Carbohydrate Polymers 106 (2014) 312–318

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation and characterization of *in-situ* crosslinked pectin–gelatin hydrogels

Bhuvanesh Gupta [a,*], Mythili Tummalapalli [a], B.L. Deopura [a], M.S. Alam [b]

[a] *Bioengineering Lab, Department of Textile Technology, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India*
[b] *Department of Chemistry, Jamia Hamdard, New Delhi 110062, India*

ARTICLE INFO

Article history:
Received 4 January 2014
Received in revised form 25 January 2014
Accepted 5 February 2014
Available online 14 February 2014

Keywords:
Oxidized pectin
Gelatin
Crosslinking
Schiff base
Hydrogel
Glycerol

ABSTRACT

Crosslinked hydrogels were developed by *in-situ* reaction of periodate oxidized pectin (OP) and gelatin. The reaction takes place through the formation of Schiff bases between aldehyde groups of OP and amino groups of gelatin. The effect of various process parameters such as reaction time, reaction temperature, pH of the reaction and composition on the efficacy of the crosslinking was investigated. Field emission scanning electron micrsocopy (FESEM) revealed that homogenous, single phase systems are obtained after the crosslinking of OP and gelatin. The swelling characteristics of the hydrogels were monitored. The equilibrium swelling varies in the range of 195–324% with a variation in the gelatin content (10–40%). Glycerol, when used as a plasticizer, improved the flexibility and the handling characteristics of the crosslinked hydrogels. Plasticized films retained good tensile strengths in the range of 19–48 MPa. By proper selection of the reaction conditions, the efficiency of crosslinking can be controlled to obtain the optimum results.

1. Introduction

Polymers derived from natural resources in the arena of human healthcare such as drug delivery (Megeed, Cappello, & Ghandehari, 2002; Elzoghby, Samy, & Elgindy, 2012), wound care (Chen, Wang, Chen, Ho, & Sheu, 2006; Vasconcelos, Pêgo, Henriques, Lamghari, & Cavaco-Paulo, 2010; Peng et al., 2013) and tissue engineering (Zander, Orlicki, Rawlett, & Beebe, 2012; Van Vlierberghe, Dubruel, & Schacht, 2011; Gomes, Leonor, Mano, Reis, & Kaplan, 2012). Most proteins are hydrogels by nature, non-toxic, biocompatible, and biodegradable. These features render proteins extremely attractive for healthcare applications. Wound dressings represent an innovative domain of medical technology where proper healing of a wound is assisted by the use of a thin coating consisting of different polymers. Moist environment, high exudate absorption and scar prevention are the features of a proper wound dressing. Hydrogels based on chitosan and polyvinyl alcohol have been developed into dressings which are antimicrobial in nature and show good exudate absorption (Agarwal, Alam, & Gupta, 2013; Gupta, Arora, Saxena, & Alam, 2009).

Gelatin can undergo gelation and has a tamponading effect. It has been reported that gelatin sponges are used for inducing hemostasis in bleeding wounds (Balakrishnan, Mohanty, Umashankar, & Jayakrishnan, 2005). Kanokpanont et al. fabricated an innovative bi-layered wound dressing comprised of a fibroin woven fabric and a sericin/gelatin sponge (Kanokpanont, Damrongsakkul, Ratanavaraporn, & Aramwit, 2012). These dressings exhibited controlled biodegradation and accelerated wound healing. Electrospun mats of the polysaccharide chitosan and proteinous gelatin were prepared (Rujtanaroj, Pimpha, & Supaphol, 2008). The incorporation of silver nanoparticles conferred antibacterial activity and these mats have potential applications in wound management. *In-situ* gellable wound dressings of oxidized alginate and gelatin were synthesized by Balakrishnan et al. which exhibited very good wound healing efficacy when tested on full thickness wounds on rat models. Random and aligned polycaprolactone/gelatin electrospun scaffolds which encouraged nerve differentiation and proliferation were used successfully as supports for nerve regeneration (Ghasemi-Mobarakeh, Prabhakaran, Morshed, Nasr-Esfahani, & Ramakrishna, 2008). Although gelatin possesses several excellent properties, its mechanical properties pose a problem and a variety of modification techniques, both physical and chemical, are being employed to improve the mechanical properties of gelatin gels (Lee & Mooney, 2001). Recently, a lot of interest has been generated in fabricating *in-situ* gellable, non-toxic hydrogels based on proteinous materials and polysaccharides (Cortesi et al., 1999; Dawlee, Sugandhi, Balakrishnan, Labarre, & Jayakrishnan, 2005; Gao, Yan, Dai, & Wan, 2012; Li, Wan, Li, Liang, & Wang, 2009; Liao, Zhang, & Chen, 2009).

* Corresponding author. Tel.: +91 11 26591416; fax: +91 11 26581061.
E-mail address: bgupta@textile.iitd.ernet.in (B. Gupta).

http://dx.doi.org/10.1016/j.carbpol.2014.02.019
0144-8617/

Pectin is an anionic polysaccharide hydrogel, poly(1,4-galacturonic acid), found in the cell walls of terrestrial plants. Although it has traditionally been used in food industry as a gelling agent, it has become a material of interest from the biomedical point of view in recent years. Tripathi et al. fabricated chitosan/poly(vinyl alcohol)/pectin ternary films that possessed excellent antimicrobiality for food packaging applications (Tripathi, Mehrotra, & Dutta, 2010). Pectin aerogels possess higher biodegradation rates than wheat starch, which is used as a standard for biodegradation (Chen, Chiou, Wang, & Schiraldi, 2013). Pectin/chitosan/Eudragit® RS ternary films capable of sigmoidal drug delivery were developed (Ghaffari et al., 2007). Oxidized citrus pectin was coupled with an anticancer drug, doxorubicin, for targeted drug delivery (Takei, Sato, Ijima, & Kawakami, 2010). Cipriani et al. suggested that chemically sulfated citrus pectin fractions possess good antithrombogenic properties and therefore could be potentially used in wound care systems. A novel spray-on formulation of pectin and papain was synthesized which exhibited 20% faster wound healing efficacy in the first 4 days alone (Jáuregui et al., 2009). Under ambient conditions, pectin and gelatin form reversible polyion-complex hydrogels due to ionic interactions between positively charged gelatin and negatively charged pectin (Farris et al., 2011).

