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Investigations of antibacterial activity of chitosan in the polymeric composite coatings

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ABSTRACT

Chitosan and its derivatives can be called environment purification functional materials that they can effectively control the growth, reproduction of hazardous bacteria and also control toxic pollutants. In this study, an acrylic resin was converted to an antibacterial composite material by using chitosan. Different states of chitosan; solid state (powders) and colloid state, were inserted to the polymer matrix individually. Glass substrates were coated with this polymeric matrix. Obtained samples were characterized by Fourier Transform Infrared (FTIR), Scanning Electron Microscope (SEM) and scratch test. Antibacterial activity against Staphylococcus aureus (*S. Aureus*) was studied by applying entitled in vitro test. Zones of inhibition were estimated on the nutrient agar plates, and also percent decreasing tests were performed. It was concluded that chitosan can be considered an effective antibacterial additive. Colloid chitosan reinforced composites demonstrated much better antimicrobial activity comparing with powder chitosan reinforced ones.

1. Introduction

Antibacterial and antimicrobial agents, and the well-prepared systems are becoming important day by day. They have been studied for possible use in a variety of the healthcare applications, industries, laboratories and environments, in addition to houses [1]. As is known, the most important use is to set medical environments and equipment for the sterilization in order to prevent thousands of deaths resulted from hospital-associated infections. Besides, linens and its derivatives also allow bacteria to grow in contact with the human body. Therefore, a suitable environment should be provided in order not to let infectious diseases spread with fungus and viruses.

First of all, chitosan is one of those antibacterial materials as an active biomolecule that have a dangerous role in our life. The largely used natural biopolymer material is produced from wastes of crab and shrimp shells. Chitosan and its derivatives can be called as functional and environmental purification materials which can effectively control the growth, reproduction of hazardous bacteria and toxic pollutants. They are defined as non-toxic, biodegradable, biocompatible and hydrophilic, in addition to having antimicrobial and antibacterial characteristics [2].

Next, chitin is generally represented as a linear polysaccharide composed of $(1 \rightarrow 4)$ linked units of *N*-acetyl-2-amino-2-deoxy-D-glucose [3]. As for chitosan, it is a linear polysaccharide comprised of extensive deacetylation of chitin. It is mainly composed of two kinds of $(1 \rightarrow 4)$ linked structural units viz. 2-amino-2-deoxy-D-glucose and *N*-acetyl-2-amino-2-deoxy-D-glucose. In Fig. 1, a completely deacetylated chitosan's chemical structure is demonstrated. Actually, it is difficult to make chitin deacetylate completely. Essentially, chitosan is a family of chitins with different/low degrees of acetylation. Soluble capacity of chitosan in dilute aqueous solutions is a widely accepted criterion to differentiate from chitin [4,5].

In comparison, chitosan is more soluble and has a better antimicrobial activity than chitin because chitosan has a positive charge on the C2 of the glucosamine monomer below pH 6 [6]. Although the complete mechanism of the antimicrobial action of chitin, chitosan, and their derivatives have not been elucidated yet, but varied ideas have been suggested [7]. Chitosan has positively charged amino group which interacts with negatively charged microbial cell membranes. This leads to the leakage of proteinaceous and other intracellular constituents of the microorganisms. That may be one of the reasons of having antimicrobial feature of chitosan [8]. Another reason is related to the moves of chitosan on the outer surface of bacteria. At a lower concentration (0.2 mg/mL), polyca-

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1 http://www.ikc.edu.tr.

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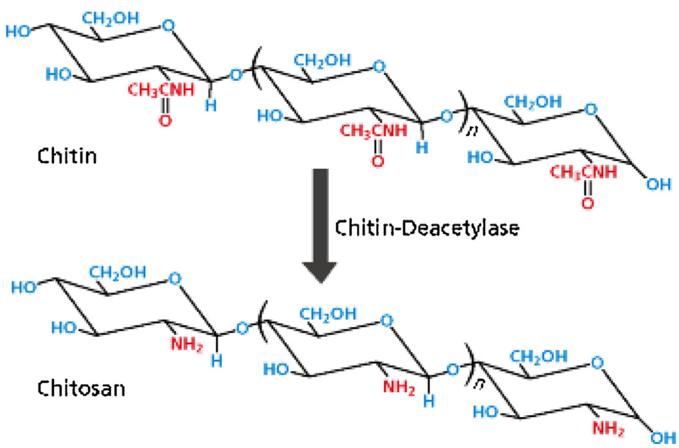

Fig 1. Structure of (a) chitin and (b) chitosan [5].

tionic chitosan can be probably bound to the negatively charged bacterial surface for causing agglutination. The larger number of positive charges may impart a positive charge to the bacterial surfaces in order to keep them in suspension at a higher concentration [6].

