

# One-step preparation and surface activation of magnetic iron oxide nanoparticles for bio-medical applications

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This paper describes the one-step preparation and surface functionalization with 3-aminopropyltriethoxysilane (APTS) of magnetite nanoparticles during their synthesis by using only  $\text{Fe}^{2+}$  in alkaline medium. Mean crystal size calculated from X-ray diffraction spectra was 10.8 nm for uncoated magnetite, while magnetic particle diameter calculated from magnetization loops varied between 9.1 nm - 11 nm, depending on the APTS concentration. Activation of magnetite nanoparticles with amino-silanes led to the decreasing of saturation magnetization from 41.4 emu/g for uncoated magnetite to 18-20 emu/g for coated magnetite.

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## 1. Introduction

The functionalized magnetic iron oxides can be successfully used for biomedical applications such as antibody or enzyme immobilization.

Iron oxides are well-known compounds and can be easily prepared in laboratories. They are composed of Fe along with O and/or OH [1]. The most important and used iron oxides are  $\text{Fe}_3\text{O}_4$  (magnetite) and  $\gamma\text{-Fe}_2\text{O}_3$  (maghemite). Both compounds are ferrimagnetic on the bulk state. When prepared as nanoparticles of certain size they present high magnetization saturation and superparamagnetic behavior [2]. Based on these properties they can serve as excellent physical support for the immobilization of different (bio)molecules and can be used in magnetic separation, as magnetic markers in biosensors etc. Also,  $\alpha\text{-Fe}_2\text{O}_3$  (hematite) can be used as physical support, but taking into account its weakly ferromagnetism, it can be only used in processes that would not require high magnetization saturation of the support.

When used in biotechnology or medical applications, the magnetic materials should present biocompatibility, superparamagnetic behavior at room temperature [2] and increased stability in appropriate solutions.

The activation of support's surface can be achieved by using various organic substances, the most spread, beside polymers, being organosilanes and carbodiimides [3, 4]. Organosilanes, known also as coupling agents or adhesion promoters, are bifunctional molecules composed by a trialkoxysilane or trichlorosilane group and organic head-group functionality (e.g.  $-\text{NH}_2$ ,  $-\text{SH}$ ).

Generally, the available literature describes the activation with organosilanes of the iron oxides after their synthesis. However, due to the high magnetic dipole-dipole interaction, these bare magnetic particles easily

interact to agglomerate and form clusters [5]. Therefore, sometimes it is highly required to control and maintain the initial size of the particles, avoiding their clustering before surface-functionalization. To solve the above problems, surface-functionalized magnetite nanoparticles were synthesized by  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  coprecipitation in medium of  $\text{NH}_3$  and water-soluble 3-aminopropyltriethoxy-silane (APTS) [5]. However, in order to obtain magnetite by using  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ , one should take into account the optimum ratio between iron salts. Therefore, it is more convenient to use only  $\text{Fe}^{2+}$  for obtaining magnetite. In order to optimize the functionalization process of the magnetic iron oxides, here we describe a modified chemical method to obtain in one step magnetite nanoparticles functionalized with APTS by using only  $\text{Fe}^{2+}$ .

## 2. Experimental

All reagents were of reagent grade and used without further purification. Ferrous chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , > 99 %), water-soluble 3-aminopropyl-triethoxysilane (50 % in water) and sodium hydroxide were used.

X-ray diffraction spectra were recorded using a Bruker AXS D8 Advance diffractometer ( $\text{CuK}\alpha$  radiation,  $\lambda = 0.1541$  nm).

FT-IR spectroscopic studies were recorded on a Jasco 6100 spectrometer.

The magnetic properties were investigated with a Lake Shore 7410 vibrating sample magnetometer (VSM).

Thermomagnetic curves were obtained with a VSM at 15°C/min heating rate.

### 2.1. Preparation of magnetite (Mag1)

3 g ferrous chloride tetrahydrate, dissolved in 10 ml ethylic alcohol and heated at 45°C, were slowly dropped into a solution of 25 ml ethylic alcohol, 2 ml double distilled water, 10 g NaOH, under vigorous magnetic stirring at room temperature. Color of the solution gradually changed from orange to black, leading finally to black precipitate – the specific color for magnetite [6]. The suspension was further maintained under stirring for 30 min. Subsequently, the particles were washed four times with ethylic alcohol and double distilled water and separated by using a NdFeB magnet.

### 2.2. Preparation of APTS-functionalized magnetite

3 g ferrous chloride tetrahydrate, dissolved in 10 ml ethylic alcohol and heated at 45°C, were slowly dropped into a solution of 25 ml ethylic alcohol, 2 ml double distilled water, 10 g NaOH, 0.5 ml (Mag2), and 1 ml APTS (Mag3) respectively, under magnetic stirring. Color of the solution quickly changed from orange to brown precipitate. The suspension was kept under stirring for 30 min. Then, the precipitate was washed with alcohol and water.

