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

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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).

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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

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