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Extrusion/Spheronization of Pectin-Based Formulations. I. Screening of Important Factors

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ABSTRACT This study investigated the possibility of producing pectin-based pellets by extrusion/spheronization. The study also identified factors influencing the process and the characteristics of the resulting product. Three types of pectin with different degrees of amid and methoxyl substitution were studied in combination with different granulation liquids (water, calcium chloride, citric acid, and ethanol) and/or microcrystalline cellulose. Pellets were prepared in a power-consumption-controlled, twin-screw extruder; then they were spheronized and dried. The products were characterized by image analysis, sieving analysis, and disintegration and dissolution tests. The results were evaluated by multivariate analysis. Different additives, either in the granulation liquid or in the powder mixture, influenced the ability of the extruded mass to form pellets (the processability) with this technique. However, the various pectin types responded to modifications to a different extent. Short, nearly spherical pellets are obtained with granulation liquids, such as ethanol, that reduce the swelling ability of pectin. Pellets produced with ethanol are, however, mechanically weak and tend to disintegrate. Pectin molecules with a high degree of free carboxylic acid groups seem to be more sensitive to changes in the granulation liquid. Addition of microcrystalline cellulose as an extrusion aid generally resulted in improvements in shape and size. It was demonstrated that the processability of pectin as well as the characteristics of the products can be influenced in different ways during the process (eg, adding substances to the granulation liquid or to the powder mixture).

Keywords: Pectin, Pellets, Extrusion, Spheronization, Multivariate analysis.

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INTRODUCTION

Successful pelletization of a hydrophilic polymer by the extrusion/spheronization technique, without pretreatment of the polymer or admixture of an extrusion-aiding polymer, has not been reported. Law and Deasy [1] have produced pellets from spray-dried mixtures of hydrophilic polymers (Na-CMC, HPMC, HPC, PVP) and microcrystalline cellulose (MCC) by extrusion/spheronization. They found that the more adhesive polymers (Na-CMC and HPMC) were less suitable for pelletization by this technique. Goskonda and Upadrashta [2] reported successfully manufacturing spherical pellets from a combination of Avicel RC-591 and chitosan by extrusion/spheronization; Avicel RC-591 was found to be necessary.

To obtain more knowledge about the behavior of a swelling hydrocolloid in the extrusion/spheronization process, pectin was chosen as an example because of abundant availability. Pectin is a nontoxic, partly water-soluble, gel-forming polysaccharide extracted from apple pomace or citrus peel. This polymer has been suggested as a carrier for colon-specific delivery as well as for sustained-release purposes [3-5] . Pectin has been studied in several drug delivery systems, from matrix tablets [6-8] to press-coated [7,9,10] and film-coated tablets [11-13] . Several reports have been published on pectin in multiple-unit dosage forms, mainly on hydrogel beads produced by ionotropic gelation of pectin in the presence of calcium ions [14-17] . Another interesting approach to multiple-unit pectin systems are the hydrogel-coating of pellets by immersion of calcium acetate-containing pellets of MCC in a pectin solution [18,19] .

The objective of this study was to investigate the possibility of producing pectin-based pellets by extrusion/spheronization. In the future, such pectin pellets may be used for colon-specific delivery. As no

earlier studies regarding extrusion of pectin have been reported, this study represents the first steps in identifying some of the factors influencing the process of pectin extrusion and the characteristics of the resulting product. The study is consequently not intended to be an optimization of a pectin pellet formulation for colon-specific delivery.

The degree of substitution (methoxylation and amidation) was expected to have a strong influence on the properties of pectin. Consequently, both high-methoxylated (HM) and low-methoxylated (LM) pectin were evaluated. An amidated LM pectin was included in the study because earlier reports have shown interesting properties for this modification [6] .

MATERIALS AND METHODS

Materials

An HM pectin with a degree of methoxylation (DM) of 69% (USP pectin, batch no. 6596/02 from Citrus Colloids, Hereford, UK) and an LM pectin with a DM of 35% (Pectin Classic CU701, batch no. 0811754 from Herbstreith & Fox GmbH, Neuenburg, Germany), as well as an amidated LM pectin with a DM of 25% and degree of amidation (DA) of 23% (pectin type 920, batch no. 6753/01 from Citrus Colloids) were evaluated.

