Preparation of Antibacterial Silver Containing Nanocomposites

A. Kuzminova, J. Kratochvíl, A. Shelemin, O. Kylián, and H. Biederman

Charles University Prague, Faculty of Mathematics and Physics, Prague, Czech Republic.

J. Beranová

Charles University in Prague, Faculty of Science, Viničná 5, 128 44 Prague 2, Czech Republic.

Abstract. Silver containing nanocomposites receive increasing attention as antibacterial coatings. In this study we report on production of such materials using combination of gas aggregation sources of Ag nanoparticles and plasma enhanced chemical vapour deposition performed in HMDSO/O₂ working gas mixtures. The main attention is devoted to the investigation of solubility of Ag nanoparticles in water in dependence on the properties of matrix material and determination of antibacterial properties of produced films.

Introduction

Increasing resistance of certain bacterial strains to common antibiotics becomes serious problem in public health. Because of this there is an urgent demand to develop alternative bactericides. One of the materials that experiences renewed and increasing attention is silver, whose good antibacterial properties are known since ancient times. Although the exact bactericidal mechanism of silver is still not fully understood, it is supposed that the antibacterial nature of silver is predominantly connected with its ability to release silver ions that subsequently interact with vital enzymes or bacterial DNA or are capable to destroy irreversibly cell membranes of pathogenic organisms and hence inhibit their growth [Morones et al., 2005; Marambio–Jones and Hoek, 2010; Eckhardt et al., 2013].

Recently, various nanocomposite coatings consisting of silver nanoparticles (AgNPs) inside a matrix of plasma polymers have been intensively investigated [e.g., Zaporojchenko et al., 2006, Sardella et al., 2006; Körner, et al., 2010; Lischer et al., 2011; Saulou et al., 2012]. In this case the plasma polymers serve as a reservoir for the out-diffusion of silver ions that are produced in aqueous environment by oxidative dissolution process involving protons and dissolved oxygen [Liu and Hurt, 2010]:

\[ 2\text{Ag}(s) + \frac{1}{2}\text{O}_2(aq) + 2\text{H}^+(aq) \rightarrow 2\text{Ag}^+(aq) + \text{H}_2\text{O} \quad (1) \]

The advantage of use of plasma polymers as matrix material is connected both with their very good adhesion to various substrates and possibility to regulate Ag⁺ ion release by properties of plasma polymerized matrix, e.g. its cross-link density, wettability or chemical structure [Vasilev et al., 2011].

The common ways of production of Ag/plasma polymer nanocomposites are co-sputtering or sputtering combined with plasma-enhanced chemical vapor deposition (PE-CVD) of plasma polymers. In this study we investigate an alternative method, which is based on combination of PE-CVD process used for production of matrix material with deposition of AgNPs by means of a gas aggregation nanocluster source. The produced films are examined with respect to their chemical composition, wettability as well from the point of view of their solubility in water, which is determined by analyzing the changes in the UV-Vis spectra. Finally, antibacterial properties of received coatings are investigated by means of biological tests.

Experimental details

Samples deposition

Deposition system, which is schematically presented in Figure 1a, was used for the preparation of thin films of plasma polymerized hexamethyldisiloxane (HMDSO). It consisted of high vacuum chamber pumped by diffusion and rotary pumps, RF planar electrode operated at frequency 13.56 MHz and load-lock system for introduction of samples into the deposition chamber. The precursor HMDSO (Sigma) was thermally stabilized and vaporized outside the apparatus. The flow of HMDSO was in all experiments 0.25 sccm. When using oxygen, the O₂ was mixed with HMDSO and fed into the deposition chamber. O₂ flow was varied from 0 sccm up to 15 sccm, which corresponds to HMDSO/O₂ ratios up to 1:60. Pressure during deposition was 40 W and applied RF power during the depositions was 40 W. Optical emission spectroscopy was used for characterization of processing plasma. Optical signal emitted by the plasma was collected through the diagnostics window and analyzed by Avantes spectrometer in the range 250–850 nm.

A gas aggregation source (GAS) was used (Figure 1b) for deposition of silver nanoparticles. It consisted of water cooled aggregation chamber ended with conical lid with orifice 2 mm in diameter. Inside the gas aggregation chamber DC planar magnetron equipped with Ag target was placed. The pressure inside the gas
aggregation chamber was 30 Pa, magnetron current was 0.1 A and deposition time was 2 min. Under these conditions sub-monolayer of Ag nanoparticles with mean diameter 12 nm was deposited. More details about employed GAS can be found in previous study [Polonskyi et. al., 2012]. In case of fabrication of nanocomposite coatings we used sequential deposition of layers of plasma polymers and Ag nanoclusters.

