

Carbon coated core-shell multifunctional fluorescent SPIONs

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Experimental

Materials

Ferrocene, hydrogen peroxide and Phosphate buffer solution (PBS) were purchased from Sigma Aldrich. Solvents used in synthesis and others studies were of analytical chemical grade. All chemicals were used without further purification.

Synthesis of multifunctional magnetic and fluorescent core shell nanoparticles (MFCSNPs)

A slight improvement in an earlier described synthesis procedure was used [1]. In a typical synthesis of MFCSNPs, ferrocene (0.20 g) was dissolved in acetone solution (60 mL). After intense sonication for 1 hour, 5 mL of hydrogen peroxide solution was slowly added in the ferrocene solution, which was then vigorously stirred for 30 minutes under magnetic stirring. The solution was transferred to a 100 mL Teflon-lined stainless steel autoclave. After sealing, the autoclave was heated to and maintained at 220 °C for 36 hours. The autoclave was then cooled naturally to room temperature. The supernatant was carefully discarded using a magnet. The precipitate was then washed with acetone three times to remove excess ferrocene. Finally, the black precipitate was dried at 30 °C in a vacuum oven and kept for further characterization.

Characterization

Powder X-ray diffraction (PXRD) pattern was recorded on a Rigaku Smart Lab diffractometer, using CuK α radiation from 5 to 80 degree 2 θ with scanning rate of 2 degree/min. The particle morphology and elemental mapping of MFCSNPs samples were obtained by transmission electron microscopy (TEM) using FEI Tecnai TEM equipped with a LaB6 source operating at 200 kV. Scanning transmission electron microscopy-high angle annular dark field (STEM-HAADF) imaging was also performed on the TEM. The morphology and energy dispersive x-ray analysis (EDS) of MFCSNPs were observed using a field emission scanning electron microscopy FESEM (NOVA NanoSEM 450) at an accelerating voltage of 10 kV with spot size 3. Atomic force microscopy (AFM) imaging was done using dimension ICON (Bruker) AFM in tapping mode at scanning rate of 0.9 Hz (Scan area-800 $\times$ 800 nm²) (Vertical scale-30 nm). The hydrodynamic diameter of MFCSNPs was attained by measuring through dynamic light scattering method using Malvern Zetasizer instrument. UV-Vis absorption spectra were measured

with Shimadzu U-2450 UV-Vis spectrophotometer from wavelength range of 200-800 nm. Fluorescence spectra and lifetime measurements were recorded on Horiba spectrophotometer from wavelength range of 200 to 800 nm. Absolute quantum yield of MFCSNPs was similarly calculated in Horiba spectrophotometer at excitation wavelength of 400 and emission wavelength of 450 nm respectively. Fourier transformed infrared (FTIR) spectra were recorded with Agilent Technologies Cary 6000 series FTIR spectrometer at wavenumber from 400 to 4000 cm^{-1} . Thermal properties were measured by Perkin Elmer Pyris Thermogravimetric (TGA) analyser under nitrogen atmosphere from room temperature to 700°C with the heating rate of 10°C min^{-1} . X-ray photoelectron spectra (XPS) was recorded on a VG ESCALAB250 electron spectrometer with a monochromatic Al K α (1486.6 eV) at 15 kV and 10 mA, and all binding energies were referenced to the C 1s peak (284.6 eV). Magnetic properties were analysed through vibrating sample magnetometer-superconducting quantum interference device (VSM-SQUID) under the vibrating magnetic field of 4T at 300 K.

Fluorescence lifetime measurements of MFCSNPs

The average lifetime of MFCSNPs was calculated from fluorescence lifetime decay measurements using excitation wavelength of 400 nm and emission wavelength of 450 nm. The measurements were performed with aqueous solution of MFCSNPs with concentration of 0.2 mg/ml in triplicates. The obtained data was fitted in 3 exponential fitting to calculate average lifetime.

Confocal laser scanning microscopy studies (CLSM) of MFCSNPs

MFCSNPs were spin coated on a glass slide by gently spin coating for 10s (2000rpm) to performed confocal imaging. The glass slides were cleaned by sonication in ethanol, followed by incubation in HNO₃ and KOH solution for 20 min each, and were washed in MiliQ water after every step and finally dried by Nitrogen. A Nikon Eclipse Ti microscope was used for the confocal microscopy and images were acquired using Nikon Nis-Element software. MFCSNPs were excited by four laser channels, 401 nm, 488 nm, 561 nm, and 639 nm, with corresponding filters. For movie recording, the aqueous dispersion of MFCSNPs was drop casted on clean glass slide and imaged under four laser channels sequentially.

Phantom Preparation for MRI scanning

For MRI scanning, base solution with 1.5% agarose was prepared. This base solution was put into a large (2L) cylindrical beaker as well as in nine 15 mm small test tubes. In small test tubes different iron concentration were added to get concentrations of 0.005 mM, 0.01 mM, 0.015 mM, 0.02 mM, 0.03 mM, 0.04 mM, 0.05 mM, 0.1 mM, 0.25 mM, 0.5 mM respectively. These small phantoms, 1 to 9, were inserted vertically (circumscribed) inside large phantom as shown in figure 3.

Magnetic resonance imaging (MRI) and relaxivity studies of MFCSNPs

MRI experiments were performed at 3T whole-body MRI system (Ingenia, Philips Healthcare, The Netherlands) using a 16 channel receive only coil. Data for T2 map was acquired using vendor supplied T2 mapping pulse sequence with option of multiple echoes. MRI protocol consisted of FOV=200*200, matrix size =256*256, slice thickness =6 mm, number of slices =12, TR =6000ms and TE = 30, 60, 90, 120, 150, 180, 210 ms. MRI data for T1 map was also acquired using inversion recovery based pulse sequence. For this sequence we used TI = 100, 300, 500, 700, 1000, 1500, 2000, 2500, 3000 and 3500 ms.

