Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 347 (2008) 9–15
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

# *Ex vivo* mucoadhesion of different zinc-pectinate hydrogel beads

Ellen Hagesaether [a,*], Ragnar Bye [b], S. Arne Sande [a]

[a] *Department of Pharmacy, School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway*
[b] *Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway*

Received 3 May 2007; received in revised form 8 June 2007; accepted 13 June 2007
Available online 26 June 2007

**Abstract**

The objective of this study was to investigate the mucoadhesive properties of pre-swelled hydrogel beads made of six types of pectin from three manufacturers. The types of pectin differed mainly in the degree of methoxylation and degree of amidation. Zinc ions were used as cross-linking agent. The mucoadhesive properties were tested on an inverted fresh porcine small intestine attached to a rotating cylinder. Beads made of pectin with a high degree of methoxylation (70%) showed superior mucoadhesive results compared to the other formulations, which could be correlated to the lower amount of zinc in this formulation, subsequently leading to a lower amount of cross-linking and higher mobility of the polymer chains of these beads. This study therefore also indicated the importance of doing mucoadhesive measurements on relevant formulations, and not basing the understanding solely on investigating polymer solutions. Samples from different manufacturers produced the same results.


*Keywords:* Mucoadhesion; Pectin; Gel beads; Crosslinking; Zinc

## 1. Introduction

Achieving mucoadhesion of a formulation in the gastrointestinal tract could lead to a higher bioavailability of the entrapped drug due to increased residence time and a closer contact between the absorptive membrane and the formulation (Yin et al., 2006). 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.

The dominant feature of pectin ((1 → 4) α-D-galacturonic acid units) is shown in Fig. 1, indicating the different types of substitution investigated in this article. In a previous study, we showed that only pectin with a degree of methoxylation (DM) of about 35% showed a selective interaction with mucin (probably through hydrogen bonding), whereas the general (unspecific) adhesion of pectin generally increased with increasing degree of total substitution (Hagesaether and Sande, 2007). This work was conducted on pectin solutions versus a mucin dispersion using a tensile test. Work on free films confirmed the selective interaction of pectins with a high ability to engage in hydrogen bonds (Hagesaether and Sande, submitted for publication). But, in contrast to the findings on solutions, the general adhesion was higher for the films of pectin with a DM of about 35%. This was considered to be caused by the higher cohesion of this film. This indicated that the properties of the formulation influenced the mucoadhesive properties, and is in line with (Koffi et al., 2006), showing a correlation between the viscoelastic properties of gels and their bioadhesiveness. In addition, as pure pectin formulations dissolve too rapidly, it is important for realistic pectin formulations that the solubility of the polymer in water is reduced, by cross-linking.

Particulate systems are generally considered to be advantageous for mucoadhesive formulations, and small sized particles are often preferred, as the size enables them to make intimate contact with a larger mucosal surface area (Sudhakar et al., 2006).

It is well known that the experimental set-up could influence on the results obtained from testing of mucoadhesion (Sandri et al., 2005), and a method mimicking the *in vivo* situation should therefore be employed.

The aim of this study was consequently to test the mucoadhesive properties of relevant particulate pectin formulations using a set-up mimicking the environment in the small intestine. The pectin types chosen differ i.a. in functional groups and consequently the molecular weight and viscosity. All of these properties are known to affect the mucoadhesion (Dodou et al., 2005).

* Corresponding author. Tel.: +47 22 85 79 05; fax: +47 22 85 44 02.
*E-mail address:* ellen.hagesather@farmasi.uio.no (E. Hagesaether).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.06.034

