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POLYOL SYNTHESIS OF Ag NANOWIRES AND BIOCIDAL ACTIVITY OF THE OBTAINED PRODUCT

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Silver nanowires were prepared by the polyol process with ethylene glycol as a solvent and reducing agent, silver nitrate as a silver source, polyvinylpyrrolidone as a growth-directing agent, stabilizer and agglomeration prevention agent, and copper chloride as a growth control agent. The product was characterized by UV–Vis spectroscopy, scanning electron microscopy, and X-ray diffraction. The biocidal activity of silver nanowires was investigated using the disc diffusion method on the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa* and the fungi *Aspergillus niger* and *Candida albicans*. X-ray diffraction and scanning electron microscopy showed that, in addition to silver nanowires, the product also contains quasi-spherical silver nanoparticles and some silver chloride. Based on the results of UV–Vis spectroscopy and scanning electron microscopy, it was estimated that the diameter of the nanowires is 200–300 nm. Nanowires have shown weak bactericidal and good fungicidal activity. Silver nanowires showed particularly good activity in inhibiting the growth of *Candida albicans*.

Keywords: polyol method; silver nanowires; biocidal activity

ПОЛИОЛНА СИНТЕЗА НА Ag НАНОЖИЦИ И БИОЦИДНА АКТИВНОСТ НА ДОБИЕНИОТ ПРОИЗВОД

Извршена е синтеза на сребрени наножици со полиолен процес со етиленгликол како растворувач и редукционо средство, сребронитрат како извор на сребро, поли(винилпиролидон) како средство за насочување на синтезата, стабилизатор и како средство за спречување на агломерација и бакархлорид како средство за контрола на синтезата. Производот беше карактеризиран со UV–Vis спектроскопија, скенирачка електронска микроскопија и рендгенска дифракција. Биоцидната активност на сребрените наножици беше испитана со примена на методот на дифузија на диск на бактериите *Bacillus subtilis* и *Pseudomonas aeruginosa* и на фунгите *Aspergillus niger* и *Candida albicans*. Рендгенската дифракција и скенирачката електронска микроскопија покажаа дека покрај сребрените наножици производот содржи и квазисферни сребрени наночестички и малку сребрен хлорид. Врз основа на резултатите од UV–Vis спектроскопија и скенирачката електронска микроскопија беше проценето дека пречникот на наножиците изнесува 200–300 nm. Наножиците покажуваат слаба бактерицидна и добра фунгицидна активност. Сребрените наножици покажуваат особено добра инхибициона активност врз растот на *Candida albicans*.

Клучни зборови: полиолен метод; сребрени наножици; биоцидна активсот

1. INTRODUCTION

Microbial infectious diseases present a serious threat to human health, especially due to the resistance of bacteria to antibiotics because of their excessive use. Alternative biocidal products in the form of various organic and inorganic compounds used in the past proved to be toxic and harmful to

the environment. The development of nanotechnology has brought the improvement of old and the emergence of new solutions to this problem.[1,2] Owing to its strong bactericidal effect and low toxicity for human cells, nanosilver has been used as an antibacterial agent since the end of the 19th century.[3,4] In addition to the form of colloidal silver, which is still used today to treat skin infections, conjunctivitis, and allergic rhinitis, nanosilver is used to impregnate bandages and plasters and to prepare ointment intended to reduce the microbial load of wounds and burns, thereby reducing the risk from infection.[5] As many as three mechanisms for the bactericidal effect of silver nanoparticles have been proposed.[2] The mechanism based on the creation of silver ions by oxidation with oxygen or in some other way is most commonly mentioned. Free silver ions further act on the cell membrane of the bacteria, disrupting the integrity of the cell. According to another mechanism, reactive oxygen species are formed in the presence of nanosilver, which further act on bacterial cells. The third mechanism is reduced to direct damage caused by the adhesion of silver nanoparticles and silver ions to the surface of the bacterial cell.

