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## Infrared Spectroscopy of Functionalized Magnetic Nanoparticles

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### 1. Introduction

Nanotechnology development has allowed that nanomaterials can be used in biomedical applications, and nanometer sized objects can interact with biological entities like cells, virus, protein, enzyme, etc. For this reason, many research projects has been focused in the development of nanosystems, nanoparticles and nanodevices for this applications. This area is relatively new, according to the ISI web of knowledge, the publications of the nanoparticles for biomedical applications started on 2000 year, and since that time they have increased exponentially (Figure 1). The nanoparticles (NPs) used for biomedical purposes generally include zero-dimensional nanospheres and one-dimensional nanowires and nanotubes.

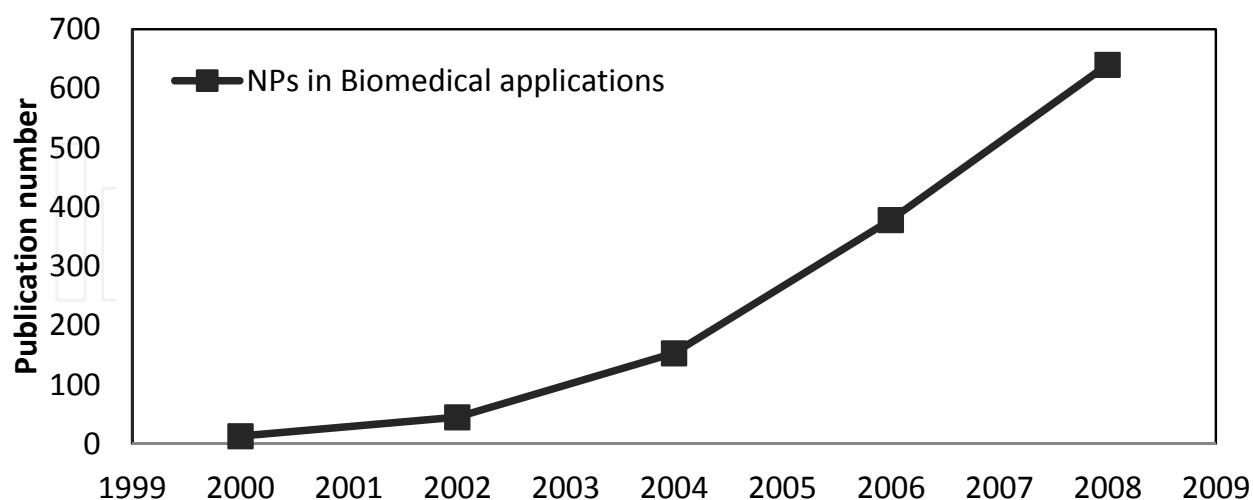


Fig. 1. Trends of Nanoparticles (NPs) in Biomedical application, information extracted from ISI Web of Knowledge.

Figure 2, extracted from ISI Web of Knowledge, shows the publications related to gold, silver, iron and magnetic nanoparticles for biomedical application. Magnetic nanoparticles

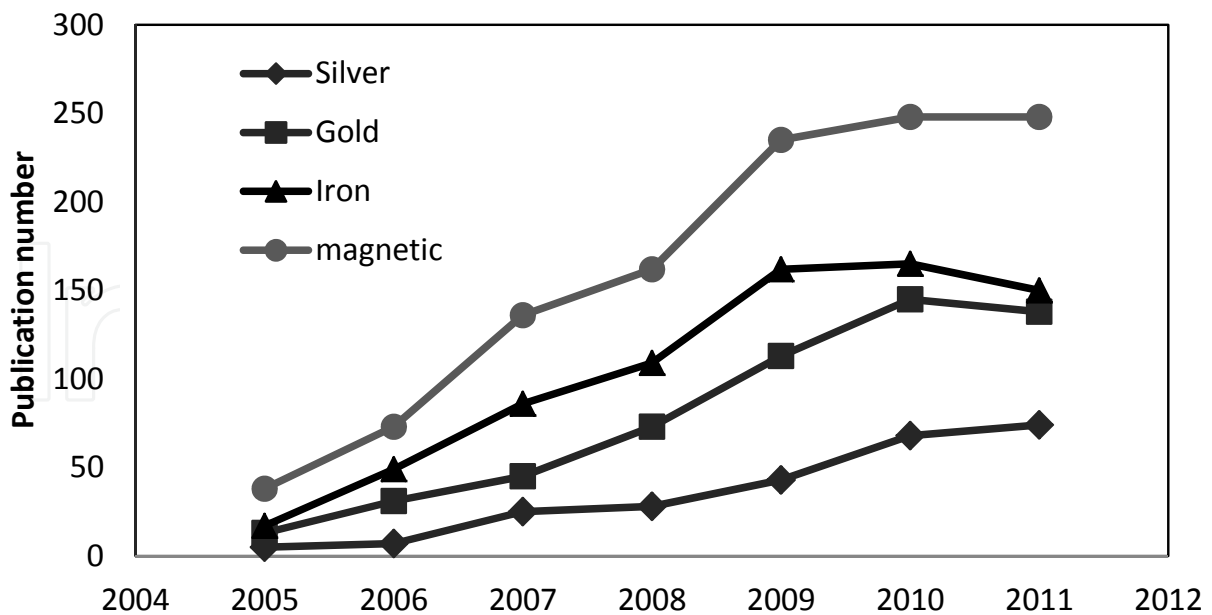


Fig. 2. Trends of silver, gold, iron and magnetic nanoparticles used in Biomedical applications, information extracted from ISI Web of Knowledge

are at the forefront as the most promising materials for clinical diagnostic and therapeutic applications. Magnetic nanoparticles (MNPs) are widely used for labeling and manipulating biomolecules, targeting drugs and genes, magnetic resonance imaging, as well as hyperthermia treatment (Varadan et al., 2008; Cornell and Schwertmann, 2003). Magnetite Nanoparticles ( $\text{Fe}_3\text{O}_4$ ) are the most used magnetic material for biomedical applications because they have a high enough saturation magnetization to allow its manipulation with an external field, superparamagnetic behavior and ability to bond with different molecules to surface functionalized (Cullity and Graham, 2009; Neuberger et al., 2005). In biomedical applications, the characteristics of the magnetite nanoparticles have a significant advantage when they interact with biological molecules, therefore, many methods of synthesis have been developed in order to control surface morphology, particle size, particle distribution, and chemical stability among others (An-Hui et al., 2007; Gao and Gu, 2009).

