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Preparation and characterization of bio-nanocomposite films of agar and silver nanoparticles: Laser ablation method

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ABSTRACT

Silver nanoparticles (AgNPs) were prepared by a laser ablation method and composite films with the AgNPs and agar were prepared by solvent casting method. UV–vis absorbance test and transmission electron microscopy (TEM) analysis results revealed that non-agglomerated spherical AgNPs were formed by the laser ablation method. The surface color of the resulting agar/AgNPs films exhibited the characteristic plasmonic effect of the AgNPs with the maximum absorption peaks of 400–407 nm. X-ray diffraction (XRD) test results also exhibited characteristic AgNPs crystals with diffraction peaks observed at 2θ values of 38.39°, 44.49°, and 64.45°, which were corresponding to (1 1 1), (2 0 0), and (2 2 0) crystallographic planes of face-centered cubic (fcc) silver crystals, respectively. Thermogravimetric analysis (TGA) results showed that thermal stability of the agar/AgNPs composite films was increased by the inclusion of metallic silver. Water vapor barrier properties and surface hydrophobicity of the agar/AgNPs films increased slightly with the increase in AgNPs content but they were not statistically significant ($p > 0.05$), while mechanical strength and stiffness of the composite films decreased slightly ($p < 0.05$). The agar/AgNPs films exhibited distinctive antimicrobial activity against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*Escherichia coli* O157:H7) bacterial pathogens.

1. Introduction

Silver has long been recognized as an effective antimicrobial agent with a broad spectrum of antimicrobial activity against not only both Gram-positive and Gram-negative pathogenic bacteria but also viruses and other eukaryotic microorganisms (Russell & Hugo, 1994). Accordingly, with the advent of nanotechnology, silver nanoparticles (AgNPs) have been emerged in the development of an antibacterial, antifungal, antiviral, and anti-inflammatory agent (Rai, Yadav, & Gade, 2009; Vaidyanathan, Kalishwaralal, Gopalram, & Gurunathan, 2009). Especially, in food packaging sectors, AgNPs have been exploited for the preparation of antimicrobial active food packaging films due to their strong antimicrobial activity with high thermal stability (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012).

The antimicrobial efficiency of AgNPs-included antimicrobial packaging films is greatly influenced by various factors such as particle size, and its distribution, degree of particle agglomeration, silver content, and interaction of silver surface with the base polymer (Kim et al., 2007). Above all, AgNPs should be well dispersed through polymer matrix without agglomeration. Therefore, it is essential to obtain AgNPs with proper dimensions and to choose proper polymeric materials for the preparation of efficient antimicrobial packaging films with AgNPs.

Conventionally, AgNPs have been produced by the reduction of silver nitrate ($AgNO_3$) using chemical reducing agents such as sodium borohydride, dimethyl formamide, triethanolamine, and hydrazine (Yoksan & Chirachanchai, 2010). However, such chemical reduction method is not recommended since the chemicals are highly reactive and known to pose a potential environmental hazard and biological risks. Instead, a variety of green technologies for the preparation of AgNPs have been developed (Habbalalu, Lalley, Nadagouda, & Varma, 2013). For example, biological materials such as plant extracts, bacteria, fungi, and yeast have been used as mediators for the synthesis of AgNPs (Rhim, Wang, & Hong, 2013). Recently, another type of green technology has been tested using various carbohydrates such as glucose, sucrose, starch, chitosan, and marine polysaccharides. In these technologies, the biopolymers act as both reducing and stabilizing agents and also as polymer matrix for carrying AgNPs (Rhim et al., 2013; Venkatpurwar & Pokharkar, 2011). Furthermore, these approaches using biopolymers are safe, biocompatible, nontoxic

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and environmentally friendly (Sharma, Yngard, & Lin, 2009). Also, various physical methods such as UV, γ-ray, and microwave irradiation, thermal treatment, photochemical process, and sonochemical process have been used for the preparation of AgNPs without hazardous chemical concerns (Yoksan & Chirachanchai, 2010).

Although there are many routes available for the synthesis of nanoparticles, there is an increasing need to develop high-yield, low-cost, non-toxic and environmentally friendly procedures. One of the newest such approaches for preparing AgNPs is a laser ablation method (Christopher, Alee, & Rao, 2011; Herrera, Padilla, & Hernandez-Rivera, 2013). This is based on the ablation of a solid target, i.e., metallic silver, by a pulsed laser beam. The target is usually located in a liquid environment and the ablated AgNPs are collected in the form of colloidal solution. This is a fast, straightforward, and easy method for preparing AgNPs compared to other methods, as it does not need multistep chemical synthetic procedures, long reaction times, and high temperatures. However, most of the published works are mainly focused on the preparation and characterization of AgNPs using the laser ablation method (Hajiesmaeilbaigi, Mohammadalipour, Sabbaghzadeh, Hoseinkhani, & Fallah, 2006; Zamiri, Zakaria, Ahangar, Sadrolhosseini, & Mahdi, 2010, 2011a, 2011b). Though several research works have been published about the preparation of antimicrobial nanocomposite films by incorporation of AgNPs, produced by the reduction of $AgNO_3$, into the biopolymers such as agar and chitosan (Rhim et al., 2013; Tripathi, Mehrotra, & Dutta, 2011), to date, there is no report on the synthesis of bionanocomposite films blended with polymer and AgNPs produced by the laser ablation method.

