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10-year stability of magnetite nanopowder prepared by the exploding wire method: is it a useful feature for environment safety and biomedical applications?

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ABSTRACT

Aim. To analyze the structural, magnetic, and cytotoxic features of magnetite nanoparticles (MNPs) prepared by the exploding wire method and stored in a dark place at ambient temperature (65 ± 15% humidity, air pressure 760 ± 20 mm Hg., temperature 22 ± 4 °C) for 10 years.

Materials and methods. The properties of MNPs were analyzed by X-ray diffraction (XRD), transmission electron microscopy (TEM) and selected area electron diffraction (SAED), and vibrating-sample magnetometer (VSM). Viability of human blood mononuclear leukocytes was determined using 0.4% trypan blue staining after 24-hour culture with the nanopowder.

Results. The calculated size of the particles remained almost unchanged after 10 years of storage. The XRD and SAED patterns showed that crystallinity was preserved for 10 years. The diameter of the crystalline component of MNPs (D_{XRD}) was close to the particle size determined by TEM. It confirms high crystallinity of the tested nanoparticles. Saturation magnetization (M_S) of the MNP powder after 10 years of storage was unexpectedly higher than that of the as-prepared MNP powder. Reduced remanent magnetization (M_R / M_S) was equal for both samples within the margin of error. No cytotoxic effect of MNPs *in vitro* was detected in the long-term study.

Conclusion. No dramatic changes in the structural, magnetic, and cytotoxic features of MNPs were noted after 10 years of storage. It indicated 10-year stability of MNP powder that may be a useful feature for environment safety and biomedical applications.

Keywords: magnetite nanoparticles, ambient conditions, nanopowder stability, human blood mononuclear leukocytes, *in vitro* cytotoxicity

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Десятилетняя стабильность нанопорошка магнетита, приготовленного методом электровзрыва проводников: полезное свойство для экологической безопасности и биомедицинского использования?

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РЕЗЮМЕ

Цель. Провести анализ структурных, магнитных и цитотоксических свойств наночастиц магнетита (МНЧ), приготовленных методом электровзрыва проводников и хранящихся в защищенном от света месте при комнатной температуре (влажность 65 ± 15%, атмосферное давление 760 ± 20 мм рт. ст., температура 22 ± 4 °С) в течение 10 лет.

Материалы и методы. Свойства МНЧ изучали с помощью рентгеноструктурного анализа (РСА), просвечивающей электронной микроскопии (ПЭМ) с исследованием электронной дифракции на выбранной области (SAED) и магнитометрии с вибрирующим образцом (VSM). Жизнеспособность мононуклеарных лейкоцитов крови человека определяли окрашиванием 0,4%-м раствором трипанового синего после 24-часового сокультивирования с нанопорошком.

Результаты. Расчетный размер частиц практически не изменился после 10 лет хранения. Картины электронной и ренгеновской дифракции показали, что кристаллическая природа сохранялась в течение 10 лет. Диаметр кристаллической части МНЧ, определяемый, как область когерентного рассеивания ренгеновского излучения ($D_{ОКР.}$) был близок к размеру частиц, определенному с помощью ПЭМ ($D_{ТЕМ}$), что свидетельствует об их высокой кристалличности. Намагниченность насыщения (M_S) для порошка МНЧ после 10 лет хранения оказалась неожиданно выше, чем для свежеприготовленного порошка МНЧ. Приведенная остаточная намагниченность (M_R/M_S) была одинаковой в пределах погрешности измерений для обоих образцов. При длительном исследовании *in vitro* цитотоксическое влияние МНЧ не установлено.

Заключение. Кардинальные изменения структурных, магнитных свойств МНЧ после 10-летнего хранения не обнаружены. Сделан вывод о 10-летней стабильности электровзрывного нанопорошка магнетита, которая может быть полезна в плане его экологической безопасности и биомедицинских приложений.

