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CHEMISTRY
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Antibacterial effect of silver nanoparticles synthesized on polyvinyl alcohol

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Abstract: Silver nanoparticles synthesized on PVA (compound **1**) and on PVA/silica (compound **2**) were tested for antibacterial effect on *E. coli*, *P. aeruginosa* and *S. aureus* strains. The bactericidal effects of silver nanoparticles were compared based on diameter of inhibition zones in discs diffusion method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) as well as medium supplemented with different concentration of nanoparticles. The results showed the strong antibacterial effect of silver nanoparticles against tested bacteria, the MIC values for silver on PVA (compound **1**) were 3.1 $\mu g \cdot ml^{-1}$ for *E. coli* and 6.25 for *P. aeruginosa* and *S. aureus*. For silver nanoparticles on the PVA/silica complex (compound **2**), MIC values were 6.25 and MBC 12.5 $\mu g \cdot ml^{-1}$ for all investigated strains. Both tested silver nanoparticles compounds caused complete inhibition of growth of *E. coli*, *P. aeruginosa* and *S. aureus* at concentration 5 $\mu g \cdot ml^{-1}$ in medium.

Keywords: silver; nanoparticles; antibacterial effect; disinfectants; inhibition

1. Introduction

Silver as a metal and its ions are known since antiquity as very good disinfectants. One of the most disinfectant and protective species against infection was the lapis or silver nitrate. It was noted that both the bacteria and fungi in the presence of such solution died very quickly. However, the mechanism of action of silver nitrate was a mystery for contemporary scholars [1].

The use of silver ions in a traditional way is difficult due to their susceptibility to reductive influence of light and their deactivation before contact with pathogen. Synthesis of nanoparticles seems to solve this problem, since they act very much like silver ions, but this technique allows to obtain them in already reduced form [1,2].

Nanosilver ions act on the cells of bacteria and fungi in two ways.

(1) Bacterial cell, in contrast to the eukaryotic cell keeps its genetic material directly in the cytoplasm. In the cytoplasm silver ions form with oxygen the unstable silver oxide which undergoes decomposition. In this process, the formed atomic oxygen combines with DNA resulting in its degradation. Moreover, silver ions show a strong affinity to pyrimidine bases; this behavior causes the disruption of replication processes [1,3].

(2) Silver also heavily damages the cell walls of bacteria and fungi. The construction of cell walls of these organisms is different from the cell walls and membranes of eukaryotic organisms. While in eukaryotes the cell membrane consists mainly of lipids with protein channels that allow transport of ions, sugars and fats, in prokaryotes the main building block of the cell walls is peptidoglycan. This compound consists of long polysaccharide chains connected by peptide bridges. Bridges are made of amino acids, one of them is cysteine, which contains sulfur, namely the thiol group at the end of the side chain. Oxidation of the thiol groups leads to formation of –S–S– units. Silver ions and nanoparticles show a strong affinity to sulfur, present in cysteine. The reaction of silver with –SH groups inhibits formation of –S–S– units. Cysteine is in one of the respiratory enzymes of bacteria. If these enzymes are blocked, *e.g.* by silver ions or nanoparticles, bacteria must die [2,4–6].

2. Experimental

2.1. Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by chemical method, silver nanoparticles on PVA as compound **1** and on PVA/silica complex as compound **2** with the procedures:

2.1.1. Compound 1

Silver nitrate (1.8 g) was treated with distilled water (18 ml), 40% PVA solution (100 g), n-butanol (20 g), 2-propanol (30 g) and propylene glycol (30 g). The resulting solution was magnetically stirred, and added dropwise (1 drop/minute) to 0.02% ascorbic acid solution (50 ml). Then the reaction mixture was left for one hour. After this time 2-propanol (150 ml) and distilled water (100 ml) were added, the whole was centrifuged and supernatant was decanted and treated with 2-propanol (100 ml) to precipitate. After centrifugation, decantation and drying, the silver nanoparticles on PVA (0.83 g) of a 15–40 nm grain size were obtained. For microbiological tests they were prepared as a water dispersion of a concentration of 4000 ppm of pure silver [7,8].

2.1.2. Compound 2

Silver nitrate (1.8 g) was treated with distilled water (44 ml), 40% PVA solution (50 g), silica flame of a 20–30 nm grain size (4 g), butanol (30 g), 2-propanol (50 g) and propylene glycol (30 g). The resuting solution was magnetically stirred and added dropwise (1 drop/min) to 0.02% ascorbic acid solution (60 ml). Then the reaction mixture was left for one hour. After this time distilled water (250 ml) was added,

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precipitate was filtered off, washed with ethanol (100 ml) and distilled water (200 ml) and dried. The silver nanoparticles on the PVA/silica complex (4.5 g) of a 20% silver content were obtained. For microbiological tests they were prepared as a water dispersion of concentration of 2000 ppm of silver in 1000 ml.