Functionalization of pectin offers enormous possibilities to transform this material into a wide range of interesting products such as in wound care systems. A complex set of properties is required for an efficient wound dressing, including exudate absorption capacity, good porosity for the permeation of water vapor and gases, biocompatibility and antimicrobial nature. In the current work, we aim at developing pectin and gelatin coatings on cotton fabric. Pectin confers hydrogel nature (Jung, Arnold, & Wicker, 2013; Moreira et al., 2014) while gelatin, being a denatured protein, offers a good medium for cell culture and growth (Li et al., 2013; Thirupathi Kumara Raja, Thiruselvi, Sailakshmi, Ganesh, & Gnanamani, 2013). The porosity is due to the cotton fabric. The aldehyde groups introduced in pectin *via* periodate oxidation would help in *in-situ* reduction of silver nitrate to nanosilver, which is known as an excellent antimicrobial agent (Choi, Yu, Esteban Fernández, & Hu, 2010; Gupta, Tummalapalli, Deopura, & Alam, 2013). The whole approach is therefore used to develop effective and biodegradable wound dressings. 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; Li, Wu, Mu, & Lin, 2011; Vicini et al., 2004; Balakrishnan & Jayakrishnan, 2005; Gupta, Tummalapalli, et al., 2013). It has been reported that the aldehyde groups generated can react with the amino groups of lysine and hydroxylysine of gelatin to form Schiff bases (Balakrishnan et al., 2005; Fang, Takahashi, & Nishinari, 2005; Dash, Foston, & Ragauskas, 2013; Draye et al., 1998). In the current study, we have attempted to examine the effect of various reaction conditions, *viz*. reaction time, reaction temperature, reaction pH and composition on the efficiency of this *in-situ* crosslinking reaction so that a plasticized material may be developed.

2. Experimental

2.1. Materials

Citrus pectin ($Mw \sim 30{,}000$ g/mol, degree of esterification ~ 72%) and 2,4-dinitrophenylhydrazine (DNPH) were purchased from CDH Fine Chemicals, India. Gelatin, from porcine skin (high gel strength) and acid orange 7 were procured from Fluka Analytical, Germany and Sigma Chemicals, India, respectively. Periodic acid was purchased from Merck Chemicals, India. Glycerol and isopropanol were obtained from Fisher Scientific, India. All other chemicals used were of analytical grade. Millipore water was used for all the experiments.

2.2. Crosslinking of oxidized pectin with gelatin

Oxidized pectin was synthesized according to the procedure reported in our earlier work (Gupta et al., 2013). Briefly, a known amount of gelatin was dissolved in 50 mL deionized water. Subsequently, a solution of oxidized pectin (aldehyde content 2.1 mmol/g) was prepared by dissolving a predetermined amount of material in deionized water. The two solutions were mixed and the reaction was allowed to take place under constant stirring for specific time periods (0.5–24 h) at different temperatures (60–90 °C). The pH (2–9) was maintained using dilute hydrochloric acid and sodium bicarbonate solution. At the end of the reaction, the crosslinked hydrogel (OP-Gel) was precipitated out using excess isopropanol. The crosslinked product was separated by vacuum filtration.

2.3. Plasticization of OP-Gel crosslinked system with glycerol

Crosslinked OP-Gel was mixed with appropriate amounts of glycerol (10–40 wt% of polymer) under constant stirring for 16 h at 60 °C. At the end of the reaction, the solutions were poured onto disposable polystyrene Petri dishes and then dried at ambient room temperature to produce air dried films.

2.4. Determination of the aldehyde content

The amount of aldehyde consumed and the fraction of free aldehyde were determined according to the procedure reported in our earlier work (Gupta et al., 2013). Briefly, 100 μL of 0.3% oxidized pectin–gelatin gel 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 λ = 326.4 nm using a Perkin Elmer Lambda 35 UV–VIS spectrophotometer. The amount of aldehyde consumed was calculated according to

$$\text{aldehyde content}\left(mmol/g\right) = \left[\frac{\text{reacted DNP}\left(mmol/g\right)/198.14}{3\times10^{-4}}\right]$$

where 198.14 is the molecular weight of DNP. The initial aldehyde content in OP has been found to be 2.1 mmol/g.

2.5. Determination of the amino content

The concentration of amino groups consumed in the crosslinking reaction was measured using the uptake of an acidic dye, according to the method adopted by Saxena et al. (Saxena, Ray, & Gupta, 2010). Briefly, 100 μL of 0.3% OP-Gel hydrogel was allowed to react with 20 mL acid orange 7 (AO7) (0.1 mg/mL) at a pH of 3. The reaction was allowed to continue for 5 h at a temperature of 30 °C. At the end of the reaction time, the solution was neutralized by the addition of NaOH solution. The entire solution was centrifuged at 7000 rpm for 10 min. The dye content was determined from the optical density of the supernatant solution at λ = 486 nm using a Perkin Elmer Lambda 35 UV–VIS spectrophotometer. The amount of amino consumed was calculated according to

$$\text{amino content}\left(mmol/g\right) = \left[\frac{\text{reacted AO7}\left(mmol/g\right)/350.32}{3\times10^{-4}}\right]$$

