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# Development of Dextran Coated Zinc Oxide Nanoparticles with Antimicrobial Properties

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**Abstract:** Dextran coated zinc oxide nanoparticles with various zinc concentration have been developed in this study. Various characterization techniques were used in order to study the physical-chemical properties of the obtained samples. The structure of the samples was investigated using X-Ray diffraction (XRD), while the morphology was studied by scanning electron microscopy (SEM). Information regarding the porosity of the samples were obtained with the aid of Brunauer-Emmett-Teller (BET) method. The results of the physico-chemical characterization depicted the obtaining of a nanocomposite with homogenous and uniform morphology. Furthermore, the antimicrobial activity of the samples was also investigated against Gram-positive bacterial strains (*Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis*), Gram-negative bacterial strains (*Pseudomonas aeruginosa* 1397, *Escherichia Coli* ATCC 259220) and against fungal strain *Candida albicans* ATCC 10231. The results of the antimicrobial assay showed that the nanocomposites exhibited good inhibitory effects against all the tested microorganisms making them suitable candidates for the further development of antimicrobial agents for biomedical applications.

**Keywords:** Zinc oxide, Nanoparticles, Dextran, Antimicrobial properties.

## INTRODUCTION

Nowadays, microbial related infections pose a significant health threat, giving rise to potential economic and social complications. The increase of cases involving pathogenic strains, escalating instances of outbreaks, bacterial antibiotic resistance, the emergence of new bacterial mutations, as well as the absence of sufficient vaccines in underdeveloped regions, and the prevalence of hospital-associated infections collectively constitute a global hazard to human well-being. In this context, there is an imperative need to new advances in the development of novel antimicrobial agents [1-4]. Bacterial cells are typically comprised of a cell membrane, a cell wall, and cytoplasm [5]. The bacterial cell wall is predominantly composed of a uniform peptidoglycan layer formed of amino acids and sugars and is positioned outside the

cell membrane. The cell wall usually has the role to uphold both the osmotic pressure within the cytoplasm and also to preserve the distinctive shape of the cell. Gram-positive bacteria have a cytoplasmic membrane with multiple layers of peptidoglycans and a denser cell wall measuring between 20 to 80 nanometers [6]. On the other hand, Gram-negative bacteria presents a cell wall which is comprised of two membranes, an outer membrane and a plasma membrane which is accompanied by a slender layer of peptidoglycans having a thickness between 7-8 nanometers [7]. It was reported by numerous researchers that zinc oxide nanostructures exhibit significant antibacterial efficacy against a wide spectrum of bacterial species [8-10]. Currently, researchers are exploring developing ZnO as an antibacterial agent in both macro and nano dimensions. Several proposed mechanisms elucidate the antibacterial impact of ZnO nanoparticles, encompassing the generation of reactive oxygen species, zinc ion release, disruption of cell membranes, penetration of bacterial cell walls, and internalization of nanoparticles. Among these mechanisms, the most prevalent involve the production of reactive oxygen

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species, such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and superoxide ( $\text{O}_2^-$ ), at the surface of zinc oxide, as well as the gradual release of zinc ions [11-13]. Zinc, is well known as an essential trace element. More than that, zinc ions play an important role in various physiological processes within the human body. Beyond its well-known functions in immune system modulation and wound healing, zinc has recently gained attention for its remarkable antimicrobial properties [14-17]. On the other hand, dextran is also a natural polysaccharide composed of glucose molecules whose biological properties made it of exceptional use in biomedical applications. Dextran is generally well-tolerated by the human body, making it biocompatible and due to its solubility in water, makes it suitable to be used in various pharmaceutical and medical applications. Dextran has been reported to be suitable for applications, including but not limited to drug delivery, as antithrombotic agent, in wastewater treatment, and acting as a blood supplement [18-22]. The results reported by Hussain, M.A. *et al.* [21] showed that dextran can effectively function as a stabilizing agent for silver nanoparticles. Furthermore, a study conducted by Predoi, D. *et al.* [16] concluded that the addition of dextran on the surface of zinc-doped hydroxyapatite nanoparticles does not affect their biological properties. On the other hand, it is well known that globally, antibiotic-resistant microorganism represent an important public health problem involving high costs and a decrease in patients' quality of life [23]. Taking into consideration all of these aspects, the development of dextran coated zinc oxide nanoparticles may be of interest to the scientific community due to its special physico-chemical and antimicrobial properties, potentially representing a suitable candidate for the development of new antimicrobial agents. In this study, we report for the first time preliminary results regarding the development and characterization of dextran coated zinc oxide nanoparticles.

The aim of present research was to develop zinc oxide coated with dextran by a simple and cheap method. The preliminary characterization of the structural and morphological properties as well as the evaluation of the antimicrobial properties was also a goal of this research.

