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Functionalization of pectin by periodate oxidation

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ABSTRACT

High methoxy citrus pectin was oxidized by periodic acid to prepare a dialdehyde functionalized material. The effect of various reaction conditions, viz., reaction time, reaction temperature, pH of the medium, periodic acid concentration and solvent composition on the oxidation process was investigated. With an increase in the reaction time, the aldehyde content increased. However, the intrinsic viscosity of the system decreased indicating that degradation takes place simultaneously with oxidation. The amount of aldehyde generated also increased with an increase in reaction temperature and the concentration of periodic acid. Due to the polyanionic behaviour of pectin, greater aldehyde contents were obtained at lower pH. Keeping all other reaction conditions constant, greater aldehyde contents were obtained in water–ethanol system than in pure aqueous medium. Increase in the ethanol content increased the amount of aldehyde generated. FTIR spectra of oxidized pectin systems show a carbonyl peak at 1734 cm^{-1}. They further reveal that partial ionisation of—COOH groups takes place leading to a peak at 1614 cm^{-1}.

1. Introduction

Natural materials have created enormous interest as biomaterials for human healthcare systems. They are being widely investigated as coating materials (Fu et al., 2005; Saxena et al., 2011), wound dressings (Gupta, Arora, Saxena, & Alam, 2009) and in tissue engineering (Letourneur, Champion, Slaoui, & Jozefonvicz, 1993). Pectin is a natural polysaccharide, poly (1,4-galacturonic acid), which is obtained from the cell walls of terrestrial plants and exhibits polyanionic behaviour. Pectin has traditionally been used in food industry as a gelling agent (Farris et al., 2011; May, 1990) for jams and jellies. Li et al. synthesised saponified citrus pectin and used it effectively for the adsorption of heavy metals, such as lead, zinc and cadmium from waste water (Li, Yang, Zhao, & Xu, 2007). Tripathi et al. developed an antimicrobial chitosan/poly (vinyl alcohol)/pectin ternary film for food packaging applications. It exhibited good antimicrobial activity against various pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pesudomonas* and *Candida albicans* (Tripathi, Mehrotra, & Dutta, 2010). Pectin/chitosan/Eudragit® RS ternary films were developed (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007). These films were found to be capable of sigmoidal drug delivery and therefore could be potentially used as targeted drug delivery vehicles. Liu et al. developed pectin/poly(lactide-co-glycolide) double network composite matrices for tissue regeneration (Liu et al., 2004). Pectin–NH_2 and chondroitin sulphate polyelectrolyte complexes were synthesized with potential applications as biomaterials (Fajardo, Lopes, Pereira, Rubira, & Muniz, 2012). Pectin has been crosslinked with diazoresin by photo irradiation to fabricate cell culture supports (Plewa et al., 2011). Human bone stromal cells were successfully seeded upon this medium. In another study, Munarin et al. investigated pectin based injectable biomaterials for bone tissue engineering (Munarin et al., 2011). Pectin grafted with arginylglycylasparticacid (RGD) peptide was used in lieu of an extracellular matrix. MC3T3-E1 preosteoblasts were immobolized on these scaffolds and exhibited a constant viability and good differentiation. Takei et al. coupled doxorubicin, an anticancer drug, with periodate oxidized citrus pectin and used it as an injectable drug delivery system (Takei, Sato, Ijima, & Kawakami, 2010). Pectin was blended with poly (vinyl alcohol) to improve its mechanical properties and used for colonic drug delivery systems (Mishra, Datt, & Banthia, 2008). Cipriani et al. found that chemically sulphated citrus pectin fractions possessed good antithrombogenic properties and hence offers excellent applications in healthcare systems (Cipriani et al., 2009). The hydrocolloid nature of pectin makes it attractive for usage in wound dressing applications. DuoDERM®, Granuflex®, Hydrocoll® and CitruGel® are a few commercial wound dressings based on pectin. Granulflex® and Hydrocoll® are formulations of pectin, gelatin and carboxymethylcellulose while DuoDERM® and CitruGel® are based on pectin and carboxymethylcellulose. In one study, papain was immobilized on pectin and the films were found to accelerate wound healing without any adverse secondary effects

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(Segura-Ceniceros et al., 2006). Pectin was used as a coating on chitosan/Tencel nonwoven mesh to fabricate a composite wound dressing, which was found to exert a positive influence on wound healing capability (Lin, Lou, Lu, Huang, & Lin, 2008). Pectin has been modified by various means to introduce different groups.