MCC (Avicel PH101, FMC, Philadelphia, PA, USA) was used as an extrusion aid (0%-80% of the total powder mixture). Acetylsalicylic acid (ASA) (NMD, Oslo, Norway) was chosen as a model drug substance (20% of the total powder mixture).

Different granulation liquids were used (concentrations given as % wt/wt): pure demineralized water, 10% aqueous solution of citric acid, 5% calcium chloride, and 20% ethanol. All chemicals were analytical grade (Merck, Darmstadt, Germany).

Methods

Experimental designs

The factors investigated were the different pectin types and granulation liquids and the content of MCC in the powder mixture. Levels for each factor are presented in **Table 1** . The experimental design is a mixed-level, full-factorial design. Pellets without pectin consisting of 80% MCC and 20% ASA, granulated with each of the 4 granulation liquids, were produced as references.

Preparation of pellets

Pellets were prepared using a power-consumption-controlled, twin-screw extruder (type ZE25x18D, Berstorff AG, Hannover, Germany) at a power consumption of 180 W [20] . The extruder had 48 dies 1 mm in diameter and 2.5 mm in length. The powder feed rate was 25 g to 26 g depending on the formulation. The extruded mass was rounded in a spheronizer (Type S-320, Nica, Molndal, Sweden) with a crosshatched plate 320 mm in diameter at 800 rpm for 5 minutes. The pellets were dried in a fluid-bed dryer at 50°C for 30 minutes (Glatt, Binzen, Germany).

The moisture content of the extrudate was gravimetrically determined after drying at 105°C for 24 hours.

Processability

To obtain an approximate characterization of the production process ("processability"), 3 categories were defined on the basis of sensory examination of the product. Category 3 represents formulations with high processability, meaning that the extrudate appeared to be nonsticking and easily spheronized. Category 2 represents a slightly sticky extrudate that still is breakable into short rods. Combinations that either were impossible to pass through the extruder or produced extrudate too sticky to be spheronized were classified as category 1.

Table 1. Experimental Levels for the Variables

Factors	Experimental Levels
Pectin type	HM, LM, amidated LM
Concentration of MCC	0%, 20%, 50%
Additive to the granulation liquid	none, EtOH, $CaCl_2$, citric acid

Note: MCC = microcrystalline cellulose; HM = high methoxylated; LM = low methoxylated, none = pure water.

Shape and size characteristics of the pellets

All products (categories 2 and 3) were characterized according to shape, size, and size distribution using an image analysis system (Leica Q500MC, Qwin, Cambridge, UK). Prior to processing of the images, care was taken to ensure that all pellets were detected as single entities. One pixel corresponds to 54 µm. Six feret diameters were measured around each individual particle for 400 ± 100 items. The length was defined as the longest feret diameter, and the breadth was defined as the shortest. The area (total number of detected pixels) and roundness (perimeter^2 /{4*p*area*1.064}) were calculated for each particle. The median and the upper and lower quartile (D75 and D25) as well as the interquartile range (D75-D25) were calculated for all image parameters.

Mechanical stability

The mechanical strength of the pellets could not be measured by a crushing test due to the large variation in shape and size. A standard friability test (from the European Pharmacopoeia with glass beads included for testing of pellets) did not produce significant differences between the formulations, although very different amounts of fines were produced during the fluid-bed drying of the pellets.

To evaluate mechanical stability, the number of fine particles produced in the drying process was employed as a measure of the mechanical strength of the products. This was determined by sieving the product after drying. Particles smaller than 0.3 mm were regarded as fines. The higher the number of fines, the less mechanically stable were the products.

Disintegration

A disintegration test (Ph Eur) was performed as modified and described previously [21] . Cylinders of Plexiglas (made at the workshop, Dept. of Pharmaceutics and Biopharmacutics, University of Kiel, Germany) closed with sieves (mesh size of 710 µm) at both ends were filled with pellets and placed in the disintegration test apparatus. The cylinders were kept in position during the test by metal plumbs. Of the total pellet fraction of each batch, 100 mg of pellets were tested, and the test was repeated 6 times for each batch. The disintegration was classified as totally (category 3), partly (category 2), or not disintegrated (category 1) within 15 minutes in 0.1 M HCl at 37ºC.

