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Chemically modified nanoparticles surface for sensing bacterial loading—experimental study based on fluorescence stimulation by iron ions

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ABSTRACT

The influence of iron ions supplied from magnetite nanoparticles with chemically modified surface on *Pseudomonas aeruginosa* germ was aimed—with experimental and theoretical approach of the intensity of the fluorescent signal emitted by the pyoverdine like siderophores. As the coated magnetic nanoparticles could function as probes, the possibility of designing a chemical device was considered based on the sensing of iron reduction from Fe^{3+} into the more soluble Fe^{2+}, for detecting various levels of contamination ($10 \div 10^8$ cell/ml) of biological specimens and environmental samples. The proposed mathematical model estimated the fluorescence intensity due to siderophore synthesized by *Pseudomonas*, considering that the parameter describing the ion–bacteria interaction depends differently on the cell density for different magnetite nanoparticle coatings: linear dependence was found in the case of sodium oleate coating while power function was revealed for tetramethyl ammonium coating of magnetite nanocores, in both cases magnetite suspension being supplied in the same concentration (0.1 μl/ml). The calculated values of fluorescence intensity fitted the experimental data corresponding to magnetite supplied bacteria with graph slopes close to the unit and correlation coefficients of 0.999 and 0.996, while for the control samples, where that parameter was zeroed, correlation coefficient was found of 0.999.

1. Introduction

The bacterial loading of liquid media, either environmental or biological ones, could be detected through various methods, based on microorganism features as well as on the interactions developed with chemical or physical factors. Due to the fact that conventional methods for pathogen detection have limitations and require complex and time-consuming investigation procedures, biosensors—including those based on iron ions, were imposed as powerful tools with high-sensitivity and real-time detection of pathogens.

Having the latest technology at hand, researchers developed innovative approaches of using iron ions from magnetic nanoparticles in the designing of biosensing devices, in order to obtain higher sensitivity and specificity [1–4]. It is already known that these unique types of nanostructured matter have many advantages such as increased ratio surface/volume, controllable size, enhanced magnetic properties and they are easy to immobilize to an electrode surface by applying a magnetic field. Thus, various types of nanostructures have been engineered in order to detect specific targets, including bacteria [5–9]. For example, Koets et al., 2009 [10] developed a magneto-resistant sensor using superparamagnetic particles as labels for the detection of *Escherichia coli* and *Salmonella*. Recently, Haik et al., 2008 [11] reported the design of specific immunoassay techniques for the immobilization and rapid detection of pathogens, Mejri et al.(2011)[12] successfully developed an electrochemical immunosensor for bacteria detection based on functionalized magnetic nanoparticles immobilized onto bare gold electrode, while Maalouf et al.(2008)[13] described an immunomagnetic biosensor for *E. coli*, based on functionalized paramagnetic nanobeads attracted to a gold-electrode surface via a magnetic field.

Many optical biosensors for detecting pathogenic bacteria are based on the measurement of the fluorescence emission of various molecules [14,15]. For instance, Mechery et al., 2006 [16] have used a fluorescence-detection system based on dye-doped silica nanoparticles, which provided significant signal amplification in *E. coli* O157:H7 antibody–antigen recognition, while Chang et al., 2001 [17] have reported a 350% enhancement of the detectability of *Pseudomonas aeruginosa* using a PAMAM dendrimer film in an optical biosensor. The interaction of *Pseudomonas* bacteria and iron ions from magnetic nanoparticles has been studied by several research teams focused on the magnetite nanoparticle antimicrobial features [18–20], while others developed some biotechnological applications. Liu et al.(2009) have proposed a method for targeting bacteria from clinical samples based on the interaction between the galactophilic lecithin from *P. aeruginosa* membrane receptors and the magnetic nanoparticles coated with phosphoproteic containing Galα(1-4)Gal units [21], while Heyd et al.(2009) reported on the investigation of

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magnetic immobilizates of *P. aeruginosa* on magnetite for the application in continuous biosurfactant production of rhamnolipids [22]. *Pseudomonas delafieldii* labeled with magnetic Fe_3O_4/ammonium oleate nanoparticles and immobilized by external magnetic field was found able of greater desulfurization activity than free cells or cells immobilized on celite (Shan et al., 2005) [23]. Since in previous studies [24–26] the magnetite nanoparticles ability of changing the intensity of the fluorescent signal released by *P. aeruginosa* cells was revealed, the influence of magnetite nanoparticles surface on the green-bluish emission of bacterial cells was chosen as the target of the present investigation.

2. Materials and methods

2.1. Biological material

P. aeruginosa standard strain ATCC 17503 was cultivated in glass tubes of 3 ml in Oxoid nutrient broth, the samples being seeded with equal aliquots from the stock inoculums; three series of samples were prepared with five replies for each inoculum (10, 10^2, 10^4, 10^6 and 10^8 cell/ml). Concentrations of 0.1 μl/ml from two different magnetic nanoparticle suspensions were added in two tube arrays to supply iron ions. Overnight incubation (of 18 hours) was accomplished in INCUCELL thermostatic room (37.0 ± 0.5 °C). After the turbidimetric assay, the tubes were thermally inactivated at 100 °C and centrifuged for 10 min at 3500 min^{-1}, the supernatant being used for fluorescence measurements.