Ключевые слова: наночастицы магнетита, комнатные условия, стабильность нанопорошка, лейкоциты крови человека, цитотоксичность *in vitro*

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Для цитирования: Хлусов И.А., Омельянчик А.С., Родионова В.В., Шуплецова В.В., Хазиахматова О.Г., Юрова К.А., Литвинова Л.С., Норкин И.К. Десятилетняя стабильность нанопорошка магнетита, приготовленного методом электровзрыва проводников: полезное свойство для экологической безопасности и биомедицинского использования? *Бюллетень сибирской медицины.* 2023;22(1):96–102. https://doi.org/10.20538/1682-0363-2023-1-96-102.

INTRODUCTION

Since 1989, about 100 nanomedical applications and products have been approved by the US FDA and entered the global market. Since 2010, the development of nanomedicines and the number of marketed nanomedicines have significantly increased due to the resulting healthcare benefits [1, 2]. Magnetic nanoparticles (MNPs) are of interest due to their unique magnetic properties and are widely used in different fields, such as nanomedicine, engineering, agriculture, energy, and environmental remediation [3].

MNPs are a class of materials with unique physical and chemical properties, which may affect the environment [4] and biological systems. Therefore, determining hazards and risks associated with their application is relevant. Risk assessment is crucial for developing recommendations on MNP effects and application [5]. Circulation of nanochemical agents in the industry, environment, and among customers, as well as their wastes and storage time are essential for understanding the balance between efficiency and safety of nanotechnologies.

In this regard, more attention should be paid to concerns related to long-term stability of magnetic iron oxide nanoparticles *ex vivo*, since nanoscale magnetite (Fe_3O_4) oxidizes to maghemite (γ-Fe_2O_3) in ambient conditions. However, the duration of this process has not been well described. There are very few studies focused on MNP aging, for example, one study showed that the process lasts 18 months in an aqueous suspension [6]. We analyzed the structural, magnetic, and cytotoxic properties of MNPs prepared by the exploding wire method and then stored in a dark place at ambient temperature (65 ± 15% humidity, air pressure 760 ± 20 mm Hg., temperature 22 ± 4 °C) for 10 years.

MATERIALS AND METHODS

The nanopowder was obtained by V.S. Sedoi (the Institute of High Current Electronics, Siberian Branch of the Russian Academy of Sciences, Tomsk, Russia) by the exploding wire method (EWM) in 2007. A detailed methodology and experimental setup used in the present study for structural and magnetic evaluation were described earlier [7, 8]. EWM makes it possible to obtain nanoparticles of various compositions, including metallic iron (α-Fe) and its oxides with good crystallinity, which is confirmed by the high saturation magnetization value of the obtained nanoparticles [9].

The crystal structure and phase composition of the samples were examined by X-ray diffraction (XRD). The morphology and microstructure were determined by transmission electron microscopy (TEM) with selected area electron diffraction (SAED). Magnetic properties were studied using a vibrating sample magnetometer (VSM) at room temperature (295 K) in a field range of 1.1 T.

The cytotoxicity of MNPs was tested *in vitro* using human blood mononuclear leukocytes (MNLs) collected from the venous blood of healthy volunteers (Protocol No. 948 of 09.02.2009; the local Ethics Committee of Siberian State Medical University, Tomsk, Russia; Protocol No. 5 of 16.05.2016; the local Ethics Committee of Immanuel Kant Baltic Federal University, Kaliningrad, Russia) [10].

Aseptic MNP ferrofluid in isotonic (0.9%) saline (30 mg / l, pH = 6.9) was prepared by ultrasonication for 30 min at a power output of 100 W. No stabilizers (sodium citrate, chitosan, etc.) were added because of their possible effect on the cells. Moreover, even stabilized ferrofluids demonstrated unequal distribution of MNPs in the cell culture [11].

