Electronic Supplementary Material (ESI) for RSC Advances.

Synthesis and *In-Vitro* Cytotoxicity Evaluation of Ruthenium Polypyridyl-Sensitized Paramagnetic Titania Nanoparticles for Photodynamic Therapy

Mohammad H. Sakr,[a] *Najeeb M. Halabi,*[b,c] *Leen N. Kalash,*[a] *Sara I. Al-Ghadban,*[b] *Mayyasa K. Rammah,*[b,d] *Marwan E. El Sabban,*[b*] *Kamal H. Bouhadir,*[a*] *Tarek H. Ghaddar*[a*]

Nanoparticles Preparation

Synthesis of Polyacrylic-Acid-Capped Iron Oxide Nanoparticles (PAA-Fe_3O_4), (A-NPs): Stable Fe_3O_4 nanoparticles were synthesized using the method of coprecipitation of Fe(II) and Fe(III) salts in the presence of PAA oligomer as a surfactant following a reported procedure with modifications.(1, 2) A 500 ml 3-necked round bottom flask (RBF) was charged with 5.57 g (21.24 mmol) of $FeCl_3.6H_2O$, 2.01 g (10.21 mmol) of $FeCl_2.4H_2O$ and 100 ml of water. A solution of 1.74 g of PAA in 50 ml of water was added to the mixture. The mixture was probe sonicated for 15 min, followed by purging with nitrogen gas for 20 min while heating it up to 60°C. The mixture with stirred vigorously and excess NH_4OH (10 ml, 28 wt%) were transferred to the reactor using a syringe. The temperature was raised to 90°C and the mixture was stirred for 45 min, cooled down to room temperature and dialyzed against water until it became neutral (the *p*H of the dialysate was periodically checked using a litmus paper). The final concentration was found to be ≈ 32 mg/ml of water.

Preparation of Fe_3O_4@SiO_2, (B-NPs): A modified Stober method(3) of coating the magnetite nanoparticles with silica was carried out in a basic ethanol/water mixture at 25°C. In a 5-liter 3-necked RBF, 20 ml of PAA-Fe_3O_4 solution were diluted in a 2560 ml of 1:3 water:ethanol solution, then probe sonicated for 30 min. Next, excess NH_4OH (48 ml, 28% by weight) was added to the solution before it was deaerated with nitrogen for 30 min. Then, under vigorous mechanical stirring, 4.8 g (28.5 mmol) of TEOS diluted in 320 ml of ethanol were added drop-wise from a separatory funnel as slow as possible; and stirring was continued for 12 h. The resultant solution was probe sonicated for 30 min and dialyzed against ethanol to a final concentration of ≈ 12 mg/ml of ethanol.

Preparation of Fe_3O_4@SiO_2@TiO_2, (C-NPs): Coating of nanoparticles with titania was carried out following a reported procedure with modifications.(4) Briefly, 71 ml of silica-coated NPs (12 mg/ml in ethanol) were diluted with 3270 ml of ethanol and sonicated in a 5-liter RBF for 30 min. With vigorous mechanical stirring at 50°C, a solution of titanium isopropoxide (8.54 ml titanium isopropoxide in 430 mL isopropanol) was added drop-wise to the mixture over a period of 3 hours. The mixture was left to age at 50°C for 4 hours. Water (30 ml) was added and the reaction was stirred at 60°C for 30 min. After 24 h, the mixture was sonicated for 30 min followed by exhaustive dialysis against water to remove the alcohol. The final volume of the solution was adjusted to 80 ml followed by hydrothermal treatment at 200°C for 24 h. The final nanoparticle concentration was 19.6 mg/ml in water.

Anchoring Ruthenium to Fe_3O_4@SiO_2@TiO_2@Dye (D-NPs): The inorganic dye that was used in this study was (dc-bpy)$_2$Ru(dm-bpy).2PF$_6$ (Dye) where dc-bpy is 4,4′-dicarboxy-2,2′-bipyridine, dm-bpy is 4,4′-dimethyl-2,2′-bipyridine. This dye was prepared following a procedure we have reported earlier.(5) To anchor it to the titania-coated NPs (C-NPs), a solution of the latter was dialyzed against ethanol until it became water free. Excess dye (0.5 mM) in ethanol was added to the dialyzed C-NPs solution, bath sonicated for 30 min, and then left in the dark overnight. The solution was washed multiple times by centrifugation until the supernatant solution obtained after centrifugation was dye free. The final NPs concentration was 9.5 mg/ml in ethanol.

