Interaction of silver nanoparticles with biological objects: antimicrobial properties and toxicity for the other living organisms

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Abstract. This paper presents several examples of the biological effects of small-sized silver nanoparticles (10.5±3.5nm) observed in experiments on bacteria, slim mold, unicellular alga and plant seeds. The nanoparticles were prepared by the biochemical synthesis, based on the reduction of metal ions in reverse vicielles by biological reductants - natural plant pigments (flavonoids). It is found that, except for the plant seeds, silver nanoparticles (SNP) act as a strong toxic agent, both in water solution and as part of liquid-phase material. It is shown also that the biological action of silver nanoparticles can not be reduced to the toxic action of silver ions in equivalent concentrations or to that of the surfactant (the SNP stabilizer) present in the SNP water solution. Possible SNP applications are suggested.

1. Introduction

Silver nanoparticles (SNP) are widely used now for the creation and production of modified materials with special properties. However, the mode of their action on the living organisms and the conditions providing the safety of their applications remain poorly understood. Therefore, of particular interest are studies allowing to reveal the effects of nanoparticles on various functions of the biological systems of various levels of organization. In particular, it is important to test the SNP antimicrobial activity, in order to define their minimal concentrations providing the 100% death of a given microorganism and thus to estimate their effectiveness for the use as disinfectant. At the same time, it is clear that it is desirable to avoid the pathological changes in the plant, animal or human organisms caused by their contact with SNP present in air, water or on various surfaces after treatment with SNP-containing disinfectants. Hence it is reasonable to assume that studies of the antimicrobial properties of silver nanoparticles should be combined with the tests of their biological action allowing to determine the concentration limits within which the nanoparticles are not toxic for a given higher organism subjected to their influence.
It is well-known today that size, form, stability and properties of metal nanoparticles depend on the method used for their synthesis. So it seems justified to test the action of silver nanoparticles on various biological objects using the SNP preparations obtained by one and the same method. Such an attempt was made by us in studies of the biological effects of SNP obtained by the original method of biochemical synthesis [1,2], based on the reduction of metal ions by natural plant pigments (quercetin, rutin, morin) in reverse micelles formed by the anionic surfactant AOT. As shown earlier [2-4], the method allows to obtain SNP small in size (below 25 nm) and stable on air in micellar solution for a long time (up to several years). For the studies of the nanoparticles interaction with biological objects we used water solutions of SNP obtained by their transfer from micellar solution into the water phase.

In this paper we briefly describe the results of studies of the SNP effects on bacteria species in water solution and in the biodegradable polymer films, as well as of the toxicity exhibited by these nanoparticles towards some other biological systems. The experiments were carried out on small nanoparticles (about 10 nm in size) for which one could expect that specific properties of the nanosized state are clearly expressed.

2. Materials and Methods

Silver nanoparticles were obtained by the biochemical synthesis in AOT reverse micelles. Here the reduction of metal ions to atoms and subsequent formation of nanoparticles takes place in the micelle water core at small hydration extent \( w = [H_2O]/[AOT] < 4 \). From micellar solutions, water dispersions of SNP were prepared by the specially developed procedure [5]. The SNP concentration in micellar or water solutions was found from optical absorption spectra recorded on spectrophotometer Helios-\( \alpha \) (Thermo Electronics, GB) in 1 mm quartz cell. Particle sizes in micellar or water solution were determined by transmission electron microscopy (TEM) on LEO912 AB OMEGA microscope (Carl Zeiss, Germany). From electron micrographs particle size distributions were found for no less than 300 particles using Gauss approximation.

![TEM image and size distribution of silver nanoparticles in standard water solution.](image_url)
The spherical SNP 10.5 ± 3.5 nm in size (see Figure 1) were used in studies of the biological effects both as water solutions and as a part of liquid-phase (e.g. paints or polymer films) or solid materials (activated carbon, cloths, polyamide filtering membranes covered with SNP). The SNP concentration in solution is expressed either in the corresponding molar concentration of silver salt or as equivalent weight content of metallic silver (in mg/liter or in µg/ml). The details of experimental procedures in various biological tests are given in [6].

