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Published in the Russian Federation
Nanotechnology Research and Practice
Has been issued since 2014.

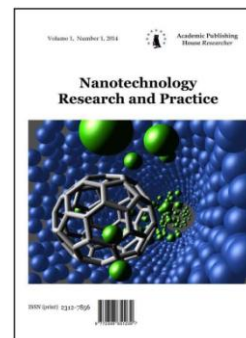
ISSN: 2312-7856

E-ISSN: 2413-7227

Vol. 8, Is. 4, pp. 140-150, 2015

DOI: 10.13187/nrp.2015.8.140

www.ejournal13.com



UDC 541.144; 544.77

The Electron Microscopy of Micro-Dispersed Colloid Silver Nanoparticles

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Abstract

In this review article are studied the methods for preparation of micro dispersed colloid silver nanoparticles and the electron microscopy, as well as the scopes of their practical application in nano-industry, bionanotechnology and water processing and water purification techniques. The mechanisms of bactericidal action of colloid silver on a microbial cell are discussed from the point of view of absorptive, electrostatic, enzyme and mutagen theories. Silver exerts both bactericidal and bacteriostatic effect against more than 500 species of microorganisms. The bactericidal effect of silver is 1,500 times more than the same concentration of phenol and 3.5 times more than the action of mercuric chloride. It is shown that the effects of colloid silver are defined by the concentration, the sizes of micro dispersed nanoparticles and their stability in water solutions. The micro dispersed silver nanoparticles may be prepared by using various physical-chemical, biochemical and biotechnological methods.

Keywords: colloid silver, nanoparticles, bionanotechnology, water treatment.

Introduction

Silver (Ag) – is a heavy metal with an atomic mass of 107,87 a.u.e. relating to the sub-group of the first group of the periodic system, having a pronounced physiological effect and antimicrobial action.

A wide range of antimicrobial action of silver, no resistance toward Ag by the majority of pathogens, low toxicity, the absence of allergens and their being well tolerated by patients contributed to the increased interest toward silver in this country and the creation of various anti-inflammatory medications having antiseptic and bactericidal action based on it as progotol, collargol etc. [1].

At the end of XIX century K. Nagel [2] found that the cause of bactericidal effect of silver on cells of microorganisms are ions Ag⁺. This phenomenon is denoted oligodynamic effect (from the Greek. “oligos” – small, the trace, and “dynamos” – action). Silver exerts oligodynamic effect only

in dissolved, e.g. ionized form. Further it was established that silver exerts the most strong bactericidal action in the following row of heavy metals “copper–silver–gold”, and less – copper and gold [3]. This effect is explained by dependence of biological properties of trace elements on the position they occupy in the periodic table. In the works of L.A. Kulsky (Ukraine) it was shown that the destruction of microorganisms cause not only Ag^+ ions, but their dissociated compounds – substances capable of disintegrating in aqueous solutions to charged ions [4].

Ag^+ ions and colloidal solutions (suspensions of micro dispersed silver nanoparticles) possess bactericidal, bacteriostatic, antiviral, antifungal and antibacterial activity against more than 500 pathogenic microorganisms, yeast, fungi and viruses [5]. Their antimicrobial effect of Ag^+ is slightly stronger than the penicillin, biomitsin and other antibiotics due to the inhibitory effect on antibiotic resistant strains of bacteria [6]. Thus, the bacteria-annihilating effect of silver is 1500 times higher than that one of the phenol concentration in the same concentration, and 3,5 times higher than that of mercuric chloride. Thus, the bacteria-annihilating effect of silver is 1500 times higher than that of the phenol concentration in the same concentration, and 3,5 times higher than that of mercuric chloride. Ag^+ ions exert an antimicrobial effect from bacteriostatic (ability to inhibit microbial growth) to bactericidal (the ability to destroy microbes) on many pathogen microorganisms as *Staphylococcus aureus*, *Streptococcus*, *Proteus vulgaris*, *Pseudomonas* and *Escherichia coli* [7]. Ag^+ has pronounced antifungal activity at concentration of 0,1 mg/l: at the microbial load of 100,000 cells per 1 liter the destruction of pathogenic yeast of *Candida albicans* occurs within 30 min after the direct contact with the metallic silver.

