Carbohydrate Polymers 154 (2016) 77–85

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Synthesis and characterization of non-toxic and thermo-sensitive poly(*N*-isopropylacrylamide)-grafted cashew gum nanoparticles as a potential epirubicin delivery matrix

Clara M.W.S. Abreu[a], Haroldo C.B. Paula[b], Vitor Seabra[c], Judith P.A. Feitosa[a], Bruno Sarmento[c,d,e], Regina C.M. de Paula[a,*]

[a] *Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Caixa Postal 6021, Rua Humberto Monte s/n, Campus do Pici, CEP 60455-760 Fortaleza, Brazil*
[b] *Departamento de Química Analítica e Físico-Química, Universidade Federal do Ceará, Caixa Postal 6021, Rua Humberto Monte s/n, Campus do Pici, CEP 60455-760 Fortaleza, Brazil*
[c] *CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde and Instituto Universitário de Ciências da Saúde, Rua Central de Gandra 1317, 4585-116 Gandra, Portugal*
[d] *INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal*
[e] *i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal*

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form 9 August 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
N-isopropylacrylamide
Nanoparticles
Thermo-sensitive
Cytotoxicity
Epirubicin

ABSTRACT

Cashew gum (CG) was grafted with *N*-isopropylacrylamide (NIPA) by radical polymerization to originate a stimuli-sensitive copolymer for drug delivery purposes. NMR and IR spectroscopy confirmed the insertion of NIPA onto the cashew gum chains. The graft copolymer (CG:NIPA) demonstrated thermal responsiveness. The critical aggregation concentration of the copolymers at 25 °C was higher than at 50 °C. At temperatures lower than the LCST, the nanoparticle size ranged from 12 to 21 nm, depending on the CG:NIPA ratio, but above the LCST the particles aggregated, increasing the particle size. Regarding the potential for future oral application, the nanoparticles showed no cytotoxic activity against the Caco-2 and HT29-MTX intestine cell lines. Epirubicin was encapsulated into nanoparticles of CG-NIPA (1:1), resulting in a 64% association efficiency and 22% loading capacity. Thus, the CG:NIPA graft copolymer demonstrates good potential for used in controlled drug delivery systems.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The increasing use of nanoparticles as drug delivery systems has resulted in significant improvements in the pharmacokinetics of therapeutic active molecules. Nanoparticles have been particularly efficient in increasing the solubility and stability of drugs, enhancing the administration safety and improving the biodistribution pattern, leading to more physiologically compatible and less toxic profiles (Luo et al., 2012a).

Core-shell type (hydrophilic shell - hydrophobic core) nanopolymer aggregates, in the form of micelles, may be more suitable as drug delivery systems because of their greater structural stability gained from the intertwining of their chains and the intermolecular interactions between hydrophobic segments (Kang, Na, & Bae, 2003). These nanosystems are generally produced from amphiphilic copolymers, which spontaneously organize into micelles through intra or intermolecular associations when in contact with an aqueous medium (Bigot et al., 2010).

Polysaccharides appear to be appropriate for preparing nanoparticles, especially those for biomedical purpose, due to their unique physicochemical properties and excellent biocompatibility (Sosnik, das Neves & Sarmento, 2014). Moreover, they are very safe and highly biodegradable and there are abundant sources in nature which are associated with low cost processing (Yang, Han, Zheng, Dong, & Liu, 2015). Furthermore, the relatively high molecular weight of polysaccharides means that the release rate of the loads can be controlled and allows biodegradation to occur before rapid renal clearance (Basu, Kunduru, Abtew, & Domb, 2015).

