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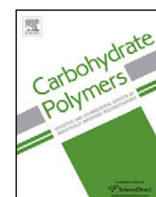

Hydrophobic lappaconitine loaded into iota-carrageenan by one step self-assembly

Wenxiu Sun[a,b,c,*], Marleny D.A. Saldaña[b], Yujia Zhao[b], Lingling Wu[a,b], Tungalag Dong[a], Ye Jin[a], Ji Zhang[c]

[a] College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot 010018 China
[b] Department of Agricultural, Food and Nutritional Science (AFNS), University of Alberta, T6G 2P5 Edmonton, AB, Canada
[c] College of life sciences, Northwest Normal University, Lanzhou 730070 China

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ABSTRACT

New data on the loading of pH-sensitive lappaconitine loaded into iota-carrageenan (LA-ICG) is provided. This LA-ICG ionic biopolymer was prepared by one step self-assembly. The LA-ICG was characterized in terms of the loading capacity, lappaconitine (LA) releasing behavior, pH-sensitivity, and analgesic properties. Iota-carrageenan (ICG) high loading capacity reached up to 26.18% (w/w). Also, the LA, loaded with ICG, was released faster in an acidic environment than that in neutral or alkaline environments. Animal analgesic experiments showed that the LA-ICG of low molecular weight had earlier onset time and longer duration than the LA. These results suggest that the ICG of low molecular weight has great potential to achieve the synergistic effect of LA. In addition, the ICG can be used as a novel natural polymeric carrier for loading a hydrophobic alkaloid.

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1. Introduction

In the past few years, marine microorganisms, such as bacteria, microalgae and seaweeds, have been widely investigated as they represent a largely undeveloped field of valuable materials. Among them, seaweeds are the most abundant sources of polysaccharides, such as carrageenan (CG), alginate and agar (De Ruiter & Rudolph, 1997; Jiao, Yu, Zhang, & Ewart, 2011; Michel, Nyval-Collen, Barbeyron, Czjzek, & Helbert, 2006). The CG is the generic name for a family of high molecular weight sulfated polysaccharides obtained from a number of sources, including seaweeds, bacteria, fungi, insects, crustacea, and even humans, and can be structurally tuned through genetic engineering (Colquhoun et al., 2001; Coviello, Matricardi, Marianecci, & Alhaique, 2007; D'Ayala, Malinconico, & Laurienzo, 2008; Laurienzo, 2010). CG, which is a natural polymer, is inert, safe, non-toxic, biocompatible, biodegradable, low cost, eco-friendly and abundantly available in nature (Guo, Skinner, Harcum, & Barnum, 1998). It is composed of galactose and hydrogalactose units linked by glycosidic unions (Coviello et al., 2007; Jiao et al., 2011). The disaccharide units are variably sulfated, resulting in a sulfate content of 22–38% by weight in commercial carrageenan (Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002). Other cations, such as ammonium, calcium, magnesium, potassium, and sodium, are also often presented in the form of galactose esters (U.S. Pharmacopeia, 2010).

Additionally, CG is a good source of soluble fiber (Burtin, 2003; Li, Ni, Shao, & Mao, 2014). There are several different CGs with slightly varied chemical structures and properties. The three most relevant, with the highest commercial interest, CG polysaccharides are iota, kappa, and lambda CG (U.S. Pharmacopeia, 2010; van de Velde, Lourenço, Pinheiro, & Bakker, 2002).

The US Food and Drug Administration approved CG as an additive that is "Generally Recognized as Safe" (GRAS) for consumption and topical applications. CG is a versatile ingredient suitable for food and nonfood applications (van de Velde, Lourenço, et al., 2002). Iota-carrageenan (ICG) with no nutrient value has been widely used in the food industry (Heertje, 2014), most recently in the pharmaceutical industry as an excipient in pills and tablets (Campo, Kawano, da Silva, & Carvalho, 2009; Gupta, Hariharan, Wheatley, & Price, 2001) and as potential raw material for hydrogels (Hoffman, 2012). However, compared with commonly used pharmaceutical excipients, such as hydroxypropyl methylcellulose, chitosan, carbomer and alginate, the utilization of ICG is still limited.

