Published in final edited form as:

J Magn Magn Mater. 2015 August 1; 387: 96–106. doi:10.1016/j.jmmm.2015.03.085.

Impact of magnetic field parameters and iron oxide nanoparticle properties on heat generation for use in magnetic hyperthermia

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Abstract

Heating of nanoparticles (NPs) using an AC magnetic field depends on several factors, and optimization of these parameters can improve the efficiency of heat generation for effective cancer therapy while administering a low NP treatment dose. This study investigated magnetic field strength and frequency, NP size, NP concentration, and solution viscosity as important parameters that impact the heating efficiency of iron oxide NPs with magnetite (Fe_3O_4) and maghemite ($\gamma\text{-Fe}_2\text{O}_3$) crystal structures. Heating efficiencies were determined for each experimental setting, with specific absorption rates (SARs) ranging from 3.7 to 325.9 W/g Fe. Magnetic heating was conducted on iron oxide NPs synthesized in our laboratories (with average core sizes of 8, 11, 13, and 18 nm), as well as commercially-available iron oxides (with average core sizes of 8, 9, and 16 nm). The experimental magnetic coil system made it possible to isolate the effect of magnetic field parameters and independently study the effect on heat generation. The highest SAR values were found for the 18 nm synthesized particles and the maghemite nanopowder. Magnetic field strengths were applied in the range of 15.1 to 47.7 kA/m, with field frequencies ranging from 123 to 430 kHz. The best heating was observed for the highest field strengths and frequencies tested, with results following trends predicted by the Rosensweig equation. An increase in solution viscosity led to lower heating rates in nanoparticle solutions, which can have significant implications for the application of magnetic fluid hyperthermia *in vivo*.

Keywords

Magnetic fluid hyperthermia; iron oxide nanoparticles; magnetic field strength; magnetic field frequency; specific absorption rate

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Introduction

Magnetic fluid hyperthermia (MFH) using localized iron oxide NPs offers a significant benefit over whole body and regional hyperthermia, which can lead to several vascular and cardiac disorders [1-3]. MFH has also been shown to kill cells faster as compared to traditional hyperthermia methods, which can play an essential role in reducing the therapy administration time for cancer treatment [4]. MFH can reduce side effects in patients while amplifying treatment of cancer using superparamagnetic NPs, which can be specifically targeted using antibodies or peptide sequences [5,6] and directed to cancerous tissue through the enhanced permeation and retention (EPR) effect [7]. Magnetic fields in the kHz to MHz range have been investigated for heat generation in various MFH systems using superparamagnetic and ferromagnetic iron oxide NPs [8,9]. To be used effectively for cancer treatment, the least possible dose of NPs should be introduced in the human body to avoid possible side-effects and bioaccumulation. Thus, it is essential to understand the factors that affect heat generation in NP dispersions to maximize the therapeutic effectiveness of MFH.

Some NPs based on iron oxide have been approved for medical use by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [6]. While magnetic NPs that contain cobalt ferrites and nickel ferrites may have better magnetic properties for heat generation, the medical use of these materials is generally infeasible. NPs made of nickel ferrite have been shown to have an adverse effect on cell viability and replication, while it was demonstrated that cobalt ferrite NPs can be toxic to mammalian cells at concentrations needed for cancer hyperthermia treatment [10 - 12]. In addition to their acceptability for medical use, iron oxide NPs feature good colloidal stability when coated with appropriate surfactants or polymers which can also provide a linkage to cell-targeting moieties [13]. Iron oxide NPs have been widely investigated for magnetic heating, and have also proven to be useful as MRI contrast agents [6, 14]. These properties make iron oxide NPs attractive for use in cancer theranostics.

To characterize the heating of magnetic nanoparticles under AC magnetic field exposure, specific absorption rate (SAR) values are determined from temperature-time profiles and computed as heat generation per mass of NPs or iron (Fe) content of NPs in W/g [15-18]. NPs with high SAR are largely favored for cancer treatment as administration of NPs to patients can be kept to a minimum while using brief durations of magnetic field exposure that still achieve the temperature rise essential to induce cell death. SAR is calculated as:

$$SAR (W/g) = \frac{m_s * c_p}{m_{np}} * \left(\frac{\Delta T}{\Delta t} \right) \quad \text{Eq. (1)}$$

Here m_s is the mass of solution, m_{np} is either the mass of NPs or the mass of Fe in the NPs, c_p is the heat capacity of the solution, and $(\Delta T / \Delta t)$ is the initial slope of the temperature rise vs. time curve for NP heating. The SAR value serves as guidance for comparing the heating rates of NPs with different compositions and concentrations, at different magnetic field settings.

