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Making PVA-Based Antimicrobial Food Packaging Film: An Incorporated Association with AgNPs-Immobilized Cellulose Nanospheres

Hong-Phuong Phan[a], Chau-Giang Tran[a], Quang-Dong Tu[a], Minh-Tam K. Nguyen[a], Hoa-Hung Lam[a], Uyen P.N. Tran[b], Trung Dang-Bao[a,*]

[a]Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, Ho Chi Minh City, Vietnam
[b]Faculty of Engineering and Technology, Van Hien University, Ho Chi Minh City, Viet Nam
dbtrung@hcmut.edu.vn

As the most universal biopolymer, nanocelluloses have been emerged with distinctive features such as biodegradability, renewability, non-toxicity and applicable feasibility in large-scale industry. In an effort to diversify the practical application of such natural origin materials, the surface modifications of nanocelluloses based on hydroxyl groups have been widely addressed. In the context of metal nanoparticles loaded on nanocelluloses, such above-mentioned modifications required complicated multiple-step procedures and used external toxic reagents, limiting their applications in humans. The aim of this study reported a simple and eco-friendly chemical approach in immobilizing silver nanoparticles (AgNPs) on corn leaf-derived nanocellulose using non-toxic reagents. Taking into account the native surface hydroxyl groups of nanocellulose in its dispersing in water and favouring the immobilization of metal nanoparticles, the nanospheres were obtained with a mean diameter of 22.5 nm as proven via transmission electron microscopy (TEM), the exclusive presence of crystalline AgNPs was evidenced via X-ray diffraction (XRD), and the Ag percentage of 4.1 % was explored on the surface via energy-dispersive X-ray (EDX). The complete antimicrobial performances against *E. coli* and *S. aureus* were achieved within 6 h. The findings of this work were achieved in a green context, widening a preliminary trial in strawberry packaging; for example, a PVA-based film blended with 2 % AgNPs-immobilized cellulose nanospheres remained strawberries intact and no appearance of microorganisms.

1. Introduction

The current progress of nanotechnology has widened many opportunities in fabrication of nanoparticles and nanostructured materials and their potential application in diverse fields (Dang-Bao et al., 2022); within this framework, natural origin materials have been more attractive in regard to valorisation of biomass sources (Ning et al., 2021). As the most universal biopolymer, cellulose and cellulose-derived materials have been emerged with distinctive features such as biodegradability, renewability, non-toxicity and applicable feasibility in large-scale industry (Thomas et al., 2018). At a nanometer-size, nanocelluloses (mainly referring cellulose nanocrystals and cellulose nanofibrils) possess intense physical and chemical properties, in particular more flexible functional groups on their surface. In fact, the size, shape and crystallinity have been really decided by the cellulosic sources, and their corresponding isolation conditions (Noremylia et al., 2022). In nature, cellulose can be eliminated from the plant cell wall via the removal of lignin, hemicellulose and other natural impurities using alkali agents. Subsequently, acid agents can attack the amorphous regions, breaking the glycosidic bonds composed of two glucose units and releasing the nano-sized fragments of cellulose, called as nanocelluloses (Huang and Fu, 2013).

Possessing the surface hydroxyl groups, nanocelluloses can be efficiently functionalized, such as aldehyde (Zhang et al., 2018), carboxyl (Gopiraman et al., 2018), thiol (Dang-Bao et al., 2023), toward the uptake of metal ions via electrostatic interaction or metal chelation. Metal nanoparticles (MNPs) could be well-dispersed on the

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surface of nanocelluloses via the in-situ chemical reduction of metal ions anchored on such entities (Thach-Nguyen and Dang-Bao, 2022). In principle, the formation of MNPs on nanocelluloses have been admitted via the two strategies (i) the surface functionalized groups can act as reductants; (ii) external reductants should be utilized like $NaBH_4$, N_2H_4, H_2, etc. In all the cases, nanocelluloses play an important role in stabilizing MNPs due to their natural supramolecular arrangement and preventing their agglomeration (Thach-Nguyen et al., 2022). On the other side, the additional cationic surfactants were reported in order to disperse metal nanoparticles via steric effects, such as CTAB (An et al., 2017), TMA (Jebali et al., 2018).

