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Fe Doped CdTeS Magnetic Quantum Dots for Bioimaging†

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Abstract

A facile synthesis of 3–6 nm, water dispersible, near-infrared (NIR) emitting, quantum dots (QDs) magnetically doped with Fe is presented. Doping of alloyed CdTeS nanocrystals with Fe was achieved *in situ* using a simple hydrothermal method. The magnetic quantum dots (MQDs) were capped with NAcetyl-Cysteine (NAC) ligands, containing thiol and carboxylic acid functional groups to provide stable aqueous dispersion. The optical and magnetic properties of the Fe doped MQDs were characterized using several techniques. The synthesized MQDs are tuned to emit in the Vis-NIR (530–738 nm) wavelength regime and have high quantum yields (67.5–10%). NIR emitting (738 nm) MQDs having 5.6 atomic% Fe content exhibited saturation magnetization of 85 emu/gm[Fe] at room temperature. Proton transverse relaxivity of the Fe doped MQDs (738 nm) at 4.7 T was determined to be 3.6 mM⁻¹s⁻¹. The functional evaluation of NIR MQDs has been demonstrated using phantom and *in vitro* studies. These water dispersible, NIR emitting and MR contrast producing Fe doped CdTeS MQDs, in unagglomerated form, have the potential to act as multimodal contrast agents for tracking live cells.

1. Introduction

Development of multimodal contrast agents has attracted attention recently due to their potential use in biomedical imaging applications. These probes can be used as contrast agents for complementary imaging modalities, optical and magnetic resonance imaging (MRI), thus integrating the advantages of three dimensional anatomic imaging with highly sensitive fluorescence imaging^{1, 2}. Although numerous multimodal contrast agents have been developed^{3–9}, research on magnetically doped quantum dots (QDs) or magnetic quantum dots (MQDs) has become particularly attractive^{10, 11}. The advantages of MQDs include size tunable fluorescence, high quantum yields, photo stability, resistance to photo-degradation, ability to multiplex and extremely small size^{12, 13}. MQD probes emitting in the near-infrared (NIR) window (650–900 nm) are even more attractive^{14–17} for imaging of biological specimens, as they generate minimal absorbance and auto fluorescence, thus

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enabling deep tissue non-invasive *in vivo* imaging¹⁸ e.g. bio-distribution, tumor targeting and lymph node mapping¹⁴. The integration of optical and magnetic character into bimodal probes has been accomplished in multiple ways by forming asymmetric heterostructures (CdS-FePt)¹⁹, CdSe-Fe₂O₃²⁰; developing fluorescence semiconductor shell on magnetic core e.g. Core/shell Co/CdSe²¹; co-embedding magnetic and fluorescent nanocrystals e.g. CdSe/ZnS with Fe₂O₃ in polymer nanoparticles²², Fe₃O₄-CdTe²³, γ -Fe₂O₃-CdSe in silica²⁴, CdSe/ZnS-Fe₃O₄ encapsulation in mesoporous silica²⁵; magnetic core-multiple shell particles e.g. γ -Fe₂O₃-SiO₂-CdTe-SiO₂²⁶; incorporation of chelated paramagnetic Gd to the QDs e.g. CdSe/ZnS in lipidic micelles²⁷, silica coated CdS:Mn/ZnS²⁸, CdSe/ZnS²⁹; biomagnetic nanoparticles conjugated to QDs e.g. ferritin protein conjugated QDs³⁰. In all the above examples, bimodal contrast agents were obtained by combining different probes possessing fluorescent and magnetic functionalities. QDs doped with magnetic ions or MQDs, offer an elegant solution by incorporating both functionalities in a single probe. Synthesis of magnetically doped core and core/shell MQDs, emitting in the visible regime and synthesized using non-aqueous solvents have been reported, for e.g. Co and Mn doped CdSe³¹, Cr doped CdSe³², Gd doped in CdSe³, Mn doped in CdSe³³, Mn doped in the core of CdS/ZnS³⁴ and in the shell of core/shell CdSe/Zn_{1-x}Mn_xS³⁵, Mn doped InP^{36, 37}, Ni doped CdTeSe/CdS³⁸. Many reports on the development of MQDs emitting in the visible regime are available, but very few exist³⁹ for bi-functional MQDs that emit in the NIR. In addition to their NIR emission characteristics, MQDs that are water dispersible are also desirable for biological imaging applications. Biological applications of MQDs synthesized using non-aqueous techniques require further surface modifications to render aqueous dispersibility, which increases the processing steps and may adversely affects optical properties. The MQDs discussed so far were mostly synthesized using the non-aqueous organometallic process. Santra et. al³⁴ developed CdS:Mn/ZnS QDs using the aqueous based inverse micelle method but the emission wavelength was limited to visible region. Overcoming the above challenges, we have successfully synthesized water dispersible, high quantum yield, NIR emitting and MR effective, Fe doped CdTeS MQDs. Here we report the synthesis of NIR (>700nm) emitting MQDs, achieved through hydrothermal method, along with *in vitro* characterizations and functional evaluations.

