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Effect of low and high methoxyl citrus pectin on the properties of polypyrrole based electroactive hydrogels

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ABSTRACT

Electroactive hydrogels were prepared using commercial citric pectin, either raw (PC) or purified through dialysis (dPC), and chemically synthesized polypyrrole (PPy). ^{1}H NMR analyses showed that PC is a low methoxyl pectin (degree of methoxylation, DM = 46%) and dPC is a high methoxyl pectin (DM = 77%). The pyrrole polymerization was monitored through UV–vis spectroscopy and both samples were observed to be good stabilizers for PPy in aqueous medium. The dispersions were used to prepare the hydrogels h-PC-PPy and h-dPC-PPy. The hydrogel h-dPC-PPy has a higher swelling index (SI ≈ 25%) at pH 1.2 than the hydrogel h-PC-PPy (SI ≈ 7%). Contrastingly, at pH 6.8 both hydrogels lost their mechanical integrity. Raman spectroscopy revealed that PPy is more oxidized in h-PC-PPy. Nevertheless, both hydrogels are electroactive and therefore can be considered for applications in which the control of the degree of swelling is desired.

1. Introduction

Hydrogels are excellent in biomedical applications since they can exhibit stimuli responsive behavior. Certain factors induce interesting properties such as pH, ionic strength, temperature, electric signals, and others (Qiu & Park, 2001). Furthermore, the swelling degree is dependent on the chemical structure, molar mass, composition and degree of crosslinking of the polymer matrix, as well as the presence of low molar mass compounds (Peppas, Bures, Leobandung, & Ichikawa, 2000). Due to their versatility and technical advantages over other classes of materials, the use of hydrogels has been expanded to enable desired applications.

Polysaccharides are excellent candidates to prepare stimulus responsive hydrogels since they generally contain a diversity of functional groups that respond differently depending on the environment. Among the polysaccharides that can form gels, pectins have the advantage of being obtained from the primary cell walls of many plants like from the peels of citrus fruit, which are considered agroindustrial residues. Pectins are a family of heterogeneous polysaccharides with structures varying from linear homogalacturonans (HG) to highly branched and complex rhamnogalacturonans (RGI and RGII), which can be classified according to branching degree and type of side-chains monosaccharides (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996). HG have linear chains of (1 → 4)-linked α-galacturonic acid (Gal*p*A) units. RGs have main-chains of (1 → 4)-linked α-galacturonic acid (Gal*p*A) units interspersed with (1 → 2)-linked α-L-rhamnopyranosyl (Rha*p*) units, which are substituted by side-chains of arabinans, galactans or arabinogalactans (RG-I) or complex side-chains containing rare sugars, such as apiose, aceric acid (3-*C*-carboxy-5-deoxy-L-xylose), 2-*O*-methylfucose, 2-*O*-methylxylose, and DHA (3-deoxy-D-lyxo-2-heptulosaric acid) (RG-II) (Carpita and McCann, 2000; Moreira et al., 2014). Pectins can be partly methyl esterified at CO_2H-6 and according to the degree of methoxylation (DM), they are classified as low methoxyl (LM) when DM < 50% or high methoxyl (HM) when DM > 50%.

Although polysaccharide hydrogels can be used in a series of applications, their combination with organic conducting polymers extends their usage as smart materials for the biomedical field. Many research groups have prepared polysaccharide-conducting polymers composites (Molino et al., 2015; Quintanilha, Orth, Grein-Iankovski, Riegel-Vidotti, & Vidotti, 2014). The intrinsic characteristics of the polysaccharide impact the physical-chemical properties of the composite such as the water solubility, rheolog-

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ical and surface behavior, and the capacity to form gels (Stephen, Phillips, & Williams, 1995).

Polypyrrole (PPy) is a conductive polymer and has received considerable attention due to its high electrical conductivity and electrochemical stability (Scrosati, 1995). In addition to being studied for biosensing, energy storage, and microelectronic devices, PPy has also been tested for biomedical applications (Otero, Martinez, & Arias-Pardilla, 2012; Zhang, Wang, Xing, & Yu, 2015). Polypyrrole has been combined with heparin, a potential anticoagulant, forming a composite that was found to be an excellent substrate for endothelial cell growth (Garner, Georgevich, Hodgson, Liu, & Wallace, 1999; Stewart, Liu, Clark, Kapsa, & Wallace, 2012). Electrically synthetized PPy was studied to stimulate nerve regeneration. Rat PC-12 cells demonstrated to be highly interactive with PPy films when electrically stimulated and caused little adverse tissue response according to *in vivo* experiments (Schmidt, Shastri, Vacanti, & Langer, 1997).

In this work, for the first time, it is presented the preparation of pectin-PPy hydrogel composites that combine the electrochemical properties of PPy with the swelling behavior of pectin hydrogels. Low and high DM pectin were obtained by a simple dialysis process. The properties of the hydrogels depend on the pectin properties, whereas the properties inherent to the PPy were not drastically affected by the nature of the polymer matrix.

2. Materials and methods

2.1. Materials

Ammonium persulfate (APS, 98 wt%), sodium hydroxide (NaOH, 97 wt%), calcium chloride ($CaCl_2$, 99 wt%), potassium dihydrogen phosphate (KH_2PO_4, 99 wt%) and sodium phosphate monobasic ($NaHPO_4$, 98.5%) were purchased from Synth (São Paulo, Brazil). Pyrrole (98%, Sigma-Aldrich) was distilled under low pressure before use. The commercial citrus pectin (PC) was bought locally (Mercado Municipal de Curitiba, Curitiba, Brazil). All solutions were prepared using ultrapure water (18.2 MΩ cm at 25 °C).

