

Fluid Dynamics and Heating of Magnetic Nanoparticles in Simulated Blood Vessels

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Magnetic nanoparticles (MNPs) can be used to locally target therapies and offer the benefit of using an AC magnetic field to combine hyperthermia treatment with the triggered release of therapeutic agents. Here, we investigate localization of MNPs in a simulated environment to understand the relationship between magnetic field intensity and bulk fluid dynamics to determine MNP retention in a simulated blood vessel. As MNPs travel through blood vessels, a therapeutic agent can be released locally as the particles are slowed or trapped by application of an external magnetic field. Magnetic cobalt ferrite nanoparticles were synthesized and labeled with a fluorescent rhodamine tag to visualize flow patterns in a flow cell, as monitored by a fluorescence microscope. Particle retention was determined as a function of flow rate, concentration, and magnetic field strength. Understanding the relationship between magnetic field intensity, flow behavior and nanoparticle characteristics will aid in the development of therapeutic systems specifically targeted to diseased tissue. The project also explored the heat generating capabilities of cobalt ferrite magnetic nanoparticles with respect to particle concentration and magnetic field strength; thereby, examining the particle properties relevant to localized hyperthermia treatment.

Background

The American Cancer Society estimates that in 2007 more than one million people were diagnosed with some form of cancer and more than half a million people died from cancer in the United States alone. While the cancer mortality has decreased somewhat since the 1950s due to better diagnostic techniques, a significant decrease has not been seen compared to other prevalent diseases such as heart attacks and other cardiovascular diseases. Currently cancer is treated with radiation, surgery, chemotherapy, or any combination of these. However, each of these generally causes severe side effects in patients. Another form of cancer treatment is hyperthermia, which is clinically described as heating cancerous tissue to temperatures greater than 42°C. Cancerous tissue does not possess a very advanced vascular system; therefore, it does not cool itself as effectively as healthy tissue. When cancer cells are heated past 42°C they begin to die, while healthy cells are predominantly unharmed up to 48°C. However, at present, hyperthermia treatment is performed on entire regions of the body, which also results in negative patient side effects.

The ideal cancer treatment would be a combination of therapies that are administered locally to cancerous tissue; thereby, minimizing negative patient side effects, while increasing treatment efficacy. One such combination therapy of interest is a localized form of hyperthermia treatment partnered with localized drug delivery. The objective of this research is to characterize the flow behavior of MNPs in a simulated blood vessel to investigate the possibility of particle retention at a specified location.

Magnetic nanoparticles offer a unique opportunity to administer hyperthermia treatment at the cellular level, and many of these particles also possess properties that are applicable for advanced imaging. Hyperthermia would be achieved via exposure of these MNPs to an AC magnetic field due to the superparamagnetic properties of these MNPs. Therefore, this study also investigates the heat generation of these particles with respect to MNP concentration and magnetic field strength. The retention and heat generation study focuses on the cobalt ferrite (CoFe₂O₄) MNPs. Cobalt ferrite was chosen for three reasons: it has been shown to be non-toxic [3], it is a good contrast agent for Magnetic Resonance Imaging (MRI) [6], and it has a fairly high

specific absorption ratio (SAR), which directly correlates to the magnitude of heat generation seen in a specified amount of time [4].

Experimental

Synthesis of monodispersed cobalt ferrite magnetic nanoparticles was accomplished by an organic phase process of two metal precursors and a long chain alcohol. The various properties of these nanoparticles were controlled by varying the reaction temperature, time, and solvent [7]. These MNPs were characterized for particle size, saturation magnetization, and composition. An external AC magnetic field was generated by an induction hyperthermia chamber, and the particle heating was observed via an infrared camera with respect to particle concentration and magnetic field strength.

The MNPs were later tagged with rhodamine B, a fluorescing dye, and subsequently observed via fluorescence microscopy. The blood vessel was simulated using a Glycotech® Flow Chamber. The concentration of MNPs retained within the flow cell directly correlates to the intensity of fluorescence observed, and the magnitude of such was confirmed by means of UV/Vis spectrophotometry. Microscopy visualized the overall flow pattern of the MNPs with respect to concentration, flow velocity, and magnetic field strength.