## 2. Magnetic nanoparticles

Many magnetic nanoparticles such as magnetite, strontium and cobalt ferrites, lanthanum-zinc ferrites, nickel, iron and some compounds with a rare earth like  $\text{SmCo}_5$  have been developed (Pankhurst et al., 2003). Magnetite,  $\text{Fe}_3\text{O}_4$  is the magnetic material most used in biomedical application due to its several interesting properties such as great chemical stability, low toxicity, and its magnetic saturation mentioned above for being manipulated with an external field, biocompatibility and the heating ability in presence of a field, which made it an interesting candidate for hyperthermia treatment (Sun et al., 2004) for this reason in recent years, much effort has been focused in the design and controlled synthesis of this material with certain shape and particle size. Many methods for synthesis of magnetite nanoparticles have been developed like co-precipitation, microemulsion, sol-gel, sputtering,

thermal decomposition, etc. With this variety of methods particles with different morphologies such as spheres, rods, wires and tubes has been obtained (Palla et al, 1999; Joralemon et al, 2005; Terrazas et al, 2010). However, the coprecipitation method is still the most popular method for their simplicity and easy way to manipulate the size and morphology of the particles by the use of templates, besides being a method whose raw materials are relatively inexpensive.

Recent publications have been emphasized on the particle size control of magnetite nanoparticles, because under some critical value, the material exhibits a superparamagnetic behavior, this means that there is no hysteresis in the magnetization curves, which implies that the retentivity and coercivity are close to zero (Cullity and Graham, 2009). Biomedical applications involve strict requirements on particle size, and it can be by using a chemical coprecipitation through the control of nucleation and growth process. Magnetite nanoparticles obtained by chemical coprecipitation method are produced by the precipitation of divalent ( $\text{Fe}^{+2}$ ) and trivalent ( $\text{Fe}^{+3}$ ) iron salts in an alkaline medium. The size and the number of nucleus are influenced by the alkaline medium and the addition velocity, which results in a nucleation and growing process; a fast nucleation will form high concentration of nuclei and small particles, while a slow nucleation will form low nuclei concentration generating larger nanoparticles (figure 3).

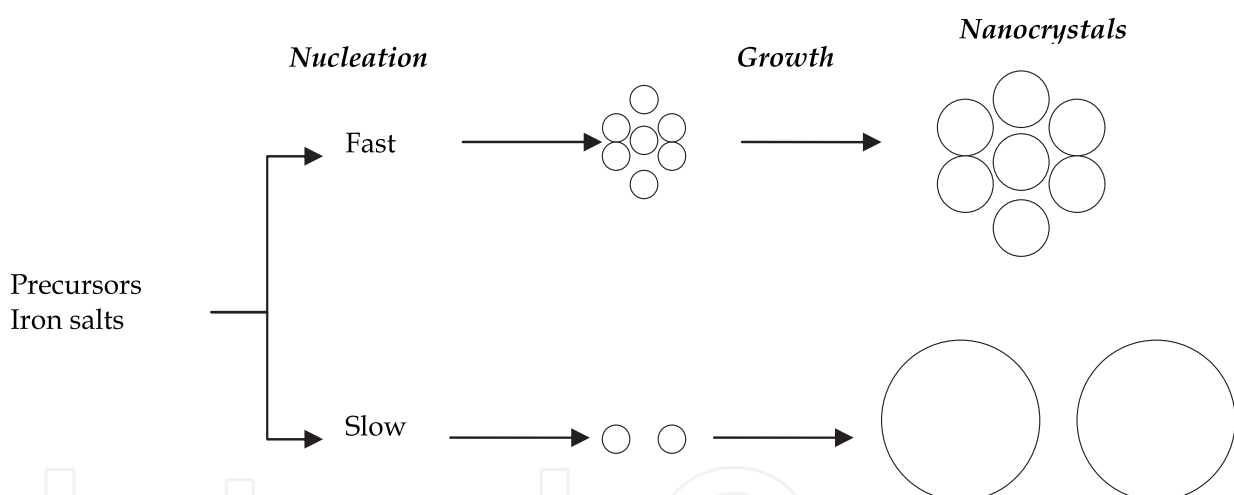


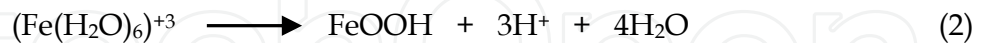
Fig. 3. Schematic representation of nucleation and growth kinetics.

## 2.1 Chemical coprecipitation

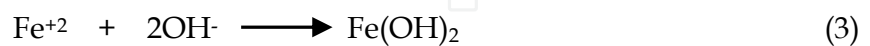
As mentioned above, chemical co-precipitation consists in the precipitation of divalent ( $\text{Fe}^{+2}$ ) and trivalent ( $\text{Fe}^{+3}$ ) iron salts in an alkaline medium, maintaining a molar ratio of 1:2, by using ammonium hydroxide, ammonia or some other alkaline solution to increase pH reaction that is required to magnetite formation. Commonly the addition of alkaline solution to divalent and trivalent iron solution is made slowly, drop by drop (titration) under vigorous agitation using a magnetic agitator. The initial solution of divalent and trivalent iron cations had acidic pH and after the titration is close to 12, a black precipitate is formed which indicates that the reaction has been completed. The chemical reaction that takes place during magnetite formation from iron salts solutions by increasing the pH can be represented in the following overall chemical equation (Cornell et al, 2003; Gnanaprakash et al, 2007):



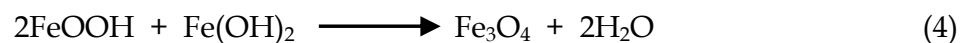
In general, the solubility of trivalent iron oxide ( $\text{Fe}^{+3}$ ) is smaller than the one observed on divalent iron oxides ( $\text{Fe}^{+2}$ ). The trivalent iron hydrolyzes and forms hydroxide species. The hydrolysis can be induced by heating up the solution. The complete hydrolysis corresponds to the formation of a trivalent iron oxide-hydroxide and it is represented according to the following chemical reaction:



The divalent iron cation in solution ( $\text{Fe}^{+2}$ ) reacts to form the divalent iron oxide in basic conditions (presence of hydroxyl ion  $\text{OH}^-$ ), which is presented in equation 3:



Under the reaction conditions, divalent iron hydroxide and trivalent iron oxide-hydroxide species were likely to be formed. This being established, it is suggested that the following chemical reaction mechanism occurred: trivalent iron cation hydrolyzes forming ( $\text{FeOOH}$ ) as pH increases; under alkaline conditions divalent iron cation forms  $\text{Fe}(\text{OH})_2$ . Both chemical species reacted to each other at pH values of around 10 to 11, forming magnetite according to equation 4:



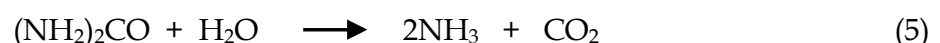
## 2.2 Chemical coprecipitation with fast injection

The chemical coprecipitation with fast injection differs from conventional coprecipitation in the speed at pH of the reaction pH solution is increased; in order to favor magnetite formation abruptly. Divalent and trivalent iron salt solutions have an initial pH of 0 to 1. On the conventional coprecipitation method, the pH of the solution is increased by the addition of an alkaline solution drop by drop, which is considered slow speed; while on the rapid injection method, the pH of the solution is increased by adding the salt solution directly to ammonium hydroxide solution, speed to be considered rapid and explosive. The difference between both methods is schematically shown in figure 4.

## 2.3 Chemical coprecipitation with reflux and aging conditions

A trivalent iron solution is placed into a bowl flask and heated up to 80 °C under refluxing conditions for a period of time of 2 hours. A precipitate is formed and separated from supernatant. Trivalent iron cation is hydrolyzed due to an increment of temperature promoting the hydrolysis and forming a trivalent iron oxide-hydroxide ( $\text{FeOOH}$ ). After the 2 hours of hydrolysis reaction, a yellowish precipitate is obtained.

Another solution is prepared with divalent iron and urea. This solution is mixed with the previous precipitate and heated up to 90°C-96°C for 20 hours under refluxing conditions. The required pH condition is obtained through the slow decomposition of the urea when the temperature increases above 90°C, this condition will increase the pH uniformly favoring a more slower nucleation in the solution (Terrazas et al., 2010).



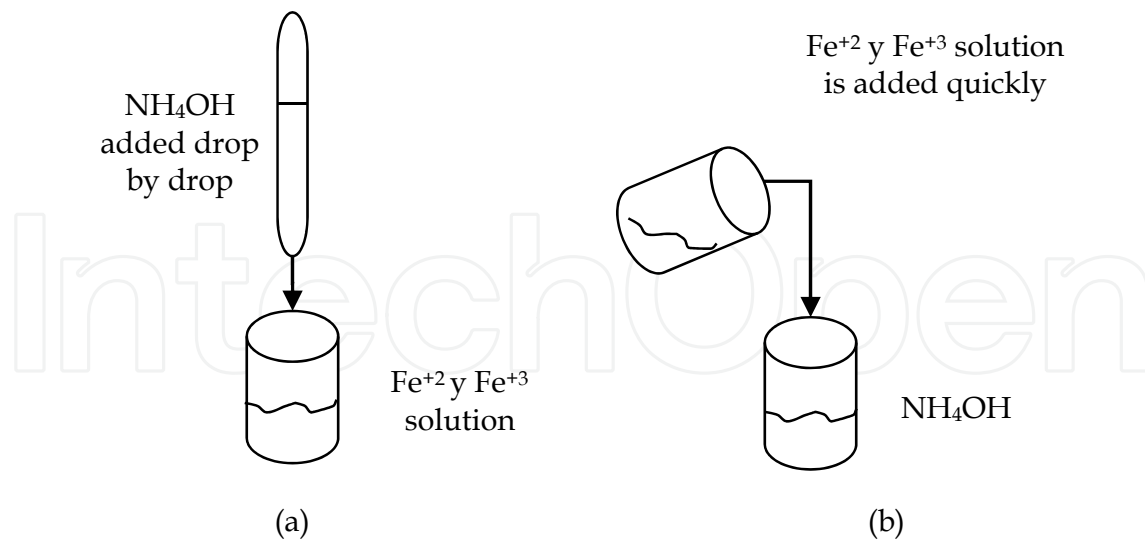
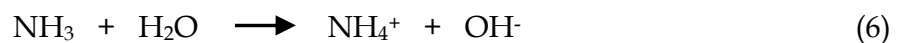


Fig. 4. Difference between conventional coprecipitation (a) and coprecipitation with fast injection (b).



By the use of urea in the chemical coprecipitation with reflux and aging conditions method, the speed of pH increment is considerably slower than the one observed in the slow injection and rapid injection co-precipitation methods. The condition of slow and uniform pH increment and the reacting time of 20 hours under favorable conditions for magnetite formation, affect the nucleation and particle growth process making it very slow, which is reflected in the formation of bigger particles than those promoted in slow injection and rapid injection methods.

The difference of these methodologies lies in the particle size and its distribution as is shown in figure 5. Magnetite obtained by coprecipitation with reflux and aging shows higher particle size and a wide particle size distribution. According to these results, magnetite obtained by coprecipitation with fast injection shows the smallest average particle size and the closest particle size distribution; differing from magnetite obtained by common coprecipitation, Table 1.

The magnetite obtained using these three methodologies have a spherical particle shape with a superparamagnetic behavior, and their saturation magnetization is influenced by the particle size. Values of 55.9, 64.3 and 78.2 emu/g were obtained from nanoparticles with an average particle size of 16 nm, 27 nm and 200 nm respectively. The saturation magnetization increases when the particle sizes are larger. Hysteresis loops of the synthesized nanoparticles are shown in figure 6.