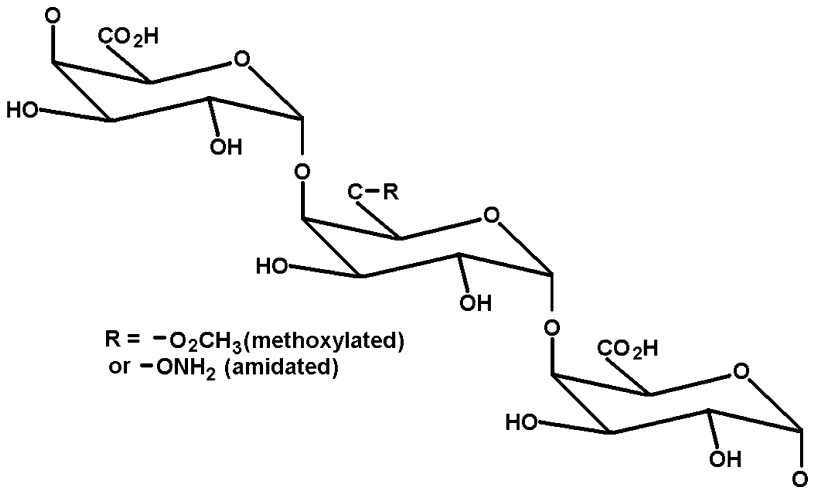


Fig. 1. A schematic illustration of the dominant feature of pectin.

Pectin types were obtained from several manufacturers in order to investigate if products from different manufacturers display varying 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 possessing excellent mucoadhesive properties (Duchêne et al., 1988).

## 2. Materials and methods

### 2.1. Materials

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

Six types of citrus pectin (purified and characterized by capillary viscometry (Hagesaether and Sande, 2007)), listed in Table 1, were studied. A fractional factorial design was used. The factors varied among the pectin types were: DM (two levels; high and low), degree of amidation (DA) (two levels; amidated or non-amidated) and manufacturer (three levels). Due to the manufacturing process, the molecular weight will be correlated with the DM.

All other chemicals used were of analytical grade.

Fresh porcine small intestine was delivered by Fatland slaughter house, Oslo, the same day as the experiments were performed.

### 2.2. Manufacturing of hydrogel beads

A standard ionotropic gelation technique was used for the manufacturing of the hydrogel beads. 3.0 wt.% polymer solutions were added dropwise, using a constant force, with a disposable syringe (a nozzle of 1.0 mm inner diameter) to a 200 ml of a gently agitated solution of the crosslinking agent (1.0 wt.% $ZnCl_2$). The falling distance was 5 cm. The gelled particles thus formed were allowed to remain in the crosslinking solution for 30 min. The particles were subsequently washed twice for 10 min in purified water, in order to remove $Cl^-$- and excess of $Zn^{2+}$ ions. The particles were dried for 20 h at room temperature and stored at a constant relative humidity of 22.5% at room temperature.

### 2.3. Characterization

#### 2.3.1. Weight, size and shape characteristics of hydrogel beads

Ten individual hydrogel beads of each formulation were weighed on a Sartorius weight (ME235S, Germany) with a reproducibility of 0.024 mg (standard deviation), leading to an

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

| | Pectin classic | Genu® pectin | | Grindsted® pectin | | Pectin classic |
|---|---|---|---|---|---|---|
| | CU 701 | X-920-02 | X-917-02 | LA 410 | RS 400 | CU 201 |
| Batch* | 00501087 | BA-2005-28 | 4450099 | 4010175812 | 0412744 | 00412079 |
| DM* | Low (35%) | Low (36%) | Low (31%) | Low (27%) | High (70%) | High (70%) |
| DA* | No (0%) | No (0%) | Yes (19%) | Yes (21%) | No (0%) | No (0%) |
| Total substitution* (%) | 35 | 36 | 50 | 48 | 70 | 70 |
| Manufacturer* | Herbstreith & Fox KG, Germany | CPKelco, Denmark | CPKelco, Denmark | Dansico, Denmark | Dansico, Czech Republic | Herbstreith & Fox KG, Germany |
| Intrinsic viscosity | 3.8 | 3.5 | 3.9 | 4.3 | 5.1 | 5.4 |
| Huggins′ constant | 0.23 | 0.44 | 0.42 | 0.63 | 0.76 | 0.85 |

approximate relative standard deviation of 3.5%. Ten individual hydrogel beads of each formulation were characterized according to projected equivalent diameter (the diameter of a circle with the equivalent area) and aspect ratio (the longest Feret's diameter (length)/the shortest Feret's diameter (width)) using an image analysis system (Leica Q500MC, Qwin, Cambridge, UK). One pixel corresponds to 54 μm. The height (perpendicular to the projection) was tested using a TA-XT2i texture analyzer (stable micro systems, Surrey, UK) in a "measure force in compression" mode.