Functionalization of pectin offers enormous possibilities to transform this material into a wide range of interesting products. The vicinal diols present at the C2 and C3 carbons of the anhydro D-glucopyranose ring in pectin undergo oxidation by periodic acid to yield a dialdehyde structure (Kim, Kuga, Wada, Okano, & Kondo, 2000; Kim, Wada, & Kuga, 2004; Li, Wu, Mu, & Lin, 2011; Vicini et al., 2004). During this process, degradation of the polymer chains takes place, leading to shorter chain lengths and reduction in the molecular weight (Balakrishnan, Lesieur, Labarre, & Jayakrishnan, 2005). A precise study involving the correlation of the structure with the reaction parameters still needs to be analysed. The reaction conditions play a very crucial role in determining the yield, reaction kinetics and the characteristics of the end product. In the current study, we have studied the effect of the reaction conditions, i.e. time of reaction, temperature of reaction, pH of the reaction medium, periodic acid concentration and solvent composition, on the oxidation of pectin to obtain the maximum amount of usable product.

2. Experimental

2.1. Materials

Citrus pectin ($M_w \sim 30{,}000$ g/mol, degree of esterification ~ 72%) and 2,4-dinitrophenylhydrazine were purchased from CDH Fine Chemicals, India. They were used as received. Periodic acid, absolute ethanol, and isopropanol were obtained from Merck Chemicals, India. All other chemicals used were of analytical grade. Millipore water was used for all the experiments.

2.2. Oxidation of pectin by periodic acid

2.2.1. Oxidation in aqueous medium

To a 2% solution of pectin in distilled water, 3 mL of periodic acid of varying concentration (0.1–1.2 M) was added. The pH (2–8) of the medium was maintained using dilute hydrochloric acid and sodium bicarbonate solution. The reaction was allowed to take place under constant stirring for specific time periods (10–240 min) at different temperatures (20–60 °C). To prevent autooxidation due to light, the reaction vessel was wrapped in several layers of aluminium foil and the reaction was carried out in the dark. At the end of the reaction, the oxidized pectin was precipitated out using excess isopropanol. The oxidized product was separated by vacuum filtration. Unless otherwise specified, all the oxidation reactions were carried out in aqueous medium.

2.2.2. Oxidation in water–ethanol mixture

2 g of pectin were dissolved in water–ethanol mixtures of varying compositions (5–20% ethanol). To these solutions, 3 mL of 0.5 M periodic acid was added. The pH of the solutions was maintained at 3.5 using dilute hydrochloric acid and sodium bicarbonate solution. The reaction vessel was wrapped in several layers of aluminium foil to prevent auto oxidation due to light. The reaction was carried out under constant stirring for a period of 2 h at 40 °C. After 2 h, the oxidized pectin was precipitated out in excess isopropanol. The oxidized product was separated using vacuum filtration.

2.3. Determination of the aldehyde content

Hydrazines are known to react with aldehydes to yield hydrazones (Shannon & Barany, 2004; Lynch, Lim, Thomas, & Peters, 1983; Igawa, Munger, & Hoffmann, 1989; Andreoli, Manini, Corradi, Mutti, & Niessen, 2003). Dinitrophenylhydrazine (DNPH) can therefore react with oxidized pectin to produce dinitrophenylhydrazone. Hence, the amount of DNPH consumed is equivalent to the amount of aldehyde generated. Briefly, 100 μL of 0.3% oxidized pectin was added to 10 mL of freshly prepared DNPH solution. The reaction mixture was allowed to stand for 1 h and then centrifuged at 7000 rpm for 10 min. The absorbance of unreacted DNPH in the supernatant fluid was measured at $\lambda = 326.4$ nm using a Perkin Elmer Lambda 35 UV-VIS spectrophotometer. The amount of aldehyde generated was calculated according to:

$$\text{Aldehyde conc. (mmol/g)} = \left[\frac{\text{Reacted DNP (mmol/g)}/198.14}{3 \times 10^{-4}}\right]$$

where 198.14 is the molecular weight of DNPH.

