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Zinc oxide nanoparticle-coated films: fabrication, characterization, and antibacterial properties

Yunhong Jiang[1], Alex J. *O'*Neill[2], Yulong Ding[13,]

1. Institute of Particle Science and Engineering, University of Leeds, LS2 9JT, UK
2. School of Molecular and Cellular Biology, University of Leeds, LS2 9JT, UK
3. School of Chemical Engineering, University of Birmingham, B15 2TT, UK

The email address of the corresponding author: y.jiang@leeds.ac.uk

ABSTRACT:

In this article, novel antibacterial PVC-based films coated with ZnO nanoparticles (NPs) were fabricated, characterized and studied for their antibacterial properties. It was shown that the ZnO NPs were coated on the surface of the PVC films uniformly and that the coating process did not affect the size and shape of the NPs on the surface of PVC films. Films coated with concentrations of either 0.2 g/L or 0.075 g/L of ZnO NPs exhibited antibacterial activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria, but exhibited no antifungal activity against Aspergillus flavus and Penicillium citrinum. Smaller particles (100 nm) exhibited more potent antibacterial activity than larger particles (1000 nm). All ZnO-coated films maintained antibacterial activity after 30 days in water.

Keywords: ZnO NPs, Escherichia coli, Staphylococcus aureus, Aspergillus flavus, Penicillium citrinum, antibacterial activity.

Introduction

The unique physico-chemical properties of ZnO NPs have made them one of the most commercialized nanomaterials used in a variety of applications such as sunscreen products and textiles (Stoimenov et al. 2002; Huang et al. 2008, Jones et al. 2008; Akhavan et al 2009). ZnO NPs show activity as antibacterial materials against both Gram-positive and Gram-negative bacteria, including spores. The study of antibacterial activity of ZnO NPs has focused on a wide range of pathogenic and non-pathogenic microorganisms such as Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Bacillus subtilis (Sawai et al. 1998; Yamamoto et al. 2001; Lok et al. 2007; Zhang et al. 2007; Padmavathy et al. 2008; Zhang et al. 2010). The development of methods for the fabrication and deposition of nanomaterials onto polymer and glass surfaces would potentially enable additional applications for ZnO NPs, for example as antibacterial surfaces in medical and food industry. However, to date there have been few reports of the fabrication and use of these materials in the form of antibacterial coatings for protection of food and for preventing transmission/infection in hospital settings (Applerot et al. 2009; Li et al. 2007). In addition, the cytotoxicity of ZnO NPs is another issue awaiting resolution. Reddy reported that identical ZnO NPs had minimal effects on primary human T cell viability at concentrations toxic to both Gram-negative and Gram-positive bacteria (around 0.25 g/L). It suggests that ZnO NPs have potentially proved useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimes (Reddy, Feris et al.2007). However, Kang et al found that cytotoxicity exhibited dose- and time-dependent effects for ZnO NPs. It was observed the cell activity is significant different between the concentration of 0.0125 and 0.050 g/L for 24 h against Caco-2 cells (Kang Guan et al. 2013). Sharma et al found that ZnO NPs

(0.008-0.02 g/L) can reduce the human epidermal cell (A431) viability for 24 and 48 h in a concentration and time dependent manner (Sharma, Shukla et al. 2009). These results suggest the evaluation of NPs should be carried out on a case by case basis.

There is a general agreement that the antibacterial effects of NPs are determined by a set of physico-chemical characteristics, e.g. size, shape, colloidal stability if in a liquid suspension form and surface chemistry. It is essential to understand the appropriate characterization of the NPs in vitro, due to the particles can remain dispersed as primary particles, agglomerate, aggregate, or any combination of these. Thus, a set of well-defined particles in terms of purity, size, shape, and degree of agglomeration was essential to evaluate the toxicity of NPs. Several European Union projects (Quality-Nano, Marina et. al) are aimed to establish the standards in NP processing and characterisation to ensure biological reproducibility. However, it is difficult to trace and measure the size distribution and physico-chemical properties changes of NPs in a bacteriological growth medium during the experimental process. Since the size and shape of particles is unlikely to change after the coating process. ZnO NPs coated onto a polymer surface would be to facilitate the evaluation of the relationship between different physico-chemical properties and antibacterial properties.

In this article, the ZnO NPs were coated on PVC films and characterized by using a scanning electron microscope (SEM) for the surface morphology and particle size and a Brunauer–Emmett–Teller (BET) device for the surface area. The NPs were found to firmly fix onto the film surfaces and no changes were found to the particle size during the process. The approach used in this work represents a simple and cost effective method to coat ZnO NPs onto PVC surface without the aid of binders. The resulting ZnO NPs coated films exhibit an antibacterial activity that are stable over a prolonged

period, and could potentially be employed in hospital settings or in food industry to reduce risks of microbial contamination.

Materials and Methods

Commercially-produced ZnO NPs were purchased from Nano-Structure & Amorphous Materials (USA). The primary size of the NPs given by the manufacturer was 90 – 200 nm. However, SEM (model LEO 1530 FEGSEM) analyses revealed that some of the particles were micro-size and agglomerated (Figure 1a). In order to reduce the size of the large primary particles and to break the NP agglomerates, NP stock suspensions were prepared by dispersing ZnO NPs in water and vigorously stirring the resulting suspension by sonication for 30 min in an ultrasonicatior (Clifton, UK) followed by milling the suspension in a Dyno-Mill (Willy A. Bachofen, Switzerland) for 2 h at the room temperature to produce a master suspension (Damonte et al. 2004). All the NP suspensions were kept at 4 ^{o}C in the dark. The hydrodynamic diameters and zeta potentials of particles in the suspension were determined with a Zetasizer (Malvern Instruments, UK).

