Sys Rev Pharm 2020;11(9):592-596
A multifaceted review journal in the field of pharmacy

Enhancing Antimicrobial Properties of Food Packaging Sheets by Incorporating ZnO- Nanoparticles (NPs)

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ABSTRACT

Packaging sheets characteristics play very important role in food packaging industries. To enhance their antimicrobial properties, ZnO nanoparticles (NPs) are added to polyvinyl chloride sheet synthesized via phase precipitation This enhancement would provide antibacterial properties and extend shelf life of the wrapped food products. The effect of increasing ZnO-NPs concentrations (i.e. 0.1- 0.4 g) on sheet properties was explored. The characteristics of the bespoke packaging sheets were investigated via scanning electron microscopy (SEM) and biomass measurements. The results show that ZnO-NPs clearly changed the sheet structural morphology and its addition gives a positive effect on the general morphology of the packaging sheet. In addition, more than 95% of biomass reduction and 5±0.7 mm inhibition zone was recorded. The results of the study have great ramifications in packaging technology through enhancing the antimicrobial properties of the wrapping sheets as well as extending shelf life of the food products.

Keywords: Packaging sheets, antimicrobial properties; ZnO-Nanoparticles

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INTRODUCTION

Food spoilage and food safety are the most visible issues in food industry in the recent years. Bacterial foodborne outbreaks have been frequently reported worldwide. The supply chain of food is real concern in the world as it directly affects consumers lives and health. However, the reported outbreaks have led to increased customers concerns and disintegration in public confidence of safety of the food supply (1). In addition, food spoilage leads to loss colour and texture of foods and reduce their nutritive value, leading to encourage growth of pathogenic bacteria and make it non-edible. Contamination of food occurs while food products expose to the environment during slaughtering, food packaging and processing (2). Preservation techniques such as heating, freezing and salting are regularly used to extend shelf life of food products, but this does not prevent recontamination and that would risk customers' safety. Packaging systems with antimicrobial activities have drawn much attention, as a novel development in food packaging, which incorporate antimicrobial agents within a film of polymers to repress targeted contaminated microbes (3). Antimicrobial activities can be achieved by integrating agents with antimicrobial activities to the packaging films that can extend lag phase of contaminated microbes and reduce their live counts, leading to prevent their growth and/or substantially decrease their growth rate (4). These antimicrobial agents can be added directly into food in process of formulation and this process is highly regulated by FDA to specify the safety levels of the added antimicrobial substances. Another approach is adding these agents to wrapping films, which would result instant inhibition of desired contaminated microbes. However, some survived microbes can continue to grow as soon as these agents get depleted. This depleted may be due to the natural degradation of these substances with time and would eventually lead to shorten the shelf life (5-7).

Polyvinyl chloride (PVC) has drawn great attention by researchers due to its great properties such as stability at high temperatures, its low price and resistance to acids. This polymer has physical property, rigid thermoformed foil, which makes it suitable to be used preferred packaging a wide range of foodstuffs (8). To enhance PVC properties, nanoparticles can be incorporated into the polymer, making it possess better antimicrobial properties. Many materials with antimicrobials properties such as inorganic materials, ZnO, have drawn much interest due the rising issue of antibiotics resistant microbes. Addition of zinc oxide offers many advantages such as it shows constant antibacterial activity with a wide range of pH, ranging from 3 up to 10, and this is a very important feature for different kinds of food in packaging industry (9).

Zinc oxide is mediated in mechanism of generation of hydrogen peroxide (H_2O_2) and this mechanism is thought to be responsible for antimicrobial activity (9).

The aim of the current work is to prepare PVC sheets with increasing concentrations of ZnO-NPs as an antibacterial additive to enhance the ability to inhibit *E. coli* bacterial growth.

Zinc oxide-coated PVC sheet with antimicrobial activity as a potential food packaging option. Different ZnO-NPs concentrations were added to the PVC sheet and the antibacterial activity against *E. coli* was examined in vitro.