The parameters that govern power loss in magnetic hyperthermia are defined by the Rosensweig equation [19], where the power generation (P) in iron oxide NPs when subjected to an AC magnetic field is defined as:

$$P = \pi \mu_o \chi_o H^2 f \frac{2\pi f \tau}{1 + (2\pi f \tau)^2} \quad \text{Eq. (2)}$$

Here, μ_o is the permeability constant of free space ($4\pi \times 10^{-7}$ T-m/A), χ_o is the magnetic susceptibility of the particles, H is the magnetic field strength, f is magnetic field frequency, and τ is the relaxation time for reorientation of magnetic moments in NPs, either through whole NP motion (Brownian relaxation) or spin relaxation (Néel relaxation) [19]. The power generated through application of an AC magnetic field results in thermal energy, and for a given set of superparamagnetic NPs the quantity of heating is a function of the square of magnetic field strength when all other factors are held constant. Frequency can also be used to tune the heat generation, as the power generation reaches an asymptote when frequency is increased. The application of the Rosensweig equation, and contribution of different relaxation mechanisms to MFH has been well described [19 – 24], and further relationships between magnetic heating and NP properties are manifest in the magnetic susceptibility and relaxation time.

By changing the properties of the applied magnetic field (through field intensity and frequency), heating in superparamagnetic NPs can be optimized. The power input by the magnetic field can also be tuned by adjusting the time course of field application. The field can be applied for different durations of time or using variable field intensity, for example through the use of a feedback control loop where the field is adjusted to maintain a fixed temperature. One such system has been proposed by Tseng et al. using a thermocouple and a temperature processing unit to maintain a constant hyperthermia temperature [25]. A number of studies have investigated MFH to determine preferred parameters that lead to high SAR values [26-31]. In most published studies, MFH magnetic field frequencies are applied in the range of 80 to 700 kHz, while field strength usually lies between 1 and 50 kA/m [15, 26 – 31]. A wide range of SAR values have been reported for NPs of different compositions, sizes, and size distributions, for many different field strengths and frequencies which are often fixed by the geometry and electrical configuration of the magnetic coils. Additional complications that make comparison of experimental results between groups challenging include the reliability of NP characterization and differences in SAR reporting, which is normalized by either NP mass or the mass of Fe in the NPs, but is often not clearly reported due to difficulties in distinguishing the oxidation state of Fe in the NPs. These variables make it difficult to reach conclusions about optimal NP structures and magnetic field parameters to achieve effective heating. SAR values for commercial and custom-synthesized iron oxide NPs have been reported covering a range from lower than 10 W/g Fe to higher than 2000 W/g Fe [15, 26 – 31]. Some of the highest reported SAR values of 2452 W/g of Fe for cubic iron oxide NPs and 1650 W/g for spherical iron oxide NPs were obtained by Guardia et al. and Fortin et al., respectively [26, 27].

Heat generation in magnetic NPs under application of a high frequency magnetic field is governed by Néel relaxation, Brownian relaxation, and a hysteresis loss mechanism [19].

Néel relaxation occurs due to the flipping of magnetic moments inside each NP, whereas Brownian relaxation occurs due to the rotation of entire particles along with the magnetic moment. Néel and Brownian relaxations are theorized to be the dominant heat loss mechanisms for particles that are superparamagnetic in nature, while hysteresis losses that occur due to movement of domain walls under application of magnetic field are responsible for heating in larger sized ferromagnetic particles [19]. There is, however, disagreement over the maximum size for single domain NPs, and where this transition occurs. The critical NP size range separating superparamagnetic and ferromagnetic domain varies based on structure and composition of NPs. In a study by Bakoglidis et al. where NPs investigated were a mixture of maghemite and magnetite, it has been suggested that particles beyond 13 nm in size lie in the ferromagnetic domain, whereas smaller particles lie in the superparamagnetic domain [32]. Krishnan has determined by mathematical modeling that the maximum size for a particle to be single domain and superparamagnetic is in the range of around 35 nm for maghemite and 25 nm for magnetite, while that for being single domain and ferromagnetic is approximately 90 nm for maghemite and slightly larger than 80 nm for magnetite [22]. Vergés et al. surveyed the results of other researchers and reported that the transition range from single domain to multi domain is around 50 nm for magnetite NPs [33]. Thus, while the mechanism of heating is expected to depend largely on particle size, there are two complicating factors that make attributing heat generation to a particular heating mechanism difficult: (1) the particles may have single or multiple crystal domains, and (2) the size distribution of NPs can be widely disperse for a given sample.