In this context, metal nanoparticles loaded on nanocelluloses were previously reported in catalysis, such as AgNPs immobilized on corn leaf-derived nanocellulose (Thach-Nguyen et al., 2022) and palladium nanoparticles immobilized on corncob-derived nanocellulose (Dang-Bao et al., 2023). In an effort to diversify their application, it seems to be foreseeable that AgNPs immobilized on nanocelluloses promise as antimicrobial agents in various templates. For example, thiol-functionalization of cotton fabric could be obtained via an esterification with thioglycolic acid, and followed by a chemical reduction of Ag^+ ions toward AgNPs (using $NaBH_4$ as a reductant) (Xu et al., 2018); AgNPs were dispersed in a cellulose nanocrystal suspension with the assistance of $NaBH_4$ (Xu et al., 2013). Although focusing on antimicrobial applications, the above-mentioned reports utilized $NaBH_4$ (a toxic chemical) and a complicated functionalization procedure involving uncontrolled chemicals, resulting in possible harmful risks to human.

In this report, the fabrication of zero-valent AgNPs immobilized on cellulose nanospheres due to their natural hydroxyl groups, excluding any external surfactants. Using a green reducing agent such as glucose, their high antimicrobial activity toward *E. coli* and *S. aureus*, permitting making PVA-based antimicrobial film for food packaging application.

2. Experimental

2.1 Fabrication of AgNPs immobilized on cellulose nanospheres and PVA-based film

The chemicals were supplied from Aldrich, at analytical grade levels. Cellulose nanospheres derived from corn leaf could be successfully obtained via a simple chemical procedure (including alkaline and bleached pretreatments, and acid hydrolysis) from our previous report (Thach-Nguyen et al., 2022). Being derived from 5.0 g of raw material, as-prepared cellulose nanospheres were then dispersed in 50.0 mL of distilled water. A 5.0 mL solution of 0.1 M $AgNO_3$ was poured to the suspension to yield the homogeneous mixture, before dropwise adding 5.0 mL solution of 1.0 M NaOH at 80 °C for 15 min. The chemical reduction of $AgNO_3$ toward AgNPs was then performed using 1.8-g of glucose at 80 °C, continuously stirred at 500 rpm for 6 h. The grey solid was separated by centrifugation, washed using distilled water, and finally air dried at 80 °C overnight.

In order to make PVA-based film, PVA (5.0 g) was completely dissolved in 50.0 mL of distilled water at 80 °C until reaching a transparent solution. An amount of AgNPs-immobilized cellulose nanospheres (1 %, 2 %, and 3 %, w/w) was added into the above solution, then stirred at 500 rpm at 80 °C for 2 h. After pouring the mixture into a petri dish (10.0 mL in a 9.0 cm inner-diameter dish) and cooling down to room temperature, the solvent evaporation was performed at 60 °C overnight. The dried film was kept in a sealed bag for further use.

2.2 Antimicrobial activity and food packing of PVA-based film

The antimicrobial test of as-prepared AgNPs-immobilized cellulose nanospheres against (−) *Escherichia coli* (*E. coli*) ATCC 25922 and (+) *Staphylococcus aureus* (*S. aureus*) ATCC 29213 was performed via the colonies-forming unit (CFU) method using 3M™Petrifilm™ Aerobic Count Plates. The bacteria were precultured at 37 °C for 24 h in Mueller Hinton Broth (MHB) medium. The bacterial suspensions were then diluted in MHB, vortexed evenly and adjusted to a density of 10^8 CFU/mL (OD600 ~ 0.08–0.1). The diluted bacterial suspensions (100 μL) were added into 20.0 mL of sterile physiological saline containing 250 mg of nanomaterial; the mixture was vortexed evenly and then shaken at 150 rpm. After every interval time, the samples (100 μL) were diluted and inoculated on the petrifilm, incubated at 37 °C for 24 h, and counting the number of colonies appeared on the petrifilm. The control experiments were carried out in the same procedure, using nanocellulose instead of AgNPs-immobilized cellulose nanospheres.

Taking an example for food packaging application, PVA-based films were fabricated with the different compositions of AgNPs-immobilized cellulose nanospheres varied from 0 %, 1 %, 2 % to 3 % (w/w). Undamaged and homogeneous-sized strawberries were fully wrapped by PVA-based films, and stored under an ambient environment. During taking the photos each time, it must ensure not to touch the samples. After 7 d, the wrappers were removed to observe the visible sign of strawberry spoilage.

