Electronic Supplementary Material (ESI) for Soft Matter

Chelating oleyl-EDTA amphiphiles: self-assembly, colloidal particles, complexation with paramagnetic metal ions and promise as magnetic resonance imaging contrast agents

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Experimental Details

EDTA dianhydride and Oleyl alcohol were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). All other reagents and solvents were of analytical grade and obtained from Sigma-Aldrich. NMR spectra were recorded either on a 200 mHZ or 400 mHZ Bruker Spectrometer.

Synthesis of N-mono-(carboxy- *cis*-9-Octadecenyl)- ethylene diamine-N, N′, N′-triacetic acid (EDTA-MO), and N, N′-Bis -(carboxy- *cis*-9-Octadecenyl)- ethylene diamine-N-N′-diacetic acid (EDTA-BO)

To a solution of anhydrous dimethyl formamide (DMF), 40mL, was added EDTA-dianhydride (2.56 g, 10 mmol) and the reaction mixture was heated up to 100 ˚C until dissolved. The reaction mixture was cooled to 50 ˚C and oleyl alcohol (5.04 g, 18 mmol) dissolved in 10 mL of tetrahydrofuran (THF), was added. It was stirred for additional 3 h. 10 mL of water was added and the reaction mixture was stirred for another hour, followed by evaporation of the solvents under reduced pressure. The oily residue was re-dissolved in ethanol/sodium acetate solution and applied to a C18 prep HPLC column (50 mm X 200 mm, Waters, Madison, USA). The pure conjugates were separated and eluted

by a stepwise gradient method from solvent A: H_2O/ethanol 90/10, to solvent B: ethanol. The mono-oleyl conjugate eluted on 85% buffer B and the bis-oleyl conjugate eluted at 100% buffer B. The pure title compounds were dried under reduced pressure to obtain 1.0 g of EDTA-MO and 3.9 g of EDTA-BO conjugate with an overall yield of 61%.

EDTA-MO, MS: 542.36. ^{1}HNMR in DMSO-d_6 : 5.33(m, 2H, =C*H*-CH_2), 4.01 (t, 2H, CH_2-O), 3.53 (s, 2H, N- CH_2 –CO), 3.44 (s, 6H, N- CH_2 –CO), 2.75(t, 4H, N- CH_2), 1.98 (m, 4H, =CH-CH_2), 1.55 (m, 2H, O- CH_2-CH_2), 1.25 (m, 22H, CH_2), 0.85 (t, 3H, CH_3).

EDTA-BO, MS: 792.63, ^{1}HNMR in DMSO-d6: 5.33 (t, 4H, =C*H*-CH_2), 4.01 (t, 4H, CH_2-O), 3.52 (s, 4H, N- CH_2 –CO), 3.44 (s, 4H, N- CH_2 –CO), 2.73 (t, 4H, N- CH_2), 1.99 (m, 8H, =CH-CH_2), 1.55(m, 4H, O- CH_2 CH_2), 1.24 (m, 44H, CH_2), 0.85 (t, 6H, CH_3).

Mn-EDTA-MO

To a solution of EDTA-MO (400 mg, 0.738 mmol) in 10 mL of water, was added 2 mL of 2M Na-acetate solution. Manganese acetate (181 mg, 0.738 mmol) dissolved in 4 mL of water was added dropwise. The reaction mixture turned to a milky brown solution. The complexation reaction was continued overnight and then tested by ESI/MS which showed complete disappearance of the peak due to the parent amphiphile and the appearance of the peak at MS=625 related to Mn-EDTA-MO. The solvent was removed under reduced pressure and 40 mL additional water was added. The slurry mixture was stirred overnight. The precipitate was filtered and dried under vacuum to obtain the title compound in 80% yield.