3. Results and discussion

We present here three examples of the antimicrobial activity of SNP and three examples of their effect on the other biological objects – acellular slim mold, unicellular alga and plant seeds. Antimicrobial activity is illustrated by the tests with (1) SNP water solution and (2) biodegradable polymer film containing SNP.

(1) In the tests fulfilled on pathogen bacteria species, Escherichia coli and Staphylococcus aureus, bacteria cell suspensions with $2 \times 10^5$ CFU/ml were incubated with various SNP concentrations obtained by dilution of the initial SNP water solution (Table 1). The SNP concentration in cell suspension, C(SNP) lied in the range 0.27 - 6.5 µg/ml. The initial SNP concentration was 216 µg/ml. It was found that both bacteria species were fully destroyed at C(SNP) ≥2.88 µg/l SNP (≥1.5% of initial concentration) after 30 min of incubation.

Table 1. Action of SNP water solution on the bacteria species Escherichia coli 1257 and Staphylococcus aureus 906 in aqueous medium. Tests of the antibacterial activity were carried out by means of incubation of bacteria with silver nanoparticles at various dilutions of SNP initial water solution. (-) – absence and (+) – presence of bacterial growth. The data obtained by Moscow Disinfection Center.

| Microorganism | Exposition (hours) | Bacteria growth at various C(SNP). Upper row - % of initial SNP concentration, lower row – SNP concentration in the medium (µg/ml) |
|---------------|--------------------|------------------------------------------------------------------------------------------------------------------|
|               | 3                  | 2                   | 1,5                 | 1,0               | 0,75              | 0,5               | 0,25              | 0,125             |
| E.coli 1257   | 0,5                | -                   | -                   | +                  | +                 | +                 | +                 | +                 |
|               | 1,0                | -                   | -                   | -                  | +                 | +                 | +                 | +                 |
|               | 2,0                | -                   | -                   | -                  | +                 | +                 | +                 | +                 |
|               | 24,0               | -                   | -                   | -                  | -                 | +                 | +                 | +                 |
| S.aureus 906  | 0,5                | -                   | -                   | -                  | +                 | +                 | +                 | +                 |
|               | 1,0                | -                   | -                   | -                  | -                 | +                 | +                 | +                 |
|               | 2,0                | -                   | -                   | -                  | -                 | +                 | +                 | +                 |
|               | 24,0               | -                   | -                   | -                  | -                 | +                 | +                 | +                 |

(2) Figure 2 shows the results observed in the tests on E.coli in water suspension. It is seen that, at the high initial concentration of bacteria cells ($3 \times 10^8$ CFU/ml) the high level of inactivation (> 90%) is achieved after incubation for 30 min in the whole range of SNP concentrations (3 – 30 µg/ml). Beginning from 5 µg/ml, total death of bacteria (100 % inactivation) is registered.
Figure 2. The effect of SNP on inactivation level of E.coli cells in water suspension. The SNP concentration in suspension was varied by means of dilution of the initial SNP water solution. Initial concentrations of SNP – 60.5 μg/ml, AOT – 20 mM. At each SNP concentration the suspension was incubated for 30 min before quenching the SNP activity. Initial concentration of bacteria cells – 3*10⁸ CFU/ml. Data from the Institute for Genetics and Selection of Industrial Microorganisms of the State Scientific Center (Moscow).

Polymer films with SNP were obtained by mixing the SNP water solution with that of biodegradable polymer (carboxymethyl chitin); details of the film preparation are given in [7]. Such films were tested on antimicrobial activity against Staphylococcus aureus and Salmonella typhimurium (Table 2). It was found that the films containing small SNP additions (0.03-0.06 wt%) possess a high antimicrobial activity, at high concentrations of bacteria cells in the films (~4*10⁷ CFU/cm³). The results obtained testify to the strong bactericidal effect of the films with SNP. It is supposed that such films can be applied in medicine, for example, in the treatment of skin injuries.

