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ZnO-PLA nanocomposite coated paper for antimicrobial packaging application

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ABSTRACT

In this study, ZnO nanoparticles were incorporated in PLA (Polylactic Acid) coating layer for antimicrobial packaging application. The SEM images show that the nanoparticles were homogenously distributed across the surface thanks to its surface modification. The antimicrobial assay indicates that the active material was effective in inactivating *E. coli* and *S. aureus*. Furthermore, *E. coli* was found to be more susceptible to this type of agent, showing 3.14 log reduction for 0.5 wt% agent loading in the PLA coating layer. This result was compared across the publications using the same type of agent for treating both Gram-positive and Gram-negative microorganisms. The discrepancy between the results can be explained by an important fact that ZnO nanoparticle has multiple action mechanisms, and different antimicrobial testing methods may activate only part of the action mechanisms. In addition, nano-enabled packaging material for food contact application and its market prospect were analysed.

1. Introduction

Choosing appropriate active agent is key to the development of antimicrobial packaging system. Compared with the organic agents (e.g. organic acid, essential oil component, nisin, etc.), metallic-based nanoparticles (NPs) as antimicrobial agent offers a few advantages, such as superior antimicrobial efficacy, no negative impacts on the food sensory properties, and compatibility with harsh polymer processing conditions (Duncan, 2011; Liu et al., 2009; Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012; Martins et al., 2013). Due to the NP' strong antimicrobial activity, it holds potential application not only in food spoilage control (Fernández, Picouet, & Lloret, 2010; Li, Xing, Jiang, Ding, & Li, 2009; Maneerat & Hayata, 2006), but also in food safety control by inactivating foodborne pathogens. The latter aspect has been explored in a number of research works, for example, impressive inhibitory effect was observed in treating *E. coli* O157:H7 with ZnO NPs (Liu et al., 2009); PLA coating containing ZnO NPs was found to be effective in inactivating *Salmonella* inoculated in liquid egg albumen (Jin & Gurtler, 2011); in the test with ready-to-eat chicken, 2 log reduction in the inoculated bacteria (*S. aureus* and *S. typhimurium*) was observed after 24 h applying the ZnO NP-containing active packaging, and complete inhibition after 6–8 days (Akbar & Anal, 2014).

Survey of literature shows that the relevant research has mainly concentrated on four nanoparticles: silver (Ag), titanium dioxide (TiO_2), zinc oxide (ZnO) and copper (Cu) (Llorens et al., 2012). Among them, there is an increasing interest to use ZnO NPs for food contact application, which derives from the following merits:

1) The non-nano form of ZnO is already authorised by the EFSA (European Food Safety Authority) as an additive for plastic materials and articles, with a SML (Specific Migration Limit) of 25 mg/kg food (EFSA CEF Panel on Food Contact Materials, 2015).
2) ZnO NPs exhibit low toxicity to the biological systems (Reddy et al., 2007). Furthermore, zinc is an essential element for human's physiological activity; c.a. 10 mg/person/day is needed (EFSA CEF Panel on Food Contact Materials, 2015). Toxicity

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studies recommend an upper limit of 25 mg/person/day (EFSA CEF Panel on Food Contact Materials, 2015).

3) In food packaging, transparency remains a determining factor for choosing the packaging material. On the other hand, some foods are susceptible to UV light. To solve this problem, UV-blocking agent is used in polymer processing. Studies show that ZnO NP loading as low as 1 wt% enables good UV-blocking performance without impairing the host polymer's transparency (El-Feky, Hassan, Fadel, & Hassan, 2014; Murariu et al., 2011; Therias et al., 2012).

In this study, we proposed a paper-based packaging material, which is coated with ZnO-PLA nanocomposite. The application can be a paper wrap for deli foods since there is a relatively high risk of microbiological contamination in such cold processed foods. The focus is placed on the *in vitro* assessment of the antimicrobial activity of ZnO NPs.

2. Materials and methods

2.1. Coating recipe and coating on paper substrate

ZnO NPs (Zano® 20 Plus-3) were kindly supplied by Umicore, Belgium. The NPs are surface coated with organosilane ([3-(methacryloxy)propyl] trimethoxysilane) for improved dispersibility in polymer processing. The NPs have an average particle size of 30 nm (Murariu et al., 2011). As the NPs were supplied in powder form, first they were dispersed in the solvent: adding a proper amount of NPs (0.075 g, 0.15 g, and 0.45 g in respective beakers) into 100 mL ethyl acetate, stirring vigorously for 10 min, and then applying 5 min ultrasonic treatment (Sonics, microtip CV 334, 750 w 20 kHz) to break up the agglomerates/aggregates. Afterwards, 15 g PLA pellets (Polylactic Acid, 4060D, Natureworks) were added into each NPs dispersion under vigorous stirring at room temperature until full dissolving. In this way, three coating solutions were prepared, in concentrations of 0.5 wt%, 1 wt% and 3 wt% (NP over PLA in dry solids weight).