In vitro dissolution

The in vitro dissolution was tested according to the paddle method (Ph Eur). We exposed 500-mg pellets to 2 different test media: 1 liter 0.1 M HCl and 1 liter phosphate buffer saline solution (pH 6.8, ionic strength 0.11) at 37°C for 120 minutes at 50 rpm (n = 3-6). Each test was performed until complete release of the active substance occurred. As the model drug is susceptible to degradation during processing and in the dissolution test, the amounts of ASA and the degradation product salicylic acid (SA) were measured by UV absorbance at 2 wavelengths (? = 260 and 303 nm) using a Shimadzu Photometer, Kyoto, Japan). The reported amount released is the sum of ASA and SA.

Pseudoplasticity index/solubility assessments

Pectin solutions of 3% (wt/wt) were prepared from aqueous solutions at the given concentration of each selected additive. Samples of 500 µL were tested in a plate-cone rotational viscometer (Haake PK100, Karlsruhe, Germany) at 30°C. Within 60 seconds, the shear rate was linearly increased from 0 to 3000 s^{-1} . Prior to the run, each sample was tempered for 60 seconds in the gap. For each pectin preparation, 10 measurements were performed and the average and standard deviation calculated.

As polysaccharides are known to exhibit pseudoplastic flow, the Herschel-Bulkley equation

$$\tau = \tau_0 + K\,D^n \qquad (1)$$

was fitted to the measured data. t represents the shear stress (Pa), t_0 the yield stress, D the shear rate (s^{-1}), K the viscosity constant, and n an index of pseudoplasticity, describing the deviation from Newtonian behavior. The pseudoplasticity index (n) for pectin in water was compared with the index for pectin in aqueous solutions of all the selected additives. An n-value above 1 indicates a solution containing particles.

Multivariate analysis

To evaluate the data and identify the most important factors for preparation of high-quality pellets, principal component analysis (PCA) and projection to latent structures (PLS) regression were applied (The Unscrambler, Camo ASA, Trondheim, Norway). A description of the PCA and PLS methods as well as an introduction to interpreting typical graphs as score and loading plots can be found elsewhere [22,23] . For

evaluation of the nonspectral data, the variance of each variable was scaled to unit variance (1/SD.). All models were calculated employing cross validation. The approximate uncertainty variance of the PLS regression coefficients was estimated by "jack-knifing" as described by Martens and Efron [24] .

RESULTS

Evaluation of the manufacturing process

The manufacturing of the pectin formulations was not as smooth and uncomplicated as was the manufacturing of the MCC reference pellets. **Figure 1** illustrates how the products varied in shape and size-from spherical to dumbbell-shaped to rods of different lengths.