Preparation of FITC-labeled C-NPs: The FITC-labeled C-NPs were prepared in two steps; anchoring dopamine to the surface and labelling the dopamine with fluorescein isothiocyanate. Dopamine was anchored to the NPs by incubating C-NPs with excess dopamine overnight at room temperature. The NPs were magnetically captured with the aid of a Neodymium magnet and washed multiple times with water to remove the remaining free dopamine. The FT-IR spectrum of the resulting NPs confirmed the presence of dopamine on the nanocomposites. In the second step, excess fluorescein isothiocyanate in ethanol was added to the resulting NPs solution, and was left overnight. The next day, the remaining free FITC was removed as before by successive washes with ethanol and water. The final supernatant was discarded and the FITC-C-NPs were reconstituted in water.

Results

The magnetite cores (A-NPs) were electrostatically stabilized and dispersed by the addition of polyacrylic acid. Infrared, energy-dispersive X-ray and X-Ray diffraction measurements of the multilayered NPs confirmed the presence of the surfactant polymer and the three oxides. Figure S1 shows the FT-IR spectra of PAA alone, and those of the NPs at different stages of their synthesis. The strong band at ca. 1710 cm^{-1} in PAA spectrum is attributed to the carbonyl group stretching vibration.(6) The appearance of the latter in the spectrum of A-NPs with a lower intensity is indicative of the presence of PAA and its binding to the surface of the magnetite NPs forming carboxylate groups.(7) The spectrum of A-NPs also exhibits the characteristic absorption band of Fe-O stretching vibration at 582 cm^{-1} as previously reported.(1) The spectrum of B-NPs show the symmetric and asymmetric vibrations of Si-O-Si at around 800 and 1080 cm^{-1}, respectively.(8) The broad band in the spectrum of C-NPs between 500 and 800 cm^{-1} is that of Ti-O-Ti vibration.(8)

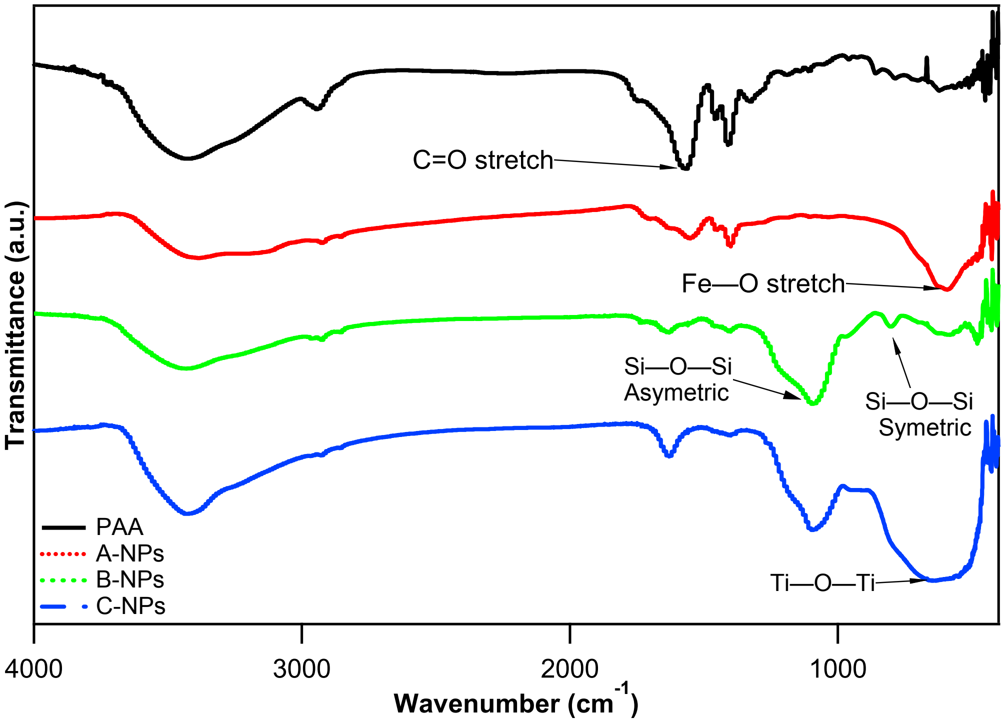

Figure S1: : FT-IR spectra of PAA (solid-black), A-NPs (dotted-red), B-NPs (dashed-green), and C-NPs (dashed-dotted-blue).