Procedures for preparing coated PVC films

PVC films (Department of Biological Science of Tianjin University of Science and Technology) were cut into different sizes and cleaned in an ultrasound bath for 5 min. The films were attached on the substrate, and care was taken to ensure the films were flat. An appropriate amount of ZnO suspension was applied drop-wise onto the surface of the films using a pipette. The amounts of ZnO NPs per film disc ranged from 100 µg/cm^2 to 500 µg/cm^2. The films were then left in a clean environment (Laminar flow cupboard) for air drying, before heating at 110 ^{o}C temperature for 10

minutes. During heating, an appropriate load was placed on the top of the film to help consolidate coating. The content of ZnO NPs was determined by thermo gravimetric analysis (TGA) on thermoanalyzer (Netzsch Co., Germany). Samples were heated up to 900 ^{o}C from room temperature at the speed of 10 ^{o}C per minute.

Antimicrobial susceptibility testing

The agar diffusion method was used in initial experiments to evaluate the antibacterial activity ZnO coated film against Escherichia coli DH5α and Staphylococcus aureus SH1000 (obtained from the department of Biological Science of University of Leeds, UK). The films were cut into 10 mm diameter discs and placed on Luria-Bertani (LB) agar seeded with 100 µL of bacterial culture containing approximately 10^5-10^6 colony forming units/mL (CFU/mL). The plates were incubated at 37 °C for 24 h, and the diameters of the zone of inhibition around the discs were measured with a calliper. Equivalent experiments were conducted using the fungal species Aspergillus flavus (A. flavus) and Penicillium citrinum (P. citrinum) (Department of Biological Science of Tianjin University of Science and Technology). However, in this case PDA agar was employed, and the plates were incubated at 28 °C for 72 h. All experiments were done in triplicate.

The antibacterial properties of the coated films were further evaluated during the bacterial growth cycle in liquid culture as follows. E. coli and S. aureus were incubated in LB broth (Sigma-Aldrich, UK) at 37 ^{o}C in the dark for 18 h in a shaking incubator (New Brunswick Scientific, USA) at 200 rpm, to yield approximately 5×10^9 CFU/mL. The culture was then diluted to give approximately 1×10^6-10^7 CFU/mL. Three replicate tubes were prepared for each treatment. In a typical experiment for construction of the growth curves, 50 µl of the diluted culture of either E. coli or S.

aureus was inoculated into 20 mL LB broth containing the ZnO coated films. Cultures were incubated at 37 ^{o}C with vigorous aeration. The growth curve was determined by monitoring the optical density (OD) at 600 nm in a 10 mm optical path length quartz cuvette. The same amount of E. coli in LB broth medium cultured under the same conditions was used as a control. Viable cell numbers were followed by plating diluted cultures onto LB agar, incubating the plates for 48 h at 37 ℃, and then enumerating colonies.

Durability test: The antibacterial durability of the coated films was further evaluated by evaluating the effect of prolonged immersion (5, 20 and 30 days) in water on the films' antibacterial activity against E. coli. At room temperature, ZnO coated films were immersed in water for 5, 20, and 30 days respectively. They were washed by stirring with constant velocity at 200 rpm for 15 min. After washing, the coated films were put into LB broth containing E. coli. Cultures were incubated at 37 ^{o}C with vigorous aeration. The growth curve was determined by monitoring the optical density (OD) at 600 nm in a 10 mm optical path length quartz cuvette.

Experimental data about the inhibition of ZnO-coated film against E. coli and S. aureus was analysed using OriginPro 8.0 software. The mean values were calculated and reported as the mean ±SD (n = 3).

The method used to assess the antifungal effect of ZnO coated PVC films was similar to that reported by Manerat et al. (2006). Briefly, aliquots (25 mL) of A. flavus and P. citrinum were adjusted to a concentration of 2.0×10^{5} conidia/mL and were then mixed with the sample films. The blank films were used as control. Then, 1 mL of mixture suspension after storing in the dark for 24 h was added to potato dextrose agar (PDA)

plate (Sigma-Aldrich, Gillingham, UK) and culture at 28 ℃ for 72 h before enumerating colonies.

Results and discussion

Fabrication and characterization of ZnO NPs suspensions and ZnO coated PVC films

Figure 1a shows the primary sizes of the NPs provided by the manufacturer; the same samples post-milling are shown in Figures 1b and 1c. It is apparent from these images that various sizes of particle were present in the original sample before milling, covering the range between 200 nm and micro sized. The shapes of particles were irregular, including rod-like, ball, and rhomboid shapes. After milling, the particle sizes were more homogeneous and sphere-like with a mean diameter of 50-100 nm. However, the SEM results also indicated that some milled particles were in the form of agglomerates. The Malvern Nano-Sizer showed results in terms of a hydrodynamic diameter distribution and average size of ZnO NPs during milling. Figure 2a showed that the peaks were generally observed for the manufactured sample, which was in a good agreement with the SEM results. During the milling, the average size of ZnO NPs was gradually reduced with the increasing time of milling. Finally, only one peak was left and the average size was reduced to 100 nm (Figure 2b). This result was a little different with SEM results, because the milled particles were still in the form of agglomerates to some extent. Figure 3 shows the TEM image of ZnO NPs after milling. It can be seen that the average particle sizes were 50 ± 35 nm in diameter. Some of them were nearly spherical in shape, with a Wurtzite crystal structure, whilst others were irregular in shape. There were some agglomerations among the NPs.

