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MAGNETIC NANOFLUID WITH ANTITUMORAL PROPERTIES

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Abstract

The present study deals with the synthesis and characterization of magnetic nanofluid and it's in vitro anti-cancer activity against HEp2 cells. The magnetic nanofluid with an average size of 10 nm was synthesized via a modified precipitation technique and characterized by FT-IR, XRD, DTA-TG and TEM. After 24 h incubation of HEp2 with the magnetic nanofluid, significant changes in the cell morphology were discernible in fluorescent microscopy. Cytotoxicity assay shows that the magnetic nanofluid exhibits significant cytotoxicity against HEp2, 50% of thee cells being killed after 24 hours incubation with magnetic nanofluid without any external alternating magnetic field.

Keywords *Magnetic nanofluid, magnetite, HEp2 cells, antitumoral activity*

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Introduction

Magnetite is currently investigated because of their numerous applications such as nanofluids [1], drug delivery, improve sensibility of microorganisms against different beta-lactamic drugs [2], bone cancer treatment [3], magnetic resonance imaging [4], stabilization of essential oils [5], inhibition of microbial biofilms [6], antipathogenic surfaces [7] and water purification [8]. Fe_3O_4 has attracted attention in biomedical applications because of their biocompatibility and low toxicity in the human body [9,10]. It is necessary to modify magnetite surface with an organic or inorganic shell, in order to protect them from chemical degradation or agglomeration according to the environments in which they will be used [11,12]. Magnetic hyperthermia is a technique by which magnetic nanoparticles are either introduced or sent within tumors and heated under the application of an alternating magnetic field [13].

Hyperthermia, as an adjuvant therapy of cancer, is appealing because of its fewer side effects than chemotherapy and radiotherapy, and feasible repeated treatments without concern for cumulative toxic side effects [14]. In this paper is reported the synthesis and characterization of magnetic nanofluid with antitumoral properties without application of any alternating magnetic field.

Experiment Details

Synthesis. The magnetic nanofluid was synthesized by a modified precipitation method [1,15,16]. Half gram of myristic acid (C_{14}) was solubilized in a known volume of ultrapure water, corresponding to a 1.00% (w/w) solution, under stirring at room temperature. Then, 4 mL of a basic aqueous solution consisting of

28% NH_3 were added to C_{14} solution. Thereafter, 100 mL of $FeSO_4/FeCl_3$ (1.2/0.6 %/%) were dropped under permanent stirring up to pH = 9. The product was repeatedly washed with methanol and separated with a strong NdFeB permanent magnet.

Characterization.

FT-IR. A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain the FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25^{o}C). FT-IR spectra were collected in the frequency range of 4000–650cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were ratioed against a background of an air spectrum.

XRD. X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle *2θ* range of 10-80.

HR-TEM. The transmission electron images were obtained on finely powdered samples using a Tecnai^{TM} G2 F30 S-TWIN high resolution transmission electron microscope (HRTEM) from FEI. The microscope was operated in transmission mode at 300 kV with TEM point resolution of 2 Å and line resolution of 1 Å. The finely MNPs powder was dispersed into pure ethanol and ultrasonicated for 15 minutes. After that diluted sample was put onto a holey carbon coated copper grid and left to dry before it was analyzed through TEM.

DTA-TG. The differential thermal analysis (DTA) coupled with thermo gravimetric analysis (TGA) was performed with a Shimadzu DTG-TA-50H, at a scan rate of 10^{o}C/min, in air.

Bioevaluation. For the quantification of eukaryotic cell viability, propidium iodide (PI) and fluorescein diacetate (FdA) stains were used. Briefly, magnetic nanofluid was coated on glass slides [3]. Each coated slide was transferred into 3,5 Petri dish and 2 mL of complete medium containing 3 x10^5 HEp2 cells were added. The effect of coated substances on cell viability was evaluated after 24 hours by adding 100 µL PI (0.1 mg/mL) and 100 µL FdA (0.1 mg/mL) and fluorescence was quantified using Observer.D1 Carl Zeiss microscope. All cells from several fields were counted and the cell viability was established by the ratio between viable cells number (green) and number of total cells (viable cell - green and dead cells - red).

Results and Discussions

The purity and crystalline properties of the sample was investigated by X-ray diffraction. The XRD pattern of sample is shown in Figure 1.

