Slava
Technological Park

General Manager of
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_____________ V.I. Shkredov
"   " March 2015

PROTOCOL

of the colloidal homogeneous dispersion of silver nanoparticles laboratory tests

The study of the colloidal homogeneous dispersion of silver nanoparticles provided by LLC “Iso-lab” was made in the Technology Center for collective usage in the branch of “Nanotechnologies and Nanomaterials” JSC “Technopark Slava”. The purpose of the study was to determine the composition of the colloidal homogeneous dispersion: specification of the presence of silver nanoparticles in the colloidal homogeneous dispersion, as well as other substances, including SAS, sizing and concentration of silver nanoparticles and the recommendations on the perspective areas of application of the above mentioned colloidal homogeneous dispersion.

Spectrophotometric research of the colloidal homogeneous dispersion

The presence of silver nanoparticles, their concentration and the presence of other substances in the colloidal homogeneous dispersion was determined by the optical spectrophotometry using a spectrophotometer SF-2000 (manufactured by CSC “Spectrum”, Russia). Figure 1 shows the spectra of absorbance D in the range of wavelengths 300 - 800 nm for the initial colloidal homogeneous dispersion, as well as for the homogeneous dispersion obtained by the diluting of the initial homogeneous dispersion with distilled water. The figure shows that the spectra for all the homogeneous dispersions containes one peak at a wavelength of 390 nm. The peak at a wavelength of 380 - 420 nm is the characteristic of silver nanoparticles and is caused by the plasmon resonance phenomenon. Other peaks on the spectra of the optical density are not observed that indicates that the analyzed homogeneous dispersion is a colloidal suspension of silver nanoparticles in water and does not contain other substances, including SAS.

Figure 2 shows that the height of the original peak of plasmon resonance and the dilute of the homogeneous dispersions are proportional to the concentration of silver nanoparticles in the homogeneous dispersion. The comparison of the absorbance spectrum of the initial homogeneous dispersion with those of the other brands on the market of colloidal homogeneous dispersion of silver nanoparticles (see Figure 3) allows to make an assessment of the nanoparticle concentration in the tested homogeneous dispersion. It is about 0.07 g / L. Figure 2 may be recommended to the potential consumers of the colloidal homogeneous dispersion for the input control of silver nanoparticles concentration in the homogeneous dispersion and also for the output control of the silver nanoparticles concentration in the material, if the material is optically transparent - varnishes, plastics, shampoos etc.

The research of the colloidal homogeneous dispersion by the transmissive electronic microscopy (TEM)

The dimensions of nanoparticles after evaporation of solvent (water) from the sample of the colloidal homogeneous dispersion, their structure and composition were researched by TEM with the help of electron microscope LEO-912 AB OMEGA (Germany). Figure 4 is a microphotography, obtained by the mentioned microscope. The figure shows that the majority of the nanoparticles has a size from 5 to 15 nm, though there are particles up to 25 nm. The particles have a shape close to spherical. The electron-diffraction pattern depicted in Figure 4 shows that the nanoparticles consist of crystalline silver without appreciable doping materials of oxides and salts. Crystallite size coincides with the size of the particles, thus silver particles are monocrystal. The difference between the sizes of nanosilver particles obtained by dynamic light scattering and transmissive electronic microscopy is not surprising, as the first method explores the particle size directly in the homogeneous dispersion, and the second - after removal of the solvent (water).

Research of the effect of a colloidal homogeneous dispersion to some opportunistic mold fungi

The test of the medication on the fungicidal properties were made with the disk diffusive method. The filter paper disks impregnated with the medication were placed in Petri plates with pre-seeded “lawn” of mold fungi. As the test cultures the following mold fungi were used: Aspergillius niger, Aspergillius flavus, Penicillium funiculosum, Ulocladium atrum, Chaetomium globosum. The proposed test species of mold fungi are known as strong human allergens, they can cause a variety of fungal infections and are biodestructors of the organic materials. Three days later we observed the development of tested crops on the labored disks and around them. According to the growth character and the zone of the inhibition biological activity of the medication deposited on the paper disk was measured.