MATERIAL AND METHODS

Sheet preparation

The moisture content in PVC polymer (Georgia gulf company, USA) was removed by drying it out for 4 h at 60 °C in the oven. 13 % of the PVC polymer and 87 % of DMAc solution (Sigma Aldrich, UK) was used to prepare casting solution. Using a magnetic stirrer for 2 days at 200 rpm and 40°C, was conducted to achieve a homogeneous solution. To the obtained homogeneous solution, ZnO Nanoparticles (Skyspring, USA) were added at weights of (0, 0.1, 0.2, 0.3, and 0.4g to obtain the final casting solution. (10). The final casting solutions with different ZnO weights were kept for 15 min in a water bath to prevent the ZnO-NPs from aggregation. The

polymer solutions with 200 µm thickness under atmospheric conditions were cast using a motorized film applicator (Elcometer 4340, USA). PVC sheets prepared for this study were kept at room temperature in a nonsolvent deionized water bath for deposition. The nascent sheet was kept in distilled water for 48 hrs to remove the residual DMAc. Finally, the sheet was taken out from the distilled water and 30 % glycerol solution for 48 hrs was used to keep the sheets. This process is vital to prevent the sheet structure from cracking and collapsing.

Properties of the sheet surface

Scanning Electron Microscopy (SEM)

The top surface of the flat sheets was examined using scanning Electron Microscopy (TESCAN VEGA3 SB instrument, Germany) at 30kV voltage acceleration.

Measurement of the biomass concentration

Biomass concentration correlates with optical density (OD) was measured by a spectrophotometer (6705 UV/ Vis. JENWAY, UK) to determine optical density at 600 nm with sampling done on hourly basis.

Study of antibacterial properties of packaging sheet

Antibacterial performance was investigated by agar well diffusion and broth cultures methods using *E. coli* bacteria as a model microorganism (11). One mL Inoculum of *E. coli* bacteria (containing 10^7-10^9 cfu/ml grown overnight at 37 ℃) was spreaded on Mueller-Hinton agar plates with a sterile swab moistened with the bacterial suspension. Wells of 8 mm diameter were punched and filled with 100 µl of different ZnO-NPs concentrations (i.e. 0.1- 0.4 g/L) and left two hours to diffuse at room temperature. The plates were then incubated in the upright position at 37° for 24 h. Negative controls were conducted by filling wells with distilled water. Alternatively, PVC sheet samples (about 10 mm diameter) were sterilized by autoclaving and then placed and incubated at 37℃ overnights. If the bacterium is susceptible to the ZnO-NPs suspension, it does not grow around the ZnO-NPs filled wells neither around PVC sheet disks thus forming a zone of inhibition. Otherwise, bacteria grow up to the margin of disk if there are not affected. Inhibition's zones diameters were measured and reported in mm.

Also, the ZnO-NPs concentrations (i.e. 0.1- 0.4 g/L) were prepared with Mueller-Hinton broth and inoculated with 10% of *E. coli* bacteria (containing 10^7-10^9 cfu/ml grown overnight at 37 ℃) and distributed in 125-ml Erlenmeyer flasks on a shaking platform (150 rpm). Three replicates were carried out for each experiment and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Surface changes after ZnO-NPs addition

Figure 1 shows the SEM pictures for the sheet's top surface with ZnONPs 0 and 0.4g, in the casting solution. It was observed that the ZnONP's had a significant impact on the structure of PVC/ZnONP flat sheet. In Figure 1A, dense surface with very limited pores was seen on the sheets surface. Whereas, adding 0.4 g of ZnO-NPs increases the porosity of the surface as clearly observed in Figures 1B.

