Synthesis of the newly developed Core-Shell Au/Fe3O4 Magnato-plasmonic nanocomposite in Cancer Cells

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Abstract: Background: Fe3O4-gold-Polyethyleneimine core-shell nanostructure can be used in biotechnological and biomedical applications.This research was conducted to assess the biocompatibility of the core–shell Fe3O4@ Au nanocomposite, which have potential application in biomedical imaging. **Results:** Magnetite nanoparticles with an average size of 14 nm in diameter were synthesized using the chemical co-precipitation method. A gold-coated Fe3O4 monotonous core-shell nanostructure was produced with an average size of 21 nm in diameter by PEI reduction of Au3+. The results of analyses with electron diffraction, particle size, zeta potential,Transmission Electron Microscopy (TEM),) and vibrating sample magnetometer (VSM) indicated that the nanoparticles were regularly shaped, and agglomerate-free, with a narrow size distribution. We use the Laser Induced Breakdown Spectroscopy (LIBS) as a promising non-destructive technique for the spectral analysis of Gold – coated magnetic nanoparticles (Fe3O4). The biocompatibility of the obtained NPs, at concentration, was evaluated via MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay and the results showed that the NPs were non-toxic at concentrations 50µg /mL. **Conclusions:** A rapid, mild method for synthesizing Fe3O4-gold nanoparticles using Polyethyleneimine was investigated. A magnetic gold core-shell- Polyethyleneimine nanocomposite, including both the supermagnetic properties of iron oxide and the optical characteristics of colloidal gold nanoparticles, was synthesized.

1. Background:

Nanoparticles are nanostructures with at least one dimension being less than 100 nm. Gold-coated magnetic nanoparticles are a class of nanoparticles that have attracted much attention because of their advantageous characteristics, such as their inertness, non-toxicity, super magneticity, ease of detection in the human body, a magnetic core that is protected against oxidation, their facilitated bioconjugating ability, catalytic surface, and their potential for a variety of biological applications [1,2]. Gold-coated nanoparticles have great biocompatibility with the human body with the ability to interact with biomolecules such as polypeptides, DNA, and polysaccharides [3]

Polyethyleneimine (PEI)-capped gold nanoparticles (AuNPs) are successfully manufactured using PEI as the reducing agent and stabilizer low toxicity and high biocompatibility.

The production of core-shell Fe3O4- gold-biopolymer nanocomposites has attracted much attention over the past several years as they can be used in biotechnological and biomedical areas, including bio targeting for cancer treatment, drug delivery, biodetection, and downstream processing (i.e., the purification and bioseparation of biomolecules). Gold nanocomposites utilizing Polyethyleneimine (PEI) offer several potential benefits using the magnetic core for controllability, as well as the immobilization of biomolecules and other optical properties through their gold shell [4-6].

In this work, we report on study of LIBS application for in vitro qualitative identification of two types of magnetic nanoparticles (Gold – coated iron oxide nanoparticles) uptake on cell. In this context, laser – induced breakdown spectroscopy (LIBS) is a potential alternative to other spectroscopic, mass spectrometric, or X-ray techniques used in biomedical applications. It is a practically non-destructive as well as rapid elemental analysis technique with the critical advantage of being applicable in situ, thereby avoiding sampling and sample preparation.

This paper describes a simple and rapid method for synthesizing controllable, agglomerate-free Fe3O4-gold Polyethyleneimine (PEI) nanocomposites. Polyethyleneimine (PEI) was used as the reducing agent. Additionally, the spectral properties of core-shell Fe3O4-gold nanoparticles synthesized by this method have been evaluated by modern analytical techniques (LIBS) and the results are discussed.

2. Material and methods
Synthesis of Fe3O4 core shell gold nanoparticles:

Briefly, the composite particle was prepared by the reduction of Au3+ with hydroxylamine in the presence of Fe3O4 particles as seeds. First, the seeds were co-precipitated Fe (II) and Fe (III) ions in alkaline medium, followed by rinsing with water several times until the pH reached 6–7. Second, Fe3O4 particles were dispersed in 0.1 mol/L (25 mL, 14mM) HAuCl4. 4H2O solution in a beaker, and slowly mixed in a shaking incubator to allow adsorption of Au3+ onto the Fe3O4 surface.PEI solution ofl % (w/w) PEI (1.44 mL) was then added to the system. The mixture was incubated with shaking for at room temperature for 24 h. The core/shell structure of Fe3O4/Au was formed. Finally, the particles were washed with water until the pH was 6.7.

