

Synthesis of Micrometric Single Crystalline Magnetite with Superparamagnetic Properties for Biomedical Applications

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ABSTRACT

We synthesized micrometric magnetite crystals with average size of 10 μ m (along the <001> axis) with superparamagnetic behavior at room temperature by hydrothermal method. Without changing the concentrations of the precursors, we have investigated the possibility of obtaining single phase magnetite between 2h and 40h of high pressure–temperature treatment time in temperature range: 140°C to 270°C. A low remanent magnetization (≤ 5 emu/g) for magnetite was observed at high level magnetic field intensity (4kOe) in all cases, uncommon at room temperature for single crystalline magnetic particles having dimensions in the micrometric domain.

Keywords: hydrothermal, superparamagnetic, magnetite, micrometric, biomedical.

1. INTRODUCTION

Extending superparamagnetic behaviour from nanometric to micrometric scale is a real challenge, but it could be extremely useful in environmental and some biomedical applications. Several studies have demonstrated that ultrasmall superparamagnetic iron oxide-type (USPIO) nanoparticles increase the intensity of the MRI signal [1, 2-5] and improve the monitoring of cellular migration [6-8]. These researches outline the fact that in order to improve the MRI signal several millions of USPIO nanoparticles are necessary to mark a single cell, which is a difficult task [9]. However, Hinds *et al.* [10] have shown that micrometric iron oxide particles (MPIO) can be used as an alternative for the same purpose; such particles may efficiently be endocytosed by various cells. Saphiro *et al.* [9] demonstrated for the first time that a single MPIO particle can be used for cellular marking and then detected by MRI. Most of the current research attempts to solve this problem by increasing the number of nanoparticles; however, increasing particle size to micrometric scale is expected to produce a better effect [10]. The literature indicates [11-16] that massive efforts were involved in synthesising single

crystalline microparticle with superparamagnetic properties but, to our knowledge, at micrometric level (much bigger than magnetic domains have) this was attained only through the agglomeration of millions of nanoparticles, each of these nanoparticles having lower dimensions than the magnetic domain has (10nm). The major disadvantage of magnetic microparticles composed of clusters of nanoparticles is that the saturation magnetization is small compared to single crystalline microparticles. On the other hand, the literature contains a few reports [12, 17] about the synthesis of single crystalline micrometric magnetite, but none refers to the superparamagnetic behaviour of the crystals. However, the crystals of Liu *et al.* [12] exhibit magnetic properties significantly less convenient (10kOe saturation field, 80Oe coercivity, 6.6emu/g remanence compared to 3.6kOe saturation field, 18.6Oe coercivity, 3.4emu/g remanence, respectively) than those reported by us [18-20].

Based on the hydrothermal decomposition of the Fe-EDTA complex we have reported the successful formation of magnetite monodisperse microcrystals with superparamagnetic behaviour with size of 40-45 μ m after 40 hours of autoclavation at 230°C [18] and 38 hours at 250°C [19]. Also, we developed a short time experimental procedure (2 hours of autoclavation at 250°C) for synthesizing magnetite microoctahedrons in the 10-15 μ m range (along the <001> axis), with superparamagnetic behavior at room temperature (RT) [20]. In order to elucidate some issues related to the growing mechanism of these particles we extended our previous studies and we investigated the possibility of obtaining magnetite in single phase between 2h and 40h of autoclavation within a temperature range of 140°C to 270°C.

2. EXPERIMENTAL METHODS

2.1. Synthesis

The chemicals: FeNH₄(SO₄)₂·12H₂O (FAS), Na₄EDTA and urea, all of analytical purity, were supplied by Fluka (Sigma-Aldrich). An aqueous solution 1.05x10⁻¹M of FAS, 1.05x10⁻¹M Na₄EDTA, 9.71·10⁻¹ M urea was also prepared.