Relaxivity mapping

MRI images were processed using in house written programs in MATLAB. Voxelwise T₂ map was generated by fitting signal intensity data corresponding to different TEs to following mono-exponentially decaying function:

$$S(TE) = A \times e^{-\frac{TE}{T_2}} \quad [1]$$

ROIs were drawn over small phantoms and average T₂ values were computed for each ROI. R₂ = 1/T₂ values were computed. Transverse Relaxivity (r₂) of contrast agent is estimated using following equation:

$$R_2 = R_{20} + r_2 \times C \quad [2]$$

Where R₂ is concentration dependent relaxation time and R₂₀ is relaxation time of base solution or agarose without contrast agent.

Single molecule imaging (time trace and photon counts) studies of MFCSNPs

MFCSNPs were spin coated on a clean glass slide to analyse the single-molecule time

traces. The diffraction limited spots produced due to single-molecule blinking were observed using 100× Nikon total internal reflection fluorescence (TIRF) objectives [2]. For excitation, a 532 nm diode laser was used. An oil immersion Nikon TIRF objective (100× magnification and 1.49 NA) was mounted on a custom-built inverted optical microscope. To separate the excitation and emission light, a 532 nm high pass Dichroic (AHF Analysentechnik) was used for imaging. Further, to record the single molecule photon counts, an Andor electron multiplication charge coupled device (EMCCD) iXon Ultra was used at a readout rate of 17 MHz with exposure time 50 ms. The time trajectories were analysed by Andor Solis Software. Typically, the incident photons were converted to electrons and subsequently counted by the EMCCD camera during imaging. In addition, the ‘analyse region of interest (ROI)’ tool of Andor solis was significantly used to extract the total counts along with the maximum, mean and standard deviation values using a group of pixels. The movies were recorded under the kinetic mode of the EMCCD. The time/frame trajectories of the counts/intensity at given pixel were obtained for the given exposure time to record the movies. These time trajectories were further used for analysis. The image area was taken as only 128 × 128 pixels from the total 512 × 512 pixels of the whole sensor of the EMCCD. Thus, we used a total image area of 64 × 64 pixels (10.24 μm × 10.24 μm) with a pixel size of 160 nm using 100× magnification. The Andor iXon Ultra EMCCD camera has a pixel size of 16 μm × 16 μm, so on a system with a 100× objective lens with the pixel size of 160 nm (16 μm/100). During the measurement, the background was calculated from the image area where no molecule were present. A background free signal was obtained after subtracting the mean value of the background from the original trajectory. The background signals (after complete photobleaching) were also set to zero level for further correction. The blinking experiment with cyanine dye molecules were also performed and were compared.

Material Characterization

Fabrication of MFCSNPs

A magnetic core consisting of small iron oxide nanocrystals and fluorescent carbon shell were formulated into multifunctional magnetic and fluorescent core shell nanoparticles (MFCSNPs) prepared in single step solvothermal method. The reaction conditions were

optimized by varying the different parameters such as precursor concentration, reaction temperature and amount of oxidizing agent to yield core shell nanoparticles. Detail insight in synthesis showed that change in reaction parameters yielded different nanostructures with iron oxide core and amorphous carbon shell structures. Reaction temperature and amount of oxidizing agent during synthesis made a strong impact on particles morphology. Among these synthesised nanostructures, spherical nanostructures showed better contrast as core shell in TEM and STEM images (figure 1) and opted monodispersity in terms of particle morphology. To briefly understand the synthesis mechanism, two steps are given here. Initially, ferrocene and H_2O_2 are dissolved in acetone and heated to $220\text{ }^\circ\text{C}$ in sealed chamber which results the decomposition of ferrocene in iron and cyclopentadiene. Thereafter, cyclopentadiene decomposes and carbon free radicals are generated. Due to high surface energy of these carbon radicals; they get agglomerated and forms large carbon radicals under the high temperature inside the sealed chamber. Meantime, H_2O_2 decomposes and generate O_2 which simultaneously oxidize the iron atoms to form iron oxide nanoclusters. During oxidation process, Fe^{2+} cations are generated which again catalyse H_2O_2 decomposition and resulting new free hydroxyl and carboxyl radicals. These free radicals accelerate the oxidation of iron to yield iron oxide nanoclusters and simultaneously react with free carbon radicals making them hydrophilic hydroxyl and carboxyl groups. These reactions happens at very high temperature and pressure in gaseous state. As reaction proceeds to supersaturation, iron oxide nanoclusters get agglomerated and yield core structure followed by the deposition of carbon based free radicals and hydroxyl and carboxyl groups as a shell on the surface on iron oxide core. These surface adsorbed carbon radical species enable uniform carbon coating on iron oxide core. Chemical bonding in iron oxide nanocrystals and surface adsorbed groups was successfully confirmed by XPS and FTIR spectra.

Table S1. Detailed parameters used for the synthesis of MFCSNPs.

Entry	Precursor concentration (gm)	Amount of H ₂ O ₂ (ml)	Reaction temperature (°C)
Sample 1	0.20	5 ml	220
Sample 2	0.30	5 ml	220
Sample 3	0.50	5 ml	220
Sample 4	0.80	5 ml	220
Sample 5	1.0	5 ml	220
Sample 6	0.20	5 ml	200
Sample 7	0.20	5 ml	200
Sample 8	0.20	10 ml	220
Sample 9	0.20	15 ml	220

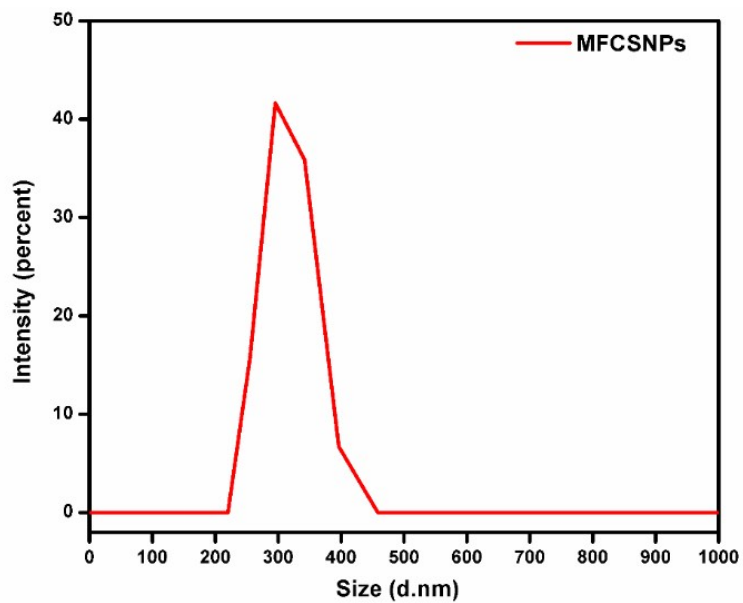


Figure S1a. The hydrodynamic diameter of MFCSNPs as determined from dynamic light scattering (DLS) measurements in aqueous solution.