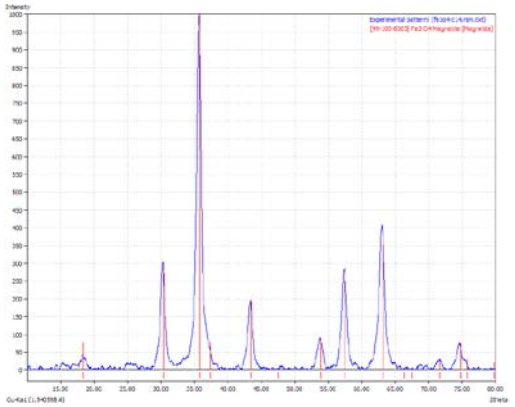

Figure 1: *XRD patterns of prepared sample*

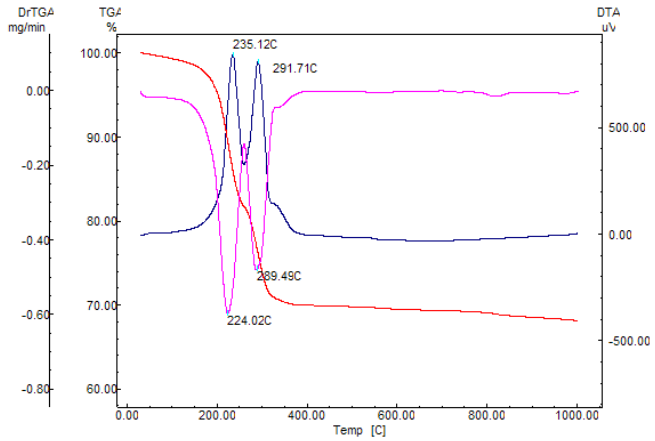

Figure 2: *Thermal analysis of Fe_3O_4@C14*

No additional peaks have been observed indicating the formation of pure and single phase without impurities

that remain from the un-reacted precursors of the formation of other phases such as Fe_2O_3.

The complex thermal analysis of the obtained material confirms the existence of $C_{13}H_{27}COO^-$ adsorbed on the surface of magnetite core (Figure 2). The degradation of the organic shell can be relieved by both DTA and TGA. The DTA curve exhibit two intense exothermic effects at 235 ^oC and 292 ^oC and a less intense exothermic effect at ~330 ^oC.

