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Chitosan/silver nanocomposites: Synergistic antibacterial action of silver nanoparticles and silver ions

Siva Kumar-Krishnan [a], Evgen Prokhorov [a], Monserrat Hernández-Iturriaga [b], Josué D. Mota-Morales [a], Milton Vázquez-Lepe [c], Yuri Kovalenko [a], Isaac C. Sanchez [d], Gabriel Luna-Bárcenas [a,*]

[a] Centro de Investigación y de Estudios Avanzados del IPN, Querétaro 76230, Qro., Mexico
[b] Universidad Autónoma de Querétaro, Querétaro 76010, Mexico
[c] Departamento de Ingeniería de Proyectos, Universidad de Guadalajara, 44430, Mexico
[d] Department of Chemical Engineering, The University of Texas at Austin, Austin, TX 78712, United States

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ABSTRACT

In the present work chitosan–silver (CS/Ag) nanocomposites, either in the form of nanoparticles (AgNP) or as ionic dendritic structures (Ag^+), are synthesized by a simple and environmentally friendly *in situ* chemical reduction process. The antibacterial activity of the resulting nanocomposites in the form of films is studied against two bacteria, Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. The relationship between electrical, structural and antibacterial properties of CS/AgNP and CS/Ag^+ nanocomposites are studied by transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction, and UV–Vis, impedance, infrared and X-ray photoelectron spectroscopies. The results demonstrate that in contrast to CS/Ag^+ ion films, the CS/AgNP composites films (average particle size less than 10 nm) showed a significantly higher antibacterial potency. The collective action of AgNP and Ag^+ ions facilitate the enhancement and synergetic antibacterial activity below certain critical concentration. The bactericide activity of both CS/AgNP and CS/Ag^+ ion composite films increases by increasing the concentration of Ag. The composites containing 1 wt.% of silver nanoparticles and about of 2 wt.% of silver ions exhibit a maximum antibacterial activity, which is close to their electrical percolation threshold. The concentration of AgNP and Ag^+ ions above the threshold level greatly diminish the antibacterial potential.

1. Introduction

In recent years the development of efficient and greener routes for metal nanoparticles syntheses has gained considerable interest in various areas of nanotechnology. Among metal nanoparticles, silver nanoparticles (AgNP) have attracted much attention due to their potential as antimicrobial agent; they are widely applied in many biological and medical fields such as biosensors, wound healing, treat burns and cancer therapeutics [1–4]. Several AgNP based composites have been demonstrated enhanced performance through the stabilization and support of nanoparticles [5,6]. Regarding silver-based nanocomposites, chitosan–silver nanoparticles (CS/AgNP) nanocomposites represents an emerging group of bio-nanostructured hybrid materials because of its biocompatibility and biodegradability. CS is considered a non-toxic biopolymer, aside of its excellent antimicrobial and antifungal activities against a wide range of

* Corresponding author. Tel.: +52 (442)211 9900.
E-mail address: gluna@qro.cinvestav.mx (G. Luna-Bárcenas).

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microorganisms when compared to other polymers and biopolymers [7,8].

Several physical and chemical approaches have been established to prepare CS/AgNP composite films. Recent studies reported about direct dispersion of AgNPs into chitosan matrix for antibacterial applications [9]. However, common methods involve the chemical reduction of silver salts by different reducing agents such as $NaBH_4$, sodium citrate, or ascorbic acid [10–12]. However, in such chemical processes the nanoparticles tend to aggregate due to the high surface energy inherent to the synthesis. The reducing agents used for the preparation might exhibit environmental toxicity. Residuals of the chemical reduction processes could also affect the interaction with OH and NH_3^+ (or NH_2) functional groups of chitosan biopolymer. Therefore the composite's physicochemical and electrical properties can be greatly affected, and in consequence its antibacterial properties may eventually be affected as well. It was found that AgNP prepared from sodium borohydride reduction process causes cytoprotective effect on human immuno-deficiency virus infected cells [13]. Hence, a greener and environmental friendly approach for the synthesis and stabilization of nanoparticles using biopolymers present clear benefits. Biopolymers such as chitosan offer control over the rate of the chemical reduction process, thus enabling the synthesis and stabilization of nanoparticles with different size and shapes without the use of additional capping agents [14]. The stability of silver nanoparticles against agglomeration is considered the most important factor for their antibacterial efficacy in nanocomposites; the interaction of bacteria with AgNPs is more intensive when AgNPs are well dispersed [15]. It was reported in previous studies with phosphotriazine/diamine polymers that such type of stabilization together with an *in situ* greener synthesis route of polymer/silver nanocomposites exhibited higher antibacterial efficacy against many bacterial species [16]. On the other hand, some reports show that fabrication of asymmetric or porous chitosan film impregnated with AgNPs enhances the controlled release antibacterial activity [17–19].

