

# Multifunctional Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles for photodynamic therapy

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## ABSTRACT

We synthesized Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell structure as a multifunctional platform for photodynamic therapy (PDT). The magnetic properties of these nanostructures will enable magnetically driven targeting as well delivery of photosensitizer deep in tumor tissues. Reactive oxygen species (ROS) generation capability of ZnO will help to kill cancer cells through oxidative stress. Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanostructures were synthesized via a simple method in aqueous phase. X-ray diffraction (XRD) patterns showed the presence of both, Fe<sub>3</sub>O<sub>4</sub> and ZnO phases that suggests the development of a core-shell. Photoluminescence (PL) measurements displayed the excitonic emission of ZnO, at around 380 nm, in co-existence with a weak and broad defect-related green emission. Energy Dispersive X-ray Spectroscopy (EDS) indicated the presence Zn species on the crystal surface at variable amounts, which were dependent on the shell thickness. The generation of singlet oxygen species under UV irradiation has been verified using DPBF (1,3-diphenylisobenzofuran) as quencher for singlet oxygen. Quantum yield (QY) of singlet oxygen generated by core-shell nanoparticles was found to be 28%, suggesting the potential application of these core-shell nanostructures in PDT.

**Keywords:** magnetite, zinc oxide, heterostructured nanoparticles; singlet oxygen, photoluminescence.

## 1 INTRODUCTION

PDT is an emerging cancer treatment modality that involves the use of a photoactive drug, a photosensitizer (PS) and light. The light interacts with photosensitizer and generates singlet oxygen, responsible for killing of cancer cells selectively [1-2]. The possibility of promoting the singlet oxygen quantum yield by using QDs alone or combined with conventional photo-sensitizers, opens new possibilities for cancer therapy [3]. Recent studies on QDs for PDT applications have been centered on CdSe or CdTe and CdSe/ZnS core/shell QDs. The enhanced generation of singlet oxygen from CdSe QDs linked to Pc4

PS has been verified along with the capability of bare CdSe to sensitize molecular oxygen [4]. Derfus et al. also verified the QDs cytotoxicity mediated by UV irradiation [5]. Bakalova's group has confirmed the potential of CdSe QDs anti-CD conjugates to sensitize leukemia cells to UV irradiation and/or to reinforce the effect of conventional PS trifluoperazine sensitizer [6]. These studies suggested that semiconductor QDs dots and QDs-PS platform will enhance the effectiveness of PDT generation and application in selective destruction of cancer cells. However, these most promising Cd-based QDs structures are considered to be most cytotoxic. Therefore, design and development of biocompatible, non-toxic QDs and their evaluation as direct sensitizers or QD/PS conjugate for PDT in cancer become indispensable. In the present work, we have synthesized multifunctional Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles and evaluated their capability to generate singlet oxygen. The photochemical activity of Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles has been investigated in presence of 1,3-diphenylisobenzofuran (DPBF), a singlet oxygen quencher, in methanol [7].

## 2 EXPERIMENT

### 2.1 Materials

Iron (II) chloride tetrahydrate FeCl<sub>2</sub>·4H<sub>2</sub>O, Iron (III) chloride hexahydrate, FeCl<sub>3</sub>·6H<sub>2</sub>O, sodium hydroxide NaOH, zinc acetate dihydrate Zn(OOCCH<sub>3</sub>)<sub>2</sub>·H<sub>2</sub>O, lithium hydroxide monohydrate LiOH·H<sub>2</sub>O, zinc nitrate hexahydrate, Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, 1,3-diphenylisobenzofuran (DPBF, 97%), Methylene Blue (MB) 99%, Rose Bengal (RB) 95%, nitric acid ACS reagent 70%, hydrochloric acid ACS reagent 37%, and ammonium hydroxide NH<sub>4</sub>OH (14.5 M) were of reagent grade and used without further purification. Acetone and ethanol were of chemical grade.