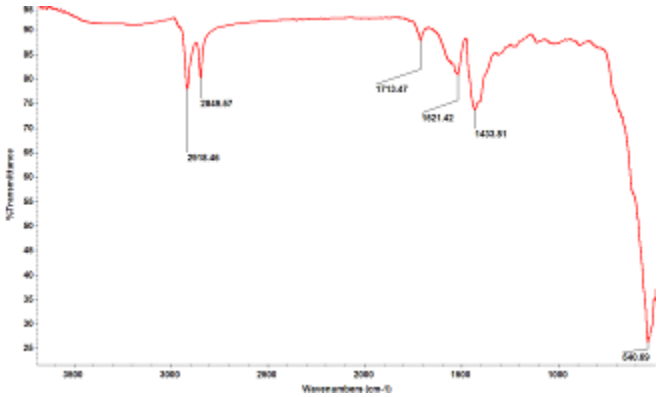

Figure 3: *FT-IR spectra of nanofluid*

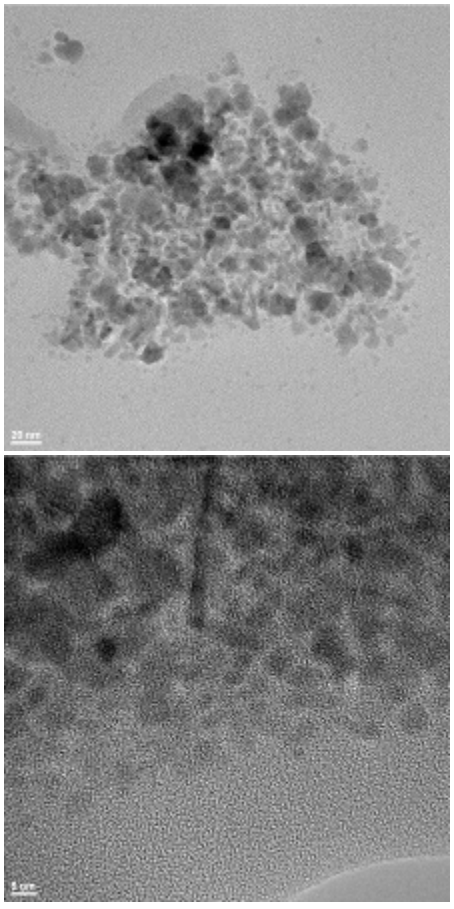

Figure 4: *TEM images of magnetic nanofluid*

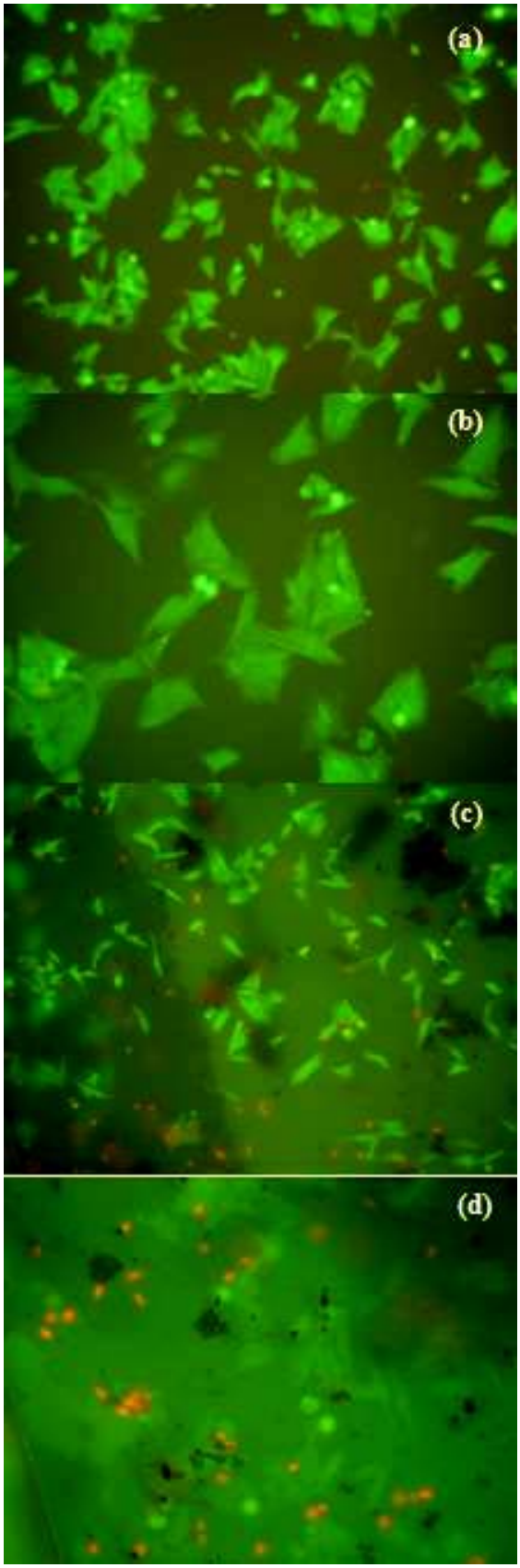

Figure 5: *HEp2 cells line (a,b) control; (c,d) treated with nanofluid (200x)*

From the point of view of weight loss the adsorbed miristate is lost, in three successive steps, corresponding to the three above mentioned exothermic effects. The total lost of the organic components is ~30%. It has to mention that no free water can be identified by DTA and TGA. Based on TGA data, Fe_3O_4:C_{14} ratio was estimated to be 7:3. The FT-IR analysis (Figure 3) identified the organic coating on the surface of the magnetite nanoparticles. The peak recorded at about 1713 cm^{-1} at FT-IR spectrum of the nanofluid show the C=O stretching vibration of fatty acids. The peaks at 2918 and 2849 cm^{-1} were assigned to stretching vibration of C–H. The peak at 540 $cm^{-1}$was assigned to magnetite. All these bands are in concordance with reported literature [17,18]. TEM images of the obtained nanofluid are shown in figure 4. The particles are very small (nanometric size) with homogenous particles size distribution. Moreover, it can be observed that the particles posses a quasi-spherical shape without aggregation. The average size of nanoparticles obtained from TEM images is about 10 nm. Figure 5 shows the morphology of HEp2 cell line grown with and without nanofluid. Antitumoral effects of Fe_3O_4@C_{14} treatment for 24 h on the HEp2 cell line were evaluated by double fluorescent staining with PI and FDA. Nanofluid showed antitumoral activity on the tested eukaryotic human epidermoid cancer cells (Figures 4c and 4d), as revealed by the high cytotoxicity rate, demonstrating the potential use of this nanostructured system for biomedical applications.

Conclusions

This report describes the preparation of a magnetic nanofluid with antitumoral properties, without any external alternating magnetic field. Myristic acid was used as organic shell. XRD pattern identify the magnetite as unique crystalline phase. FT-IR proved the presence of C_{14} in the structure of nanofluid. According to thermal analysis the containing of organic component in magnetic nanofluid is ~30%. The biological cytotoxcity assay on the tumoral Hep 2 cells highlights the potential useful of this magnetic nanofluid in the development of antitumoral treatments.

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