Promising nanocompounds based on carbon and ultrafine metal particles with antibacterial properties in the development of a new generation of nano-disinfectants

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Abstract. Using the unique properties of nanomaterials leads to new and original solutions to the problems that are currently emerging, for example, to solving the problem of the development of various infections caused by bacterial strains that are resistant to traditional antibacterial drugs. This fully applies to bacterial strains—pathogens of animal and human infections with resistance to drugs—as well as to bacteria on the surfaces in laboratories and hospitals that acquired new resistance to a wide range of chemicals and compounds that were previously used against them. In this regard, the development of a new generation of nanodisinfectants based on fundamentally different active components, in particular, on the basis of carbon nano compounds and ultrafine metal particles, is of particular interest. Evaluation of such nanoscale compounds and/or particles was carried out using a recombinant strain of Escherichia coli K12 TG1 with the cloned luxCDABE genes of Vibrio fischeri by bioluminescent analysis. The compounds of nanocarbon and metal nanoparticles that can be used as an active principle in the creation of antibacterial drugs used in medicine and veterinary medicine are defined.

1. Introduction
The unique properties of nanomaterials—physical, chemical, and biological—determine their large-scale use in various fields of economic activity, including agriculture [1–5], in the field of creating alternative antibacterial drugs [6, 7], which is associated with the formation of multiresistant strains that cause “hospital” epidemic outbreaks, as well as uncontrolled infections with aerogenic and water spread. In this regard, it is advisable to conduct research on the development of new tools and methods for the prevention of bacterial infections based on fundamentally new active substances. In these circumstances, the use of nanocarbon compounds, in particular, derivatives of C60-fullerene functionalized by various chemical groups [8] and metal nanoparticles as an active agent, will be widely used for a wide range of bacteria, including pathogens of human and animal diseases [9], in particular, with respect to strains that are widely resistant to traditional antibacterial drugs (or, for example, multiresistant strains of Staphylococcus aureus and Escherichia coli) [10]. As for the mechanisms of their antibacterial activity, they are fundamentally different from previously used agents. At the same time, the possibility of using these compounds with the combination of its action with the photodynamic inactivation of microorganisms makes them even more in demand on the
modern market [11]. However, one should take into account the nature of the functionalization of carbon nanocompounds, because not every functionalization, including leading to an increase in the water solubility of C60-fullerene, is accompanied by an increase in its antibacterial activity [12]. As for ultrafine metal particles, an important parameter is the refinement of the size of the nanoparticles of each specific substance [13]. Thus, existing studies show that a decrease in the size of metal nanoparticles leads to an increase in their bioavailability and a more intense manifestation of antibacterial properties [14]. However, technically reducing the size of metal nanoparticles is a rather laborious and economically disadvantageous process, in connection with this, the use of a photodynamic increase in the antibacterial characteristics of drugs gives more opportunities for their use.

In this regard, the aim of the work was to develop a new generation of disinfectants for the effective disinfection of various surfaces based on highly structured carbon nanocompounds and ultrafine metal particles evaluated using the recombinant strain *Escherichia coli* K12 TG1 with the cloned *luxCDABE* genes of *Vibrio fischeri*.

2. Materials and Methods

In our studies, we used three samples of compounds based on nanocarbon, which are water-soluble derivatives of C60-fullerene (DC60-1, DC60-2, DC60-3), covalently functionalized by various chemical groups (addenda). These fullerenes were synthesized as a result of the transformation of halogen fullerenes into polyfunctional derivatives as a result of the interaction of C60Cl6 chlorinated fullerene with primary and secondary amines in the presence of a base. The degree of purification of the obtained compounds from technological impurities was studied by an inductively coupled plasma mass spectral method using an Elan-6100 quadrupole mass spectrometer (PerkinElmer, USA). Moreover, the use of this method made it possible with a sensitivity of at least 0.1 wt % to determine the quantitative presence of 62 chemical elements in them and to ascertain a high degree of purification of the obtained preparations (99.0–99.9 %), which subsequently made it possible to explain the detected biological activity, namely by the intrinsic properties of C60-fullerene derivatives. The composition and structure of the obtained compounds were proved using a set of physicochemical research methods: 1H and 13C NMR spectroscopy, two-dimensional correlation spectroscopy, infrared spectroscopy (IR spectroscopy) and optical absorption spectroscopy.

The line of ultrafine particles of metals was represented by three samples, including ultrafine particles of silver (UFP Ag), molybdenum (UFP Mo) and zinc (UFP Zn). These particles were synthesized as a result of plasma-chemical synthesis (Platina Moscow), passed material science certification by electron scanning JSM 7401F and transmission JEM-2000FX microscopy (JEOL, Japan), as well as by X-ray diffraction analysis using a multifunctional diffractometer DRON-7 (NPP Burevestnik, Russia). All particles were represented by a spherical shape with a size in the range of 50-98 nm.

Samples of nanomaterials were placed in sterile penicillin vials, and distilled water was added to obtain concentrations of 1 M in each vial. These suspensions of C60-fullerene derivatives and ultrafine metal particles were processed by ultrasonic washing at a frequency of 35 kHz for 30 hours to achieve the greatest separation of the conglomerates of the mixture.