To the best of our knowledge this is the first time water-dispersible magnetic and vis-NIR emitting Fe doped CdTeS MQDs have been synthesized by aqueous hydrothermal technique for bioimaging applications and imaged using MRI. Characterization of the resulting MQDs was performed using XRD, TEM, XPS, ICP and SQUID. Photoluminescence (PL) emission properties of the Fe doped MQDs were studied as a function of size. MRI, optical absorbance and PL measurements of the MQDs demonstrate their utility for bimodal magnetic resonance and optical imaging. The synthesized MQDs emit in the 530-738 nm wavelength regimes and have a PL efficiency of 10-67.5%. By this process synthesis of stable MQDs emitting up to 738 nm was successful. The Fe doped MQD is an example of a model system for bimodal probes, but the methodology can be extended to other doped QD compositions.

2. Experimental

Reagents and Materials: In the experiment the chemicals used were CdCl₂ (99.7% Fisher), FeCl₂.4H₂O (99% Sigma), N-Acetyl-Cysteine (NAC) (99% Sigma), tellurium powder (200 mesh, 99% Acros), sodium borohydride (99% Acros) and sodium hydroxide (99% Fisher).

2.1 Synthesis of the Fe doped CdTeS MQDs

The Fe doped CdTeS MQDs were synthesized using a process similar to Zhao et al⁴⁰ with some modifications. In a typical process, sodium hydrogen telluride (NaHTe) was prepared from NaBH₄ and tellurium powder in argon purged distilled (DI) water. The molar ratio of

NaBH₄ to Te used was 2:1. The as prepared NaBH₄/Te solution was aged at 4 °C for the reaction to be completed. The time period allowed for reaction was more than 8 hrs. In a separate 250 ml round bottom flask CdCl₂ (30 mM) solution in 100 ml DI was prepared. N-Acetyl-Cysteine (NAC) and FeCl₂.4H₂O were added to the solution so that Cd:NAC and Cd:Fe molar ratios were 1:2.5 and 8.5:1 respectively. The solution was Argon purged for more than 30 min with subsequent addition of NaHTe resulting in a wine red color solution. The pH of the precursor solution was adjusted to (8.1 – 8.4) by using 2M NaOH followed by heating the solution in several 23 ml polytetrafluoroethylene (PTFE) lined autoclaves (Parr acid digestion bombs) in batches of 10 ml, for different time intervals, and at a constant temperature of 180 °C. The autoclaves were cooled by a hydro-cooling process. The MQDs thus prepared were taken out and characterized after washing. Washing of the MQDs was achieved by centrifugation at 2600 rpm for 4-5 minutes, using Amicon Ultra-15 Centrifugal Filter Unit with membranes suitable for 30 kDa protein filtration.

2.2 Characterization

Vis-NIR PL—SpectraMax® M5 Multi-Mode Microplate Reader was used for collecting Vis-NIR absorption and emission spectra of the MQDs at room temperature over a wavelength range of 400 – 850 nm and plotted using SoftMax Pro v5.3 software. The MQD samples were measured against water as reference. The excitation wavelength was set at 300 nm and the emission spectra obtained over 430 – 850 nm. The PL quantum yields (QYs) of the MQDs were determined at room temperature by comparing with a solution of Rhodamine 6G in ethanol (QY 95%)⁴¹. The absorbance or optical density (OD) of Rhodamine 6G and the MQDs were adjusted to less than 0.1 for QY determination. For the QY measurements, the excitation wavelength used was 500 nm.

TEM—JEOL 2010F operating at 200 kV was used for acquiring HRTEM images of the MQDs. The TEM sample preparations were done by drying a dispersion of MQDs on carbon coated Cu grids (01813-F, Ted Pella, Inc., USA), overnight at room temperature. The same TEM and samples were used for EDS analysis of the MQDs.