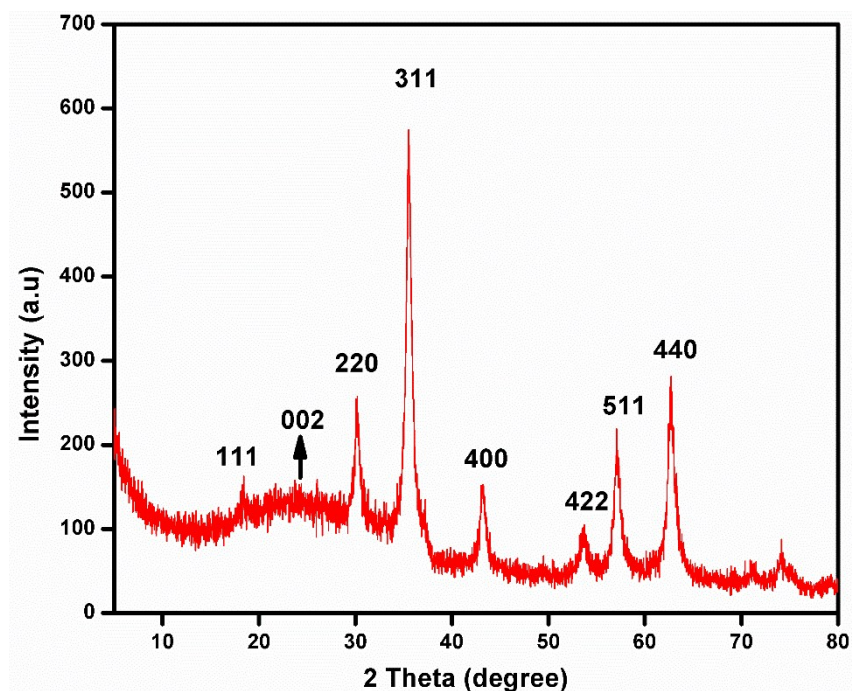


Figure S1b. PXRD spectra of MFCSNPs showing the reflections of diffraction peaks (311), (400), (422), (511), and (440)] observed for Fe_3O_4 and amorphous graphitic carbon (002) respectively.

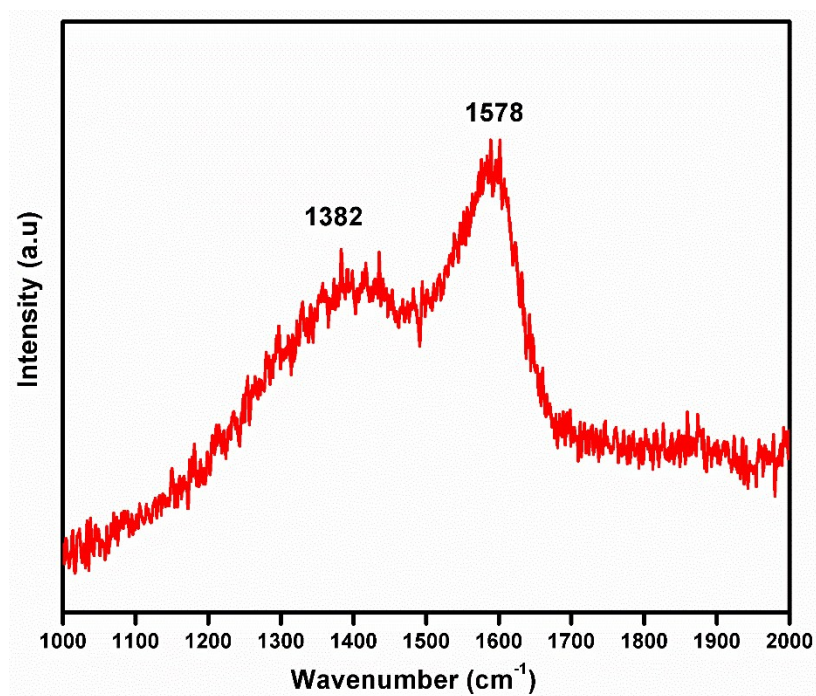


Figure S2. Raman spectra of MFCSNPs showing two vibrational bands D band (sp^3) and G band (sp^2) for amorphous graphitic carbon.

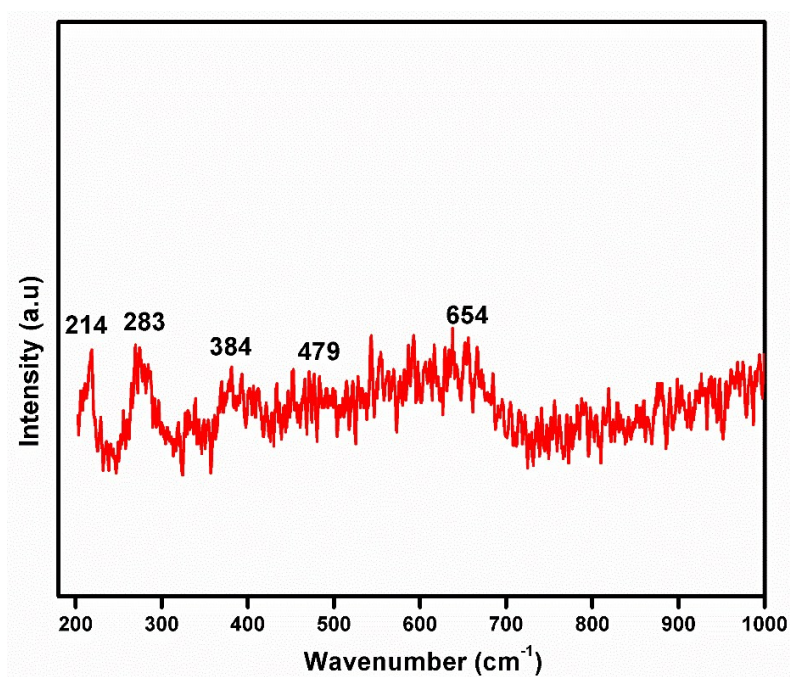


Figure S3. Raman spectra of MFCSNPs showing vibrational bands observed at 214, 283, 384, 479, 654 cm⁻¹ correspond to different bending modes of magnetite phase of iron oxide.