## MATERIALS AND METHODS

### Materials

In this study, zinc acetate dehydrates ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ), 37% hydrochloric acid (HCl), 28%

ammonia ( $\text{NH}_3$ ) and dextran ( $[\text{C}_6\text{H}_{10}\text{O}_5]_n$ , Mr ~40,000) were used. All products were purchased from Merck.

### Synthesis of Dextran Coated Zinc Oxide

Zinc oxide was obtained using a cheap method, namely the co-precipitation method. Zinc acetate dehydrates powder ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ) was mixed with 0.5M hydrochloric acid (HCl) solution under vigorous stirring for 30 minutes. After that, ammonia ( $\text{NH}_3$ ) 5M was added drop by drop at room temperature until the pH of the solution reached 9. The resulting solution was further stirred at a temperature of 85 °C for 8 hours. After the mixture reached room temperature, it was washed with double-distilled water several times to remove residual impurities. After the last wash, the mixture was centrifuged. From the resulting product after centrifugation, different quantities (5, 7 and 10 g) were added to 100 mL of 5% dextran solution and left to stir together for 16 hours. After 16 hours, the three solutions based on ZnO and dextran were centrifuged. The resulting product was dried in an oven at a temperature of 100 °C in air. In the end, three samples resulted (10ZnODx, 7ZnODx and 5ZnODx) which were analyzed.

### Physico-Chemical Characterization

The crystal structure of the dextran coated zinc oxide (ZnODx) samples was characterized by X-ray diffraction (XRD) with a Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany) using  $\text{CuK}\alpha$  ( $\lambda = 1.5418 \text{ \AA}$ ) as incident radiation. The range of the diffraction angle ( $2\theta$ ) was 10- 80° using 34 s measuring time per step and a step of 0.02°. The scanning electron microscopy (SEM) was performed to assess morphology of ZnODx samples using a Hitachi S4500 scanning electron microscope. A Micromeritics ASAP 2020 Physisorption Analyzer (Micromeritics Instrument Corp., Norcross, GA, USA) was used for  $\text{N}_2$  adsorption-desorption measurements. The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface area on the basis of nitrogen adsorption isotherm measurements at 77 K [24].

### Antimicrobial Activity

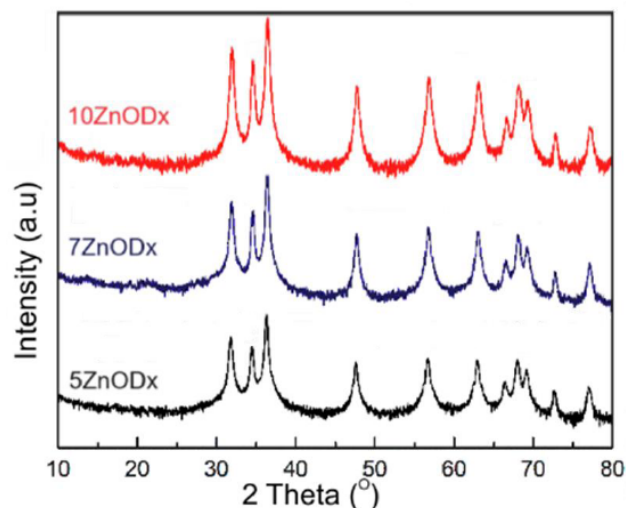
Preliminary studies regarding the antimicrobial activity of the samples were carried out through a qualitative analysis of the antimicrobial properties of the samples against ones of the most common Gram positive (*Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* 1397, *Escherichia*

*Coli* ATCC 259220) and fungal strain *Candida albicans* ATCC 10231. The antimicrobial assays were performed using an adapted diffusimetric method. The modified diffusimetric approach involved dispersing the chemical compound within a designated "spot" on the Muller-Hinton medium, followed by plate seeding in the initial phase. Incubation of the plates occurred at a temperature of 37°C for 24 hours, after which they were allowed to equilibrate at room temperature. The inhibition zones' diameters, formed in the surrounding medium due to the interaction of microorganisms with the samples, were measured using a ruler. These inhibition zones served as indicators of the antimicrobial activity of the samples. Dimethyl sulfoxide (DMSO) was employed as a standard and subjected to comparative testing. The bactericidal impact of the samples was quantified based on the development of an inhibition zone.

