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Photochemical synthesis of silver nanoparticles on chitosans/montmorillonite nanocomposite films and antibacterial activity

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ABSTRACT

Silver nanoparticles (AgNPs) were synthetized on chitosans/montmorillonite nanocomposite films by photochemical method. Nanocomposites were prepared using chitosans with different molar masses and deacetylation degrees, as well as modified with diethylaminoethyl (DEAE) and dodecyl groups. AgNPs formation on the films was followed by the appearance of the plasmon band around 440 nm as a function of irradiation time. TEM images revealed AgNPs with spherical morphology for all nanocomposites. For nanocomposites using modified chitosans, the AgNPs synthesis occurred quickly (1.5 h) while for the others films it was above 11 h. The film of modified chitosan with dodecyl and DEAE groups presented smaller and more uniform nanoparticles size along mixture of exfoliated and intercalated structures. This modified chitosan is an amphiphilic compound that can act controlling the size/shape of the AgNPs. The results of antibacterial activity suggested that all nanocomposite-AgNPs films inhibited the growth of *Escherichia coli* and *Bacillus subtilis*.

1. Introduction

Over the past few years, silver nanoparticles (AgNPs) have become a target of great interest due to their remarkable electronic, optical, mechanical, magnetic, chemical and antimicrobial properties (Dick, McFarland, Haynes, & Van Duyne, 2002; Ghosh & Maiti, 1996; Kanmani & Rhim, 2014; Severin, Kirstein, Sokolov, & Rabe, 2009; Thuc et al., 2016). In regard with size and shape-based of AgNPs, several applications ranging from catalysts, biomedical field, disinfectant sprays, ink, food package and textile products (Chernousova & Epple, 2013; Emam & Ahamed, 2016; Thuc et al., 2016; Zoya, 2012). Hence, a myriad of methods for AgNPs synthesis have been reported, including biological, chemical, photochemical and electrochemical processes (Bhaduri et al., 2013; Lengke, Fleet, & Southam, 2007; Patra et al., 2014; Thuc et al., 2016). However, the chemical reduction using hazardous reducing agents, such as sodium borohydrate, formaldehyde and ammonia is typically employed for AgNPs preparation, limiting the use of AgNPs for biological or medical applications (Emam & Ahamed, 2016; El-Nour, Eftaiha, Al-Warthan, & Ammar, 2010; Lombardo, Poli, Castro, Perussi, & Schmitt, 2016; Wojtysiak & Kudelski, 2012).

On the other hand, the number of publications about green synthesis of the AgNPs that use environmentally friendly compounds as reducing agents has risen. Among these processes, the green irradiation methods including laser, gamma, ultrasonic wave, ion and ultraviolet (UV) radiation of silver salts in aqueous solution have been used as alternative routes to broaden the range of applications (Shameli et al., 2010; Son, Youk, & Park, 2006; Zhou et al., 2012). In addition, the synthesis of AgNPs was also described using clay suspension, in which the clay lamellae behave as nanoreactors of Ag^{+} to Ag^{0} (Patakfalvi & Dékány, 2004). Furthermore, several polysaccharides, such as glucose, dextrose, starch and chitosan (Ji, Liu, Zhang, Xiong, & Sun, 2016; Oluwafemi et al., 2016; Thomas et al., 2009) have been investigated as reducers and/or stabilizing agents for AgNPs preparation owing to their ability for coordinating with metal ions (Emam & Ahamed, 2016). These polymer-Ag^{+} complexes can be reduced by different experimental conditions, to produce smalls AgNPs with narrow size distribution (Emam & Ahamed, 2016). Regarding natural polymers, chitosan, the main derivative of chitin, has been extensively studied as a cationic biopolymer of high bioactivity, biodegradability, biocompatibility and low toxicity, in addition to its antimicrobial activity (Araiza, Alcouffe, Rochas, Montembault, & David, 2010; Epure, Griffon, Pollet, & Avérous, 2011; Youssef, Yousef, El-Sayed, & Kamel, 2015). These properties

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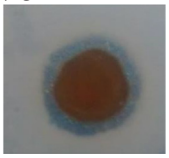
Table 1
Inhibition zones for nanocomposite films and examples of absence (a) and presence (b) of these zones. These images were obtained to Ch83 and Ch83/10%SWy-2/AgNPs films, respectively, against *B. subtilis*.

E. coli		B. subtilis	
Sample	d (mm)	Sample	d (mm)
Ch83	N.I	Ch83	N.I
Ch83/10%SWy-2	N.I	Ch83/10%SWy-2	N.I
Ch30/10%SWy-2	N.I	Ch30/10%SWy-2	N.I
Ch30d/10%SWy-2	N.I	Ch30d/10%SWy-2	N.I
Ch30-DEAE/10%SWy-2	N.I	Ch30-DEAE/10%SWy-2	N.I
Ch30-DEAE-Dod/10%SWy-2	N.I	Ch30-DEAE-Dod/10%SWy-2	N.I
Ch83/AgNPs	8.7 ± 0.6	Ch83/AgNPs	8.5 ± 0.7
Ch83/2.5%SWy-2/AgNPs	7.6 ± 0.3	Ch83/2.5%SWy-2/AgNPs	8.3 ± 0.5
Ch83/5%SWy-2/AgNPs	8.7 ± 0.6	Ch83/5%SWy-2/AgNPs	8.7 ± 0.6
Ch83/10%SWy-2/AgNPs	8.3 ± 0.6	Ch83/10%SWy-2/AgNPs	7.3 ± 0.6
Ch30/10%SWy-2/AgNPs	7.5 ± 0.5	Ch30/10%SWy-2/AgNPs	8.0 ± 0.3
Ch30d/10%SWy-2/AgNPs	7.8 ± 0.2	Ch30d/10%SWy-2/AgNPs	8.3 ± 0.5
Ch30-DEAE/10%SWy-2/AgNPs	8.3 ± 0.3	Ch30-DEAE/10%SWy-2/AgNPs	8.0 ± 0.2
Ch30-DEAE-Dod/10%SWy-2/AgNPs	8.0 ± 0.3	Ch30-DEAE-Dod/10%SWy-2/AgNPs	9.0 ± 0.3
a)  b) 			