According to the test results of the fungicidal effect of the medication showed its highly effectiveness according to the mold fungi Aspergillius niger, Penicillium funiculosum, Ulocladium atrum, Chaetomium globosum; and good effectiveness according according to the mold fungus Aspergillius flavus. These facts allows the medication to be perspective to the usage of its fungicidal properties.

Conclusion.

1. The research has shown that the colloidal homogeneous dispersion is a suspension of silver nanoparticles in water and does not contain other substances, including SAS.
2. The toxicity of the tested homogeneous dispersion is 9 times lower than the same concentration of the silver ion homogeneous dispersion. According to the research made in the Institute of Nutrition of the RAMS, the maximum permissible concentration (quantitatively expressing the toxicity to the human body) of silver nanoparticles is 450 mg / l, instead of 50 mg / l, like in silver ions. Long-term studies made by the leading RAMS Institutes showed that silver nanoparticles destroy a large number of bacteria, viruses, fungi, but has a low toxicity to humans, so they are used for disinfection of upholstered furniture, buildings, toys, to impart biocidal properties to air filters and water filters, they are used as biocidal additives in textile and paint industries.
3. As the properties of the nanoparticles depend mainly on their shape and size, it can be said that the studied colloidal homogeneous dispersion of silver nanoparticles has a good correlation with biocidal and toxicity.
4. The tested homogeneous dispersion does not contain SAS and may be used in those areas where biocidal agent foaming is not acceptable and where the contact of biocidal medication with the mucosa may occur.

5. Taking into account the properties of the tested homogeneous dispersion of silver nanoparticles, and the lack of impurity substances in its composition, including SAS, as perspective areas of application we the following can be recommend:

   a. Water-based paints for medical and child institutes and crowded places. The tested homogeneous dispersion can be used as a biocidal additive in paints. The ratio between the amount of additive and the amount of paint must be determined experimentally by microbiological testing. The estimated ratio is from 1:40 to 1:20.
   b. Water-based paints for places with high humidity (bathrooms, boiler rooms, swimming pools, baths). The tested homogeneous dispersion can be used as a biocidal additive in paints to prevent mold. The ratio between the amount of additive and the amount of paint must be determined experimentally by microbiological testing. The estimated ratio is from 1:20 to 1:10.
   c. Filters for air conditioners and ducts. The tested homogeneous dispersion can be used for the silver nanoparticles deposition on filters to protect from fungi and legionellosis. For treatment it is recommended to use undiluted original homogeneous dispersion. The amount of the homogeneous dispersion per 1 m^2 surface of the filter should be determined experimentally by microbiological testing.
   d. Biocidal filters for drinking water. The tested homogeneous dispersion can be used for the deposition of the silver nanoparticles on charcoal to kill bacteria, viruses and fungi in the filtered water. For processing it is recommended to use the initial homogeneous dispersion diluted in 5 - 15 times. The multiplicity of the dilution should be determined experimentally by microbiological testing.
   e. Biocidal fabrics for sportswear, underwear, socks. The tested homogeneous dispersion can be used for the deposition of the silver nanoparticles on the fabric by spraying or impregnation, followed by squeezing and drying. For processing it is recommended to use the initial homogeneous dispersion diluted in 3 - 10 times. The multiplicity of the dilution and the amount of the homogeneous dispersion per 1 m^2 on the surface of the fabric must be determined experimentally by microbiological testing.
   f. Wet biocidal wipes. The tested homogeneous dispersion can be used as a biocidal additive to the liquid impregnating wipes. The ratio between the amount of the additive and the amount of the liquid must be determined

experimentally by microbiological testing. The estimated ratio is from 1: 5 to 1:15.

