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Chemical structure analyses of phosphorylated chitosan

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ABSTRACT

Chemical modification of chitosan to generate new bio-functional materials can bring more desirable properties depending on the nature of the groups introduced. Phosphorylated chitosan has attracted interests in recent years. The literature has reported that the phosphorylation of chitosan could be achieved through three different reaction routes, namely, in the presence of H_3PO_4/urea, $H_3PO_4/Et_3PO_4/P_2O_5$, or P_2O_5/CH_3SO_3H. However, the exact chemical structure of phosphorylated chitosan synthesized by different reaction routes has not been systematically studied and compared. Meanwhile, the most common opinion is that the hydroxyl group in chitosan is the main substitution site. In this work, phosphorylated chitosan was synthesized using three different reaction routes, and the chemical structures of the products were studied by infrared, X-ray photoelectron and ^{13}C NMR spectroscopic characterization. It was observed that in the reaction routes using H_3PO_4/urea and $H_3PO_4/Et_3PO_4/P_2O_5$, the amino groups were substituted instead of the hydroxyl groups. In the reaction route using P_2O_5/CH_3SO_3H, the amino groups were shielded by the ionic binding with CH_3SO_3H, and the C-6 hydroxyl groups were phosphorylated. Different structures of the phosphorylated chitosan were proposed based on the characterization results.

1. Introduction

Chitosan, the fully or partially deacetylated form of chitin, is known to be present widely and abundantly in nature as an essential supporting structure for several living organisms including fungi, crustaceans, insects and arthropods.[1,2] Due to its advantageous properties of being nontoxic and enzymatically biodegradable, chitosan is widely used in agriculture, biotechnology, production of cosmetics, medicine and food industry as a biologically active polymer.[3] The biomedical applications of chitosan are governed by its antibacterial property, and the polymeric film prepared by chitosan is possibly permeable for physiologically active substances and decompose in a living body.[4] Chitosan based growth-regulating, protecting, stimulating, and immune-modulating agents are used in agriculture.[5]

In recent years, much attention has been focused on the synthesis of new bio-functional chitosan derivatives through chemical modifications. This is because the modification would not change the fundamental skeleton of chitosan, but would keep the original physicochemical and biochemical properties and would bring new properties depending on the nature of the groups introduced.[6–9]

At the same time, phosphorylated chitosan derivatives are known as promising materials for the development of polyfunctional plant growth regulators and resistance inductors of prolonged action. And several techniques to synthesize phosphate derivatives of chitosan have been proposed due to such interesting biological and chemical properties. Among others, such modified chitosan could exhibit bactericidal and metal chelating properties.[10–14]

According to the previous studies, the phosphorylation of the hydroxyl functional groups of chitosan to generate phosphonate was mainly through three pathways (Fig. 1). The modification reaction is carried out respectively in the presence of H_3PO_4/urea,[15,16] $H_3PO_4/Et_3PO_4/P_2O_5$[17,18] (in reference 18 chitosan films were used for the phosphorylation reactions) and P_2O_5/methanesulfonic acid.[19–21] However, the results of our study showed different results from what had been reported by the above mentioned research work. Therefore, in this paper, three phosphorylated chitosan samples were synthesized using the procedures mentioned above, and their chemical structures were characterized using infrared, X-ray photoelectron and ^{13}C NMR spectroscopy.

2. Experimental

2.1. Materials and reagents

Chitosan (MW 100,000–300,000, degree of deacetylation >90%), methanesulfonic acid (99% extra pure), triethyl phosphate (Et_3PO_4) (99% extra pure) and 1-butanol (99% extra pure) were all received from ACROS Organics Canada. Phosphorus pentoxide (P_2O_5), urea, *N*,*N*-dimethyl formamide (DMF) and phosphoric acid (H_3PO_4) were

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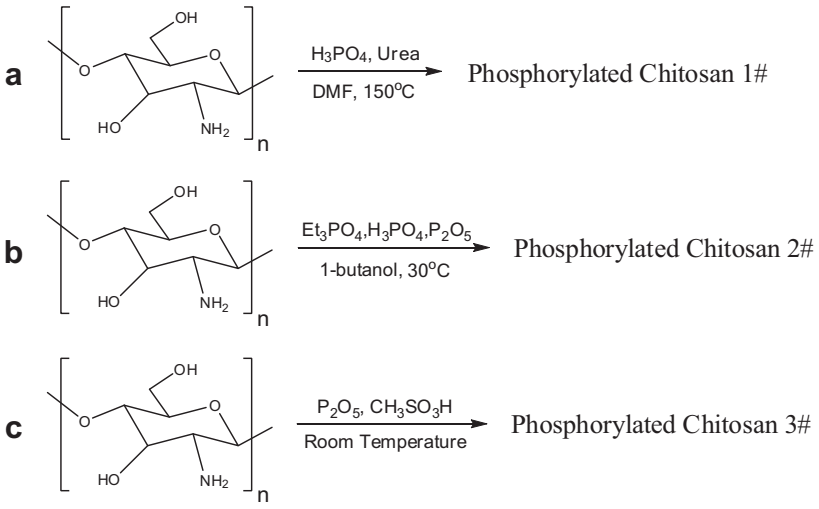