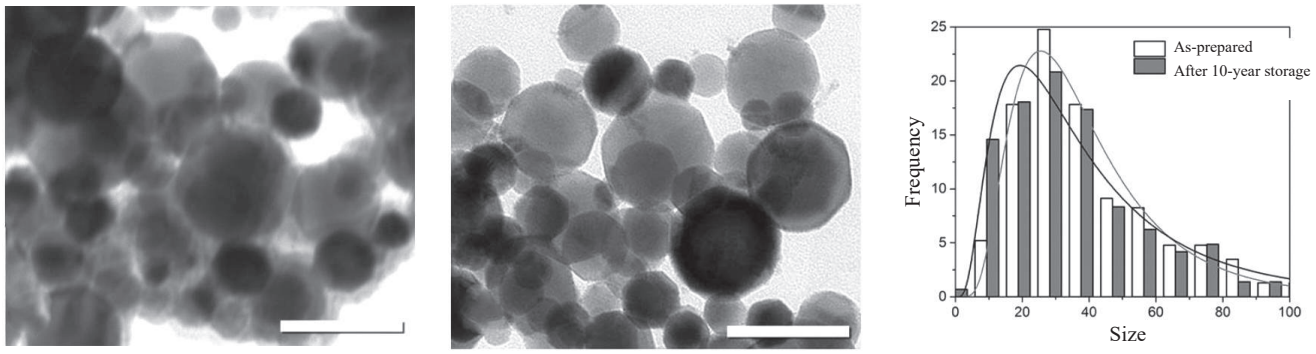
The MNL suspension in plastic tubes contained 1×10^6 viable cells per 1 ml of the synthetic nutrient medium (Sigma-Aldrich, USA) containing RPMI-1640, 10% fetal bovine serum, 50 mg / l gentamicin, and 280 mg / l L-glutamine. This suspension was mixed with the MNP ferrofluid immediately after ultrasonication at a ratio of 10 : 1 to preserve the pH value of 7.2–7.3 in the cell culture. The final MNP concentration in the cell culture was ten maximum tolerated doses (10 MTDs = 3 mg / l) of MNPs in relation to iron ions. One MTD of iron in water is equal to 0.3 mg / l. Cell viability was calculated on the Countess Automated Cell Counter (Invitrogen, USA) using 0.4% trypan blue solution (Invitrogen, USA) according to ISO 10993-5 after 24 hours of culturing at 5% CO_2, 100% humidity, and 37 °C. The main antigen profile of MNLs (CD45, CD3, CD14) was analyzed by flow cytometry [10].

The results were analyzed using Statistica 13.3 for Windows. The data were presented as the mean and the standard deviation $M \pm SD$. Due to non-normal distributrion of variables (the Shapiro – Wilk test), the Mann – Whitney U-test (p_U) was used to evaluate statistical differences in the samples.

RESULTS AND DISCUSSION

The values for average particle size distribution (D) are presented in Table 1. According to TEM, MNPs have a spheroid or polyhedral shape with rounded edges (Fig.

1). TEM images for the as-prepared samples performed in 2007 showed that the particles had smoothed edges, which may be attributed to poorer crystallinity of the surface or to the fact that the equipment used 10 years ago had lower resolution. TEM images were processed using ImageJ software. To analyze MNP size distribution, the obtained distribution was fitted with the lognormal distribution [11].



a *b* *c*

Fig. 1. TEM images of iron oxide powder: *a* – as-prepared; *b* – after 10 years of storage in ambient conditions, *c* – histogram of size distribution: scale bar is 100 nm.

The calculated MNP size remained almost the same after 10 years of storage. MNPs demonstrated lognormal size distribution with similar average diameters for the as-prepared and long-stored samples (Table 1).

The SAED pattern showed that crystallinity persisted for 10 years (Fig. 2, *a*). Bright rings are formed by spots related to the (111), (220), (311), (400), (422), (511), and (440) axis of the face-centered cubic structure of Fe_3O_4. One weak intensity ring marked as 110_a was associated with a small content of metallic a-Fe.

The quantitative phase analysis was performed with Rietveld refinement (Table 1). The results of the XRD measurements of iron oxide powder demonstrated that the predominant phase in the particles was magnetite (X-ray scan 04-015-3102), with a value of approximately 96−97 wt.%. In addition, a small amount of metallic a-Fe (X-ray scan 04-014-0360) was observed with a value of approximately 2−3 wt.% (Fig. 2, *b*, Table 1).