2.4. Determination of intrinsic viscosity

The intrinsic viscosity measurement was carried out according to the procedure followed by Muhidinov et al. (2010). Pectin solutions of varying concentrations were prepared using 0.1 M NaCl as the solvent. The viscosity measurements were carried out using an Ubbelohde viscometer at a temperature of 30 ± 0.1 °C. This temperature was maintained using a Julabo 31A constant temperature water bath. All the measurements were carried out in triplicate. The solvent flow time was found to be 82.7 ± 10 s. The intrinsic viscosity [η] was calculated according to the following equations

$$\eta_{sp} = \frac{ti - t_o}{t_o}$$

$$[\eta] = \lim_{c \to 0}\left(\frac{\eta_{sp}}{c}\right)$$

where, t_i, t_o, η_{sp} and c are the elution time of solution, elution time of solvent, specific viscosity and concentration of the solution in g/dL, respectively.

The intrinsic viscosities were calculated by extrapolating the η_{sp}/c vs. c plots to infinite dilution.

2.5. FTIR spectroscopy

FTIR spectra of virgin pectin and oxidized pectin films were recorded using a Perkin Elmer Spectrum-BX FTIR system. The samples were scanned in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. X-ray diffraction (XRD)

Wide angle X-ray diffraction patterns were obtained using a PANalytical X'Pert PRO instrument, operating at 40 kV and 40 mA using sealed-tube Cu $K\alpha$ radiation ($\lambda = 1.54$ Å).

3. Results and discussion

The aim of the work is to oxidize pectin into a dialdehyde structure so that it can be used to prepare wound care materials. Pectin undergoes oxidation by periodic acid according to the Malaprade mechanism (Kim et al., 2000, 2004; Li et al., 2011; Vicini et al., 2004). The vicinal diols present on the C2 and C3 carbons of the anhydro-D-glucopyranose ring undergo oxidation to yield a dialdehyde structure. Complete oxidation is not possible, either in aqueous medium or in water–ethanol systems, due to the formation of hemiacetals between the aldehyde and neighbouring hydroxyl groups (Balakrishnan et al., 2005; Kim et al., 2004; Li et al.,

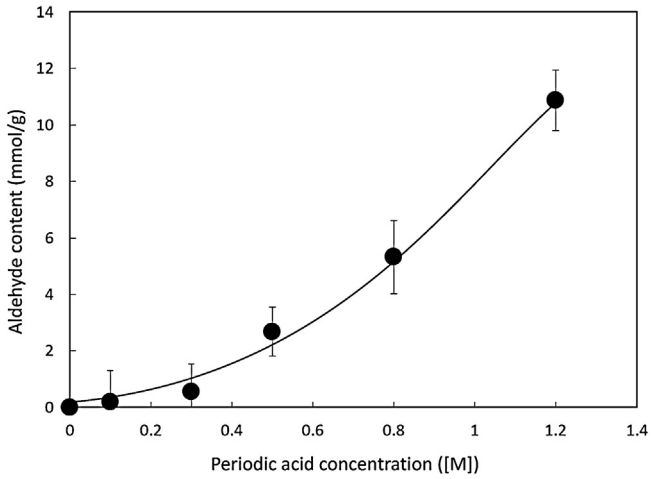

Fig. 1. Effect of the periodic acid concentration on the aldehyde content of oxidized pectin. Reaction time 2 h; reaction temperature 40 °C; pH 3.5.

2011; Scott, Tigwell, Phelps, & Nieduszynski, 1976; Vicini et al., 2004).