g. Spray for the disinfection of furniture, children's toys, rooms. The analyzed colloidal homogeneous dispersion diluted in 5 - 10 times can be used to fill aerosol cans. The multiplicity of the dilution should be determined experimentally by microbiological testing.
h. Aerosol for protection the inner surface of the shoe from the fungi. The analyzed colloidal homogeneous dispersion diluted in 3 - 8 times can be used to fill aerosol cans. The multiplicity of the dilution should be determined experimentally by microbiological testing.
i. Antiseptic and medicinal product for medical applications (treatment of wounds, burns, skin and mucous membrane affected by fungal diseases). As the antiseptic it is considered to use pure analyzed colloidal homogeneous dispersion. This area requires large-scale microbiological, pharmacokinetic and morphological researches.
j. The biocidal polymer packaging for food products. As biocidal additives into polymer it is considered to use pure colloidal homogeneous dispersion. The ratio between the amount of the additive and the amount of the polymer is to be determined experimentally by microbiological testing. The estimated ratio is from 1: 100 to 1:50.

The Head of the Shared Technology Centre in the branch of “Nanotechnologies and Nanomaterials”

JSC "Technopark Slava"

Cand. Sc. I.A. Chmutin

Exhibit 1

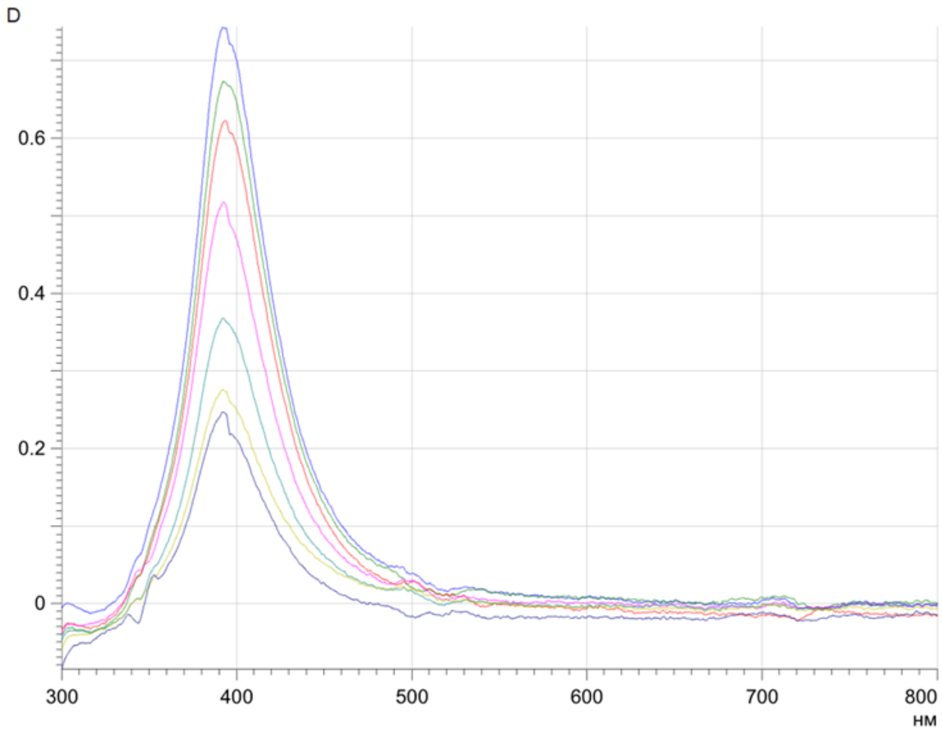
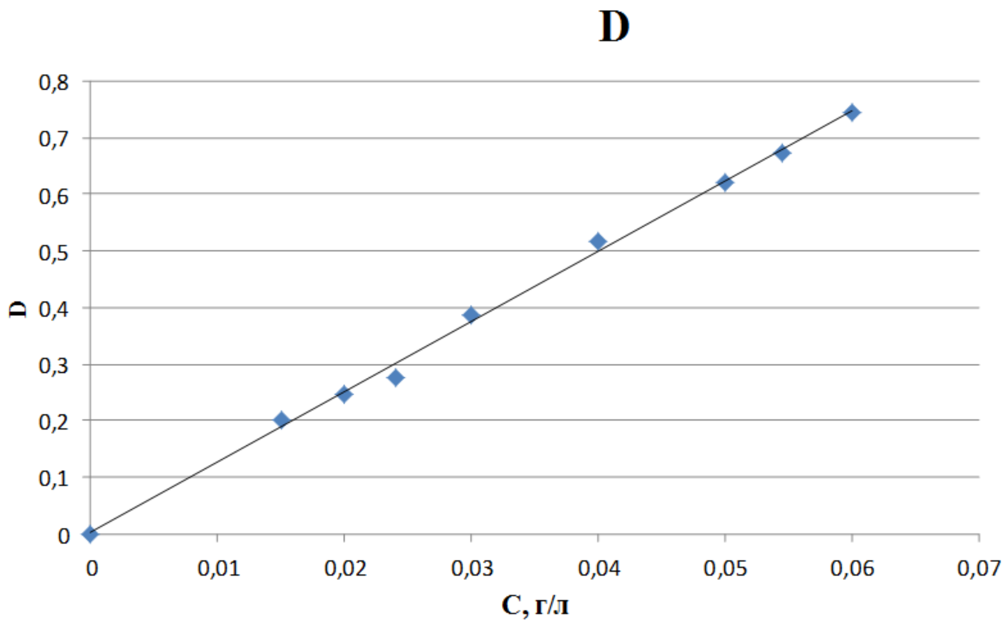


