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Radiotracer Methods and Neutron Activation Analysis for the Investigation of Nanoparticle Biokinetics in Living Organisms

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Abstract—The physical foundations and methodological issues of nuclear activation methods (radiotracers and neutron activation analysis) have been discussed in regards to a study of assimilation of a number of inorganic nanoparticles (NPs) by living organisms and their distribution in organs and excretion. The methods of activation of NPs within a colloidal solution or biological samples have been analyzed. Advantages of the radioactive tracer method and neutron activation analysis have been compared. A brief overview of a number of successful works using nuclear methods for studying the interaction of silver, gold, selenium, and zinc and titanium oxide NPs with living organisms has been given. As an example, a description and results of the experiment on long-term (28 days) injection of silver NPs into laboratory mice have been given. The abilities of neutron activation analysis to estimate the mass of residual blood (and the NPs in it) in biological samples and to confirm the penetration of the NPs through the blood–brain barrier in quantitative terms have been demonstrated.

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INTRODUCTION

The production of nanomaterials and their application in different areas of human activity steadily broaden. The output rate of consumption goods based on nanomaterials [1] and the application of nanoparticles (NPs) in medicine also grow rapidly [2–5]. In connection with this, a study of the interaction of NPs with living organisms becomes especially urgent. The measurement of biokinetic parameters describing the behavior of NPs in the organism such as assimilation, organ and tissue distribution, metabolism, and excretion is necessary above all for an estimation of the potential danger of NPs of a certain type for a living organism. These parameters are necessary both in fundamental studies of the effect of NPs on the nature and practical applications of NPs, for example, in pharmacology, veterinary science, food industry, cosmetology, etc.

In practice, the biokinetic parameters of NPs are determined, as a rule, by measuring the mass and the number of the NPs in biological samples of organs, tissues, blood, and discharges of laboratory animals after the administration of NPs into the organism of the laboratory animals [2, 6–11]. If these measurements are performed for the biosamples taken at different periods after the administration of NPs into the organism, then it makes it possible to obtain characteristics of the assimilation, distribution, and excretion depending on time, i.e., the time history of the parameters studied.

In general, the methods of measuring the content of NPs in biological samples depend on the chemical composition and peculiarities of the structure of the NPs, and there is no universal technique to date suitable for all nanomaterials. Considering methods that are sufficiently widespread in practice, it is necessary to mention electron and optical microscopy, quantitative chemical analysis, different methods of spectroscopic elemental analysis (including different types of optical [12] and mass spectrometry [13, 14]), neutron activation analysis [2, 15, 16], chromatography, and methods based on the introduction of labels into the NPs studied [11]. Thus, choosing a technique to measure the number of NPs in biological samples, there are certain options. Criteria for the selection, together with the obvious accuracy and sensitivity requirements, include parameters such as labor-intensiveness and representativeness as well. The requirement of low labor intensiveness is connected with the great number of samples necessary for measurements of biokinetic parameters (the typical number of samples for mea-

surements in the experiment for one type of NPs is about 200). Representativeness is the possibility of obtaining a reliable integral value of the number of NPs in the biological sample having macroscopic sizes (from units to tens of mm) at possible nonuniform distributions of NPs with respect to the sample volume per one measurement.

According to [1], commercial materials require the most the NPs based on inorganic materials (in particular, silver, carbon, titanium and zinc oxide, silicon, and gold). This determined the direction of the studies described in the present paper and the choice of inorganic NPs. Analyzing the methods of measuring biokinetic parameters of inorganic NPs, it has been concluded that the methods based on the introduction of labels into the NPs studied have significant advantages. It has been found the set of the requirements (accuracy, sensitivity, low labor intensiveness, and representativeness of measurements) is satisfied the most by some nuclear physical methods using radioactive labels–markers introduced into the NPs studied (radioactive tracer technique and neutron activation analysis). The nuclear physical methods of the analysis of substances are based on the irradiation of samples studied by fluxes of excitation radiation (neutrons, charged particles, and γ quanta) and further recording of the secondary radiation generated by atoms of the sample. They have been used for a long time in biology and other fields of research and have started to be used in the research of NPs since the 1990s [17, 18]. In Russian practice, these methods studying NPs were either not applied at all or applied rarely and unsystematically.

Theoretically, nuclear physical methods make it possible to use the radioactive tracer technique for quantitative measurements of nanomaterials of any origin, both organic and inorganic, since radioactive, isotropic, spin, or any other labels could be introduced in any substance; however, for NPs of different origins and compositions, different methods of introducing radioactive tracers and measurement of their activity in the samples are optimal. For the inorganic NPs considered, the introduction of a radioactive tracer is implemented most easily by neutron [2, 11] or proton [19] radiation of the NPs or biosamples with further quantitative measurements of activity of the tracer isotope by gamma-ray spectrometric methods.

