

Supplementary information

One-step synthesis of monodisperse water-soluble ‘dual-responsive’ magnetic nanoparticles

Ian Robinson,^a Cameron Alexander,^b Le T Lu,^a Le D Tung,^c David G Fernig^{d,e} and Nguyen
TK Thanh^{*a,d,e}

^a Department of Chemistry, University of Liverpool, Crown Street, Liverpool, L69 7ZD, UK.

^b School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK.

^c Department of Physics, University of Liverpool, Crown Street, Liverpool, L69 7ZE, UK.

^d School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK.

^e Liverpool Institute for Nanoscale Science, Engineering and Technology, University of Liverpool,
Liverpool, L69 7ZB, UK.

Experimental section

All chemicals were purchased from Aldrich Ltd (Gillingham, UK) except dicobalt octacarbonyl (Strem Ltd, Royston, UK) and α,α' -azobis(isobutyronitrile) (Molekula Ltd, Wimbourne, UK) and used without further purification, apart from, N-isopropylacrylamide and N-tert-butylacrylamide, which were re-crystallised from hot hexane.

Using standard oxygen-free conditions, the thermal decomposition of dicobalt octacarbonyl or iron pentacarbonyl, in organic solvent, in the presence of the thermo-responsive polymer was used to synthesise NPs consisting of Co and γ -Fe₂O₃, respectively. In a typical synthesis of Co NPs, the polymer (0.04 mmol) was dissolved in 1,2-dichlorobenzene (DCB) (8 ml) and heated to reflux with vigorous stirring before a solution of dicobalt octacarbonyl (3.56 mmol) in DCB (2 ml) was rapidly injected into the solution. The solution was refluxed for 10 min then cooled to room temperature. For the γ -Fe₂O₃ NPs, the polymer (0.04 mmol) was dissolved in dioctyl ether (10 ml) at 100 °C and iron pentacarbonyl (3.56 mmol) was added with vigorous stirring. The solution was heated to reflux and held at that temperature for 30 min, then cooled to room temperature. A permanent magnet was

used to concentrate the NPs and remove excess polymer in the supernatant. After removal of the solvent the NPs were redispersed in hexane. This process was repeated three times before NPs were dried *in vacuo* at 20 °C for 30 min. The residue was re-dispersed in water and sonicated for 15 min. A permanent magnet was again used to concentrate the NPs and the water was removed. This process was repeated three times before the NPs were suspended in water.

Transmission electron microscopy (TEM) samples were prepared by adding 5 µl of sample in water to a carbon-coated copper TEM grid at room temperature and allowed to evaporate slowly in air. Images were obtained using an FEI Tecnai G2 120 kV TEM, operated at 100 kV and visualised using analySIS software. The diameter (d) of the NPs was taken as the mean of a minimum of 200 (n) NPs measured using Bersoft Image Measurement software.

To establish the behaviour of the polymer-coated NPs across ranges in pH, the NPs were diluted ten-fold in water and the pH adjusted with either 0.1 M HCl or 0.1 M NaOH to give a pH range from 1 to 11.8 and the ζ-potential of the NPs was measured. Measurements were taken on three separate samples using a ZetaPlus Zeta Potential Analyzer (Brookhaven Instruments Corp.) using ZetaPALS software and the average values are presented.

To study the effect of temperature upon the stability of the NPs in solution, 0.5 ml aliquots of aqueous nanoparticle suspension was mixed with either water (2 ml) or phosphate buffered solution (PBS) (2ml; 10 mM, pH 7.2). The diluted suspensions were then exposed to a range of temperatures from 5 °C to 50 °C.

Magnetic measurements including the zero-field-cooled, field-cooled magnetisation were carried out in a MPMS SQUID magnetometer on the diluted samples in aqueous solution (approximately 6 mg of Co NPs and 2 mg of γ-Fe₂O₃ diluted in 200 µl of water).

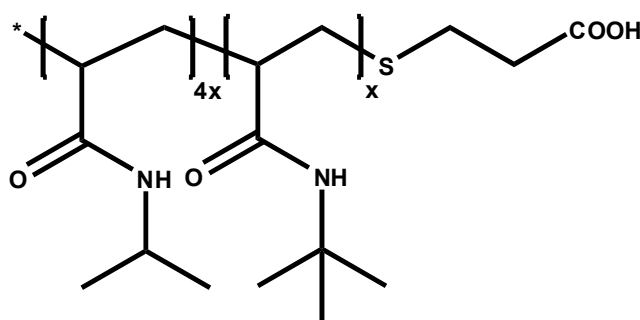
For γ-Fe₂O₃ NPs, the structure were further characterised by ⁵⁷Co Mössbauer spectroscopy (data not shown). At 77 K, the spectra showed both sextet and doublet components characteristic of slow and fast relaxing superparamagnetic relaxation, respectively. Values of magnetic hyperfine field

and isomer shift for the sextet component are consistent with γ -Fe₂O₃ phase but modified slightly due to the small particle nature of the sample.

Polymer synthesis

In a typical polymer synthesis, *N*-iPAm and *N*-*t*-BAm were dissolved in propane-2-ol (40 ml) with 3-MPA and ACVA or AIBN (for exact amounts see Table S1). The solution was degassed by freeze-thaw cycles under vacuum at least three times. The tube was then placed in an oil bath at 65 °C for 24 h. After cooling to room temperature, the mixture was concentrated under reduced pressure and the residue added to diethyl ether (250 ml). The precipitated polymer was filtered and the residue was re-dissolved in the minimum amount of tetrahydrofuran and re-precipitated into diethyl ether (250 ml) three times. The purified polymer was dried in *vacuo* at 20 °C for 16 h.

Table S1 *The amounts of monomers used and free radical initiators to make the poly(alkylacrylamide) polymers P1 and P2. (N-iPAm = N-isopropylacrylamide; N-t-Bam = N-tert-butylacrylamide; 3-MPA = 3-mercaptopropanoic acid; ACVA = 4,4'-azo-bis(4-cyanovaleric acid; AIBN = α,α' -azobis(isobutyronitrile; LCST = Lowest Critical Solution Temperature; Mw = molecular weight).*



Poly(*N*-isopropyl-co-*t*-butylacrylamide)

Polymer	<i>N</i> -iPAm (g)	<i>N</i> - <i>t</i> -Bam (g)	3-MPA (ml)	ACVA (g)	AIBN (g)	LCST (°C)	Mw (g/mol)
P1	8.40	1.79	0.19	-	0.19	23.5	6800
P2	9.01	1.12	0.19	0.33	-	28.0	6000

The Mw of the polymers was determined by titration of the dissolved polymer (approx. 0.1 g) in water (50 ml) with freshly prepared sodium hydroxide solution (10 mM). The values are shown in Table 1. The end point was determined by using phenolphthalein solution (1 % w/v in ethanol) as an indicator.

The LCST was determined by dissolving the polymers in water at a concentration of 10 mg/ml and cooling to 5 °C then heating to 50 °C at 0.5 °C/min in a heating block of a UV spectrometer. The LCST was taken at the onset of a sharp increase in absorption at 500 nm, which is indicative of an increase in the turbidity of the solution due to the phase transition of the polymer.