Transmission electron microscopy (TEM) images on a JEM-2100 transmission of these Fe3O4@Au nanoparticles were digitized with a high-magnification. Absorption spectra of the Fe3O4/Au core/shell nanoparticles were measured and compared with bare Fe3O4 nanoparticles by Scan UV/Vis–near-IR spectrophotometer. Also measured size distribution, and zeta potential measurements of PEI-cappedFe3O4-AuNPs in aqueous solution were carried out by DLS and on a Malvern Zetasizer Nano ZS90 instrument with a He/Ne laser (633 nm) and 908collecting optics.
 Cell culture

A human liver cancer cell line (HepG2) purchased from Cancer National Institute was cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated new-born calf serum, penicillin G 100 U/mL, and streptomycin 100 μg/mL at 37°C in a humidified environment containing 5% CO2.

Cell proliferation assay

Cytotoxicity was determined by MTT assay. Exponentially growing HepG2 cells were seeded in 96-well plates in RPMI 1640 medium containing 10% new-born calf serum at a density of 5×10^3 cells/well for 24 hours. The cells were treated with concentration 50 μg/ mL of magnetic gold nanoparticles core shell containing Fe3O4 for 12, 24, and 48 hours. Meanwhile, RPMI 1640 medium was used as the blank control. Then, 20 μL of MTT 0.5 mg/mL were added to each well and cultured for another 4 hours. Thereafter, the formazan was dissolved with 150 μL of dimethyl sulfoxide after blotting the culture medium. Finally, the plates were shaken lightly for 10 minutes and the reduction of MTT was quantified by absorbance at a wave length of 570 nm using a microplate reader (Model-sunrise, Tecan AusturiaG mbh 5082Gordig, Austuria). The cell inhibition ratio (%) was calculated as (1 – A treated group/A control group) × 100. Each assay was repeated at least three times.

LIBS measurements were carried out by focusing a Nd-YAG laser (Continuum, Surlite II)at 532 nm with pulse width of 6 ns and 10 mJ pulse energy on the surface of the sample by a 50-mm focal length lens. The plasma emission was collected by telescopic system and transferred through a fiber to Echelle spectrometer (PI - Echelle, Princeton Instruments, USA) attached to an ICCD camera (ICCD I-MAX, Princeton Instruments). The focal spot diameter is found to be in the range of 50-100 μm. which is small enough to consider this technique as a non-destructive technique.

3. Results and Discussion

The self-prepared Fe3O4@Au nanocomposites were approximately spherical, particle size analyzer, and uniform in size as observed by TEM (Figure 1 (a,b)). The size and size distribution of metallic and oxide colloidal NPs are significantly influenced by the type of reduction reagents used in the process. Generally, a strong reduction reaction promotes a fast reaction rate and favors the formation of smaller NPs [7]. Meanwhile, we have found that the concentration of ammonium hydroxide has a marginal influence on the size of SPIONs in the range of 25%–30% v/v.
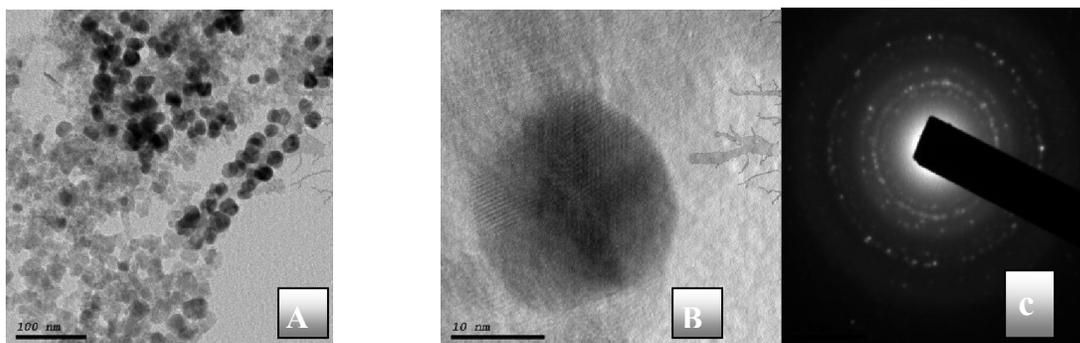

