Hot-Injection Synthesis of Iron/Iron Oxide Core/Shell Nanoparticles for T_2 Contrast Enhancement in Magnetic Resonance Imaging

David A. J. Herman,[a] Peter Ferguson, [b] Soshan Cheong, [a,c] Ian F. Hermans, [b] Ben J. Ruck, [a] Kathryn M. Allan, [a] Sujay Prabakar, [a] John L. Spencer, [a] Conrad D. Lendrum [c] and Richard. D. Tilley*[a]

[a]*School of Chemical and Physical Sciences and The MacDiarmid Institute of Advanced Materials and Nanotechnology, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand*
[b]*Malaghan Institute of Medical Research, P.O. Box 7060, Wellington, New Zealand*
[c]*Industrial Research Ltd. P.O. Box 31-310, Lower Hutt, New Zealand*

Corresponding Author
richard.tilley@vuw.ac.nz

Supporting Information

Experimental Section

Syntheses were carried out using commercially available reagents. Iron (III) chloride ($FeCl_3$), iron (II) chloride ($FeCl_2$, 98%), aluminium (III) chloride ($AlCl_3$), tetrahydrofuran (THF), mesitylene (98%), oleylamine (OLA, 98%), 1-octadecene (ODE, 90%), *meso*-2,3-dimercaptosuccinic acid (DMSA, 98%) were all purchased from Sigma-Aldrich. Methyllithium (1.6M in diethyl ether) was purchased from Acros Organics.

Synthesis of bismesitylene iron(II) hexafluorophosphate $[(\eta^6\text{-mesitylene})_2Fe](PF_6)_2]$ (1)

Bismesitylene iron(II) hexafluorophosphate $[(\eta^6\text{-mesitylene})_2Fe](PF_6)_2$, was prepared according to literature method with modifications.[1] $FeCl_3$ (10.00 g, 0.062 mol) and $AlCl_3$ (25.00 g, 0.19 mol) were mixed with mesitylene (200 cm^3, purged with N_2) for 24h under ambient conditions. The reaction mixture was cooled to 0°C and quenched with distilled water. After filtration the aqueous phase, containing the dication, was separated from the organic phase and washed several times with drum petroleum ether. The dication was then precipitated with aqueous $K^+PF_6^-$ solution (KPF_6, 20 g in distilled water, 200 cm^3), and the resulting orange solid filtered at the pump, washed with drum petroleum ether and dried to give **(1)** (31.06 g, 85% yield).

Synthesis of bis(η^5-1,3,5-exo-6-tetramethylcyclohexadienyl) iron(II), $[Fe(\eta^5\text{-}C_6H_3Me_4)_2]$ (2)

Bis(η^5-1,3,5-*exo*-6-tetramethylcyclohexadienyl) iron(II), $[Fe(\eta^5\text{-}C_6H_3Me_4)_2]$ was prepared according to literature method with modifications.[1] **(1)** (2.10g, 3.6 mmol) was suspended in THF (100 cm^3, purged with N_2) and cooled to -77°C in a dry ice/acetone complex bath. Under a stream of N_2 gas, MeLi (7.3 cm^3, 10.2 mmol) was injected yielding a red-brown mixture within seconds. The reaction mixture was warmed to room temperature while stirring, and subsequently quenched with 20 cm^3 distilled water to remove any excess MeLi. The solution was filtered and all the THF was removed under reduced pressure to yield an orange aqueous suspension. Extraction with petroleum ether gave an orange solution, which following solvent evaporation under reduced pressure yielded an orange solid. The solid was redissolved in toluene, filtered to remove impurities, and subsequently the solvent was removed under reduced pressure to yield a red/orange solid **(2)** (0.82 g, 67% yield). ^1H NMR (toluene-d_8, 30ºC): δ 3.37(s, 4H), 2.33(q, 2H), 2.06(s, 6H), 1.61(br s, 12H), 0.43(d, 6H)

