UDK 678.7:66.017:620.3
Original scientific article/Izvirni znanstveni članek

ISSN 1580-2949
MTAEC9, 49(1)55(2015)

ANTIBACTERIAL COMPOSITE BASED ON NANOSTRUCTURED ZnO MESOSCALE PARTICLES AND A POLY(VINYL CHLORIDE) MATRIX

PROTIBAKTERIJSKI KOMPOZIT NA OSNOVI NANOSTRUKTURNIH DELCEV ZnO IN OSNOVE IZ POLIVINIL KLORIDA

Jakub Sedlák[1,2], Pavel Bažant[1,2], Jiří Klofáč[1,2], Miroslav Pastorek[1,3], Ivo Kuřitka[1,2]

[1]Centre of Polymer Systems, University Institute, Tomas Bata University in Zlin, Nad Ovcirnou 3685, 760 01 Zlin, Czech Republic
[2]Polymer Centre, Faculty of Technology, Tomas Bata University in Zlin, Namesti T.G. Masaryka 275, 762 72 Zlin, Czech Republic
[3]Department of Polymer Engineering, Faculty of Technology, Tomas Bata University in Zlin, Namesti T.G. Masaryka 275, 762 72 Zlin, Czech Republic
j1sedlak@ft.utb.cz

Prejem rokopisa – received: 2013-10-04; sprejem za objavo – accepted for publication: 2014-03-10

The microwave-assisted solvothermal synthesis of inorganic ZnO nanoparticles is a facile method yielding a broad variety of active fillers with specific properties. The synthesis of hierarchical nanostructured zinc oxide mesoscale particles was carried out under continuous microwave irradiation from soluble zinc acetate as the precursor in a diethylene glycol solvent. The prepared ZnO particles were characterized with X-ray diffractometry, scanning electron microscopy and UV-VIS spectrometry. A composite was obtained by mixing these particles with the softened medical-grade poly(vinyl chloride) as a model polymer matrix to develop an antibacterial polymer system with a possible application for plastic medical devices. The testing of the antibacterial activity of the composite confirmed an excellent performance against *Escherichia coli* (gram-negative) and sufficient activity against *Staphylococcus aureus* (gram-positive) bacteria according to ISO 22196: 2007. In addition, no adverse effects of the filler on the mechanical properties of the composite were observed in comparison with the neat PVC resin. Therefore, the prepared composites can be considered as suitable candidates for application in plastic medical devices and other industries.

Keywords: antibacterial, PVC, composite, ZnO, nanoparticles

Solvotermična sinteza anorganskih delcev ZnO v mikrovalovni pečici je lahka metoda za izdelavo različnih polnil s posebnimi lastnostmi. Sinteza delcev cinkovega oksida s hierarhično nanostrukturo je bila izvršena s kontinuirnim mikrovalovnim obsevanjem v mikrovalovni pečici iz predhodno raztopljenega cink acetata v raztopini dietilen glikola. Pripravljeni delci ZnO so bili karakterizirani z rentgensko difrakcijo, vrstično elektronsko mikroskopijo in z UV-VIS-spektrometrijo. Kompozit je bil izdelan z mešanjem teh delcev v zmehčan medicinski polivinil klorid kot modelno polimerno osnovo za razvoj protibakterijskega polimernega sistema z možnostjo uporabe za plastične medicinske naprave. Preizkušanje protibakterijske aktivnosti kompozita je pokazalo odlično vedenje pri *Escherichia coli* (gram-negativne) in zadovoljivo aktivnost proti bakterijam *Staphylococcus aureus* (gram-pozitivne) skladno z ISO 22196: 2007. Dodatno pa v primerjavi s čisto PVC-smolo ni bil ugotovljen neželen učinek polnila na mehanske lastnosti kompozita. Zato je pripravljeni kompozit primeren kandidat za uporabo v plastičnih medicinskih napravah in v drugih industrijah.

