

**SYNTHESIS AND CHARACTERIZATION OF CORE-SHELL
MAGNETITE NANOPARTICLES FOR BIOMEDICAL APPLICATIONS**

Sergiu SORA¹, Rodica Mariana ION^{1,2}

1. Universitatea Valahia Targoviste, 2. ICECHIM Bucuresti

Abstract.

This work aims to establish and to optimize the conditions for chemical synthesis of nanosized magnetic core-shell iron oxide. The core is magnetite and for the shell we used gold in order to obtain different nanoparticles. Iron oxides was synthesized by sonochemical process using ferrous salts, favoring the synthesis at low-temperature, low costs, high material purity and nanostructure control. After synthesis, some investigation techniques as: X-ray diffraction (XRD), atomic force microscopy (AFM), Termogravimetric analysis (TGA), Fourier-Transform Infrared Spectroscopy (FTIR) and UV-Vis absorbance spectroscopy, have been used to see the characteristics of the nanoparticles. For in-vitro applications, it is important to prevent any aggregation of the nanoparticles, and may also enable efficient excretion and protection of the cells from toxicity. For biomedical applications like magnetic biofunctional material vectors to target tissues, the particles obtained have to be spherical with 10 nm average diameter.

Key words: magnetite, nanocomposite, core-shell, sonochemical method.

1. INTRODUCTION

Particles with diameters in the range of 2 to 100 nm, so-called nanocrystalline materials, have become a major interdisciplinary area of research during recent decades. Besides noble metal nanoparticles, nanoparticles with novel magnetic properties are attracting more and more attention with regard to bioapplications. Materials where the magnetization can reach the saturation state and is larger than paramagnetic ones, are called as superparamagnetic. This characteristic allows a promising future in application of the magnetic nanoparticles in biomedicine, particularly in magnetic resonance imaging (MRI), tissue engineering, and drug delivery^[1-4]. Magnetic nanoparticles, for instance those composed of magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃), have unique thermal, chemical, and magnetic properties that make them particularly well suited for medical applications. However, materials that are used in medical diagnoses or as therapeutic agents require specific characteristics, such as colloidal stability, biocompatibility, resistance against environmental degradation, and nontoxicity. For these applications, nanoparticles must be passivated with an external inert shell in order to protect the magnetic core against oxidation. Since gold is an inert element from the viewpoint of chemical reactivity, it is very useful as a coating for protecting magnetic nanoparticles.

In this paper, magnetite Fe₃O₄ nanoparticles within 6 and 20 nm in diameter were synthesised by sonication method at low temperature (50 °C) temperature. Core-shell system with gold is created, too. Their analytical characterization are evaluated and discussed.

2. EXPERIMENTAL PART

2.1. Synthesis of Fe_3O_4 nanoparticles

The chemical reactions driven by intense ultrasonic waves that are strong enough to produce cavitation are oxidation, reduction, dissolution, and decomposition [5]. It has been known that three different regions are formed during the aqueous sonochemical process these are; (a) the inner environment (gas phase) of the collapsing bubble, where elevated temperatures (several thousands of degrees) and pressure (hundreds of atmospheres) are produced, causing water to vaporize and further to pyrolyze into H and OH radicals; (b) the interfacial liquid region between the cavitation bubbles and bulk solution, the temperature in this region is lower than that of the interior of the bubbles, however, it is still high enough for thermal decomposition of the solutes to take place, in addition, greater local hydroxyl radical concentrations in this region have been reported; and (c) the bulk solution, which is at ambient temperatures and where the reaction between reactant molecules and OH or H take place. Among the above mentioned three regions, it appears that the sonochemical reaction occurs within the interfacial region.

The synthesis of pure nanometer-size Fe_3O_4 particles has been carried out as follows, 0.4g of $FeCl_2 \cdot 4H_2O$ was dissolved in 200ml of 1:1 solution of ethanol-water that was previously bubbled for 30 minutes with Ar gas. The obtained solution was then ultrasonic irradiated for 30 minutes, after this in the solution it was added NaOH 2N until the pH reaches at 12.5-12.6, the nanoparticles precipitated immediately as the NaOH solution come in contact with the ultrasonic irradiated solution of $FeCl_2$, the ultrasonic irradiation was continued for 10 minutes, the obtained nanoparticles was filtered and dried at 60°C for 1 hour. The laboratory set-up for magnetite synthesis, is shown in Figure 1.

