Available online at www.sciencedirect.com

ScienceDirect

Ceramics International 41 (2015) 397–402

CERAMICS INTERNATIONAL

www.elsevier.com/locate/ceramint

Synthesis and characterization of maghemite nanoparticles for hyperthermia applications

E.M. Múzquiz-Ramos[a,*], V. Guerrero-Chávez[a], B.I. Macías-Martínez[a], C.M. López-Badillo[a], L.A. García-Cerda[b]

[a]*Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Blvd. V. Carranza y José Cárdenas Valdés, C.P. 25280, Saltillo, Coah. México*
[b]*Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna Hermosillo No.140, C.P. 25294, Saltillo, Coah. México*

Received 26 July 2014; accepted 22 August 2014
Available online 1 September 2014

Abstract

Magnetic nanoparticles for hyperthermic treatment of cancers have gained significant attention in recent years. In this work, biocompatible maghemite nanoparticles were synthesized by the oxidation of magnetite nanoparticles, using a coprecipitation method followed by heat treatment at different temperatures ranging from 200 to 300 °C for 3, 5 and 8 hours. The samples were analyzed by X-ray diffraction, vibrating sample magnetometry and transmission electron microscopy. The heating ability was evaluated under a magnetic field using a solid state induction heating equipment. Additionally, hemolysis test was performed. The obtained nanoferrites showed a particle size within the range of 10–11 nm and superparamagnetic behavior. The maghemite obtained at 250 °C for 5 hours was able to heat in concentrations of 13 mg/2 ml under a magnetic field (10.2 kAm^{-1} and frequency 362 kHz), increasing the temperature up to 49 °C. Hemolysis test, evaluated as release of hemoglobin, revealed that all the samples showed no hemolytic effects up to 3 mg/ml, indicating no damage of the red blood cell membranes. The results indicated that the maghemite nanoparticles obtained might be potential materials for cancer treatment by hyperthermia.

Keywords: Maghemite nanoparticles; Hyperthermia; Hemolysis test; Biomaterials

1. Introduction

Hyperthermia is a therapeutic procedure in which tissues are heated above normal physiological ranges. It is most often considered as an alternative therapy for cancer treatment, where a notable lack of side effects makes it an attractive substitute for chemotherapy and radiation. Adequate increases in temperature (41–46 °C) may alter the functionality of intracellular proteins, leading to cellular degradation and ultimately inducing apoptosis. However, in moderate hyperthermia, longer treatment about hours are generally required for effective treatment, often in combination with additional treatment modalities such as radiation or chemotherapy [1].

Hyperthermia can be induced using magnetic nanoparticles, in a treatment known as magnetic hyperthermia. The nanoparticles can be introduced into the human body in the region surrounding the cancer tumor and then heating them up by using an external magnetic field. The dimensions of nanoparticles used in the hyperthermia method must be less than 100 nm [2].

In the clinical application of magnetic hyperthermia, magnetic field strength is limited by physiological considerations since large amplitude. High-frequency magnetic fields may induced local heating in non-magnetic tissues due to induced eddy currents [3]. Frequencies as high as 300 kHz and field amplitudes of 10–30 kA/m have been used [1].

All biomedical and bioengineering applications require that these ferrites must have not only high magnetization values and narrow particle size distribution, but they also have to be nontoxic and biocompatible [4]. Apart from acute toxicity, the

*Corresponding author. Tel.: +52 844 4159534x220; fax: +52 844 4390511.
E-mail address: emuzquiz@uadec.edu.mx (E.M. Múzquiz-Ramos).

http://dx.doi.org/10.1016/j.ceramint.2014.08.083
0272-8842/

toxicity of degradation products, stimulation of cells with subsequent release of inflammatory mediators and toxic effects through the particulate system have to be seriously considered [5].

The large number of blood contact interactions, as well as the intensity of the adverse physiological outcome (severe hemolysis may lead to life threatening conditions such as anemia), makes an examination of hemolytic activity an important aspect of preclinical characterization of nanoparticles [6].

The *in vitro* cytotoxicity assays are the primary biocompatibility screening tests for a wide variety of materials used in medical devices. Current experience indicates that a material that is judged to be nontoxic *in vitro* will be nontoxic *in vivo* assays [7].

Several types of iron oxides exist in nature and can be prepared in the laboratory, but nowadays only maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) are able to fulfill the necessary requirements for biomedical applications. These requirements include sufficiently high magnetic moments, chemical stability in physiological conditions and low toxicity, not to mention the easy and economical synthetic procedure available for the preparation of these materials [8].

