MIDDLE POMERANIAN SCIENTIFIC SOCIETY OF THE ENVIRONMENT PROTECTION

Rocznik Ochrona Środowiska

Volume 22. Year 2020 ISSN 1506-218X 40-50

Research of Aggregatic Stability and Bactericidal Activities of Nanosilver Colloidal Solutions

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1. Introduction

The bactericidal properties of silver and its compounds are known for a long time. Argentum nitrate is indicated in the Pharmacopoeia of Pliny, issued back in 69 BC. Nevertheless, the active use of silver in medicine began only in the XIX century. Significant contribution in this regard was made by the American surgeon J.M. Sims, who in 1852 used silver threads as suture material during operations on vesicouvaginal fistulae. In addition, he also used catheters covered with silver for urine removal (Alexander 2009). At the same time, 1% solution of nitrate argentum in the treatment of neonatal ophthalmic infections was used in clinical practice. The efficiency of 0.25% and 0.1% solutions of argentum nitrate against typhoid sticks and anthrax was proved. However, with the discovery of antibiotics in the 40's. XX century the interest in the development of silver preparations has declined significantly.

Over time, with an increase in the number of antibiotics, most bacteria developed resistance to them, and researchers begin to look for other substances that have bactericidal properties. In many cases, previously forgotten, the important role belongs to silver preparations in the form of soluble and water-soluble salts (for example, argentum iodide), metallic silver in the form of nanoparticles with sizes ranging from several to hundreds of nanometers (Bernavsky 2006).

Thus, known works (Savchenko 2012, Litvinova 2016), where argentum nitrate was used as an antibacterial substance. The disadvantage of using silver salts as a bactericidal agent is their short-term effect, the instability of the solution, which manifests itself in the formation of a black color for the action of light,

as well as the restoration of nitrate to nitrite, which leads to oxidative stress of cells.

Compared with silver compounds, the mechanism of bactericidal action of silver nanoparticles remains unexplored until the end. Some researchers have discovered a greater toxic effect of silver nanoparticles related to gram-positive and gram-negative bacteria compared to its classical chemical compounds (Lok et al. 2007). The antibacterial activity of silver nanoparticles is mainly studied *in vitro* experiments. Their bactericidal action against ampicillin-resistant are established *Staphylococcus aureus* (MRSA) and *Escherichia inch* (Shahverdi 2007).

Antibacterial activity of silver nanoparticles depends on their size, shape, area, chemical composition and charge of the surface. Another factor in the toxicity of silver nanoparticles is the presence of ligands in aqueous solution, in particular, Cl^-, PO_4^{3-}, S^{2-}, SO_4^{2-}, which can interact with Ag^+ ion or with nanoparticles, causing aggregation (Choi et al. 2009). In addition, the presence of molecular oxygen contributes to the allocation of Ag^+ ions from the surface of the nanoparticle and increases their toxicity (Liu et al. 2011).

It is established (Xiu et al. 2011) that AgNPs (Ag nanoparticles) are covered with amorphous carbon in the size of 35.4±5.1 nm were in 20 times less toxic than Escherichia coli, than Ag^+ ions (EC 50: 2.04±0.07 vs. 0.10±0.01 μg/ml). However, their toxicity increased 2.3 times for the influence of air (EC 50: 0.87±0.03 mg/ml), which contributed to the allocation of Ag^+ ions from the surface of nanoparticles.

In the works (Baker et al. 2005, Kim et al. 2007) antimicrobial effect of silver nanoparticles at concentration of 0.35 ng/ml for *Escherichia coli* 3.5 ng/ml for *Staphylococcus aureus.* Also synergistic antimicrobial activity of silver nanoparticles with ampicillin, penicillin G, amoxicillin, kanamitsin, erythromycin, clindamycin, tetracycline, chloramphenicol and vancomycin have been established. In addition, the functional silver nanoparticles conjugated with ampicillin enhanced the bactericidal effect of cell wall lysis and interaction with DNA, resulting in the rapid death of bacteria.

Silver nanoparticles can directly interact with the proteins and phospholipids of the cell membrane, violating its integrity and permeability (Li et al. 2010). Unlike Ag^+ ions, silver nanoparticles, thanks to a larger surface area, provide better contact with bacteria. After attaching nanoparticles to the cell membrane, they pass inside the cells of the bacteria. Like the ions of the argentum, nanoparticles can interact with sulfur-containing proteins and phosphorus-containing components, in particular DNA, RNA, inhibiting their functions. In addition, silver nanoparticles violate the respiratory chain of mitochondria of bacteria, which inevitably leads to cell's death (Holt & Bard 2005).