Magnetite structure is an inverse spinel with a face center cubic unit based on 32 O<sup>2-</sup> ions with a regularly cubic close packed along the [111] direction. There are eight formula units per unit cell. Magnetite differs from other iron oxides in that it contains both divalent and trivalent iron. Its formula is written as Fe<sup>III</sup>[Fe<sup>II</sup> Fe<sup>III</sup>]O<sub>4</sub> and the brackets denote octahedral sites, tetrahedral Fe spins are directed antiparallel to octahedral Fe<sup>3+</sup> and Fe<sup>2+</sup> spins so

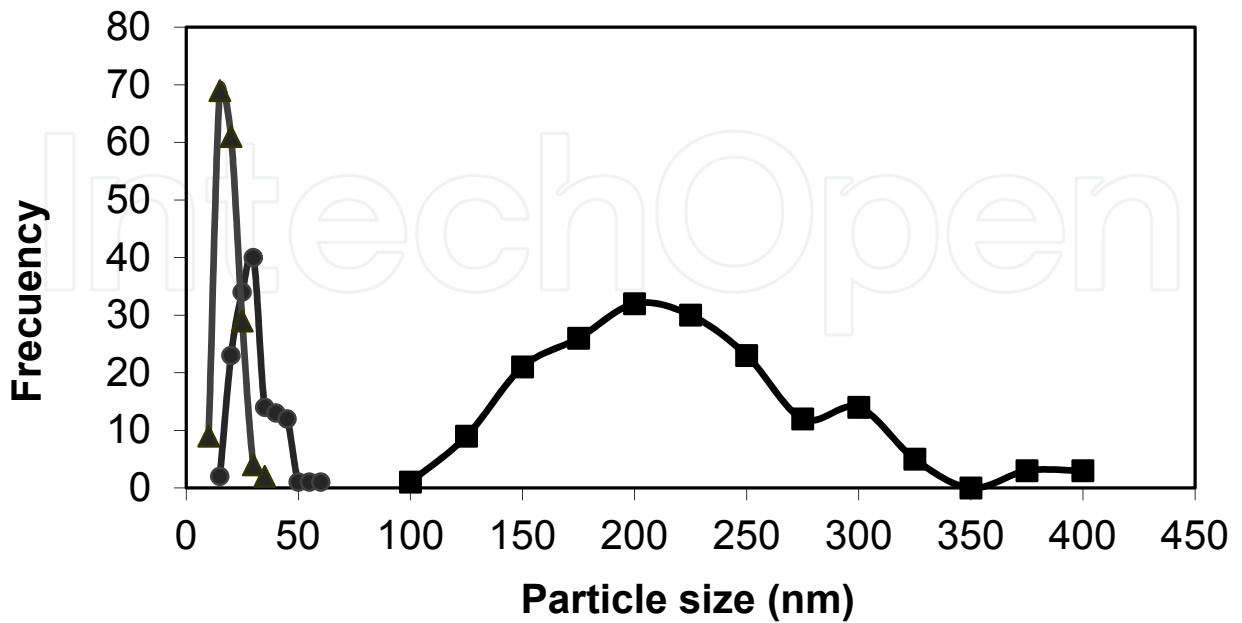


Fig. 5. Comparison of magnetite particle sizes and their distribution obtained by common coprecipitation (●), coprecipitation with fast injection (▲) and coprecipitation with reflux and aging (■)

	Common coprecipitation	Coprecipitation with fast injection	Coprecipitation with aging and reflux
Average particle size (nm)	27.6	16.2	206.9
Standard Deviation (nm)	8.2	4.4	58.9

Table 1. Average particle size of magnetite obtained by different methodologies

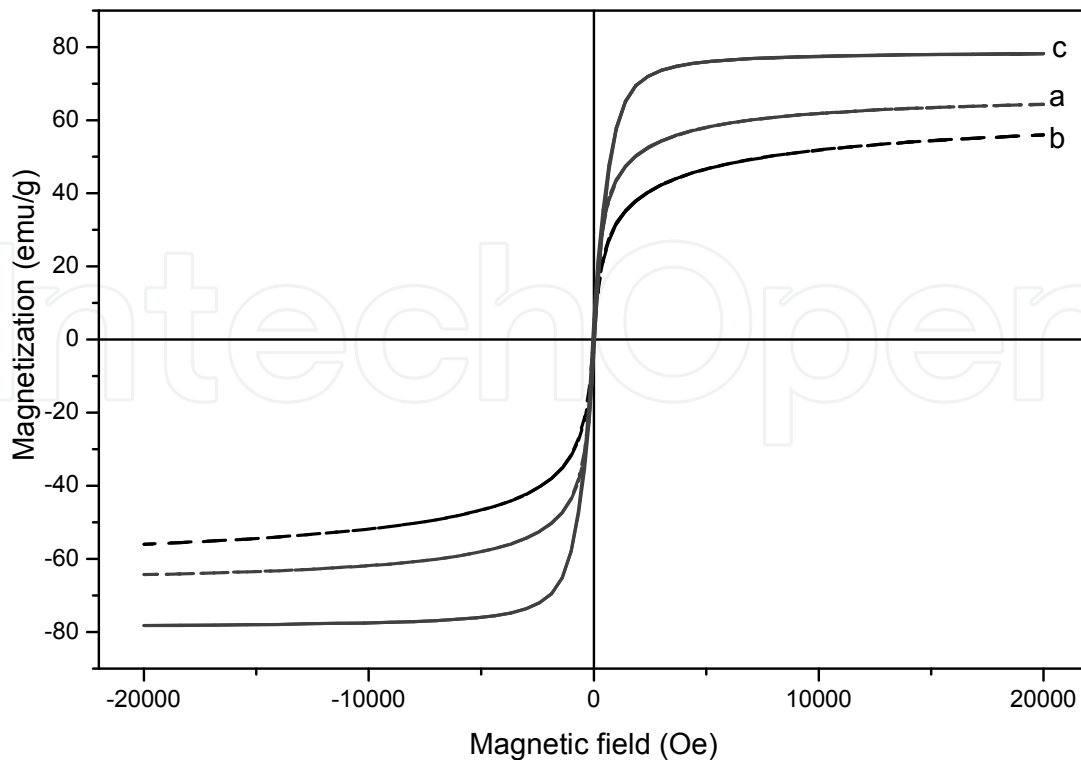


Fig. 6. Hysteresis loops of magnetite obtained by common coprecipitation (a), with a fast injection (b) and aging and reflux(c)

