# Evaluation of the Heating Capability and *in vitro* Hemolysis of Nanosized $Mg_xMn_{1-x}Fe_2O_4$ (x = 0.3 and 0.4) Ferrites Prepared by Sol-gel Method

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***Abstract*—**Among the different cancer treatments that are currently used, hyperthermia has a promising potential due to the multiple benefits that are obtained by this technique. In general terms, hyperthermia is a method that takes advantage of the sensitivity of cancer cells to heat, in order to damage or destroy them. Within the different ways of supplying heat to cancer cells and achieve their destruction or damage, the use of magnetic nanoparticles has attracted attention due to the capability of these particles to generate heat under the influence of an external magnetic field. In addition, these nanoparticles have a high surface area and sizes similar or even lower than biological entities, which allow their approaching and interaction with a specific region of interest. The most used magnetic nanoparticles for hyperthermia treatment are those based on iron oxides, mainly magnetite and maghemite, due to their biocompatibility, good magnetic properties and chemical stability. However, in order to fulfill more efficiently the requirements that demand the treatment of magnetic hyperthermia, there have been investigations using ferrites that incorporate different metallic ions, such as Mg, Mn, Co, Ca, Ni, Cu, Li, Gd, etc., in their structure. This paper reports the synthesis of nanosized $Mg_xMn_{1-x}Fe_2O_4$ (x = 0.3 and 0.4) ferrites by sol-gel method and their evaluation in terms of heating capability and *in vitro* hemolysis to determine the potential use of these nanoparticles as thermoseeds for the treatment of cancer by magnetic hyperthermia. It was possible to obtain ferrites with nanometric sizes, a single crystalline phase with an inverse spinel structure and a behavior near to that of superparamagnetic materials. Additionally, at concentrations of 10 mg of magnetic material per mL of water, it was possible to reach a temperature of approximately 45°C, which is within the range of temperatures used for the treatment of hyperthermia. The results of the *in vitro* hemolysis assay showed that, at the concentrations tested, these nanoparticles are non-hemolytic, as their percentage of hemolysis is close to zero. Therefore, these materials can be used as thermoseeds for the treatment of cancer by magnetic hyperthermia.

***Keywords*—**Ferrites, heating capability, hemolysis, nanoparticles, sol-gel.

## I. Introduction

THE search for a better quality of life has led to the development of more efficient treatments for diseases that affect a significant percentage of the population worldwide. In the specific case of cancer, one treatment that has attracted attention is hyperthermia, mainly due to the overcoming of some of the disadvantages that are present in the conventional approaches for treating this disease (surgery, radiation and chemotherapy). Generally, hyperthermia is a technique that utilizes heat to destroy or damage cancer cells. This procedure involves a moderate increase of temperature in the body or a specific region of it, above the normal value that is established by the thermoregulation system of an organism in a particular moment [1], [2]. The aim of increasing the temperature is achieving the eradication of cancer cells or provoke a higher sensitization of them to the effects of radiation and chemotherapy [1]–[3]. The range of temperatures used in this procedure varies between 41°C and 46°C, and these are maintained for periods of one hour or more for the majority of the tissues without causing damage to normal cells [1], [4], [5]. The increase of temperature in the tissues can be accomplished by several means, depending on the location, depth and staging of the tumor [1]. One approach that is being highly studied nowadays is the use of magnetic nanoparticles, mainly due to several attractive characteristics, which include dimensions smaller than or comparable to cells, viruses, proteins and gens, the possibility of coating them to interact with biological entities and the capability of these to produce heat under the action of an external magnetic field [6]. This technique is specifically termed magnetic hyperthermia and consists in the supply of the magnetic nanoparticles to a target tissue and then the application of an external magnetic field that results in the heating of the nanoparticles. Within the magnetic nanoparticles that are used for medical applications, the most used is magnetite [6]–[8]. However, recent research explores the possibility of incorporating different cations in the crystalline structure of ferrites [4], [9]; this is in order to improve either their magnetic properties or biocompatibility. The synthesis of mixed ferrites can be accomplished by several chemical methods, and among these sol-gel method offers the possibility of obtaining high purity and homogeneous nanoparticles [10], [11]. In this study, the synthesis of $Mg_xMn_{1-x}Fe_2O_4$ (x = 0.3 and 0.4) ferrites was carried out by sol-gel method, and then the obtained products were evaluated in terms of heating capability and *in vitro* hemolysis to determine their potential use as thermoseeds for the treatment of cancer by magnetic hyperthermia.

