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RESEARCH PAPER

Synthesis and Enzymatic Degradation of Epichlorohydrin Cross-Linked Pectins

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ABSTRACT

The water solubility of pectin was successfully decreased by cross-linking with increasing amounts of epichlorohydrin in the reaction media. The initial molar ratios of epichlorohydrin/galacturonic acid monomer in the reaction mixtures were 0, 0.37, 0.56, 0.74, 1.00, 1.47, and 2.44. The resulting epichlorohydrin cross-linked pectins were thus referred to as C-LP0, C-LP37, C-LP56, C-LP75, C-LP100, C-LP150, and C-LP250, respectively. Methoxylation degrees ranged from $60.5 \pm 0.9\%$ to $68.0 \pm 0.6\%$, and the effective cross-linking degrees, determined by quantification of the hydroxyl anions consumed during the reaction, were 0, 17.8, 26.0, 38.3, 46.5, 53.5, and 58.7%, respectively. After incubating the different cross-linked pectins (0.5% w/v) in 25 mL of 0.05 M acetate–phosphate buffer (pH 4.5), containing 50 μL of Pectinex® Ultra SP-L (pectinolytic enzymes), between 60 and 80% of the pectin osidic bounds were broken in less than 1 hr. Moreover, increasing the cross-linking degree only resulted in a weak slowing on the enzymatic degradation velocity.

Key Words: Epichlorohydrin; Cross-linked pectins; Colonic drug delivery; Enzymatic degradation.

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INTRODUCTION

Pectins, hydrophilic linear polysaccharides extracted from plant cell walls and chiefly consisting of partially methoxylated poly-α-(1→4)-D-galacturonic acids, are generally regarded as nontoxic material, mainly used as gelling agents.[1] Being specifically, completely, and rapidly digested by colonic bacteria,[2] pectins—like other natural polysaccharides degraded by the intestinal microflora—have been studied in view to be used for carrying drugs to the colon.[3]

Although their potential as specific colonic drug delivery carriers has been reported,[4,5] pectins and generally the other native degradable polysaccharides are soluble and swell in aqueous media. Colonic delivery systems based on the use of these polymers alone are therefore unable to prevent the release of drugs during transit through the stomach and small intestine. The reduction of pectin water solubility, which is therefore necessary, has been achieved up to now either by formation of pectin-polycation [calcium, chitosan, trimethylaminoethylmethacrylates (Eudragit® RS, Eudragit® RL)] complexes or by blending pectins with cellulosic or acrylic insoluble polymer coatings.[6–14]

Unfortunately, the in vitro evaluation of matrices and coating formulations based on such complexes or blends have shown that these systems are enable to perform properly specific delivery of drugs to the colon: the release of model drugs in the dissolution media containing commercial pectinolytic enzymes was lower or slightly higher than that observed in the absence of enzymes. Thus, it has been thought that chemical modifications, intended to decrease the aqueous solubility and swelling properties of pectins while maintaining their degradation by pectinolytic enzymes, could be an interesting alternative.

The aim of this work was to prepare water-insoluble epichlorohydrin cross-linked pectins intended for targeting drugs to the colon. Pectin was dispersed in water–95% ethanol (1:3) containing 1.25 M sodium hydroxide and cross-linked with increasing amounts of epichlorohydrin. Furthermore, the resulting cross-linked pectins were characterized by determining their cross-linking degrees, the percentage of methoxylation, and aqueous solubility. Finally, degradation kinetics of the cross-linked derivatives, incubated in 0.05 M acetate–phosphate buffer (37°C, pH 4.5) in the presence of pectinolytic enzymes (Pectinex® Ultra SP-L), were determined.

EXPERIMENTAL

Materials

Epichlorohydrin (Aldrich Chemical Co., Gillingham, UK) was used for the cross-linking of pectin HM from apple (Fluka, Busch, Switzerland). Methanol (for high-performance liquid chromatography) and 95% ethanol were supplied from Labscan Ltd. (Dublin, Irland) and Stella s.a. Laboratories (Liège, Belgium), respectively. Pectinex Ultra SP-L was purchased from Novo Ferment (Dittingen, Switzerland). All the other materials used were of analytical reagent grade.

Methods

Synthesis of Epichlorohydrin Cross-Linked Pectins

Synthesis of epichlorohydrin cross-linked pectins was carried out in three steps.[15] The methoxyl groups of pectin were first hydrolyzed to obtain sodium pectate, which was then cross-linked in alkaline media with increasing amounts of epichlorohydrin. Cross-linked sodium pectates were finally methoxylated into cross-linked pectins.