### 2.2 Synthesis of Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles

Fe<sub>3</sub>O<sub>4</sub> superparamagnetic nanocores were prepared and treated with sodium citrate according to available methods

in the literature [8, 9], with minor modifications. These as-treated nanoparticles were dispersed into deionized water at pH 5.0. Then aqueous solution of  $Zn(NO_3)_2$  was added dropwise into this suspension keeping the magnetite/Zn molar ratio at 1:2 and contacted for 1h under vigorous stirring at room temperature. After the pH value was increased up to 9.0 at  $80^\circ C$ , the resulting suspension was stirred for additional 3 hours to promote the dehydration and atomic rearrangement involved with the formation of ZnO on the surface of magnetite. Finally, the solids were magnetically collected, washed with ethanol and abundant deionized water and dried.

### 2.3 Determination of singlet oxygen

The generation of singlet oxygen (SO) was verified by photooxidation of DPBF and monitored by absorption spectroscopy. Photooxidation reaction involves the formation of singlet oxygen via energy transfer from the excited nanoparticle to ground state molecular oxygen. A stock solution of  $5.0 \times 10^{-4}$  M QDs and  $6.0 \times 10^{-5}$  M DPBF were prepared and mixed immediately in a quartz cuvette in dark before exciting it with a light source. The optical absorption spectra were recorded as a function of the irradiation time.

### 2.4 Characterization techniques

X-Ray Diffraction (XRD) measurements were carried out with a Siemens Diffractometer D5000 using Cu-K $\alpha$  radiation. Luminescence properties were determined by photoluminescence (PL) spectroscopy in a spectrofluorometer fluoroMax-2 at room temperature with a 150mW continuous ozone-free Xe lamp as the excitation source. Optical absorption measurements were carried out in an UV-Visible spectrophotometer (DU 800, Beckman Coulter). Energy dispersive X-ray spectroscopy (EDS) was carried out using LEO35 Field Emission by Zeiss. The magnetic properties were measured at room temperature with a Vibrating Sample Magnetometer (VSM, Lakeshore 7400).

## 3 RESULTS AND DISCUSSION

XRD analyses of the as-synthesized isolated and heterostructured  $Fe_3O_4/ZnO$  powders confirmed the development of crystalline cubic magnetite and hexagonal ZnO wurzite phases (figure 1 a&b). The XRD pattern of figure 1-c clearly shows the diffraction peaks corresponding to both  $Fe_3O_4$  and ZnO phases that would suggest the formation of a core-shell like arrangement. The shell thickness was calculated by comparing the average crystallite size using the (400) peak of pure and  $Fe_3O_4/ZnO$  nanoparticles and estimated to be 2nm.

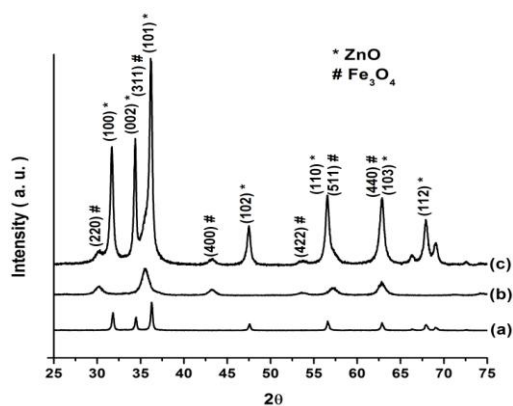


Figure 1: X-ray diffraction spectra for ZnO (a),  $Fe_3O_4$  (b) and  $Fe_3O_4/ZnO$  (c) nanoparticles.

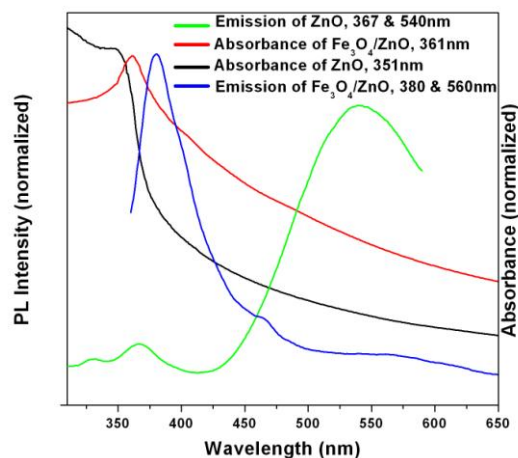


Figure 2: PL and Uv-Visible measurements for pure ZnO  $Fe_3O_4/ZnO$  core shell nanoparticles.