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## II. Materials and Methods

### A. Materials

Nanosized $Mg_xMn_{1-x}Fe_2O_4$ (x = 0-1) particles were synthesized by sol-gel method, using ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$), magnesium nitrate ($Mg(NO_3)_2 \cdot 6H_2O$), manganese nitrate ($Mn(NO_3)_2 \cdot 4H_2O$) and ethylene glycol ($C_2H_6O_2$) as precursors.

*B. Synthesis of the Magnetic Nanoparticles by Sol-Gel Method*

Stoichiometric amounts of ferric nitrate, magnesium nitrate and manganese nitrate were dissolved into 5 mL of ethylene glycol in a 100 mL beaker. This solution was stirred for 2 h at 40 °C, and the obtained sol was then heated up to 80°C and kept at this temperature until a brown gel was formed. The gel was aged for 2 h at room temperature and then dried at 95°C for 72 h. Subsequently, the dry gel was heat treated at 50°C in air for 60 min. The obtained products were milled and washed several times with ethanol, in order to remove the ethylene glycol excess. Finally, the powders were dried at room temperature.

*C. Sample Characterization*

The characterization of the products was carried out by X-ray diffraction (XRD), vibrating sample magnetometry (VSM) and transmission electron microscopy (TEM). The phase structures of magnetic nanoparticles were characterized by XRD, using a X'Pert Philips diffractometer, with Cu Kα radiation (λ= 1.5418 Å) at a scanning rate of 0.02° $s^{-1}$ (2θ scale) into the 20-80° interval. The magnetic properties were evaluated using a vibrating sample magnetometer PPMS (Physical Property Measurement System) of Quantum Design model 6000, with an option to measure the hysteresis loop in a measurement time of 60 min. TEM images were acquired using a Titan 80-300 microscope. For the evaluation of the heating capability of the nanoparticles an AMBRELL magnetic induction equipment model EasyHeat was used, with a power input of 200.2 A and a magnetic field of 10.2 KA/m with a frequency of operation of 354 KHz. The hemolysis assay was carried out according to the procedure described in the Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles E2524 – 08.

## III. Results and Discussion

*A. XRD Analysis*

Fig. 1 shows the XRD patterns of $Mg_xMn_{1-x}Fe_2O_4$ (x = 0, 0.3, 0.4 and 1) nanoparticles synthesized by sol-gel method and heat treated at 500°C for 1 h. In these, it can be observed for all the cases the presence of a single crystalline phase, which reflections correspond to a cubic inverse spinel structure and are similar to those of $MgFe_2O_4$ (JCPDS 88-1935).

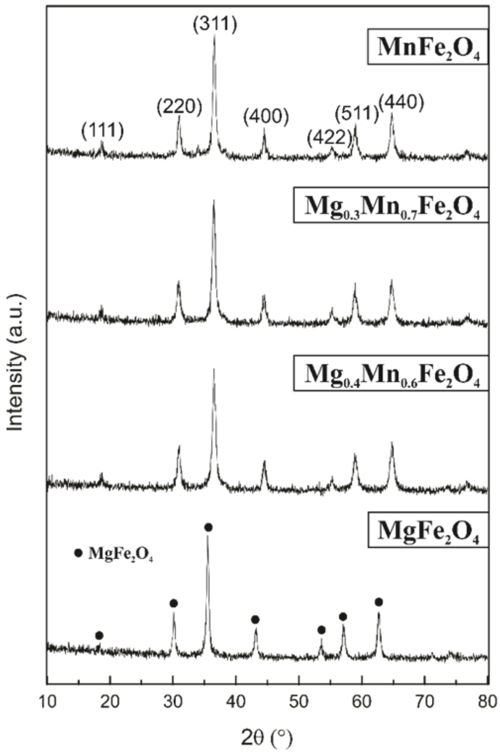


Fig. 1 XRD patterns of $Mg_xMn_{1-x}Fe_2O_4$ (x = 0, 0.3, 0.4 and 1) nanoparticles synthesized by sol-gel method and heat treated at 500°C for 60 minutes

