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A facile microwave synthetic route for ferrite nanoparticles with direct impact in magnetic particle hyperthermia

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ABSTRACT

The application of ferrite magnetic nanoparticles (MNPs) in medicine finds its rapidly developing emphasis on heating mediators for magnetic hyperthermia, the ever-promising "*fourth leg*" of cancer treatment. Usage of MNPs depends largely on the preparation processes to select optimal conditions and effective routes to finely tailor MNPs. Microwave heating, instead of conventional heating offers nanocrystals at significantly enhanced rate and yield. In this work, a facile mass-production microwave hydrothermal synthetic approach was used to synthesize stable ferromagnetic manganese and cobalt ferrite nanoparticles with sizes smaller than 14 nm from metal acetylacetonates in the presence of octadecylamine. Prolonging the reaction time from 15 to 60 min, led to ferrites with improved crystallinity while the sizes are slight increased. The high crystallinity magnetic nanoparticles showed exceptional magnetic heating parameters. *In vitro* application was performed using the human osteosarcoma cell line Saos-2 incubated with manganese ferrite nanoparticles. Hyperthermia applied in a two cycle process, while AC magnetic field remained on until the upper limit of 45 °C was achieved. The comparative results of the AC hyperthermia efficiency of ferrite nanoparticles in combination with the *in vitro* study coincide with the magnetic features and their tunability may be further exploited for AC magnetic hyperthermia driven applications.

1. Introduction

Magnetic nanoparticles (MNPs) dispersed in several media are currently a subject of basic and applied research that lead to a wide variety of applications such as magnetic storage systems, waste water treatment, catalysis, contrast enhancement in magnetic resonance imaging (MRI), site-specific drug delivery and magnetic particle hyperthermia [1–12]. In particular, magnetic particle hyperthermia represents a least-invasive modality in cancer treatment in good synergy with standard modalities, currently under clinical trials [13]. Heat dissipation can be exploited from MNPs under an AC magnetic field in specific tumor sites, resulting to a therapeutic outcome and driving malignant cells to destruction as cancer cells are generally more susceptible in regional temperature variations than normal cells [11].

Ferrites of the general formula MFe_2O_4 where M = Fe, Mn, Co have been of particular interest from magnetic applicability point of view based on their facile fabrication, chemical stability and magnetic tunability, showing more recently great potential in biomedical applications [14]. Optimization of magnetic, morphological and surface properties of MNPs is pertinent in bioapplications and challenging task. It is widely accepted that intrinsic properties of MNPs are governed by the synthetic conditions. MNPs size is strictly related to the metal precursors, the reaction temperature and the surfactant that during the decomposition process is adsorbed, desorbed or covalent bonded at the surface of the nuclei influencing the nucleation and growth phenomena and finally influence the physicochemical characteristics of the NPs. Moreover, magnetization in ferrites can vary following the divalent/trivalent metal stoichiometric ratio [15] and in that way can be designed to have the prerequisites response to AC field variations that constitutes the key issue for magnetic particle hyperthermia. Up to date, various MNPs have been tested in effort to optimize hyperthermia's outcome [1,16]. Cobalt ferrite ($CoFe_2O_4$) represents a typical hard magnetic material with considerable potential for biomedical applications as strong binding with serum albumin proteins have been found [17,18]. Nanocrystalline manganese ferrite ($MnFe_2O_4$) is also one of the most promising magnetic nanomaterials that exhibits good chemical stability and biocompatibility [15], higher magnetization (M_s) and low coercivity (H_c) than other doped ferrite members and in some cases even higher M_s comparing with the most studied and currently available iron oxide MNPs (*e.g.* Feridex and Resovist) [19].

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Despite a lot of work carried out on synthetic strategies, reaching a very high level of sophisticated methods, a "green" economical mass-production synthesis of high reproducibility MNPs needs to be developed. Among rapid and environmentally benign method, microwave irradiation namely also as MW-assisted reactions has been proved efficient for several NPs as metallic, binary transition metal oxides, multimetal oxides *etc.* [20–26]. Meanwhile, to our knowledge there are few reports on the hydrothermal microwave synthesis of ferrites; $CoFe_2O_4$ and $MnFe_2O_4$ using surfactant free hydrolysis reactions of chloride [25] and sulfate salts [27] have been prepared respectively while surfactant assisted hydrolysis reactions of nitrate [28,29] salts with oleic acid as the dispersing agent for both ferrites were also reported.

In view of the above together with our ongoing interest in the synthesis of ferrite MNPs as enhanced performance candidates for theranostics [15,19,30–32] in the present study we chose to investigate the application of microwave heating to the preparation of manganese and cobalt ferrite nanoparticles. In specific, a hydrothermal-surfactant assisted microwave procedure (H-MW) is followed where metal acetylacetonates have been used as precursors while as a surfactant octadecylamine (ODA) was employed. Microwave-assisted heating has been selected as a greener approach to synthesize ferrite MNPs in shorter time intervals (several minutes compared to hours) and with lower power consumption (hundreds of Watts) as a consequence of directly and uniformly heating the contents. The synthetic conditions that have been chosen were analogue to those performed previously by us [32] for the preparation of $CoFe_2O_4$ MNPs in an autoclave with subcritical water, firstly for comparison reasons with the H-MW method and secondly because the previously resulted MNPs fulfilled very good characteristics for bioapplication. In so, a comparison is discussed. To go further, the H-MW procedure under the same synthetic conditions performed for the preparation of $MnFe_2O_4$ MNPs. The relative good hydrophilic properties of the resulted MNPs gave us effort for magnetic hyperthermia experiments.