METHODS OF ACTIVATING NANOPARTICLES

The most adequate way to incorporate a radioactive tracer into inorganic NPs is their activation by thermal (or epithermal) neutrons during neutron capture with the transformation of part of the nuclei in the NPs studied into radioactive isotopes. Since the chemical properties of the isotopes (defined by the electron shells of the atoms) are completely identical to the initial atoms in the NPs activated, an isotopic tag does not change the biokinetic characteristics of the NPs labeled with respect to the initial ones. The low energy of thermal and epithermal neutrons does not impair the arrangement of the molecules (atoms) in the internal structure of NPs and, moreover, causes significant absorption cross sections of such neutrons by the atoms activated. The activity of an isotopic tag in the samples studied could be measured by the intensity of beta and gamma radiation generated during the nuclear beta decay of the isotopic tag or by the intensity of X-ray radiation generated during the decay of some instable isotopes as a result of the K-capture of electrons. In contrast to the beta radiation, the gamma and X-ray radiation of beta decays have a linear energy spectrum, which is individual for every isotope. This makes it possible to use during measurements of the high energy selectivity of modern gamma spectrometric devices and to noticeably increase the sensitivity and accuracy of the measurements. Moreover, gamma and X-ray radiation are weakly absorbed by the material of samples (in contrast, to beta radiation) and their measuring equipment is technically developed and commercially available. Due to this, it is desirable to choose as radioactive tracers isotopes emitting during decay gamma and/or X-ray radiation. The activation of inorganic NPs by thermal neutrons of a nuclear reactor was successfully used studying the distribution and tropism of NPs to different organs and tissues in a number of studies [2, 17, 18], as well as by the authors of the present study carrying out experiments on the biokinetics of NPs of zinc oxide [6–8], silver [9, 10], gold, and selenium [9, 11].

An important parameter in choosing a radioactive tracer is its lifetime, which should not be too short to carry out the measurements before there is an unacceptable decrease in its activity or too long, since an extremely long period of half-decay proportionally decreases the activity of the radioactive tracer due to a decrease in the number of decays per time unit. In general, the acceptable time of half-decay is from several days to several years. Outside this range, technical difficulties of the measuring sharply increase. In such cases, other more complicated methods of activation could be used instead of radiation with thermal neutrons. Possible variants are activation with fast neutrons in the course of the reaction (n, p); protons, (p, n), (p, 2n); and others with nuclei of new elements being formed in the course of the reaction from the initial nuclei (transmutation of the initial nuclei) [11, 19]. During transmutation, the radioactive nuclei of the elements neighboring the initial elements in the periodic table and having acceptable lifetimes could be formed. A great diversity of nuclear reactions using fast neutrons and protons makes it possible to choose the activation method required for every certain type of NPs and samples studied; in general, the variant of the method should be chosen individually.

The source of fast protons could be represented by a cyclotron or other accelerators of charged particles;

the source of fast neutrons could be both a nuclear reactor (putting the samples irradiated near fuel uranium rods) and beryllium target bombarded by fast protons from a cyclotron.

Forming a radioactive tracer in the NPs of titanium dioxide, the activation of titanium by thermal neutrons in the course of the reaction of neutron capture is not reasonable, since it produces isotopes with an extremely short (on the order of ms) lifetime. At the same time, during the (n,p) reaction irradiating titanium by neutrons with energy higher than 2.2 MeV, gamma-emitting isotopes of scandium, ^{46}Sc and ^{47}Sc, are formed with half-lives of 88.3 and 3.4 days, respectively. This method was successfully used by the authors in the experiment studying the biokinetic parameters of NPs of titanium dioxide (rutile) [10].

It should be noted that, in the case of the use of nuclear transmutation, the NPs labeled are not completely identical to the initial NPs, since they contain atoms of the radiotracer different from the atoms of the initial NPs. This strictly speaking affects the biokinetic characteristics of the NPs labeled. Nevertheless, a change in the biokinetic properties of the NPs labeled due to changes in part of the nuclei (activated) and, correspondingly, in their chemical properties is negligibly small: during the activity of samples formed in practice of tens and hundreds of Bq, the number of activated nuclei uniformly distributed within the NPs constitutes about 10^{-8}–10^{-6} of the total number of the molecules/atoms in the composition of one NP. Approximately the same portion of the atoms labeled is on the surface of NPs; i.e., the chemical and biokinetic properties of the surface of inorganic NPs change insignificantly.

