

## Extremely low level of Ag nanoparticle excretion from mice brain in *in vivo* experiments

A Antsiferova<sup>1,3,6</sup>, Yu Buzulukov<sup>1,3</sup>, V Demin<sup>1,3</sup>, P Kashkarov<sup>1,3,4</sup>, M Kovalchuk<sup>1,5</sup>, E Petritskaya<sup>2</sup>

<sup>1</sup>National Research Centre “Kurchatov Institute”, 1, Academica Kurchatova sq., Moscow, Russia

<sup>2</sup>M.F. Vladimirovsky Moscow Regional Research Clinical Institute, 61/2, Schepkina st., Moscow, Russia

<sup>3</sup>Moscow Institute of Physics and Technologies, Institutskii lane, Dolgoprudny, Moscow Region, Russia

<sup>4</sup>Lomonosov Moscow State University, GSP-1, Leninskie Gory, Moscow, Russia

<sup>5</sup>Saint Petersburg State University, 7-9, Universitetskaya emb., St. Petersburg, Russia

Email: antsiferova\_aa@nrcki.ru

**Abstract.** Silver nanoparticle accumulation in mice organs as well as the excretion processes from them were studied. The investigation included a one-time oral administration of silver nanoparticles and a series of prolonged oral administrations of the same nanoparticles to study the long-term impact of the nanoparticles. In these experiments, the mice had been fed with colloid silver and in these prolonged experiments, administrations lasted for 2 months. The nanoparticle administration was then cancelled for one month. The elemental composition of tissue samples was studied by Nuclear Physical technique, which allowed us to obtain the masses of the key element, namely silver. It was demonstrated that silver concentrations in tissues were redistributed with time. The main result of this work was the discovery of extremely low level of silver nanoparticle excretion from mice brain (just 6% per month) following the cancellation of NP administration. However, the rates of excretion from blood and liver appeared to be rather high (about 80% per month). Thus, the accumulation effect of silver nanoparticles in the mice brain was observed, which is of great practical importance. It changes the approach to the toxicity assessment of silver nanoparticles as a result of the prolonged injection of colloidal silver.

### 1. Introduction

Due to the increased practical use of nanoparticles (NPs) in various areas of industry, including the food industry, medicine, pharmacy and others, their interaction with living organisms and recycling processes are of significant interest for applied science as well as for fundamental one. Thus, silver NPs are widely used as food supplements, antibiotics, antiviral agents and hygiene agents [1-10]. However, the data on the influence of silver on mammal organisms either demonstrates a pronounced cytotoxic effect, in particular in nanoform [11-22], which is likely due to the high penetrability of NPs. Another problem is the pollution of the environment from the NPs released from consumption goods. In this, case NPs eventually enter to the food chain reaching human organisms as a result. Having in mind both the reasons, it seems to be significant to study their natural ways of exposure.



The main objective of this work is to study the biokinetics of silver NPs in organs and the biological fluids of laboratory animals, i.e. their biodistribution in the organisms as a result of natural ways of injection, penetration through various histohematogenous barriers of the organism and NP following transport via blood. The bio-objects under investigation in this study are blood, the liver and the brain. The answers to the important questions concerning these organs could be beneficial in the formation of the basis of toxic and hygienic standards, imposing certain restrictions on the use of silver NPs as consumer goods.

It has been already proven that silver injected into an organism in nanoform is able to penetrate through the blood-brain barrier [23, 24], perhaps into the cells of the neural system. At least by additional measurements of the Fe isotope activity in blood and the whole brain, it was fairly confirmed that the major fraction of silver of the same type as described in the present paper concentrates not in the blood vessels of brain but in the cellular or perhaps intracellular medium of brain [23]. It makes the problem of silver nanosafety extremely topical and important. Therefore, in the present research we especially focused on the ability of silver NPs to penetrate into the brain.