that the Fe  $3^+$  moments cancel, leaving a spontaneous magnetization equivalent to one Fe  $2^+$  moment per molecule, eight tetrahedral sites are occupied by trivalent iron, and the divalent and trivalent cations occupies the sixteen octahedral sites (Cornell and Schwertmann, 2003). X-ray diffraction is the most widely used technique to determine the crystalline structure of a material. However in the case of magnetite it can be confusing because the magnetite has the same crystalline structure that maghemite, but this one has a interstitial voids, therefore by using XRD is not conclusive. The difference between the two materials is that some of the interstitial atomic positions of the maghemite are not fully occupied, and consequently having atomic holes. In the case of magnetite, the infrared spectroscopy is very useful because this technique arises as a result of divalent and trivalent cations interaction with electromagnetic radiation, this interaction involves excitation for vibration or rotation of molecules in their ground electronic state, and they are associated with stretching deformation of the interatomic bonds and bending deformation of the interbond angles. FTIR spectroscopy provides a fast mean of identification.

Infrared spectra of the magnetite shows the chareacteristic bands at  $590$  and  $450\text{cm}^{-1}$  approximately due to the Fe-O bond in tetrahedral and octahedral positions. Figure 7 shows the infrared spectra of the magnetite with a different particle size, the band at  $600\text{cm}^{-1}$  approximately is broadening when the particle size decreases. According to Nasrazadani (1993) this effect indicates an increment of cation vacancy in the lattice, this behavior corroborated with the decreased value of the lattice parameter, which is shown in table 2. These values are minor than the lattice parameter of defect free magnetite ( $8.396\text{ \AA}$ ), this small reduction is assumed to be due to the prevalence of a small amount of cation deficiency.



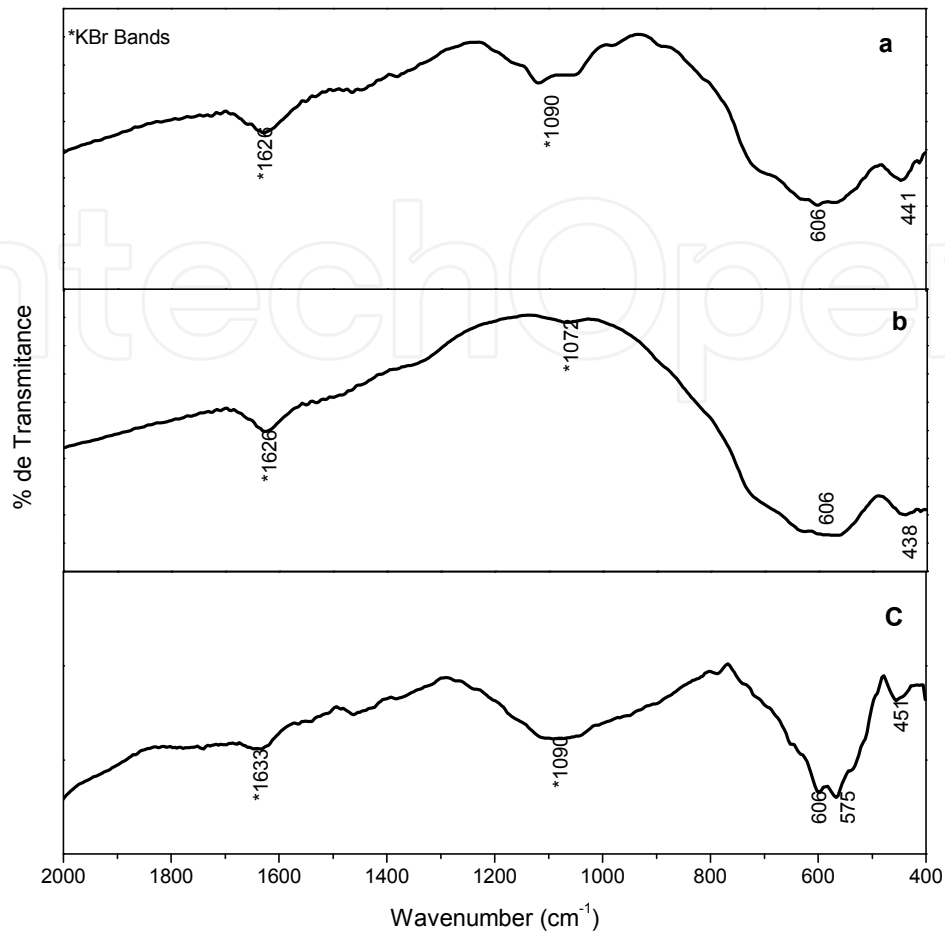


Fig. 7. Infrared spectra of the magnetite obtained by common coprecipitation (a), with a fast injection (b) and aging and reflux(c).

Sample	Method of Synthesis	Particle size (nm)	Lattice cell (Å)
Magnetite	Coprecipitation with aging and reflux	$206.9 \pm 58.9$	8.34468
Magnetite	Common coprecipitation	$27.6 \pm 8.2$	8.34270
Magnetite	Coprecipitation with fast injection	$16.2 \pm 4.4$	8.33475

Table 2. Variation of the magnetite lattice cell with a determined particle size

Substitution of the cation on magnetic structures has been studied in order to improve the magnetic properties and FTIR spectroscopy is one of the techniques used in this kind of studies. In magnetite structure, the divalent iron is totally or partially replaced for strontium, cobalt, copper, nickel, manganese, cadmium, aluminum and gadolinium (Brabers et al., 1998). Figure 8 shows the infrared spectra of magnetite doped with cobalt; this cation occupies octahedral sites without changing inverse spinel crystal structure of magnetite.

