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Research paper

Mucoadhesion and drug permeability of free mixed films of pectin and chitosan: An *in vitro* and *ex vivo* study

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ABSTRACT

The objective of this study was to identify the important factors for the drug permeability and mucoadhesion of casted free pectin/chitosan combination films. The factors varied were: the type of pectin (low and high methoxyl pectin) and the ratio pectin:chitosan (25:75, 50:50 and 75:25). The model drug used for measuring drug permeability was paracetamol. A texture analyzer was used for measuring mucoadhesion by using two different setups: (1) *in vitro* tensile tests measuring the detachment force of films versus a mucin dispersion and (2) *ex vivo* shear tests measuring the friction forces between pre-hydrated films and fresh porcine small intestine, with the system immersed in phosphate buffer, pH 6.8.

The type of pectin used in the combination films did not have a significant effect on the drug permeability. The *ex vivo* mucoadhesion test revealed significant differences between low and high methoxyl pectin only for the 50:50 pectin:chitosan films. For that type of film, the peak and friction forces were highest for high methoxyl pectin. Both the mucoadhesion and drug permeability generally increased with decreasing amounts of pectin relative to chitosan in the films.

1. Introduction

The concept of mucoadhesion is appealing because, if successfully achieved for a formulation in the gastrointestinal tract, a higher bioavailability of the entrapped drug would be expected [1]. The reason is increased residence time and a closer contact between the membrane and the formulation. This could also allow drug release over sustained periods of time, reducing the need for re-administration and/or reducing the amount of drug needed.

In most cases, achieving mucoadhesion in the gastrointestinal tract is meaningless without a corresponding sustained drug release. The theories that describe the mechanism of mucoadhesion are well known [2]. According to the diffusion theory, mucoadhesion occurs as a consequence of molecular diffusion, leading to interpenetration and entanglements. Based on this theory, an inherent paradox for mucoadhesive formulations may be detected: the factors leading to an increased mucoadhesion can possibly lead to a faster drug release. This effect has been reported by some authors, for example, for pectin tablets upon adding xylitol [3] and for glyceryl monooleate matrices upon adding additives with different polarities [4]. Our earlier work has shown that the mucoadhesion of zinc-pectinate hydrogel beads increased with decreasing amount of zinc and thereby decreased cross-linking of the beads [5] which is likely to result in higher drug release.

Pectin is a polysaccharide very abundant in nature. The dominant feature of pectin is the (1 → 4) α-D-galacturonic acid units partially esterified with methanol. The degree of methoxylation (DM) is used to classify pectin as low methoxyl pectin (DM < 50) and high methoxyl pectin (DM > 50) [6]. Recent studies have shown the mucoadhesive potential for pectin, and especially pectin with a low DM, both for solutions [7], gels [8] and casted free films [9], which was demonstrated to be due to hydrogen bonding between pectin's free acid groups and mucin [10]. On the other hand, the values of diffusion coefficients of mucin [11] and rheological synergy with mucin [12], both in simulated intestinal fluid, have been reported to increase when the DM increased.

Chitosan is a polysaccharide containing amino groups and is soluble in acidic media. The mucoadhesive properties of chitosan have been extensively studied, and chitosan is currently generally accepted as a mucoadhesive agent [13] due to either ionic interactions between the positively charged amino groups and the negatively charged mucin at low pH or due to hydrogen bonding via the unionized amine groups and mucin at higher pH values [14]. However, the mucoadhesive properties of chitosan have also been questioned [15].

Unintended fast drug release is a challenge related to the use of pectin and chitosan alone as mucoadhesive formulations [16]. To promote prolonged drug release in the small intestine, mixtures of chitosan and pectin have been used extensively. Promising results have been achieved for casted mixed pectin/chitosan films

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dissolved in acidic solution [17]. The polymer complex could develop as a consequence of hydrogen bonding between chitosan and pectin in acidic medium [18], or of ionic interactions arising as the pH starts to change when introduced to the neutral dissolution medium [19].

Coating is a convenient way of applying a mucoadhesive polymer to a formulation, and testing free films is generally considered to be useful for early predictions and formulation optimization, which were previously demonstrated for drug release [20] and mechanical properties [21]. Films as patches can also be used *per se* as a formulation for the gastrointestinal tract [22,23], but in that case the introduction of a drug to the mucoadhesive matrix may change the mucoadhesive properties.