## 3. Results and discussion

The magnetic iron oxide nanoparticles were prepared by adding the solution of Fe<sup>2+</sup> in ethylic alcohol to the mixture of ethylic alcohol, water, NaOH and, in the case of Mag2 and Mag3, APTS. In order to increase the super saturation of the system, a great amount of OH<sup>-</sup> was introduced in the reaction system by using a higher than required quantity of NaOH. The non-dissolved NaOH crystals (about 2 g) were removed before washing the black precipitate.

Since water can oxidize magnetite particles to  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> [7], in order to reduce magnetite oxidation during its synthesis, a solution based mainly on ethylic alcohol and a small amount of double distilled water - required for dissolving NaOH crystals - was used. Moreover, in preliminary observations we noted that when using only water for preparation, the magnetization saturation of the dried precipitate was very small, indicating that other than Fe<sub>3</sub>O<sub>4</sub> or  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> iron oxides were formed. For washing the

precipitates, a solution of 50 % (v/v) ethylic alcohol in double distilled water was used.

There were prepared two samples of APTS-functionalized magnetite in order to evaluate mainly the influence of the APTS concentrations on the magnetic properties of the functionalized nanoparticles.

The hysteresis loops of nanoparticles are shown in Fig.1. All three samples show superparamagnetic behavior at room temperature.

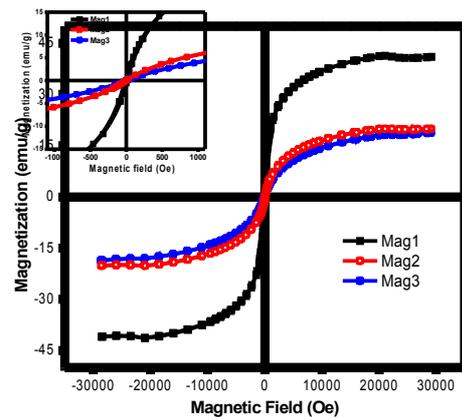


Fig. 1. Magnetization loops of all samples. Inset: detail of the hysteresis loops near zero.

The saturation magnetization,  $\sigma_s$ , of uncoated magnetite nanoparticles (Mag1) is 41,4 emu/g which is in good agreement with the reported value [4], whereas the saturation magnetizations of the APTS-coated Mag2 and Mag3 nanoparticles are 20.1 emu/g and 18.7 emu/g, respectively.

From the slope of the magnetization curve near  $\sigma = 0$ , the diameter ( $d_{VSM}$ ) of the particles can be calculated for materials which present superparamagnetic behavior [8]. For all samples,  $d_{VSM}$  was calculated using the equation:

$$d_{VSM} = \left( \frac{18kT \left( \frac{d\sigma}{dH} \right)_0}{\pi \rho \sigma_s^2} \right)^{1/3}, \quad (1)$$

where  $k$  is the Boltzmann constant,  $T$  is the temperature in Kelvin,  $(d\sigma/dH)_0$  is the slope of the magnetization curve near  $\sigma=0$ ,  $\rho$  is the density of Fe<sub>3</sub>O<sub>4</sub> (5.24 g/cm<sup>3</sup>), and  $\sigma_s$  is the saturation magnetization. Thus, the calculated mean particle sizes were 11 nm (Mag1), 10.4 nm (Mag2) and 9.1 nm (Mag3).

From the VSM data one can observe that the saturation magnetization,  $\sigma_s$ , of sample Mag1 is about half of the bulk magnetite. This is due to the fact that magnitude of  $\sigma_s$  is affected by particles size and correlates with their size [8]. The reduced saturation magnetization

of Mag2 and Mag3 in comparison with Mag1 is due to the diamagnetic contribution of the APTS coating.

The X-ray diffraction spectra of the sample Mag1 is shown in Fig. 2.

By using Scherrer formula, the mean crystal size ( $d_{XRD}$ ) was calculated from the broadening of the (311) reflection of the spinel structure. Thus, the mean crystals diameters were about 10.8 nm.

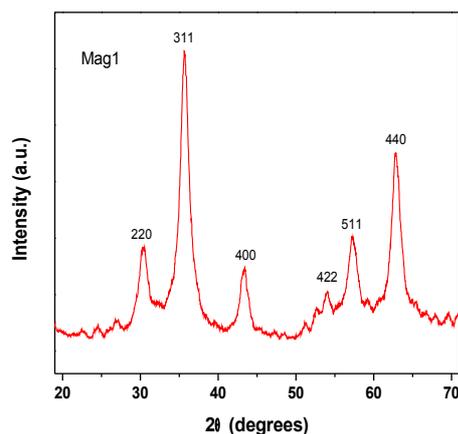


Fig. 2. X-ray diffractogram of samples Mag1 and Mag3.