Since they are the easiest to prepare, spherical nanoparticles are most often used for bactericidal purposes, while silver nanowires have recently attracted more attention. Since they have excellent conductivity and are suitable for the production of thin films, their potential use in the production of electronic circuits is investigated most often.[6] In contrast, the use of silver nanowires for biomedical purposes is much less investigated. However, recent studies indicate the potential use of silver nanowires in the impregnation of textiles for the treatment of wounds[7] and the preparation of thin films for the surface treatment of medical supplies such as catheters.[8] Due to their one-dimensional structure, silver nanowires have a larger width-to-length ratio than other nanosilver types and, therefore, somewhat different physicochemical properties. For example, they are less toxic, and it is believed that this could be why they may be more suitable for use in biomedicine.[2]

There are several methods of synthesis of nanowires, such as hydrothermal synthesis, redox synthesis, self-assembly, and the template method.[9] Template methods are particularly diverse, the initial approach was based on using hard templates such as various nanoporous structures, for example, porous aluminum oxide.[10] The use of hard templates makes it possible to obtain nanowires of targeted and uniform length and diameter, but the dissolution of the template often leads to damage to the nanowires.[11] In order to overcome the limitations of the hard template method, soft template methods were developed, and the most commonly used method from this group is the polyol method. Pioneering works on this procedure are those of Sun et al.[11,12] Ethylene glycol is the polyol (alcohol with more than one OH group) most commonly used in this process. The role of the polyol is twofold; it is both a solvent and the precursor of a reducing agent. Namely, at elevated temperatures, the polyol is oxidized to aldehyde, which reduces silver.[13] $AgNO_3$ is used as a silver precursor, while polyvinylpyrrolidone), PVP, is used as a soft template. The mechanism by which PVP acts as a soft template has not yet been fully elucidated.[2] It is believed that PVP, by binding carbonyl groups to {100} planes of initially formed silver particles, prevents the growth of these planes, while the growth of {111} planes is unhindered.[14] Due to limited growth in two spatial dimensions, silver particles grow in only one dimension. The polyol method is relatively simple and fast and gives good yields. Since the result of the synthesis is a suspension of silver nanowires, the product is a suitable agent for impregnation. The mentioned advantages of the polyol method compared to all others make this method the most common process for obtaining silver nanowires.

The original process of the polyol method consisted of dissolving PVP in ethylene glycol and then adding $AgNO_3$ to the mixture at a controlled rate, which is crucial for controlling the dimensions of the nanowires. In order to simplify the process, Korte et al.[15] proposed a method of slowing down the growth rate by adding trace amounts of salt. $CuCl_2$ proved to be a suitable salt since the Cu^{2+} ion binds oxygen atoms that would otherwise block the growth of silver nanowires, while the Cl^- ions control the rate of adding Ag^+ ions to the nanowires by forming AgCl whose slow decay defines the availability of Ag^+ ions.[16] The molar ratio between PVP and $AgNO_3$, the length of the PVP molecule, and the reaction temperature and time are also important for the outcome of the process.[13]

Unlike spherical silver nanoparticles, whose biocidal properties are relatively well studied, research on the biocidal properties of silver nanowires is still in progress. Silver nanowires proved to be effective antibacterial agents against *Escherichia coli* and *Staphylococcus aureus*. Biocidal activity, as well as the mechanism of action for these two microorganisms, can mostly be found in studies regarding Ag nanowires.[2,17] Silver nanowires are often loaded onto graphene oxide to prevent their aggregation in solution and increase

antibacterial activity. Some studies show that while silver nanowires delay and decrease the growth of *Escherichia coli*, silver nanowires loaded onto graphene completely inhibit the growth of *E. coli*, showing results similar to the commonly prescribed antibiotics.[2] In this paper, the bactericidal and fungicidal properties of silver nanowires were investigated using the disk diffusion method on microorganisms commonly found in the environment and relatively resistant to different conditions: the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa* and the fungi *Aspergillus niger* and *Candida albicans*. Silver nanowires were prepared using the polyol method, and the synthesis product was characterized using UV–Vis spectroscopy, scanning electron microscopy, and X-ray diffraction.