The ZnO coated PVC films were prepared by the method described in the experimental section. The SEM image of the PVC blank films and ZnO coated films

are shown in Figure 4. The ZnO NPs were coated on the surface of the PVC films uniformly, and the size and shape of ZnO NPs on the surface of PVC films had not changed upon coating. Figure 5 shows the stability of ZnO NPs on the surface of PVC films following vigorous stirring in an aqueous solution and measuring the turbidity of the solution at the wavelength of 300 nm (the maximum absorption wavelength and which did not locate on the forbidden transition wavelength). As seen in Figure 5, the OD value of the aqueous solution containing the ZnO coated films was significantly lower than the OD value of a comparator ZnO suspension containing 0.1 g ZnO/L. Furthermore, the OD values obtained for a solutions containing blank (uncoated) film were similar to that observed for the coated films. This suggested that no ZnO NPs was released from the surface of the coated films, implying that the particles have been stably embedded on the surface. Ultrasonication and TGA were used to estimate the binding between particles and PVC films. TGA thermogram (50-900 ℃) obtained from blank films and ZnO coated PVC films with sonication time of 10 min were shown in Figure 6. The TGA thermogram obtained from blank films showed two consecutive weight losses around 345 and 490 ℃ as a result of decomposition of DOP plasticizer and Carbon-carbon scission. No further weight loss was observed at higher temperature. Total weight loss was 90.75% and remaining 6.8% could be attributed to binders and pigments used during the polymer making process. ZnO coated PVC films sonicated for 10 min showed that total weight losses of 76.15% and remaining 19.65%. The differences in weight losses (13%) compared to blank films correspond to the amount of ZnO NPs deposited on the film surface, indicating that certain amount of ZnO NPs had been firmly coated on PVC film surface even with sonication for 10 min.

Two sizes of sample films (1×1 and 2×2 cm^2), coated with same amount of ZnO NPs (concentration of 2 g/L), were used in subsequent experiments to investigate the antibacterial effect of surface area of ZnO coated PVC films (see below). The content of ZnO and surface areas of ZnO coated films are listed in Table 1. The surface areas of coated films were 26.38 m^2/g and 33.07 m^2/g respectively. The surface area of the coated films (33.07 m^2/g) was higher than that the blank films (15.33 m^2/g), suggesting that the increase of specific surface area is a consequence of the presence of the NPs coated on the PVC films.

Determination of the antibacterial activity of ZnO coated PVC films

The inhibitory effects of ZnO coated PVC films against S. aureus (zone of inhibition of 13.5 mm) and E. coli (zone of inhibition of 12.7 mm) showed that ZnO coated films exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria, as shown in Figure 7. However, for fungi, no inhibitory zone could be seen on the plates after treating with the same ZnO coated PVC films (Figure 8). To further examine the antibacterial activity of the ZnO coated PVC films, the inhibitory effect of ZnO coated films against actively-growing bacterial cultures was examined by following absorbance measurements. The ZnO coated PVC films demonstrated substantial inhibition of bacterial growth (Figure 9, 10) whilst the blank films lacking NPs had no antibacterial effect. The antibacterial activity of ZnO coated PVC films increased with increasing concentration of ZnO coated on the films.

Effect of size of ZnO coated PVC films on bacterial growth inhibition

The functional properties of NPs are heavily influenced by the size of the particles. Therefore, it was important to determine whether the particle size had an effect on the antibacterial properties of the ZnO coated films. Two different sizes of ZnO NPs were

coated on PVC films. ZnO ultrafine powder-coated films (> 1µm) and ZnO NP (mean particle size 50 -70 nm) coated films were used for growth inhibition assays using E. coli. The data established that sample films with smaller particle size showed a stronger inhibition of bacterial growth compared to the sample films coated with the larger particle (Figure 11).

Effect of surface area of ZnO coated PVC films on bacterial growth inhibition

In chemical reactions, the surface area to volume ratio is an important factor for the reactivity; materials with large surface area to volume ratios (small diameter or porous) react at much faster rates than monolithic materials, because more surface is available to react. To examine whether the surface area of ZnO coated PVC films impacted the antibacterial properties of the coated film, two different sizes of films with same amount of ZnO NPs were prepared by the above described method and used for a growth inhibition assay with E. coli. The content of ZnO and BET surface areas of ZnO coated films are listed in Table 1. Figure 12 shows the growth curves of E. coli in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of ZnO coated films with different specific surface area (same amount of ZnO NPs). The coated film with the larger surface area (1.24 m^2) showed a stronger antibacterial activity than the smaller surface area of coated films (0.93 m^2). These results are in agreement with those of Yamamoto 2001 and Zhang et al. 2007, who found that larger surface area and higher concentration of ZnO NPs in suspension were associated with greater antibacterial activity. Jones et al. and Padmavathy et al. reported that the minimum inhibitory concentration (MIC) for ZnO NPs (50-70 nm) in solution was 0.08 g/L against E. coli in LB medium (Jones, Ray et al. 2008, Padmavathy and Vijayaraghavan 2008) . In Figure 13, the antibacterial effects of ZnO NPs at a concentration of 0.2 g/L in solution and on the surface of PVC films were compared.