Fig.1

The optical density spectra D for the researched original colloid homogeneous dispersion (blue line) and the homogeneous dispersion diluted with distilled water in various proportions.

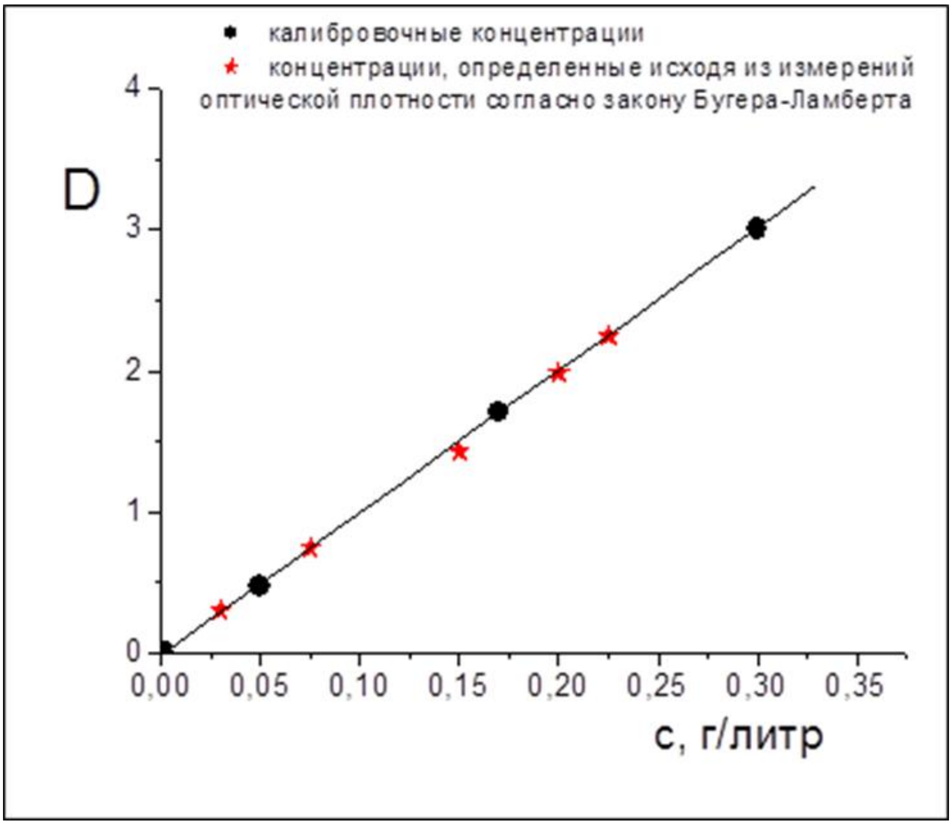


S, g/l

Fig.2

The dependence of the optical density D for the researched colloidal homogeneous dispersion of silver nanoparticles from the concentration of the nanoparticles.