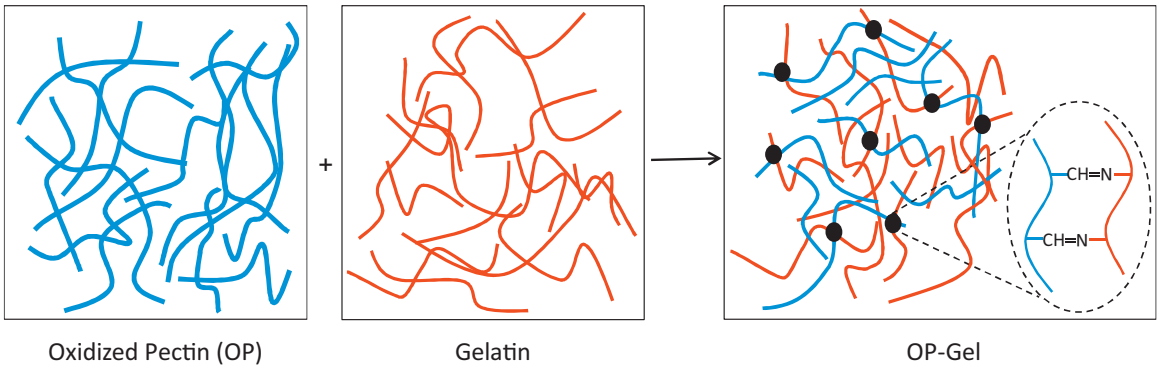

Fig. 1. Schematic representation of the *in-situ* crosslinking reaction between oxidized pectin and gelatin.

where 350.32 is the molecular weight of acid orange 7. The initial amino content has been found to be 1.9 mmol/g.

2.6. Brookfield viscometry

The viscosities of OP-Gel crosslinked solutions were measured by a Thermosel Brookfield Digital Viscometer using a spindle (Spindle number RV-1 and spindle factor 5) at 20 rpm. The viscosity η of the solution was calculated as

$$\eta\,(cp) = reading \times 5$$

2.7. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of oxidized pectin and OP-Gel crosslinked 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 2 cm^{-1}.

2.8. Field emission scanning electron microscopy (FESEM)

Vacuum dried OP-Gel crosslinked films were fractured under cryogenic conditions using liquid nitrogen. The fragments were mounted on a specimen stub with the fracture surfaces pointed upwards and coated with a thin layer of gold using a Hitachi E 1010 Ion Sputter coating machine. The surface morphology was examined by using Hitachi S4800 field emission scanning electron microscope operated at 20 kV. Magnification was maintained at 20kX.

2.9. Swelling studies

The swelling studies of the swollen hydrogels were conducted gravimetrically. The swelling temperature was set as 37 °C and the pH was maintained at 7.4 using a phosphate buffer saline (PBS) buffer for exploring potential biomedical uses and also other applications. At predetermined time intervals, the hydrogel samples were taken out from the buffer solution and weighed after removing the excess water on the surfaces with filter paper. The equilibrium swelling was determined as

$$equilibrium\ \ swelling\,(\%) = \left[\frac{W_s - W_d}{W_d} \times 100\right]$$

where W_d is the weight of the dry hydrogel and W_s is the equilibrium weight of swollen hydrogel (Raafat, Eid, & El-Arnaouty, 2012).

2.10. Tensile strength

Films were cut into rectangular strips of 5 mm width and 70 mm length. Tensile tests were performed on an Instron model 4202 mechanical tester. It was operated with a crosshead speed of 60 mm/min and gauge length of 50 mm at 27 ± 2 °C and 65 ± 2% relative humidity. Tenacity, modulus and ultimate elongation were obtained from the stress–strain curves which are an average for 10 specimens.

3. Results and discussion

Pectin is an anionic polysaccharide containing active carboxyl groups that can undergo ionic interaction with the negatively charged gelatin chains (Farris et al., 2011). However, these interactions are reversible, physical and weak in nature and do not confer any beneficial mechanical properties. Complex coacervation has been reported between pectin and gelatin for controlled drug delivery (Saravanan & Rao, 2010). The drawback in such systems is that an external crosslinker, such as formaldehyde or glutaraldehyde, is required for hydrogel formation. In order to eliminate the toxicity introduced by the use of these crosslinkers, the present work was carried out using an *in-situ* crosslinking methodology. In our previous work (Gupta et al., 2013), we have reported the functionalization of pectin by periodate oxidation to introduce aldehyde functional groups in the pectin backbone structure, which can undergo reaction with gelatin. The *in-situ* crosslinking reaction between oxidized pectin and gelatin is predominantly because of the Schiff base formation between the aldehyde groups of oxidized pectin and the amino groups present on the lysine and hydroxylysine components of gelatin (Gao et al., 2012). The schematic diagram in Fig. 1 elucidates this theory of linking between the two chains. The network thus obtained by the formation of C=N bonds helps in retaining the structural integrity and swellability of the fabricated hydrogel.

To understand the behavior of the crosslinking reaction, we have varied several reaction parameters, *viz.* reaction time, reaction temperature, pH of the reaction and gelatin content and studied their effect. In Fig. 2, the effect of reaction time on the crosslinking process in terms of the aldehyde and amino consumption is shown. Initially, with an increase in reaction time, the amount of consumed aldehyde and amino increased. After a time period of 16 h, this seems to level off at 1.21 mmol/g without any further appreciable change. This indicates that a saturation state has been reached where the maximum possible crosslinking takes place. Hence, 16 h was deemed to be the optimum reaction time for subsequent experiments. However, a significant amount of aldehyde and amino groups is left behind as unreacted ones. This may be due to the viscous reaction medium which diminishes the accessibility

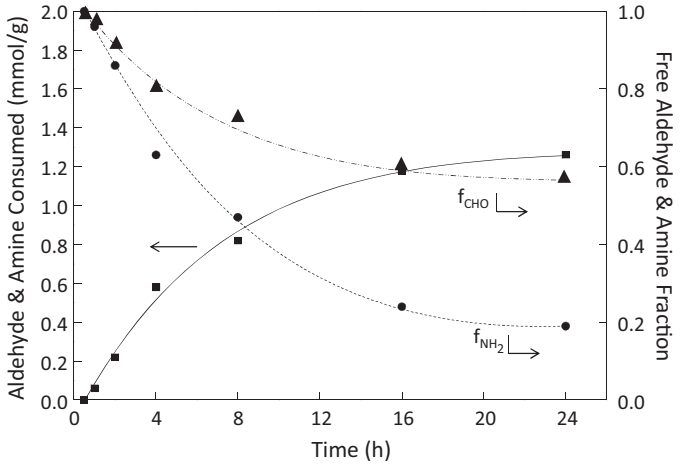

Fig. 2. Effect of reaction time on the aldehyde and amino consumption. Reaction temperature 60 °C; pH 4.3; gelatin content 20%.