Preparation of Sodium Pectate

Synthesis of epichlorohydrin cross-linked pectins was started with the preparation of sodium pectate, which is much more stable than pectins. Indeed, contrary to pectates, the latter is intensely and rapidly depolymerized by *trans*-elimination in alkaline aqueous media.[16]

Sodium pectate was prepared at 4°C from pectin HM as follows: about 100 g of pectin HM was dispersed in 500 mL of water–95% ethanol (1:3). This mixture was mechanically stirred and, at 1 hr intervals, four equal portions of a total volume of 500 mL of 2 M sodium hydroxide in water–95% ethanol (1:3) were added. After stirring the suspension for 3 hr, sodium pectate powder was collected by filtration through a Bückner funnel. The powder was resuspended in 1 L of 0.5 M sodium hydroxide in water–95% ethanol (1:3) and stirred for 15 hr. It was then collected by filtration, washed successively with water–95% ethanol (1:3) until neutrality and finally with 95% ethanol.

The resulting sodium pectate powder was dried at room temperature for two days and then at 60°C until constant weight. The powder was ground in a mortar, and the particle size fraction below 315 μm was collected for the cross-linking reaction by sieving. The sodium galacturonate content in sodium pectate, determined by acid-base titration,[17] was 76.1 ± 0.8% w/w (mean ± SD, $n = 3$).

Cross-Linking of Sodium Pectate with Increasing Amounts of Epichlorohydrin

Amounts of about 5 g of sodium pectate, exactly weighed, were introduced in 50 mL glass-stoppered erlenmeyer flasks. Fifteen milliliters of 95% ethanol, containing the required amount of epichlorohydrin, were added while stirring at room temperature. After homogenizing the slurries, 5 mL of 5 M sodium hydroxide aqueous solution were added while stirring, and the resulting reaction mixtures (Table 1) were incubated at 40°C, in a horizontal shaking water bath (GFL 1087, Vel, Belgium), set at 200 shakes/min. After 4-hr incubation, the reaction was stopped by the addition of 0.5 M acetic acid aqueous solution until neutrality (pH 7.0), determined by using a pH meter. The molar quantity of acetic acid used, referred to as Ni, will be used in the cross-linking degree. The different cross-linked pectates obtained were collected by filtration, washed with 10 fractions of 20 mL of water–95% ethanol (1:3) and, finally, with two fractions of 20 mL of 95% ethanol.

Methoxylation of Epichlorohydrin Cross-Linked Pectates

Epichlorohydrin cross-linked pectates were chemically methoxylated to obtain degrees of methoxylation ranging from 60% to 68%, according to the method of Heri et al.[18]: the cross-linked pectates were suspended in 250 mL of methanol containing 1 M of sulfuric acid and stirred for eight days at 4°C. The resulting cross-linked pectins were collected by filtration, washed with methanol until complete elimination of sulfates, and dried at 100°C until constant weight. Figure 1 shows the structure of original pectin and that of the theoretical resulting cross-linked pectins.

The molar ratios of epichlorohydrin/sodium galacturonate monomer present in the initial reaction mixtures were 0.0, 0.37, 0.56, 0.74, 1.00, 1.47, and 2.44. These ratios correspond to 0.0, 13.1, 19.6, 26.1, 34.5, 52.0, and 86.3% w/w of epichlorohydrin/sodium pectate, respectively. The resulting cross-linked pectins were referred to as C-LP0, C-LP37, C-LP56, C-LP75, C-LP100, C-LP150, and C-LP250, respectively (Table 1).

Characterization of Cross-Linked Pectins

Solubility of the cross-linked pectins has been evaluated semiquantitatively, at 20°C or 100°C. Quantities of 50 mg of each pectin derivative were dispersed in 5 mL of 0.05 M acetate–phosphate buffer solutions of pH 4.5 or 6.0.

Methoxylation degrees (molar percentages of methoxylated galacturonic acids) of the different

Table 1. Composition of the reaction media used for synthesis of the different epichlorohydrin cross-linked pectins.

Cross-linked pectins	C-LP0	C-LP37	C-LP56	P-LP75	C-LP100	C-LP150	C-LP250
Sodium pectate (g)	5.0004	5.0095	5.0013	5.0059	5.0075	5.0023	5.0002
Epichlorohydrin (g)	0	0.6545	0.9825	1.3062	1.7265	2.6020	4.3161
95% ethanol (mL)	15	15	15	15	15	15	15
5 M NaOH aqueous solution (mL)	5	5	5	5	5	5	5
ECH/GA (molar ratio)	0	37	56	0.74	0.98	1.47	2.44
ECH/sodium pectate (weight ratio, %)	0.0	13.1	19.6	26.1	34.5	52.0	86.3

ECH, epichlorohydrin ($M_r = 92$, density = 1.18 g/mL). Sodium pectate consists of 76.1% (m/m) sodium galacturonate (GA), which has a molecular weight (M_r) of 199. Therefore, 5 g of sodium pectate contain 19.12×10^{-3} mol of sodium galacturonate. Concentration of NaOH and the molar ratio of NaOH/sodium galacturonate in the initial reaction mixtures is equal to 1.25 M and 1:0.765, respectively.