2.2. Test methods of antibacterial activity

For study of effects of compounds **1** and **2** following strains were used: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* MRSA strains (obtained from the hospital). The applied methods were: (a) the disc diffusion method [9,10], (b) the determination of MIC (Minimal Inhibitor Concentration), (c) MBC (Minimal Bactericidal Concentration) [9], and (d) culture on solid medium containing the tested compounds [10,11].

2.2.1. Disc method

The bacterial suspension (100 µl of *ca* $3 \cdot 10^6$ ml^{-1} cells) with optical density OD 600, was deposited on the surface of the solid Muller-Hinton (MH) medium and Nutrient Agar (AO) before placing sterile paper discs (one per a plate). After that, on each disc the compound **1** or **2** was deposited; the quantity of deposition was 8 µg per disc. After incubation at 37°C for 24 hours the average diameter of the inhibition zone surrounding the disc was measured [9,10].

2.2.2. MIC

The Minimum Inhibitory Concentration defined as lowest concentration of compound that inhibits the growth of an organism [9] was determined on 1 ml of MH liquid medium containing different concentrations of tested compounds: 400, 200, 100, 50, 25, 12.5, 6.25, 3.1 and 1.6 $\mu g \cdot ml^{-1}$. The tubes were inoculated with 100 µl of *ca* $3 \cdot 10^6$ ml^{-1} cells bacterial suspension. After incubation at 37°C for 24 hours tubes were tested for growth or no growth of bacteria.

2.2.3. MBC

The Minimum Bactericidal Concentration defined as the lowest concentration of compound that kills 99.9% of the bacteria was determined as follows: a calibrating spoonfull (10 µl) from each tube was inoculated on nutrient agar and incubated at 37°C for 24 hours. The nanoparticle concentration causing bactericidal effect was selected basing on absence of colonies on the agar plate [9].

2.2.4. Medium diluted method

Solid MH medium was dissolved and supplemented by tested silver nanoparticles at final concentration 1–50 µg, than medium was poured into sterile Petri dishes. As inoculum 0.05 ml bacterial suspension with optical density of OD_{600} equal to 0.1 was used. After incubation at 37°C for 24 hours the grown colonies were counted [10].

3. Results and Discussion

Using disc diffusion method the antibacterial activity of compound **1** and **2** was tested against Gram-negative strains *E. coli* and *P. aeruginosa* and Gram-positive strain *S. aureus* MRSA by measuring the diameter of inhibition zone (Figure 1, Figure 2).

The diameter of inhibition zone exemplifies susceptibility of microorganisms, the strains susceptible to compounds exhibit larger zone, whereas resistant strains exhibit smaller zone. We observed in the disc method that *E. coli* were more resistant to silver nanoparticles than *S. aureus* (Table 1); analogous data were reported in [9].

Using the MIC test, similar results were obtained as in the discs diffusion method, *E. coli* were less resistant than *P. aeruginosa* and *S. aureus* for silver nanoparticles on PVA while for silver nanoparticles on PVA/silica complex the MIC value was equal to 6.25 µg/ml for all strains (Table 2).

Using the MBC method, we have found for all strains the MBC value equal to 6.25 µg/ml for silver nanoparticles on

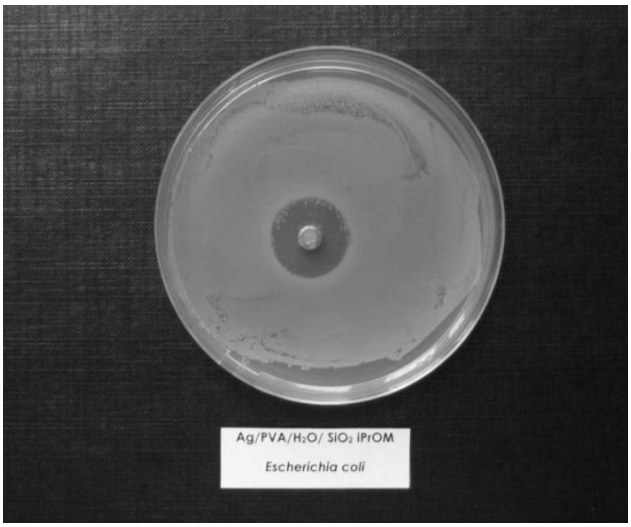

Figure 1. Disc method. Inhibition zone of *Escherichia coli* growth in the presence of silver nanoparticles on PVA/silica complex (**2**).

Figure 2. Disc method. Inhibition zone of *Staphylococcus aureus* growth in the presence of silver nanoparticles on PVA/silica complex (**2**).