Before autoclavation the pH of the mixture was adjusted to a lower value of 5.15 using HCl. The mixture was transferred into several autoclaves (teflon-lined stainless-steel) and heated up between 2 and 40h (every 2 hours) within a temperature range of 140°C to 270°C by 20 to 20 Celsius degree. After cooling, the final pH of the solutions was between 9.2 and 9.45. Particles were collected by filtration. The mixed product obtained by this procedure contained hematite (α -Fe₂O₃) nanocrystals, iron carbonate (FeCO₃) microcrystals and magnetite (Fe₃O₄) microcrystals in different proportions. The micrometric magnetite was further separated by decantation and magnetic separation. They were washed with bidistilled water and dried at 60°C in air. We further present the complete characterization of the sample synthesized at 270°C at 2h of autoclavation.

2.2. Characterization

An X'Pert PRO MPD diffractometer (PANalytical) with Cu K α radiation ($\lambda=0.15418$ nm, Ni filter), θ configuration, assisted by the X'Pert Data Collector program was used for the analysis of crystal structure at room temperature. The PDF 4+ Database of Joint Committee on Powder Diffraction Standards (JCPDS) was used for phase identification. SEM and FEI (Inspect S) were employed for the morphology of the particles and energy-dispersive X-ray spectroscopy (EDAX) analysis for the identification of chemical components. Infrared (IR) spectrum was recorded in the wave numbers ranging between 4000 and 400 cm⁻¹, with JASCO 4200 Fourier transform infrared (FT-IR) Spectrophotometer using a KBr wafer. An AC hysteresigraph [21] was used to test the magnetic properties of the particles.

3. RESULTS AND DISCUSSION

Figure 1 presents the diffraction pattern that allowed for unequivocal identification of hematite and magnetite; using the ICSD (Inorganic Crystal Structure Database) reference

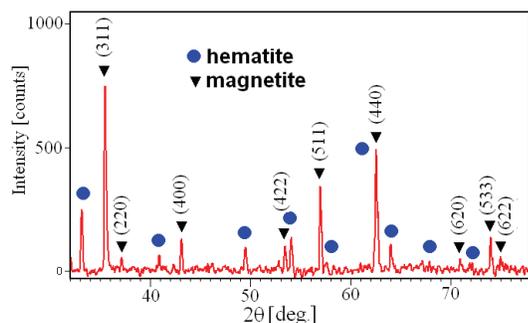


Fig. 1. Magnetite/hematite, XRD pattern

code 01-079-0007 for hematite and 01-076-1849 for magnetite the diffraction peaks were identified. The black powder (after separation) was also analyzed by XRD. Figure 2a shows the diffraction pattern that allowed for unequivocal identification of magnetite having the reference code presented above (01-076-1849). The presence of iron and oxygen only is revealed in the EDAX spectrum presented in Figure 2b. No traces of Na, S, C and N have been observed, which confirms the high purity of the final product. The little bit higher than theoretically predicted O/Fe atomic ratio can be attributed to the hydration and superficial oxidation of magnetite.

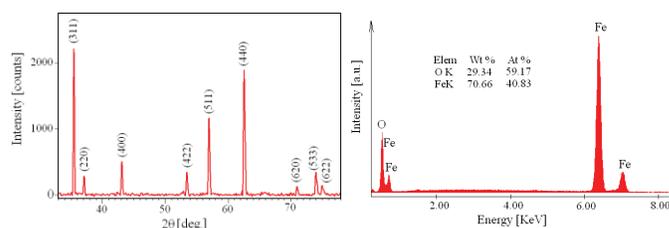


Fig. 2 a. Magnetite, XRD pattern; b. EDAX spectrum

The morphology of the magnetite microcrystals representing a combination of octahedral and dodecahedral faces of 10-15 μ m in size (along the <001> axis) is revealed in Figure 3.

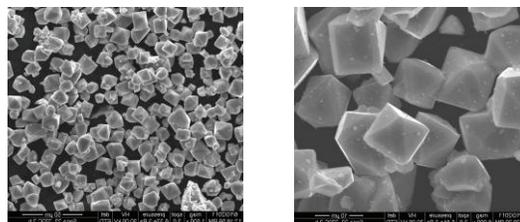


Fig.3. SEM image of magnetite microcrystals.

