Supplementary information

High performance multi-core iron oxide nanoparticles for magnetic hyperthermia: microwave synthesis, and the role of core-to-core interactions

Cristina Blanco-Andujar^{abc}, Daniel Ortega^{cde}, Paul Southern^{bc}, Quentin A. Pankhurst^{bc*} and Nguyen Thi Kim Thanh^{ab*}

^aDepartment of Physics and Astronomy, University College London, Gower Street, London,
WC1E 6BT, UK. ^bUCL Healthcare Biomagnetics Laboratory, 21 Albemarle Street, London,
W1S 4BS, UK. ^cInstitute of Biomedical Engineering, University College London, Gower
Street, London, WC1E 6BT, UK. ^dInstituto Madrileño de Estudios Avanzados en
Nanociencia (IMDEA-Nanociencia), Cantoblanco 28049, Madrid, Spain. ^eCentro Nacional
de Biotecnología (CNB-CSIC) - IMDEA Nanociencia Associated Unit "Unidad de
Nanobiotecnología", Cantoblanco, 28049 Madrid, Spain.

Corresponding authors:

Nguyen Thi Kim Thanh <u>ntk.thanh@ucl.ac.uk</u>

Quentin Andrew Pankhurst <u>q.pankhurst@ucl.ac.uk</u>

Reproducibility studies

The reproducibility of the synthesis of citric acid coated iron oxide NPs through the described microwave method was investigated to assess its feasibility towards massproduction. The sources of variation that could affect the repeatability of the results were studied. The rate of injection of sodium carbonate was identified as the "Achilles heel" of the reaction, as it is subjected to human error. To overcome this limitation, a syringe pump was used to provide reproducible injection conditions. Each reaction was repeated three times at four different injection rates. As expected, the obtained results revealed a direct link between the rate of injection and the quality of the obtained nanoparticles. DLS measurements for the series of samples showed an increase on the average hydrodynamic diameter (D_H) with the rate of addition of Na₂CO₃ (Fig. S1) as attested by the one-way ANOVA test (F(3, 8) =18.90, p < 0.001). Tukey post-hoc comparison for the four rates of injection indicated significant differences ($\alpha = 0.05$) with a rate of injection of 1 ml/min; however, no significant differences were found between the rates of injection of 2 to 4 ml/min. All samples were stable over time; however, a rate of addition greater or equal to 3 ml/min led to a size distribution with a maximum hydrodynamic diameter of nearly 300 nm. The corresponding particle size distributions from TEM images were also affected by the injection rate (Fig. S2). Whereas the obtained particles had spheroidal morphology independently from the rate of addition, particle average size and polydispersity varied with the injection rate. Core size increased from 12.7 ± 2.1 nm to 16.9 ± 2.9 nm upon increasing the injection rate from 1 to 2 ml/min (Fig. S2) with no detrimental effect on the reproducibility of D_H between the repeats (Fig. S1). Conversely, a further increase on the injection rate had a detrimental effect on the nanoparticle size distribution (Fig. S2), as seen by the increased coefficient of variation with injection rate.



Fig. S1. DLS measurement of citric acid coated iron oxide nanoparticles synthesised by coprecipitation in a microwave reactor at a rate of injection of sodium carbonate ranging from 1 to 4 ml/min (left to right panel order).

A ferromagnetic-like curve was obtained for all samples (Fig. S3). Saturation magnetisation (σ_s) at 300 K varied with a maximum 81.0 Am²kg⁻¹ and minimum 70.1 Am²kg⁻¹ at an injection rate of 1 and 2 ml/min, respectively (Table S1). Magnetic saturation was close to the bulk value of maghemite (78 Am²kg⁻¹ at 300 K),¹ which could indicate that the contribution of citric acid to the overall NP mass was negligible.² The decrease in σ_s may be the result of finite size and surface effects, since no significant particle morphology differences were detected. (Fig. S2). Particle size distribution widened upon increasing the injection rate (Fig. S2), which led to the presence of big particles over the typical superparamagnetic size (17.5 nm for maghemite and 12.2 nm for magnetite nanoparticles) threshold of iron oxide³ and became blocked at 300 K (Fig. S3). Consequently, H_c and σ_r at 300 K increased with injection rate (Table S1). At 5 K, σ_s of all samples was higher compared to the 300 K values as a result of the blocking of the spins at lower temperatures.³ The increase on σ_s was significantly higher for the sample prepared at 1 ml/min (Table S1), amounting to 12.5 Am²kg⁻¹. This larger net increase indicates a higher population of superparamagnetic nanoparticles at RT becoming blocked at lower temperatures (Fig. S2).



Fig. S2. TEM images and particle size distribution of CA-IO nanoparticles synthesised with 2 mmol CA and step time for the reaction of 10 min with Na₂CO₃ with injection rates of: A-C) 1 ml/min, D-F) 2 ml/min, G-I) 3 ml/min and J-L) 4 ml/min.

