COMPOSITE MATERIAL BASED ON HYBRID MICRO-SIZED Ag-ZnO FILLER FOR ANTIBACTERIAL APPLICATIONS

Pavel BAŽANT, Zuzana KOŽÁKOVÁ, Ondřej HUDEČEK, Michal MACHOVSKÝ, Miroslav PASTOREK, Ivo KUŘITKA

Centre of Polymer Systems, Faculty of Technology, Tomas Bata University in Zlín, T. G. Masaryka 5555, 760 01 Zlín, Czech Republic, bazant@uni.utb.cz

Department of Polymer Engineering, Faculty of Technology, Tomas Bata University in Zlín, Nam. T. G. Masaryka 275, 762 72 Zlín, Czech Republic

Abstract

Microwave hydrothermal synthesis is a fast, simple, and reproducible method which does not require necessarily any template, catalyst, or surfactant and can control the morphology of Ag-ZnO crystals from simple to complex. Here, it was used for preparation of micro-sized hybrid filler. Zinc acetate and silver nitrate were used as starting material for microwave-assisted synthesis and hexamethylenetetramine (HMTA) was chosen as a precipitation and reduction agent. Ag-ZnO structures were obtained in a good yield at a relatively low temperature within several minutes. This hybrid Ag-ZnO filler was mixed up in PVC matrix. A series of materials with different mass concentration of filler was prepared ranging up to 5 wt%. The antibacterial activity of the final product was tested against Staphylococcus aureus and Escherichia coli according to ISO 22196 standard. Relation of content of filler and antibacterial activity against representative strains of Gram-positive and Gram-negative bacteria was investigated. X-ray diffraction (XRD) method and scanning electron microscopy (SEM) were used for characterization of composition, structure and morphology of Ag-ZnO microparticles.

Keywords: microwave synthesis, hybrid filler, antibacterial activity, Ag-ZnO

INTRODUCTION

A strong pressure on advanced materials development is evolved in medicine due to enormous increase of infection rate and mortality related to nosocomial infections caused by bacteria adherent on medical devices. The use of antimicrobial agents as additives in plastic materials for medical devices and other applications is conceptualised in so called Antimicrobial polymer systems (APS), which are materials consistent from a polymer matrix loaded with mineral and organic additives providing the antimicrobial function while the matrix gives the shape and mechanical properties to the item. APS must comply different properties e.g.: low toxicity to human, animals, compatibility with processing aids and other additives, no negative impact on the properties and appearance of the plastic article, storage stability and long-lasting efficacy. [1]

As various bacterial strains have acquired drug resistance against antibiotics and other organic bactericidal or bacteriostatic agents, the attention is focused on inorganic materials as additives. Among them, hybrid metal-semiconductor materials have arisen a great interest because of their complexity of optical, electrical, magnetic, antibacterial and other properties. Recently, various techniques are studied for preparation of novel hybrid systems with specific design of their functional properties, thus new application fields are opened [2-4]. Several works have been published on synthesis of various Ag-ZnO nanocomposites and on their antibacterial activity against gram-positive and gram-negative bacteria. [2, 5-7]. The strong antibacterial activities of both metallic Ag and Ag⁺ ions have been known for a long time [8-10]. Zinc oxide (ZnO) is another inorganic antibacterial agent which, in form of nanoparticles, exhibit strong antibacterial activities on a broad spectrum of bacteria although its effect on bacteria is not fully understood yet. [11]. Ag shows better antibacterial activity against gram-negative than gram-positive bacteria, while ZnO shows better antibacterial performance against gram-positive than gram-negative bacteria. [12-14] Moreover, the combination of these
two materials in nanocomposite form exhibit enhanced activity due to synergetic effects between both kinds of nanoparticles. [5]

Hence, we offer an original method for preparation of hybrid Ag-ZnO filler by microwave assisted synthesis (MW). Preparation of hybrid nanostructures Ag-ZnO by MW was already reported by Bhattacharyya et al 2008 [15], where they used microwave polyl synthesis in a 15 min reaction conducted under argon atmosphere. Another attempt to use MW was presented by Karunakaran et al. [2] where they work with microwave domestic oven in cycle mode: on for 30 s and off for 30 s. However, in this paper is present preparation of hybrid Ag-ZnO nanoparticles by microwave domestic oven with reflux cooling system for 5 minutes. This method is very easy, safe and feasible open vessel solvothermal synthesis from water solutions of simple chemicals.

