Green-synthesized silver nanoparticle coating on paper for antibacterial and antiviral applications

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Abstract
The use of active packaging has attracted considerable attention over recent years to prevent and decrease the risk of bacterial and viral infection. Thus, this work aims to develop active packaging using a paper coated with green-synthesized silver nanoparticles (AgNPs). Effects of different silver nitrate (AgNO3) concentrations, viz. 50, 100, 150, and 200 mM (AgNPs-50, AgNPs-100, AgNPs-150, and AgNPs-200, respectively), on green synthesis of AgNPs and coated paper properties were investigated. A bio-reducing agent from mangosteen peel extract (ex-Garcinia mangostana (GM)) and citric acid as a crosslinking agent for a starch/polyvinyl alcohol matrix were also used in the synthetic process. The presence of AgNPs, ex-GM, and citric acid indicated the required synergistic antibacterial activities for gram-positive and gram-negative bacteria. The paper coated with AgNPs-150 showed complete inactivation of virus within 1 min. Water resistance and tensile strength of paper improved when being coated with AgNPs-150. The tensile strength of the coated paper was found to be in the same range as that of a common packaging paper. Result revealed that the obtained paper coated with AgNPs was proven to be effective in antibacterial and antiviral activities; hence, it could be used as an active packaging material for items that require manual handling by a number of people.

Keywords Silver nanoparticles · Green synthesis · Active packaging · Antibacterial activities · Antiviral activity

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Introduction

Paper is a cellulosic material, which has been used for versatile purposes such as packaging, printing/writing, household products, filtration, and healthcare items because of its biodegradability, light weight, reusability, and recyclability [1, 2]. In improving and uncovering new functions to paper, a common and simple technique such as coating has been applied to achieve new properties [3, 4]. Substances such as bioactive agents, wax, and biopolymers have been used for paper coating to extend its applications [5–7].

The use of nanotechnology to create nanomaterials has been quite common in coating applications because of the high surface area of nanoparticles with regard to their volume, low utilization of chemical agents, and less consumption of energy [8, 9]. Among available nanomaterials, silver nanoparticles (AgNPs) are widely used because of their outstanding properties such as antimicrobial activities [10, 11] and less toxicity for mammalian cells [12, 13]. Coating of AgNPs on various substrates has been used in personal care products [2], food packaging [14, 15], and medical instruments [16]. The preparation of AgNPs can be achieved by various techniques. Among the techniques, the green synthesis of AgNPs is more ecofriendly than other methods because of the use of materials with low toxicity [17–19]. Several bio-based compounds such as terpenoids, glycosides, alkaloids, and phenolics can be used as bio-reducing agents [10, 20]. Recently, the new trend of using bio-based extracts from agricultural [21, 22], food, and beverage wastes [23] has attracted considerable attention. Ounkaew et al. [7] prepared the paper coated with green-synthesized AgNPs using spent green tea. They found that the coating of AgNPs on paper enhanced water resistance and inhibited the growth of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The presence of extracted spent green tea in AgNP synthesis could reduce the reaction time from 2 h to 20 min. Panzella et al. [23] proposed the green synthesis of AgNPs using spent coffee grounds (SCG). The obtained phenolic compounds in SCG such as chlorogenic acid and caffeic acid served as bio-reducing agents. The SCG containing 0.3%wt of AgNPs could inhibit the growth of *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *S. aureus*. Ounkaew et al. [22] observed that the use of agricultural waste such as sugarcane leaf combined with 20 wt% citric acid for in situ green synthesis of AgNP nanocomposite films provided a synergistic effect of antibacterial activities. In addition, the presence of citric acid as a crosslinker in nanocomposite films based on starch and polyvinyl alcohol (PVA) created ester linkages, which led to an increase in mechanical properties and water resistance. Apart from the use of wastes from agriculture and beverage as bio-reducing agents, the waste from fruit peels would be an attractive alternative source because of the availability of enriched phenolic compounds [24, 25]. *Garcinia mangostana* (GM) or purple mangosteen peel consists of phenolic compounds such as xanthones, which can be applied in medical applications [26]. Lee et al. [27] observed that the use of GM peel for AgNP synthesis in an acidic condition with pH of about 4 yielded nanoparticles in spherical shapes and a uniform dispersion of particles. Green-synthesized AgNPs using
GM peel could be applied as an anticancer drug nanocarrier. Although a number of studies have focused on green synthesis of AgNPs for various applications, particularly antibacterial activities, little information is found on antiviral activities and other properties. At present, considering that microbial pathogens such as viruses are also a major public health concern [28], the development of coating materials for the inhibition of microorganism growth could be a new and effective approach to prevent bacterial and viral infection [29, 30].