N.I.: No Inhibition.
The error was obtained by three independent experiments. The results were reported as mean ± standard deviation.

combined with its ability to form films, allows the application of this polysaccharide in food packaging, bone substitutes and artificial skin (Shameli et al., 2010).

In order to obtain superior stability of chitosan-based nanocomposites, the addition of a natural multilayer silicate known as montmorillonite clay significantly enhances the chemical and/or mechanical stability, in comparison with the polymer itself (Wang et al., 2005; Xie et al., 2013; Xu, Ren, & Hhanna, 2006). Montmorillonite clay is formed by a single octahedral sheet of magnesia or alumina located between two silica tetrahedral sheets (Xu et al., 2006). Moreover, the characteristics of chitosan/montmorillonite nanocomposites coupled with AgNPs properties can result in versatile materials for a wide range of applications.

Several approaches concerning photochemical synthesis of AgNPs in solutions of polymers or nanocomposites have been extensively reported in the literature (Krishnan et al., 2015; Shameli et al., 2010; Thomas, Yallapu, Sreedhar, & Bajpai, 2009). However, the metal nanoparticles synthesis can also be performed in solid material directly on films using irradiation, as described by Sakamoto, Fujistuka, and Majima, 2009, which studied the fabrication of Au/Cu bimetallic nanoparticles in poly(vinyl alcohol) films by UV light irradiation.

Herein we report AgNPs synthesis on chitosan/clay nanocomposite films by UV irradiation after the films preparation. The influence of clay concentration and modification of chitosan structure was also evaluated in the AgNPs formation. The characterization of samples was performed using X-ray diffraction analyses (XRD), UV–vis spectroscopy and transmission electron microscopy (TEM). Finally, the antibacterial activities of materials were investigated against *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*).

2. Materials and methods

2.1. Materials

The chitosan (Ch83) used in this work was purchased from Aldrich Chemical Co with a deacetylation degree of 85% and a viscosity-average molar mass of 83,000 g mol^{-1}. Chitosan with low molecular weight (Ch30) 30,000 g mol^{-1} (deacetylation degree of 85%) was obtained according to the method reported by Tommeraas, Varum, Christensen, and Smidsrod, (2001). It was also prepared chitosan with a deacetylation degree of 98% (Ch30d) and a viscosity-average molar mass of 30,000 g mol^{-1} (Tiera et al., 2006).

Modified chitosans (Fig. 1) used in this work were prepared according to the method reported earlier (Gabriel, Tiera, & Tiera, 2015). Ch30-DEAE is a hydrophilic chitosan derivative with 40% of substitution degree by diethylaminoethyl groups (DEAE). Ch30-DEAE-Dod is an amphiphilic chitosan derivative with 40 and 6% of substitution degree by DEAE and dodecyl groups (Dod), respectively. The substitution degrees were determined by ^{1}HNMR spectroscopy (data not shown).

The SWy-2 montmorillonite clay was kindly supplied by Source Clays Repository of Clay Minerals Society, University of Missouri (Columbia, MO). The SWy-2 clay was purified according to the method described by Gessner, Schmitt, and Neumann, (1994). Glacial acetic acid (Synth, Brazil), silver nitrate (TEC-LAB, Brazil) and distilled water were also used in this work.

2.2. Nanocomposite films preparation

SWy-2 dispersions with 2.5 wt%, 5 wt% and 10 wt% based on chitosan were prepared by dispersing appropriate amounts of SWy-2 into 15 mL of 0.25 mol L^{-1} aqueous acetic acid solution under stirring for 24 h. Then, 0.2 g of Ch83 powder and 1.0 mL of silver nitrate solution (0.05 mol L^{-1}) were added into the clay dispersions. The mixtures were stirred continuously for 24 h. Afterwards, these solutions were cast onto polystyrene Petri dishes with dimensions of 60 mm × 15 mm. The films were dried in an oven at 30 °C, and peeled from the Petri dishes.

Additionally, the nanocomposite films containing Ch30, Ch30d, Ch30-DEAE and Ch30-DEAE-Dod were prepared using only 10 wt% of SWy-2 based on polymer by the methodology described above.

Accordingly, AgNPs were synthesized by photochemical method. The nanocomposite films were placed in a UV light irradiation chamber containing sixteen UV germicidal lamps at 25 °C up to 24 h. At the same position, the emission of lamps was 254 nm