The aim of this work was to identify the important factors for the drug permeability and mucoadhesion of casted free films. The free films consisted of either low methoxyl (LM) or high methoxyl (HM) pectin in combination with chitosan at different ratios. Both *in vitro* and *ex vivo* mucoadhesions were investigated. The rationale behind the project was that the cross-linked mixed network consisting of both pectin and chitosan might be tight enough to slow down the drug release, but at the same time that the two mucoadhesive polymers might be flexible and mobile enough to allow for interpenetration with mucin. To our knowledge, this is the first time the mucoadhesive properties of mixed films of chitosan and pectin have been investigated.

2. Materials and methods

2.1. Materials

Mucin from porcine stomach, type II, batch 075K0676, was purchased from Sigma–Aldrich (Schnelldorf, Germany), and used as received.

Chitosan of medium molecular weight (190,000–310,000), Batch 09303PE, 75% deacetylation (information provided by the manufacturer), was purchased from Sigma–Aldrich (Wisconsin, USA) and used as received.

The LM and HM pectins derived from citrus were kindly provided by the manufacturer (Herbstreith & Fox KG, Germany) and purified prior to use. The characteristics of the two types of pectin (purified and characterized by capillary viscometry as described in [10]), are listed in Table 1. Due to the manufacturing process, the molecular weight will decrease with the DM.

All other chemicals used were of analytical grade.

Fresh porcine small intestine was delivered by Fatland Slaughter House, Oslo, on the same day as the experiments were performed.

2.2. Preparation of films used for the swelling and permeability studies

Free films were prepared by casting and evaporation. The solvent was 0.1 M HCl, pH 1.0, and chitosan, LM and HM pectins are soluble at this pH. Solutions with a polymer concentration of 2.0 wt% were made. Thereafter, mixtures were prepared: 75:25, 50:50 and 25:75 of LM/HM pectin:chitosan, yielding a total of six different films. Glycerol was used as plasticizer and was added at an amount of 30% relative to the dry polymer weight. Glycerol has earlier been used to plasticize pectin films [24,25]. An amount of 25 g of the respective polymer mixtures was cast into a glass Petri dish with a diameter of 9.5 cm. Subsequently, evaporation of the solvent was carried out at a temperature of 25 °C at 25% RH for 22 h. In addition, plasticized films consisting of just chitosan, LM or HM pectin were prepared in the same way.

Table 1
The types of pectin classic investigated (*information provided by the manufacturer)

	Pectin classic	
	LM	HM
Brand*	CU 701	CU 201
Batch*	00609007	00701043
DM* (%)	36	70
Galacturonic acid content* (%)	89	86
Intrinsic viscosity (η) (dl/g)	3.4	4.5
Huggińs constant, k'	0.47	0.95

2.3. Preparation of films used for the in vitro and ex vivo mucoadhesion testing

When casting films to be tested for mucoadhesion, some modifications were made to the procedure as described in Section 2.2. (1) In order to be able to distinguish between a cohesive and adhesive failure of the films, the colour Indigo was dispersed in the film solutions at a concentration of 5.0% relative to the dry polymer weight. (2) Films of larger dimensions were made. A total of 400 g of solution with a polymer concentration of 0.75 wt% was cast into polystyrene Nucleon™ Δ Dishes (22.5 cm × 22.5 cm, VWR.com). The amount and concentration were chosen in order to avoid technical problems during manufacturing and at the same time produce films of approximately equal thickness as in Section 2.2.

The films manufactured from both procedures Sections 2.2. and 2.3. were removed from the dish and were cut using a sharp scalpel, after complete drying. The films were stored in a desiccator at room temperature with 22.5% RH for at least 2 days before testing, so that the moisture content was 6–9% as determined gravimetrically by drying at 130 °C until constant weight. The thickness of the polymer films was determined using a micrometer.

2.4. Swelling and erosion

Pieces of film 1.25 cm × 1.25 cm were weighed ($V1$) (Sartorius weight ME235S, Germany, STD 0.025 mg) and immersed in 5.0 ml phosphate buffer, pH 6.8, for 5 min. After removal of excess water, the hydrated films were re-weighed ($V2$). Results are expressed as $V2/V1$, indicating the amount of swelling relative to the original weight. The film pieces were subsequently dried for 22 h at 25 °C at 25% RH, and weighed ($V3$) to determine whether any erosion had taken place. Results are expressed as $V3/V1$, indicating the film weight still remaining after the swelling experiment relative to the original weight. The procedure was repeated 10 times for each system. In addition, the erosion of the mixed films was tested after 24 h in buffer (three replicates).