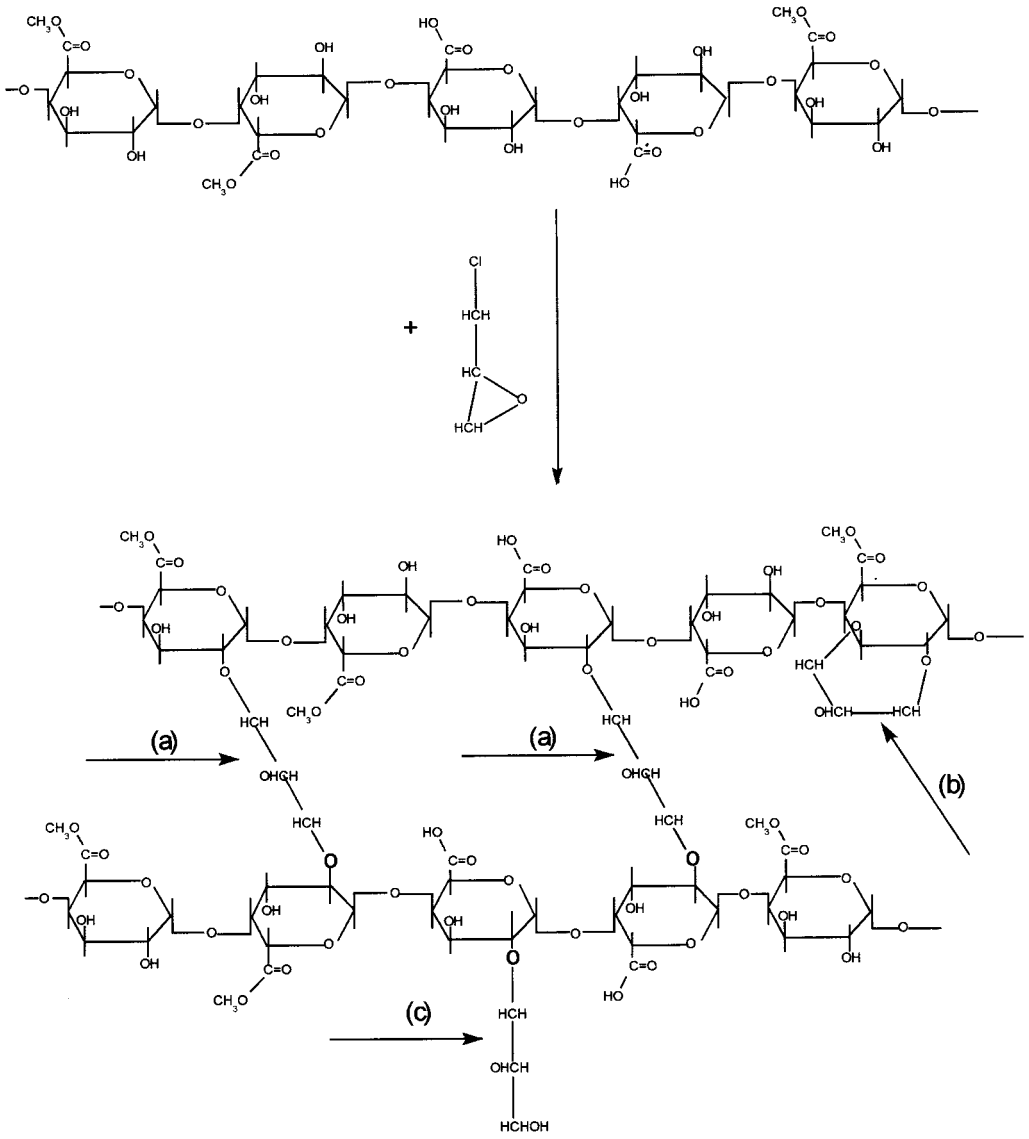

Figure 1. Theoretical schematic representations of the cross-linking reaction between pectin and epichlorohydrin. Arrows indicate the different possible modifications: bridges between two polymeric chains of pectin (a), internal bridges on the same polymeric chain (b), and finally pendent groups (c).

cross-linked pectins were determined by acid–base titration in aqueous media, according to the method of USP XXIII (1995).[17]