The presence of several functional groups, such as hydroxyl, carboxyl, sulfate and amino, allows polysaccharides to be modified through the insertion of hydrophobic or hydrophilic groups. The insertion of a polymer chain or the polymerization of a monomer onto a polysaccharide can promote the formation of self-assembled nanoparticles. These nanoparticles may be responsive to stimuli and drug release can be promoted through a change in the nanoparticle volume in response to variations in the pH, or temperature or

* Corresponding author.
E-mail address: rpaula@dqoi.ufc.br (R.C.M. de Paula).

http://dx.doi.org/10.1016/j.carbpol.2016.08.031
0144-8617/© 2016 Elsevier Ltd. All rights reserved.

ionic strength or the presence of a magnetic field (Maya et al., 2013; Nitta & Numata, 2013). In this regard, the use of the polysaccharide obtained from cashew gum shows promise.

Cashew gum is a heteropolysaccharide composed of galactose, glucose, arabinose and glucuronic acid (de Paula & Rodrigues, 1995; De Paula, Heatley & Budd, 1998). It presents no toxicity (Quelemes et al., 2013) and low viscosi ty (de Paula & Rodrigues, 1995) and its production is feasible, since its exudation is simple, often spontaneous and provides high yields (Cunha, de Paula, & Feitosa, 2009). In fact, cashew gum offers good potential for commercial production, since the cashew tree (*Anacardium occidentale* L.) is extensively cultivated in tropical regions of the world. The literature also shows that it has potential for biomedical applications and provides good yields in chemical modifications (Das, Nayak, & Nanda, 2013; Moura Neto, Maciel, Cunha, de Paula, & Feitosa, 2011; Gowthamarajan, Jawahar, Wake, Jain, & Sood, 2012; Quelemes et al., 2013).

Cashew nanoparticles have been prepared via different routes, for instance, acrylic acid-grafted-cashew gum has been prepared by radical polymerization using Ce (IV) ions as the initiator (Silva, Feitosa, Paula, & de Paula, 2009), by polyelectrolyte complexation of carboxymethylated cashew gum and chitosan (Silva, Maciel, Feitosa, Paula, & de Paula, 2010) and by the self-assembly of acetylated cashew gum prepared through the dialysis of an organic solution (DMSO) against a non-solvent (water) (Pitombeira et al., 2015).

Poly-*N*-isopropylacrylamide (PNIPA) is a thermoresponsive material with a critical solution temperature (LCST) close to 32 °C. This polymer has therefore been extensively investigated for use in biomedical applications, including drug delivery, mainly in association with biodegradable materials (Chen, Chen, Nan, Wang & Chu, 2012; Lizundia, Meaurio, Laza, Vilas & León Isidro, 2015; Lv et al., 2011; Zhang et al., 2009). Although PNIPA is not biodegradable and may raise toxicity concerns related to its continued residence in the intraocular cavity (Lai & Hsieh, 2012), it is reported that PNIPA particles present no adverse geno- or cytotoxicological effects toward many cell lines (Naha et al., 2010; Rejinold, Sreerekha, Chennazhi, Nair & Jayakumar, 2011), indicating that once formulated into nanoparticles it can be considered as biocompatible.

Poly-*N*-isopropylacrylamide grafting onto another polymer, such as a polysaccharide, is of greater interest than the copolymerization of NIPA with other synthetic polymers, since the continuous stream of the PNIPA chain can be broken, reducing the interaction between isopropyl groups above the LCST (Fundueanu, Constantin & Ascenzi, 2008; Fundueanu et al., 2010).

In this context, the aim of this study was to synthesize and characterize thermoresponsive nanoparticles of cashew gum grafted with *N*-isopropylacrylamide for potential biomedical applications. No reports of cashew gum grafted with *N*-isopropylacrylamide could be found in the literature and this is the first time that the toxicity of cashew gum and it derivatives against Caco-2 and HT29-MTX cells has been evaluated. As a proof of concept for the application of CG-NIPA nanoparticles as a delivery matrix, the association efficiency and loading capacity of epirubicin (EPI) were evaluated. EPI is an anthracycline and a clinically useful chemotherapy agent, but it shows poor aqueous solubility and severe side effects.