Thus, the possibility of activation of the nuclei of elements–markers without changes in their charges is preferable to transmutation; however, in the case of transmutation, the change in the biokinetic characteristics is negligibly small. At the same time, the activation by transmutation significantly broadens the applicability of the radiotracer method, making it suitable for most elements in the periodic table.

METHODS OF MEASUREMENTS OF BIOKINETIC PARAMETERS OF NANOPARTICLES UPON THEIR INTRODUCTION INTO A LIVING ORGANISM

The activation methods to measure the biokinetic parameters of NPs upon their introduction into a living organism could be implemented in two main ways. In the first variant, NPs—preliminarily labeled with radioactive isotopes using one of the methods described in the previous section—are introduced into the organism of laboratory animals by a method suitable for the tasks of the experiment (orally or parenterally, including inhalation, transdermal or intranasal introduction, etc.). In the second variant, animals have nonradioactive NPs introduced, which are activated at the further stage in the composition of the taken biosamples irradiated with neutrons as a whole [2, 10, 17, 18]. This variant of the method per se is classic neutron activation analysis and would be further referred to as such, in contrast to the first variant, which we would refer to as a radiotracer method. It should be noted that, in literature, neutron activation analysis using gamma spectroscopy is often referred to as instrumental neutron activation analysis, highlighting its difference from neutron activation analysis with the radiochemical analysis of activation products, which is rarely used at present.

A serious limitation of neutron activation analysis is difficulties related to the identification of the NPs labeled when they contain biogenic elements with a high natural content in the organism (iron, zinc, selenium, and others) that are activated together with the nanomaterials studied of the same elements. A high natural level of radiation typical for the tracer applied appears in the sample, which does not reliably reflect the number of NPs entered the organism. For example, neutron activation analysis could not be applied for iron and zinc oxide NPs, while these NPs are successfully identified and measured by the radiotracer method.

It should be underlined that a serious advantage of neutron activation analysis is the possibility of introducing nonradioactive NPs into the animals, which is of great significance for experiments with long-term introduction (weeks and months). In practice, the experiment with neutron activation analysis could be carried out in two stages. At the first nonradioactive stage, the administration of a preparation with NPs to animals and sample preparation are carried out. At the second radioactive stage, the biosamples are activated and the mass of the NPs in them is measured. This makes it possible for research institutions without a special infrastructure for dealing with radioactive substances to conduct the nonradioactive part of the experiments and then to pass the biosamples for activation and measurements into institutions having the corresponding infrastructure. This variant of collaboration was successfully used in practice in [10] and in the experiment described below.

According to the problems posed, preparations for administration containing radiolabelled or nonactivated NPs (usually a colloid solution of the NPs studied in distilled water) are prepared. One peculiarity of the numerous types of colloid solutions with low zeta-potential is their liability to agglomeration with the formation of clusters of several NPs and with an increase in their linear sizes. In these cases, to maintain the experimental integrity with respect to a study of the effect of just individual NPs and not their agglomerates, NP solutions sterically and electrostatically stabilized should be used. In the first variant, the surface of NPs is covered with special stabilizing poly-

mers (for example, polyvinylpyrrolidone) introduced into the solution. In the case of electrostatic stabilization, the pH of the aqueous phase of a NP preparation is adjusted. In both variants, it is necessary to painstakingly control the sizes and size distributions of the NPs studied. If necessary, the suspensions (preparations) used could be subjected to treatment by ultrasonic dispersers, which sufficiently effectively destroys the NP clusters. In the case of the radiotracer method, it is preferable to activate initial superdispersed NP powders, which are not affected by an increased temperature and intensive gamma radiation in the reactor while, activating ready suspensions of NPs, the temperature and gamma radiation in the reactor could affect stabilizing substances and agglomeration.

Modern measuring gamma spectrometric devices at present have high sensitivity and significant energy selectivity, which makes it possible to measure samples with activity of about several Bq with high accuracy (order of unities of percent and higher) and under conditions of sufficiently low radioactive background (for example, in the case of the radiotracer method and low-background gamma spectrometry) with activity of tenths of Bq. This makes it possible to use preparation doses with activity lower than several hundreds of kBq, which does not exceed the level of the minimal significant activity, for introduction to laboratory animals. The latter is determined in the Russian radiation safety criteria in force, NRB-99/2009, as activity which is so small that it is not covered by NRB-99/2009 and the corresponding restrictions on the use of radioactive substances [20]. Thus, the introduction of preparations of labeled NPs is safe for staff and the radiation influence of the dose of the labeled NPs introduced on the laboratory animals is negligibly small.