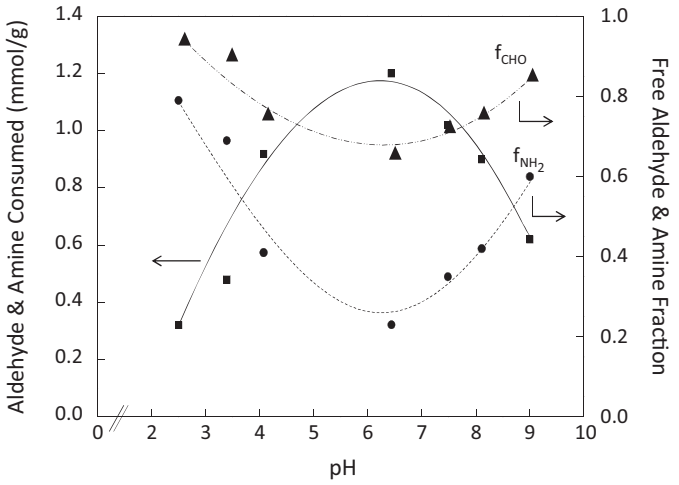

Fig. 4. Effect of pH on the consumption of aldehyde and amino groups. Reaction time 16 h; reaction temperature 60 °C; gelatin content 20%.

of the chains to link together by covalent crosslinking. Interestingly, almost half of the aldehyde groups remain unreacted.

Fig. 3 depicts the effect of reaction temperature on the crosslinking reaction between oxidized pectin and gelatin. Gelatin tends to gel out of the solution at temperatures below 50 °C, producing a phase separated system. In order to avoid such a circumstance, all the reactions were performed above 50 °C. One can observe that as the reaction temperature increases from 60 °C to 90 °C, the consumption of the aldehyde and amino functionalities decreased. This inversely proportional relationship can be explained by the fact that at higher temperatures, more energy is available to the system in which state the polymer chains can slip past each other rapidly. This leaves a very short window of time for interaction between the aldehyde and amino groups, leading to limited crosslinking.

Another important process parameter that affects the rate of reaction is the pH of the medium. This study is depicted in Fig. 4. While oxidized pectin is polyanionic in nature, gelatin is amphoteric by virtue of the presence of both carboxylic groups and amino groups. This implies that these macromolecules are extremely susceptible to even minute changes in the pH. In the pH range of 2–6.5, the consumption of aldehyde and amino is gradually increasing after which it steadily reduces. In the acidic pH region, the amino groups of gelatin are present in the protonated form and this hinders the crosslinking phenomenon (Schacht, Nobels, & Vansteenkiste, 1993). Within the pH range of 8–8.5, the isoelectric point of gelatin occurs where there is no net change on the molecule and phase separation takes place. It has been reported that rapid gelation takes place before the isoelectric point (Schacht et al., 1993). Above the isoelectric point, a net negative charge is developed on the gelatin chains which repels the electric field of oxidized pectin. This results in a net reduction in the extent of crosslinking. Ergo, the pH of 6.4 seems to be the most suitable for the crosslinking reaction.

The amount of gelatin was varied and the effect of this variation on the crosslinking reaction was studied as shown in Fig. 5. There is always a trade-off between the number of aldehyde groups and the number of amino groups necessary for optimum crosslinking. Too high or too low a quantity of either moiety leads to reduced interactions. The effect of composition on the viscosity of the OP-Gel crosslinked systems is presented (the Supplementary information). As the gelatin content increases, the viscosity of the system increases. Due to an increase in the viscosity, the mobility of the chains is reduced and the possibility of interaction decreases. However, it has to be kept in mind that not only the viscosity but the overall quantity of functional groups also affects the crosslinking reaction. Therefore, it can be stated that a gelatin content of 30% is optimum for network formation.

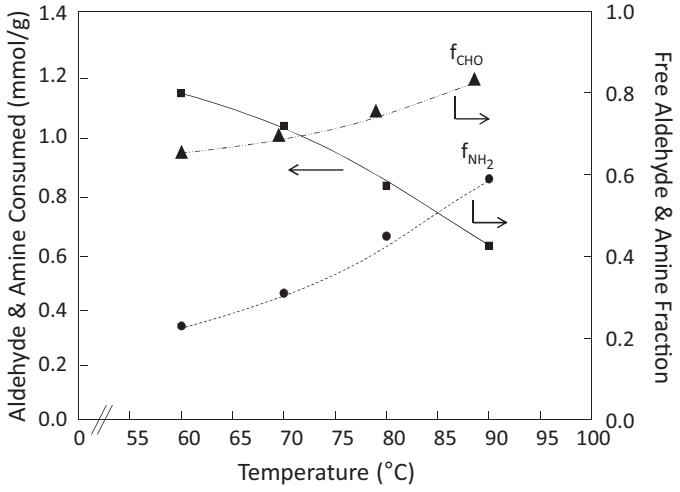

Fig. 3. Effect of reaction temperature on the aldehyde and amino consumption. Reaction time 16 h; pH 4.3; gelatin content 20%.