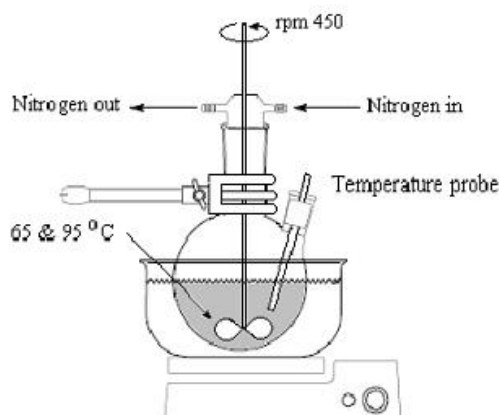


Figure 1

2.2. Apparatus

Fourier transformed IR spectroscopy (FT-IR) standard spectra were collected by using a Perkin Elmer Spectrum GX spectrometer. A range of 400–4000 scans were accumulated for each spectrum at a spectral resolution of 4 cm^{-1} .

The samples were examined by thermal analysis systems, too, by means of Mettler 4000 TA, TG 50 analyzer system at a rate of 10°C min^{-1} in a static air atmosphere;

X-ray diffraction (XRD) was used to determine the lattice spacing, iron oxide structure of the complexes and of the obtained product. The XRD patterns were recorded on a DRON 2 diffractometer

using $\text{CoK}\alpha$ and $\text{CuK}\alpha$ radiation with an iron filter.

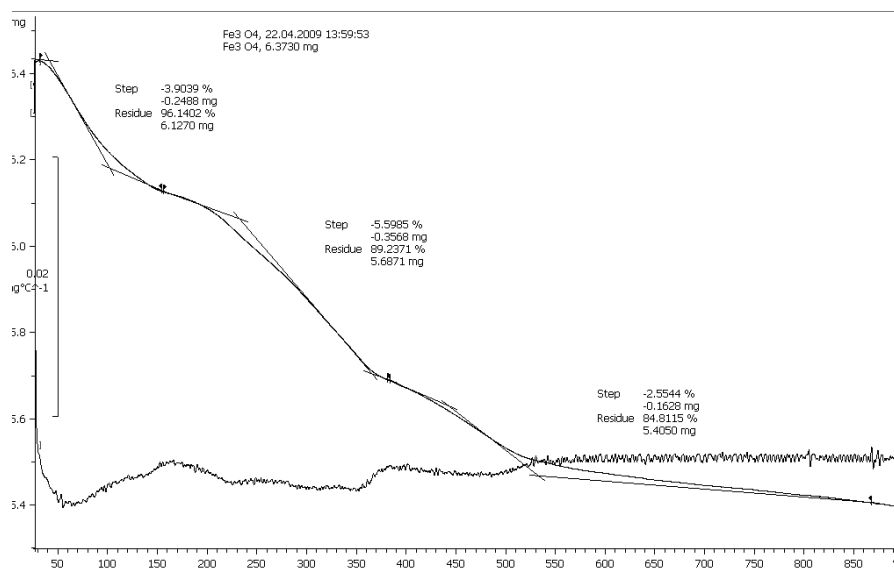
A confocal electron microscopy was performed using a Zeiss 900 and a Zeiss EM10 microscope. The tested cells (epitheloid carcinoma of human cervix (HeLa) (ATCC CCL-2; American Type Culture Collection, Rockville, MD), was used for testing.