Characterization of samples
Various techniques were employed for the characterization of prepared thin films of plasma polymers, Ag nanoclusters and their nanocomposites. The thickness of films was determined by means of spectroscopic ellipsometry using a variable angle spectroscopic ellipsometer (Woolam M-2000DI) in the wavelength range of $\lambda = 192$–1690 nm at an angle of incidence $\text{AOI} = 55$–75° in air and at room temperature. The chemical composition of deposited coatings was analyzed by X-Ray Photoelectron Spectroscopy (XPS, Phoibos 100, Specs) using an Al Kα X-ray source (1486.6 eV, Specs). The XPS spectra were acquired in the energy range 0–900 eV and recorded using 40 eV pass energy (step 0.5 eV, dwell time 0.1 s). Wettability of prepared samples was evaluated by means of a sessile water drop method. The optical properties were studied by UV-Vis spectrophotometer (Hitachi U-2900) in the spectra range 325–900 nm. The changes in UV-Vis spectra may be used as simple and fast method to estimate silver ion release from the coatings [Alissawi et al., 2012].

Evaluation of antibacterial character of prepared films
Gram negative bacterium *E. coli*, strain K12 (laboratory stock) was employed as experimental microorganism for assessment of antibacterial activity of produced nanocomposite materials. Bacteria were cultivated in Luria broth (LB) at 37 °C until mid-exponential phase of growth (optical density at 450 nm ca. 0.5). The culture was then diluted 1:10 in sterile distilled water and 1 ml was placed on the surface of LB agar plate. After all liquid soaked into the agar, a glass disc coated by silver containing nanocomposites (sterilized by UV light for 30 min from each side) was placed onto the inoculated agar surface (the coated side facing down). Glass coated with films of plasma polymerized HMDSO and SiO$_x$ served as negative controls. After overnight incubation at 37°C, the plates were photographed and the inhibition zones (clear zones where bacteria did not grow) around discs were measured.

Results and discussion
Deposition of thin films of plasma polymerized HMDSO and SiO$_x$ and their characterization
The first step in this study was characterization of plasma sustained in different working gas mixtures as well as determination of properties of deposited coatings.

It was found that changes of working gas mixture had strong impact on the composition of plasma as monitored by means of optical emission spectroscopy. As can be seen in Figure 2, only spectral lines of atomic hydrogen and weak CH molecular system were present in the emission spectra of plasma sustained in pure
Absence of oxygen lines or bands belonging to oxygen containing species (e.g. CO and OH) in this case suggests very low level of fragmentation of HMDSO molecule in the plasma bulk [Lamendola et al., 1997]. Addition of oxygen to HMDSO resulted in rapid disappearance of CH molecular band from the emission spectra and appearance of bands belonging to CO, OH a CO$_2$ and lines of atomic oxygen. This behaviour may be explained by interaction of oxygen either with HMDSO molecule or its fragments produced in the plasma. However, further increase of oxygen fraction in the working gas mixture caused gradual decrease of intensities of spectral bands of CO, OH a CO$_2$. This is most likely the result of formation of oxygen containing species (e.g. CO$_2$, SiO$_2$) difficult to be detected by optical emission spectroscopy.

Above described changes in plasma composition naturally influenced also the chemical composition of coatings deposited using different working gas mixtures. This is demonstrated in Figure 3, for limiting cases of films prepared in pure HMDSO and in mixture HMDSO/O$_2$ 1:60. As can be seen, films deposited in pure HMDSO have structure typical for plasma polymers with high fraction of carbon, whereas coatings prepared in mixture HMDSO/O$_2$ 1:60 exhibit glass-like structure with negligible carbon content and Si to O$_2$ ratio close to 0.5. These results are in agreement with previous studies using mixture of HMDSO and oxygen [Hegemann et al., 1999; Zajickova et al., 2001].

The different chemical composition of produced films subsequently leads to their different wettability: whereas films produced in pure HMDSO (these coatings will be denoted as pHMDSO in subsequent text) are hydrophobic with static water contact angle close to 100°, films produced in oxygen rich working gas mixtures (denoted as SiO$_x$) are highly hydrophilic with static contact angle of water around 15° (see Figure 4).
Deposition of silver containing nanocomposite films

The second step was fabrication of nanocomposite coatings. As mentioned above, the nanocomposites had layered structure with alternating layers of Ag nanoparticles and layers of pHMDSO or SiOx. In all cases the first and the last layer was either pHMDSO or SiOx. The presence of Ag nanoparticles in prepared nanocomposites was confirmed by presence of strong anomalous absorption peak of silver in recorded UV-Vis spectra (see Figure 5). Since SEM analysis of morphology of Ag nanoparticles deposited on pHMDSO or SiOx did not indicated any significant difference in their sizes and shapes (data not shown), the variations in position of Ag anomalous absorption peak in Ag/pHMDSO (405 nm) and Ag/SiOx nanocomposites (460 nm) may be ascribed to different dielectric constants of pHMDSO and SiOx, respectively.

