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. Fernigd,e and Nguyen T. K. Thanhade

Monodisperse water-soluble Co and γ-Fe₂O₃ nanoparticles have been prepared in a single-step method using stimuli-sensitive polymers.

Magnetic nanoparticles (NPs) have been attracting great interest due to their potential in vivo applications in areas such as magnetic resonance imaging (MRI), targeted drug delivery and hyperthermia treatment of solid tumours.1 However, a major problem when considering these applications is the stability of the magnetic NPs in aqueous environments. Current solutions are the synthesis of the NPs either in situ with a suitable stabilising ligand (one-step method) or a two-step method using ligand exchange. The one-step synthesis method is relatively simple, although when carried out in aqueous solution, it has so far proved more difficult to control the size and the monodispersity of the NPs. On the other hand, in the two-step method, monodisperse NPs are first synthesised in organic solvent, e.g., via pyrolysis of organometallic compounds.2 Then, in the second step, the NPs are transferred to aqueous solution using a process of ligand exchange. Regardless of ligand, this second step is rather complex and in many cases very difficult to realise.

Here we report a facile one-step synthesis of monodisperse magnetic NPs coated with a ‘smart’ thermo-responsive polymer using the thermal decomposition of organometallic compounds in organic solvent. The key to this strategy is the use of polymers that have switchable solubility between organic and aqueous phases, such that the NPs can be surface-stabilised by a single polymer component under two normally mutually-incompatible environments. The polymers are based on poly(N-isopropyl-co-t-butylacrylamide), synthesised to contain a thioether terminated with a carboxylic acid for interaction with nanoparticle surfaces. In water these polymers undergo a sharp coil-to-globule phase transition at a Lowest Critical Solution Temperature (LCST).3 The coil-to-globule transition means this polymer is water soluble and hydrophilic below the LCST but is hydrophobic above it, and, dependent on the co-monomer composition, is also soluble in an organic solvent. As a consequence, it is possible to dissolve the amphiphilic co-polymer in hot organic solvents, facilitating the synthesis of monodisperse NPs by thermal decomposition. Upon cooling, the nanoparticle suspension precipitates and the organic solvent can be removed and replaced with water. In this way, it is possible to produce functional ‘smart’ magnetic NPs with dual-controllable associative properties in a single step. Previously, a thermo-responsive polymer was used to coat magnetic NPs (Fe₃O₄) in a multi-step method in which the NPs were synthesised and coated with a double layer of surfactant that was then used as seeds in a polymerisation reaction.4 In our approach, the selection of an appropriate thermo-responsive polymer coating allows the synthesis of the NPs to be carried out in just one step to achieve the monodispersity, stability and water solubility of the NPs.

We have used ten different polymers in the synthesis of Co and γ-Fe₂O₃ NPs. However, in terms of stability and monodispersity, polymer P1 (Table 1) was found to be most suitable for coating Co NPs and polymer P2 for γ-Fe₂O₃ NPs (details of the synthesis of polymers P1, P2 and polymer coated NPs can be found in the ESI†).

Table 1 The amounts of monomers and free radical initiators used to make the poly(alkylacrylamide) polymers P1 and P2

<table>
<thead>
<tr>
<th>N⁻PAm/g</th>
<th>N⁻r-Bam/g/m</th>
<th>ACVA/g</th>
<th>AIBN/g</th>
<th>Mw/g mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 8.40</td>
<td>1.79</td>
<td>0.19</td>
<td>—</td>
<td>0.19</td>
</tr>
<tr>
<td>P2 9.01</td>
<td>1.12</td>
<td>0.19</td>
<td>0.33</td>
<td>—</td>
</tr>
</tbody>
</table>

The Co and γ-Fe₂O₃ NPs were spherical with a mean diameter of 7 ± 1 nm and 8 ± 1 nm, respectively, as observed by TEM (Fig. 1). The reason why Co and γ-Fe₂O₃ NPs are better formed with different types of polymers may be related to the different solvents used in the synthesis (1,2-dichlorobenzene for Co and dioctyl ether for γ-Fe₂O₃ NPs) and the slight differences in hydrophilic/hydrophobic balance of the two polymers. Two

† Electronic supplementary information (ESI) available: Experimental section and polymer synthesis. See DOI: 10.1039/b713528a
different mechanisms have been suggested to explain the formation of polymer-coated NPs. In the ‘polymer capping’ model, the polymer chains adsorb onto the growing nanoparticle and form a layer that can inhibit any further growth.\(^5\) The other mechanism suggests a combination of the capping mechanism and particle formation kinetics in which the formation of the NPs is affected by the surface tension between the NPs and the polymer solution as well as the energy involved in the nanoparticle formation.\(^6\)

The results of the zero-field-cooled (ZFC) and field-cooled (FC) magnetisation showed that both the Co and \(\gamma\text{-Fe}_2\text{O}_3\) NPs were superparamagnetic at room temperature with a blocking temperature of about 6 K and 92 K, respectively (Fig. 2).