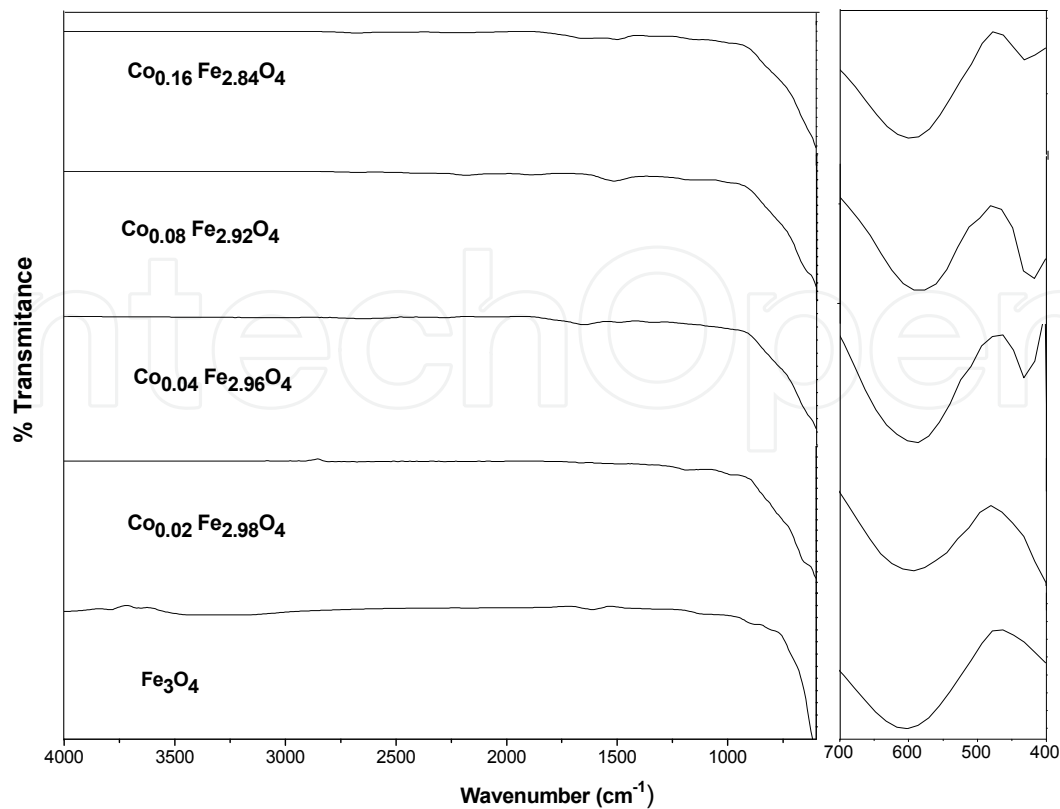


Fig. 8. Infrared spectra of cobalt doped magnetite.

### 3. Functionalization of magnetic nanoparticles

One of the most important aspects of the nanoparticles for biomedical applications is the surface preparation of the nanoparticles in order to improve their biocompatibility with biological entities and provide chemical stability. The nanoparticles surfaces can be modified with a biocompatible or/and biodegradable polymeric coating. The polymer can be natural such as chitosan ( $C_6H_{13}NO_5$ ), collagen, folic acid ( $C_{19}H_{19}N_7O_6$ ) or synthetic as dextran ( $H(C_6H_{10}O_5)_xOH$ ), tetraethyl orthosilicate ( $SiC_8H_{20}O_4$ ), N-(2-aminoethyl-3-aminopropyl) trimethoxysilane ( $C_8H_{22}N_2O_3Si$ ), poly-lactic-co-glycolic acid (PLGA), polyethylene glycol ( $C_{2n}H_{4n+2}O_{n+1}$ ), etc. This surface modification needs to have a functional groups like: carboxyl ( $-COOH$ ), hydroxyl ( $-OH$ ), amine ( $-NH_2$ ), etc, with the capability to bond with a biological molecules. Table 3 shows a summary of recent publications of the most used coating materials for magnetite nanoparticles.

One of the most used techniques to ensure that the functionalization of magnetic nanoparticles has occurred, is the Fourier infrared spectroscopy (FTIR) because of its simplicity and availability. This technique provides the information about the excitation of vibration or rotation of molecules in their ground electronic State. In magnetite structure, these vibration, are associated with the stretching deformation of the interatomic bond of the iron with other molecules. Magnetite with a silica (figure x, MS sample) shell is confirmed by the characteristic adsorption band at  $1090\text{ cm}^{-1}$  due to silane group presence. When a aminosilane is used like a coating, the spectra (figure, MA sample) show the band at  $2943\text{ cm}^{-1}$  due to the stretching of C-H from methyl group ( $-CH_2$ ,  $-CH_3$ ), the band at  $1072\text{ cm}^{-1}$  is due to the Si-O bond and the bands at  $3309$  and  $1654\text{ cm}^{-1}$  are attributed to the amine

Material	Particle size (nm)	Application	Reference
Silica	20-300	DNA separation, Drug delivery, Metal separation in waste	Schweoger et al, 2011; Chen et al, 2011; Del Campo et al, 2001; Ajay and gupta, 2005
Dextran	10-200	Drug delivery NMR imagines	Liu et al, 2011; Quin et al, 2011; Catherine et al, 2003; Zhang et al, 2007
Polyethylene glycol (PET)	10-50	NMR imagines, Gen delivery	Kami et al, 2011; Phadatare et al, 2011; Zang et al, 2008;
Polyvinyl alcohol (PVA)	10-50	NMR imagines, Drug delivery,	Pardoe et al, 2001; Morteza et al, 2009.
Polyvinyl Pyrrolidone (PVP)	10-20	Drug delivery	Young-Lee et al, 2006
poly-lactic-co-glycolic acid (PLGA)	250	Tissue engineering, Cell targeting	Schliehe et al, 2011; Chih-Hang et al, 2011; Mu andFeng,2001; Yoshida and. Babensee, 2006;
Polystyrene (PS)	10-20	NMR imagines DNA separation	McCarthy et al, 2011; Ramirez et al, 2003
Methyl polymethacrylate	10-50 o	NMR imagines, Entities separation	Gao et al, 2010;
Polypyrrole	20-100	Protein separation, Metal separation	Madhumita et al, 2011; Ammar et al, 2004; Andrevia et al, 2006;
Cellulose	20-50	Drug delivery	Huixia et al, 2011
Chitosan	20-100 o	Cell targeting, Tissue engineering, Drug delivery, hyperthermia	Coroto et al, 2011; Arami et al, 2011; Del campo et al, 2001;
Gelatin	50-100	DNA separation, drug delivery	Gaihre et al, 2009
Starch	10-20	Cell Separation	Dong-Hyun et al, 2009

Table 3. Materials used in functionalization of magnetite nanoparticles.

group (-NH<sub>2</sub>). A sample with a double coating silica-aminosilane (Figure 9, MSA sample) shows the band of both materials, and a new band is shown at 802 cm<sup>-1</sup> due to Si-O-Si bond. Using this information, a suggested mechanism of coated particles can be proposed (Scheme 1) in magnetite-aminosilane shell, the silicon is bonded with the iron through the deprotonation of magnetite; when a silica shell is added before the aminosilane groups, the silicon is bonded in the same way with the magnetite and the silicon bonded with aminosilane trough S-O-S bond.