The activity of samples of colloidal silver depends directly on the concentration of Ag^+ ions and the amount of silver nanoparticles in aqua solutions: at concentration of Ag^+ 1 mg/l *Escherichia coli* get killed after 3 min, at 0,5 mg/l – after 20 min, at 0,2 mg/l – 50 min, and at 0,05 mg/l – 2 hours. The sensitivity to silver pathogenic and nonpathogenic microorganisms is varied; the first group of microorganisms has higher levels of sensitivity toward silver [8]. With keeping this in mind, in this country, a method for treatment of dysbiosis of various etiology is developed based on abdominal electrophoresis of Ag^+ ions (concentration – 500 mg/l) that allows to achieve a stable therapeutic effect [9].

There is the evidence that Ag^+ ions have the ability to inactivate the viruses of pox, influenza A1, B, and some enteric adenoviruses *in vitro* [10], as well as to exert a therapeutic effect in the treatment of viral enteritis, and inhibit the HIV virus at an early stage of development [11]. Thus, 1 mg/l of colloid micro-dispersed silver in the aqueous solution within 30 min causes the inactivation of influenza viruses A1, B, Mitra and Sendai. Therefore, it become obvious the significant advantage of using colloidal silver Ag^+ in comparison with conventional antibiotic therapy, because silver has no significant side effects.

Silver and its colloid solutions may also be applied as effective antibacterial agents in direct contact with the surface wounds and inflammation and bacterial infection suppurating portions [12]. The results of the application of colloidal silver solutions show the effectiveness of their use in the treatment of gastrointestinal diseases, cholecystitis, infectious hepatitis, cholangitis, pancreatitis, duodenitis and intestinal infections [13]. The solution of colloidal silver can also be used for stomach ulcers and duodenal ulcers, because in this case is destroyed bacterium *Helicobacter pilori*, populating the mucous membranes of the stomach and intestines, responsible for erosive and ulcerative processes in the gastrointestinal tract [14]. Unlike ionic silver that while getting into the digestive tract, forming insoluble salts precipitated and loses its bactericidal activity, the colloidal silver nanoparticles stabilized in aqueous solutions, are more resistant and can operate for long periods of time.

It should be noted that silver as a microelement is included in the body tissues, endocrine glands, liver and brain. Thus the human body contains approximately 20 μg of Ag per 100 g of dry matter [15]. According to various sources, the physiological norm of silver in organism compiles from 20 mg to 40 mg [16].

The high biological activity of microelements in the human body is associated with their participation in the synthesis of enzymes and hormones. Depending on the concentration of ions in aqueous solutions, Ag^+ ions can both stimulate and inhibit the activity of some enzymes. Under their influence the intensity of amplified oxidative phosphorylation in mitochondria in brain tissues is increased on almost twice, as well as the nucleic acid content is increased that improves the supply of oxygen to brain cells [17].

During the incubation of various cellular tissues in normal saline containing 0,001 μg of Ag^+ , the brain tissue absorbs on 24 % more oxygen, myocardium – 20 %, liver – 36 % kidney – 25 %. A further increase in the concentration of Ag^+ ions to 0,01 μg reduces the absorption of oxygen by cells of these organs, indicating about the regulation of energy metabolism by Ag^+ ions according to the type of feedback mechanism. In studying the effects of drugs based on colloidal silver on the human body it was noted their stimulating effect on the composition of blood: neutrophils forms in blood are disappeared; it was shown some increase in the number of lymphocytes and monocytes, red blood cells and hemoglobin while deceleration of erythrocyte sedimentation rate [18].

