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In situ coupling of chitosan onto polypropylene foils by an Atmospheric Pressure Air Glow Discharge with a liquid cathode

D. Nikitin[a,b], A. Choukourov[b,*], V. Titov[a], L. Kuzmicheva[a], I. Lipatova[a], E. Mezina[a], V. Aleksandriiskii[a], A. Shelemin[b], I. Khalakhan[c], D. Slavinska[b], H. Biederman[b]

[a] *G. A. Krestov Institute of Solution Chemistry of the Russian Academy of Sciences, Akademicheskaya 1, 153045 Ivanovo, Russia*
[b] *Charles University in Prague, Faculty of Mathematics and Physics, Department of Macromolecular Physics, V Holešovičkách 2, 18000 Prague, Czech Republic*
[c] *Charles University in Prague, Faculty of Mathematics and Physics, Department of Surface and Plasma Science, V Holešovičkách 2, 18000 Prague, Czech Republic*

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ABSTRACT

Atmospheric air plasma treatment of chitosan solutions leads to degradation of chitosan molecules by OH radicals and is accompanied by a predominant cleavage of glycosidic linkages and by a decrease of the molecular weight. The degradation proceeds via first order kinetics with the rate constant of $(5.73 \pm 0.22) \times 10^{-6}\ s^{-1}$ and the energetic yield of chitosan bond scission of $(2.4 \pm 0.2) \times 10^{-8}$ mol/J. Products of degradation together with intact chitosan molecules adsorb and form coatings on polypropylene foils immersed into the solution that is being plasma treated. The plasma treatment results in strong binding of chitosan to polypropylene due to the formation of covalent bonds between the activated polymer surface and chitosan molecules. Plasma-driven crosslinking is responsible for the accumulation of compressive stress which leads to the development of buckling instabilities in the chitosan coatings.

1. Introduction

Carbohydrates of natural origin are highly attractive for use in various applications including food industry, waste water treatment, paper industry and agriculture. Recent years witnessed an increase of scientific interest in biomedical applications of naturally-derived polymers, in particular chitosan. Chitosan is produced by deacetylation of chitin, a naturally occurring polyaminosaccharide present in exoskeleton of many living organisms. Among naturally-derived polymers, chitosan is distinguished for its nontoxicity and biodegradability. Its median lethal dose LD_{50} is very high and similar to that of salt and sugar (Reis et al., 2008). Biocatalysis, controlled drug delivery, blood coagulation and wound healing are only a few of numerous fields where chitosan was found to be effective. However, poor mechanical strength of materials made on its basis (powders, foils, 3D scaffolds) limits the use of chitosan in applications where strict requirements on mechanical performance should be met. In these applications, the use of conventional materials, such as synthetic polymers, with precisely defined bulk properties is inevitable. These, however, often face problems with insufficient biocompatibility and fail to perform properly in contact with tissues unless their surface is specifically modified. Hence, a recognized strategy has become to attach a thin layer of chitosan to synthetic polymers with retention of their useful bulk properties and with improvement of biocompatibility.

When a solid comes into contact with a chitosan solution, a chitosan layer may adsorb on the solid surface. In this case, macromolecules are held on the surface by very weak van der Waals forces and can be easily removed by simple rinsing with water, making this method impracticable. In recent years, polyelectrolyte multilayer deposition was developed (del Hoyo-Gallego et al., 2016; Richert et al., 2004; Serizawa, Yamaguchi, & Akashi, 2002) which makes use of electrostatic interactions. These are also prone to degradation when in contact with physiological medium (Picart et al., 2005). For stronger chitosan anchoring, covalent binding was considered. Among different methods, low-temperature plasma treatment is known to create radicals in synthetic polymers that can be subsequently used for covalent binding of various molecules. Low-temperature plasma is a highly nonequilibrium medium in which a minor population of energetic electrons with a mean energy of several electronvolts coexists with a much more abundant population of less energetic species (neutrals, ions, metastables) with the mean energy close to the ambient environment. As a result, even temperature-sensitive materials can

* Corresponding author.
E-mail address: choukourov@kmf.troja.mff.cuni.cz (A. Choukourov).

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be subject to low-temperature plasma treatment without affecting their bulk properties and with significant modification of their outermost layers.

Chitosan was immobilized from solutions on polymer foils preliminarily treated by low pressure plasma and grafted with polyacrylic acid (Aiping & Tian, 2006; Pandiyaraj et al., 2015; Xin et al., 2013). Carboxyls of polyacrylic acid served as chemical linkers for covalent attachment of chitosan via reactions with primary amine groups, and an improvement of biologically nonfouling properties of the polymers was demonstrated. Alternatively, direct binding of chitosan from solutions without the use of any chemical linkers was performed on low pressure plasma treated wool (Wang et al., 2015), viscose fabric (Zemljič, Peršin, & Stenius, 2009) or nonwoven cellulose (Vosmanská, Kolářová, Rimpelová, Kolská, & Švorčík, 2015). Improvement of the dyeability and shrinkage of wool, and the antibacterial and wound healing activity of viscose and cellulose were shown.

Low-temperature plasma can be maintained at atmospheric pressure as well. The activation of polyethylene (Theapsak, Watthanaphanit, & Rujiravanit, 2012) and nonwoven polypropylene fabric (Černáková et al., 2014; Theapsak et al., 2012) by atmospheric pressure dielectric barrier discharge, and pretreatment of cotton by atmospheric pressure plasma jet (Zhou & Kan, 2014) were found effective for subsequent binding of chitosan from water solutions with no linkers used. Improvement of antibacterial performance of these materials was also demonstrated.