The FT-IR analysis was performed to characterize the surface nature of the 2 h resulting product. The FT-IR spectrum of the particles (Fig. 4) exhibits characteristic peaks of substoichiometric magnetite at around 578 cm⁻¹ [22, 23]. Otherwise, the peaks observed on FT-IR spectrum at 515cm⁻¹ could be considered as characteristic peaks of magnetite [24]. A wide and strong band at 3100–3630 cm⁻¹ centered at 3461cm⁻¹ could be assigned to O–H vibrations [25, 26] or from the adsorbed water molecules [27, 28], which is consistent with EDAX findings. The weak peak situated at approximately 2879 cm⁻¹ could be attributed to symmetric C–H vibrations [29]. The peak at 1632 cm⁻¹ may also show the existence of Fe–O (1624 cm⁻¹ as Lu said) [30], or could be a signal of EDTA carboxyl groups when bound to surfaces, according to [31]. The 1397 cm⁻¹ IR feature could be due to both vs(COO⁻) free (1405 cm⁻¹) and vs(COO⁻), as Nowak and coworkers observed [32].

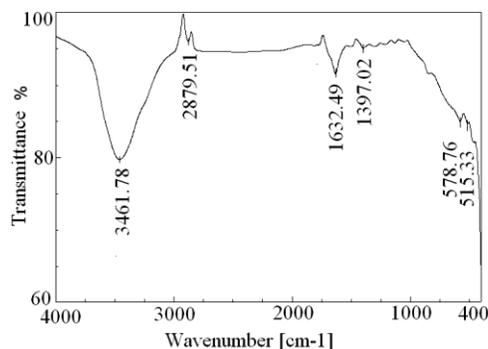


Fig.4. The FT-IR spectrum of magnetite

As Figure 5 reveals, saturation magnetization can be estimated to approx. 93 emu/g. The values for coercivity ($H_c=26\text{Oe}$) and magnetic remanence (5emu/g- Fig. 5-inset) are very low, indicating a superparamagnetic-like behavior, which is rather uncommon for the micrometric range of magnetic particles size. A possible explanation of this behavior can be correlated with a low level of imperfections, involving a low level of internal stresses, which leads to a decrease in coercivity, confirming that our final product has good crystallinity. The large dispersion in the crystallite axes is consistent with low remanence.

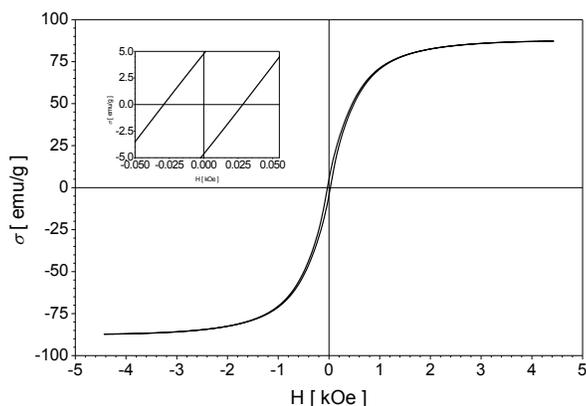


Fig.5. RT magnetic hysteresis loop of the Fe_3O_4 crystals.

The chemistry of Fe(III)-EDTA decomposition is largely described in [33-35]. In our previous work [19] we suggested a possible mechanism for magnetite microcrystals synthesis at 230°C and 38h of high pressure-temperature treatment time. We further present shortly the results of our investigations at 230°C , 250°C and 270°C between 2 and 40h of high pressure-temperature treatment time (maintaining the concentration unchanged for the precursors). What is significantly different between syntheses at 230°C and 250°C is that, in the first case, the iron carbonate and nanometric hematite coexist in mixed phases between 4 and 26h. In the second case, hematite completely disappears after 8h of thermal treatment, and sometimes magnetite appears in a very small quantity, to coexist with siderite between 8 and 18h of hydrothermal

treatment. In both cases after high pressure-temperature treatment time longer than 26h, mixed phases of siderite and magnetite coexist until 40h of hydrothermal treatment, when micrometric monodisperse magnetite with unusual superparamagnetic behavior (remanent magnetization between 3 and 5 emu/g) at room temperature appears in the single phase.

