Synthesis of Ultrasmall Superparamagnetic Iron Oxide Nanoparticles for fMRI

Colin M. Wilson

Imaging Probe Development Center, National Heart, Lung, and Blood Institute, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, ipdc@nhlbi.nih.gov

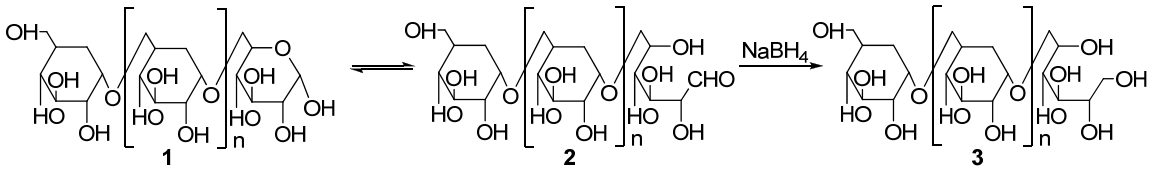
Background

The macaque monkey is an important model for studying information processing in the human visual system. In order to bridge the information gap that exists between human neuro-imaging and macaque physiology and anatomy, functional magnetic resonance imaging (fMRI) techniques have been developed using commonly available 1.5 Tesla scanners to map activity in the visual cortex of the awake macaque. Iron oxide contrast agents with long blood half-lives have been shown to increase the functional sensitivity of fMRI ~5fold compared with blood oxygen level-dependent (BOLD) technique. These agents are injected intravenously prior to scanning, which increases the T1 contrast and allows blood volume signals to be isolated. The increased signal-to-noise ratios so derived effectively reduce scanning time while revealing improved maps of activity.[1] Ultrasmall superparamagneitc iron oxide nanoparticles (USPIO-NP) were synthesized for this purpose.

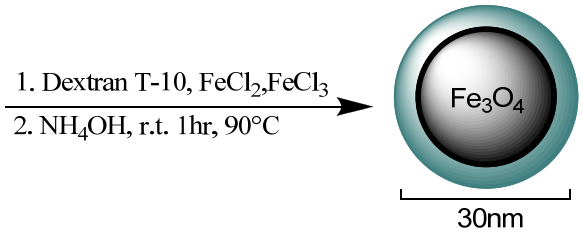
Chemistry

Dextran-coated USPIO-NP were prepared using the co-precipitation method, based on known synthetic procedures with minor modifications.[2-4] Briefly, dextran T10 (**1**, **2**) was reduced with sodium borohydride, diafiltered and lyophilized to afford the linear polyol form of the polysaccharide (**3**) in a 86% yield. The reduced dextran T10 (**3**) was reconstituted in water and combined with ferrous chloride tetrahydrate and ferric chloride hexahydrate in a molar ratio of 1: 13.6: 27.1(polysaccharide: $FeCl_2{\cdot}4H_2O$:$FeCl_3{\cdot}6H_2O$) under argon atmosphere. Ammonium hydroxide was added to precipitate the iron particles, which were heated before being purified by ultracentrifugation and tangential flow filtration (TFF).

Scheme 1. Reduction of dextran T10.



Scheme 2. Synthesis of reduced dextran coated UPSIO-NP.



Experimental

General. Iron(II) chloride tetrahydrate and Iron(III) chloride hexahydrate were purchased from Sigma Aldrich (St. Louis, Missouri). Dextran T10 was purchased from Pharmacosmos A/S (Holbaek, Denmark) and used without any additional purification. Size exclusion-high performance liquid chromatography (SE-HPLC) analysis was performed on an Agilent 1200 series instrument equipped with a refractive index detector and a Waters Ultrahydrogel 250 column. The mobile phase was deionized water and the flow rate was 0.4mL/min. Diafiltration was performed on a Millipore labscale TFF system. Dynamic light scattering (DLS) hydrodynamic size characterization was completed using a Malvern Zetasizer ZS (Nanocomposix). Transmission electron microscopy (TEM) analysis was performed on a JEOL 1010 running at 100 kV with an AMT XR41B (2k x 2k resolution) camera (Nanocomposix). Inductively Coupled Plasma optical emission spectrometry (ICP-OES) analysis of iron content was performed on a Thermo Jarrell Ash ICAP 61 at a wavelength of 260 nm (Columbia Analytical Services).

Reduced Dextran T10. Dextran T10 (10 g) was dissolved in 100 mL of water, 1 g of sodium borohydride was added and the mixture was stirred for 12 h at room temperature. The solution

was adjusted to pH 7.0 using 4 M HCl and diafiltered using a 5-kDa NMWL Pellicon XL50 ultrafiltration membrane (PXC005C50, Millipore). The solution was then passed through a 0.22µm syringe filter (Millex-GS, Millipore) and lyophilized to afford a white solid in 86% yield. Following TFF purification, a small decrease in retention time for the reduced dextran (19.2 min) was observed relative to the starting native dextran (19.7 min). This was consistent with previous observations.[4]

Reduced Dextran T10-Coated Iron Oxide Particles. Reduced dextran T10 (8.07 g) was dissolved in 200 mL of deionized water. The solution was cooled to 4°C on ice and degassed with argon for 1 h. Ferrous chloride tetrahydrate (2.19 g) and ferric chloride hexahydrate (5.91 g) were each dissolved in 12 mL of degassed dH_2O and kept on ice for approximately 15 min. The two iron solutions were added to the dextran solution and allowed to stir for several minutes before 15 mL of ammonium hydroxide was slowly added. The resulting black viscous solution was heated to 90°C for 1h and allowed to cool overnight. Large pellets were removed by ultracentrifugation at 20 k relative centrifugal force (rcf) for 30 min. The supernatant was exhaustively diafiltered against deionized water using a 100-kDa NMWL Pellicon XL50 ultrafiltration membrane (PXB100C50, Millipore) and exchanged into 0.02 M citrate, 0.15 M sodium chloride buffer, pH 8.3. The particles were concentrated to 1.36 wt% Fe, passed through a 0.22µm syringe filter (Millex-GS, Millipore) and stored at 4°C.