In this study, the coating of AgNPs on paper was established by using extracted GM peel as a bio-reducing agent and starch/PVA copolymer as a binder. Different concentrations of silver nitrate for the green synthesis of AgNPs were studied. Moreover, antibacterial and antiviral activities of paper coated with AgNPs, water resistance, and tensile strength were investigated.

**Experimental**

**Materials**

Cassava starch with 17% amylose and 12.17% moisture was purchased from Bangkok Interfood Ltd., Thailand; PVA with an average molecular weight of 1700–1800 was purchased from Laboratory Reagents & Fine Chemical. Citric acid with purity of 99.5% was purchased from RCI Labscan Limited. GM peel powder with a particle size of 180–250 µm was supplied from local shops in Khon Kaen, Thailand.

**Preparation and characterization of ex-GM**

The ex-GM was prepared by using the ratio of GM peel powder to deionized water at 1:60. The mixture of GM peel powder and deionized water was heated at 90 °C for 5 min with constant agitation. Then, the mixture was filtered through a qualitative filter (no. 1) to obtain ex-GM sample. The total phenolic content (TPC) of ex-GM was determined using the Folin–Ciocalteu method and gallic acid as standard according to Tronchuen et al. [31]. A UV–vis spectrophotometer (Agilent Cary 60 UV–Vis Spectrophotometer) was used in the analysis to obtain TPC. Ex-GM had a TPC of 37.48 ± 1.06 mg GAE/g of GM peel powder.

**Preparation of paper coated with AgNPs**

First, the solutions of cassava starch and PVA were prepared separately. 2.5 g of cassava starch was dissolved in 50 mL of ex-GM by using a magnetic stirrer for 20 min at 90 °C. 2.5 g of PVA was also dissolved in ex-GM at 95 °C for 60 min. Afterward, the solutions of starch and PVA were mixed for another 5 min at 90 °C. Silver nitrate solutions with concentrations of 0, 50, 100, 150, and 200 mM were added into the starch/PVA solution and stirred for 12 h at 90 °C. The obtained solution was cooled down to room temperature, and then 5 g of citric acid was added and stirred for another 5 min. The homogenous mixture solution was cast on a piece of filter paper
using a doctor blade with a gap of 200 µm to evenly spread the mixture solution. The coated paper was then dried at 50 °C for 30 min.

Characterization of paper coated with AgNPs

A UV–Vis spectrophotometer (Perkin-Elmer, Lambda 35, spectrometer) was used to characterize the green-synthesized AgNPs at 350–510 nm. The effect of reaction time and concentration of AgNO₃ on the synthesis of AgNPs was measured in triplicate.

The morphology of AgNPs was observed using transmission electron microscopy (TEM, FEI, model: TECNAI G2 20). One milliliter of sample solution was diluted 20 times in deionized water, deposited onto 400-mesh carbon-coated Cu grids, and dried at ambient conditions for 24 h. Micrograph analysis was performed at 200 kV acceleration voltage. The surface of the coated paper with AgNPs and high-resolution energy-dispersive X-ray spectroscopy (EDS) mapping were characterized using a scanning electron microscope (SEM, Hitachi Miniscope model TM-3000). All samples were coated with gold using an ion sputtering device.

X-ray diffraction of sample was investigated by using a SmartLab X-ray diffractometer over a diffraction angle range of 0° ≤ 2θ ≤ 80°; the diffractometer was equipped with a Cu Kα radiation source (wavelength λ = 1.542 Å) operated on a scan rate of 10° (2θ) at 40 kV and 30 mA.

The structural interaction of samples was analyzed by attenuated total reflection infrared (ATR-FTIR) spectroscopy (Jasco 4200). The spectra of all samples were tested in the range of 4000–400 cm⁻¹ with 64 scans at a resolution of 2 cm⁻¹.