Eventually, $MnFe_2O_4$ MNPs are further proceeded to *in vitro* hyperthermia tests by examining their effect on human osteosarcoma cell line (Saos-2). Osteosarcoma is the most common bone tumor and the third most common malignancy in children and adolescents [33]. Saos-2 cells possess several osteoblastic features and could be useful as a permanent line of human osteoblast-like cells and as a source of bone-related molecules. Among their advantages are the well-documented characterization data, the possibility to obtain large amounts of cells in a short time, and the fact that they can be fully differentiated in a manner that the osteoblastic cells naturally do. Therefore, they are incorporated as a valuable model for studying events associated with the osteoblastic differentiation stage in human cells produced by diverse scenario hyperthermia treatments [30,34–36]. The *in vitro* performance induced by manganese ferrite nanoparticles on a human osteosarcoma cell line (Saos-2) that possesses osteoblastic features demonstrates the important role that these systems may have in magnetic particle hyperthermia application [37].

2. Materials and methods

2.1. Microwave–hydrothermal synthesis

For the preparation of $MnFe_2O_4$ and $CoFe_2O_4$ MNPs a microwave-hydrothermal synthesis was employed using a commercial Microwave Accelerated Reaction System, Model MARS 6-240/50-CEM [38]. This system operates at a maximum frequency of 2450 MHz and a power of 1800 W. Although, similar frequency is used in domestic microwave ovens, additional features such as full automation, fine-tuning of the most important reaction parameters (pressure, power, temperature and time) and a twist board, yield this method quite challenging. All the reagents were of analytical grade and were used without any further purification. Iron(III) acetylacetonate [$Fe(acac)_3$] and octadecylamine (>90.0%) were purchased from Fluka; manganese (III) acetylacetonate [$Mn(acac)_3$ ≥ 99.9%] and cobalt(III) acetylacetonate [$Co(acac)_3$, ≥99.9%] were supplied by Aldrich. Cetyltrimethylammonium bromide (CTAB, 98.0%) was purchased from Alfa Aesar. All aqueous solutions were prepared with water from a Milli-Q water purification system.

The reaction was carried out in a double-walled vessel consisting of an inner Teflon container liner and an outer composite sleeve of high strength polymer. Multiple vessels have been simultaneously used, while one served as reference, equipped with the temperature and pressure sensors, the other was used to equilibrate the rotating system; 0.9 mmol of manganese(III) acetylacetonate ($Mn(acac)_3$) and 0.9 mmol of cobalt(III) acetylacetonate ($Co(acac)_3$) in case of manganese and cobalt ferrites respectively, together with 1.8 mmol of iron(III) acetylacetonate ($Fe(acac)_3$) both in the presence of 1 mmol of ODA $C_{18}H_{39}N$ were dissolved in 60 mL of distilled water. Afterwards, the aqueous solution was stirred for 15 min with magnetic stirrer at room temperature. In the sequence, the mixture was transferred into a Teflon autoclave.

Each H-MW process was repeated for two different intervals (15 and 60 min) at 200 °C with initial ramp time heating step (from 25 °C to 200 °C) set to be 10 min under a stabilized pressure of 294 kPa inside the autoclave. After MH processing, the autoclave was left to cool down for ~30 min till room temperature. The pressure limit was also set at 14 bars and the power limit at 800 W, keeping all the time the system under vigorous agitation. Finally, the products were washed and separated from the solution by repeated centrifugation cycles (5000 rpm) and ethanol washing stages to eliminate impurities and finally dried at 40 °C under vacuum to receive particles in a powder form.

2.2. Physical and chemical features

Manganese and cobalt ferrite nanoparticles were structurally characterized by X-ray powder diffraction (XRD), which was carried out in a SIEMENS D500 X-ray diffractometer using the K_α line of Cu as a radiation source in the 2Θ range from 30° to 90° with scanning step width of 0.05° and 3 s scanning time per step. The magnetic properties included hysteresis loops and ZFC-FC (zero field cooled–field cooled) cycles were measured by using vibrating sample (VSM – 1.2H/CF/HT Oxford Instruments VSM) and also with a SQUID magnetometer (Quantum Design MPMS with field range of ±5 T at the temperature range of 5–300 K). Fourier transform infrared (FTIR) spectroscopic data was taken using advanced direct surface contact method in the range from 4000 to 250 cm^{-1}. Thermogravimetric analyses (TGA) were performed on the samples with a heating rate of 5 °C min^{-1} from room temperature up to 800 °C using a Material Analysis and Characterization TG-DTA system to observe the weight loss/gain during heating.

2.3. Heating efficiency

The heat dissipation of the MNPs ferrite suspensions was evaluated with an alternating current magnetic hyperthermia device. The aqueous solutions were placed in the center of a two-loop water cooled induction coil connected to an AC field generator (SPG-10: Ultrahigh Frequency Induction Heating Machine, Shuangping Corporation) that operates at fixed frequency of 765 kHz together with an applied magnetic field of 24 kA m^{-1}. Data acquisition for hyperthermia was started at ambient temperature. In order to maintain a magnetic fluid with stable dispersion, synthesized nanoparticles were converted to hydrophilic by addition of a positively charged ligand, cetyltrimethylammonium bromide (CTAB), an approach that studied before by us [39] where the effect of surface modification has been examined. Temperature increase was measured for ample time (600 s for each sequence) using a temperature optical fiber probe dipped into the solution with an accuracy of ±0.5 °C. It has been reported [40] that AC magnetic fields where the product $H \times f$ (applied magnetic field and frequency respectively) does not exceed the value ~5×10^8 A m^{-1} s^{-1} is well-tolerated by a human patient. Although, in our case, the corresponding value was