Fig. 1. TEM images of magnetic nanoparticles core shell gold.The processing condition(''run'')is reported in (a)–(b). Selected area electron diffraction pattern showing the magnetite core shell crystal lattice of bare FE3O4 -gold (C).

Figure1(c) shows that the typical selected area diffraction pattern (SADP) of Fe$_3$O$_4$@AuNPs.Basically as explained with Qianghua *et al.* [8], for gold containing composites with sizes higher than18 nm. The SAED pattern is the same as that of pure Au nanoparticles, however for nanoparticles with mean sizes lower this size range, the diffraction pattern is different for both pure iron oxide and pure gold NPs. In our case the ring pattern diameters is match very well with diffraction pattern of pure gold which is confirming the existence of gold atoms on the surface of SPIO NNPs. This observation is probably due to the heavy atom effect of gold NPs on the surface of SPION NPs.

The average diameter detected by Malvern laser particle size analyzer was about 21.04 nm, with a narrow diameter distribution for Fe$_3$O$_4$@Au composite MNPs (Figure 2a) and about 78.82 nm for Fe3O4-MNPs (Figure 2(b) inset). The zeta potential of Fe3O4@Au composite MNPs at pH 7.4 was 45.4.2 ±3.99 mV (Figure2 (c)) while it was −25.3± 4.1 mV for Fe3O4-MNPs (Figure 2(d) inset). The absorption spectra of Fe3O4/Au core/shell nanoparticles and bare gold nanoparticles is shown in Figure 3. A surface plasmon resonance band was observed at 590 nm for the Fe3O4/Au nanoparticles. The surface plasmon resonance band for the Fe3O4/Au core/shell bimetallic nanoparticles showed a red-shift and broadening of the peak, which is commonly observed in other Au bimetallic systems.[9].
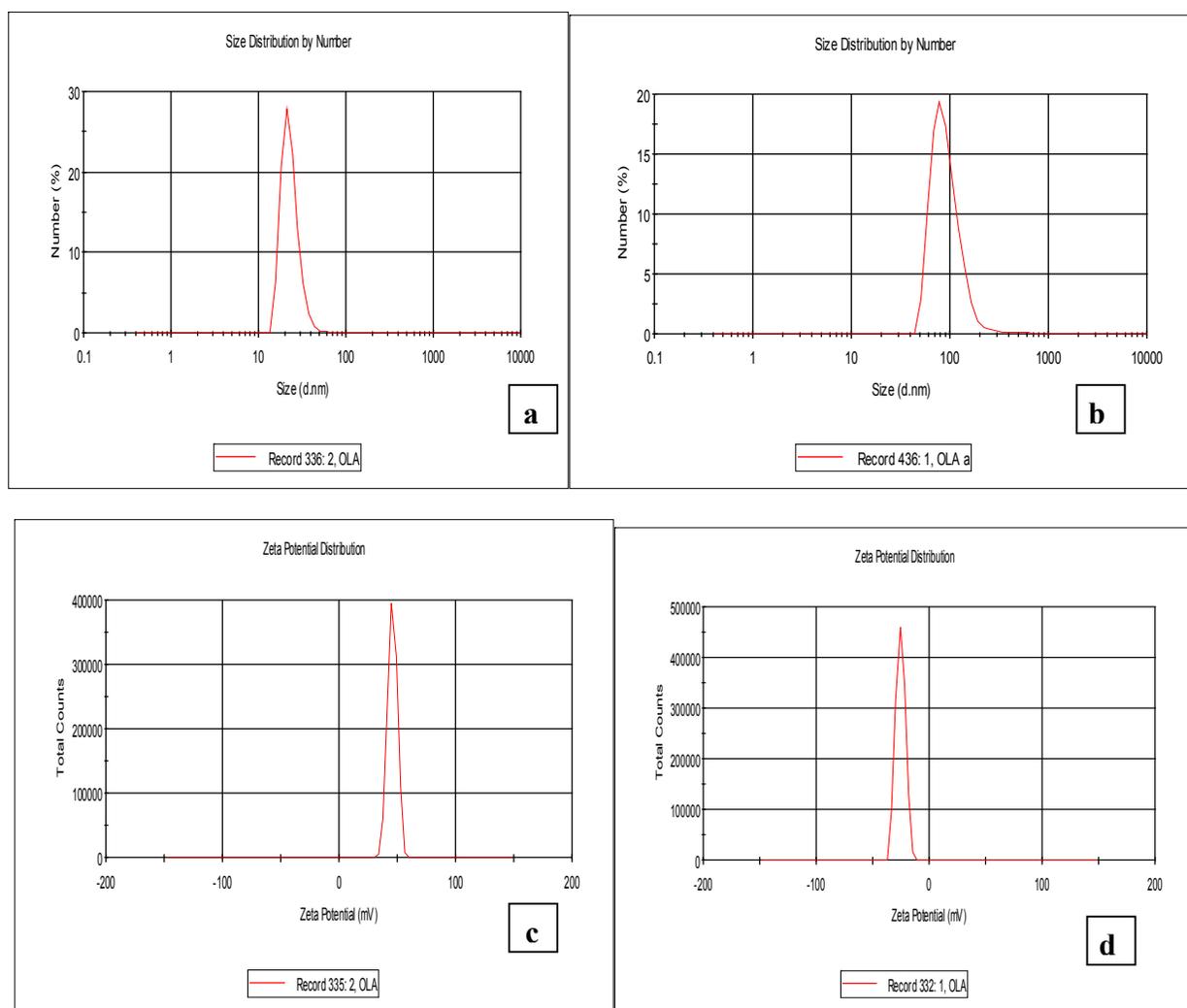

