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14. ABSTRACT The investigation on the interaction of superparamagnetic nanoparticles with cell membranes was divided into three phases: the synthesis and characterization of the magnetic and structural properties, surface functionalization of the particles, and ferrofluid studies of the particles in the presence of a slowly varying magnetic field. Two methods were explored to synthesize the superparamagnetic nanoparticles used in this investigation to achieve a distribution in particle size. Chemical synthesis and microwave plasma spray, a proprietary technique developed by Materials Modification Incorporated (MMI). Chemical synthesis yielded the most uniform particle size distribution in the 4 - 14 nm range, while plasma spray was more successful in synthesizing particles in the 14 - 40 nm range. Most of the discussions in this report will be centered on the chemical synthesis process. The magnetic and fluid studies on the nanoparticles showed excellent superparamagnetic behavior. The surface functionalization phase focused on binding bipolar organic compounds to nanoparticles to make them hydrophilic as a precursor to cell membrane interaction studies. Theoretical techniques were developed to analyze the the magnetization results will be presented in thesis report.					
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Investigation of the Interaction of Superparamagnetic Nanoparticles with Cell Membranes

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The investigation on the interaction of superparamagnetic nanoparticles with cell membranes was divided into three phases: the synthesis and characterization of the magnetic and structural properties, surface functionalization of the particles, and ferrofluid studies of the particles in the presence of a slowly varying magnetic field. Two methods were explored to synthesize the superparamagnetic nanoparticles used in this investigation to achieve a distribution in particle size. Chemical synthesis and microwave plasma spray, a proprietary technique developed by Materials Modification Incorporated (MMI). Chemical synthesis yielded the most uniform particle size distribution in the 4 - 14 nm range, while plasma spray was more successful in synthesizing particles in the 14 - 40 nm range. Most of the discussions in this report will be centered on the chemical synthesis process. The magnetic and fluid studies on the nanoparticles showed excellent superparamagnetic behavior. The surface functionalization phase focused on binding bipolar organic compounds to nanoparticles to make them hydrophilic as a precursor to cell membrane interaction studies. Theoretical techniques were attempted to determine the magnetization of the individual particles, however, those calculations are incomplete and will be discussed in the completed thesis.

Synthesis:

The nanoparticles in this study were synthesized using a modified co-precipitation method developed by Shouheng Sun et al. at IBM¹. The apparatus developed to synthesize the particles using this method is shown schematically in Figure 1. This apparatus consist of a round bottom 250 ml attached to a water-cooled condenser tube. The condenser is capped with a barbed tube adapter connected to a bubbling and argon/nitrogen supply. The process is controlled using a stirrer/heater, thermometer, and silicone oil bath to ensure temperature uniformity throughout the process. An appropriate size-heating mantle for round bottom flask with voltage modulator was used for refluxing. The magnetic particles were precipitated out of solution using a K&J Magnetics neodymium iron boron permanent magnet-2 inches in diameter and 1-inch thick.

In the Shouheng Sun et al. method, oxygen from long chain diols bond with metal acetylacetonate in a two-step process in the presence of oleic acid and oleylamine to produce ferrite nanoparticles. The advantages of this process are that it can be scaled and the particle size distribution is reasonably narrow. The Shouheng Sun et al. method was used to synthesize superparamagnetic magnetite nanoparticles in this study by reacting 6 mmols of iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$) with 30 mmol of 1,2-dodecanediol (1,2-DDD), 18 mmol of oleic acid, and 18 mmol oleylamine in the 80 ml of phenyl ether. The mixture was heated to 200° C for period of thirty minutes in a nitrogen atmosphere. The mixture was refluxed for thirty more minutes yielding a black mixture. Ethanol was added at 2:1 ratio by volume of ether to the mixture that enabled the particles to be precipitated with a neodymium iron boron magnet. After decanting with the magnet held to the flask, the nanoparticles were rinse two more times with ethanol. Finally, the particles were dispersed in hexane using oleic acid and oleylamine as the ligand intermediary. The process yielded ~700 mg of 4 nm nanoparticles. We found that substituting the phenyl ether with benzyl ether could produce larger grains. The high

boiling point of benzyl ether ($\sim 300^{\circ}\text{C}$) allowed the reaction to be conducted at a higher temperature, producing nanoparticles in the 6-7 nm range with similar yields (700 mg); however, the time of the initial step had to be lengthened to two hours and the reflux for one hour. We also discovered that 14 nm particles could be synthesized by using the 6-7 nm particles as seeds and the benzyl ether as a substitution for the phenyl ether.

