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Total reflection x-ray fluorescence spectroscopy as a tool for evaluation of iron concentration in ferrofluids and yeast samples

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ABSTRACT

In this study, total reflection x-ray fluorescent (TXRF) spectrometry was applied for the evaluation of iron concentration in ferrofluids and biological samples containing iron oxide magnetic nanoparticles obtained by the laser target evaporation technique. Suspensions of maghemite nanoparticles of different concentrations were used to estimate the limitation of the method for the evaluation of nanoparticle concentration in the range of 1–5000 ppm in absence of organic matrix. Samples of single-cell yeasts grown in the nutrient media containing maghemite nanoparticles were used to study the nanoparticle absorption mechanism. The obtained results were analyzed in terms of applicability of TXRF for quantitative analysis in a wide range of iron oxide nanoparticle concentrations for biological samples and ferrofluids with a simple established protocol of specimen preparation.

1. Introduction

In the last decades, the growing interest in biomedical research has led to the creation of multidisciplinary groups that include not only chemists, physicists and engineers, but also biologists and biochemists. This trend is caused by the high involvement of novel materials and techniques coming from independent branches of science and consolidating into the emerging fields of biophysics and nanotechnology [1,2]. One of the addressed subjects in both basic and applied biomedical research is magnetic nanoparticles (MNPs). Their applications vary from magnetic biosensing, magnetic separation, magnetic resonance imaging to selective cell destruction using hyperthermia, drug delivery and many others [3–6]. The problems related to toxicity and accumulation of MNPs in specific parts of the body become very important. The last trends have led to special requests: big size of the batch for thorough characterization of MNPs, due to the fact that their properties can vary from batch to batch even with well controlled fabrication techniques [7] and a precise control of the MNPs concentration in tissues and biofluids. Magnetite (Fe_3O_4) is one of the most versatile biocompatible magnetic materials with a high saturation magnetization and relatively weak magneto-crystalline anisotropy [8]. On the other hand, the long-term instability of the properties of magnetite MNPs is encouraging researchers to study maghemite (Fe_2O_3) for particular applications. One of the most productive techniques for obtaining big batches of spherical maghemite MNPs is laser target evaporation (LTE) technique which doesn't suffer from the well known disadvantages of chemical synthesis, such as low production rates, limited purity, deviations from the sphericity, wide size distributions and a high environmental cost of the final product [9].

It is important to characterize each batch by different techniques, i.e. to measure the same quantity with multiple methods caused a new tendency to develop protocols for characterization of the properties of biological samples by techniques initially developed for different kind of the samples. One of such techniques is the total reflection x-ray fluorescent spectrometry (TXRF) [10]. TXRF is a relatively new method of elemental analysis that can be applied to samples in the form of thin near-surface layer including dried drops of homogenized solutions, suspensions of fine particles, or even a thin layer of whole cells. High sensitivity and reliability of the analysis results, in case of proper sample preparation, combined with the availability of highly effective desktop spectrometers lead to wider environmental and biomedical

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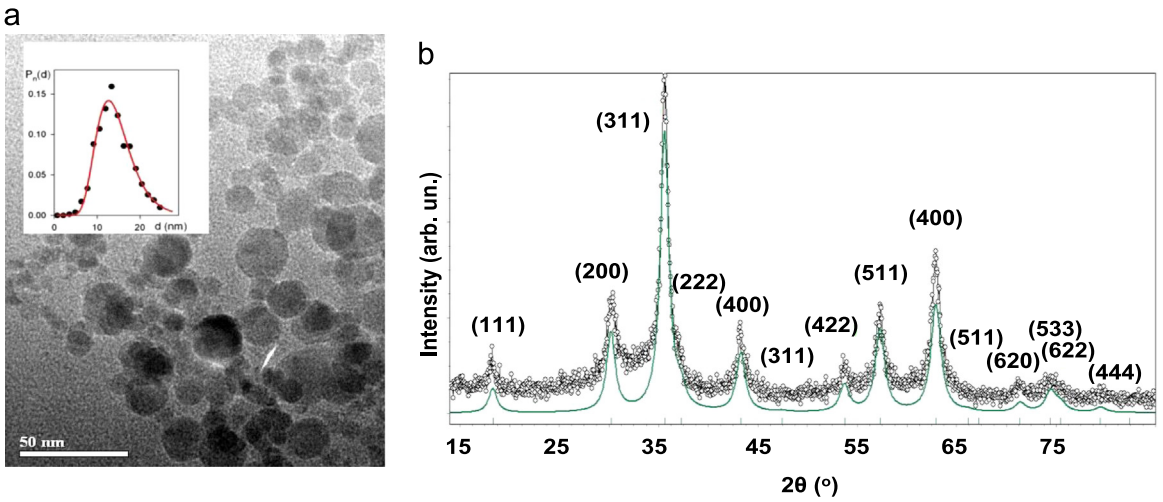

Fig. 1. TEM image of iron oxide MNPs. Inset: particle size distribution (2040 particles) (a). XRD plots of as-prepared MNPs with bright peaks Miller indexes. Points – experimental diffraction values, line – fitting by the database magnetite structure (b).

research involving TXRF spectrometry [10]. Although the TXRF method without a pressure controlled chamber proved to be limited for low Z (such as carbon, nitrogen, and oxygen) elements' quantification [11], it can be successfully applied for the determination of elements with higher-energy characteristic lines.