Researchers have found that silver ions can produce free radicals, inducing oxidative stress (Danilczuk et al. 2006). However, there was no significant difference in the toxicity of silver nanoparticles between anaerobic and aerobic conditions, which undermines oxidative stress and the formation of active forms of oxygen (AFO) as an important antibacterial mechanism of Ag^{+}.

In (Lok et al. 2007), the resistance of strains of bacteria of the genus *Salmonella* to silver nanoparticles was discovered. It was established that the mechanisms of resistance are not related with chemical detoxification, but are the result of the membrane protein, which is ATP-azo and chemiososmotic cation: proton an anti-porter. This system, using the energy of ATP, removes silver ions from the cell.

It was also found in resistant to the action of silver *E coli* strains over expression of the Cus C, Cus F, Cus B and Cus A genes encoding the chemosmotic system involved in the transport of copper and silver ions from the cell (Lok et al. 2008).

It has been established that silver ions penetrate the ion channels into *E. coli* cells without damaging the membranes. Analysis revealed a reduction in the expression of genes coding forribosomal subunit S2, succinyl-CoA-synthetase, and maltose conveyor. An expression reduced of the ribosomal S2 gene. The subunits are critical to bacteria, as they lead to the suppression of other proteins, in particular those involved in the synthesis of ATP (Yamanaka et al. 2005). As a result, various metabolic pathways are violated and replication is inhibited.

Silver has antimicrobial properties against a wide range of microorganisms, not only bacteria, but also some fungi, as well as viruses. However, several mechanisms for the formation of resistance to silver in bacteria have been identified (Priskoka 2016).

1. *Salmonella* resistance *thyphimurium* can be formed due to plasmid pMG101, which is also responsible for the resistance to mercuric chloride, ampicillin, chloramphenicol, tetracycline, streptomycin and sulfanilamides. Plasmid pUPI199 in some strains *Acinetobacter baumannii* contains genes that are relevant for the resistance to argentum nitrogen.
2. Resistance to silver in the E. coli (*Escherichia coli*) are associated with the mechanisms of porous transport caused by porine proteins, which provide specific passive transport of sugars, ions and amino acids through the outer membrane, as well as mutations of the genes of proteinpores.
3. Resistance can be generated by applying silver in small concentrations, such as ½ MIC (half the maximum inhibitory concentration) or whole MIC, but not at the level of bactericidal concentrations.

Methods for obtaining such solutions are basically oxidation-reduction reactions in which cations Ag^+ recovered to metallic silver. In this case, the main problem of these methods, regardless of the reducing agent, is the aggregate stability of the solutions which were obtained.

Stabilizers are added in the lyophobic system to create a potential barrier of repulsion of colloidal particles and to provide the same aggregative stability. As stabilizers, inorganic and organic substances and also synthetic and natural polymers can be used (Shirokobokov 2009).

However, it is known that the stabilization of nanoparticles is more effective when using surfactants. They may contain various functional groups such as -SH, -CN, -COOH, $-NH_2$. One of the most commonly used stabilizers is cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS). In addition, surface-active substances may have their own bactericidal activity. Therefore, the purpose of this work was to investigate the effect of surfactants of different chemical nature on the aggregate stability of colloidal solutions of nanosilver and to determine the bactericidal activity of such multicomponent systems.

Purpose is study of the influence of various chemical nature SAS on the aggregate stability of nanosilver colloidal solutions and the determination of bactericidal activity of such multi-component systems.

2. Experiment methodology

Synthesis of colloidal solutions of silver nanoparticles was carried out by Ostwald's reaction, restoring 0,001 n. argentum nitrate solution (p.f.a.) 1% solution of tannin (chem.p.). The process was implemented in a practically neutral medium (pH from 5.7 to 6.8), which was provided by the addition of 1% sodium carbonate solution, by the reaction (1):

$$6AgNO_3 + C_{76}H_{52}O_{46} + 3Na_2CO_3 \rightarrow 6Ag + C_{76}H_{52}O_{49} + 3CO_2 + 6NaNO_3 \qquad (1)$$

To the obtained solutions of nanosilver surface-active substances of different chemical nature: cationic surfactant – alkamone (GOST 10106-75); anionic surfactants – sodium dodecyl sulfate (GOST 8748-2006) and sulfanol (TU 6-01-1001) and nonionic surfactants − OS-20 (GOST 10730-82) were added. The final concentration of surfactant in solutions was 0.1%.