2.5. Permeability studies

The permeability of dissolved paracetamol through the films was tested as described previously [25], with only small modifications. A test unit was prepared by dissolving 12 mg paracetamol in 8 ml of phosphate buffer, pH 6.8, in a glass vial. Film samples with a thickness of 0.06 ± 0.01 mm and a diameter of 1.9 cm were cut and put on top of the vial. The test unit was sealed with a rubber ring and an aluminium capsule, so that a film area with a diameter of 1.0 cm was exposed to the dissolution media. The test unit was placed in a USP paddle apparatus at 37 °C at 50 rpm in 1.0 L of phosphate buffer, pH 6.8. Samples of 3 ml were drawn every 15 min for spectrophotometric ($\lambda = 243$) determination of the amount of paracetamol released through the films, and circulated back to the dissolution container. Each type of film was tested six times. Results are expressed as K_{app}, representing the slope of the release profile for the first 6 h. K_{app} can be used for comparing

the films to each other, since the thickness of the films, area of exposed film and concentration gradient are similar.

2.6. In vitro mucoadhesion test

Mucin dispersion (3.0 wt%) in phosphate buffer, pH 6.8, was evenly spread on two different filter papers (35 μl on each paper) with an inert backing layer (Watman® Benchkote) of 1.25 cm × 1.25 cm. One of the filter papers 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 other was attached to a specially designed larger, upper, movable part (11 cm × 11 cm). A piece of the free film to be tested (1.25 cm × 1.25 cm, thickness: 0.07 ± 0.01 mm) was placed in between, and the upper, movable part was lowered until it reached contact with the film sample. Based on our previous study [10], 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.

The force measurement was repeated five times for each formulation. In addition, the intermediate precision was tested by repeating the measurements the following day, leading to a total of 10 measurements per formulation. The measurements were also repeated for the filter paper soaked with pure buffer instead of mucin dispersion.

Displacement and force of detachment were recorded. Based on the obtained force versus time curve, peak force (F_{max}, g) and area of work (AUC, g s) were obtained. The values obtained from testing the films versus mucin dispersions are referred to as the general mucoadhesion, and the values obtained from testing films versus buffer are referred to as the unspecific adhesion. To estimate the mucin interaction for the films, the unspecific adhesion of the films was deducted from the general mucoadhesion.

2.7. Ex vivo mucoadhesion test

The friction forces between films and mucous were measured by using a shear test setup. A fresh porcine small intestine was cleaned, opened longitudinally and cut into pieces of 5 cm × 5 cm, and thereafter attached to a specially designed block of 2.0 cm × 2.0 cm, height 1.5 cm and weight 45.7 g, with an elastic rubber band. The mucosal side was facing outwards, and was kept hydrated throughout the experiment. The block was attached to a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, England) with a thread via a pulley, so that raising the stationary part of the texture analyzer would lead to a movement in the horizontal direction for the block. Dry film pieces of 10 cm × 10 cm and thickness of 0.07 ± 0.01 mm were attached to a container placed on the lower stationary part of the texture analyzer by clamps. The film was allowed to swell for 1 min by completely immersing it in an excess of phosphate buffer, pH 6.8. The block with the mucosa was placed on the film, and the film was allowed to swell for another 1 min, this time with part of it covered by the mucous block. Thereafter, the block was moved in the horizontal direction with a speed of 0.5 mm/s, and the friction forces arising between the pre-hydrated film and the mucosa were measured.

The intestines were divided into a total of 12 different intestinal segments. Every type of film was tested one time on each intestinal segment (a total of 12 parallels for each type of film), so that varying properties of the intestinal segment employed would not influence the results. The testing sequence for the films was randomized. The experiments were carried out on five different days using five different intestines.

Displacement and force of detachment were recorded. Based on the obtained force vs. time curve, peak force (F_{max}, g) was obtained. In order to obtain a measure of the average friction force between mucosa and the sliding films, the area under the curve for 20 s starting from 5 s after peak force (AUC_{20}, g s) was calculated. F_{max} and AUC_{20} were adjusted for the varying properties of the intestinal segments by dividing by the sum of F_{max} and AUC_{20}, respectively, for that segment and multiplying by the average sum of F_{max} and AUC_{20}, respectively, for all segments.

2.8. Statistical analysis

The mean and standard errors for all values were calculated. Measurements outside the quartiles ±1.5 times the interquartile range of the repeated measurements were excluded as outliers ($Q1 - 1.5 \times IQR$; $Q3 + 1.5 \times IQR$). Standard errors for differences and quotients were calculated using error propagation theory. For group comparisons a one-way analysis of variance (ANOVA) followed by post hoc Tukey's test (SAS 9.1., SAS Institute Inc., Cary, NC, USA) was applied. Films consisting of the same ratio but different types of pectin, and films consisting of the same type of pectin but different ratios were compared to each other. Whenever differences are discussed or claims made about their relative ranking to each other, the difference was statistically significant ($p < 0.05$), unless otherwise stated.