ICP—Inductively coupled plasma optical emission spectrometer (ICP-AES Perkin Elmer Optima 3200 RL) was used for the elemental analysis of the MQDs and compared with the data obtained from EDS analysis. WinLab32TM ICP software was used for plotting the data. The read delay was set at 15 s, and the optical wavelengths used to analyze the elements were 228.8 nm, 214.3 nm and 238.2 nm for Cd, Te and Fe respectively. ICP sample preparation was done by drying a dispersion of 250 µl of the MQDs overnight at room temperature, followed by dissolving them in 2-3 ml aqua regia and diluting the resulting solution to a volume of 10 ml using DI water.

XRD—The powder diffraction patterns for the MQDs were recorded using a Philips X-ray diffractometer APD 3720 (Philips Scientific, Mahwah, NJ) at 40 kV and 20 mA using Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$) and scanned at a rate of 0.04 deg/s over a range of $20^\circ < 2\theta < 130^\circ$.

XPS—The XPS characterizations of the MQDs were obtained using Perkin Elmer 5100 XPS system using Mg K α x-ray ~1253.6 eV. Samples were prepared by dropping MQD suspensions on 1 mm $\times$ 1 mm silicon wafers at room temperature and allowing the samples to dry in oven for a few minutes. The MQD samples were sputtered using Ar beam at 4 KeV.

SQUID—The magnetic hysteresis properties of the samples were obtained using a Quantum Design (MPMS-5S) SQUID at 10 K and 300 K respectively. Non-magnetic filter paper was

soaked with 15 μ L liquid sample and placed inside non-magnetic empty gelatin capsules. These gelatin capsules were further mounted inside a non-magnetic measuring straw for insertion into the SQUID. Two types of measurements were made: in the first magnetization loops, $M(H)$ were obtained by cyclically sweeping the field (H) from negative to positive values at fixed temperatures (T) and in the second temperature-dependent magnetization, $M(T)$ was determined in both field-cooled (FC) and zero-field-cooled (ZFC) conditions. The external field could be varied to values as high as 50 kOe.

MRI—MRI experiments for Fe doped MQDs in water and in cells incubated with MQDs were performed using a 12 mm solenoid RF coil for signal transition and reception on an Agilent 4.7 T system at room temperature. MRI phantoms using glass tubes were prepared from Fe doped MQDs having concentrations of 9.7, 4.9, 2.4, 1.2, 0.6, 0.3 mM Fe and DI water as blank. T_2 weighted MR images of the samples were acquired using a multi-slice, multi-echo spin-echo (T_R and T_E) sequence with the following parameters: repetition time T_R 11000 ms, echo time T_E 10 ms with echo spacing of 10 ms for a total of 66 echoes, a matrix 128×64 , FOV $15 \times 10 \text{ mm}^2$ and slice thickness of 1.0 mm. Decay of signal intensity as a function of T_E was utilized to calculate the T_2 values by single exponential fitting of the echo train from regions of interest (ROI). $1/T_2$ values were plotted against increasing Fe concentrations and transverse relaxivity (r_2) of the MQDs was determined from the slope of the plot.

Optical imaging—The glass tubes containing MQDs having concentrations 9.7, 4.9, 2.4, 1.2, 0.6, 0.3 mM Fe and DI water as blank were imaged using Xenogen IVIS® Spectrum Biophotonic Imager with 675 nm excitation and 740 nm emission filters. The 9.7 mM Fe containing solution had a particle concentration of 13.8 mg ml⁻¹. The glass capillary containing 9.7 mM Fe was further imaged inside a mouse phantom, XFM-2 Fluorescent Phantom (PN 121365), using a 675 nm excitation and 740 nm emission filter at 7.2 mm and 18.2 mm dorsal depths. The imaging software used was Living Image® 4.1.

MQD uptake by J774 cells—Mouse macrophage/monocytic J774 cells were grown using standard Dulbecco's modified eagle's medium (DMEM), supplemented with 10% fetal bovine serum, glutamax and penicillin/streptomycin. Prior to labeling, 1×10^6 cells/ml DMEM were seeded into 4-well chamber slides and allowed to attach before subsequent incubation at 37 °C with 200 μ g/ml each of MQD738 and Feridex® I. V. NPs in water for 6 hours.