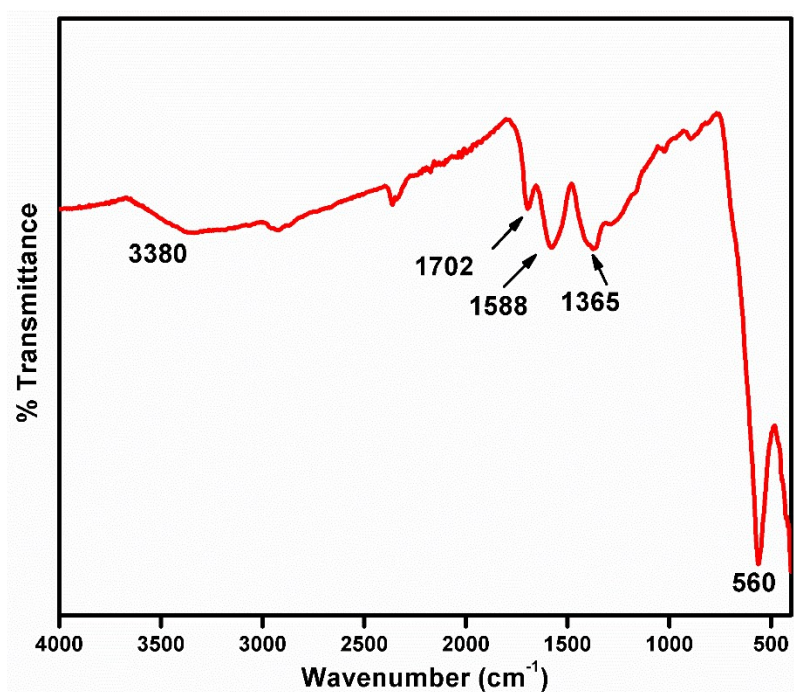


Figure S4. FTIR spectra of MFCSNPs shows absorption peak for OH group at 3380 cm⁻¹, C=O group at 1702 cm⁻¹ and C=C stretching at 1588 cm⁻¹ respectively.

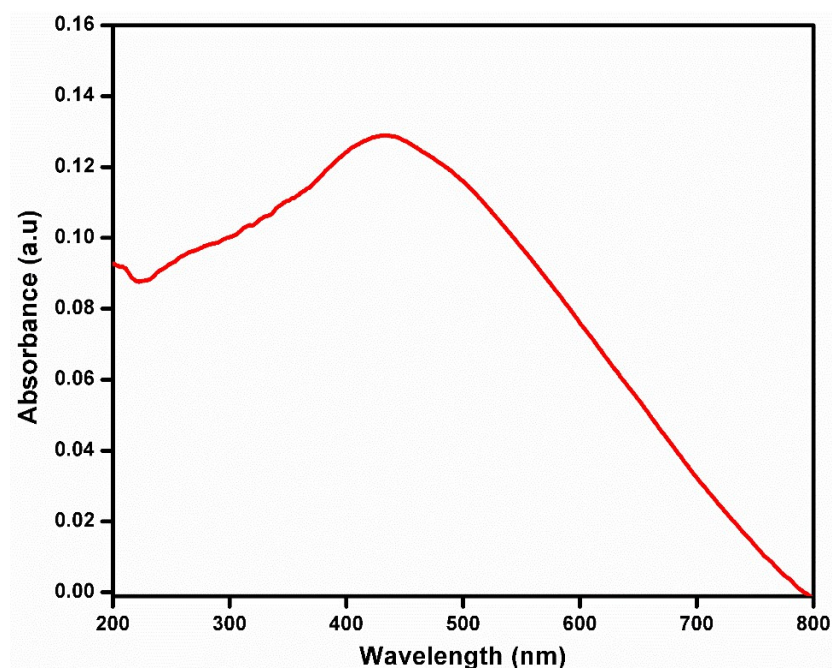


Figure S5. UV-Vis absorbance spectra of MFCSNPs as obtained in aqueous solution with a broad absorption peak ranging from 200-800 nm having absorption maxima at 450 nm and a shoulder peak at 225 nm respectively.

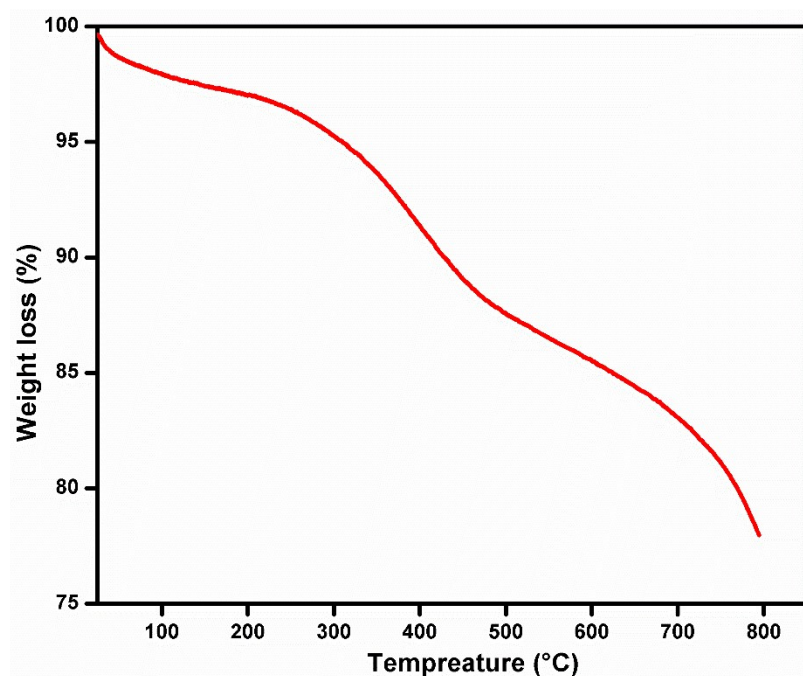


Figure S6. TGA spectra of MFCSNPs analyzed under nitrogen atmosphere upto 800 °C showing two step weight loss attributed to removal of surface adsorbed water molecules followed by degradation of carbon content present in sample.