3. Results and discussion

3.1. Swelling and erosion

The film consisting of merely chitosan as the film forming polymer disintegrated during the swelling experiment, hence no data is presented for this type of film. This indicates that films consisting of this type of chitosan alone are not suitable for obtaining a combination of mucoadhesion and sustained release in the small intestine. The swelling results for the films containing just pectin as the film forming polymer are given in Table 2. The pure LM pectin film swelled about 50% more than the pure HM pectin film. This is probably due to the higher hydrophilicity of LM pectin than to HM pectin as illustrated by the lower Hugginś constant (Table 1).

Hydrogen-bonded interpolymer complexes exhibit properties entirely different from the parent polymers [26]. Hence, they must be regarded as a separate system with its own unique properties, and not as a system with properties representing the relative amount of the individual polymers. The swelling of the films consisting of 75:25 LM/HM pectin:chitosan illustrates this (Table 2), as the swelling is lower than for any of the individual polymers. For the mixed films, the swelling increased with decreasing amount of pectin relative to chitosan in the films for both LM and HM pectins. This is in line with earlier work showing that films consisting of 2:1 LM pectin:chitosan swelled more than films consisting of 3:1 LM pectin:chitosan at pH 7 [19], and that the weight gain of films of 1:2 HM pectin:chitosan is considerably higher than 2:1 HM pectin:chitosan at pH 5.0 [27]. Turbidimetry and viscosity measurements showed that at pH 5.4, the optimal ratio for strong interaction was between 2:1 and 3:1 HM pectin:chitosan [28]. In

Table 2
Swelling presented as *V2*/*V1*, indicating the amount of swelling relative to the original weight (means ± SD)

Ratio pectin:chitosan	25:75	50:50	75:25	100:0
LM pectin	9.0 ± 1.0^{a} $n = 10$	4.3 ± 0.3^{b} $n = 10$	2.7 ± 0.3^{c} $n = 10$	6.9 ± 0.6 $n = 10$
HM pectin	$9.4 \pm 0.6^{\alpha,*}$ $n = 10$	$5.7 \pm 0.5^{\beta}$ $n = 9$	$2.5 \pm 0.3^{\gamma,*}$ $n = 10$	4.4 ± 0.6 $n = 10$

Means with the same letter are not significantly different (ANOVA, post hoc Tukey's test, significant level = 0.05).

* Not significantly different ($p > 0.05$) from the value displayed by the corresponding film of LM pectin.

addition, hydrogen bonds and ionic interaction between pectin and chitosan were confirmed by Fourier transform infrared spectroscopy, wide angle X-ray diffraction and thermogravimetric analysis. This indicates that the amount of pectin relative to chitosan should be high for extensive interactions between pectin and chitosan at these pH values, either for ionic interactions or for intramolecular H-bonding. Oscillatory shear experiments conducted on mixtures of LM pectin and chitosan at pH 1 revealed more extensive interactions between 75:25 pectin:chitosan than between 25:75 pectin:chitosan [29]. Turbidimetry and dynamic light scattering measurements indicated that the interaction between LM pectin and chitosan at pH 1 was more pronounced with high amounts of pectin relative to chitosan [18].

Only the 50:50 pectin:chitosan films showed significant differences between LM and HM pectins when comparing films with the same pectin:chitosan ratio. The film with LM pectin swelled the least. It is the interactions between pectin and chitosan that govern the properties of the mixed films. Since LM pectin is more hydrophilic and hence more likely to swell than HM pectin, the fact that the mixture with LM pectin swelled less than the mixture with HM pectin indicates that the interaction between pectin and chitosan is favoured by a high amount of free carboxylic acid groups. This is expected, whether the interaction is mainly by hydrogen bonding at low pH or ionic interactions occurring as the pH is increased. In both cases free acid groups should be the main contributor. This is also in line with [25] demonstrating that drug permeability was lower through mixed pectin/chitosan films when the pectin type had more free acid groups.

The erosion of all pectin containing films was low: 81–89% of the films remained at the end of the swelling experiments ($V3/V1$: 0.81–0.89). The weight loss might be due to some of the plasticizer leaking out of the films. This has been demonstrated previously [30]. After 24 h of swelling of the mixed films, the lowest $V3/V1$ observed was 0.85, confirming low erosion.

3.2. Permeability studies

Testing of drug release through the films is of utmost importance when the purpose is mucoadhesive formulations for the small intestine. The films with just chitosan as the film forming polymer were not tested for drug release, as the films dissolved very quickly in phosphate buffer, pH 6.8. The films with just pectin as film forming polymers were tested, but the permeability of paracetamol through these films was high: all the drug was released within the first hour for the HM pectin film, and within the first 2.5 h for the LM pectin film. Results are therefore shown only for the mixed films in Table 3.

