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Self-limiting Deposition of *N*-(3-Trimethoxysilylpropyl)ethylenediaminetriacetic Acid for the Production of Aqueous-stable Iron Oxide Nanoparticles

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This paper presents a novel self-limiting deposition of a carboxysilane functional group to the surface of maghemite iron oxide nanoparticles (IOP). Self-limited deposition of *N*-(3-trimethoxysilylpropyl)ethylenediaminetriacetic acid on iron oxide is achieved by rapid injection of the basic silane into acid-stabilized iron oxide. The instant pH shift from 2.3 to 11 through neutral promotes silane deposition on the iron oxide surface but is rapid enough to prevent aggregation and free condensation. Carboxysilane iron oxide particles (CSIOP) are rendered aqueous stable in all pH >5.95. Silane coating is characterized by FTIR, TEM, and DLS.

Over the past several years, iron oxide nanoparticles (Fe_2O_3 and Fe_3O_4) have taken a central role in the field of biological imaging.[1,2] Requirements of such particles include biocompatibility, aqueous stability, superparamagnetism, and a host of flexible properties such as specific targeting, drug delivery, and immune evasion. For the superparamagnetic domain of iron oxide ($< \approx 20$ nm), the most salient of these properties is aqueous stability. Methods of production of iron oxide nanoparticles do not readily lead to hydrophilic surfaces as organic phase methods (thermal decomposition and microemulsion) require hydrophobic capping ligands and aqueous methods (alkaline coprecipitation and hydrothermal synthesis) result in bare iron oxide surfaces (isoelectric point, pH 7) that aggregate at physiological pH. The principle means of rendering hydrophilic surfaces include lengthy ligand exchange reactions (in excess of 72 h) in the case of organic synthesis, or various coatings of small molecules, polymers, and biological molecules such as proteins, metals, or silica.[1,3–5]

This paper presents a novel, rapid, and self-limiting method of producing aqueous-stable iron oxide nanoparticles employing *N*-(3-trimethoxysilylpropyl)ethylenediaminetriacetic acid as a capping ligand in a single instantaneous step. The advantages of this ligand include: covalent binding to iron oxide, biocompatibility, and simple bioconjugation through standard carbodiimide chemistry. The aqueous process employs a rapid pH shift to limit the window of silane deposition resulting in extremely thin silica coatings and highly stable particles.

Fe_2O_3 iron oxide nanoparticles (IOP) are produced (Figure 1) by standard alkaline coprecipitation followed by oxidation to Fe_2O_3 in the presence of iron(III) nitrate and nitric acid.[5] Briefly 86 mM $FeCl_3$ and 43 mM $FeCl_2$ are prepared in 25-mL deionized water to which 2.5-mL NH_4OH is rapidly injected under ambient conditions. The black precipitate is washed several times and particles are refluxed with magnetic stirring in 0.2 M $Fe(NO_3)_3$ and 0.8 M nitric acid for 1 h. The black particles gradually turned bright orange/brown and precipitated. Particles settled on the magnet and excess nitric acid was removed. Particles were immediately redispersed in water and dialyzed overnight against 10 mM nitric acid, sample was recovered in a final volume of 10-mL nitric acid. The transparent red Fe_2O_3 solution is stable in 10 mM nitric acid pH 2.3 by virtue of the $–OH_2^+$ groups on the surface and particles are monodispersed at 8 ± 2 nm in diameter. X-ray diffraction patterns of the product of this synthetic protocol indicate an intermediate lattice parameter between maghemite and magnetite.[5] 3 mL of this solution were vigorously stirred and 200 µL 45% (w/v) *N*-(3-trimethoxysilylpropyl)ethylenediaminetriacetic acid (Na^+ salt) was rapidly injected. The solution instantly became turbid and reclarified as the pH shifted from 2.3 to 11 and the reaction stopped. The carboxysilane-functionalized iron oxide particles (CSIOP) were magnetically collected, washed in pH 11 NaOH to clear excess silane and dialyzed overnight against water.

