

PHYSICO-CHEMICAL PROPERTIES OF AGAR/SILVER NANOCOMPOSITE FILMS INTENDED FOR FOOD PACKAGING APPLICATION

Sladana Z. Davidović¹, Miona G. Miljković¹, Neda R. Radovanović¹, Suzana I. Dimitrijević¹,
Aleksandra R. Nešić²

¹University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, Serbia

²University of Belgrade, Vinča Institute of Nuclear Sciences, Mike Petrovića-Alasa 12-14, Belgrade, Serbia

Abstract

In this work, antimicrobial agar based nanocomposite films containing silver nanoparticles were prepared. For the synthesis, a masterbatch of in situ stabilized silver nanoparticles produced in agar solution was used. The incorporation of silver nanoparticles improved the mechanical and water vapor barrier resistance of films, in comparison to the neat agar film without silver. Agar/Ag films showed high antimicrobial activity against Staphylococcus aureus, Escherichia coli and Candida albicans. The migration of silver nanoparticles from the agar films, assessed by food contact tests, was less than 0.05 mg/g which is under the legal limits. These results indicated that the agar films incorporated with silver nanoparticles have potential to be used as packaging material.

Key words: silver nanoparticles, agar film, food package material

1. INTRODUCTION

The development of biodegradable active packaging has gained attention as an alternative for petroleum-derived polymers due to its versatility and environmentally friendly nature. The biodegradable films can be made from biopolymers, including proteins, polysaccharides, and lipids or combination of those materials. Among natural polymers, agar is one of the most promising biocompatible and biodegradable material, which has received attention due to low hydrophilicity, great film-forming properties, wide availability and low cost. Agar is a fibrous carbohydrate extracted from a group of marine algae of the class *Rhodophyceae* and mainly composed of alternating repeating units of D-galactose and 3, 6-anhydro-L-galactopyranose (Rhim, 2011; Atef et al., 2014). However, the poor mechanical properties of agar films limited their utilization in a packaging application. Therefore, the research works have been focused on the improvement of such properties by reinforcing agar films with nanofillers such as nano clay, nanometals, and crystalline nanocellulose. The application of nanotechnology has been emerging in the food packaging industry to develop bio-nanocomposite packaging materials by homogeneous blending biopolymers with various types of nano-sized filler materials to improve the properties such as mechanical and gas barrier properties with extra functional properties like ultraviolet light screening and antimicrobial properties. Among all nanofillers, silver nanoparticles (AgNPs) have long been recognized as one of the most 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 eukaryotic microorganisms. Conventionally, AgNPs have been produced by the reduction of silver nitrate (AgNO₃) 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 et al., 2013). For example, biological materials such as plant extracts, bacteria, fungi, and yeast have been used as mediators for the synthesis of AgNPs (Rhim et al., 2013). Recently, another type of green technology has been tested using various carbohydrates such as glucose, sucrose, starch, chitosan, and marine polysaccharides (Huang, Yang, 2004; Shukla et al., 2012; Venkatapurwar, Pokharkar, 2011). In these technologies, the biopolymers act as both reducing and stabilizing agents

and also as polymer matrix for carrying AgNPs. Furthermore, these approaches using biopolymers are safe, biocompatible, nontoxic and environmentally friendly.

In this study, we have prepared agar/AgNPs composite film using an environmentally-friendly method, i.e., reduction of AgNO₃ in the presence of agar solution. The main objective of the present work was to prepare agar-based nanocomposite films containing nano-sized silver particles synthesized by an environmentally friendly method and their mechanical, barrier and antimicrobial properties were also examined.