Mn-EDTA-BO

To a solution of EDTA-BO (793 mg, 1 mmol) in 10 mL of ethanol, was added 1 mL of 2M sodium acetate solution. Manganese acetate (245 mg, 1 mmol), dissolved in 6mL of water, was added, and the reaction mixture turned into a milky solution. The complexation reaction was stirred overnight and then was tested by ESI/MS, which showed complete disappearance of the peak due to the parent amphiphile and the appearance of the peak at 905.27, equivalent to the mass of the salt of the complex. Ethanol was removed under reduced pressure and 50 mL additional water was added to the precipitate and stirred overnight. The precipitate was filtered and dried under vacuum to obtain the title compound in 48% yield.

^{1}H-NMR showed broadening and nearly disappearance of the EDTA ^{1}H peaks due to the proximity to the paramagnetic Mn ions.

High Performance Liquid Chromatography (HPLC)

Analytical HPLC was performed on Waters HPLC equipment (Waters Corporation, Milford, MA, USA), comprised of a 600 solvent delivery system with a 600 automated gradient controller using a Phenomenex Gemini C18 column (5 μM, 4.6 X150 mm) and an Alltech 2000 Evaporative Light scattering Detector (ELSD). The mobile phases consisted of A) 50% H_2O, 50% AcCN and 0.05% (v/v) trifluoroacetic acid (TFA) and B) 60% THF, 40% AcCN and 0.05% TFA. The samples were analysed on a linear mobile phase gradient from 80% buffer A to 100% buffer B within 5 min, followed by 100% buffer B within the next 3 min and an extra 6 min for equilibration of the column to the initial condition. A flow rate of 1 mL/min was used to elute all the samples. The ELSD detector nebulizer temperature and nitrogen gas flow were set to 103 °C and 2.9 L/min respectively.

Preparative HPLC

Separation of the mono and bis alkylated of EDTA conjugates were performed on a Waters PrepLC 4000 system using a PrePak C_{18} reverse phase column (100X 40 mm) eluted with a linear gradient with water and ethanol as the mobile phase and at a flow rate of 20 ml/min. Detection of the peaks was performed on a Waters 2487 Dual λ absorbance UV detector, with the wavelength set at 220 nm.

Electro-spray ionization mass spectroscopy (ESI-MS)

ESI-MS of the chelating amphiphiles and their complexes was performed on a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA, USA) equipped with ESI and APCI interface. Samples were infused using a syringe pump or using the LC/MS mode.

Differential Scanning Calorimetry (DSC) and Thermogravimetry Anlaysis (TGA)

Glass transition temperatures and melting points of the samples were studied by using a Mettler Toledo DSC 822 system with a Mettler TSO 801RO sample robot (Mettler Toledo; Melbourne, Australia). Samples were run at a scan rate of 2.5 °C/min and recorded using the STARe software package (Mettler Toledo; Melbourne, Australia). Temperature and enthalpy calibration was performed using indium as the standard. The samples were run in aluminum crucibles in a sealed

furnace and were cooled to -130 °C before heating at a rate of 2.5 °C /min up to 100, 200 °C or 250 °C.

Thermogravimetry analyses were recorded by using a Mettler Toledo TGA SDTA851 with a Mettler TSO801RO sample robot (Mettler Toledo; Melbourne, Australia). Samples were run at a scan rate of 10 °C/min from room temperature to 500°C. The samples were run in the aluminum crucibles in a sealed furnace and in a stream of air. STARe software package (Mettler Toledo; Melbourne, Australia) was used for measurement and analyses of the samples.

