The method of radioactive tracer for measuring the amount of inorganic nanoparticles in biological samples

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Abstract. The method to measure the mass of inorganic nanoparticles in biological (or any other samples) using nanoparticles labeled with radioactive tracers is developed and applied to practice. The tracers are produced in original nanoparticles by radioactive activation of some of their atomic nuclei. The method of radioactive tracers demonstrates a sensitivity, specificity and accuracy equal or better than popular methods of optical and mass spectrometry, or electron microscopy and has some specific advantages. The method can be used for study of absorption, distribution, metabolism and excretion in living organism, as well as in ecological and fundamental research. It was used in practice to study absorption, distribution, metabolism and excretion of nanoparticles of Ag, Au, Se, ZnO, TiO\textsubscript{2} as well as to study transportation of silver nanoparticles through the barriers of blood-brain, placenta and milk gland of rats. Brief descriptions of data obtained in experiments with application of this method included in the article. The method was certified in Russian Federation standard system GOST-R and recommended by the Russian Federation regulation authority ROSPOTREBNADZOR for measuring of toxicokinetic and organotropy parameters of nanoparticles.

1. Introduction
The production of nanotechnology-based consumer goods and commercial applications of nanoparticles (NP) develops quite quickly [1-3]. Data base on consumer goods (made with using of NPs) includes 1824 items up to beginning of 2015. During 2014 the number of names of consumer goods increases by 10 % and continue to grow. Almost all NPs in consumer goods are inorganic ones. The most popular are NPs of Ag (24% of all goods), Ti and TiO\textsubscript{2} (10%), C (4%), Si and SiO\textsubscript{2} (3%), ZnO (2%), Au (1%). Other NPs are used sufficiently less [4].

Potential health and environmental risks of engineered nanoparticles (NPs) should ensure environmental and health safety (EHS) of nanotechnologies, especially of nanotechnology-based consumer goods. It is necessary to control mass or concentration of NPs inside living organism, plants, food, agriculture raw materials, consumer goods etc.[5]. In order to estimate potential danger and EHS of NPs it is important to study processes of absorption, distribution, metabolism and excretion (ADME) of NPs in living organism. The ADME process studies are performed usually by model experiments with administration of engineered NPs in living organism and measuring of their
mass or concentration in organs and biological liquids. Thus it is obvious that developing the enhanced methods to measure mass/concentration of engineered NPs in biological samples is actual problem for estimation of safety of products containing NPs as well as for other EHS problems.

2. Methods to measure mass of inorganic NPs in biological samples

There are not universal methods to measure mass of any NPs in biological samples nevertheless there are a lot of specific methods which are applicable for any specific NPs [5]. As shown in section 1, consumer goods containing NPs are developed on base of short set of inorganic NPs mentioned above, and so they are the most actual for practice applications of EHS research. The most popular methods to measure in samples mass of these NPs are elemental analysis, e.g. optical spectroscopy (atomic absorption or atomic emission spectroscopy) and mass spectrometry, as well as transmission electron microscopy (TEM) and methods of labeled NPs.

TEM method is quite expensive and has a low representativity. Methods of elemental analysis are applicable only to non-biophile elements (i.e. elements with low natural mass concentration in organism) and need laborious preparing of samples before measuring. One of the most efficient alternative methods is one of labeled particles, which is applicable to variety of model experiments, e.g. to study ADME of NPs administered to laboratory animals [6], or transportation of NPs along trophic chains in ecological systems, or transportation of NPs from packing with NP into the food [7, 8]. Fluorescent labels may visualize NPs in biological samples, e.g. by using of confocal microscopy. The uptake of fluorescently labeled NPs in cells may be measured by flow cytofluorometry. Labels which produce a characteristic signal in the Raman spectra also may be used to measure mass of engineered NPs in samples or just in the body of a laboratory animal [9]. The main requirements to labels are minimal influence on chemical and physical properties of NPs under investigation and simple way to register labeled NPs in samples. Hence the ideal labels are isotopic tracers, as they produce very low changes in chemical/physical properties of NPs and don’t influence biological and surface properties of labeled NPs [5, 12]. Isotope tracers may be stable or radioactive. One can measure an amount of isotopes in samples using mass-spectroscopy (for stable isotopes) or gamma- or beta spectrometry (for radioactive ones). In specific situations the other methods of registration of isotope labels may be available, e.g. nuclear magnetic resonance spectroscopy. Generally radioactive isotopes may be registered in living organism (e.g. by methods similar to positron-emission tomography) without disturbing the vital activity of organism. Really radioactive tracers are popular in biology, e.g. many experiments were performed with $^3$H tracer [10, 11], experiment with labeled NPs of ZnO described in [12].

3. Incorporation of radioactive tracers in NPs

Radioactive tracer method (RTM) in applications aimed at measuring of mass/concentration of NPs in biological samples implies administration to laboratory animals NPs labeled with radioactive tracers.

The simplest way to incorporate isotope tracer into NP is transformation (activation) of some stable nuclei in original NPs to their radioactive isotopes by means of neutron irradiation. The transformation may be performed by irradiation of original NPs by neutrons of very low energy in the course of nuclear reaction of neutron capture, e.g. $^{109}$Ag (stable) (n, $\gamma$) $\rightarrow ^{110m}$Ag (radioactive).