In recent years in the scientific literature are appeared data on the immunomodulatory properties of silver [19]. It was demonstrated that, depending on the concentration silver may stimulate or inhibit cell phagocytosis. Under the influence of silver the amount of immunoglobulin classes of *A*, *M*, *G* increases, as well as increases the amount of *T*-lymphocytes.

The significant factor in the use of colloidal silver is that the concentration of Ag^+ at 50–250 mg/l, close to the maximum permissible has no adverse effects on the organism [20]. At prolonged intake of Ag^+ ions in the diet occurs the accumulation of Ag^+ in tissues of the human body – argyria, which is the result of photochemical reduction of Ag^+ ions [21]. The essential influence on the development of argyria has individual susceptibility of organism to silver, the body's immune status and other factors.

The above-mentioned factors suggest that silver plays a significant role in the biochemical processes and can be considered as a trace element necessary for normal functioning of internal organs and systems, as well as an enhancing agent to the immune system, which has a strong impact on numerous pathogens. Therefore, the development and improvement of new bionanotechnology approaches for preparation of micro-dispersed silver nanoparticles for medicine, nanotechnology industry and water treatment is promising, as well as the study of the nanostructure of silver nanoparticles and their physical-chemical properties.

The aim of the research was to examine the mechanisms of the biological action of silver on microbial cell, the methods of preparation of micro-dispersed silver nanoparticles, as well as the searching for possible areas of practical application of silver nanoparticles.

Experimental Part

Material

The study was performed with samples of micro-dispersed nanoparticles obtained from Russian Joint Company “Nano-Bio-Technology” (Moscow, Russia). The samples were taken *and* analyzed in solid samples according to National standard of the Russian Federal Agency of Technical Regulation and Metrology.

Analytical methods

Transmission electron microscopy

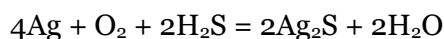
The structural studies were carried out with using transmission electron microscopy (TEM) on JSM 35 CF (JEOL Ltd., Korea) device, equipped with X-ray microanalyzer “Tracor Northern TN”, SE detector, thermomolecular pump, and tungsten electron gun (Harpin type W filament, DC heating); working pressure: 10^{-4} Pa (10^{-6} Torr); magnification: 300000, resolution: 3,0 nm, accelerating voltage: 1–30 kV; sample size: 60–130 mm.

Results and Discussion

The mechanism of action of Ag^+ ions on the microbial cell

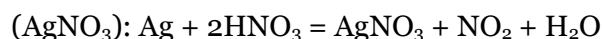
The basic physical properties of the metallic silver are demonstrated in Table below.

Due to the presence of hydrogen sulfide in the air the metallic silver eventually get oxidized, covered with a dark touch of silver sulfide (Ag_2S):



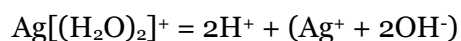
From all silver oxides are stable only oxide (I) Ag_2O and oxide (II) AgO . Silver oxide (I) is formed on the silver surface as a thin film due to the oxygen adsorption, which increases with increasing the temperature and pressure. The suspension of Ag_2O has antiseptic properties. At a temperature of $+200\text{ }^\circ\text{C}$ Ag_2O is decomposed.

The metallic silver exhibits stability in many acids. Hydrochloric acid, dilute sulfuric acid and “aqua regia” (the mixture of nitric acid and hydrochloric acid in volume ratio 1:3) do not act on the metallic silver, because the metal surface forms a protective film of silver chloride (AgCl). However, silver is good soluble in nitric acid to form soluble sodium nitrate:



Hot concentrated sulfuric acid dissolves metallic silver to form silver sulfate (Ag_2SO_4) (solubility of Ag_2SO_4 in water makes up 0,79 wt.% at $t = +20\text{ }^\circ\text{C}$).