The antimicrobial activity of the sample was evaluated by agar diffusion. Gram-negative and gram-positive bacteria, i.e., E. coli and S. aureus, respectively, were selected as representative strains of food pathogens. The bacteria were cultured overnight in brain heart fusion broth at 37 °C. The bacterial culture was diluted in a medium to obtain turbidity of approximately 10⁸ CFU/mL. The bacterial inoculums were seeded on Muller Hinton Agar plates using the swab plate technique. After 24 h of incubation at 37 °C, the inhibition zone (mm) around the sample disk was measured in triplicate.

Dengue virus serotype 3 (DENV-3) strain P12/08 and Vero cell were used to determine the antiviral activity of the coated paper. The antiviral activity of the sample was investigated using immunofocus assays. Vero cells were seeded in 96-well plates at 2 × 10⁴ cells/well in Dulbecco’s modified eagle medium (DMEM) supplement with 10% fetal bovine serum (FBS) and incubated at 37 °C in 5% CO₂ overnight. Samples were cut (1 cm × 1 cm) and sterilized by pressurized saturated steam at 121 °C for 15 min using Autoclave. The sterile coated paper was placed into the bottom of sterile 1.5-mL microcentrifuge tubes. One hundred microliters of DENV-3 were added to each coated paper sample. After adsorption, 200 µL of DMEM without FBS was immediately added into the coated paper samples and incubated at room temperature for 0, 15, 30, 60, and 120 min. After being incubated at various time points, the coated paper was discarded. The viral treated supernatant was serially diluted in DMEM without FBS from 10⁻¹ to 10⁻⁴ in tenfold increments.
Each dilution was inoculated into Vero cell (100 μL/well) and incubated for 2 h at 37 °C in 5% CO₂. After 2 h of incubation, the cells were overlaid with Eagle minimal essential medium containing 1.5% methylcellulose (FUJIFILM, Wako Pure Chemical Corporation) and 2% FBS. Three days after infection, the cells were fixed with 3.7% v/v formaldehyde/phosphate-buffered saline (PBS) for 1 h and permeabilized with 0.5% v/v Triton X-100/PBS (Sigma, USA) for 10 min. After 1 h of incubation in a blocking buffer containing 1% FBS and 0.05% Tween-20 in PBS, the cells were treated with a mouse monoclonal antibody 4G2 for 1 h and washed three times with PBS. Then, the cells were incubated with Alexa Fluor 488 goat anti-mouse IgG for 1 h in blocking buffer. Finally, the cells were washed three times with PBS, and the number of foci was counted under a fluorescence microscope (Olympus).

The hydrophobicity of the coated paper with AgNPs was measured by using an optical contact angle meter (OCA 15EC) with 5 μL of deionized water droplet at ambient temperature [32], and an average of five measurements per sample was reported. Water vapor transmission rate (WVTR) of the coated paper was also tested. A sample with 10 mL of distilled water was weighed and kept in a desiccator at 25 °C and 52% RH. The weight gain of sample was measured as a function of time. A slope of linear portion of the weight gained versus time was a value of WVTR. The difference in partial pressure of water vapor across the sample (ΔP) was also determined. The water vapor permeability (WVP) was then calculated following Eq. (1).

\[
WVP = \frac{WVTR \times \text{Thickness}}{\Delta P}
\]  (1)

The tensile strength of the coated paper was tested using a universal testing machine (Shimadzu SCG) equipped with a 5 kN load cell following the ASTM D 882-10 method (2015). The initial distance among the grips was 50 mm, and a crosshead speed of 5 mm s⁻¹ was used.