## RESULTS AND DISCUSSIONS

Figure 1 shows the XRD patterns of the zinc oxide samples coated with dextran (10ZnODx, 7ZnODx and 5ZnODx) obtained in this study. All the diffraction peaks of the three analyzed samples belong to zinc oxide according to the JCPDF card (No. 36-1451). The pattern suggests that the 10ZnODx, 7ZnODx and 5ZnODx samples with different zinc oxide concentration are constituted in hexagonal wurzite structure. In the XRD pattern, all three samples show a strong (101) diffraction peak. This behavior is in agreement with the studies presented previously [25]. In the analyzed XRD pattern, no additional maxima were observed, which shows a high purity of the three obtained samples. It is observed that the diffraction maxima are more intense as the concentration of zinc oxide in the sample is higher. This behavior shows that

the degree of crystallinity is higher in samples with a higher concentration of zinc oxide.

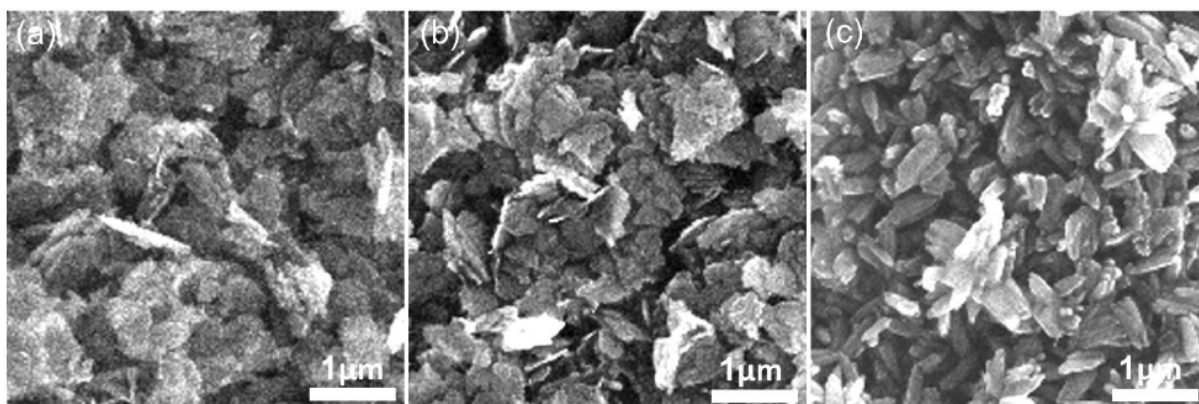


**Figure 1:** The XRD patterns of dextran coated zinc oxide samples.

The specific surface area of the 10ZnODx, 7ZnODx and 5ZnODx samples was estimated using the Brunauer - Emmett - Teller (BET) method. Thus, it was observed that the specific BET surface area was the highest in the case of the 5ZnODx sample (32.687 m<sup>2</sup>/g). The 10ZnODx sample had an estimated BET specific surface of 28.295 m<sup>2</sup>/g while for the 7ZnODx sample it was 25.413 m<sup>2</sup>/g.

The morphology of the samples was investigated using SEM. The results of the SEM studies are depicted in Figure 2.

The SEM images emphasized that the particles have a predominant morphology platelet-like and having a polyhedral shape. More than that, the SEM images also showed that the morphology of the



**Figure 2:** SEM micrographs of 5ZnODx (a), 7ZnODx (b) and 10ZnODx (c).

**Table 1: The Susceptibility Spectrum of Gram-Positive Strains *Staphylococcus Aureus* 0364, *Enterococcus Faecalis* ATCC 29212 and *Bacillus subtilis* Towards 10ZnODx, 7ZnODx and 5ZnODx Samples**

Bacterial strain\Sample	10ZnODx	7ZnODx	5ZnODx
<i>Staphylococcus aureus</i> 0364	+++ <sup>1</sup>	++	+ <sup>2</sup>
<i>Enterococcus faecalis</i> ATCC 29212	++ <sup>3</sup>	++	± <sup>4</sup>
<i>Bacillus subtilis</i>	++	++	+

**Table 2: The Susceptibility Spectrum of Gram-Negative Strains *Pseudomonas Aeruginosa* 1397, *Escherichia Coli* ATCC 259220 Towards 10ZnODx, 7ZnODx and 5ZnODx Samples**

Bacterial strain\Sample	10ZnODx	7ZnODx	5ZnODx
<i>Pseudomonas aeruginosa</i> 1397	+++	++	+
<i>Escherichia Coli</i> ATCC 259220	++	++	±

samples that varies is influenced significantly by the concentration of zinc oxide. It can be observed that with the increase of the zinc oxide content, the morphology of the nanoparticles changed considerably having nanorods like shapes with variable thicknesses. These results are in agreement with previous reported studies regarding the effect of various dopants on the morphology of the nanoparticles [26-27]. In their study, Ramirez *et al* [26], determined that the zinc ions concentration is critical to the obtaining ZnO. Their findings reports that the zinc ions concentration determines either the obating of bulk ZnO or nanoroads that have the behavior of self-assemble ZnO.