Synthesis of iron/iron oxide core-shell nanoparticles

All procedures were performed under standard Schlenk techniques. **(2)** (0.1 g, 0.31 mmol), OLA (2.5 cm^3, 7.5 mmol) and ODE (1.5 cm^3) were mixed and degassed via freeze-pump-thaw method (FPT), while separately ODE (8 cm^3) was degassed via FPT in a 3-necked flask and then heated to 300°C, where the mixture containing **(2)** was injected instantly resulting in a black solution within two seconds. Upon injection the reaction temperature immedietly dropped to 260°C and was left at that temperature for 2h and then cooled to room temperature before being exposed to air and purified via magnetic separation and redispersed in toluene for TEM analysis, then dried under vacuum to yield a black powder (50mg, 50% yield)

Preparation of water dispersible iron/iron core-shell nanoparticles

Ligand exchange to replace OLA with DMSA was carried out based on known methods.[2] 30 mg of the as-synthesised nanoparticles were dispersed in degassed and deionised (DDI) water (10 cm^3). Separately, DMSA (20 mg, 0.11 mmol) was added to DDI water (20 cm^3). The pH of both solutions was adjusted to 3.0 with HCl, upon which the DMSA solution was added to the nanoparticles solution and the pH was again adjusted to 3.0 while stirring vigorously. The resulting mixture was centrifuged at 3500 rpm for 10 minutes yielding a black precipitate which was redispersed in DDI water (10 cm^3) and the supernatant containing OLA was discarded. The pH of the dispersion was stirred and pH adjusted to 9.25-9.5 with NaOH and was maintained for 30 minutes before lowering the pH to 7.4 with HCl to deprotonate and reprotonate the DMSA functional groups for ease of ligand exchange. The resulting solution was centrifuged at 3500rpm for 10 minutes and supernatant discarded. The nanoparticles were dispersed in DDI water.

Preparation of iron oxide nanoparticles

The nanoparticles were synthesised following literature method.[3] Briefly, under vigorous stirring two solutions (i) $FeCl_3$ (5.406 g) in 20 cm^3 DDI water, and (ii) $FeCl_2$ (1.988 g) in 5 cm^3 of HCl (2 M), were added to 100 cm^3 DDI water, to which 120 cm^3 ammonia solution was added and remained under stirring for 5 minutes. The black precipitate was purified by centrifugation at 3500 rpm for 20 minutes, and redipsersed in DDI water. The iron oxide nanoparticles were coated with DMSA as per the procedure used to coat the iron/iron oxide core-shell nanoparticles.

Physical measurements

Transmission electron microscopy (TEM) images were taken on a JEOL 2010 TEM microscope at an acceleration voltage of 200 keV. Powder XRD measurements were obtained from a Pan Analytical X'pert Pro MPD X-ray diffraction System using Cu Kα radiation. Magnetization measurements were obtained from a dried iron/iron oxide core/shell nanoparticle sample, pressed and secured in a cylindrical gelatin holder and performed on a Quantum Design MPMS 2 SQUID magnetometer. Measurements were made using a Quantum Design MPMS 2 SQUID magnetometer. The variation of the magnetic moment was carried out by altering the applied field from 60,000 Oe (6 T) to -60,000 Oe (-6 T) at 300 K. The diamagnetic contribution of the gelatin capsule was corrected for by subtracting from the data sets the magnetic moment measured for the empty gelatin capsule and sample holder, but considered neglible due to the high magnetization values obtained from the NP sample.

Preparation of nanoparticle dispersion in agar

A stock solution was diluted with PBS to give concentrations of 4, 10, 20 μg.mL^{-1} of Fe in PBS. To 100 μL of each dilution, 100 μL of 2% agar was added to give concentrations of 2, 5 and 10 μg(Fe).mL^{-1} in 1% agar. The control was prepared by adding 100 μL of 2% agar to 100 μL PBS.

MRI of nanoparticles

MRI was performed using a Brucker Instruments AVANCE400 nuclear magnetic resonance spectrometer, equipped with a Brucker Micro 2.5 imaging module. MR images were acquired at 9.4 T using a 2D multi-slice spin-echo sequence, at room temperature, with the following parameters: echo time (TE): 12 ms, repetition time (TR): 1000.00 ms, pixel size: 100 μm x 100 μm, slice thickness: 1 mm, number of echoes: 4, 4 averages, total experiment time: 5 minutes.