3. Results and Discussion

3.1 Physicochemical features of AgNPs immobilized on cellulose nanospheres

The formation of AgNPs was observed on the colour change from white (cellulose nanospheres) into grey. The crystal structures of both cellulose and AgNPs were evidenced via XRD technique (Figure 1). Regarding to cellulose crystals, the diffraction peaks at 2θ in a range of 10–35° were attributed to the cellulose type I with the planes of (1$\bar{1}$0), (110), (200) and (004) (Gong et al., 2017). In a range of 35–80°, other diffraction peaks confirmed the presence of face-centred cubic (fcc) silver crystals according to the standard pattern (JCPDS 65-2871). There are no crystalline phases of silver hydroxide, peroxide and chloride that are usually obtained in the presence of plant entities. As estimated from the Scherrer equation at the plane of (111), the silver crystallite site was 17.6 nm.

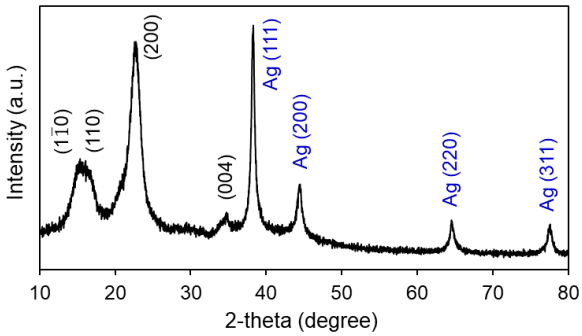

Figure 1: XRD pattern of AgNPs-immobilized cellulose nanospheres.

The surface of such a nano-system was scanned by EDX mapping (Figure 2a), detecting the compositions of C (yellow), O (blue) and Ag (red) with a homogeneous distribution. This result evidenced a uniform distribution of AgNPs on the nanocellulose's surface. Accordingly, the Ag percentage was estimated to be around 4.1 % by EDX spectroscopy (Figure 2b).

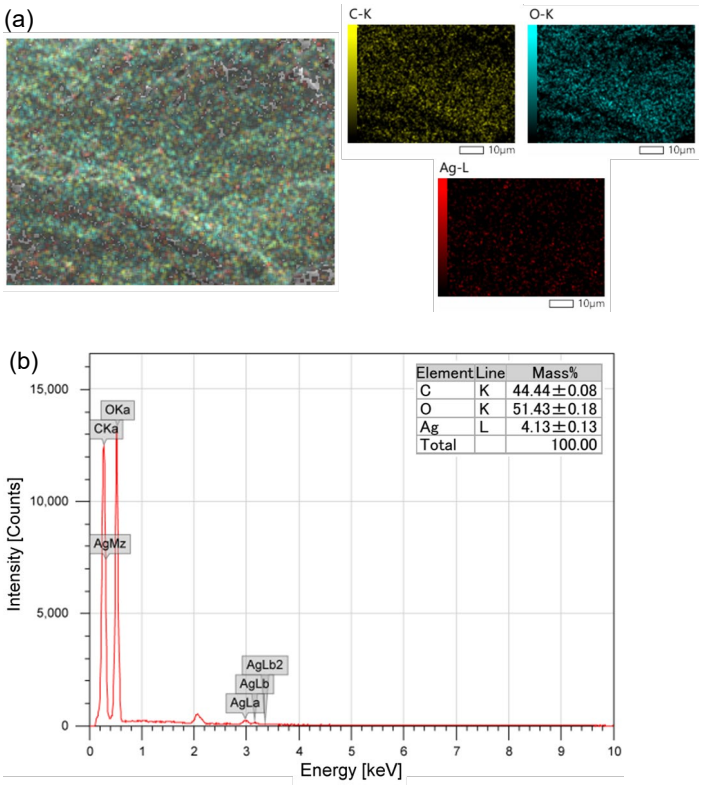

Figure 2: (a) EDX mapping, and (b) EDX spectrum of AgNPs-immobilized cellulose nanospheres.

The thermal behaviour of AgNPs-immobilized cellulose nanospheres was evaluated via thermogravimetric analysis (TGA) (Figure 3). At the first step, the weight loss of adsorbed H_2O was approximately 10 % under 250 °C. The thermal decomposition of cellulosic components was previously admitted around 250 °C (Thach-Nguyen et al., 2022). The followed weight losses involving two sequential series were 50.8 % (250–350 °C) and 35.3 % (350–600 °C), probably due to a caramelization effect (Somseemee et al., 2022). During the thermal decomposition, the caramelization effect could be occurred on the cellulose surface that could coat its core, decelerating its decomposition. The residual left from 650 °C was around 4 %, being close to the silver percentage on the surface using EDX analysis.