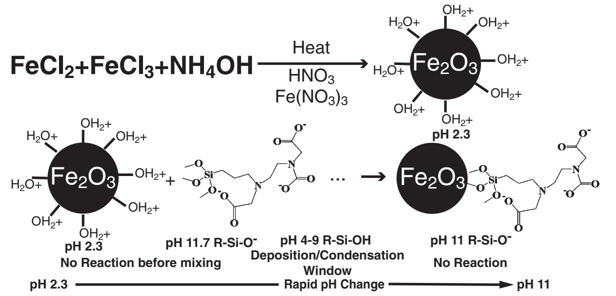

Figure 1. Proposed reaction mechanism of self-limiting deposition of carboxysilane onto iron oxide surface. The rapid pH shift brings particles through their isoelectric point without aggregation and functionalizes the surface to be stable at neutral pH.

Silanes are known to covalently bond to iron oxide via surface –OH groups.[6] The Fourier transform infrared spectrum seen in Figure 2 shows this functional bond. The strong absorbtion near 650 cm^{-1} is characteristic of the Fe–O bond in iron oxide and bands at 3434, 1632, and 1378 cm^{-1} are stretching, deformed vibration, and bending, respectively of the –OH bond. The functional group is seen by the appearance of a band at 1040 cm^{-1}, identifying the Si–OH, Si–O–Si, or Si–O–Fe stretch.[7] It is proposed that the silane functionalization is relatively sparse given the weakness of the peak by FTIR and the fact that TEM cannot resolve a coated layer (Figure 3).

The self-limiting functionalization is a result of the pH shift during synthesis. The stock carboxysilane salt (45% in water) is stabilized in the siliconate form by the high pH of 11.7 resulting from the equilibrium of COO^- groups absorbing H^+ ions. Upon

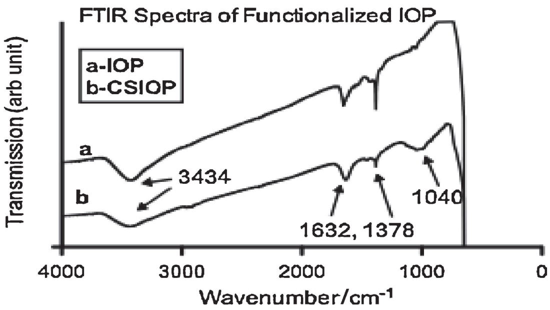

Figure 2. FTIR spectra of IOP and CSIOP.

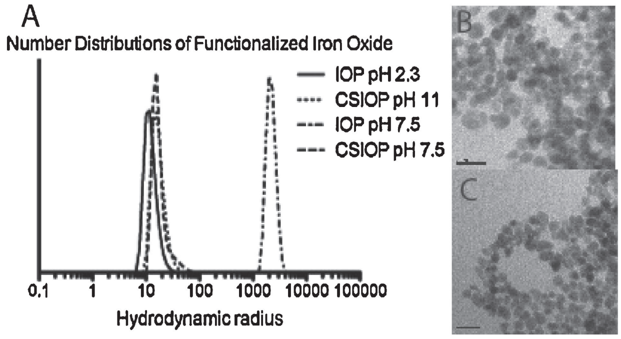

Figure 3. Dynamic light scattering distributions of IOP before/after silane functionalization and at neutral pH (10 mM Tris-Cl) (A), TEM images of IOP (B) and CSIOP (C) showing no morphological differences, scale bar is 20 nm.