The aggregate stability of the resulting colloidal solutions was determined by the spectral method (spectrophotometer SF-56, RF), measuring the spectra of the surface Plasmon resonance of silver nanoparticles immediately after their receipt and after 24 hours.

The bactericidal properties of solutions of silver nanoparticles were investigated using reference strains of the *Enterobacteriacea* family *Escherichia coli.* For the study of E.coli initial solution (10^8 units), a control sample was prepared by sequential dilution with a physiological solution: $1.5 \cdot 10^1$ units (maximum number of colonies-15). An experimental series of solutions was prepared for sowing according to Table 1. In this case, to each test tube, in addition to the control sample, added 0,1 ml of the studied solution. The test had 18-20-hour cultures of fresh test strains that did not pass more than three passages on nutrient media. Daily culture of test strains was washed off with a physiological solution and subjected to an optical turbidity standard corresponding to 1 billion cells in 1 ml.

With Densi La Meter determined the McFarlant's turbidity standard (unit) to the suspension concentration (Table 1).

Table 1. Turbidity standard

McFarland's Turbidity Standard (op.)	0.5	1	2	3	4
Number of International Turbidity Units (MO)	1.7	3.3	6.7	10	3.3
The appropriate concentration of the suspension x10^8 CFU/ml	1.5	3	6	9	12

Turbidity of the *E.coli* bacteria suspension was 0,5 (McFarland unit), corresponding to a concentration of $1.5 \cdot 10^8$ CFU/ml.

From the original standard suspension, ten times dilution was prepared by transfer of 0.5 ml of culture in 8 test tubes from 4.5 ml of physiological solution, mixing thoroughly, changing the pipette after each transfer. We received a series of serial dilutions from 10^8 to 10^0 *E. coli* cells at 1 ml. To each test tube with dilute suspension, 0.1 ml one of the studied solutions was added, namely:

Control sample (dilution $1.5 \cdot 10^1$),
Solution of argentum nitrate (0.001 n),
Solution of silver nanoparticles (NS),
0.1% alkamone solution for surfactant,
0.1% solution of surfactant: Sodium dodecyl sulfate,
0.1% solution of surfactant sulfanol,
0.1% solution of surfactant OS-20,
NS + alkamone solution,
Solution NS + Sodium dodecyl sulfate,
Solution of NS + sulfanol,
Solution NS + OS-20.

Control samples of dilutions were used for the study of bactericidal properties: $1.5 \cdot 10^1$ (maximum number of colonies − 15) and 10^o (no growth of bacteria).

Suspensions of microorganisms have been left at room temperature (22-23°C); in 24 and 48 hours. The hanging was carried out according to the standard method (Lok et. al. 2007) by the horizontal method. By 0.1 ml. from the investigated mixtures were placed *in the* center of the meat – pepper broth in Petri dishes, distributed by a spatula on the entire surface, after application, the material was placed in a thermostat at 36°C for 24 hours.

The total microbial number was determined by direct counting the number of bacteria. Colonies that grew both on the surface and in the depth of the agar were counted with a magnifying glass with an increase of 2-5 times. The number of viable cells of microorganisms was expressed by the number of colony-forming units (CFU), followed by a recount of 1 g sediment The results of bactericidal action were expressed as a percentage of the number of CSFs in the investigated solutions to the number of CFU in the control sample (the maximum number of CRU = 15).

3. Results and discussion

The aggregate stability of colloidal solutions of nanosilver was determined by using the phenomenon of the surface plasmon resonance inherent in nanoparticles of metals. Fig. 1 shows the spectra of colloidal solutions of nanosilver without the addition of surfactant and surfactants of different chemical nature, which were registered immediately upon receipt.

