MAGNETIC NANOPARTICLES INFLUENCE ON SOME BACTERIAL CULTURES

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Abstract. Two suspensions of magnetic nanoparticles were prepared based on chemical coprecipitation of magnetite (MN1) and cobalt ferrite (MN2) from iron and respectively iron and cobalt salts, the magnetic powders being coated in sodium oleate for stabilization in deionized water. Microstructural and magnetic characterization was accomplished in both cases by applying standard methods. Two bacterial strains: *Staphylococcus aureus* – a Gram positive germ, and *Escherichia coli* – a Gram negative microorganism were cultivated in nutrient broth by adding 0.1 μL/mL magnetic nanoparticle suspension. Cell density was assessed by means of optical density measurements and calibration curve evidencing the inhibitory effect of magnetic nanoparticles on the bacterial cell growth. The diminution of *S. aureus* cell density was the most remarkable – more than 50% decrease compared to the control level for MN1 and MN2 test variants. *E. coli* response was consistent with smaller negative variations of the cell density following the treatment with magnetic nanoparticles – the cell density decreasing up to 93% for MN1 and MN2 samples.

Key words: metal oxides, human pathogens, cell density.

INTRODUCTION

The antimicrobial action of various types of nanoparticles was reported in the literature for both magnetic and non-magnetic nanosized material. Nanoparticles of non-magnetic metals such as Ag, Au, CuO and ZnO were found to have antimicrobial action against the beneficial soil microbe, *Pseudomonas putida* [6, 11]. Silver nanoparticles are the most known for their negative effects on microorganism proliferation as shown in [5, 14, 17]. Some experiments revealed the zinc oxide nanoparticles antimicrobial action in some microorganisms as reported in [2, 3, 9, 10, 12]. Magnetic nanoparticles were also found to be potential antimicrobial agents according to [1, 2, 3, 4, 7, 8, 13].

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In the frame of our experiments we have investigated the behavior of two well known bacteria in the presence of two types of magnetic nanoparticles.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

Two bacterial strains: *Staphylococcus aure*us ATCC 10832 – a Gram positive pathogen germ and *Escherichia coli* ATCC 12435 – a Gram negative microorganism were cultivated in 3 mL volumes of Oxoid nutrient broth at 37.0±0.5°C (in INCUCELL thermostatic room). The inoculation was accomplished by seeding with equal aliquots from the stock inoculum adjusted at the density of 10^8 CFU/mL (colony forming units/mL).

MAGNETIC NANOPARTICLES

Co-precipitation of Fe_3O_4 and $CoFe_2O_4$ nanoparticles (NP) from the mixtures of $FeCl_3$ solution with either $FeCl_2$ or $CoCl_2$ solutions in the presence of boiling 25% NaOH under constant stirring at 80°C. The magnetic NPs, collected and separated in magnetic field gradient, were washed several times with distilled water at room temperature; then the sodium oleate solution was added and magnetically stirred for about 2 hours at over 80°C temperature to ensure NP coating and dispersion in deionized water; this way the MN1 and MN2 magnetic nanoparticle suspensions were prepared – the biocompatibility of sodium oleate coated magnetite being demonstrated in [15]. For transmission electron microscopy (TEM) investigation a TESLA device with the resolution of 1.0 nm (sample deposition of collodion sheet after 10^{-4} dilution and drying) was used. X-ray diffraction (XRD) was applied using Shimadzu XRD 6000 device with CuKα radiation while magnetization measurements were accomplished on magnetic NP samples using a vibrating sample magnetometer (VSM) Quantum Design, model 6000 [16].

METHODS

Shimadzu spectrophotometer with quartz cells of 0.2 cm width was used to assess bacteria cell density, based on the measurements of light extinction at the wavelength of 560 nm, after 18 hours incubation with deionized water as reference. Student t-test was applied to assess the statistical significance of the differences between the average values corresponding to magnetic nanoparticle samples and the control ones, by taking into consideration the measurement results from four repetitions of every experimental variant.

RESULTS

The analysis of TEM images (Figs. 1, 2) has shown fine granularity in both magnetic NPs (Fe_3O_4 and $CoFe_2O_4$) with the mean physical diameter of 12.3 nm and respectively 13.1 nm; some aggregated NPs were observed with up to 25 nm size. XRD recordings have shown the typical diffraction lines confirming that both NP samples are well crystallized with a spinel structure, without significant line broadening or detectable signals of any other crystalline or amorphous phase.

