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Influence of thickness and coatings morphology in the antimicrobial performance of zinc oxide coatings

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ABSTRACT

In this research work, the production of undoped and silver (Ag) doped zinc oxide (ZnO) thin films for food-packaging applications were developed. The main goal was to determine the influence of coatings morphology and thickness on the antimicrobial performance of the produced samples. The ZnO based thin films were deposited on PET (Polyethylene terephthalate) substrates by means of DC reactive magnetron sputtering. The thin films were characterized by optical spectroscopy, X-Ray Diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and Scanning Electron Microscopy (SEM). The antimicrobial performance of the undoped and Ag-doped ZnO thin films was also evaluated. The results attained have shown that all the deposited zinc oxide and Ag-doped ZnO coatings present columnar morphology with V-shaped columns. The increase of ZnO coatings thickness until 200 nm increases the active surface area of the columns. The thinner samples (50 and 100 nm) present a less pronounced antibacterial activity than the thickest ones (200–600 nm). Regarding Ag-doped ZnO thin films, it was verified that increasing the silver content decreases the growth rate of *Escherichia coli* and decreases the amount of bacteria cells present at the end of the experiment

1. Introduction

More natural, safer and higher quality consumable products have been seen as a market demand of huge importance in what concerns the Food Packaging Industry. Foodborne illnesses and death is rising worldwide, particularly in developed countries [1]. Indeed, data from the Foodborne Diseases Active Surveillance Network (Food Net) states that comparing 2007 with 2004–2006, the estimated occurrence of infections caused by *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* did not diminished significantly, and above all, the incidence of *Cryptosporidium* infections even increased by 44% [2]. At the same time, foodborne illness-outbreaks create tremendous social and economic burdens bringing the fear for the re-emergence of infections diseases. Furthermore, the development of antibiotic resistance continues to draw public attention to food safety [3,4]. The combination of the supra-mentioned reasons with the current awareness for environmental conservation and protection has empowered the development of edible coatings and films from biodegradable materials to maintain the quality of both fresh and processed food [5].

Silver-based thin films [6,7] and zinc oxide nanoparticles [8–10] have emerged as promising candidates for active food packaging systems, particularly due to their antibacterial activity. However, due to the lack of knowledge regarding the interactions of nano-sized materials at the molecular and physiological levels and their potential effects on human body, a major concern related to safety of nanoparticles for consumer's health is raising [11,12]. Titanium dioxide (TiO_2) has been used as a self-cleaning and self-sterilizing material to coat different tools, including sanitary ware, food tableware and cooking ware [13,14]. The antimicrobial effects of TiO_2 are activated by its photocatalytic behaviour, which is totally dependent on ultraviolet and/or visible light irradiation. However, for food packaging industry this is a huge drawback since the antimicrobial activity will only be effective in irradiated packages. An alternative to overcome this limitation could be the use of Zinc oxide (ZnO). ZnO presents antimicrobial activity, can act as a permeation barrier coating [15] and is Generally Recognized as Safe material (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). In this sense, the development of zinc oxide thin films can be considered of great added value to food packaging

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industry. There are many scientific publications describing the interaction between ZnO nanoparticles and bacterial cells. However, as far the author's knowledge there are limited reported works concerning the evaluation of the antimicrobial activity of zinc oxide coatings deposited by means of reactive magnetron sputtering. In this sense, the main goal of this research work is to access the antimicrobial activity of zinc oxide coatings and study the influence of coating thickness, morphology and silver content in the antimicrobial activity. Its applicability for food packaging systems is also discussed.

To the author's knowledge, this work is the first to study the influence of thickness and coatings morphology on the antimicrobial activity, contributing to the knowledge of important physical variables in the thin films production.

2. Materials and methods

2.1. Thin films production and characterization

Zinc oxide thin films were deposited on Polyethylene Terephthalate (PET) substrates from Goodfelow and silicon (Si) substrates from Silllicon Materials by reactive DC magnetron sputtering (Advanced Energy Pinnacle Plus, 5K, DC Pulsed). The thin films deposited on PET were used for the evaluation of the optical properties and the antimicrobial activity and the ones deposited on Si were used for structural, morphological and compositional characterization. It was used a circular zinc target (Φ = 75 mm) with a purity of 99,9% and a thickness of 4 mm acquired from Goodfellow Before deposition, the sputtering chamber [16] was pumped down to 10^{-3} Pa. A gas atmosphere composed by argon (Ar, working gas) and oxygen (O_2, reactive gas) was used in the deposition processes. The Ar and O_2 flows were kept constant at 70 sccm (standard cubic centimetre per minute) and 18 sccm, respectively. Each deposition was carried out with a working pressure of 0.6 Pa, a current of 0.35 A and a target/substrate distance of 80 mm. Prior to each deposition the zinc target was pre-sputtered during 5 min. All other deposition parameters were kept constant and the depositions were performed at room temperature (40 °C measured inside the chamber). In order to study the influence of the coating thickness and morphology in its antimicrobial activity, the thickness was controlled by changing the deposition time. Ag pieces with purity of 99.99% were placed on the zinc target in order to promote the film doping effect.