The film-based ZnO NPs exhibited reduced antibacterial activity compared to solutions containing the same concentration of ZnO. This was likely due to the reduced available surface area of ZnO in suspension compared to coated films.

Durability testing of ZnO coated PVC films

An ideal antibacterial material should be durable, preferably effective for the entire lifetime of the coated product. We performed antibacterial durability test on ZnO coated PVC films by evaluating the effect of prolonged immersion (5, 20 or 30 days) in water on the films' antibacterial activity against E. coli. All the coated films retained over 90% of their antibacterial activity, even after 30 days (Figure 14). These results indicate that even in the absence of a chemical binder, ZnO NPs on a polymer surface are stably affixed and not readily washed away.

Conclusions

ZnO coated PVC films exhibited antibacterial activity against both Gram-positive bacteria (S. aureus) and Gram-negative bacteria (E. coli). The films lacked antifungal activity, indicating a degree of selective toxicity against prokaryotes versus eukaryotes. Concentration and size were shown to be two important factors affecting the antibacterial properties of ZnO coated PVC films. Quantitative antibacterial tests revealed an excellent and durable antibacterial activity, with little reduction in the activity of ZnO coated PVC films observed after 30 days in water. The antibacterial activity and stability/durability of ZnO coated films suggest that they would represent an effective antibacterial material for surfaces involved in medicine and food handling situations.

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Table list

Table 1 BET surface areas of the ZnO NPs coated on various sizes of PVC films

Sample	Surface area BET (m^2)	Area Per Gram (m^2/g)
Blank film (2×2 cm^2)	0.32	15.33
ZnO coated films (1×1 cm^2/ 0.1 g/l)	0.93	26.38
ZnO coated films (2×2 cm^2/0.1 g/l)	1.24	33.07

Figure List

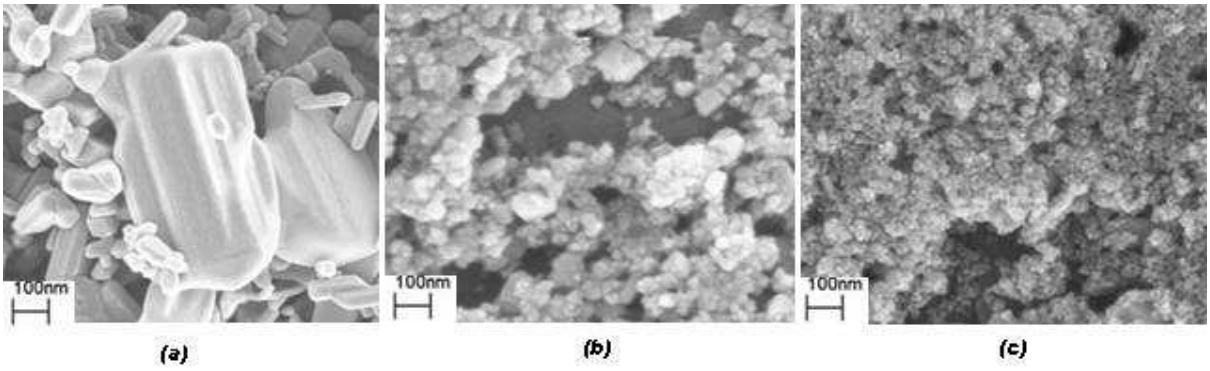
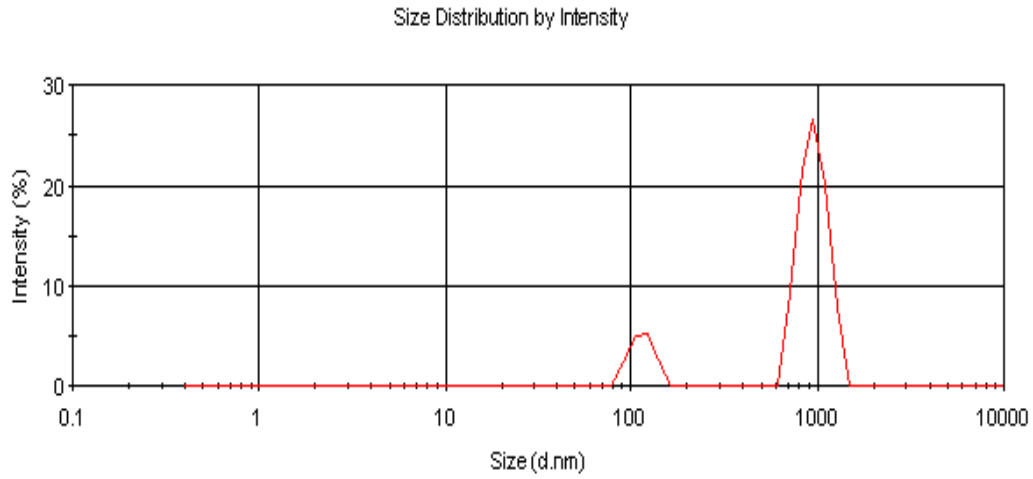
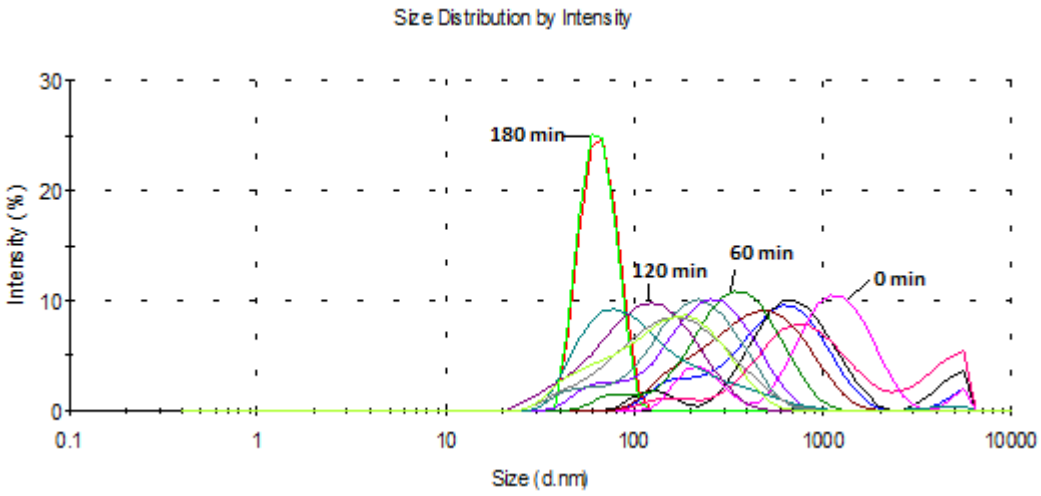