In aqueous solutions (solubility of $\text{Ag} - 0,04\text{ mg/l}$) silver ions Ag^+ form stable for a long time maintaining stability the hydrated ions:



This process is due to the properties of polar water molecules to interact with each other by intermolecular dipole-dipole interactions and hydrogen bonds. As a result, H_2O molecules are formed around Ag^+ ions the hydrated aqueous layer, capable of screening off oppositely charged ions from the interaction that is why those ions acquire higher stability in aqueous solutions.

Table: The basic physical properties of the metallic silver

Physical property	Value
Atomic mass, a.u.e	107,87
Density, g/cm^3	10,49
Melting point, $^\circ\text{C}$	960,5
Boiling point, $^\circ\text{C}$	2210,0
Latent heat of fusion, cal/g	25,0
Specific heat, $\text{cal}/(\text{g}\cdot^\circ\text{C})$	0,056
Electrical resistivity, $\mu\text{Om}\cdot\text{cm}$	1,62
Thermal conductivity, $\text{cal}/(\text{cm}\cdot\text{s}\cdot^\circ\text{C})$	0,974

Among the numerous theories (enzymatic, adsorption, electrostatic, mutagenic) explaining the mechanism of action of Ag^+ ions on the bacterial cell, the most common today is currently the adsorption theory. It consists in that the bacterial cell loses the viability as a result of the interaction of electrostatic forces generated between the negative-charged cell membrane and the positively charged Ag^+ ions during the adsorption of Ag^+ by the membrane of the bacterial cell [22].

The effectiveness of silver in comparison with other disinfection agents is shown in Fig. 1.

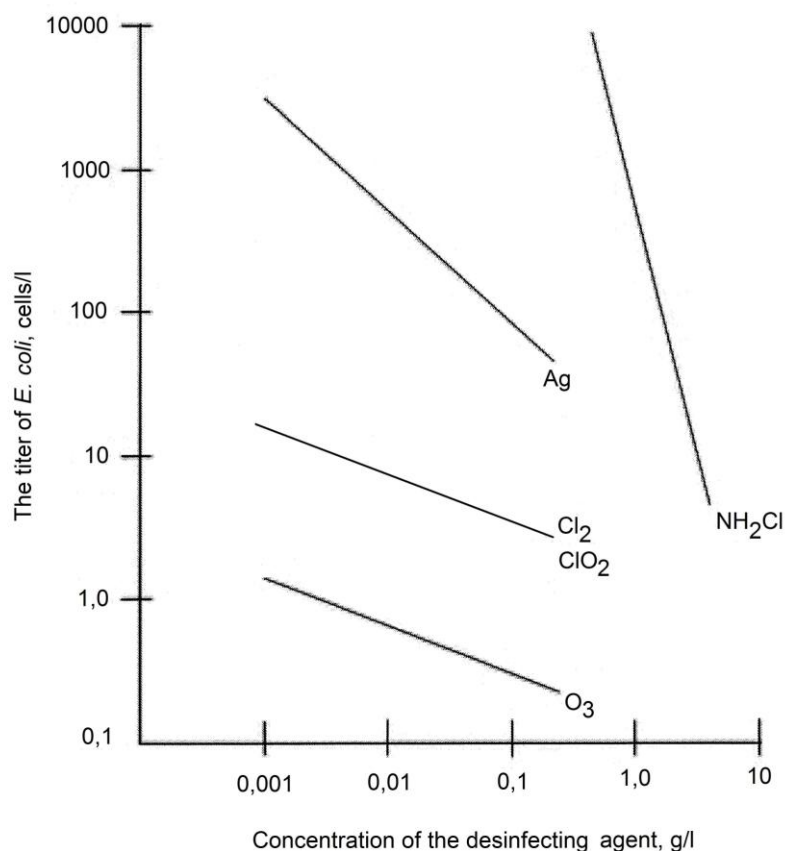


Figure 1. The rate of deactivation of *E. coli* by various disinfection agents [20]

It is believed that Ag^+ ions are absorbed by the bacterial membrane and further are capable of reacting with the membrane and bacterial proteins consisting of peptidoglycan – a heteropolymer of N-acetylglucosamine and N-acetylmuramic acid, providing the mechanical strength and ensuring the structural and functional properties of cell membranes of bacteria and protozoa (Fig. 2).

