Synthesis of Amine-stabilized Aqueous Colloidal Iron Oxide Nanoparticles

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Abstract

We demonstrate a simple one-step process for the synthesis of iron oxide nanoparticle aqueous colloids using the multifunctional molecule, dodecylamine (DDA), that electrostatically complexes with aqueous iron ions (one precursor Fe^{2+} from $FeCl_2$), reduces them, and subsequently caps the nanoparticles. The iron oxide particles thus synthesized are of the face-centered cubic (FCC) phase with high degree of monodispersity with appropriate concentration of amine capping molecular layer. The aqueous magnetic nanocrystalline colloids were characterized by TEM, XRD, XPS, TGA/DTA and FTIR spectroscopy techniques. The relaxivity, stability, and hydrodynamic size of the nanoparticles were investigated for potential application in magnetic resonance imaging (MRI). The magnetic properties were also studied by using a superconducting quantum interference device (SQUID) magnetometer at room temperature. We believe that such simple one-step synthesis of biocompatible aqueous nanomagnetic colloids will have viable applications in biomedical imaging, diagnostics and therapeutics.

Iron oxide is one of the most popular multi-functional magnetic materials with diverse applications including multi-tera bit storage device[1–3], catalysis[4, 5], sensors[6] as well as a platform for high sensitivity biomolecular magnetic resonance imaging (MRI) for medical diagnosis and therapeutics[7–10]. One of the main shortcomings of conventionally synthesized and polymer-functionalized Fe_3O_4 for biomedical applications is the presence of thick polymer layer which increases the hydrodynamic radii considerably (up to 100 nm) causing the dampening of the MR signal. Introduction of a cationic surface layer with ($-NH_2$) amine on top of iron oxide nanoparticles surface is believed to have very good uptake by the cancer cells, and provide sharper in-vitro and in-vivo MRI signal as compared to the conventional cross-linked iron oxide (CLIO) nanoparticles[9,10]. However, it is not clear if these nanoparticles will have better bio-stability (including buffer saline, different pH conditions) with higher loading and enhanced biocompatibility and optimal magnetism as compared to dextran coated CLIO nanoparticles hence the need is to optimize surface functionality of these magnetic particles to create a versatile platform for variety of biomedical and bioclinical applications.

Iron oxide colloidal suspensions with discrete size deviation and a superparamagnetic behavior (size regime of 5–20 nm) are now envisioned as building block for many biomedical applications. Several approaches have been developed to synthesize Fe_3O_4 nanoparticles. However, compared to the well-known alkaline oxidation route[11] which is believed to not be

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suitable for many in-vivo applications, the more promising routes are thermal decomposition of various oragnometallic iron precursors. For example, very recently Sun et al[12] reported highly uniform magnetite nanoparticles through Fe-acetylacetonate decomposition in phenyl ether and the most promising one for large scale synthesis of Fe_3O_4 structure is the Fe-oleate precursor decomposition by annealing at 320 °C in high boiling point octadecene[13]. More recently a modified synthetic approach in highly polar solvent was proposed by Gao et al[14] which renders particles water-soluble as opposed to previously mentioned thermal decomposition methods which require further surface modification to pull organic-soluble particles into water, which results in lower yield and poor dispersion. From the synthesis point of view, an important consideration will be to prepare nanoparticles economically using relatively low-grade reagents under aerobic conditions. The motivation for our work stems from the need for aqueous synthesis of iron oxide colloidal nanoparticles under ambient conditions.

In this article we report one-step aqueous approach to synthesize Fe_3O_4 nanoparticles from a single $FeCl_2$ precursor using long-chain amine (dodecylamine, DDA) as reducing and surface-functionalizing agent. Capping of iron oxide nanoparticles with the amine molecules stabilizes the particles in solution nearly covalently and renders them water-dispersible. The procedure is reported in the references section[15]. We illustrate through structural characterization that control of amine concentration with respect to Fe^{2+} concentration gives control of the size and morphology of the product. The amine-capped nanoparticles can be obtained in the form of a highly air-stable dry powder after evaporation of the aqueous component that is readily redispersible in water as well as nonaqueous solvents. Amine-group functionalized Fe_3O_4 nanoparticles may offer an excellent platform for next generation nanobiocomposites and bio-tracers. As an example, this surface functionality is used for the covalent binding of a fluorescent dye, 4-chloro-7-nitrobenzofurazan (NBD), and Oregon Green to the nanoparticles[16].