The X-ray diffraction patterns of the different NPs are presented in Figure S2. The XRD pattern of A-NPs was in agreement with the JCPDS card No. 19-0629, presenting the characteristic peaks at 2θ: 30.4° (220), 35.7° (311), 43.4° (400), 53.8° (422), 57.4° (511) and 63.0° (440) of cubic spinel structure of magnetite.(9) Coating the Fe_3O_4 cores with SiO_2 didn't change the structure of the former as can be seen in the diffractogram of the B-NPs. The XRD pattern of the latter show the attenuation of the Fe_3O_4 peaks, and a new broad peak between 20° and 28° which is indicative of the amorphous nature of the SiO_2 layer.(9) The diffractogram of C-NPs comprises peaks for TiO_2 layer along with those of the magnetic core. The peaks at 25.4° (101) and 48.1° (200) are characteristic of the anatase polymorph of TiO_2 (JCPDS card No. 21-1272).(10)

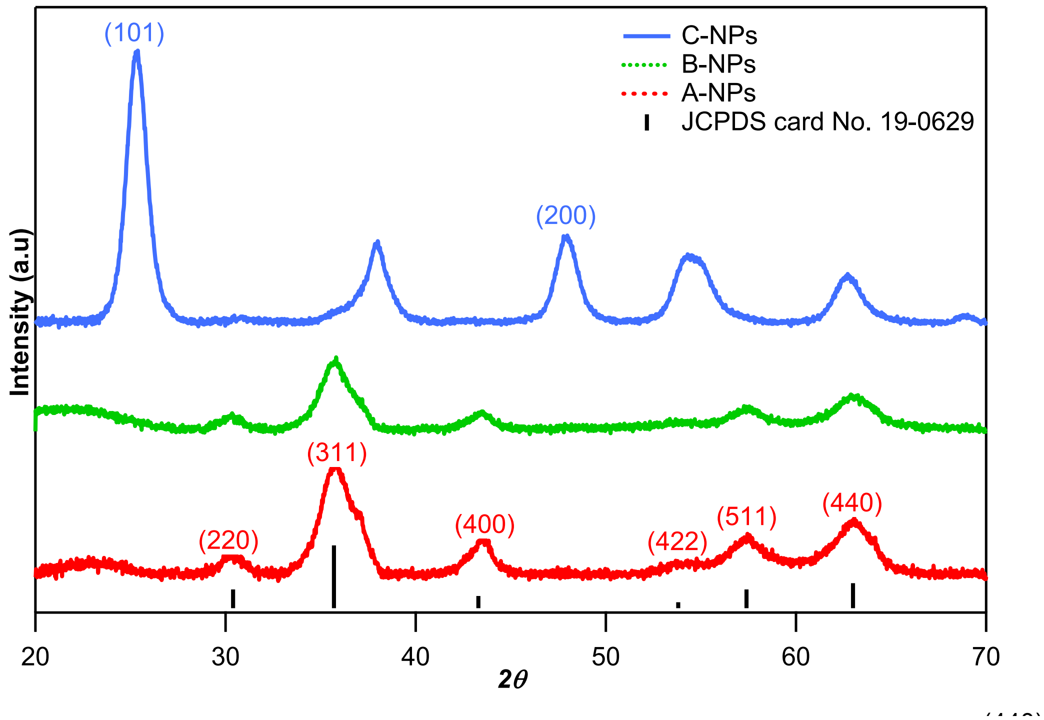

Figure S2: : XRD diffractogram of A-NPs (dashed-red), B-NPs (dotted-green), and C-NPs (solid-blue) in addition to JCPDS card No. 19-0629 of Fe3O4.