2. EXPERIMENTAL PART

2.1. Materials

2.2. Preparation of antimicrobial films

Silver nanoparticles (AgNPs) synthesis was carried out in situ in the film suspensions. This procedure allows coupling both processes, taking advantage of the agar film suspension as dispersive medium of the synthesized AgNPs. For this purpose 1.5% wt agar suspensions containing 0.4 wt% of MgCl₂, 0.1 wt% of glucose and 0.1 wt% of CaCO₃ were mixed and autoclaved at 120 °C for 20 min. After that, the glycerol as plasticizer was added in a concentration of 30% wt to the respect of agar and mixture was stirred at 60 °C for 1h. The pH was set to 9 using 1M NaOH. To obtain nanocomposites systems 10 mg/mL solution of AgNO₃ was added to obtain concentration of 1.5x10⁻⁴ M of Ag colloid. Control films (without AgNPs) were prepared in a similar way replacing AgNO₃ solution by ultrapure water in order to simulate the whole process. The system was kept under constant stirring for 20 min at 60 °C, to achieve the formation of AgNPs. The films were cast in silicone molds and left to dry for 24h at room temperature.

2.3. Characterization of films

2.3.1. Mechanical analysis

The mechanical properties of each film sample were measured by analyzing the tensile strength (TS), Young's modulus (YM) and percent elongation at break (EAB) according to ASTM standard method 828-88 (ASTM 1989). Samples were cut into 3x0.5 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 1185 operated in tensile mode with crosshead speed set 2 mm/min. For each film, 6 samples were tested and the average values were presented.

2.3.2. Light transmittance

The light transmittance of films was measured at the ultra-violet and visible range (280, 350, 400, 500, 600, 700 and 800 nm) using a UV-visible spectrophotometer according to the method of Fang et al. (2002).

2.3.3. Water vapor permeability

The water vapor permeability (WVP) of the films was determined, gravimetrically, according to the standard method of ASTM E96-95. The CEAST cups with an average depth of 3 cm and width of 3 cm were used to measure the WVP of the films. Each sample was placed on the top of a cup containing water. The cups were covered with a topper and tightened using screws. The cups were placed in a humidity chamber set at 25 °C and 50% RH. The weight loss of each cup was measured at 1 h intervals for 8 h. Three replicates were measured for each composite film. WVP was calculated from the following equation:

$$WVP = \frac{WVTR \times L}{\Delta p}$$

where WVTR (mg/ m² s) indicates the water vapor permeability rate calculated by dividing the slope of weight vs. time plot by the surface area of the cup, L (m) is thickness of pectin films and Δp is the water pressure difference between both sides of the film (Pa).

2.3.4. Antimicrobial assays

The antimicrobial activity of films was assessed against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10259. The overnight broth cultures of all microorganisms were streaked on the sterile tripton soy agar plates. The agar diffusion test was performed by placing the 6 mm diameter discs of films previously sterilized under UV light for 30 min on seeded plates. The plates were incubated for 24h at 37 °C. Quantitative test was performed according to Stanić et al. (2014) with some modification. The overnight broth cultures of all microorganisms were diluted in saline to obtain the number of cells $\sim 10^6$ CFU/mL (adjusted using McFarland turbidity standard). The 1x1 cm pieces of sterile film samples were placed in 10 mL saline containing 10^6 CFU/mL of tested pathogen and incubated at 37 °C. The 0.1 mL aliquots were taken after 2, 4 and 8 h to determine the number of viable cells. The percentage of viable cell reduction (R %) was calculated according to equation:

$$R(\%) = \left[\frac{C_0 - C}{C_0} \right] \times 100$$

where C_0 , is the initial number of microorganisms (inoculum) and C is the number of microorganism colonies of the samples.

2.3.5. Determination of silver content in film and release of silver

An accurately weighed amount of film was digested with hydrogen peroxide (30%, w/w) and nitric acid (70%, w/w), in a microwave oven. Upon cooling, the solution was made up to 25 mL in a volumetric flask using deionized water. Appropriate dilutions were carried out and the silver ion content in the film was determined using ICP-MS assay.

6 cm² of the films were cut and immersed in 30 mL of aqueous solutions. Since many vegetables have a pH of around 6, the pH of these aqueous solutions was adjusted to 6 in order to mimic the real conditions and correlate release of silver ions with antimicrobial performance. Samples were kept at 25 °C for 24 h, 72 h and 168 h to allow silver release from the films. Before each measurement, the aqueous solutions in the test tubes were removed and nitric acid was added to stabilize silver in its ionic form. The silver ion content for each measurement was determined by ICP-MS assay.