In the UV absorption studies, it was detected that chitosan causes the considerable losses of proteinic material for Pythium oaroecandrum at pH 5.8 [9]. Chitosan can bind tracing metals like a chelating agent and this can prevent the formation of toxic materials and the growth of microbes. By activating defensive processes in the host tissue, it can act as a water binding agent, and also prevent various enzymes. Due to the penetration toward the nuclei of the microorganisms and the interference with the synthesis of mRNA and proteins, binding of chitosan with DNA and inhibition of mRNA synthesis take part [6,10].

Some innate factors such as structure of chitosan, its degree of polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates, and the environmental conditions, can impress the antimicrobial activity of chitosan. Coating materials including antimicrobial agents have been attractive areas for researchers due to preventing the growth of pathogenic bacteria. Chitosan has been still pointed out as an antimicrobial film or a forming agent because of its biodegradability, biocompatibility, cytotoxicity, and antimicrobial activity [6].

In contrast to neutral and alkaline conditions, acidic solutions lead to dye grasping higher [11]. Yoshida et al. [12,13] showed that at a lower pH, more protons are prosperous for protonation amino groups of chitosan molecules to form groups of $-NH_3^+$. Hence, it could be possible to see increasing electrostatic attractions between negatively charged dye anions and positively charged adsorption sites. Therefore, this will bring about an increase in dye adsorption. Chiou and Li [14] presented similar explanations for the adsorption of RR 189 (reactive dye) on cross-linked chitosan beads. The adsorption was lower than acidic solution for example they can go down from pH 10.0 to 13.0. They expressed this action by the fact that chemical cross-linking reduces either the total number or the diameter of the pores in chitosan beads. Thus, the transferring the dye molecule was more difficult [11].

In this study, an acrylic resin was converted to an antibacterial coating material by using chitosan. Different states of chitosan, solid state (powders) and colloid state, were inserted to the polymer matrix individually. Glass substrates were coated with this polymeric matrix. Then, the samples were characterized by Fourier Transform Infrared (FTIR), Scanning Electron Microscope (SEM) and scratch test. In addition, antimicrobial properties of the samples against *Staphylocoocus aureus* were determined.

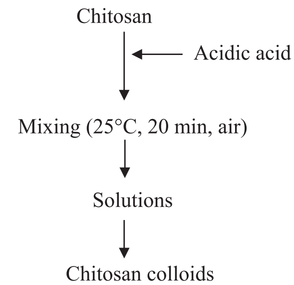

Fig. 2. Preparing method of chitosan colloids.

Table 1
Description and sample codes of composite coatings.

Sample codes	Chitosan	Chitosan percentage in the composites (%)
CH00	None	0
CHP1	Ground Powder	1
CHP2	Ground Powder	5
CHC1	Colloid	0.01
CHC2	Colloid	0.05
CHC3	Colloid	0.10

2. Materials and methods

2.1. Chitosan powders and colloids

Chitosan was used in two different ways; powder and colloid. For obtaining the powders, chitosan (Poly-(D) glucosamine, Sigma) was grounded in a grinding mill at 25 °C for 5 h in the air. The aim of the comminution is to obtain homogeneous distribution and to increase the effect of the particles by increasing contact points. Chitosan colloids were prepared as presented in Fig. 2. Different amounts of chitosan were dissolved in the acid solution. After a complete mixture by using magnetic stirrer at 25 °C for 20 min, homogeneous chitosan colloids were obtained.

2.2. Coating preparation

Acrylic composites were prepared by adding chitosan powders and colloids to the polymeric matrix. For the manufacture of the composites, acrylic resin (polymethyl acrylate) was used as a polymeric matrix supplied from DYO, Turkey. Chitosan powders and colloids were incorporated into the acrylic resin with different loading levels to assess the concentration dependence of material's antimicrobial affect. Glass substrates were coated with those polymeric composites. After the obtained composite coatings were subsequently dried for 24 h at the room temperature in the air, no more curing process was performed. The sample codes and descriptions coatings are indicated in Table 1.

2.3. Characterisation

FTIR (Perkin Elmer Spectrum BX) absorption spectra of the composite materials were exclusively measured over the range of 4000–400 cm^{-1} a components in the samples, FTIR spectra of chitosan reinforced composites were enrolled. With 20 scans at the speed of 1 scan per 2 s, the spectra shown were acquired. Films used in the infrared tests were about 10 μm thick. The microstructural cross-sectional area of chitosan and the chitosan reinforced with coatings were examined through SEM with a JEOL JJM 6060 model. Size factors and wettability features of the coating were calculated by SEM. Shimadzu Scanning Scratch Tester SST-W101 equipped with a standard off-line Zeiss metallographic microscope was used to examine the adhesion properties. Critical force (Wc) was settled