#### 2.3.2. Moisture content

The moisture content of ten individual particles of each type was determined gravimetrically by drying at 130 °C until constant weight.

#### 2.3.3. Swelling

Ten individual particles of each type were swelled in 20 ml of phosphate buffer pH 6.8 for 10 min. Thereafter, the weight and projected equivalent diameter were measured as described in Section 2.3.1. The height after swelling was not tested, as the texture analyzer was unsuitable for the purpose.

#### 2.3.4. SEM-micrographs of the hydrogel beads

The beads were mounted on aluminium stubs using double-sided sticky tape sputter-coated with Au/Pd in combination 60/40 (Polaron E500 Sputter-coater, UK) and examined using scanning electron microscope (JMS-6400 SEM, JEOL, Japan).

#### 2.3.5. Determination of zinc content in the beads

Ten individual beads of each formulation were weighed and dissolved in nitric acid 65% at approximately 70 °C, and the amount of zinc released was determined by atomic absorption spectroscopy (AAS). The procedure was repeated with beads allowed to swell in 20 ml of phosphate buffer pH 6.8 for 10 min, before dissolution.

### 2.4. Mucoadhesion

Mucoadhesion was tested using a modified version of the rotating cylinder method (Grabovac et al., 2005). A fresh porcine small intestine was cleaned, cut into pieces of 7 cm, inverted and threaded on a cylinder (diameter: 2 cm). 25 hydrogel beads of the same formulation were allowed to swell for 10 min in 50 ml of phosphate buffer pH 6.8 and subsequently placed gently onto the mucosa without application of any force within 2 min. The cylinder with the mucosa was placed in a chamber containing 500 ml of phosphate buffer pH 6.8 at 37 °C, and rotated with 300 rpm for 10 min. The amount of beads remaining attached to the intestine was counted, and as a control, the amount that had fallen off. 11 parallels of each bead formulation were tested and one parallel of each bead formulation were tested on every intestine (a total of 11 intestines), so that varying properties of the intestines employed would not influence the results. The testing sequence for the formulations was randomized.

The means and standard errors for all values were calculated. 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. A difference was considered to be statistically significant when $p < 0.05$.

## 3. Results and discussion

### 3.1. Manufacturing of hydrogel beads

The acid groups of pectin are known to engage in coordination bonds with divalent cations forming the well known egg-box structure. Stability is improved when there are at least seven consecutive carboxyl groups on each participating chain. Pectin with a low DM will consequently be expected to show a more extensive bonding. Amidation of pectin is known to increase the sensitivity towards gelation by calcium (Sande, 2005), and reduced diameter has been observed for amidated pectin beads (Bourgeois et al., 2006). This observation was explained by reduced hydrophilicity and internal hydrogen bonding between the amide groups, leading to the formation of a more compact network. Calcium-ions are commonly used for these systems, however, pectin with a DM as high as 70% will not form a gel with calcium-ions. (El-Gibaly, 2002) has reported a lower drug release from Zn-pectinate gel microparticles compared to calcium pectinate beads. According to (Pillay et al., 2005), this observation is due to the different crystal structures and coordination numbers of Zn and Ca. Consequently zinc ions were used as cross-linking agent, and indeed, hydrogel beads were formed from all pectin qualities. To our knowledge, this is the first time manufacturing of hydrogel beads with pectin DM 70% has been reported.

### 3.2. Characterization of hydrogel beads

The moisture content of the beads varied from 5–9% (data not shown), and no correlation could be observed between type of pectin and moisture content. The weight, projected equivalent diameter and height of the different beads are shown in Table 2. The beads made of alginate have a slightly lower weight than the other types (about 0.63 mg). The beads made of the different types of pectin had a comparable size and the weight was about 0.66–0.68 mg. The lower diameter for beads made of amidated pectin reported in (Bourgeois et al., 2006), was not detected. The aspect ratio for the tested beads was $1.19 \pm 0.08$ indicating acceptable roundness. However, the height (0.62–0.82 mm) was somewhat lower than the equivalent diameter (1.07–1.20 mm), indicating that some deformation occurred in the vertical direction during the drying process.