In next, a new APS based on prepared Ag-ZnO filler was developed using medical grade Poly (vinyl chloride) (PVC) as the matrix. PVC is the most widely used material use in medical devices. Major medical uses of PVC include intravenous fluid bags and tubing, blood and plasma bags, enteral feeding and dialysis equipment, catheters, and gloves [16, 17]. This material is well processable and relative cheap. The broad application window of medical-grade PVC as a leading biomedical polymer underlies the motivation of choosing this material.

EXPERIMENTAL

2.1 Materials

The starting materials silver nitrate AgNO₃ (purum, ≥99.5%) and zinc acetate dehydrate Zn(CH₃COO)₂•2H₂O (purum, ≥99%) were purchased from Penta (Prague, Czech Republic), polyvinylpyrollidon PVP (Mw = 40,000) was purchased from Sigma-Aldrich (Prague, Czech Republic). Hexamethylenetetramine (HMTA) C₆H₁₂N₄ (purum, ≥99%, Fluka) was purchased from Sigma-Aldrich (Prague, Czech Republic) and used both as precipitation agent and growth modifier. Demineralised water was used overall in these experiments. Medical grade plasticized PVC RB3 (Modenplast Medical, Italy) was used as polymer matrix.

2.2 Synthesis of Ag-ZnO particles

A domestic oven (CWR-TECH, 1150W/230V-50Hz) was modified by drilling a hole on the ceiling for open vessel solvothermal synthesis with external cooler. In the typical procedure, the starting materials were dissolved in water as follows: The solution (I) 10.8 g Zn(CH₃COO)₂•2H₂O was mixed together with solution (II) 0.699 g AgNO₃, and then with solution (III) 0.721 g PVP. The total amount of used water was 100 mL. The obtained solution (I+II+III) was placed in a 250 mL reaction bottle into the microwave oven cavity. The reaction mixture (I+II+III) was heated for 2 minutes then a solution of 6.998 g C₆H₁₂N₄ in 50 mL water was added quickly through the dropping system and microwave heating continued for another 3 minutes. The system was left to cool down naturally after switching of microwaves. Finally, the particles were collected by microfiltration and washed by demineralised water. Obtained powder was dried in a laboratory oven.

2.3 Characterization

The phase structure of filler particles was characterized by X-ray diffractometer PANalytical X’Pert PRO (PANalytical, The Netherlands) using Cu Ka1 radiation (λ = 1.540598 Å) operating at 40 kV and 30 mA. The crystalline phase composition was evaluated by the software PANalytical X’Pert High Score using normalized RIR method. The RIR is the ratio between the integrated intensities of the peak of interest and that of a known standard [18]. The morphology of the products was investigated by scanning electron microscope Vega II LMU (Tescan, Czech Republic) with beam acceleration voltage set at 10 kV.
2.4 Compounding of filler and polymer

Various amounts of prepared fillers were melt-mixed with PVC by the use of micro compounder HAAKE MiniLab II (Thermo Scientific) so the final filler loads were 0.5, 1, 3, 5 wt%. PVC pellets were mechanically premixed with the required amount of filler and fed into the compounder at the rate of 2 g of material per minute. The process was performed at 160 °C and 70 rpm. By running the instrument in circulation mode, the mixture homogenisation was controlled by measuring the torque of the drive motor and the pressure in the backflow channel. The compounding time of 10 min was sufficient for achievement of constant torque and pressure signals in all compounded samples. At the end of the mixing, the bypass valve was opened and the sample was extruded as a strand. Then, prepared PVC mixtures were compression molded for 3 minutes at 160 °C and then cooled in a nother press. Obtained 0.5 mm thick sheets were used for the preparation of samples for measurements. Reference (blank) samples without filler were prepared in the same way.