The amount of aldehyde generated during the oxidation reaction was estimated by monitoring the amount of unconsumed DNPH after reaction with oxidized pectin. The variation in aldehyde content with the concentration of periodic acid is shown in Fig. 1. It is seen that there is an exponential increase in the aldehyde concentration with an increase in the periodic acid concentration (Balakrishnan et al., 2005; Varma & Kulkarni, 2002). As the concentration of periodic acid increases, a larger number of periodate ions are available which can attack multiple number of vicinal diols, simultaneously. The probability of hemiacetal formation is reduced, leading to an increase in the number of aldehyde groups formed.

Unlike cellulose which is an uncharged polysaccharide, pectin exhibits polyanionic behaviour. This implies that the pH of the reaction medium plays an important role in the oxidation process. The effect of pH on the aldehyde concentration is presented in Fig. 2. As the pH is increased, the aldehyde concentration is reduced. The negative electrostatic field of anionic polysaccharides repels the negatively charged periodate ions from entering the domain of the polysaccharide (Scott & Harbinson, 1968). This can be countered by reducing the pH of the reaction medium. Therefore, when the pH is increased from 2.37 to 6.23, we see a marked difference in the values of the aldehyde concentration, 3.6 mmol/g and 0.84 mmol/g, respectively.

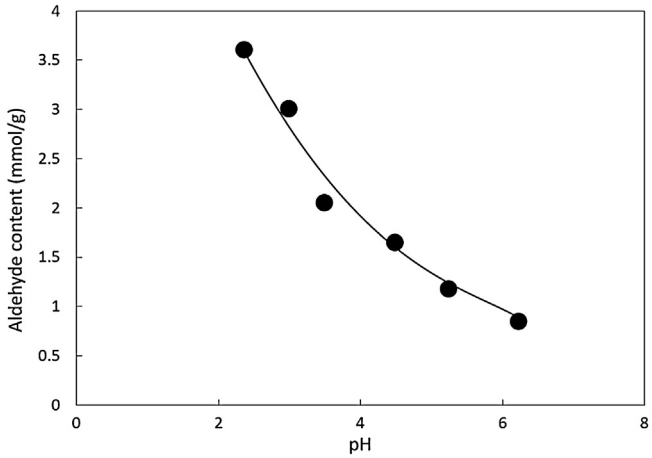

Fig. 2. Effect of pH on the aldehyde content of oxidized pectin. Reaction time 2 h; reaction temperature 40 °C; periodic acid concentration 0.5 M.

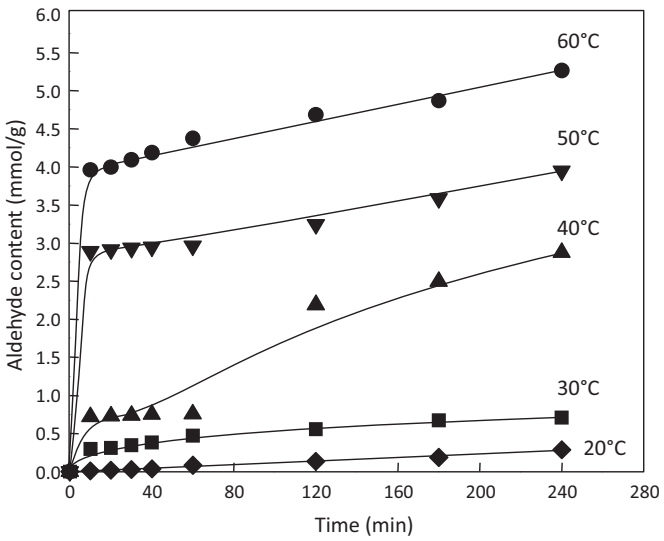

Fig. 3. Effect of the reaction time on aldehyde content at various reaction temperatures. pH 3.5; periodic acid concentration 0.5 M.