In different periods of time after the introduction of the preparation, determined by the conditions of the experiments, the animals have discharge samples taken and then samples of organs, tissues, and blood of interest taken. For a quantitative determination of the activity of the radiotracers in the biological samples, measurements of the intensity of the spectrum lines in one or several energy regions typical for the given radiotracer are carried out using low-background gamma spectrometric devices. To move from the measured activity of the trace to the mass content of the NPs in the biosamples, the method of comparison with the activity of the reference sample containing a known amount of the preparation of the NPs under study introduced to the animals is used [2, 6, 8, 11]. For a number of years (from 2008), specialists from the National Research Centre "Kurchatov Institute," together with those of the Institute of Nutrition of the Russian Academy of Medical Sciences, MONIKI, and other research and medical institutions have performed studies on the biokinetics of NPs in living organisms by the nuclear physical methods described above. In the course of these works, for silver, gold, zinc, and selenium NPs, parameters describing the assimilation of these NPs by the organism of laboratory animals (rats and mice) at single oral administration, organ and tissue distributions of the NPs introduced, and the excretion of the NPs from the organism were measured [6–11]. For silver NPs, the assimilation and organ distribution parameters at long-term repeated oral administration into the organism of rats were also measured [10]. For silver and zinc oxide NPs, these techniques were certified in the GOST-R system [21, 22]. Also, the methods were recommended by a normative document of the Ministry of Health and Social Development of the Russian Federation for determining the organotropy and toxicokinetic parameters of artificial nanomaterials [23].

New results of the long-term introduction of silver NPs to mice are given below. Quantitative measurements of the mass of the NPs in the biological samples were performed comparing the activity of the biosamples measured by gamma spectrometry with the reference samples as it was made in [2, 11]. Additionally, much attention was paid to measuring the linear sizes of the NPs used.

APPLICATION OF NEUTRON ACTIVATION ANALYSIS FOR MEASURING BIOKINETIC PARAMETERS OF NANOPARTICLES

At present, nanomaterials based on silver NPs are the most in-demand among the mass consumer goods [1]. In connection with this, a study of biokinetic characteristics of silver NPs, especially in regards to the inner protective barriers of the organism, is very urgent.

In a series of experiments using nuclear physical methods, the biokinetic characteristics of silver NPs stabilized sterically [9, 11] together with the results, which have no analogs in the world, on the transport of silver NPs through the blood–brain barrier in the experiments taking into account the presence of residual blood in the biosamples [10] were obtained. Studies concerning the long-term administration of silver NPs are of particular interest for human safety and corresponding normalization. It is this route of entry which represents the greatest potential danger under the conditions of increasing application of silver NPs in pharmacology, veterinary medicine, packaging material, dietary supplements, and hygienic and cosmetic products [9]. The results and description of the experiment carried out recently studying the biokinetic parameters of silver NPs during the long-term (30 days) oral introduction of NPs into the organism of laboratory mice are given below.

MATERIALS AND METHODS

The bioaccumulation of silver NPs of an Argovit-C preparation commercially produced in Russia by VEKTOR-VITA NPTs and the preparation of silver

NPs produced by MATI, Russian State Technological University, was studied. The Argovit-C NPs are a colloid solution with a rated concentration of NPs of about 10 mg/mL dispersed in distilled water and coated with a stabilizing cover of polyvinylpyrrolidone. The second type of silver NPs used was obtained by a dispersion and condensation method resulting in NPs being suspended in distilled water with concentration of 50 mg/L. The agglomeration was prevented by a very low concentration of NPs in the solution, minimizing the interaction of the NPs with each other (moreover, weak electrostatic stabilization of NPs is possible according to the manufacture's description). This type of Ag NPs we will further refer to as unstabilized silver NPs.

To determine the sizes of the NPs, dynamic light scattering (DLS) was used on a Malvern DLS spectrometer. The initial colloid solution of the Argovit-C preparation with a concentration of 10 mg/mL was highly concentrated and opaque. Due to this reason, to prevent multiparticle scattering during measurements, optimal concentrations of 100, 50, and 10 mg/L were chosen. For size determination of unstabilized silver NPs the original solution was used as well as diluted solutions with concentrations 12.5 and 25 mg/L. The dilution of the solutions was performed using distilled deionized water. Before each of the measurements, the solutions were thoroughly stirred and treated in an ultrasonic bath for 15 min. Further DLS measurements were carried out 2 min after treatment.

To study the bioaccumulation, the silver NPs were administered to male white laboratory mice of the SHK strain with initial weights of 25–30 g. The experiment was performed together with MONIKI, where the animals were kept and introduced with the NPs daily for a month and the brain, liver, and blood samples were prepared for the further activation and measurements of the mass of silver NPs in the samples. Working with animals was carried out according to the requirements of the Decree of the Ministry of Health of the Soviet Union No. 755, August 12, 1977, On Measures for Further Improving the Forms of Working Using Experimental Animals and the Decree of the Ministry of Higher and Secondary Education No. 742, November 13, 1984 On the Approval of Rules of the Work Using Experimental Animals.

