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13. ABSTRACT (Maximum 200 Words) We have developed a productive alliance with Drs. Kaminski and Mertz from the Argonne National Laboratories and with Dr. Rosengart from the University of Chicago, who have designed a unique, portable, blood detoxification device with the goal of "magnetically filtering" undesirable components from the blood stream. Our group has supported their DARPA-AFOSR project entitled Biohazard Detoxification Method Utilizing Magnetic Particles by providing materials and materials' guidance. Our focus during the current year has been on achieving biodegradable nanospheres in the 100-500 nm diameter size range which contain high concentrations of magnetite. The current status of this work is that we have poly(D,L-lactide) nanospheres which contain 40 weight percent magnetite. The average nanosphere diameter is approximately 80-90 nm. We have incorporated one weight percent of a 2k-20k Mn poly(ethylene oxide-b-D,L-lactide) block copolymer into the nanospheres in efforts to achieve hydrophilic surfaces. The current focus is on (1) learning how to increase the nanosphere size slightly to achieve 100-500 nm particles, (2) achieving even higher concentrations of magnetite, and (3) understanding how to achieve surfaces sufficiently covered with poly(ethylene oxide) with this nanosphere process to avoid immune response. We will collaborate with Kaminski/Mertz/Rosengart to relate nanosphere composition and processing to immune response.				
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**Toward High Magnetic Moment, Blood Dispersible Nanoparticles: An
Enabling Technology for Biomagnetic Interfacing Concepts**

Final Report submitted to
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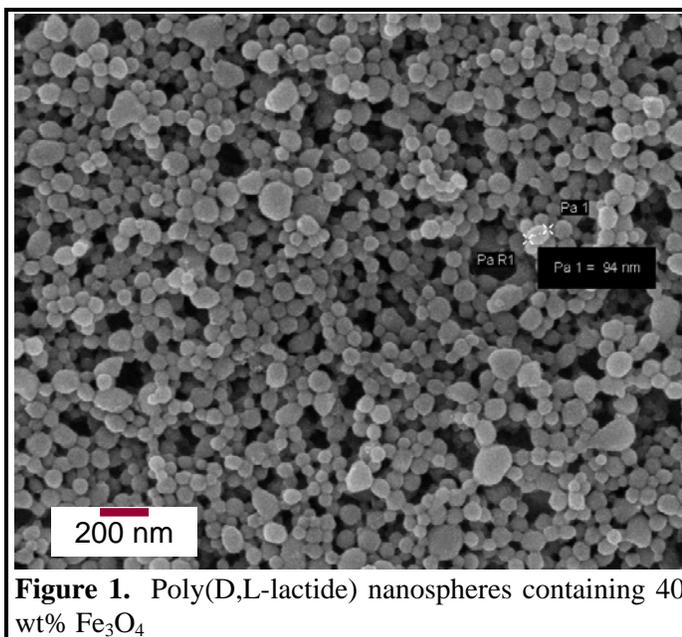
In collaboration with
Dr. Michael Kaminski and Dr. Carol Mertz
Argonne National Laboratories
and
Dr. Axel Rosengart
The University of Chicago

March 2, 2006

This project addresses preparative methods for fine dispersions of magnetite nanoparticles in biocompatible/biodegradable polymeric matrices, and the fabrication of controlled size nanospheres of such materials. We have developed a productive alliance with Drs. Kaminski and Mertz from the Argonne National Laboratories and with Dr. Rosengart from the University of Chicago, who have designed a unique, portable, blood detoxification device with the goal of “magnetically filtering” undesirable components from the blood stream. Our group has supported their DARPA-AFOSR project entitled *Biohazard Detoxification Method Utilizing Magnetic Particles* by providing materials and materials’ guidance. Our focus in the current year is on achieving biodegradable nanospheres in the 100-500 nm diameter size range which contain high concentrations of magnetite.

Synopsis

The status of this work is that we have developed a method for preparing poly(D,L-lactide) nanospheres which contain 40 weight percent magnetite (figure 1). The average nanosphere diameter is approximately 80-90 nm. We have incorporated one weight percent of a 2000-20,000 M_n poly(ethylene oxide-*b*-D,L-lactide) surface modifying copolymer into the nanospheres to achieve hydrophilic surfaces, but we have not yet characterized those surfaces well. The current focus is on 1) learning how to increase the nanosphere size slightly to achieve 100-500 nm particles, 2) incorporating even higher concentrations of magnetite, and 3) understanding how to achieve surfaces sufficiently covered with poly(ethylene oxide) using this particular nanosphere fabrication process to avoid immune response. We will collaborate with Kaminski/Mertz/Rosengart to relate nanosphere composition, processing parameters, and surface structure to interactions with blood.



Synthesis

Obtaining the desired nanospheres requires several synthetic processes.