Figure 1 SEM images of ZnO NPs before milling (a), after 1 h milling (b) and after 3 h milling (c)



a



b

Figure 2 Particle size distributions by intensity before (a) and during milling (Interval: 20 mins b)

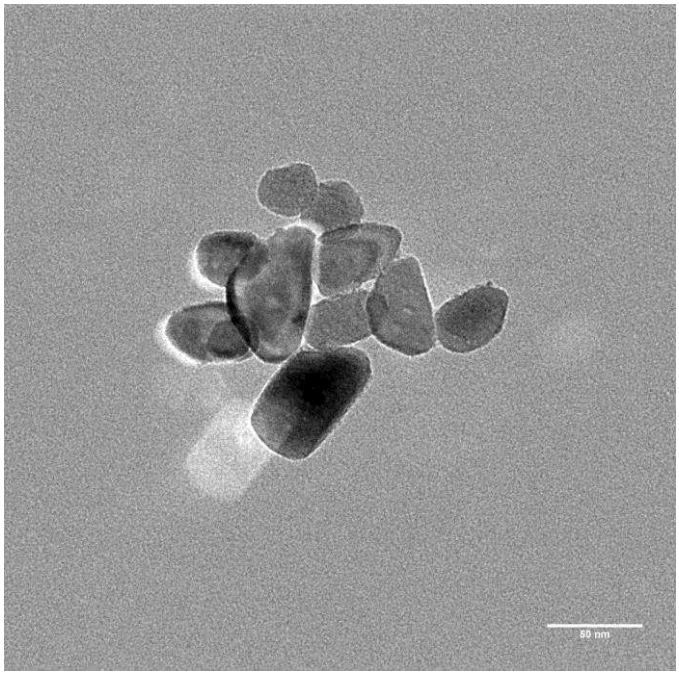


Figure 3 TEM images of ZnO NPs after milling

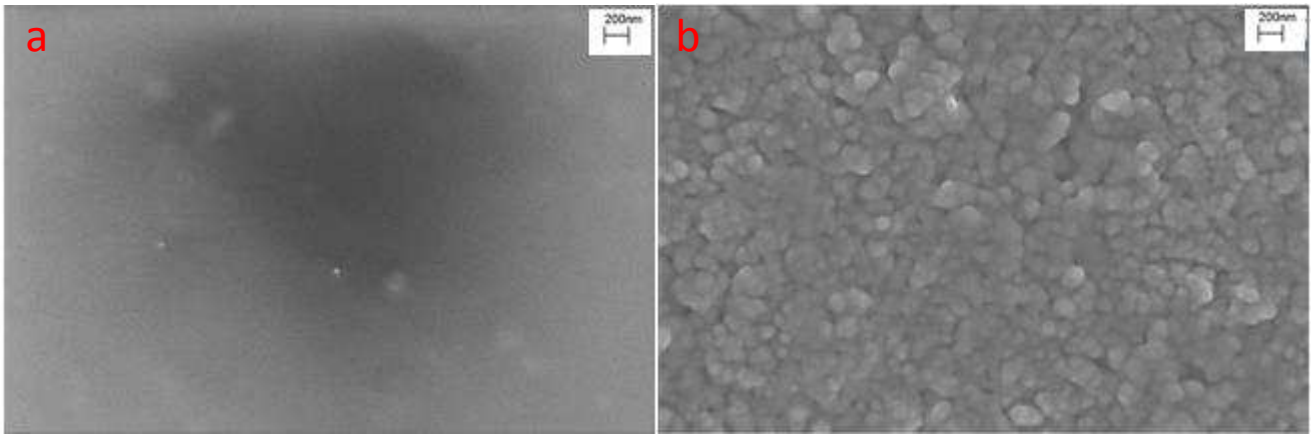


Figure 4 SEM images of PVC films: (a) blank film and (b) ZnO coated films

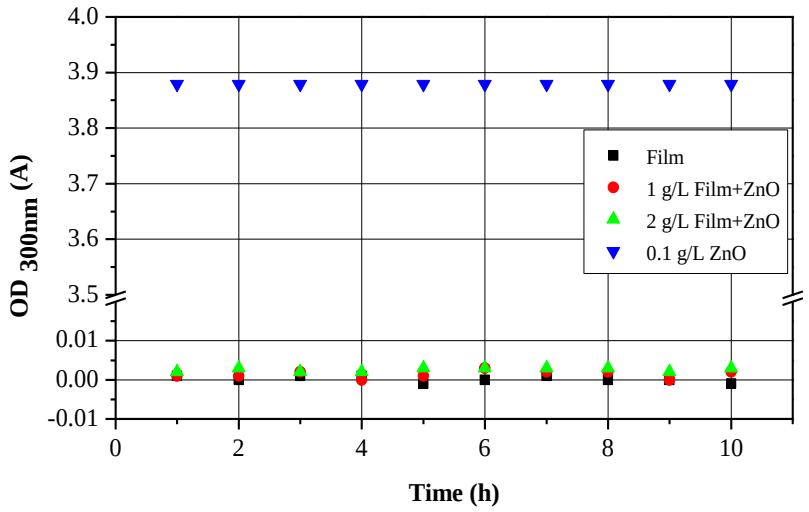


Figure 5 Optical Density of ZnO coated PVC films by vigorously stirring into an aqueous solution at the wavelength of 300 nm