Figure 3 shows the photoluminescence and Uv-Visible spectra for for pure ZnO and  $Fe_3O_4/ZnO$  core-shell nanoparticles. The photoluminescence spectra of were recorded the with excitation wavelength of 350 nm. The emissions corresponding to excitonic recombination at 380 nm and oxygen vacancy or surface defects related at 540 nm are observed for  $Fe_3O_4/ZnO$  and pure ZnO nanoparticles. However, the emission at 540 nm is significantly reduced in core-shell nanoparticles. A plausible explanation for the quenching of defect-related peak in the core-shell nanoparticles would consider the reduction of the density of oxygen vacancies near the crystal surface in the ZnO layer deposited onto the magnetite core.

Energy dispersive X-ray spectroscopy analyses confirmed the presence of Zn, O, Fe in as-synthesized core-shell nanopowders (Figure 3).

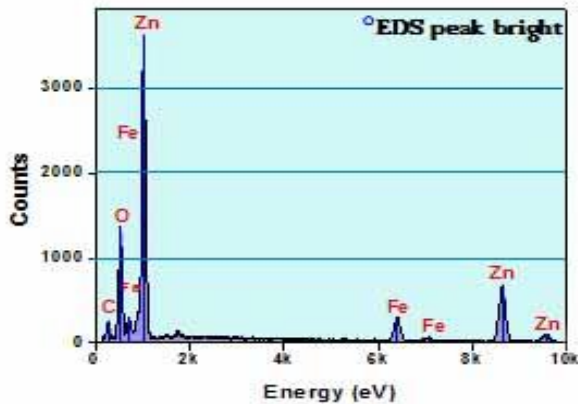


Figure 3: EDS spectra for Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles.

UV-visible spectroscopy was used for the indirect measurements and quantification of singlet oxygen generation by Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles. PDT efficiency depends on the efficacy of the NPs-PS platform to generate SO that could be determined by measuring SO quantum yield ( $\Phi_{\Delta}$ ). The quantum yield is defined as the number of <sup>1</sup>O<sub>2</sub> molecules generated per absorbed photon. Kostka et al. used a method [10] based on the change in absorbance of a chemical scavenger caused by its time-dependent decomposition by SO. In our case we have used DPBF as singlet oxygen scavenger. The singlet oxygen quantum yield is calculated using following equation.

$$\Phi_{\Delta \text{ sample}} = \Phi_{\Delta \text{ standard}} (k_{\text{standard}}/k_{\text{sample}})$$

Where,  $\Phi_{\Delta \text{ sample}}$  is the SO quantum yield of Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles,  $\Phi_{\Delta}$  is the standard SO quantum yield of a standard photosensitizer (rose Bengal). The  $k_{\text{standard}}$  and  $k_{\text{sample}}$  are determined from the slopes of the time-dependent decrease of DPBF plots, expressed as the decrease of absorbance A at 409 nm,  $d(\ln(A_0/A_t))/d(t)$ , of the standard photosensitizer and the measured sample, respectively.

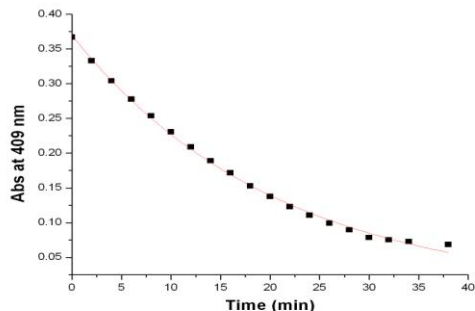


Figure 4: Time-dependent photooxidation of DPBF by singlet oxygen produced by 1x10<sup>-5</sup> M of rose bengal in methanol