* Corresponding author at: Room 407, College of Food Science and Engineering, Inner Mongolia Agricultural University, No. 306 Zhaowuda Road, Saihan District, Hohot, Inner Mongolia 010018, China.
E-mail address: wenxiu_sun@imau.edu.cn (W. Sun).

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LA is an alkaloid with excellent analgesic activity found in the root of *Aconitum sinomantanum Nakai*. However, its application has been restricted due to its hydrophobicity, slow onset and short duration (Sun, Hu, Ding, Duan, & Jia, 2011; Sun, Wang, Zhang, & Wang, 2014; Sun, Dong, & Ding, 2012).

In this study, to improve the solubility of LA, ICG was investigated not only as a drug-loaded polymer but also as an ionic polymer to increase the LA poor solubility. The strategy use was based on the characteristics of ICG, a natural anionic sulfated linear polysaccharide. A series of ICG with different molecular weights were prepared, targeting the following advantages. Firstly, according to the polyelectrolyte ability, ICG could be loaded with LA via self-assembly to improve the LA solubility in water. Secondly, LA-loaded ICG could be used as a pH-sensitive product due to its pH-sensitive sulfur group. Finally, the ICG could be used as a model matrix for alkaloid loading, and further application in the pharmaceutical field. Therefore, this study focuses on the degradation, loading and self-assembly capacity of ICG. In addition, the LA release behavior, pH-sensitivity and analgesic properties of LA-ICG were investigated.

2. Materials and methods

2.1. Materials

Iota-Carrageenan was purchased from Sigma–Aldrich Canada Co. (Oakville, ON, Canada). Lappaconitine was extracted from *Aconitum sinomantanum Nakai* in the laboratory with 98% ethanol, which details are reported elsewhere (Zhang et al., 2007). A 732 medical ion exchange resin with strong acid form was obtained from Shanghai Zhenxing Co. (Shanghai, Shanghai, China). Other reagents, such as ethanol, and N,N-dimethylformamide were purchased from Sigma–Aldrich Canada Co. (Oakville, ON, Canada) and directly used without further purification.

2.2. Characterization

UV spectra were recorded on a UV-2501PC/2550 (Shimadzu Corporation, Japan). The FTIR spectra were recorded using a Bruker Vector-22 FT-IR spectrometer from 4000 to 500 cm^{-1}. ^{13}C NMR spectra were recorded on a Bruker DRB-300WB spectrometer operating at 75.48 MHz at 90 °C. Intact samples (50 mg/mL) were dissolved in 1:1 D_2O:H_2O and transferred into 5 mm o.d. NMR tubes. Deuterated dimethylsulfoxide (DMSO-d6) was added as an internal reference. Chemical shifts were relative to internal standard DMSO-d6 (39.4 ppm). Elemental analysis (C, H, N and S) was performed using a PE-2400 analyzer. X-ray photoelectron spectra were obtained on an X-ray photoelectron spectrometer (ESCALab MKII), using Mg KR radiation (1253.6 eV) as the exciting source. The morphology was observed using environmental scanning electron microscopy (Philips XL-30 ESEM) operated at an accelerating voltage of 20 kV. Molecular weight determination HPSEC-LLS measurements were carried out on a high pressure size-exclusion chromatography combined with multi-angle laser photometer (MALLS, λ = 690 nm; DAWN EOS, Wyatt Technology Co., USA). Ultrahydrogel^{TM} column (7.8 mm × 300 mm, Waters, USA) was used as SEC instrument. An optilab refractometer (DAWN, Wyatt Technology Co., USA) was simultaneously connected. The samples with desired concentrations were prepared and optical clarification of the samples was achieved by filtration into a scattering cell. The injection volume was 50 μL and the flow rate was 0.5 mL/min. The analgesic properties assays were carried out using protocols approved by the Health Sciences Animal Welfare Committee at the Inner Mongolia Agricultural University.