(The OD of 0.1 g/L ZnO solution was used a sample to compare with the optical density of solution contained coated films)

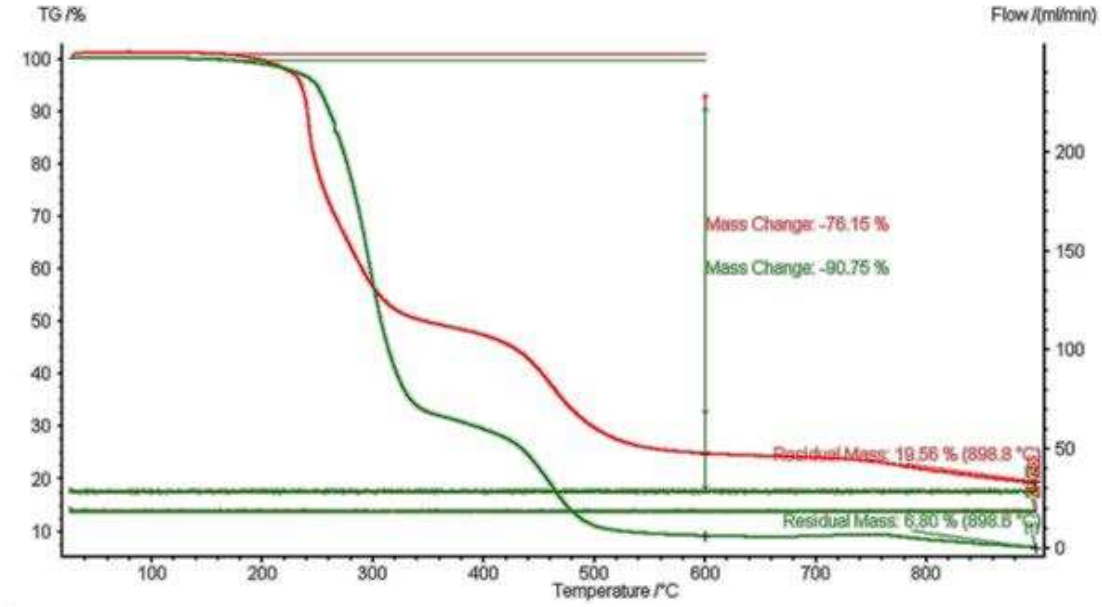


Figure 6 TGA of ZnO NPs coated PVC films

(a) (b)

Figure 7 Inhibitory zone of ZnO coated film against S. aureus (Figure a: C, the blank film; P, PVC film with 93.75 μ g/cm^2 ZnO NPs. B, the PVC film with 187.5 μ g/cm^2 ZnO NPs) and E. coli (Figure b: C, the blank film; d, PVC film with 93.75 μ g/cm^2 ZnO NPs. g, the PVC film with 187.5 μ g/cm^2 ZnO NPs. The diameters of all film discs were 10.0 mm).