Table 2. Dynamics of the interaction of bacteria strains with polymer films made from carboxymethyl chitin with or without SNP. Bacterial culture was introduced into the segment (1/4) of polymer film (d=50 mm, thickness 50 μ). After incubation for a given time films were resuspended in the nutrient broth and the CFU number was calculated after cultivation in the standard conditions. Data obtained from the Gamaleya Institute of epidemiology and microbiology (RAMS).

| Bacteria species, Dose | Films without (control) and with various concentrations of SNP | Log of the number of living cells in the films after the time periods (hours) |
|-----------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|                       |                                                               | 1 | 3.0 | 6.0 | 24.0 |
| Salmonella typhimurium | Initial bacteria culture                                      | 6.0 | 6.0 | 6.0 | 6.0 |
| TMLR66                | Chit - 10. A (control)                                        | 6.0 | 5.8 | 5.5 | 3.0 |
From the results of experiments described above and other similar tests (see [6]) it was concluded that SNP water solutions possess a high antimicrobial activity and can be considered as basic solutions for the creation of disinfectants of a new type, effective and less dangerous for users than those containing chlorine and its derivatives or amino compounds.

The effect on living organisms of the higher levels of organization was studied on the objects of various types, including plasmodium of the slim mold, unicellular algae, plant seeds, animal cells in vitro and animal organisms. In the control experiments, SNP action was compared to that of Ag⁺ ions in equivalent concentrations and of the AOT water solution added to the concentration equal to that introduced with SNP water solution. Here we give three examples of the in vitro studies of the SNP effect.

(1) Plasmodium of the slim mold Physarum polycephalum is a popular object used in studies of chemotactic phenomena. It is a multinuclear protoplasm surrounded by the mutual membrane capable of growth and amoeboid movement. The aim of our experiments (made in collaboration with Institute of theoretical and experimental biophysics of RAS) was to determine the effect of SNP on the Plasmodium motive activity and growth. For the description of experimental procedure see [8]. Example of the result obtained on Petry dishes showing the SNP effect on the Plasmodium growth is presented in Figure 3.

| 10⁶ KOE          | Chit – 10. B (0,03 wt% Ag) | 5,1 | 4,2 | 3,0 | 0 |
|------------------|----------------------------|-----|-----|-----|---|
| Chit – 10. C (0,06 wt% Ag) | 0   | 0   | 0   | 0  |
| Staphylococcus aureus | Initial bacteria culture  | 6,0 | 6,0 | 6,0 | 6,0 |
| 10⁶ KOE          | Chit - 10. A (control)    | 6,0 | 5,7 | 5,3 | 3,5 |
| Chit – 10. B (0,03 wt% Ag) | 5,2 | 4,4 | 3,2 | 0  |
| Chit – 10. C (0,06 wt% Ag) | 0  | 0  | 0  | 0  |
Figure 3. Spreading of Plasmodia on agar substrate in 6h. SNP (НЧС), AOT and Ag⁺ concentrations are, respectively, 10⁻⁵ g-ion/l (1.08 μgAg/ml), 2*10⁻³ M and 10⁻⁵ M (upper row) and ten times higher (lower row). Scale bar, 1 cm. Reproduced from [8] with permission from Springer.

It is seen that, at C(SNP) = 10⁻⁴ M no growth is observed in all cases. At C(SNP) = 10⁻⁵ M, there is a marked difference in growth in the medium containing SNP and in three media containing AgNO₃, AOT and AOT + AgNO₃ mixture. The data obtained allowed to work out the row of effectiveness: AgNO₃ << AOT < AgNO₃ + AOT << SNP. These results indicate that, in case of SNP, toxic action is strongly amplified, and this effect can not be reduced to the destabilizing action of AOT, or to the release of Ag⁺ ions from the surface of nanoparticles. Similar conclusion was made in our studies of the SNP effect on mice in vivo and (for Ag⁺ ions) in studies of SNP antimicrobial activity on E.coli [6].