Water Penetration into chelating amphiphiles

The water penetration behaviour of the bulk amphiphiles was analysed by using cross polarized optical microscopy (POM). Namely, 1- 2 mg of each amphiphile was placed on a microscope slide and the sample was heated until melted or used as the original neat amphiphile (for EDTA-MO). A cover-slip was placed on the neat EDTA-MO amphiphile or the melted EDTA-BO sample and the sample was allowed to cool to room temperature. The microscope slide was placed into the Linkam PE94 hot stage (Linkam Scientific Instruments Ltd; Surry, England) equipped with temperature control via a central processor PE94. Water or sodium acetate solutions were added to the edges of the cover-slips by a syringe. This process generates an instant water penetration into the sample by capillary action between the glass slide and the cover slip and creates a concentration gradient from 100% water to 100% neat amphiphile. The edges of the cover slips were then sealed with transparent glue to seal the sample and avoid evaporation of the water during heating and phase examination. The samples were then heated at 2.0 °C/min and phase transitions were examined. Water penetration scans were performed between ambient temperature until the neat amphiphile was melted or otherwise, up to 100 °C. Textures were observed with an Olympus GX51 inverted optical microscope (Olympus Australia Pty. Ltd.; Melbourne, Australia) in the presence or absence of crossed polarizing lenses. Images were captured with a Nikon DS-Ri1 Cooled Cooler Camera (Coherent Scientific Pty.Ltd. Inc, South Australia).

X-Ray Diffraction (XRD). XRD analyses were performed on a PANalytical X'pert PRO X-ray diffractometer. Incident X-ray radiation was produced from a line-focused PW3373/00 Cu X-ray tube operating at 45 kV and 40 mA. Solid sample was placed and pressed into thin film in the middle of a glass slide with 1cm^2 area. Sample stage was a Spinner PW3064 with reflection but not moving. The diffracted beam was detected by an X'Pert data collector. Samples were analysed at room temperature over a range of 2-80° 2θ with a step size of 0.05° 2θ, with each step measured for 60s.

Cryogenic Transmission Electron Microscopy (Cryo-TEM)

A laboratory-built humidity-controlled vitrification system was used to prepare the samples for Cryo-TEM. Humidity was kept close to 80% for all experiments, and ambient temperature was normally 22 °C. A. In one case the sample was pre-incubated at 40°C. Prior to plunging, the pipette tips and filter paper were also warmed to 40°C, and the vitrification chamber was also kept at approximately 40°C. 4µl aliquot of the sample was pipetted onto a 300-mesh copper grid coated with lacy formvar-carbon film (ProSciTech, Thuringowa, Queensland). After 30 seconds adsorption time the grid was blotted manually using Whatman 541 filter paper, for between 2 and 10 seconds. The grid was then plunged into liquid ethane cooled by liquid nitrogen. Frozen grids were stored in liquid nitrogen until required. The samples were examined using a Gatan 626 cryoholder (Gatan, Pleasanton, CA, USA) and an FEI Tecnai 12 cryo-TEM, The Netherlands) operating at 120 kV and equipped with a Megaview IIICCD camera and AnalySIS imaging software(Olympus). Alternatively, a Tecnai TF30 cryo-TEM operating at 200 kV and equipped with a Gatan US 1000-2K CCD camera and digital micrograph imaging software was used for imaging the samples. At all times low dose procedures were followed, using an electron dose of 8-10 electrons/$Å^2$ for all imaging.

Assessment of T_1 and T_2 relaxivities

The proton longitudinal and transverse relaxation time (T_1 and T_2) for each sample were measured at 20 MHz (0.47 T) and 25ºC using a Bruker MINISPEC or a 23 MHz MARAN ultra benchtop low field NMR (Oxford instrument, UK). T_1 was measured with an inversion recovery sequence. The recycle delay time was set to five times the T_1 value. In general, 10 points were taken for each T_1 measurement. T_2 was measured with a Carr-Purcell-Meiboom-Gill (CPMG) sequence and were fitted into multiple or distributed exponentials.

