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ToC figure: The formation of iron oxide NPs in scaled-up conditions is monitored in situ using a handled magnetic portable sensor.
The evolution of magnetic properties during the formation of iron oxide NPs by coprecipitation in aqueous media and in scaled-up conditions is monitored in situ using a portable sensor measuring magnetic signal that is proportional to third derivative of magnetization. A detailed tracking of subsequent changes is also performed ex situ using more conventional analytical and physical methods.

Iron oxide NPs are highly exciting nanomaterials both from fundamental research point of view considering the dependence of the magnetic properties with size, shape, composition, surface and interaction, and for their numerous potential applications in biomedical and nanotechnologies ranging from diagnosis, therapies, in byproducts and the possibility of obtaining large amount of NPs.

The precipitation method, present significant batch to batch performance differences. This is also related to the fact that the formation of iron oxide NPs is a very fast process, and that no sensitive, standardized easy to use measurement techniques exist for the validation and quality control of this synthesis. Finally, despite the considerable number of publications on the synthesis of iron oxide NPs, there is still lack of reports dealing with the magnetic properties evolution at different points of the synthesis from the same initial precursor solution. This should lead to the optimization of iron oxide NPs synthesis with controllable and reproducible process, and should allow to progress towards good manufacturing practice standards and towards regulatory approval.

Here we study the nucleation and growth mechanism of iron oxide NPs formed by water co-precipitation, scaled-up for industrial production, using an innovative magnetic device (called MIAtek), measuring the third derivative of the magnetization around zero field. Consequently, this sensor is very sensitive to superparamagnetic (SPM) or weakly ferromagnetic (small coercive field) NPs behavior at room temperature. In previous works, we have shown that the MIA technologies, firstly devoted to magnetic immunoassays, feature sensitivity, rapidity and ease of use compared to conventional magnetic ones (e.g. superconducting quantum interference device (SQUID) or Vibrating Sample Magnetometer (VSM) in order to characterize the magnetic properties of the synthesized nanomaterial or to quantitatively monitor in situ NP cell internalization. This work shows the suitability of MIAtek to achieve in situ metrological monitoring of iron oxide NPs synthesis in aqueous media and at a pilot scale. Detailed tracking of subsequent changes is performed using both MIAtek and more conventional analytical and physical methods: TEM, DLS and VSM. In addition to the work of Baumgartner et al., we showed that the primary particles present SPM behavior. The set of results provides valuable information that can be used either to improve existing preparation methodologies or elaborate new ones, more appropriate for volume production.
The iron oxide NPs synthesis is performed within controlled physicochemical conditions scalable for industrial level production (see Experimental Section). Briefly, NPs are prepared in a laboratory two-stage continuous flow reactor, Figure 1.a. The ammonium hydroxide and iron chloride solutions (Fe(II)/Fe(III)=1/2) are mixed at constant inflow in first reactor (nucleation reactor) and NPs growth process is performed in second reactor stage (growth reactor). Magnetic measurements are recorded using handled portable MIAtek reader and performed at the nucleation reactor outlet (sample labeled “t0”) and in situ and continuously at the second reactor outlet, Figure 1.a. The first acquisition in the growth reactor is performed 2 min after nucleation stage up to 40 min, with an acquisition interval of 1 min. Moreover, the reaction mixture was aliquoted and frozen at different reaction times and collected samples were then characterized “ex-situ” using MIAtek, VSM, DLS and TEM. Iron content was estimated by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis.

Figure 1.b shows a typical time series of the nucleation and growth process followed with MIAtek reader. Black points and red stars are in situ and ex situ aliquoted samples MIAtek measurements respectively. Green diamonds are the blocking temperature values of the aliquoted samples.