Figure 5 shows the absorption decay curves of 6.0 x10<sup>-5</sup> M solution of DPBF as function of irradiation time in presence of 7x10<sup>-5</sup> M and 5x10<sup>-4</sup> M suspensions of Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles in methanol. The results revealed the higher activity for higher concentrations of quantum dots as could be seen from exponential decay in absorbance at 409 nm. The DPBF absorbance followed the exponential decay trend similar as shown in figure (4) for rose bengal, a well known singlet oxygen generator[11]

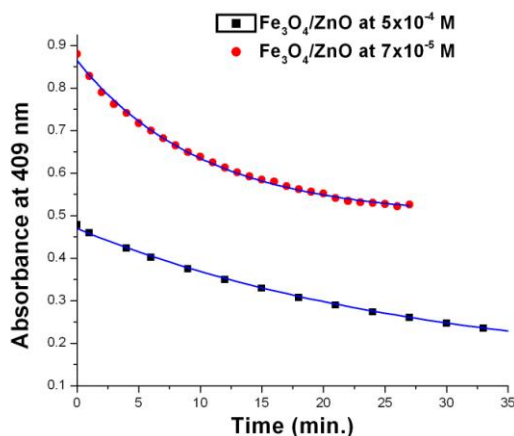


Figure. 5 Kinetics for photo-oxidation of 6.0x10<sup>-5</sup> M DPBF using 7x10<sup>-5</sup> M and 5.0x10<sup>-4</sup> M Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles

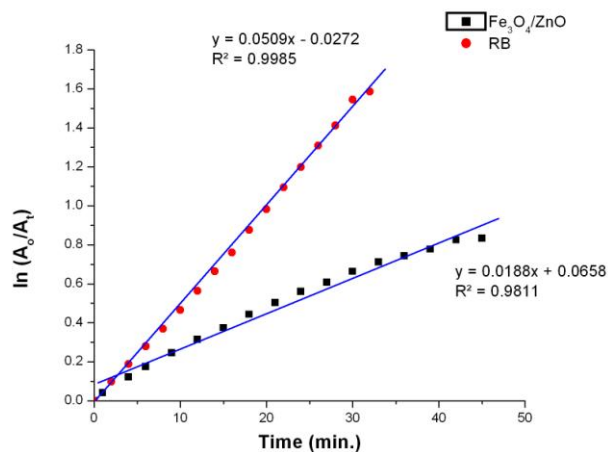


Figure 6: Time-dependent decomposition of DPBF by singlet oxygen produced with Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles at 7x10<sup>-5</sup> M in methanol.

Figure 6 shows the plot  $\ln(A_0/A_t)$  vs time for 7x10<sup>-5</sup> M Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles and rose bengal. The QYs for Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell and rose bengal were calculated to be 0.28 and 0.76 respectively. Table 1 shows the comparison of quantum yield for synthesized core-shell

nanoparticles with other reported results suggesting the potential application of them for PDT.

**Table 1: Comparison of Singlet Oxygen QY**

QDs/PS	PS	$^1O_2 \Phi_A$	Ref.
	Rose bengal	0.76	Rosa et al [7]
	Methylene blue	0.5	Rosa et al [7]
CdSe		0.05	Samia et.al[4]
CdTe/dye		0.43	Shi et al [12]
<u>CdSe@CdS@ZnS</u> /phitochelatin		0.31	Hsieh et al [13]
Fe <sub>3</sub> O <sub>4</sub> /ZnO core-shell		0.28	Present work

## 4 CONCLUSIONS

Water-based superparamagnetic Fe<sub>3</sub>O<sub>4</sub>/ZnO core-shell nanoparticles have been successfully synthesized using a simple aqueous solution method. XRD results confirmed the formation of nanosize crystalline magnetic Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles. The photoluminescence measurements revealed the feasibility in the band gap engineering as a result of ZnO shell formation onto Fe<sub>3</sub>O<sub>4</sub>cores. The possibility and capability of the Fe<sub>3</sub>O<sub>4</sub>/ZnO nanoparticles to generate singlet oxygen has also been verified through the continuous oxidation of DPBF. The quantum yield of synthesized core-shell structures to generate singlet oxygen was estimated to be 0.28 suggesting their potential application in PDT for cancer treatment

## 5 ACKNOWLEDGEMENTS

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