Cobalt Ferrite Synthesis

A high temperature phase reaction of iron (III) acetylacetonate, $\text{Fe}(\text{acac})_3$, and cobalt (II) acetylacetonate, $\text{Co}(\text{acac})_2$, with 1,2 hexadecanediol in the presence of oleic acid and oleylamine leads to cobalt ferrite nanoparticles. $\text{Fe}(\text{acac})_3$ (2 mmol), $\text{Co}(\text{acac})_2$ (1 mmol), 1,2 hexadecanediol (10 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20mL) were mixed and magnetically stirred under flow of nitrogen. The mixture was heated to 200°C for 2 hours and then heated to reflux at ~300°C for an hour. The mixture was then cooled and dispersed into ethanol. After sufficient washing and centrifugation to remove solvent and undispersed residue, the mixture is readily dispersed into hexane [7]. It is then necessary to disperse the nanoparticles in water. The aqueous dispersion method used requires a mixture of meso-2,3-dimercaptosuccinic acid (DMSA), dimethyl sulfoxide, and the hexane

dispersed CoFe_2O_4 particles. This solution is then subjected to an ultrasonication bath for 24-72 hours. Next, the mixture was washed and dispersed in water, if necessary more DMSA was added and the mixture was again placed in an ultrasonication bath for another 24-72 hours. These particles were then analyzed using X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), and Vibrating Sample Magnetometry (VSM).

CoFe_2O_4 Heat Generation

The heat generation of aqueously-dispersed cobalt ferrite nanoparticles was determined by recording temperature data observed from Infrared Thermacam™ during exposure to an AC magnetic field. This AC magnetic field was created using a custom-designed induction hyperthermia chamber (Induction Atmospheres, Rochester, NY). The magnitude of heat generation was observed with respect to varying MNP concentration and magnetic field strength. A concentration of ~7 mg/mL aqueous CoFe_2O_4 MNPs were used to find the magnitude of heat generation at 350 Oe, 563 Oe, and 700 Oe. The magnitude of heat generation was recorded over a period of 15 minutes. The magnitude of heat generation was also investigated with respect to concentration of cobalt ferrite at 1, 1.5, 2, and 5 weight percents.

Fluorescent Tagging

Fluorescent tagging of the MNPs was achieved using the method described by Bertorelle *et al.* [2]. The process utilizes the strong interaction between the positive MNP surface charge and the dimercaptosuccinic acid (DMSA). Coupling of the MNP and fluorescent dye, rhodamine B, occurs via the thiol and carboxyl groups. Rhodamine B and cystamine were coupled by an amide bond, where 17 mg of rhodamine B, 7 mg of 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDC), and 4 mg of cystamine were mixed in 25 mL of water and then stirred for 3 hours [2]. Afterwards, 25 mg of DMSA and 1 M of MNPs were added to the mixture, subsequently stirred for 2 hours. Magnetic separation was used to remove the MNPs from the solution, and DI water was used to wash the MNPs. Finally, TMAOH was used to redisperse the MNPs into water.

Flow Behavior and Retention

The flow behavior of the MNPs and particle retention with the simulated blood vessels were characterized as a function of MNP concentration, flow velocity, and magnetic field strength. The blood vessel was simulated using a rectangular flow kit purchased from Glycotech. The flow diameter may be manipulated using gaskets of various sizes allowing different sized “blood vessels” to be analyzed. These flow cells are also for use on an inverted microscope making them ideal for characterizing flow by fluorescence microscopy. The ferrofluid was pulled from through the flow cell by a peristaltic pump and the MNP concentration was then measured downstream using UV-visible spectrophotometry. Neodymium magnets were used to induce a static magnetic field to influence the particles in flow. These magnets were placed at various distances from the center of flow to alter the strength of the static magnetic field. The particles were then returned to the initial stock solution creating a closed loop system. The ferrofluid contains water, MNPs tagged with rhodamine B, untagged MNPs, and free rhodamine B. Therefore, a spectral analysis was needed to determine an appropriate wavelength to observe the concentration of tagged particles active in flow. A wavelength of 445 nm was chosen.

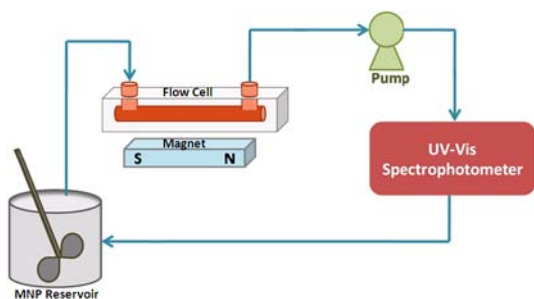


Figure 1: Flow Cell Experimental Set-Up

Results

CoFe₂O₄ Particle Analysis

The cobalt ferrite MNPs were successfully synthesized and characterized by XRD, TEM and VSM analysis. The XRD analysis confirmed the presence of cobalt ferrite nanoparticles. The particles were determined to range from 7-10 nm based on TEM analysis [Figure 2]. Finally, VSM determined the magnetization of the particles, which directly correlates to the magnitude of heating seen from the

particles. The magnetization is also directly related to particle size [6]. A magnetization of 41.4 emu/g was observed.

