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Assessment of the capability of maghemite nanoparticles for hyperthermia application and study their cytotoxicity on cancer cell lines

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ABSRACT: Highly monodispersed maghemite nanoparticles with sizes around 13.58 nm were successfully synthesized by co-precipitation method. The morphology of maghemite nanoparticles was studied by scanning electron microscope (SEM). The capability of maghemite to act as heat nano-sources upon application of an alternating magnetic field (AMF) was investigated viaexposing them to AMF and the specific absorption rate (SAR) was calculated from temperature-time curve. SAR values proved to be concentration dependent with SAR of 135 watt/g at a concentration of 10 mg/ml of maghemite. Furthermore, the cytotoxicity of maghemite was evaluated against human colorectal adenocarcinoma cells (Caco-2 cells). The results reveal the potential of maghemite nanoparticles for application in cancer treatment.

KEYWORDS: maghemite, hyperthermia, specific absorption rate (SAR), cytotoxicity

I. INTRODUCTION

With the development of nanotechnology, resources to various applications in the medical field has been provided, leading to significant advances in terms of diagnosis, biological detection, therapy and drugdelivery [1].In this context, magnetic nanoparticles possess important characteristics that make them attractive for various biomedical applications including tissue repair, magnetic resonance imaging(MRI)[2],bio-separation, cell tracking [3],drug delivery[4] and hyperthermia[5]. Specifically, iron oxides nanoparticles have attracted significant attention, due to their superior properties like biocompatibility, low toxicity, stability, availability for surface modification, and higher relaxation values, thus presenting unique characteristics for clinical applications[6].

Hyperthermia is known as an alternative treatment that can be delivered alone or as an adjunct to radiation and/or chemotherapy to treat cancer. The temperature is locally raised, causes a change of the physiology of diseased cells, finally leading to cell death. On the basis of the degree of temperature raise, hyperthermia treatment can be categorized into different types namely; moderate hyperthermia and thermablation[7]. In thermo ablation, a tumor is exposed to high temperatures ≥46 °C up to 56 °C, which cause cells to undergo direct tissue necrosis, coagulation or carbonization. Whereas, moderate hyperthermia (41 °C <T<46 °C), causes the cells to undergo physiological changes leading to induction and regulation of apoptosis. However, the conventional hyperthermia has many limitations asnonselective heating of the health tissues which results in burns, blisters and discomfort in addition to limited penetration of heat into deep tissues leading to insufficient dosage and ultimately, inefficient treatment. Consequently, magnetic fluid hyperthermia is utilized to overcome all these limitations. Magnetic fluid is a stable colloid based on maghemite nanoparticles, suspended in water. The fluid is assumed to be injected into the tumor and can be targeted by applying static magnetic field. When these nanoparticles are in the vicinity of tumor and exposed to AMF, the heat is generated to kill the tumor cells avoiding heating of healthy tissues. The current study focuses on the assessment of the feasibility of maghemite as a candidate for magnetic hyperthermia.

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Conflicting evidence regarding the toxicity of maghemite has been investigated in vitro and in vivostudies. For instance, changes in nanoparticle size and shape proved to influence cell toxicity, with rod-shaped or nano-sized maghemite being more toxic than sphere-shaped and micrometric particles, respectively [8].Furthermore, the surface charge of maghemite was found to affect cell cytotoxicity;positively charged maghemite showed to be more toxic, since they produce nonspecific interactions and adsorptive endocytosis with the negatively charged cell membrane, thus increasing their intracellular accumulation and affecting cell membrane integrity [9]. Other parameters such as concentration, type of coating, mode of administration, as well as the cell line may explain the different results for maghemite toxicity. The present work studies the effect of concentration of maghemite nanoparticles on toxicity of Caco-2 cell lines besides the assessment of the hyperthermia capability of maghemite.