3. Results and discussion

The synthesis of vis-NIR emitting, magnetic and water dispersible Fe doped MQDs using hydrothermal route is outlined in Figure 1. The synthesis of magnetically doped MQDs is typically carried out in oil^{3, 31, 35-38, 42} with the aid of surface coordinating agents and surfactants. Although the as prepared organometallic MQDs usually have high photoluminescence and quantum yields (QYs), further surface treatments to make them water dispersible leads to significant QY reduction. In comparison, hydrothermal route possesses two main advantages over conventional methods of MQD synthesis. First, this method allows the synthesis of magnetic NIR QDs without using the toxic precursors and reagents commonly used in the organometallic route⁴². Secondly, the as prepared MQDs are highly water dispersible, they do not require further surface treatments to modify dispersion. Moreover, the capping of these MQDs with a non-toxic N-Acetyl-Cysteine (NAC) stabilizes aqueous dispersion and also allows bioconjugation⁴³. NAC can protect cells from MQD induced cytotoxicity and oxidation because of its known antioxidant⁴⁴ property. Hydrothermal synthesis allows high temperature synthesis of the MQDs in aqueous media, thus significantly accelerating nanocrystal growth rates and improving the efficiency of the

synthesis technique. Fe doped CdTeS MQDs emitting at 530-738 nm were prepared by reacting CdCl_2 with NaHTe and NAC at a constant temperature of 180 °C in teflon-lined autoclaves maintaining the Cd:Te:NAC molar ratio at 2:1:2.5. The Cd:Fe molar ratio was kept constant at 8.5:1. Addition of excess Fe led to complete fluorescent quenching of the MQDs. The pH of the precursor solution was maintained within the range of 8.1 to 8.4, throughout the synthesis. For the Fe doped MQDs, the absorption and emission peaks gradually red shifted (Figure 2) with increase in heating time accompanied with changing full width at half maximum (FWHM). Change in reaction time from 70 to 99 minutes produced first absorption peak shift from 600 to 676 nm, while the PL emission peak shifted from 625 to 738 nm. These results illustrate that the size of the MQDs and their corresponding emission wavelengths can be tuned by simply varying the heating time of the precursor solution. Detailed information about the optical properties of the Fe doped MQDs at different reaction times viz. first absorption peak, PL emission peak, Stokes Shift, FWHM and QY are listed in Table 1.

It is noted that the Stoke shift, defined as the difference in wavelength between first absorption peak and the emission peak, increased for NIR emitting MQDs. Higher stoke shift is desirable for bioimaging applications, since it minimizes excitation-emission wavelength overlap. Also, the MQD emission spectra obtained are narrow and symmetric for visible emitting MQDs and become broader for those that emit in the NIR. The FWHM for the MQDs varied within 49 nm to 76 nm, similar to those observed in case of thiol capped CdTe/CdS QDs⁴⁰. The QY for the Fe doped CdTeS MQDs emitting in the 530-738 nm (samples were labeled as MQD530-MQD738) region varied within 10-67.5%. It has been observed, that by the hydrothermal method, Fe doped NIR MQDs can be synthesized much faster compared to some of the reported techniques adopted for NIR emitting MQD synthesis in aqueous solutions that required several hours to even days for the reaction to be completed⁴⁵. Heating the MQDs for more than 99 minutes produced agglomeration and subsequent quenching of the MQD fluorescence. This phenomenon may be attributed to hydrolysis and subsequent decomposition of the thiols, resulting in the depletion of ligands required to stabilize the MQDs at the later stage of the reaction^{40, 45}.

The MQDs were characterized using XRD, TEM, EDX and Xenogen IVIS® Spectrum Biophotonic Imager to investigate their size, structure and chemical composition. From the TEM images, sizes of the MQDs were found to lie between 3-6 nm. The measured size of Fe doped MQD738 is 4.9 (± 0.7) nm. The crystalline nature of the MQDs is demonstrated by the presence of well-resolved lattice fringes in the TEM image (Figure 3). DLS characterizations were performed to investigate the MQD particle sizes and size distributions. MQD537 and MQD642 were determined to have hydrodynamic diameters of 2.8 nm and 12.2 nm respectively (Figure S1) and have narrow size distributions. The hydrodynamic diameter is usually larger than the actual diameter measured using TEM, the difference attributed to the thickness of the capping agent, NAC, surrounding the MQDs.

The MQDs emitting above 720 nm, however, exhibited relatively broader particle size distributions and larger hydrodynamic diameters compared to those emitting at lower wavelengths. This indicates the possibility of MQD agglomeration, as discussed earlier, due to thiol hydrolysis and subsequent degradation of the stabilizing NAC, when heated for longer times. The XRD pattern (Figure 4) exhibits three main peaks which correspond to the (111), (220) and (311) planes indicating zinc blende structure.