Ključne besede: protibakterijski, PVC, kompozit, ZnO, nanodelci

1 INTRODUCTION

Nosocomial infections have become a serious problem of today's hospital environment. The prevalence of medical-devices-related infections (MDIs) rapidly grows among hospital-acquired infections and high health risks are especially associated with the devices which are in close contact with the inner parts of a patient's body.[1] As the polymers are the most frequently used materials in medical devices, the prevention against bacterial adhesion and growth on their surfaces is an absolute imperative.[2] There are two principally different strategies of how to resolve this problem. The first approach uses organic antimicrobial additives to plastics. They are very efficient; however, their mechanism of action requires diffusion out of the plastic surface and they can be classified as migratory additives. In contrast, the second approach uses inorganic additives with a very low solubility which ensures sufficient surface antibacterial activity and a minimum leakage of the active species into the patients body.[3] Therefore, a utilization of inorganic antibacterial nanopowders by compounding them with commercial polymer resins seems to be the simplest way leading to an enhanced antibacterial activity of a polymer composite system.

In recent years, a great effort has been made by the community of researchers for the synthesis and utilization of nanomaterials in the field of medical materials.[4] Zinc oxide is one of the promising candidates from the group of metal oxides (e.g., CaO, MgO, TiO_2, Cu_2O) that can be obtained with various properties depending on the nanoparticle size and shape as the crucial parameters.[5] ZnO is a biocompatible material exhibiting antimicrobial properties against the gram-negative as well as gram-positive bacteria. The mechanisms of the antibacterial

action are still not perfectly understood, but there are several proposals for the explanation of the microbial inhibition such as the generation of reactive oxygen species (ROS) like hydroxyl radicals and singlet oxygen, or the release of Zn^{2+} cations.[6,7]

Up to now, a large variety of physical and chemical methods have been developed for the ZnO-nanoparticle preparation such as thermal decomposition, hydrothermal, solvothermal or microwave-assisted syntheses. The last mentioned microwave energy exclusively reduces the reaction time as well as the energy demands of the process.[8–12]

Here we report on a fast and simple solvothermal microwave-assisted synthesis of hierarchical nanostructured mesoscale ZnO aggregates, their compounding into medical-grade plasticized PVC as a model polymer matrix suitable for utilization in medical devices and an evaluation of the surface antimicrobial and mechanical properties of the final composites.

2 EXPERIMENTAL WORK

2.1 Materials

All the chemical reagents used in the experiment were of the analytical grade and used as received without further purification: zinc acetate dihydrate, p.a., (a purity of 99 %, Penta, The Czech Republic), diethylene glycol, p.a., (a purity of 99 %, Penta, The Czech Republic). Azeotropic denatured ethanol (The Czech Republic) was used for washing the product. The medical-grade softened PVC resin RB-3 (Modenplast Medical, Italy) was used as the polymer matrix.

2.2 Synthesis of the ZnO antibacterial filler

At first 2.195 g of $Zn(CH_3COO)_2 \cdot 2H_2O$ was completely dissolved in 100 mL of diethylene glycol at a temperature of about 100 °C during a 30 min agitation on a magnetic stirrer. The obtained solution was transferred to a 250 mL boiling flask and irradiated under reflux for 5 min using microwaves. The product was cooled down naturally after turning off the microwave oven. The powder was collected with centrifugation, washed with ethanol and air-dried up to the constant mass. The microwave reaction was performed in a microwave open-vessel system MWG1K-10 (Radan, The Czech Republic, 800 W, 2.45 GHz), based on modifying a domestic microwave oven by drilling a hole in the ceiling for external cooling and equipped with an external source of microwave energy that allows a control of the duty cycle. The reaction mixture was heated in a quasi-continuous mode at the maximum heating power.

2.3 Compounding the ZnO filler with the PVC matrix

The PVC-filler compounds were prepared with a melt-mixing process using a Brabender Plasti-Corder machine (Brabender, Germany) equipped with a 50 cm^3 mixing chamber and the mixing elements working in a contra-rotating mode. In this way, four mixtures differing from each other with respect to the load of the filler in the polymer matrix were compounded with the compositions as follows: w = (0.5, 1, 2 and 3) %. The compounding process consisted of three continuous phases. The first started by feeding the mixing chamber with PVC pellets and synthesized filler in the form of a fine powder at 20 r/min for two minutes. In the next step, after the feeding of the chamber was finished, the engine speed was continuously increased from 20 r/min up to 50 r/min within 1 min. The last part of the mixing was done at 50 r/min for 5 min. The mixing process was monitored by measuring the torque of the drive engine. The processing time of 8 min was enough to obtain a constant torque value for all the samples. No resin discoloration or other signs of a polymer deterioration connected with the use of the ZnO filler were observed under the used mixing conditions. The prepared compounds were compression molded to square-shaped sheets with the dimensions of 50 mm × 50 mm × 1 mm at 180 °C for 2.5 min. Blank samples without any filler were prepared in the same way as the filled ones.