In order to evaluate stability of prepared nanocomposites in aqueous environment, samples were immersed for 1 day into deionized water. Based on the results presented in Figure 5, following conclusions may be drawn.

First, only a slight red shift of absorption peak was observed for pHMDSO/Ag/pHMDSO nanocomposites (4 nm and 2 nm for 10 nm and 20 nm thick overcoats). Second, blue shift (10 nm) and decrease of intensity of Ag anomalous absorption peak was observed in case of SiOx/Ag/SiOx nanocomposite with 10 nm thick top layer of SiOx.

Since the position and shape of anomalous absorption peak of silver is very sensitive to size, size distribution and density of Ag nanoparticles in nanocomposites, observed changes may be interpreted as follows. In case of pHMDSO/Ag/pHMDSO nanocomposites, the red shift indicates increase of the mean size of Ag nanoparticles, which may be due to partial agglomeration of smaller Ag nanoparticles. In contrast, blue shift observed in case of SiOx/Ag/SiOx nanocomposite is connected with decrease of the mean size of Ag nanoparticles. This is most likely connected with oxidation of Ag nanoparticles by oxygen dissolved in water, which leads to release of Ag+ ions and consequently to partial dissolution of Ag nanoparticles. In addition, it was found that the effect of aqueous environment diminishes with increasing thickness of the top layer, which is in agreement with recent results [Alissawi et al., 2013]. These observations are highly important for design of the nanocomposite structure for optimal antibacterial performance that is strongly connected with capability of coatings to provide sufficient amount of bactericidal Ag+ ions. According to obtained results it can be expected that higher antibacterial effect will be exhibited SiOx based nanocomposites as compared to pHMDSO ones.

Preliminary evaluation of antibacterial effect of silver containing nanocomposite films

Final step in this study was evaluation of antibacterial properties of prepared coatings. Taking into account the results presented in the preceding section, i.e. dependence of the Ag+ ion silver release on the thickness of the matrix material, silver containing nanocomposites were prepared with different number (up to 5) of inter-layers of Ag nanoparticles separated by 7 nm thick layers of pHMDSO or SiOx. Figure 6 shows representative photographs of glass discs placed on LB agar surface inoculated with E. coli after overnight incubation. Distinct differences in antibacterial properties were observed in dependence on used matrix material as well as in dependence on the number of Ag inter-layers. Whereas for Ag/pHMDSO nanocomposites only an indication of formation of inhibition zone was detected, in case of Ag/SiOx nanocomposites the inhibition zone was clearly visible and its diameter increased with increasing content of Ag nanoparticles.

Conclusion

Layered nanocomposites containing Ag clusters embedded in hydrophobic (pHMDSO) and hydrophilic (SiOx) matrix were prepared by PE-CVD method combined with deposition of Ag nanoparticles by gas aggregation source. It was found that both the thickness and chemical structure of the matrix layer influence solubility of Ag clusters, and thus also silver ion release, in aqueous environment. More pronounced changes in UV-Vis spectra indicating faster release of Ag+ as well as better antibacterial performance were observed for
Figure 5. UV-Vis spectra of as deposited coatings and coatings immersed for 1 day in water. The graphs show UV-Vis spectra of pHMDSO/Ag/pHMDSO coatings with thickness of the top pHMDSO layer (a) 10 nm and (b) 20 nm and spectra of SiO\textsubscript{x}/Ag/SiO\textsubscript{x} nanocomposites with thickness of the SiO\textsubscript{x} layer (c) 10 nm and (d) 20 nm.

Figure 6. Photographs of glass discs placed on the LB agar surface inoculated with \textit{E. coli}, after overnight incubation. (a) discs coated with pHMDSO and Ag/pHMDSO nanocomposites and (b) discs coated with SiO\textsubscript{x} and Ag/SiO\textsubscript{x} nanocomposites. The diameter of discs was 12 mm.

SiO\textsubscript{x} matrix as compared to pHMDSO. This behaviour may be ascribed to different chemical structure and with it connected wettability of these two materials. Whereas SiO\textsubscript{x} is highly hydrophilic material, pHMDSO is hydrophobic. Hydrophobicity of pHMDSO may represent higher barrier for water penetration through it to Ag nanoparticles, which in turn limits the Ag\textsuperscript{+} ion release and antibacterial efficiency of pHMDSO/Ag coatings.

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References