As the surface roughness could be a factor influencing the mucoadhesive properties, magnified and SEM-pictures of the beads were taken (Fig. 2). There were no obvious differences between the formulations, neither with respect to polymer type nor manufacturer. All the beads appeared smooth on the surface, with no pores or cracks.

The increase of weight and size of the beads after swelling are presented in Table 2. As can be seen, there are pronounced differences between the beads. The increase of weight for beads

Table 2
Weight and size of hydrogel beads before- and after swelling (results are expressed as the mean ± S.D., $n = 10$)

| | Pectin classic, DM 35% | Genu® pectin, DM 36% | Genu® pectin, DM 31%, amidated | Grindsted®, DM 27%, amidated | Grindsted®, DM 70% | Pectin classic, DM 70% | Alginic acid |
|---|---|---|---|---|---|---|---|
| Weight (mg) | 0.68 ± 0.02 | 0.68 ± 0.06 | 0.67 ± 0.01 | 0.66 ± 0.02 | 0.66 ± 0.04 | 0.68 ± 0.02 | 0.63 ± 0.02 |
| Equivalent diameter (mm) | 1.20 ± 0.02 | 1.12 ± 0.08 | 1.18 ± 0.02 | 1.10 ± 0.01 | 1.11 ± 0.05 | 1.18 ± 0.04 | 1.07 ± 0.05 |
| Height (mm) | 0.72 ± 0.04 | 0.62 ± 0.07 | 0.70 ± 0.03 | 0.68 ± 0.01 | 0.82 ± 0.03 | 0.65 ± 0.04 | 0.70 ± 0.12 |
| Average percent weight gain of 10 individual beads after swelling | | | | | | | |
| | 76 ± 10 | 63 ± 24 | 67 ± 14 | 64 ± 16 | 253 ± 40 | 235 ± 36 | 100 ± 23 |
| Average percent increase in equivalent diameter of 10 individual beads after swelling (%) | | | | | | | |
| | 26 ± 4 | 31 ± 12 | 26 ± 3 | 34 ± 6 | 69 ± 6 | 63 ± 7 | 37 ± 7 |

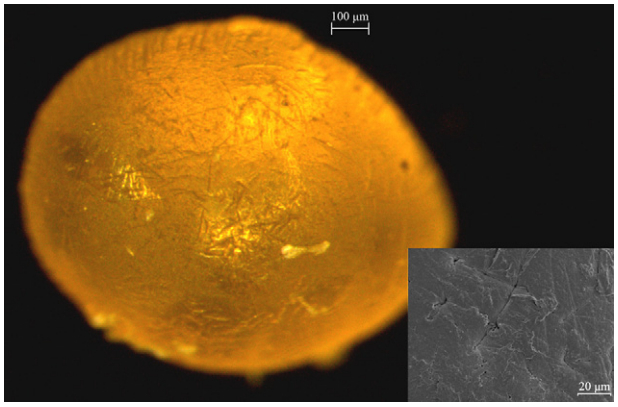


Fig. 2. Magnified and SEM-picture of a representative hydrogel bead consisting of Genu® pectin, DM 31%, DA 19%.

of pectins with DM 35 and 36% as well as the amidated pectins varied from 63–76%, whereas the weight increase for beads made of pectin DM 70% was 235 and 253% (Grindsted® and pectin classic, respectively). The increase of weight for the beads made of alginate was 100%. Despite the fact that pectin with a high DM is more hydrophobic, the beads made of this type of pectin swelled to a larger extent than the other types. It is reasonable to assume that this is due to the weaker cross-linking between pectin DM 70% and zinc.

The results from the AAS-analysis are presented in Table 3. Beads of pectin DM 70% had the lowest amount of zinc (4.3 and 5.1 wt.% for Grindsted® and classic pectin, respectively) followed by beads made of amidated pectin (6.8 and 6.9 wt.% for Genu® and Grindsted® pectin, respectively) closely followed by beads made of pectin DM 35 and 36% (7.7 and 8.3 wt.% for classic pectin and Genu® pectin, respectively). Beads made of alginate had the highest amount of zinc (13.4 wt.%). This is probably related to the amount of unsubstituted acid groups. Only beads made of pectin DM 70% had a significantly lower amount of zinc after swelling for both types (a decrease of 14 ± 5% and 18 ± 7% for Grindsted® and classic pectin, respectively). This again indicates a weaker cross-linking for this type of pectin.