Figure (2) the particle size and zeta potential (a,c) magnetite core shell gold Fe$_3$O$_4$@Au (b, d)the magnetic nanoparticle.
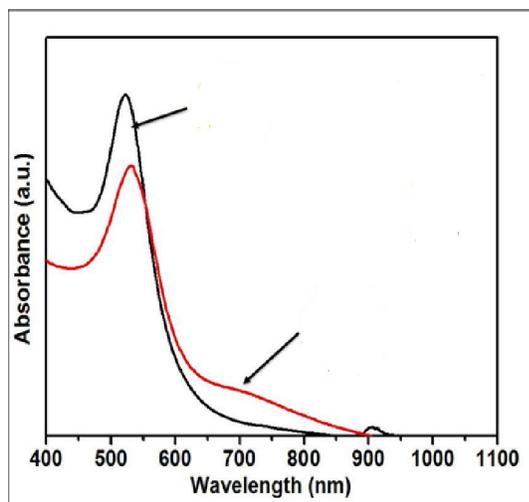
Figure 3. UV–vis spectracomparingAu and Fe3O4@Au.

The magnetization versus the magnetic field of optimized bare Fe3O4 and Fe3O4@Au are shown in Figure 4 (a, b). Display magnetic data illustrates the major differences between Fe3O4 and Fe3O4@AuNPs. First, the magnitude of the magnetization for Fe3O4@Au is much smaller than that of Fe3O4. The maximum magnetization values are 22 emug^{-1}(Fe3O4)