addition to iron oxide at pH 2.3 (stabilized in 10 mM nitric acid by a strong positive ζ-potential resulting from OH_2^+ surface groups), the siliconate groups become protonated to silanols and the basic solution drives the pH up initiating silanol condensation at the positively charged iron oxide surface.[8] The simultaneous addition of COO^- groups to the iron oxide surface, coupled to the solution pH rapidly shifting to 11 quickly stops both surface and solution based silanol condensation and excess siliconates are restabilized at high pH. The reaction is so rapid that iron oxide particles are taken through neutral ζ-potential without aggregation and free silica condensation is prevented, resulting in a transparent solution with no increase in total absorbance at 600 nm (data not shown), indicating no new particle formation or substantial growth. The extreme pH shift is essential as smaller volumes of injected basic silane resulted in more neutral equilibrium pH and aggregated particles. The resulting silica deposition is very thin (presumably a monolayer), and cannot be resolved by TEM images of bare and silane-functionalized particles (Figure 3). Vibrating sample magnetometry (300 K) indicate that both IOP and CSIOP are superparamagnetic with respective saturation magnetizations at 65.1 and 48.2 emu g^{-1}. Dynamic light scattering measurements indicate a net increase in hydrodynamic radius of 4.7 nm, which may be attributed to a thicker solvation layer that accompanies a higher charge density (3, $-COO^-$ replaces 1, $-OH_2^+$) and the different solution conditions. The surface charge conferred to the iron oxide by the tricarboxylic acid is sufficient to grant the nanoparticles stability at all pH >5.95 in water for at least 14 days (Figure 4) and in Tris-Cl buffer, in contrast to bare Fe_2O_3 which aggregate in Tris-Cl at pH 7.5 (Figure 3). Observations have seen stability for several months on the bench (data not shown). At pH <5.95, the particles quickly aggregate in agreement with a pK_a ranging up to ≈5 for carboxyl groups. Particles are stable in phosphate buffered saline (PBS) for days but aggregate and precipitate in time (Figure 4). Increased "absorbance" is in reality increased scattering as particles aggregate and grow in size, with the subsequent decrease resulting from their precipitation. The stability of these carboxysilane-functionalized iron oxide particles can be compared to reports on stability of iron oxide with other aqueous silane functional groups, the most common being (3-aminopropyl)triethoxysilane which have had reports using similar functionalization methods of stability over only 26 h.[9] It is of note that carboxysilanes used in ligand-exchange reactions of organic synthesized iron oxide have demonstrated long-term stability, but this reaction takes substantially more time and involves organic solvents that must be disposed of such as toluene or hexanes.[4]

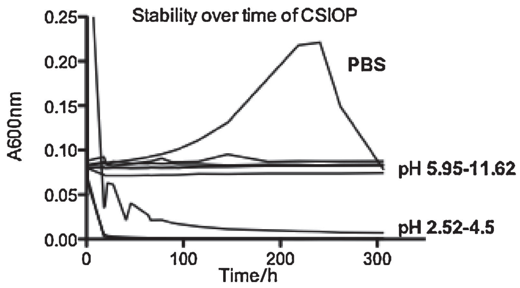

Figure 4. Absorption at 600 nm over time from CSIOP in different pH showing stability over pH 5.95–11.62.

In conclusion, we have demonstrated the production of carboxysilane-functionalized iron oxide nanoparticles measuring 8 ± 2 nm in diameter in a simple and rapid manner that possess long-term colloidal stability under a wide variety of pH. The coating layer is extremely thin and possesses functional groups for easy functionalization. The ease and speed of preparation, stability, and chemical flexibility of these particles make them ideal candidates for a variety of biological applications.

References and Notes

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1 V. I. Shubayev, T. R. Pisanic, II, S. Jin, *Adv. Drug Delivery Rev.* **2009**, *61*, 467.

2 M. Mahmoudi, S. Sant, B. Wang, S. Laurent, T. Sen, *Adv. Drug Delivery Rev.* **2011**, *63*, 24.

3 W. Wu, Q. G. He, C. Z. Jiang, *Nanoscale Res. Lett.* **2008**, *3*, 397.

4 R. De Palma, S. Peeters, M. J. Van Bael, H. Van den Rul, K. Bonroy, W. Laureyn, J. Mullens, G. Borghs, G. Maes, *Chem. Mater.* **2007**, *19*, 1821.

5 M. Chastellain, A. Petri, H. Hofmann, *J. Colloid Interface Sci.* **2004**, *278*, 353.

6 X. Huang, A. Schmucker, J. Dyke, S. M. Hall, J. Retrum, B. Stein, N. Remmes, D. V. Baxter, B. Dragnea, L. M. Bronstein, *J. Mater. Chem.* **2009**, *19*, 4231.

7 I. J. Bruce, T. Sen, *Langmuir* **2005**, *21*, 7029.

8 R. C. Merrill, R. W. Spencer, *J. Phys. Chem.* **1950**, *54*, 806.

9 X.-C. Shen, X.-Z. Fang, Y.-H. Zhou, H. Liang, *Chem. Lett.* **2004**, *33*, 1468.