2.3. Sample preparation

Degradation with sulfuric acid: ICG (1 g) was added into 100 mL of deionized water, and then stirred well until completely dissolved at 60 °C, followed by the addition of 1 mL H_2SO_4 (2 mol/L). After 4 h, 0.1 mol/L NaOH was used to adjust pH to 7.0. The solution was dialyzed using deionized water for 3 days and the degraded ICG was obtained by freeze-drying.

Static state degradation: ICG (1 g) was added into 100 mL of deionized water, and stirred well until completely dissolved at room temperature (22 °C). The aqueous ICG was slowly pumped at 1 mL/min through the glass column (D 2 cm × H 30 cm) loaded with 25 g cation exchange resin. Then, the solution collected was freeze-dried to obtain the degraded ICG.

Dynamic state degradation: ICG (1 g) was added into 100 mL of deionized water, and stirred well until completely dissolved at room temperature (22 °C). Then, cation exchange resin (25 g) was added and stirred at 250 rpm for 100 min. Finally, the resin was separated by filtration from the liquid solution (deionized water and ICG), which was later freeze-dried to obtain the degraded ICG.

To obtain a suitable ICG molecular weight, the resin amount, reaction temperature and time were investigated.

Determination of resin amount: ICG (1 g) was dissolved in 100 mL of deionized water, and the aliquot was divided into four equal parts with 1, 1.5, 2, and 2.5 g cation exchange resin added, respectively. Stirring continued for 2 h at 60 °C. Products obtained after filtering were named as I-2 h-1 g, I-2 h-1.5 g, I-2 h-2 g and I-2 h-2.5 g.

Determination of reaction temperature: ICG (1 g) was dissolved in 100 mL of deionized water, and the aliquot was divided into four equal parts with 2.5 g cation exchange resin added in each part and stirred for 2 h at 30, 40, 50, and 60 °C, respectively. Products obtained after filtering were named as I-2 h-30 °C, I-2 h-40 °C, I-2 h-50 °C, and I-2 h-60 °C.

Preparation of degraded ICG (Scheme 1a): ICG (1 g) was dissolved in100 mL of deionized water and then divided into four equal parts with 2.5 g cation exchange resin added in each part and stirred for 1, 2, 3, and 4 h at 60 °C. Products obtained after filtering were named as I-60 °C-1 h, I-60 °C-2 h, I-60 °C-3 h, and I-60 °C-4 h.

Preparation of LA-ICG (Scheme 1b): The degraded ICG visualized in Scheme 1a was followed by the addition of 0.05 g LA separately. Extra LA was removed by the use of chloroform. LA-ICG was obtained after freeze-drying: I-60 °C-1 h + LA – high molecular weight LA-ICG, I-60 °C-2 h + LA – middle molecular weight LA-ICG, and I-60 °C-4 h + LA – low molecular weight LA-ICG.

2.4. LA content

The LA-ICG was diluted into six different concentrations by 0.1 mol/L HBr, and then filtered by micro-filtration membrane (0.45 μm). The absorbance of the solution was measured at 298 nm using a UV-spectrometer (Zhang et al., 2007). The content of LA in LA-ICG was calculated using LA standard curves.

2.5. Drug release assays

The LA-ICG (10 mg) was suspended in PBS (5 mL) at selected pH values (pH = 6.8, 7.0 and 7.4). The solutions were then transferred into dialysis bags (MWCO 3500) and immersed into 45 mL of PBS at 37 °C for drug release (Scheme 1c). The release medium of 10 mL was withdrawn periodically and replaced with equivalent volume of fresh PBS. The LA concentrations in buffer solutions were determined by HPLC (Agilent 1100, USA, C18 column). A mixture of 70% methanol and 30% water at a flow rate of

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