At the end of the cross-linking reactions, epichlorohydrin that did not react with sodium pectate passes into the filtrate after filtering and washing the cross-linked pectates with water–95% ethanol (1:3). Epichlorohydrin in the filtrate could be assayed to determine the quantity that has effectively reacted with sodium pectate. Indeed, epichlorohydrin can be converted in alkaline media into glycerol, which can be finally assayed. For instance, in the presence of ATP, NAD^+, glycerokinase, and glycerol dehydrogenase, glycerol is converted into glycerophosphate that is then oxidized, resulting in a release of NADH quantifiable by UV-Visible spectrophotometry, at 340 nm.[19] On the other hand, glycerol could be assayed after a chemical oxidation with an excess of periodate.[15]

However, according to the mechanism of the cross-linking reactions between polysaccharides and epichlorohydrin in alkaline media (Fig. 2), a new,

simple, and easy method has been used in this study to determine directly the quantity of epichlorohydrin that effectively reacted with sodium pectate. As shown in Fig. 2(I), the reaction between epichlorohydrin with galacturonate acid monomers results in the release of an equal quantity of hydrochloride acid, which reacts afterward with the sodium hydroxide present in the reaction media.

At the end of the cross-linking reaction, the molar quantity of acetic acid used for stopping the cross-linking reaction (Ni), determined with a pH meter (pH 7.0), has allowed us to determine the percentage of epichlorohydrin incorporated in sodium pectate and then the cross-linking degrees of pectins (Table 2, Fig. 3). A blank reagent, consisting of a reaction mixture without epichlorohydrin (Table 1; C-LP0), has been carried out in the same conditions. The amount of acetic acid (No) necessary to neutralize the reaction mixture (pH 7.0) after 4 hr incubation has also been determined.

The total amount of epichlorohydrin that has been incorporated in sodium pectate is therefore equal to (No − Ni), which is the actual molar quantity of sodium hydroxide of the reaction media

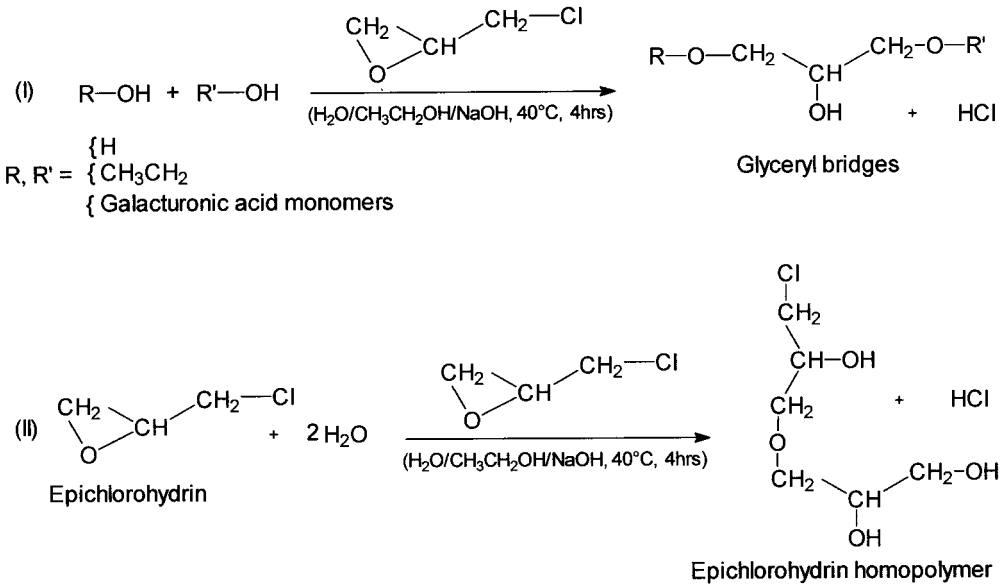

Figure 2. Schematic representation of the possible reactions that can theoretically occur in alkaline media: epichlorohydrin could react with sodium pectate, ethanol, water (I), or with itself [homopolymerization (II)]. All of these potential reactions result in a release of hydrochloric acid.

Table 2. Summary of characteristics of the different epichlorohydrin cross-linked pectins.