Table 1

Phase composition, particle size, and magnetic properties of the MNP powder, *M (SD)*

Year	Phase composition, wt.%			Structural properties		Magnetic properties (295 K)	
	Fe_3O_4/ γ-Fe_2O_3	α-Fe	Unidentified phases	D_{XRD}, nm	D_{TEM}, nm	M_R/M_S	M_S, Am²/kg
2007	96	3	~1	31(3)	34 (0.6)	0.14 (0.01)	71.1 (0.9)
2017	97	2	~1	32(4)	33 (0.7)	0.16 (0.01)	79.8 (0.4)*

* $P_U < 0.05$; the measurements were carried out in Tomsk in 2007, as well as in Tomsk and in Kaliningrad in 2017.

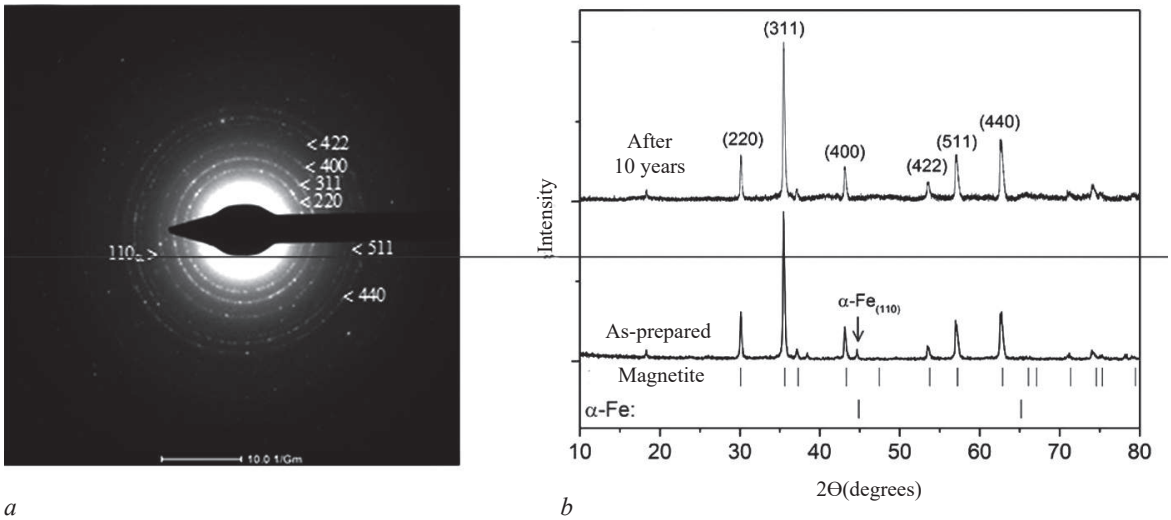
It should be noted that two ferrimagnetic iron oxides magnetite and maghemite both have an inverse spinel structure with similar lattice constants, and due to peak broadening at the nanoscale, they cannot be distinguished with conventional X-ray diffraction techniques [12]. The average size of the coherent scattering domain (D) was calculated with the Scherrer equation (1).

$$D_{XRD} = k\lambda / (\beta \times \cos\theta),$$

where the shape factor *k* is 0.9 for spherical shape, λ is the wavelength of Cu Kα radiation (1.54178 Å) and β is the full width at half-height [13]. For nanoparticles with good crystallinity, this size was close to the particle size determined by TEM (Table 1).

The saturation magnetization (M_S) for the aged MNPs calculated according to Fig. 3 was slightly greater than that for the as-prepared MNPs (Table 1). Reduced remanent magnetization (M_R / M_S) was equal for both samples within the margin of error.

Two magnetic iron oxides, magnetite and maghemite, have different values of saturation magnetization ($M_S^{Fe_3O_4} = 92-100$ and $M_S^{\gamma\text{-}Fe_2O_3} = 60-80$ Am / kg [14]).



a *b*

Fig. 2. SAED pattern of MNP powder stored for 10 years (*a*); XRD pattern of the as-prepared sample and the sample after 10 years of storage (*b*) in ambient conditions