and 3.5emug^{-1} (Fe3O4) for optimized Fe3O4 and Fe3O4@Au, respectively. The mass of Fe3O4 was obtained from the loading of magnetic particles in the plastic capsules. It is believed that the big difference between the two values reflect the decreased coupling of the magnetic moments as a result of the increased spacing between the particles of the cores, which is due to formation of the gold shells. On the basis of the data near zero magnetization,we can identify another important difference in coercivities between Fe3O4 and Fe3O4@Au NPs. The Hysteresis plots showed a clear increase in coercivity for Fe3O4@Au NPs. This observation likely reflects the fact that coercivity of a superparamagnetic NP is inversely related to the particle size and less effective coupling of dipole moment due to increase in the inter particle distance [10]. The increase in the coercivity of Au-coated Fe3O4 can be attributed to the increasing of inter particle distance between magnetic cores due to non magnetic effect of gold shell that leads to a less-effective coupling of the magnetic dipole moments.In contrast, the magnetic dipole moments in the case of uncoated Fe3O4 NPs are coupled more effectively,and hence the particles tend to have a lower coercivity [11].
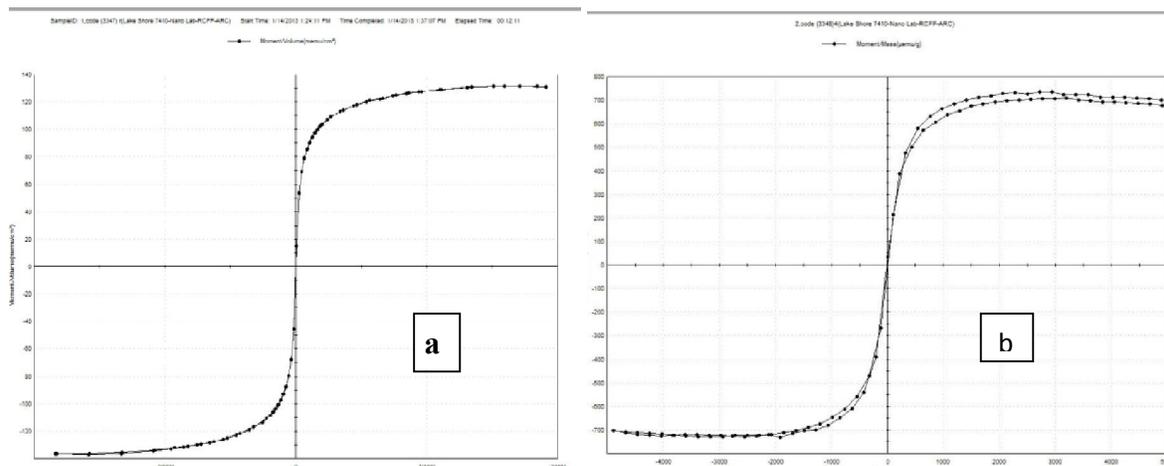

Figure (4) Magnetization hysteresis for optimized Fe3O4 (a) and Fe3O4@Au(b)

Gold provides stability for the magnetic nanoparticles in solution as well as providing a good inert surface for assisting the binding of various biomolecules [12-14]. The gold shell was synthesized by the reduction of Au3+ with PEI as a nontoxic, biocompatible reducing agent in the presence of Fe3O4 nanoparticles.

***In-vitro* biocompatibility**

Fig. 5 shows the results of MTT assay at different incubation time of Fe3O4@Au NPs. Viability greater than 80% after 24h incubation at concentration

50 μg /mL reveals acceptable *in-vitro* biocompatibility [15]. The results of the present study are in agreement with Karlsson *et al.* [16] and Taylor *et al.* [17] that reported Fe3O4 /gold are safe and non-cytotoxic at concentration 50 μg/ mL. Meanwhile,it is noteworthy to mention that toxicity pathway of Fe3O4 gold could be different due to the size and morphology variations [18].

LIBS spectral

Using the described LIBS technique, the element content of the cells was identified uptake of

iron magnetic nanoparticles(Fe3O4) into cells and gold iron magnetic nanoparticles (Au- Fe3O4). Specific spectral lines of elements had been chosen as the finger print lines (FPL) characterizing two specific magnetic nanoparticles types, namely iron magnetic nanoparticles (Fe3O4) and gold iron magnetic nanoparticles (Au- Fe3O4). Spectral lines of only two elements had been selected as FPL for the identification of iron magnetic nanoparticles(Fe3O4) and gold iron magnetic nanoparticles (Au- Fe3O4). Figure. 6a shows zoomed segments of the spectrum where Fe spectral lines appear. Fe lines at 261.2, 271.8 and 272.7nm were the finger print lines for uptake of

iron magnetic nanoparticles(Fe3O4) into cells. On the other hand figure 6b shows the spectral lines of Au at 267.6 nm and Fe at at 261.2, 271.8 and 272.7nm were the finger print lines for uptake of Gold Fe3O4 into cell .

