STUDY OF THE ANTIBACTERIAL ACTIVITY OF COMPOSITES STEVENSITE/ZnO

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Abstract

During the past few years there has been a remarkable growth of research activities exploring properties and biomedical applications of nano-sized materials. Size, shape, size distribution, morphology, surface functionalization and stability of nanomaterials have an influence on resulting biological and other effects of nanomaterials. Due to the unnecessary overuse of antibiotics in the second half of the 20th century, mankind must face the incidence increase of resistant and/or multiresistant bacterial strains, which may significantly decrease the efficiency of current medical treatment. Nanoparticles or nanomaterials could potentially represent the new medicines because no acquired bacterial resistance after application of nanomaterials has been observed yet. In this study, ZnO nanoparticles(NPs) were synthesized by the reaction between zinc chloride and sodium hydroxide. Stevensite/ZnO nanocomposite (SEZN) was thus prepared by addition of the aqueous mixture of zinc chloride and sodium hydroxide solutions into the aqueous suspension of stevensite. The prepared samples were characterized using X-ray powder diffraction. The modification of the standard microdilution method was used for the evaluation of the antibacterial activity of ZnO NPs and composite stevensite/ZnO. Antibacterial assay using common human pathogen bacterial strains showed that ZnO NPs and SEZN composite have antibacterial potency.

Keywords: antibacterial activity, ZnO, clay, nanocomposites, XRD.

1. INTRODUCTION

Due to the large surface/volume ratio and thus enhanced reactivity, nanomaterials are perspective materials which can give favorable opportunities for medial or environmental applications due to their unique physical and chemical properties which are caused by their size in nano dimensions [1]. Nanomaterials based on metal oxide are being used in products e.g. for disinfection. Antibacterial properties of those nanomaterials were also observed [2]–[5].

Among metal oxide powders, ZnO demonstrates significant growth inhibition of broad spectrum of bacteria [6]–[8]. The proposed mechanism for the antibacterial activity of ZnO is based on catalysis of formation of reactive oxygen species (ROS). Cell wall and cell membrane of bacteria are damaged upon the contact with nanoparticles of ZnO, which cause inhibition of bacterial growth [3]. Moreover, Zn^{2+} can bind to the membranes of microorganisms and thus prolong the lag phase of the microbial growth cycle [9].

Phyllosilicates have unique crystallochemical properties; therefore they may act as a suitable matrix for anchoring ZnO nanoparticles [10]–[12]. If e.g. ZnO nanoparticles are chemically bound to a suitable matrix (e.g. stevensite) they still demonstrate photodegradable properties, however potential environmental risks are lowered due to the decreased mobility in the environmental media.

The aim of the study was to prepare, and characterize stevensite/ZnO composite and explore its antibacterial activity for selected human pathogens in relation to the extent of daylight irradiation.

MATERIALS AND METHODS

1.1. Preparation of the samples

ZnO nanoparticles (NPs) were prepared by the reaction between zinc chloride (anhydrous pure, Lach-Ner, Czech Republic) and sodium hydroxide (G.R. Micropearls, Lach-Ner, Czech Republic) at the molar ratio of Zn^{2+}:OH^- being 1:5. Stevensite/ZnO nanocomposite (further assigned as SEZN) was prepared by addition of the aqueous mixture of zinc chloride and sodium hydroxide solutions into the aqueous suspension of stevensite SE (Morocco) followed by continuous stirring at 100 °C. The mixture was allowed to react for 5 h. Resulting solid phase was washed with distilled water and dried at 105 °C for 24 h. The precursors/clay ratio was chosen so that the nanocomposite contained 50 wt.% of ZnO.

1.2. X-ray powder diffraction

The XRPD patterns were recorded under CoKα irradiation (λ = 1.789 Å) using the Bruker D8 Advance diffractometer equipped with a fast position sensitive detector VÅNTEC 1. Measurements were carried out in the reflection mode, powder samples were pressed in a rotational holder. Phase composition was evaluated using ICDD PDF 2 Release 2014 database.