2.4 Characterization

The synthesized powder was characterized with X-ray powder diffraction (XRD) using an X´Pert PRO (PANalytical, The Netherlands) with a Cu K_α radiation source (λ = 0.1540598 nm). The micrographs of the prepared powder and cross-section surfaces of the composites were taken with a scanning electron microscope Vega II (Tescan, The Czech Republic) and the UV-VIS absorption spectra were collected with an Avaspec UV-VIS spectrometer (Avantes, The Netherlands) with an AvaLight-DHS-DUV source type and an integrating sphere ($BaSO_4$ coated) was used for the diffuse reflectance measurement. The mechanical properties of the composites were evaluated with the tensile tests performed on a Testometric universal testing machine M350-5CP (LABOR machine Ltd., The Czech Republic) according to the ISO 37: 2005 standard with a type-2 testing specimen. The speed of the moving clamps was 250 mm/min. All the sample measurements were replicated six times.

2.5 Antibacterial testing

An evaluation of the surface antibacterial activity of the composites against bacterial adherence and growth was performed according to the ISO 22196: 2007 (formerly known as JIS Z-2801) standard. Gram-positive bacteria were represented by *Staphylococcus aureus* ATCC 6538P and gram-negative by *Escherichia coli* ATCC 8739, both obtained from The Czech Collection of Microorganisms (The Czech Republic). The size of the test specimens was 50 mm × 50 mm × 1 mm. A HERAcell 150i incubator (Thermo Scientific, USA) was

used for the cultivation. The antibacterial activity R was calculated using Equation 1:

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (1)$$

where R is the antibacterial activity; U_0 is the average of the common logarithm of the number of viable bacteria cells in 1/cm^2, recovered from the untreated test specimens immediately after the inoculation; U_t is the average of the logarithm of the number of viable bacteria cells in 1/cm^2, recovered from the untreated test specimens immediately after 48 h; and A_t is the average of the logarithm of the number of viable bacteria cells in 1/cm^2, recovered from the treated test specimens immediately after 48 h. The colonies were counted after 24 h and checked after 48 h for a presence of slowly growing colonies on both untreated and treated test specimens, improving the original standard protocol. All the tests were repeated in triplicates.

3 RESULTS AND DISCUSSION

The crystalline-phase structure of the synthesized filler is shown on the diffractogram in **Figure 1**. By comparison with JCDD PDF-2 entry 01-079-0207, diffraction peaks were labelled as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) and (104) and assigned to the hexagonal ZnO wurtzite structure. Moreover, the evident broadness of diffraction lines implies that the synthesized particles are of nano-scale dimensions.

Figure 2 shows the diffuse reflectance UV-VIS (DR-UV-VIS) spectrum of the prepared ZnO nanocrystals that exhibit a strong absorption with the maximum below 380 nm. This peak is slightly blue-shifted from the typical position of bulk ZnO[13] which points towards the nanosized particles, similarly as with the XRD analysis. The morphology of the prepared filler is presented in the SEM image in **Figure 3a**. As can be seen, the material consists of globular-like particle aggregates with a diameter ranging from 200 nm up to 1 μm. According to the XRD analysis and DR-UV-VIS measurement it can be concluded that these aggregates are assembled from much smaller nanocrystals.

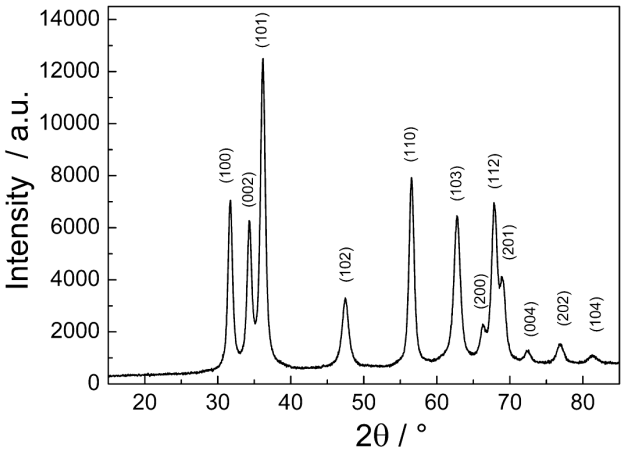

Figure 1: X-ray diffractogram of the prepared ZnO filler
Slika 1: Rentgenski difraktogram pripravljenega ZnO-polnila