*B. VSM Analysis*

Table I shows the magnetic properties and crystallite size (calculated by the Scherrer equation) of the $Mg_xMn_{1-x}Fe_2O_4$ (x = 0, 0.3, 0.4 and 1) nanoparticles. These results show that the manganese ferrite ($MnFe_2O_4$) and magnesium ferrite ($MgFe_2O_4$) show the highest and lowest values of saturation magnetization ($M_s$) respectively, and the mixed ferrites ($Mg_xMn_{1-x}Fe_2O_4$, x = 0.3 and 0.4) have values of $M_s$ between these two. The value of $M_s$ decreases when the content of Mn also decreases, this due to the fact that Mn has a higher magnetic moment ($\mu_B$=5) than Mg ($\mu_B$=0) [12], and their distribution in the interstitial sites of the spinel structure has a major influence on the values of this property [13]. In addition, in all cases the values of remanence ($M_r$) and coercivity ($H_c$) are close to zero, which results in a behavior near to the superparamagnetic regime. This can be observed also in Fig. 2, where the hysteresis loops of the ferrites show that these materials have a soft ferrimagnetic behavior that tends to the superparamagnetism, as the typical sigmoidal curve shape occurs.

TABLE I
MAGNETIC PROPERTIES (SATURATION, REMANENCE AND COERCIVITY) AND CRYSTALLITE SIZES (CALCULATED BY THE SCHERRER EQUATION) OF $MG_XMN_{1-X}FE_2O_4$ (X= 0, 0.3, 0.4 AND 1) NANOPARTICLES SYNTHESIZED BY SOL-GEL METHOD AND HEAT TREATED AT 500°C FOR 60 MINUTES.

| $Mg_xMn_{1-x}Fe_2O_4$ (x = 0, 0.3, 0.4 and 1) | Saturation Ms (emu/g) | Remanence Mr (emu/g) | Coercivity Hc (Oe) | Crystallite Size Scherrer equation (nm) |
|---|---|---|---|---|
| $MnFe_2O_4$ | 49.7117 | 1.0156 | 10 | 18 |
| $Mg_{0.3}Mn_{0.7}Fe_2O_4$ | 41.2598 | 0.7843 | 10 | 15 |
| $Mg_{0.4}Mn_{0.6}Fe_2O_4$ | 38.6570 | 0 | 0 | 15 |
| $MgFe_2O_4$ | 24.3022 | 0.5681 | 10 | 21 |

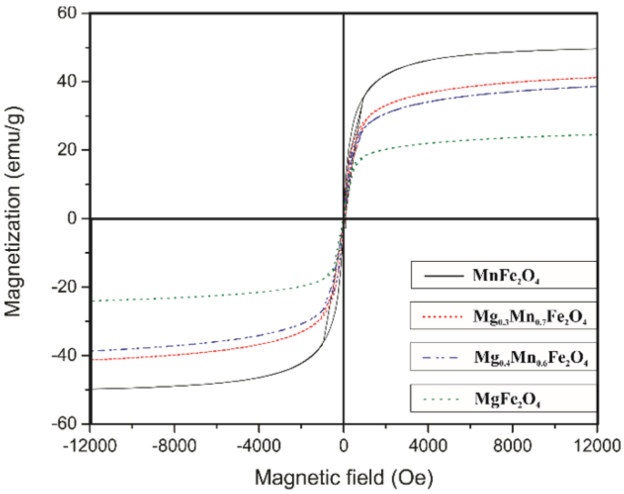


Fig. 2 Hysteresis loops of $Mg_xMn_{1-x}Fe_2O_4$ (x = 0, 0.3, 0.4 and 1) nanoparticles synthesized by sol-gel method and heat treated at 500°C for 60 minutes

*C. TEM Analysis*

Fig. 3 shows the TEM images of the $Mg_{0.3}Mn_{0.7}Fe_2O_4$ and $Mg_{0.4}Mn_{0.6}Fe_2O_4$ nanoparticles. It can be observed for both samples a spherical-like morphology and sizes in accordance to those calculated by the Scherrer equation. Furthermore, due to the soft ferrimagnetic behavior of the particles and their nanometric size, agglomerates can be observed. This also as a result of the permanent magnetic moments that experience small particles with single domains, which causes them to be permanently magnetized and agglomerate, as reported by Iftikhar et al. [14].