The low values obtained for K_{app} indicate that there are interactions between chitosan and LM and HM pectins at pH 6.8. Only the curve up to 6 h was investigated, as after this point, the curves were no longer linear, but levelled off due to the decreasing concentration gradient. However, for the first 6 h the linearity was good ($R^2 > 0.99$), indicating zero-order kinetics and swelling of the films followed by diffusion-controlled drug release. As expected from the swelling experiments, K_{app} generally increased with decreasing amount of LM and HM pectins in the films, rendering the 75:25 LM/HM pectin:chitosan films with K_{app} significantly lower than the other types of film. Again this is an indication of the extensive interaction occurring between chitosan and pectin at a high pectin:chitosan ratio. This finding is also in line with [25] reporting lowest drug permeability through films with a high content of pectin to chitosan. Comparing LM and HM pectins, there were no significant differences between films of the same ratio.

3.3. In vitro mucoadhesion test

The area under the peak correlated well ($R^2 = 0.97$) with the peak force for all systems tested, therefore only peak force will be discussed further. The peak force for films versus buffer (unspecific adhesion) and for films versus mucin (general mucoadhesion) for all films can be found in Fig. 1. The film consisting of just chitosan as a film forming polymer experienced a cohesive instead of an adhesive failure, that is easily seen due to the dark blue colour of the films. The values for the chitosan film are therefore included in Fig. 1 only for reference, and will not be discussed any further. All of the other types of films underwent an adhesive failure.

For the mixed films, the unspecific adhesion was higher for the films of HM pectin than for the films of LM pectin, and the unspecific adhesion was higher when the amount of pectin in the films was low. Having in mind the results from the swelling experiments, this indicates that for these non-dissolving films, some swelling is advantageous for adhesion, leading to a viscous surface that will adhere unspecifically. This illustrates the importance of the wetting theory of mucoadhesion [2], where a mucoadhesive should have the ability to swell and spread on the mucus layer.

The measured general mucoadhesion is a function of both the unspecific adhesion and the mucin interaction. Estimated mucin interaction is a complicated concept, influenced both by degree of hydration, the ability to diffuse and engage in entanglements and intramolecular bonding between the polymer and mucin. When assessing the filmś mucin interaction, the unspecific adhesion of films (obtained from testing film versus buffer) must be deducted from the general mucoadhesion (from testing films versus mucin). As can be seen from Fig. 1, in all cases the measured general mucoadhesion was higher than the measured unspecific adhesion, demonstrating interactions between the polymer systems involving pectin and mucin.

In this study, the differences between films of LM and HM pectins were only marginal. Therefore, neither the superior ability of LM pectin to engage in hydrogen bonding with mucin, compared to HM pectin reported earlier [10], nor the reported increase in values for parameters indicating mucoadhesion with increase in DM [11,12] could be confirmed in this experiment. However, the film consisting of 100% LM pectin as film forming substance displayed significantly higher mucin interaction than the corresponding HM pectin film ($p < 0.05$), when only these two types of films were compared to each other. Comparing the mixed films to each other, the overall highest mucin interaction in this experiment was detected for a film containing LM pectin, namely, 25:75 LM pectin:chitosan. However, this value was not significantly different

Table 3
Drug permeation through mixed films: K_{app} for the first 6 h (means ± SD)

Ratio pectin:chitosan	LM pectin			HM pectin		
	25:75	50:50	75:25	25:75	50:50	75:25
K_{app} (mg/h)	0.44 ± 0.05^{a} $n = 6$	0.51 ± 0.06^{a} $n = 5$	0.35 ± 0.04^{b} $n = 6$	$0.51 \pm 0.07^{\alpha^*}$ $n = 6$	$0.48 \pm 0.07^{\alpha^*}$ $n = 5$	$0.37 \pm 0.06^{\beta^*}$ $n = 5$

Means with the same letter are not significantly different (ANOVA, post hoc Tukey's test, significant level = 0.05).
* Not significantly different ($p > 0.05$) from the value displayed by the corresponding film of LM pectin.