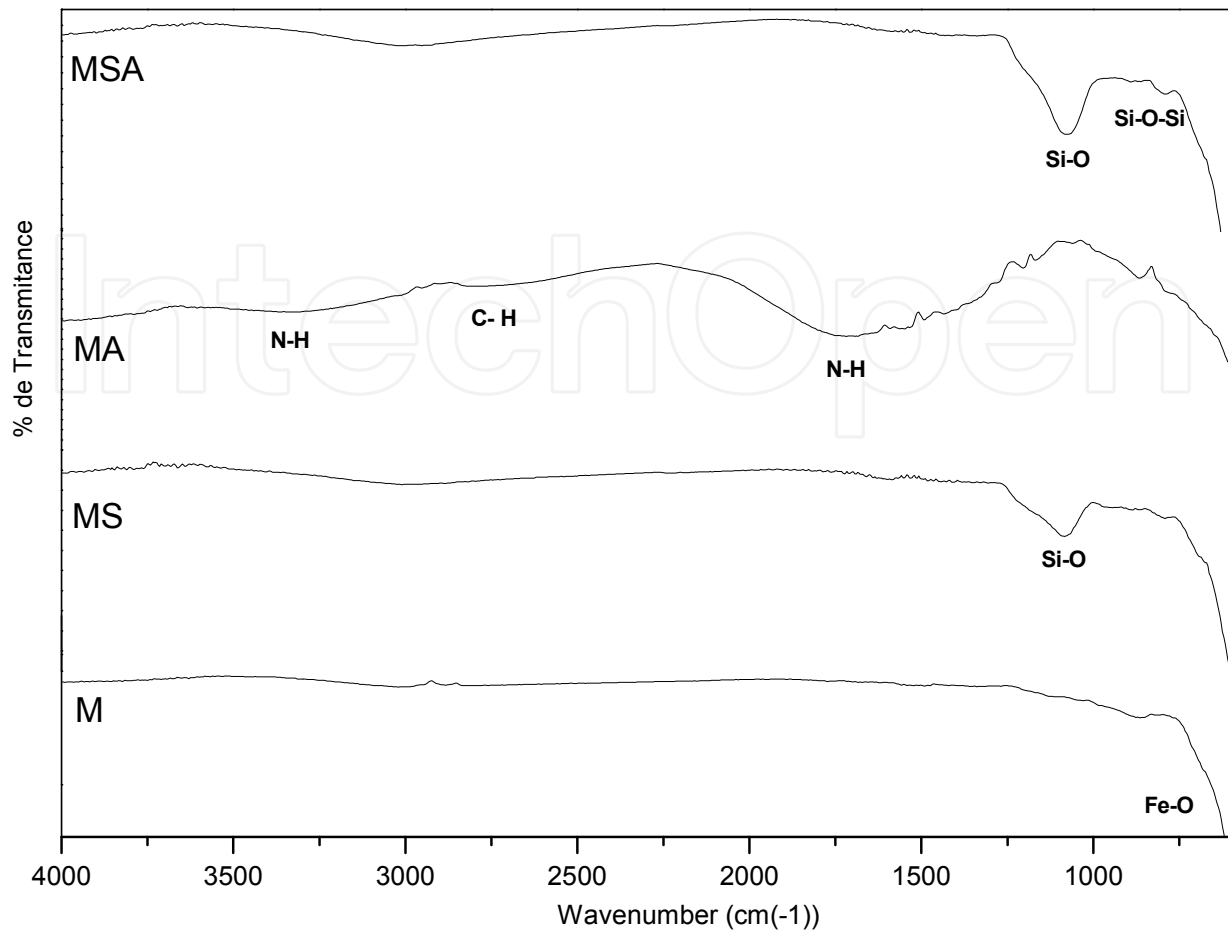
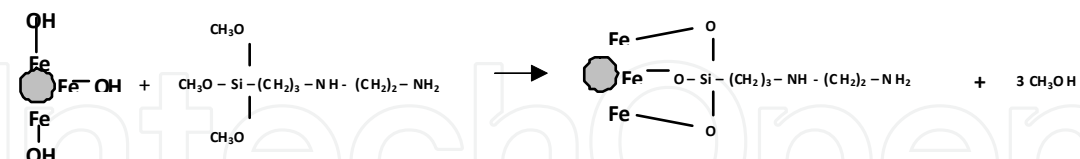
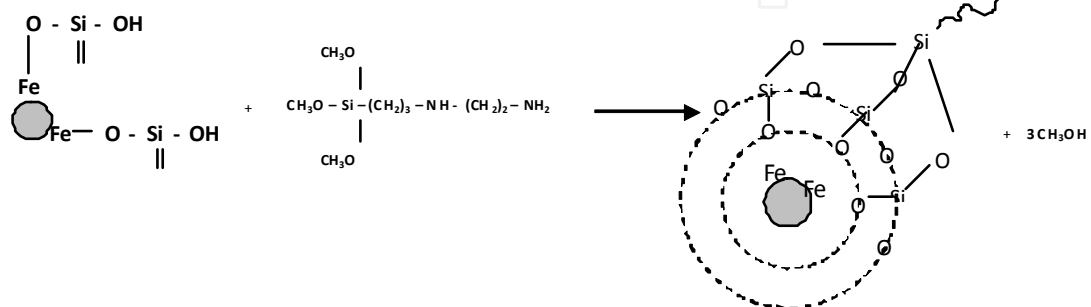


Fig. 9. FT spectra of magnetite (M), magnetite-aminosilane (MA) and magnetite-silica-aminosilane (MSA) obtained by reflux and aging method.