The nanocrystalline nature of the MQD samples was evident from the broad nature of the XRD peaks. XRD on both undoped QDs and Fe doped MQDs was performed and a small shift of the diffraction peaks for Fe doped MQDs was observed towards lower 2θ when compared to undoped MQDs. The XRD peaks of the Fe doped MQDs are relatively less

sharp than those of the undoped MQDs. The d value for (111) in undoped MQDs was found to be 3.59 Å, whereas for Fe doped MQDs it is 4.71 Å. All of these observations, viz. change in lattice parameter along with XRD peak shift toward lower 2θ and decrease in peak sharpness indicate structural modification of the MQDs with Fe doping. No diffraction peak related to Fe precipitates or iron impurity phase was detected, demonstrating the formation of Fe:CdTeS solid solution and doping of Fe into the crystal lattice of CdTeS. The crystallite size of the Fe doped MQDs was calculated using the Scherrer formula:

$$D = (K\lambda) / (\beta \cos \theta) \quad (1)$$

where, D is the grain size, λ is the wavelength of x-ray radiation (0.1541 nm), K is the constant usually taken as 0.89, and β is the peak width at half maximum height. From equation (1) the grain size of Fe doped MQD738 was estimated to be 5.2 (± 0.1) nm which is in agreement with the diameter obtained from the TEM image.

Sizes obtained from TEM and XRD for another sample, MQD730 were also compared. For MQD730, sizes measured from TEM and XRD were found to be 4.4 ± 0.3 and 4.4 ± 0.1 nm, respectively. As in the case of MQD738, for this sample too the TEM and XRD sizes are in close agreement.

The elemental composition of Fe doped MQDs 738 as determined by EDS (Figure S2) and ICP are given in Table S1. The amount of Fe present in MQD738 is 5.6 atomic% as determined by ICP, which is close to the value obtained using EDS. ICP measurements (Table S2) for MQD730 showed Fe content of 3.1 atomic%. EDS could not detect the presence of Fe in MQD730, probably due to the small amount present. A close quantitative agreement for the Cd, Te, and S values was observed for the two different methods. ICP analysis of the undoped samples illustrates (Table S2) alloyed QD compositions, indicating S incorporation into the QDs during nanoparticle growth obtained from the hydrolysed thiols. The addition of thiols primarily served to stabilize the nanoparticles and in passivating the surface trapping states, that originate from the dangling bonds, thus improving the QY of the QDs⁴⁶. Following this reasoning, although, thiol hydrolysis seems to be detrimental to the QY of the QDs, the substitution of some of the Te atoms with S atoms favoured not only the improvement of the QY but also in achieving higher emission wavelengths⁴⁶. Further characterization of the surface structure with XPS provided information regarding the type and nature of the elements constituting the MQDs. The XPS results are illustrated in Figure 5. The typical peaks of the elements Cd, Te, Fe and S are distinguishable. Cd and Te peaks with the following binding energies Cd 3d_{5/2} (404.9 eV), Cd 3d_{3/2} (411.8 eV), Te 3d_{5/2} (572.2 eV) Te 3d_{3/2} (582.6 eV) were obtained (Figure S3). The Fe 2p_{3/2} peaks were identified at 708.9 eV, 711.4 eV and 714.3 eV (Figure 5b) along with the S 2p peaks at 161.5 and 162.7 eV (Figure 5c). From the NIST database the 708.9 nm and 711.4 nm peaks are attributed to the presence of Fe⁴⁺ and Fe²⁺ ions respectively in the MQDs while S 2p_{3/2} peaks at 161.5 eV and 162.7 eV are due to the presence of S²⁻ and S⁴⁻ respectively, indicating the presence of Fe-S₂ and Fe-S type units in the MQDs.

To further characterize the Fe doped MQDs depth profile experiments with XPS were conducted. MQD679, MQD687, MQD698 and MQD711 were analyzed with XPS after sputtering with Ar for 0, 5 and 10 minutes. With increase in sputtering time (Figure 6a) the Fe atomic% increases, demonstrating that the MQDs have Fe rich core and the Fe content diminishes toward the periphery of the MQDs. The XPS spectra of Fe2p_{3/2} peak after sputtering MQD711 with Ar for 0, 5 and 10 minutes is illustrated in Figure 6b. From the figure, it is evident that there is substantial amount of Fe present toward the centre of MQD711. Xie et al⁴⁷ have reported similar observations for Fe doped ZnSe MQDs, where more Fe was present in the interior of the MQDs than on the surface.

