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Characterization and potential applications of gamma irradiated chitosan and its blends with poly(vinyl alcohol)

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ABSTRACT

Naturally available chitosan (CHI), of high molecular weight, results in reduced efficiency of these polymers for antibacterial activity. In this regard, irradiation is a widely used method for achieving reduction in molecular weight of polymers, which may improve some of its characteristics. Chitosan was extracted from crab shells and degraded by gamma radiations. Effect of radiation dose on chitosan was analyzed by Fourier transform infrared (FTIR) spectroscopy. Furthermore, the irradiated chitosan was blended with poly(vinyl alcohol) (PVA) and crosslinked with tetraethylorthosilicate (TEOS) into membranes. The membranes were found to be smooth, transparent and macroporous in structure, exhibiting high tensile strength (TS: 27-47 MPa) and elongation at break (EB: 292.6-407.3%). The effect of molecular weight of chitosan and chitosan blends on antibacterial activity was determined. Irradiated low molecular weight chitosan and membranes showed strong antibacterial activity against *Escherichia coli* and *Bacillus subtilis*.

1. Introduction

Poly [beta (1,4)-2-amino-2-deoxy-D-glucose], known as chitosan, is obtained from chitin by a deacetylation process [1]. Chitin is the principal structural polysaccharide of the shells of crustaceans and is the second most abundant, naturally occurring polysaccharide after cellulose [1,2]. Crosslinked chitosan membranes are three dimensional networks that can absorb and retain a large amount of water while maintaining their structures. Chitosan membranes have various applications in the field of medicine (such as tissue engineering, dressings for burns and controlled drug release systems) and food packaging [3–8]. The molecular weight (MW) and degree of deacetylation (DDA) of chitosan varies greatly depending on the source of chitin and the method of deacetylation. The DDA of chitosan is typically over 70%, making it soluble in acidic aqueous solutions. Due to its biodegradability, biocompatibility and lack of toxicity, much attention is being paid to chitosan, particularly for biomedical applications [9–11].

The antibacterial activity of chitosan is affected by molecular weight and degree of deacetylation. Low molecular weight chitosan has strong antibacterial properties and is also harmless to human body [12–16]. Being a cationic polymer, most chitosan blends have the ability to respond to external stimuli such as temperature, pH, and electric fields [13]. Recently the antibacterial and antifungal activities of chitosan have attracted much interest [14,17,18]. For the food packaging industry, food quality and safety to human health are the two major concerns as consumers prefer fresh and minimally processed products. Particularly, bacterial contamination of ready to eat products constitute is of concern [12,19]. Chitosan has proven a useful antimicrobial agent in food processing, particularly for improving the shelf life of food materials [20,21].

As a blending agent, polyvinyl alcohol (PVA) has excellent miscibility and film forming properties. It contributes better tensile strength and flexibility to the blends. It is synthetic and inexpensive polymer used to produce membranes. PVA blended chitosan has been employed in many biomedical applications due to its biodegradable, non-carcinogenic and biocompatible nature [3,4,21–23]. The antibacterial properties of numerous chitosan blends, including cellulose/chitosan [15], PVA/chitosan [22,23], starch/chitosan [24,25], banana flour/chitosan [26], gelatin/chitosan [27], pullulans/chitosan [28], guar gum/chitosan [29], have been investigated.

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In this study, molecular weight of chitosan was reduced by gamma radiations at different doses. The chitosan of variable molecular weight was mixed with PVA and crosslinked with tetraethylorthosilicate (TEOS) as biocompatible and non-toxic crosslinker to form membranes. The mechanical, water barrier and contact angle values for chitosan and chitosan blends were recorded and effects of change in molecular weight on these properties were investigated. Furthermore, the antibacterial activity of chitosan and membranes was studied against *Escherichia coli* and *Bacillus subtilis*.

2. Materials and methods

2.1. Materials

Chitosan was obtained from crab shells having 75% DDA. PVA (degree of hydrolysis 98.0-98.8 mol%, degree of polymerization ~4300, MW 195 kDa) was obtained from Aldrich (Germany). Tetraethylorthosilicate (TEOS) (d = 0.933 g/ml) was obtained from Fluka (Germany) and glycerol (98% reagent grade) from Fisher Scientific (Pittsburg, PA, USA). All other chemicals (NaCl, $BaCl_2$, NaOH, HCl, CH_3COOH etc.) were of analytical grade.

