Using the method of biotesting to assess the toxicity of waste medical and biological practices containing nanomaterials

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Abstract. One of the current modern scientific directions is the research and introduction of products obtained using nanotechnologies into various spheres of human activity. Among the variety of nanostructures, metal nanoparticles are found to be the most widely used, which, due to the uniqueness of their properties and small size, are considered as the most promising agents in the composition of bactericidal agents. They are used as specific carriers of drugs and as components in the composition of antibacterial agents. In addition, it has been proven that the bacteriolytic and bacteriostatic properties of some metals are greatly enhanced with decreasing size. Nanoparticles of various metals, among which silver nanoparticles are the most widely used, can be used independently; however, their high ability to aggregate requires the choice of stabilizers, which usually use natural and synthetic polymers. However, their use is limited due to the high toxicity of metallic nanoparticles. Therefore, the search for the safest options for metal nanoparticles is relevant. This article presents data on the toxicity study of prototypes of silver nanoparticles stabilized by synthetic and natural polymers on biological test objects.

1. Introduction

Today, the pharmaceutical industry is the main component in maintaining the health of the population, which leads to an increase in the quality and duration of life. But at the same time, there is a downside – it is the waste of the pharmaceutical industry, which have an impact on the environment. Pharmaceutical contamination of wildlife is closely associated with increased consumption of medicines, which, in turn, contributes to demographic aging, the increasing prevalence of chronic diseases, the availability of low-cost treatment and the emergence of new drugs [1]. Currently, about 4000 active drugs have been registered [2, 3].

Pharmaceutical substances entering the environment can vary in a certain way under the influence of both biotic and abiotic factors. However, a number of compounds are not only resistant to natural destruction, but also able to change their activity with the formation of physicochemical modifications that pose a potential danger to living organisms and humans [3, 4]. These include, in particular, nitrogen-containing heterocyclic compounds exhibiting antimicrobial activity, some analgesics, as well as drugs used in sleep disorders, epilepsy and other diseases [2].

Nanomaterials are considered as the basis for the formation of a new technological element. Their essence consists of the purposeful manipulation of material objects and the creation of structures in the range of sizes from units to tens of nanometers. They are characterized by unique physicochemical
properties associated with a high ratio of their surface to volume, which indicates a high efficiency of their action [5-9]. Nanotechnologies are used to create biocompatible materials for surgery, antiseptic dressings, for targeted drug delivery to tissues [10-21].

Effective vaccines of a new generation are being developed, which are hybrid nanostructures formed by nanometers of artificial origin together with recombinant DNA molecules and virus-like particles [22, 23]. Therefore, nanoparticles should be carefully investigated not only for the toxicity of a particular nanoparticle, but also for the degree of exposure to its compounds, as well as their effect depending on the concentration of the substance in the medium and the ability to react with other compounds. The main risk factor for the use of nanotechnology in medicine is the lack of information about the interaction of specific nanoparticles with the human body [24-26].

At the same time, to assess the safety of nanomaterials, it is necessary to develop new toxicological approaches, since the inapplicability of traditional dosimetry to them [27, 28].

At present, it is necessary to change the previously established views on the problem of utilization of pharmacological waste in connection with anthropogenic changes occurring in the environment. There are four main ways of utilization of pharmaceutical trades, regulated by 87 and 155 instructions of the Ministry of Environment: discharge into industrial sewage system; burning; accommodation on specially equipped sanitary landfills; splitting up. None of the ways is environmentally friendly. The development of a unified integrated approach to the search for environmentally friendly methods of disposal and recycling of waste today is an important issue that requires a consistent and focused decision [29-32].

One of the methodological techniques that allows you to evaluate the effect of toxic environmental factors on the organism as a whole, or on its individual function is biotesting. Using biotesting methods, it is possible to model the effects of a particular factor with a general biological effect on a living organism, which contributes to a rapid assessment of the toxicity of the studied factors.

Due to the relevance of the above stated material, the purpose of the study was to evaluate the acute toxicity indicators of silver nanoparticles stabilized by various polymeric compounds on the model of a biotest object Daphnia magna Straus.

2. Objects and methods of research

The acute toxic effect of prototypes of silver nanoparticles stabilized by natural and synthetic polymers was estimated by the mortality of Daphnia magna Straus after 48 hours of exposure according to the standard method [33].

As prototypes, silver nanoparticles solutions stabilized with natural polymers were used: carboxymethyl cellulose (Ag / CMC), sodium oleate (Ag / OleNa) and synthetic polymer: dioxyl sodium sulfosuccinate AOT (AgW), sodium dodecyl sulfate (Ag / SDS), polyazolidine ammonium, hygrochloride, sodium dodecyl sulfate (Ag / SDS), polyazolidine ammonium, hydrochloride, sodium dodecyl sulfate (Ag / SDS), polyazolidine ammonium, polymerase, sodium dodecyl sulfate (AgW) hydrate ions of iodine (Ag / PG-m), polyvinyl alcohol (Ag / PVA). The working concentrations of the test samples were 3%, 2%, 1%, 0.5%, 0.25% and 0.125%.

According to the method, the assessment of the impact of experimental samples of silver nanoparticles presented in the test medium was carried out according to the daphnia mortality rates (D. magna Straus) in comparison with the control culture in samples that did not contain the dispersions studied (control). The criterion of the acute toxic effect of the compound was the death of 50% or more of the biotest objects for 48 hours in the test sample, as compared with the control samples in which all crustaceans maintained their viability.