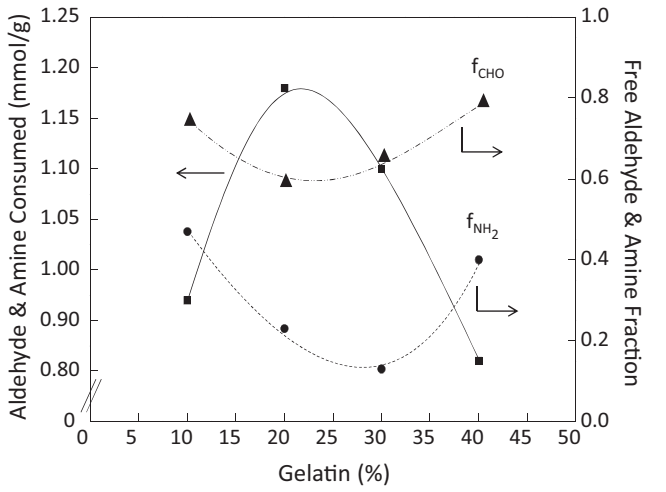

Fig. 5. Effect of composition on the consumption of aldehyde and amino groups. Reaction time 16 h; reaction temperature 60 °C; pH 6.4.

The FTIR spectra of the crosslinked networks are presented in Fig. 6. The nature of the interaction can be clearly elucidated. At a wavelength of 1734 cm^{-1}, the aldehyde peak stretching occurs (Gupta et al., 2013). This peak, although present in the crosslinked hydrogel, is of lower intensity indicating that the aldehyde groups are diminishing due to the reaction with gelatin. As the gelatin content in the polymer system increases, the carbonyl peak at 1734 cm^{-1} diminishes and a new peak at 1548 cm^{-1} appears. This corresponds to the amide II (–NH bending) peak of gelatin (Chang, Ko, & Douglas, 2003). Although C=N stretch seems to be present in the range of 1615–1650 cm^{-1}, it has similar conjugation effects to C=O stretch which is already present in oxidized pectin. Therefore, this interaction could not be clearly delineated. The optical density of the carbonyl stretch for the spectra in Fig. 6 was calculated relative to the –CH bending at 1452 cm^{-1} [$-CHO_{1734\,cm^{-1}}/-CH_{1452\,cm^{-1}}$] (Supplementary information). As mentioned in the earlier paragraph, there is a compromise between the number of aldehyde and amino functionalities necessary for effective crosslinking. The optical density of aldehyde groups initially reduces from 1.46 at 0% gelatin composition to 0.8 at 30% gelatin composition, beyond which it increases, indicating that more –CHO remains unreacted. Therefore, it can be stated that the maximum extent of crosslinking takes place at a 30% gelatin composition of OP/Gel when the reaction conditions were 16 h, 60 °C and pH 6.4.

Cryo-fractured surfaces of the crosslinked hydrogels were analyzed by the FESEM technique. The morphologies are shown in Fig. 7. The system is very homogenous without any phase separation for all compositions. This suggests that the interaction between oxidized pectin and gelatin is smooth leading to structural integrity.

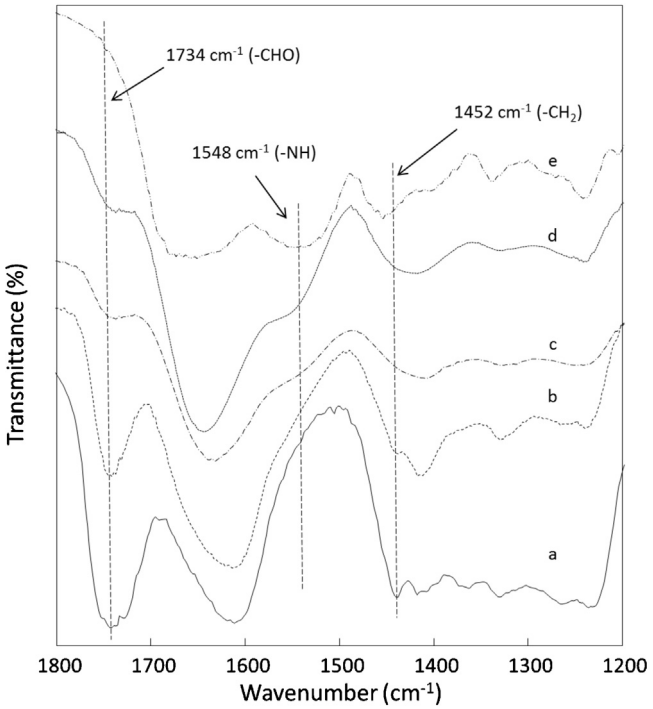

Fig. 6. FTIR spectra of (a) OP and OP-Gel with gelatin content of (b) 10%; (c) 30%; (d) 40%; (e) gelatin.

