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Xyloglucan nano-aggregates: Physico-chemical characterisation in buffer solution and potential application as a carrier for camptothecin, an anti-cancer drug

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ABSTRACT

In this work, native xyloglucan was extracted from *Tamarindus indica* seeds (XGT), and its properties in phosphate buffer solution (PBS) were evaluated in comparison with a commercial tamarind kernel powder (TKP). The physico-chemical characteristics of the polysaccharides such as molar mass, critical concentration and intrinsic viscosity were determined. Furthermore, using spectroscopic and microscopy techniques, it was observed that the XGs tested can be considered macromolecules able to aggregate as nano-entities of 60–140 nm. The XGT tended to an ordered and compact spherical conformation determined by the Huggins constant, circular dichroism, atomic force microscopy and transmission electron microscopy. After the determination of the properties in PBS the XGs, at concentrations of 25% above their critical aggregation concentration, were used to encapsulate camptothecin, an anti-cancer drug. The XGT sample showed an encapsulation efficiency of 42% and first-order drug delivery kinetics. These results demonstrated the importance of knowledge of the physico-chemical properties of polysaccharides, for example, to better conduct their biotechnological applications as drug carriers.

1. Introduction

Polysaccharides are the most important source for a broad variety of advanced polymeric materials and have emerged as an immense renewable resource for biopolymers. These features have led to an outstanding increase in interest among scientists, research institutes and industrial companies (Castro, Silva, Carmona-Ribeiro, Kappl, & Petri, 2007; Heinze et al., 2005; Jelinek & Kolusheva, 2004; Sierakowski, Castro, Lucyszyn, & Petri, 2007; Silva, Carmona-Ribeiro, & Petri, 2007; Ueta & Diniz, 2008).

Polysaccharides are abundant and readily available from renewable sources such as the algal and plant kingdoms, microbial cultures of selected strains and through recombinant DNA techniques. Thus, they have a large variety of compositions and properties that cannot be mimicked easily in the laboratory. Their ease of production makes many polysaccharides cheaper than synthetic polymers (Coviello, Matricardi, Marianecci, & Alhaique, 2007).

The xyloglucans (XGs) are a group of storage or structural heteropolysaccharides from plants, and their structure is composed of a 1,4-linked β-D-glucan main chain that is partially substituted with α-D-Xyl side-chains at the O-6 atoms. Depending on the source, the side-chains can be β-D-Gal-1,2-α-D-Xyl or α-L-Fuc-1,2-β-D-Gal-1,2-α-D-Xyl (Carpita & Gibeaut, 1993; Fry, 1989; Hayashi, 1989; McNeil, Darvill, & Fry, 1984; Varner & Lin, 1989) or more complex chains (Hantus, Pauly, Darvill, Albersheim, & York, 1997; Freitas et al., 2005; Jia, Qin, Darvill, & York, 2003; York, Kumar-Kolli, Orlando, Albersheim, & Darvill, 1996).

The branches present in the structure of xyloglucans may also contribute to the variation in viscosity as well as its solubility and conformational flexibility of the main chain; therefore, different branches may give different properties to the polysaccharide (Sims et al., 1998).

The XGs are water-soluble, but the individual macromolecules tend not to fully hydrate, and, consequently, aggregated species remain present even in very dilute solutions. The biopolymer shows a balance between hydrophobic and hydrophilic character, and the substantial chain stiffness of the cellulose-like backbone facilitates

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intermolecular interactions (Nishinari & Takahashi, 2003; Picout, Ross-Murphy, Errington, & Harding, 2003).

Due to its unique rheological properties, the xyloglucans have potential usage in the food (Bhattacharya, Bal, Mukherjee, & Bhattacharya, 1991; Maeda, Yamashita, & Morita, 2007; Yamanaka et al., 2000), pharmaceutical, medical and cosmetic industries (Burgalassi, Panichi, Saettone, Jacobsen, & Rassing, 1996; Itoh et al., 2008), among others.

Another important characteristic of the XGs are that they do not present toxicity (Shirakawa, Yamotoya, & Nishinari, 1998) and can be used, for example, to administer certain drugs. Coviello et al. (2007) indicated that this polysaccharide is a promising protector, especially in the delivery of oral drugs that can be stomach irritants. Furthermore, xyloglucan can also be considered as a suitable polymer for coating nanocomposites of LDH-enalapril (Ribeiro, Arizaga, Wypych, & Sierakowski, 2008), mucoadhesive formulations (Yoo et al., 2005) and for drugs that are administered intraperitoneally (Suisha et al., 1998), rectally (Miyazaki et al., 1998), percutaneously and topically (Takahashi et al., 2002). They are also suitable for ocular applications (Burgalassi, Chetoni, Panichi, Boldrini, & Saettone, 2000; Miyazaki et al., 2001).

Camptothecin (CPT) is a potent cytotoxic alkaloid, which was first isolated and characterised by Wall et al. (1966) from the oriental tree *Camptotheca accuminata*. CPT has a unique mechanism of action as a topoisomerase I inhibitor (Hsiang, Hertzberg, Hecht, & Liu, 1985), and it is believed to act by stabilising a topoisomerase I-induced single strand break in the phosphodiester backbone of DNA, thereby preventing re-ligation, which leads to the production of double-strand DNA breaks during replication and, if not repaired, results in cell death (Hsiang et al., 1985; Liu, 1989). CPT has shown potent antitumour activity against a wide spectrum of human malignancies, including human lung, prostate, breast, colon, stomach and ovarian carcinomas, melanoma, lymphomas and sarcomas (Dancey & Eisenhauer, 1996; Potmesil & Pinedo, 1995; Takimoto, Wright, & Arbuck, 1998).