2.2. Magnetic nanoparticles aqueous suspensions

The aqueous suspensions of magnetite submicron particles were obtained by suspending in deionized water the magnetite powder freshly prepared by Massart's adapted co-precipitation method [27]. In order to obtain stable suspensions, the nanopowders were coated with two types of organic shells—tetramethyl ammonium hydroxide (electrostatic stabilization) and sodium oleate (steric stabilization), to facilitate their uniform dispersion in water, resulting in Fe_3O_4/T and Fe_3O_4/S colloidal suspensions.

The microstructural investigation of the magnetic core/organic shell systems was carried out—the average crystallite diameter (of 11.4 nm for Fe_3O_4/T [28] and respectively 8.2 nm for Fe_3O_4/S [29]) being evaluated by X-ray diffractometry (XRD), the volume fraction of magnetite being of about 2% in both magnetite suspensions.

The saturation magnetization was measured by vibrating sample magnetometry (VSM) and resulted in 49.6 emu/g for Fe_3O_4/T [28] and, respectively, 50.3 emu/g for Fe_3O_4/S [29].

Scanning electron microscopy (SEM) was carried out using VEGA\TESCAN device (SE detector, HV: 30.00 kV) which resulted in average physical diameter of core/shell systems of about 20.0 nm for Fe_3O_4/T and respectively 42.0 nm for Fe_3O_4/S (Images 1 and 2); sample dilution was of 10^4.

2.3. Spectral investigation

Cell density was assessed by turbidimetric assay (Fig. 1), using Shimadzu UV-1700 spectrophotometer at $\lambda = 560$ nm and calibration curve. The recording of fluorescence spectra (Fig. 2) was performed using LS 55 Perkin Elmer spectrofluorimeter device, with excitation radiation of $\lambda_{ex} = 300$ nm; 1:10 sample dilution of supernatant resulted from thermally treated samples was carried out for avoiding the quenching of the fluorescent signal. Measurements of fluorescence intensity were carried out at 410 nm, in the maximum of the large band recorded in the green bluish emission light.

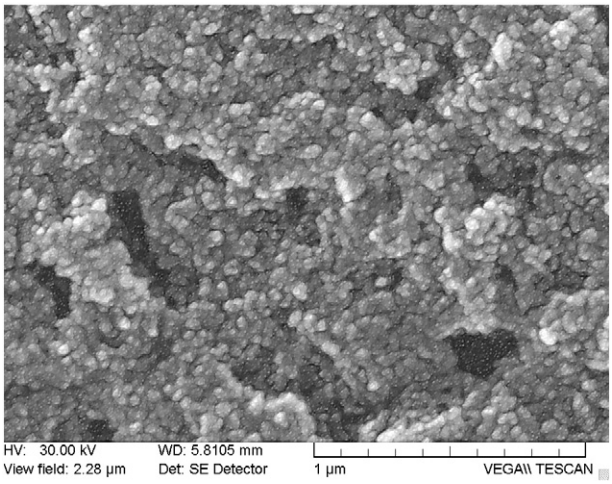

Image 1. SEM recording of Fe_3O_4/T core/shell systems.

2.4. Statistical analysis

Average values and standard deviations resulted from five repetitions of every sample measurement were taken for graphical plots. Student *t*-test was applied in order to assess the statistical significance of the differences between control and samples relatively to the significance threshold of 0.05.

3. Results and discussion

The experimental data are given in the figures below. Turbidimetric measurements have resulted in the graphs from Fig. 1 (cell density being assessed based on calibration curve). It can be seen that the same array of initial inoculum density values (10, 10^2, 10^4, 10^6, 10^8 cell/ml) resulted in higher values of cell density in the cases when iron ions were supplied in the bacteria culture medium—except for the lowest value of inoculum corresponding to Fe_3O_4/T colloidal nanoparticles (standard deviation of about 7%). The most remarkable stimulatory effect of iron ions was obtained in the samples inoculated with 10^4 and 10^6 cell/ml, where almost 30% supplementary increase in cell density was noticed for the aqueous suspensions of Fe_3O_4/S, and especially for Fe_3O_4/T. Statistical significance of the differences between iron ions supplied samples and control ones was assured ($p < 0.05$) in all cases except for the lowest and highest inoculum densities corresponding to Fe_3O_4/S data. Linear approach revealed the highest slope in the case of Fe_3O_4/T colloidal nanoparticles (58.98(±3.27) compared to 52.69(±4.88) and

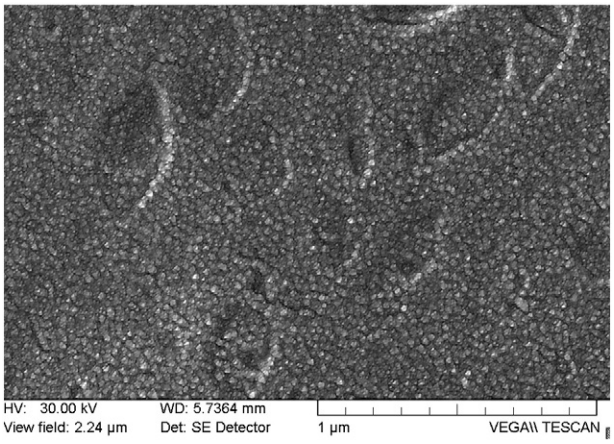

Image 2. SEM recording of Fe_3O_4/S core/shell systems.