Green synthesis of zinc based nanoparticles zinc ferrite by *Petroselinum crispum*

A M Korotkova\(^1,2\), O B Polivanova\(^3\), I A Gavrish\(^1\), E N Baranova\(^4\), S V Lebedev\(^1,2\)

\(^1\)Federal Research Centre of Biological Systems and Agro-Technologies of the Russian Academy of Sciences, 29, 9 Yanvarya Str., Orenburg, 460000, Russia  
\(^2\)Orenburg State University, Institute of Bioengineering and Biotechnology, 8, Yanvarya Str., Orenburg, 460200, Russia, \+7(3532)776770  
\(^3\)Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Department of Genetics, Biotechnology, Breeding and Seed, 49, Timiryazeva Str., Moscow, 127550, Russia, \+7(499)9764072  
\(^4\)All-Russian Research Institute of Agricultural Biotechnology, Laboratory of Cell Biology, 42, Timiryazevskaya Str., Moscow, 127550, Russia, \+7(499)9771636

**Abstract.** Zinc ferrite nanoparticles (ZnFe\(_2\)O\(_4\)) are being increasingly investigated due to their unusual properties different from those of bulk materials. In this study, Zn ferrite nanoparticles were synthesized by green method using *Petroselinum crispum*. The synthesized magnetic nanoparticles were characterized using TEM technique. The biological activity of the preparations produced was studied on a test plant of wheat *Triticum vulgare* L. Wheat plants were grown in hydroponic medium with the addition of ZnFe\(_2\)O\(_4\) nanoparticles in concentrations ranging from 10\(^{-5}\) to 10\(^{-4}\) M. On the third day, the germination rate of wheat seeds was calculated. The results showed that powders of acidic nature (pH=2) significantly reduced seed germination relative to the intact samples at concentrations of 10\(^{-3}\) and 10\(^{-4}\) M (by 100 and 47% relative to the control, respectively). ZnFe\(_2\)O\(_4\) synthesized in alkaline medium reduced the germination rate to a lesser extent, and at similar concentrations – to 7.3 and 22.8%. Moreover, at the minimal concentration of 10\(^{-3}\) M, there was a slight increase in germination up to 19.3%. Cell viability of seedling roots was evaluated by transformation of the water-soluble tetrazolium salt to formazan (WST-test). Higher cell viability was observed after exposure to zinc ferrite produced in alkaline medium as compared to preparations produced in acidic medium. Particles synthesized in alkaline medium at the concentration ranging from 10\(^{-1}\) to 10\(^{-3}\) M decreased cell viability to 77% relative to the control samples, and the preparation produced in alkaline medium under similar conditions reduced the rate only at maximum concentrations of 10\(^{-1}\) and 10\(^{-2}\) M to 53%. In the case of treatment of 10\(^{-5}\) M ZnFe\(_2\)O\(_4\) of alkaline nature, cell viability was observed to grow up to 23% relative to the control samples.

1. **Introduction**

Among numerous nanomaterials, MFe\(_2\)O\(_4\) nanopreparations (M – Mg, Zn, Ni, Co, Cd, etc.) that belong to spinel-type magnetic products are relevant in biomedical practice [1]. Spinel ferrites are used in drug delivery as biosensors for magnetic resonance imaging and magnetic hydrothermia [2–4]. ZnFe\(_2\)O\(_4\) is one of the most commonly studied materials due to lower toxicity of Zn\(^{2+}\). ZnFe\(_2\)O\(_4\) exhibits a structure with octahedral B-sites occupied by Fe\(^{3+}\) and tetrahedral A-sites mainly occupied by Zn\(^{2+}\). Nanoparticles are currently of particular focus due to their unusual properties different from those of bulk materials [5].
It is well known that properties of the material mainly depend on the synthesis method. Physical and chemical approaches such as sol-gel, instantaneous combustion, citrate gel, coprecipitation, hydrothermal synthesis, sol-gel method of automatic combustion, etc. are employed for synthesis of spinel ferrite. Among synthesis techniques, the green synthesis used to produce powders from plant extracts provides high environmental friendliness and economic efficiency.

