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NUCLEAR PHYSICAL METHOD FOR THE DETECTION OF CHEMICAL ELEMENTS IN BIOLOGICAL AND OTHER SAMPLES USING ACTIVATION BY CHARGED PARTICLES*

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Ядерно-физический метод детектирования химических элементов в биологических и других образцах на основе активации заряженными частицами*

ABSTRACT

Purpose: To develop a method of radioactive tracers by the activation by charged particles for the studying quantitative content of chemical elements and nanoparticles in biological samples and in the environment.

Material and methods: Theoretical analysis and test experiment were carried out to study the possibility of using various nuclear methods for detection of chemical elements and nanoparticles in biological and other samples, using the activation of different isotopes by a charged particles flux. The characteristics of the products and the various nuclear reactions, taken from the IAEA's nuclear databases, have been considered. The irradiation of natural isotopes of titanium by fast neutron flux produces radioactive isotopes ^{46}Sc and ^{47}Sc (with half-life $T_{1/2}$, respectively, equal to 83.8 and 3.35 days), by fast protons flux — ^{48}V ($T_{1/2}$ = 16 days) and by alpha-particles flux — ^{51}Cr ($T_{1/2}$ = 27.7 days). The flux of fast protons after interaction with the natural isotopes of platinum mixture generates radioactive isotope ^{195}Au ($T_{1/2}$ = 186 days), with the isotopes of iron — ^{56}Co ($T_{1/2}$ = 77.7 days), with the isotopes of manganese — ^{54}Mn ($T_{1/2}$ = 312 days), with europium isotopes — ^{151}Gd ($T_{1/2}$ = 124 days) and ^{153}Gd ($T_{1/2}$ = 241.6 days). We also consider the possibility of exposure to iron isotopes by fast deuterons flux with the formation of isotope ^{56}Co. All radioactive isotopes are gamma-emitters and are suitable for the measuring on gamma-spectrometers. Particular attention is paid to the detection of nanoparticles of titanium dioxide, which takes one of the first places in the list of priority nanomaterials. For estimate the proportion of silver nanoparticles or another nanoparticles passing through the blood-brain barrier, evaluation of the content of iron in the blood can give a key information.

Results: The use of such methods in addition to the traditional neutron activation analysis expands the list of chemical elements, which can be successfully detected by the nuclear activation. This expansion includes such elements as titanium, iron, platinum, manganese, europium and some others.

Key words: *nuclear-physical methods, radioactive tracer, charged particles, biokinetics, laboratory animals, the environment, nanoparticles*

РЕФЕРАТ

Цель: Разработка метода радиоактивных индикаторов на основе активации заряженными частицами для исследования биокинетики химических элементов и наночастиц в биологических образцах и окружающей среде.

Материал и методы: Проведен теоретический анализ и тестовый эксперимент по исследованию возможности применения различных ядерно-физических методов детектирования химических элементов в биологических и других образцах, в том числе в составе наночастиц, с использованием активации различных изотопов потоком заряженных частиц. Проанализированы продукты различных ядерных реакций. При облучении природных изотопов титана потоком быстрых нейтронов образуются радиоактивные изотопы ^{46}Sc и ^{47}Sc (с периодами полураспада $T_{1/2}$ равными соответственно 83,8 и 3,35 дня), потоком быстрых протонов — ^{48}V ($T_{1/2}$ = 15,98 дня), а альфа-частицами — ^{51}Cr ($T_{1/2}$ = 27,7 дня). При облучении быстрыми протонами природной смеси изотопов платины образуется радиоактивный изотоп ^{195}Au ($T_{1/2}$ = 186,12 дня), изотопов железа — ^{56}Co ($T_{1/2}$ = 77,7 дня), изотопов марганца с образованием изотопа ^{55}Mn ($T_{1/2}$ = 312,1 дня), изотопов европия — изотопы гадолиния ^{151}Gd и ^{153}Gd ($T_{1/2}$ составляет, соответственно, 124 и 241,6 дня). Рассмотрена также возможность облучения изотопов железа быстрыми дейтронами с образованием того же изотопа ^{56}Co. Все образующиеся радиоактивные изоптопы являются гамма-излучателями и имеют удобные для целей измерения на гамма-спектрометре энергетические линии. Особое внимание уделяется детектированию наночастиц из двуокиси титана, занимающими одно из первых мест в списке приоритетных наноматериалов. При количественной оценке доли наночастиц серебра или других наночастиц, проходящих через гематоэнцефалический барьер, оценка содержания железа в крови может дать недостающую ключевую информацию. При выборе оптимальной процедуры проведения эксперимента получаемые радиоактивные продукты будут иметь активность ниже минимально значимой активности.