II. EXPERIMENTAL WORK

A. Synthesis of maghemite nanoparticles

Maghemite nanoparticles were synthesized by co-precipitation method of an aqueous solution containing Fe^{2+} ($FeCl_2{\cdot}4H_2O$, 99%) and Fe^{3+} ($FeCl_3{\cdot}6H_2O$, 97%) salts in the molar ratio Fe^{2+}/Fe^{3+}of0.65 with sodium hydroxide (NaOH, 10M). After stirring for 15 minutes at 80 ˚C, the black precipitate was separated by strong magnet and washed several times with distilled water. Finally maghemite nanoparticles were dried under vacuum at room temperature overnight.

B. Characterization

The crystal phase of the as-prepared maghemitenanoparticles was determined by powder X-ray diffractometry (Bruker D8Advanced Diffractometer System) with a Cu Ka (1.5418 Å) source. The grain size and morphology of maghemite nanoparticles were examined using scanning electron microscope (SEM). The elemental composition ofmaghemitenanoparicles was investigated using energy dispersive X-ray (EDX) spectrometer (OXFORD INCA Penta FETX3- England). Specific absorption ratio (SAR) of maghemite sample was measured by induction heater (DW-VHF 10 KW, China at 198 kHz, 9.4 kA/m), a constant volume of the solution of the sample (5ml of 3 and 10mg/ml solution of magnetic colloid) was placed in glass vial that was inserted in Styrofoam jacket (insulator) and the temperature was measured by alcohol thermometer. The experimental setup is shown in **Fig. 1**

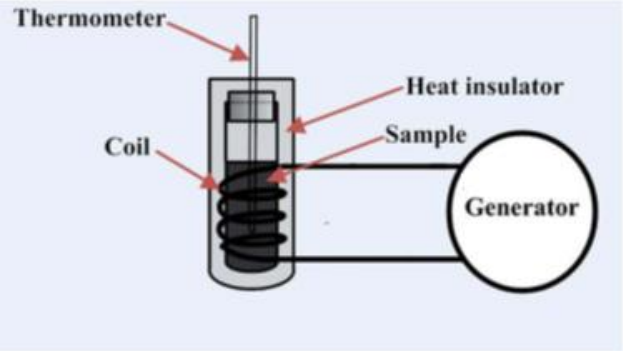

Fig.1 Experimental setup for hyperthermia measurements

Cytotoxicity of maghemite nanoparticles was investigated by MTT assay. Caco-2 cell lines (1 X 10^5 cells / ml) were cultured as monolayer in Roswell Park Memorial Institute (RPMI) 1640 medium in 96 well tissue culture plate. Cells were kept in a humidified atmosphere of 95% air and 5% CO_2 at 37°*C* for 24 hours. Sample was two folds diluted in (RPMI) 1640 Medium with 2% serum (maintenance medium). Cells were treated with 0.1 ml of each dilution of the tested samples leave 3 wells as control (receiving only maintenance medium). Tissue culture Plate was incubated at 37°C. MTT solution was prepared (5 mg/ml in PBS) and 20µl of MTT solution were added to each well.The plate was incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C for 1-5 hours to allow the MTT to be metabolized. The medium was removed and formazan crystal (metabolic product of MTT) was dissolved in 200µl DMSO. The plate was placed on a shaking table (150 rpm) for 5 minutes to thoroughly dissolve the formazan crystal. Optical density was measured at 570 nm and subtracts the background at 620nm.

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III. RESULTS AND DISCUSSIONS

A. Structure and morphology

Fig.2 illustrates the XRD patterns of maghemite nanoparticles. The identical diffraction peaks typical of maghemite observed at 2θ = 30.169◦, 32.054, 35.452◦, 43.254◦, 56.783◦, 62.728◦ and 73.997◦. The data were indexed and showed to be consistent with ICDD card 89-0688. The sample was crystallized in single phase cubic structure with lattice parameter a =8.40 Å was calculated by **Equation 1.** The average crystallite sizes of Maghemite sample was calculated using the Debye–Scherrer's[10]**Equation 2**

$$d_{hkl}^2 = \frac{a^2}{h^2+k^2+l^2} \quad (1)$$

$$T = \frac{C\lambda}{\beta \cos\theta} (2)$$

where T is the average crystallite size, λ is the wavelength of the X-ray radiation, β is the full-width at half-maximum intensity of the powder pattern peak and θ is the Bragg angle. The obtained value of crystallite size is13.58 nm. This size range is convenient for biomedical applications[11].