Injection rate (ml/min)	$\sigma_{s 300K}$ (Am ² kg ⁻¹)	μ ₀ H _{c 300K} (T)	σ _{r 300K} (Am ² kg ⁻¹)	$\sigma_{s 5K}$ (Am ² kg ⁻¹)	μ ₀ Η _{c 5K} (T)	σ_{r5K} (Am ² kg ⁻¹)
1	81.0	1.2x10 ⁻⁴	0.2	93.5	1.98x10 ⁻²	21.6
2	70.1	1.9x10 ⁻⁴	0.3	78.6	1.81x10 ⁻²	18.8
3	74.6	2.5x10 ⁻⁴	0.5	84.0	2.05x10 ⁻²	20.6
4	72.4	6.7x10 ⁻⁴	1.1	80.7	2.02x10 ⁻²	21.6

Table S1. Overview of the magnetic parameters for CA-IO nanoparticles.



Fig. S3. Magnetisation curves for citric acid coated iron oxide nanoparticles synthesised by coprecipitation in a microwave reactor at a rate of injection of sodium carbonate ranging from 1 to 4 ml/min at A) 300 K and B) 5 K.

The reproducibility of the heating performance of the synthesised particles was varied with the rate of injection of sodium carbonate. Changes on core (Fig. S2) and hydrodynamic size (Fig. S1) were translated on the heating capability of the generated samples. A one-way ANOVA was used to test for heating performance differences among the rates of addition. Heating performance differed significantly across the four injection rates used, F(3, 8) =26.65, p < 0.001. Nonetheless, Tukey post-hoc comparison indicated no significant differences ($\alpha = 0.05$) in the heating performance between injection rates of 2 and 3 ml/min. Nevertheless, an injection rate of 2 ml/min offered a higher control on the final properties of the nanoparticles, as attested by the reproducibility studies on morphology and hydrodynamic size and the smaller coefficient of variability.



Fig. S4. Changes on ILP with the rate of addition of Na₂CO₃. Samples were measured at a frequency of 950 kHz and a field amplitude of 10.5 kA.m⁻¹.

Surface functionalisation – ATR-FTIR

Surface functionalisation was assessed by the ATR-FTIR spectrum. The chemisorption of CA to the surface of the iron oxide nanoparticles was confirmed from the shift of the characteristic peaks for free CA (Fig. S5). The 1065, 1247 and 1391 cm⁻¹ peaks were assigned to the OH group, the symmetric stretching of C-O and the symmetric stretching of COO⁻ of CA, respectively.⁴ The vibration for the asymmetric stretching of the COOH groups of CA, shifted from 1700 to 1601 cm⁻¹ due to the binding of the carboxylic groups to the surface of the nanoparticles.⁵ Charge delocalisation gave a single-like bond character to the C=O bond, which reduced the vibration frequency. The 2871 cm⁻¹ peak is due to CH₂ stretching. The band observed around 3300 - 3500 cm⁻¹ may correspond to unbound OH groups from CA; however, it is also associated to coordinated OH groups from the

physisorbed water on the nanoparticle surface,^{6, 7} and thus it cannot be used for identification purposes.



Fig. S5. ATR-FTIR spectra of IONPs before and after CA coating. CA spectrum is included for reference. The 1800 - 2700 cm⁻¹ range has been excluded as no significant information was found.

TEM images and particle size distributions for the complete CA-io sample series.







Fig. S6. TEM analysis of CA-io nanoparticle series. Particle size distributions were obtained from the measurement of at least 300 particles per sample and fitted to a log normal function.

XRD patterns and agreement indices for the Rietveld refinement.

The room temperature XRD patterns from the complete set of freeze-dried samples primarily show a single phase corresponding to an iron oxide with an inverse spinel structure (Fig. S7 and Table S2). After the corresponding analysis, it is not possible to unambiguously distinguish whether the diffraction patterns correspond to either maghemite (γ -Fe₂O₃) or magnetite (Fe₃O₄) or a mixture of both due to the well-known similarity between the typical diffraction profiles of these structures. In addition, peak broadening due to the small average particle size throughout the series further complicates an accurate phase characterisation. As the concentration of citric acid increases in solution, Fe(II) from the inverted spinel structure is slowly removed from the framework, with the subsequent increment of vacancies in the structure. The unit cell parameters obtained from the Rietveld refinement showed a reduction on the unit cell to a maximum of 8.378 Å for CA-ioA. which corresponds to non-stoichiometric or partially oxidised magnetite (magnetite and maghemite reference unit cells 8.397 Å and 8.334 Å, respectively).⁸ The average decrease on the unit cell parameter with the increase of citric acid in solution suggests the displacement of Fe(II) from the structure.







Fig. S7. X-ray powder diffraction patterns and agreement indices from Rietveld refinement calculated for the CA-io series (D = crystallite size, a = lattice parameter, GoF = Goodness of Fit, R_W = weighted R). The (*) indicates a peak corresponding to sodium carbonate remaining in the sample.

Table S2. Crystallite size (D_{XRD}) changes for the CA-io nanoparticle series obtained from theRietveld refinement of the room temperature XRD patterns.