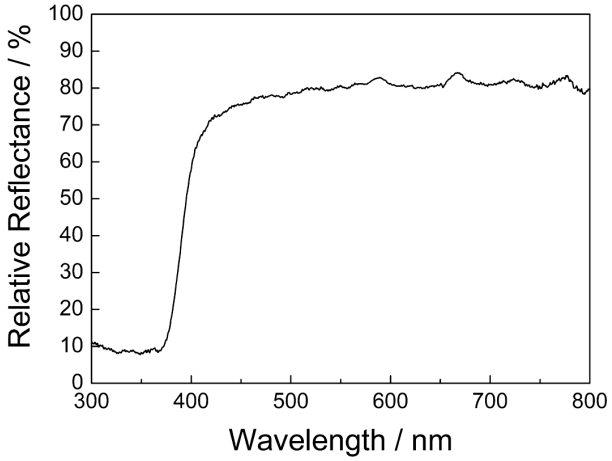

Figure 2: UV-VIS diffuse-reflectance spectrum of the prepared ZnO filler. Relative reflectance is plotted versus wavelength.
Slika 2: UV-VIS-spekter razpršene odbojnosti pripravljenega ZnO-polnila. Relativna odbojnost je prikazana v odvisnosti od valovne dolžine.

The filler dispersion in the prepared composites was achieved at the aggregate level and its good distribution in the PVC matrix is shown in the SEM image in **Figure 3b**, for the sample with w = 2 % of the filler. There are no visible agglomerates of the globular aggregates within the polymer matrix. On the other hand, the aggregates were not disaggregated during the mixing procedure.

The mechanical properties of the prepared composites were characterized with tensile tests, and the Young's modulus, elongation at break and tensile strength were chosen as the three representative quantities for evaluating the filler-amount effects on the composite performance. As summarized in **Table 1**, no significant changes in the mechanical properties were observed across all the samples. Thus, it can be concluded that (i) the incorporation of the inorganic ZnO filler up to w = 3 % did not change the properties of the

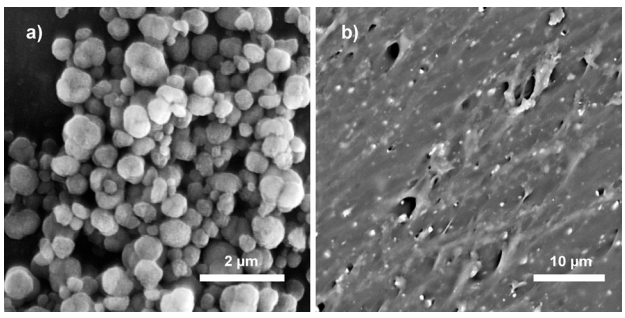

Figure 3: SEM images of: a) prepared ZnO filler and b) cross-section of the prepared composite material with w = 2 % of ZnO filler
Slika 3: SEM-posnetka: a) pripravljeno ZnO-polnilo in b) prerez pripravljenega kompozitnega materiala z masnim deležem ZnO-polnila 2 %

neat medical-grade plasticized PVC matrix which was already designed by its producer to be an optimally flexible material for medical devices such as urinal catheters or blood bags, and that (ii) the filler had no adverse influence on the application potential of the prepared antibacterial composite.