### 3.3. Mucoadhesion

In order to avoid false positive results, the method used was intentionally designed to provide poor conditions for mucoadhesion. This was done by swelling the beads prior to carrying out the experiments, placing the beads on the mucosa without using any force, carrying out the experiments with a large amount of water present and using a rotational speed as high as 300 rpm.

The use of fully hydrated dosage forms when testing mucoadhesion, is uncommon. As stated above, this will probably lead to poorer conditions for mucoadhesion. This is due to the elimination of adhesion as a consequence of the formulation extracting water from a hydrated mucosa. Differences between the pectin types that might otherwise be camouflaged by the dominating hydration processes could then hopefully be detected. In addition, 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.

Results showing percent of beads still adhering to the intestine after 10 min are shown in Fig. 3. For beads made of pectin DM ~35% and amidated pectin, the results showed only insignificant differences between similar pectin types from different manufacturers. For beads made of pectin DM 70%, the classic pectin from Herbstreith & Fox KG showed significantly higher mucoadhesion (86% adherence) than Grindsted® pectin from Danisco (79% adherence).

The mucoadhesiveness was significantly different for all three types of pectin. The mucoadhesiveness was highest for Grindsted® and classic pectin both DM 70%, followed by the amidated pectins Genu® and Grindsted® pectin (52% of adhering beads for both types) while classic pectin DM 35% and Genu® pectin DM 36% showed poorest results (35 and 24% still adhering, respectively). The alginate beads performed on

Table 3
Amount of zinc before- and after swelling (results are expressed as the mean ± S.D., $n = 10$)

| | Pectin Classic, DM 35% | Genu® pectin, DM 36% | Genu® pectin, DM 31%, amidated | Grindsted®, DM 27%, amidated | Grindsted®, DM 70% | Pectin classic, DM 70% | Alginic acid |
|---|---|---|---|---|---|---|---|
| Zn (wt.%) | 7.7 ± 0.4 | 8.3 ± 0.4 | 6.8 ± 0.5 | 6.9 ± 0.5 | 4.3 ± 0.2 | 5.1 ± 0.3 | 13.4 ± 0.9 |
| Zn (wt.%) swelled | 7.5 ± 0.4 | 7.7 ± 0.4 | 6.5 ± 0.3 | 7.2 ± 0.4 | 3.7 ± 0.0 | 4.2 ± 0.2 | 13.8 ± 0.5 |

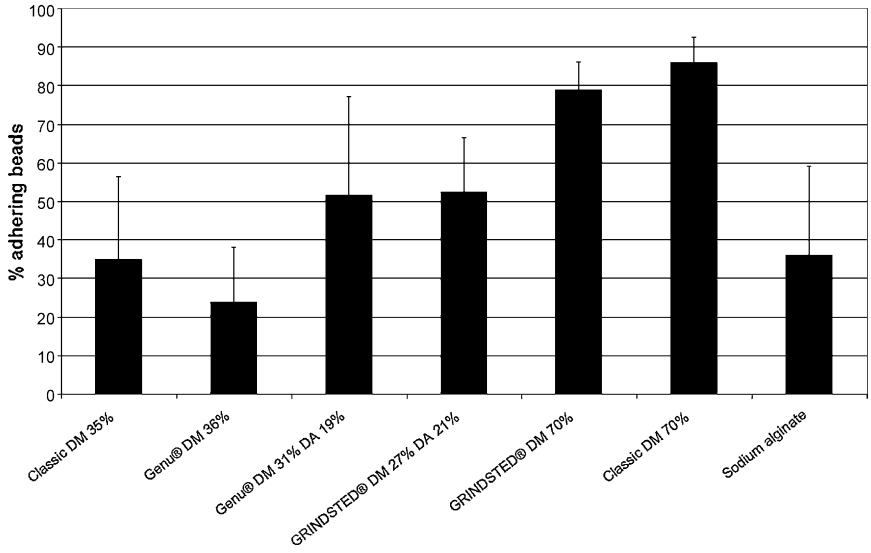


Fig. 3. Percent adhering beads. Results are expressed as the mean with the bar showing S.D. ($n = 11$).

the average comparable to the pectin DM ∼35% beads (36% adherence), but due to a large variance, their adhesion was not significantly different from neither pectin DM ∼35% beads nor amidated beads.

