectins and mixtures of mucin and pectin
were, by adding urea, demonstrated to be caused by hydrogen-
bonding. The importance of hydrogen-bonding in mucoadhesion
has been highlighted several times, and the presence of –COOH
and –OH groups are generally regarded as beneficial with respect to
mucoadhesion. Mucin will be negatively charged at pH > 3, and
the free carboxylic acid groups of pectin have a pKa of about 3.
The fact that both mucin and pectin are negatively charged, and
that water itself is a strong hydrogen bonding substance, may make
the results and explanation seem paradoxical. However, it has pre-
viously been suggested that the electrostatic repulsion with the
same charges may cause an uncoiling of polymer chains, which may
facilitate chain entanglement and bond formation (Sriamornsak
et al., 2010). Even more, some surprising hydrogen-bond attrac-
tion forces have been measured between carbohydrates and mucin
in solution. This was attributed to matching active site patterns,
thereby being able to compete with the strong hydrogen-bonding
properties of water (Huang and Peppas, 2007). The negative charges
of mucin and pectin make them a stronger electron donor than water,
and hydroxyl groups can act as electron acceptors.

Mucoadhesion was found to be low or non-existing for NH pectin
for both methods. HM pectin displayed an inconsistent change of AFM roughness parameters, but during the Biacore®
experiment, values in-between those displayed by LM and NH
pectin were observed. During these experiments, the concentra-
tions were 100 times higher than during AFM. Therefore, despite
clear differences in the thermodynamic conditions during the dif-
terent types of experiments, some contribution from the higher
viscosity to the intermediate mucoadhesion observed, cannot be
ruled out.

Solutions of mucin, pectins and mixtures of mucin and pectin
were scanned with AFM in a recent study (Sriamornsak et al.,
2010). From the pictures they concluded that NH pectin is the

--Fig. 4. Sensorgram of the Biacore measurements when LM pectin was injected onto
a BSM covered Au-surface (baseline value set to 0 RU). Each injection time point of
polymer is indicated by an arrow turning upwards (↑) and flushing is indicated by an
arrow turning downwards (↓). Thus there is a constant flow of polymer between sets of
upwards and downwards arrows and of water between downwards and upwards
arrows.

3.3. General discussion

The different theories and mechanisms behind mucoadhesion have been reviewed many times, e.g. (Smart, 2005). For example, for a dry formulation, mucoadhesion can be viewed as a step-wise process, with a first step consisting of establishing an intimate contact between the mucoadhesive and the mucous membrane, and a second consolidation step, where various molecular interactions of physicochemical nature occur to consolidate and strengthen the adhesive joint. During the first step, at least two processes can occur that may lead to adhesion. One is related to swelling of the formulating, liberating the polymer chains. In this way, the optimum in viscosity at the surface, leading to a general adhesion, tack, may be reached. The other is related to the formulation extracting water from the underlying mucosa, leading to adhesion by capillary attraction or as a result of the increased strength of the mucosa (Marshall et al., 2004). However, as the hydration of the initially dry formulation gradually increases with time, the adhesion established during step one will decline, unless compensated by another mechanism. It is therefore step two that is responsible for prolonged adhesion, which in some cases is beneficial for drug treatment. We therefore decided to focus on step two. This was achieved by studying the molecular interactions between a surface that mimics the buccal mucosa and test polymer solutions of a low concentration. In that way, step one can be disregarded, and it can be assumed that the test polymers are fully hydrated, possess maximum flexibility and minimal intra- and intermolecular bonding, i.e. they are in their most uncoiled configuration.

The test polymers used in this study all possess a different Mw. This is often the case for different types of polymers, especially those of a natural origin. The Mw of a polymer is a parameter that can influence the adhesive properties, in addition to chemical structure. Therefore, assessing the impact of differences in Mw is important. In general, for highly soluble polymers like pectin, a high Mw will increase the viscosity of a polymer solution, leading to a higher tack and hence a general tendency for adherence to most types of surfaces, including those covered by mucus. For the polymers in this study, the positive control, PAA (Carbopol 980) is known to possess a very high Mw of a few million Da, while the negative control, PVP (Povidon 25) has a Mw of about 15–30 kDa. The intrinsic viscosities for the different types of pectin can be seen in Table 1. Using the Mark–Houwinks relationship, \[ [\eta] = K \times M^a \]
and the constants for K and a obtained from (Christensen, 1954) or (Anger and Berth, 1986), the Mw can be estimated to vary from about 70 kDa for LM pectin to about 115–135 kDa for HM pectin, with amidated pectin having a Mw in between. This method has been used previously (Hiorth et al., 2003). The results are reasonable, as the manufacturing process used to produce LM pectin from HM pectin is also known to invoke hydrolysis of the pectin backbone. Either because of this difference in Mw or, alternatively, due to the higher number of hydrophobic groups, 3.0 wt.% solutions of HM pectin generally displayed a higher viscosity and hence a higher tack. This further lead to a higher unspecific adhesion and hence a higher general mucoadhesion than LM pectin (Hagesaether and Sande, 2007). The same effect was observed for casted films (Hagesaether and Sande, 2008).

