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Mechanical and antibacterial properties of novel high performance chitosan/nanocomposite films

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ABSTRACT

Zinc oxide (ZnO) nanoparticle was successfully synthesized using hydrothermal method as well as, silver (Ag) nanoparticle was direct prepared during chitosan nanocomposites preparation. Chitosan films were investigated by recognized the effect of kinds of acids (formic or acetic acid). Correspondingly, using of concentrated NaOH was checking for facilitating de-casting of the films. Utilization of formic acid arisen higher quality films than those films produced using acetic acid as solvent. Optimization was based on the mechanical properties for both types of acids solvent. Furthermore, the elasticity of the prepared films was enhanced by blending hydroxyl ethylcellulose (HEC) with chitosan. Loading the films by silver and zinc oxide nanoparticles (Ag-NPs and ZnO) was achieved during the preparation of films under the optimum conditions. Characterizations of the prepared ZnO nanoparticles and the prepared nanocomposites films were investigated by FT-IR, XRD, SEM, TEM and EDAX. Chitosan nanocomposite films displayed good Antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhamrium*, *Bacillus cereus*, and *Listeria monocyte*. Therefore, these films can be used for packaging applications.

1. Introduction

There has been a growing interest over the past few years in the progress of biopolymers relatively to their sustainable, renewable, abundant sources, friendliness to the environment, and potential to substitute for some petrochemicals [1]. Chitosan, a deacetylated derivative of chitin is structurally similar to cellulose, which is composed of only one monomer of glucose. It is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine being obtained by the partial deacetylation of chitin. Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids such as nitric, perchloric, phosphoric [2,3], hydrochloric, formic, acetic, lactic and citric acid solutions [4]. Chitosan differs from cellulose where chitosan has NH_2 group in C-2 position instead of OH group in cellulose (Scheme 1).

Chitosan have shown great capacity to be used in food industry as preservative owing to its high antimicrobial activity against various microorganisms [5] and inhibits the growth of a wide variety of fungi, yeasts and bacteria [4]. Chitosan has been largely employed in many areas, such as photography, biotechnology, cosmetics, treatment of industrial effluents for removal of metallic and coloring ions. It forms good films and membranes and these films have a possible to be employed for packaging, mainly as an edible packaging [6]. This is attributable to its excellent oxygen and carbon dioxide barrier properties and interesting antimicrobial properties. The film properties of chitosan rely on its morphology, which is affected by molecular weight, degree of N-acetylation, solvent evaporation, and free amine regenerating mechanism [7].

Chitosan has previously been investigated as matrix to incorporate ZnO or Ag nanoparticles [8–10]. Especially, diverse silver compounds were incorporated into various forms of chitosan matrix, including solution [11], gel [12] and film [13,14]. Most of these papers mostly cope with the morphology of the prepared nanocomposites and with the study of their antimicrobial and antibacterial activity. Owing to the biocompatible, eco-friendly, and low cost chitosan/ZnO composites can be used for food packaging and biomedical applications [15].

Hydroxyethylcellulose (HEC) is water soluble cellulose ether, non-ionic polymer and compatible with a wide range of other water-soluble polymers [16], as well as it can also form homogeneous blends with CMC and chitosan. Current nanotechnology

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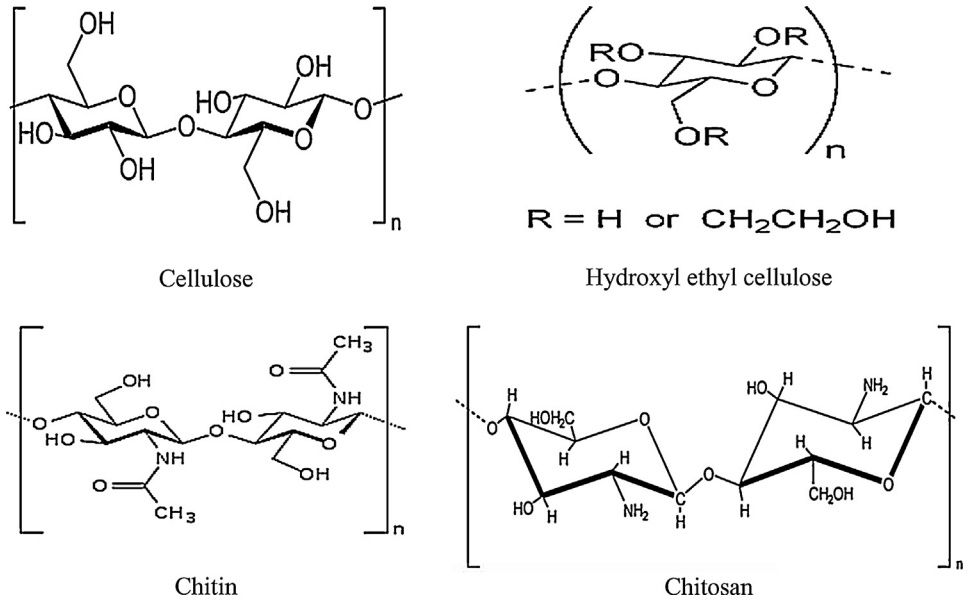