In this experimental setup, debonding between the bead and intestine may occur via three different modes of failure: (1) a failure within the intestine. (2) a mucoadhesive failure between the mucosa and formulation/bead. (3) a cohesive failure within the formulation. The beads were rather cohesive, in addition a higher amount of zinc should lead to higher cohesion. As the most cohesive beads (beads with the highest amount of zinc) displayed the lowest mucoadhesion, it is unlikely that the fracture occurred within the formulation. The intestine showed some shedding, which could indicate a mucosal failure. But if this was the primary mechanism of failure, no differences between the formulations should be observed. Thus, the results indicate that a mucoadhesive failure did occur.

Different theories or mechanisms for mucoadhesion exist (Dodou et al., 2005). As mucoadhesion occurs as a combined result of all of these theories, no single theory gives a complete description of the process. It is well accepted that the first stage of mucoadhesion can be related to wetting leading to intimate contact between the two substances. Then the molecules can diffuse, leading to interpenetration and entanglements (diffusion theory). Following this process, secondary chemical bonds like electrostatic attractions (electronic theory) and/or other attractions resulting from hydrophobic interactions, hydrogen or van der Waals bonds could occur (adsorption theory). Hydrogen bonding is especially often used to explain mucoadhesion (Saiano et al., 2002; Mortazavi, 2003).

In this experiment, the beads were fully hydrated before the study started. In addition, a large amount of water was present. It is therefore unlikely that the formulations extracted water from the mucosa thereby leading to attractions as a result of wetting. Having in mind the results from the testing of polymer solutions (Hagesaether and Sande, 2007), showing that only pectin DM 35 and 36% selectively bonded with mucin, it is also unlikely that the electronic or adsorption theory is relevant for explaining the strong mucoadhesion of pectin DM 70% beads. The diffusion theory is then probably the most important theory for explaining the results from this experiment.

The mucoadhesive properties of the pectin beads can be related to the amount of zinc in the beads after swelling, as illustrated in Fig. 4. Swelling and AAS-analysis revealed that the formulations of pectin DM 70% had the lowest amount of zinc and the weakest zinc interaction. Formulations of pectin DM 70% also showed the highest mucoadhesion. For the formulations of pectin DM ∼ 35%, the situation was vice versa; these formulations had the highest amount of zinc and the lowest mucoadhesion. Cross-linking pectin with zinc will probably reduce the polymer mobility, and thereby impede their ability to diffuse and interpenetrate the mucin molecules.

This finding is in line with other work showing that cross-linking a polymer will disturb its mucoadhesive properties. This was shown for films based on blends of poly(acrylic acid) and (hydroxypropyl)cellulose by (Dubolazov et al., 2006) and for alginate/chitosan microparticles by (Wittaya-areekul et al., 2006). (Goto et al., 2006) working on microparticles of poly(methacrylic acid-grafted-ethylene glycol) and (Limer et al., 2006) synthesising star polymers of dimethylaminoethyl methacrylate, showed that an increased flexibility of polymer chains will lead to better mucoadhesive properties, even though the theory has been questioned by (Lehr et al., 1992).

The high selective interaction of pectin DM ∼ 35% with mucin, as observed for pectin solutions (Hagesaether and Sande, 2007), was hence not recognised as an important factor for explaining the results for hydrogel beads. Measured mucoadhesion of hydrogel beads was on the other hand mainly explained by degree of cross-linking. This study therefore indicates the importance of doing mucoadhesive measurements on relevant formulations, and not basing the understanding solely on investigating polymer solutions.