The core size and size distribution of the amine-stabilized iron oxide nanoparticles were examined by transmission electron microscopy (TEM). Figure 1 shows the TEM images of iron oxide nanoparticles with three different sizes. As the molar ratio of dodecylamine to $FeCl_2$ increased up to 1, the particle size increased. The higher the molar ratio was above 3, the smaller the nanoparticles. This trend agreed with that reported in the literature[17]. The inset in Figure 1A is the HRTEM image of corresponding nanoparticles.

Product of reaction1 (Fe/amine concentration − mole ratio 1:1) shows that the nanoparticles consist of two distinct broad size distributions typically in the range of 10 nm to 40 nm (Figure 1A). The improved uniformity in particle size distribution in reaction2 (Fe/amine concentration − mole ratio 1:4) and reaction3 (Fe/amine concentration − mole ratio 1:7) is clearly evident from the TEM images of Figure 1B and 1C respectively. It can be seen that at higher amine concentrations, the particle size becomes more uniform. A closer look at these particles is shown in figure 1E and figure 1F and these images are used to estimate the particle size distribution. Figure 1D shows the particle size distribution plot of about 60 particles for reaction2 and 150 particles for reaction3. These plots further corroborate the results from TEM and show that the particle size distribution is much narrower after reaction2 (Fe/amine mole ratio 1:4) with a standard deviation of within 15% compared to over 40% for reaction1 (Fe/Amine − mole ration 1:1). Moreover the average particle size after reaction2 is controlled well with respect to amine concentration (13 ± 1 nm and 8.5 ± 1 nm respectively). The control of core size with respect to amine concentration is similar to the well-known 'Au-thiol' system, where the cores of thiol-stabilized Au nanoparticles decrease in size and dispersity with increasing thiol concentration[18]. This is not surprising considering that higher amine surfactant would have finer iron oxide particles with narrower size distribution. At present, we understand

that there is a simple correlation between amine concentration and particle size. The particle size increases with a decrease in the concentration of DDA.

While the TEM images demonstrate the uniformity of particles with respect to DDA concentration and the high resolution image reveal the necessary crystallinity, the Fe_3O_4 phase formation was corroborated by the x-ray diffraction (XRD) patterns. It showed broad peaks assigned to the[11], {311}, {400}, (511) and (440) planes of a face centered cubic (fcc) lattice of iron oxide (Fig. 2 A). The corresponding lattice constant is $a = 0.82$ nm, which matches to the reported standard data (JCPDS File No. 19–629; $a = 0.8393$ nm). The crystallographic form of Fe_3O_4 nanoparticles was also verified by its structural transformation to maghemite upon oxidation at 300 °C. Noticeable change in peak positions are shown in figure 2B, in addition to new peaks appearing after oxidation (Please refer to supporting information Figure S2, the full XRD spectra of as synthesizes sample, annealed at 300 and 600 °C). The resulting patterns after annealing at 300 and 600 °C match well with maghemite and hematite phases, respectively. The average particle size of the nanoparticles (11.9 nm and 8.2 nm) calculated by the Scherrer formula, using the full width half maximum (FWHM) of the intense (311) reflection (Figure 2C and 2D), was comparable with the value obtained from TEM images (reaction2 and reaction3: Figure 1B and 1C respectively). Three different curves in Figure 2C and 2D demonstrate the growth kinetics of the nanoparticles after 3 h, 6 h and 12 h reaction time, while XRD patterns in Figure 2E illustrate two distinct particle sizes revealing a sharp peak for the bigger particle size achieved with lower amine concentration. In addition to concentration, the particle size and distribution were affected by the time variation (supporting info). For instance, reaction time 3 h [supporting info (Figure 1S (A & B: Fe/Amine mole ration 1:1)) afforded irregular shaped nanoparticles with rods ranging from 20–30 nm diameter. After 12 h reaction time, the particle size became very large (average diameter 30 ± 10.5 nm) and the particle size distribution was relatively broad (Figure 1S (C & D)). Thus, the particle size and distribution of iron oxide nanoparticles prepared in this work were strongly controlled by the DDA: iron ion molar ratio.

