SILVER NANOPARTICLES STABILIZED BY CARBONATE IONS AS ANTIBACTERIAL MATERIAL

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Abstract

The «pure» colloidal solution of silver (hydrosol) preparation method was developed. This hydrosol contains only silver NPs of the average size (20-25 nm) and stabilizing carbonate ions. Preparation method consists in the photo-chemical reduction of silver ions by oxalate-ions, which generate stabilizing carbonate ions. The hydrosol was studied by methods of physicochemical analysis (spectrophotometry, dynamic light scattering, and transmission electron microscopy, scanning electron microscopy). The hydrosol is aggregative stable for several weeks in water. The Bacteria Escherichia coli (gram-negative, family Enterobacteriaceae) is chosen as biological object to study the bactericidial properties NPs It has been found that silver NPs suppress the development of microflora. However, the antimicrobial effect is lower in comparison with silver in ionic form.

Keywords: Silver nanoparticles, antibacterial material, carbonate ions, «pure» hydrosol, Escherichia coli

1. INTRODUCTION

Silver nanoparticles (NPs) find wide use as effective antimicrobial agents in industrial applications and in medicine [1, 2]. One of the most common groups of nanomaterials is silver nanoparticles of various shapes and sizes. Silver NPs have wide antimicrobial action spectrum, the lack of resistance to it from the majority of pathogenic microorganisms and allergenic properties contribute to the increased attention to this nanomaterial. Silver in a form of nanoparticles is used in food packaging, sports clothes, paints, enamels and objects of common use, also in the perfume production and medical preparations. The antimicrobial activity increase multiple by reason of the large specific surface area. The small sizes (≤ 10 nm) allow influencing on the intracellular processes [3].

One of the most common ways of silver NPs preparation is reduction silver ions on the water solution [4]. Sodium borohydride, hydrazine, citrate, ascorbate usually are used as reduction agents [5, 6]. In water NPs show hydrophobic properties. Stabilizing organic and inorganic agents added into the solution to prevent the aggregation of nanoparticles. Polymeric compounds and polar organic molecules cap the metallic core and the large micelle formed as a result. A layer of stabilizer weakens the mutual attraction of the metallic particles. On the one hand the NPs surface modification by the stabilizer provides aggregative stability, on the other hand physicochemical properties change significantly. Used stabilizing and reducing agents and their dissociation products as a rule are toxic. For this reason, the disinfecting silver action is complicated by these toxic supplements and solvate layer, obstructing the contact silver NPs with biological object. Consequently, there are actually to develop a methodology of synthesize silver NPs without toxic compounds and to the greatest extent matches to the natural water composition, containing carbonate ions and stabilize it by electrostatic methods using ions with small sizes, which are harmless for the people health and the environment. On the NPs surface the carbonate ions are forming electrical double layer. The micelle size with electrostatic mechanism of stabilization is comparable with size of nanoparticle and NPs surface is much more available for biological object in comparison with polymeric mechanism of stabilization [7]. The task was to obtain the “pure” silver hydrosol, containing only NPs of small sizes and stabilizing it carbonate ions.

The Bacteria Escherichia coli (gram-negative, family Enterobacteriaceae) was chosen as biological object to study the antimicrobial activity of produced silver NPs. Silver ions’ activity was studied to compare the results.

Different concentrations of silver NPs and silver ions have been inoculated into the liquid nutrient media with bacteria cells. Silver activity consists in inhibition of cells quantity increase as a result of cells division impairing.

2. MATERIAL AND METHODS

Monohydrate of the silver perchlorate ($AgClO_4 \cdot H_2O$, 99%) by Aldrich Chemical and the potassium oxalate ($K_2C_2O_4$, 99.9 % special purity grade) by Reakhim were used in experiments. Preparation of “pure” silver hydrosol, contained only silver NPs and carbonate ions is based on reducing silver ions in solution in the presence of oxalate ions under the pulse UV-radiation. As the source of silver ions, 3×10^{-4} mol/L aqueous silver perchlorate ($AgClO_4$) was mixed with 1×10^{-4} mol/L potassium oxalate ($K_2C_2O_4$) as carbonate ions source (ionic stabilizing agent). Irradiation was carried out on the air in the quartz cuvette (2 mL) with the optical path length 5 mm. Solutions have been irradiated by pulse xenon lamp with the total radiation flux intensity I_{UV} = 6.0×10^{20} quantum/s. Optical spectra were measured using a Cary 100 Scan (Varian Inc.) spectrophotometer, equipped with thermostatic Peltier cell, with temperature T = 20 ^{0}C. Hydrodynamic size and ζ-potential of colloidal particles were determined by method of dynamic light scattering on Delsa Nano C (Beckman Coulter, Inc.), the wavelength of the scattered laser radiation λ = 658 nm. The sizes and polydispersity of the nanoparticles were determined with a JEM-2100 transmission electron microscope (TEM) (JEOL, Japan) operating at an accelerating voltage of 200 kV.