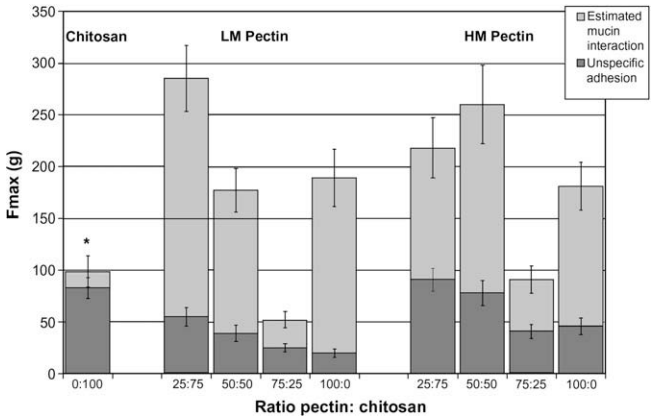

Fig. 1. The peak force obtained from the *in vitro* tensile tests for the films versus buffer (unspecific adhesion) and the estimated mucin interaction. The total height of the columns is the measured general mucoadhesion (films versus mucin). Results are expressed as the mean with the bar showing SD ($n = 10$). *Cohesive failure.

from the second highest value ($p = 0.082$) that is displayed by the 50:50 HM pectin:chitosan film.

Generally, an increased pectin:chitosan ratio reduced the mucin interaction. As can be seen from Table 2, films of low pectin:chitosan ratio also swelled more than the films of high ratio. This indicates that swelling and polymer mobility are important prerequisites for mucin interaction. Extensive swelling can enable entanglements between the polymers and mucin, and expose the chemical entities able to engage in secondary interactions, such as hydrogen bonding. The exception to the general correlation was the 25:75 HM pectin:chitosan film. Mixed films containing HM pectin displayed a maximum of mucin interaction at the ratio of 50:50 pectin:chitosan, and further lowering the amount of pectin led to lower values of mucin interaction. From the swelling data and the literature discussed in 3.1., the 25:75 HM pectin:chitosan film is probably the least cohesive film. It is well known that swelling and hydration favours mucoadhesion up to a certain point, after which the cohesion is compromised. This has been thoroughly discussed earlier [31], and is probably the explanation for the optimum observed when decreasing the ratio of HM pectin:chitosan.

The general adhesion is affected by both the unspecific adhesion and mucin interaction. Therefore, differences in adhesion between LM and HM pectins indicated for mucin interaction were further reduced. The effect of varying the pectin:chitosan ratio was, however, different for the two types of pectin. For the mixed films with LM pectin, there was a general increase in peak force upon decreasing the amount of pectin relative to chitosan. For the mixed films with HM pectin, no further increase was observed when decreasing the pectin:chitosan ratio from 50:50 to 25:75. The explanation for this has already been discussed in relation to mucin interaction: high flexibility and mobility of the polymer chains in the films are advantageous for mucoadhesion, but only to some extent, after which the film cohesion is possibly compromised.

3.4. Ex vivo mucoadhesion test

Films consisting of only one film forming polymer were not tested for *ex vivo* mucoadhesion due to high water solubility and high drug permeability (see Sections 3.1. and 3.2). Their use as mucoadhesive dosage forms for sustained drug delivery in the small intestine is therefore not realistic. Consequently, only the mixed films were tested.

Mucoadhesion is usually tested using a tensile test setup for measuring the detachment force. The use of a shear test setup for measuring the friction forces between a formulation and a mucosa is uncommon, even though this setup is regarded as more realistic with respect to the processes going on during an *in vivo* situation [2,32]. Generally, smaller forces are expected to be measured for this setup compared to the usual tensile tests [32]. Among the work using this procedure [2,32–35], dry formulations have been tested with limited amounts of fluid present. In this work we propose a method for testing the friction forces of pre-hydrated films completely immersed in water. This is probably a more realistic setup for an *in vivo* situation, as it is unlikely that the formulations will be able to reach the mucosa of the small intestine in a dry form. In addition, the suggested setup will probably lead to poorer conditions for mucoadhesion, due to the elimination of adhesion as a consequence of the formulation extracting water from a hydrated mucosa. Differences between the films, which might otherwise be camouflaged by the dominating hydration processes, could then hopefully be detected.

All types of films showed an adhesive failure, as the blue film was visually observed as intact during the whole experiment. The curves obtained for the different mixed films were investigated and characterized. Representative examples of the six different mixed films can be found in Fig. 2a and b. Generally, for the 75:25 LM/HM pectin:chitosan films, the measured friction forces reach a plateau level without a detectable peak force. For the 50:50 LM/HM pectin:chitosan films, a well-defined peak force could be detected followed by a plateau level of friction force. The 25:75 LM/HM pectin:chitosan films generally displayed more complex curves. A well-defined peak occurred, but the forces did not drop to a steady plateau level. The peaks were generally broad, and the force tended to increase again after a drop from the observed main peak (see Fig. 2a and b). This could indicate that forces were developed during the shear test for the 25:75 LM/HM pec-

