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# ZnO/graphite composites and its antibacterial activity at different conditions

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ABSTRACT

The paper reports laboratory preparation, characterization and in vitro evaluation of antibacterial activity of ZnO/graphite nanocomposites. Zinc chloride and sodium carbonate served as precursors for synthesis of zinc oxide, while micromilled and natural graphite were used as the matrix for ZnO nanoparticles anchoring. During the reaction of $ZnCl_2$ with saturated aqueous solution of $Na_2CO_3$a new compound is created. During the calcination at the temperature of 500 °C this new precursors decomposes and ZnO nanoparticles are formed. Composites ZnO/graphite with 50 wt.% of ZnO particles were prepared. X-ray powder diffraction and Raman microspectroscopy served as phase-analytical methods. Scanning electron microscopy technique was used for morphology characterization of the prepared samples and EDS mapping for visualization of elemental distribution. A developed modification of the standard microdilution test was used for in vitro evaluation of daylight induced antibacterial activity and antibacterial activity at dark conditions. Common human pathogens served as microorganism for antibacterial assay. Antibacterial activity of ZnO/graphite composites could be based on photocatalytic reaction; however there is a role of $Zn^{2+}$ ions on the resulting antibacterial activity which proved the experiments in dark condition. There is synergistic effect between $Zn^{2+}$ caused and reactive oxygen species caused antibacterial activity.



## 1. Introduction

Microorganisms are ubiquitous in the biosphere. Some on them do not affect humans or animals, some of them are even useful in several food productions, but there are lots of microorganisms which could have negative impact on human health. Therefore it is important to find the balance between benefits and negative effects, to have the negative effects under control. For this purpose commercially available antibiotics are being used. Nevertheless due to the huge overuse of antibiotics in the second half of twentieth century multidrug-resistant bacterial strains have originated. Researchers all over the world currently try to find new medicine to overcome these resistant strains [1].

Due to the unique physico-chemical properties nanomaterials are being deeply studied for their antibacterial properties in the last decade. It has been discovered that several nanomaterials exhibit strong antibacterial activity. Metal and metal oxide nanoparticles such as silver (Ag) [2], silver oxide ($Ag_2O$), titanium dioxide ($TiO_2$) [3], gold (Au) [4,5], silica (Si) [6] and copper oxide (CuO) [7] have been described to possess antimicrobial activity.

Bare nanoparticles may pose some environmental risks due to their enhanced reactivity in comparison with bulk materials [8,9]. When nanoparticles are tightly chemically bonded to a suitable matrix (e.g. clay minerals or graphite) they still demonstrate unique e.g. antibacterial properties but their environmental risks are decreased due to the limited mobility in the environmental media. Graphite is layered structured allotrope carbon made from stacked graphene sheets. It exhibits anisotropy in electrical and mechanical properties. It is a common and widely used material with relatively low damaging effects for human health or the

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environment. In this work graphite was chosen as a matrix for the nanostructured composite material due to its wide use and environmental safety.

The aim of the study was to prepare, characterize ZnO/graphite nanostructured composite material and explore its antibacterial activity against selected human pathogens in relation to the exposure to daylight irradiation in comparison with dark conditions.

## 2. Materials and methods

### 2.1. Studied nanocomposites

$ZnCl_2$ p.a. and $Na_2CO_3$ (Lachema) were used for the synthesis of ZnO precursor. Two graphite substrates, micromilled – further assigned as Gra(1) and high purity natural graphite – further assigned as Gra(2) (Graphite Týn, spol. s.r.o., Czech Republic), were used as matrices for ZnO precursor nanoparticles.

The synthesis includes two main parts. In the first step, the ZnO precursors, $Na_2Zn_3(CO_3)_4{\cdot}(H_2O)_3$ and $Zn_5(OH)_6(CO_3)_2$, was prepared by the reaction of $ZnCl_2$ with $Na_2CO_3$ saturated solution stirring at room temperature in the presence of graphite substrate. During the second step, the ZnO precursor was dried at 100 °C and thermally decomposed at 500 °C to form ZnO nanoparticles. Composites with 50 wt.% of ZnO were prepared and designated as ZinGra(1)5X and ZinGra(2)5X, where X corresponds to thermal treatment temperature (1 – drying at 100 °C and 5 – calcination at 500 °C) [10].

### 2.2. Microscopic and phase analysis

The prepared samples were sintered in an electrical laboratory furnace LH15/13 (LAC, s.r.o.) with the heating rate of 5 °C $min^{-1}$ up to the final sintering temperature of 1000 °C and keeping that temperature for 1 h. The weight after burning (i.e. the weight of pure ZnO) divided to the weight of the sample before burning gives the weight percents of ZnO determined.