Figure 1. Three major synthesis routes for phosphorylated chitosan. (a) H_3PO_4/urea,[15,16] (b) H_3PO_4/Et_3PO_4/P_2O_5,[17,18] (c) P_2O_5/methanesulfonic acid.[19–21]

purchased from Fisher Scientific Canada. All chemicals used in this work were of analytical grade. The KBr powder used in the DRIFTS-FTIR measurements was of spectroscopic grade purchased from PIKE Technologies, USA.

2.2. Purification of chitosan

Chitosan was purified by the re-precipitation method. Briefly, the chitosan was dissolved in 3% (v/v) aqueous acetic acid overnight and, after complete dissolution, the resulting gel was filtered through sintered glass funnel, to remove undissolved and gelatinous particles. Chitosan was then precipitated through the dropwise addition of 1 M aqueous NaOH solution, with stirring. Finally, the regenerated chitosan was thoroughly washed with distilled water until neutrality, vacuum dried and ground in a laboratory agate mortar to yield a fine powder.

2.3. Phosphorylation of chitosan

Route 1:1 g powdered chitosan, 5 g urea, and 10 mL phosphoric acid were added into 40 mL DMF solution. The mixture was stirred at 150 °C in a sand bath for 1 h. After cooling, the reaction mixture was filtered. The precipitate was respectively washed thoroughly with distilled water and anhydrous ethanol, and vacuum dried. The obtained product was designated phosphorylated chitosan 1#. The reaction scheme is shown in Figure 1a.[15,16]

Route 2: 1 g powdered chitosan was suspended in 20 mL 1-butanol in a round bottom flask. The reaction mixture was prepared by respectively adding 20 mL H_3PO_4 and 20 mL Et_3PO_4, followed by the step-by-step addition of 2 g P_2O_5 with stirring. The reaction was allowed to proceed under a N_2 atmosphere with constant magnetic stirring in a water bath at 30 °C. After 3 h, the reaction mixture was filtered. The precipitate was rinsed with anhydrous ethanol and suspended twice in this solvent for 30 min, then rinsed by distilled water several times to remove free inorganic phosphate, and finally, the phosphorylated chitosan 2# was obtained after vacuum drying. The reaction scheme is shown in Figure 1b.[17,18]

Route 3: 1 g powdered chitosan was first thoroughly dissolved in 14 mL methanesulfonic acid in a round bottom flask. Then, 2 g P_2O_5 was added step-by-step with constant stirring, and the flask was sealed later to avoid the absorption of moisture from air. After 3 h of reaction at room temperature, the reacted slurry was poured into a vigorously stirred ether solution, and a white precipitate immediately appeared. After filtration, the residue was rinsed several times with ether and acetone, and then, dissolved or suspended in distilled water (50 mL) and dialyzed completely. The suspension was then poured into fiercely stirred acetone solution and white flocs were formed, which were collected through centrifugal settling. After rinsing several times with acetone, the solids were vacuum dried to obtain phosphorylated chitosan 3#. The reaction scheme is shown in Figure 1c.[19–21]

2.4. The NaOH treatment to phosphorylated chitosan

The phosphorylated chitosan was first added to a 0.1 M NaOH solution and with stirring for 30 min. Then, the precipitation was filtrated and rinsed by distilled water for three times. After vacuum drying, the NaOH-treated phosphorylated chitosan was finally obtained.

2.5. Characterization methods

Infrared, X-ray photoelectron and nuclear magnetic resonance spectroscopic measurements were made to analyze the chemical structure of phosphorylated chitosan. One sample of the phosphorylated chitosan was synthesized by each route and was characterized by these three techniques.

2.5.1. Infrared spectroscopy

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was used to investigate the chemical structure of the synthesized phosphorylated chitosan.

DRIFTS-FTIR spectra were obtained using a Nicolet 8700 (Thermo) instrument with a 'Smart Collector' accessory for DRIFTS. A spectral resolution of 4 cm^{-1} was used, and 256 scans per sample were accumulated. Samples (0.05 g ± 0.002 g) were intimately mixed with previously dried spectroscopic-grade KBr (0.8 g ± 0.002 g) by gentle grinding to minimize particle break up. The sample-KBr mixtures were placed in the Smart Collector sample cell and the DRIFTS spectra were obtained against a KBr background which was used as a reference.

2.5.2. X-ray photoelectron spectroscopy (XPS)

The XPS spectra were obtained on a Kratos Analytical Axis-165 X-ray Spectrometer, using dual (Mg and Al) X-ray radiation as the excitation source (15 kV), operated at 300 W. The analyzer was run in the constant analyzer transmission mode. The emitted