2. EXPERIMENTAL

2.1. *Synthesis*

The synthesis of silver nanowires was carried out according to the modified procedure of Naz et al.[18] Ethylene glycol, $HOCH_2CH_2OH$ (Carlo Erba, rpe), was used as solvent and reductant. Silver nitrate, $AgNO_3$ (Merck, pa), was used as the source of silver. Polyvinylpyrrolidone) $(C_6H_9NO)_n$ (Alfa Aesar, mw 58000) was used as a growth directing agent, stabilizer, and agglomeration prevention agent, while copper chloride dihydrate, $CuCl_2 \cdot 2H_2O$ (Merck, pa), was used as a growth controlling agent.

The synthesis procedure is shown schematically in Figure 1. First, 150 ml of ethylene glycol (denoted as EG in the figure) was added to a round-bottomed flask into which a condenser was inserted. The flask was immersed in an oil bath that was placed on a heating and stirring plate. The system was heated and stirred (260 rpm) at 160 °C under reflux for one hour. Then 2.3 mg of $CuCl_2$ was added to the flask. A solution of 1.936 g of polyvinylpyrrolidone) (PVP) in 5 ml of ethylene glycol was prepared separately. This solution was added to the flask 15 min after the addition of $CuCl_2$. The solution was added to the flask drop by drop using a separatory funnel; the addition lasted approximately half an hour. A solution of 0.48 g of $AgNO_3$ in 5 ml of ethylene glycol was also prepared separately. This solution was added to the flask drop by drop with a funnel. The addition lasted approximately half an hour. The solution was then allowed to stand at room temperature for two days. During this time, there was a separation of the phases into an upper yellowish phase and a lower cloudy white phase. According to the literature,[19] the upper phase consists predominantly of silver nanoparticles and the lower phase predominantly of silver nanowires. The phases were separated by decantation. The lower phase was then centrifuged at a speed of 4000 rpm for 10 min, and the precipitate was washed with acetone. The procedure was repeated once more, and then the sediment was transferred to a Petri dish to dry.

2.2. *Characterization*

The suspension of silver nanowires in ethylene glycol was analyzed by UV– Vis spectroscopy, and the dry precipitate was analyzed by scanning electron microscopy (SEM) and X-ray diffraction (XRD).

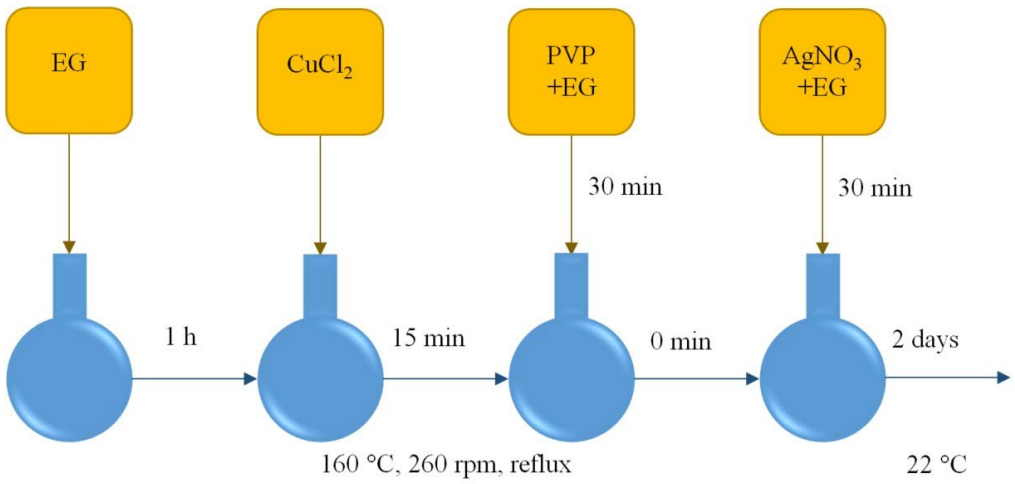

Fig. 1. Diagram of the synthesis procedure of silver nanowires using the polyol method

