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Antibacterial activity of diisocyanate-modified chitosan for biomedical applications

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ABSTRACT

A diisocyanate-modified chitosan (DIMC) was synthesized via a cross-linking reaction with chitosan and diphenyl methane diisocyanate. The structural and thermal properties of the DIMC were systematically characterized by FTIR, UV–vis, TGA, DSC, XRD and SEM. In addition, the optical properties were evaluated by photoluminescence. Finally, the antibacterial activities of the synthesized DIMC were examined against *Escherichia coli* and *Staphylococcus pyogenes* bacteria by agar plate diffusion method. The DIMC showed better degree of bacterial growth inhibition against *E. coli* as compared with unaltered chitosan. These results suggest that the synthesized chitosan xerogel could be used as a novel biodegradable material with improved antibacterial properties for biomedical applications.

1. Introduction

Chitosan is the deacetylated form of chitin, an abundant polysaccharide found naturally in the hard shells of crustaceans and cell wall of fungi. Chitosan consists of 2-amino-2-deoxyβ-D-glucopyranose units linked through a β(1 → 4) linkage. Biodegradable chitosan biopolymer has many potential benefits including physicochemical and biological properties like biocompatibility, biodegradability and hemocompatiblity [1–5]. Owing to its unique properties, chitosan can be directly modified for a wide range of applications including but not limited to gene delivery, cosmetics, antimicrobial, tissue engineering, and biotechnology [6–13]. Accumulating lines of evidence suggest antimicrobial action of chitosan based derivatives [14,15]. According to recently published reports, shrimp chitosan shows antimicrobial activity against gram-negative bacteria such as *Escherichia coli* by disrupting the outer membrane barrier properties [16,17]. Antimicrobial effects of chitosan oligomers against *Bacillus megaterium*, *Bacillus cereus* and *Entrerobacter sakazakii* have also been reported [18]. Chitosan may have antibacterial activities depending on several factors such as (i) its charge and solubility [19], (ii) its nutrient chelating property [20], or (iii) its anti-transcriptional properties [21]. Several lines of evidence suggest antimicrobial action of chitosan against yeasts and fungi [22,23]. Recently, a lot of interest has been shown for the usage of chitosan in the food industry for preservation and processing [24]. Bio-films based on chitosan combined with materials such as proteins, polysaccharides and antimicrobial peptides have been successfully probed at an experimental level on food products such as eggs, fruits, vegetables, dairy products and meat [19,25,26].

Xerogels are low density porous materials consisting of a three-dimensional open-cell network with in-filling air in the interfaces [9,27]. The interconnected structure with continuous nanopores can provide efficient mass transfer of the liquid or gaseous substances. Xerogels have exceptionally high specific area, high porosity and surface properties and are attracting strong interest for applications in biomedical fields. Xerogels are expected to have excellent performances as biosensors, gene delivery, antimicrobials and as scaffolds for tissue regeneration [27–31]. Yim et al. reported the co-gelation of tetramethylorthosilicate and polymeric methylene diisocyanate (MDI) yielding silica polyurethane hybrid aerogels [32]. Preparation of chitin based polyurethanes with phenyl isocyanate has also been reported [33,34]. Xu and Wu have synthesized organic bisurea compounds from phenyl isocyanate and a variety of diamines and their roles as crystallization nucleating agents of poly(L-lactic acid) [35].

In this paper, we describe simple preparation, physicochemical evaluation, optical study and specific agar diffusion bioassay method of DIMC.

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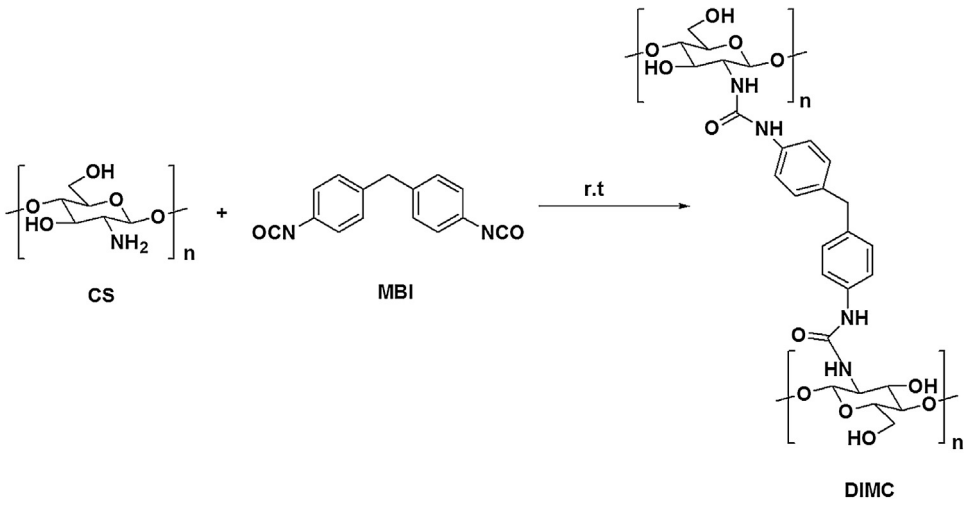

Scheme 1. Schematic representation of the preparation of diisocyanate-modified chitosan (DIMC).