However, the pectin concentrations used in this study were only 0.001 wt.% during the AFM measurements and 0.1 wt.% during the Biacore measurements. Using the same values for intrinsic viscosity, the concentration when the pectins start to overlap can be calculated to be ca. 0.2 wt.% and hence higher than the concentrations used in this study. Additionally, the differences in viscosity of the pectin solutions are negligible as determined by capillary viscosimetry. In that way, the mucoadhesion of pectins was tested here on a molecular level, without interference from the viscoelastic properties nor the intra-molecular interactions between the polymer chains themselves.

In this study, both the AFM and Biacore measurements indicate that LM pectin was more mucoadhesive than amidated and HM pectin, despite having a lower Mw. Reasons for this can be that LM pectin interpenetrated the mucin network easier or displayed intermolecular interaction with the mucin molecules. This is in line with our earlier studies, where LM pectin showed a particularly strong estimated specific interaction with mucin (i.e. the adhesion remaining after the unspecific adhesion have been mathematically subtracted), which was demonstrated for 3.0 wt.% solutions and casted films by tensile tests (Hagesaether and Sande, 2007, 2008). For solutions, the superior mucoadhesive properties of LM pectin were, by adding urea, demonstrated to be caused by hydrogen-bonding. The importance of hydrogen-bonding in mucoadhesion has been highlighted several times, and the presence of –COOH and –OH groups are generally regarded as beneficial with respect to mucoadhesion. Mucin will be negatively charged at pH>3, and the free carboxylic acid groups of pectin have a pKa of about 3. The fact that both mucin and pectin are negatively charged, and that water itself is a strong hydrogen bonding substance, may make the results and explanation seem paradoxical. However, it has previously been suggested that the electrostatic repulsion with the same charges may cause an uncoiling of polymer chains, which may facilitate chain entanglement and bond formation (Sriamornsak et al., 2010). Even more, some surprising hydrogen-bond attraction forces have been measured between carbohydrates and mucin in solution. This was attributed to matching active site patterns, thereby being able to compete with the strong hydrogen-bonding properties of water (Huang and Peppas, 2007). The negative charges of mucin and pectin make them a stronger electron donor than water, and hydroxyl groups can act as electron acceptors.
most mucoadhesive. They did not consider the change in roughness parameters, which was shown in this study to be useful. In fact, already in 2000 Patel et al. assessed the arithmetic roughness average when testing the mucoadhesion of polycarbophil, chitosan and hydroxypropyl methylcellulose towards a buccal cell surface, and in 2006 Svensson et al. observed a change in roughness of the surface when BSM and chitosan was deposited layer by layer in an AFM liquid cell.

The usefulness of using the change in roughness parameters as an indication of mucoadhesion was indicated by the negative and positive control polymers, PVP and PAA, respectively. PVP displayed insignificant while PAA displayed significant changes. PAA is also a polymer with many acidic groups and hence possesses negative charge in a pure water solution. However, in contrast to pectins, PAA lacks electron acceptors. The influence of the extremely high Mw and viscoelastic properties can therefore not be completely ruled out as an explanation.

In conclusion, the promising mucoadhesion of LM pectin in solution, previously reported by us using estimated specific mucin interaction, was here demonstrated directly on a molecular scale by two independent methods. A high charge density due to free acid groups was found more important for high mucoadhesion than high Mw or amidation. Due to the low concentrations used, neither the viscoelastic properties of pectin solutions nor the intra-molecular interactions between the polymer chains themselves interfered with the results.

SPR and roughness parameters from AFM allowed to explicitly measure the specific mucin interaction, i.e. the second adhesion step, without subtracting the tack and unspecific adhesion. The results for pectin obtained during the AFM experiments were compared to the behavior of PAA as positive and PVP as negative control, respectively. Additionally, the results for pectin generally correlated with those given by an independent method; SPR. The AFM method can therefore be recommended as a starting point when choosing between different mucoadhesive polymers, also for polymers intended for mucoadhesion in the gastrointestinal tract, as the interaction was studied in an aqueous environment.

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References