(2) Toxic effects of AgNP on microalga *Chlorella vulgaris* were studied by means of microelectrophoresis in collaboration with laboratory for biotesting of the Ministry of Natural Resources (Izhevsk, RF). The use of microelectrophoresis technique for the estimation of toxicity of various agents towards algae is based on the fact that the viability of living cells may be characterized by the surface charge of their plasma membrane, namely, the decrease of surface charge reflects the decrease of cell viability. Hence the toxicity of a given agent may be estimated from the decrease in membrane surface charge which is determined by measuring the changes in the amplitude of cell vibration in the alternative electric field.

Toxic effect is characterized by the toxicity index \( T = (A_c - A_{cell})/A_c \) where \( A_c \) and \( A_{cell} \) are the average amplitudes of cell vibration found for the control and experimental cell suspension, respectively. Apart from SNP, toxic effects of Ag⁺ ions and mixture AOT+Ag⁺ were estimated independently.

Figure 4. The effect of SNP(AgNP), Ag⁺ ions and mixture Ag⁺ + AOT on the viability of *Chlorella vulgaris*. The toxicity index \( T \) was estimated after 25 min incubation of alga cells in distilled water with the agents studied added to concentrations indicated.
The results are shown in Figure 4. It is seen that the toxicity of SNP for chlorella increases with SNP concentration and then remains constant at C(SNP) from 0.108 μg/ml (10^{-6} M) to 10.8 μg/ml (10^{-4} M), the achieved level of toxicity being equal to approximately half of the maximal (T = 1). At almost all concentrations studied the SNP are more toxic for chlorella than Ag+ ions or Ag+ + AOT mixture added to the same total concentrations as those introduced with nanoparticles. Hence here the toxic action of AgNP is unlikely to be caused by only Ag+ ions released from the nanoparticles surface or by the combined effect of Ag+ ions and AOT molecules present in the nanoparticles solution. This is in accordance with observations made on Plasmodium Physarum. At the highest concentration (10^{-4} M), the equal toxicity of all three agents studied is, most likely, the result of the cell death or near death state, similar to that observed for Plasmodium at the same concentration (Figure 3).

(3) The effect of SNP on the germination of plant seeds (arabidopsis and soy-bean) and on the lifetime of mice was studied in Vavilov Institute of General Genetics RAS. The influence of SNP water solution was compared to that of AOT and AgNO3 water solutions. The required concentrations of reagents were achieved by dilution of the initial solutions, for details see [6]. The results for arabidopsis are presented in Figure 5.

![Figure 5](image_url)

**Figure 5.** The effect of AgNP, Ag+ ions and AOT water solutions on the germination of plant seeds (*Arabidopsis thaliana*). The seeds were soaked in distilled water for 24 hours, then washed and placed into Petry dishes with SNP(AgNP), AOT or AgNO3 water solution for another 24 hours. Afterwards the seeds were washed with distilled water and placed for germination in the standard conditions.

As issues from these data, in the wide range of concentrations (5*10^{-7} - 1*10^{-3} M) the nanoparticles in water solution are not toxic for the seeds studied. This shows that SNP solution may be used for the protection of seeds from infection by the pathogen bacteria. Unexpectedly, a strong toxic effect was observed for Ag+ ions, contrary to what was found in experiments with Plasmodium, alga and animal organisms [6]. The origin of this effect remains to be elucidated.
4. Conclusions

1. Silver nanoparticles 10.5±3.5 nm in size act as a strong toxic agent towards both microorganisms and some other living systems, excluding plant seeds studied. With microorganisms, the SNP effect is registered both in the water medium and for the SNP-modified liquid-phase polymer films;
2. In most cases studied, the effect of silver nanoparticles exceeds significantly that of Ag+ ions in equivalent concentrations. This allows to suggest that the effect of silver nanoparticles can not be regarded as only the consequence of the action of Ag+ ions released from the nanoparticle surface.

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