Результаты: Применение рассмотренных ядерно-физических методов в дополнение к традиционному нейтронно-активационному анализу существенно расширяет список химических элементов, на такие как титан, железо, платина, магний, европий и др., для детектирования которых они могут быть успешно применены.

Ключевые слова: *ядерно-физические методы, радиоактивный индикатор, заряженные частицы, биокинетика, лабораторные животные, окружающая среда, наночастицы*

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Introduction

Accelerated development of nanotechnology increases the amount of products containing nanoparticles (NPs). Only in the food industry currently more than 200 products with nanomaterials are used. Exponential growth of the amount of products with nanomaterials requires an assessing not only their useful properties, but also the degree of risk to humans and the environment that creates the problem of ensuring their safety for human health and the environment.

A key way in solving this problem is to study biokinetics (absorption, biodistribution, metabolism and excretion) of nanoproducts in animals and humans. The basis of this research is a quantitative measurement of the NPs mass content in biological samples.

Quantitative measurement of NPs content in living organisms and material / waste of nanotechnology presents considerable difficulties because of the high demands for sensitivity and accuracy. These difficulties in the most concentrated form manifest in quantitative measurements of NPs content in complex, multi-component and polydisperse systems, such as tissues of living organisms.

One of the most promising methods for measuring the mass of inorganic NPs is a method of radioactive tracers, based on neutron activation of the atomic nuclei. In previous years, NRC "Kurchatov Institute" in collaboration with the Moscow Institute of Nutrition performed a significant amount of work on the development of the method and its application to the study of biokinetics of NPs, such as silver, zinc dioxide, gold and selenium. For the first two types of NPs the techniques were developed and certified in GOST R system [1]. These techniques are recommended for use in Rospotrebnadzor' document MR 1.2.0048-11 [2].

Method of detecting chemical elements contained in the NPs based on thermal neutron activation is applicable to a limited number of such elements. They include silver (Ag), zinc (Zn), selenium (Se), cerium (Ce), lanthanum (La), iron (Fe), gold (Au), iridium (Ir). The most serious limitation is imposed on the number of elements in the biokinetics' study in the experiments on laboratory animals due to possible requirements on their duration. In studies of trace elements and hazardous pollutants in the environment, agricultural and food products, this list may be expanded considerably. Practical needs in quantifying the content of trace elements in the various materials demands significant expansion of this list.

In different practical fields (scientific research, medicine, nanotechnology) there is a need for methods of detecting other elements such as titanium (Ti), platinum (Pt), europium (Eu) and some others. In addition, not for all elements, listed in the first list, and not in all options of research the high sensitivity of the measurement in the application of the neutron activation method can be ensured. For example, such situation may occur in measuring the iron content in the samples due to the low content of isotope ^{58}Fe (0.28 %) in the natural isotope mixture. The measurement sensitivity may fall due to long time isotope activation with relatively short half-life, for example, $^{195}Pt_{78}$ with $T_{1/2} = 4$ days. In connection with this, we started the study of the possibility of using the elements' activation in nanomaterials by irradiation with fast charged particles (p, d, ^{3}He, ^{4}He) paying particular attention to proton irradiation. Preliminary theoretical analysis and experimental research using beams of fast charged particles of the cyclotron in NRC "Kurchatov Institute" promises the hope of the viability of this method of activation as applied to biological and environmental research.