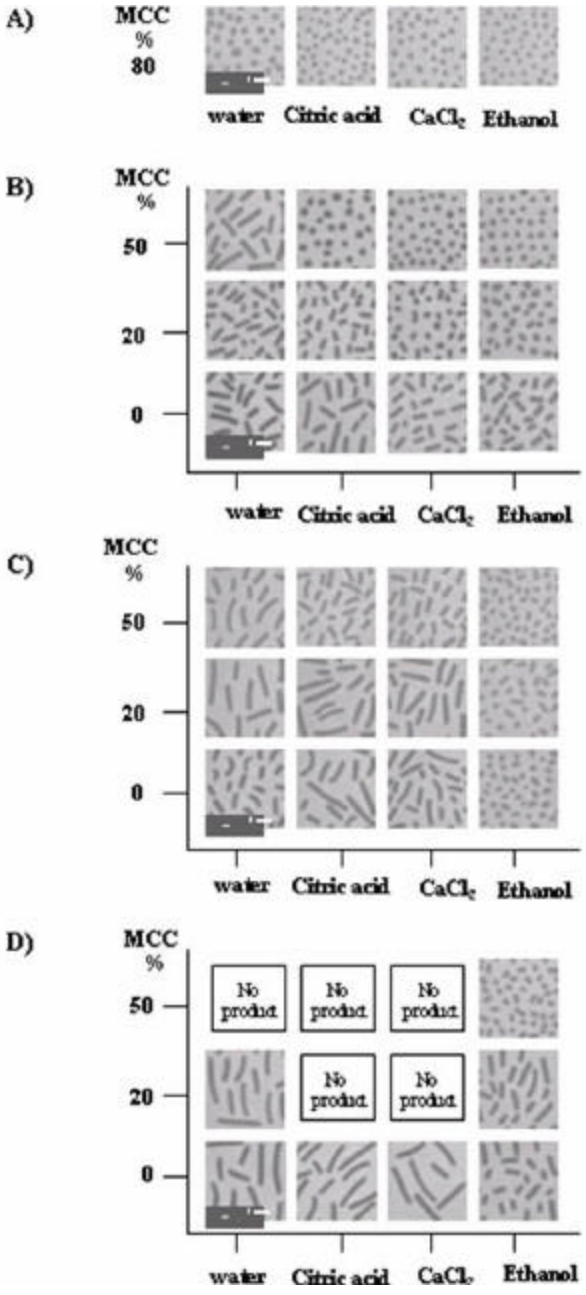

Figure 1. (below, left) Shape, size, and distribution of the pectin products and the reference pellets: A) microcrystalline cellulose (MCC) (no pectin), B) amidated low-methoxylated pectin, C) low-methoxylated pectin, D) high-methoxylated pectin.

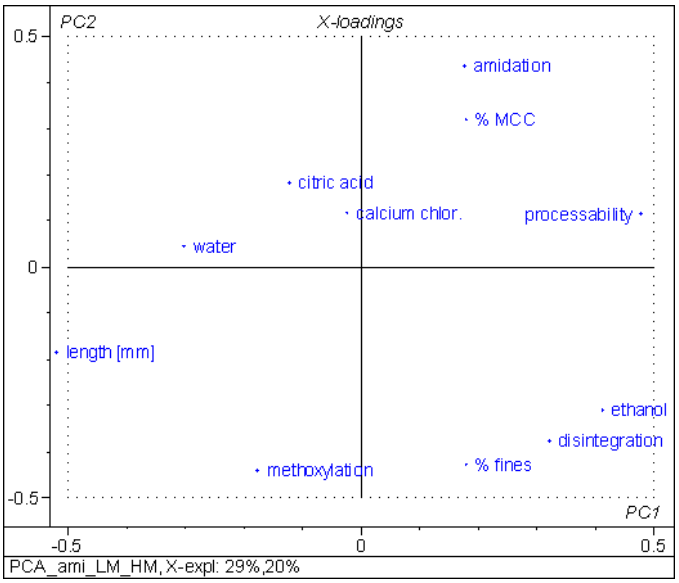

Figure 2. Loading plot from principal component analysis of all data (49% of the variance explained on PC1 and PC2)

Figure 2 presents the loading plot of a PCA of the data matrix. Forty-nine percent of the variation is explained by 2 principal components. The processability is primarily explained by the first principal component (PC1). Pellet length has approximately the same location on the opposite side of origin, indicating negative correlation. As can be seen in Figure 1D, HM pectin failed to yield a product in many experiments. In the further evaluation, the experiments with HM pectin were excluded.

Shape and size characteristics of the pellets

Combinations producing products with long, rod-shaped particles tend to have a broader size distribution than combinations with short, nearly spherical products (**Figure 1**). The factors that contribute to producing shorter pellets were identified in a PLS analysis of pellet length. **Table 2** presents the characteristics of the prediction models. In **Table 3** , the trends of the significant regression coefficients of the main factors are illustrated. Amidated pectin, addition of ethanol to the granulation liquid, and a high concentration of MCC are the variables that significantly reduce the pellet length. Water without any additive shows a significant positive regression coefficient.