3. RESULTS AND DISCUSSION

3.1. Mechanical analysis

Mechanical parameters such as tensile strength (TS), elastic modulus (E), elongation at break (e) are shown in Figure 1. The slight increase in tensile strength and Young modulus after addition of AgNPs was observed, whereas elongation at break of nanocomposite films decreased. This result could be explained by interaction formed between AgNPs and functional hydroxyl groups from agar. The mechanical properties of the films are closely related to the distribution and density of the intra and intermolecular interactions between the polymer chains in the film matrix. Hence, further investigation of structural properties of films should be examined.

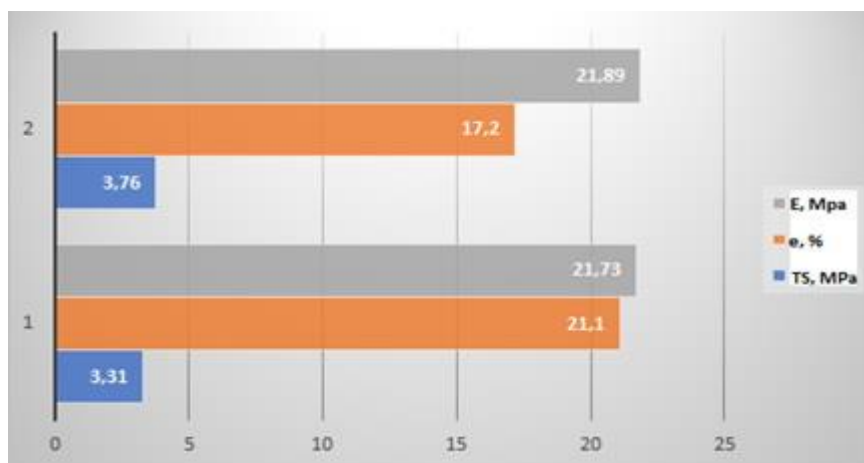


Figure 1. Mechanical parameters of: 1) control agar film, 2) agar/Ag film.

3.2. Light transmittance

It is important to investigate the percentage of light transmitted in both UV and visible light regions in order to design packaging materials for storage of precise food products. The control film was a transparent one with high transmittance value at 800 nm (70%), and had the moderate UV barrier property as indicated by the low transmittance value at 280 nm (35.4 %). The addition of nanosilver into agar films led to significant decrease in light transmittance in UV (280–400 nm) and visible range (400–800 nm) through agar films. It is found that agar/Ag film sample allowed 59.4% of visible light to pass through the film at 700 nm and 28.8% of UV light at 280 nm. These results suggested that AgNPs played significant role in blocking both UV and visible light in the films due to opaqueness of nanosilver and hindrance of light passage or light scattering by nanosilver dispersed in agar matrix. These results are favorable, as food packaging with a barrier in the UV range can prevent the lipid oxidation of food products.

Table 1. Light transmittance through control agar film and Ag/agar film on selected wave lengths

λ (nm)	Transmittance, %	
	Control agar film	Agar/Ag film
280	35.4	28.8
350	51.5	39.95
400	58.8	47.8
500	60.9	50.2
600	61.4	49.1
700	66.2	54.5
800	70	59.4

3.3. Water vapor permeability

Water vapor permeability (WVP) should be as low as possible, since one of the main roles of food packages is to provide good barrier and avoid transfer of moisture between the food and environment. Figure 2 summarizes the WVP values of control agar film and agar/Ag film. As it can be seen, incorporation of AgNPS caused reduction in the WVP. This behavior could be attributed to the

increased tortuosity of the polymeric matrix. A similar trend was observed by Cheviron, Gouanve and Espuche (2015) and Ortega et al. (2017) working on starch based films containing AgNPs and Rhim et al. (2011) working on agar/Ag films. They established pronounced decrease in WVP value with an increase in concentration of silver nanoparticles into films and explained this trend by increased tortuous pathway of water vapor diffusion which was caused by the impermeable hydrophobic silver nanoparticles into polymer matrix.