Material and methods

The method of radioactive tracers based on neutron activation was developed many decades ago for the purpose of biochemical and physico-chemical analysis.

Radiotracer method based on the neutron activation was developed several decades ago to conduct biochemical and physico-chemical analysis. This method is unique in its features of sensitivity and accuracy (see review paper [3]). It is one of the widely used technologies from the standpoint of its sensitivity [1, 3–7].

Originality and features of modern development of the radiotracer method are as follows:

- the scope of the application nanoparticles / nanomaterials with specific chemical elements, support research on their bio- and toxico-kinetics;
- the application of modern highly sensitive gamma-spectrometric equipment;
- development of standardized measurement techniques to ensure the reliability and accuracy of measurement; one embodiment of this method (the method of comparison with a standard sample) allows to receive the result of measurement with a high precision (with a relative error less than 15 %);
- it is possible to determine the mass content of biophilic elements (e.g., zinc, selenium) in biological tissues and organs, in contrast to many other methods;
- it is possible to measure directly the mass content in solid samples, while many other methods require prior sample transformation into a liquid or gaseous state;
- determination of the mass content of nanomaterials can be produced both in micro- and macro-samples up to several centimeters in all three dimensions;
- if the activated isotope has a sufficiently long half-life (a few ten-day periods), this method can be applied to biological and other experiments lasting for tens — hundreds of days, commensurate with the time of process of transport of nanomaterials in experimental animals or objects of the environment;

- the possibility of using not only thermal neutrons flow but also charged particles flow for the isotope activation significantly expands the scope of the nuclear-physical methods.
- NRC "Kurchatov Institute" has a certified equipment necessary for the application of nuclear-physical methods of measuring the mass of chemical elements (research nuclear reactor IR-8 with a thermal neutron flux of at least 10^{12} neutrons/s×cm^2, cyclotron, modern multichannel analyzers of gamma-spectra, etc.).

Activation analysis options

As in the embodiment of neutron activation analysis two modifications of the method are used: the absolute and relative measurements [1, 4, 7]. In absolute method, the mass content of the investigated element in the sample is calculated by the known nuclear properties of the target and activated radioactive isotope and the value of the flux density of charged particles during irradiation and others. In this case, the measurements can contribute significantly to systematic and random components of uncertainty into the results.

The absolute method is used for the preliminary assessment of activity of the samples. Using the results of this evaluation, a scenario of the experiment using nuclear-physical methods of detection of nanomaterials in the samples is determined. In particular, the exposure time of the sample by neutrons or charged particles, the holding time after the exposure and the time of measuring the activity on a gamma-spectrometer are determined.

In the relative method of measuring, the content of the element in the sample is determined by comparison of the radiation activity of the test sample and the standard sample with a known content of an element after simultaneous exposure of test and standard samples to the activating particles' flow. In this case, the measurement' result does not depend on the variation of the value of the particle flux density. Thus, the measurement uncertainty in the relative activation analysis method is much smaller. To use it, the standard samples with known content of the element must be prepared.

Depending on the goals and objectives of the study, as well as on the biological properties of the sought-for element (whether it is a biophil element, i.e., is it contained in the body in a natural amount, or it is not biophil) one of two experiment' variants on laboratory animals is chosen:

1) co-activating irradiation of the test and standard samples with subsequent gamma-spectrometric analysis;
2) preliminary preparation of the radiolabeled material containing a tested chemical element in a particular physico-chemical form by irradiation of primary non-radioactive material; administration of radioactively labeled material in the test medium (animal' organism or some other sample); gamma-spectrometric analysis of the sample with the tested radiolabeled element.

For such elements as Pb, Ag, Au, As, etc. one may use both types of the experiments. For biophilic elements (Fe, Zn, Se, Ti, etc.), when a varying amount of the element is contained in the animal organs, in the biological studies only the second type of the experiment may be recommended — with the radiolabel material.

