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Rheological properties of gallic acid-grafted-chitosans with different substitution degrees

Minhao Xie [a], Bing Hu [a], Yuhua Yan [a], Li Zhou [a], Shiyi Ou [b], Xiaoxiong Zeng [a, *]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[b] Department of Food Science and Engineering, Jinan University, Guangzhou 510632, Guangdong, China

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ABSTRACT

A method to graft gallic acid (GA) onto chitosan (CS) with the assistance of 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC) and hydroxybenzotriazole has been previously developed, affording GA-grafted-CS (GA-*g*-CS). In the present study, the optimal reaction conditions for the synthesis of GA-*g*-CS with highest gallic acid substitution were determined as chitosan 10.1 mg/mL, mole ratio of gallic acid to EDC fixed at 1, and temperature ranging from 15 to 30 °C. The amount of gallic acid grafted onto chitosan had considerable influence on viscosity of GA-*g*-CS. Viscosity of GA-*g*-CS had shear-thinning behavior, and had concentration, temperature and pH-dependent manner. Free gallic acid and NaCl showed little impact on GA-*g*-CS viscosity, while $CaCl_2$ and Na_2SO_4 aggregated GA-*g*-CS and decreased the viscosity rapidly. GA-*g*-CS with $MgSO_4$ exhibited a three-region (shear thinning - plateau or shear thickening - shear thinning) viscosity profile. GA-*g*-CS shared similar rheological properties with chitosan, but the grafting of gallic acid endowed the polymer with some properties different from plain chitosan. The grafting of gallic acid altered macromolecular structure and provided more possibility for the polymer to interact with environment. GA-*g*-CS, a chitosan derivative, is a potential material in food industry.

1. Introduction

Chitosan is a linear copolymer consisting of *N*-acetyl-D-glucosamine and D-glucosamine units linked by β-(1→4) glycosidic bonds with different proportions depending upon the degree of acetylation (DA) (Dash, Chiellini, Ottenbrite, & Chiellini, 2011). It is the only semi-natural amino polysaccharide and its cationic characteristic in acidic medium is unique among polysaccharides. It exhibits excellent properties such as biocompatibility, biodegradability, non-toxicity, non-allergen, adsorption property, and film and fiber forming ability (Kumar, 2000; Ngo et al., 2015). Furthermore, it has diverse biological activities such as antibacterial and antioxidant activities, elicitation of plant defense, cholesterol lowering effect and wound-healing property (Kasaai, 2010). It also has the potential to be exploited as a lipogenesis-suppressing diet supplement (Chiu, Chan, Yang, Liu, & Chiang, 2015) and a gastrointestinal microecology-modulating agent (Vernazza, Gibson, & Rastall, 2005). In this respect, chitosan is recommended as a functional material with interesting properties in food, medicine, cosmetics, agriculture, and chemical industries. Chitosan is employed in food industry for coating fresh fruits, clarification and de-acidification of fruit juices, recovery of solid materials from food processing wastes and water, food preservative, emulsifying agent, and preparation of edible films (Kaur & Dhillon, 2014; Shahidi, Arachchi, & Jeon, 1999). The biomedical-pharmaceutical applications of chitosan are tissue engineering, wound healing, drug delivery, gene therapy, and bioimaging in forms of micro/nanoparticles or hydrogels (Dash et al., 2011).

As a result of its rigid crystalline form and intense intra- and inter-molecular hydrogen bonding, chitosan is normally insoluble in aqueous solution whose pH is above 7.0; however, the protonated free amino groups on glucosamines of chitosan facilitate its solubility in acidic conditions (pH < 6.0) (Di Martino, Sittinger, & Risbud, 2005). Due to its poor solubility, many modifications of chitosan have been attempted and many chitosan derivatives have been synthesized (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). In addition, grafting is an attractive way to impart functional groups to a polymer, combine the advantages of both grafted molecules and polymers and endow them with new characteristics for specific applications (Bhattacharya & Misra, 2004; Hu & Luo, 2016). The attachment of side groups onto chitosan

* Corresponding author.
E-mail address: zengxx@njau.edu.cn (X. Zeng).

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backbone provides versatile materials with specific functionality and alters biological or physical properties (Dash et al., 2011). Grafting polyphenols such as gallic acid (GA), caffeic acid (CA), ferulic acid (FA), chlorogenic acid (CGA), epigallocatechin gallate (EGCG) onto chitosan or modified chitosan received much attention recently, and GA-, CA-, EGCG-, CGA-chitosan and FA-carboxymethyl chitosans have been synthesized via enzyme-, free radical- or activated ester-mediated reactions (Aljawish et al., 2012; Aytekin, Morimura, & Kida, 2011; Bozic, Gorgieva, & Kokol, 2012; Lei, Wang, Liang, Yuan, & Gao, 2014; Liu, Lu, Kan, Tang, & Jin, 2013b; Pasanphan & Chirachanchai, 2008; Wei & Gao, 2016a; Woranuch & Yoksan, 2013). The polyphenol-chitosan conjugates exhibit antioxidant, antibacterial, anti-diabetic, anti-inflammation, biological membrane protection, and Fe-adsorption activities (Ahn et al., 2015; Aytekin et al., 2011; Lee & Je, 2013; Liu, Lu, Kan, & Jin, 2013a; Liu et al., 2015; Mertins, Mathews, Gomide, Baptista, & Itri, 2015; Woranuch & Yoksan, 2013). They can be employed as novel biomaterials to prepare drug-delivering nanoparticles with specific properties (Hu et al., 2015, 2016).