TEM and DLS were used to determine the size of the iron core (Figure 1) and hydrodynamic diameter (Figure 2), respectively (Nanocomposix). The core size was 4.37 ± 0.59 nm and the Z-average hydrodynamic diameter was 30.29 nm with a polydispersity index of 0.139.

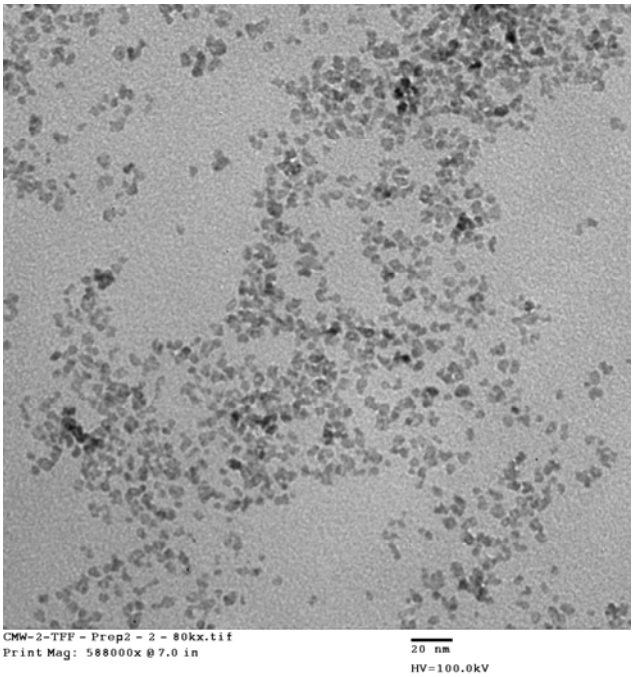


Figure 1. TEM image of USPIO-NP

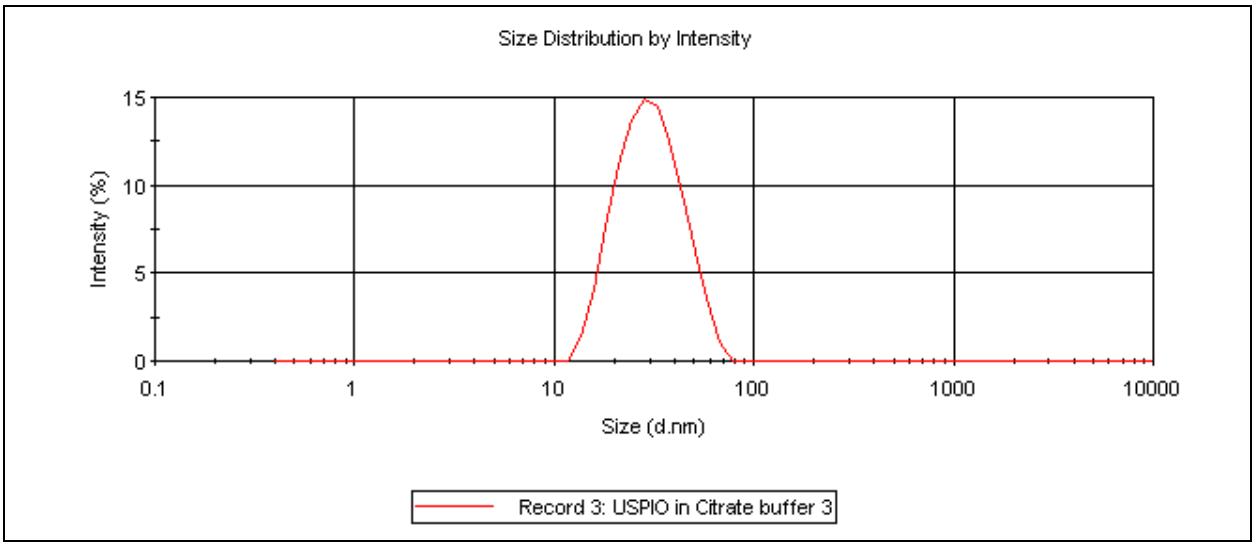


Figure 2. DLS size distribution by intensity of USPIO-NP

References

1. Vanduffel W, Fize D, Mandeville JB, Nelissen K, Van Hecke P, Rosen BR, Tootell RB and Orban GA. Visual motion processing investigated using contrast agent-enhanced fMRI in awake behaving monkeys. Neuron. 2001 Nov 20;32(4): 565-77. PMID 11719199

2. Thorek DL and Tsourkas A. Size, charge and concentration dependent uptake of iron oxide particles by non-phagocytic cells. Biomaterials. 2008 Sep;29(26): 3583-90. PMID 18533252

3. Jarrett BR, Frendo M, Vogan J and Louie AY. Size-controlled synthesis of dextran sulfate coated iron oxide nanoparticles for magnetic resonance imaging. Nanotechnology. 2007 Jan 24;18(3): 035603

4. Paul KG, Frigo TB, Groman JY and Groman EV. Synthesis of ultrasmall superparamagnetic iron oxides using reduced polysaccharides. Bioconjug Chem. 2004 Mar-Apr;15(2): 394-401. PMID 15025537