# Synthesis and characterization of magnetic nanoparticles for cancer therapy

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## ABSTRACT

Magnetic nanoparticles are widely used in biomedical applications such as in magnetic resonance imaging, targeted drug delivery and cancer therapy. These particles have been actively investigated as the next generation of purpose-specific drug delivery for more than thirty years. In this context, magnetite nanoparticles, being bio compatible, have also been investigated owing to their immense potential for use in cancer therapy. In this project, sol-gel based chemical synthesis route have been employed to prepare single phase nano-sized magnetite particles. The crystal structure of the samples was investigated by X-ray diffraction. The structural morphology was determined using a scanning electron microscope. Electrical characteristics have been analyzed using a four probe technique. These nanoparticles are then functionalized by polymers to achieve better bio-compatibility and then inserted into cancer cell. It has been observed that the cancer cell decayed due to heat produced by magnetic nanoparticles upon the application of AC magnetic field.

Keywords: Magnetite nanoparticles; superparamagnetism; anti-cancer drug delivery

### 1) Introduction

The development of nanoparticles has been increased tremendously because of their unique electrical, magnetic and chemical properties which are quite different from their corresponding bulk materials. Magnetic nanoparticles (i.e.,  $Fe_3O_4$ ) have found their applications such as in magnetic sensors (Zeng *et al.* 2002), high density magnetic recording media (Black *et al.* 2000), printing ink (Atarashi *et al.* 1990), ferrofluids (Raj *et al.* 1995), magnetic resonance imaging (Tifenauer *et al.* 1996), catalysis (Huang *et al.* 2006) and especially in biological labeling, tracking, imaging, detection, and separations (Nam *et al.* 2003) because of their bio-compatibility and chemical stability. Various methods have been devised to synthesize  $Fe_3O_4$  such as sol-gel auto combustion (Hua *et al.* 2012), co precipitation (Wei *et al.* 2012), sono-

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chemical approach (Vijaykumar *et al.* 2000), micro emulsions technique (Zang *et al.* 2008), hydrothermal synthesis (Haw *et al.* 2010) and thermal decomposition (Vijaykumar *et al.* 2000). In the present study,  $Fe_3O_4$  nanoparticles have been synthesized in order to investigate their potential applications in the field of anti-cancer drug delivery i.e., hyper thermic treatment. The basic principal of hyper thermic treatment by nanoparticles lies in the fact that during magnetization and demagnetization of nanoparticles, energy is lost in the form of heat which is then used to destroy the cancer cells. Several attempts have been made to use magnetic nanoparticles in hyper thermic treatment.

Magnetic iron oxide (Fe<sub>2</sub>O<sub>3</sub>) nanoparticles in the range of 20-100 nm were used by Gilchrist (1957) to treat metastatic cancer by injecting into the cells. The particles were exposed by an alternating radiofrequency magnetic field (AMF) to induce the heat. Hyperthermia treatment by magnetic fluid has also been proposed by Jordan et al. (2005) in various comprehensive in-vitro studies. Biocompatibility, nontoxicity, injectability, high level accumulation in the target tumor and effective absorption of the energy of AMF are the important required properties. Modified dextran magnetite and its consequences on the hyper thermic by testing several human carcinoma cell lines in vitro. The specific absorption rate (SAR), used to indicate the heat extraction rate in hyperthermia is low as compared to that of conventional dextran magnetite. Dextran magnetite acts as a super-paramagnetic particle rather than a ferromagnetic due to its small size. Therefore, it has very low hysteresis loss. Hilger et al. (2005) used magnetic nanoparticles for the treatment of breast cancer and it was found that the magnetic hyperthermia treatment could be implemented successfully in breast cancer and Cole et al. (2011) reviewed the use of magnetic nanoparticles in cancer diagnosis, targeted drug delivery and cancer treatment. It has been found that there is a lot of potential in magnetic hyper thermic treatment. So by going through all the reported work this fact has been established that the magnetic nanoparticles can be very useful in hyperthermia. In this work we have synthesized iron oxide nanoparticles in order to investigate their potential applications in the treatment of cancer cells. The magnetic nanoparticles were prepared using sol-gel auto combustion method as this novel technique has emerged as an inexpensive, energy and time efficient. Besides, we can also have better size and shape control which is very important regarding the application point of view (Hua et al. 2012).