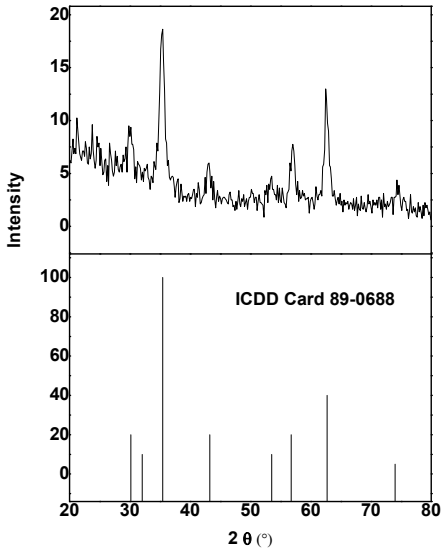

Fig.2 XRD pattern of maghemite nanoparticles

The morphology of the investigated sample was studied using scanning electron microscope (SEM). **Fig.3(a**) illustrates the SEM image of the maghemite sample. The average grain size of the nanoparticles is 26.16 nm. The value of grain size is greater than the average crystallite size from the XRD results (13.58 nm) due to The aggregation of the particles owing to the magnetic interactions [12]. **Fig.3 (b**) showed the morphology of the nanoparticles at high magnification scale. The EDX spectrum of the maghemite is shown in **Fig.3(c)**which confirms the homogenous mixing of the Fe and O ions. The chemical composition of the maghemite is illustrated in Table 1. The values of experimental and theoretical compositions are consistent.

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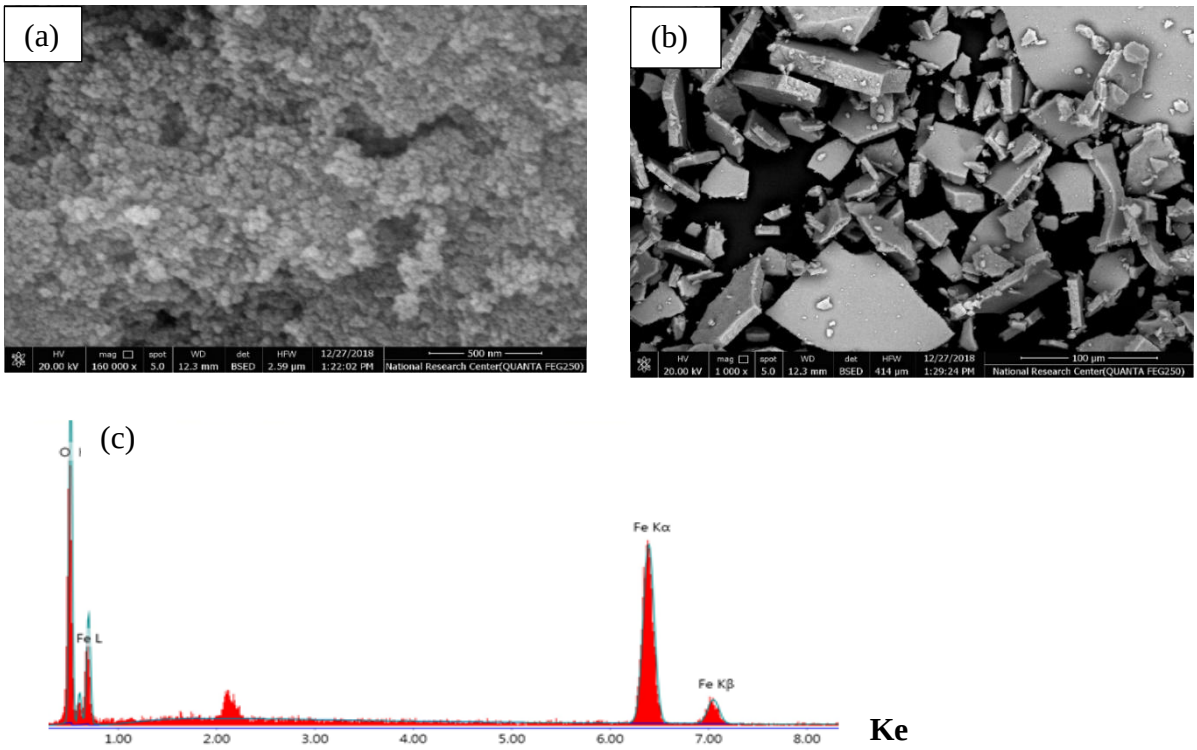