The reaction mechanism of nanoparticle formation from a single precursor Fe2+ to Fe_3O_4 is not very well understood. However, on the basis of the results with the amine based chelation and subsequent reduction, we believe and propose that reaction mechanism is simple and proceeds with amine reduction that involves a proton abstraction from an Fe(II)-bound amine to yield $Fe(III)(RNH)^-$ species. These amine chelates then react with water to form the iron oxide, still containing the amine surfactant at the surface. One of the advantages of amine matrix is that it accelerates the hydrolysis of metal ion in the presence of amine matrix and it occurs readily without addition of a base because amine provides the basic media [Kandori, K.; Yasukawa, A.; Ishikawa, T. *Journal of Colloidal and Interface Science* **1996**, *180*, 446.] On the basis of these facts, we may conclude that amine acts as a reductant in N2 atmosphere and Fe3O4 may originate from the $Fe^{2+/3+}$-chelates reduced by amine with the oxygen originating from the –OH ligands. A thermogravimetric analysis (TGA) of the magnetite nanoparticles obtained after drying the colloidal solution was performed over the range of temperature 20–600 °C in nitrogen atmosphere. TGA of DDA-coated Fe_3O_4 nanoparticles indicates chemisorption of DDA at the nanoparticle surface and its multilayer deposition at higher DDA concentration (Fe/Amine mole ratio 1:7). The first and brief event of weight loss occurs around 100 °C and is due to evaporation of the adsorbed solvent. The TGA data (Figure 3A) demonstrated that regardless of the atmosphere, the major mass loss in DDA-coated nanoparticles occurs between the range 210–350 °C, which is higher than that for the pure DDA (248 °C, b.p.). This shift in the temperature could be due to multilayered adsorption of DDA on the iron oxide surface, requiring higher temperature for the vaporization of bound DDA. This is followed by a minor weight gain between 350 and 450 °C. Perhaps, this complex behavior results from the protective nature of amine molecules on the iron oxide surface which distinguishes quite well the dehydration of the magnetite powder and ultimately causes very

slow and delayed oxidation of Fe^{2+} around 300 °C. The transformation of magnetite into maghemite corresponds to the feature at around 200°C. [Cornell, R. M.; Schwertmann, U. *The Iron Oxides*; VCH: New York, 1996; p 135.] This is reported for DTA/TGA experiments conducted on maghemite: no feature is observed on any curves except the weight loss due to dehydration of powders. [Cornell, R. M.; Schwertmann, U. *The Iron Oxides*; VCH: New York, 1996; p 135.] We believer that the amine monolayer protects the magnetite structure well the and hence there is no competition between dehydration and oxidation of Fe^{2+} as had been seen earlier for magnetite structures. [Daou, T. J.; Pourroy, G.; Begin-Colin, S.; Greneche, J. M.; Ulhaq-Bouillet, C.; Legare, P.; Bernhardt, P.; Leuvrey, C.; Rogez, G. *Chem. Mater.,* **18** (18), 4399–4404, 2006.] The final phase transformation of maghemite into hematite is observed at around 500 C.[[3] Xisheng, Y.; Dongsheng, L.; Zhengkuan, J.; Lide, Z. *J. Phys. D: Appl. Phys.* **1998**, *31*, 2734.]