Results and discussion

Synthesis and characterization of AgNPs for paper coating

The formation of AgNPs as a function of reaction time was monitored using UV–vis spectroscopy (Fig. 1). The characteristic surface plasmon resonance (SPR) peaks were observed around 420–424 nm at different reaction times, which indicated the formation of AgNPs [21]. Moreover, the change in peak intensities was observed when the reaction time increased from 2 to 12 h. The increase in peak intensity indicated the increase in the concentration of AgNPs [33]. After 12 h of reaction, no increase in peak intensity was observed. The broadened SPR peak and decrease in magnitude were observed. This phenomenon implied that longer reaction time could lead to the aggregation of AgNPs [34, 35]. Therefore, the reaction was completed within 12 h. This suitable reaction time was selected when synthesizing AgNPs at various silver nitrate concentrations. Figure 2 shows the UV–Vis spectrum of
green-synthesized AgNPs at 0–200 mM of silver nitrate. The shift of the absorbance peak to a shorter wavelength was observed in AgNPs-100 when compared with AgNPs-50. The blue shift of the wavelength indicated the decrease in particle size [36]. For AgNPs-150 and AgNPs-200, the absorbance peaks shifted to a longer wavelength, which referred to the red shift and implied the increase in particle size [37]. Dutta et al. [38] suggested that the change in reducing environment during the green synthesis of AgNPs resulted in the shift of absorbance peak. However, the absorbance peak of AgNPs-150 showed a red shift, and it still had the highest peak
intensity. A higher peak intensity indicated higher formation of AgNPs [37]. The decrease in peak intensity was found in AgNPs-200. The use of high silver nitrate concentration in green synthesis resulted in a large number of AgNPs formed with a high surface area and surface tension, which led to the agglomeration of AgNPs [39]. The obtained UV–Vis spectrum results were consistent with the TEM analysis (Fig. 3). Particles with an almost spherical shape were also found in all AgNP samples (Fig. 3a). The amount of AgNPs tended to increase with the increase of silver nitrate concentration. The slightly aggregated particles were found in AgNPs-150, whereas AgNPs-200 had densely aggregated particles. Figure 3b exhibits the particle-size distribution of AgNPs. The average particle size could be divided into two ranges. The first range was 2.36 ± 0.87–3.74 ± 1.11 nm for AgNPs-50 and AgNPs-100, whereas the second range was 189.96 ± 71.27–294.73 ± 106.46 nm for AgNPs-150 and AgNPs-200.

The crystalline structure of AgNPs on the coated paper was characterized using XRD. The XRD pattern of Ag crystals was observed in the 2θ range of 30°–80°, which represented indexed reflections of (111), (200), (220), and (311) from the face-centered cubic (FCC) unit cell [40]. Figure 4 shows that 2θ values of the paper coated with AgNPs were found at 38.05°, 47.36°, and 64.77° corresponding to the (111), (200), and (220), respectively [41, 42]. Some unknown peaks (marked with a star) might be related to bio-organic phases on the surface of AgNPs [43, 44]. Consequently, the obtained AgNPs on the coated paper had an FCC structure.

**Attenuated total reflection infrared characterization of paper coated with AgNPs**

The ATR-FTIR spectra of papers coated with ex-GM, ex-GM combined with citric acid, and AgNPs-150 are depicted in Fig. 5. The paper coated with ex-GM showed characteristic peaks at 1013 and 1066 cm\(^{-1}\) (C-O stretching) [45], 1342–1413 cm\(^{-1}\) (CH or CH\(_2\) bending) [46], and 3263 cm\(^{-1}\) (OH stretching) [45]. The presence of citric acid in paper coated with ex-GM and AgNPs-150 showed a new peak at 1706 cm\(^{-1}\), which corresponded to carboxyl (C=O) groups and ester linkages of citric acid [22]. The shift of absorbance peaks at 3263–3306 cm\(^{-1}\) was also found. This phenomenon was attributed to intermolecular hydrogen bonding among reactants such as PVA, starch, ex-GM, citric acid, and AgNPs. This observation was consistent with the finding of a previous report [45].