The study of biological samples (cells or tissues) has certain testing limitations when physical methods are used. These limitations are caused by the wide variety of complex processes that exist in the living matter and the wide variety of morphologies of each particular sample. To make biophysical research more effective, one can select model systems, which reproduce the main features and properties of more complex living system. Single-cell yeasts are good model subjects [4] that can be used not only for the study of the MNPs' accumulation mechanism and analysis of MNPs' toxicity, but also as a system potentially suitable for graduating a magnetic biosensor [12]. We applied TXRF spectrometry to evaluate the concentration of iron oxide MNPs in simple biological samples having a light matrix as well as ferrofluids of different concentrations.

The aim of the present work is to estimate the applicability and limitations of TXRF spectrometry for iron quantification in ferrofluids and simple single-cell organisms elaborating a simple established protocol of specimen preparation in a wide range of MNP concentrations.

2. Samples and techniques

Iron oxide MNPs were synthesized by LTE method based on the evaporation of the solid pellet by the laser beam followed by condensation of vapors in the gas phase [4,9]. These conditions provided the formation of spherical particles with controlled dispersion parameters. For synthesis of individual MNPs with similar size the evaporation was performed in a constant gas flow, which prevented particles from agglomerating. The LTE laboratory setup provided continuous synthesis of iron oxide MNPs with a production rate of 50 g/h. Evaporation was performed in pulsed regime with a 5 kHz frequency and 60 μs pulse duration favoring the formation of uniform fine MNPs and narrow particle size distribution.

For MNPs structural characterization, X-ray diffraction (XRD) studies were performed by DISCOVER D8 (Bruker) diffractometer using Cu-Kα radiation ($\lambda = 1.5418$ Å). Bruker software TOPAS-3 with Rietveld full-profile refinement was employed for a quantitative analysis. The average size of coherent diffraction domains was estimated using the Scherrer approach [13]. Transmission electron microscopy (TEM) was performed by JEOL JEM2100 microscope operating at 200 kV. The specific surface area (S_{sp}) of MNPs was measured by the low-temperature sorption of nitrogen Brunauer–Emmett–Teller physical adsorption technique (BET) [14] using the Micromeritics TriStar3000 analyzer.

Suspensions of iron oxide MNPs were prepared in distilled water with the addition of sodium citrate (electrostatic stabilizer) in 5 mM concentration. Coarse suspensions in an initial concentration of 5 g/L were treated by a Cole-Parmer CPX-750 ultrasound processor at 300 W average power output level. De-aggregation of the suspension was performed in the same way as described in our previous reports [9]. During the ultrasound treatment the diminishing of an average hydrodynamic diameter of aggregates in suspension was monitored by dynamic light scattering. When a constant value of the hydrodynamic diameter was achieved, the suspensions were centrifuged using a Hermle Z383 centrifuge. The supernatant was separated with a syringe and used for further studies. The aggregation state of MNPs in water suspension (both with and without living cells) was evaluated by dynamic light scattering using the Brookhaven Zeta Plus analyzer. The electrokinetic zeta potential of the suspensions was measured by electrophoretic light scattering (ELS) using the same analyzer.

For the study of maghemite MNP's accumulation process two nonpathogenic strains of yeasts were used: *Exophiala nigrum* (black yeasts) and a mutant strain of red yeasts which were isolated from Lake Baikal and the Schumak River in Russia respectively [15]. A liquid and gelatinous agar nutrient medium was used for cultivation. Maghemite MNPs in form of a stabilized aqueous suspension were introduced in a liquid and gelatinous nutrient medium directly with a final MNPs concentration of 0.5 g/l and 0.3 g/l consequently. The samples for TXRF measurements were prepared by diluting the yeasts colony of known mass (2–4 mg for yeasts grown on gel) in 0.5 ml of deionized water. The volume of the specimen placed onto the polyimide-coated substrate was 40 μl, including 20 μl of the yeasts suspension and 20 μl of the vanadium internal standard (200 ppm). The presence of a small quantity of iron in the nutrient media and, consequently, in the yeasts samples was taken into account by subtracting the iron concentration obtained on the reference yeast colony grown in similar condition but without adding MNPs to the nutrients. TEM images of cell cultures were obtained with a Philips EM208S electron microscope at an accelerating voltage of 70 kV. Cell morphology was also studied by optical and scanning (Hitachi