3. RESULTS AND DISCUSSION

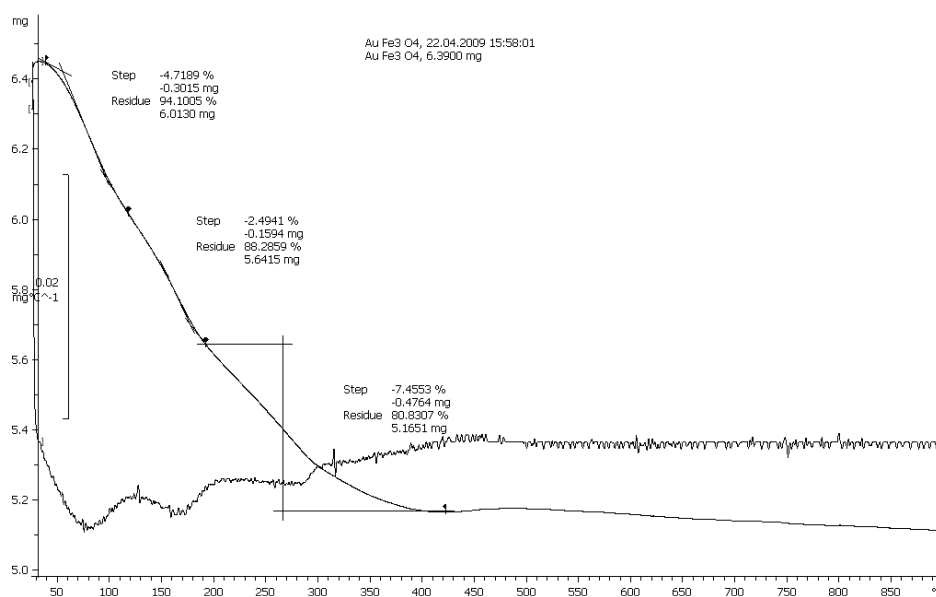
Thermal analysis of the unloaded and the loaded magnetite is done here using Differential scanning calorimeter. The thermograms of different Fe_3O_4 nanoparticles synthesized with or without Au are shown in Fig. 2. Below 100 degree C the weight losses of the nanocomposite are quite small because of the removal of absorbed physical and chemical water. All the thermograms show an endothermic peak seen at 100 °C which is due to release of adsorbed water and not due to any dehydroxylation reaction as magnetite does not have structural OH. The second peak which is exothermic in nature shows transformation of magnetite to maghemite.

DSC/TGA results showed that on addition of gold the exothermic transformation peaks of magnetite to maghemite shifted to higher temperatures. They weakened down and broadened. This suggests retardation of magnetite to maghemite transformation in presence of gold by substitution of gold in the magnetite structure.

DSC curves for synthetic magnetite samples are shown in Figure 2,a) for magnetite produced at 275, 425, and 485 °C, attributable to magnetite- maghemite transition.



a) Unloaded magnetite



b) gold loaded magnetite

Figure 2

FTIR is used here to understand structure, bonding and reactivity of magnetite and gold sorbed on its surface. The adsorbate will influence the symmetry and thus the vibrational spectrum of the surface atoms to which it is bound upon sorption. Observations are made on how the location and intensity of the absorption bands of magnetite are affected by substitution of gold atoms if any or what any other effect adsorbate atoms have on these vibrations. It is also used to study structure of surface complexes. Based on splitting of stretching frequency due to metal coordination, the surface complex of gold and the iron oxide could be identified.

FTIR spectrum of magnetite exhibits two strong infrared absorption bands at 570 cm^{-1} (ν_1), and 390 cm^{-1} (ν_2). These bands can be assigned to Fe–O stretching mode of the tetrahedral and octahedral sites for the ν_1 band at 570 cm^{-1} and the Fe–O stretching mode of the octahedral sites for the ν_2 band at 390 cm^{-1} , provided that Fe^{3+} ion displacements at tetrahedral sites are negligible. Oxides other than magnetite have also been given to record peaks corresponding to each of them in order to observe later if any kind of transformation is occurring in the presence of gold ions or if the transformation is hindered when these ions are adsorbed. The entire spectrum recorded from 4000 cm^{-1} to 400 cm^{-1} showed the following peaks which are attributed to specific functional groups. The general range of $3600 - 3100\text{ cm}^{-1}$ is due to the antisymmetric and symmetric OH stretching. The IR bands in region $1670 - 1600\text{ cm}^{-1}$ are due to bending vibrations of OH in the water molecule. FTIR spectroscopy (figure 3) was further used to measure the product. For the magnetite sample, the IR band at 3394 cm^{-1} can be assigned to the stretching modes of H_2O molecules or OH groups on the surface of magnetite particles; the IR band at 1547 cm^{-1} is assigned to the H_2O bending vibrations. The IR bands at 795 cm^{-1} and 891 cm^{-1} were detected only for the uncoated magnetite, and these IR bands should be attributed to the Fe–O–H bending vibrations in goethite ($\alpha\text{-FeOOH}$)^[6,7]. Since this bands are not detected for the gold coated magnetite we can assume that the core-shell structure was formed. The same conclusions could be deduced from EDXRF spectra figure 4.