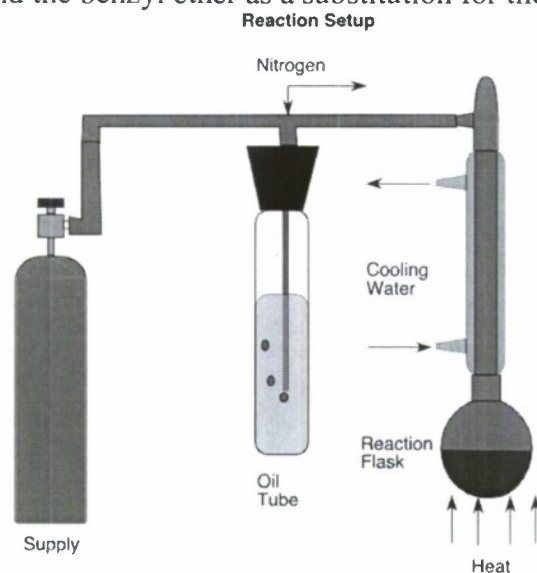


Figure 1. Shows the synthesis apparatus used to precipitate the superparamagnetic nanoparticles

Particle Size Measurements

The particle size and uniformity were characterized using a Hitachi S-5500 Scanning Tunneling Electron Microscope (STEM). For these studies, the nanoparticles were dispersed in a hexane solution and dropped onto carbon coated copper grids supplied by Structure Probe Inc. The nanoparticles were imaged at 30 KV power output in the bright-field mode. The particles appeared as neatly arrayed opaque objects. Afterwards, the diameters were measured with Quartz imaging software provided by Hitachi where the length is calibrated to number of pixels.

Typically, many images are collected from different regions of the grid to generate a large sampling. Particle diameters were measured for the particles and the mean and standard deviations were calculated. The images from these studies are shown in Figures 2a, 2b and 2c for average particle sizes in the 5 nm, 6.7 nm, and 13.3 nm ranges, respectively.

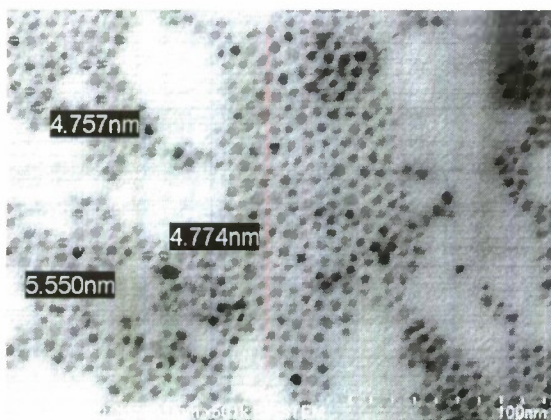


Figure 2a.

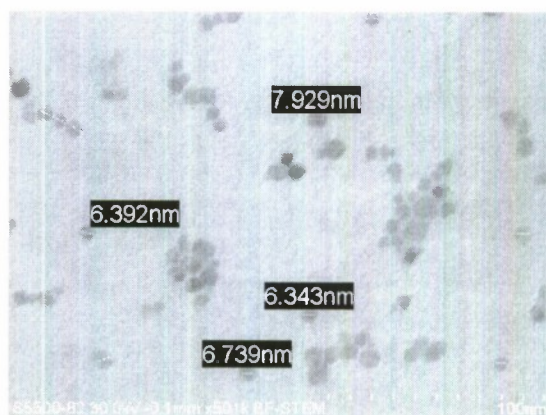


Figure 2b.

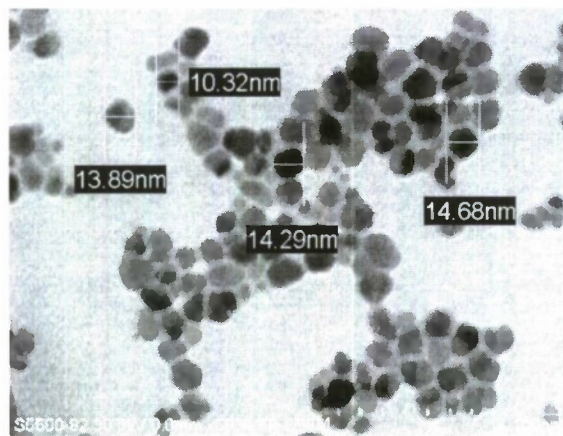


Figure 2c.