A high molecular weight is generally considered favourable for mucoadhesion. A high molecular weight could hypotheti-

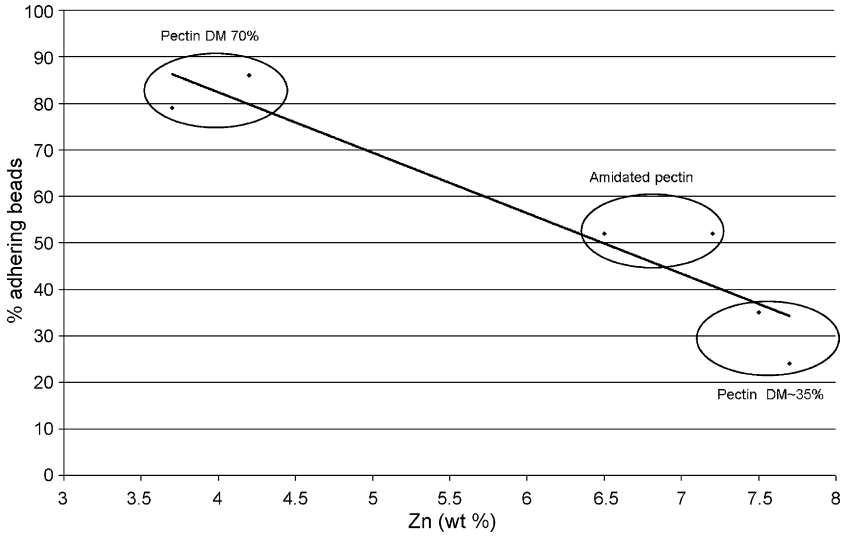


Fig. 4. Percent adhering pectin beads and the amount of Zn in the beads after swelling.

cally lead to a deeper penetration of the mucoadhesive polymer, but at the same time, the flexibility of the chains will be reduced (Dodou et al., 2005). The molecular weight of pectin will decrease with decreasing DM, since it is known that the manufacturing process used to manufacture low DM pectin from high DM pectin also invoke hydrolysis of the pectin backbone. The high molecular weigh of the pectin types with a DM of 70% could therefore be an additional factor favouring mucoadhesion.

When placing the beads on the mucosa, they were already swelled and therefore of different sizes (Table 2). The beads of pectin DM 70% weighed about twice as much as the other beads with a corresponding increase in the feret diameters. When developing mucoadhesive formulations, small sized particles are often preferred enabling them to make intimate contact with a larger mucosal surface area (Sudhakar et al., 2006). This was further investigated by (Goto et al., 2006), who showed that smaller sized poly(methacrylic acid-grafted-ethylene glycol) microparticles displayed strongest mucoadhesive capacities, which was explained by easier and deeper interpenetration into the mucous layers. With particles in the mm range deep interpenetration into the mucous layer is, however, an unlikely mechanism. On the contrary, the larger size of the pectin DM 70% beads will provide them with a higher area available for interaction, possibly promoting adhesion. On the other hand, they will also have a higher area for friction forces against the aqueous surroundings and a larger weight which should lead to a lower adhesion. It is therefore unclear how the larger size of the pectin DM 70% beads would affect mucoadhesion. However, the pronounced increase in mucoadhesive properties for amidated beads over beads of DM 35 and 36% pectin without differences in size, points at amount of zinc and cross-linking as the main explanation for the different mucoadhesive properties of the three types of pectin beads.

The release of drug from these systems has not been the focus of this work, but has been investigated by (Chambin et al., 2006; Dupuis et al., 2006) for amidated pectin beads in hard capsules. Even though the drug release was found to be slow when an enteric coated capsule was used, it is reason to believe that drug release from beads made of pectin DM 70% will be substantially faster, due to a lower and weaker cross-linking. Our findings therefore indicate that an inherent paradox for mucoadhesive formulations exist, with the factors leading to an increased mucoadhesion on one hand, inevitably leading to a faster drug release on the other. Prior to development of a successful mucoadhesive product based on pectin, efforts should therefore be put into reducing the solubility of this system. Alternatively, a fast release may be acceptable for buccal/nasal/ocular formulations for which the main purpose of bioadhesion would be to provide intimate contact with the mucosa and avoid immediate formulation detachment.

## Acknowledgments

All the pectin types were kindly provided by the manufacturers. The authors are grateful to Torill Marie Rolfsen at Department of Molecular Biosciences, University of Oslo, for carrying out the SEM measurements, and to Mr. Scheie and coworkers from the slaughterhouse Fatland Oslo A/S for supply of porcine intestinal mucosa.