Table 1. Disc method. Size of inhibition zones of bacterial growth in the presence of **1** and **2** (mm).

	1 (on PVA)		**2** (on PVA/silica complex)	
	Muller-Hinton medium	Nutrient agar medium	Muller-Hinton medium	Nutrient agar medium
Concentration of compounds (μg/disc)	8	8	8	8
Escherichia coli	21	22	20	21
Pseudomonas aeruginosa	30	26	20	20
Staphylococcus aureus	37	42	32	35

Table 2. Minimal Inhibitory Concentration (MIC) (μg/ml) for silver nanoparticles on PVA (**1**) and on PVA/silica complex (**2**).

	Strain	*E. coli*	*P. aeruginosa*	*S. aureus*
1	MIC μg/ml	3.10	6.25	6.25
2	MIC μg/ml	6.25	6.25	6.25

Table 3. Minimal Bactericidal Concentration (MBC) (μg/ml) for silver nanoparticles on PVA (**1**) and on PVA/silica complex (**2**).

	Strain	*E. coli*	*P. aeruginosa*	*S. aureus*
1	MBC μg/ml	6.25	6.25	6.25
2	MBC μg/ml	12.50	12.50	12.50

PVA (**1**) and 12.5 μg/ml for silver nanoparticles on PVA/silica complex (**2**) (Table 3).

The comparison of influence of silver nanoparticles on growth of *E. coli*, *P. aeruginosa* and *S. aureus* was also detected by using the medium diluted method. We did not observed any differences between tested strains reaction. At the 2.5 μg/ml dose, the plates were uncountable and at the 5 μg/ml dose.

Kora *et al*. [10] obtained MIC for *E. coli* 40–140 μg of silver nanoparticles/ml and 140 μg of silver nanoparticles/ml for *S. aureus*. The higher activity of our compounds is probably caused by their different structure. Our synthesis of nanoparticles is based on reduction of silver ions complexed with polymer structure in strongly alkaline environment.

As a carrier we use PVA polymer or silica, which are growth matrices for nanoparticles. When reaction is complete carriers are washed, and silver nanoparticles are the final products.

Ruparelia [9] observed MBC values of 60–220 μg/ml for four different strains of *E. coli*, and 160 μg/ml for three different strains of *S. aureus*. However our tested compounds are significantly more effective, which is probably caused by their sizes, allowing them to penetrate bacteria cell wall [11]. Silver nanoparticles sythetized on PVA (**1**) is more effective than silver nanoparticles sythetized on PVA/silica complex (**2**). The activity of **1** and **2** is influenced by the release of silver into solution around nanoparticles. When this happens in bacteria cytoplasm, and if the silver release is strong, the higher concentration of Ag results in more effective deactivation of bacteria enzymes by binding of Ag with thiol groups, *e.g.* in methionine [12,13]. We observed the complete growth inhibition of bacteria on the MH agar medium sup-

Table 4. Growth of bacteria on the MH agar medium supplemented with silver nanoparticles on PVA (**1**) and on PVA/silica complex (**2**).

Concentration μg/ml		*Escherichia coli*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
1	2.5	uncountable	uncountable	uncountable	uncountable	uncountable	uncountable
	5	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited
	7.5	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited
	10	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited
2	2.5	uncountable	uncountable	uncountable	uncountable	uncountable	uncountable
	5	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited
	7.5	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited
	10	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited	growth inhibited

plemented with both silver compounds complex at concentration 2.5–10 μg/ml (Table 4). Similar results was obtained by Kora *et al*. [10] for *P. aeruginosa* while Yoon *et al*. [11] observed the complete growth inhibition of *E. coli* at 60 μg/ml concentration of silver nanoparticles.

4. Conclusions

It was found that silver nanoparticles on PVA (**1**) and on PVA/silica complex show higher antibacterial activities than compounds reported up to now in the literature. Compound **1** basing on PVA showed higher antibacterial activity than compound **2**, basing on silica support. This fact results from structure of nanoparticles, since nanoparticles bound with PVA support release silver ions in a shorter time than those bound with PVA/silica.

Measurements of inhibition zone have shown more effective antibacterial activity of compound **1** than **2**; results of tests of MIC and MBC indicate availability of compounds **1** and **2** for bacteria. In the experiments the correlation of size of nanoparticles with their antibacterial effect was observed: size of nanoparticles of **1** allows their penetration into bacteria cell walls, causing the antibacterial activity of **1**. Both compounds **1** and **2** in concentration of 5 μg/ml completely inhibited the growth of tested bacteria.

The results of disc method have shown that *Escherichia coli* are more sensitive strain to silver nanoparticles than *Staphylococcus aureus*.

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