Magnetization Studies:

The magnetization studies were performed using an Oxford Scientific Vibrating Sample Magnetometer (VSM). The magnetometer has a sensitivity 10^{-5} emu and can measure the magnetization over a temperature range between 4.2 K and 1000 K in fields up to 9 Tesla with a superconducting magnet.

The samples for the VSM studies were dried to powder form and weighed. The powder was then packed in a gelatin capsule with cotton. Figure 3. shows a typical magnetization curve for 4 nm superparamagnetic magnetite (Fe_3O_4) nanoparticles. The magnetic moment for superparamagnetic particles is zero in zero applied field. The competition between exchange anisotropy and the shape anisotropy of nanoparticle leave the magnetic spins randomly distributed resulting in a net zero magnetic moment. However, as the applied magnetic field (H) is increased the spins align with the field and a magnetic moment is observed.

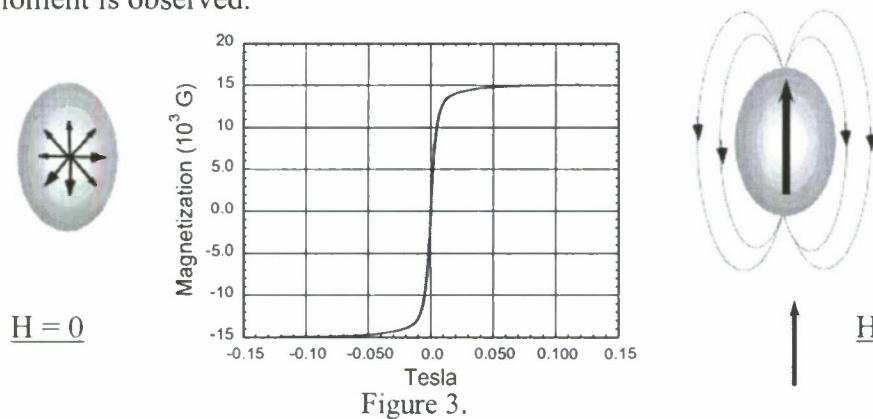


Figure 3.

The maximum particle size for superparamagnetic magnetite is 40 nm. In general, the relationship between the spin distribution and particle size is given by $K_s V = 25 k_B T$, where K_s is the shape anisotropy, V is the volume of the particle, k_B is the Boltzmann constant T is the temperature. The shape anisotropy, $K_s = 1/2(N_{||} - N_{\perp})M^2$ depends on the magnetization M and the difference between perpendicular and parallel demagnetizing factors. The relationship between particle size and spin direction in a demagnetized particle is shown in Figure 4. The spin directions are shown in a closure domain configuration for the larger particle on the left; as the particles become increasingly smaller the spin directions can no longer sustain a closure configuration and aligns in one direction. However, when the particle size reaches the critical dimensions, defined by the

above conditions, for superparamagnetism the spin direction become randomly distributed and no domain structure is observed.

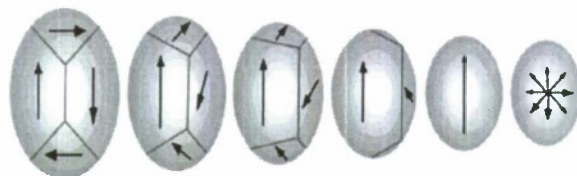


Figure 4. Schematically shows the domain structure of demagnetized single particle as the particles become progressively smaller from left to right.

Functionalization

For pharmaceutical applications, functionalized superparamagnetic nanoparticles are an ideal target delivery vehicle. The functionalization reduces the electrostatic attraction and this coupled with the zero magnetic interaction between superparamagnetic particles in zero field enables the particles to remain suspended in solution until precipitated by the application of a magnetic field at the target site.

In colloids, ferrite nanoparticles require an appropriate ligand to remain in a stable suspension. The ligand is typically an organic compound that contains at least two groups. At one terminal, a group that binds covalently to the nanoparticle surface and the other that interacts with the medium or electrostatically with coatings around other particles⁴. The nanoparticles produce are bound to oleic acid and oleylamine that aid in suspension in only nonpolar solvents. The investigator studied methods to exchange those nonpolar coatings with one that could facilitate stable aqueous suspensions. According to the literature on the subject, dopamine offered the best solution because its hydroxyl groups create stronger bonds to ferrite nanoparticles and the amino group can accept a positive charge creating a shell that could electrostatically repel the particles³. The repulsion force between particles would prevent aggregation.

The investigator tested three methods to create aqueous colloidal suspension. An original method adding drop wise the non-polar suspension to dopamine ethanol solution produced the best results. Two other methods – one using an amino acid model-11-aminoundecanoic acid and the other using micro emulsion with dopamine HCl – were less successful.