Concomitantly, the full width at half maximum of the \( D_h \) distribution increases, as shown in Table 1. The TEM sample “t2” is characterized by branched network of very small particles (≈3-4 nm), not well crystallized, but not amorphous. Within further reaction time, the network disappears to finally obtain predominantly well crystallized iron oxide nanoparticles with an average size of about 7 nm. The diffraction patterns show the evolution of the NP crystallinity. The number of “final” NPs increases with time at the expense of the branched network. Considering the various step rule scenarios presented in Baumgartener publication\(^7\), our experimental conditions are in accordance with their considerations relating to the phase transformation occurring from rapid agglomeration of nanometric ferrihydrite primary particles without formation of intermediate amorphous bulk precursor phase. Hence, the estimated ratio of free energy gain per unit volume \( \gamma_{Fh}/\gamma_{Mt} \) on formation of ferrihydrite (Fh) versus magnetite (Mt) nuclei formation \( \gamma_{Fh}/\gamma_{Mt} \) is in the range of 0.7-0.94 (see supplementary information for detailed calculation). Considering a range for surface energy ratio \( \gamma_{Fh}/\gamma_{Mt} = 0.25-0.5 \), this corresponds to the boundary window (stability) for which the formation of ferrihydrite primary particles is favored and induce magnetite nucleation and growth. In addition, our work shows that these primary particles present SPM behavior as characterized with MIAtek (Figure1.b) and highlight up to 2 hours, no significant differences in magnetic signal occurs (Supplementary Figure S1). Considering that the variation of magnetic properties variation is correlated mainly to the size evolution and magnetic dipolar interactions, this seems to indicate that optimal size is achieved and the end of the reaction is after about 20 min. This is in accordance with iron yields measured with ICP-MS, as in Table 1, showing that the reaction is complete within 20-30 min.
Figure 3 a depicts magnetization vs. temperature curves after zero field cooling (ZFC) and after field cooling (FC) measured at 100 Oe for the various aliquoted samples. All of the samples show SPM behavior\(^{19-21}\), i.e. showing for ZFC curve a gradual increase of the magnetization until blocking temperature \(T_B\) and for higher temperatures a 1/T Curie-type decrease. Furthermore, the FC curve overlaps with the ZFC curve for high temperatures and then splits from the ZFC curve due to remanent magnetization acquired by particles while blocking in an external field. The magnetizations in ZFC curves have been normalized to the blocking temperature and are reported as green diamonds in Figure 1b and in Table 1.

A \(T_B\) increase is observed continuously with time, from 95 K to 145 K and after 30 min, a constant value 150 K is measured. Such an increase in \(T_B\) has to be correlated to the increase of the NP diameter, as in Table 1. The temperature dependence in FC particle size and the dipole–dipole interaction energy depending on the particle size, inter-particle spacing, and the presence of a spin disorder layer on NP surface.\(^{16,26-27}\)

As previously discussed, primary particles (sample “t0”) were considered in the first approximation as ferrihydrite NPs based on TEM characterization (Fig. 3) and the publication of Baumgartener et al.\(^{2}\). In another work, the publication of Gugyodo et al. reported SPM behavior at room temperature for ferrihydrite NPs with an average size between 3 to 5.5 nm\(^{28}\). The \(T_B\) and \(M_s\) (300 K) values are in the range of 45K to 80 K and 6 to 13 Am\(^2\)kg\(^{-1}\) respectively. In our case, \(T_B\) and \(M_s\) values for sample “t0” are 95K and 33 Am\(^2\)kg\(^{-1}\). This indicates that sample “t0” cannot be pure ferrihydrite NPs. On the other hand, Song et al. showed that magnetite NPs, with an average size of 3 nm and 5 nm, are characterized by \(T_B\) and \(M_s\) values of 40 K , 56 Am\(^2\)kg\(^{-1}\), and 96 K, 59 Am\(^2\)kg\(^{-1}\) respectively\(^{26}\). Moreover, maghemite NPs with size distribution from 3 to 5 nm exhibit \(T_B\) and \(M_s\) values of 94 K and 30 Am\(^2\)kg\(^{-1}\)\(^{29}\). On the basis of these different works and our own results, this suggests that the sample “t0” is a mixture of ferrihydrite and maghemite phases.