The impact of C60-fullerene derivatives and ultrafine particles on *Escherichia coli* K12 TG1 with the cloned *luxCDABE* genes of *Vibrio fischeri* was evaluated using the bioluminescent analysis technique [21].

The luminescent strain of *Echerichia coli* K12 TG1, constitutively expressing *luxCDABE* genes of the natural marine microorganism *Vibrio fischeri*, was used as an object of study. The used bacterial strain is produced by NVO Immunotech (Russia) in a lyophilized state under the commercial name "Ekolyum" and is a lyophilized culture of luminescent bacteria contained in an inert gas medium in glass vials. The lyophilized form of microorganisms was diluted with distilled water to obtain a bacterial suspension.
For bioluminescence, an opaque plastic microplate was used for an Infinite PROFI200 microplate analyzer (Tecan Group, Ltd, Switzerland). 200 μl of a suspension of C60-fullerene derivatives or ultrafine particles of metals were added to the plate cells and a number of double dilutions were carried out in the subsequent cells of the series, adding 100 μl of distilled water. After the addition of C60-fullerene derivatives or ultrafine metal particles into the microplate, 100 μl of the bacterial biosensor was added to each cell. To study the effects of derivatives of C60-fullerenes and ultrafine particles upon exposure to UV irradiation, they were incubated in the cells of a tablet using a broadband mercury-quartz lamp (Osram, Germany) from a distance of 10 cm, so that the energy illumination of the samples measured using a UV radiometer TKA-PKM (Russia) was 24.8 W/m², and the exposure time varied from 0 to 15 minutes with a resolution of 5 minutes (in some cases, less). The calculated doses of UV radiation were 4.48, 8.99 and 13.47 J/m². After the samples were additionally incubated for 15 minutes to stabilize the effect of UV on the studied samples. The control sample contained 100 μl of distilled water and 100 μl of bacterial suspension. The tablets were placed in the measuring unit of a microplate analyzer, recording the luminous intensity for 180 min with an interval of 5 min. Based on the data obtained, the toxicological parameters of the EC20 and EC50 were calculated corresponding to the concentrations of C60-fullerene derivatives or ultrafine metal particles causing 20 or 50 % inhibition of the biosensor glow compared to the control. The results of the influence of nanomaterials on the intensity of bacterial bioluminescence (I) were evaluated by changing the luminosity of the control and experimental samples at the zero and n-th second of the experiment [16].

The main data obtained in the studies were performed in triplicate and processed using the Excel and Statistica V8 programs (StatSoft Inc., USA).

3. Evaluation of the effect of derivatives of C60-fullerene and ultrafine metal particles on *Escherichia coli* K12 TG1

The biological effects of the studied compounds were evaluated after 180 minutes of contact with the luminescent strain of *Escherichia coli* K12 TG1. Moreover, further interaction was impractical due to a decrease in the intensity of bioluminescence in the control samples due to the lack of energy substrates for the bioluminescence reaction. An earlier registration did not give a complete picture of the development of biological effects, which in most cases were fully manifested in the time-dependent dynamics of a decrease in bioluminescence by 180 min of contact.

Moreover, all representatives of derivatives of C60-fullerenes had a fairly pronounced antibacterial effect. So, for the C60-fullerene derivative DC60-1, the values of the toxicological parameters EC20 and EC50 amounted to 0.021 and 0.086 M, respectively, while the development of the antibacterial effect, expressed in quenching of the sensor strain, developed gradually and reached maximum values only by the 3 hour of the experiment (Figure 1). In contrast, DC60-2 in two maximally used concentrations from the first minutes of contact led to a pronounced and complete suppression of the luminescence of the test strain, and the calculated values of the parameters EC20 and EC50 were 0.008 and 0.023 M. DC60-3 behaved in a similar way with DC60-2 and achieved the maximum possible the effect occurred already at the 90-th minute of contact; however, the development of the antibacterial effect reduced and the corresponding values of 0.012 and 0.028 M were found in the EC20 and EC50 samples. Thus, the study allows stating the derivative of C60-fullerene DC60-2 as the most promising drug used for the stated purposes.

The study of the antibacterial properties of ultrafine metal particles showed the presence of a sufficiently pronounced inhibitory effect on the luminescent strain. In this case, a change in the glow of the sensor strain in the presence of ultrafine silver particles occurred within 20 % of the control values and allowed calculating the toxicological parameters of EC20 and EC50 at the levels of 0.001 and 0.005 M, respectively. Ultrafine zinc particles also caused a complete suppression of the constitutive bioluminescence of the test object, starting with the minimum used concentrations, and the calculated toxicological parameters were 0.006 and 0.010 M, respectively. Moreover, for Zn nanoparticles, a toxicological effect at concentrations of 0.0004 M was observed already at the fifth minute of the experiment. A further increase in concentration led to a decrease in the intensity of the
glow. No less pronounced, but later toxic effect was exerted by ultrafine particles of molybdenum. The toxicological parameters EC20 and EC50 calculated for them were 0.008 and 0.016 M, respectively. Based on this, all three samples of these ultrafine particles can be characterized as compounds with a pronounced antibacterial effect, and its development was mostly observed in the early stages of incubation, which counts in favor of using these samples as antibacterial agents [17, 18].