The main advantages of this technique are rapid identification uptake of iron magnetic nanoparticles (Fe3O4) into cells and need little or no sample preparation where the time required for a identification was between 15 and 25 sec which is extremely short time compared with the conventional or even other laser based analytical techniques.
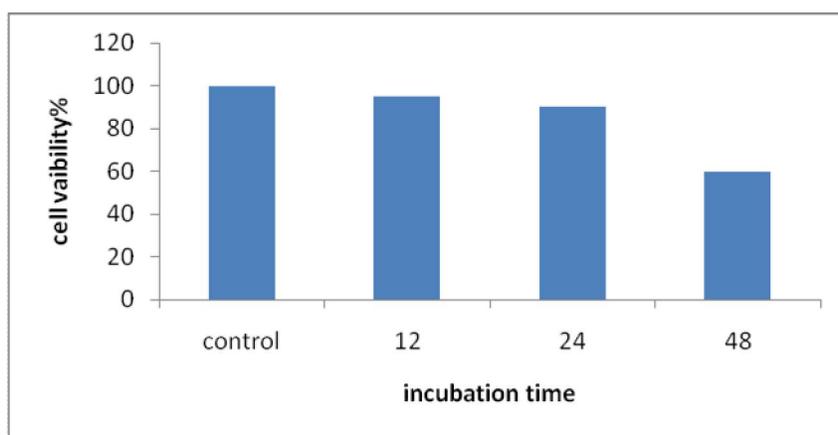

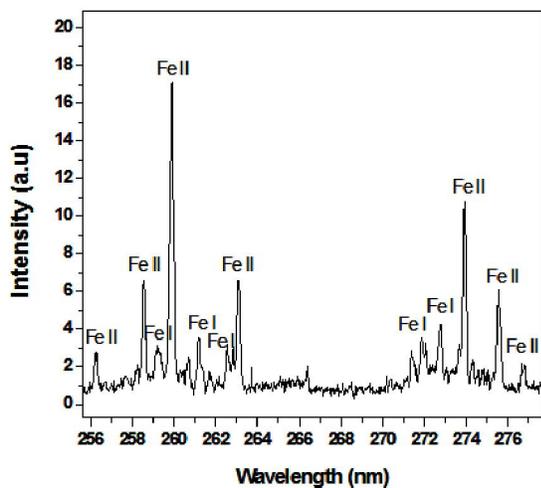**Figure 5: The percentage viability of cells at different incubation time of the Fe3O4 /gold nanoparticles**

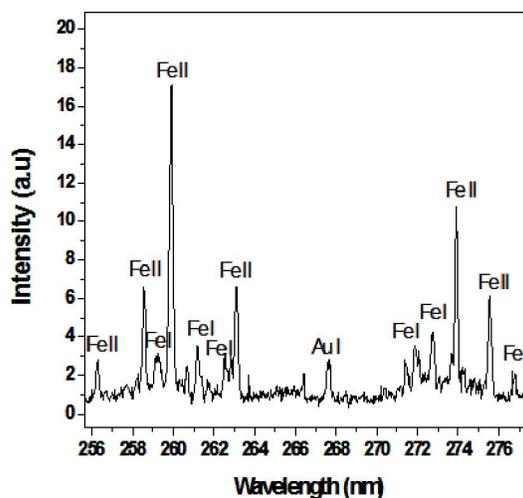**Figure. 6a LIBS Spectrum of Cells uptake Fe3O4 nanoparticles**

Figure. 6b LIBS Spectrum of Cells uptake Au- Fe3O4

Conclusion:

This study demonstrates the potential of magnetited gold nanocomposed was synthesized, A rapid, simple, agglomerate free method was reported for the production of monodisperse gold-coated Fe3O4 nanoparticles using biopolymer PEI as a stabilizing agent and reducing agent. Magnetic Fe3O4-gold- Core-

shell PEI nanostructures show a great potential for biotechnological and biomedical applications in the near future. The technique of laser induced breakdown spectroscopy (LIBS) offers a simple and fast method for elemental analysis, which can be very beneficial in many nanotechnology and biomedical applications.

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