The interaction of the prepared suspension of nanowires with ultraviolet and visible radiation was investigated using a UV–Vis spectrophotometer, Cary 1E, Varian, USA, in the 300–700 nm wavelength range. The morphology of the sample was determined by scanning electron microscopy using a Tescan Vega 3 EasyProbe scanning electron microscope with a voltage of 10 kV. The obtained micrographs were analyzed using the ImageJ freeware. The phase composition of the prepared sample was determined using an X-ray diffractometer, Shimadzu XRD 6000. CuKα radiation, tube voltage of 40 kV, and current of 30 mA were used. Data were collected in the range of 2θ angles between 25° and 80° with a step of 0.02° and a dwell time of 0.6 s. The unit cell parameter, *a,* of the cubic crystal lattice of silver was calculated using the Unit Cell program.[20]

2.3. *Biocidal effects assessment*

The disc diffusion method was used to evaluate the biocidal effect.[21] This method, also known as the Kirby-Bauer test, is routinely used to determine the sensitivity of bacteria to antibiotics because of its speed and simplicity. The procedure consists of suspending a pure microbial culture in a saline solution and applying it to a solid nutrient medium in a Petri dish. A disk of filter paper is impregnated with a bactericidal agent at a certain concentration and placed on the surface of the agar. During the incubation time of 24 h at 37 °C, the agent diffuses into the substrate, and its concentration is the highest immediately next to the disc and decreases with increasing distance from the disc. If the bactericidal agent is at a certain concentration, it will effectively prevent the growth of microorganisms near the disc. The area where growth is stopped is called the zone of inhibition. Measuring the width of the zone of inhibition makes it possible to evaluate the effectiveness of a certain agent at a certain concentration for a certain type of microorganism. In the case of an ineffective biocidal agent, the inhibition zones will not be visible. A highly effective agent will create a wide ring without the growth of microorganisms.

For the analysis, the following test microorganisms were used: Gram-positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Pseudomonas aeruginosa,* and the fungi *Aspergillus niger* (a type of mold) and *Candida albicans* (a type of yeast). These microorganisms typically occur in the environment and are relatively resistant to various conditions, e.g., by forming biofilms, spores, etc. This makes them suitable for biocidal activity tests. Twenty milliliters of nutrient medium, whose thickness was about 3 mm, was poured into Petri dishes with a diameter of 90 mm. Microbial culture suspension (100 µl) was applied to the substrate surface. Filter paper disks with a diameter of 9 mm were then placed on the surface, impregnated with 20 µl of silver nanowires suspension in ethylene glycol with a concentration of 100, 20, and 10 $mg \cdot l^{-1}$, denoted A, B, and C, respectively. Three more suspensions with lower concentrations were prepared and denoted D, E, and F, but they were excluded from the further study since they showed no biocidal activity. Petri dishes were incubated at a temperature of 37 °C for 24 h. The bactericidal and fungicidal activity was evaluated by measuring the diameter of the zone of inhibition.

3. RESULTS AND DISCUSSION

Metal nanoobjects have the ability to selectively absorb visible radiation due to surface plasmon resonance. Surface plasmons are electron waves that propagate in a direction parallel to the phase boundary of metals and dielectrics. In the interaction of photons with surface plasmons, excitation of electrons occurs, which is called surface plasmons resonance. This process results in the absorption of visible radiation of certain wavelengths.[22] Considering the wide availability of UV–Vis spectrometers, absorption spectra are often used as the first indication of the formation of nanoparticles and also for a more complex analysis of the obtained product. The UV–Vis spectrum of the newly prepared nanowires suspension is shown in Figure 2.