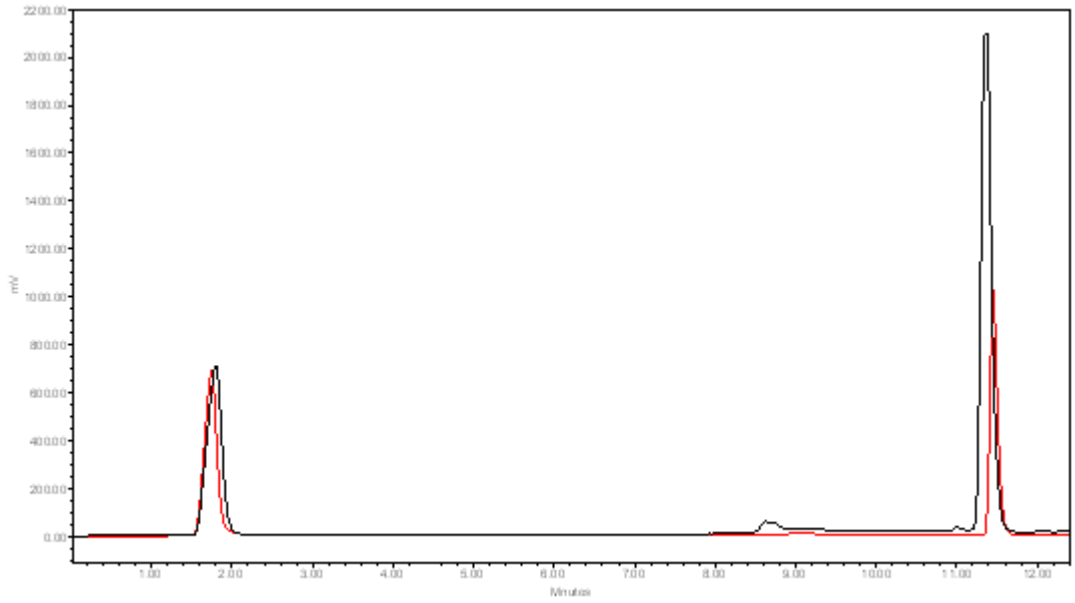

Figure 1S HPLC of EDTA-BO (black) and Mn-EDTA-BO (red) in dispersion. The same volume of the samples at equivalent concentration was injected into HPLC with an ELSD detector. The EDTA-BO had a peak at a retention time of 11.36 min while that of Mn-EDTA-BO showed a slightly increased retention time of 11.40 min. The peaks at 1.8 min were due to the Na-acetate in the solution. The integration of the peak at 11.40 min indicated that nearly one third of the original sample stayed in the dispersion and the rest was precipitated.

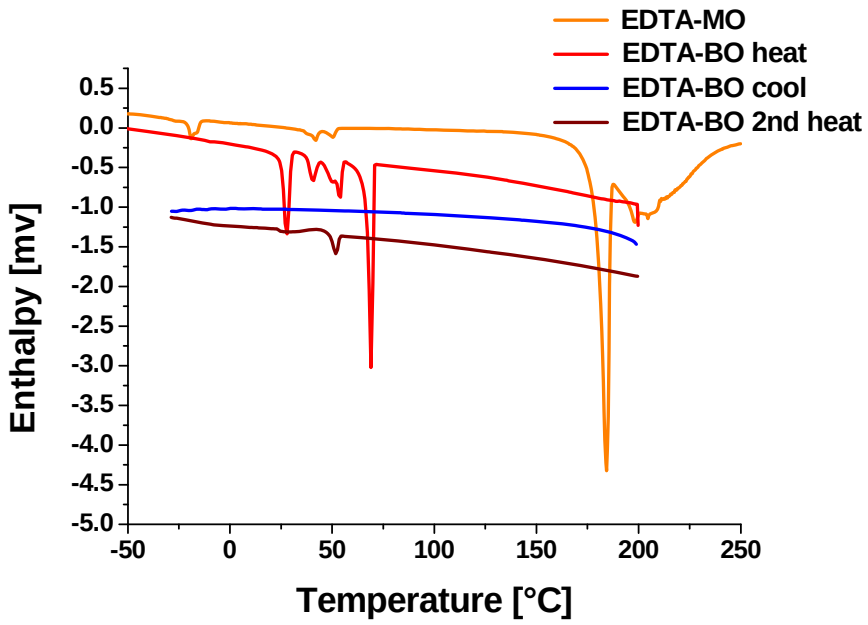

Figure 2S DSC scans of EDTA-MO and EDTA-BO (heat, cool, and heat cycles) at a scan rate of 2.5 °C/min.