1.3. 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 120M 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. 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. Suspensions of the SEZN, stevensite and ZnO NPs 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 SEZN, stevensite and ZnO NPs 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 $1.1x10^9$ (*S. aureus*), $1.2x10^9$ (*E. faecalis*),$1.3x10^9$ (*E. coli*) and $1.4x10^8$ (*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 bulbwith intensity of 2.4 mW/cm^2, which was already used in our previous experiments [13] was placed 10 cm above the reaction plate to induce photo activation of the SEZNand ZnO NPS samples, and 8 hours 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 placed 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 24h and then the MIC values were determined according to visible growth inhibition.

2. Results and discussion

XRPD patterns of the stevensite (SE) and prepared samples (pure ZnO NPs, SEZN) are shown in **Fig. 1**. Reflections intensity in the XRD pattern of SE is relatively low suggesting lower crystallinity of the clay.

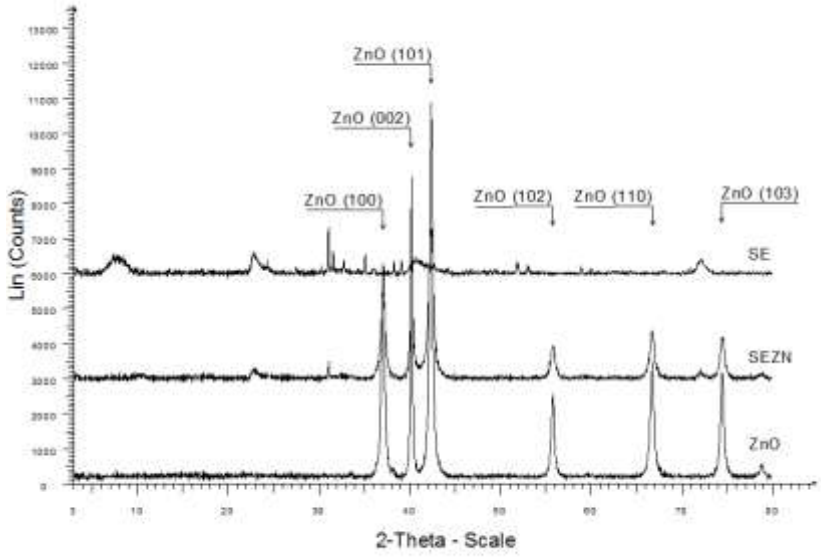

Fig. 1 XRPD patterns of the studied samples: stevensite (SE), ZnO NPs (ZnO) and stevensite/ZnO (SEZN).

The zinc oxide with hexagonal structure (PDF number 36-1451) was found in all prepared samples and for individual ZnO reflections also Miller indices are presented (**Fig. 1**). The ZnO crystallite sizes (L_C) were calculated according to the (1 0 1) ZnO diffraction peak using the Scherrer formula [14]. Lanthanum hexaboride (LaB6) was used as a standard and the calculated L_C values for pure ZnO NPs and ZnO in SEZN are 27 nm, and 21 nm, respectively.

Antibacterial activity, expressed as the MIC values of the SEZN and ZnO NPs was evaluated using four bacterial strains. Values of MIC for the samples against all bacterial strains are summarized in (**Table 1**). Pure stevensite did not exhibit antibacterial activity; therefore the MIC values could not be determined and are not included in the tables. When the MIC values could not be determined for the SEZN composite or ZnO NPs, it could be caused by the MIC value being higher than the concentration range used.