Hypothesis. Polymers to be grafted with chitosan may be immersed directly into chitosan solutions for the *in situ* treatment with atmospheric pressure plasma. Chitosan molecules were already shown to undergo structural modifications in solution plasma systems (Molina, Jovancic, Vilchez, Tzanov, & Solans, 2014; Tantiplapol et al., 2015). Oxidation of polymeric surfaces with simultaneous formation of radicals was also demonstrated (Choi, Shikova, Titov, & Rybkin, 2006). Therefore, *in situ* chitosan coupling onto polymers from solutions being treated with plasma can be expected and it may provide a single-step, linker-free process for the enhancement of biocompatibility of synthetic polymers.

2. Experimental

2.1. Materials

Chitosan, poly[β-(1-4)-2-amino-2-deoxy-D-glucose], with the molecular weight of 195 kDa was obtained by deacetylation of chitin from crab exoskeleton (Bioprogess Ltd.) An 82% degree of deacetylation was determined by potentiometry. Aqueous solutions were prepared by dissolving 1 g of chitosan in 100 ml of 2% acetic acid in water (Chimmed Ltd, GOST 61-75) at 50 °C under constant stirring for 2 h. After the stirring, the batch was left for 20 h at room temperature and 1% (w/v) chitosan solution was obtained after the subsequent filtration.

Polypropylene (PP) foils with 20 μm thickness (TU RB 00204079.164-97, Mogilevkhimvolokno Ltd) were used as substrates. PP was chosen because of its widespread use and the ability to be modified in roll-to-roll processes. Prior to the treatment, the foils were cleaned with ethanol and de-ionized water.

2.2. Plasma processing

Fig. 1 shows the experimental setup that was used as described elsewhere (Choi et al., 2006). A PP foil was fixed in a massive PP frame so that a 3 × 3 cm area was exposed. The entire assembly was placed at the bottom of a liquid cell. A chitosan solution (25 ml) was poured into the cell to a 3 mm distance between the foil and the liquid surface. A homemade DC power supply was used to ignite a glow discharge. A copper rod electrode with 1.2 mm diameter was placed in the center of the liquid cell 2 mm above the solution surface. A graphite electrode was immersed into the solution at the lateral distance of 25 mm from the copper electrode to close the electric circuit. The polarity was chosen for the liquid surface to serve as a cathode. The glow discharge was ignited in ambient air with the current of 30 mA, voltage of 673 V and power of 20.2 W. The temperature of the solution reached 74 °C in the first 5 min of the plasma processing, and then it stayed constant. For comparison, a series of identical experiments were performed on PP foils with 2% acetic acid solutions without chitosan.

2.3. Analysis of chitosan solutions

The average molecular weight (M) of chitosan was determined from the intrinsic viscosity of its solutions by a Kuhn-Mark-Houwink equation $[\eta] = KM^{\alpha}$, where $[\eta]$ is the intrinsic viscosity, K and α are constants. The intrinsic viscosity was measured experimentally by a capillary based method using an Ubbelohde viscometer with a capillary 0.56 mm in diameter. The chitosan solutions with different concentrations were prepared by a sequential dilution of the initial solution with 2% acetic acid in a buffer of sodium acetate (0.2 M, pH = 5.4). The specific viscosity of these solutions was measured at 25 °C and the intrinsic viscosity was determined by performing the extrapolation. The values of $K = 1.464 \times 10^{-4}$ and $\alpha = 0.885$ for chitosan in acetate buffer solutions were taken from (Wang, Bo, Li, & Qin, 1991).

The chemical changes induced by the action of the plasma onto the chitosan solutions were studied by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy. The FTIR spectra were recorded by a Vertex 80 V (Bruker) spectrometer in a Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy mode. The products of decomposition were precipitated from solutions by NaOH and the sediments were rinsed with distilled water. After rinsing, the solid products were dissolved in 2% HCl to transfer the COO^- ions into the nondissociated carboxyl groups, and the solutions then were dried. The spectra of thus-prepared sediments were obtained on their 1:100 pellets with KBr. The assignment of the absorption bands was performed in accordance with (Kasaai, 2008).

The NMR ^1H spectra of 0.5% HCl in D_2O solutions of 1% (w/v) chitosan were recorded at 500.17 MHz by an Avance III (Bruker) spectrometer equipped with a 5 mm TBI z-GRD probe at 294 K. The HCl solution was chosen to make sure that the CH_3 signal from the acetic acid molecules does not interfere with that of chitosan. The spectra were referenced to external standards of hexamethyldisiloxane. The assignment of the spectral shifts was performed according to (Hirai, Odani, & Nakajima, 1991; Lavertu et al., 2003).

Complementary tests were performed to establish the concentration of H_2O_2 in the 2% acetic acid water solution without chitosan after its treatment with the glow discharge under the conditions described above. The concentration of H_2O_2 was determined by an iodometric titration with an addition of ammonium molybdate which served as a selective catalyst for the reaction of H_2O_2 with iodide ions (Skoog, West, Holler, & Crouch, 2013).

2.4. Characterization of chitosan grafted onto polypropylene foils

The samples with chitosan immobilized on the PP foils were removed from the liquid cell and rinsed in 2% acetic acid water solution for 15 min and then in de-ionized water for 15 min to remove loosely bound macromolecules from the surface. Finally, the samples were dried in ambient atmosphere for at least 12 h.

The chemical composition of chitosan immobilized on PP was analyzed by X-ray Photoelectron Spectroscopy (XPS) and FTIR. An Al Kα X-ray source (1486.6 eV, Specs) with a multi-channel