Daphnids mortality counts were taken every 24 hours in experimental and control samples. The experiment was stopped if more than 50% of the crustaceans were observed to die within 24 hours in all the test tubes. Motionless individuals were considered dead if they did not begin to move for 15 seconds after lightly rocking the tube.
The number of dropped ephippia was also taken into account, since it was established that as a result of the reaction to the toxicant, daphnids molt and ephippium drops.

In experiments to determine the acute toxic effect of experimental samples of silver nanoparticles were established:

- lethal concentration causing death of 100% test organisms (LC$_{100-48}$);
- the average lethal concentration causing death of 50% and more test organisms (LC$_{50-48}$);
- the harmless concentration, causing the death of not more than 10% of the test objects (HC$_{10-48}$);
- the minimum concentration of a harmless compounds, when were not observed death of organisms (HC$_{0-48}$).

Statistical processing of the data was performed in the program "Statistica 6.0".

3. Results and Discussion

In the course of the research, it was found that according to the calculation of the percentage of dead daphnia relative to the control, all nanoparticle samples showed a toxic effect on the biotest-object under study (table 1).

Based on the data obtained, the acute toxicity indicators for the investigated aqueous dispersions of metal nanoparticles were determined, which are presented in Table 2.

As a result of the research, it was found that solutions of silver nanoparticles were characterized by different levels of toxicity with respect to test objects, which depended on the stabilizer content in the sample.

The study of the toxicity indicators of silver nanoparticles stabilized by carboxymethyl cellulose made it possible to establish an acute toxic effect of the drug against daphnia in the concentration range of 0.5–3%, for which the toxicity criteria of 0.125% and 0.25% toxicity were exceeded, had no toxic effect.

### Table 1. Daphnia survival rate in test samples of silver nanoparticles under study (exposure 48 hours)

| Laboratory codes of prototypes | Compound concentrations, % | control |
|-------------------------------|-----------------------------|---------|
|                               | 3  | 2  | 1  | 0.5 | 0.25 | 0.125 |
| AgW                           | 100 | 80 | 50 | 20  | 10   | 0      |
| Ag/OleNa                      | 90 | 90 | 70 | 40  | 25   | 5      |
| Ag/PG-m                       | 80 | 80 | 70 | 70  | 50   | 40     |
| Ag/PVA                        | 90 | 90 | 75 | 70  | 50   | 15     |
| Ag/SDS                        | 90 | 90 | 75 | 70  | 50   | 15     |

### Table 2. Indicators of the acute toxicity of silver nanoparticles (%)

| Prototypes | LC$_{100-48}$ | LC$_{50-48}$ | HC$_{10-48}$ | HC$_{0-48}$ |
|------------|---------------|--------------|--------------|-------------|
| AgW        | 3             | 1-2          | 0.25         | 0.125       |
| Ag/Coll     | -             | 3-0.5        | 0.25         | 0.125       |
| Ag/OleNa   | -             | 3-0.25       | 0.125        | -           |
| Ag/PG-m    | -             | 3.1          | 0.5-0.25     | 0.125       |
| Ag/PVA     | -             | 3-0.25       | -            | 0.125       |
| Ag/SDS     | -             | 3-0.25       | -            | 0.125       |

A high level of toxicity was established for a solution of silver nanoparticles stabilized by sodium oleate, since the toxicity criteria were exceeded for five working concentrations (3%, 2%, 1%, 0.5%, 0.25%). Acute toxic effect on the biotest object was absent only when the drug concentration was 0.125%.
An aqueous solution of silver nanoparticles exerted a toxic effect in the concentration range of 0.5–3%. Harmless for test objects were working concentrations of 0.25% and 0.125%.

Evaluation of the effect of working dilutions of silver nanoparticles stabilized by sodium dodecyl sulfate showed that concentrations from 0.25% to 3% exceeded the toxicity criteria for the biotest object and only the concentration of 0.125% was harmless.

When used as a stabilizer of metal nanoparticles of polyazolidine ammonium modified with iodine hydrate ions, it was found that a solution of silver nanoparticles had an acute toxic effect on biotest objects in the concentration range of 1-3%. For the remaining working dilutions, the toxicity criteria were not exceeded.

The effect of silver nanoparticles stabilized by polyvinyl alcohol, in the concentration range of 0.5–3% on daphnia, was characterized as acute toxic, since the toxicity criteria were exceeded for them. Concentrations of 0.25% and 0.5% were harmless when exposed to a biotest object.

The absence of toxic action for most working dilutions of silver nanoparticles solutions, stabilized by polyazolidine ammonium, modified by iodine hydrate ions, makes it possible to consider this stabilizer as the most promising.

### 4. Conclusions

Thus, it was shown that silver nanoparticles, stabilized by various versions of polymers, had an acute toxic effect, which depended on their concentration. The use of biotest objects makes it possible to reliably estimate the degree of nanomaterials toxicity, in particular, silver nanoparticles. The conducted studies allowed us to establish basically a similar pattern of manifestation metals nanoparticles of toxicity, stabilized by various polymeric compounds, with respect to *Daphnia magna* Straus.

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