In this process, Ag^+ ions interacting with the carboxyl- and amino-groups of peptidoglycans, form metal complexes of proteins with Ag^+ that has a consequence for changes in the structure and stability of peptidoglycans and is detrimental for the cell's growth. This deprives peptidoglycans the ability to transport the oxygen inside the bacterial cell, leading to hypoxia and subsequent death of microorganisms in the presence of Ag^+ . A similar inhibitory effect Ag^+ exerts on the respiratory enzymes of microorganisms by embedding in the reaction center of the enzyme and further changing it. It should be noted that since the cellular membrane of mammalian cells does not contain peptidoglycans, Ag^+ ions do not affect them.

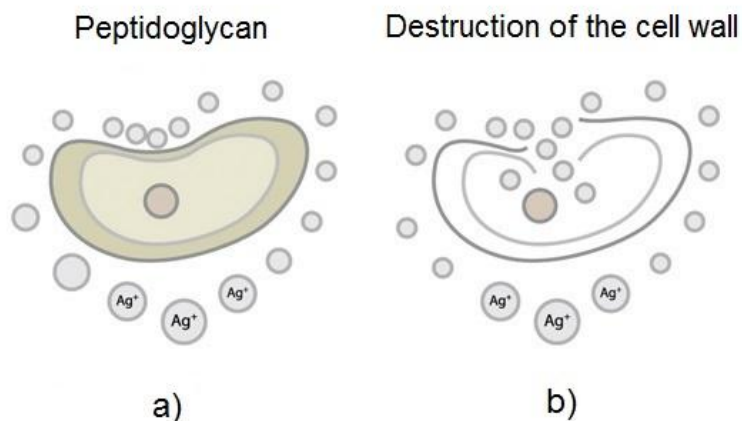


Figure 2. Schematic representation of the interaction of Ag⁺ ions with the microbial cell: a) – the interaction of Ag⁺ ions with the membrane protein peptidoglycan; b) – the process of destruction by Ag⁺ ions the cell wall with further penetration into the microbial cell

It is known that silver can inactivate certain enzymes by binding to thiol groups, resulting in formation of silver sulfide (Ag₂S). Silver also reacts with amino-, carboxyl-, imidazole- and phosphate- groups of enzymes inhibiting the activity of glucose oxidase, β-galactosidase, lactate dehydrogenase and glutathione-peroxidase [23].

In the mechanism of the bactericidal effects of silver on the microbial cell an important role apparently also plays the biochemical reactions catalyzed by Ag⁺ ions, including oxidation of the bacterial cytoplasm and its subsequent degradation by oxygen in the presence of Ag⁺ ions. Some authors explain the antimicrobial action of silver by interruption of structural and functional properties of enzymes containing interacting with Ag⁺ ions SH-, NH₂- and COOH groups, others – by interruption of the osmotic pressure in the cells as a result of interaction of Ag⁺ ions with the cytoplasm [24].

There is evidence on mutagenic properties of Ag⁺ – the formation of metal complexes of nucleic acids with Ag⁺ ions and other heavy metals as gold, whereby it is interrupted the spatial structure of the DNA and the ability of bacteria to divide [25]. Also it is assumed that one of the causes of antimicrobial action of Ag⁺ is the inhibition by Ag⁺ ions the transmembrane transport of Na⁺ and Ca²⁺ ions in microbial cells [26].

Thus, the interaction of Ag⁺ ions with the bacterial cell is a complex, multi-factorial character affecting not only the inactivation of enzymes, but biochemical reactions, metabolism and the ability of cells to divide in the presence of Ag⁺.

