Nano-Structures & Nano-Objects 15 (2018) 40–47

Contents lists available at ScienceDirect

Nano-Structures & Nano-Objects

journal homepage: www.elsevier.com/locate/nanoso

Shell-dependent antimicrobial efficiency of cobalt ferrite nanoparticles

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HIGHLIGHTS

- Cobalt ferrite nanoparticles against multidrug resistance pathogens.
- Synthesis and characterization of L-lysine and oleic acid stabilized cobalt ferrite nanoparticles.
- While $CoFe_2O_4$@Ole Nps are more efficient antimicrobial agents than $CoFe_2O_4$@Lys Nps.

ARTICLE INFO

Article history:
Received 10 July 2017
Received in revised form 9 March 2018
Accepted 10 March 2018

Keywords:
Magnetic nanoparticles
Antimicrobial efficiency
Cobalt ferrite
Co-precipitation
Thermal decomposition

ABSTRACT

Uniformly shaped and sized superparamagnetic nanoparticles (NPs) have recently received increasing attention due to their prospective applications in theranostics, sensing and as antimicrobials. However, utilization of magnetic NPs in nanomedicine largely depends on their properties, which, in turn, are influenced by the size, structure and composition of the core and the nature of stabilization shell. This paper highlights the significant influence of stabilizing shell of superparamagnetic cobalt ferrite NPs on the antimicrobial efficacy against several kinds of pathogenic microorganisms. Two very popular preparation methods of biocompatible magnetic NPs, namely, co-precipitation from alkaline solutions containing Co(II) and Fe(III) salts and L-lysine (Lys) as well as thermal decomposition of organometallic Co(II) and Fe(III) precursors in the presence of oleic acid were applied. The properties of resulting NPs are characterized and discussed herein. Surprisingly, highly efficient bactericidal behaviour of cobalt ferrite NPs capped with oleine shell compared to that of $CoFe_2O_4$@Lys, was ascribed to the differences in their surface charge and more grained structure of the former.

1. Introduction

Current research in fabrication of nano-sized materials is motivated by the idea that unique, considerably changed physical, chemical or biological properties of materials can be obtained when their size is reduced to nanometre dimensions [1,2]. According to the recent publications, except for well-known antimicrobial behaviour of silver nanoparticles (Nps) [3–5], the Nps of some metal oxides such as TiO_2, ZnO, MgO [6,7], Cu_2O [8], and ferrite, $MeFe_2O_4$, where Me = Co, Zn, Cu, Ni [9,10], are capable to fight human pathogens more effectively than antibiotic agents do [11]. The toxicity of nanosized TiO_2, ZnO, and CuO Nps have been studied by Heinlaan et al. [12]. Wang's group reported that the shape of Cu_2O crystals affects their antimicrobial activity: octahedral Cu_2O crystals bounded by {111} facets exhibited higher activity in killing *Escherichia coli* than cubic ones bounded by {100} facets, presumably due to stronger electrostatic interaction of the former [13]. As reported, the antimicrobial properties of NPs can easily be altered by changing their size, shape, and crystallinity. Size-dependent interaction of silver [14–16], ZnO [17], as well as cobalt ferrite NPs [18] has been reported emphasizing effectiveness of ultra small-sized species due to 4 times higher efficacy of ultra-small NPs against *S. cerevisiae* and several kinds of Candida microorganisms in comparison with the 15-nm sized ones [18].

Recently, the emphasis has been placed onto understanding the role of the NP stabilizing shell because the specific properties can be rendered *via* smart functionalization of NP surface. For example, it has recently been reported that $CoFe_2O_4$ NPs capped with folic acid and hematoporphyrin fragments are effective anticancer reagent [19]. Moreover, the surface composition of silver NPs

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https://doi.org/10.1016/j.nanoso.2018.03.007
2352-507X/

strongly influences their interaction with bacteria membrane [20]. Nevertheless, according to review of Soenen et al. [21], surface functionalization of NPs does not alter the intrinsic NP toxicity, but may reduce the extent of cell-NP interaction. However, to the best of our knowledge, until now no studies have been devoted to investigation of the effect of the shell of magnetic NP on their antimicrobial properties.

