Carbohydrate Polymers 181 (2018) 812–817

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Synthesis, characterization and antioxidant activity of chitosan-chromone derivatives

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ARTICLE INFO

Keywords:
Chitosan derivatives
Chromone
Terephthalaldehyde
Antioxidant activity

ABSTRACT

In this study, a new chromone-functionalized chitosan Schiff base and its cross-linked derivative were synthesized and characterized by FT-IR, UV–vis, ^{13}C CP/MAS solid-state NMR, TGA, XRD-powder and SEM measurements and elemental analysis data. Degrees of substitution (DS) were determined from the elemental analysis by using the C/N ratios. The *in vitro* antioxidant activity of high molecular chitosan and its chromone derivatives was evaluated as radical scavengers against 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH·). The results showed that both of the chitosan-chromone derivatives have good antioxidant potential which might be due to the phenolic group introduced after chemical modification of chitosan with a chromone derivative. Chromone-chitosan Schiff base (CSCH) had a better ability to scavenging DPPH radical (IC_{50}, 0.88 mg/mL) than that of its cross-linked derivative (CSCH-TP) obtained by using terephthalaldehyde (IC_{50}, 1.32 mg/mL).

1. Introduction

Chitosan (**CS**) is the second-most abundant biopolymer derived from the N-deacetylation of chitin. The design and synthesis of chitosan-based materials has been attracted by researcher groups due to their unique biological and physicochemical properties such as biocompatibility, biodegradability and film-forming ability. Various novel chitosan derivatives have been obtained by chemical modifications of reactive hydroxyl and amino/acetamido groups attached to the macromolecule backbone (Kumar, 2000; Jiao et al., 2011; Muzzarelli, 1977). For instance, Schiff bases that can be synthesized by condensation of amino groups of chitosan with aldehydes or ketones have been received extensive interests for their expanded biofunctional properties such as antibacterial, antitumor and antioxidant activities (Elshaarawy, Refaee, & El-Sawi, 2016; Tamer et al., 2017). Chitosan and especially self-assembled chitosan materials have therefore become of great interest as new functional biomaterials with significant potential in numerous fields (Guo et al., 2015; Jiao et al., 2015; Zhao et al., 2015). In numerous studies, chitosan was reported as a potential antioxidant agent (Kim & Thomas, 2007; Vinsova & Vavrikova, 2011; Kaya et al., 2014).

Natural compounds isolated from plants possess biological activities can be used to improve the antioxidant effect of chitosan (Pasanphan & Chirachanchai, 2008). Chromone and its derivatives are an important class of heterocycles, mainly present in natural products and exhibit a wide range of pharmacological properties such as anti-bacterial, anti-fungal (Grindlay and Reynolds, 1986; Jovanovic, Steenken, Tosic, Marjanovic, & Simic, 1994), anti-cancer (Martens and Mithöfer, 2005), anti-HIV (Zhou, Shi, & Lee, 2010), anti-ulcers (Parmer, Tariq, & Ageel, 1987), antiinflammatory (Gabor, 1986), wound healing (Maho & Yoshiyuki, 2010), and anti-oxidant (Kuroda, Uchida, Watanabe, & Mimaki, 2009). It was reported that the chemical modification of chitosan molecule with a chromone derivative led to an enhancement in the biological activities such as antimicrobial (Kumar & Koh, 2012), fungicidal and insecticidal (El Badawy, 2008). But there is no any previous report on the antioxidant activity of a chitosan-chromone derivative.

In this study, a new chitosan-chromone Schiff base (CSCH) and its crosslinked derivative (CSCH-TP) were synthesized by the condensation reaction of 6-formyl-7-hydroxy-5-methoxy-2-methylbenzopyran-4-one (CH) with chitosan (CS) and terephthalaldehyde (TP) was used as a crosslinking agent. Their structural characterization was carried out by means of UV–vis, FT-IR, ^{13}C NMR spectroscopy, XRD Diffraction, TGA, SEM imaging techniques and elemental analyses. Furthermore, the antioxidant activities of the obtained materials were evaluated by using DPPH scavenging assay.

2. Materials and methods

2.1. Materials

Chitosan with high molecular weight (> 75% deactylated) was

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https://doi.org/10.1016/j.carbpol.2017.11.074
Received 2 October 2017; Received in revised form 15 November 2017; Accepted 20 November 2017
Available online 24 November 2017
0144-8617/

purchased from Sigma-Aldrich. Glacial acetic acid, terephthalaldehyde (TP), methanol, ethanol and the organic solvents were purchased from Merck Co. The anti-oxidant reagent 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. All reagents were of analytical grade and were as received without further purification.

Elemental analyses (C, H and N) were performed using a Thermo Scientific Flash 2000 CHNS/O Analyzer. The FT-IR and UV–vis spectra were recorded on a Perkin Elmer Spectrum Two with U-ATR FTIR spectrometer and on a OPTIZENα UV–vis Spectrometer, respectively. 0.02 g of sample was dissolved in 25 mL of acetic acid 1%. The electronic absorbance of samples in a quartz cell scanned from 230 to 500 nm was recorded. ^{13}C NMR spectra were obtained by a Bruker Superconducting FT/NMR Spectrometer Avance TM 300 MHz WB with CP/MAS technique (cross-polarization, magic-angle-spinning). The X-ray diffraction patterns of samples were recorded at room temperature on a using a Rigaku System RadB X-Ray Diffractometer, using monochromated Cu Kα radiation in the range 2–40° (*2θ*), at 25 °C. The surface morphology of chitosan and derivatives were examined by using a JEOL JSM 5500 scanning electron microscope (SEM) at 10 kV. The thermogravimetry analyses (TGA) were carried out in nitrogen atmosphere using METTLER TOLEDO, heated from 30 °C to 1000 °C at a heating rate of 10 °C min^{-1}.