The energy dispersive X-ray spectroscopy analysis of the different NPs showed the presence of the 5 expected elements: C, O, Fe, Si, and Ti. The relative percentage of iron to silicon was consistent at 2:3 in both the silica coated and the titania coated samples, Table 1.

Table 1: mass percentage of the different elements in B and C NPs.

Sample	Iron	Silicon	Titanium
B-NPs	39.37	60.63	-
C-NPs	12.10	17.55	70.35

Brunauer-Emmett-Teller (BET) surface areas for the different nanocomposites are summarized in table 2. The surface area increased with the addition of each coating layer but then decreased following the hydrothermal treatment. This suggests that the increased crystallinity of TiO_2 (rutile → anatase) achieved by the treatment was accompanied by a deterioration of its porous structure.

Table 2: BET surface area of the different NPs.

Sample	BET Surface Area (m^2/g)
A-NPs	95
B-NPs	145
C-NPs (before hydrothermal treatment)	233
C-NPs (Hydrothermally treated)	126

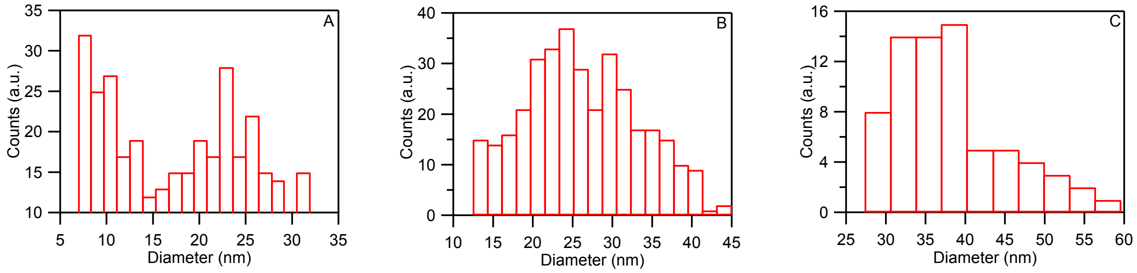

Figure S3: The particle size distributions obtained from SEM images of A, B and C.

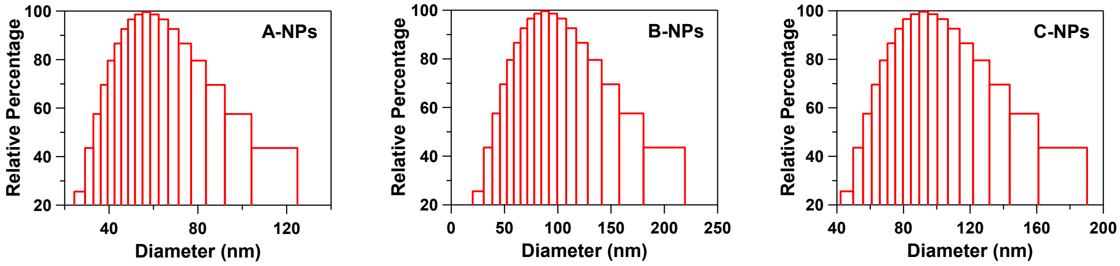

Figure S4: DLS intensity-weighed hydrodynamic diameter of the different NPs.