3. RESULTS AND DISCUSSION

3.1. Silver NPs characterization

UV-light action on aqueous solution of silver ions (3×10^{-4} mol/L) containing oxalate ions (1×10^{-4} mol/L) initiates the formation of active reducing radicals CO_2^-, silver ions reduction and formation of NPs and carbonate according to following scheme:

$$(CO_2)_2^{2-} \rightsquigarrow 2\,CO_2^- \quad (1)$$

$$Ag^+ + CO_2^- \rightarrow Ag^0 + CO_2 \quad (2)$$

$$nAg^0 \rightarrow Ag_n \quad (3)$$

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^{2-} \quad (4)$$

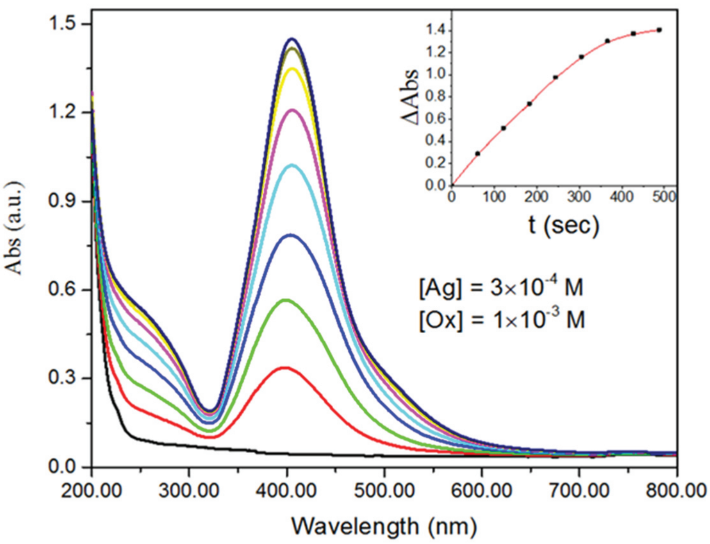

Figure 1 Optical absorption spectra of solution containing Ag^+ ions (3×10^{-4} mol/L) and ions (1×10^{-3} mol/L) after different time of UV irradiation. The insert shows variations in the SPR peak intensity (I_{SPR}) as depending on the irradiation time

It is substantiated by appearance and growing of optical absorption of surface plasmon resonance (SPR) on range 390-410 nm, which is characteristically for silver NPs [8]. The shift of SPR band from 410 nm to 390 nm occurs on the reduction process. This is due to the silver ions reduction on the NPs surface and their “pumping” by extra electrons as result of radical ions CO^{2-} discharge. The SPR absorption band increase and its shift to the short waves are result of free electrons concentration increase in the particle. The process is completed with full reduction of silver ions. This is manifested on achievement of optical absorbance stationary level of metal particles.