Table 2. Predictions Obtained for PLS Models for Amidated LM and LM Pectin

Property	Explained Variance in X	Explained Variance in Y	Prediction Uncertainty CV[a]
Pellet length	32	80	28
Number of fines[b]	33	90	29
Number of disintegrated pellets[c]	39	100	1
Drug release after 15 min in 0.1 M HCl at 37°C[d]	41	56	20
Drug release after 15 min in pH 6.8 at 37°C[d]	38	41	20

Notes: PLS = projection to latent structures, LM = low methoxylated, CV = coefficient of variation. Number of principal components was 2. The effect of the regression coefficients can be seen in Table 3.
[a]CV = Root mean square error of prediction/mean (given in %)
[b]Measure of the mechanical strength
[c]Disintegration in 0.1 M HCl within 15 minutes at 37°C
[d]PLS2 for 15 minutes

Table 3. Trends of the Regression Coefficients of the Main Factors Obtained in the PLS Models

X-Variables	Pellet Length	% Fines	Disintegration	Drug Release After 15 Min in 0.1 M HCl	Drug Release After 15 Min in Buffer (pH 6.8)
Amidation	↓	↓	--	↑	↑
Granulation liquid					
- no additive	↑	↓	↓	↓	↓
- ethanol	↓	↑	↑↑	↑↑	↑↑
- calcium chloride	--	↓	↓	↓	↓
- citric acid	--	--	↓	↓	↓
Microcrystalline cellulose	↓	↓	--	↑	↑

PLS = projection to latent structures
Note: See also Table 2. Number of principal components was 2 for all properties; ↑ = significant positive, ↓ = significant negative, -- = not significant, ↑↑ = extra large coefficient. The significance of the regression coefficients has been determined by jack-knifing and corresponds to p = 0.05.

Mechanical stability

The results of the PLS analysis of percentage of fines produced are shown in **Table 2**. **Table 3** shows that the only variable with a significant positive regression coefficient was ethanol, indicating pellets made with ethanol are mechanically weak.

Disintegration

All pellets granulated with ethanol disintegrated to some degree within the duration of the test, regardless of the pectin type used. The combination of amidated LM pectin granulated with calcium chloride containing no MCC was removed as an outlier because the behavior was very different from what was predicted by the model and could not be explained by the same factors. The results of the PLS analysis (**Tables 2** and **3**) indicate that ethanol was the only significant factor in favor of disintegration.

In vitro dissolution

The dissolution rate of the model drug in acidic media is generally faster from pectin pellets than from pure

MCC pellets. Typical examples of dissolution profiles from pellets of LM pectin are shown in **Figure 3** . The results using phosphate buffer saline solution (pH 6.8) demonstrate that LM pectin granulated with calcium chloride had a significantly slower dissolution rate than did the corresponding MCC pellets.

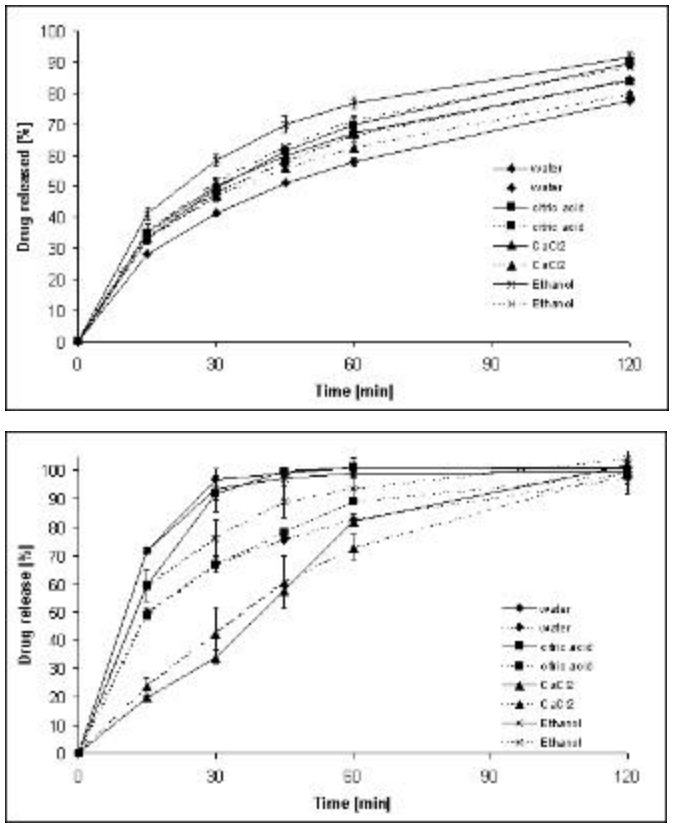

Figure 3. Examples of dissolution profiles for pellets of low-methoxylated pectin (whole lines) and pure microcrystalline cellulose (dotted lines): A) in 0.1 M HCl, B) in phosphate buffer saline solution (pH 6.8) (n = 3-6).

