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# Magnetic nanoparticle with high efficiency for bacteria and yeast extraction from contaminated liquid media

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ABSTRACT

Magnetic nanoparticles with a disordered cation vacancies show a rapid and high efficiency extraction for microorganism, such as bacteria Gram-positive (*Staphylococcus aureus* and *Bacillus Cereus*) or Gram-negative (*Escherichia Coli*) and yeast (*Candida albicans*). The magnetic nanoparticles were obtained from variable molar ratio of ferrite and ferric ions. X-ray diffraction, Raman spectroscopy, Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy has been used to identify the structure and the composition of the iron oxides. The as prepared nanoparticles show better efficiency of bacteria and yeast extraction than commercial magnetite or maghemite nanoparticles. A removal as high as 95% of the nanoparticles for bacteria Gram-positive and yeast was achieved without any need of the surface functionalization. The pH of the media has no effect of the bacteria-magnetic nanoparticle binding. This suggested that the link created is not electrostatic.



## 1. Introduction

Nosocomial infections are infections acquired in hospital settings; these infections are caused by different pathogenic bacteria. The most common types of these infections are urinary tract, surgical wound, lower respiratory tract, and digestive system infections as well as systemic infections (sepsis). Nosocomial infections increase the patients' mortality rate, lead to extended hospital stays and participate in raising healthcare cost [1]. Although enhancing hand hygiene of healthcare personnel could help in the prevention of nosocomial infections, necessary invasive surgical procedures significantly increase the risk of these infections, in particular in immuno-depressed patients. Therefore, a rapid and efficient procedure for the diagnosis of the bacteria type is required. Identifying the causal bacteria type is essential for prescribing the right drug treatment for the patient in order to reduce the mortality rate which results from nosocomial infections.

The identification of bacteria in a contaminated medium is carried out in two steps. The first one is the extraction of the bacteria from the contaminated fluid and the second step is identifying the bacteria's genetic profile. The genetic profiling of bacteria is performed using well-established techniques such as Polymerase Chain Reaction (PCR) or mass spectrometry (Maldi). However, the bacteria extraction from the medium (*e.g.* blood) is a limiting step, since several hours of incubation are often required prior to extraction. Use of magnetic materials was proposed as a good approach for the microorganism extraction, compared to traditional techniques such as centrifugation which generate shear forces which may disrupt cells or degrade nucleic acids. However, using magnetic nanoparticles presents some challenges, for instance (i) the reaction between the nanoparticles and the microorganism typically requires specific functional groups grafted at the surface *e.g.* antibodies, polyelectrolytes and carboxyl *etc.* [2–5]. (ii) Another issue is that functional biological compounds such as antibodies or proteins require suitable storage conditions to avoid denaturation of the three-dimensional structures, which would significantly reduce their bioactivity [6]. (iii) Moreover, antibodies are costly and their specific storage conditions increase even further the cost of their transport [7]. As an alternative, synthetic functional groups grafted at the surface of magnetic nanoparticles have been proposed; these groups have the advantage of binding affinity to both Gram-negative and Gram-positive bacteria [8]. However, the elaboration of the corresponding materials uses sophisticated methods. Indeed, more often the magnetic nanoparticles (mostly magnetite) used are covered by a silica protective coating where functional

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groups are easily grafted for diagnostic and therapeutic purposes [9,10]. These synthesis procedures are complex and often raise the price of the material and consequently the cost of the therapeutic process.

In this study, we propose a low cost and easy to make magnetic nanoparticles as a viable alternative for universal microorganism extraction. These magnetic nanoparticles exhibit (1) faster kinetics of association with the microorganisms (Gram-negative *Staphylococcus aureus*, Gram negative *Escherichia coli*, Gram-positive *Bacillus Cereus*, *Candida albicans*) compared to commercial magnetite and maghemite and (2) reduce incubation times needed prior to separation to not more than a few minutes. These gains could significantly make the clinical application easier. Although the use of magnetite particles has been described for the isolation of bacteria, the interaction between the particle and the microorganism was very weak [11]. The bacteria extraction method thus often requires a minimum of 3 h incubation to bind bacteria to the magnetic particle. This delay is a significant problem for the detection of microorganisms in a clinical setting, where timely analysis is required to benefit patients with infections.

## 2. Experimental section

### 2.1. Preparation of iron oxide using a variable ratio of $F^{2+}$ and $Fe^{3+}$

A solution containing 0.36 mol iron II sulfate heptahydrate ($FeSO_4$,$7H_2O$) and 0.18 mol iron III sulfate hydrate ($Fe_2(SO_4)_3$,$xH_2O$) in 300 ml of deionized water was bubbled 30 min with air. The mixture was heated to 85 °C, continuously stirred, bubbled with air, and 1000 ml of 6 N sodium hydroxide were added dropwise over a period of 60 min. The solution was heated and bubbled 2 additional hours then cooled to 20 °C. After an overnight incubation at room temperature, the precipitate was centrifuged, the supernatant eliminated and replaced by water. The process was repeated until the pH of the supernatant reached 7. After a last centrifugation, the resulting iron oxide particles were placed in solution in one volume Phosphate Buffered Saline solution (PBS) (Lonza Ltd.) and were ready to use as a solid support.