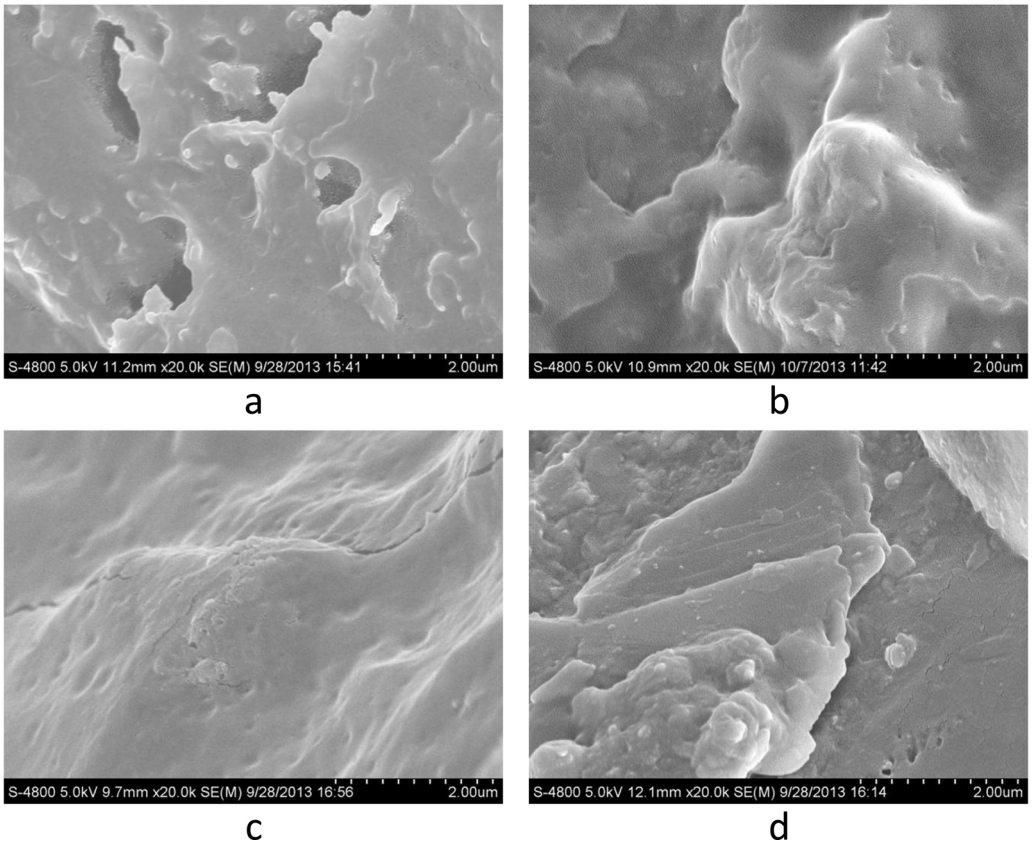

Fig. 7. FESEM analysis of *in-situ* crosslinked OP-Gel hydrogels with varying gelatin content of (a) 10%; (b) 20%; (c) 30%; (d) 40%. Reaction time 16 h; reaction temperature 60 °C; pH 6.4.

Table 1
Effect of glycerol content on the tensile properties of OP-Gel hydrogels. Gauge length 50 mm; strain rate 60 mm/min; load cell 10 kg.

Plasticizer content (%)	TS (MPa)	Elongation at break (%)	Young's modulus (MPa)
20	48.3 ± 0.7	4.6 ± 1.2	1808
30	35.2 ± 0.9	6.3 ± 2.4	1524
40	19.2 ± 0.5	8.9 ± 2.7	825

It is important to mention that the OP-Gel crosslinked hydrogels had reasonably good swelling without any dissolution. The equilibrium swelling values of OP-Gel hydrogels with gelatin content of 10%, 20%, 30% and 40% were 324%, 290%, 195% and 240%, respectively. It is observed that the swelling decreases from 324% to 195% when the gelatin content increases from 10% to 30%, and then increases. Conversely, the crosslink density increases with increase in the gelatin content up to 30% and further reduces. The swelling behavior of any system is governed by the hydrophilic–hydrophobic interactions. When the polymer–polymer interactions are lesser than the polymer–solvent interactions, swelling and eventually dissolution occurs. The crosslinks act as tethering points between different chains of the hydrogel and do not allow them to move away from each other. Since the crosslinking density is maximum at 30% gelatin content, the swelling ratio is the least. The large degree of swelling indicates that this hydrogel material is suitable in biological applications, especially as wound care materials.

Pectin is an inherently rigid polymer and has traditionally been modified with different materials to improve its mechanical properties (Mishra, Datt, & Banthia, 2008). To make the system flexible and easy to handle, glycerol was incorporated as the plasticizer. It has been reported that glycerol is a biocompatible and biodegradable material which does not inhibit cell growth and viability (Zhang, Doll, Beckman, & Hollinger, 2003) and therefore should be a good candidate for flexible films for biomedical applications. The mechanical properties of the plasticized hydrogels were analyzed in terms of their elongation at break, modulus and tensile strength. The tensile properties are elaborated in Table 1 and Fig. 8. The tensile properties of the non-plasticized hydrogels and 10% glycerol loaded hydrogels are not reported since they were too brittle to handle. It can be observed that on increasing the glycerol content from 20% to 40%, the Young's modulus drastically reduces from 1808 MPa to 825 MPa. The elongation at break, on the other hand, increases leading to the plasticized effect. This is beneficial in handling the hydrogel membranes as wound care materials, tissue regeneration scaffolds *etc*. Researchers have hypothesized that glycerol reduces the number of physical crosslinks between the macromolecular chains and introduces flexibility into the matrix (Coffin & Fishman, 1994).

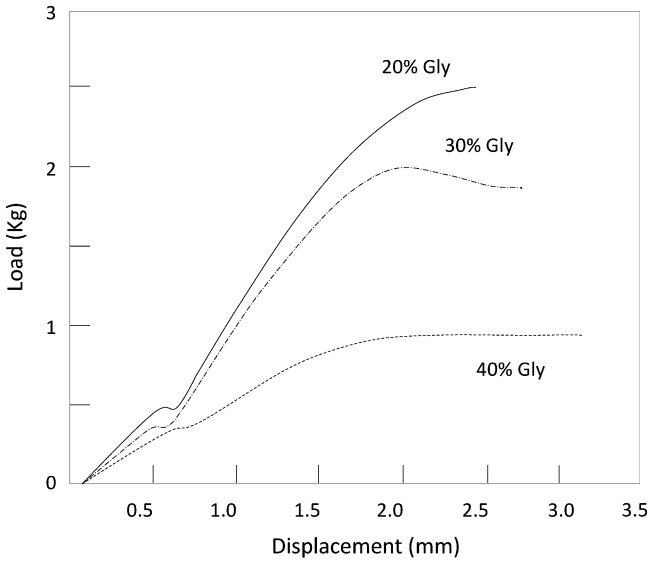

Fig. 8. Load–displacement curves of glycerol plasticized OP-Gel hydrogels under tensile stress. Gauge length 50 mm; strain rate 60 mm/min; load cell 10 kg. Reaction time 16 h; reaction temperature 60 °C; pH 6.4; gelatin content 30%.