In our previous study, we have already developed a one-pot method to graft gallic acid onto chitosan in the presence of 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt). The incorporation of gallic acid enhanced the antioxidant activity of the conjugate (Xie, Hu, Wang, & Zeng, 2014). The synthesized GA-*g*-CS presented a higher apparent viscosity than plain chitosan (Xie et al., 2014). CGA-CS covalent adduct also showed higher viscosity and shear stress than chitosan (Wei & Gao, 2016a). In addition, EGCG-CS and CGA-CS conjugates exhibited excellent emulsifying activity and superior emulsifying stability as compared with chitosan (Lei et al., 2014; Wei & Gao, 2016b). The rheological properties of polyphenol-*g*-chitosan conjugates are distinct from chitosan, and they are worthy of more attention and research. Comprehension of their characteristics is fundamental to the applications of phenolic-*g*-chitosan conjugates. In the present study, therefore, the effects of reaction conditions on the amount of gallic acid grafted onto chitosan were investigated, and GA-*g*-CS with different gallic acid substitutions were then prepared. Finally, the thixotropic properties and linear viscoelastic region of GA-*g*-CS, effects of concentration, temperature, pH, salt ion, and free gallic acid on viscosity of GA-*g*-CS were investigated.

2. Materials and methods

2.1. Reagents and chemicals

Chitosan (viscosity-average molecular weight ~ 1.5×10^5, DA $\geq$ 90.0%), EDC and Folin-Ciocalteau reagent were purchased from Kayon Biological Technology Co., Ltd. (Shanghai, China). GA and HOBt were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All of other reagents were of analytical grade.

2.2. Preparation of GA-g-CS

The synthesis of GA-*g*-CS was performed as described as our previous report (Xie et al., 2014) with slight modification. For each experiment, chitosan (0.061–0.906 g) and equal mol of HOBt were stirred in deionized water (30.0 mL) overnight to a uniformly dispersed system. The calculated amount of gallic acid (0.031–0.622 g) and EDC (0.036–1.065 g, ethanol solution) were successively introduced into the chitosan solution. The reactions were conducted for 24 h at controlled (0, 15, 30, and 45 °C) or room temperature (20–25 °C) and atmosphere. The resultant reaction system was diluted with acetic acid solution (20.0 mL/L) if necessary and centrifuged (15,300*g*, 20 min) before introducing the supernatant into dialysis tubes (MWCO 8000–14000 Da). The samples were dialyzed against deionized water for more than 72 h with eight changes of deionized water, and thin layer chromatography was used to check no free gallic acid remaining in the product. The dialyzed solution was lyophilized to afford solid GA-*g*-CS. Blank chitosan acting as a control was prepared in the same conditions but without gallic acid. The grafted gallic acid content in GA-*g*-CS was determined by the Folin-Ciocalteau method as previous reported (Liu et al., 2009; Xie et al., 2014). Briefly, 0.5 mL of GA-*g*-CS solution was mixed with 1.0 mL of Folin-Ciocalteau reagent for 5 min in the dark, and then 2.0 mL sodium carbonate (Na_2CO_3) solution (200.0 g/L) was added. The mixture was shaken and kept at 30 °C for 1 h, and the absorbance (Abs) at 747 nm was measured by using a spectrophotometer (Shanghai Jinghua Science & Technology Instruments Co., Ltd., Shanghai, China). Gallic acid was used as a standard. The gallic acid content in GA-*g*-CS was expressed as milligram gallic acid equivalent per gram of conjugate (mg GAE/g).

2.3. Measurement of rheological properties of GA-g-CS

Different batches of GA-*g*-CS sharing the approximate gallic acid substitution degrees were combined to afford sufficient sample. Three representative samples of GA-*g*-CS were prepared, with low, medium and high average GA substitution of 20.3, 94.1, and 159.6 mg GAE/g, respectively. All the measurements were carried out using GA-*g*-CS with the medium gallic acid substitution, except the test of the effect of substitution on viscosity, in which all the three kinds of samples were tested.

Rheological behavior of sample was measured on a MCR 302 Rheometer (Anton Paar GmbH, Graz, Austria) equipped with a 50 mm diameter parallel plate (PP50) with the gap fixed at 1 mm. All the samples were dispersed in acetic acid solution (10.0 mL/L) and homogenized by shearing at 10,000 rpm for 3 min (IKA T18 homogenizer, Staufen im Breisgau, Germany) before measurement. The value at each point was recorded five times, and each sample was tested in triplicate to ensure reproducibility.

The viscosity against the shear rate (0.1–1000 s^{-1}) was recorded at 20 °C at concentrations from 2 to 20 mg/mL. The effects of substitution degree, temperature, ionic strength, free gallic acid and pH on the viscosity curve were investigated at GA-*g*-CS concentration of 10 mg/mL. Ionic strength was adjusted by corresponding solid NaCl, and the pH was adjusted from 3.55 to 8.55 by NaOH solution (1.0 mol/L). Thixotropic properties of GA-*g*-CS and chitosan were measured using a 3 interval thixotropy test (3ITT).

The linear viscoelastic region (LVR) was detected at 1 Hz by an amplitude sweep test from 0.1% to 100% at 20 °C. Oscillatory measurements of the storage modulus (G′) and loss modulus (G″) were performed by a frequency sweep from 0.1 to 100 Hz at 20 °C with a shear strain of 0.1%.

2.4. Statistical analysis

The data are expressed as the mean ± standard deviation (SD) of triplicates. The least significant difference (LSD), Duncan's multiple range test, and one-way analysis of variance (ANOVA) were used for multiple comparisons by SPSS 16.0. The difference was considered to be statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Preparation of GA-g-CS with different substitutions

GA-*g*-CS was synthesized by conjugating gallic acid with