The mice obtained feed balanced in regards to all main macro- and micronutrients and water of 2.5 cm^3 per a day.

The animals were divided into three groups in regards to the feeding method and type of NPs introduced:

(i) group 1 (5 mice): control, the absence of NP preparations, and time of keeping is 1 day;

(ii) group 2 (5 mice): single enteral intragastric administration of the Argovit-C NPs at a dose of 100 μg;

(iii) group 3 (5 mice): oral introduction of unstabilized silver NPs at a dose of 125 μg for 30 days.

The NP preparations tested were introduced every day in the composition of drinking water for 30 days of the experiment (group 3) or once intragastrically using a probe (group 2). The control group had unlimited access to distilled deionized water.

After the end of the experiment, the mice were subjected to anesthesia by the intraperitoneal introduction of 0.6% urethane solution and exsanguinated using the inferior vena cava. The abdominal cavity was opened aseptically, and the liver, brain, and blood (0.5 cm^3) were taken by sterile instruments into disposable polyethylene containers.

The samples were dried in a ventilated thermostat for 24 h at temperature of 75°C up to a state close to the consistency of dried foodstuff. This significantly reduced their volume and made it possible to keep them durably (up to several weeks) in a cooler with a temperature not exceeding +5°C before their transportation into the National Research Centre "Kurchatov Institute" and further measurements. The determination of silver content in the organs and tissues studied was performed using the neutron activation analysis described above. For this, the biosamples of the organs, tissues, and blood prepared in MONIKI were packed into sealed polyethylene vessels, which, in turn, were placed into aluminum containers for further activation in the reactor channel.

The neutron activation of silver NPs in the composition of the biosamples was performed by irradiation with a flux of thermal neutrons in the VEK-9 vertical experimental channel of the IR-8 nuclear reactor of the National Research Centre "Kurchatov Institute." The neutron flux density during irradiation was, on average, constant and constituted 5.7×10^{12} neutrons per cm^2 per s. The irradiation of the biological samples with silver NPs was carried out in 24 h.

At the same time as the samples studied, the activation of reference samples with a silver content of 1 μg was conducted. The reference samples represented a solution dried using cotton wool, which was obtained by the dilution of the state standard reference samples (GSO) of silver nitrate placed into polyethylene sealed vessels with geometry approximately corresponding to that of the biological samples.

Once the irradiation and removal of samples from the core of the reactor was performed, they were kept inside the biodefence for 14 days to decrease the background of gamma radiation of short-living isotopes appearing during the activation of the nuclei of the container and samples (mainly, ^{24}Na, ^{31}Si, and ^{29}Al, with half-lives $T_{1/2}$ = 14.8 h, 2.6 h, and 6.5 min, respectively) to a safe level. This having been done, the samples were subjected to gamma spectrometric analysis.

The activity of the samples studied was measured by a Canberra gamma spectrometer (United States) having in its composition a GC4018 germanium semi-

Table 1. Main characteristics of initial nonradioactive and radioactive silver isotopes and those of a parasitic isotope of biogenic sodium

Element	Target isotope				Radioactive isotope			
	isotope	content in natural mixture, %	atomic mass, g/mol	σ, 10^{-24} cm^2	isotope	$T_{1/2}$, days	irradiation type	E_γ, keV
Ag	^{109}Ag	48.2	108.9	4.4	^{110m}Ag	249.8	β^-, γ	657
Na	^{23}Na	100	23	0.53	^{24}Na	0.625	β^-, γ	1369 2754

conductor detector, DSA-1000 analyzer, and Genie-2000 program software (Genie S501, Genie S502). The recording of gamma radiation was performed within the energy regions corresponding the most to the most intensive spectrum lines of the elements analyzed (Table 1).

During gamma spectrometric measurements, the recording of gamma quanta in a range from 60 keV to 1.5 MeV was performed, which made it possible, together with measuring the amount of ^{110m}Ag in the biosamples, to assess the content of the number of isotopes formed from the biogenic elements naturally present in the samples, including the iron isotope ^{59}Fe. The additional information obtained herewith was used to assess the amount of residual blood in the brain biosamples, since it is significant, assessing the possibility of penetration by the NPs the blood–brain barrier (see below). The quantitative determination of the content of silver NPs was carried out using the reference method according to the formula

$$m_x = m_r A_{\gamma,x}/A_{\gamma,r} \quad (1)$$

where m_r is the known mass of the element in the reference sample; $A_{\gamma,x}$ and $A_{\gamma,r}$ is the gamma activity measured in the sample under study and the reference sample, respectively.