Increasing temperature at 270°C generates dramatic changes in synthesis time (decreasing 19 times from 38h to 2h) without significant changes in microparticle properties. Also the absence of the iron carbonate crystalline phase and the presence of hematite only indicate a different mechanism of synthesis. It is possible that the decomposition of the Fe(III)-EDTA complex through an internal electron transfer [34, 35] so that a part of Fe^{3+} is reduced to Fe^{2+} creates the conditions for the ratio of $\text{Fe}^{3+}/\text{Fe}^{2+}$ to be 2:1. Maintaining the 270°C temperature constant and increasing autoclavation time results in pure iron carbonate appearing in the system.

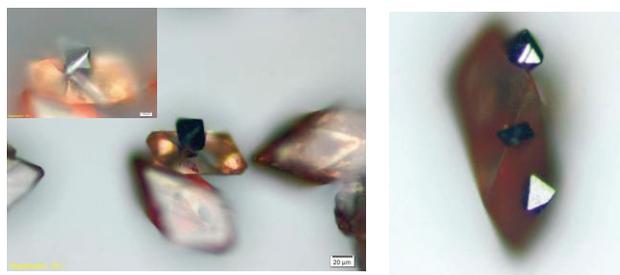


Fig.6. Optical microscope images of our synthesized iron carbonate/magnetite mixture 230°C (left) and 250°C (right).

As far as the growing mechanism of the magnetite is concerned, optical microscopy of the intermediate synthesis stages between 230°C and 250°C has shown that magnetite grows from iron carbonate: with increasing synthesis time, magnetite crystals begin to form and grow, while the iron carbonate crystals begin to disappear, vanishing after 38h of autoclavation.

4. CONCLUSIONS

We developed a new procedure for synthesizing highly crystalline Fe_3O_4 microparticles with superparamagnetic behavior by hydrothermal decomposition of the Fe(III)-EDTA complex in the presence of urea, starting from ferric ammonium sulfate and Na_4EDTA as the main precursors. Without changing the concentrations of the precursors we investigated the kinetics of phase transitions in the interval between 2 and 40h of high pressure-temperature treatment and temperatures between 140°C and 270°C . At temperatures between 140°C and 200°C , only single phase hematite is present. At temperatures between 230°C and 250°C after 38-40h of autoclaving, single phase magnetite ($40\text{-}45\mu\text{m}$ in size) is present. For these temperatures, our investigations (in optical) indicate unequivocally that magnetite crystallites grow on iron carbonate crystals. When

increasing synthesis temperature between 250°C to 270°C, synthesis time drops from 40h to 2h and mixed phases of hematite and magnetite appear in the autoclaves. These crystals have 10µm in size; they can be easily separated from nanometer hematite. Taking into account the dimensions of the cells (10µm -100µm), the choice of a particle of 10µm dimensions could be appropriate for some medical applications such as intracellular hyperthermia, controlled drug delivery system (site specific drug delivery), cellular Magnetic Resonance Imaging (MRI), monitoring cell migration for cell therapy, multimodal cancer therapy, immunomagnetic separation of cells, detection, immobilization and modification of biologically active compounds, cell labelling; magnetic separation of cells, magnetic resonance contrast agents, gene delivery, multi-modal cancer therapy.

ACKNOWLEDGEMENTS

This work was supported by program PN 09 34 01 01 of the Ministry of Research and Education in Romania. Helpful discussions and technical support from Radu Banica and Paula Sfarloaga (NIRDECM) and Cecilia Savii (Chemistry Institute of the Romanian Academy, Timisoara) are kindly acknowledged.

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