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Physicochemical properties of gelatin/silver nanoparticle antimicrobial composite films

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ABSTRACT

Active nanocomposite films were prepared by blending aqueous solutions of gelatin with different concentrations of silver nanoparticles (AgNPs) using a solvent casting method. Formation of silver nanoparticles in the solution and films was confirmed with the surface plasmon resonance (SPR) band at 400–450 nm, measured by UV–vis absorption spectroscopy. The incorporation of AgNPs slightly affected the physical and mechanical properties of the films. Increase in the concentration of AgNPs resulted in a substantial decrease in water vapour permeability (WVP) and tensile strength (TS) of the gelatin films. Energy dispersive X-ray (EDX) spectroscopy and X-ray diffraction (XRD) analysis confirmed the presence of elemental silver and crystalline structure of the AgNPs in the gelatin film. Microscopic surface structure and thermal properties of the films were also examined by FE-SEM and thermogravimetric analysis. Gelatin/AgNPs nanocomposite films exhibited strong antibacterial activity against food-borne pathogens. Gelatin/AgNPs nanocomposite films are expected to have high potential as an active food packaging system to maintain food safety and to extend the shelf-life of packaged foods.

1. Introduction

Microbial contamination can reduce the shelf life of food, increase the risks of various food-borne infections, and cause serious illness (Devlieghere, Vermeiren, & Debevere, 2004). Traditionally, various physical and chemical preservation methods have been used in the food industry to reduce the spoilage of food, to maintain food quality, and to extend shelf life. However, recent increased consumer demands for minimally processed and ready-to-eat fresh foods have motivated researchers to develop alternative new technologies for securing food safety and providing healthy food. Recently, novel active food packaging systems have been introduced to meet consumer demands and to extend the shelf life of food. Antimicrobial packaging (AMP) is one of the promising active food packaging technologies which is often achieved by incorporation or immobilization of potent antimicrobial agents into the packaging system. Various organic and inorganic materials have been used as antimicrobial agents for packaging. However, less heat-stable organic materials could limit their wide use in food packaging systems. By contrast, inorganic nano-size metallic particles, such as gold, silver, zinc, and copper, are not only more stable but also possesses a high surface to volume ratio with increased surface reactivity (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012). Therefore, metallic nanoparticles (NPs) have been widely used industrially for the past two decades.

Among the metal NPs, silver nanoparticles (AgNPs) have attracted especial attention in the food packing sector because of their remarkable and broad spectrum of antimicrobial effect against food-borne pathogens. The incorporation of AgNPs into the food packaging system could effectively inhibit the growth of pathogenic microorganisms (Llorens et al., 2012). Various physical and chemical methods have been utilized for the production of silver nanoparticles (Bankura et al., 2012). Generally, chemical methods have been widely used for the preparation of nano-scale metallic particles. However, some toxicological effects have been reported, after the use of hazardous chemicals as reducing agents. Thus, various alternative methods have been developed to synthesize AgNPs, using eco-friendly, biocompatible, and safe biological resources. Biological systems, such as bacteria, fungi, yeasts, plant extracts, actinomycetes, and certain biological components, have also been listed as safe, to produce AgNPs at extracellular and intercellular level (Bankura et al., 2012). Currently, there is a growing interest in the production of active AgNPs with potent antimicrobial effects, using polysaccharides such as starch (Mohanty et al., 2012), chitosan (Vimala et al, 2010), agar (Rhim, Wang, & Hong, 2013), pullulan, dextran (Bankura et al., 2012), guar gum (Pandey, Goswami, & Nanda, 2012), and alginate (Seo et al., 2012). However, there is no report available for the production of AgNPs using protein such as gelatin as reducing and stabilizing agents.

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Gelatin is an animal protein obtained by a controlled hydrolysis of the fibrous insoluble collagen from bones and skins of animals and fish. It is well known for its film-forming properties (Ma et al., 2012). In addition, it is abundantly available, with low cost, and is easily biodegradable and biocompatible. Gelatin films have excellent barrier properties against gas, oxygen and aromas at low or intermediate relative humidity (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009). Gelatin has frequently been used as a suitable raw material to make edible or biodegradable films for effective food packaging (Ma et al., 2012). Several researchers have used this material to make composite films by blending with other polymers or nanoclays, such as chitosan (Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011), whey protein (Jiang, Li, Chai, & Leng, 2010), pectin (Farris et al., 2011), and shellac (Soradech, Nunthanid, Limmatvapirat, & Luangtana-anan, 2012). In general, gelatin is composed of non-polar aliphatic amino acids, such as glycine (33.0%), proline (13.2%), and alanine (11.2%), and hydroxyproline (9.1%) (Gennadios, McHugh, Weller, & Krochta, 1994). In such systems, the hydroxyl group of hydroxyproline is expected to help reduction of $AgNO_3$ and the non-polar amino acids are expected to help stabilization of AgNPs.