Type of cross-linked pectin	C-LP0	C-LP37	C-LP56	C-LP75	C-LP100	C-LP150	C-LP250
Initial molar ratio of ECH/GA	0	0.37	0.56	0.74	0.98	1.47	2.44
Initial weight ratio of ECH/sodium pectate (%)	0.0	13.1	19.6	26.1	34.5	52.0	86.3
Molar % of ECH incorporated in pectate	—	96.2	93.5	86.5	81.7	64.0	42.9
Theoretical C-LD (molar %)	0	18.5	27.8	36.9	48.8	73.6	122.1
Effective C-LD (molar %)	0	17.8	26.0	38.3	46.2	53.5	58.7
DM (mean ± SD, $n = 3$)	65 ± 3	66.9 ± 0.5	63 ± 2	68.0 ± 0.6	67 ± 2	60.5 ± 0.9	63 ± 1
Solubility	S	S	IS	IS	IS	IS	IS

ECH, epichlorohydrin; GA, galacturonic acid; C-LD (molar %), degree of cross-linked; theoretical C-LD (molar %), degree of cross-linked expected; DM, degree of methoxylation. Solubility of cross-linked pectins has been evaluated at 20°C and 100°C, in 0.05 M acetate–phosphate buffer solutions of pH 4.5 and 6.0 (50 mg/5 mL): S, soluble; IS, insoluble.

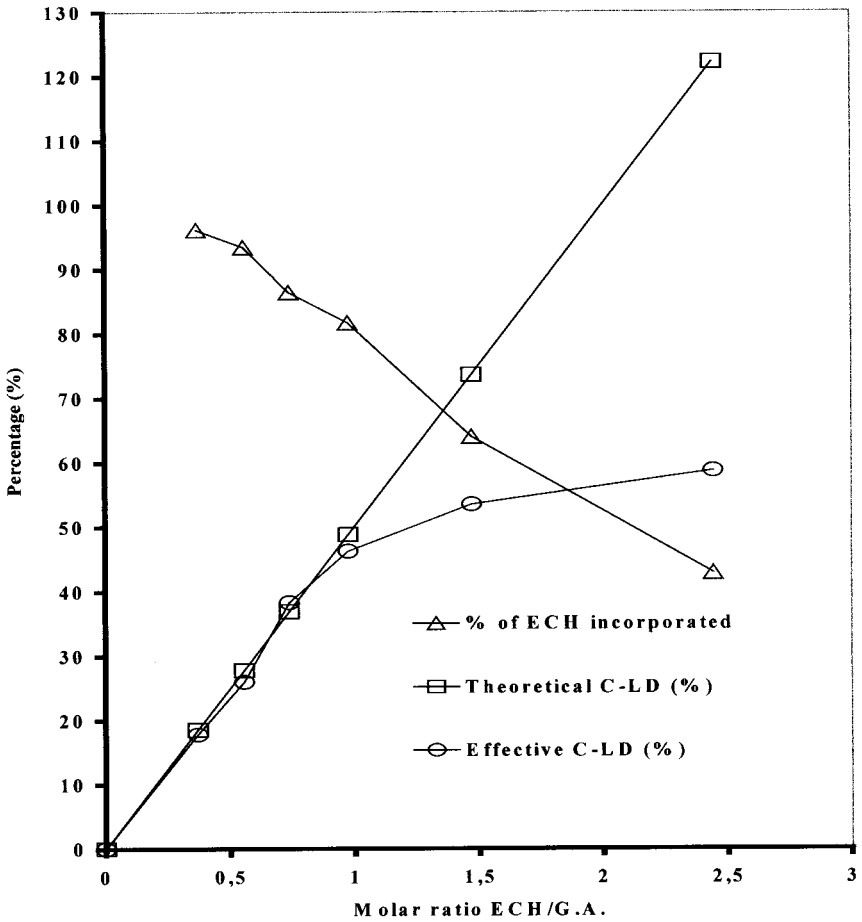

Figure 3. Influence of the molar ratio of epichlorohydrin/galacturonic acid (ECH/GA) initially present in the reaction media on the molar percentages of epichlorohydrin incorporated in sodium pectate (% of ECH incorporated), and on the theoretical and effective cross-linking degrees (C-LD, in %) of the different epichlorohydrin cross-linked pectins.

consumed during the cross-linking reaction. This value has allowed us to determine the percentages of epichlorohydrin incorporated in sodium pectate and the effective cross-linking degrees of pectins (Table 2, Fig. 3).

It has been noted that a reaction mixture, identical to that used for synthesizing C-LP250 but without sodium pectate, has allowed us to verify the absence of reaction between epichlorohydrin and the reaction medium or with itself. Indeed, according to some authors, epichlorohydrin can theoretically react with water, ethanol, and with itself (homopolymerization), resulting in the formation of glycerol, ethanol–glyceryl ether, or glyceryl homopolymer, respectively.[20] All of these reactions, which can occur in stronger alkaline media, also result in the release of hydrochloride acid in the reaction media [Fig. 2 (I and II)], detectable by the titration method used.

