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Hydrophobically modified chitosan: A bio-based material for antimicrobial active film

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ABSTRACT

The objective of the present research was to improve the hydrophobicity of chitosan, while retaining its antibacterial activity, through the grafting of dodecenyl succinyl chains onto phthaloyl chitosan, mainly at the C-6 position. Dodecenyl succinylated phthaloyl chitosan (DS-*g*-PHCTS) was synthesized via phthaloylation–dodecenyl succinylation–hydrazinolysis. The obtained derivatives were characterized by FTIR, ^{1}H NMR and XRD. Hydrazinolysis time was found to be a key factor in controlling the substitution of dodecenyl succinyl chains and phthalimido groups of the final product. DS-*g*-PHCTS – with a grafting degree of dodecenyl succinyl chains and a substitution degree of phthalimido groups of 0.73 and 0.39, respectively – exhibited an anhydrous crystal structure and the same solubility behavior as native chitosan. The introduction of hydrophobic alkyl chains provided DS-*g*-PHCTS with enhanced antibacterial activity against Gram-positive bacteria. In addition, DS-*g*-PHCTS film showed more effective bacterial growth inhibition and better water vapor barrier property under neutral pH condition than chitosan film. The results suggested that DS-*g*-PHCTS film could be potentially used as antibacterial active film.

1. Introduction

Hydrophobic derivatives of chitosan have received much attention over the past decades due to their ability to resist water/moisture permeation when they are in the form of film, which is important for packaging applications, and to form self-assembled nanoparticles in aqueous media, which is useful for water-insoluble drug delivery systems. Several studies have focused on the preparation of hydrophobic chitosan from long-chain acyl chlorides, glycidyl ethers, aldehydes, carboxylic acids and anhydrides [1–9]. Succinic anhydride is one of the most effective conjugating agents for polysaccharides bearing hydroxyl and amino groups. Many efforts have been made to conjugate certain substances – such as cholesterol, poly(ethylene glycol) monomethyl ether, acyclovir, metronidazole and prednisolone – to chitosan via succinyl linkages/spacers [5,10–13]. However, this involves a two-step reaction. The direct grafting of succinic anhydride derivatives such as 2-(dodecen-1-yl) succinic anhydride onto the biopolymer backbone via ring-opening reaction has attracted much attention due to its one-step preparation [2]. Previously, the grafting reaction of succinic anhydride derivatives onto chitosan has taken place at the C-2 position, as found in the preparation of *N*-/2(3)-(dodec-2-enyl)succinoyl chitosans [2,3] and lauryl succinyl chitosan [4]. However, the chemical modification of chitosan at amino groups may alter its original fundamental characteristics in terms of cationicity, biological activity and chelating power.

In order to preserve a certain amount of amino groups for positive charge density, antimicrobial activity and progressive chemical modification, the introduction of hydrophobic chains primarily at the C-6 position of chitosan macromolecules is proposed. The present research thus focuses on the grafting of dodecenyl succinyl chains onto chitosan, mainly at the C-6 position through phthaloylation–dodecenyl succinylation–hydrazinolysis. Characterization by FTIR, ^{1}H NMR and XRD techniques, solubility and antibacterial activity of the obtained derivatives were investigated. In addition, antibacterial properties and water vapor permeability of the final product in the form of film were studied and compared with those of chitosan film.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation = 0.90, Mw = 200,000 Da) was purchased from Seafresh Chitosan Lab Co., Ltd. (Thailand). Dodecenyl succinic anhydride was obtained from Sigma-Aldrich (USA). Phthalic anhydride was supplied by Ajax Chemicals (Australia). Hydrazine

Abbreviations: DS-*g*-PHCTS, dodecenyl succinylated phthaloyl chitosan.

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monohydrate was purchased from Carlo Erba Regenti (Italy). *N,N*-dimethylformamide (DMF) was obtained from Lab-Scan (Ireland). Pyridine was supplied by May and Baker Co., Ltd. (England). Bacto™ peptone and Mueller Hinton broth were purchased from Becton, Dickinson and Company (USA). All commercially available solvents and reagents were used without further purification.