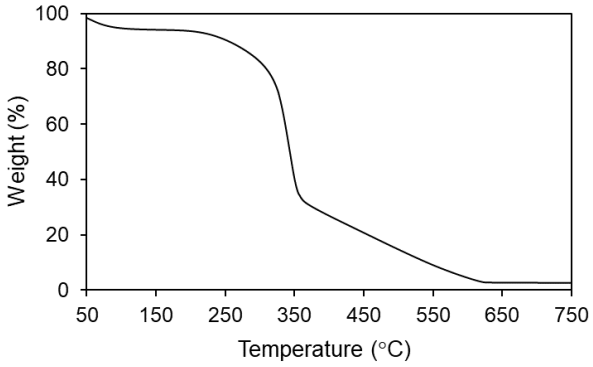

Figure 3: TGA pattern of AgNPs-immobilized cellulose nanospheres.

TEM micrograph indicated the existence of nanospheres with a mean diameter of 22.5 nm (Figure 4). The well-dispersion of nanospheres could be achieved due to the native supramolecular network of nanocellulose, preventing their agglomeration. The surface hydroxyls can act as silver chelator or hold an electrostatic interaction with Ag^+ ions before the electron transfer occurred (Thach-Nguyen and Dang-Bao, 2022). The present protocol evidenced its simplicity and efficiency in immobilizing AgNPs on cellulose nanospheres, without using any additional surfactants. This eco-benign route to fabricate AgNPs was of vital importance for further food packing film application.

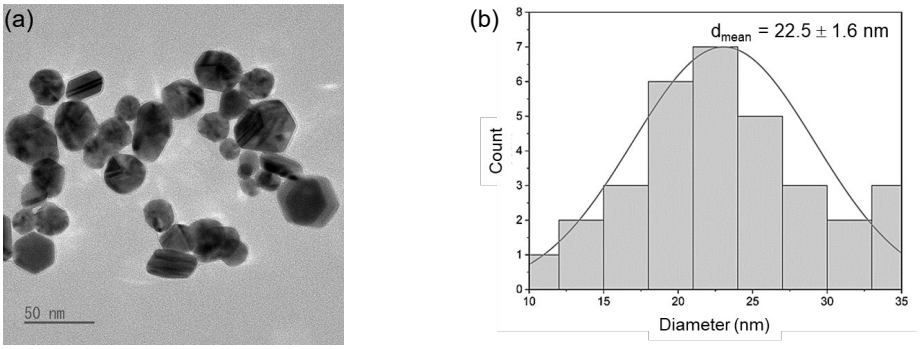

Figure 4: (a) TEM micrograph, and (b) size distribution of AgNPs-immobilized cellulose nanospheres.

3.2 Antimicrobial activity and food packing of PVA-based film

The antimicrobial resistances toward *E. coli* and *S. aureus* were tested via the CFU method (Figure 5). In contrast to the unchanged densities of CFU observed on the control samples during 24 h, AgNPs showed the complete antimicrobial performances within 6 h for both types of bacteria. This effect refers to the direct bacterial surface attachment of AgNPs that compromises the structural integrity of the membrane (Okaiyeto et al., 2019). The generation of Ag^+ ions can destroy bacterial cells via a denaturement of microbial proteins and a hamper in the replication of DNA (Hsueh et al., 2015).

The feasibility of PVA-based antimicrobial food packaging films were preliminarily examined on wrapping strawberries under an ambient environment. The growth of microorganisms on the surface of strawberries could be observed by naked eyes during storage until 7 d (Figure 6). During taking the photos each time, it must ensure not to touch the samples. After 7 d, the wrappers were removed to observe the visible sign of strawberry spoilage. In contrast to the almost decayed strawberries wrapped in pure PVA film, it could be much better to

utilize antimicrobial films blended with AgNPs-immobilized cellulose nanospheres (1 %, 2 % or 3 %, w/w). It seemed to be that the more the Ag percentage was, the better the food packaging performance was (referring to their antimicrobial activity as above discussed); in which, using 2 % AgNPs-immobilized cellulose nanospheres, the fruits remained intact and no appearance of microorganisms. It was noted that these assays were carried out under the same conditions (the temperature changed depending on the surrounding environment), but not the optimal conditions for the storage of fruits.