X-ray diffraction (Philips PW 1710 X-ray diffractometer) analysis was used to investigate the crystallographic structure of ZnO thin films. The thickness and morphology of ZnO thin films were obtained by the observation of the cross section of the fractured thin films by SEM (NanoSEM-FEINOVA 200). The optical transmittance was measured by visible spectroscopy (Shimadzu UV-310PC scanning spectrophotometer). The chemical characterization was carried out by using a XPS spectrometer (12 kV, 20 mA) from VG (ESCALAB 210), with the hemispherical electron energy analyser working in the constant pass energy mode at 50 eV and unmonocromatized Mg Kα (1253.6 eV) radiation as excitation source. Depth profiling was carried out by sequential ion bombardment with Ar ions of 3 keV of kinetic energy. Since samples are bad electronic conductors, all spectra were calibrated against the position of the $Zn2p_{3/2}$ at 1022.2 eV.

2.2. Thin films antimicrobial activity

In this research work, the *Escherichia coli* was the bacteria species chosen and it was obtained from the Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho. A pre-culture was prepared for each individual batch experiment. One colony of *E. coli* strain HB101 was picked and loop inoculated into a 125-mL Erlenmeyer flask, containing 20 mL of Luria Bertani (LB) broth (10 g/L tryptone, 10 g/L NaCl, and 5 g/L yeast extract). This pre-culture was incubated at 37 °C, for 12–15 h. On the day after, cells were transferred into different 250-mL Erlenmeyer flasks containing 50 mL of LB broth medium at a starting optical density (OD) of 0.1 measured at a wavelength of 640 nm. A coated PET circular sample (diameter of 6 cm), previously sterilized with 70% ethanol for 1 h and rinsed in sterile water, was deposited on the bottom of the flask. Flasks were then shaken at 80 rpm in a temperature-controlled incubator at 37 °C, and the OD was monitored every hour. OD measurements were made using a Spectronic 20 instrument at 640 nm and the background (turbidity due to growth medium) was eliminated by taking blank readings. The specific growth rate (μ) was calculated from the exponential phase, according to the following equation:

$$\mu = \frac{1}{t_2 - t_1} \cdot \ln\left(\frac{OD_2}{OD_1}\right) \quad (1)$$

where OD_1 and OD_2 are the optical densities corresponding to time instants t_1 and t_2, respectively. The generation time (t_g) can be calculated according to the equation:

$$t_g = \frac{\ln 2}{\mu} \quad (2)$$

3. Results and discussion

3.1. Thin films structure and morphology

The produced ZnO thin films in this research work are highly transparent, presenting a transmittance of about 80% in the visible region of the electromagnetic spectrum. The remainder 20%, is lost by reflection at the air/ZnO interface, by dispersion and by absorption of light in the substrate.

In order to study the influence of the coating thickness and morphology in its antimicrobial activity, the thickness was controlled by changing the deposition time. With this methodology it was observed that the film thicknesses ranged from 50 to 600 nm. X-ray diffraction analyses were performed in representative samples and the measurements were done between 20° and 70° (2θ) for all thin films. Fig. 1 shows the undoped zinc oxide X-Ray Diffraction patterns of the thin films having 50, 200 and 600 nm thicknesses.

In XRD patterns it is observed the presence of the (0 0 2) diffraction peak of the ZnO wurtzite structure (JPCDS36-1451 [17]) in all the samples, indicating a preferential orientation to the c-axis

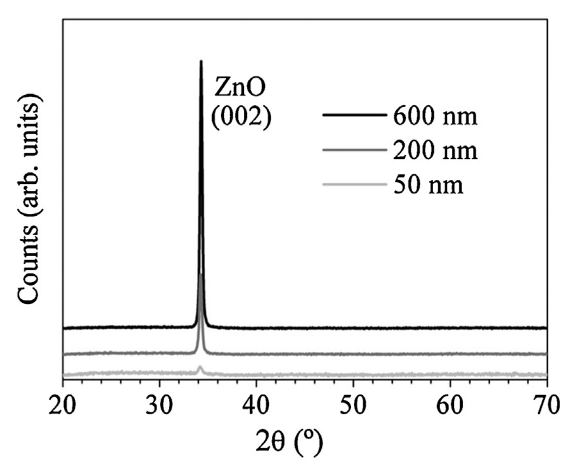

Fig. 1. XRD patterns of ZnO thin films with 50, 200 and 600 nm thickness, deposited in PET substrate.