1- Caliber concentration
2- Concentration determined according to the optical density measurements based on Bouguer-Lambert law



S, g/l

Fig.3

Dependence of the plasmon resonance peak height in the spectrum of the optical density to the colloidal homogeneous dispersion of silver nanoparticles, marketed under the trademark AgBion.

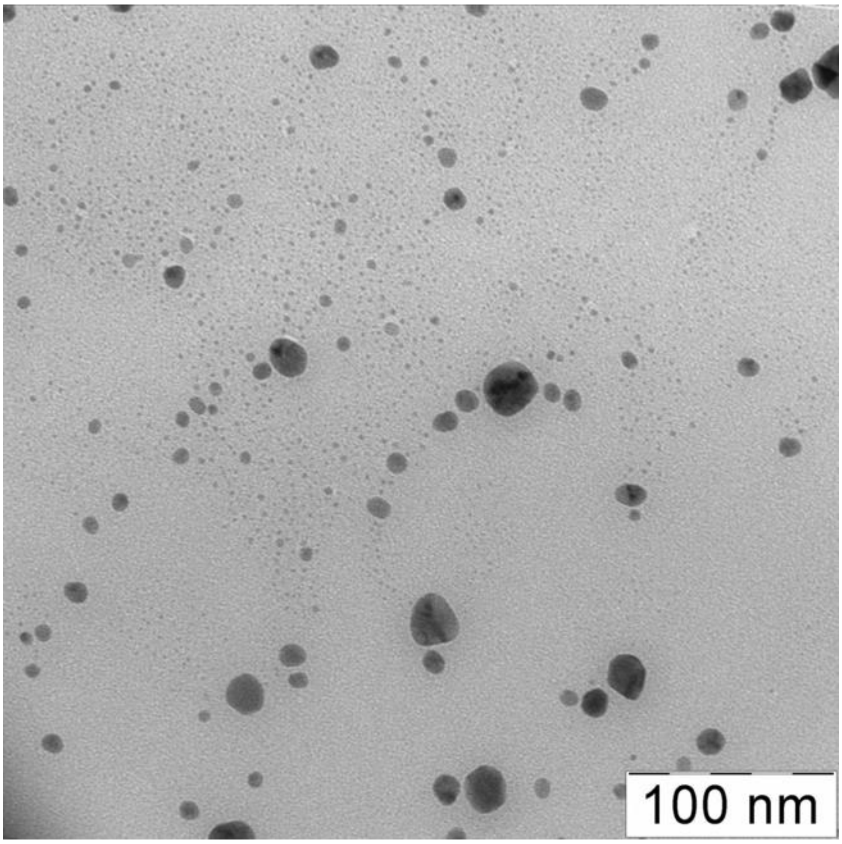


Fig. 4

The electronic micrograph of nanoparticles obtained by TEM method after the solvent evaporation from the colloidal homogeneous dispersion.