Scheme 1. Chemical structure of cellulose, hydroxyl ethyl cellulose, chitin, and chitosan.

facilitates to reproducibly manufacture a range of specific nanoparticles in accord with their variations in individual size, shape and surface structure properties. These include metals for instance (gold, silver, nickel and iron). It is similarly possible to produce metallic oxides as nanoparticles, include oxides of (iron, titanium, and zinc), or silicate minerals for example talc and mica [17].

Silver nanoparticles (Ag-NPs) have been produced by different methods, one of which is the chemical reaction method [18,19]. In this method, Ag ions are reduced by UV or γ-irradiation, ultrasound, prolonged reflux, or chemicals to produce silver nanoparticles [20]. Silver has been recognized to exhibit strong cytotoxicity towards a broad range of micro-organisms, based on oligodynamic effect, silver ions are capable of causing a bacteriostatic (growth inhibition) or even a bactericidal (antibacterial) impact [21,22]. Up to now, several mechanisms have been postulated for the antimicrobial property of Ag-NPs: (1) adhesion of nanoparticles to the surface altering the membrane properties. Ag-NPs have been reported to degrade lipopolysaccharide molecules, accumulate inside the membrane by forming "pits", and cause huge increases in membrane permeability [23]; (2) Ag-NPs penetrating inside bacterial cell, resulting in DNA damage; (3) dissolution of Ag-NPs releases antimicrobial Ag ions [24,25].

Alternative category of nanoparticles for antimicrobial activity is ZnO nanoparticles. The antibacterial mechanism of ZnO is still under investigation. The photocatalytic generation of hydrogen peroxide was suggested to be one of the primary mechanisms [26]. In addition, penetration of the cell envelope and disorganization of bacterial membrane upon contact with ZnO nanoparticles were also indicated to inhibit bacterial growth [27]. However, the role of Zn^{2+} ion released from dissolution of ZnO is not clear. It has been suggested that Zn^{2+} ion binding to the membranes of microorganisms can prolong the lag phase of the microbial growth cycle. Contrary results have been reported about the impact of particle size on the antibacterial activity of ZnO nanoparticles. Jones et al. [28] detected that smaller ZnO particles were more toxic than bigger particles, but no size related effect was found in another study by Franklin et al. [29].

The present study aims to optimize the preparation condition of chitosan nanocomposites to get high performance chitosan films. In order to achieve this task, different parameters have been investigated such as the influence of chitosan solvents, handling of film removal, and the effect of blending with another natural polymer (HEC). Furthermore, this study focused to improve the antimicrobial activity of chitosan nanocomposites film through incorporation nanoparticles such as (Ag and ZnO) inside the film matrix against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhamrium*, *B. cereus*, and *Listeria monocytes*.

2. Experimental

2.1. Materials

Chitosan (Purity 90%, Viscosity 50–300 cps) provided from Bio Basic Canada Inc. Acetic acid and formic acid were used to fix the pH of the prepared solutions of chitosan. Low molecular weight polyethylene glycol (PEG-400) was obtained from Sigma–Aldrich Chemical Co. and used as plasticizer. Hydroxyethyl cellulose (HEC) was purchase from Sigma–Aldrich Chemical Co. used as blending material with chitosan. Silver nitrate ($AgNO_3$) was used as a source for silver nanoparticles through reduction process by using tri-sod-citrate. Zinc-acetate (puriss, Reanal, Hungary) ($Zn(CH_3COO)_2{\cdot}2H_2O$) was used to prepare ZnO nanoparticle.

2.2. Preparation of ZnO nanoparticles

In order to fabrication ZnO nanoparticles, stock solutions of Zn $(CH_3COO)_2{\cdot}2H_2O$ (0.2 M) as prepared in 50 ml methanol under stirring. To this stock solution 50 ml of NaOH (varying from 0.4 M to 0.8 M) in methanol was added under constant stirring in order to get the pH value of reactants between 8 and 11. These solutions were transferred into Teflon lined sealed stainless steel autoclaves and continuous at various temperature in the range of 100–200 °C for 6 and 12 h under autogenously pressure. It was then allowed to cool naturally to room temperature. After the reaction was complete, the resulting white solid products were washed with methanol, filtered and then dried in a laboratory oven at 60 °C.

2.3. Film preparation

A typical method for film preparation was carried out by casting (2% weight) chitosan solution prepared using formic or acetic acids. A known amount of poly ethylene glycol (PEG) was added to