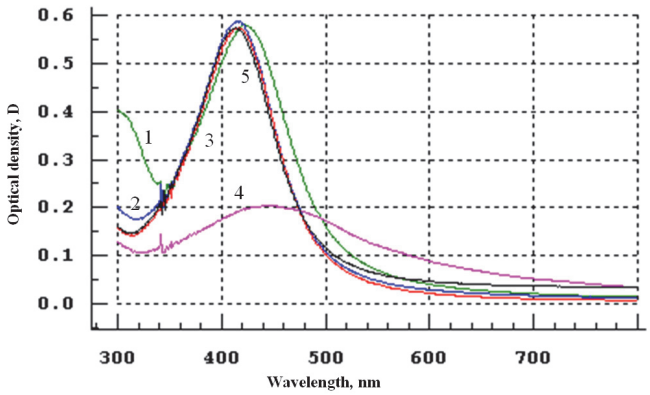


Fig. 1. Spectral characteristics of colloidal solutions of nanosilver with different surfactants immediately after preparation: (1 – without surfactants; 2 – Sulfanol; 3 – OC-20; 4 – Alcamone; 5 – Sodium dodecyl sulfate)

Fig. 2 shows the spectral characteristics of the same solutions that were recorded after 24 hours.

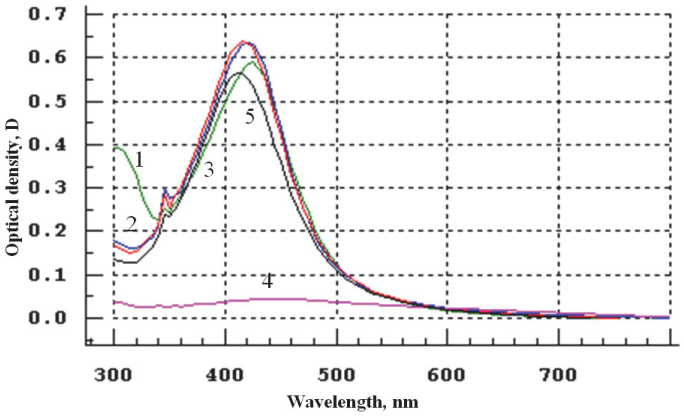
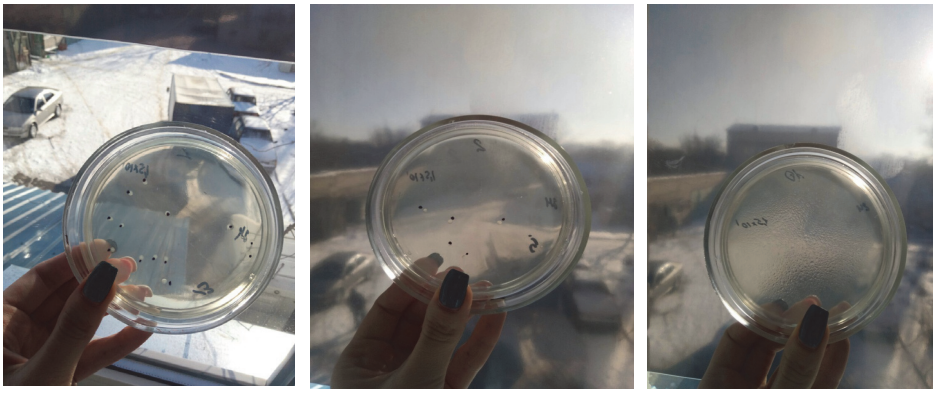


Fig. 2. Spectral characteristics of colloidal solutions of nanosilver with different surfactants immediately after after 24 hours: (1 – without surfactants; 2 – Sulfanol; 3 – OC-20; 4 – Alcamone; 5 – Sodium dodecyl sulfate)

It can be seen that anionic surfactants- sulfanol and sodium dodecyl sulfate and nonionic surfactants − OS-0 practically do not affect the spectra of surface plasmon resonance of silver nanoparticles. There is only a slight shift of maximum absorption from 420 nm for a solution of nanosilver without a surfactant up to 410 nm in the presence of surfactants. The high stability of silver nanoparticles without SAS can be explained by stabilizing properties of tannin, which has been used in exceed. At the same time, thecationic surfactant − Alcamone − greatly changes the spectral indices, also affecting the magnitude of the maximum absorption − its displacement occurs in the long wave region of 420 nm to 450 nm, which affects the optical density, which essentially decreases.

It is seen that the Alkamone solution does not actually contain nanoparticles of silver, which is characterized by a superficial Plasmon resonance. Their aggregation and precipitation dropped out. This fact is the actual proof of the negative charge presence on the surface of silver nanoparticles. The interaction of cationogenic SAS - Alkamone – with such particles leads to their electroneutrality and, consequently, to further aggregation. Spectral characteristics of other solutions have changed slightly. It should be noted that colloidal nanosilver solutions obtained with the use of tannin as a reducing agent, as opposed to other reducing agents investigated by us (ascorbic acid, hydrogen peroxide, hydroquinone with sodium citrate) exhibit high aggregate stability over a significant period of time (up to six months). Nevertheless, the study of bactericidal action of

nanosilver drugs (NS), stabilized by surfactants of different chemical nature has an independent interest, which was made at the second stage of our experimental study (Fig. 3).