FTIR of amine functionalized Fe3O4 particles further supports the chemisorption of DDA and is especially valuable to confirm the expected presence of distinctive vibrations due to the presence of amine on the particles. FTIR spectra of the DDA precursor and DDA-Fe_3O_4 nanoparticles are shown in Supporting Information (Figure S2: superimposed and magnified view, respectively) and selected band assignments are indicated. The spectrum of DDA is similar to the published spectrum[19]. The peak at 3325 cm^{-1}, which corresponds to the N-H stretching vibration mode, appears very weak and shifts to lower wave number when the DDA molecules adsorb on the nanoparticle surface, giving strong evidence that DDA anchors on the iron oxide surface through the N atom of the amine group in DDA. From the XPS measurements, Fe $3p_{3/2}$ and Fe $3p_{1/2}$ peaks observed at 711 and 724.8 eV, respectively, were identified with DDA-Fe_3O_4 nanoparticles (Supporting info: Figure 3S)[20]. Fe, N and O peaks are corrected with reference to C 1s peak at 284.5 eV. A symmetric N 1s peak for Fe3O4-DDA nanoparticles appears at 400 eV, indicating the presence of N as a charged species (Figure 3B). If there is a contribution from the iron amine complex, a very low intensity peak at 397.3 eV appears[21]. The BE region corresponding to the free amine group or bonded amine falls in the range 398–400 eV. This peak suggests the involvement of electrostatic interactions, particularly with a nanoparticle surface, and is in agreement with earlier observations[22].

Figure 4A shows the room-temperature hysteresis magnetization of dried magnetite nanoparticles obtained by SQUID magnetometry. Both 9- and 13-nm DDA-Fe_3O_4 nanoparticles exhibit superparamagnetic characteristics, indicating that the thermal energy can overcome the anisotropy energy barrier of a single particle and the net magnetization of the particle assemblies in the absence of an external magnetic field is zero. The saturation magnetization was 60 and 70 emu/g, for 9- and 13 nm size particles, respectively. The difference in saturation magnetization of the samples is mainly attributed to the differences in particle size. In addition, saturation magnetizations of our samples are slightly lower than those of similarly sized nanoparticles prepared by other methods[23], which might be caused by the surface spin canting effects created by the amine molecule on the nanoparticles surface[24]. Figure 4B shows the zero field cooling curves of 9- and 13- nm particles. Curve (i) decreases rapidly with temperature and suggests that 9 nm particles are superparamagnetic with little interaction among the particles[25]. Curve (ii) decreases slowly with temperature and indicates that 13 nm particles are superparamagnetic with some interactions among the particles.

With respect to the preparation of amine capped magnetite nanoparticles, the amine plays an important role in the reaction process. DDA did not dissolve in water at room temperature, but the reaction proceeded immediately after 35°C was reached, affording a highly dispersive liquid of Fe_3O_4 nanoparticles in a short time. On the other hand, the DDA molecules did not react with $FeCl_2$ at 100 °C, and they phase separated as an oily layer. This suggests that the intermediate Fe (II)–amine complex is stable in the aqueous condition and its reactivity strongly depends on the nature of the amine, indicating that the iron oxide nanoparticles are produced

via the Fe (II)-amine complex with the first step and the second step is its thermal decomposition. Thus, thermal decomposition of the Fe(II) - amine complex formed in water was so rapid that the Fe_3O_4 nanoparticles were subsequently strongly protected by the multilayers of amine molecule to avoid aggregation of nanoparticles. In practice, the particle size was influenced by the reaction time. Therefore, particle size is determined by thermal decomposition of the Fe(II)- amine complex formed in aqueous condition and is affected by changing the amine concentration. A similar reaction was reported for the preparation of iron oxide nanoparticles by the reaction of Fe-acetylacetonate ($Fe(acac)_3$) and 2-pyrrolidone at high temperature[14].

The effect new nanoparticles have on the magnetic relaxation of water protons in tissues (relaxivity) is an important property for use as MRI contrast agents. The transverse relaxivity (r_2, defined as the slope of $1/T_2$ vs. mM Fe) was determined for the DDA-Fe_3O_4 nanoparticles. Transverse relaxation times (T_2) were measured at 60 MHz and 37 °C using both Carr-Purcell-Meiboom-Gill (CPMG) and spin-echo pulse sequences. The r_2 values for the reaction conditions measured are shown in Table 1. During the relaxivity experiment, the T_2 relaxation times at higher iron concentrations were stable over time at 37 °C, while at lower concentrations the relaxation times became longer over time. The relaxivity values reveal no apparent trend in regards to reaction time or Fe/amine molar ratio, but further investigation is needed. The relaxivity values for all the nanoparticles remain constant within the error range.