![Figure 1](image-url)

**Figure 1.** Kinetics of the development of the antimicrobial effect of C60-fullerene derivatives DC60-2 (a) and DC60-1 (b) in all concentrations used. X axis is incubation time, sec; Y axis is the intensity of bioluminescence, rel.u.

Then, the photosensitizing properties of C60-fullerene derivatives were studied when irradiated with UV light, traditionally used to disinfect media and surfaces, which opens up the prospect of increasing the effectiveness of such an action without fundamentally changing existing techniques and technologies. The data obtained established that there was a significant decrease in EC50 values depending on the dose (time) of irradiation (Table 1) and indicated the possibility of a significant modification of the damaging effect of UV irradiation on bacterial target cells in the presence of certain derivatives of C60-fullerene, the severity of which depended on their concentration in the analyzed sample. Moreover, in comparison with the intrinsic effect of UV irradiation, the most typical was a decrease in the relative luminescence indices of *E. coli* K12 TG1, which makes it possible to characterize these compounds as UV photosensitizers.

**Table 1.** Toxicological parameter EC50 (M) calculated for derivatives of C60-fullerene in the absence and presence of UV-irradiation

| C60-fullerene derivatives | No irradiation | UV-irradiation during 5 mins | UV-irradiation during 10 mins | UV-irradiation during 15 mins |
|---------------------------|---------------|------------------------------|------------------------------|-----------------------------|
| DC60-1                    | 0.086         | 0.078                        | 0.054                        | 0.026                       |
| DC60-2                    | 0.023         | 0.018                        | 0.016                        | 0.003                       |
| DC60-3                    | 0.028         | 0.022                        | 0.019                        | 0.009                       |
Figure 2. Kinetics of the development of the antimicrobial effect of ultrafine metal particles UFP Ag (a) and UFP Mo (b) in all concentrations used. X axis is incubation time, min; Y axis is the intensity of bioluminescence, rel. u.

Similar results were obtained in the presence of ultrafine metal particles, which, during the irradiation process, began to significantly reduce the determined parameters of the EC20 and EC50. Moreover, with respect to ultrafine particles, the effect of the temporary shift, during which the formation of the bactericidal effect occurs, was more pronounced than when using derivatives of C60-fullerenes. Moreover, the effect of a complete decrease in luminescence occurred with a shorter duration of UV irradiation. So, a decrease in the luminescence intensity of the sensor strain to background values occurred already at 2-10 minutes of exposure to light in the UV range (Table 2).

Thus, it can be concluded that both before and after irradiation at certain concentrations, all the samples studied had an antibacterial effect, however, irradiation with UV light shortened the time period for its formation.

Table 2. Toxicological parameter EC50 (M) calculated for ultrafine metal particles in the absence and presence of UV-irradiation

| Ultrafine metal particles | No irradiation | 1 min | 2 mins | 5 mins | 10 mins | 15 mins |
|--------------------------|---------------|-------|--------|--------|---------|---------|
| UFP Ag                   | 0.005         | 0.004 | 0.0026 | 0.0009 | < 0.0001| < 0.0001|
| UFP Zn                   | 0.010         | 0.008 | 0.0034 | 0.0015 | < 0.0001| < 0.0001|
| UFP Mo                   | 0.016         | 0.012 | 0.008  | 0.004  | 0.001   | < 0.0001|

The significance of the combined effect of the studied compounds and UV irradiation on the cells of the sensor microorganism *E.coli* K12 TG1 was additionally confirmed by the results of two-way
analysis of variance. It was shown that under the experimental conditions used, the most significant (P < 0.001) factor causing quenching of the bioluminescence of bacterial target cells was UV irradiation: the strength of its influence on the resultant parameter ranged from 49.34 to 62.26 %.

Against this background, three of the six samples used (DC60-2, UFP Ag, UFP Mo) showed an additive UV irradiation effect, which consisted in significantly enhancing the inhibitory effect of ultraviolet radiation on the level of *E. coli* K12 TG1 bioluminescence.

4. Conclusions
As a result of the work using bioluminescent analysis methods, the biological activity of the samples was characterized, the possibility of enhancing the antibacterial effect of nanomaterials when combined with ultraviolet irradiation was determined, and samples were identified on this basis to create a drug and its possible use for effective surface disinfection with a stable and pronounced biological activity against a wide range of pathogenic and conditionally pathogenic microorganisms. At the same time, the original drug developed and the method of its use for effective disinfection of media and surfaces seem to be an effective means of combating hospital infections and preventing infectious diseases. Moreover, these samples can be used for treating the skin and, in combination with UV radiation, for treating the surfaces of laboratories and hospitals. In this case, it is possible to create a drug based on derivatives of C60-fullerene and ultrafine metal particles in the complex treatment of acute and chronic inflammatory processes of the skin and mucous membranes, including after thermal, chemical and sunburn, for treating the wound surface, treating abrasions, diaper rash, bedsores and various inflammatory and allergic skin diseases.

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