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ANTIBACTERIAL PROPERTIES OF POLYPROPYLENE PVD-COATED WITH COPPER OXIDE

Key words: antibacterial coatings, antibacterial plastics, MS-PVD, material functionalization.

Abstract: Microbial biofilm formation called "bio-fouling" causes many both epidemiological as well as technological problems by increasing material and energy demand. Due to the fact of growing microbial resistance, currently used methods of prevention have become less effective. This paper focuses on the antimicrobial properties of copper oxide deposited on the most common synthetic polymer (polypropylene). The authors succeeded to coat raw as well as processed polymer with CuO by MS-PVD treatment without damages. Functionalization with CuO resulted in very effective antibacterial activity resistant to environmental conditions. Materials functionalized this way are considered as stable, non-specific, and widely effective. The activity observed by the authors supports this thesis.

Antybakteryjne właściwości polipropylenu pokrytego tlenkiem miedzi metodą PVD

Słowa kluczowe: pokrycia antybakteryjne, tworzywa antybakteryjne, MS-PVD, funkcjonalizacja materiałów.

Streszczenie: Powstawianie biofilmu mikrobiologicznego w przemyśle zwane biofoulingiem implikuje szereg problemów epidemiologicznych i technologicznych związanych ze wzrostem materiało- i energochłonności. Wzrost lekooporności mikroflory sprawia, że dotychczasowe metody przeciwdziałania drobnoustrojom stają się coraz mniej efektywne. Niniejszy artykuł skupia się na właściwościach antybakteryjnych tlenku miedzi osadzonego na powszechnie stosowanym syntetycznym polimerze – polipropylenie. W ramach przeprowadzonych prac z powodzeniem powierzchniowo zmodyfikowano badany polimer zarówno w formie surowca, jak również wykonanego z niego komercyjnego produktu. Modyfikator – tlenek miedzi (CuO) osadzono metodą MS-PVD oraz proces modyfikacji nie spowodował uszkodzenia badanych próbek. Przeprowadzona modyfikacja skutkowała bardzo skutecznym działaniem antybakteryjnym o długim czasie działania. Materiały zmodyfikowane w ten sposób są uważane za stabilne, nietoksyczne i niespecyficzne, co czyni je bardzo efektywnym rozwiązaniem. Uzyskane przez Autorów rezultaty są tego potwierdzeniem.

Introduction

Plastics are the group of synthetic materials combining low cost with good mechanical properties and ease of processability. They are widely used for the manufacturing of a wide range of products, e.g., food packaging, plumbing installations, biomedical devices, etc. In some applications, antibacterial properties are useful or even desirable. To obtain such plastics, there are three general approaches: plastics with bond antibacterial moieties, blends with antibacterial substances, and antibacterial coatings of plastics [1]. On the basis on their functionalized use against microorganisms, antimicrobial plastics can be classified into two different categories: anti-adhesive and biocide releasing surfaces [2]. The anti-adhesive plastics prevent microbial adhesion to the surfaces, such as coating the surfaces with a layer of polyethylene glycol (PEG) [3].

Even though these surfaces strongly reduce microbial adhesion, they never are 100% efficient [2]. Materials modified with triclosan, silver, and copper are most commonly used to prevent microorganisms from growth through the release of bioactive substances. Triclosan acts more as a disinfectant rather than an antibiotic, and its activity is not stable in time [4]. Silver releases ions or act as a contact active material, and this way it expresses its antimicrobial activity [2]. However, the unnecessary release of biocides may cause an increase in both environmental pollution and overall microbial resistance.