Preliminary studies regarding the antimicrobial activity of the 10ZnODx, 7ZnODx and 5ZnODx samples were also conducted against the most common microorganisms that were deemed responsible for the apparition of infections, Gram positive (*Staphylococcus aureus* 0364, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* 1397, *Escherichia Coli* ATCC 259220) and fungal strain *Candida albicans* ATCC 10231.

The results obtained regarding the sensitivity of the tested Gram-positive bacterial strains to the action of 10ZnODx, 7ZnODx and 5ZnODx samples are presented in Table 1. The preliminary qualitative assays highlighted that all the tested bacterial strains

were susceptible to the 10ZnODx, 7ZnODx and 5ZnODx samples. More than that, the results of the antibacterial assays depicted that the inhibitory effects of the samples were correlated both with the zinc oxide concentration form the sample as well as the bacterial strain that was tested against. Therefore, the results suggests that the highest inhibitory activity was obtained for the 10ZnODx sample for all the tested bacterial strains but on the other hands, the data also depicted that the most susceptible bacterial strains was *S. aureus* in comparison to *E. faecalis* and *B. subtilis*.

The results of the antibacterial activity of the 10ZnODx, 7ZnODx and 5ZnODx samples against Gram-negative strains *Pseudomonas aeruginosa* 1397, *Escherichia Coli* ATCC 259220 are presented in Table 2. The results showed that the samples inhibited the growth of the bacterial cells and that the inhibition was also determined by the zinc oxide concentration from the sample.

Furthermore, the data depicting the sensibility of *C. albicans* fungal cells to the 10ZnODx, 7ZnODx and 5ZnODx samples is presented in Table 3. The data suggested that the samples exhibited a strong antifungal activity against the *C. albicans* growth. The results also emphasized that the antifungal effect of the samples was depended on the zinc oxide concentration.

In addition, the findings of the antimicrobial assays determined that the Gram-positive bacterial strains were more susceptible to inhibition compared to the Gram-negative ones and that the strongest susceptibility was exhibited by the fungal cells. The results obtained from the preliminary qualitative antimicrobial assays are in agreement with previously reported studies regarding the antimicrobial properties

<sup>1</sup> very strong inhibition<sup>2</sup> clear inhibition<sup>3</sup> strong inhibition<sup>4</sup> low inhibition

**Table 3: The Susceptibility Spectrum of Fungal Strain *Candida Albicans* ATCC 10231 Towards 10ZnODx, 7ZnODx and 5ZnODx Samples**

Bacterial strain\Sample	10ZnODx	7ZnODx	5ZnODx
<i>Candida albicans</i> ATCC 10231	+++	+++	++

of zinc oxide and zinc oxide derivative samples [28-35]. In their study regarding the antibacterial activity of ZnO nanofluids, Zhang *et al.* [35] reported that ZnO nanofluides exhibited a bacteriostatic activity against *E. coli* which increased with the increase of the ZnO-NPs concentrations and the decrease of the nanoparticles size. Jalal *et al.* [30] also reported that a strong antibacterial activity was obtained at an increased zinc oxide concentration. In a related study, Jenng *et al.* [28] determined that ZnO also exhibited bacteriostatic effects on two bacterial strains: *Lactobacillus salivarius* and *Streptococcus sobrinus* out of the five that were studied. Moreover, the bactericidal activity of ZnO-NPs toward four foodborne pathogens was also reported by Xie *et al.* [33] and their findings concluded that the bactericidal activity was concentration-dependent. These results are in agreement with our preliminary findings and clearly show that the growth inhibition of the microbial strains occurred at higher ZnO concentrations. The preliminary findings obtained in our study emphasized that the dextran coated zinc oxide (ZnODx) samples could be considered for being used in biomedical applications as antimicrobial agents.

## CONCLUSIONS

This study presents a simple and low-cost way of obtaining zinc oxide coated with dextran. Also, preliminary studies on the structure and morphology of the obtained samples were presented. The XRD pattern did not present additional maxima, which shows that the samples have a high degree of purity. It was observed that the degree of crystallinity of the samples increases with the concentration of zinc oxide. It was also observed that the specific BET surface area of the samples increases as the concentration of zinc oxide decreases. The results of the qualitative antimicrobial assays highlighted that the dextran coated zinc oxide (ZnODx) samples exhibited strong inhibitory activity against all the tested microbial strains. Moreover, the data suggests that the antimicrobial activity was strongly dependent on the zinc oxide concentration from the sample and also on the type of the investigated microorganisms. The findings showed that the most susceptible to the ZnODx samples was the fungal strain followed by the Gram-positive bacterial strains and then by the Gram-negative ones. These

results highlight that the dextran coated zinc oxide (ZnODx) samples could be successfully used as antimicrobial agents in medical applications.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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