4. Conclusions

The crosslinking of oxidized pectin and gelatin is predominantly due to the formation of imine bonds between aldehyde and amino moieties. The crosslinking reaction is affected by various process parameters—reaction time, reaction temperature, pH of reaction and composition. The degree of crosslinking increases with reaction time up to a certain limit and then stabilizes. An increase in reaction temperature leads to a decrease in the extent of crosslinking due to thermodynamic instability. The degree of crosslinking increases until a certain limit with an increase in pH or gelatin content, beyond which it reduces. The swellability of hydrogels with the highest degree of crosslinking is the least. The fabricated OP-Gel hydrogels are homogenous and do not exhibit any phase separation. Plasticization by glycerol introduces flexibility into the OP-Gel system, thus improving the handling ability. This comprehensive study would help in understanding the effect of various process parameters on the *in-situ* crosslinking of gelatin with oxidized pectin and in optimizing them to obtain an efficient product for further use in biomedical technology.

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.2014.02.019.

References

Agarwal, R., Alam, M. S., & Gupta, B. (2013). Preparation of curcumin loaded poly(vinyl alcohol)–poly(ethylene oxide)–carboxymethyl cellulose membranes for wound care application. *Journal of Biomaterials and Tissue Engineering*, *3*(3), 273–283.

Balakrishnan, B., & Jayakrishnan, A. (2005). Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials*, *26*(18), 3941–3951.

Balakrishnan, B., Mohanty, M., Umashankar, P. R., & Jayakrishnan, A. (2005). Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials*, *26*, 6335–6342.

Chang, M. C., Ko, C. C., & Douglas, W. H. (2003). Preparation of hydroxyapatite–gelatin nanocomposite. *Biomaterials*, *24*(17), 2853–2862.

Chen, H. B., Chiou, B. S., Wang, Y. Z., & Schiraldi, D. A. (2013). Biodegradable pectin/clay aerogels. *ACS Applied Materials & Interfaces*, *5*(5), 1715–1721.

Chen, R. N., Wang, G. M., Chen, C. H., Ho, H. O., & Sheu, M. T. (2006). Development of *N,O*-(carboxymethyl)chitosan/collagen matrixes as a wound dressing. *Biomacromolecules*, *7*(4), 1058–1064.

Choi, O., Yu, C. P., Esteban Fernández, G., & Hu, Z. (2010). Interactions of nanosilver with *Escherichia coli* cells in planktonic and biofilm cultures. *Water Research*, *44*(20), 6095–6103.

Cipriani, T. R., Gracher, A. H. P., de Souza, L. M., Fonseca, R. J. C., Belmiro, C. L. R., Gorin, P. A. J., et al. (2009). Influence of molecular weight of chemically sulfated citrus pectin fractions on their antithrombotic and bleeding effects. *Thrombosis and Haemostasis*, *101*(5), 860–866.

Coffin, D. R., & Fishman, M. L. (1994). Physical and mechanical properties of highly plasticized pectin/starch films. *Journal of Applied Polymer Science*, *54*(9), 1311–1320.

Cortesi, R., Esposito, E., Osti, M., Menegatti, E., Squarzoni, G., Spencer Davis, S., et al. (1999). Dextran cross-linked gelatin microspheres as a drug delivery system. *European Journal of Pharmaceutics and Biopharmaceutics*, *47*(2), 153–160.

Dash, R., Foston, M., & Ragauskas, A. J. (2013). Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers. *Carbohydrate Polymers*, *91*(2), 638–645.

Dawlee, S., Sugandhi, A., Balakrishnan, B., Labarre, D., & Jayakrishnan, A. (2005). Oxidized chondroitin sulfate-cross-linked gelatin matrixes: A new class of hydrogels. *Biomacromolecules*, *6*(4), 2040–2048.

Draye, J. P., Delaey, B., Van de Voorde, A., Van Den Bulcke, A., Bogdanov, B., & Schacht, E. (1998). In vitro release characteristics of bioactive molecules from dextran dialdehyde cross-linked gelatin hydrogel films. *Biomaterials*, *19*(1–3), 99–107.

Elzoghby, A. O., Samy, W. M., & Elgindy, N. A. (2012). Protein-based nanocarriers as promising drug and gene delivery systems. *Journal of Controlled Release*, *161*(1), 38–49.

Fang, Y., Takahashi, R., & Nishinari, K. (2005). Protein/polysaccharide cogel formation based on gelatin and chemically modified schizophyllan. *Biomacromolecules*, *6*(6), 3202–3208.

Farris, S., Schaich, K. M., Liu, L., Cooke, P. H., Piergiovanni, L., & Yam, K. L. (2011). Gelatin–pectin composite films from polyion-complex hydrogels. *Food Hydrocolloids*, *25*(1), 61–70.

Gao, C., Yan, T., Dai, K., & Wan, Y. (2012). Immobilization of gelatin onto natural nanofibers for tissue engineering scaffold applications without utilization of any crosslinking agent. *Cellulose*, *19*(3), 761–768.

Ghaffari, A., Navaee, K., Oskoui, M., Bayati, K., & Rafiee-Tehrani, M. (2007). Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit® RS intended for sigmoidal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *67*(1), 175–186.

Ghasemi-Mobarakeh, L., Prabhakaran, M. P., Morshed, M., Nasr-Esfahani, M. H., & Ramakrishna, S. (2008). Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials*, *29*(34), 4532– 4539.

Gomes, S., Leonor, I. B., Mano, J. F., Reis, R. L., & Kaplan, D. L. (2012). Natural and genetically engineered proteins for tissue engineering. *Progress in Polymer Science*, *37*(1), 1–17.

Gupta, B., Arora, A., Saxena, S., & Alam, M. S. (2009). Preparation of chitosan–polyethylene glycol coated cotton membranes for wound dressings: Preparation and characterization. *Polymers for Advanced Technologies*, *20*(1), 58–65.

Gupta, B., Tummalapalli, M., Deopura, B. L., & Alam, M. S. (2013). Functionalization of pectin by periodate oxidation. *Carbohydrate Polymers*, *98*(1), 1160–1165.

Jáuregui, K., Cabrera, J., Ceniceros, E., Hernández, J., & Ilyina, A. (2009). A new formulated stable papin–pectin aerosol spray for skin wound healing. *Biotechnology and Bioprocess Engineering*, *14*(4), 450–456.