The other way to incorporate radioactive tracers into original NPs is transmutation of stable nuclei in original NPs to radioactive ones of other elements, e.g. transmutation of stable isotopes of Ti to radioactive $^{48}$V or $^{47}$Sc by irradiation of original Ti in NPs with fast protons or neutrons. The main parameters of isotopes which can be used as radioactive tracers in experiments with Ag, Au, Se, ZnO are shown in table 1 [5,14,16].
Table 1. Main parameters of isotopes of Zn, Ag, Au, Se used as tracers.

| Nano-material | Original stable isotope in NP composition | Corresponding radioactive isotope |
|---------------|------------------------------------------|----------------------------------|
|               | isotope | Natural abundance [%] | isotope | T_{1/2} [days] | type of radiation | E_γ [MeV] |
| Silver        | ^{109}\text{Ag} | 48.2 | ^{110m}\text{Ag} | 249.8 | β, γ | 0.6577 |
|               |         |                 |         |             |                | 0.8847 |
| Selenium      | ^{74}\text{Se} | 0.9 | ^{75}\text{Se} | 119.8 | γ | 0.1360 |
|               |         |                 |         |             |                | 0.2647 |
| Gold          | ^{197}\text{Au} | 100 | ^{198}\text{Au} | 2.7 | β, γ | 0.4118 |
|               |         |                 |         |             |                |          |
| Zinc          | ^{64}\text{Zn} | 48.6 | ^{65}\text{Zn} | 244.3 | β\text{ }^+, γ | 1.115 |

Note. Designations: T_{1/2} is half-life of radioactive decay, E_γ is energy of main γ - radiation spectral line.

4. Measurements of mass of inorganic NPs in biological samples by radioactive tracer method
Measurements of the mass of NPs labeled with radioactive tracers may be performed by measurements of nuclei radiation of radioactive tracer. Nuclei radiation from the tracer can be gamma-radiation and beta radiation, both of them are caused by radioactivity (activity) of tracer. Usually isotopes have both kinds of radiation and power of radiation is strongly proportional to activity of tracers in the sample and so to mass of labeled NPs in the sample. In principle activity of tracers may be measured by measuring gamma or beta radiation, but in practice usage of gamma radiation has sufficient advantages. The main advantage is good penetration ability of gamma-rays and very low level of absorption and reducing in matter of samples. Beta radiation reduces sufficiently inside a sample and an operator should perform special treatment of the samples and calculate correction on self-absorption of beta radiation inside sample. Also modern gamma-spectrometer equipment is less expensive in comparison to beta-radiometrical equipment. High sensitivity and selectivity of modern gamma-spectrometers needs very low activity of samples, which is comparable with natural activity of human body (about 7 kBq). It is absolutely safe and sufficiently lower than level of maximum significant activity nominated by International Committee on Radiological Protection.

The method of RTM was certified in RF standard system GOST-R for measurements of mass concentration of NPs of Ag and ZnO in biological samples and recommended by RF regulation authority ROSPOTREBNADZOR for measuring of toxicokinetic and organotropy parameters of engineered nanomaterials in experiments with laboratory animals[15]. Some experiments on in vivo study of ADME of commercially popular NPs were performed with using of RTM [5, 14, 16] and results are briefly reported briefly in next section.

5. Results and discussion
The RTM was used for ADME measurements of NPs of Ag, Au, Se, ZnO and TiO\textsubscript{2} [13] as well as for study of transportation of NPs with milk of lactating rats and through placenta barrier of rats [16]. Sensitivity of RTM method is limited by parameters of radiation spectrometer equipment and radiation background in area of spectrometer detector. It is limited also by mass and content of the samples. In particular sensitivity depends on the value of minimal detection activity (MDA) in the form of quantitative measurements limit L_q which is described and recommended by ISO [17]. In experiments with single peroral administration of NPs of Ag, Au and ZnO [5] sensitivity was different for different samples, but in the worst case it was better than one ng per sample. Radioactive tracer ^{75}\text{Se} has low energy of γ - radiation (see Table 1) and consequently has large background in energy area of measurements, which caused by Compton scattering of γ -rays. Thus sensitivity for NPs of Se
was lower and was better than 6 ng per sample. In agree with the research described in [14] accuracy of RTM method in application to NPs of Ag is better than ±15% under P=0.95, the methods for measuring of NPs Au, Se and ZnO are similar and accuracy of measurements assumed to be the same. Some examples of data obtained in experiments with using of RTM are shown in Tables 2, 3.

Data obtained in experiments with study of ADME of NPs of Ag in rats after single peroral administration are shown in Table 2.