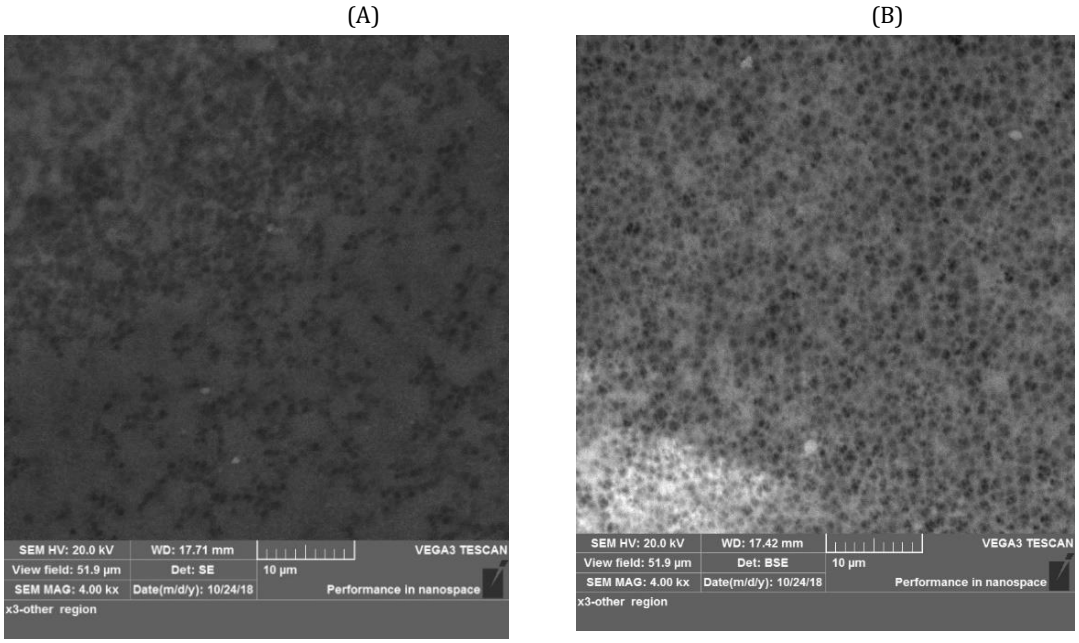

Fig 1. SEM images of the surface of the PVC sheets at two ZnO concentrations: (a) 0 g ZnO-NPs; (B) 0.4 g ZnO-NPs.

It was clear, that adding the ZnO-NPs to the dope solution significantly affected the phase inversion process and eventually the sheet morphology (12) (13). Forming a denser and highly porous sheet is mainly dependent on the interchange's rate between solvent and non-solvent solutions during phase inversion process. Increased porosity can be achieved by moving the non-solvent solution in the casting film faster than the solvent solution bypassing (14) (15)

Inhibitory effect of ZnO-NPs

Figure 2 shows the inhibitory effect of various ZnO-NPs concentrations on the *E. coli* growth, when the bacterial inhibition has proportional increase with increasing ZnO-NPs concentrations. The same results have been observed when agar well diffusion method was used (Table 1),

suggesting that ZnO-NPs has toxic effect on the bacterial system. Toxicity of ZnO nanoparticles to gram-negative bacteria was reported previously by Reddy et al. (16). ZnO-NPs prevented growth of *E. coli* at concentrations 0.3 and 0.4 g, whereas there was a limited growth observed at concentrations 0.1 and 0.2 g (Fig 2). *E.coli* is a common food contaminant and is a strong indicator of contamination of the food products and the observed reduction in *E.coli* population in (Figure 2) and (Table 2) is similar to that reported by Reddy et al.(16) and Brayner et al (17). However, the experiments were not designed to establish whether it was bacteriocidal (e.g., inducing cell death) or bacteriostatic (e.g., preventing cell growth) and thus, more research needs to be done to establish this. *E. coli* requires zinc in their growth which is usually supplied within the cultivating media and prefers it with a concentration around of ~1 mM. Zinc plays crucial role in the regulation of multiple cellular functions and fulfills several important functions e.g. as both catalytic and structural cofactors for enzymes and proteins respectively (18). Therefore, Zn at low concentrations may be used in bacterial growth but increasing its concentrations to higher range would lead to express Zn 2+efflux mechanism, indicating undesirable and potentially toxic conditions for *E. coli* (16). This inhibition may cause as result of substantial loss of cell viability/membrane integrity, which would lead eventually to bacterial cell death (16). In addition, Zheng et al., (19) found that a significant change in the regulations of glycolysis caused by ZnO; thus, a decrease in reducing powers was resulted. Further, a substantial inhibition of metabolic activities and gene expressions was observed when free zinc was used (19).