Jung, J., Arnold, R. D., & Wicker, L. (2013). Pectin and charge modified pectin hydrogel beads as a colon-targeted drug delivery carrier. *Colloids and Surfaces B: Biointerfaces*, *104*(0), 116–121.

Kanokpanont, S., Damrongsakkul, S., Ratanavaraporn, J., & Aramwit, P. (2012). An innovative bi-layered wound dressing made of silk and gelatin for accelerated wound healing. *International Journal of Pharmaceutics*, *436*(1–2), 141–153.

Kim, U. J., Kuga, S., Wada, M., Okano, T., & Kondo, T. (2000). Periodate oxidation of crystalline cellulose. *Biomacromolecules*, *1*(3), 488–492.

Lee, K. Y., & Mooney, D. J. (2001). Hydrogels for tissue engineering. *Chemical Reviews*, *101*(7), 1869–1880.

Li, J., Wan, Y., Li, L., Liang, H., & Wang, J. (2009). Preparation and characterization of 2,3-dialdehyde bacterial cellulose for potential biodegradable tissue engineering scaffolds. *Materials Science and Engineering C*, *29*(5), 1635–1642.

Li, H., Wu, B., Mu, C., & Lin, W. (2011). Concomitant degradation in periodate oxidation of carboxymethyl cellulose. *Carbohydrate Polymers*, *84*(3), 881–886.

Li, S. S., Shi, J., Liu, L., Li, J. J., Jiang, L. M., Luo, C. X., et al. (2013). Fabrication of gelatin nanopatterns for cell culture studies. *Microelectronic Engineering*, *110*(0), 70–74.

Liao, H., Zhang, H., & Chen, W. (2009). Differential physical, rheological, and biological properties of rapid in situ gelable hydrogels composed of oxidized alginate and gelatin derived from marine or porcine sources. *Journal of Materials Science: Materials in Medicine*, *20*(6), 1263–1271.

Megeed, Z., Cappello, J., & Ghandehari, H. (2002). Genetically engineered silk-elastinlike protein polymers for controlled drug delivery. *Advanced Drug Delivery Reviews*, *54*(8), 1075–1091.

Mishra, R. K., Dutt, M., & Banthia, A. K. (2008). Synthesis and Characterization of Pectin/PVP Hydrogel Membranes for Drug Delivery System. *AAPS PharmSciTech*, *9*(2), 395–403.

Moreira, H. R., Munarin, F., Gentilini, R., Visai, L., Granja, P. L., Tanzi, M. C., et al. (2014). Injectable pectin hydrogels produced by internal gelation: pH dependence of gelling and rheological properties. *Carbohydrate Polymers*, *103*(0), 339–347.

Peng, L. H., Wei, W., Qi, X. T., Shan, Y. H., Zhang, F. J., Chen, X., et al. (2013). Epidermal stem cells manipulated by pDNA-VEGF165/CYD-PEI nanoparticles loaded gelatin/β-TCP matrix as a therapeutic agent and gene delivery vehicle for wound healing. *Molecular Pharmaceutics*, *10*(8), 3090–3102.

Raafat, A. I., Eid, M., & El-Arnaouty, M. B. (2012). Radiation synthesis of superabsorbent CMC based hydrogels for agriculture applications. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *283*, 71–76.

Rujtanaroj, P., Pimpha, N., & Supaphol, P. (2008). Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. *Polymer*, *49*(21), 4723–4732.

Saravanan, M., & Rao, K. P. (2010). Pectin–gelatin and alginate–gelatin complex coacervation for controlled drug delivery: Influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. *Carbohydrate Polymers*, *80*(3), 808–816.

Saxena, S., Ray, A. R., & Gupta, B. (2010). Chitosan immobilization on polyacrylic acid grafted polypropylene monofilament. *Carbohydrate Polymers*, *82*(4), 1315–1322.

Schacht, E., Nobels, M., & Vansteenkiste, S. (1993). Some aspects of the crosslinking of gelatin by dextran dialdehydes. *Polymer Gels and Networks*, *1*(4), 213–224.

Takei, T., Sato, M., Ijima, H., & Kawakami, K. (2010). In situ gellable oxidized citrus pectin for localized delivery of anticancer drugs and prevention of homotypic cancer cell aggregation. *Biomacromolecules*, *11*(12), 3525–3530.

Thirupathi Kumara Raja, S., Thiruselvi, T., Sailakshmi, G., Ganesh, S., & Gnanamani, A. (2013). Rejoining of cut wounds by engineered gelatin–keratin glue. *Biochimica et Biophysica Acta (BBA)—General Subjects*, *1830*(8), 4030–4039.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2010). Preparation and physicochemical evaluation of chitosan/poly(vinyl alcohol)/pectin ternary film for food-packaging applications. *Carbohydrate Polymers*, *79*(3), 711–716.

Van Vlierberghe, S., Dubruel, P., & Schacht, E. (2011). Biopolymer-based hydrogels as scaffolds for tissue engineering applications: A review. *Biomacromolecules*, *12*(5), 1387–1408.

Vasconcelos, A., Pêgo, A. P., Henriques, L., Lamghari, M., & Cavaco-Paulo, A. (2010). Protein matrices for improved wound healing: Elastase inhibition by a synthetic peptide model. *Biomacromolecules*, *11*(9), 2213–2220.

Vicini, S., Princi, E., Luciano, G., Franceschi, E., Pedemonte, E., Oldak, D., et al. (2004). Thermal analysis and characterisation of cellulose oxidised with sodium methaperiodate. *Thermochimica Acta*, *418*(1–2), 123–130.

Zander, N. E., Orlicki, J. A., Rawlett, A. M., & Beebe, T. P. (2012). Qunatification of protein incorporated into electrospun polycaprolactone tissue engineering scaffolds. *ACS Applied Materials & Interfaces*, *4*(4), 2074–2081.

Zhang, J., Doll, B. A., Beckman, E. J., & Hollinger, J. O. (2003). A biodegradable polyurethane-ascorbic acid scaffold for bone tissue engineering. *Journal of Biomedical Materials Research Part A*, *67A*(2), 389–400.