Enzymatic Degradation of the Cross-Linked Pectins

About 250 mg of the cross-linked pectins, exactly weighed, were dispersed at 37°C in 25 mL of 0.05 M acetate–phosphate buffer (pH 4.5). Then, 50 μL of Pectinex Ultra SP-L were added, and the resulting mixtures were incubated at 37°C in a horizontal shaking water bath (GFL 1087, Vel, Belgium) set at 100 shakes/min. At predetermined time intervals, samples of 1 mL were withdrawn and diluted with the buffer. The reducing groups (aldehydic sugars), released in the incubation media following the clea-

vage of the pectin osidic bounds by the pectinolytic enzymes, were then quantified, using the method of Nelson.[21]

The reducing sugars released in the buffer reduce, in alkaline media and at 100°C $CuSO_4 \cdot 5H_2O$ into Cu_2O, which reacts afterward with an arsenomolybdate reagent to give a colored product, whose absorbance at 520 nm is proportional to the concentration of the reducing sugars present in the sample. Calibration graphs with galacturonic acid standards (50–650 μg/mL) were used to determine the amounts of the reducing sugars released in the buffer. Galacturonic acid was used as standard reducing sugar because the pectin derivatives consist mainly of this uronic acid (see Fig. 2). The percentages (w/w) of the reducing sugars (in equivalents of galacturonic acid) released were plotted against the incubation time.

RESULTS AND DISCUSSION

Discussion About the Method of Synthesis

Synthesis of the cross-linked pectins from pectin HM was carried out in three steps (demethoxylation, cross-linking, and methoxylation). During the synthesis process, pectin or its derivatives (sodium pectate, cross-linked, or not) were always in the dispersed state and not in solution. Although the water part of the solvent mixture can allow a sufficient solvation of pectate particles, such reactions require intimate contact between the different reacting substances. For this purpose, solvents, anion hydroxyl groups, epichlorohydrin, and methanol molecules must penetrate deeply inside the pores present in the sodium pectate particles, so that the different reactions likely take place inside of the sodium pectate particles, not only at the interface of the dispersed systems. Consequently, as attested by optical microscopic examination, the macroscopic aspects and particle sizes of synthesized cross-linked pectin powders are similar to those of the original pectin used.

It has been noted that other methods of synthesis, in which pectins or sodium pectate are in aqueous solution, have been also reported.[22,23] In these methods, the efficiency of the cross-linking reaction is compromised because not only is epichlorohydrin very slightly soluble in water, but also pectins and sodium pectate form lumps in contact with aqueous media. Moreover, in these conditions, extended gelation occurs during the cross-linking reaction. As a result, the reaction, the purification, and the handling of synthesized products are generally much more difficult.

Degrees of Cross-Linking

Some studies have been already carried out to synthesize and use cross-linked pectates or pectins as chromatographic supports for the separation of pectinolytic enzymes.[15,22,23] Theoretically, the reactions of epichlorohydrin with sodium pectate can result in the formation of pectin with a pendent glycerol moiety (Fig. 1c) or internal diether bridges (cross-linking in the same polymeric chain; Fig. 1b) or finally, the formation of bridges between two polymeric chains (Fig. 1a). Rombouts et al.[15] have found that the quantity of epichlorohydrin incorporated in sodium pectate in the form of internal bridges or pendent groups [Fig. 1 (b and c)] ranges from 11 to 16.1% of the total quantity of epichlorohydrin involved in the reaction. In other words, the reaction between epichlorohydrin and sodium pectate produces mainly diether cross-linked products (Fig. 1a).

Considering these findings, the total molar quantity of epichlorohydrin incorporated in sodium pectate, as determined by the titration method previously described, has been used to determine the degrees of cross-linking pectins. The cross-linking degree is defined as the percentage of free hydroxyl groups of pectin, located in positions C_2 and C_3 of the galacturonic acids, that have reacted with epichlorohydrin. Because each galacturonic acid carries two functional groups, the effective cross-linked degree is the half percentage of the molar ratios of epichlorohydrin effectively reacted with sodium pectate/sodium galacturonate content of sodium pectate. It has been noted that the theoretical or expected cross-linked degree (in percentage) is equal to: $1/2 \times 100 \times$ the molar ratio of epichlorohydrin/sodium galacturonate initially present in the reaction mixtures.

Figure 3 shows the molar percentages of epichlorohydrin incorporated in sodium pectate; theoretical and effective cross-linking degrees are plotted vs. molar ratios of epichlorohydrin/sodium galacturonate initially present in the reaction mixtures. These and other characteristics of the different cross-linked synthesized pectins are also given in Table 2.

As expected (Table 2, Fig. 3), the effective cross-linking degree increases as function of the initial molar ratio of epichlorohydrin/sodium galacturonate. However, it levels off when the molar quantity

of epichlorohydrin initially present in the reaction mixture exceeds that of sodium galacturonate. The maximal effective cross-linking degree obtained is equal to 59%, when the initial molar ratio of epichlorohydrin/sodium galacturonate is equal to 2.5.