When studying the penetrability of the blood-brain barrier by investigated NPs it is necessary also to determine the iron content in the blood and brain samples. Knowing the NPs concentration in the peripheral blood one can estimate the NPs content in the blood veins of the brain of laboratory animals. And then the NPs fraction passing through the blood-brain barrier can be determined. Determination of iron in the blood is carried out by the same method of radioactive tracers: activation of iron isotopes.

Theoretical analysis of the activation method with charged particles

This analysis was performed for a number of elements relevant from scientific and practical points of view.

Titanium. One of the first places in the list of priority nanomaterials is taken by titanium Ti, used usually in the form of titanium dioxide TiO_2 [8]. For this element there are no radioactive isotopes, activated by the thermal neutron flux, with the required properties. Because of the special needs in developing a method of detecting nanomaterials with this element the method of activation of titanium in fast neutrons flux was investigated.

Upon irradiating natural isotopes of titanium with fast neutrons, radioisotopes ^{46}Sc and ^{47}Sc are produced in the reaction (n, p) with the necessary properties for gamma-spectrometry measurements ($T_{1/2}$ are 83.8 and 3.35 days respectively). In this study the secondary beam of fast neutrons, generated in the cyclotron of NRC "Kurchatov Institute", was used. The study has shown that it was impossible to obtain high detection sensitivity with a titanium content of nanomaterials due to the relatively small cross section of the (n, p) reaction and insufficient power of the secondary beam of fast neutrons.

In this regard, the development of another method of detecting the content of titanium nanomaterials was initiated. That method is based on the activation of titanium by fast protons to form radioactive isotope ^{48}V in reactions (p; n, 2n, ..) on natural titanium isotopes. Radioisotope ^{48}V has a sufficiently long half-life ($T_{1/2}$= 16 days) and two gamma-lines 1.31 and 0.98 MeV with a single output for each decay. Preliminary theoretical analysis of this method showed its satisfactory characteristics.

Both methods of analysis — absolute and relative ones — are equally applicable to activation of the target by fast protons.

The evaluation of the titanium activation with the absolute method has demonstrated the possibility of the radioactive label ^{48}V formation with sufficient activity. In this evaluation the characteristics of the proton beam of the cyclotron and the available literature' nuclear data on the target nuclei and radioisotope ^{48}V were considered. Nuclear data were taken from the IAEA's nuclear databases, see fig. 1 with the data on the cross section of proton activation of natural titanium isotopes. Thus, the possibility to achieve a sufficient sensitivity of detection of nanomaterials with titanium can be real.

We also considered the option of activating titanium with fast alpha particles. Fig. 2 shows the cross section of the reaction ^{nat}Ti(α,x)^{51}Cr with the release of the radioactive isotope ^{51}Cr ($T_{1/2}$ = 27.7 days, E_γ = 0.32 MeV). The cross section for this reaction is higher than for the activation by protons and ^{51}Cr radiolabel has almost two times longer half-life, than ^{48}V.

Platinum. When irradiating natural platinum isotope mixture in the fast protons beam, reactions (p; n, 2n, ..) form radioisotope ^{195}Au, having a half-life $T_{1/2}$ = 186.1 days and gamma-line with energy 0.099 MeV. For a sufficiently long time' experiments (tens — hundreds of days), this activation option can give the desired results in the terms of sensitivity and accuracy.

Iron. The irradiation of a natural mixture of iron isotopes by fast protons produces radioactive isotopes of cobalt in the reactions ^{nat}Fe(p,x)^{56,57,58}Co. Radioisotope ^{56}Co presents the greatest interest. It is formed from the main natural isotope ^{56}Fe (91.75 % content in the natural isotope mixture), has a half-life $T_{1/2}$ = 77.7 days and gamma ray line of energy 0.847 and 1.24 MeV (respectively with the quantum yields per unit of radioisotope decay n_γ = 1; 0.67).