**Magnetite -Aminosilane shells**



**Magnetite-Silica-aminosilane shells**



Scheme 1. Suggested mechanism of coated magnetite nanoparticles

Chitosan is a natural polymeric material widely used in biomedical applications as coating in magnetic nanoparticles for biomedical applications. The spectra of this material, Figure 10 show bands at  $1400\text{ cm}^{-1}$  due to C-O of the primary OH groups; at  $1600\text{ cm}^{-1}$  due to the N-H; at  $2943\text{ cm}^{-1}$  due to the stretching of C-H from methyl group (-CH<sub>2</sub>, -CH<sub>3</sub>) and the band at  $1100\text{ cm}^{-1}$  of the hydroxyl group of the piranonic ring of the chitosan beside the Fe-O bond due to octahedral sites of the magnetite. The band at  $2250\text{ cm}^{-1}$  is due to carbon dioxide air.

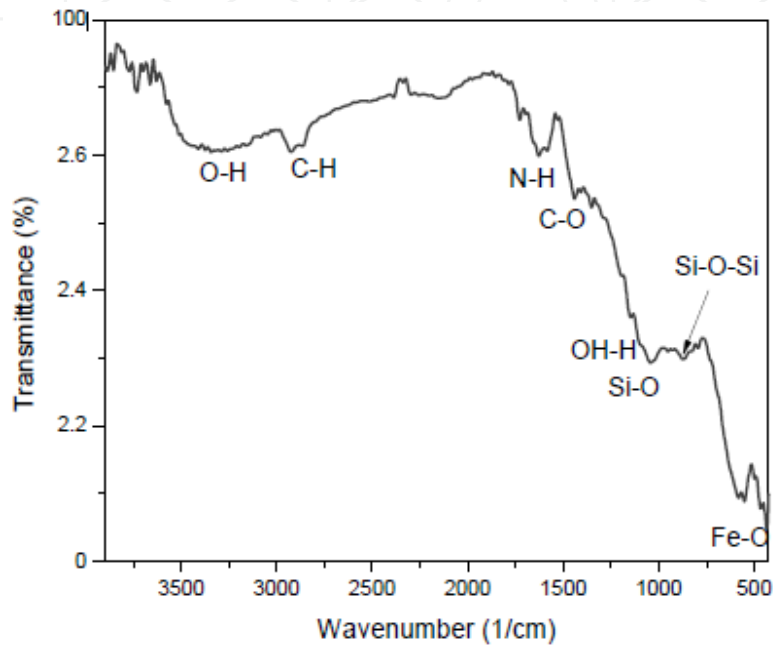


Fig. 10. FT spectra of magnetite coated with a chitosan shell.

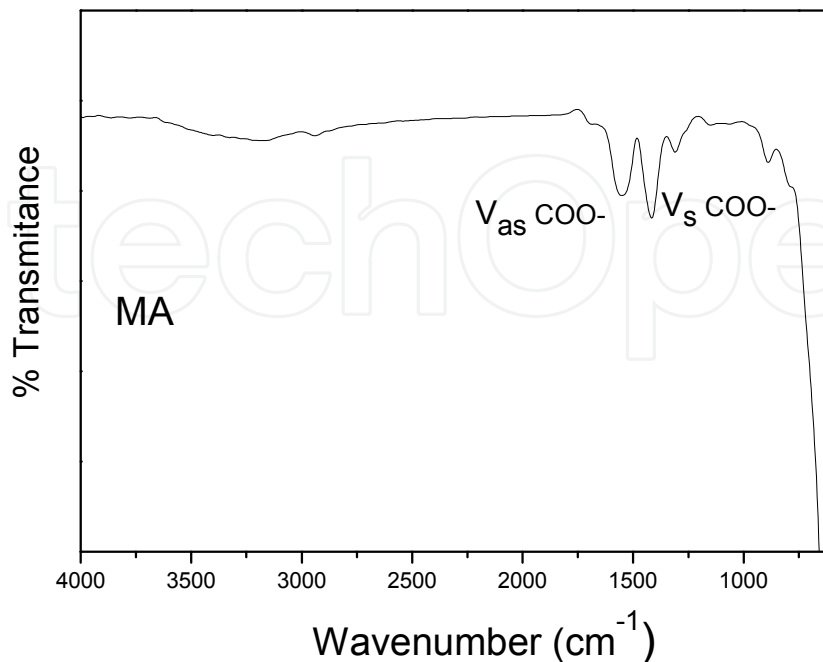


Fig. 11. FT spectra of magnetite coated with an adipic acid shell.

On the other hand adipic acid is a materials that has not been widely studied like a coating shell on magnetic nanoparticles; however, these materials have been widely used in drug delivery systems. In figure 11, the spectrum of the magnetite coated with this polymer shows the bands at 1415 y 1550 $\text{cm}^{-1}$  due to the symmetric and asymmetric carboxylate ion ( $\text{COO}^-$ ) and approximately at 600 $\text{cm}^{-1}$  the band due to the Fe-O bond in octahedral sites of the magnetite.

#### 4. Conclusions

One of the most important aspects of nanoparticles in biomedical applications is their surface functionalization in order to improve their biocompatibility with biological entities, and Fourier infrared spectroscopy (FTIR) is very useful technique that provides information about iron oxides in their ground electronic state, and when this material is bonding with a polymeric coating provides information about mechanism of functionalized magnetic nanoparticles. This technique is widely used in characterization nanoparticles due to its simplicity and availability. In magnetite structure it provides information about the excitation of vibration or rotation of the trivalent and divalent iron cations and allows knowing the occupied sites when the divalent iron is replaced with other cations.

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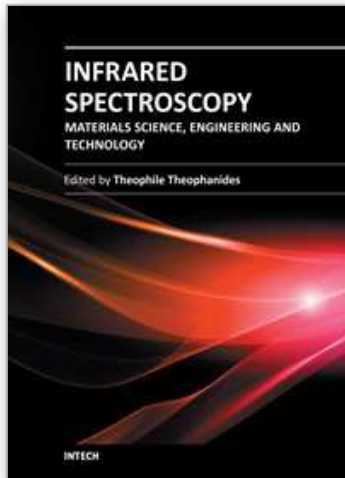
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