The results obtained are in accordance with those reported by other authors working on pectins or other polysaccharides. For instance, Rombouts et al.[15] have observed that the cross-linking degrees of cross-linked sodium pectate intended for chromatographic purposes were 0.18, 0.36, 0.46, and 0.57 when the initial molar ratios of epichlorohydrin/sodium galacturonate were 0.37, 0.56, 0.82, and 2.34, respectively. Renard et al.[20] also obtained, at 30°C, an incorporation of 14.3 mol of epichlorohydrin per mol of β-cyclodextrin (an oligosaccharide of 7 osidic units) when the initial molar ratio was 10.[20]

Solubility of Cross-Linked Pectins

Semiquantitative solubility tests have shown that solubility of the cross-linked pectins depends on the cross-linking degree (Table 2). The C-LP0 and C-LP37 derivatives whose effective cross-linking degrees are, respectively, 0 and 17.8, are soluble in 0.05 M acetate–phosphate buffer pH 4.5 and 6.0, whereas the other cross-linked products (C-LP56, C-LP75, C-LP100, C-LP150, and C-LP250) are insoluble in the same buffer solutions, even at 100°C. Other workers, using higher or lower concentrations of epichlorohydrin, have also reported similar solubility behaviors of some cross-linking polysaccharides (sodium pectate and cyclodextrins).[15,20] Cross-linking of starches or cellulose, which are water-insoluble polysaccharides, always result in the formation of insoluble gels, even with a low quantity of epichlorohydrin.[24,25]

Methoxylation Degree of the Cross-Linked Pectins

It can be observed from Table 2 that the methoxylation degree of the various cross-linked pectins are close to each other and range from $60.5 \pm 0.9\%$ to $68.0 \pm 0.6\%$ as in the original pectin HM. Therefore, the difficulty for methanol molecules to access galacturonic acid carboxyl groups, which probably increases with the cross-linking degree of sodium pectate, did not affect methoxylation of cross-linked pectates.

Enzymatic Degradation of the Epichlorohydrin Cross-Linked Pectins

Pectinex Ultra SP-L is a commercial enzymatic preparation extracted from *Aspergillus niger*. At room temperature and at pH 3.5, its activity is equal to 26,000 PG/mL (PG = milliequivalents of reducing groups released from pectic acid per minute and per unit of enzymes). Pectinex Ultra SP-L consists of three types of pectinolytic enzymes, similar to those specifically excreted by colonic bacteria: the pectinesterases that remove the methoxyl groups from pectins, the pectinases that hydrolyze the $\alpha 1\text{–}4$ glycosidic linkages of pectins, and the pectin lyases that cleave the $\alpha 1\text{–}4$ glycosidic linkages by β-elimination, resulting in the formation of double bounds between C_4 and C_5 of the galacturonic acid monomer. Therefore, the presence of Pectinex Ultra SP-L in the incubation medium results in the rapid depolymerization of pectin derivatives and the release of reducing sugars.

Figure 4 shows the degradation kinetics of the epichlorohydrin cross-linked pectins (C-LP0, C-LP75, C-LP100, and C-LP250), incubated at 37°C in 25 mL of 0.05 M acetate–phosphate buffer (pH 4.5), in the presence of 50 μL of Pectinex Ultra SP-L. Although the pH value of the colon is ranged from 5.5 to 7.5, the pH value of media was set at 4.5 to be closer to the optimal pH activity of the pectinolytic enzymes of Pectinex Ultra SP-L. Indeed, it has been previously observed that degradation of native pectins by Pectinex Ultra SP-L occurred at pH 6.0, but much more slowly.[26] As observed, concentration of the reducing sugars released in the buffer increases with the incubation time, thus confirming the hydrolyze of the $\alpha 1\text{–}4$ glycosidic linkages of the various cross-linked pectins. Degradation is very rapid because 60–80% (w/w) of reducing sugars, calculated in equivalents of galacturonic acid, are released in the incubation media in less than 1 hr. Moreover, the enzymatic degradation rates of the cross-linked pectins by Pectinex Ultra SP-L are only slightly decreased (differences were significant; analysis of variance, $p > 0.1$) when the cross-linking degree increases (Fig. 4). All the cross-linked pectins tested were rapidly degraded after the same profile, indicating that all these products can be potentially used for the specific delivery of drugs to the colon.