The option of activating iron isotopes by fast deuterons is also of interest. In reactions ^{nat}Fe(d,x)^{56}Co radioisotope ^{56}Co is generated. Fig. 3 shows the dependence of the reaction cross section on the energy of the deuterons. In these options with the existing technical characteristics of the cyclotron one can achieve better results than with irradiation by thermal neutrons.

Europium. Upon irradiation of natural europium with fast protons, reactions ^{151}Eu(p,n)^{151}Gd and ^{153}Eu(p,n)^{153}Gd generate radioactive isotopes of gadolinium ^{151}Gd and ^{153}Gd with half-life, respectively, 124 and 241.6 days and gamma ray lines 0.154; 0.243 and 0.097, 0.103 MeV respectively. This option is suitable for the detection of europium in various nanomaterials.

Manganese. Manganese has the single natural isotope, ^{55}Mn. Its irradiation by fast protons creates in the reaction ^{55}Mn(p;p,n)^{54}Mn radioisotope ^{54}Mn with a half-life $T_{1/2}$= 312.1 days and gamma line with energy 0.835 MeV.

Description of the experiment. Test experiment was performed with irradiation of TiO_2 NPs in the form of

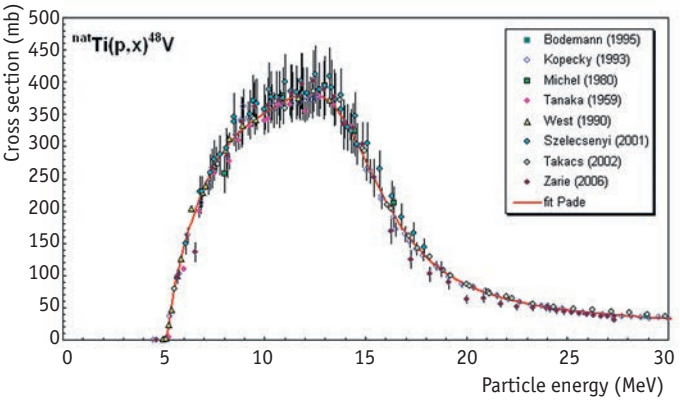

Fig. 1. Cross section of reaction Ti(p,x)^{48}V on the natural mixture of isotopes of titanium depending on the energy of the protons (IAEA, Nuclear Databases)

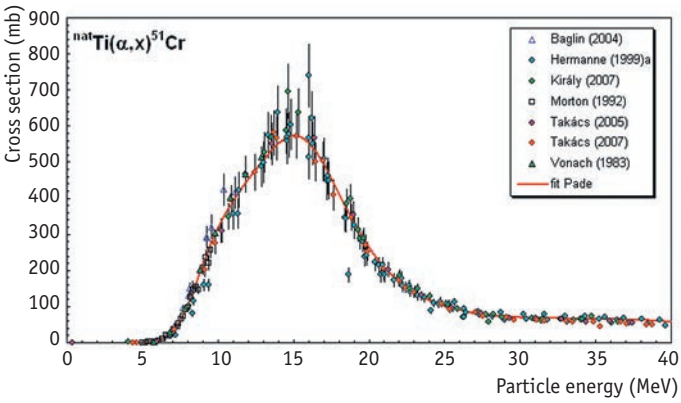

Fig. 2. Cross section of reaction Ti (α,x)^{51}Cr on the natural mixture of isotopes of titanium depending on the energy of the alpha-particles (IAEA, Nuclear Databases)