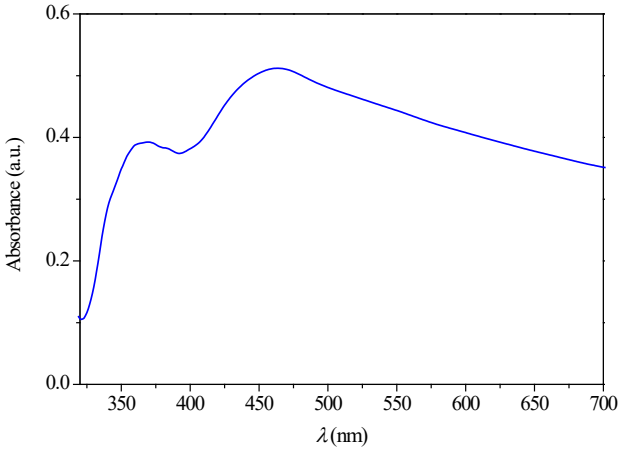

Fig. 2. UV–Vis spectrum of the prepared suspension of silver nanowires

For the case of nanoparticles, the wavelength of the absorption maximum of the nanoparticle suspension depends on the size, and it is greater the larger the particles are. Therefore, it is routinely used to estimate particle size. For example, absorption at 395 nm corresponds to nanoparticles with a size of 10 nm.[5] The absorption spectrum of nanowires is somewhat more complex since two absorption maxima appear.[17] In the spectrum shown in Figure 2, two absorption maxima are indeed noticeable, at approximately 370 and 460 nm, indicating the formation of nanowires. The maximum at 370 nm is considered to be a consequence of the plasmon resonance of macrosilver.[10] However, in the case of nanowires, it is associated with the resonance of surface plasmons in the longitudinal direction of the nanowires. On the other hand, the maximum at approximately 460 nm is attributed to surface plasmon resonance in the transverse direction of the nanowires.[23] Although it is not clearly visible, the existence of a maximum due to the plasmon resonance of spherical silver nanoparticles hidden under more intense maxima appearing due to nanowires cannot be ruled out. It follows that the prepared product contains silver nanowires, and the presence of silver nanoparticles cannot be dismissed.

Just as the position of the absorption maximum of the resonance of surface plasmons of silver nanoparticles depends on their diameter, the position of the maximum associated with the resonance of surface plasmons in the transverse direction depends on the diameter of the nanowire.[9] On the other hand, since the length of the nanowires is significantly longer than the diameter, it does not affect the position of the absorption maximum due to the resonance of surface plasmons in the longitudinal direction. Chen et al.[24] defined an equation that relates the plasmon resonance maximum of silver nanowires in the transverse direction, λ_{max}, to the diameter of the nanowire, d_{NW}, and it reads as follows:

$$\lambda_{max} = 361.3 + 0.41\, d_{NW} \qquad (1)$$

Todd and Chen[25] gave a slightly different equation:

$$d_{NW} = 999.6 + 2.791\, \lambda_{max} \qquad (2)$$

Placing the maximum value of 460 nm into the first equation gives an average nanowire diameter of approximately 240 nm, while using the same value in the second equation gives an average nanowire diameter of approximately 280 nm.