Silver is commonly used in both ionic form and silver nanoparticles as antibacterial agents. Several studies have previously reported on the antibacterial activity of silver nanoparticles and the effect of size, concentration, temperature or ionic strength over its antibacterial properties [20–22]. Those properties are extensive to polymer–silver nanocomposites. For instance, Triebel et al. [23] reported polyurethane/silver nanocomposites with enhanced silver ion release using multifunctional invertible polyester. Tamboli et al. [24] evaluated the antibacterial activity of Ag–PANI nanocomposites against *B. subtilis*. The releasing of silver ions plays a crucial role in the antibacterial activity of AgNP by generating reactive oxygen species (ROS) upon exposure to the cells [25]. In addition, silver ions alone have profound antibacterial action towards Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* [26]. Recently reported by Greulich et al. [27], there is evidence that the antibacterial activity of AgNP and Ag^+ ions (such as silver acetate) occurs in the bacteria and human cells in the same concentration range. Sotiriou and Pratsinis [28] showed the antibacterial activity of silver ions and silver nanoparticles immobilized into SiO_2 support. Additionally, several studies propose that metallic AgNP may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell [29,15]. Another scenario is that metallic AgNP not only interact with the surface of membrane but they can also penetrate the bacteria [30]. However, the mechanism of antibacterial action of AgNP and Ag^+ ions are poorly understood and there is a few knowledge on the toxicity of metallic AgNP by direct comparison with Ag^+ ions due to difficulties in controlling size, shape and agglomeration effects.

In this work we report a simple and efficient greener route to synthesize CS/metallic AgNP and CS/Ag^+ ions (silver acetate form) composite films using dilute acetic acid as a reducing agent, mediated by the biopolymer chitosan. The antibacterial activity of both silver metallic nanoparticle and silver ion containing chitosan films with different concentration were tested on Gram-positive and Gram-negative bacterias, *S. aureus* and *E. coli* respectively. Our method allows the *in situ* synthesis and stabilization of CS/AgNP and Ag^+ ions in a chitosan matrix while providing a direct comparison of antibacterial activity of silver metallic nanoparticles and silver ions in the same concentration range. To the best of our knowledge, this is the first report where the bactericidal effect of silver ions dendritic structures and silver nanoparticles reduced by dilute acetic acid and chitosan-mediated is evaluated through direct comparison. Also, a correlation between AgNP and Ag ions concentration in the nanocomposites with electrical properties and its antibacterial activity is proposed.

2. Materials and methods

2.1. Materials

Chitosan medium molecular weight (M_w = 300 kDa and 82% of degree of deacetylation) was purchased from Sigma–Aldrich (St. Louis, MO). Silver nitrate ($AgNO_3$) and acetic acid (glacial, 99–100%: Merck) were also purchased from Sigma Aldrich. Chitosan solution was obtained by dissolving 1 wt.% of CS powder in 1% aqueous acetic acid solution and was subsequently stirred to promote dissolution. All reagents are analytical grade and used without further purification. All glass containers were washed and rinsed with deionized water.

2.1.1. Preparation of CS/AgNP composite film

The synthesis was carried out by adding 100 μl of different concentrations of silver precursor $AgNO_3$ (0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 M) to 10 ml of chitosan solution in acetic acid (1%) and the final solution was magnetically stirred at 95 °C, then allowed to react for an additional 8 h. The color of the solution progressed from colorless to light yellow within 30 min, and finally to dark yellow after the reaction completed. This change of color indicates the formation of AgNP. The temperature of the reaction plays an important role as it has a strong influence on the particle size and dispersion of AgNP in the chitosan solution. CS/AgNP films were obtained by