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Physicochemical properties and biological activities of novel blend films using oxidized pectin/chitosan

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ABSTRACT

Pectin has been widely used in a variety of biomedical applications. In this study, it was modified with sodium periodate as an oxidant and characterized by physicochemical methods Periodate oxidation increased the contents of dialdehyde units and carboxyl groups in pectin, and a decrease in pectin viscosity was measured. The oxidization reaction led to a significant decrease in all values of molecular weight and size (Mn, Mw, [η] and Rh) as determined by size exclusion chromatography (SEC), which allowed the selection of the oxidized pectin to be added to chitosan. Chitosan-based films were characterized by infra-red spectroscopy (FTIR), X-ray diffractometry (XRD), and differential scanning calorimetry (DSC) measurements. Thermal behaviour studies demonstrated that interactions existed between chitosan and oxidized pectin. The haemolysis percentages of films were found to be less than 5%, which indicated their good blood compatibility. Finally, the antibacterial activity was clearly improved. Cross-linking reactions between pectin and chitosan through ionic bonds and amide bonds and between chitosan and oxidized pectin through Schiff base formation were evidenced, which opens the way to extend applications of these polysaccharides; notably, the biocompatibility and biodegradability of these new networks is convenient for pharmaceutical, biomedical or cosmetic applications.

1. Introduction

In recent years, numerous implant biomaterials, including synthetic and natural materials, have been widely used in the biomedical and pharmaceutical fields [1]. Smart hydrogels consisting of a three-dimensional polysaccharide network, which have the capability to respond to small external stimulus changes, such as temperature, pH and antigens, have attracted significant attention from both academia and industry [2]. Polysaccharide-polysaccharide interactions play an important role in the control of the architecture of animal and plant cells. Electrostatic interactions between polyions and counterions and their ionic selectivity have been studied, leading in some cases to gel formation [3].

Chitosan is a partially deacetylated polymer of acetyl glucosamine obtained after the alkaline deacetylation of chitin. It comprises copolymers constituted of glucosamine and *N*-acetyl glucosamine varying in molecular weight (from approximately 10^4–2×10^6 g/mol) [4]. Most commercial polysaccharides (e.g., cellulose, dextran, pectin, alginic acid, agar–agar, agarose, starch, carrageenan, and heparin) are either neutral or acidic, while chitosan is a basic polysaccharide. Under neutral or basic pH, chitosan contains free amino groups and is insoluble in water, while under acidic pH, chitosan is soluble in water owing to the protonation of amino functions. The solubility of chitosan depends on the distribution of free amino and *N*-acetyl groups within the copolymer [5]. Chitosan is a very useful polymer for biomedical applications due to its biocompatibility, biodegradability and low toxicity [6]. Currently, chitosan receives great interest for its commercial applications in the biomedical, food [7], cosmetics [8] and chemical industries [9]. Chitosan has been extensively employed in bone tissue engineering because of its promotion of cell growth and mineral rich matrix deposition by osteoblast cells in culture [10]. It has also been used in oral drug formulations to provide sustained release of drugs [11]. Chitosan induces wound healing on its own and produces less scarring [10].

Pectin is a polysaccharide composed of 1,4-linked α-D galacturonate residues, some of which are esterified to form methyl esters, with little branching [12]. Due to its biocompatibility,

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biodegradability, and non-toxicity, it is applied in pharmaceutical and biomedical applications, particularly in engineering drug carriers for oral drug delivery [3,13]. Pectin has been found to favourably impact cholesterol levels in blood and to behave as a natural prophylactic substance against poisoning with toxic cations [13]. Recently, pectin has attracted attention as a carrier for colon targeting due to its total degradation by colonic bacteria and its non-digestibility by gastric or intestinal enzymes [2]. Several oxidizing agents have been applied to pectin, including sodium periodate, [14,15] permanganate [16] hydrogen peroxide [17] and ammonium persulfate [18]. The oxidation reaction is based on the opening of the galacturonic acid ring leading to the formation of two reactive aldehyde units per ring, which can further interact with the second polysaccharide to form a hydrogel.

In this article, the preparation of a hydrogel composed of chitosan and pectin or oxidized pectin is proposed from the blending of the two polysaccharides. Cross-linking reactions between chitosan and oxidized pectin have been evidenced, avoiding the use of toxic crosslinking reagents. Ionic bonds and amide bonds between chitosan and pectin and the formation of a Schiff base between chitosan and oxidized pectin were considered. The compatibility and interactions between the two polysaccharides were investigated by FTIR spectroscopy, X-ray diffraction and DSC measurements and by blood compatibility and antibacterial activity, with the aim of using such hydrogels as wound-dressing materials.

2. Experimental

2.1. Materials

Pectin from apple with a degree of esterification of approximately 70–75%, chitosan (viscosity of 800.000 cps) and sodium metaperiodate ($NaIO_4$) were purchased from Sigma-Aldrich, USA.

Phosphate-buffered saline (PBS, pH 7.4, 0.1 M) was prepared by dissolving 17.97 g of disodium hydrogen phosphate, 5.73 g of monosodium hydrogen phosphate and 9 g of sodium chloride in 1 L of distilled water. Other reagents used were of analytical grade.