Preparation of micro-dispersed silver nanoparticles

The methods for preparation of metal nanoparticles having a specified size and physical-chemical properties at the nanoscale is today one of the fastest growing areas of modern nanotechnology. The main objective is the possibility of obtaining micro-dispersed nanoparticles with specified dimension and the necessary stability in water solutions towards various external factors. At present time there have been various physical, chemical, biochemical and mixed methods for the preparation of colloidal silver micro-dispersed nanoparticles (including cryochemical synthesis, cryochemical reduction, vacuum evaporation, the use of pulsed lasers etc.), the main of which is the method of chemical reduction of silver derivatives. Silver nanoparticles may be prepared by chemical reduction of silver nitrate with sodium borohydride in the presence of quaternary ammonium disulfide at +200 °C. The stable colloidal silver solutions may be prepared by reduction of silver salts with hydrogen, borohydrides and hydrazine in the presence of surfactants, e.g., sodium dodecyl sulfate. As a nucleating agent in the preparation of colloidal nanosilver (Fig. 3) is used a mesoporous aluminosilicate Na₂O·Al₂O₃·nSiO₂·mH₂O, where n = 1–10, m = 1–2, which is obtained by the hydrolysis of Si(OC₂H₅)₄ and Al(OC₃H₇)₃ in the presence of

$C_{16}H_{33}(CH_3)_3NBr$ [27]. The next stage is the complete removal of organic components through annealing the reaction mixture in an oxygen stream. At the final stage the aluminosilicate is impregnated with a solution of $AgNO_3$ and further is reduced in a stream of hydrogen. The resulting nanocomposite exhibits high catalytic activity in the oxidation reaction of methanol.

The most commonly the method of chemical reduction is used in the preparation and stabilization of micro-dispersed nanoparticles of heavy metals as silver and gold in liquid phase, in aqueous and non-aqueous media. As metal compounds are generally used their salts as reductants, such as aluminum hydrides, borohydrides, hypophosphites and formaldehyde. This method is widespread due to its technical simplicity and accessibility. By this method may be synthesized the silver nanoparticles having size $\sim 3,5-4,8$ nm.

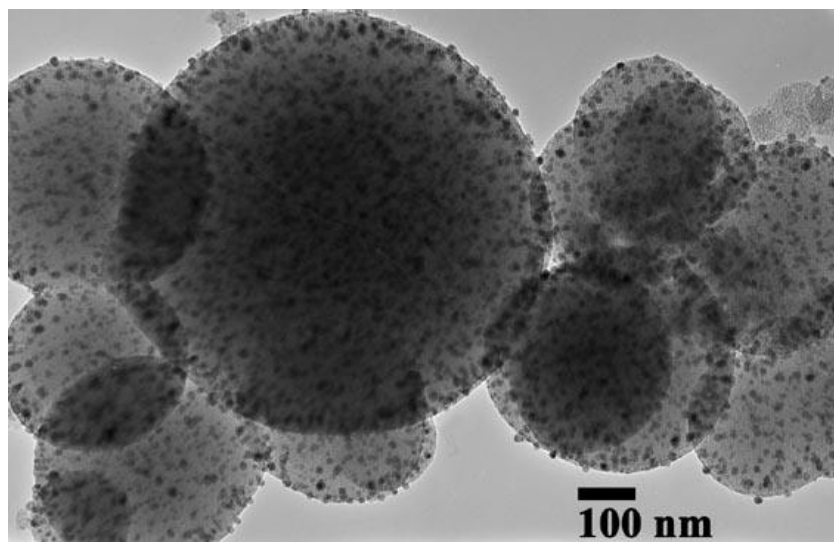


Figure 3. Electron micrographs of micro-dispersed silver nanoparticles fixed on the surface of spherical particles of the mesoporous aluminosilicate $Na_2O \cdot Al_2O_3 \cdot nSiO_2 \cdot mH_2O$, where $n = 1-10$, $m = 1-2$ [27]

For the preparation of micro-dispersed silver nanoparticles with an average size ~ 7 nm is used the photochemical reduction of $AgNO_3$ by irradiating the reaction mixture with a mercury lamp in the presence of dendrimers – highly branched macromolecule consisting of a central core and the intermediate terminal repeating units of the amino ($-NH_2$) and a carboxyl ($-COO^-$) groups.