However, the ability to penetrate different tissues strongly depends on experimental conditions such as the route of ingestion, NP size, surface functionalization [25], which designate the specific pathway of cellular uptake. Significant variety of these characteristics as well as the application of methods with diverse sensitivity and accuracy leads to controversial experimental data. In order to obtain unanimous meaning about such aspects as biokinetics and biodistribution, as well as toxicity assessment, experiments are supposed to be conducted with metrologically assured high-precision methods designed for NP content measurements and advance characterization of the key NPs.

## 2. Methodological basis of the work

Table 1. Characteristics of techniques for NP tracing

#	Method	Advantages	Disadvantages
1.	Electron Microscopy	Visualization of measurement results	Low representativeness (microsections and microsamples), complexity of sample preparation, impossibility of biophilic nanoparticle analysis
2.	Optical Spectroscopy and Mass-Spectrometry	Relatively high accessibility	Relatively low representativeness (small and microsamples), difficulties in converting of solid samples into liquids, destructive techniques, low accuracy, impossibility of biophilic nanoparticle analysis
3.	Nuclear-Physical methods	Possibility of nondestructive macrosample analysis (whole tissues or their parts), possibility of biophilic nanoparticle analysis (Zn, Fe, Se etc.), high sensitivity (up to 10 <sup>-11</sup> g) and metrological accuracy (~1-10%)	Relatively low accessibility

There are a few techniques by which it is possible to estimate the concentration of NPs in complex biological tissues. Nuclear-Physical methods demonstrate a number of advantages in comparison with other techniques: high accuracy, representativeness, integrity, possibility of concentrations measurements of biophilic NPs. Table 1 demonstrates the comparative analysis of different techniques designed to trace nanoparticles in complex biological matrices. There are two major types of Nuclear-

Physical technique: Radiolabelling technique and Neutron Activation Analysis. The first method implies administration of radiolabelled nanoparticles and tracing their transport via blood to different organs and tissues with time. In the second one, nonradioactive nanoparticles are administrated into animal organisms and then after irradiation of the tissues containing unknown amounts of NPs, elemental analysis of the tissues is conducted [26].

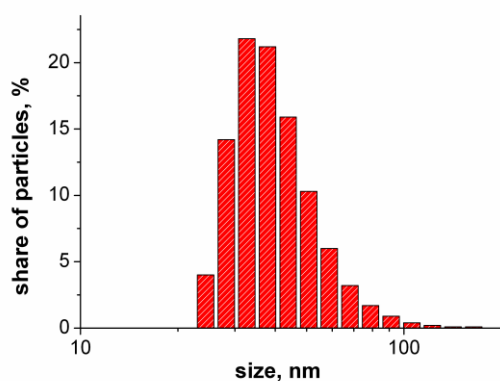
In the present work Instrumental Neutron Activation Analysis (INAA) to study biokinetics of silver NPs in biological tissues was applied. INAA is held in conjunction with gamma spectrometry which allows to obtain information about radioactive isotopes in the measured probes, and the levels of their activity are converted into concentrations of the key elements.

There are two possible pathways which can be applied for obtaining values of masses and concentrations of the key element. In absolute measurements, calculations are performed using different known nuclear-physical constants and measured values of activities. Otherwise, in more clear and reliable approach activities of measured samples are compared with activity of reference material containing the known amount of the key element, which is irradiated simultaneously with the measured samples at the same conditions.