There is a visible difference between the onsets of the daylight induced antibacterial activity of both samples where the irradiated composites exhibited faster onset of the antibacterial activity than the non-irradiated ones.Generally, lower values of MIC were achieved for irradiated composites in comparison with non-irradiated. Extension of the reaction time causes decrease of the MIC values. The lowest MIC values were achieved for both irradiated samples against *S. aureus*, *E. facealis* and *E. coli* (0.14 mg/ml).There are several proposed mechanisms of the antibacterial activity of ZnO nanoparticles. One of them is based on the release of Zn^{2+} ions and consequent diffusion of these ions into the cytoplasm [15]. Other is based on the interaction of ROS with cells or biomolecules. The study previously published [16] dealing with the antibacterial activity of ZnO [16]suggested mechanism of antibacterial activity based on the direct contact of nanoparticles with the cell wall, their deposition on the cell wall which leads to the changes in the permeability of cell wall. The results obtained in this study indicates the synergic effect of the daylight-induced production of ROS and antibacterial activity under dark conditions probably caused by the release of Zn^{2+} ions into growth media.The outcome of the day light-induced antibacterial activity of the composite SEZN is relevant in terms of potential applications of this nanocomposite for antibacterial modification of various surfaces. Our previous studies focused on the daylight induced antibacterial activity of composites ZnO/kaoline [3] and ZnO/graphite [18]. The obtained MIC values for ZnO NPs and SEZN are significantly lower than in the case of ZnO/kaoline and ZnO/graphite. It has to be pointed out that the matrix in the nanocomposites affects the resulting biological activity.From this perspective it has to be concluded that the

nanocomposites including ZnO do not cause inhibition of bacterial growth in that wide range as several antibiotics and there are several factors affectingtheresultingbiologicalproperties.

Table 1 Experimental MIC values (mg/ml)

	SEZN_15							
	S. aureus		*E. faecalis*		*E. coli*		*P. aeruginosa*	
Reaction time	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT
30 min	>100	>100	>100	>100	>100	>100	>100	>100
60min	>100	>100	>100	>100	>100	>100	>100	100
90 min	>100	100	>100	100	>100	100	>100	100
120 min	>100	100	>100	100	>100	100	>100	100
180 min	100	100	>100	100	100	100	100	100
240 min	100	100	100	100	100	100	100	100
300 min	100	100	100	100	100	100	100	100
1day before irradiation	3.7	0.14	33.3	0.14	100	0.14	100	100
1day after irradiation	3.7	0.14	33.3	0.14	100	0.14	100	100
	ZnO							
	S. aureus		*E. faecalis*		*E. coli*		*P. aeruginosa*	
Reaction time	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT	DARK	LIGHT
30 min	>100	>100	>100	>100	>100	>100	>100	>100
60min	>100	>100	>100	>100	>100	>100	>100	>100
90 min	>100	100	>100	100	>100	100	>100	100
120 min	>100	100	>100	100	>100	100	>100	100
180 min	>100	100	>100	100	>100	100	>100	100
240 min	100	100	100	100	100	100	100	100
300 min	100	100	100	100	100	100	100	100
1day before irradiation	1.2	0.14	11.1	0.14	33.3	0.14	33.3	100
1day after irradiation	1.2	0.14	11.1	0.14	33.3	0.14	33.3	100

3. CONCLUSION

ZnO NPs and the stevensite/ZnO nanocomposite were laboratory synthesized by the reaction between zinc chloride and sodium hydroxide. XRD proved the formation of hexagonal structure of ZnO nanoparticles and revealed the crystallite size for pure ZnO NPs and ZnO in SEZN are equal to 27 nm, and 21 nm, respectively. Antibacterial assay using common human pathogen bacterial strains showed that ZnO NPs and SEZN composite have antibacterial potency under dark conditions and under the artificial daylight irradiation where the onset of the antibacterial activity is faster under the irradiation. Lower MIC values were achieved against *S. aureus*, *E. facealis* and *E. coli* (0.14 mg/ml) for ZnO NPs and SEZN after daylight irradiation. Based on the preliminary findings presented above, the SEZN composite could potentially find applications in the biomedical field such as surface modifications of various materials to decrease their bacterial contamination.

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