### 2. Experimental

Three samples of iron oxide magnetic nanoparticles were prepared by using a novel sol-gel auto-combustion technique. In this method, a fuel agent is used which plays a significant role in determining the crystal structure of the material. In the present study, citric acid has been used as a fuel and its contents have been varied to affect the pH of the solution, and the consequent effect on the structure has been studied. All the reagents used were of analytical grade purity. The stoichiometric amounts of ferric nitrate [Fe(NO<sub>3</sub>)<sub>3</sub>.9H<sub>2</sub>O, Panreac Quima SA] and citric acid [C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·H<sub>2</sub>O, Panreac Quima SA] were taken as the starting material. The metal nitrate (MN) to citric acid (CA) ratio used was 1:1, 1:2 and 1:3, to make three samples, named as S1, S2 and S3,

repectively. The reagents were dissolved separately in 50 ml of de-ionized water and then mixed to make a total volume of 100 ml, in each case. The solution was placed on a hot plate at a temperature of 90°C and stirred continuously using a magnetic stirrer. The whole set up was placed in an ESCO fume hood.

After the continuous stirring of about 1 h, the solution was converted into a gel. At this point, the stirrer was taken out of the beaker and the temperature of the gel was increased to 250°C. In about 20 min, the gel was burnt in a self-propagating exothermic reaction. The final product was a loose and fluffy powder which was grinded using an Agate mortar and pestle to make the grain size uniform. The powder was sintered in an inert atmosphere for 10 h at 600°C in order to ensure complete combustion. All the samples were prepared following the same procedure.

Phase analysis and crystal structure was determined using X-ray diffraction (XRD). The structural morphology was analyzed using scanning electron microscopy (SEM). The dielectric properties, i.e., dielectric constant, tangent loss and dielectric loss factor were obtained using a Wayne Kerr Impedance analyzer.

These precipitates were annealed under Argon (Ar) atmosphere for 10 hours so as to assure full combustion. Same process was repeated for the other compositions (S2 and S3) in which the concentration of citric acid was varied from 4 g to 6 g. Phase composition and crystal structure was determined by X-ray diffraction (XRD). The morphology and size of nanoparticles were examined by scanning electron microscopy (SEM) whereas for magnetic properties vibrating sample magnetometer (VSM) has been done. The dielectric properties i.e., dielectric constant, tangent loss and dielectric loss factor the impedance analyzer was used. The powder samples were pelletized using an Apex hydraulic press for the microscopic and dielectric characterizations.

#### 3. Results and discussions

Fig. 1 shows the diffraction patterns of all the three samples obtained using XRD. Fig. 1(a) shows the diffraction pattern of the sample (S1), prepared using MN to CA ratio of 1:1. The peaks at 20 = 30.15, 35.43, 43.23, 57.06 and 62.69 were matched with the ICSD ref. no. 01-072-2303, corresponding to the cubic structure of magnetite (Fe<sub>3</sub>O<sub>4</sub>), with space group Fd3m and lattice parameter, a = 8.400 Å. v-phase of Fe<sub>2</sub>O<sub>3</sub> known as maghemite has lattice parameters and some other physical characteristics very close to magnetite. The peak at  $2\theta$  = 31.85 could be indexed as (221), related to y-Fe<sub>2</sub>O<sub>3</sub>, according to the ICSD ref. no. 00-24-0081. The peak at  $2\theta$  = 45.67 could not be indexed. It might be due to an unknown phase of iron oxide as this material has quite a lot of phases with lattice parameters very close to each other. This peak and the peak indebted to maghemite, both decreased in intensity as the concentration of the fuel agent was increased in the sample, S2. In addition, the peaks corresponding to the magnetite phase were increased in intensity. Further increase in the peaks corresponding to the Fe<sub>3</sub>O<sub>4</sub> and decrease in the peaks related to  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> were observed when the fuel concentration was further increased, keeping MN to CA ratio of 1:3. The results are guite in agreement with some previous reports (Deshpande et al. 2004).