A B C

Fig. 3. Growth of bacteria on meat-peptone broth; A − control; B – SAS OS-20; C − solution NS+OS-20

Results of research on bactericidal action of nanosilver solutions in relation to the E.coli are presented in Table 2.

Table 2. Growth of the E.coli in solutions of nanosilver and surfactants

Sample number	Description of the sample	Percentage of CFU, (%)
1	Control sample (dilution $1.5 \cdot 10^{1}$)	100
2	Solution nitrate (0.001 n)	50
3	Solution of Silver Nanoparticles (NS)	not found
4	0.1% solution of surfactant: Alcamone	50
5	0.1% solution of surfactant: Sodium dodecyl sulfate	70
6	0.1% solution of surfactant: sulfanol	100
7	0.1% solution of surfactant: OS-20	40
8	Solution of NS + Alcamone	not found
9	Solution NS + Sodium dodecyl sulfate	10
10	Solution of NS + Sulfanol	20

11	Solution NS + OS-20	not found

One can see that solutions of silver nanoparticles without surfactant and in the presence of OS-20 and Alcamone completely inhibit the growth of the intestinal rod. Silver ions solutions (Ag^+) reduce the growth of E.coli colonies by half. Some of the surfactant agents negatively affect the growth of the E.coli, but do not have complete bactericidal action.

According to the results of the studies, it was found that compared with the control of Ag^+ partially suppresses the growth of bacteria, due to surface oxidation, thus exhibiting a toxicological effect in relation to *E. coli*.

Alkamone and OS-20 superficial substances independently partially inhibit the growth of bacteria (5 and 8 colonies). SAS Sodium dodecyl sulfate and Sulfanol alone and in the colloidal solution of silver nanoparticles do not exhibit bactericidal properties. Nevertheless, in the paper (Shulgina et al. 2012) using of *E.coli* as a test object, it was proved that the use of another anionic surfactant (such as Aerosol OT (AOT) is effective in terms of the bactericidal action of nanosilver-stabilized solutions of this substance.

Complete inhibition of growth of bacteria *E. coli* showing a colloidal solution of silver nanoparticles (Ag^o) that was synthesized without SAS, and also synthesized in the presence of cationic surfactant (Alcamone) and a colloidal solution synthesized in the presence of a nonionic surfactant (OS-20). The complete suppression of the growth of *E. coli* bacteria by the colloidal solution of silver nanoparticles is, in our opinion, related to the destruction of the surface of the cell wall, since nanosilver particles have an extremely large relative surface, thereby increasing their contact with bacteria and greatly improving their bactericidal effectiveness.

4. Conclusions

The influence of surface-active substances on the aggregate stability of colloidal solutions of nanosilver obtained by the restoration of the argentum nitrate by tanine in a neutral environment was studied. It has been proved that anionic surfactants – sulfanol and sodium dodecyl sulfate and nonionic surfactants − OS-20 can be used as stabilizers of colloidal solutions of nanosilver, whereas cationic surfactant alkalomone has a coagulating effect on the solutions of silver nanoparticles, reducing their aggregate resistance. It is proved that the solutions of nanoparticles are obtained silver exhibit bactericidal action against the E. coli and may be recommended as antiseptic agents for different purposes.

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Abstract

The influence of SAS of different chemical nature on aggregate stability and bactericidal action of nano silver colloidal solutions is investigated. Colloidal solutions of silver were obtained by restoring the argentum nitrate agent in a neutral medium. With the help of spectrophotometric method, it has been proved that such solutions are characterized by high aggregate stability compared with the use of other traditional reducing agents. Anionic SAS (sodium dodecylsulfate and sulfanol) and nonionic SAS (OS-20) increase aggregate stability of nano silver solutions, while cationogenic SAS − alcamone promotes rapid coagulation and aggregation of nano silver particles. The study of bactericidal action of the solutions to the E.coli are showed that the nano silve colloidal solution with or without presence of OS-20 and alcamone completely inhibit the growth of colonies of E.coli, that is, it's have high bactericidal properties.

Keywords:
colloidal solutions of nanosilver, surface-active substances (SAS), aggregate resistance, bactericidal action