The colloid stability and biocompatibility of nanoparticles is of great importance. In order to investigate the effects of buffer, pH, and ionic strength on the DDA-Fe_3O_4 nanoparticles, we exposed them to buffered solutions of phosphate, citrate, acetate, MOPS, HEPES, and Tris. The buffered solutions were in the pH range of 5–8 and ion strength was adjusted using 0–500 mM NaCl. While the DDA-Fe_3O_4 particles showed excellent stability in pure water, the stability in buffered solutions was surprisingly low. DDA-Fe_3O_4 particles synthesized with 3 h reaction times, while the reaction is incomplete (reaction1A , 1:1 ratio and reaction2A, 1:4 ratio) were tested in Tris pH7 and pH 8, however completely precipitated within 18 h. DDA-Fe_3O_4 particles from reaction2B (1:4 ratio, 12 h reaction time) were found to be stable in sodium acetate pH 5, Tris pH 7, and Tris pH 8 for at least 3 weeks, while in other buffered conditions the nanoparticles either immediately precipitated upon addition or completely precipitated within several hours. Particles from reaction1B (1:1 ratio, 12 h reaction time) were stable in Tris pH 7 and Tris pH 8 for 8 days before minimal precipitation began to occur. When particles are cleaned by both filtration and dialysis, this leads to particles that were stable for longer time (18 h) in buffered solutions.

Hydrodynamic size of the colloidal suspension was obtained using dynamic light scattering (DLS) and shown in Table 1. These data corroborate TEM measurements showing that reaction1 (3 h) yields nanoparticles that are an agglomerated mixture of rod-like and spherical particles. The other three conditions yield nanoparticles that are spherical and more monodispersed. For comparison, dextran-coated crosslinked iron oxide nanoparticles were investigated. The r_2 for the dextran-coated particles was found to be ~113 $mM^{-1}s^{-1}$ and the hydrodynamic size was ~25 nm. When particles are more uniform in size (reaction 1B and 1C), the DDA-Fe_3O_4 particles have a relaxivity ~2 times higher than the dextran-coated particles, but have similar hydrodynamic sizes.

In conclusion, the synthesis reported here is a novel reaction, affording uniform magnetite nanoparticles capped by amine ligands from a single precursor $FeCl_2$ which renders amine-capped water-dispersible particles. The results indicate that higher DDA concentrations and longer reaction times lead to more stable, monodisperse nanoparticles with high relaxivity for use in biomedical applications. We are currently investigating these particles with DNA linkages, cell viability as amine is a "backbone functional group" in biological molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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15. A quantity of 100 ml of 10 mM concentrated aqueous solution of ferrous chloride (FeCl2.4H$_2$O) was reduced by 40 mM of dodecyl amine under mild heating at 85 °C to yield stable iron oxide nanoparticles in water. While the mixture turns black immediately after mixing indicating nanoparticles are produced by the room temperature reaction of amine with FeCl$_2$, we have observed that the rate of reduction of Fe^{2+} ions by amine is considerably enhanced at around boiling point of water and consequently, all nanoparticle syntheses were carried out at this temperature. The amine-reduced iron oxide nanoparticle solution was subjected to magnetic separation and the resulting aggregate was washed with copious amounts of acetone and deionized water to remove any uncoordinated amine molecules. The aggregate was then redispersed in deionized water for further studies.

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Table 1

Relaxivity and Hydrodynamic Size of DDA-Fe$_3$O$_4$ Nanoparticles

Reaction Conditions	CPMG r$_2$ (mM^{-1}s^{-1})	Correlation Coefficient	Spin-echo r$_2$ (mM^{-1}s^{-1})	Correlation Coefficient	Hydrodynamic Size (nm)
Fe/amine molar ration 1:1 3 h reaction time	177	0.9946	173	0.9942	1000
Fe/amine molar ratio 1:1 12 h reaction time	87	0.9975	80	0.9954	29
Fe/amine molar ratio 1:4 3 h reaction time	213	0.9937	212	0.9964	33
Fe/amine molar ratio 1:4 12 h reaction time	141	0.9995	137	0.9982	31
Fe/amine molar ratio 1:7 12 h reaction time	233	0.9997	232	0.9994	39