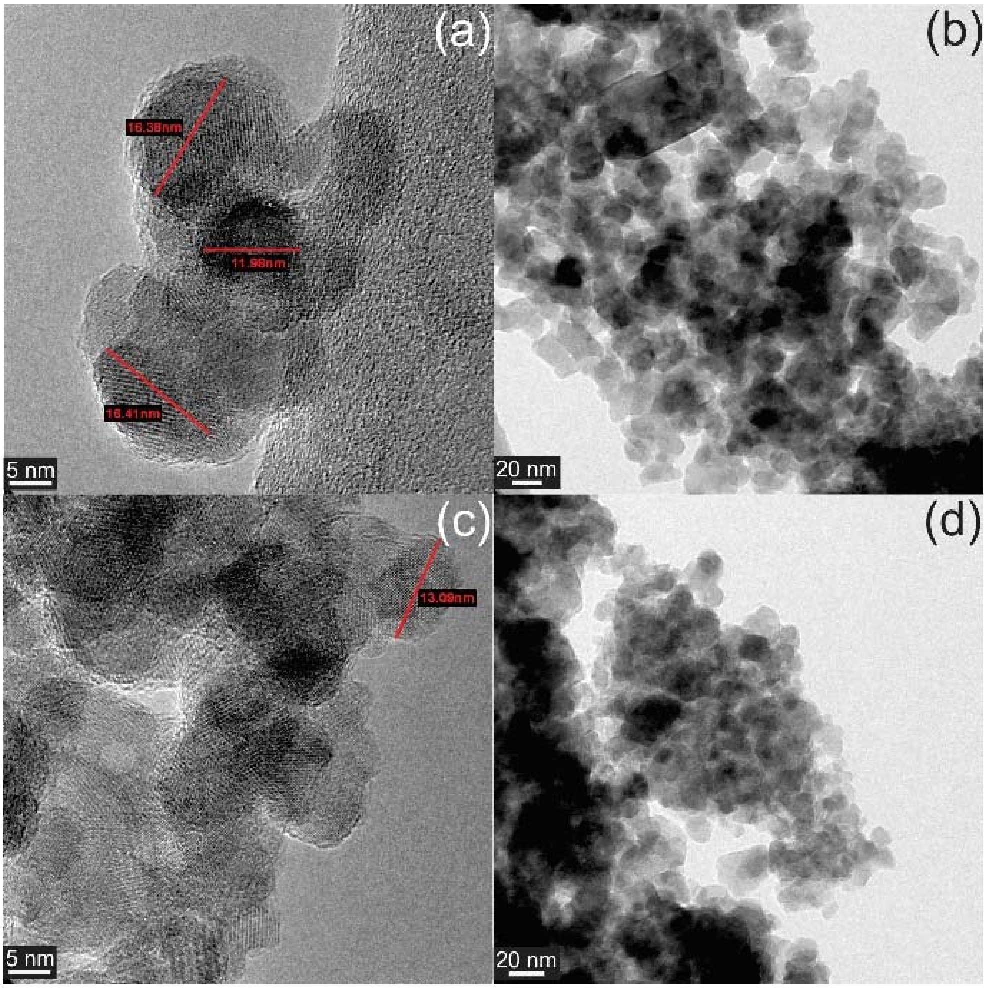


Fig. 3 TEM images of (a, b) $Mg_{0.3}Mn_{0.7}Fe_2O_4$ and (c, d) $Mg_{0.4}Mn_{0.6}Fe_2O_4$ nanoparticles synthesized by sol-gel method and heat treated at 500°C for 60 minutes

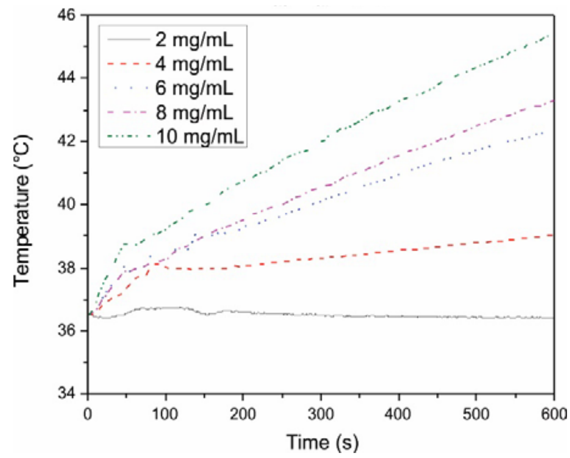


Fig. 4 Magnetic induction heating curves of the $Mg_{0.3}Mn_{0.7}Fe_2O_4$ nanoparticles dispersed in water at concentrations of 2,4,6,8 and 10 mg/mL