Magnetic nanoparticles are most preferential; being biocompatible, they are relatively less toxic and display magnetic properties [6]. Nevertheless, potential applications of nanoparticles can be considered only when their toxic effect is understood. Various ferrite nanoparticles have been synthesized and characterized, however their cytotoxicity is not well understood. To date, limited information on the toxicity of magnetic nanoparticles is available [7, 8]. Recent studies are focused on the improvement of magnetic properties of nanomaterials by changing their chemical structure, size and shape. In this regard, we have successfully synthesized new ZnFe₂O₄ nanoparticles at various pH levels in aqueous plant extracts and evaluated their structural and cytotoxic properties.

2. Materials and Methods

Synthesis of zinc ferrate nanoparticles. Fresh vegetable raw of parsley Petroselinum crispum was washed under flowing water, then twice washed with distilled water, dried on filter paper to remove moisture from the surface of leaves. After that, it was crushed to powder using liquid nitrogen. 2.5 parts of distilled water (by weight) was added to 1 part of the extract. Then it was heated for 30 minutes at 90 °C. After heating, it was filtered through 2 layers of gauze to remove plant residues. For purification, the extract was subjected to centrifugation at 15,000 rev. within 15 min. The resulting supernatant was filtered through millipore with a 0.45 μm pore size. The purified extract was diluted with distilled water (1:3) for further use in synthesis [9]. Synthesis in alkaline medium was distinguished by the fact that 10% NH₃·H₂O solution was added dropwise to ferrous sulfate solution with constant stirring until pH≥9 was determined titrimetrically.

Characterization of nanoparticles. The nanoparticle preparations were deposited onto a double-sided adhesive carbon tape (2SPI, USA) and examined using a Zeiss Merlin microscope equipped with Gemini II Electron Optics (Zeiss, Oberkochen, Germany). The measurements were carried out at accelerating voltage of 1–5 kV and probe current of 25–80 pA without any conductive coating on the sample surface.

Evaluation of biological activity of nanoparticles. To assess the biological activity of the ZnFe₂O₄ powders synthesized at pH=2 and pH≥9, the seeds of soft wheat Triticum vulgare were germinated in a climatic chamber (Agilent, USA) under 12-hour illumination at 22±1°C and humidity of 80±5% within 48 hours. After that, 5 ml of suspensions of nanoparticles at concentrations varying from 10⁻¹ to 10⁻⁵ M were added to equally germinated seeds. On the 3rd day, the seed germination rate was calculated. Next, on days 7 and 14, cell viability was measured using the Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich, USA). The roots of seedlings (40 mg) were ground in phosphate-buffered saline and centrifuged for 5 minutes at 10,000 rpm. Next, 100 μl of the supernatant was collected and mixed with 10 μl of the CCK-8 dye in a 96-well plate. The produced samples were incubated for 1 h at 37 °C, and the absorbance at λ=450 nm was measured using a microplate reader (Tecan, Austria) in the shaking and heating mode at 37 °C. The number of viable cells (in %) was calculated by the formula presented in our previous studies [10–14]. All experiments were performed in 3 replications. The obtained data were processed using Microsoft Excel and Statistica V8.

3. Results and discussion

SEM was employed to show that the synthesis environment also affects the morphology and size of the ZnFe₂O₄ bimetallic powders (Fig. 1). The preparation synthesized in weakly acidic medium (pH=2) consisted of granular particles with a size of ~250–400 nm, which contained smaller particles with a diameter of ~50 nm (A). The powders of alkaline nature were lamellar in structure and larger in size (B).
Figure 1. SEM of ZnFe$_2$O$_4$ nanopowders synthesized in an aqueous extract of P. crispum leaves: A – synthesis at pH=2, B – synthesis at pH$\geq$9, bar – 100 nm

Analysis of the biological activity of the synthesized particles using the test seedling of T. vulgare showed a difference in seed germination rate depending on both the concentration of preparations and the synthesis medium. Acidic powders (pH=2) of the entire concentration range significantly reduced seed germination relative to that of the intact samples. Significant inhibition of the index was recorded at the minimum concentrations of $10^{-3}$ and $10^{-4}$ M by 100 and 47% relative to the control, respectively (P$\leq$0.05) (Fig. 2). At the same time, ZnFe$_2$O$_4$ synthesized in alkaline medium reduced the rate to a lesser extent: at concentrations of $10^{-3}$ and $10^{-4}$ M to 7.3 and 22.8%. Moreover, at the minimal concentration of $10^{-5}$ M germination was slightly stimulated up to 19.3%.