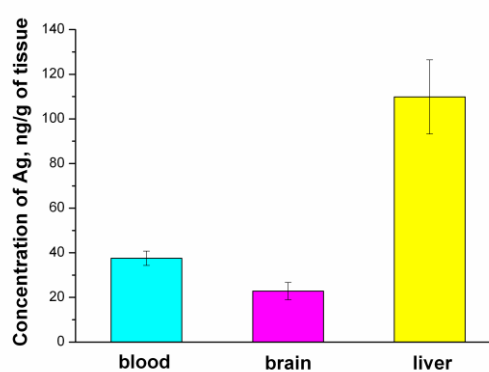
### 3. Experiment

#### 1. Materials

In the present work, biokinetics of silver nanoparticles stabilized with polyvinylpyrrolidone (PVP) performed by colloidal solution of food supplement “Argovit-C” with initial concentration of 10 mg/ml were investigated. Size and aggregation kinetics were studied with the use of Dynamical Light Scattering (DLS). Solutions of the food supplement were diluted down to 0.5 mg/ml and sonicated for 15 minutes in an ultrasonic bath then sizes of NPs were measured by DLS technique. DLS data showed that the NPs were not aggregated and the average size of NPs was about 34 nm, which is in a good agreement with manufacturer’s data. The histogram of size distribution by number of particles is demonstrated in figure 1.



**Figure 1.** Size distribution of silver NPs “Argovit-C” obtained by DLS.



**Figure 2.** Ag concentrations in different organs after once-time exposure to NPs.

## 2. Scheme of the experiments

Diluted with distilled water solutions of “Argovit-C” were administrated orally into white mice SHK digestive tract. The mice were divided into 6 groups:

- group 1: control group, 5 mice, 1 day of maintaining;
- group 2: control group, 2 mice, 2 months of maintaining;
- group 3: control group, 2 mice, 3 months of maintaining;
- group 4: once-time oral administration of 100 micrograms of silver NPs, 5 mice, 1 day of maintaining;
- group 5: repeated oral administration of silver NPs (100 microgram per day), 6 mice, 2 months of maintaining.
- group 6: repeated oral administration of silver NPs (100 microgram per day) for 2 months and administration of distilled water for 1 following month after cancelling of silver injection, 6 mice, 3 months of maintaining.

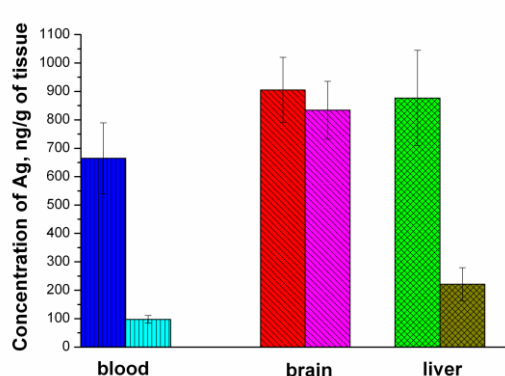
After the end of each experiment the mice were decapitated and a number of the following organs were taken, weighted and then dried for 24 hours at 70°C (liver, brain, blood).

## 2. Instrumental Neutron Activation Analysis (INAA)

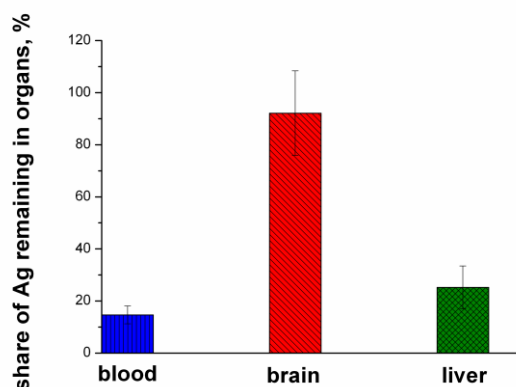
Simultaneously with the control and experimental samples, the reference samples containing known amount of silver (100 micrograms) were prepared. The reference samples were irradiated together with the experimental ones. Preparation of the reference samples was necessary for the determination of amounts of silver in experimental samples based on comparison of their activities.

All the samples were put into aluminum containers and irradiated in vertical channels VEC-9 and VEC-10 of a nuclear research reactor IR-8 at the National Research Centre “Kurchatov Institute” with thermal neutron flux approximately  $3 \cdot 10^{12} \text{ c}^{-1} \text{ cm}^{-2}$  for 12 hours. As a result, experimental samples and reference materials were labeled with  $^{110\text{m}}\text{Ag}$  radioactive isotope with half-life around 250 days. It is obvious that during such the irradiation many the elements in the samples are activated possessing the radioisotopes formed this way. After decay of high energy isotopes with short half-life samples were analyzed by measuring of  $^{110\text{m}}\text{Ag}$  activities in each sample using gamma spectroscopic device.