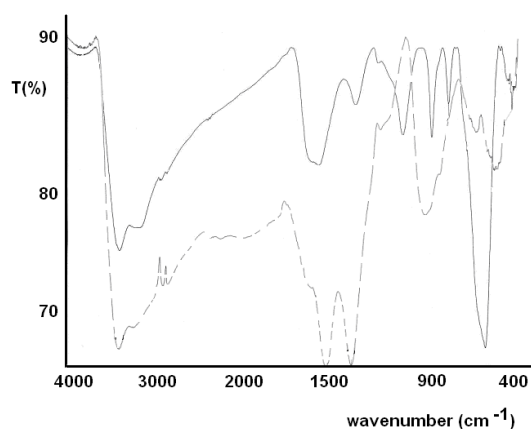


Figure 3. FTIR spectra of magnetite (—) and magnetite/gold (- - - -)

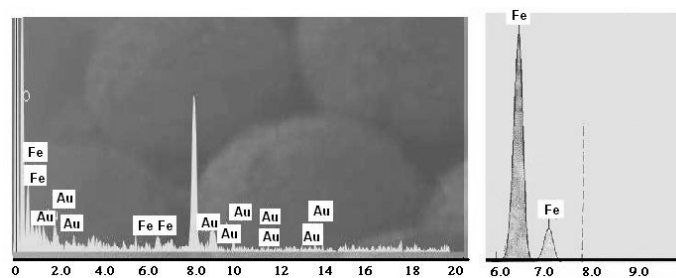


Figure 4. X-Ray fluorescence of the as obtained magnetite and magnetite/gold

Magnetite's X-ray diffraction pattern is shown in figure 5. The peak intensity can be used to quantify the proportion of iron oxide forms in a mixture by comparing experimental peak and a reference peak intensity.

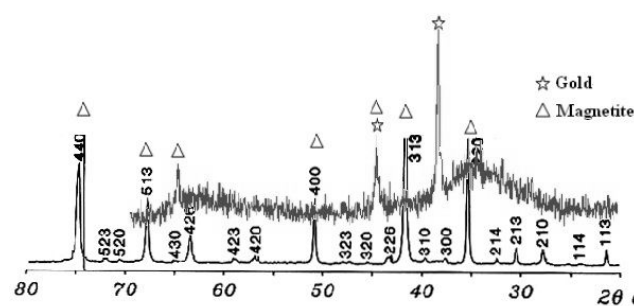


Figure 5. X-ray diffraction pattern of pure magnetite and magnetite/gold (intensity versus 2θ)

Iron oxide nanoparticle systems offer great potential in a diverse number of biomedical applications due to their guidable nature. The nanomagnetic particle surface can influence both material durability in biological environments and also toxicity issues.

The UV–Visible spectra were examined before and after separation of the magnetic nanoparticles from the gold nanoparticles (Figure 6). Plasmon resonance peaks detected after removing the magnetic nanoparticles from the reaction mixture established the formation of magnetite-gold nanocomposites. It should be noted that pure iron-oxide did not show a significant plasmon resonance peak in the absence of gold nanoparticles. As seen in Figure 6, the highest absorbance was observed for gold nanoparticles located at 530 nm (solid line curve), whereas Au-Fe₃O₄ composite nanoparticles showed a maximum peak at 608 nm (dotted line) which agreed with other previous reports. This wavelength shift in the gold surface plasmon, known as red-shift, is a probable occurrence and has been reported previously.