Table 1: Summary of the selected mechanical properties of the prepared composites and their standard deviations
Tabela 1: Povzetek izbranih mehanskih lastnosti pripravljenih kompozitov in njihov standardni odmik

Concentration of ZnO filler (*w*/%)	Young's modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
0	9.9 ± 0.2	501 ± 17	19.1 ± 0.9
0.5	9.8 ± 0.2	494 ± 30	19.0 ± 1.1
1	10.3 ± 0.3	500 ± 20	19.4 ± 0.8
2	10.1 ± 0.3	497 ± 9	19.4 ± 0.8
3	9.2 ± 1.0	520 ± 20	18.4 ± 0.4

The quantitative evaluation of the surface antibacterial activity of the as-prepared ZnO/PVC composites is summarized in **Table 2**. The obtained *R* values representing the antibacterial activity of the composites against both bacteria are high enough for the composites filled with $w = 2$ % and 3 %. The *R* value should not be lower than 2 for the materials that can be classified as the ones exhibiting an antibacterial surface suitable, for instance, for hygienic applications or less demanding applications. However, medical devices are more demanding with respect to the antibacterial performance of the used materials. The *R*-values of 5 or even 6 are expected for current high-end commercial materials, especially those with organic additives intended to be used in indwelling medical devices.[3] The surface antibacterial activity of the composites containing $w = 2$ % or 3 % of the filler against *E. coli* meet these highest requirements, while the activity against *S. aureus* is not so high. A similar antibacterial performance was recently obtained for the antibacterial systems containing hybrid Ag/ZnO filler employing nanosilver in addition to nanostructured ZnO.[14] On the other hand, *S. aureus* is a very vital and resistant bacterium, so it is difficult to suppress it and inhibit its growth. Concurrent studies reported reductions with respect to the control samples by about 70–95 % for much higher nano-ZnO loadings in PVC matrices, which means that the *R* values of less than 2 were claimed as success by their authors.[4] However, other strains were used throughout these studies and the surface-activity-estimation methods were similar but not identical to ISO 22196: 2007. From this point of view, and with a full awareness of the peculiar comparability among different published results, the performance of our prepared composites seems to be sufficiently high. Next, as the bacteria gain antibiotic resistance, the organic molecular additives in antimicrobial polymer systems may be found to be inefficient or ineffective and the inorganic nanofillers will serve as the last line of defense.

4 CONCLUSIONS

The present study describes a microwave-assisted synthetic route leading to the development of a hierarchical nanostructured ZnO mesoscale filler. The synthesized powder was clearly identified to have a ZnO hexagonal wurtzite structure and globular morphology of the aggregated nanocrystals. The composite materials compounded of medical-grade softened PVC and an as-prepared filler showed excellent antibacterial-activity values against *E. coli* and satisfactory antibacterial-activity values against *S. aureus*. Moreover, the obtained composites kept the mechanical properties of the neat PVC resin suitable as they were deteriorated neither by the processing nor by the effects of the nanofiller. These facts suggest that the prepared nanostructured mesoscale ZnO/PVC composite has an application potential in medicine as the material for medical devices being in the direct contact with the human body, besides many other possible utilizations.

Acknowledgment

The authors wish to thank for the internal grant of TBU in Zlin, No. IGA/FT/2013/026, funded from the resources for specific university research.

This article was written with the support of the Operational Program "Research and Development for Innovations" co-funded by the European Regional Development Fund (ERDF) and the national budget of the Czech Republic, within the Centre of Polymer Systems project (reg. number: CZ.1.05/2.1.00/03.0111).

This article was written with the support of the Operational Program "Education for Competitiveness"

Table 2: Summary of the surface-antibacterial-activity-evaluation results obtained for the prepared composites
Tabela 2: Povzetek rezultatov ocene površinske protibakterijske aktivnosti za pripravljene kompozite

Concentration of ZnO filler (*w*/%)	Treated specimens after 48 h *E. coli* *N*/(cfu cm^{-2})	Treated specimens after 48 h *S. aureus* *N*/(cfu cm^{-2})	Antibacterial activity (lg CFU) *E. coli*, $R = U_t - A_t$	Antibacterial activity (lg CFU) *S. aureus*, $R = U_t - A_t$
0	4.0×10^6	7.5×10^4	$U_t = 6.6$	$U_t = 4.9$
0.5	1.5×10^5	8.0×10^4	1.4	0
1	1.7×10^5	1.0×10^0	1.4	4.9
2	< 1	2.2×10^0	> 6.6	4.5
3	< 1	1.3×10^0	> 6.6	4.8

co-funded by the European Social Fund (ESF) and the national budget of the Czech Republic, within the Advanced Theoretical and Experimental Studies of Polymer Systems project (reg. number: CZ.1.07/2.3.00/20.0104).

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