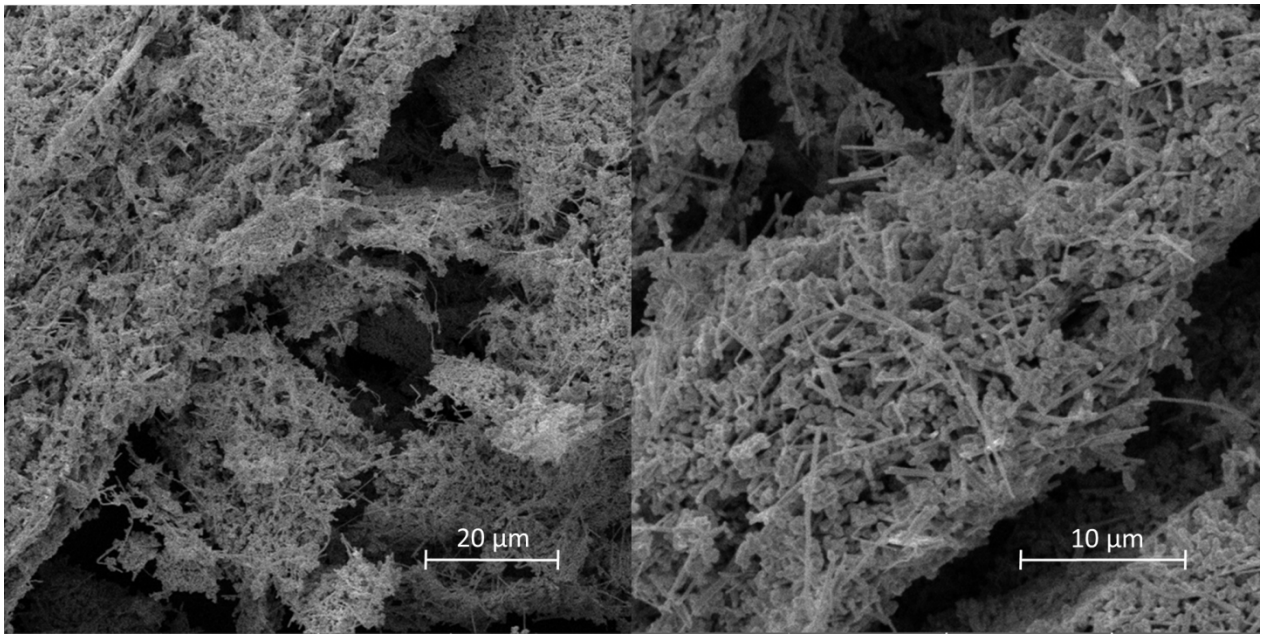

Fig. 3. SEM micrographs of the precipitated product

Figure 3 shows SEM micrographs of the prepared product. Nanowires are clearly visible and abundant. However, quasi-spherical nanoparticles are also present, and the observed nanoobjects form agglomerates. The magnification does not allow a precise determination of the diameter of the nanowires, but it can be estimated that the diameter is in the range of 200 to 300 nm. Due to

entanglement and agglomeration, the length is even more difficult to estimate, but it can be said that nanowires are mostly longer than 10 μm. It follows that the results of SEM analysis agree with the results of UV–Vis spectroscopy and confirm the presence of quasi-spherical nanoparticles.

The phase composition of the prepared sample was determined by X-ray diffraction, and the obtained pattern is shown in Figure 4. Four strong diffraction peaks at $2\theta = 38.1°$, 44.28°, 64.44°, and 77.40° are observed on the diffraction pattern. These are attributed to the reflections from the crystal planes (111), (200), (220), and (311) of the face-centered cubic crystal lattice of silver (ICDD PDF 4–783). The unit cell parameter, *a*, determined on the basis of four diffraction peaks, was calculated using the Unit Cell program and yields 4.08652 ± 0.00015 Å, which is in accordance with the ICDD data (4.0862 Å). The intensity ratios of the diffraction peaks are also consistent with the ICDD data. Weak diffraction peaks of the synthesis intermediate, silver chloride, are also observed (ICDD PDF 31-1238). Based on the reference intensity ratio method, the amount of AgCl was estimated to be 2 %.

The occurrence of silver chloride intermediate and silver nanoparticles alongside the silver nanowires in the sample is most likely the consequence of an excessive amount of $CuCl_2$ used in the synthesis. A study by Coskun et al.[26] regarding the influence of different parameters in the polyol synthesis method on the properties of the final product suggests that the addition of chloride ions is crucial for the formation of silver nanowires. With a lack of chloride ions, silver nanoparticles will form instead of nanowires due to the fast reduction of Ag^+ to Ag^0. The addition of chloride ions results in the formation of a AgCl intermediate, which slows down the reduction process and enables the growth of nanowires. However, the presence of chloride ions in an excess amount again results in the formation of nanoparticles and the precipitation of AgCl particles. In order to avoid both of these undesired outcomes as much as possible, fine tuning of the synthesis procedure is necessary.

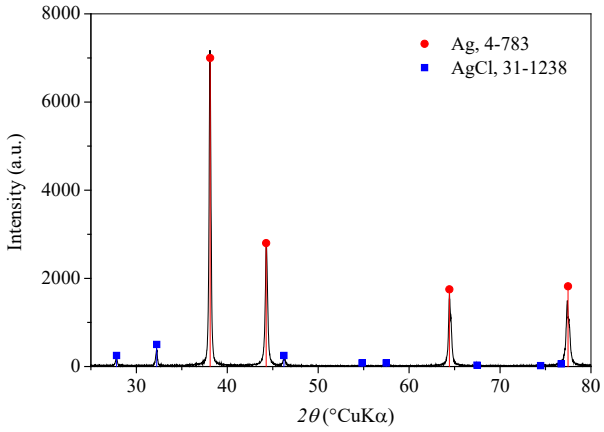

Fig. 4. XRD pattern of the precipitated product

The bactericidal and fungicidal activity of the prepared material was evaluated by the disc diffusion method for two bacteria, *Bacillus subtilis* and *Pseudomonas aeruginosa*, and the fungi *Aspergillus niger* and *Candida albicans*. Several different microorganisms were used in order to gain a better insight into the activity of the prepared nanowires. The results of testing the bactericidal and fungicidal activity of suspensions of prepared silver nanowires using the disc diffusion method are shown in Figures 5a–d. The diameters of inhibition zones after 24 h of incubation for different pathogens and concentrations of nanowire suspension are given in Table 1.