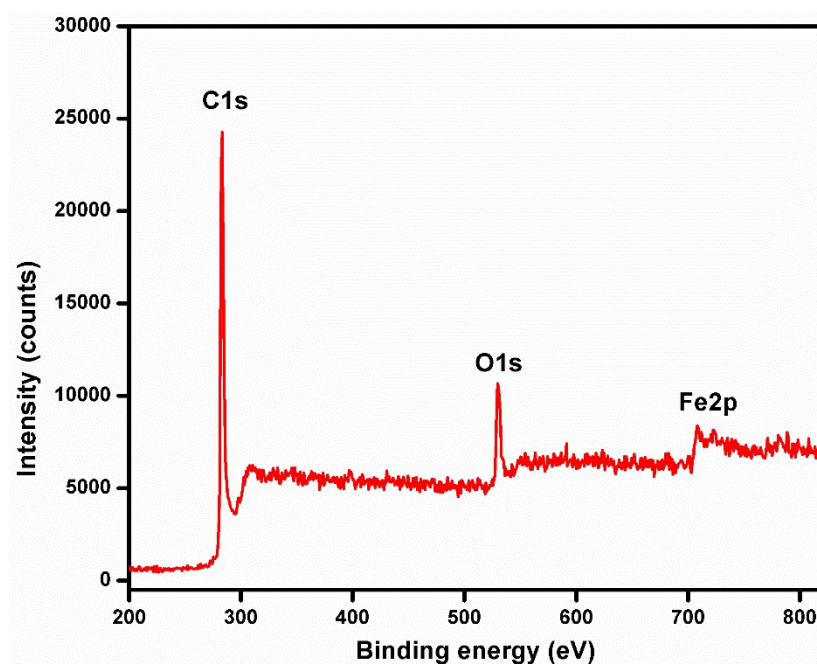


Figure S7. Full survey XPS spectra of MFCSNPs showing the presence of C1s, O1s and Fe2p in scan profile respectively.

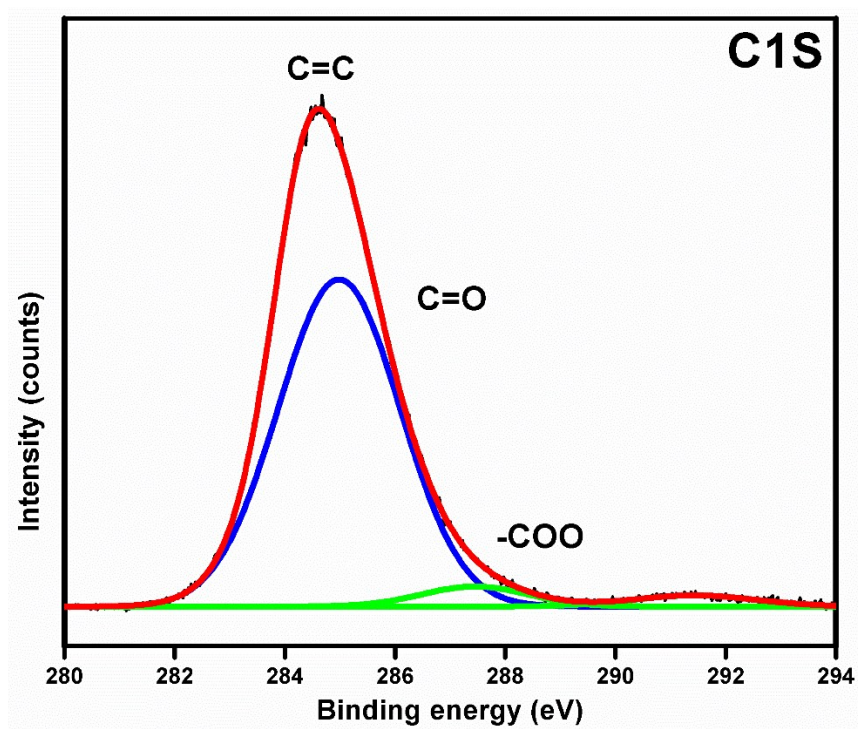


Figure S8. High resolution deconvoluted C1s XPS spectra show various carbon species C=C, C=O and -COO present in MFCSNPs respectively.