Therefore, it is inferred that the organic fuel contents play a vital role in the evolution



of magnetite phase using sol-gel auto-ignition route. Nevertheless, the presence of

Fig. 2 Crystallite size as function of MN to CA ratios

some impurity peaks at still higher contents of fuel agent reveals that it is very difficult to produce pure  $Fe_3O_4$  nanoparticles by auto-combustion mechanism because nanoparticles oxidize at the combustion temperature and pressure (Toniolo *et al.* 2007). The crystallite size was evaluated using Scherrer's formula given as

Crystallite size = 
$$0.9\lambda/B\cos\theta$$
 (1)

where ' $\lambda$ ' is the wavelength of the Cu  $K_{\alpha}$  radiation used during X-ray diffraction, 'B' is the full width at half maximum in radians and ' $\theta$ ' is the Bragg's diffraction angle in degrees. The crystallite size was decreased from 27.96 ± 0.05 to 21.72 ± 0.05 nm as the fuel concentration was increased in the series of samples, as shown in Fig. 2. Hence, it is perceived that increased fuel concentration helps in obtaining the nanoparticles in reduced size which ultimately favors the utilization of these nanoparticles in anti-cancer drug delivery.

Fig. 3 reveals the morphology and microstructure of the samples (S1, S2 and S3) obtained using SEM at an applied potential of 25 kV. First micrograph reveals the SEM image of the sample, S1, which reveals the irregular shaped grains, embedded into each other and does not exhibit real sharp boundaries. However, with increased fuel concentration, it is seen that grains become more regular in shape and ordered, although the grain sized could not be estimated from this micrograph, as well due to blurred grain boundaries. Fig. 3(c) shows the microstructural morphology of the sample, S3, synthesized using maximum fuel concentration in the series. The image demonstrates the homogenous and nicely dispersed grains with well-defined and sharp grain boundaries. The grain sizes were estimated to be in the range of 3-4  $\mu$ m. These evenly distributed grains could be easily functionalized using a suitable polymer for smooth and orderly injection into the diseased cells.

10 mm in diameter pellets were used for the determination of dielectric properties of samples using an impedance analyzer. The corresponding thickness of the pellets was 0.90, 0.97 and 1.26 mm, respectively. The results of the dielectric constant, dielectric tangent loss and the dielectric loss factor as a function of frequency have been plotted in Figs. 4-6, respectively.



Fig. 3(a), 3(b), 3(c) SEM micrographs of Fe<sub>3</sub>O<sub>4</sub> at different concentrations of fuel

It can be easily interpreted from the plots that like other ferrites, the  $Fe_3O_4$  nanoparticles exhibit the same trend as having high values of dielectric constant, tangent loss and dielectric loss factor at low frequencies and decrease with the increase in frequency while reaching to a constant saturated value at high frequencies, depicting a frequency independent behavior. This phenomenon can be explained on the basis of Koop's theory (Cullity 1979). According to this theory, the dielectric structure was said to be composed of grains and grain boundaries in which grains were

conductor while grain boundaries were non-conductive. This was also suggested by



Fig. 4 Dielectric constant as the function of frequency function of sample S1, S2 and S3



Fig. 5 Dielectric tangent loss as the of frequency of sample S1, S2 and S3

Maxwell (1982) and Wagner (1913). The theory states that when the electric field is applied on a dielectric material, its atoms need a finite time to align themselves in the direction of field. This time is known as relaxation time and its precise value is 10<sup>-9</sup> sec. As the frequency of electric field increases, a point is reached when charge carriers of dielectric do not align themselves in accordance with the field and so polarization cannot achieve its saturation value. The value of dielectric constant decreases and when we further increase the frequency, the dielectric constant becomes independent of frequency. To explain these trends, the effect of defects and dislocations in the

samples might also be taken into account. These defects activate interfacial



Fig. 6 Dielectric loss factor of samples S1, S2, S3 magnetization as function of frequency

polarizations at low frequencies. Due to this polarization, the dielectric constant is higher at low frequencies. The same behavior has also been reported by Haijun *et al.* (2003), Kuanr and Srivastava (1994) and Mu *et al.* (2008).