2.5 Antibacterial activity

Material was tested for its antibacterial activity against Staphylococcus aureus CCM 4516 and Escherichia coli CCM 4517 as representatives of Gram-positive and Gram-negative bacteria. The effect antimicrobial agent against bacterial grown in culture was tested according to the ISO 22196: 2007 (JIS Z-2801) standard. Size of test specimens was chosen 50 mm x 50 mm x 0.5 mm. The antibacterial activity \( R \) was calculated using Equation (1)

\[
R = (U_t - U_d) - (A_t - U_d) = U_t - A_t
\]

Where \( R \) is the antibacterial activity; \( U_d \) is the average of the logarithm of the number of viable bacteria, in cells/cm², recovered from the untreated test specimens immediately after inoculation; \( U_t \) is the average of the logarithm of the number of viable bacteria, in cells/cm², recovered from the untreated test specimens immediately after 24 h; \( A_t \) – is the average of the logarithm of the number of viable bacteria, in cells/cm², recovered from the treated test specimens immediately after 24 h.

RESULTS AND DISCUSSION

3.1 Ag-ZnO nanostructured microfillers

A SEM image of the as-prepared Ag-ZnO particles is shown in Fig. 1. The image was taken by BSE detector that allows distinguish composition of particles by material (greyscale) contrast showing heavier elements brighter. It can be seen that the zinc oxide particles are hexagonal truncated prisms with the size up to 1µm and silver particles are of spherical shape with the diameter up to 200 nm.

Fig. 1 BSEM microphotographs of the Ag⁺ and Zn²⁺.

Fig. 2 XRD patterns of the Ag/ZnO particles.
The composition of crystalline material was verified by powder X-ray diffractometry (XRD). Fig. 2 shows the XRD pattern of the Ag-ZnO particles. All peaks can be assigned to the Bragg reflections of the standard structure zinc oxide (JCPDS no. 01-079-0207) and face-centered-cubic silver (JCPDS no.01-087-0720). No other crystalline phase was found. Composition of crystalline phase was estimated as 34 %wt of Ag and 66 %wt of ZnO.

In spite of many advantages of MW synthesis described in introductory chapter, the method allows to process relatively small batches of reaction mixture. Therefore, synthesis was repeated five times in order to collect sufficient amount of filler to prepare all needed specimens for antimicrobial activity testing. The average yield was 1.2 g of dry powder per each synthesis.

### 3.2 Ag-ZnO PVC composites and their antibacterial activity

Medical grade PVC was loaded with hybrid filler Ag-ZnO 0, 0.5, 1, 3 and 5 %wt. Obtained materials showed good and homogenous dispersion of particles in polymer matrix No macroscopically visible marbled effects or even single stray streak were observed by careful inspection. Microscopic image obtained by SEM is shown in Figure 3 and testifies for good dispersion of filler too. Blank specimens were perfectly transparent with the characteristic very light tint of violet colour that is inherent to the raw PVC resin as delivered by the supplier.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Concentration Ag-ZnO filler in polymer [wt %]</th>
<th>Mean of the number of viable bacteria (CFU/ cm²) a</th>
<th>Antibacterial activity b (Log CFU reduction) c</th>
<th>% reduction d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The untreated specimens after incubation</td>
<td>The untreated specimens after 24 h</td>
<td>The treated specimens after 24 h</td>
<td>0.0081</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.5</td>
<td>3.89E+05</td>
<td>2.80E+08</td>
<td>2.75E+08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3.89E+05</td>
<td>2.80E+08</td>
<td>2.08E+08</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.57E+05</td>
<td>4.29E+08</td>
<td>2.44E+08</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.57E+05</td>
<td>4.29E+08</td>
<td>7.11E+02</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.5</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.34E+06</td>
<td>1.06E+08</td>
<td>9.98E+07</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.34E+06</td>
<td>1.06E+08</td>
<td>4.86E+07</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.34E+06</td>
<td>1.06E+08</td>
<td>1.78E+07</td>
</tr>
</tbody>
</table>