The viscosity surrounding NPs can also impact magnetic heating, primarily through increasing the relaxation time for Brownian relaxation, which reduces the Brownian contribution to heat generation. As most experimental investigations of MFH are done on aqueous dispersions of NPs with no significant additives to alter viscosity, the applicability of data to more complex *in vivo* environments may not be accurately estimated. For example, many applications of magnetic NPs involve the deployment of NPs in blood or tissue, where they will be surrounded by proteins [34], or in the core of a drug delivery device where the free motion of NPs is impeded by a polymer [13]. One recent study has shown that when a mixture of 13.9 nm magnetite and maghemite NPs was subjected to increasing solution viscosity from 0.9 cP to 43.2 cP at a magnetic frequency of 215 kHz and amplitude of 3.8 kA/m, the SAR value decreased to 70% of the original value [35]. Since the environment surrounding NP for medical uses will likely be significantly more viscous than water, experiments to determine the effect of viscosity on SAR are needed to predict heating *in vivo*. Thus, this phenomenon can affect the overall feasibility of MFH, particularly if Brownian relaxation is responsible for much of the heating.

While many research groups have contributed to the understanding of MFH, it is difficult to compare SAR values from different groups, as the magnetic induction coils in each laboratory often have fixed or narrow operational frequency ranges. Also in many research studies multiple factors affecting the NP heating may vary at the same time, making it difficult to isolate variables responsible for magnetic heating. We overcame this drawback using a power supply and magnetic coil that allows the frequency to be varied while the field strength is kept constant, and vice versa. We also studied the effect of particle size by

using different sizes of NPs synthesized using identical experimental methods. Based on the importance of material and magnetic field parameters on particle heating, we designed a comprehensive study to test the heating efficiency of different types of NPs covering a range of field strengths and frequencies, for different NP compositions, concentrations, and sizes, as well as solution viscosities.

Materials and Methods

1.1 Materials

All chemicals were purchased at reagent grade or better from the Sigma-Aldrich company (St. Louis, MO), unless otherwise noted. Iron (III) oxide nanopowder, less than 50 nm in size, was examined for hyperthermia applications, primarily as a low-cost, abundant nanomaterial. fluidMAG D with starch coating, and fluidMAG PAD with polyacrylamide coating were purchased from chemicell GmbH (Berlin, Germany), with a reported concentration of 100 mg/mL (which included the weight of the polymer coating). Both fluidMAG particles had a reported hydrodynamic radius of 50 nm. Additional iron oxide NPs dispersed in hexane were synthesized as described below, using iron(III) acetylacetonate 97%, 1,2-hexadecanediol 90%, oleic acid 90%, oleylamine 70% (Sigma-Aldrich, St. Louis, MO), benzyl ether 99% (Acros Organics, Fair Lawn, NJ), hexane, and ethyl alcohol. *Table 1* lists the types, abbreviations, sizes, and concentrations of particles used for this study. Alginic acid, sodium salt (Acros Organics, rated at 485 mPa-s for a 1% solution at 20 °C) was used to modify the viscosity of aqueous NP dispersions. Calcium chloride was purchased from Fisher Scientific (Fair Lawn, NJ) to cross-link alginic acid solutions.

1.2 Magnetic NP Synthesis

Iron oxide nanoparticles of various sizes were synthesized from iron (III) acetylacetonate and 1, 2 – hexadecanediol, in the presence of oleylamine and oleic acid using the thermal decomposition method described by Sun et al. [36]. Briefly, 2 mmol of iron (III) acetylacetonate was added to 10 mmol of 1,2-hexadecanediol in 20 mL of benzyl ether containing 6 mmol of oleic acid and 6 mmol of oleylamine. To synthesize particles of different sizes, this solution was heated to a set nucleation temperature for 2 hours under a nitrogen environment, with stirring. This was followed by increasing the temperature and refluxing for 1 hour. The mixture was then cooled to ambient temperature under nitrogen, and then precipitated with 40 mL of ethanol. The precipitated particles were separated by centrifugation and the supernatant was decanted. The product was then dispersed in hexane in the presence of ~0.05 mL of oleic acid and ~0.05 mL of oleylamine. Any remaining undispersed product was removed by centrifugation at 6000 rpm for 10 minutes followed by decantation. The product was precipitated with ethanol a final time, centrifuged, and decanted. The product was then redispersed into hexane.