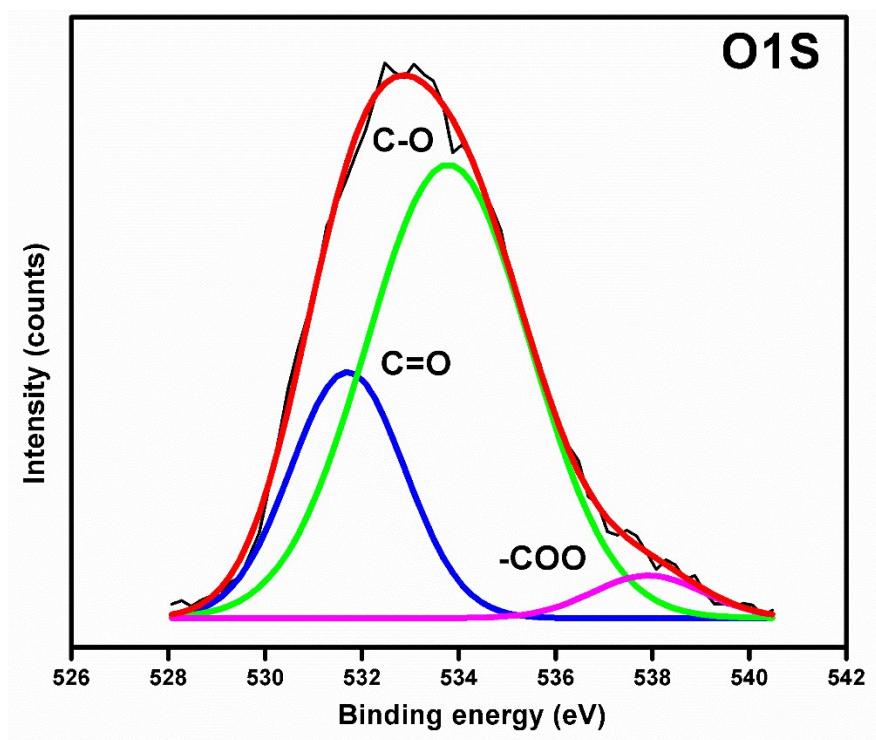


Figure S9. High resolution deconvoluted O1S XPS spectra show various oxygen species C-O, C=O and -COO present in MFCSNPs respectively.

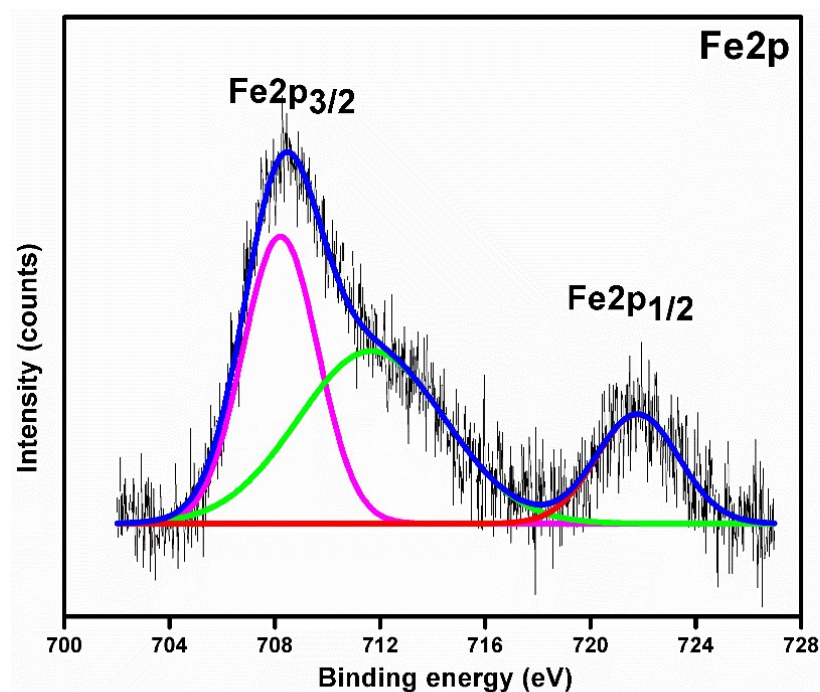


Figure S10. High resolution deconvoluted Fe2p XPS spectra shows two peaks for Fe 2p_{3/2} and one peak for 2p_{1/2} at binding energy values contributing 709, 714 and 722.5 eV respectively confirming the presence of magnetite phase of iron oxide.

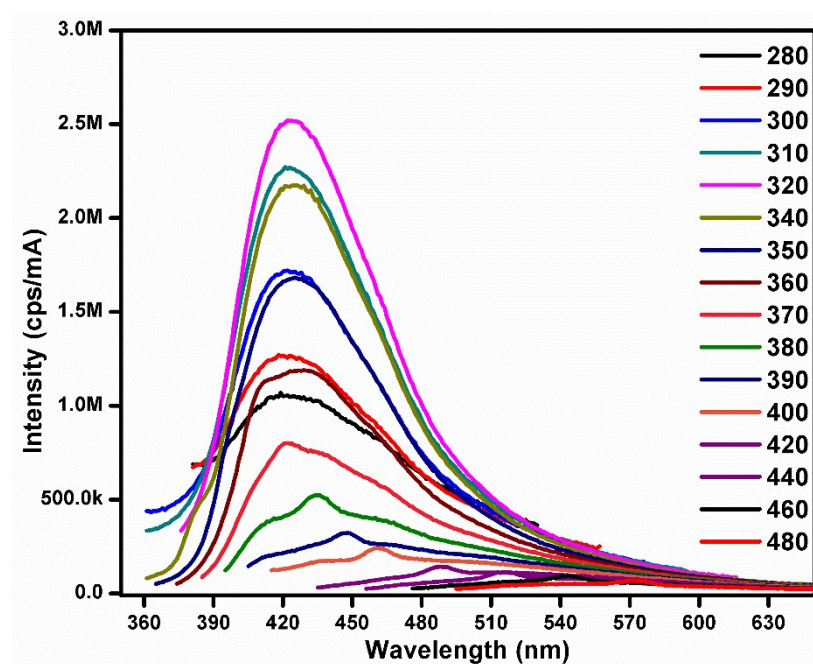


Figure S11. Excitation based emission spectra of MFCSNPs at excitation wavelength ranging from 280-480 nm obtained in aqueous solution.

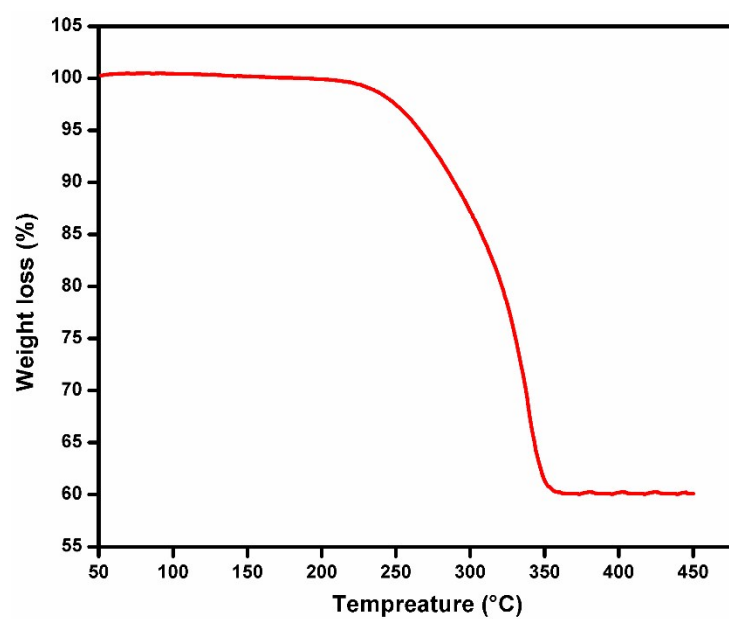


Figure S12. TGA spectra of MFCSNPs annealed upto 450 °C in air to remove carbon coating present on the surface before performing the photoluminescence Raman experiments.