However, some authors have reported a more substantial decrease of the bacterial or enzymatic degradation of cross-linked polysaccharides (metha-

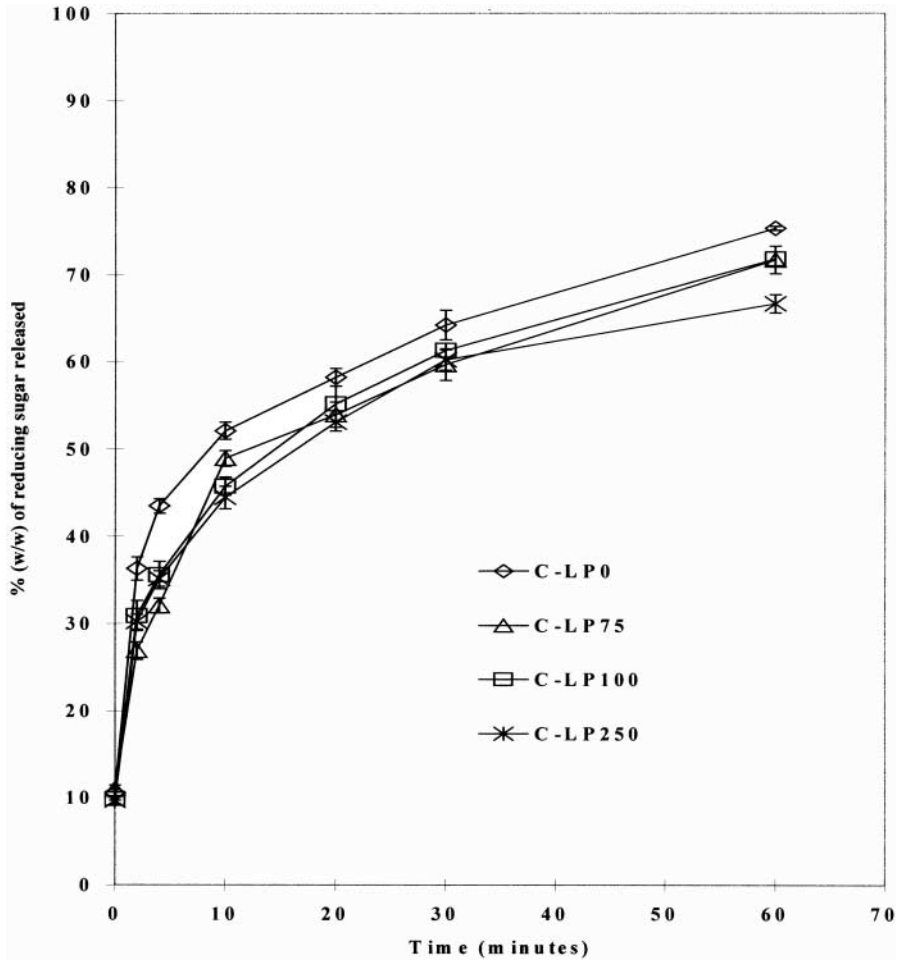

Figure 4. Percentages of reducing sugars released from epichlorohydrin cross-linked pectins (0.5% w/w) incubated in 0.05 M acetate–phosphate buffer (pH 4.5) at 37°C and in the presence of 200 μL/100 mL of Pectinex Ultra SP-L.

crylated inulin and dextrans hydrogels) when the cross-linking degree increases.[27,28] In these studies, the enzymatic degradation of original polysaccharide was much more slower than that of pectin HM by the pectinolytic enzymes. In addition, our results (rapid enzymatic degradation and low influence of the cross-linking degree) can be explained by the fact that the cross-linked pectins synthesized are only different from the natural pectin HM by the molecular weight and the presence of glycerol (Fig. 1). The cross-linking reaction, involving the incorporation of glycerol molecules in the structure of pectin, results in the formation of sufficiently hydrophilic derivatives, which can be solvated by the incubation media. The pectinolytic enzymes of Pectinex Ultra SP-L, which can easily access the osidic bounds present in solvated cross-linked pectins, can therefore rapidly hydrolyze them with a low influence of the cross-linking degree.

CONCLUSIONS

The chemical cross-linking of pectin with epichlorohydrin allowed us to prepare new hydrophilic pectin derivatives much less soluble in water. Degradation of these derivatives by commercial pectinolytic enzymes, similar to those existing in the colon, has been shown to be fast (60–80% of the pectin osidic bounds are broken in less than 1 hr) and not dramatically affected by the extent of the cross-linking degree. The results obtained show that all the epichlorohydrin cross-linked pectins can

be potentially used, under the form of matrix tablets, for the specific delivery of drugs to the colon.[29] Indeed, although the pH of the colon is higher than 4.5 units, it is reasonable to believe that matrix tablets based on the insoluble cross-linking pectins, will be able to prevent drug release in the upper parts of the gastrointestinal tract. In the colon, they will be degraded by bacterial enzymes, resulting in a huge release of the incorporated drug.

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