Thus, an efficient, economic, scalable and nontoxic monodisperse synthesis of Fe_3O_4 and γ-Fe_2O_3 nanoparticles is highly preferred for potential applications and fundamental research [9].

In this work, biocompatible maghemite nanoparticles were synthesized by the oxidation of magnetite nanoparticles, using a coprecipitation method followed by heat treatment at different temperatures ranging from 200 to 300 °C for 3, 5 and 8 hours and characterized for their potential use in magnetic hyperthermia treatment.

2. Material and methods

2.1. Preparation of magnetite nanoparticles

Magnetite nanoparticles were synthesized by a chemical coprecipitation technique [10] using two starting solutions of 0.1 M of $FeCl_3$•$6H_2O$ (Sigma-Aldrich) and $FeCl_2$•$4H_2O$ (Sigma-Aldrich). These solutions were prepared by dissolving proper amounts of the chemicals in distilled water. Appropriate amounts of solutions were mixed in a baker using a mechanical stirrer at 1000 rpm to obtain a Fe^{+2}:Fe^{+3} ratio of 2:3 while heating on a hot plate. When the temperature reached 70 °C, the stirring velocity was increased up to 5000 rpm and then NH_4OH (Sigma-Aldrich) at 10% was promptly added. After that, a black precipitate, characteristic of magnetite nanoparticles, was obtained. The precipitate was washed several times with distilled water to eliminate as much as possible the residual chlorides. The magnetite particles were dried in air at room temperature.

2.2. Preparation of maghemite nanoparticles

The obtained magnetite nanoparticles were heated in air at 200, 250 and 300 °C for 3, 5 and 8 hours to be oxidized to form maghemite. After cooling, the product was stored in a desiccator containing silica gel as a drying agent.

2.3. Characterization of maghemite nanoparticles

The maghemite nanoparticles were analyzed by X-ray diffraction (XRD) (Siemens Mod. D-5000). The magnetic properties of the samples were measured with a SQUID Quantum Design magnetometer (VSM) in applied fields from −12.5 to 12.5 KOe. The particle size and shape were studied by transmission electron microscopy (TEM) (Titan 80300 Kv). For this analysis, the samples were prepared by placing one drop of the dilute suspension of maghemite nanoparticles in acetone on a carbon-coated copper grid and allowing the solvent to evaporate slowly at room temperature.

2.4. Heating capacity

The heating capacity of selected nanoparticles was evaluated under an appropriate magnetic field (10.2 kAm^{-1} and frequency 362 kHz) using a solid state induction heating equipment (Ambrell, EasyHeat, 0224). These tests were performed during 15 min; the ferrite particles/water concentrations used were 7, 11 and 13 mg/2 ml.

2.5. In vitro hemolysis assay

The hemolysis test was performed using human whole blood from healthy non-smoking donors, following the proper guidelines for studies using human specimens. Blood, collected in heparinized-tubes, was centrifuged at 3000 rpm for 4 min at 4 °C. The pellet was washed three times with cold Alsever's solution (dextrose 0.116 M, NaCl 0.071 M, sodium citrate 0.027 M and citric acid 0.002 M, pH 6.4). The supernatant was then removed and 100 µl of the purified erythrocytes was diluted 1:99 with Alsever's solution. Then, 150 µl of this suspension was suspended in Alsever's buffer and taken for the curve-response experiments (total volume 2000 µl). This suspension of red blood cells was always freshly prepared and used within 24 h after collection.

Three concentrations of nanoparticles were tested: 0.25, 0.50 and 3.0 mg/ml. Maghemite nanoparticles were brought in direct contact with the blood samples. The tubes were gently mixed in a rotator shaker and then incubated at 37 °C ± 1 °C within a shaking water bath for 30 minutes. Alsever's solution and deionized water were used as negative (0% hemolysis) and positive (100% hemolysis) controls, respectively. Each group contained three tubes. The specimens were then centrifuged under 3000 rpm for 4 min to collect the supernatant. In order to avoid false-positive results due to nanoparticles absorbance at the assay wavelength, the iron oxide nanoparticles were removed from supernatants using permanent magnets [11].

The absorbance (A) value of the hemoglobin released from the erythrocyte cells was measured spectrophotometrically at 415 nm (Thermospectronic Genesys 5). All trials were run three times. The hemolysis rate (HR) was calculated as