The effect of the reaction time and increasing temperature on the aldehyde content is presented in Figs. 3 and 4(a). It is evident that the aldehyde concentration follows an increasing trend with an increase in the time of reaction. We can observe that there is a pronounced increase in the aldehyde concentration with an increase in the reaction temperature. This result coincides well with those reported in the literature (Calvini, Gorassini, Luciano, & Franceschi, 2006; Varma & Kulkarni, 2002). For example, the aldehyde concentration after 2 h at 20 °C is 0.13 mmol/g while it is 4.68 mmol/g at 60 °C. Generally for strong acids, to which class periodic acid belongs, the dissociation constant increases with an increase in the reaction temperature. This means that for a given concentration of periodic acid, a larger number of periodate ions are available at a higher temperature than at a lower temperature. Simultaneous attack on a greater number of vicinal diols can take place with a

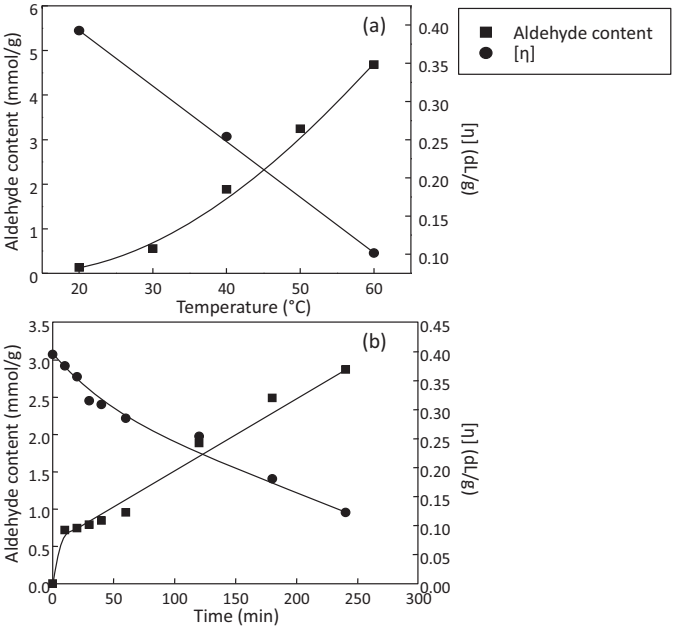

Fig. 4. Variation in intrinsic viscosity and aldehyde content with (a) reaction temperature and (b) reaction time. Reaction time 2 h; pH 3.5; periodic acid concentration 0.5 M.

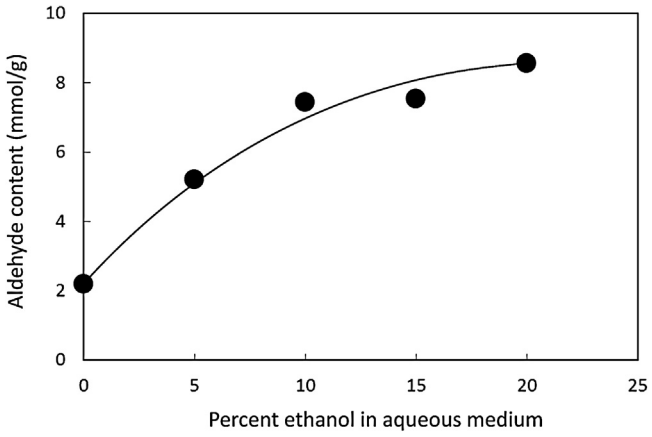

Fig. 5. Effect of solvent composition on the aldehyde content. Reaction time 2 h; reaction temperature 40 °C; pH 3.5; periodic acid concentration 0.5 M.

reduced hemiacetal formation. At the same time, the greater dissociation at higher temperatures leads to a lower pH of the system (Calvini et al., 2006). This statement implies that the pH of the medium is undergoing a decrease as the reaction progresses. As seen in Fig. 2, the aldehyde concentration increases with a reduction in the pH of the medium. This leads to a conclusion that the oxidation of pectin by periodic acid is a two way process. One route by which a larger number of periodate ions attack vicinal diols at a higher temperature and the second route in which the pH of the medium is reduced at higher temperature leading to a diminishing electrostatic repulsion between the negatively charged periodate ions and the polyanionic chain.