Particle sizes and distributions were controlled by slight variations in the reaction conditions. Slowly approaching but not exceeding the 200 °C nucleation period temperature produced particles with narrow size distributions. Refluxing gently at ~300 °C produced larger particle sizes of around 11 nm, whereas refluxing less carefully produced NPs that

had a size of around 8 nm. Still larger particles were produced using the seed growth method described by Sun et al. [36]. Using 11 nm particles as the seeds and employing the same nucleation and reflux conditions as above, 13 nm particles were produced. Larger particles of around 18 nm were produced by increasing the nucleation temperature to 210 °C for 5 minutes, then immediately cooling the mixture to 200 °C for the remainder of the nucleation period. Although the size distributions were slightly larger, this method removed the need for another seed growth reaction to produce larger particles.

1.3 Maghemite Nanopowder Dispersions

To make aqueous maghemite nanopowder dispersions, Iron (III) oxidenanopowder was mixed vigorously in DI water at 25 mg/mL and allowed to settle undisturbed for 2 days. The supernatant, which formed a stable dispersion, was collected with a Pasteur pipette and used for magnetic heating experiments. The concentration of these particles was determined by atomic absorption spectroscopy, as discussed later.

1.4 NP Characterization

Images of each of the iron oxide NPs were collected using transmission electron microscopy (TEM, FEI Technai F-20, Hillsboro, OR). The mean particle size of NPs was found by performing size analysis on TEM images using NIH Image J software. The hydrodynamic diameters of aqueous NP dispersions were measured using a Malvern ZEN3600 dynamic light scattering (DLS) device (Malvern, Worcestershire, UK).

X-ray photoelectron spectroscopy (XPS) was used to determine the oxidation state of the iron oxide NPs. Here, samples of iron oxide NPs were washed with acetone to remove the surface coating of oleic acid and oleylamine, followed by placing them under vacuum in a vacuum oven set to ambient temperature until they dried for 24 hrs. The samples were then deposited onto copper tape for XPS analysis, which was carried out on a Kratos AXIS 165 Multitechnique Electron Spectrometer (Manchester, UK) using a monochromatic aluminum source set to 10 mA, 12 kV. High resolution scans were performed with the analyzer pass energy set to 20 mA and the dwell time set to 2000 μ s. Two sweeps were performed for each scan. Because it has been shown that the C 1s peak is unsuitable for use as a reference for charge correction on iron oxide samples [37], the O 1s peak at 530.0 eV binding energy was used for this purpose.

High resolution scans of the Fe 2p region of iron oxides have been previously shown to be useful for qualitative studies of the ionic and oxidation states of iron, and the presence or absence of a satellite peak between the Fe 2p_{1/2} and Fe 2p_{3/2} has been used to differentiate between magnetite and maghemite [37], as only maghemite samples produce a satellite peak attributed to Fe 2p_{3/2}. The position of the Fe 2p_{3/2} peak is typically 711 eV, with the satellite peak located approximately 8 eV higher [37].

1.5 Characterization of Magnetic Properties of NPs

Magnetization curves were acquired using a Digital Measurement Systems vibrating sample magnetometer (VSM). The magnetometer was calibrated using a high purity nickel standard. Powder samples were carefully weighed on an analytical balance. The samples were placed

on Scotch® tape and the tape folded over to make a sandwich with the particles trapped inside the tape. The excess Scotch® tape was trimmed off with scissors. The samples were affixed to a quartz sample holder using silicon vacuum grease. Samples of fluidMAG D NPs were dried from aqueous solution to give free-standing films. The films were weighed on an analytical balance and then affixed to the quartz sample holder. Full hysteresis curves were obtained with a saturating field of 10,000 Oe. The step size was 10 Oe in the range from +1,000 to −1,000 Oe.