Magnetization loop measurements of the MQDs using a superconducting quantum interference device (SQUID) show presence of ferromagnetism at low temperature indicated by the hysteresis loop observed during M vs H measurement (Figure 7). The magnetizations are normalized to total Fe content of the MQDs. Saturation magnetization measurements (M_s) were carried out for MQDs emitting at various wavelengths in the 530-738 nm range at temperatures 10 K and 300 K. MQDs emitting between 650-738 nm exhibited significant magnetization when subjected to a varying external magnetic field over the range -50 to 50 kOe at constant temperature.

The saturation magnetization values of the Fe doped MQDs with respect to the Fe content are depicted in Figure 7 and the M_s values for MQD738 (3.9 wt% Fe) are 85 emu/gm[Fe] (10 K) and 71 emu/gm[Fe] (300 K). For comparison, the saturation magnetization of Feridex® I.V. NPs (10.4 wt% Fe) was also measured and found to be 119 emu/gm[Fe] (10 K) and 72 emu/gm[Fe] (300 K) (Figure S4). The reported M_s value for Feridex® I.V. at room temperature is 64.4 emu/gm[Fe] ⁴⁸. The M_s value obtained for the Fe doped MQD738 at room temperature is comparable to that of Feridex® I.V. NPs.

In the zero-field-cooled (ZFC) measurements the MQD and Feridex® I.V. samples were cooled without any applied magnetic field to a temperature much below the anticipated blocking temperature, T_B which is near the peaks in the ZFC curves. Then the relatively low fixed magnetic field of 80 Oe is applied and the temperature of the system was raised to obtain the ZFC magnetization. The field-cooled (FC) magnetization measurements were carried out by cooling the sample in the same magnetic field (80 Oe) and magnetization measured as a function of increasing temperature.

The temperature dependent magnetizations, M versus T curves for both FC and zero-field-cooled ZFC cases were plotted for MQD738 and Feridex® I.V. NPs. The blocking temperatures (T_B) for the NPs were determined from the peaks of the ZFC curves. As the samples were heated the transition from ferromagnetism to super paramagnetism occurred near the blocking temperature. The free movements of the magnetic moments in the samples were 'blocked' at temperatures below T_B by the anisotropy leading to their ferromagnetic behaviour. Above T_B the NPs exhibit super paramagnetic behavior. From the curves, T_B for MQD738 was determined to be 190 K (Figure 7b).

The M vs H measurements of the MQDs depicted negligible coercivity at room temperature (Figure 7a). This observation is consistent with superparamagnetic behavior shown by the MQDs. The temperature range above the blocking temperature was however insufficient to convincingly confirm superparamagnetic behaviour by demonstrating the collapse of the magnetization curves onto a single curve when plotted against the ratio H/T . However, coercivity was found to be present when their magnetic properties were measured at 10 K . The 10 K magnetization loops show hysteresis with coercive fields, $H_c \sim 240 \text{ Oe}$ for MQD738 particle (Figure 7) and $H_c \sim 90 \text{ Oe}$ for Feridex® I.V. (Figure S4).

Further analysis was done by fitting the FC curve with Bloch's $T^{3/2}$ equation (Figure 7b) in the region 10 K - 200 K ($T \ll T_c$):

$$M = M_0 [1 - (T/T_c)^P] \quad (2)$$

where, M_0 is the spontaneous magnetization at zero temperature (emu/gm), T_c the estimated Curie temperature (K) of the bulk material and P a power-law exponent. We obtain $T_c \sim 613.2 \text{ K}$ for MQD738 and $T_c \sim 283.1 \text{ K}$ for Feridex® I.V. (Figure S4). For MQD738, the Curie temperature, T_c is above room temperature. The values of parameter P obtained from equation (2) for MQD738 and Feridex® I.V. NPs are ~ 1.8 and 1.2 respectively. The ideal value for Bloch parameter P should be $3/2$, here the P values are scattered around value 1.5 .

This result indicates that all these MQDs are only qualitatively consistent with Bloch's $T_{2\rho}$ law. From these magnetization data we conclude that Fe doped MQDs show ferromagnetism at low temperatures, which is evident from their magnetization loops with non-zero H_c values and low field saturation. Magnetization measurements for MQD730 showed results similar to MQD738. Figure S5(a) shows the saturation magnetization values of the Fe doped MQD730 with respect to the Fe content. The M_s value obtained for MQD730 (2.2 wt% Fe) at both 10 K and 300 K is ~ 76 emu/gm[Fe]. The temperature dependent magnetizations, M versus T curves for MQD730 are illustrated in Figure S5(b).