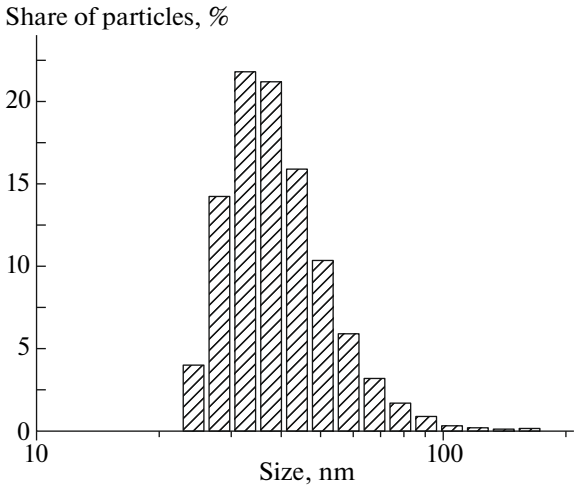

Fig. 1. Particle size distribution of the Argovit-C colloidal preparation.

It should be noted that a gamma spectrometric analysis of activated biological samples was complicated by the high level of the continuous Compton background in all regions of the measurement. The background is caused by the presence in the sample of radionuclides ^{24}Na and ^{65}Zn formed upon the activation of the biological samples from natural sodium and zinc entering into the composition of all living tissues; however, it is significantly lower than the identification threshold (critical level) [13]. The critical level represents the count rate at which the desired signal exceeds the background fluctuation with a probability of 50%. Using insignificant simplifications, the calculation formula for this threshold L_c (in units of the count rates of the decay recorded) is given by

$$L_c = 2.33\sqrt{R_b / T}, \quad (2)$$

where R_b is the sum of the count rate of the external background and Compton component of the background; T is time of measuring of the sample, equal in most cases to 3600 s.

The content of chemical elements in organs was expressed in nanogram per gram of the mass of the organ (tissue). For each of the parameters, the average value with the standard error was calculated.

RESULTS AND DISCUSSION

The data of the DLS measurements revealed that the average hydrodynamic diameter of the NPs in the Argovit-C preparation for all concentrations studied was 33.7 nm (Fig. 1). It should be noted that the treatment of the suspensions with ultrasound did not lead to a change in the sizes of the NPs, which indicates the good steric stabilization of the colloid solutions.

Figures 2 and 3 present particle size distributions for the unstabilized colloid silver at concentrations of 50 and 12.5 mg/L, respectively. It could be seen that the distribution maximum almost does not depend on the concentration, which is evidence of the absence of agglomeration in highly diluted solutions. Herewith, for silver NP concentrations of 50 and 25 mg/L, the particle size distributions were found to be almost identical, their maximum being in the region of 2.3 nm

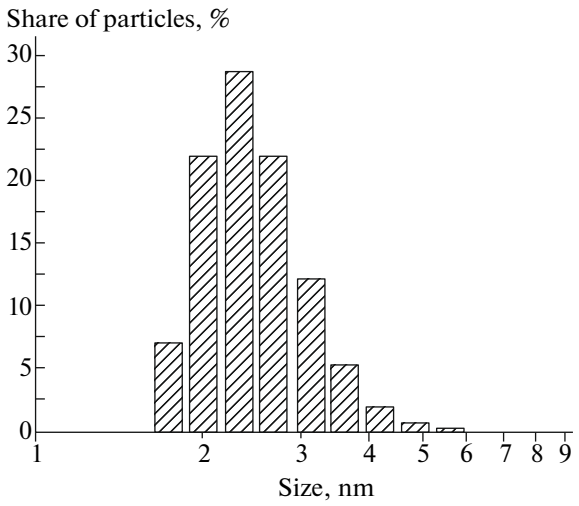

Fig. 2. Particle size distribution for the concentration of unstabilized colloidal silver of 50 mg/L.

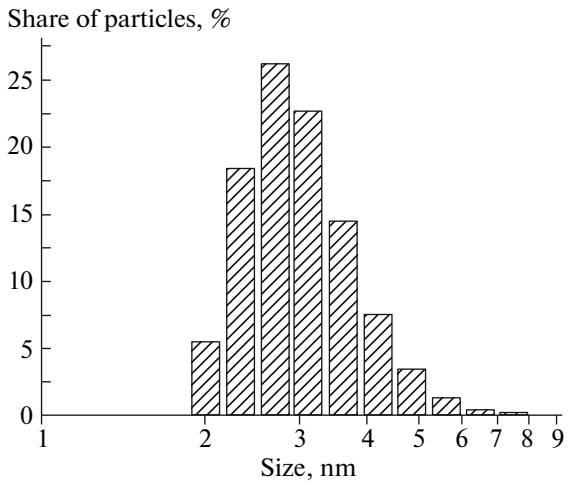

Fig. 3. Particle size distribution for the concentration of unstabilized colloidal silver of 12.5 mg/L.