Therefore, the objective of this study is to investigate and compare antimicrobial behaviour of cobalt ferrite NPs, grown by both the co-precipitation [22,23] and by thermal decomposition [24,25] methods, considering the role of their stabilizing shells. In order to form biocompatible and superparamagnetic NPs, co-precipitation synthesis was performed hydrothermally using L-lysine amino acid, as stabilizing and capping surfactant. To the best of our knowledge, the application of L-lysine has been reported earlier only for fabrication of monodisperse magnetite NPs [26]. An important finding of this study is that the antimicrobial properties are strongly dependent on the nature of the ligands capped on the Np surface, even though they have similar size. The surprisingly enhanced antimicrobial behaviour of $CoFe_2O_4$@Ole NPs with respect to $CoFe_2O_4$@Lys ones is likely to be due to the negative ζ-potential of these NPs.

2. Experimental section

2.1. Chemical reagents and equipment

In this study, all the reagents were of analytical grade at least and, except for NaOH, were used without further purification. $CoCl_2$, $FeCl_3$, $FeCl_2$, L-lyzine, Co(II) acetylacetonate, $Co(acac)_2$, ($\geq$97.0%), oleic acid, C_9H_{18}=C_8H_{15}–COOH, (72%), and Trimethylamine-N-oxide (98.0%) were purchased from Aldrich Chemicals Inc. Dibenzyl ether ($\geq$98.0%) and iron (III) acetylacetonate, $Fe(acac)_3$ ($\geq$97.0%) were obtained from Fluka Analytical. NaOH was purified by preparation of a saturated solution resulting in the crystallization of others sodium salts. After analysis, this solution was diluted to 2.0 mol L^{-1} concentration and used. Deionized/distilled water was used in all experiments.

2.2. Synthesis of NPs

Hydrophilic cobalt ferrite nanoparticles were synthesized hydrothermally at 130 °C with 10 °C min^{-1} ramp for 10 h in alkaline solution containing 25 mmol L^{-1} of $CoCl_2$, 50 mmol L^{-1} of $FeCl_3$ salts, 0.2 mol L^{-1} of L-lyzine amino acid, as chelating agent, and NaOH up to pH $\cong$ 12.35 $\pm$ 0.1. As-grown NPs were centrifuged at 7500 rpm for 5 min, carefully rinsed with pure H_2O/centrifuged several times, and finally dried at 60 °C. Similarly, magnetite (Fe_3O_4) NPs caped with L-lysine were synthesized in an alkaline solution containing $FeCl_2$ and $FeCl_3$ salts and 0.2 mol L^{-1} of L-lyzine. Hydrophobic cobalt ferrite NPs were fabricated *via* thermal decomposition of $Co(acac)_2$ and $Fe(acac)_3$ salts dissolved in the dibenzyl ether deoxygenated by bubbling with nitrogen gas. For stabilization of NPs size, oleic acid and Trimethylamine-N-oxide were used. Briefly, to form small NPs, 18 mmol L^{-1} $Co(acac)_2$, 36 mmol L^{-1} $Fe(acac)_3$, 15 mmol L^{-1} (0.051 g) Trimethylamine-N-oxide and 41.7 g L^{-1} oleic acid were dissolved in 45 mL of dibenzyl ether under the stirring and nitrogen gas purge. The temperature was increased to 230 °C and the synthesis was conducted under reflux, N_2 gas flow and stirring for 2 h followed by temperature increase to 280 °C and processing further under reflux and N_2 bubbling for 1 h. After cooling to room temperature, the crude products were centrifuged, rinsed with acetone/ethanol mixture (2:1) for several times, collected with permanent magnet and dried in air at 60 °C. For the synthesis of larger cobalt ferrite NPs, more than 15 nm in diameter, the same synthesis protocol was repeated using already synthesized NPs in a double diluted solution, compared to the first one, under the same conditions.