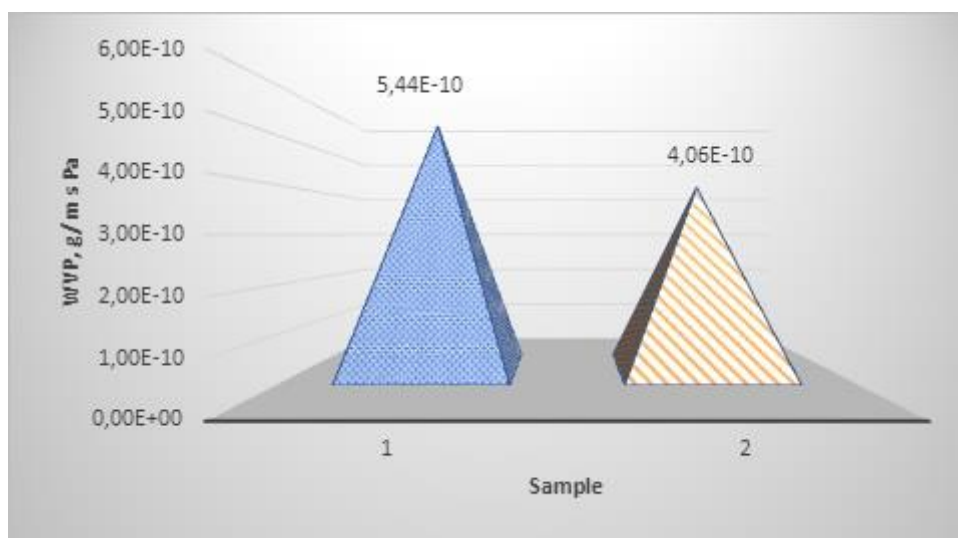


Figure 2. WVP values of: 1) control agar film and 2) agar/Ag films.

3.4. Antimicrobial activity

Antimicrobial properties of control agar film and agar/Ag film were evaluated against *S. aureus*, *E. coli* and *C. albicans*. As expected, the results revealed no inhibition zone when control agar films were used. For the film containing nano silver (Figure 3) clear zone of inhibition 12 mm in diameter around films was observed. Results of qualitative disc diffusion test show that agar/Ag films exhibit antimicrobial activity against all three tested pathogen.



Figure 3. Photograph of antimicrobial test result of agar/Ag film against *E. coli* (left), *S. aureus* (middle) and *C. albicans* (right)

Antimicrobial activity of silver is well known and described in the literature (Kim et al., 2007; Rai et al., 2009; Kang et al., 2016). The mechanism of antimicrobial activity of silver nanoparticles is still unknown, but it has been proposed that they serve as reservoir of Ag ions which interact with

negatively charged biomacromolecular components of microbial cells causing structural changes and deformation in bacterial cell walls and membranes that finally lead to cell death (Feng et al., 2000; Rai et al., 2009). Moreover, AgNPs may accumulate in the bacterial cytoplasmic membrane, causing a significant increase in membrane permeability and leading to cell death (Sondi, Salopek-Sondi, 2004).

The antimicrobial activity of agar/Ag film was approved by the quantitative test in liquid medium. According to the results of the antimicrobial assay (Figure 4), agar film with colloidal silver was very active against investigated pathogen species and caused great viable cell reduction in a relatively short time period of exposure. After 2 h viable cell number reduction was greater than 50% and increased with time of incubation. Among tested microorganisms the most susceptible was *E. coli* with 88% reduction achieved for 4 h. After 8 h almost complete inhibition (99%) was achieved for all tested pathogens.