**Antibacterial and antiviral activities of paper coated with AgNPs**

During the COVID-19 pandemic, various packaging materials have been developed with incorporated antibacterial and antiviral properties. The coating of active compounds on packaging such as film, paper, and cardboard bags could address infection-related concerns of users and promote the safety use of such products [30]. The antibacterial and antiviral activities of paper coated with AgNPs are shown in Figs. 6 and 7, respectively. Figure 6a shows the inhibition zones of *S. aureus* and *E. coli*. The paper coated with AgNPs showed a better antibacterial activity for *E. coli* than for *S. aureus*. Gram-negative bacteria (*E.
Fig. 3  A TEM micrographs of AgNPs at different concentrations of silver nitrate and B number size distribution of AgNPs in polymer matrix.
coli) had less resistance to inhibition than gram-positive bacteria (S. aureus). A thinner peptidoglycan layer of gram-negative bacteria allowed silver ions to penetrate into the cells more easily. Antimicrobial actions of AgNPs have been proposed with four mechanisms, viz. (i) adhesion of AgNPs toward the surface membrane of microbial, (ii) penetration of AgNPs into the cells resulting in the disruption of biomolecules and intracellular damage, (iii) generation of reactive oxygen species for attacking microbial cells, and (iv) disruption of the signal transduction pathways in the cells [47]. Furthermore, other bioactive agents in
the coating of the paper such as citric acid and ex-GM had antibacterial properties. The main actions of antibacterial activities for citric acid were acidulation and chelation. The acidic ions can damage the enzymes and extracellular membrane and bind essential metal ions in bacterial cells [48]. For ex-GM, the bioactive compounds such as xanthone, saponin, terpenoid, tannin, and flavonoid had an antibacterial activity. Xanthone could retard cell replication; saponin and terpenoid could destroy cell membrane, and tannin could inhibit protein transport enzyme through the cell membrane [49]. In addition, paper coated with AgNPs-150 showed a larger clear zone than paper coated with ex-GM, paper coated with

![Fig. 6](image.png)

**Fig. 6** A antibacterial activities of paper coated with AgNPs and B EDS images of coated paper with AgNPs.
This observation indicated the synergistic effect of the use of active compound combination, viz. AgNPs, citric acid, and ex-GM, on antibacterial activities. The antibacterial capability of active compounds coated on a paper was in the following increasing order: AgNPs-150-W and ex-GM < citric acid combined with ex-GM < AgNPs-150. In addition, the dispersion ability of AgNPs in a polymer matrix was considered as a factor influencing antibacterial activities. Azeredo [50] proposed that the good dispersion of AgNPs in a polymer matrix increased penetration ability into the cell wall, thereby enhancing the antibacterial activity. The dispersion of AgNPs-150 could be confirmed by EDS images (Fig. 6b). The mapping of silver and element analysis showed a homogeneous distribution of AgNPs-150 on the surface of the paper.

Figure 7 shows the influence of the paper coated with different formulas of AgNPs on antiviral activities. The titer of virus drastically decreased upon contact with paper coated with ex-GM, ex-GM combined with citric acid, AgNPs-150 without citric acid, and AgNPs-150. In addition, the virus titer of the paper coated with AgNPs-150 was found to be zero after 1 min. These results implied that the use of AgNPs combined with ex-GM and citric acid played an important role for effective antiviral properties. For antiviral activities of AgNPs, two mechanisms have been proposed, that is, (i) the binding of AgNPs to the outer surface of virus can inhibit the attachment of virus toward cell receptors, and (ii) AgNPs can bind to the DNA or RNA of the virus resulting in the inhibition of viral replication and propagation inside the host cells [47]. Citric acid can create an acidic environment to inhibit the virus growth [51], whereas the essential compounds in ex-GM could inhibit viral replication [52]. Based on the results of antibacterial and antiviral activities, the paper coated with AgNPs could be applied as packaging for various items such as foods and medical products. However, the
cytotoxicity test of this active packaging should be further studied to confirm and ensure safety.

**Physical and mechanical properties of paper coated with AgNPs**

However, the poor water resistance of paper limits its applications [53]. The wettability of materials can be investigated by water contact angle (WCA) analysis. The WCA values of paper coated with AgNPs are shown in Fig. 8. The WCA values of paper coated with AgNPs are depicted in Fig. 8. The WCA value remarkably increased from ~37° for uncoated paper to 81.4°–85.8° when paper was coated with AgNPs-100, AgNPs-150, and AgNPs-200. The increase of WCA implied the enhancement of hydrophobicity [54]. Wetting of any surface was based on two main factors, viz. chemical composition on the surface and surface roughness [55]. Figure 9 exhibits SEM images showing the roughness of the samples. The paper coated with AgNPs-100, AgNPs-150, and AgNPs-200 showed a rougher surface than the other samples. Based on Cassie’s theory, water driplet holds on the top pyramid shape, whereas the grooves will be occupied with air. This phenomenon can prevent water from filling in the grooves [55, 56]. Therefore, the roughness of the paper coated with AgNPs should be further studied to consolidate architecture and surface morphology by atomic force microscopy.