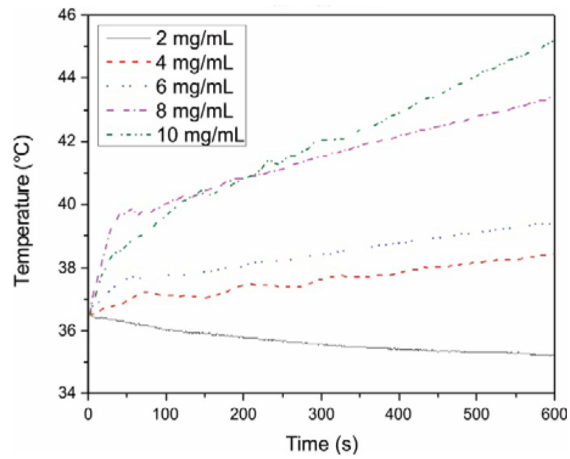


Fig. 5 Magnetic induction heating curves of the $Mg_{0.4}Mn_{0.6}Fe_2O_4$ nanoparticles dispersed in water at concentrations of 2,4,6,8 and 10 mg/mL

### *D. Heating Capability Test*

Figs. 4 and 5 present the magnetic induction heating curves of the $Mg_{0.3}Mn_{0.7}Fe_2O_4$ and $Mg_{0.4}Mn_{0.6}Fe_2O_4$ nanoparticles dispersed in water at different concentrations (2, 4, 6, 8 and 10 mg/mL), respectively. For both samples, the initial temperature was established in 36.5°C and the evaluation of the heating capability was carried out for a time of 10 minutes. It can be observed that as the concentration of the particles increases, a higher temperature is reached. This corroborates the potential of the particles to generate heat under the action of an external magnetic field. Additionally, at the concentration of 10 mg/mL it was possible to accomplish, in both cases, a temperature of approximately 45 °C. In fact, at lower concentrations and times it was possible to obtain a temperature of 39 °C, which according to Bettaieb et al. [2], between temperature range 39 °C and 45 °C it is possible to cause damage or even the death of cancer cells without causing any significant damage to healthy tissues.

### *E. Hemolysis Assay*

The toxicity *in vitro* generated by the materials when they are in contact with the erythrocytes (red blood cells) was measured according to the hemolysis assay described in the Standard Test Method for Analysis of Hemolytic Properties of Nanoparticles E2524 – 08. Generally, this procedure is based in the determination of the hemoglobin that is released when the erythrocytes are in contact with nanomaterials. Concentrations of 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL and 10 mg/mL were tested accordingly with what is reported for Pankhurst et al. [6], who found that concentrations between 5 mg and 10 mg per $cm^3$ of tumor tissue are adequate for magnetic hyperthermia in human patients. The results of this test showed that at all concentrations, for both samples, the degree of hemolysis is non-significant with values below 1%.

## IV. Conclusion

Nanoparticles of $Mg_xMn_{1-x}Fe_2O_4$ (x = 0.3 and 0.4) with a single cubic spinel structure and a soft ferrimagnetic behavior near to the superparamagnetic regime were successfully synthesized by sol-gel method. In addition, these nanoparticles exhibited a spherical-like morphology and nanometric sizes (≈ 15 nm). Both samples, at concentrations of 10 mg of magnetic material per mL of water reached in 10 minutes a temperature of approximately 45 °C, which is within the range of temperatures used for the treatment of hyperthermia. Moreover, at the concentrations tested (2, 4, 6, 8 and 10 mg/mL), the materials show a non-significant hemolytic degree. Therefore, taking into account the obtained results, these nanoparticles have a promising potential to be used as thermoseeds in the treatment of cancer by magnetic hyperthermia.

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