2. Experimental

2.1. Materials

Crude samples of the cashew tree (*Anacardium occidentale*) exudate were provided by Embrapa Agroindústria Tropical, Fortaleza, Ceará, Brazil. The cashew gum (CG) was obtained as a sodium salt using a previously described method (Rodrigues, de Paula, & Costa, 1993). *N*-isopropylacrylamide 97% (NIPA) and thiazolyl blue tetrazolium bromide were obtained from Sigma-Aldrich. Cerium (IV) ammonium nitrate (CAN) was purchased from Acrós Organics and epirubicin hydrochloride from Laboratório Químico Farmacêutico Bergamo Ltda (Brazil). All reagents were used as received.

Cashew gum used in this work has molar mass, obtained by size exclusion chromatography of 6.9×10^4 g/mol and the molar sugar ratio for galactose:glucose:arabinose:rhamnose:glucuronic acid of 1:0.2:0.08:0.05:0.06 respectively. The NMR of this purified sample is similar to previously published results on cashew gum and is shown in the Supplementary file (de Paula & Rodrigues, 1995; de Paula, Heatley & Budd, 1998).

2.2. Synthesis and purification of graft copolymers

The method used for the synthesis of the graft copolymers was adapted from a procedure involving the grafting of acrylic acid onto dextran gum (Tang, Dou, & Sun, 2006). One gram of CG was dissolved in distilled water (50 mL) at room temperature under stirring overnight. After dissolution, nitrogen was bubbled for 30 min and a solution of CAN in 0.1 M nitric acid and a designated amount of NIPA were then added successively. The molar ratio of the NIPA:CAN used in this study was 1:0.07. Three CG:NIPA ratios (1:0.5; 1:1 and 1:2) were used. The CG:NIPA molar ratio was calculated considering the monosaccharide/NIPA units. The reaction was kept for 4 h under an N_2 atmosphere at 25 ° C. In the next step, 5 M NaOH was added to neutralize the reaction system. The reaction solution was dialyzed against distilled water for 6 days using a membrane bag with a molecular weight cut-off of 12,000 to remove the unreacted monomers and the ungrafted NIPA. The final aqueous solutions were lyophilized to obtain the solid copolymers.

The method proposed to remove the homopolymer was based on the reported results for removing polymethylmethacrylate from a copolymer by washing with acetone (An, Yuan, Luo, & Wang, 2010). The copolymer (200 mg) was dispersed in acetone (50 mL) and placed under constant stirring for 48 h and the homopolymer was separated by filtration through a funnel with a fine-porosity sintered-glass plate. The extracted homopolymer was freeze-dried and quantified. The yield of purified copolymers was calculated in relation to the mass of unpurified copolymers.

2.3. Proton nuclear magnetic resonance (^{1}H NMR)

Proton spectra of a 4% (w/v) solution of CPl purified in D_2O at 25 and 70 °C were recorded on a Fourier transform Bruker Avance DRX 500 spectrometer. Sodium 2,2-dimethylsilapentane-5-sulfonate (DSS) was used as the internal standard (0.00 ppm for ^{1}H NMR).

2.4. Critical association concentration (CAC)

The CAC was determined by fluorescence spectroscopy using a methodology described in the literature (Patrizi, Piantanida, Coluzza, & Masci, 2009). The solutions of the copolymers in concentrations of 0.002–1.0 mg/mL were prepared in an aqueous solution of pyrene 5×10^{-7} mol/L. Fluorescence spectra were obtained using a QuantaMaster50 spectrometer (Photon Technology International) equipped with a thermostatic bath (Cole Parmer Polystat) linked to the cell compartment. The excitation spectrum (310–360 nm) was obtained using an emission wavelength (λ_{em}) of 374 nm. The intensity ratio (I_{338}/I_{334}) was used to determine the CAC. A slit width of 0.5 mm (entrance and exit) was used for all measurements. The experiments were performed at 25 and 50 °C.

Download English Version:

https://daneshyari.com/en/article/1384695

Download Persian Version:

https://daneshyari.com/article/1384695

Daneshyari.com