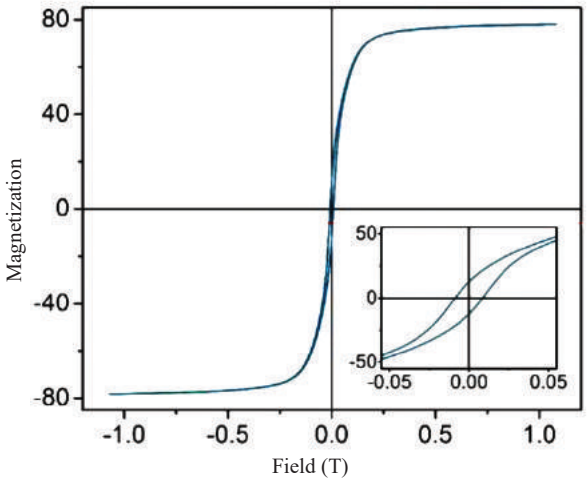
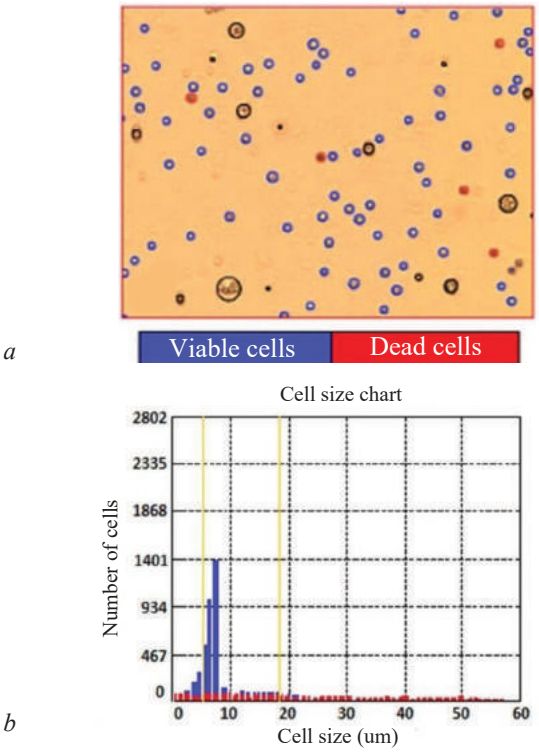


Fig. 3. Hysteresis loop of MNPs powder at T = 295 K after 10 years of storage in ambient conditions

Obtained M_S values were closer to the maghemite phase, however, some decrease in magnetization can be also related to the size effects (e.g. the presence of a fraction of canted surface spins or antiphase boundaries) [15, 16].

The cytotoxic effect of MNPs was not detected *in vitro* using a cell counter (Fig. 4) for any year of a long-term study (Table 2). The mean number of living MNLs within the min – max spectrum of diameters (7–18 μm) did not differ significantly after adding the same MNPs at 10 MTDs in 2007 and 2017.

A total of 93–95% of MNLs with sizes of 7–11 μm (Fig.4) expressed CD45CD3 T cell antigens. Large cells presented CD45CD14 markers of monocytes / macrophages in 5–7% of the cases, which did not depend on MNP use and year.



a *b*

Fig. 4. Calculation of cell viability (a) and size (b) using the Countess Automated Cell Counter

Table 2

In vitro* viability of MNLs after 24-hour co-culture with MNPs at 10 MTDs, *M* ± *SD

Nanopowder	Number of viable (not stained with tryptan blue) cells, %, n = 5	
	Control cell cultures (without MNPs)	Cell cultures with MNPs
As-prepared	86.2 ± 1.62	85.8 ± 3.02
After 10 years of storage	87.5 ± 2.06	85.6 ± 2.71

CONCLUSION

The 10-year stability of the structural, magnetic, and cytotoxic properties of electroexplosive magnetite nanopowder, which was stored in the dark place at ambient temperature, can be considered a useful property to ensure a balance between its environmental safety and biomedical properties as an inorganic carrier for the diagnosis and treatment of cancer and a wide variety of other diseases.

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Authors contribution

Khlusov I.A., Rodionova V.V., Litvinova L.S. – conception and design, interpretation of the results. Omelyanchik A.S. – carrying out of the physical part of experiments, drafting of the manuscript. Shupletsova V.V., Khaziakhmatova O.G., Yurova K.A. – carrying out of the biological experiments. Norkin I.K. – statistical analysis of the data.

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