Sample	Step time (min)	MW power (W)	CA (mmol)	D _{XRD} (nm)	a (Å)
CA-ioA	60	50	1	13.2	8.378
CA-ioB	60	50	2	13.3	8.370
CA-ioC	60	300	1	12.7	8.375
CA-ioD	60	300	2	14.3	8.369
CA-ioE	10	50	1	13.7	8.375
CA-ioF	10	50	2	14.2	8.372
CA-ioG	10	300	1	12.5	8.373
CA-ioH	10	300	2	14.7	8.359

Mössbauer spectroscopy

The standard model for analysis of Mössbauer spectral is based on Lorentzian lineshapes for the absorption lines. A less common, but very useful extension of this methodology, that is pertinent to the analysis of fine particle iron oxides, is the Voigt lineshape, which is the resultant of an intensity-based summation of Lorentzian lineshapes whose energies follow a Gaussian distribution relative to the center of the absorption. This model was used in the fitting of the room temperature Mössbauer spectra of samples CA-ioA and CA-ioH. (For details see: K. Lagarec and D. G. Rancourt, Recoil-Mössbauer spectral analysis software for Windows, University of Ottawa, Ottawa, ON (1998).)

Both spectra exhibit a sextet with broad lines. This was fitted to a purely phenomenological model comprising a superposition of three Voigtian sextets, with each component sextet comprising two or three subcomponents. The resultant fits are shown in Fig. S8A. Very little difference was distinguishable between the two spectra, as illustrated in the overlay of the raw data shown in Fig. S8B.

Regarding the stoichiometry of the sample, the mean isomer shift (spectral centroid) of 0.40 mm/s is a good deal less than the 0.53 mm/s of stoichiometric magnetite, Fe₃O₄, while also being larger than the 0.34 mm/s of stoichiometric maghemite, γ -Fe₂O₃. From this we infer that the samples may comprise a non-stoichiometric magnetite and/or a mixture of magnetite-like and maghemite-phases.



Fig. S8A. Mössbauer spectra for samples CA-ioA and CA-ioH at 300 K (Iobs = observed intensity, Icalc = calculated intensity, HFD Site = hyperfine field distribution site).



Fig. S8B. Overlay of the room temperature Mössbauer spectra of samples CA-ioA (black symbols) and CA-ioH (blue symbols).

Static magnetic properties at 300 and 5 K

Sample	σ_{s300K}	$\mu_0 H_{c\;300K}$	σ_{r300K}	σ_{s5K}	$\mu_0 H_{c5K}$	σ_{r5K}
	(Am ² kg ⁻¹)	(T)	(Am ² kg ⁻¹)	(Am ² kg ⁻¹)	(T)	(Am^2kg^{-1})
CA-ioA	71.5	4.6x10 ⁻⁴	0.5	84.5	2.4x10 ⁻²	17.8
CA-ioB	70.4	3.2x10 ⁻⁴	0.4	82.6	2.0x10 ⁻²	16.9
CA-ioC	72.9	3.7x10 ⁻⁴	0.4	84.5	2.4x10 ⁻²	19.9
CA-ioD	65.2	6.2x10 ⁻⁴	0.7	76.7	1.3x10 ⁻²	18.9
CA-ioE	71.2	5.5x10 ⁻⁴	0.6	83.5	2.1x10 ⁻²	18.1
CA-ioF	71.9	4.6x10 ⁻⁴	0.5	83.9	2.0x10 ⁻²	16.9
CA-ioG	70.7	3.1x10-4	0.3	82.9	2.2x10-2	17.5
CA-ioH	72.7	5.9x10 ⁻⁴	0.7	85.2	2.1x10 ⁻²	18.1

Table S3. Summary of the main magnetic parameters of the CA-io sample series.



Fig. S9. Hysteresis loops at A) 300 and B) 5 K for the whole CA-io sample series.

References

- 1. B. D. Cullity and C. D. Graham, *Introduction to magnetic materials*, Wiley-IEEE, 2nd edn., 2009.
- 2. P. Guardia, B. Batlle-Brugal, A. G. Roca, O. Iglesias, M. P. Morales, C. J. Serna, A. Labarta and X. Batlle, *J. Magn. Magn. Mater.*, 2007, **316**, e756-e759.
- 3. D. Ortega, in *Magnetic nanoparticles: from fabrication to clinical applications: theory to therapy, chemistry to clinic, bench to bedside*, ed. N. T. K. Thanh, CRC Press, 2012, pp. 3-44.
- 4. J.-J. Max and C. Chapados, J. Phys. Chem. A, 2004, 108, 3324-3337.
- 5. M. Răcuciu, D. E. Creangă and A. Airinei, *Eur. Phys. J. E*, 2006, **21**, 117-121.
- 6. J. T. Keiser, C. W. Brown and R. H. Heidersbach, J. Electrochem. Soc., 1982, **129**, 2686-2689.
- 7. G. Busca, V. Lorenzelli, G. Ramis and R. J. Willey, *Langmuir*, 1993, 9, 1492-1499.
- 8. R. M. Cornell and U. Schwertmann, *The iron oxides in the laboratory: Preparation and characterization*, Wiley-VCH Publishers, Weinheim, Germany, 2nd edn., 2000.