The same procedure was used (by adjusting the molar ratio of $FeSO_4$,$7H_2O$ and $Fe_2(SO_4)_3$,$xH_2O$) to synthesize the iron oxide with increasing molar ratio $2Fe^{2+}$:$1Fe^{3+}$ and $3Fe^{2+}$:$1Fe^{3+}$.

### 2.2. Magnetite and maghemite

The magnetite particles termed SA-magnetite are commercially available magnetite (99.9% Sigma-Aldrich, ref. 518158). The maghemite particles were obtained by heating pure SA-magnetite during 3 h at 250 °C in air.

The SA-magnetite and the maghemite were suspended in solution in one volume PBS and then used as a solid support with no further purification.

### 2.3. Structural characterization

The morphology of the particles was observed by Hirox SH-1500 Scanning Electron Microscope (SEM) coupled with EDX spectroscopy and equipped with Tungsten filament as an electron gun Type.

A Kristalloflex Siemens D 5000 diffractometer using the CuK$\alpha$ radiation $\lambda = 1.5418$ Å, was used. The patterns were registered for $2\theta$ values comprised between 2° and 72°, with increments of 0.02° each second. The diffracted X-rays were registered by a scintillation detector.

X-ray photoelectron spectroscopy (XPS) analyses were performed with a Kratos Axis Ultra spectrometer (Kratos Analytical – Manchester – UK) equipped with a monochromatized aluminum X-ray source (powered at 10 mA and 15 kV). The sample powders were pressed into small stainless steel troughs mounted on a multi-specimen holder. The pressure in the analysis chamber was around $10^{-6}$ Pa. The angle between the normal to the sample surface and the lens axis was 0°. The hybrid lens magnification mode was used with the slot aperture resulting in an analyzed area of 700 µm × 300 µm. The pass energy was set at 40 eV. In these conditions, the energy resolution gives a full width at half maximum (FWHM) of the Ag 3 $d_{5/2}$ peak of about 1.0 eV. The following sequence of spectra was recorded: survey spectrum, C 1s, O 1s, Fe 2p, Na 1s, Si 2p, Cl 2p, S 2p and C 1s again to check the stability of charge compensation in function of time and the absence of degradation of the sample during the analyses. The binding energies were calculated with respect to the C–(C, H) component of the C 1s peak fixed at 284.8 eV. The spectra were decomposed with the CasaXPS program (Casa Software Ltd., UK) with a Gaussian/Lorentzian (70/30) product function after subtraction of a linear baseline. Molar fractions were calculated using peak areas normalized on the basis of acquisition parameters, sensitivity factors provided by the manufacturer, and the transmission function.

Raman spectroscopy was used to identify the synthesized iron oxide phase(s), and compare it (them) to that (those) present in the commercial ferri- and paramagnetic iron oxides (magnetite purchased from Sigma-Aldrich and maghemite obtained by the calcination of the commercial magnetite). The Raman spectra were obtained from a Thermo Fisher Scientific Raman Spectrometer (model DXR). Excitation wavelength was 633 nm. The laser power was always kept at 0.9 mW at the sample, to avoid sample degradation or phase transformation. After each spectrum had been recorded, a careful visual inspection was performed in order to detect any change that could have been caused by the laser. A 50× objective lens was used with a 50 µm opening and a non-confocal (slit) mode. The spectral resolution was 7 – 9 $cm^{-1}$. Acquisition time was 150 s and the number of scans was fixed to 5 per sample. The spectral range was between 100 $cm^{-1}$ and 1500 $cm^{-1}$. Data were processed using the Software "OMNIC", Thermo Fisher Scientific Company, USA.

Fourier transform infrared spectra (FTIR) were acquired for the iron oxide samples and compared to those of the commercial magnetite and maghemite. For this analysis, the pellets were prepared by grinding ~5 mg together with ~200 mg of dry potassium bromide, and pressing the resulting mix in a pellet mold at about 10 tons/$cm^2$ pressure. Spectra were recorded with a Bruker Equinox 55 FT-IR spectrometer as a dry KBr pellet in the 400–1000 $cm^{-1}$ range, with a resolution of 4 $cm^{-1}$. The number of scans was fixed to 200. Before each measurement, a background spectrum was obtained using a pure KBr pellet.

### 2.4. Evaluation of the microorganisms extraction efficiency

A schematic of the procedure is provided in Fig. 1. In an Eppendorf tube of 1.5 ml, 110 µl of the magnetic nanoparticles (*e.g.* $1Fe^{2+}$:$1Fe^{3+}$) were added to 1 ml of a bacterial solution, in a 1.5 ml Eppendorf tube. The tube was stirred gently for 30 s. Then, the tube was placed under *a magnetic field* (Ademtech Magnetic Device Adem-Mag MSV ref. 20104). With the magnetic particles sticking on the wall of the tube, the supernatant was aseptically replaced by 1 ml sterile PBS. The pH of the solution was fixed at 7 or varied in a 3–11 range using hydrochloric acid (HCl) or sodium hydroxide (NaOH). The supernatant was subsequently added to LB agar medium on a Petri dish which constituted the "dish 1". The tube was removed from *the magnetic field*, and the bacteria-magnetic nanoparticle complexes where then suspended once again in 200 µl sterile PBS and then diluted 1000 times. 200 µl of this dilution were inoculated on LB agar medium in a separate Petri dish; this constituted the "dish 2". After an overnight

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