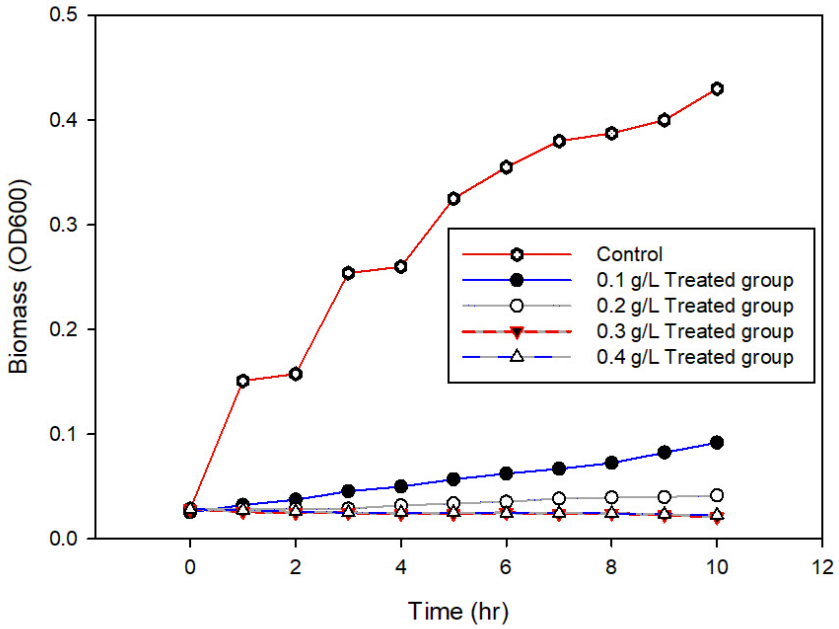

Fig 2. Bacterial performance under different ZnO-NPs concentrations

Table 1. Inhibition zones of various free and embedded ZnO-NPs concentrations

ZnO-NPs concentration (g)	Inhibition zones diameter (mm) ±SD
0	No zone observed
0.1	2.75±0.5
0.2	3±0.3
0.3	4.5±0.5
0.4	5±0.7

Further, ZnO-NPs were embedded in PVC sheets to make PVC/ZnO sheets to improve its antimicrobial characteristics. However, the results showed that inhibition zones were observed around the sheet's

sections which were placed on agar plates inoculated with bacteria. It is not expected to see a typical inhibition zone around the PVC sheet's section but instead no growth closes the edges of the sheet and under the sheet's section would be seen. However, the results showed that bacterial growth was up to the edges of the sheet. This may be due to that the free ZnO-NPs can bind with macromolecules such as proteins and carbohydrates, and all vital functions of microbe would drop and discontinue, while embedding the ZnO-NPs in PVC sheet would restrict the release of Zn^{2+} ions, toxic ions, and significantly limit or reduce its inhibitory effects (Table 1) and Figure (3). Further, ZnO-NPs were embedded in PVC sheets to make PVC/ZnO sheets to improve its antimicrobial characteristics and the results showed that inhibition was observed around the free ZnO section which were placed on agar plates inoculated with bacteria.