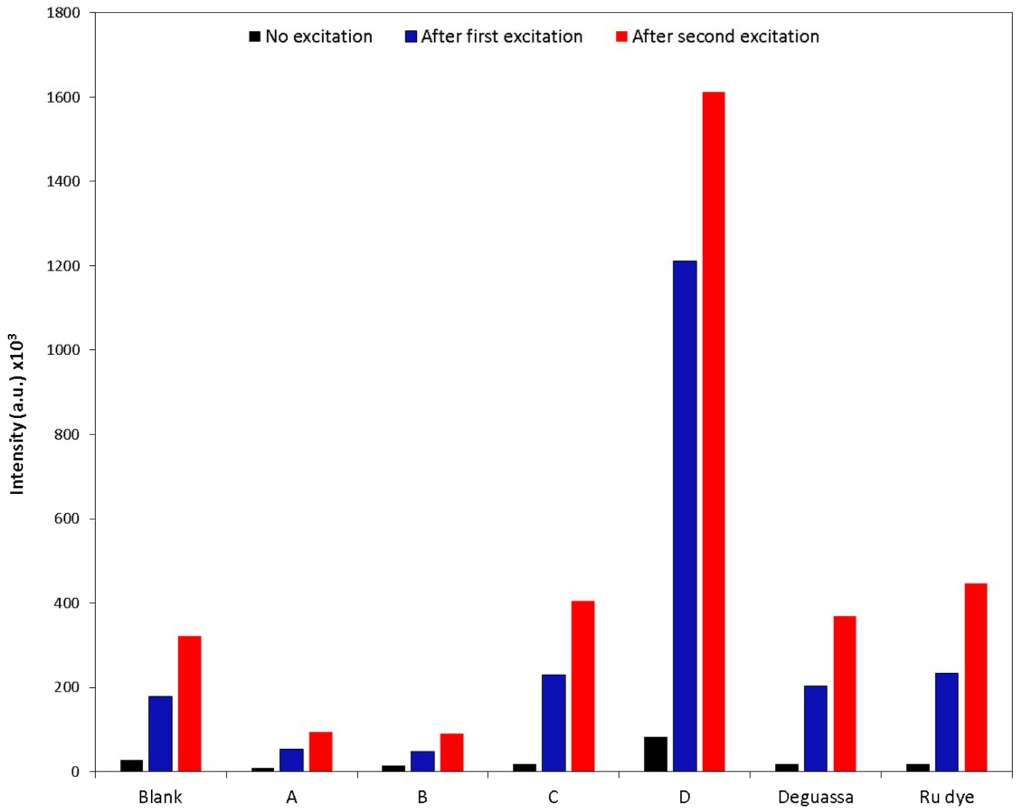

Figure S5: Fluorescence intensities of DCF at 520 nm after successive white light excitation (5 min intervals) of a blank control, A, B, C, D, Ru dye, and Deguassa NPs.

1. Lin C-L, Lee C-F, Chiu W-Y. Preparation and properties of poly(acrylic acid) oligomer stabilized superparamagnetic ferrofluid. J Colloid Interface Sci. 2005;291(2):411-20.
2. Ravikumar C, Bandyopadhyaya R. Mechanistic Study on Magnetite Nanoparticle Formation by Thermal Decomposition and Coprecipitation Routes. The Journal of Physical Chemistry C. 2011;115(5):1380-7.
3. Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. J Colloid Interface Sci. 1968;26(1):62-9.
4. Pang SC, Kho SY, Chin SF. Fabrication of magnetite/silica/titania core-shell nanoparticles. J Nanomater. 2012:427310, 6 pp.
5. Younes AH, Ghaddar TH. Synthesis and Photophysical Properties of Ruthenium-Based Dendrimers and Their Use in Dye Sensitized Solar Cells. Inorg Chem. 2008;47(8):3408-14.

6. Krimm S. The infrared spectra of complex molecules, Vol. 1 (3rd ed.), L. J. Bellamy, Halsted Press, a division of John Wiley & Sons, Inc., New York, 1975, 433 pp. $24.00. J Polym Sc, Polym Lett Ed. 1976;14(2):121-.

7. Moore RGC, Evans SD, Shen T, Hodson CEC. Room-temperature single-electron tunnelling in surfactant stabilised iron oxide nanoparticles. Phys E. 2001;9(2):253-61.

8. Wang R, Wang X, Xi X, Hu R, Jiang G. Preparation and photocatalytic activity of magnetic Fe3O4/SiO2/TiO2 composites. Adv Mater Sci Eng. 2012:409379, 8 pp.

9. Wu S-H, Wu J-L, Jia S-Y, Chang Q-W, Ren H-T, Liu Y. Cobalt(II) phthalocyanine-sensitized hollow Fe3O4@SiO2@TiO2 hierarchical nanostructures: Fabrication and enhanced photocatalytic properties. Appl Surf Sci. 2013;287:389-96.

10. Li W, Zeng T. Preparation of TiO_2 Anatase Nanocrystals by TiCl₄ Hydrolysis with Additive H_2SO_4. PLoS One. 2011;6(6):e21082.