## 4. Results



**Figure 3.** Ag concentrations in different organs after long-time exposure to NPs. Left bar is related to group 5 and the right bar is to group 6.



**Figure 4.** Fractions of silver remaining in mice organs after 1 month of washing up.

The amounts of silver in the control samples were lower than the sensitivity of the method and neglected. Results of the single administration and 2 months of exposure to NPs are demonstrated in figure 2 and 3 (left bars) respectively. The result obtained for group 6 is demonstrated in figure 3 (right bars). It can be seen that the highest concentrations related to a one-time exposure are observed in liver and blood, and in 2 months of NPs administration obvious redistribution of silver is shown. After long-time exposure the highest concentrations of silver were found in brain and liver which may be explained by accumulation of silver in brain and filtering properties of liver while blood seemed to be quite self-cleansing. After washing silver up during one month concentrations of silver in all organs reduced.

In order to estimate probable accumulation effects, average concentrations of silver in corresponding mice organs from group 5 and group 6 were compared. The obtained fractions of the rest silver in different tissues are presented in figure 4. It is seen that the rates of excretion of silver NPs from blood and liver after 1 month of washing NPs up are rather high (around 80% and 75% respectively) and the level of excretion of silver NPs from brain is extremely low (6%) which might be explained by the active functional properties of a blood-brain barrier such as mechanisms of endocytosis and exocytosis by which a NP may be taken up or extracted. Thus, no accumulation effects in liver and blood were observed. Presumably, further intake of pure water without any silver NPs and protection from other contaminating sources would bring to reducing of the amounts of silver in these organs to neglecting values. Though an extremely low level of silver excretion from brain was demonstrated, this asserts the assumption about possible accumulation of nanosilver in brain made in the previous work [23].

## 5. Conclusions

According to the results obtained, relatively high levels of excretion from liver and blood indicate no significant hazardous effect of silver NPs on these organs - NPs do not tend to accumulate there. On the other hand, low level of silver excretion from brain causing growing accumulation of the element in this organ might imply risks for humans using products containing nanosilver. The reason for such unexpected blood-brain barrier behavior in relation to Ag NPs is not understood and is the subject for further research. The condition of silver, which penetrates into brain is not yet a finally resolved question. Are the silver NPs conserved in the nanoform or solved to the ionic state when reaching the blood-brain barrier? In spite of that, silver is widely believed to be a non-metabolizable element and is hardly oxidized. We did not reach a unanimous agreement in the literature concerning the transformations of Ag NPs in living organism. Nevertheless, the discovery and confirmation of the fact of the accumulation of silver NPs in brain is of great practical importance, which can modify existing approaches of nanosilver toxicity assessment during the long-time exposure to organisms. Therefore, there is a need for further studies to determine the features of extended kinetics of silver accumulation in brain and the actual structural forms of silver in different tissues after possible transformations in living organism. Moreover, it is not known if the concentrations of silver measured in the brain (up to 1200 ng/g of tissue) can influence the cognitive functions of animals.

All questions mentioned above are the important subjects for the following research.

It is also should be noted that the use of Nuclear-Physical methods described in this work is highly suitable for tracing NPs in complex heterogeneous macroscopic samples such as the biological organs and tissues. At the same time due to its high sensitivity, selectivity and metrological accuracy this technique can be successfully applied for different puposes – elemental analysis of samples such as food products, drugs, soils and other inorganic complex materials and their certification. Thus, the use of INAA and Radiolabelling technique can help to reveal the unexpected data of different phenomena and promises to be one of the future top techniques.