The usability of a polymer is limited by its molecular weight. According to the Mark Houwink Sakurada equation, molecular weight is directly proportional to the intrinsic viscosity (Torres, Robin, & Boutevin, 2000; Beer, Wood, & Weisz, 1999; Snyman, Hamman, Kotze, Rollings, & Kotzé, 2002; Muhidinov et al., 2010). The effect of the reaction time and reaction temperature on the molecular weight and degradation behaviour of pectin can therefore be understood by examining the intrinsic viscosity. This is presented in Fig. 4(a and b). There is an inversely proportional relationship between reaction time and the intrinsic viscosity of the system. During the periodate oxidation of polysaccharides, acid catalysed cleavage of the β-1,4-glycosidic linkages holding the pyranose rings together takes place (Calvini et al., 2006; Balakrishnan et al., 2005; Li et al., 2011). Over long periods of time and at higher temperatures, physical degradation can also take place by the breaking of intermolecular and intramolecular H-bonds (Li et al., 2011). From the plot in Fig. 4(a), we can understand that an increase in the reaction temperature leads to a reduction in the intrinsic viscosity. At higher temperatures, more energy is available to the system leading to an enhanced number of collisions. Increase in reaction temperature implies a decrease in the pH of the system leading to a boost in the rate of degradation (Calvini et al., 2006).

The nature of the solvent plays a crucial role in determining the reaction kinetics, yield, and the characteristics of the end product. We utilized a mixed solvent system – water and ethanol – of varying composition and studied its effects on the periodate oxidation of pectin. The variation in aldehyde concentration with increasing ethanol content is shown in Fig. 5. Since pectin is insoluble in organic solvents, we expected a lower degree of oxidation as compared to that in aqueous medium. However, we observed that the opposite is true. In a similar work on periodate oxidation of alginate, Balakrishnan et al. (2005) suggested that the hemiacetal formation is somewhat hindered in water–ethanol

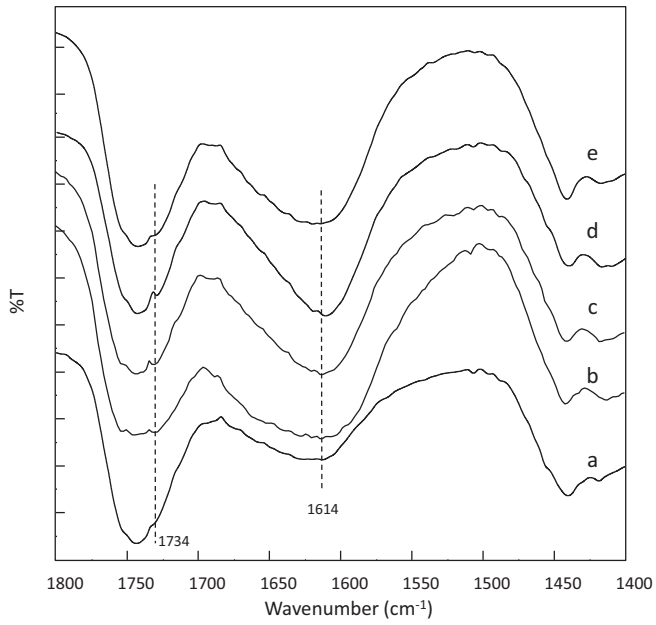

Fig. 6. FTIR spectra of (a) virgin pectin and oxidized pectin with varying aldehyde content; (b) 1.89 mmol/g; (c) 3.25 mmol/g; (d) 5.27 mmol/g; (e) 8.56 mmol/g (synthesized in 80/20 water/ethanol system).

systems due to minimal dissolution of alginate. This reasoning holds good for all polysaccharides and therefore to pectin. Beyond 80/20 water/ethanol composition, we found that pectin precipitated out and hence the solvent composition was not varied any further.