Hence, the present study was performed to synthesize AgNPs by the laser ablation method and to test the feasibility of production of biopolymer, agar-based nanocomposite films with the AgNPs. The nanocomposite films were characterized by determining their optical, mechanical, barrier, thermal, and antimicrobial properties.

2. Materials and methods

2.1. Materials

A square silver plate (2 cm × 2 cm, 1 mm thick, 99.99% purity) was procured from Sigma–Aldrich (St. Louis, MO, USA). Food grade agar was procured from Fine Agar Agar Co., Ltd. (Damyang, Jeonnam, Korea) and glycerol was purchased from Daejung Chemicals & Metals Co., Ltd. (Siheung, Gyonggido, Korea). Polyvinyl pyrrolidone (PVP) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and all other reagents were of analytical grade.

2.2. Preparation of AgNPs

A Q-switched Nd:YAG laser (Brilliant b, Quantel, Les Ulis, France) with pulse duration of 8 ns and 10 Hz repetition rate at the fundamental wavelength of 1064 nm was employed for ablation of silver. A silver plate was located in cubic glass cell containing 150 mL of 5% PVP solution. The PVP solution was used as a stabilizing agent for ablated AgNPs, in which the AgNPs are kept from agglomeration through the capping of AgNPs by the PVP (Tsuji et al., 2008; Wang, Qiao, Chen, Wang, & Ding, 2005; Wang, Liu, Ji, Ren, & Ji, 2012). The PVP is a water-soluble polymer made from monomer *N*-vinylpyrrolidone and the U.S. Food and Drug Administration (FDA) has approved this chemical for many uses in medicine, pharmacy, cosmetics and packaging industries, and it is generally considered safe (FDA).

First, the silver plate was washed using ultrasonic bath for 30 min and then immersed in the PVP solution. The solution was stirred magnetically during the ablation process to disperse the produced AgNPs. The laser output power was 200 mJ/pulse. The laser beam was focused on the silver target surface through a plano-convex lens (f = 7 cm). The ablation was carried out with different duration times, i.e., 2, 4, and 8 h. The resulting ablated AgNPs were 20, 40, and 80 mg, respectively, which were confirmed by weight difference of the silver plate before and after the laser ablation.

2.3. Characterization of AgNPs

AgNPs solution with the concentration of 20 mg of AgNPs in 150 mL of 5% PVP solution was prepared using the same ablation method for the characterizing the optical properties. UV–vis absorption spectra of the AgNPs solutions were obtained by using a pectrophotometer (Model US/Lamda 3B, Perkin Elmer Co., Santa Clara, CA, USA) in the range of 300–700 nm.

Shape and size of the prepared AgNPs were characterized using Field Emission Transmission Electron Microscopy (FE-TEM, JEM-2100F, JEOL Ltd., Japan) at accelerating voltage of 200 kV. For the TEM experiments, two different concentrations of sample (2 and 16 mg AgNPs/100 mL PVP solution) were prepared by the laser ablation. A drop of each prepared solution was deposited onto nickel grids coated with thin carbon film and left for 1 day to dry completely at room temperature.

2.4. Preparation of agar/AgNPs composite films

Film solutions were prepared by dissolving 4 g of agar powder into the AgNPs solution (0, 20, 40, and 80 mg AgNPs/150 mL) with 1.2 g of glycerol while mixing vigorously for 30 min at 90 °C using a magnetic stirrer. The film solution was cast onto a leveled Teflon film (Cole-Parmer Instrument Co., Chicago, IL, USA) coated glass plate (24 cm × 30 cm), then dried for about 24 h at room temperature following the method of Rhim et al. (2013). In the same way, the control agar film was prepared without AgNPs. The resultant film was peeled off from the casting surface.

All film samples were preconditioned in a constant temperature and humidity chamber at 25 °C and with 50% relative humidity (RH) for at least 48 h to normalize the moisture content. The thickness of films was measured using a micrometer (Dial Thickness gauge 7301, Mitutoyo, Tokyo, Japan) with an accuracy of 0.01 mm.

2.5. Color and transparency

Surface color of the films was measured using a Chroma meter (Konica Minolta, CR-400, Tokyo, Japan) following the method of Rhim et al. (2013). The optical properties of the films were tested by measuring UV–vis absorption and transmission of the films. UV–vis absorption measurements were performed in the range of 300–700 nm using a UV–vis spectrophotometer (Model 8451A, Hewlett–Packard Co., Santa Clara, CA, USA). Transparency of the film samples was expressed as the percent transmittance measured at 660 nm.

2.6. X-ray diffraction (XRD) analysis

Structures of agar and AgNPs-incorporated agar films were evaluated with XRD measurements using a PANalytical Xpert pro MRD diffractometer (Amsterdam, Netherlands), operated at 40 kV and 30 mA, equipped with Cu Kα radiation at a wavelength of 0.15406 nm. The samples were scanned over the range of diffraction angle 2θ = 10–80° with a scanning rate of 0.5°/min at room temperature.

2.7. Microstructure and element analysis

Microstructure of agar and agar/AgNPs films was tested using a FE-SEM (Field Emission Scanning Electron Microscope; S-4800,