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RF hydrazine plasma modification of chitosan for antibacterial activity and nanofiber applications

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ABSTRACT

Chitosan nano powders were modified using RF hydrazine plasma produced at low pressure (26.66 Pa) with 13.56 MHz frequency at a power of 100 W for 30 min. Characterization and investigation of the properties of plasma-modified chitosan (PMCh) and non-modified chitosan (Ch) were carried out using an optical monochromator, FTIR, florescence analysis, TGA, SEM, and X-ray techniques. FTIR spectra of PMCh indicated a band broadening at 3436 cm^{-1} that confirmed increasing functional groups based on H-bonding. The number of NH_2 groups was determined from fluorescence analysis. TGA analysis shows that the moisture absorption is three times higher in the PMCh structure. Ch and PMCh in PVA solutions were used to produce nanofibers by the electrospinning method; average fiber diameters were 480 and 280 nm for Ch and PMCh, respectively. It was found that the antibacterial effect of PMCh is better than the Ch for Gram-positive strains.

1. Introduction

Chitosan is an N-deacetylated biopolymer of chitin, and it is among the most abundant natural polysaccharides that are embedded in the protein matrix of a crustacean shell or squid pen.[1] Because of its favorable physicochemical and biological properties such as its being biocompatible, non-toxic, and antibacterial, chitosan is considered as an attractive material that can potentially be used in many biomedically related applications.[2–5] Recent researches have been focused on modification of the surface of chitosan, the key purpose of which is to alter the chemical composition and the surface properties of chitosan to suit specific applications.[6] The chemical modification of the chitosan surface using reactions between the amino groups of chitosan and carboxylic acid derivatives indicated that amino groups on the surface of chitosan are the reactive groups.[7] The surface hydrophobicity/hydrophilicity of chitosan influences properties of the chitosan for many different applications.[8]

Chitosan is insoluble in water, alkali, and inorganic acids, although it is soluble in dilute aqueous acetic and formic acids.[9] The free amino groups of chitosan contribute to its solubility. Furthermore, it is thought that an increase in amino groups included in chitosan molecules will improve its solubility for electrospinning applications, as well as render it more biocompatible for such functions as blood-clotting and antibacterial activities.[10]

Chemical modifications to increase amino group on chitosan surface have been used in limited fields of applications.[11,4]

RF-plasma can be used to modify surface properties of polymers such as hydrophilicity,[12] adhesion[13], and biocompatibility.[14] Recently, the modification of the surface properties of the chitosan by plasma treatment has attracted the attention of other research groups.[15,16]

It was indicated that surface modification by argon plasma resulted in a change in the filtration characteristics and ionic permeability of chitosan membranes.[17] The blood-clotting properties of the chitosan were improved by NH_3 plasma treatment, with and without O_2 pretreatment, by as much as 71.4% and 55.2%, respectively.[18]

In this work we have carried hydrazine RF-plasma treatment on chitosan surfaces. The effects of plasma treatment on chitosan particles have been investigated using FTIR, fluorescence, elemental, X-ray, TGA, and SEM analyses. Nanofibers of the plasma-modified and unmodified chitosan were obtained by an electrospinning method from a 2% acetic acid solution in the presence of poly(vinyl alcohol) (PVA). The nanofiber properties were compared by SEM analysis, and the antibacterial properties of PMCh were investigated by viable cell counting method on agar plates.

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2. Experimental

2.1. Materials

Hydrazine, fluorescamine spray reagent (0.05% fluorescamine in acetone) and chitosan, medium molecular weight with 1.10×10^6 g/mol and DD = 75–85, were purchased from Sigma–Aldrich. Glacial acetic acid from E. Merck and poly(vinyl alcohol) (PVA) from Sigma–Aldrich were used without purification.

Four bacterial strains were used for the antibacterial evaluations. These included two Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and two Gram-positive strains (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923). Strains were incubated overnight at 35 °C in Müller–Hinton broth (MHB).

2.2. Plasma modification of chitosan

The reactor used for plasma modification is a capacitively coupled, 13.56 MHz RF rotating plasma installation. The reactor (Fig. 1) is composed of a Pyrex tubular (internal diameter: 5 cm; lenght: 30 cm) glass chamber (Fig. 1, pt. 7). Two ferrofluidic feedthroughs (pts. 4 and 10) are at both ends of the chamber, which assures the free rotation of the reaction chamber and a good vacuum inside the rector. The metallic surfaces of the ferrofluidic sealing systems facing the interior of the chamber are protected by ceramic disks (thickness 10 mm, not shown in the diagram) against the plasma species. The rotation of the plasma chamber can be digitally controlled in the 0–50 rpm range with the aid of a driving unit composed of an electric motor (pt. 13), belt-coupling system, and angular speed controller (pt. 12). The plasma gases and vapors are supplied to the reactor through several metallic chemical reservoirs (pt. 1). The gas flow is controlled by flow meters (pt. 3) and needle valves (pt. 2). The vacuum inside the reactor is achieved through a mechanical vacuum pump and large capacity valves. A liquid nitrogen trap is inserted between the pump and reaction chamber to protect the pump from reactive plasma species. The desired flow rate and pressure, monitored by an MKS vacuum gauge, can be established in the reactor by operating the valves. The 13.56 MHz RF power (pt. 8) is transmitted into the reactor using two semi-cylindrical copper electrodes (pt. 6) located outside the chamber.