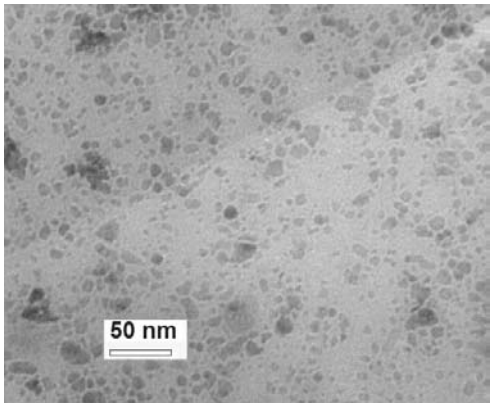

Fig. 1. TEM images of magnetic NPs of Fe_3O_4.

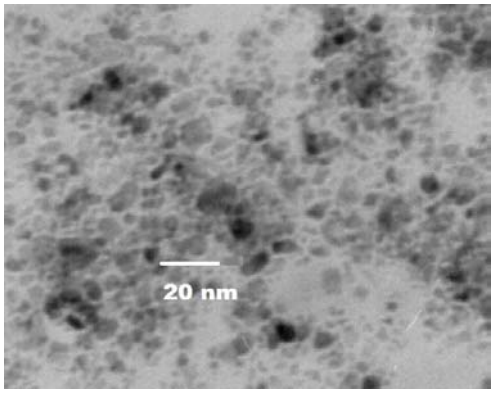

Fig. 2. TEM images of magnetic NPs of $CoFe_2O_4$.

Detailed presentation of microstructural and magnetic investigation based on XRD and VSM was done in [16] where the values of the NP magnetic core diameter were found to be 9.3 nm and 7.4 nm respectively while the saturation magnetization values were 59 and respectively 64 emu/g. In Figure 3, the cell density in the magnetic NP samples inoculated with *S. aureus* in terms of optical

density is given. In the case of both MN1 and MN2 test variants the diminution of cell density was remarkable – up to about 55% decrease compared to the control.

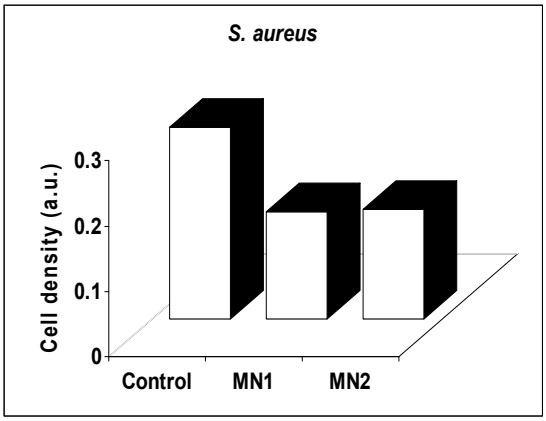

Fig. 3. Cell density in MN1 and MN2 bacterial samples of *S. aureus* (a.u. – arbitrary units).

This means that both the magnetite suspension (MN1) and cobalt ferrite one (MN2) could impede cell growth with remarkable efficacy. The statistical significance of the changes recorded between the control sample and the MN variants was ensured related to the significance threshold of 0.01. We mention that additional control samples were prepared for both studied microorganisms by supplying the bacterial culture medium only with the amount sodium oleate equal with that existing in the volume of diluted NP suspensions. No measurable differences in the optical density could be evidenced – this being the confirmation of the biocompatibility of magnetic core/sodium oleate shell colloidal nanoparticles [15]. It is possible that the NP presence could kill only part of the bacterial cells while other part was only impeded to multiply or their multiplication was considerably delayed. *E. coli* behavior was characterized by smaller negative variations (Fig. 4) of the cell density following the treatment with magnetic nanoparticles – the cell density being decreased up to 93% for MN1 and MN2 samples. The standard deviation corresponding to the four repetitions of every sample behavior investigation was of about 8% for both microorganisms so that the statistical significance of the changes induced by the magnetic NPs in the *E. coli* cultures was not necessary ensured (since the differences between the control samples and the NP ones are comparable with the standard deviation).