Copper is a reddish, ductile metal present in many natural niches like rock, soil, water, and sediments [5]. There are four possible oxidation states: solid metal Cu (0), cuprous ion (I), cupric ion (II), and rarely Cu (III) [6]. Copper has been known for its antimicrobial agent far before the discovery of microorganisms in the 19th century. Moreover, copper was used for the first time in medicine as a biocide by an Egyptian doctor, which was recorded around 2600 and 2200 BC [7]. The ancient Phoenicians used silver and copper bottles to store beverages and food. During the World War I, this metal was used to prevent wound infection as an addition to dressings [8]. Today, this metal and its alloys are widely used as chemical biocides in a wide range of purposes. There are two stable oxides: cupric oxide (CuO) and cuprous oxide (Cu_2O). Both of them are semiconductors and have band gaps in the visible or near infrared regions [9]. Copper generates many reactions resulting in the production of hydroxyl radicals through Fenton and Haber-Weiss reactions [10]. These radicals destroy cellular structures by causing numerous oxidation reactions of molecules such lipids and proteins [11]. Copper oxide is cheaper than silver, easily mixed with polymers, and exhibits a non-toxic nature, and its production costs are affordable, and there are smaller band gaps. Fungi, yeast, bacteria, and higher organisms, when exposed to high concentrations of copper, react in changes in the permeability barrier of the plasma membrane [12]. The breakdown of membrane integrity caused by copper oxides results in deteriorated cell viability or even disintegration. These reactions were proven for membranes of both Gram-positive and Gram-negative bacteria and lead to cell death [13]. Generally, Gram-negative bacteria are more sensitive to copper than Gram-positive bacteria, which is assigned to differences in cell wall construction [14].

The aim of this paper was to examine the antibacterial properties of polypropylene coated with CuO by MS-PVD plasma processing.

1. Material and methods

The experimental scope covered two types of samples to be modified and tested: raw polypropylene and capillary microfiltration membranes made of this polymer. This was to examine the behaviour in the plasma reactor for both a solid sample and a porous material of a delicate special structure. Raw polypropylene samples were outsourced and prepared by machine-cutting from the 5-mm thick sheet (made by 3M) in the form of 1” discs. Prior to the plasma treatment, the discs were washed with 96% ethanol in an ultrasonic bath (10 min.) and dried. Commercially available microfiltration membranes “Membrana” S6/2, prior to the plasma treatment, were cut into approx. 120 mm pieces. The samples were subjected to plasma treatment in a MS-PVD reactor made by The Institute for Sustainable Technologies – NRI in Radom (ITeE – PIB) shown in Fig. 1.

Fig. 1. Plasma reactor used for surface modification of polypropylene designed and built at ITeE – PIB in Radom (Poland)

The samples were inserted into suitable holders and subjected to argon plasma prior to the final coating in order to remove residues. The technological parameters of plasma treatment are shown in Table 1. On the basis of the author's experience with metal substrates to be coated, the greater current of the plasma source, the more intensive are the deposition processes, and thus the resulting coating layers are thicker. However, using the available equipment, the authors were unable to measure the thickness of the coating layers on the polymer substrate. Membranes having a complex spatial structure involving subtle polymer bands were exposed to plasma at shorter times. Longer process times might cause thermal degradation of the polymer. On the other hand, the surface-to-volume ratio in porous material (membranes) compensates thinner coating by the much greater total area of coated surface.

Table 1. Major parameters of plasma treatment processes

Sample type	Reactor pressure p [mbar]	Process gas composition	Process time t [min]	Source current [A]
CuO (PP discs)	$5x10^{-3}$	90%Ar, 10%O2	20	0.8
CuO 0,2A (PP membranes)	$5x10^{-3}$	90%Ar, 10%O2	2.5	0.2
CuO 0,4A (PP membranes)	$5x10^{-3}$	90%Ar, 10%O2	2.5	0.4
CuO 0,8A (PP membranes)	$5x10^{-3}$	90%Ar, 10%O2	2.5	0.8

After plasma treatment, prior to the testing, all the samples were washed with 96% ethanol in ultrasonic bath (10 min.) and dried. The solid PP samples were examined for the changes of the contact angle for deionized water and diiodomethane (CH_2I_2, Alfa Aesar) using the static sessile drop method by means of a goniometer made by ITeE-PIB. The values of surface free energy (SFE) were calculated with the control software provided with the goniometer according to the Owens-Wendt method. The surface free energy of a solid has a decisive effect on its wettability. Its knowledge also enables the contact angle, the work of adhesion and the interfacial tension with liquids with known properties to be roughly predicted. This information is relevant for processes such as coating, painting, cleaning, printing, hydrophobic or hydrophilic coatings, bonding, dispersion, etc.