- Synthesis of a biocompatible and biodegradable polymer that has carboxylate functional groups for strong binding to magnetite particle surfaces (completed)
- Synthesis of a block copolymer surface modifier for the nanospheres (completed)
- Synthesis of magnetite nanoparticles and adsorption of the biodegradable polymer onto the magnetite nanoparticle surfaces to form a polymer-magnetite complex (current research)
- Fabrication of the polymer-magnetite complex into larger nanospheres (100-500 nm diameter) with surface modification (i.e., poly(ethylene oxide) surfaces) (current research)

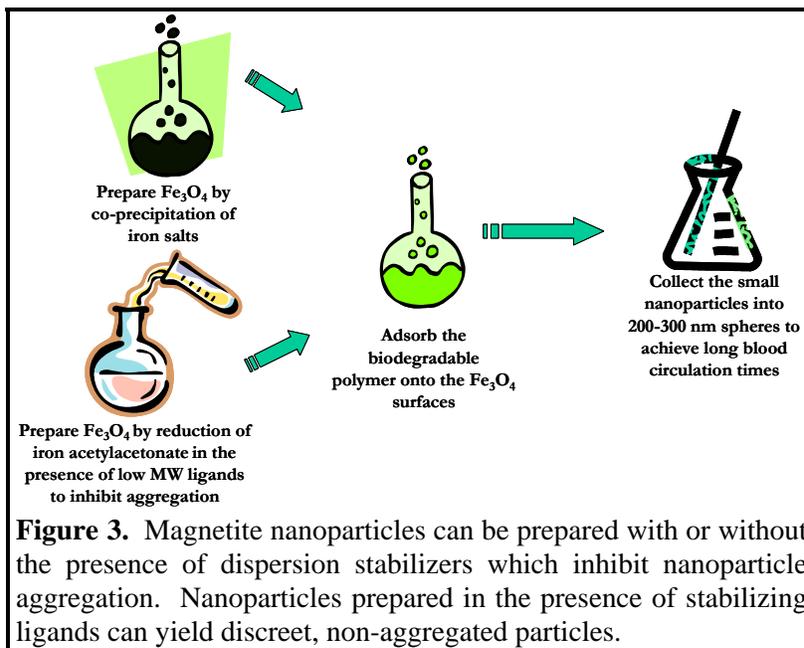
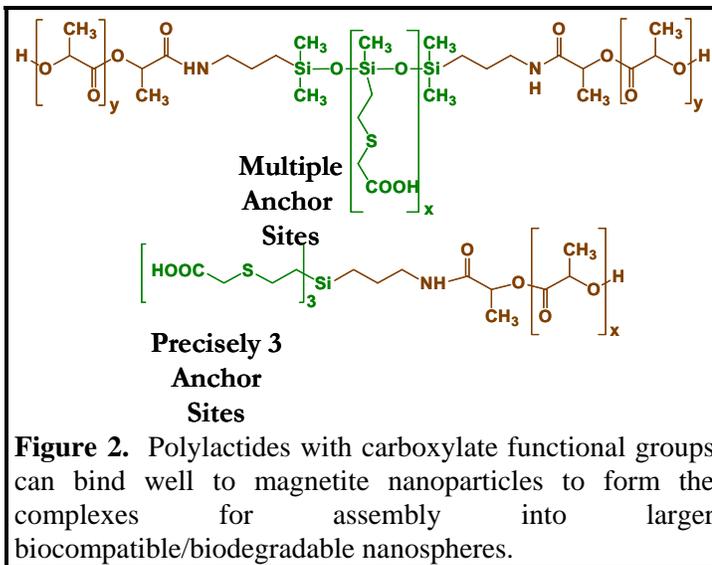
We have established methods for synthesizing two types of poly(D,L-lactide)s that contain carboxylate groups for binding to magnetite (figure 2). Poly(D,L-lactide) is well known to be biocompatible and biodegradable. Extension of this technology to produce similar poly(lactide-*co*-glycolide)s with carboxylate functionality, so that biodegradation rate can be tailored, should be straightforward.