---

a The values for the untreated specimens after 24 h and the treated specimens after 24 h were significantly different according to the Student's test (P<0.05; n=3).
b The values calculated with reference to population of bacteria recovered from the untreated specimens after inoculation (prior to incubation).
c The log 10.
d %difference in the populations prior to and after incubation

The antibacterial effects of ZnO and silver nanomaterials are not fully elucidated yet, but the prevailing paradigm suggests various combinations of mechanisms: In the case of ZnO was proposed generation of hydrogen peroxide as one of the primary mechanisms [19]. In the latter case of silver nanoparticles following mechanisms are widely accepted. (1) Silver ion release followed by cellular uptake and a cascade of intracellular reactions, (2) extracellular and intracellular generations of ROS (reactive oxygen species), and (3) direct interactions between nano-scaled silver and cell membranes [20]. For APS are important not only antibacterial properties of the selected combination of fillers but the whole system including polymer matrix...
must assure reasonable release rate of active species to the surface of the plastic article. Quantitative assessment of antibacterial activity of the tested samples, the $R$-values and % reduction are shown in Table 1. The bacterial Log reduction value should not be less than 2.0 for an APS that can be categorized as having effective antibacterial surface as it is the minimal antibacterial activity according to ISO 22196:2007 and JIS Z2801:2000 [21]. This condition comply already material PVC with 3 wt% Ag-ZnO filler for bacteria \textit{E. coli}. However, the antibacterial activity of Ag-ZnO composite material against \textit{S. aureus} is lower than Log 2 even at the highest level (5 %wt) of filler content. This low performance is in contradiction with reported efficiency of ZnO against gram positive bacteria. [22] It seems that the release of active antibacterial species is somewhat blocked by the PVC matrix or the species are deactivated prior to their release from the material. Nevertheless, obtained results create framework for further improvement of the material under development. [23,24] The performance of the material loaded by 5 %wt of filler was proved as reasonable APS with respect to \textit{E.coli} (antibacterial activity of 5.8) and its performance against \textit{S. aureus} will be subjected to further improvements with respect to medical application.

CONCLUSION

An easy, safe and feasible open vessel microwave assisted method was introduced for synthesis of hybrid Ag-ZnO filler from water solutions of simple chemicals. Prepared filler consists of zinc oxide hexagonal prisms with the size up to 1μm and adjoined silver spherical particles with the diameter up to 200 nm. Composition of crystalline phase was estimated as 34 %wt of Ag and 66 %wt of ZnO. Ag-ZnO filler was mixed with medical grade PVC in order to create an antibacterial polymer system. Its performance against representative gram-positive and gram-negative bacteria was tested. The $R$-value is higher than 2.0 at PVC with 3 wt% and 5.8 with 5 %wt of Ag-ZnO against \textit{E. coli} and can be categorized as effective antibacterial surfaces (according to ISO 22196:2007 and JIS Z2801:2000). The antibacterial activity against \textit{S. aureus} is less than required minimum for application in antibacterial polymer systems. These findings suggest that the use of the hybrid Ag-ZnO filler may have potential applications; however it requires further increase performance against gram positive bacteria.

ACKNOWLEDGEMENTS

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

\textit{The work was supported by the grant project of the Ministry of Education, Youth and Sports of the Czech Republic (reg. number: MSM 7088352101).}

LITERATURE


[10] GOGOI, SK; GONANATH, P; PAUL, A; RAMESH, A; GHOSH, SS; CHATTOPADHYAY, A. Green Fluorescent Protein expressing Escherichia Coli as a Model System for Investigating the Antimicrobial Activities of Silver Nanoparticles. Langmuir, 2006 OCT 24, Vol.22(22), P.9322-9328. ISSN:0743-7463.