The antibacterial activity was evaluated relative to the sample type: the membranes were examined using the ASTM E2149-13a standard procedure (dynamic contact), whereas solid PP discs were tested according to the method developed by the authors. The uses of different methods were caused by the fact that solid and heavy raw PP samples (> 1g) are inadequate for evaluation according to the ASTM method. In the static--contact method, each disc (autoclaved at 121˚C, 20 min) was placed into a 100 ml bottle (Duran) with a modified side facing up. 0.9 ml of the buffer (0.3 mM KH_2PO_4) was added on the side the disc ensuring proper humidity inside the bottle. 0.1 ml of bacterial overnight culture of *Escherichia coli* ATCC 25922 or *Bacillus Subtilis* ATCC 6633 in TSB (tryptic soy broth) were placed on the disc surface and the twisted bottles were carefully placed into an incubator (37˚C) for 1 hour. Then, 19 ml of sterile buffer was aseptically added to the bottle and vortexed to resuspend bacteria. The resulting suspension (20 ml) was examined for CFU (*colony forming units*) by a standard plate method and compared to the control (unmodified material) for the evaluation of antimicrobial efficiency.

To estimate the coating stability, the suspensions after ASTM-testing of capillary membranes were also examined for Cu concentration (leaching) using cuvette tests LCK329 (0.1-8 mg/L) by HACH-Lange and measured according to the manufacturer's requirements on HACH DR6000 spectrophotometer.

2. Results and discussion

Both raw polypropylene and membranes were successfully coated with CuO without any visual signs of thermal damage. Polypropylene coated with CuO revealed significantly increased contact angle values (CA) for water and diiodomethane, which resulted in the lower calculated surface free energy (SFE) as shown in Fig. 2. Depending on the specific final application, this change may be either positive or negative. From the microbiological point of view, the role of surface free energy of the material depends on the surface free energy of the bacteria and the ionic strength of liquid, because the surface free energy influences the adhesion and also depends on these two factors. Bakker et al. [15] showed the different patterns of the adhesion of different bacterial strains to materials. Some stains revealed a decreasing affinity to materials with increasing surface free energy; whereas, three strains isolated from a marine environment exhibited a stronger affinity for substrata of greater SFE.

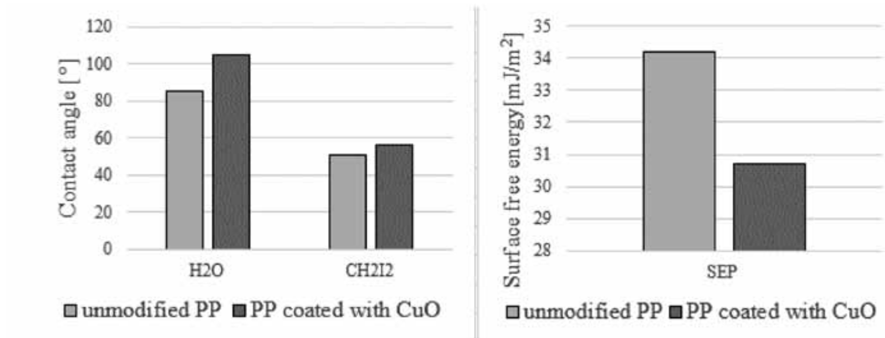

Fig. 2. Contact angle values and surface free energy of unmodified PP and PP coated with CuO

The study on antibacterial activity of PP discs coated with CuO revealed a very good efficiency in the elimination of both model G+ and G- bacterial strains (*Escherichia coli* and *Bacillus subtilis,* respectively) used in a static-contact method. The CFU measured for suspensions inoculated with *E. coli* was zero, which indicated a total reduction of bacterial growth (Fig. 3); whereas, for *B. subtilis,* the reduction was significant, but some bacterial cells survived (Fig. 4). No antibacterial effect was observed for unmodified polymer. This observation confirms the claims of greater sensitivity of G- bacteria to antimicrobial factors [14].