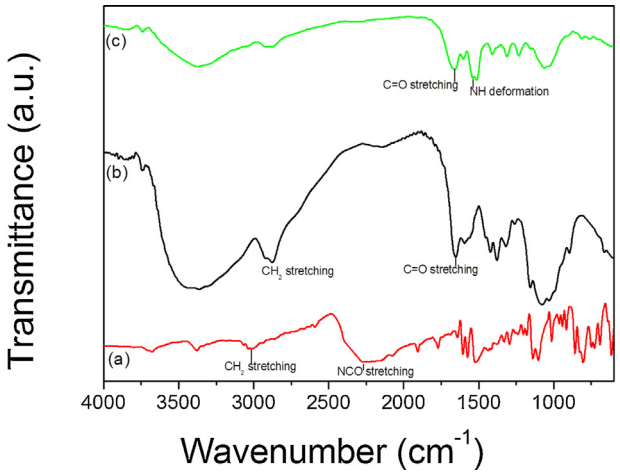

Fig. 1. FTIR of 4,4′-Methylene bis(phenylisocynate) (MBI) (a), chitosan (b), and diisocyanate-modified chitosan (DIMC) (c).

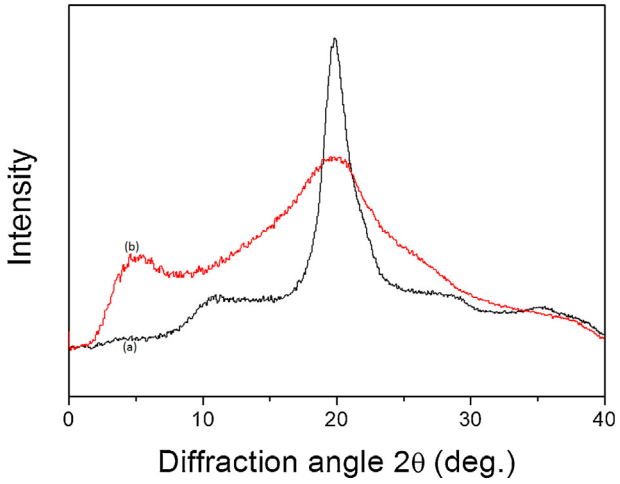

Fig. 2. XRD of chitosan (a) and DIMC (b).

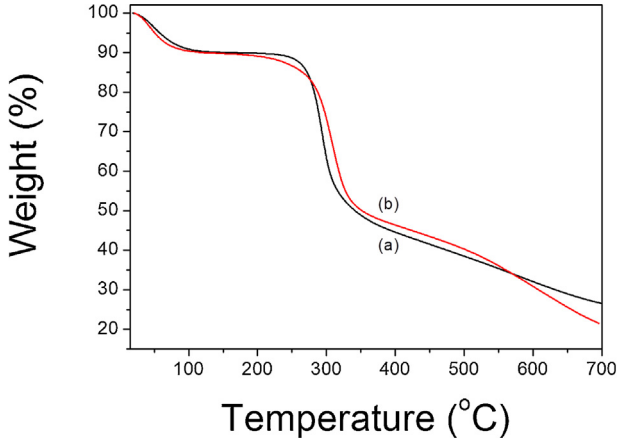

Fig. 3. TGA of chitosan (a) and DIMC (b).

2. Experimental

2.1. Materials

Chitosan (degree of deacetylation 78%), glacial acetic acid, DMF and 4,4′-methylene bis (phenylisocynate) (MBI), acetone were purchased from Sigma–Aldrich (St. Louis, MO, USA). The test strains, *E. coli* (#11775), and *Staphylococcus pyogenes* (#19615) were supplied by American Type Culture Collection (Manassas, VA, USA). All solvents and solutions were used as such, without further purification.

2.2. Preparation of diisocyanate-modified chitosan (DIMC)

Chitosan (500 mg) was dissolved in 20 mL of aqueous acetic acid (1.5% w/v), and 400 mg of 4,4′-methylene bis (phenylisocyanate) solution was added drop wise in chitosan solution under magnetic stirring for 1 h. The magnet bar was stopped after gel formation and the contents were solvent exchanged 3 times with acetone. The final product were filtered and dried under ambient pressure giving a powder (Scheme 1).

2.3. Analysis methodology

Fourier transform infrared (FT-IR) spectroscopic measurements were recorded on JASCO FT-IR spectrophotometer using KBr pellets. X-ray diffractometer (D/Max2500VB+/Pc, Rigaku, Japan) with Cu Kα characteristic radiation (wavelength λ = 0.154 nm) at a voltage of 40 kV and a current of 50 mA. Thermogravimetric analysis was carried out in a TA Q 50 system TGA. The samples were scanned at a heating rate of 10 °C/min under flow of nitrogen. Differential scanning calorimetry with DSC Q1000V7.0 Universal V3.6C TA