(Fig. 2). At the same time, the maximum of the distribution for a concentration of 12.5 mg/L is near 2.6 nm (Fig. 3), which, in contrast to the previous data, is within the accuracy of the DLS measurements.

The processed data of the measurements of the bioaccumulation of Ag NPs are given in Tables 2 and 3 and histograms in Figs. 4 and 5.

Figure 4 gives the silver concentration in the brain, liver, and blood for chronic introduction of the unstabilized NPs. The results of single (acute) introduction of the Argovit-C NP preparation are presented in Fig. 5.

We note that, in contrast to acute introduction (Fig. 5) during chronic introduction (Fig. 4), the silver concentration in all the organs and tissues considered significantly increases, which could be evidence of the accumulation effect of the NPs and could partially reflect the effect of the stabilizing polymer coating in the case of the Argovit-C colloid silver.

The greatest silver concentration is observed in the liver; the next target organ accumulating silver is the brain (which coincides with the results of [14] with the biokinetics of silver NPs with a diameter of 14 nm being studied during their oral introduction for 28 days to laboratory mice and measuring their mass by ICP-MS), whereas the Ag concentration in the blood was found to be the least. On the whole, the distributions with respect to organs obtained are close to the data of [12], and quantitative differences could be caused by the difference in the NP sizes (60 nm in [12]). In the

Table 2. Measured activity of reference samples and conversion factors from the measured silver ^{110m}Ag [Bk] activity to silver content in nanograms

No. reference sample	Ag mass, ng	Activity, Bq	Factor, ng/Bq	Average factor, ng/Bq
1	100	6.8	14.8	14.2
2	500	37.0	13.6	
3	100	5.0	19.8	19.0
4	500	27.0	18.2	

Table 3. Mass and activity of NPs measured in biosamples of experimental animals

Organ	Single administration of NPs (d = 34 nm) average values over group			Long-term administration of NPs (d = 2.5 nm) average values over group		
	mass, ng	activity, Bq		mass, ng	activity, Bq	
	Ag	Ag	Fe	Ag	Ag	Fe
Brain	11.3	0.5	15.3	203	13.3	14.7
Blood	18.7	1.0	205	109	7.8	303
Liver	165	8.7	274	1110	58.6	193

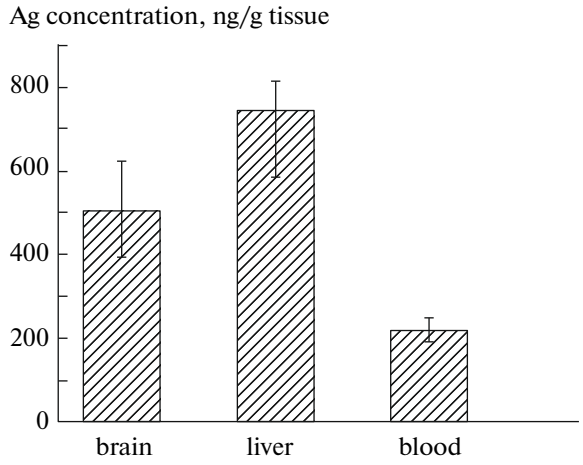

Fig. 4. Silver concentration in different organs and tissues after the long-term administration of unstabilized silver NPs to mice.

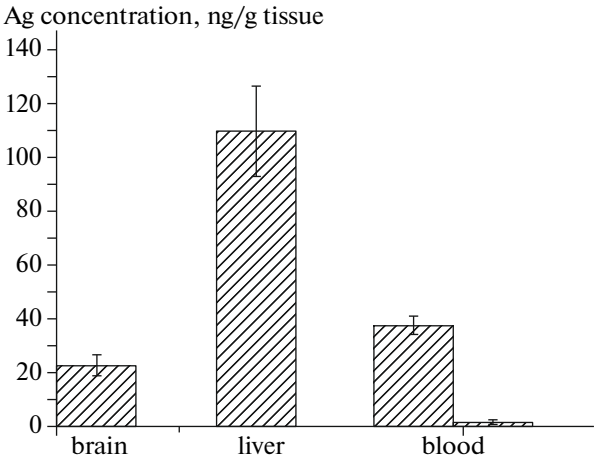

Fig. 5. Silver concentration in different organs and tissues as a result of a single administration of the Argovit-C stabilized silver NPs to mice. The rightmost column indicates the average possible content of colloid silver in the blood of control mice.

case of the acute introduction, the situation changes: the silver concentration in the blood exceeds that in the brain. This, together with the data of Table 3, could be evidence of the prevailing accumulation of Ag NPs (i.e., slow excretion as compared to the drug absorbability from the blood) in the animal brain, which is in good agreement with the data of [13], where the biokinetics of silver NPs with a diameter <20 nm at long-term (28 days as in [14]) oral introduction has been studied using one-particle ICP MS. The silver concentration in the tissues of the control group was in most cases lower than the critical level of identification (except for the blood, where it was found to be negligibly small). This result is in accordance with the literature data on the natural content of silver in the animal organism [25].