The micro-dispersed silver nanoparticles with an average size of $\sim 3-4$ nm may be prepared by either biochemical or biotechnological approaches [28] in the aqueous solution stabilized with $AgNO_3$ as a stabilizer and reducing organic compounds – gelatin, a mixture of polypeptides, obtained by partial hydrolysis of animal collagen protein, polyhydrates, dextran or the products of hydrolysis of cell walls of microorganisms (Fig. 4). Furthermore, some fungi and microorganisms are capable while growing in the presence of Ag^+ ions to produce inside the cytoplasmic space the micro-dispersed flat polyhedral silver nanoparticles with sizes $\sim 50-100$ nm. Additionally, some microorganisms are capable of extracellular microbiological reduction of aqueous silver nitrate solutions to form micro-dispersed silver nanoparticles with a size of $\sim 20-50$ nm.

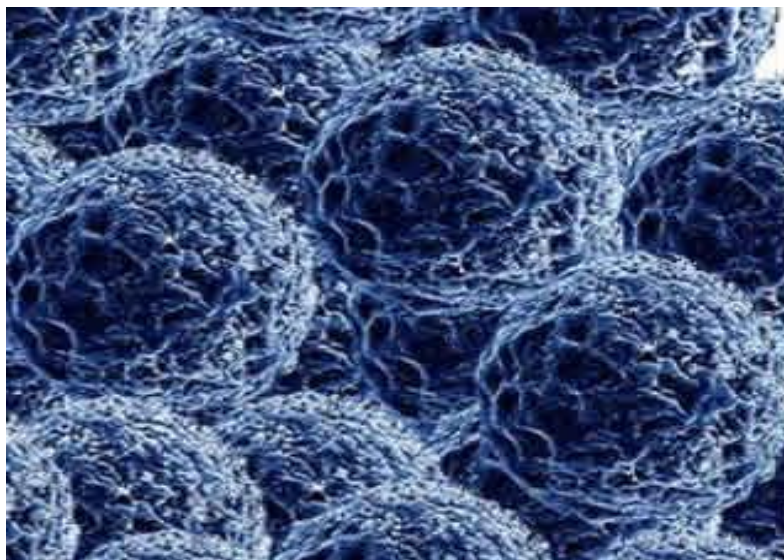


Figure 4. Electron micrographs of micro dispersed silver nanoparticles with a size of ~ 5 nm on the polymer matrix of gelatin [28]

In recent years, it became common the electrolytic method for the preparation of colloidal solutions of micro-dispersed silver nanoparticles with a size of ~ 2 – 7 nm by passing the direct electric current through the submerged silver or silver-copper electrode (anode) placed in an aprotic solvent as tetrabutyl-ammonium bromide in acetonitrile [29]. In the electrolysis process occurs the partial dissolution of the silver anode that results that the solution is saturated with Ag^+ . At a given strength of the electric current and electric voltage on the electrode the concentration of Ag^+ solution is determined by the time of the current source operation and the amount of the aqueous solution through which electric current is passed.

The physical-chemical properties of colloidal silver nanoparticles are determined by their aggregate and sedimentation stability, as well as the possibility to air oxidation. Wherein, the colloid stability of silver nanoparticles depends on the initial concentration of Ag^+ ions in solution. The dimensions of micro-dispersed silver nanoparticles obtained by different methods vary within ~ 3 – 100 nm limits. Their physical properties in the nanoscale are different from the properties of the metallic silver in particular, the reduction of particle size leads to the lower melting temperature. Furthermore, the silver nanoparticles have extremely high specific surface area, which increases the contact area of silver with bacteria or viruses, significantly improving the bactericidal action of silver, the absorption by a cell and the transport across the cell membrane.