**Conclusions**

Our work shows an original approach to validate routine monitoring and quality control of iron oxide NP synthesis using MIAtek. With this sensor and for a complete metrology approach, it is necessary in the first step to fully characterize the material properties such as size, concentration, magnetic behavior (\(T_B\), \(M_s\), \(H_c\) for SPM or slightly ferromagnetic NPs) with conventional techniques. This first step defines the standard for a specific synthesis. Hence, the reproducibility of the MIAtek signal under the same experimental conditions allows validating the quality of the process and physical-chemical NP characteristics. Evidently, a change in the experimental conditions could lead to new NP properties and so a new standard would be generated. In this work, we used classical iron oxide co-precipitation in aqueous media as a proof of concept for such innovative magnetic metrology. We thus anticipate our approach to be a possible starting point to extend to other SPM or weakly ferromagnetic NPs synthesis.

**Notes and references**

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\(^{†}\) Electronic Supplementary Information (ESI) available: Typical time series of the nucleation and growth process followed with MIAtek reader for longer reaction time, Calculation of boundary window for estimated ranges for surface and bulk-energy ratio for Fe/Fe stability and TEM images with high magnification of the various aliquoted samples. See DOI: 10.1039/b000000x/

\(^{‡}\) Experimental Section
Iron oxide nanoparticles synthesis: NPs were prepared by  

coprecipitation method at a pilot scale by addition (0.0144 mol min⁻¹) of  
an ammonium hydroxide solution (0.36 mol L⁻¹) with an iron chloride  
solution ([Fe] = 0.12 mol L⁻¹) with a stoichiometric ratio Fe(II):Fe(III) =  
0.5). The entire process is thermostated at 30°C and the pH and magnetic  
signal were measured at regular time intervals in the growth reactor (see  
Figure 1).

Ex-situ Characterization techniques: Ten milliliters samples were taken  
from the reaction mixture at different times (2, 5, 10, 20, 30, 40, 50  
minutes) and rapidly frozen in liquid nitrogen in order to stop the growth  
of nanoparticles. Obtained particles were washed several times by  
magnetic decantation with deionized water. Each time, supernatant was  
discarded, and the final precipitate was finally dispersed in water at pH =  
2.

The magnetism of collected samples was characterized using a vibrating  
sample magnetometer, VSM (Quantum Design, Versalab) and MIAteK (ex-situ)  
at room temperature. Measurements were performed on liquid samples.  
The magnetization curve was obtained with VSM by cycling the  
applied field from -2300 to +2300 kA m⁻¹ for two times with a step  
of 8 kA s⁻¹ and the temperature was set to 300 K. The ZFC curve is obtained  
by first cooling the system in zero field from 270 K to 50 K. Next, the  
field is applied and subsequently the magnetization is recorded while  
increasing the temperature gradually. The FC curve is measured by  
decreasing the temperature in the same applied field.

Bright field imaging and electron diffraction studies were carried out with  
a Philips CM200 microscope operating at 200 kV. The samples were  
prepared by dispersing NPs in ethanol then depositing it onto a carbon  
film supported by a copper grid.

The hydrodynamic diameter and the zeta potential of nanoparticles were  
determined by dynamic laser light scattering (DLS) on a Nano-ZS ZEN  
3600 device (Malvern Instruments, Malvern, UK).

Determination of iron content was performed by Inductively Coupled  
Plasma Mass Spectrometry (ICP-MS) analysis (Agilent 7500ce). The  
samples were digested in concentrated HNO₃ solution and diluted with a  
2% HNO₃ solution for analysis.

Acknowledgements

We thank L. Lenglet for discussion, Neelogy company and FUI grant  
(project 3MT) for providing scale-up laboratory, Magnisense company for  
providing MIAteK.

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