Figure 2. Effect of biosynthesized zinc ferrite nanopowders under various conditions of the medium on seed germination rate of T. vulgare

Analysis of the enzymatic activity of reductases on accumulation of T. vulgare formazan in root cells after 7 and 14 days of exposure to ZnFe$_2$O$_4$ revealed a higher resistance of test plants to nanopowders synthesized in acidic medium. The particles synthesized in acidic medium in concentrations ranging from $10^{-1}$ to $10^{-5}$ M on days 7 and 14 decreased the indicator to 71.5 and 77% of the control, respectively (Fig. 2).

The preparation produced in alkaline medium under similar conditions reduced cell viability on day 7 only at concentrations of $10^{-1}$ and $10^{-2}$ M (53 and 23%) and on day 14 at $10^{-1}$ M (37%). In the case of alkaline treatment with ZnFe$_2$O$_4$, a smooth growth of viable cells in the roots of the test seedlings was
observed at concentrations of $\leq 10^{-3}$ M. A significant increase in cell viability was observed on experimental day 7 at maximum concentration of $10^{-5}$ M by 23% relative the control (P≤0,05).

Thus, the difference in the biological activity of zinc ferrate nanopowders can be associated with the conditions of particle synthesis. Acidic medium led to lower tolerance of the plants probably due to enhanced processes of extraction of metal ions from the nanoparticle nucleus [15]. The mechanism of these nanoparticles can be explained by generation of reactive oxygen species (ROS) due to increased intracellular $\text{Zn}^{2+}$, which leads to subsequent redox imbalance [16]. In addition, biological effects could also be associated with the size and character of the particle surface due to the synthesis method. SEM images showed that particles synthesized at acidic pH were spherical and oval; however, they were smaller in size compared to particles produced in alkaline medium, which exhibited sharper edges of the plates [17], but had lower inhibitory effect on plants.

Previous studies also showed the dose-dependent cytotoxicity of $\text{ZnFe}_2\text{O}_4$ [18–21]. Recently, it was reported that $\text{ZnFe}_2\text{O}_4$ nanoparticles coated with protein exhibit lower cytotoxic effects with improved therapeutic effects [22, 23]. Moreover, Meidanchi et al. [24] reported on the possible use of nano-$\text{ZnFe}_2\text{O}_4$ in cancer therapy. Therefore, nanoparticles produced in our study are promising and require further testing on other living organisms.

4. Acknowledgments

The research was carried out with financial support by RFBR 18-316-00116.