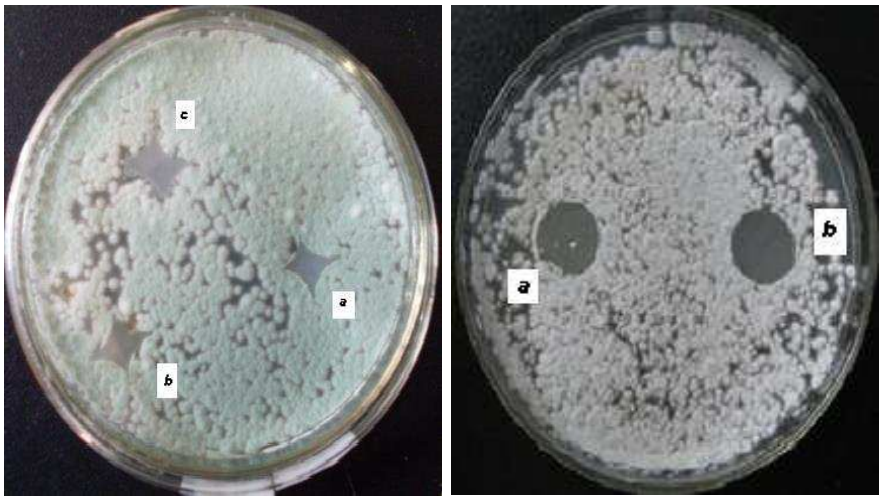


Figure 8 Inhibitory zone of ZnO coated film against *Aspergillus flavus* (a, the blank film; b, PVC film with 93.75 μ g/cm^2 ZnO NPs. c, the PVC film with 187.5 μ g/cm^2 ZnO NPs) and Penicillium Chrysogenum (a, PVC film with 93.75 μ g/cm^2 ZnO NPs. b, the PVC film with 187.5 μ g/cm^2 ZnO NPs)

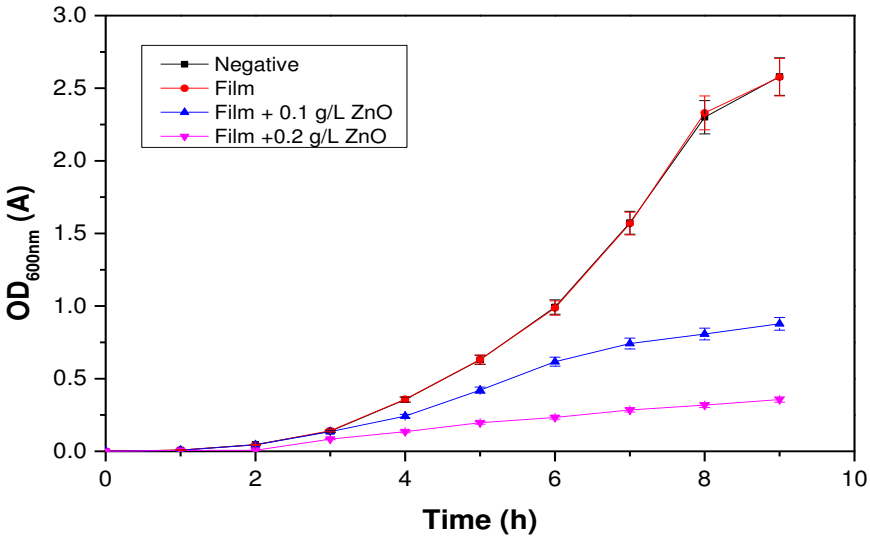


Figure 9 Growth curves of E.coli in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of two different concentrations of ZnO coated films.

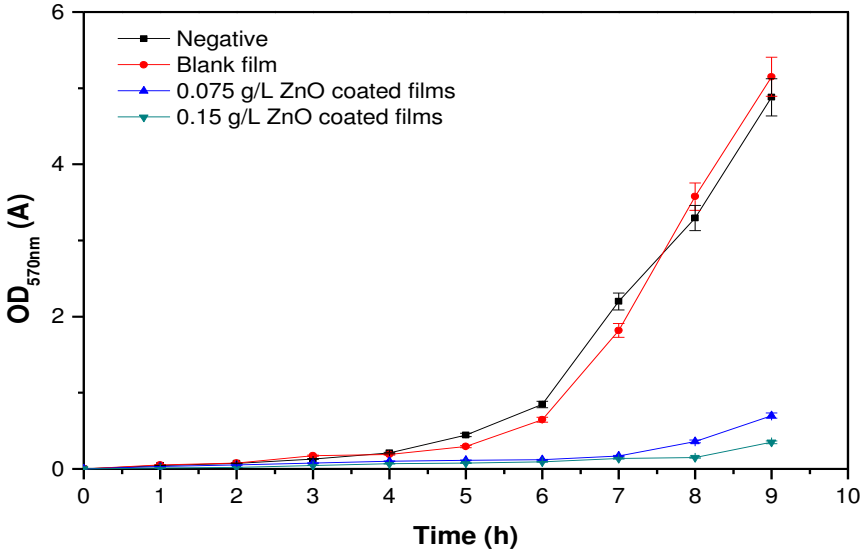


Figure 10 Growth curves of S. aereus in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of two different concentrations of ZnO coated films

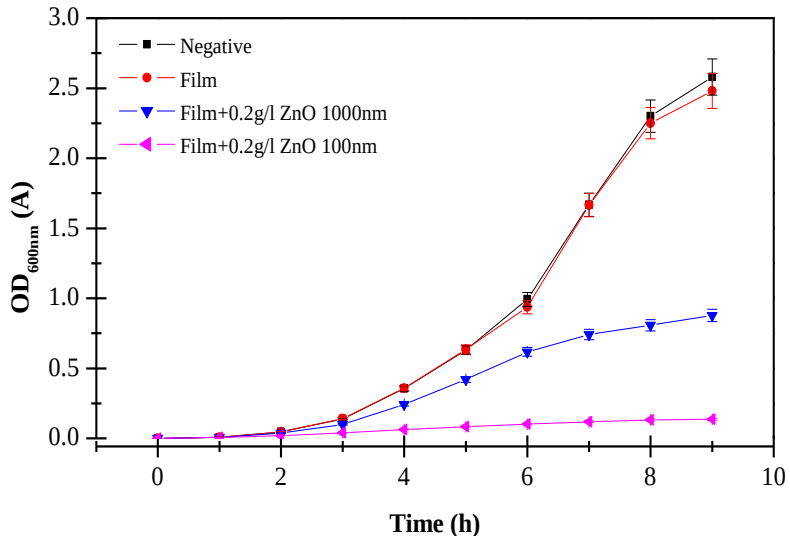


Figure 11 Growth curves of E.coli in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of ZnO coated films of different particle size