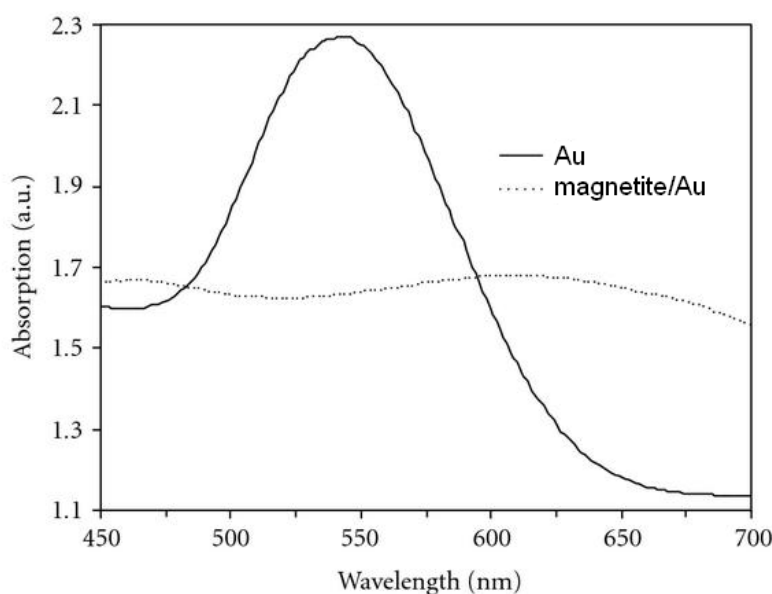


Figure 6: Absorbance spectra of gold nanoparticles (solid line) and magnetite-gold composite nanoparticles (dotted line).

For biomedical applications we tested our synthesized compounds on He La tumor cells.

The ultrastructural morphologic aspect of the HeLa cells phototreated with Fe₃O₄/Au, reinforces the results that a great number of cells with plasma membrane damage, characteristics of irreversible cellular lesion, and karyorrhexis nucleus were observed. These are characteristics of the cell necrosis process. Despite the knowledge that Fe₃O₄/Au localizes preferentially in the mitochondrial membrane the initial localization of the photosensitizer in the plasma membrane and the interaction time in our experiments suggest a process of cell death with necrosis like characteristics. This was probably due to the free radicals generated after PDT, leading to oxidation of the side chains of the amino acid residues that promote proteins cross-linking and oxidation of the protein structure that results in its fragmentation.

After Fe₃O₄/Au treatment the following features could be observed: (i) cytoplasmatic extensions branching out towards the periphery and contacting the neighboring preelongations and membranes, (ii) swellings on the cells surface, and finally (iii) some breaks (ruptures) at the cells periphery (Figure 7).

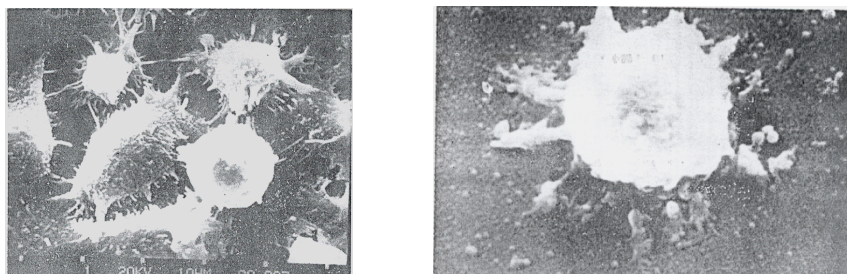


Figure 7. Microgramm of laser irradiated HeLa tumor cells

The main explanation resides in the fact that cisplatin induces apoptosis in HeLa tumor cells implying attack on mitochondria.

Fluorescence images of HeLa cells incubated with Fe₃O₄/Au are represented in Figure 8.

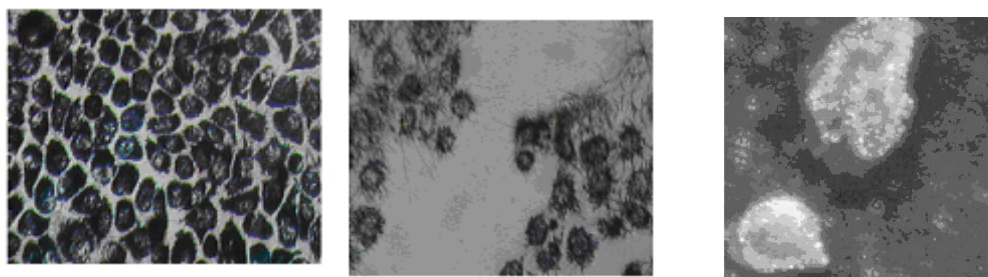


Figure 8. Fluorescence images of HeLa cells incubated with Fe₃O₄/Au

4. CONCLUSIONS.

This paper outlines a facile strategy for the sonochemical synthesis of Fe₃O₄-Au core-shell nanoparticles. The size and shape of the particles can be controlled and modified by adjusting only a few parameters as irradiation time, concentration of FeCl₂ or the proportion of ethanol-water mixture. The core-shell structure of composite particles was confirmed by the FT-IR spectroscopy. The composite particles offer advantages in both magnetic separation using the property of their magnetic core, and in immobilization of bio-molecule through their gold shell.

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