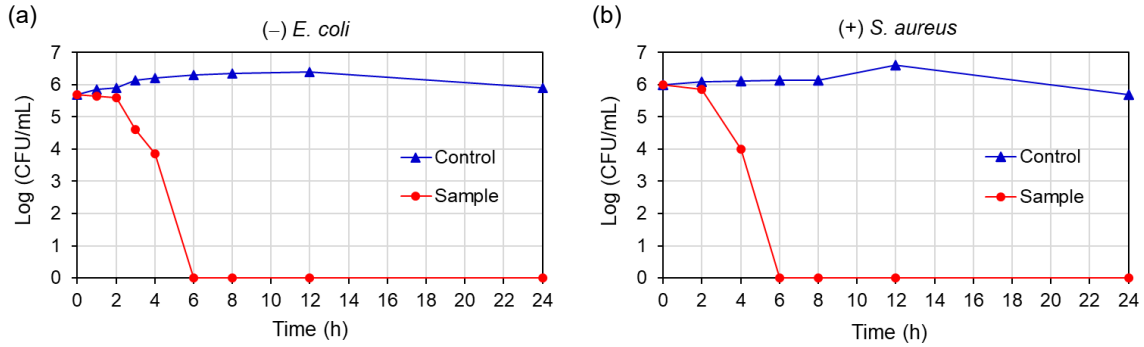

Figure 5: Antimicrobial efficiency of AgNPs-immobilized cellulose nanospheres on (a) E. coli, and (b) S. aureus.

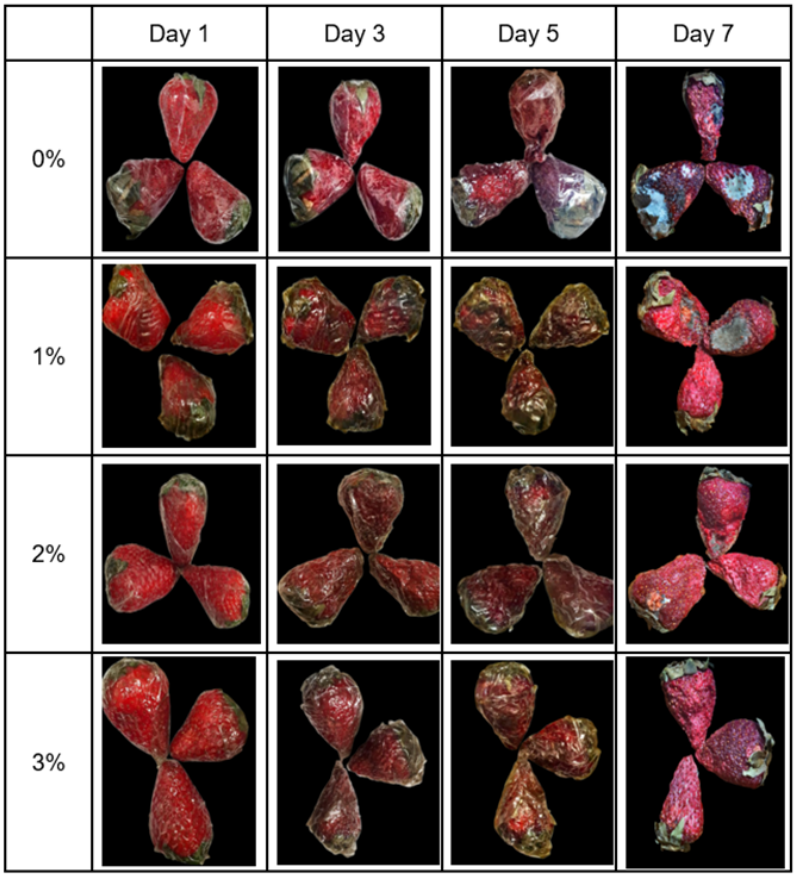

Figure 6: Photos of strawberries wrapped by PVA-based films with various compositions of AgNPs-immobilized cellulose nanospheres varied in 0–3 % (w/w) taken on different times.

4. Conclusions

A facile and efficient procedure to fabricate AgNPs immobilized on corn leaf-derived cellulose nanospheres were established with some preferences: (i) an immobilizer derived from an agro-residue; (ii) less surface modification, and without using any external stabilizers or surfactants; (iii) an eco-benign route using a non-toxic

reducing agent. Such a nano-system (4.1 %wt. Ag, 22.5 nm in diameter) showed the complete antimicrobial performances within 6 h, against both *E. coli* and *S. aureus*. The PVA-based antimicrobial packaging film showed strawberries with no decay and no microorganisms, promising a realistic application in food preservation in future.

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