In addition, it has intensively been investigated that the dielectric properties of ferrites are dependent upon several other factors, including the method of preparation, chemical composition, grain structure and grain size of the ferrite nanoparticles (Murthy and Viswantham 1990). This may be explained qualitatively by the fact that electronic exchange between ferrous and ferric ions in ferrites cannot follow the frequency of externally applied alternating field beyond a critical frequency value (Kozo 1971).

Another very interesting point is that the decrease in dielectric constant is related to the hooping of electrons between  $Fe^{+2}$  to  $Fe^{+3}$ , so electric field does not provide enough energy for hooping of electrons at lower energy but this energy can be provided when the frequency is increased.

The condition for maximum dielectric loss is given by (Ata and Attia 2003).

$$\omega \tau = 1 \tag{2}$$

where ' $\omega$ ' is the angular frequency given by ( $\omega = 2\pi f_{max}$ ) and ' $\tau$ ' is the relaxation time. The relaxation time can be given ( $\tau = \frac{1}{2}p$ ) where 'p' is jumping probability per unit time or hopping probability. This jumping probability is directly related to frequency ( $f_{max}\alpha p$ ). This relation predicts that if frequency is increased then jumping probability will also increase and vice versa. This increase in jumping probability is responsible for the decrease in dielectric constant at high frequencies (Reddy *et al.* 1999).

Magnetic hysteresis (M-H) loops of all the samples were obtained from a vibrating sample magnetometer at an applied field of  $\pm$  10 kOe, as plotted in Fig. 7. The M-H

loops revealed that the saturation magnetization (M<sub>s</sub>) was increased from 42.21 emu/g



Fig. 7 Saturation magnetization and remanence at different MN to CA ratios

to 56.70 emu/g while magnetic remanence ( $M_r$ ) was decreased from 6.16 emu/g to 3.81 emu/g, in the series with increase in the fuel concentration. The value of coercivity ( $H_c$ ) was varying between 70 to 170 Oe, for the three samples. The results are in quite agreement with the previously reported results (lonas *et al.* 2012) and (Kim *et al.* 2001). Interestingly, the value of  $M_s$  is quite low from the corresponding pure and bulk Fe<sub>3</sub>O<sub>4</sub>. This can be explained on the basis of small grain size and also due to non-magnetic impurities related to the other phases of iron oxide in the samples (Liu *et al.* 2010). The magnetic behavior is in accordance with the phase analysis as described by the diffraction patterns.

### 4. Conclusions

 $Fe_3O_4$  nanoparticles were successfully synthesized by sol-gel auto-combustion route method. The crystal structure, microstructural morphology, dielectric and magnetic properties were investigated by using XRD, SEM, impedance analyzer and VSM, respectively. The diffraction analysis revealed that fuel concentration plays a vital role in synthesizing the magnetite phase of the iron oxide. Size and shape of the grains were visualized using scanning electron microscope. The sample prepared with maximum fuel concentration showed nicely arranged and well-dispersed grains quite suitable for polymer-based functionalization. The dielectric parameters exhibited high values at low frequencies and a decreasing trend was witnessed for the increased values of frequencies. The value of  $M_s$  was increased while that of  $M_r$  was decreased as the samples were prepared with enhanced value of fuel concentration. The superparamagnetic nature of the samples was evident from the M-H loops. This feature makes these nanoparticles extremely useful in biomedical applications especially in targeted anti-cancer drug delivery. **References** 

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