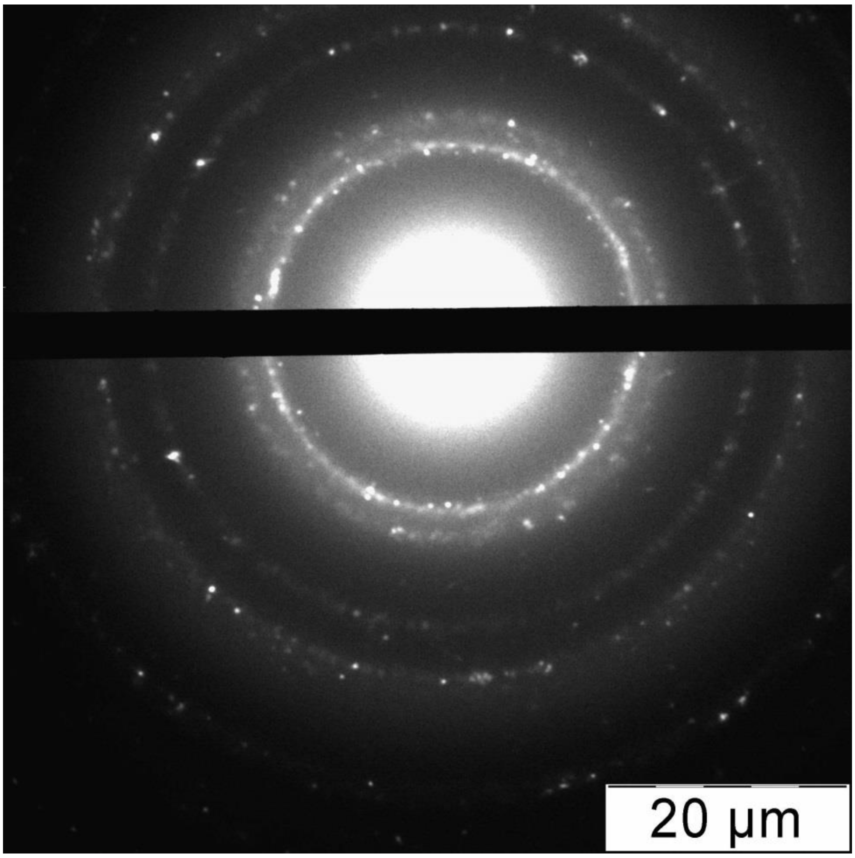


Fig. 5

The electron-diffraction pattern of the studied nanoparticles obtained after removing of the solvent from the colloidal homogeneous dispersion.

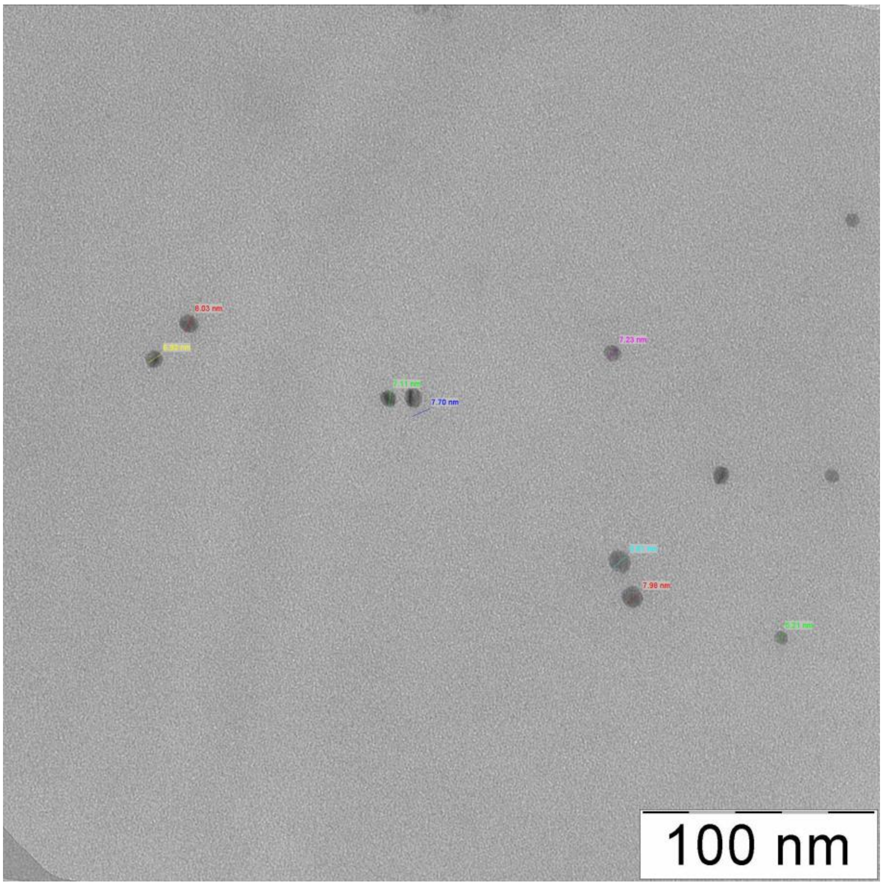


Fig.6

The electronic micrograph of the nanoparticles obtained by TEM method after the solvent evaporation from the colloidal homogeneous dispersion.