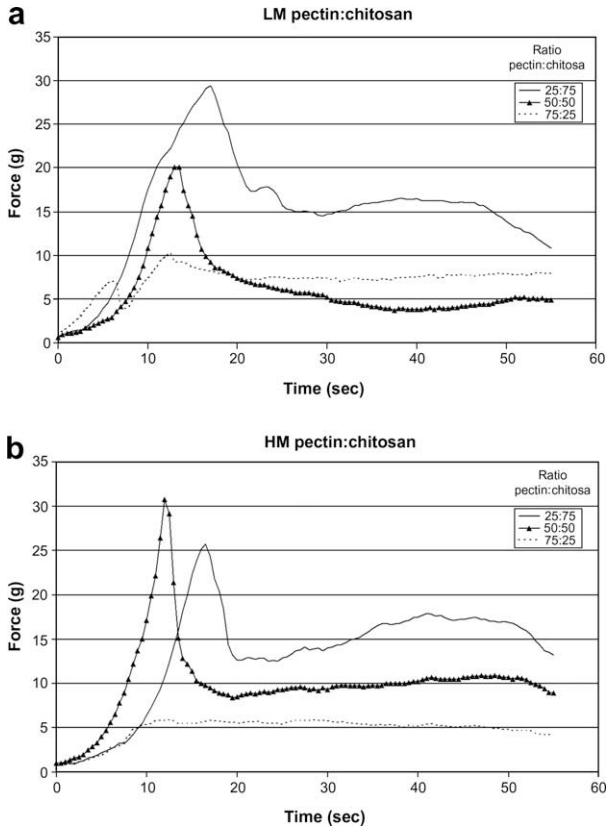

Fig. 2. Force versus times curves for representative examples of the different mixed pectin/chitosan films. (a) LM pectin:chitosan and (b) HM pectin:chitosan.

Table 4
Peak force (F_{max}, g) and area under the curve for 20 s starting from 5 s after peak force (AUC_{20}, g s) obtained from the *ex vivo* shear tests measuring the friction forces (means ± SD)

Ratio pectin:chitosan	LM pectin			HM pectin		
	25:75	50:50	75:25	25:75	50:50	75:25
F_{max} (g)	27.2 ± 6.1^{a} $n = 10$	18.6 ± 4.0^{b} $n = 10$	9.3 ± 2.4^{c} $n = 10$	$24.7 \pm 4.3^{\alpha *}$ $n = 10$	$27.5 \pm 4.8^{\alpha}$ $n = 10$	$6.8 \pm 2.5^{\beta *}$ $n = 10$
AUC_{20} (g s)	274 ± 59^{a} $n = 12$	121 ± 35^{b} $n = 10$	125 ± 29^{b} $n = 9$	$298 \pm 45^{\alpha *}$ $n = 12$	$190 \pm 22^{\beta}$ $n = 10$	$116 \pm 46^{\gamma *}$ $n = 9$

Means with the same letter are not significantly different (ANOVA, post hoc Tukey's test, significant level = 0.05).
* Not significantly different ($p > 0.05$) from the value displayed by the corresponding film of LM pectin.

tin:chitosan films, and consequently that the interaction with the mucosa was more extensive than for the other films.

The peak force and AUC_{20} for the different mixed films are summarized in Table 4. The peak force and AUC_{20} did not correlate, which is not surprising due to the different curve shapes obtained as discussed above.

The peak force from the *ex vivo* measurements correlated with the peak force of general mucoadhesion measured in the *in vitro* measurements (Fig. 3). The overall R^2 was 0.93. For the LM pectin:chitosan films evaluated separately, the R^2 was 1.00 and for the HM pectin:chitosan films the R^2 was 0.99. This indicates that the simple *in vitro* method is capable of giving good estimates of the peak forces developing under the more complex *ex vivo* conditions, and therefore proves to be a useful method for providing additional information about the mucoadhesive mechanisms involved.

In a recent article, the detachment force of various non-pre-hydrated chitosan/PVP films versus a sheep buccal mucosa immersed in buffer was related to the *ex vivo* mucoadhesion time at 50 rpm stirring rate [36]. Relating the data obtained for the mixed pectin/chitosan films to the correlation obtained for the chitosan/PVP films, a residence time of about 7 h could be expected for the pectin/chitosan films with the highest detachment force, and a residence time of about 3 h for the least adhesive. The *in vivo* relevance of these estimations is, however, unclear, and needs verification, but the estimates might give some indication of the mucoadhesive potential. The small intestine is about 6 m long, and since the films did not erode in 24 h (Section 3.1.), the possibility of film detachment followed by re-attachment also exists.