One important factor determining the stability of the NPs is their surface charge which can be measured through the \(\zeta\)-potential. A higher surface charge (or high value of \(\zeta\)-potential) means the NPs are more electrostatically repulsive to one another and thus the NPs are more stable in solution. We have investigated the \(\zeta\)-potential of the NPs over a wide range of pH and the results are displayed in Fig. 3. At pH 1 the \(\zeta\)-potential was found to be 22 mV for the Co NPs and 14 mV for the \(\gamma\text{-Fe}_2\text{O}_3\) NPs. As the pH increased, the \(\zeta\)-potential became more negative to reach a value of \(-21\) mV at pH 12 for the Co NPs and \(-45\) mV at pH 10.5 for the \(\gamma\text{-Fe}_2\text{O}_3\) NPs. At neutral pH, the \(\zeta\)-potential was found, by interpolation, to be approximately \(-5\) mV and \(-24\) mV for the Co and \(\gamma\text{-Fe}_2\text{O}_3\) NPs, respectively.

The dependence of the \(\zeta\)-potential upon pH can be attributed to an increase in the ionisation of the nanoparticle surface; some contribution may also have arisen from the carboxylic terminal groups of the polymer chains losing a proton in increasingly basic conditions. The overall effect was an increase in negative charge on the outer shell of the NPs. Importantly, however, irrespective of the \(\zeta\)-potential, the NPs remained stable in aqueous solutions, indicating the strong colloidal stabilising effect of the pendant polymers when chain-extended. In comparison with the poly(N-isopropylacrylamide) brushes reported by Yim et al.,\(^7\) the polymer chains attached to the NPs are likely to extend no more than a few nm in solution, but this still represents a substantial ‘corona’ compared to the diameters of the magnetic NPs. We reason that, at the physiological pH, 7.4, the combination of charge and steric shielding afforded by the polymer chains would ensure increased stability below the LCST, but would not be

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**Fig. 1** TEM micrographs of the NPs produced using a one-step synthesis method and dispersed in water. (A) 7 ± 1 nm Co NPs and (B) 8 ± 1 nm \(\gamma\text{-Fe}_2\text{O}_3\) NPs.

**Fig. 2** ZFC (symbols) and FC (lines) magnetisation of the Co and \(\gamma\text{-Fe}_2\text{O}_3\) NPs as a function of temperature.

**Fig. 3** The effect of pH on the \(\zeta\)-potential of (A) Co and (B) \(\gamma\text{-Fe}_2\text{O}_3\) NPs. The results are the mean value of three separate experiments and the error bars represent the standard deviation of the mean.
causing the NPs to disaggregate. Because of the swelling/deswelling or ‘smart’ behaviour of the polymer in response to external stimuli, polymer coated magnetic NPs can be exploited for use in a therapeutic application by binding therapeutic agents to the polymer\(^9\) (e.g. anionic DNA\(^{10}\) or hydrophobic anticancer drugs\(^{11}\)). The magnetic NPs can then be targeted to a specific site by using a magnetic field and the induction of a change in temperature and/or pH would allow the controlled release of the therapeutic agent.\(^{12}\)

In terms of toxicity, pN-pPAm polymers have been dosed in previous studies at up to 4000 mg kg\(^{-1}\) in mice without causing acute cytotoxicity,\(^{13}\) and other studies have indicated good tolerance by Caco-2 cells at temperatures below pN-pPAm LCST and for incubation times of up to 3 h above LCST.\(^{14}\) Whereas low levels of Co are beneficial to human health,\(^{15}\) there are currently no data on the toxicity of Co in the form of nanoparticles.

In conclusion, we have synthesised a series of monodisperse ‘dual-responsive’ NPs with a Co or \(\gamma\)-Fe\(_2\)O\(_3\) core and a shell of thermo-responsive polymer in a single-step process. The NPs are superparamagnetic at room temperature, water soluble and exhibit reversible aggregation behaviour with changes in temperature. The response of these NPs to external stimuli may thus be exploited for use as therapeutic delivery agents and the stability of the NPs in water and PBS suggests they have potential use in applications such as MRI contrast agents, cell tracking and as reporters for immunosays.

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Notes and references