Fig.3: a), b) Scanning electron microscope images of maghemite nanoparticles showing their grain size and morphology respectively; c) EDX spectrum of the maghemite nanoparticles

Table 1 iron and oxygen weight ratios in maghemite from EDX analysis

Elements	Weight percentage (wt. %)		Atomic percentage (at.%)	
	Theoretical	EDX analysis	Theoretical	EDX analysis
O K	28.12	27.64	57.73	57.14
Fe L	71.88	72.36	42.27	42.86

B. Hyperthermia measurements

In order to evaluate applicability of maghemite for hyperthermia,the magnetic fluid was exposed to alternating magnetic field with amplitude of 9.4 kA/m and frequencyof 198 kHz.The specific absorption rate of the ferrofluid is calculated by Equation 3

$$SAR = \frac{\sum m_i \; c_i}{m_{mag}} \frac{\Delta T}{\Delta t} \quad (3)$$

Where c_i is the individual specific heat of water and glass vial that are 4.18 and 0.793 $JK^{-1}g^{-1}$ respectively, m_i is the individual mass of water and glass vial; 5 and 5.2758 grams respectively, $\frac{\Delta T}{\Delta t}$ is the initial slope of the time-dependent temperature curve and $m_{mag}$is the mass of maghemitein the solution in grams[13].**Fig.4** shows the temperature rise with time curve for two concentrations of the magnetic fluid (3 and 10mg/ml) exposed to AMF, as the concentration increases, SAR value and hence the potential of maghemite nanoparticle for hyperthermia increases as reported from literature[14]. The calculated SAR values for 3 and 10 mg/ml are 76.77 and 187.66 Watt/g respectively. SAR increases

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with concentration due to increasing the magnetic dipolar interactions between the particles. From the curve, the temperature reaches the therapeutic temperature (≥42℃) in few minutes even at the low concentration (3mg/ml).

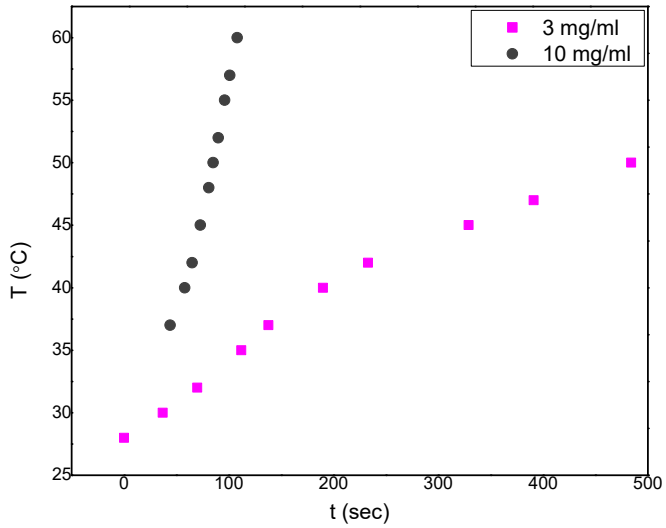

Fig.4 time-temperature curve for maghemite nanoparticles

C. Cytotoxicity of maghemite nanoparticles

In the present work, the cytotoxicity of maghemite was evaluated against human colorectal carcinoma cell linebased on MTT assay. **Fig.5** illustrates a decrease in the viability of Caco-2 cancer cells against the increasing concentration of maghemite. IC 50%of maghemite is 1.533 mg/ml; this concentration caused 50% death of cancer cells. High concentrations of maghemite nanoparticles resulted in complete cytotoxicity of Caco-2 cells as listed in **Table 2**, whereas, low concentrations has low cytotoxicity as was shown in previous studies [15–17]. The high SAR value of the prepared magnetic fluid, even at low concentration, can be efficiently manifested in cancer treatment by hyperthermia.