The PC was purified by dialyzing against tap water through 12–14 kDa M_w cut-off (Spectra/Por® Cellulose Ester) for 24 h, followed by lyophilization. The resulting powder was named dPC (dialyzed pectin) and contains 5.3 ± 0.1 wt% of ash. The values corresponding to the weight-average molar mass, M_w, and the homogeneity, M_w/M_n, of the samples PC and dPC were obtained through Viscotek GPC/SEC equipment (Malvern) connected to a Shodex SB-806M HQ-column. Refractive index, light scattering LALLS (7°), RALLS (90°), and differential viscometer were used as detectors. The injection volume was 100 μL at a flow rate of 0.4 mL min^{-1}. Samples were prepared at a concentration of 1 mg mL^{-1} in $NaNO_3$ 0.1 mol L^{-1} (also used as the mobile phase) containing 200 ppm NaN_3 and filtered through a cellulose ester membrane with porosity of 0.22 μm (Millipore). The value used for *dn/dc* was 0.147 mL g^{-1} (Fishman, Chau, Kolpak, & Brady, 2001).

2.2. Characterization of pectins

The monosaccharide composition of PC and dPC was determined as follows. Pectin samples (2 mg) were hydrolyzed with 2 mol L^{-1} TFA (trifluoroacetic acid) (1 mL) for 8 h at 100 °C. The product was successively reduced with $NaBH_4$ (Wolfrom & Thompson, 1963a), acetylated with Ac_2O–pyridine (1:1, v/v) (Wolfrom & Thompson, 1963b), and the resulting alditol acetates were examined by GC–MS. This was performed with a Varian model 3800 gas chromatograph coupled to a Saturn 2000R mass spectrometer using a DB-225 capillary column (25 m × 0.25 mm i.d.). Temperature used was 50 °C during injection, then programmed at 40 °C min^{-1} to 220 °C (constant), with He as carrier gas. The products were identified by their typical retention times and electron impact profiles. Uronic acid contents were determined by the colorimetric method of Filisetti-Cozzi and Carpita (1991).

^{1}H NMR spectroscopy was employed to confirm the DM (Grasdalen, Bakoy, & Larsen, 1988). Samples were deuterium-exchanged three times by freeze-drying with D_2O, then finally dissolved in D_2O (at 10 mg mL^{-1}) and transferred into 5 mm NMR tubes. The ^{1}H NMR spectra were acquired at 70 °C with 256 scans on a Bruker AVANCE III 400 NMR spectrometer observing ^{1}H at 400.13 MHz. Chemical shifts were expressed as δ ppm. The DM was obtained using the equation below.

$$DM\,(\%) = \frac{IA - IB}{IA + IB}100 \quad (1)$$

IA refers to the intensity of resonance signals of H-1 of all GalA units (–COO^- and —$COOCH_3$ forms) and of H-5 of all esterified GalA units (∼δ 5.12 to δ 4.76). IB refers to the intensity of resonance signals from H-5 of non-esterified GalA units (∼δ 4.58 to δ 4.52).

The protein content of the pectins was determined by the colorimetric method described by Hartree (1972).

2.3. Preparation of the electroactive hydrogel composites

To obtain the composite dispersions PC-PPy and dPC-PPy, the pectin sample (0.25 g) was first solubilized in 15.5 mL of water in a double walled glass reactor kept at 45 °C under magnetic stirring. After complete solubilization, 50 μL of pyrrole and 1.5 mL of 1.7 mol L^{-1} sulfuric acid solution was added to this solution (final pH 3.0). The polymerization was induced by adding 20 μL of ammonium persulfate (APS, 1.0 mol L^{-1}) at intervals of 5 min for 85 min (total of 360 μL). The successive addition method was chosen since the one-pot method caused the appearance of precipitates in the dispersion. The PPy formation was monitored by UV–vis absorption spectroscopy using an Agilent 8453 spectrophotometer. The blank was obtained using the corresponding pectin dispersion. Aliquots of 300 μL were taken at intervals of 5 min, diluted in 2.7 mL of ultrapure water, and transferred to a quartz cuvette. At the end of the polymerization reaction, the dispersions were dialyzed against distilled water for 24 h to remove low molar mass molecules including unreacted Py and PPy oligomers.

The hydrogels were prepared from the dialyzed dispersions by adding 0.2 g of pectin followed by 0.3 mL of aqueous solution of $CaCl_2$ (5 w/v%), under magnetic stirring (Yoshimura, Sengoku, & Fujioka, 2005). The dispersions were left to rest for 24 h. Afterwards, 5 mL of NaOH (10% w/v) was added and the dispersions were left to rest again for another 24 h in a circular mold. The prepared hydrogels as described above were named h-PC-PPy and h-dPC-PPy. They were removed from the molds and washed thoroughly with distilled water several times until pH 7. Afterwards, they were lyophilized and macerated for further characterization.

2.4. Swelling behavior in gastric and intestinal pH

To determine the swelling index (SI) of the hydrogels (in wt%, Eq. (2)), approximately 0.3 g of previously dried hydrogel (W_i) was immersed in 100 mL of the solution. Gastric (pH 1.2) and intestinal (pH 6.8) pH solutions were obtained by preparing buffer solutions of KCl/HCl (ionic strength = 0.40 mol L^{-1}) and KH_2PO_4/NaOH (ionic strength = 0.25 mol L^{-1}), respectively. The mass of the swollen hydrogel (W_f) was monitored up to 72 h. Before weighing, the hydrogels were slightly pressed against an adsorbent paper to remove the excess of fluid.

$$SI\,(\%) = \frac{W_f - W_i}{W_i}100 \quad (2)$$