The effects of the different variables seem to be equal in both media (**Table 3**). Ethanol was again an important variable in increasing the dissolution rate. The effect of the different variables does not change when comparing the models obtained for different time intervals.

Pseudoplasticity index/solubility assessments

Solutions of the 3 pectins in water (with no additive) showed pseudoplastic behavior, as expected (**Table 4**). HM pectin showed a less pseudoplastic behavior compared with the amidated LM pectin (n = 0.18 and 0.92, respectively), while the nonamidated LM type displayed an intermediate n-value. A concentration-dependent increase in the index of pseudoplasticity was observed for all tested additives for the 2 LM pectins (LM and amidated LM). With respect to amidated LM pectin, all the tested additives resulted in precipitation (n > 1), while only calcium chloride and ethanol had this effect on the nonamidated LM pectin. For HM pectin, a significant increase was observed only for the ethanol preparation.

Table 4. Pseudoplasticity Index (n) for 3% (wt/wt) Pectin Preparations in Aqueous Solutions of Various Additives

Pectin Type	Pseudoplasticity Index				
	Water	Citric Acid	Lactic Acid	Calcium Chloride	Ethanol
HM	0.18	0.14	0.23	0.16	> 1
LM	0.38	0.43	0.71	> 1	> 1
amidated LM	0.92	> 1	> 1	> 1	> 1

Note: HM = high methoxylated; LM = low methoxylated

DISCUSSION

No "shaping" of the products took place during spheronization, the breadth remained constant, and the cylinder length was reduced as a result of mechanical weakness.

The R^2 for the correlation between length and aspect ratio is 0.972. A PCA performed on the results from the image analysis showed that all parameters except breadth are strongly correlated along PC1, which explains 97% of the variation. Based on the correlation between length, area, roundness, and aspect ratio, pellet length can be chosen as a single parameter to describe the pellet size. Length was chosen over aspect ratio to highlight that the main problem with these formulations was that they did not break into small enough pieces (1 mm). Shorter lengths consequently indicate good pellets, as spherical pellets will have a length equal to the diameter.

The combinations that failed to form products gave, as expected, a cluster in a PCA of all variables and processability. Another interesting group in this PCA is the experiments with HM pectin that gave rather poor products. These are all long, rod-shaped pellets with a median length of 6 to 7 mm. This might imply that these pellets were falsely accepted as products. Both removing the 4 combinations as outliers and recategorizing them as failures resulted in improvements in the PLS model (predicted versus

measured correlation coefficient improved from 0.76 to 0.97). On the basis of these findings, HM pectin can be regarded as having a negative influence on pellet formation within the parameters of this study.

The effect of the various additives to the granulation liquid must be related to the ability of the additive to interact with pectin. The positive effect of ethanol on the processability and size of the pellets might be explained by the fact that adding ethanol to the granulation liquid reduces the solubility of pectin. The swelling is therefore limited, which in turn leads to improved shape and size.

Pectin is hydrophilic (**Figure 4**) and has numerous sites for hydrogen-bond formation. The carboxylic acid group on the C6 can be either free, methoxylated, or amidated. The degree of substitution and the nature of the substituents determine the solubility of pectin. Pectin forms gel upon swelling. A high degree of swelling during granulation and extrusion of pectin would result in a sticky extrudate. As the solubility of gelling substances is hard to assess, the index of pseudoplasticity was used instead as an assessment of the changes occurring in pectin in the different granulation liquids. In solution, pectin chains will form entanglements together with the association of immobilized solvent. An increase in pseudoplasticity index might therefore be taken as an indication of increase in the entanglement formation. When the solubility limit is reached, the entanglements will no longer be in solution and precipitation takes place. These observations are listed in **Table 4** and confirms that the solubility of pectin is affected by various additives.