The usage of micro-dispersed silver nanoparticles

The practical usage of silver in the form of micro-dispersed nanoparticles significantly reduces the acting concentration of silver hundreds of times while maintaining bactericidal properties of silver [30]. Due to the ability of silver nanoparticles for a long time to keep the antibacterial properties, economically is justified their use as additives in water-based paints, lacquers, detergents, cosmetic creams and other materials that can improve the efficiency of their use and increase self-life and storage.

The composite nanomaterials, produced by the domestic industry, based on the stabilized in aqueous solutions the colloidal silver nanoparticles possess the inherent silver antimicrobial action in a wide range of pharmacological and in some respects (the preparation method, efficiency, availability, low cost) are superior to their foreign counterparts. In this country recently was developed a scientific basis for the creation of new domestic nanomaterials based on Ag^+ ions with desired antimicrobial properties with little change in the technological process.

The silver nanoparticles may be used to modify the traditional and new bio- and nano-materials: nanosorbents, fillers, coatings, detergents and disinfectants, including tooth pastes, detergents, soaps, creams and cosmetics. The coatings based on nanomaterials (composite, textile,

paint, nanocarbon, nanosilicates), modified with silver nanoparticles can be applied as prophylactic antimicrobial protection means in problem areas where there is a risk of spread of infection: transport, public catering enterprises in areas of agricultural and livestock companies, nurseries, sports and health facilities. In conjunction with nanocarbon and nanosilicate minerals silver nanoparticles is appropriate to include in sorbents based on activated carbon impregnated with Ag^+ , for purification of water and destruction of pathogens in the filters of air conditioning systems, swimming pools, shower rooms and other public areas.

Another promising area of using the micro-dispersed silver nanoparticles is the disinfection of water in water treatment and water treatment processes [31]. At the recent time in Russia and Bulgaria are developed effective filter sorbents based on activated carbon impregnated with Ag^+ for cleaning and disinfection of water in homes and offices. These compact devices may be used to conduct an effective water treatment and disinfection of water by Ag^+ in the domestic environment.

The content of Ag^+ in drinking water is regulated by domestic norms and standards of SanPin 2.1.4.1116-02 [32] (not more than 0,025 mg/l of Ag^+).

Conclusions

The samples based on micro-dispersed silver nanoparticles possess a broad spectrum of bactericide, bacteriostatic and antiseptic effect. They may be used in different branches of medicine for the treatment and prevention of various infectious diseases, sanitation and drinking water treatment, disinfection of swimming pools and common areas, in cosmetics – to create a stimulating creams, in the paint industry – for the production of bactericidal water-based paints and varnishes, in the manufacture of detergents, soaps and toothpastes, fillings, and as a component of the sorption filters based on activated carbon for water purification and destruction of pathogens.

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УДК 541.144; 544.77

Электронная микроскопия мелкодисперстных наночастиц коллоидного серебра

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Аннотация. В статье рассмотрены методы получения мелкодисперстных наночастиц коллоидного серебра, электронная микроскопия и области его практического применения в наноиндустрии, бионанотехнологии и водоподготовке. Рассмотрены механизмы бактерицидного воздействия коллоидного серебра на микробную клетку с точки зрения адсорбционных, электростатических, ферментативных и мутагенных теорий. Серебро оказывает бактерицидное и бактериостатическое воздействие по отношению более чем 500 видов микроорганизмов. Эффект уничтожения бактерий препаратами серебра в 1500 раз выше действия такой же концентрации фенола и в 3,5 раза выше действия сулемы. Показано, что эффекты коллоидного серебра определяются концентрацией, размерами и стабильностью мелкодисперстных наночастиц в водных растворах, которые получают с помощью различных физико-химических, биохимических и биотехнологических методов.

Ключевые слова: коллоидное серебро, наночастицы, нанобиотехнология, водоподготовка.