Dropping nanoparticles in hexane suspension in the solution provide the best mode for ligand exchange of the bound oleic acid with the free dopamine HCl. To accomplish this, the investigator prepared a 200 ml solution of dopamine/ethanol with 10 mg of dopamine. The investigator also dispersed 50 mg of nanoparticles in 200 ml hexane. The ethanol solution was heated to 70 C with the aid of an oil bath to dissolve the dopamine. While stirring, the dispersion was added a drop at a time pausing briefly to evaporate excess hexane. Subsequently, the ethanol solution containing the suspension was placed on ice to magnetically precipitate the nanoparticle and allow washing of the sample. After sample was washed, the particles were suspended in 18 MΩ water. The suspension was observed to be gravitationally stable, exhibiting no precipitation.

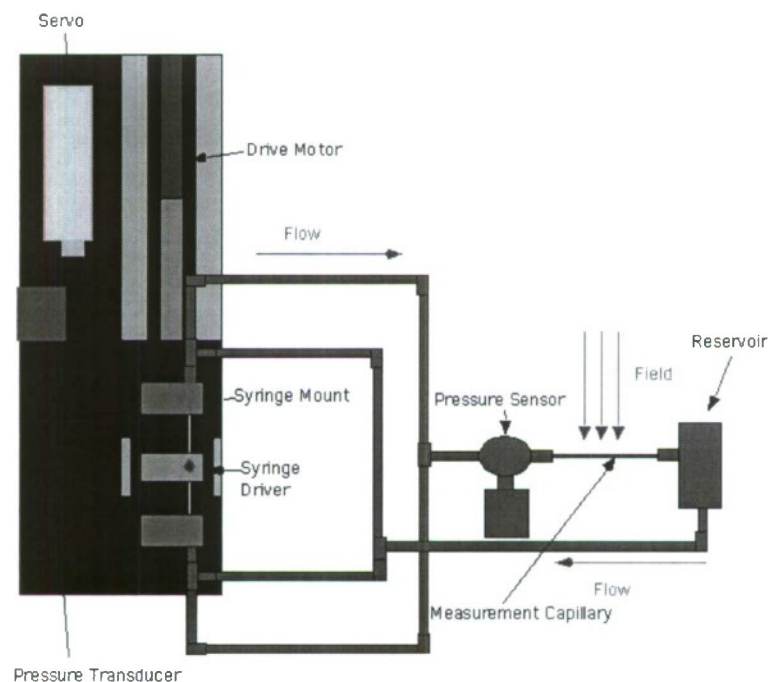
Micro emulsion of nanoparticles suspended in benzyl alcohol produce no transfer of nanoparticles. This method was suggested in the work of Chenjie Xu⁴. The investigator precipitated nanoparticles from the hexane dispersion using ethanol and a magnet. The precipitant was dispersed in benzyl alcohol. The investigator prepared 40 ml

- 5 mg/ml aqueous Dopamine HCl solution. The two mixtures were sonicated together in VWR model 50 Sonicator for 30 minutes. The emulsion was allowed to settle overnight. The aqueous layer was decanted and studied with respect to response to a magnetic field and addition of sodium chloride. The method was not successful because the sonicator use did not emulsify the non-polar fine enough to allow efficient ligand transfer.

The use of 11-aminoundecanoic acid polymerizes the nanoparticles to a single aggregate. The investigator disperse ~20 mg sample in hexane. In a round bottom flask, the dispersion and 50mg 11-aminoundecanoic acid was added to 30 ml phenyl ether. The mixture was heated to melting point of 11-aminoundecanoic acid with argon bubbling while stirring. After melting, the product was cooled and collected. Polymerization is common amongst amino acids. The polymerization could have occurred via two mechanisms. The first is excess amino acid chained the nanoparticles together. The second is that both the amino and the carboxylic groups bind without preference to the nanoparticle leaving an equal distribution of those groups at the surface. Those bind the particles together.

Fluid Measurement of Functionalized Superparamagnetic Solutions (Ferrofluids)

Prior to investigation the interaction of magnetic particles with cell membranes the investigator chose to examine the feasibility of the functionalized ferrofluids as a targeted delivery vehicle. In order to examine the dynamic ferrofluid behavior in the presence of an external magnetic field a Precision Flow Simulator was developed as per our specifications by Shaefer and Ortikes at the Georgia Institute of Technology. The main idea of the simulator is to measure the pressure response to the flow of the ferrofluid in the presence of an alternating external magnetic field.