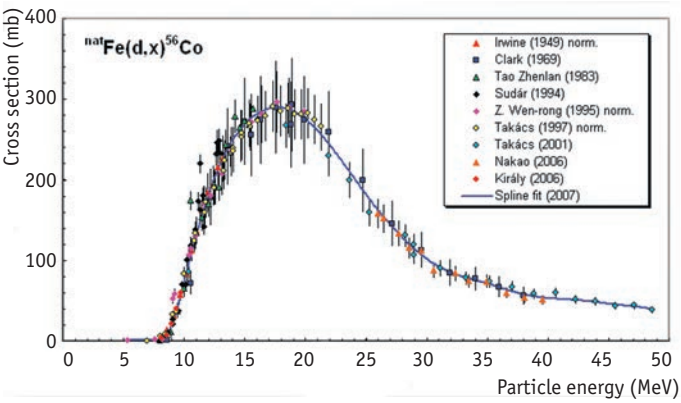

Fig. 3. Cross section of reaction Fe(d,x)^{56}Co on the natural mixture of isotopes of iron depending on the energy of the deuterons (IAEA, Nuclear Databases)

powder rutile (Sigma-Aldrich, USA — Germany) by the proton beam of the cyclotron. According to the study on the transmission electron microscope TEM, the rutile was presented partially by aggregated nanorods with a diameter of 5–10 nm and a length of 40–50 nm [6].

For planning the experiment, the theoretical calculations were made with the absolute method. Taking into account the rather high cross section of natural titanium isotopes' activation by fast protons and characteristics of the proton beam of the cyclotron, the following parameters were chosen for the irradiation of ampoules with TiO_2: proton beam power with an energy of 32 MeV — 0.1 mA, exposure time — 28 minutes. For the irradiation the sample was prepared containing 0.6 g of titanium dioxide powder (0.36 g of titanium) in a sealed tube, made of quartz glass.

Fig. 4 shows the spectrum of gamma-lines after proton activation of the sample with TiO_2 obtained on gamma-spectrometer in NRC "Kurchatov Institute".

After opening the ampoule 0.58 g of powder were dissolved in 25 cm^3 of water and, after vigorous stirring, the solution was aliquoted in the tubes in amount of 1 cm^3. Thus, each tube contained 23.2 mg of rutile NPs. Measurements on gamma-spectrometer have shown that activity of radioisotope ^{48}V in tubes corresponded to 4.4 kBq, which gives a specific activity about 190 Bq / mg. One should take into consideration a nonuniformity of irradiation of rutile mass in the tube due to relatively short free path of the protons and the narrowness of the proton beam. Homogeneity of samples was provided by dissolving them in water and stirring.

Two tubes were subjected to centrifugation at 18.000 rpm for deposition of particles and nanoparticles and evaluation of the possible content of ^{48}V ions in solution. After merging the liquid of the two tubes in one other tube, its activity was measured.

Results and discussion

In the reaction with the fast protons radionuclides ^{48}V produced possess high recoil energy. It was necessary to determine the ^{48}V amount which can be outside the nanoparticles volume. Behavior of radioisotopes ^{48}V and NPs themselves are different in the body of the animal.

Measured activity of the test tube with liquid from the two initial test tubes after centrifugation was equal to 7.1 Bq. Its initial activity was 8.8 kBq. Thus, the activity of the isotope ions did not exceed $0.8 \cdot 10^{-3}$ of initial NPs activity. This testifies to the low yield of radioisotope ^{48}V outside NPs after proton irradiation.

The main purpose of developing MRI on the basis of the activation by charged particles is to provide a reliable and sensitive method for measuring the content of NPs with TiO_2 and other substances in the studies of NPs distribution kinetics in experimental animals. For NPs loaded with silver, zinc and selenium, these experiments were carried out using thermal neutron activation [1, 5, 6]. Previous experience in this part of the experimental work with animals and of gamma-spectrometry analysis is fully applicable in experiments with activation by the charged particles.