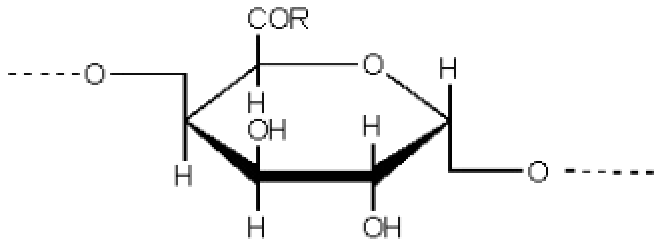

Figure 4. Structural formula of the main unit in pectin (polygalacturonic acid). Dependent on the substitution on C6, the unit has a free acid group, is methoxylated or amidated.

When each pectin type was studied separately, calcium chloride and citric acid were found to result in shorter products for the amidated LM pectin. It is possible that these additives in the granulation liquid contribute to a limitation on the swelling of pectin. Because calcium chloride is a divalent cation, it is capable of cross-linking the pectin chains, forming an insoluble network during manufacturing. From the literature it is known that amidated pectin is more sensitive toward calcium ions than is HM pectin [26] . This is reason to believe that an optimal concentration of calcium ions exists to form a proper network [26] . A suggested mechanism for the positive effect of citric or lactic acid on the swelling is that the acidic granulation liquids contribute to a more hydrophobic pectin molecule by reducing the number of pH-dependent, negatively charged groups. In this case, the concentration of acid or the pH of the granulation liquid could be an important factor.

The effect of adding MCC as an extrusion aid was found to be dependent on the pectin type. For amidated LM pectin, it seems to have a reducing effect on the shape and size of pellets as well as improving their mechanical properties. For the 2 nonamidated types, the effect on shape and size varies. The threshold for the amount of MCC necessary for improvement of the shape depends on the type of pectin, its solubility, and its swelling properties. The improvement may be an inherent effect of MCC or just a consequence of a reduced amount of pectin.

Although swelling during the manufacturing process could be reduced by adding ethanol to the granulation liquid, the products were found to be mechanically weaker. This is an effect that is also seen for MCC pellets extruded with high concentrations of ethanol [27] . It is suggested that ethanol produces more porous pellets than water does, because granulation liquid and highly porous pellets tend to disintegrate [21] . This was also found to be valid for pectin pellets.

Based on the large variations in the pellet size among the different combinations of this study, the pellet length was included as a variable in a PLS2 analysis (PLS with more than response) of drug dissolution. The length was found to be one of the most important variables determining the rate of dissolution in both test media. Because the size is correlated to the specific surface area, the results from the dissolution test cannot be directly compared without knowing the specific surface area of the different pellets.

The estimated transit time through the small intestine is 4 hours [3] . After 4 hours both in acidic medium and in buffer (pH 6.8), 100% of the model drug was released from all formulations. None of the combinations could therefore be recommended for colonic delivery.

CONCLUSION

The main finding of this study is that the processability of pectin as well as the characteristics of the products can be influenced in different ways during the process (eg, adding substances to the granulation liquid or to the powder mixture).

Using multivariate data analysis, important variables for control of product formation from pectin-containing formulations in an extrusion/spheronization process could be identified. The chemical properties of the pectin-in other words, the degree of methoxylation and amidation-are important for the processability of the formulation. A high DM is not favorable for product formation, but amidation of the LM pectin seems to have a positive impact on production of short pellets.

It is also shown that the processability can be improved by altering additives to the granulation liquid and by adding MCC to the powder mixture. The processability is also decisive for the shape, size, and size distribution of the final product. Amidated pectin extruded with ethanol-containing granulation liquid is well suited for production of short, nearly spherical pellets. These products were, however, found to be less mechanically stable and more likely to disintegrate. The dissolution rate was relatively high both in 0.1 M HCl and in phosphate buffer saline solution (pH 6.8) for all pectin types.

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