Reference

[1] Chen Q and Zhang Z J 1998 Size-dependent superparamagnetic properties of MgFe2O4 spinel ferrite nanocrystallites Applied Physics Letters 73 3156–58
[2] Pospiskova K et al 2013 Magnetic particles-based biosensor for biogenic amines using an optical oxygen sensor as a transducer Microchimica Acta 180 311–18
[3] Mohapatra J et al 2013 Surface controlled synthesis of MFe2O4 (M = Mn, Fe, Co, Ni and Zn) nanoparticles and their magnetic characteristics Cryst. Eng. Comm. 15 524–32
[4] Hoque S M et al 2013 Superparamagnetic behaviour and T1, T2 relaxivity of ZnFe2O4 nanoparticles for magnetic resonance imaging Philosophical Magazin 93 1771–83
[5] Shanmugavela T et al 2014 Synthesis and structural analysis of nanocrystalline MnFe2O4 Physics Procedia 54 159–63
[6] Sen S et al 2014 Effect of functionalized magnetic MnFe2O4 nanoparticles on fibrillation of human serum albumin J. of Physical Chem. 118 11667–76
[7] Kanagesan S et al 2014 Characteristics and cytotoxicity of magnetic nanoparticles on breast cancer cells J. of Optoelectronics and Advanced Materials 6 41–50
[8] Kanagesan S et al 2013 Cytotoxic effect of nanocrystalline MgFe2O4 particles for cancer cure. J. of Nanomaterials 8
[9] Makarov V V, Makarova S S et al 2014 Biosynthesis of stable iron oxide nanoparticles in aqueous extracts of Hordeum vulgare and Rumex acetosa plants Langmuir ACS J. of Surfaces and Colloids 30 5982–88
[10] Lebedev S V, Korotkova A M and Osipova E A 2014 Evaluation of the influence of Fe6 iron nanoparticles, Fe3O4 magnetite nanoparticles, and FeSO4 iron (II) sulfate on Triticum vulgare photosynthetic pigments content Rus. J. of Plant Physiol. 61 603–8
[11] Korotkova A and Lebedev S 2015 Influence of iron of nanoparticles on induction of oxidative damage in Triticum vulgare Ecology, Environment and Conservation 21 101–11
[12] Korotkova A and Lebedev S et al 2017 The influence metal nanoparticles (Fe, Cu, Ni) and their oxides (Fe3O4, CuO, NiO) Agricultural Biology 2(1) 172–82
[13] Korotkova A M, Lebedev S V et al 2017 The study of mechanisms of biological activity of copper oxide nanoparticle CuO in the test for seedling roots of Triticum vulgure Environmental Sci. and Pollution Res. 24(11) 10220–33
[14] Korotkova A M, Sizova E A, Lebedev S V and Zyazin N N 2015 Influence of NPs Ni° on the induction of oxidative damage in Triticum vulgure Oriental J. of Chem. 31 137–45
[15] Reidy B et al 2013 Mechanisms of Silver Nanoparticle Release, Transformation and Toxicity: A Critical Review of Current Knowledge and Recommendations for Future Studies and Applications Materials (Basel) 56(6) 2295–350
[16] Saptarshi S R et al 2015 Investigating the immunomodulatory nature of zinc oxide nanoparticles at sub-cytotoxic levels in vitro and after intranasal instillation in vivo J. of Nanobiotechnology 13
[17] Chanteau B, Fresnais J and Berret J F 2009 Electrosteric enhanced stability of functional sub-10 nm cerium and iron oxide particles in cell culture medium Langmuir 25(16) 9064–70
[18] Kanagesan S, Mansor H et al 2016 Evaluation of Antioxidant and Cytotoxicity Activities of Copper Ferrite (CuFe2O4) and Zinc Ferrite (ZnFe2O4) Nanoparticles Synthesized by Sol-Gel Self-Combustion Mathematical Methods in the Applied Sci. 6 184
[19] Alhadaq H A, Akhtar M J and Ahamed M 2015 Zinc ferrite nanoparticle-induced cytotoxicity and oxidative stress in different human cells Cell & Bioscience 9
[20] Tomitaka A, Hirukawa A et al 2009 Biocompatibility of various ferrite nanoparticles evaluated by in vitro cytotoxicity assays using HeLa cells J. of Magnetism and Magnetic Materials 321 1482–4
[21] Duskaev G K, Deryabin D G, Karimov I, Kosyan D B and Notova S V 2018 Assessment of (in vitro) Toxicity of Quorum Sensing Inhibitor Molecules of Quercus cortex J. Pharm. Sci. & Res. 10 (1) 91–5
[22] Hajipour M J, Akhavan O et al 2014 Hyperthermia-induced protein corona improves the therapeutic effects of zinc ferrite spinel-graphene sheets against cancer RSC Advances 4 62557–65
[23] Sheik A and Jain P 2016 A Thorough Study of Zinc Ferrite Nanoparticles with Reference to Green Synthesis Int.J. of Nanomedicine and Nanosurgery 2 3
[24] Meidanchi A, Akhavan O et al 2015 ZnFe2O4 nanoparticles as radiosensitizers in radiotherapy of human prostate cancer cells Materials Sci. and Engineering 46 394–9