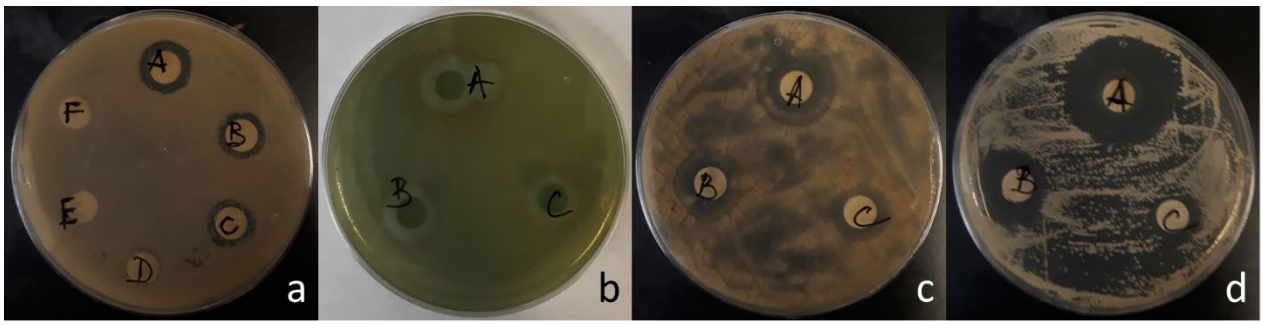

Fig. 5. Petri dishes with disks impregnated with a suspension of silver nanowires of different concentrations and inoculated with different pathogens. (a) *Bacillus subtilis*, (b) *Pseudomonas aeruginosa*, (c) *Aspergillus niger*, (d) *Candida albicans*. (A) 100 mg·l^{-1}, (B) 20 mg L^{-1}, (C) 10 mg L^{-1}. (D, E, and F) lower concentrations with no biocidal activity

T a b l e 1

Diameter of the inhibition zone for the investigated microorganisms

Concentration of Ag nanowires suspension (mg·l^{-1})	**100**	**20**	**10**
Microorganisms		**Diameter (mm)**	
Bacillus subtilis	15	14	13
Pseudomonas aeruginosa	18	16	15
Aspergillus niger	25	21	18
Candida albicans	35	23	16

As can be seen in Table 1, silver nanowires showed moderate bactericidal activity and far better fungicidal activity. Particularly good results were obtained for the fungus *Candida albicans* (inhibition zone of 35 mm at a concentration of silver nanowires of 100 mg·l^{-1}). *Candida albicans* is a highly adaptable microorganism whose resistance is enhanced by biofilm formation. Moreover, studies show that the inhibitory effect of the tested agent on biofilm formation depends on the concentration. Therefore, it can be concluded that silver nanowires interfere with *Candida albicans* forming biofilm and exhibit high antifungal activity (Fig. 5d).[27] Inhibition zones in the case of the *Aspergillus niger* fungus were somewhat smaller, but in this case, too, we can speak of good inhibitory activity.

4. CONCLUSIONS

Silver nanowires with a diameter of 200–300 nm were prepared by the polyol process in the presence of copper chloride. In addition to silver nanowires, the product also contains quasi-spherical silver nanoparticles and some silver chloride. Nanowires showed good fungicidal activity, and particularly good growth inhibition was observed for *Candida albicans* fungus. It can be expected that there will be more frequent use of silver nanowires for biomedical purposes, especially for the surface treatment of medical supplies, with the aim of preventing fungal infections.

Declaration of competing interest. The authors declare that they have no any known financial or non-financial competing interests in any material discussed in this paper.

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