WVP is a main packaging property that indicates resistance capability to water vapor transmission of packaging materials. A low WVP is required to minimize the moisture transfer from the surrounding to the packaged product, particularly under harsh moisture conditions [57]. The WVP of samples is illustrated in Table 1. The WVP decreased from $5.84 \pm 0.71 \times 10^{-8}$ g/m$^2$×kPa×h for uncoated paper to $0.17 \pm 0.68 \times 10^{-8}$–$0.20 \pm 0.31 \times 10^{-8}$ g/m$^2$×kPa×h for coated paper. Srichiangsa et al. [58] suggested that the coating of paper could reduce the number and size of

![Fig. 8 WCA values of paper coated with AgNPs](image-url)
pores on the paper surface, resulting in the poor permeability of the paper package to water vapor. Furthermore, the presence of nanoparticles in coated paper could reduce WVP by the formation of a tortuous path for water vapor transmission.

The tensile strength of paper coated with AgNPs is depicted in Fig. 10. The tensile strength indicates the maximum stress in which the paper packaging can withstand before breaking [55]. All the coated papers showed higher values in tensile strength when compared with uncoated paper. The tensile strength increased up to 69.58% when the paper was coated with AgNPs-150. The formation of strong intermolecular hydrogen bonds between the polymer matrix and AgNPs could enhance tensile strength [59, 60]. The obtained tensile strength at 35.27 MPa for paper coated with AgNPs-150 was comparable to other common packaging paper in previous reports, for example, paper coated with curdlan/chitosan (25–37 MPa) [61], paper coated with chitosan–caseinate bilayer (21–26 MPa) [62], and paper coated with carboxymethyl cellulose/cellulose nanocrystal-immobilized AgNPs (18–20 MPa) [60]. However, increasing

![Fig. 9 SEM images of coated paper with AgNPs](image)

### Table 1  WVP of uncoated and coated paper

| Sample                              | WVP (g/m² × kPa × h) |
|-------------------------------------|----------------------|
| Uncoated paper                      | 5.84 ± 0.71 × 10⁻⁸   |
| ex-GM combined with citric acid     | 0.17 ± 0.68 × 10⁻⁸   |
| AgNPs-50                            | 0.18 ± 0.19 × 10⁻⁸   |
| AgNPs-100                           | 0.19 ± 2.37 × 10⁻⁸   |
| AgNPs-150                           | 0.20 ± 2.18 × 10⁻⁸   |
| AgNPs-200                           | 0.20 ± 0.31 × 10⁻⁸   |
AgNP concentration at AgNPs-200 for coating, the tensile strength decreased to 24.93 MPa. Vaezi et al. [63] indicated that the agglomeration of nanoparticles might result in the decrease of tensile strength.

**Conclusion**

Bioactive packaging based on a paper coated with green-synthesized AgNPs having antiviral and antibacterial activities has been successfully developed. The AgNPs were prepared from different reaction times and AgNO₃ concentration using ex-GM as a bio-reducing agent. The optimal reaction time was 12 h. The result showed that the synthesized AgNPs with various AgNO₃ concentration were mostly spherical in shape with an average particle size of 2.36–294.73 nm. Based on silver mapping and element analysis, AgNPs-150 had a homogeneous distribution on the paper surface. The paper coated with AgNPs showed greater hydrophobicity because of the greater surface roughness of the samples. The highest tensile strength of the coated paper was observed in AgNPs-150. The paper coated with AgNPs-150 exhibited the highest antibacterial property against *E. coli* and *S. aureus*, and it also had antiviral properties. Furthermore, the use of combination active compounds, that is, AgNPs, citric acid, and ex-GM, provided the synergistic effect on antibacterial activities. Based on the obtained results, the coated paper with green-synthesized AgNPs integrated with citric acid and ex-GM is a potential material for bioactive packaging application.

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