The TG/DTA analysis was performed on Setaram SETSYS $18_{TM}$ device. Both samples had approximately 22 mg and they were measured in $Al_2O_3$ crucibles under air atmosphere with heat rate 5 C $min^{-1}$.

The diffractometer Bruker D8 Advance (Bruker AXS, Germany) equipped with detector VÅNTEC 1 was used to record the XRPD patterns under Co Kα irradiation ($\lambda$ = 1.789 nm). During the measurement the reflection mode was used and powder samples were pressed in a rotational holder. The database PDF 2 Release 2004 (International Centre for Diffraction Data) was used to evaluate the phase composition.

Smart Raman Microscopy System XploRA™ (HORIBA Jobin Yvon, France) which allows point analysis was used for the phase characterization of the prepared nanocomposites. Raman spectra were acquired with 532 nm excitation laser source, with 50× objective and using 1200 gr./mm grating.

Scanning electron microscope MAIA3 GMU (TESCAN) was used – ultra-high resolution SEM with Schottky field emission cathode. Images were taken by using a combination of InBeam SE + Low-Energy BSE detector at 2.5 kV. EDS analysis was performed with X-MaxN 150 (Oxford instruments) and the EDS data were processed in AZtec software.

### 2.3. Antibacterial assessment

Four different human pathogenic bacterial strains were used for the in vitro determination of antibacterial activity of the prepared samples. Glucose broth (HiMedia) was used as a growth media. Turbidity of the inoculums was measured using DEN-1 McFarland Densitometer (BioSan). Incubation of bacteria was conducted in Biological thermostat BT 120 M at 37 °C.

Standard microdilution method enabling determination of the minimum inhibitory concentration (MIC) of tested substances served as the method for evaluation of the antibacterial activity of the ZinGra composites. Disposable microtitration plates were used for the testing. Commercial solid blood agar plates for the cultivation of bacteria without any additional modifications were used. Liquid growth media were prepared according to producer's instructions and sterilized in an autoclave. Suspension of the ZinGra samples in the growth media was diluted to achieve the following concentrations of 100, 33.3, 11, 3.7, 1.2, 0.41, 0.014 mg/ml of ZinGra in the media. *Staphylococcus aureus* 3953, *Enterococcus faecalis* 4224 and *Pseudomonas aeruginosa* 1960 were acquired from the Czech Collection of Microorganisms (Czech Republic). The used bacterial inoculums had the following cell concentration of $1.1 \times 10^9$ (*S. aureus*), $1.3 \times 10^9$ (*E. faecalis*) and $1.1 \times 10^9$ (*P. aeruginosa*) CFU/ml (colony-forming units per milliliter). Each compartment of the microtitration plates was inoculated. This plate is called the reaction plate. The lamp with wide spectrum bulb with intensity of 2.4 mW/$cm^2$, which was already used in our previous experiments [11] was placed 10 cm above the reaction plate to induce photo activation of the ZinGra samples, and 8 h of irradiation of the plate was applied on the first day of the experiment. Parallel reaction plate with the same composites at the same concentrations was performed at dark conditions without any irradiation. After the defined time period present living bacterial cells were transferred from the reaction plates to the pure growth media using an inoculation hedgehog. These re-inoculated plates were incubated at 37 °C for 24 h and then the MIC values were determined according to visible growth inhibition. On the second day living bacterial cells from the reaction plate were transferred to a plate with pure media, 8 h of irradiation the same approach was applied again. After the irradiation the cells were transferred from the reaction plate to the plates with pure media. The non-irradiated reaction plate was performed at the dark conditions however the same defined time period as in the case of irradiated one was followed. On the third day the experiment continued in the same manner. Before and after the irradiation living cells were transferred from the reaction plate to a plate with pure media and 8 h of irradiation was applied again. This modification of the standard microdilution method eliminated issues with the determination of MIC caused by turbidity of dead cells or caused by the presence of the ZinGra sample particles in microtitration plate, because only living bacterial cells can be captured by needles of the inoculation hedgehog.

## 3. Results and discussion

Graphite is not stable at oxidative atmosphere at 1000 °C, it burns provably. Therefore this fact was used for determination of the content of ZnO in the composites. The weight after burning (the weight of pure ZnO) divided to the weight of the sample before burning gives the weight percent of ZnO determined (Table 1).

Pure $Zn_5(OH)_6(CO_3)_2$ has maximal mass loss at 247 °C [12], ZinGra samples have maximal mass loss at 239 °C (Fig. 1) and 244 °C

**Table 1**
Determined content of ZnO in the ZinGra composites.

| Sample | Content of ZnO [wt.%] |
|---|---|
| ZinGra(1)55 | 42.43 |
| ZinGra(2)55 | 45.44 |