Investigations in solution, using cells in culture and in mouse phantom were performed to demonstrate the utility of the Fe doped MQDs as bio-imaging agents. One of the main advantages of hydrothermal synthesis, is that no further surface modification, post coating or ligand exchange is necessary for dispersing the MQDs during biological experiments in aqueous environment. The as prepared MQDs are highly dispersible in aqueous media. MR and optical imaging experiments were performed to establish that the Fe content was sufficient for generating contrast in MRI and that the QY was sufficient for contrast in optical images. Local perturbation in the magnetic moments of the proton (1H) in water when subjected to a strong magnetic field produces the MRI signal. In clinical contrast mechanisms differences in transverse or longitudinal proton relaxation times, T_2 or T_1 respectively due to tissue-specific proton density variations give rise to the MRI contrast. In the absence of significant difference in tissue relaxation times, a paramagnetic exogenous agent may be utilized to enhance contrast. They decrease the 1H relaxation times of the surrounding water and generate MRI contrast. The effectiveness of a paramagnetic material to generate contrast is measured by a parameter, relaxivity, which is defined as the relaxation-rate change per unit concentration of contrast agent⁴⁰. Fe doped MQDs produced T_2 contrast, due to Fe functioning as T_2 contrast agent. MRI of the MQDs dispersed in water and having concentrations of 9.7, 4.9, 2.4, 1.2, 0.6, 0.3 mM Fe with water as a blank was performed. The measurement of relaxation time T_2 in 4.7 T at room temperature was done using a T_2 weighted spin-echo sequence. The T_2 -weighted images are shown in Figure 8(a).

As expected with T_2 weighting, higher Fe concentration produced larger signal intensity decrements (top row). The relaxivity, r_2 for the Fe doped MQD738 was determined to be $3.6 \text{ mM}^{-1}\text{s}^{-1}$. Although the r_2 value of Fe doped MQD738 is lower than that of Feridex® I. V. NPs ($111.5 \text{ mM}^{-1}\text{s}^{-1}$)⁴⁹, the MR images in Figure 8(a) illustrate that the amount of Fe doping in the MQDs is sufficient to produce MR contrast. Same concentration of the MQDs used for MR imaging was imaged in the Xenogen IVIS® Spectrum Biophotonic Imager using excitation of 675 nm to demonstrate that they are also brightly luminescent. As illustrated in Figure 8(b), higher concentration of Fe MQDs produced brighter luminescence. The high luminescence efficiency of the MQDs is illustrated by the high intensity of emission observed even at low concentrations of the MQDs used in this experiment. The 9.7 mM Fe (13.8 mg/ml) doped MQD738 was also imaged inside a mouse phantom. The XFM-2 Fluorescent Phantom is a mouse shaped IVIS accessory, made up of polyurethane polymer that includes particles to mimic scattering, and fluorescent dye to simulate the optical properties of live tissue. This phantom contains cavities that can accommodate thin capillaries. As depicted in Figure 8(c), bright luminescence of the MQDs (using a 740 nm emission filter) was observed inside the phantom when excited with 675 nm excitation at dorsal depths of 7.2 mm (left) and 18.2 mm (right). Fluorescent signals from both the positions were observed unmistakably with minimal interference from the tissue material. To determine the difference in the emission intensities at the two depths, same region of interest (ROI) was selected over the luminescent area. Radiant efficiency [(photons/sec/cm²/sr)/($\mu\text{W}/\text{cm}^2$)] of the ROIs was found to decrease from (4.9×10^{10}) to (6.8×10^9) for corresponding change in dorsal depth from 7.2 mm to 18.2 mm. In addition, the fluorescent emission from 18.2 mm depth was observed to be more diffused and scattered over a larger

volume. The observed decrease in radiant efficiency and diffused scattering for MQDs placed at 18.2 mm dorsal depth relative to those at 7.2 mm are due to larger scattering of the emitted photons from greater depths. These results along with NIR emission properties of the MQDs indicate their potential for deep tissue in vivo imaging inside live animals.

To assess the utility of the bimodal MQDs for biological applications, they were applied to cells in culture. J774 macrophages were incubated with MQD738 at a concentration of 0.37 mM Fe in water for 6 hours. The cells containing the MQDs were then injected into the phantoms and subjected to MR imaging. For comparison, cells without MQDs and cells with Feridex® I. V. NPs were imaged along with cells containing Fe doped MQD738. The differences in MRI contrast generated by these cells are shown in Figure 8(d). The T_2 contrast generated by cells containing the MQDs is intermediate between that generated by those containing Feridex® I. V. NPs and the blank phantom containing cells without any particles. This demonstrates the contrast generating capacity of the Fe doped MQDs. These results illustrate that the same range of applied concentrations of Fe doped MQDs can produce both optical and MR contrast and demonstrate the dual-mode imaging capability of these MQDs.