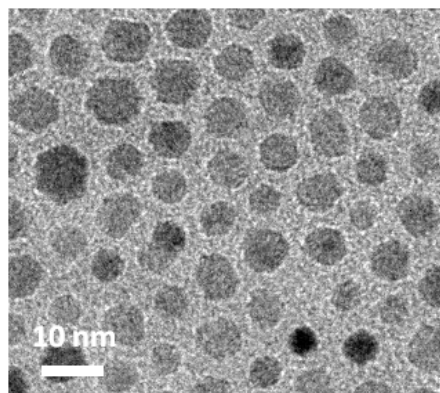
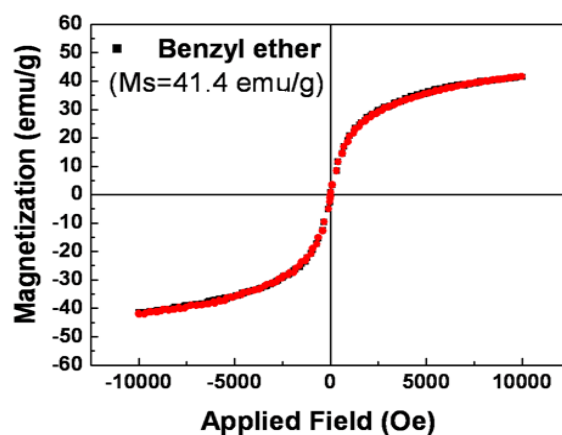


Figure 2: (Above) TEM image of CoFe₂O₄ nanoparticles. (Below) Saturation magnetization observed from VSM



Heat Generation Study

The heat observed by the Infrared Thermacam was seen to increase with an increase in the magnetic field strength. In addition, increasing the concentration of magnetic nanoparticles also showed a notable increase in the heat generation observed [Figures S1 & S2].

Particle Retention Study

The MNPs were successfully tagged with Rhodamine B and imaged by fluorescence microscopy (Nikon Eclipse 2000). Fluorescent tagging was confirmed by exposing the ferrofluid to a strong magnetic field and imaging the successive movements of the fluorescing particles [Figure 3].

The particles were seen to be very sensitive to magnetic field exposure upon visual inspection. This

confirmed the possibility of manipulated targeting via an induced magnetic field. The retention of the MNPs was determined with respect to three flow rates and two magnetic fields strengths, thus far. From these studies, it was determined that an increase in nanoparticle retention was seen with an increase magnetic field strength showing a direct relationship. However, an inverse relationship was seen between particle flow rate and particle retention. As the flow rate was increased, a decrease was seen in the nanoparticle retention rate [Figure S3].

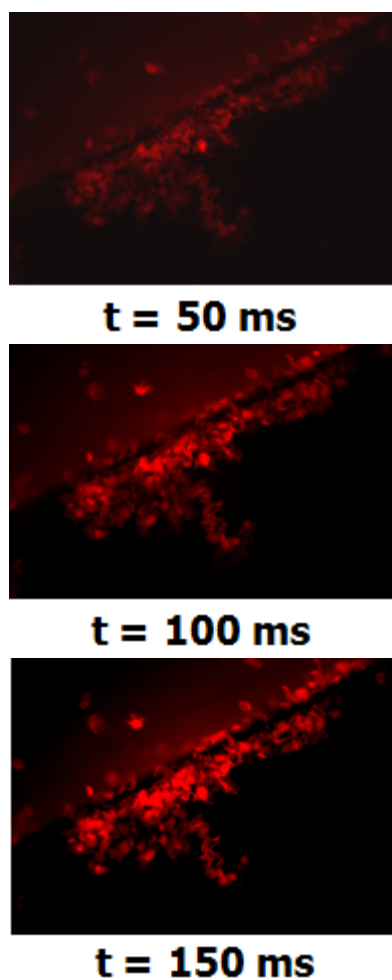


Figure 3: CoFe₂O₄ imaged by fluorescence microscopy. Note the hooking of particles from general flow as influenced by a static magnetic field.

In summary, this study shows the possibility of localized hyperthermia being achieved through the application of targeted magnetic nanoparticles. First, heat generation is possible through exposure to an AC magnetic field, where heat generation directly

correlates to both magnetic field strength and particle concentration. In addition, the particle retention rate is also influenced by both magnetic field strength and particle flow rate. However, the retention rate directly correlates to magnetic field strength, and is inversely related to particle flow rate.

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