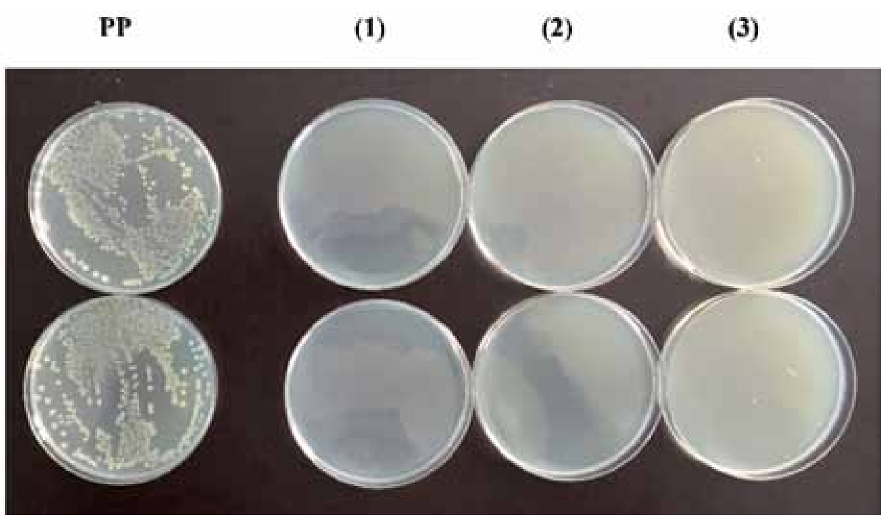

Fig. 3. The results of the plate assay for E. coli suspension after 1-hour contact with the sample (samples 1-3 in duplicates); PP (unmodified) as a control

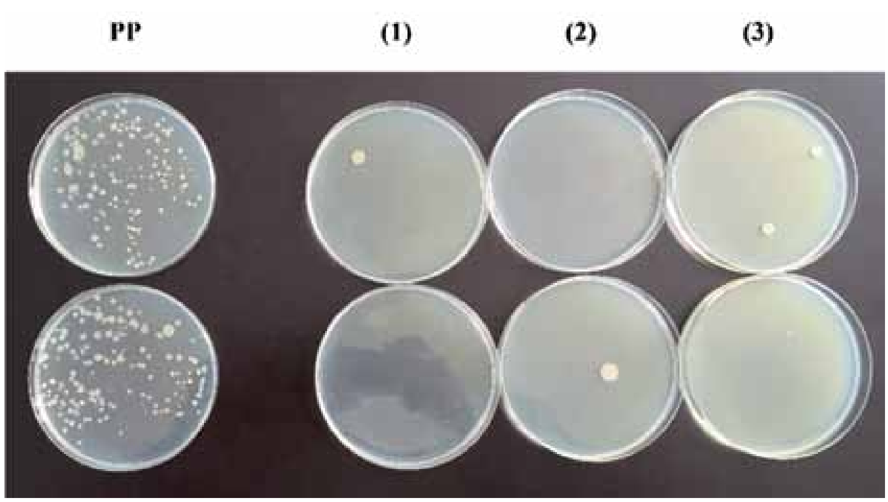

Fig. 4. The results of the plate assay for B. subtilis suspension after 1-hour contact with the sample (samples 1-3 in duplicates); PP (unmodified) as a control