In the present study, gelatin-based antimicrobial nanocomposite films with AgNPs were developed by a solution casting method. The gelatin films, with or without AgNPs, were characterized using SEM, EDX, XRD, FT-IR, and TGA analysis. Moreover, film transparency, surface colour, water contact angle, water vapour permeability, mechanical properties, and antimicrobial activities of the nanocomposite films were examined.

2. Materials and methods

2.1. Materials and microbial strains

Protein polymer gelatin was procured from Gel Tec Co., Ltd. (South Korea). Silver nitrate, brain heart infusion broth (BHI), tryptic soy broth (TSB), and agar powder were purchased from Duksan Pure Chemicals Co., Ltd. (Gyeonggi-do, South Korea). Food-borne pathogens, such as *Escherichia coli* O157:H7 ATCC 43895, *Listeria monocytogenes* ATCC 15313, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 29213, and *Bacillus cereus* ATCC 21366, were obtained from the Korean Collection for Type Cultures (KCTC) in South Korea. All the strains were cultured in BHI and TSA agar medium and subsequently stored at 4 °C for further tests. All solutions were prepared using ultra-filtered high purity deionized water.

2.2. Preparation of gelatin/AgNPs nanocomposites

Gelatin/AgNPs nanocomposite films were developed, using a solution casting method. For this, a stock solution of $AgNO_3$ was prepared by dissolving 5.0 g of $AgNO_3$ in 100 ml of distilled water with boiling for 1 h. Gelatin (5 g) was dissolved in 150 ml of water containing 3 g of sorbitol as plasticizer, as well as combined reducer, with stirring at 100 °C for 20 min. Then, pH of the solutions was adjusted to pH 8.0 using 1 N NaOH solution. Different amount of $AgNO_3$ stock solution (0, 0.3, 0.6, 0.9, and 1.2 ml which corresponds to 0, 10, 20, 30, and 40 mg of AgNPs, respectively) were added dropwise into the film solution mixtures and brought to boiling for 1 h with vigorous stirring. Colour of the solutions was changed from a transparent white to a yellow colour and then cast evenly onto a levelled Teflon film (Cole-Parmer Instrument Co., Chicago, IL, USA) coated glass plate (24 × 30 cm), and allowed to dry at room temperature (~22–25 °C) for 2 days.

2.3. Conditioning and thickness measurement

All dried film samples were peeled off from the glass plate and preconditioned at 25 °C and 50% RH for 48 h in a constant temperature humidity chamber to normalize the moisture content before further analysis.

The thickness of each film sample was measured using a hand-held micrometer (Dial Thickness gauge 7301, Mitutoyo Corporation, Kanagawa, Japan) with an accuracy of 0.01 mm. Measurements were made at 6 different points on each film sample and the average values were used for the property measurement.

2.4. Optical properties

Optical properties of the composite film samples were tested by measuring the UV–vis absorption spectrum of the films. For this, a rectangular piece of film was cut from each film sample and directly clamped between two spectrophotometer magnetic cells. The absorbance spectra were measured using a UV–vis spectrophotometer (Model 8451A, Hewlett-Packard Co., Santa Alara, CA, USA) in the range 300–700 nm.

2.5. Apparent surface colour and transmittance

The colour of the films was measured, using a Chroma meter (Minolta, CR-200, Tokyo, Japan). A white standard colour plate ($L = 97.75$, $a = -0.49$ and $b = 1.96$) was used as a background for measurements of film surface colour. Hunter colour (L, a, and b) values were averaged from five readings from each sample. The total difference in colour of the films (ΔE) was calculated according to the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{0.5} \quad (1)$$

where ΔL, Δa, and Δb are the differences between the colour of standard colour plate and film samples.

Transparency of the each film sample was expressed as the percent transmittance measured at 660 nm. The measurements were carried out in 3 replicates for each film sample and the average values were presented.

2.6. Mechanical properties

The mechanical properties of each film sample were measured by analyzing the tensile strength (TS), Young's modulus (YM) and percent elongation at break (E) according to ASTM standard method 828-88 (ASTM 1989). Samples were cut into 2.54 × 10 cm strips, using a precision double blade cutter (Model LB.02/A, Metrotech, S.A., San Sebastian, Spain). TS, YM, and EAB tests were performed, using an Instron Universal Testing Machine (Model 5565, Instron Engineering Corporation, Canton, MA, USA) operated in tensile mode with an initial grip separation and crosshead speed set at 50 mm and 50 mm/min, respectively. Each composite film sample was mounted between two grips on the machine and stretched until it broke. For each film, ten samples were tested and the average values were presented.

2.7. Moisture content (MC), water vapour permeability (WVP) and water contact angle (WCA)

The moisture content of each film sample was measured according to the method of Soradech et al. (2012). For this, each film sample was cut into 3 × 3 cm and dried in a hot air oven at 100 °C for 24 h. Before and after drying, the weight loss was measured as water content and expressed as a percentage, based on the initial weight of film.