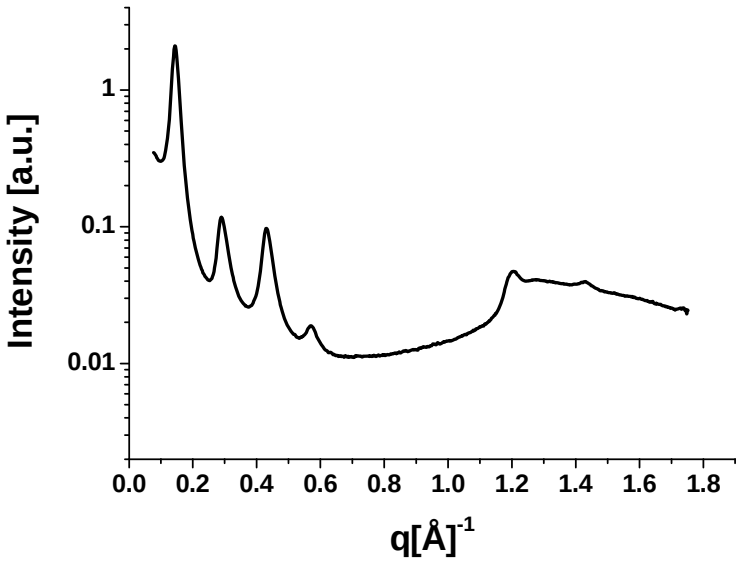

Figure 3S SWAXS pattern of Mn-EDTA-BO displaying 4 peaks in SAXS region which can be assigned to a lamellar crystalline structure with a d-spacing of 4.31±0.05 nm. The superimposed peak on the broad peak in the WAXS region implies some short range ordering either in the headgroups or hydrocarbon chains, yet less ordered compared to its non-complexed counterpart as shown in Figure 3 main text.