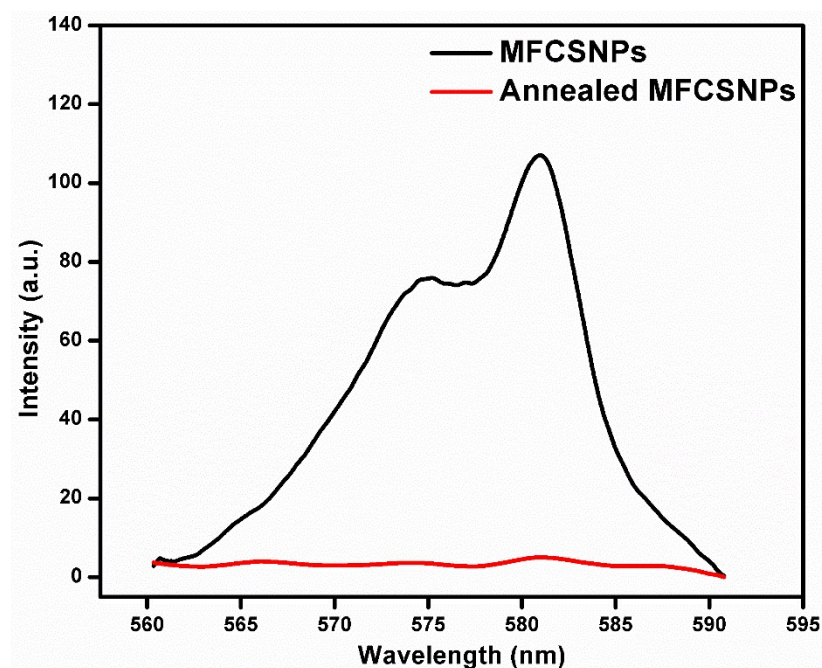


Figure S13. Photoluminescence Raman spectra of MFCSNPs before and after annealing the sample upto 450 °C in air atmosphere display the disappearance of luminescence peaks in annealed samples.

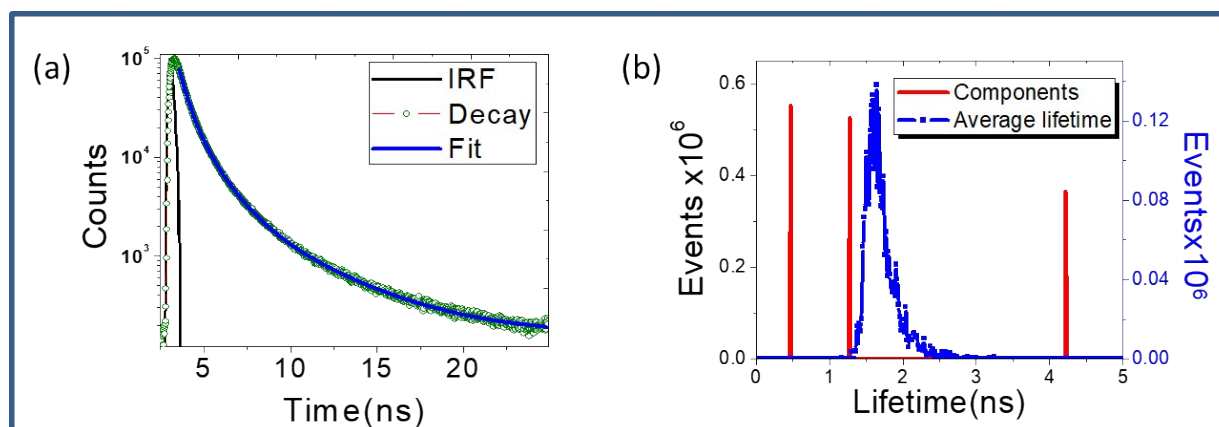


Figure S14. (a) Lifetime decay of the MFCSNPs. (b) Lifetime component and histogram calculated from the distribution of the particles.