Due to the rotation of the glass reactor chamber, fresh surfaces of the powdery samples can be constantly brought out for plasma exposures, leading to uniform modification of chitosan particles. In a typical experiment, the vacuum-oven-dried sample is loaded into the vacuum chamber, which had been cleaned previously. The required hydrazine pressure and flow rate are created in the reactor. Then the rotation of the reactor is started, and the plasma is ignited and sustained for the pre-selected reaction time. At the end of the plasma exposure, the gas-feed valves are closed, and the system is evacuated to the base pressure for 5 min followed by venting the system to atmospheric pressure. The following experimental parameters have been used: time, 30 min; RF frequency, 13.56 MHz; power, 100 W; pressure, 26.66 Pa.

Optical emission discharge spectra were taken using a Princeton Instrument Acton Series Monochromator (0.500 m focal length) during the experiment in order to understand the discharge chemistry. The monochromator optical fiber is placed close to the glass discharge chamber to take the optical emission spectra (OES) of the RF hydrazine plasma (Fig. 2). In a typical hydrazine discharge, N_2 1st positive, N_2 2nd positive, NH_3, N_2^+ 1st negative lines were observed.[19]

2.3. Fluorescence labeling technique

Evaluation of primary amine functionalities on the chitosan surface has been performed by a fluorescence labeling technique. The plasma-treated and -untreated substrates were reacted three times consecutively with 200 μL of fluorescamine solution (50 mg fluorescamine in 100 mL of dry acetone, Sigma–Aldrich) using a chromatographic syringe. Labeled samples were vacuum dried, and

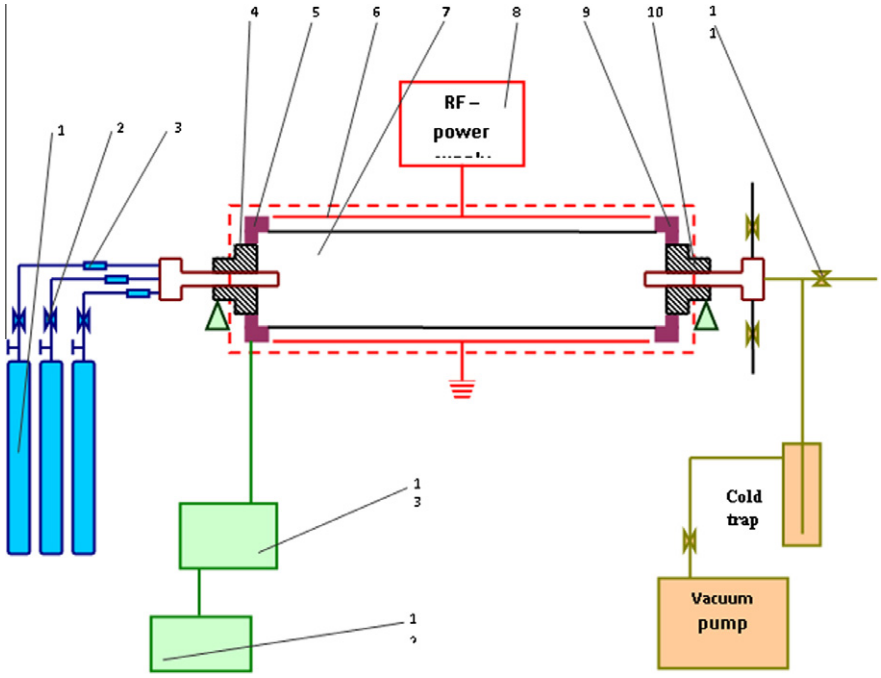

Figure 1. Diagram of the installation of the vacuum-operated plasma rotating reactor; (1) metallic chemical reservoirs or compressed gas cylinders; (2) valves; (3) flow meters; (4 and 10) ferrofluidic feed-throughs; (5 and 9) flanges; (6) electrodes; (7) glass tube; (8) power supply; (11) vent valve; (12) angular speed controller; and (13) motor.