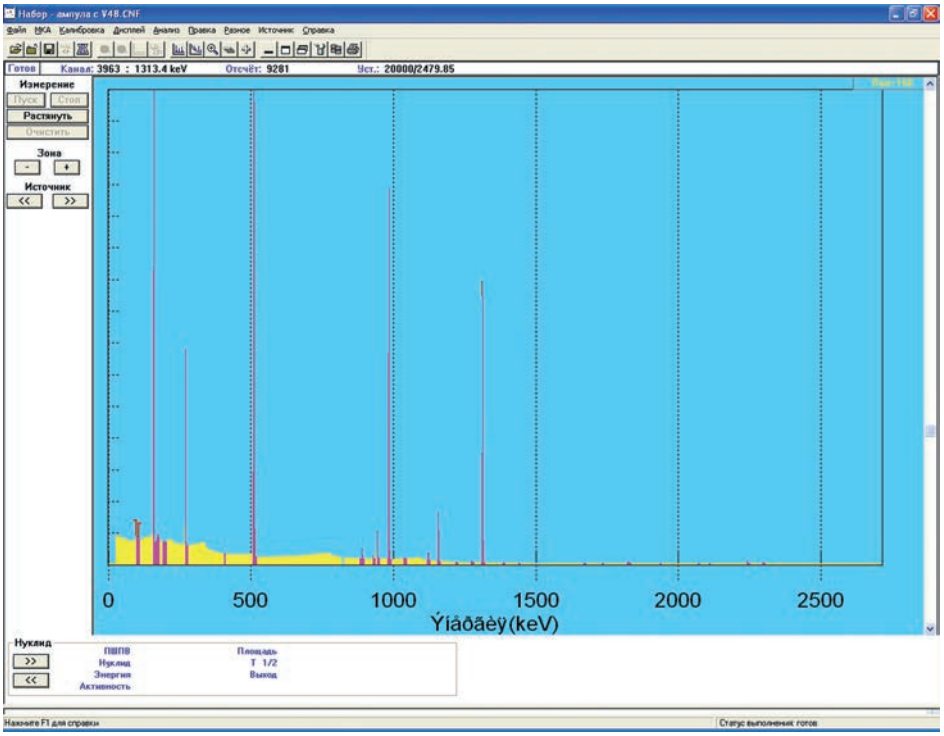

Fig. 3. Cross section of reaction Fe(d,x)^{56}Co on the natural mixture of isotopes of iron depending on the energy of the deuterons (IAEA, Nuclear Databases)

The main attention of past and planned experiments on NPs biokinetics was given to per oral way of NPs administration. It is appropriate to note that the earlier MRI method, based on the activation by accelerated protons, was applied in Germany to determination of titanium oxide distribution after its inhalation by rats [8].

During the session with the optimal proton irradiation of test samples and gamma-spectrometry analysis one can expect that the detection limit of titanium in the sample can be reduced to a few — tens of nanograms.

The sensitivity of the detection method of titanium in various samples 2–3 orders of magnitude higher with activation by cyclotron' fast protons than by fast neutrons generated in the same cyclotron.

Titanium is not biophilic element. Nevertheless, it is present in small amounts in some animal tissues [9]. For this reason, the biological experiments with titan containing materials should be made using the 2nd option (see above): pre-production of radioactive-labeled material containing the desired chemical element.

Conclusion

Summing up, it can be noted that the theoretical analysis and test experiments have shown the possibility of effective application of nuclear physics methods to measure the NPs content in biological, environmental and other samples containing a number of scientifically and practically significant elements, using the activation of source isotope by fast charged particles. Application of this method of activation, in addition to the method of thermal neutron activation extends the list of the NPs and the chemical elements, to which nuclear physical method of detection can be successfully applied.

Experimental verification of the possible release of the radioisotope ^{48}V beyond the NPs volume because of the relatively high energy recoil in the reaction (p, x) shows that this output is negligible, and its impact on the accuracy of measurement of the NPs with TiO_2 is insignificant. A similar result can be expected for other elements using activation by accelerated charged particles. Such test was carried out only for the activation of the elements by fast protons. According to available data, some of which is shown above, the reaction cross sections with other charged particles (d, ^3He, ^4He) have the same order of magnitude as the reactions with protons. This means that, if it is necessary for activation of the studied elements, these charged particles may also be used.

Nuclear-physical methods of detecting the trace elements in different samples based on neutron activation has continuously been used in various research institutes, see e.g. [3, 7]. But ecological and hygienic control of these micronutrients is still comprise an actual scientific and practical problem.

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