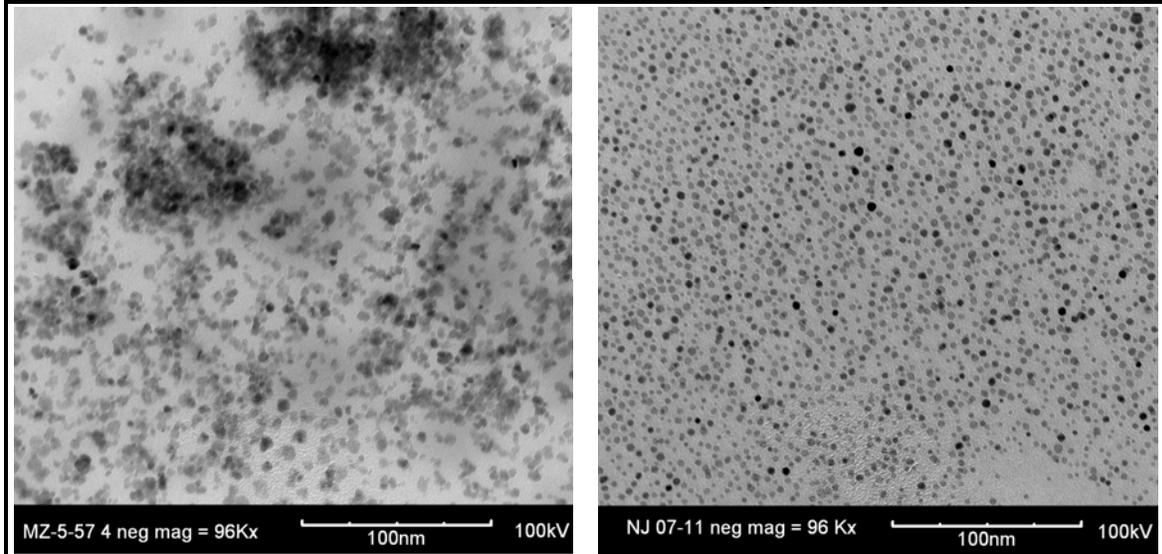
We have explored two primary methods for synthesizing small magnetite nanoparticles: 1) Reaction of stoichiometric (1:2) FeCl_2 and FeCl_3 with hydroxide in the absence of any ligands to inhibit aggregation,¹ and 2) Reductive thermolysis of $\text{Fe}(\text{acac})_3$ at 200-300 °C in the presence of ligands to inhibit aggregation (figure 3).²⁻⁴ It is quite clear that the non-aqueous thermolysis process of the organometallic ferric acetylacetonate can yield better dispersions of magnetite (figure 4). The details of how magnetite nanocrystals are formed in the thermolysis processes, however, are not yet clear and will be pursued.

Following the method of Sun et al.,³ ferric acetylacetonate was heated at 200-300 °C in the presence of hexadecanediol, oleylamine and oleic acid to produce fine magnetite nanoparticles. The hydrophobic nanoparticles with some bound oleyl components could be well dispersed in the poly(D,L-lactide) copolymer and the copolymer was adsorbed onto the nanoparticle surfaces at 50 °C. Dynamic light scattering of chloroform dispersions of the poly(D,L-lactide) coated magnetite nanoparticles indicates that the magnetite is coated with the polymer (figure 5). We have incorporated up to 40 weight

percent magnetite in these polymer-magnetite complexes, and we plan to optimize this process further to determine the upper limit of magnetite concentration. Following the method of Li et al., we are also pursuing the development of water dispersible magnetite by heating ferric acetylacetonate at 250 °C in the presence of pyrrolidone.⁴ Since the pyrrolidone is a small water-soluble ligand, it may be more easily displaceable than the oleic acid/amine components.

SQUID magnetic susceptometry measurements indicate that the magnetic properties are sensitive to the temperature schedule during thermolysis. Specific saturation magnetization of the magnetite in this early work varies from about 60-80 emu/g of magnetite in these complexes, and the values are dependent on the thermolysis times (figure 6). Longer heating times at these elevated temperatures appear to elicit some sintering, but TEM photomicrographs suggest that this sintering may be relatively controllable. Larger nanoparticles are produced with longer heating times, and it seems reasonable that the larger sizes may be related to higher saturation magnetizations.