It should be noted that the nuclear physical method in the form of neutron activation analysis makes it possible to reliably assess the number of NPs having passed through the histohematogenous barrier directly into the organ or tissue studied without taking into account the NPs from the residual blood in the biosample in the lumen or on the walls of the blood vessels. For this it is necessary to measure the specific activity of the blood sample taken individually with respect to the element labeled (in our case, ^{110m}Ag) and also with respect to the iron isotope ^{59}Fe. Further, measuring the activity of the organ studied with respect to the same isotope of ^{59}Fe, the maximum amount (mass) of the residual blood contained in this organ is determined (assuming that all the iron is in the blood and obtaining, thus, the upper estimate). Using the specific activity of the blood with respect to the element labeled, the maximum possible activity of this element in the blood vessels of the organ considered is calculated. Correspondingly, the remaining activity in the organ with respect to the element studied is caused by its content directly in the organ tissues on the other side of the blood-vessel cavities. Thus, the method described could give unambiguous quantitative proof of the penetration of NPs through biological barriers. First and foremost, this is of interest for the blood–brain barrier, since many other methods often are not capable of providing representative proof of the penetration of nanomaterials through the barrier indicated directly into the brain tissue.

Thus, as a result of the present study of the activity of the ^{59}Fe and ^{110m}Ag isotopes in the blood of the mice (Table 3), the data on the portion of silver in the vessels of the brain constituting no more than 15% of the total silver in the brain were obtained. Herewith, in the experiments performed earlier on rats, these characteristics constituted no more than 7% of the total amount of silver [10]. Thus, it could be concluded that silver NPs are capable of penetrating the blood–brain barrier from the blood vessels directly into the neuronal tissue of the brain in an amount of 1×10^{-4} of the dose introduced orally.

It should be noted that the result of the present study is one of the first direct proofs of the ability of silver NPs without a stabilizing coating to penetrate the blood–brain barrier and a demonstration of the possible accumulation of Ag NPs in the tissues (liver, brain, and blood) and potential dependence of the result on the presence of the stabilizing coating.

CONCLUSIONS

In this study, the bioaccumulation of silver NPs covered with a stabilizing coating (polyvinylpyrrolidone) and unstabilized silver NPs in the tissues and bioliquids of laboratory mice using neutron activation

analysis and gamma spectrometric analysis and geometrical characteristics of the NPs using DLS were studied. The results obtained (taking into account the difference in the size of the NPs studied) are in good agreement with the results of the studies on the bioaccumulation of silver NPs in the rat organism performed earlier [10, 13, 14]. Upon the acute administration of stabilized NPs, the high tropism to some tissues (liver and blood) was revealed. The chronic administration of unstabilized NPs, in turn, was found to be different: the brain—together with the liver—was found to be the main target organ accumulating silver. Using nuclear physical methods, it has become possible to directly establish that silver NPs penetrate the blood–brain barrier and are accumulated in the brain, potentially being able to induce cognitive changes, which should be a subject of further research.

Thus, in the present study, the ability of unstabilized silver NPs to penetrate the mouse organism and accumulate in different tissues as a result of long-term administration has been found. Herewith, the qualitative result does not depend on the presence of the stabilizing coating, corresponds to the results of the previous studies on the chronic administration of stabilized silver NPs to rats, and is invariant with respect to the 13-fold difference in the sizes of the NPs used. At the same time, the quantitative result should be considered taking into account the significant difference in the sizes of the NPs and chemical structure of their surface (with polymer coating and without it). In turn, the concentration dependences obtained as a result of the acute administration of the stabilized colloid silver to the mice have been observed to be in qualitative agreement with the characteristics of the acute experiment on the introduction of the same silver to rats [11].

An analysis of the peculiarities of the nuclear physical methods indicated (high accuracy, selectivity, possibility of identifying NPs from bioessential elements, and possibility to carry out measurements with heterogeneous macrosamples) and the set of the experimental results [2–11, 21–23] suggest the high reliability and practicality of nuclear physical methods for biokinetics studies (including toxico- and pharmacokinetics) of numerous widespread inorganic nanomaterials.

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