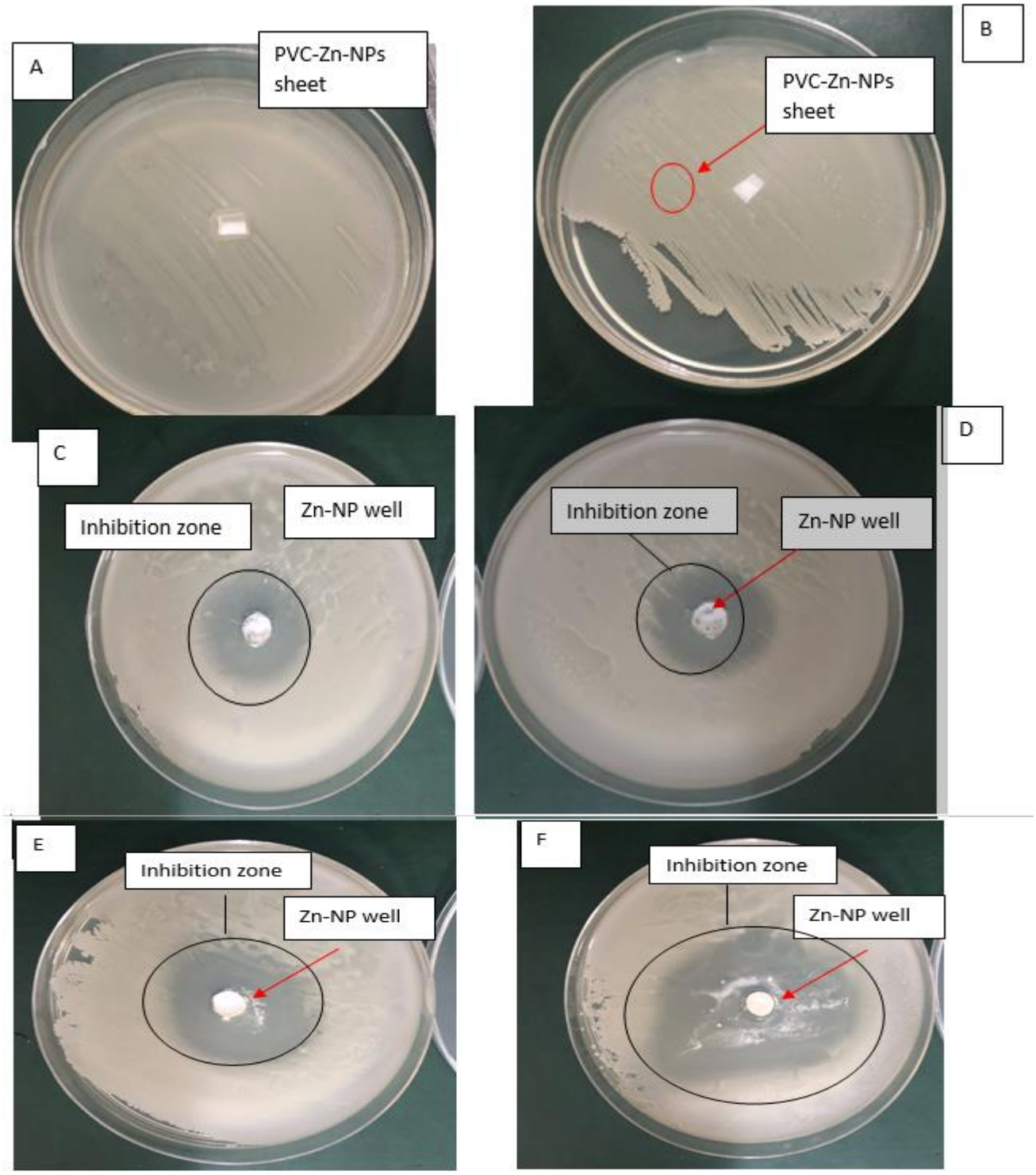

Fig 3. Images of bacterial growth with: (A)(B) (PVC/ZnO-NPs) sheets at different concentrations (0.2 and 0.3 g) of ZnO-NPs.(C)(D)(E)(F) free ZnO-NPs addition (0, 0.1, 0.2, 0.3 and 0.4g)

CONCLUSION

In this study, the prepared PVC/ZnO flat sheet with anti-microbial properties was investigated. The addition of ZnO-NPs clearly changed the structural morphology of the sheets. The inhibitory effect of various ZnO-NPs concentrations on the *E.coli* growth was obviously seen and the bacterial inhibition has proportional increase with increasing ZnO-NPs concentrations. ZnO-NPs substantially inhibited growth of *E. coli* at concentrations of 0.3 and 0.4 g, whereas there was a limited growth observed at concentrations 0.1 and 0.2 g.

FUNDING

This research received no external funding

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