## References

Bourgeois, S., Gernet, M., Pradeau, D., Andremont, A., Fattal, E., 2006. Evaluation of critical formulation parameters influencing the bioactivity of β−lactamases entrapped in pectin beads. Int. J. Pharm. 324, 2–9.

Chambin, O., Dupuis, G., Champion, D., Voilley, A., Pourcelot, Y., 2006. Colon-specific drug delivery: influence of solution reticulation properties upon pectin beads performance. Int. J. Pharm. 321, 86–93.

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.

Dubolazov, A.V., Nurkeeva, Z.S., Mun, G.A., Khutoryanskiy, V.V., 2006. Design of mucoadhesive polymeric films based on blends of poly(acrylic acid) and (hydroxypropyl)cellulose. Biomacromolecules 7, 1637–1643.

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.

Dupuis, G., Chambin, O., Génelot, C., Champion, D., Pourcelot, Y., 2006. Colonic drug delivery: influence of cross-linking agent on pectin beads properties and role of the shell capsule type. Drug Dev. Ind. Pharm. 32, 847–855.

El-Gibaly, I., 2002. Oral delayed-release system based on Zn-pectinate gel (ZPG) microparticles as an alternative carrier to calcium pectinate beads for colonic drug delivery Int. J. Pharm. 232, 199–211.

Goto, T., Morishita, M., Kavimandan, N.J., Takayama, K., Peppas, N.A., 2006. Gastrointestinal transit and mucoadhesive characteristics of complexation hydrogels in rats. J. Pharm. Sci. 95, 462–469.

Grabovac, V., Guggi, D., Bernkop-Schnürch, A., 2005. Comparison of the mucoadhesive properties of various polymers. Adv. Drug Deliv. Rev. 57, 1713–1723.

Hagesaether, E., Sande, S.A., 2007. In vitro measurements of mucoadhesive properties of 6 types of pectin. Drug Dev. Ind. Pharm. 33, 417–425.

Hagesaether, E., Sande, S.A., In vitro mucoadhesion of pectin films, effect of type of pectin and plasticizer. Pharm. Dev. Technol., submitted for publication.

Koffi, A.A., Agnely, F., Ponchel, G., Grossiord, J.L., 2006. Modulation of the rheological and mucoadhesive properties of thermosensitive poloxamer-based hydrogels intended for the rectal administration of quinine. Eur. J. Pharm. Sci. 27, 328–335.

Lehr, C.-M., Bouwstra, J.A., Spies, F., Onderwater, J., van het Noordeine, J., Vermeij-Keers, C., van Munsteren, C.J., Junginger, H.E., 1992. Visualization studies of the mucoadhesive interface. J. Control. Release 18, 249–260.

Limer, A.J., Rullay, A.K., San Miguel, V., Peinado, C., Keely, S., Fitzpatrick, E., Carrington, S.D., Brayden, D., Haddleton, D.M., 2006. Fluorescently tagged star polymers by living radical polymerisation for mucoadhesion and bioadhesion. React. Funct. Polym. 66, 51–64.

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, 413–420.

Pillay, V., Danckwerts, M.P., Muhidinov, Z., Fassihi, R., 2005. Novel modulation of drug delivery using binary zinc-alginate-pectinate polyspheres for zero-order kinetics over several days: experimental design strategy to elucidate the crosslinking mechanism drug dev. Ind. Pharm. 31, 191–207.

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.

Sande, S.A., 2005. Pectin-based oral drug delivery to the colon. Expert Opin. Drug Deliv. 2, 441–450.

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, 146–155.

Sudhakar, Y., Kuotsu, K., Bandyopadhyay, A.K., 2006. Buccal bioadhesive drug delivery – a promising option for orally less efficient drugs. J. Control. Release 114, 15–40.

Wittaya-areekul, S., Kruenate, J., Prahsarn, C., 2006. Preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. Int. J. Pharm. 312, 113–118.

Yin, Y., Chen, D., Qiao, M., Lu, Z., Hu, H., 2006. Preparation and evaluation of lectin-conjugated PLGA nanoparticles for oral delivery of thymopentin. J. Control. Release 116, 337–345.