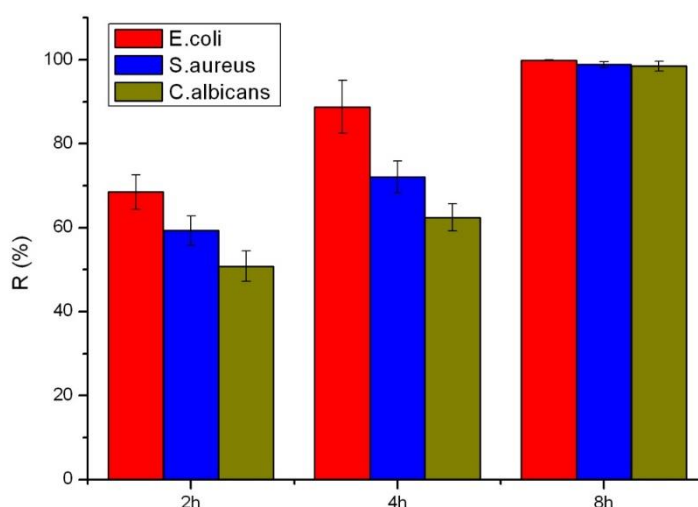


Figure 4. The percentage of viable cell reduction obtained after 2, 4 and 8 h incubation with agar/Ag film

3.5. Release of silver ions

In order to better understand the binding mechanism of silver ions to agar films, the silver ion content in the films was evaluated. The obtained results showed that the content of AgNPs in agar films was 38 ppb.

Contact tests using food simulants were performed to determine if the nanostructured agar films fulfill the recent regulations and European directives on food packaging (EN1186-1, 2002), according to the Commission Regulation No. 10/2011, which establishes the overall migration limits (OML). For plastic materials, the OML is 60 mg (of substances)/kg (of foodstuff or food simulant) for all substances as maximum migration. The migration of silver ions in food simulant after 1, 3 and 7 days was evaluated and presented in Figure 5. As it was shown, the release of silver ions after 7 days was 37 ppb (or 37 mg/kg), which proved that agar/Ag films accomplish the requirements for food packaging. The above data indicate that the produced films are safe and can be used in food contact.

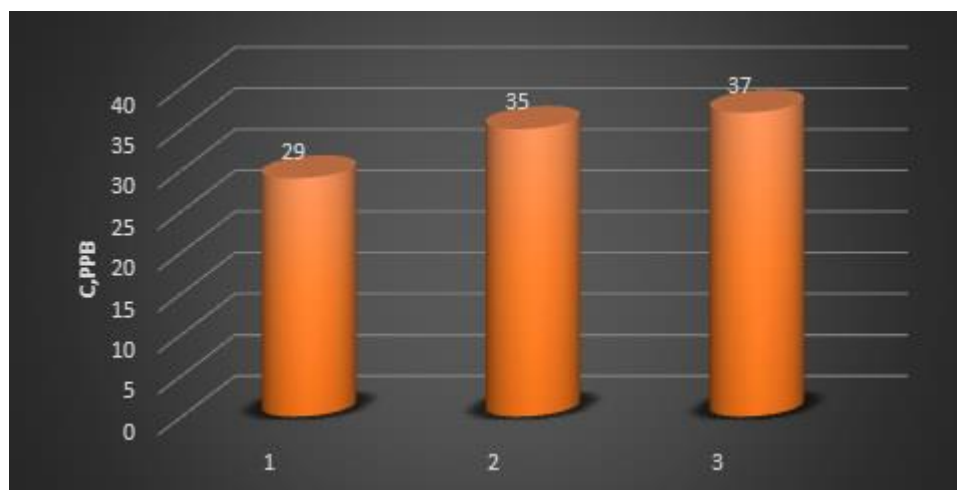


Figure 5. Release of silver ions after: 1) 1 day, 2) 3 days, 3) 7 days for agar/silver film.

4. CONCLUSIONS

To overcome the disadvantage of petrochemical based plastics, the agar-based nanocomposite films reinforced with AgNPs were investigated. Effect of AgNPs incorporation on mechanical strength, water vapor and light barrier property and antimicrobial property of films was evaluated. Developed nanocomposite films exhibited improvement in water vapor and light barrier property, whereas mechanical stability was maintained. The nanocomposite films exhibited profound antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as against yeast. These results suggest that the agar/Ag nanocomposite films can be potentially used as an eco-friendly, UV barrier and active food packaging materials to ensure food safety and prolong the shelf-life of packaged food.

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