Precision Flow Simulator

Figure 5.

The system uses a rotary motor to convert uniform circular motion into reciprocating linear motion that moves the plunges of two syringes in a push pull configuration. One syringe inputs from the reservoir containing the ferrofluid and the other syringe outputs to a tube that carries the fluid to a pressure transducer, then to a capillary in the magnetic field zone, followed by a return to the fluid reservoir. The transducer response and applied magnetic field were measured with a data acquisition system. The simulator is shown schematically in Figures 5.

The colloidal suspension was added to the reservoir and the pressure transducer dome. The pumping mechanism was not engaged until all tubes were filled with suspension. Air bubbles are meticulously removed so as to not interfere with transducer measurements. The system was run without field to generate a baseline set of measurements for comparison. The measurements were made with a slowly varying alternating magnetic field. The ferrofluid pressure dependence is shown as a function of field in Figure 6. The solid line shows one cycle variation of a 0.05 Tesla magnetic field; the pressure dependent response is shown above. We see a somewhat delayed response of the 1% ferrofluid solution, however, the maximum peaks in the pressure appear to occur at the maximum and minimum amplitudes of the applied field. The pressure maxima implies the superparamagnetic ferrofluid aggregates blocking the fluid flow, the minima indicates that due to the superparamagnetic nature of the particles there is no longer an exchange interaction between the particles and they return to solution. The overall gradual increase in pressure over the cycle suggests that some of the particle may remain attached to the capillary walls.

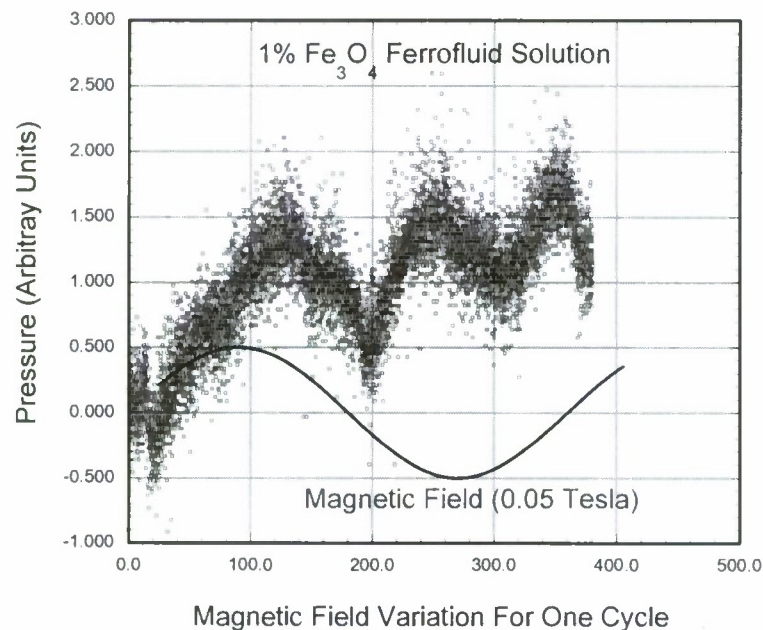


Figure 6

Summary

The overall objective was to create stable magnetite nanoparticles, to functionalize them with a biocompatible ligand, and to study the rheology of the colloidal suspension made from those particles. Using the co-precipitation method combine with strong magnets and larger reaction vessels, ~750 mg of nanoparticles could be produce in a single day.

The functionalization studies yielded stable aqueous ferrofluids and also insight on how the fluids studies could proceed. Initially, 11-aminoundecanoic as model of an amino acid polymerized the particle into a single unsuspendable mass. However, dopamine HCl as the literature suggest was a better candidate to create aqueous suspensions. Micro emulsion proves not to be an effective technique to exchange dopamine with oleic acid and oleylamine. However, the drop wise method proved to be the most effective. By dropping hexane suspensions into several orders of magnitude more dopamine/ethanol solution, the separation between particles was lengthen assuring that ligand exchange would occur before any two nanoparticles would agglomerate. The only caveat to dopamine HCl was addition of another chloride source like NaCl or even excess dopamine HCl was that particle would precipitate.

Reference

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^{*} This reported represent the latest epistle of Henry Corcoran, a Master's Student in the Department of Physics at Morgan State University. Mr. Corcoran has completed about 80% of his work. His progress has been somewhat delayed because of his full time employment at the Naval Research Laboratory. We expect Mr. Corcoran to complete his work this semester and obtain his Master's degree in May 2009. At that time we will forward a copy of his thesis.