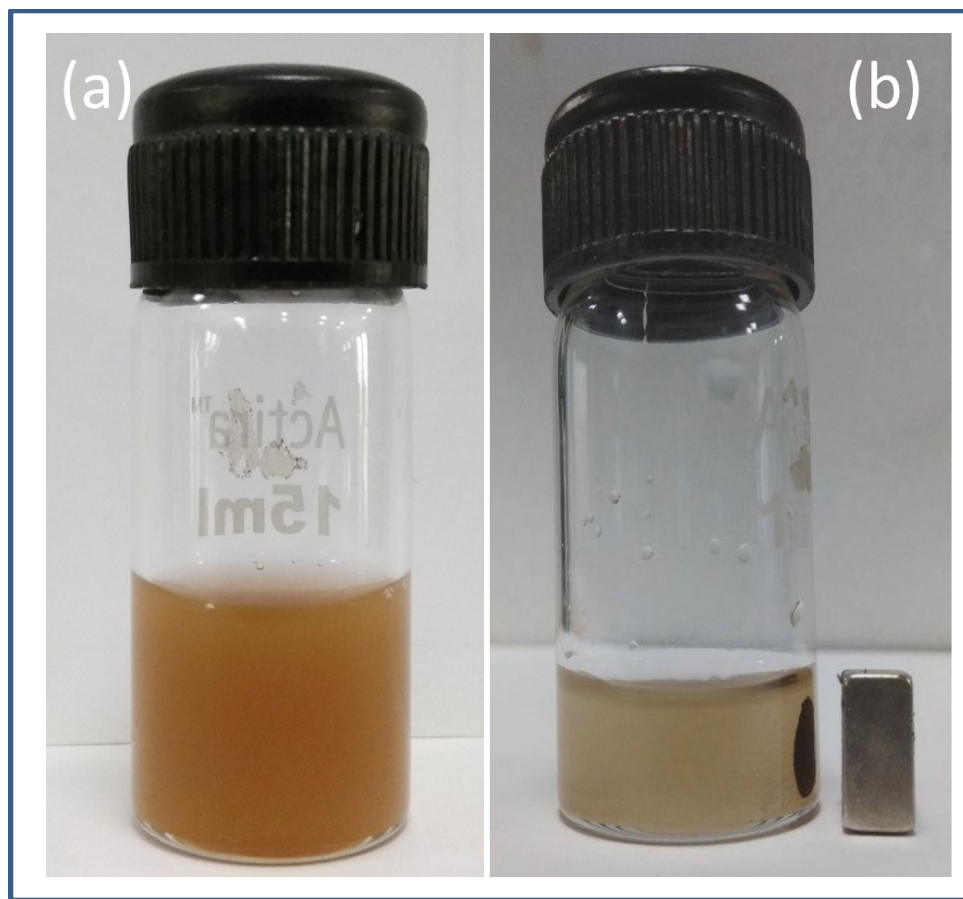


Figure S15. (a) Photographs of MFCSNPS dispersed in aqueous solution. (b) MFCSNPs in aqueous solution after magnetic separation.

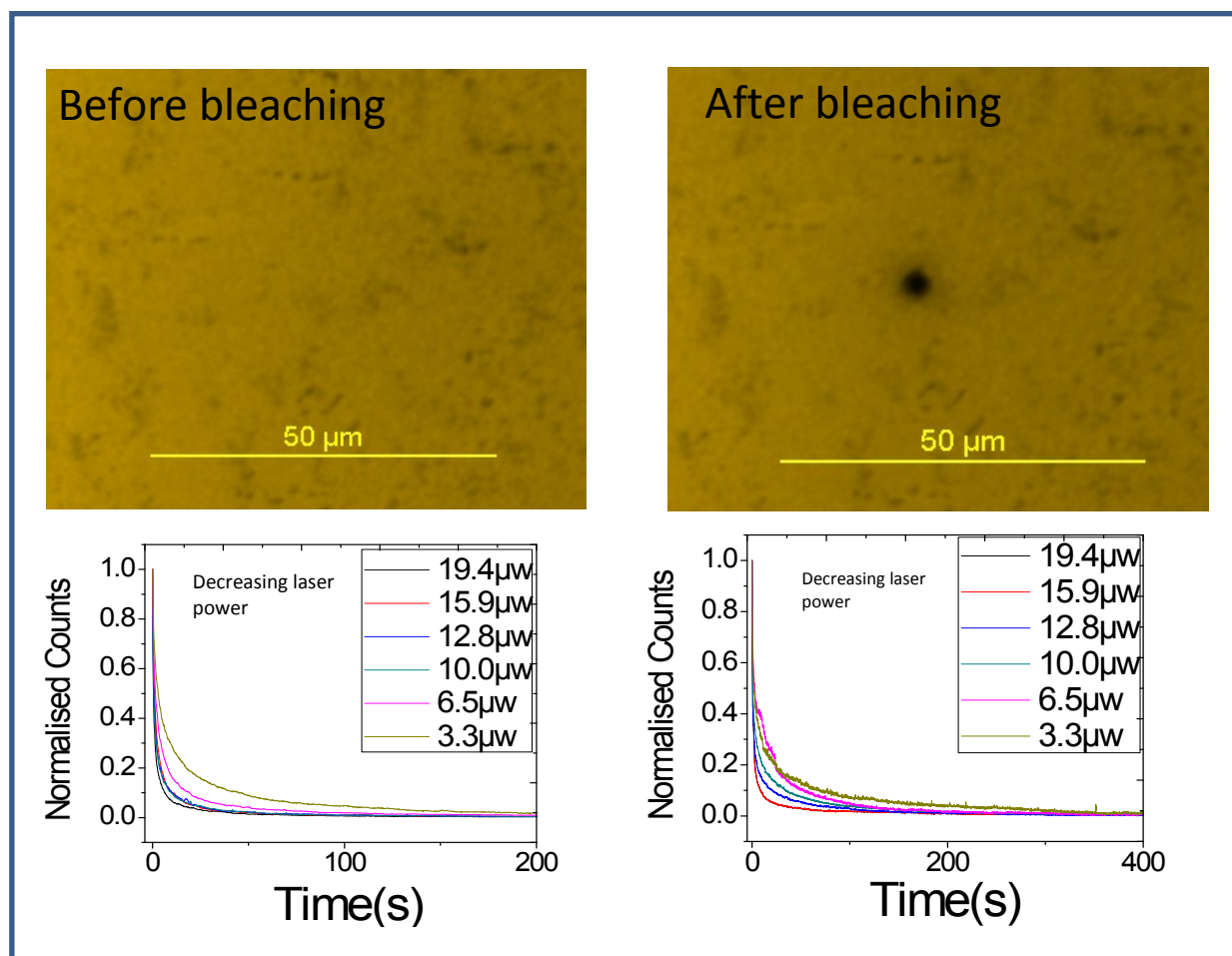


Figure S16. Photobleaching experiments of MFCSNPs with comparison to Rhodamine dye spin coated on a cover slip and excited with the same laser power.

References

- [1] Wang, Hui, et al. "Fluorescent porous carbon nanocapsules for two-photon imaging, NIR/pH dual-responsive drug carrier, and photothermal therapy." *Biomaterials* 53 (2015): 117-126.
- [2] Verma, Navneet C., Syamantak Khan, and Chayan K. Nandi. "Single-molecule analysis of fluorescent carbon dots towards localization-based super-resolution microscopy." *Methods and applications in fluorescence* 4.4 (2016): 044006.