The main interpretation can be related to the higher resistance to chemical or physical agents that characterize the Gram negative microorganisms (like *E. coli*) compared to Gram positive ones (like *S. aureus*). In Figure 5 the comparison between the behaviors of the two microorganisms can be seen. The relative cell density (normalized to the control value) is presented. It is more evident than in the

previous figures that the NP variants corresponding to *E. coli* bacteria were less affected compared to *S. aureus* variants–the corresponding values on the *E. coli* curve being higher or at least equal to the values corresponding to the *S. aureus* curve.

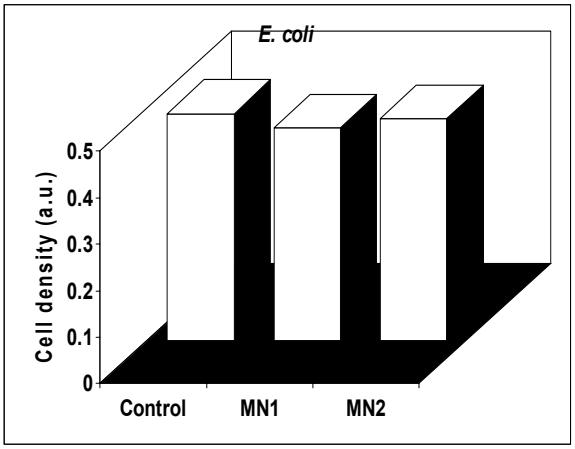

Fig. 4. Cell density in MN1 and MN2 bacterial samples of *E. coli* (a.u. – arbitrary units).

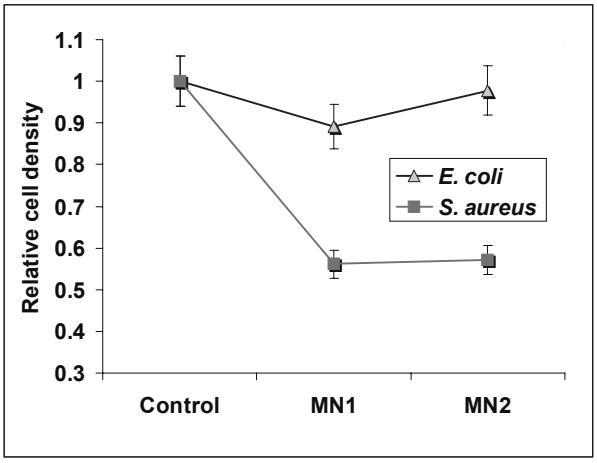

Fig. 5. Comparative behavior of *S. aureus* and *E. coli* to MN1 and MN2.

This study might be of interest for the understanding of influences of environmental uncontrolled pollution with manufactured nanoparticles released in the soil water and air by various nanotechnological industries as well as for the side effects of magnetic nanoparticles administered as contrast agents in magnetic resonance imagistic or as magnetic carriers of targeted biomolecules in the frame

of advanced medical procedures. The results provided by our experiments are concordant with those of other authors. In [8] the authors evidenced an inhibitory effect of magnetite/oleic acid colloidal nanoparticles on *S. aureus* while cobalt ferrite /oleic acid nanoparticles induced inhibitory effects on *S. aureus* and other bacteria. Also, in [4] it was showed that the magnetic nanoparticles of $CoFe_2O_4$/oleic acid and Fe_3O_4/oleic acid exhibit either stimulatory or inhibitory effects on the microbial virulence, depending on the tested nanoparticles composition and the tested microorganisms. The novelty of the above presented research is the evidence of antimicrobial effect of magnetic nanoparticles when supplied in suspension to the culture medium of the studied microorganisms – in contrast with the reports of other authors [4, 8] that focused on biofilms. Hence the practical interest is related to the possible interference of nanoparticles administered in the body fluids for medical diagnosis or therapy with possible microbial load in blood or wet tissues.

CONCLUSION

Different inhibitory effects of the two magnetic nanoparticles were evidenced in the pathogen bacteria, with higher and significant amplitude in the Gram positive germ *S. aureus* than in the Gram negative bacteria *E. coli*. In the next investigation steps the intimate nature of the interaction between the magnetic nanoparticles and the bacterial cells will be approached using complementary investigation methods.

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