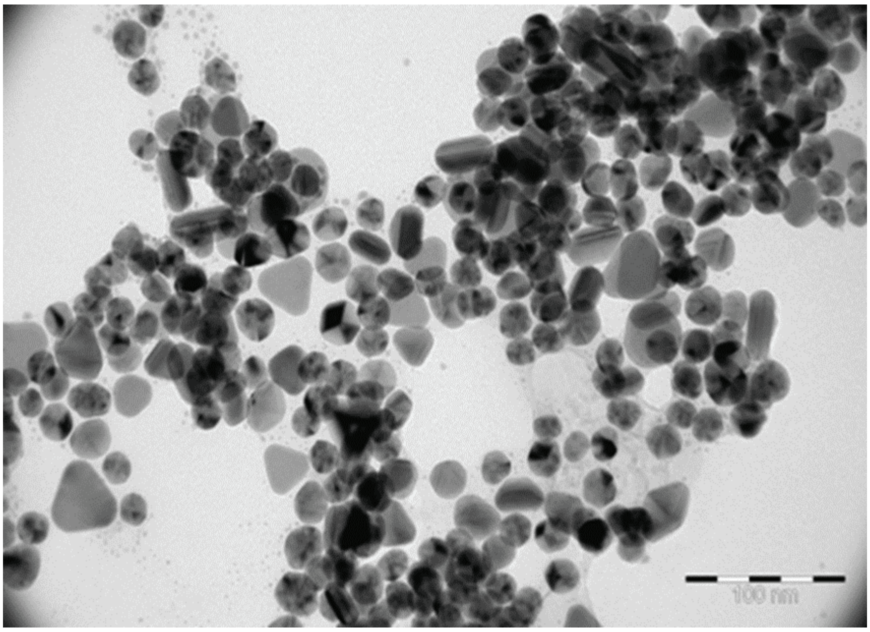

Figure 2 TEM image of obtained silver nanoparticles

TEM data indicates the nanoparticles formation mainly with spherical form and with sizes narrow distribution. The NPs medium size measured by TEM is 21.9 ± 4.6 nm and measured by DLS is 22.8 ± 5.2 nm. Thus, micelle has the approximately same size such as metallic core. Silver hydrosol is stable for several weeks. [9]. Silver NPs have thin but effective stabilizing electric double layer [10].

3.2. Antibacterial activity of silver NPs

Antibacterial activity of obtained NPs was studied.

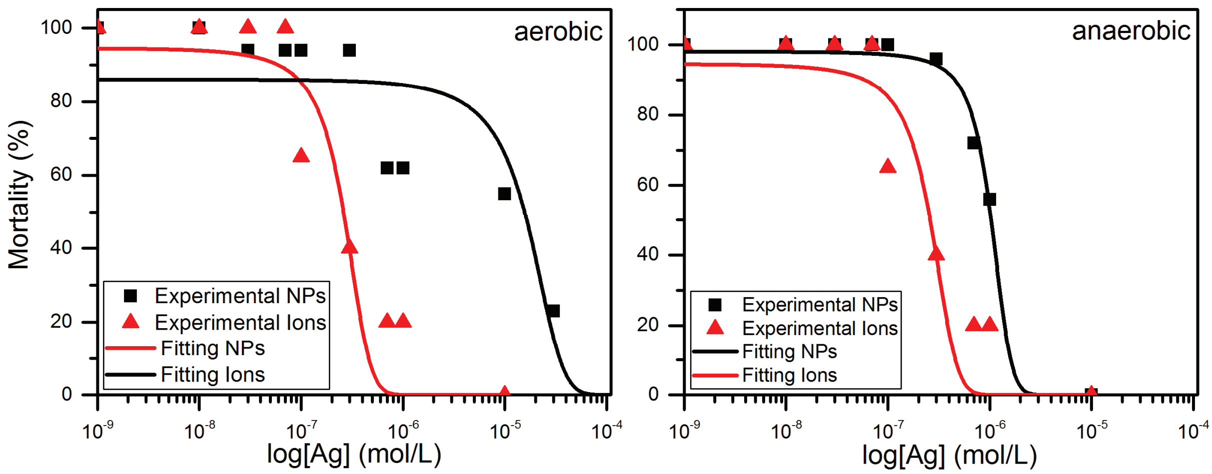

Figure 3 Quantity of cells: result of inhibition by different concentration of silver under aerobic and anaerobic conditions

The antibacterial effect of silver ions shows up at lower concentrations than nanoparticles. Suppressing of the activity of bacteria has been found at lower concentrations under aerobic conditions in comparison with anaerobic conditions.

Table 1 LD50 (mole/L) of bacteria Escherichia coli

	Ion, mol/L	NPs, mol/L
Aerobic	2.61×10^{-7}	1.56×10^{-5}
Anaerobic	2.61×10^{-7}	1.03×10^{-6}

Under anaerobic conditions LD_{50} of bacteria Escherichia coli consists 2.61×10^{-7} mol/L for silver in ionic form and 1.56×10^{-5} mol/L for silver in metallic form.

3. CONCLUSION

Thus, we developed a method for the preparation of pure silver hydrosols, which contain virtually only small nanoparticles and stabilizing carbonate ions. These hydrosols are free from compounds that are usually employed as reducing agents or stabilizing additives and the products of their decomposition, which may be toxic towards living nature or affect functioning of living organisms. The silver hydrosol that we prepared fully corresponds to the composition of natural freshwater and can be used as a disinfecting solution. It has been found that silver NPs suppress the development of microflora (Bacteria Escherichia coli). However, the antimicrobial effect is lower in comparison with silver in ionic form. Despite of lower antimicrobial effect of silver NPs compared with silver in ionic form, NPs are perspective bactericidal material with long-lasting and prolonging action. Silver nanoparticles are more effective and universal antimicrobial agent because they firmly absorbed and could be included in objects of different purpose. As well NPs are sources of silver ions for a long time and they can be used as effective disinfectants for medicine.

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