From X-ray diffraction spectra the mean crystal size for Mag1 calculated using Sherrer formula is in good agreement with that calculated from VSM data for magnetic particles.

From the FTIR spectrum (Fig. 4) of Mag1, the absorption bands at  $3440\text{ cm}^{-1}$  and  $1630\text{ cm}^{-1}$  are assignable to O–H stretching and bending vibrations, respectively, of physically adsorbed  $\text{H}_2\text{O}$  and –OH groups.

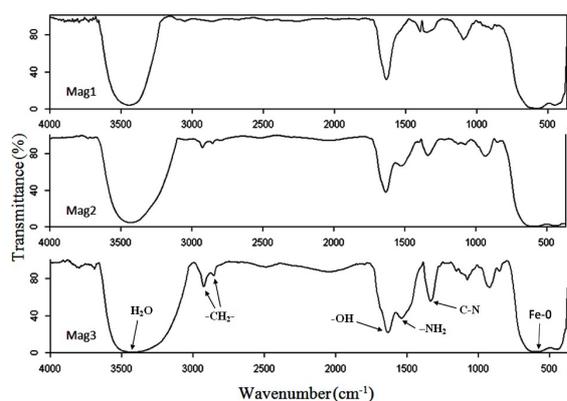


Fig. 4. FTIR spectra of coated and uncoated magnetic nanoparticles.

The presence of Fe-O bond stretching vibrations is observable at  $590\text{ cm}^{-1}$ .

In the case of Mag1 and Mag2, the absorption bands at  $2924\text{ cm}^{-1}$  and  $2852\text{ cm}^{-1}$  correspond to the stretching

vibrations of  $-\text{CH}_2-$  group of aminopropyl group, and are absent in Mag1. The absorption bands at  $1540\text{ cm}^{-1}$  and  $1341\text{ cm}^{-1}$  have been associated with the bending vibration of free  $-\text{NH}_2$  and the stretching vibrations of C-N, respectively. The absorption contribution from free  $-\text{NH}_2$  group at  $3416\text{ cm}^{-1}$  was probably overlapped by the vibration bands of water.

Data from FTIR spectra showed specific absorption bands of the amino-silane, confirming the presence of the APTS layer on the magnetite particles.

The thermomagnetic curves of Mag1 and Mag3 (Fig. 5) emphasize the presence of two main magnetic compounds that can be observed at about  $200^\circ\text{C}$  and  $580^\circ\text{C}$ , respectively.

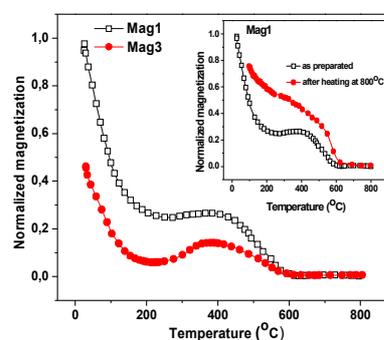


Fig. 5. Thermomagnetic curves of sample Mag1 and Mag3. Inset: thermomagnetic cooling curve of sample Mag1.

The first Curie temperature ( $T_C$ ) is characteristic for  $\text{FeOOH}$ -like compounds [1] and is due to the presence of water in samples as a consequence of the preparation process. The second ( $T_C$ ) is distinctive for magnetite [1].

The thermomagnetic cooling curve of Mag1 shows that iron oxides, other than magnetite, almost disappeared, the compound transformations being irreversible.

The black color of the Mag1 pointed out that the sample was more likely magnetite than maghemite [1, 6]. Additionally, from the thermomagnetic data, one can see  $T_C$  for maghemite is absent while the one corresponding to magnetite it is clearly present at about  $580^\circ\text{C}$ . Moreover, taking into account the relative high magnetization saturation of the uncoated magnetite nanoparticles, it is also clear that the mass ratio between magnetite and other low magnetic or non-magnetic compounds in the sample is favorable to magnetite.

## 5. Conclusions

Magnetite and APTS-functionalized magnetite nanoparticles have been synthesized in one step at room temperature by precipitation of only  $\text{Fe}^{2+}$  in alkaline medium. The thermomagnetic curves confirmed the presence of magnetite in all samples. The APTS concentration influences the magnetic properties and

particle size. The magnetization saturation,  $\sigma_s$ , of APTS-functionalized nanoparticles was about half of the obtained uncoated magnetite nanoparticles. Mean magnetic particle diameters of about 10 nm, were calculated from magnetization loops data. The FTIR spectra of APTS-functionalized nanoparticles evidenced free  $-\text{NH}_2$  groups, and demonstrated the bonding of amino-silane groups on magnetite surface.

Further work will be focused on enzyme immobilization on APTS-functionalized nanoparticles in order to be used in an electrochemical biosensor.

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