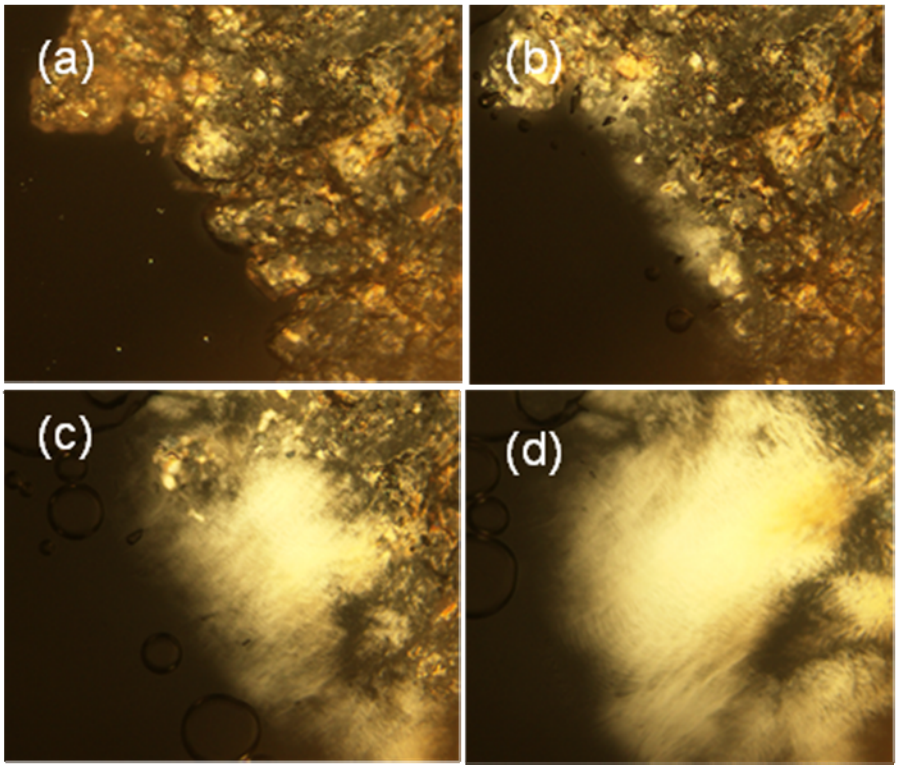

Figure 4S POM images of water penetration into EDTA-MO by adding 100 mM Na-acetate solution viewed at (a) 25 °C neat amphiphile, (b) 25 °C after addition of 100 mM Na-acetate and equilibration for 30 min, a new band of anisotropic texture characteristic of L_α was formed at the boundary with Na-acetate (c) 30 °C, L_α band expanded and (d) 50 °C, L_α band expanded further, magnification: X100.

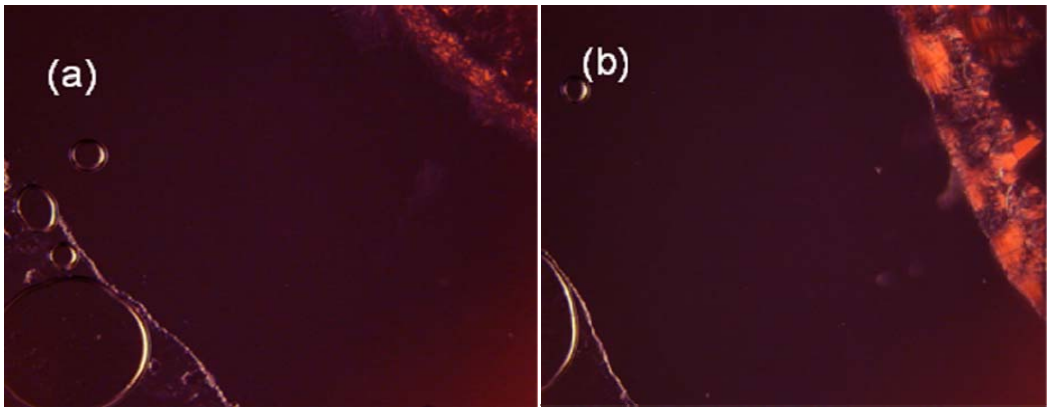

Figure 5S POM images of water penetration into EDTA-BO by adding 100 mM Na-acetate solution viewed at (a) 60 °C after equilibration for 30 min and (b) 70 °C after equilibration for 30 min, magnification: X100.