2.2. Oxidation of pectin

Pectin was oxidized as previously described by Chetouani et al. [19], using several concentrations of sodium periodate ($NaIO_4$, 0.026, 0.052, 0.078 and 0.104 M) as an oxidant at 35 °C. The use of different concentrations leads to different degrees of the oxidization of pectin. The pH level of 4 was maintained by using successively diluted solutions of sulfuric acid and of sodium bicarbonate during the oxidation reaction. Several experimental periods were selected: 1, 2, 3 and 4 h. The oxidation reaction is briefly reported here. In a 100 mL flask, 30 mL of sodium periodate was added to a solution of pectin (5%), resulting in the dissolution of 3 g of pectin in 60 mL of distilled water. To avoid light exposure initiating secondary reaction, the flask was covered by aluminium foil. After gelification with ethanol, the oxidized pectin was isolated after several cycles of filtration and washed with distilled water and ethanol until a complete removal of iodic residues was obtained. The oxidized pectin was dried at 37 °C to reach a constant mass.

2.3. Preparation of films

The biopolymer films were prepared by casting from a solution composed of chitosan and pectin (at 5 wt% of total solids) using the following procedure. Five grams of a blend of chitosan and pectin at a ratio of 60/40(%) were dissolved in 100 mL of distilled water for 30 min. The pH value of the solution was adjusted to 4.5 by addition of hydrochloric acid solution (0.1N). The solubility of chitosan in aqueous solutions is pH-dependent: the solubility of chitosan increases as the acidity of the solution increases. The solution was then placed in a water bath at 60 °C under gentle stirring for 30 min to obtain a homogeneous solution [20].

Ten millilitres of this solution was poured into a 9.5-cm-diameter polystyrene Petri dish that was exposed to air at ambient temperature in the dark for 3–4 days for solvent evaporation.

The procedure was applied to prepare films of (chitosan/pectin) at a ratio of (60/40) (%) (CH/P (60/40) film) and of (chitosan/oxidized pectin) at a ratio of (60/40) (%) (CH/OP (60/40) film). In the latter case, the pectin was oxidized for 4 h with a 0.026 M sodium periodate.

2.4. General measurements

2.4.1. Aldehyde unit content (DA%)

The aldehyde unit content was determined using a rapid quantitative alkali consumption method [21]. In a 100 mL Erlenmeyer flask, 0.15 g of dried oxidized pectin was suspended in 10 mL of sodium hydroxide (0.25 M). The flask was swirled in a water bath at 70 °C for 2 min followed by cooling under water flux for 1 min. Then, 15 mL of standardized sulfuric acid (0.125 M), 30 mL of distilled water and 1 mL of neutral phenolphthalein (0.2%) were added in turn. Titration of the acidic solution was carried out using sodium hydroxide (0.25 M). The percentage of dialdehyde units (DA) was calculated by applying the following equation:

$$\mathbf{DA}\,(\%) = (\mathbf{C_1V_1} - 2\mathbf{C_2V_2}) \times 100/\frac{\mathbf{m}}{173}1000 \quad (1)$$

where C_1 and V_1 represent the molarity and the total volume of NaOH, C_2 and V_2 represent the molarity and the volume of H_2SO_4, m is the dry weight of the oxidized pectin, and 173 is the average molecular weight of the repeating unit of the oxidized pectin. The experiments were performed in triplicate.

2.4.2. Carboxyl group content (%)

The carboxyl group content of the oxidized pectin was determined according to the modified procedure of Chattopadhyay et al. [22]. Approximately 2 g of oxidized pectin was mixed with 25 mL of HCl (0.1 N). The resulting slurry was magnetically stirred for 30 min and was then vacuum filtered through a 150 mL medium porosity fritted glass funnel and subsequently washed with 400 mL of distilled water. The pectin cake was thereafter carefully transferred into a 500 mL beaker, and the volume was adjusted to 300 mL by adding distilled water. The 500 mL beaker was placed in a boiling water bath with continuous stirring for 15 min to ensure complete gelatinization. The pH was titrated at 8.3 using a standardized 0.01N NaOH solution after adjusting the volume to 450 mL. For comparison, a blank test was performed with unmodified pectin.

The carboxyl group content was calculated as follows:

$$\textit{percentage of carboxyl content} = 0.045 \times \left(\textit{mEq of acidity}/100\textit{g pectin}\right) \quad (2)$$

$$\text{With } \textit{mEq of acidity}/100\textit{g pectin} = \frac{[(\textit{sample blank})\,\textit{in mL} \times \textit{normality of NaOH} \times 100]}{\textit{sample weight (dry basis) in g}} \quad (3)$$

2.4.3. Relative average molecular weights

Number and weight average molecular weights were determined by size exclusion chromatography (SEC) using a Shodex SB-G pre-column and two Shodex OH-pack SB HQ 804 and 806 columns. The pectin sample was solubilized in 5 mL of a $LiNO_3$ solution (2 g/L) at room temperature for 3 h under magnetic stirring.