In conclusion, we report a novel aqueous based hydrothermal technique to produce vis-NIR emitting MQDs that could be used for combined optical and MR biological imaging applications. The MQDs synthesized by the hydrothermal technique are not only fluorescent and magnetic, hence useful for bimodal imaging, but are also smaller in size. Furthermore, for producing bimodal nanoparticles, one pot hydrothermal synthesis is a relatively simpler technique in comparison to the conventional methods that employ multiple steps. The technique described here permits the incorporation of Fe ions, up to a specified amount, into NIR emitting MQDs without compromising the PL QY. This represents a significant breakthrough in the design and development of bimodal NIR emitting aqueous based MQDs. The Fe content in the MQDs is variable and can easily be modified. The maximum amount of Fe that could be doped into the MQDs without compromising the fluorescent property was found to be 5.6 atomic%. Above this amount Fe produces significant quenching of the MQD fluorescence. The MQDs in the visible region have room temperature high quantum yield of 35-67.5% while those in the NIR region exhibit 10-35% QY. The superparamagnetic nature of the MQDs was established using SQUID magnetometry measurements at 10K and 300K and their superparamagnetic property was found to be similar to Feridex® I.V. NPs. The MRI results demonstrate that these magnetic MQDs can serve as effective T_2 contrast agents. The applications of these MQDs might include but not limited to tracking of live cells, cancer diagnosis and therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Flowchart for the synthesis of magnetic Fe doped CdTeS MQDs using hydrothermal technique.

Figure 2. (a) Absorbance and (b) PL spectra for Fe doped CdTeS MQDs when excited at 300 nm, where the Cd:NAC and Cd:Fe molar ratios were set to 1:2.5 and 8.5:1 respectively ($[\text{Cd}^{2+}] = 30 \text{ mM}$).

Figure 3. TEM image of Fe doped CdTeS MQDs emitting at 738 nm. Inset shows SAD pattern.

Figure 4.

XRD of Fe doped and undoped CdTeS MQDs. The line spectra show the cubic CdS and CdTe reflections with their relative intensities.

Figure. 5.

(a) XPS spectra for the Fe doped MQDs emitting at 711 nm (b) Fe $2p_{3/2}$ peaks and (c) S $2p$ peaks after Argon sputtering for 10 min.

Figure. 6.

(a) Fe atomic% versus sputter time for Fe doped MQDs emitting at 679, 687, 698 and 711 nm. (b) XPS spectra of Fe_{2p}_{3/2} peak after sputtering MQD711 with Ar for 0, 5 and 10 minutes.

Figure 7.

(a) Magnetometry measurements using SQUID at 10K and 300K of 738 nm emitting Fe doped CdTeS per unit iron and (b) Temperature dependent magnetization of 738 nm emitting Fe doped CdTeS MQDs measured at a constant magnetic field of 80 Oe. (FC – field cooled, ZFC – zero field cooled)

Figure 8.

(a) T₂ weighted images of Fe doped MQD738 inside glass capillaries at various Fe concentrations at 4.7 T, 200 MHz. (b) Corresponding fluorescence images from the MQDs using Xenogen IVIS® Spectrum Biophotonic Imager after exciting them at 675 nm. (c) Fluorescence image of the MQDs of concentration 9.7 mM Fe (13.8 mg/ml particle concentration) inside mouse phantom at dorsal depths of 7.2 mm (left) and 18.2 mm (right), using excitation 675 nm and emission 740 nm filters (d) T₂ weighted images of J774 macrophages labeled with 200 µg/ml (0.14 mM Fe) Fe doped MQD738 and 200 µg/ml (0.37 mM Fe) Feridex® I.V. NPs imaged at 4.7 T, 200 MHz.

Table 1

First absorption peak (λ_{A_b}), PL emission peak (λ_{E_m}), Stokes shift, full width at half maximum (FWHM), quantum yield (QY) of Fe doped MQDs at different reaction times from 70 to 99 minutes.

Reaction Time (min)	λ_{A_b} (nm)	λ_{E_m} (nm)	Stoke Shift (nm)	FWHM (nm)	QY(%)
70	600	625	25	49	67.5
88	650	679	29	67	33.3
93	668	698	30	67	26.7
94	671	711	40	69	27.2
97	673	730	57	65	22.5
99	676	738	62	76	10.0