Therapeutically, CPT has not yet been fully utilised, due primarily to its poor solubility in water and its toxic effects after long administration times (Cortesi, Esposito, Maietti, Menegatti, & Nastruzzi, 1997). Attempts to deliver CPT or its derivatives efficiently have included encapsulation in microspheres, microemulsions, polymeric micelles and polymeric implants (Cortesi et al., 1997; Hatefi & Amsden, 2002; Shenderova, Burke, & Schwendeman, 1997, 1999; Storm et al., 2002).

In the present paper, we determined the physico-chemical properties of two samples of tamarindus xyloglucans (XGs) in phosphate buffer solution (PBS) by observing the aggregation characteristics, and, after this analysis, the XG solutions were tested to load and release CPT *in vitro*.

2. Materials and methods

2.1. Materials

A xyloglucan sample (coded XGT) was extracted from tamarind (*Tamarindus indica*) seeds from a market in the State of Rio Grande do Norte, Brazil and compared with a commercially available xyloglucan (coded TKP, i.e., Tamarind kernel powder) that was obtained from Balasanka Mills Co., India. We measured molar ratios of the Glc:Xyl:Gal units of 2.8:2.3:1 (XGT) and 2:2:1 (TKP) by ^{1}H NMR spectroscopy, using a Bruker Avance DRX-400 spectrometer. D_2O was used as the solvent, and the experiments were carried out at 70 °C. The chemical shifts are reported as δ (ppm) relative to acetone (δ 2.224), which was used as an internal standard. Other XG characterisation parameters have already been reported (Jo, Petri, Valenga, Lucyszyn, & Sierakowski, 2009). Camptothecin (CPT; $C_{20}H_{16}N_2O_4$, M_w = 348.4 g/mol) was purchased from Sigma–Aldrich. The other materials were of commercial grade and were used without purification.

2.2. Analysis of the xyloglucan. Its polydispersity, molar mass and critical concentration

After aqueous extraction, the samples (1 mg/mL) were solubilised in pure MilliQ water and filtered through 0.45 and 0.22 μm pore cellulose acetate membranes. The solutions (100 μL) were measured using GPC to calculate the polydispersity index ($\overline{M}_w/\overline{M}_n$) and the molar mass, $\overline{M}_w$, relative to PEO 22k (polyethylene oxide) and Dextran 70k, which were used as standards. The samples were analysed at 632.8 nm using a Viscotek system equipped with PWxl 6000, 4000 and 2500 columns (Tosoh, Japan) that were connected in series, which was coupled to a differential refractometer from Viscotek (model VE3580), a viscosimetric detector and a laser light scattering detector (model 270 Dual Detector). The setup was also equipped with low angle (7°) and right angle (90°) lasers, and the sample was eluted with pure MilliQ water at a flow rate of 1 mL/min. By determining the $\overline{M}_w$, it was possible to calculate the critical concentration (c^*), which determines the limit concentration between the diluted and semi-diluted states, using Eq. (1):

$$c^* = \frac{\bar{M}_w}{(4/3)\pi \times NA \times {R_g}^3} \quad (1)$$

where $\overline{M}_w$ is the molar mass (g/mol), NA is Avogadro's constant, and R_g is the radius of gyration (nm). The values calculated using Eq. (1) were comparable with those found using rheological analysis.

2.3. Rheological measurements

For the intrinsic viscosity determinations, we solubilised the XG samples (1.2–0.6 g/L) in PBS (28 mM phosphate buffer solution containing 123 mM NaCl, at pH 7.4) to simulate physiological conditions (Ciccoli, Signorini, Alessandrini, Ferrali, & Comporti, 1994; Ferrali et al., 1997). We used the Huggins equation to determine the intrinsic viscosity ([η]) by extrapolation of the reduced viscosity (η_{red}) to the limit at zero concentration ($c \to 0$), where the linear coefficient is represented by [η] (Huggins, 1942). All of the analyses were performed using a RheoStress1 rheometer (Haake GmbH, Germany) equipped with a DG43 spindle. A Haake DC30 bath and a thermostatic Universal Temperature Controller (UTC) were used to maintain the temperature at 25 °C.

2.4. Conformational analysis

The circular dichroism (CD) spectra were recorded over the range of 190–250 nm (measured in millidegrees (mdeg) on a Jasco J-815 spectropolarimeter (Jasco Instruments, Japan). We recorded the average of 16 scans using a 0.1 cm path length cylindrical quartz cuvette and a sample concentration of 1.0 mg/mL. The entire instrument, including the sample chamber, was constantly flushed with N_2 gas during operation. The spectra were obtained after overnight stirring (approximately 20 h of incubation in PBS). Spectra were recorded at 5, 25 and 37 °C, and the temperatures were maintained using a circulating water bath TC-100 (Jasco). At each temperature tested, the sample was incubated for 5 min before the analysis.

2.5. Determination of the critical aggregation concentration of the polysaccharides. Fluorescence measurements.

To compare the encapsulating capacity of the polysaccharides (Amiji, 1995), the samples were diluted in PBS at concentrations from 0.001 to 2.0 mg/mL. Pyrene, dissolved in methanol, was added