Determine of transverse relaxivity (r_2)

Paravision software (Bruker) was used for MR image reconstruction and analysis. A region of interest (ROI) was manually selected within the axial image of each nanoparticle dispersion in agar. A plot of the signal intensity against the echo time was fitted with an exponential function: $I(t) = I_0\exp(-t/T_2)$; where $I(t)$ = signal intensity at time t, I_0 =signal intensity at 0 s, and the transverse time T_2 was then calculated. The plot of $1/T_2$(sample) – $1/T_2$(control) against iron concentration ([Fe], in mM) was fitted with a linear function: $1/T_2$(sample) – $1/T_2$(control) = r_2 x [Fe] (see Figure S3); where the relaxivity (r_2) was determined as the slope of the fit.

Supplementary Figures

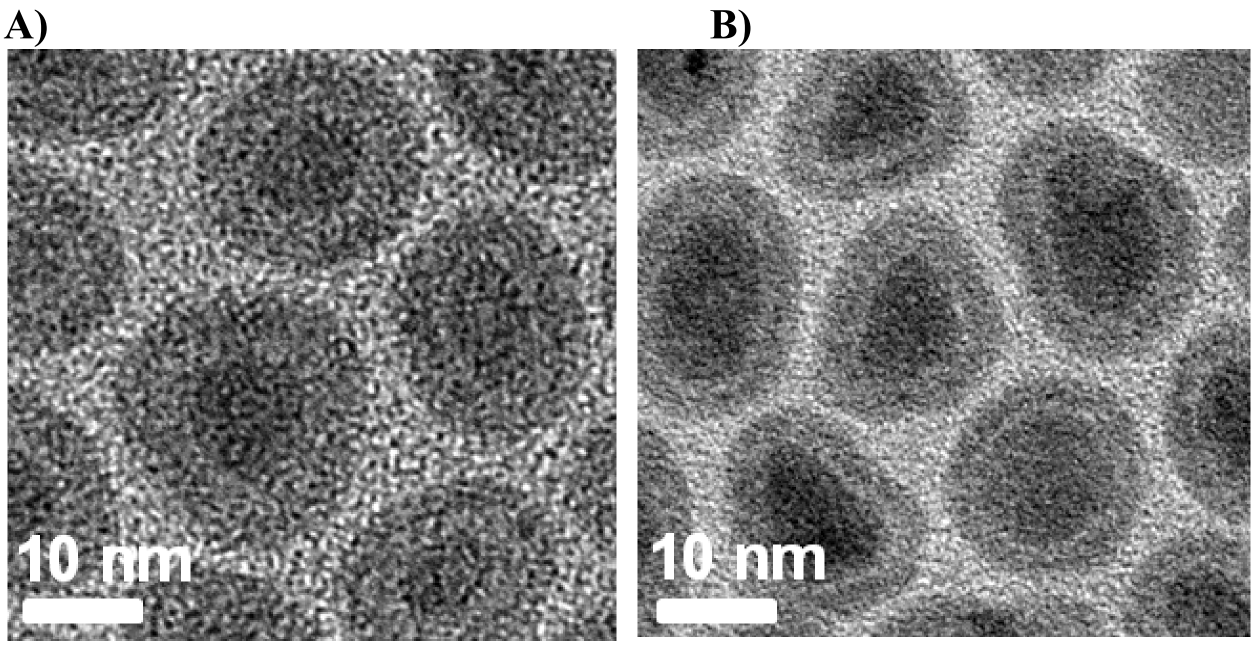

Figure S1: A) TEM image of iron/iron oxide core/shell NPs dispersed in toluene, six months after synthesis showing the stability of the core/shell structure over time, and B) TEM image core/shell NPs dispersed in water after ligand exchange with DMSA.