In Fig. 6, we present the FTIR spectra of oxidized pectin with increasing aldehyde concentration as compared to neat pectin. While the peak at ~1740 cm^{-1} is ascribed to the R–CO–R′ ester stretching, the carbonyl peak can be observed at ~1734 cm^{-1}. After an intensive study, Fan et al. concluded that the peak for the dialdehyde in periodate oxidized polysaccharides is seen in a very narrow range of 1732–1734 cm^{-1} (Fan, Lewis, & Tapley, 2001). Our finding coincides very well with this report. The broad peak at ~1637 cm^{-1} is assigned to the stretching of H_2O in neat pectin. The stretch at 1614 cm^{-1} is assigned to the free COO^- groups in oxidized pectin. As the aldehyde content increases, this peak appears to have narrowed down and become sharper. It has been reported by several authors that this is due to the partial ionization of —COOH groups on the polysaccharide backbone above a threshold pH of 2.5 (Calvini et al., 2006).

On the other hand, in the FTIR spectra of oxidized pectin synthesized in water–ethanol and pure water (Fig. 6(e)), we observe that the peak intensity at 1614 cm^{-1} is lesser in the case of the water–ethanol system when compared to the water system, even though the aldehyde concentration is greater (8.56 mmol/g). This indicates that the ionization of —COOH groups on the polysaccharide backbone is somewhat hindered in the mixed solvent system.

Pectin exhibits two characteristic crystalline peaks at $2\theta \sim 13^\circ$ and 31°. Upon oxidation, there is no shift in the peak position. However, with an increase in the aldehyde content, the relative intensity of these two peaks increases as shown in Fig. 7. During periodate oxidation, pectin undergoes degradation producing shorter chains. By virtue of their short length, these chains have greater mobility. Thus, they can diffuse faster through the polymer matrix and order themselves better leading to perfection in the crystalline structure. It can be hypothesized that while the size of the crystalline regions has increased, the length of the amorphous tie molecules between the crystal zones is reduced.

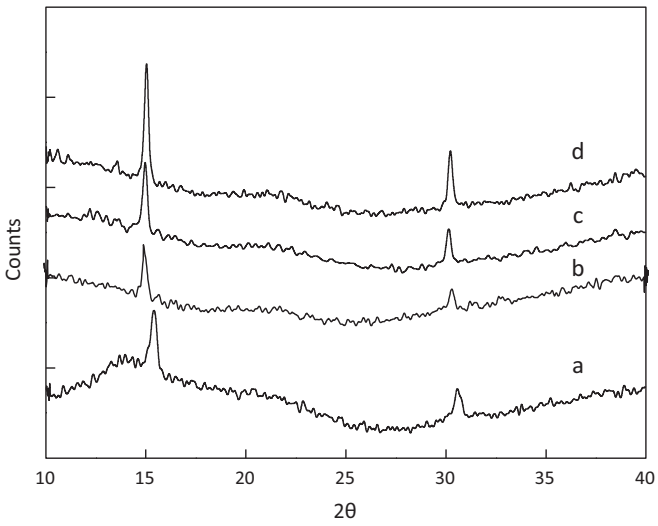

Fig. 7. X-ray diffractograms of (a) virgin pectin and oxidized pectin with varying aldehyde content (b) 3.25 mmol/g; (c) 4.18 mmol/g; (d) 5.27 mmol/g.

4. Conclusions

Oxidation of pectin, a natural polysaccharide, by periodic acid takes place according to the Malaprade mechanism to yield a dialdehyde structure. Oxidation is accompanied by degradation leading to a decrease in the intrinsic viscosity. Increase in reaction time, reaction temperature and periodic acid concentration leads to an increase in the aldehyde concentration. Due to the polyanionic behaviour of pectin, oxidation can take place more effectively at lower pH than at higher pH values. The relatively less intense electrostatic effect at lower pH values promotes oxidation process. Higher values of aldehyde concentration can be obtained using mixed water–ethanol system as the solvent. The hemiacetal formation is partially hindered in the mixed system leading to an increase in the aldehyde concentration. This comprehensive study would help in optimizing the reaction parameters to obtain the maximum yield of usable product according to the desired application.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.06.069.

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