2.2. Preparation of chitosan-chromone derivatives, *CSCH* and *CSCH-TP*

The chromone derivative (6-formyl-7-hydroxy-5-methoxy-2-methylbenzopyran-4-one, **CH**) was obtained by the oxidation of visnagin, according to the procedure described before by our group (Beyazit, Çatıkkaş, Bayraktar, & Demetgöl, 2016; Gönaydın & Beyazit, 2004).

For the preparation of **CSCH**, 0.5 g of powder chitosan was dissolved in 25 mL of 1.0% (*v/v*) glacial acetic acid. The mixture was vigorously stirred under the reflux condition until the chitosan was completely dissolved. 0.2 g of chromone derivative in 50 mL methanol solution was added drop wise into the chitosan solution and the mixture was stirred under reflux condition for 4–5 h. The yellow color product was filtered off, washed with ethanol and dried *in vacuo*.

For the preparation of **CSCH-TP**, 0.3 g of CSCH was dissolved in 40 mL of 1.0% (*v/v*) glacial acetic acid and 0.3 g of terephthalaldehyde **(TP)** in 25 mL ethanol solution were added drop wise into the CSCH solution. The mixture was stirred under reflux condition for 4–5 h. The yellow color product was filtered off, washed with ethanol and dried *in vacuo*.

The synthesis route of chitosan-chromone derivatives are summarized in Scheme 1.

2.3. Antioxidant activity

2.3.1. DPPH scavenging activity

CSCH or **CSCH-TP** (2 mL in 1.0% (*v/v*) glacial acetic acid solution) was added to a methanolic solution of DPPH (2.0×10^{-4} M, 2.0 mL) and 5 mL of methanol. The mixture was shaken for 10 s and left to stand at room temperature for 30 min. The scavenging activity of **CSCH** or **CSCH-TP** on DPPH· was determined by the absorbance at 517 nm. The percentage of scavenging activity was calculated by using the following Eq. (1):

$$\% \text{ scavenging activity} = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100 \quad (1)$$

where $Abs_{control}$ is the absorbance of the control (without the test sample) and Abs_{sample} is the absorbance of the sample (with the test sample).

3. Results and discussion

3.1. Elemental analysis

The elemental analysis data of chitosan and its chromone derivatives (CSCH and CSCH-TP) are listed in Table 1. Carbon to nitrogen (C/N) ratios of CS, CSCH and CSCH-TP were found to be 5.57, 8.41 and 10.62%, respectively. The increase in the C/N ratios of chitosan-chromone derivatives can be explained by the chemical modification of chitosan with a nitrogen-free chromone analog.

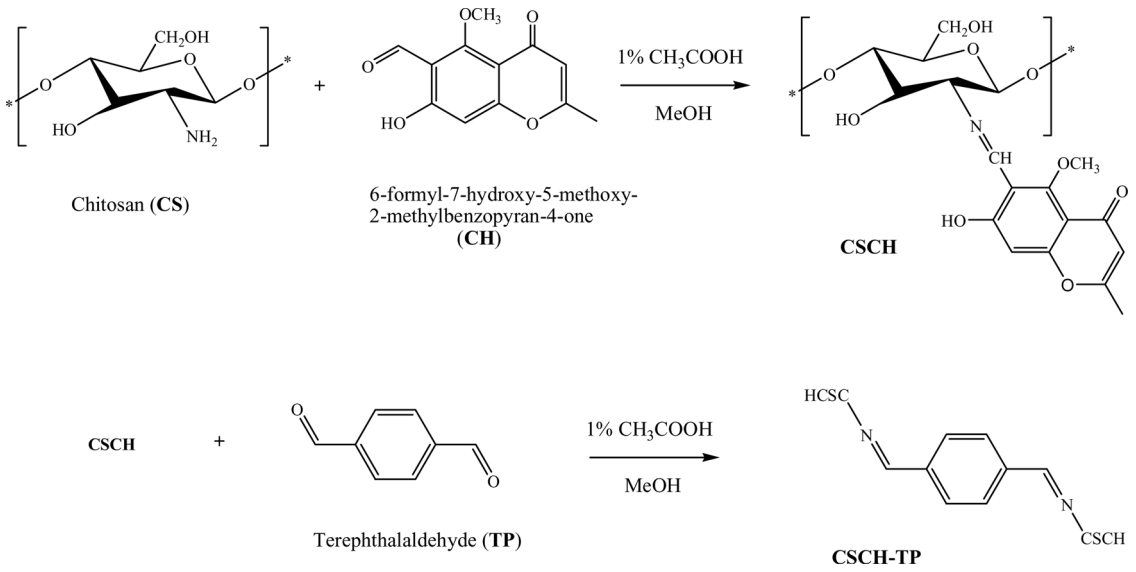
From Table 1, the degree of substitution (DS) of the chitosan derivatives to $-NH_2$ group on chitosan was calculated by following Eq. (2), modified with base in the model of Inukai et al. (Inukai, Chinen, Matsuda, Kaida, & Yasuda, 1998):

$$DS = \frac{(C/N)m - (C/N)o}{n} \quad (2)$$

Where $(C/N)_m$ is the C/N of the chitosan derivative, $(C/N)_o$ is the C/N of the original chitosan, *n* is the number of carbon introduced after chitosan modification. The DS values obtained were about 0.33 and 0.50 for CSCH and CSCH-TP, respectively.

3.2. UV–vis spectra

The electronic spectra of chitosan and its derivatives were measured



Scheme 1. The synthesis of chitosan-chromone Schiff base (CSCH) and its cross-linked derivative (CSCH-TP).