Table 2 cytotoxicity of different concentrations of maghemite nanoparticles against Caco-2 cells

ID	Conc. mg/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
Caco2	--------	0.324	0.339	0.351	0.338	0.00781	100	0	
1	50	0.018	0.017	0.019	0.018	0.000577	5.325443787	94.67455621	1.533
	25	0.02	0.018	0.018	0.018667	0.000667	5.522682446	94.47731755	
	12.5	0.019	0.018	0.017	0.018	0.000577	5.325443787	94.67455621	
	6.25	0.035	0.026	0.037	0.032667	0.003383	9.66469428	90.33530572	
	3.12	0.086	0.062	0.079	0.075667	0.007126	22.38658777	77.61341223	
	1.56	0.183	0.208	0.199	0.196667	0.007311	58.18540434	41.81459566	

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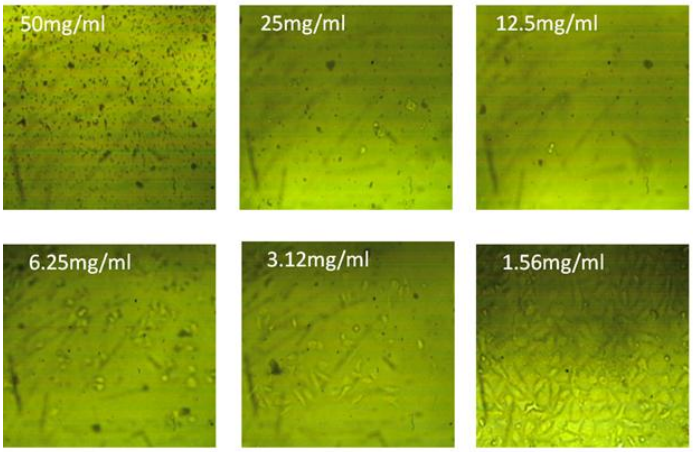

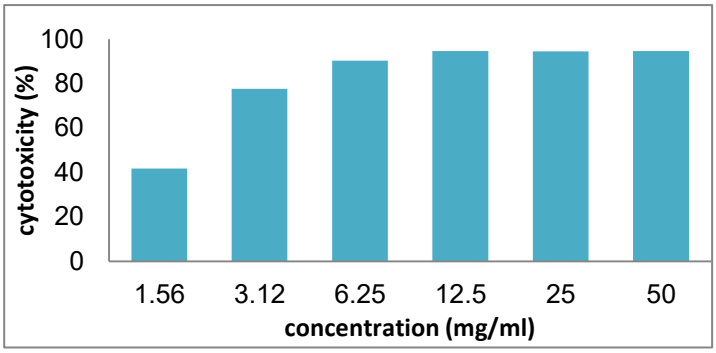

Fig.5 cytotoxicity of maghemite nanoparticles against Caco-2 cells

IV. CONCLUSION

Maghemite nanoparticles were chemically synthesized with crystal diameter of 13.58 nm, which is convenient for various biomedical applications. The influence of concentration of ferrofluid on the specific power absorption was studied. The maghemite are capable of reaching therapeutic hyperthermia temperature in few minutes even at low concentrations. The cytotoxicity of maghemite nanoparticles against Caco-2 cell lines was also investigated. The viability measurements showed that high concentrations of maghemitenanoparticles cause complete cytotoxicity for cancer cell lines. Finally, the study revealed that low concentration of maghemite nanoparticles has great potential to be applied in cancer therapy by hyperthermia.

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