2.2. Phthaloylation of chitosan

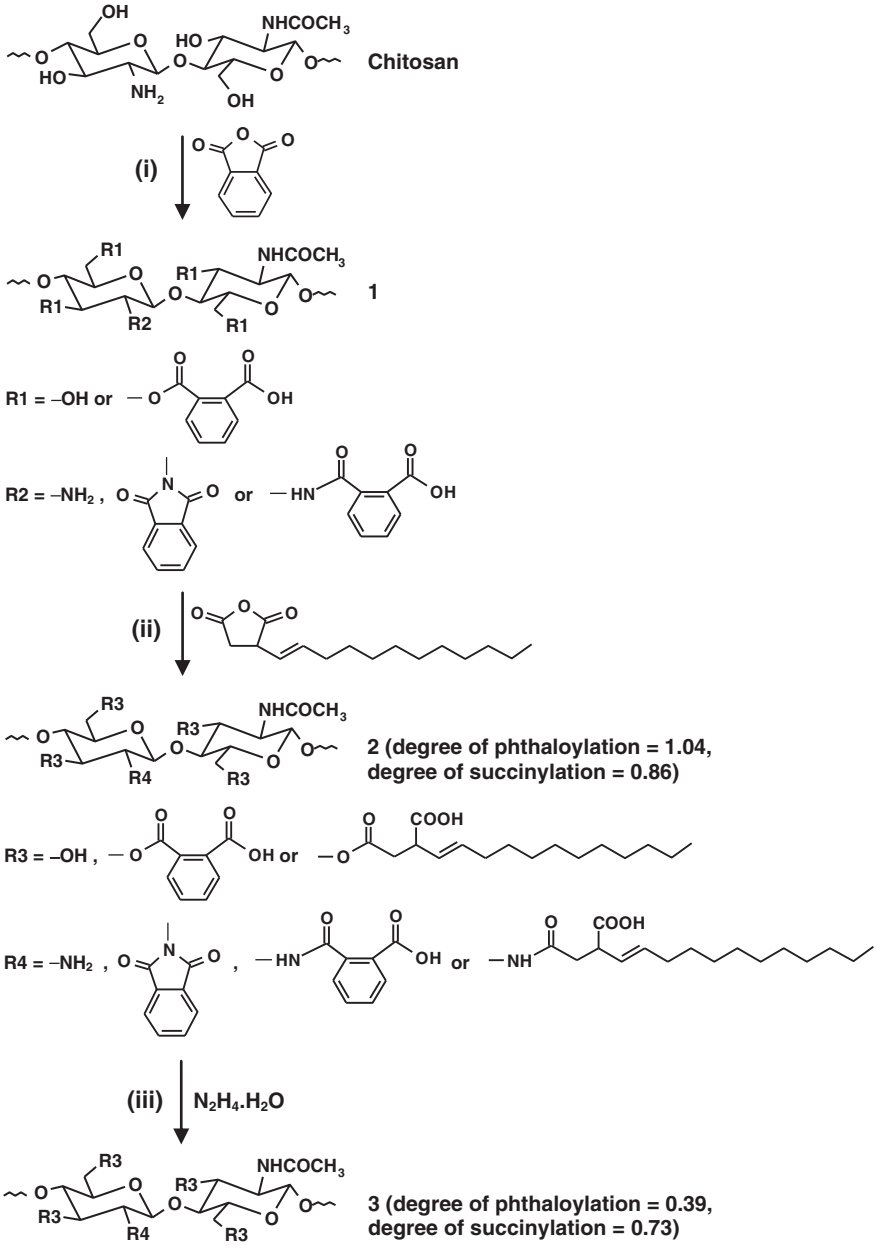
Phthaloylation of chitosan was carried out according to the method reported in our previous article [14]. Briefly, chitosan (1 g) and phthalic anhydride (4.48 g, 5 mol equiv to pyranose ring) were dissolved in DMF (20 mL). The mixture was stirred at 100 °C under reduced pressure for 6 h followed by at 60 °C under nitrogen atmosphere overnight. The supernatant obtained after centrifugation was precipitated in cold water. The precipitate was then thoroughly washed with water and methanol several times to give phthaloyl chitosan (**1**) (Scheme 1).

2.3. Dodecenyl succinylation of phthaloyl chitosan

1 (0.25 g) was dissolved in DMF (5 mL) at two different temperatures, i.e. 30 °C and 60 °C. Dodecenyl succinic anhydride (1, 2, 3 and 5 mol equivalent to **1**) and pyridine (a few drops) were then added to the homogeneous **1**/DMF solution. The mixture was agitated for 24 h at a temperature corresponding to that used for dissolving **1** in DMF (30 °C or 60 °C), then cooled to room temperature and precipitated in cold water. The precipitate was washed with distilled water and methanol several times and dried under reduced pressure to obtain dodecenyl succinylated phthaloyl chitosan (**2**) (Scheme 1).

2.4. Hydrazinolysis of dodecenyl succinylated phthaloyl chitosan

2 (0.25 g) was dissolved in hydrazine monohydrate (15 mL) by stirring at 80 °C under nitrogen atmosphere for different lengths of time (3, 6, 9 and 12 h). The solution was cooled to room temperature and dialyzed against distilled water for 3 days. The precipitate was



Scheme 1. Synthesis pathway for (i) **1** through phthaloylation of chitosan, (ii) **2** through dodecenyl succinylation of **1** and (iii) **3** through hydrazinolysis of **2**.