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## References

- [1] Suppi S, Kasements K, Ivask A, Kunis-Beres K, Shihtmae M, Kurvet I, Aruoja V and Kahru A *Journal of Hazardous Materials* 2015 **286** 75
- [2] Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M and Kahru A *Archives of Toxicology* 2013 **87**(7) 1181
- [3] Hu R, Li S, Kong F, Hou R, Guan X and Guo F *Genetics and Molecular Research* 2015 **13**(3) 7022
- [4] Ramar M, Manikandan B, Marimuthu P, Raman T, Mahalingam A, Subramanian P, Karthick S and Munusamy A *Spectrochimica Acta* 2015 **140** 223
- [5] Kujda M, Oćwieja M, Adamczyk A, Bochenska O, Bras G, Kozik A, Bielanska E and Barbasz J *Journal of Nanoscience and Nanotechnology* 2015 **15**(5) 3574
- [6] Sheehy K, Casey A, Murphy A and Chambers G *Journal of Colloidal and Interface Science* 2015 **443** 56
- [7] Sohrabnezhad S, Pourahmad A, Mehdipour Moghaddam M and Sadeghi A *Spectrochimica Acta* 2015 **136**(PC) 1728
- [8] Shameli K, Akhmad M, Jaffar Al-Mula E, Shabanzadeh P and Bagheri S *Research on Chemical Intermediates* 2015 **41**(1) 251
- [9] Ortego L, Gonzalo-Asensio J, Dolores V and Concepcion G *Journal of Inorganic Biochemistry* 2015 **146** 19
- [10] Salem W, Leitner D, Zingl F, Schratte G, Prassl R, Goessler W, Reidl J and Schild *International Journal of Medical Microbiology* 2015 **305**(1) 85
- [11] Prabhu S and Poulouse E *International Nano Letters* 2012 **2**:32
- [12] Xu L, Shao A, Zhao Y, Wang ZH, Zhang C, Sun Y, Deng Y and Lee Chou L *Journal of Nanoscience and Nanotechnology* 2015 **15**(6) 4215
- [13] Wu J, Yu C, Tan Y, Hou Z, Li M, Shao F and Lu X *Environmental Research* 2015 **138** 67
- [14] AshaRani P, Low Kah Mun G, Hande MP and Valiyaveetil S *ACS Nano* 2009 **3**(2) 279
- [15] Bar-Ilan O, Albercht R, Fako VE and Furgeson DY *Small* 2009 **5**(16) 1897
- [16] Gwinn M and Vallyathan V *Environmental Health Perspectives* 2006 **114**(12) 1818
- [17] Roco M *Environmental Science and Technology* 2005 **39**(5) 106A
- [18] Liu H, Yang H, Lin B, Zhang W, Tian L, Zhang H and Xi ZH *Toxicology Research* 2015 **4**(2) 486
- [19] Simard J, Vallieres F, de Liz R, Lavastre V and Girard D *Journal of Biological Chemistry* 2015 **290**(9) 5926
- [20] Zhang X, Choi YJ, Han JW, Kim E, Park JH, Gurunathan S and Kim JH *International Journal of Nanomedicine* 2015 **10** 1335
- [21] Heyrdnejad M, Samani R and Aghaeivanda S *Biological Trace Element Research* 2015, 6p
- [22] Korani M, Ghazizadeh E, Korani S, Hami Z and Mohammadi-Bardbori A *European Journal of Nanomedicine* 2015 **7**(1) 51
- [23] Antsiferova A, Buzulukov Yu, Demin V, Demin V, Rogatkin D, Petrinskaya E, Abaeva L and P. Kashkarov *Nanotechnologies in Russia* 2015 **10**(1-2) 100
- [24] Borm P and Kreyling W *Journal of Nanoscience and Nanotechnology* 2004 **4** (5) 521
- [25] Khlebtsov N and Dykman L *Chemical Society Reviews* 2011 **40** 1647.
- [26] Demin V, Antsiferova A, Buzulukov Yu, Demin V and Soloviev V *Medical Radiology and Radiation Safety* 2015 **60**(2) 60