A white bleached kraft paper (basis weight 106 g/m^2, ash content 7.7%, top side sized) was used as substrate for coating. The coating was done on the sized side.

Coating was carried out on a lab film applicator (Elcometer 4340) using a smooth bar for depositing 50 μm wet film onto the substrate. After coating, the samples were allowed to dry overnight at the room temperature. The structure of the final packaging material is illustrated in Fig. 1. Sample identification and description is summarised in Table 1.

2.2. Material characterization

2.2.1. Nanoparticle analysis by TGA

The NPs are surface coated with an organosilane that serves as coupling/dispersing agent to improve the agent dispersibility. The amount of surface coating was determined with TGA (Thermogravimetric Analysis, measurement carried out with TGA Q5000, TA).

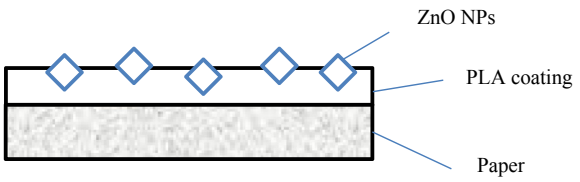

Fig. 1. Schematic illustration of the structure of the packaging material with ZnO-PLA coating.

Table 1
Sample identification and description.

Sample identification	Sample description
Control	Base paper coated with pristine PLA
0.5%-NP	Substrate coated with 0.5 wt% ZnO-PLA composite
1%-NP	Substrate coated with 1 wt% ZnO-PLA composite
3%-NP	Substrate coated with 3 wt% ZnO-PLA composite

7.36 mg ZnO NPs were loaded in a platinum sample holder. The temperature program was set as follows: hold the temperature at 80 °C for 10 min, then increase it to 600 °C at a rate of 20 °C/min, and after that, hold it at 600 °C for 10 min. Nitrogen gas of flow rate 25 mL/min was used to purge the sample atmosphere.

2.2.2. Morphology of coating surface

The morphology of the coated material and the NP distribution were examined with the SEM (Scanning Electron Microscopy). Prior to analysis, the samples were coated with a thin layer of carbon, 15 nm in thickness (sputter coating carried out with Q150T ES, Quorum). The images were captured with a SEM system (Quanta 650, FEI) equipped with detectors of secondary electrons and backscattered electrons. The presence and distribution of ZnO NPs were further confirmed with the EDX (Energy Dispersive X-ray spectroscopy, supplied by X-MAX, Oxford Instrument, coupled with the SEM).

2.3. Antimicrobial assay

The sample's antimicrobial activity was assessed with the method of JIS Z 2801, which is widely used for evaluating the antimicrobial activity of non-porous surfaces or materials, e.g. plastic. In brief, the treated and control materials were cut into squares 5 × 5 cm, the coated side was inoculated with 0.4 mL inoculum (*S. aureus* 1.1E6 CFU/mL, *E. coli* 8.4E5 CFU/mL), covered with a piece of clear film 4 × 4 cm (cleaned with 70% ethanol before use), and placed in a covered petri dish. The samples were incubated at 35 °C and RH ≥ 90% for 24 h. Afterwards, the samples were washed with 50 mL neutralizing solution (recipe: 34 g neutralizing broth base in 1 L distilled water with 5 mL Polysorbate 80, boil and autoclaved) with a stomacher (BagMixer, Interscience). The serial dilutions of the rinse liquid were plated on agar plate using an automatic plater (easySpiral, Interscience) to enumerate the viable cells in CFU (Colony Forming Unit). Controls at 0 h and 24 h of inoculation were used and denoted as C0 and C24, respectively.

Each sample was tested in triplicate (two CFU readings per assay). Data were presented as mean ± standard deviation.

The differences between the samples (the effect of NP presence and NP loading) were evaluated with one-way analysis of variance (ANOVA, StatPlus:mac). Difference was considered statistically significant when p value < 0.05.

Log Reduction (also called activity value) is an indicator to describe a material's antimicrobial activity. As shown in Equation (1), it is calculated as the difference between the number of viable cells in the control (log $CFU_{control24h}$) and in the treated sample (log $CFU_{treated24h}$) after 24 h inoculation (Martins et al., 2013).

$$\text{LogReduction} = \log CFU_{\text{control 24h}} - \log CFU_{\text{treated 24h}} \quad (1)$$

The samples were also tested with the method of ASTM E2180, which allows full contact of targeted bacteria with the test surface with the aid of agar slurry. In short, the molten agar slurry (maintained at 45 °C) was inoculated with the bacterial cells (*S. aureus*). A thin layer of the inoculated agar slurry (0.5 mL) was pipetted onto the treated and control materials (in square 3 × 3 cm) and allowed