The area under the curve (AUC_{20}), representing the average friction forces of a sliding film, is perhaps the most important parameter with respect to an *in vivo* situation, as it is unrealistic that a substance will have time to interact with the mucosa in the small intestine without being subjected to shear forces. As may be seen from Table 4, AUC_{20} generally increased with decreasing amount of pectin relative to chitosan. Significant differences between LM and HM pectins could be detected only at the ratio of 50:50 pectin:chitosan. The AUC_{20} was highest for the HM pectin film. Higher values for HM pectin compared to LM pectin are in line with the work reported for diffusion coefficients of mucin [11] and rheological synergy with mucin [12]. However, as the AUC_{20} correlated with the swelling of the films (Fig. 4) with an R^2 of 0.96, this probably again indicates the importance of swelling and polymer flexibility and mobility for interdiffusion to occur. In this case the formulation will have very limited time to spread to and interact with the mucosa, as the shear forces are applied continuously. This will probably further accentuate the importance of flexible polymer chains. The fact that the 25:75 HM pectin:chitosan film displayed higher mucoadhesion friction forces than the corresponding 50:50 film, an effect not detected for the other mucoadhesion parameters, indicates this. The favourable influence of polymer flexibility on mucoadhesion has been demonstrated earlier for chemically cross-linked cast films compared to uncross-linked films [37], hydrogels tethered with PEG chains [38] and 3-arm star polymers [39].

In conclusion, our study has shown that flexibility of the polymer chains in a formulation is the dominating factor for both *ex vivo* mucoadhesion and drug release. When incorporated into a formulation, the intrinsic properties of the polymers, for example, the ability to engage in selective bonding with mucin, will consequently be of less importance than the overall swelling properties of the formulation.

The unfortunate correlation between the mucoadhesion and drug release was confirmed in this study, in that films with a low amount of pectin relative to chitosan generally swelled more and displayed higher drug permeability and mucoadhesive forces than

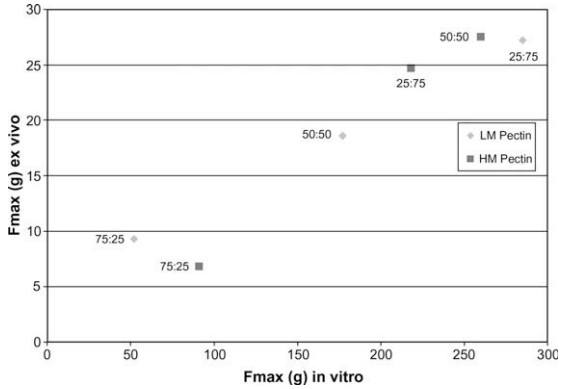

Fig. 3. The peak force obtained in the *ex vivo* experiments correlated with the measured general mucoadhesion peak force obtained from the *in vitro* experiments (the overall R^2 was 0.93). For the LM pectin:chitosan films the R^2 was 1.00 and for the HM pectin:chitosan films the R^2 was 0.99. The ratio expressed in the figure refers to the ratio of pectin:chitosan in the films.

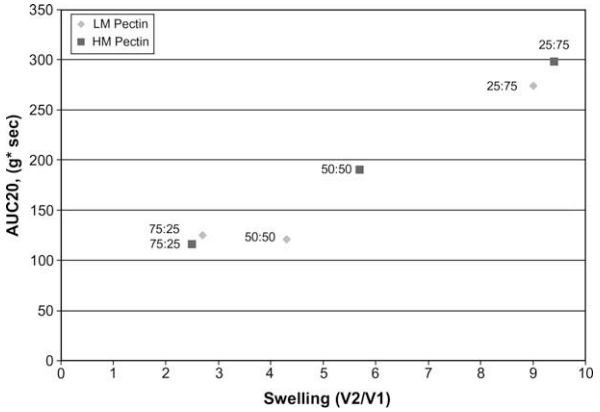

Fig. 4. The area under the curve for 20 s starting 5 s after the peak force (AUC_{20}, g s) obtained from the *ex vivo* experiments correlated with the swelling of the films (*V2*/*V1*), $R^2 = 0.96$. The ratio expressed in the figure refers to the ratio of pectin:chitosan in the films.

films with a higher pectin to chitosan ratio. The fact that the mucoadhesion tended to increase with increased swelling of the mixed films is a problem in obtaining a sustained release. According to our findings, a general guidance may be proposed: For drug molecules with a high permeation, high ratios of pectin:chitosan should be chosen. However, for drug molecules with a lower permeation, the ratio could be decreased to achieve better mucoadhesion. Among the ratios investigated in this study, the ratio of 50:50 could not be recommended. The reason is lower AUC_{20} compared to 25:75 pectin:chitosan, without a corresponding significant decrease in drug permeability of the films.

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