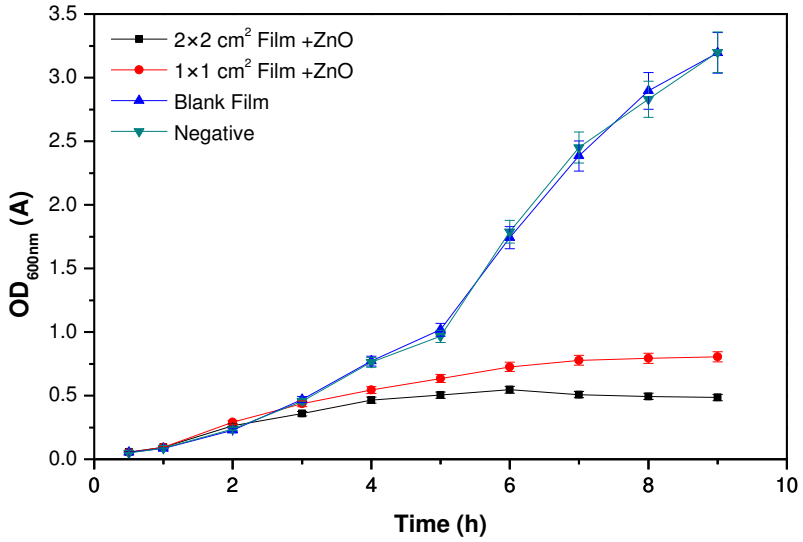


Figure 12 Growth curves of E.coli in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of ZnO coated films of different specific surface area

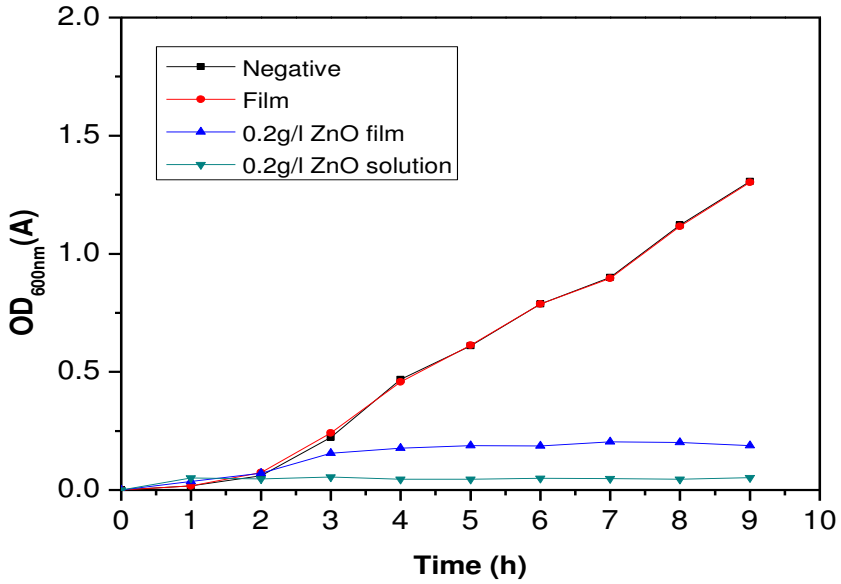


Figure 13 Growth curves of E.coli in LB medium inoculated with 10^7 CFU/mL of bacteria in the presence of ZnO coated films and ZnO suspension

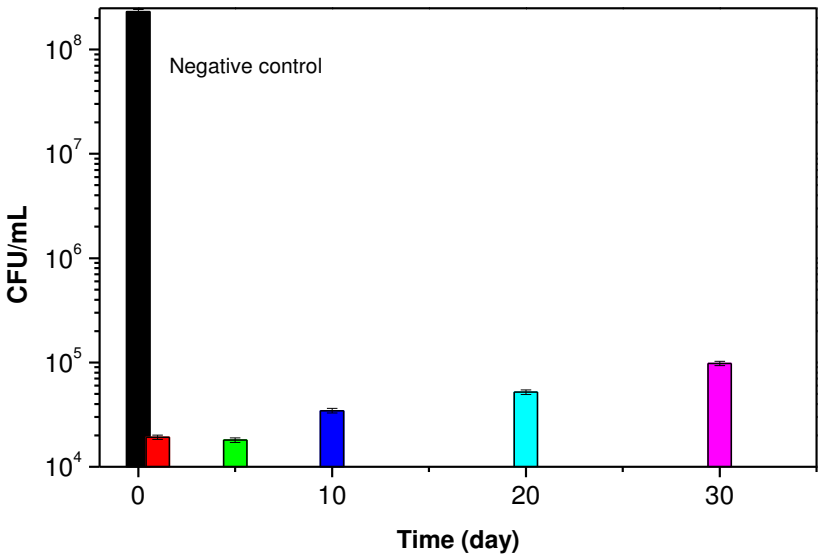


Figure 14 Durability testing of ZnO coated PVC films against E. coli