2.2. Degradation of chitosan by irradiation

A chitosan solution (2%, w/v in acetic acid) was mixed with hydrogen peroxide (1%, v/v) and irradiated under cobalt-60 gamma radiations at dose rate of 1.02 kGy/h up to maximum of 75 kGy. The irradiated chitosan was precipitated, filtered and washed in distilled water until a neutral pH was achieved. The precipitates were vacuum dried at 50 °C and used for further studies. Un-irradiated chitosan and chitosan irradiated at 25 kGy, 50 kGy and 75 kGy will be referred to as CHIC, CHI25, CHI50 and CHI75, respectively. The molecular weights of extracted chitosan were determined using an Ubbelohde viscometer [30]. The viscosity average molecular weight (Mv) of irradiated chitosan samples is given in Table 1.

2.3. Preparation of membranes

Each of the chitosan samples was dissolved in acetic acid (0.5 M) to yield a 2% (w/v) chitosan solution. PVA was separately dissolved in deionized water at 80 °C to make 4% (w/v) solution. The two solutions were mixed and an appropriate amount of glycerol was added. The mixture was stirred thoroughly for 30 min at 55–60 °C and crosslinker (TEOS) was then added drop wise. After half an hour, the resulting mixture was poured into plastic dishes and dried in oven at 40 °C. Membranes will be referred to as MemC through Mem75, depending upon the amount of radiation used on the chitosan, as detailed in Table 1. All formulations contained fixed mass ratio of CHI:PVA – 5:95.

2.4. Infrared spectroscopy (IR)

The IR spectra of samples were determined in the range of 4000 to 400 cm^{-1} using a Fourier transform infrared (FTIR) spectrophotometer (Thermo Electron Corp., Nicolet 6700, Waltham, Massachusetts, USA) at room temperature. Spectra were recorded with 200 scans at 6.0 cm^{-1} resolution using an attenuated total reflectance (ATR) technique with a diamond crystal tip.

2.5. Scanning electron microscopy (SEM)

The surface morphology of membranes was examined using a model JSM-7500F field emission scanning electron microscope (Jeol, Japan). The membranes were prepared by freeze-drying samples after swelling in distilled water. The images were examined at different magnifications.

2.6. Water absorption studies

Small pieces of membranes were weighed and immersed in deionized water (30 mL), until equilibrium was reached. The swollen membranes were taken out and extra water was carefully removed. The water absorption capacity (WAC) was calculated using the equation

$$WAC\,(\%) = \left[\frac{Ws - Wd}{Wd}\right] \times 100$$

where "Wd" is the weight of the dry membrane and "Ws" is the weight of swollen membrane at equilibrium [31]. The results were reported as the average of three readings.

The swelling response of membranes was also measured at different temperatures, pH and in ionic solutions.

2.7. Mechanical properties

The tensile strength (TS) and elongation at break (EB) of the membranes were measured on a TA.XT2 Texture Analyzer (Texture Technologies, New York, USA) at a speed of 0.1 mm/s. The appropriate sized membranes (1.0 × 10.0 cm) were cut using a razor blade and the gauge length was set at 10.0 mm. For each sample, the measurements were replicated four times.

2.8. Water vapor transmission rate (WVTR) and water vapor permeability (WVP)

The WVTR and WVP of the membranes were analyzed according to the ASTM Method E96/E96 M. Circular test cups were filled with calcium chloride (10.0 ± 0.5 g) as desiccant at 0% relative humidity (RH), and sealed with the test membranes. The membranes were tightly attached and the initial weights of the cups were recorded. The cups were placed in an environmental desiccator set at 25 °C and 53% RH. After reaching equilibrium state in the desiccators, cups were weighed daily for 14 days. The WVTR was calculated as the slope of the regression line drawn between elapsed time and the weight change of the test cups. The actual WVTR and WVP of the membranes were calculated using to the following equations

$$\mathrm{WVTR} = \frac{G/t}{A}$$

$$\mathrm{WVP} = \frac{\mathrm{WVTR}}{\Delta P} = \frac{\mathrm{WVTR}}{S(R_1 - R_2)}$$

where "G" is weight change (g), "t" is time (h), "A" is the test area (m^2), and "ΔP" is vapor pressure difference (Pa).

2.9. Contact angle measurements

Static water contact angles of membranes were determined at room temperature by the drop method using a VCA Optima XE Dynamic Contact Angle Analyzer (AST Products Inc., Billerica, MA). The rate of change of surface wettability was taken at different points on the membranes. A CCD camera was used to record images, immediately after the water drop was deposited onto the membrane surface. The measurements were repeated 10 times for each membrane with the results presented as a mean of these readings.