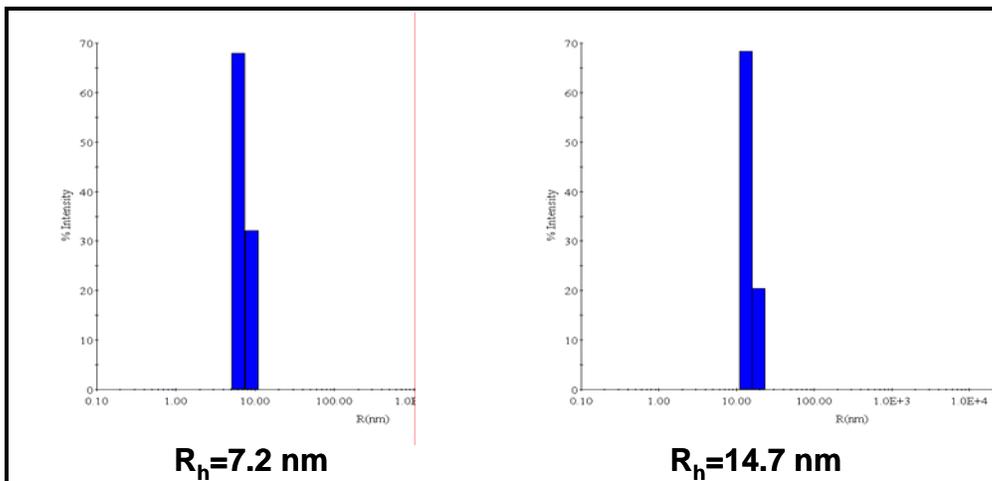




FeCl₂/FeCl₃ method

Fe(acac)₃ method

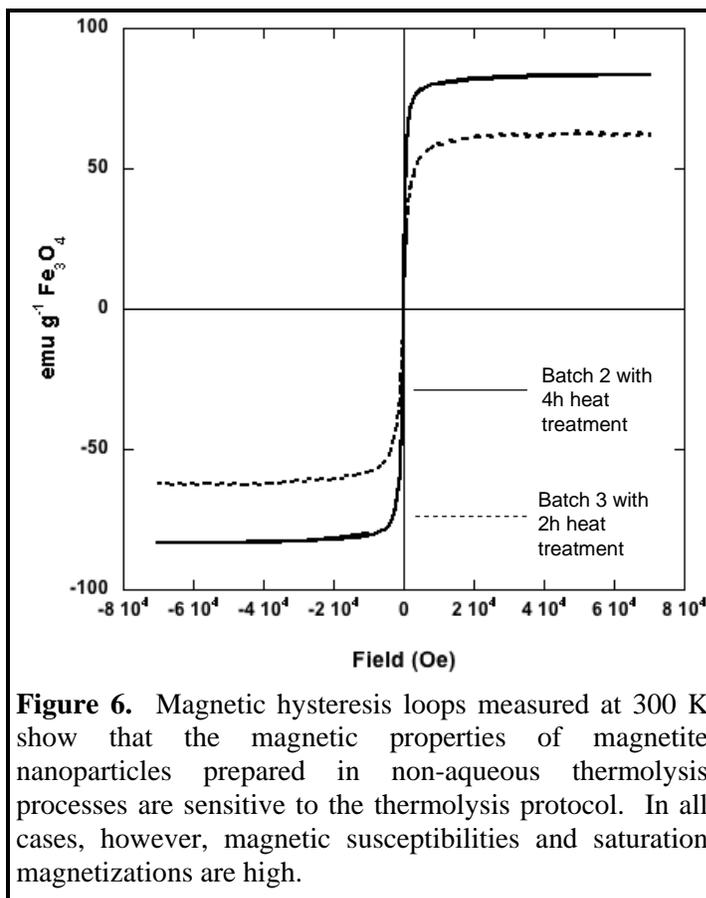
Figure 4. Comparison of the state of dispersion of magnetite nanoparticles dispersed in a 20,000 M_n poly(D,L-lactide) when the magnetite is prepared by different methods. The TEM samples were prepared by placing one drop of a dilute chloroform dispersion of each complex onto carbon paper-coated copper grids, and air-drying the materials. Significantly improved dispersions can be obtained by reduction of iron acetylacetonate in the presence of ligand to inhibit aggregation. This enables high concentrations of magnetite to be incorporated into the PDLA complexes. We have attained 40 wt % magnetite and it appears that this process will allow us to increase this further.



R_h=7.2 nm

R_h=14.7 nm

Figure 5. Comparison of hydrodynamic radii of (left) magnetite nanoparticles prepared by reduction of ferric acetylacetonate prepared by the method of Sun et al. in chloroform (the nanoparticles are coated with some oleyl components from the thermolysis procedure), and (right) the magnetite nanoparticles after adsorption of a 20,000 g/mole poly(D,L-lactide) having carboxylate binding groups. Note the increase in hydrodynamic size after coating the magnetite with the biodegradable polymer.



Once the magnetite-poly lactide complexes have been formed, these materials are assembled into larger nanospheres.⁵⁻⁶ Two methods have been investigated for obtaining controlled size nanospheres in the 100-500 nm diameter range. One process, modified from Bilati et al.,⁵ involves dissolving the magnetite-polymer complex, and optionally adding a relatively low concentration of a surface modifying block copolymer. The blended dispersion is then syringed into a rapidly stirring (~9000 rpm) non-solvent. It is important that the two solvents are miscible so that the solvent containing the complex can be rapidly extracted upon addition into the non-solvent. When a complex containing 40 wt% magnetite and 1 wt% of a 2000-20,000 g/mole PEO-PDLA surface modifier was dissolved in DMSO, then precipitated into a 75/25 v/v methanol/water mixture, the nanospheres depicted in figure 1 resulted. The alternative method of Gref et al.⁶ involves dissolving the complex in dichloromethane, then precipitating into water containing a poly(vinyl alcohol-co-vinyl acetate) dispersant. We are presently investigating methods to really control nanosphere size by both processes.

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