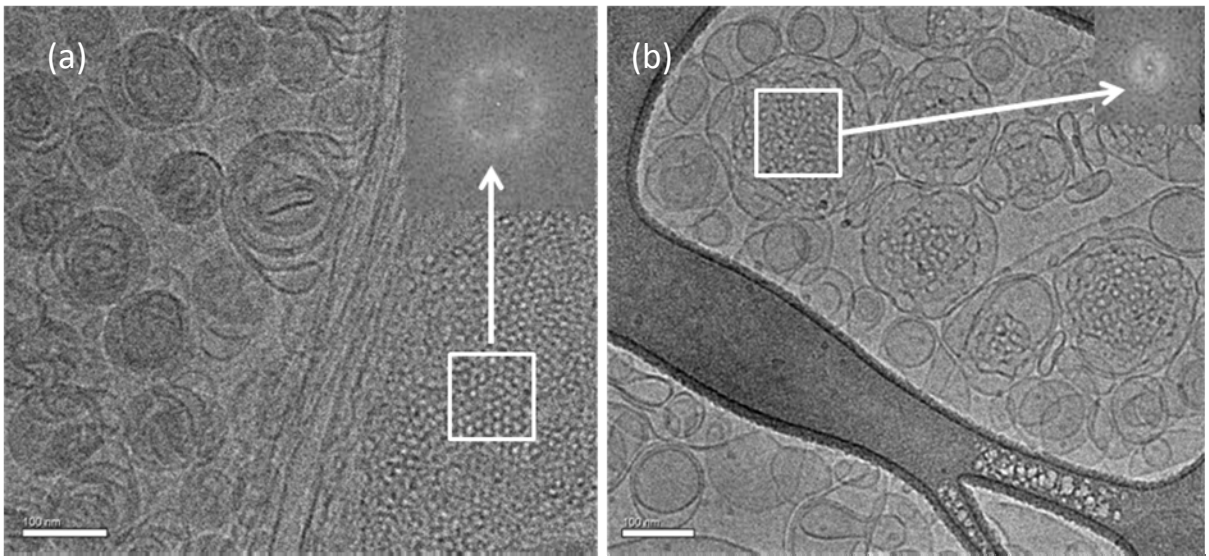

Figure 6S Cryo TEM micrographs of EDTA-BO particles, snap frozen from 40 °C. Scale bars are 100 nm. Insets show the fast Fourier transform (FFT) of the internal nanostructures, reflecting hexagonal arrangement in (a) and less ordered arrangement in (b).

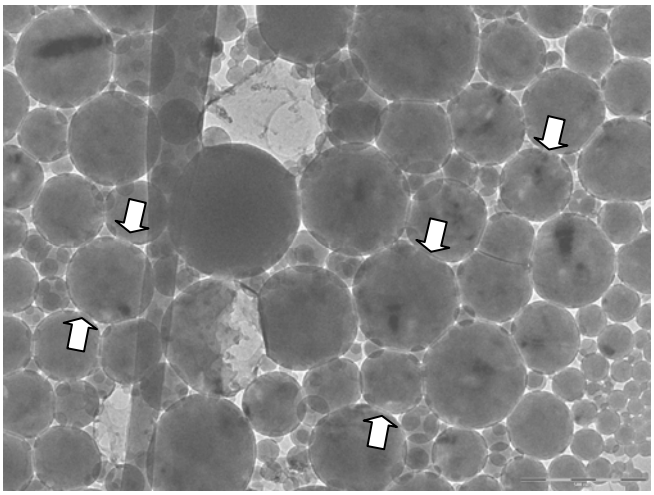

Figure 7S Cryo TEM micrographs of Gd-EDTA-BO particles. Arrows show fused colloidal dispersed particles. Scale bars are 500 nm.

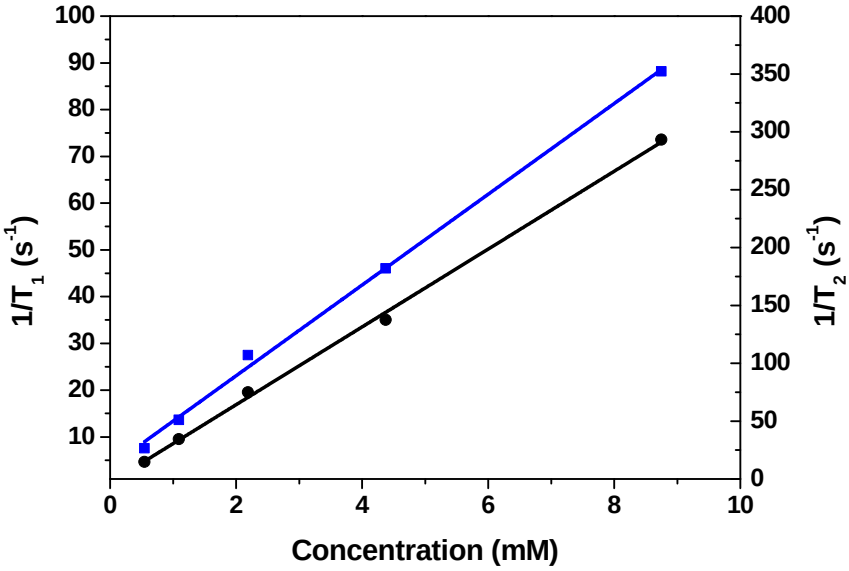

Figure 8S Reciprocal longitudinal and transverse relaxivity ($1/T_1$(black) and $1/T_2$(blue)) of Mn-EDTA-BO dispersed MWLNTs at different concentrations, showing a linear correlation within the concentration range examined.