2.3. Nanoparticle characterization

The morphology of the as-grown products was investigated using a transmission electron microscope (TEM, model MORGAGNI 268) operated at 72 kV. The NPs subjected to TEM observations were dispersed in ethanol, drop-casted onto a carbon-coated copper grid, and dried naturally. The average size of NPs was estimated from at least 150 species observed in their TEM images. High resolution transmission electron microscopy (HRTEM) studies of the as-grown products were performed using a LIBRA 200 FE at an accelerating voltage of 200 kV. Hydrodynamic size of NPs in water was determined by dynamic light scattering (DLS) tests at 25 °C under ambient conditions using Zetasizer Nano S (Malvern Instruments, UK) equipment. Magnetization measurements were conducted using the vibrating sample magnetometer calibrated by Ni sample of similar dimensions as the studied sample of NPs placed in the plastic tube. The magnetometer was composed of the vibrator, lock-in-amplifier, and electromagnet. The magnetic field was measured by the teslameter FH 54 (Magnet-Physics Dr. Steingrover GmbH). *X*-ray powder diffraction experiments were performed on a D8 diffractometer (Bruker AXS, Germany), equipped with a Göbel mirror as a primary beam monochromator for CuK_α radiation (λ = 1.5418 Å).

Infrared spectra were recorded in transmission mode on an ALPHA FTIR spectrometer (Bruker, Inc., Germany) equipped with a room temperature detector DLATGS. The spectral resolution was set at 2 cm^{-1}. Spectra were acquired from 100 scans. Samples were dispersed in KBr tablets. Parameters of the bands were determined by fitting the experimental spectra with Gaussian–Lorentzian shape components using GRAMS/A1 8.0 (Thermo Scientific) software.

2.4. Antimicrobial tests

Antimicrobial assessment of the synthesized cobalt ferrite NPs against prokaryotic (*E. coli, Staphylococcus aureus*) and eukaryotic (*Candida parapsilosis, Candida albicans*) microorganisms was performed using the serial dilution method. Antimicrobial tests against eukaryotic microorganisms *C. albicans* and *C. parapsilosis* were done in sterile Sabouraud CAF media composed of 10 g L^{-1}peptomycol, 40 g L^{-1} glucose, and 0.5 g L^{-1} chloramphenicol; pH = 5.6 $\pm$ 0.2 at 25 °C. Antimicrobial assessment of the synthesized cobalt ferrite NPs against prokaryotic microorganisms *E. coli* and *S. aureus* was carried out in sterile Nutrient Broth (1 g L^{-1} glucose, 15 g L^{-1} peptone, 6 g L^{-1} sodium chloride and 3 g L^{-1} yeast extract); pH = 7.5 $\pm$ 0.2 at 25 °C. Following these investigations bacteria and yeast strains were propagated in Nutrient and Sabouraud CAF agar medium at (30 $\pm$ 1) °C for 24 h and (27 $\pm$ 1) °C for 48 h, respectively. The fresh cultures were harvested and diluted in sterile nutritional media to yield colony-forming units (CFU) inoculum of 1–5 $\times$ 10^6 for yeast and (6.4–8.0) $\times$ 10^8 for bacterium cells, based on optical density at 530 nm (OD_{530}) and 600 nm (OD_{600}). The range of OD_{530} and OD_{600} was obtained between 0.12–0.15 and 0.08–0.1, respectively. Then 19 mL of the diluted microorganism suspensions, which were collected at the logarithmic stage of growth, were transferred in a 50-mL glass flask. Finally, 1 mL of distilled water, containing 20 mg black NPs was added to the liquid medium and further incubated at room temperature for 72 h with 150 rpm shaking. L-lysine and magnetite (Fe_3O_4) NPs were used as a negative control. Tween 80 was used as a solvent for oleic acid. During the cultivation, 1 mL of the suspension was taken from each reaction mixture, diluted in the glass tubes *via* broth dilution method and spread on the Nutrient Broth and Sabouraud agar media plates using the stainless steel spreader. The growth of microorganisms was tested after incubation at 30 $\pm$ 1 °C and 27 $\pm$ 1 °C for 2–3 days.