The same good results were recorded for membranes made of polypropylene and coated with CuO in three plasma processes differing in the source current (0.2; 0.4 and 0.8A), evaluated according to the ASTM E2149--13a using only one reference strain of *Escherichia coli* defined in the procedure. After the 1-hour shaking with samples, there was no antibacterial activity observed for unmodified PP compared to the control (no sample) and 100% bacterial reduction for all samples modified with CuO, irrespective of the plasma treatment parameters (Fig. 5). In order to evaluate the stability of such promising properties, the authors decided to repeat the test two more times, using the same samples (rinsed and autoclaved in between). Even in the third consecutive test run, all samples modified with CuO exhibited the same total antimicrobial activity against *E. coli* (Fig. 5), with almost no visual change of the tested material.

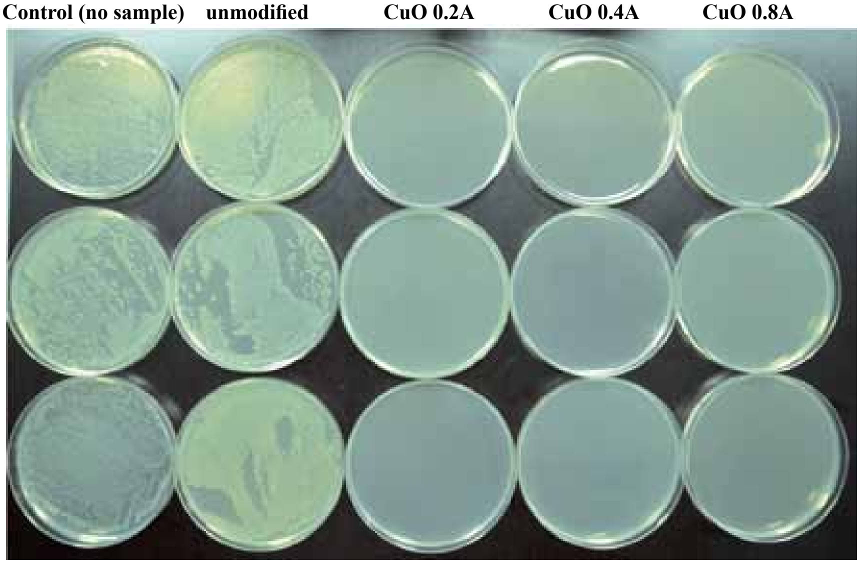

Fig. 5. The results of the ASTM E2149-13a test for PP membranes coated with CuO using different source currents

The high efficiency of antimicrobial activity recorded in both static and dynamic conditions suggested the release of biocide from the material rather than contact action. In order to evaluate the intensity of leaching, the bacterial suspensions used in ASTM E2149 tests were immediately examined with cuvette tests measuring the concentration of total cuprum. The analysis showed the strong relationship between the thickness of coating and the Cu concentration in suspension after 1h of shaking with samples (Table 2). It may be concluded that, in some purposes, in order to limit the leaching while maintaining good antimicrobial activity, the thin layers of CuO are sufficient, which is additionally less invasive to the material and the products made of it (no deformation and degradation of the modified PP discs and membranes).

Table 2. The intensity of Cu release by the coatings deposited under different plasma source currents

Coating type:	Concentration of Cu ions in the test buffer after ASTM E2149-13a test run [mg/L]
(unmodified)	0
CuO 0.2A	0.893
CuO 0.4A	1.110
CuO 0.8A	2.270

Conclusions

Polypropylene as one of the most popular synthetic polymers, and when modified with CuO, it exhibits very promising antibacterial properties that may be implemented in many market products. High efficiency, moderate toxicity and the ease of introduction of this compound makes it especially attractive in those applications where undesired (and often pathogenic) microflora may get in contact with the material decreasing the epidemiological risk. Because of its non-specific properties against different species (which was also widely reported in the literature), the functional coatings based on CuO are in-line with the current worldwide trend for sustainable solutions for preventing drug-resistant microorganisms.

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