Table 2. Distribution of Ag NPs in organs of rats after single intragastric administration. Data are shown in percent of administered dose of 1 mg per animal.

| Biological specimen | Time after administration [hours] |
|---------------------|----------------------------------|
|                     | 24                               | 48                       | 72                       |
| GIT+feces (the sum) | >98                             | >98                      | >99                      |
| Carcass             | 0.36±0.17                       | <0.6                    | 0.23±0.09                |
| Liver               | 0.6±0.2                         | 0.8±0.3                 | 0.18±0.10                |
| Kidneys             | 0.014±0.002                     | 0.029±0.008             | 0.007±0.003              |
| Blood               | 0.13±0.05                       | 0.20±0.05               | 0.05±0.02                |
| Lungs               | 0.009±0.003                     | 0.016±0.003             | 0.006±0.003              |
| Heart               | 0.004±0.002                     | 0.006±0.002             | 0.0032±0.0007            |
| Pancreatic gland    | 0.008±0.002                     | 0.012±0.005             | 0.004±0.001              |
| Spleen              | 0.05±0.02                       | 0.06±0.03               | 0.010±0.004              |
| Gonads              | 0.016±0.003                     | 0.033±0.007             | 0.010±0.004              |
| Brain               | 0.003±0.001                     | 0.012±0.002             | 0.005±0.002              |
| Urine               | 0.012±0.002                     | 0.032±0.009             | 0.048±0.037              |

*Note. Designation GIT means gastrointestinal tract.*

RMT was also used in an experiment on study of transportation of Ag NPs with milk of lactating rat females to suckling mammary pups of rats [6]. The single peroral dose for lactating female was about 1.8 mg of Ag NPs per body weight. Time of lactation was 48 hours. The total accumulation of Ag NPs into the milk exceeded 1.94 ±0.29 % of the administered dose over 48 hours of lactation. Main data are shown in Figure 1. It was the first time experimental evidence of the transfer of NPs from mother to offspring through the breast milk.
In precise experiments there is always the risk of influence of external contaminations of samples. In the experiment with mammary infant rats there was a risk of external contamination of rat pup body from feces of mother female. And therefore additional experiments were performed to verify data shown at Figure 1 against external contaminations of infant rat bodies with Ag NPs by feces of lactating female. As shown in Table 3, feces contain about 98% of administrated dose of Ag NPs (after peroral administration). Thus additional measurements of several rat pup internal organs and carcass were performed to clarify the localization of main part of NPs in rat pup bodies. The data obtained are shown in the Table 3.

Table 3. Distribution of NPs of Ag in the body of mammary infant rats, which were drinking milk from lactating mother rat exposed with NPs of Ag.

| Biological sample | Mass of NPs of Ag in organs of mammary infant rats (in percent of dose of 1.8 mg administered to lactating female rat) |
|-------------------|------------------------------------------------------------------------------------------------------------------|
|                   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | Average |
| Carcass           | 0.0155 | 0.0101 | 0.010 | 0.0119 | 0.0144 | 0.0081 | 0.0135 | 0.0134 |
| GIT               | 0.095 | 0.141 | 0.164 | 0.111 | 0.132 | 0.098 | 0.161 | 0.129 |
| Liver             | 0.0245 | 0.0251 | 0.0259 | 0.0239 | 0.0283 | 0.0305 | 0.0245 | 0.0266 |
| Kidneys           | 0.0009 | 0.0010 | 0.0011 | 0.0010 | 0.0014 | 0.0016 | 0.0017 | 0.0013 |
| Sum of sample weights | 0.1102 | 0.1510 | 0.2010 | 0.1478 | 0.1761 | 0.1366 | 0.2007 | 0.1605 |
| Wholebody         | 0.149 | 0.1520 | 0.166 | 0.1451 | 0.146 | 0.152 | 0.164 | 0.153 |
| Difference        | 0.0388 | 0.0010 | -0.0350 | -0.0028 | -0.0301 | 0.0154 | -0.0367 | -0.0071 |
| Difference %      | 26.0 | 0.7 | -21.1 | -1.9 | -20.6 | 10.1 | -22.4 | -4.6 |

Note: specimens were obtained in 24 hours after intragastric administration of 1.8 mg of Ag NPs to mother rat.

Data of table 3 shows that main part of NPs registered in the infant rats is localized inside body and surface contaminations are negligible. Also the sum of masses of NPs detected in organs and carcass
was compared to the mass of NPs registered in the whole rat pup bodies. The differences were shown in table 3 (last two rows). The average deviation does not exceed measurement uncertainties of the RMT [15] which confirms the reliability of the obtained data.

6. Conclusion
It can be concluded that RTM is quite appropriate to model biological experiments with inorganic NPs on ADME study as well as for broad variety of EHS investigations. It has several serious advantages in comparison with conventional methods of determination mass/concentration of NPs in biological samples: by the side of optical and mass-spectrometry methods of elemental analysis the RTM can measure mass/concentration of NPs of biophile elements at their natural background in organism, RTM does not need any preparing of samples before measuring and thus is sufficiently less labor-consuming. Also by contrast to conventional methods (including TEM) RTM determinates mass of NPs in whole sample and interpretation of obtained data is more transparent. The experimental data obtained in experiments with ADME study demonstrate sensitivity and accuracy comparable or better than conventional methods. The RTM was used successful in the model experiments on in vivo study of ADME of some commercially actual NPs, for the first time were obtained experimental evidences of the transfer of Ag NPs from mother to her off-spring through placenta and breast milk.

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