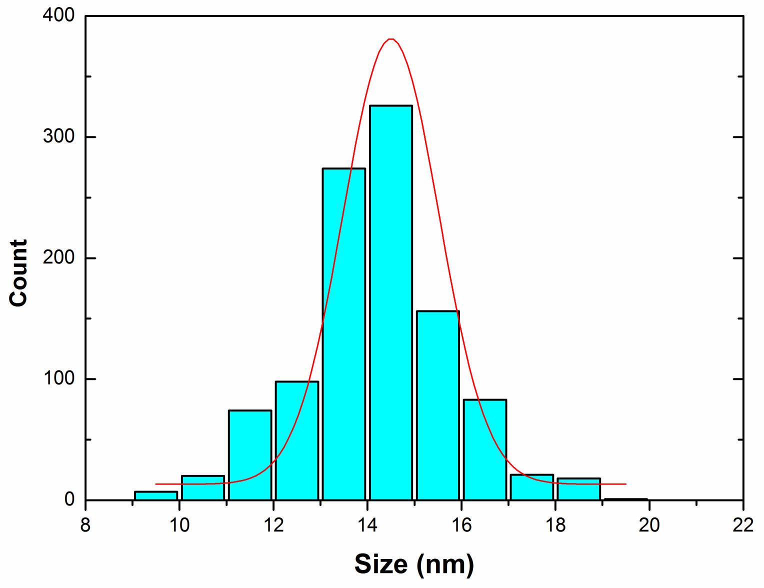

Figure S2: Size distribution histogram of 1000 iron/iron oxide core/shell NPs selected from random areas of different TEM grids. Mean size = 14.0 nm, standard deviation = 1.9 nm (13%)

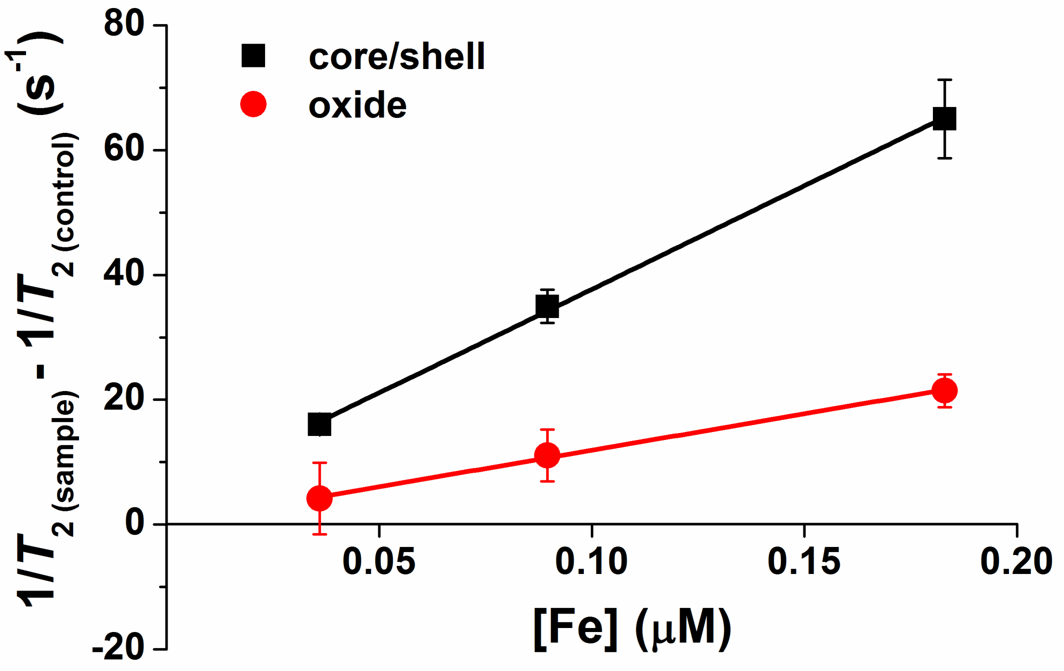

Figure S3: Sample plot using T_2 ,measurements of nanoparticles at varying iron concentrations to determine relaxivity.

References

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2. Fauconnier, N.; Pons, J. N.; Roger, J.; Bee, A., *Journal of Colloid and Interface Science*, **1997,** *194*, 427-433.
3. Pisanic, T. R.; Blackwell, J. D.; Shubayev, V. I.; Finones, R. R.; Jin, S., *Biomaterials*, **2007,** *28*, 2572-2581.