1.6 Magnetic Heating of NPs

All magnetic heating experiments were performed using a custom-designed hyperthermia coil (Induction Atmospheres, Rochester, NY) (Figure 1). A 5 kW power supply (Ameritherm, Model Novastar 5kW, Scottsville, NY) is connected to a heat station to which the coils are attached. The hollow coils are designed to allow circulating chilled water to pass through, in order to minimize temperature rise in the coils. A circulating chiller bath (Koolant Koolers Model JT1000, Kalamazoo, MI) was set to circulate water through the coils at 18 °C. A 4 cm inner diameter coil with two turns in a distance of 1.2 cm was separated from two identical turns by a gap of 2 cm. This 4-turn coil was used for all heating experiments, as it had a sufficiently wide coil diameter to allow insulation to fit between the coil and samples to minimize non-specific heating of samples due to the heating of the coil surfaces. An additional 6-turn coil (4 cm inner diameter) could be placed in series with the magnetic heating coil, and was used to modulate the frequency applied to the NP dispersions (frequency modulator coil shown on left side in Figure 1). A copper bar can be placed across any number of the coils to allow a total of seven testing frequencies at each voltage setting (including one with the frequency modulator removed).

Each NP dispersion was taken at a measured volume of 1.2 mL and filled in a plastic micro-centrifuge tube, which was positioned in the axial and radial center of the top two turns of the coil and surrounded by a layer of insulating foam. The magnetic field strength was adjusted by altering the voltage setting in the heating station circuitry, which in turn changed the current supplied to the coil. The frequency was modified by placing the frequency modulating coil in series with the hyperthermia coil and adjusting the copper bar so that the circuit would bypass from zero to all six loops. For experiments to determine the effect of field strength on magnetic heating, the frequency was maintained at a constant value by using a fixed coil setup and adjusting the voltage supplied to the coil. In experiments to investigate the effect of field frequency on heating, the voltage was appropriately changed so that the field strength remained constant while the frequency was changed due to addition of the frequency modulator coil. Field strength settings ranging from 15.1 to 47.7 kA/m were used for experiments, while the frequency was varied from 123 to 430 kHz (Table 2). To test the effect of magnetic field strength on NP heating, four different field strength settings of 15.1 kA/m, 22.8 kA/m, 35.8 kA/m, and 47.7 kA/m were obtained at a set frequency of 194 kHz by changing the voltage applied to produce the magnetic field. Additional field strengths of 32.9 kA/m and 27.2 kA/m were obtained at maximum applied voltage when the frequency was reduced to 143 kHz and 123 kHz, respectively, by addition of the frequency modulator coil. To study the effect of frequency on hyperthermia, field strengths were set at 38.2 kA/m and tested at four different frequencies: 123, 143, 194 and

430 kHz. Several combinations of these magnetic field parameters were tested on commercial fluidMAG D particles, whereas selected parameters were used to test the heating of other magnetic particles listed in *Table 1*.

An infrared camera (FLIR systems, North Billerica, MA) was used to record the temperatures of samples contained in the micro-centrifuge tube. The FLIR camera was capable of measuring temperatures with a resolution of 0.1 °C.

1.7 Measurement of Magnetic Field Strength

The magnetic field strengths generated by the hyperthermia coil were measured using a magnetic field probe (AMF Life Systems, Auburn Hills, MI). The axial and radial components of the AC magnetic field were measured as a function of position within the coil for several input voltages and coil configurations. For magnetic heating experiments, samples were placed in the axial and radial center of the coils, where the field strength was found to be highest.

1.8 Preparation of Viscous Alginate NP Solutions

To determine the effect of viscosity on SAR, 2.0 and 4.0 wt % alginate solutions were prepared by adding measured quantities of sodium alginate to aqueous NP dispersions. The mixtures were vigorously mixed and formed a viscous solution after leaving it overnight. A cross-linked alginate gel containing dispersed NPs was made by adding 0.5 mL of 2 wt % calcium chloride in water to the 4 % alginate-NP solution. For each of these solutions, care was taken not to vary the net NP concentration. The viscosities of the 2 and 4 wt % alginate solutions were measured by using a Zahn cup-style Boekel viscosimeter (Philadelphia, PA). Heating experiments were conducted using the same 1.2 mL sample size as for dispersed NPs.

1.9 Experimental Data Collection

All heating experiments were performed for 10 minutes, by placing the micro-centrifuge tube in the center of the coil and applying the selected magnetic field parameters. Experiments were performed in triplicate, starting at room temperature. Non-specific heating of NP solutions was subtracted from each run by performing identical heating experiments on pure solvents (i.e., water, hexane) using the same magnetic field parameters and subtracting the $T(t)$ profile for the pure solvent from the $T(t)$ profile for the NP solution.

Results and Discussion

2.1 Characterization of NPs

TEM and DLS were used to determine mean iron oxide NP diameters, and the hydrodynamic diameters in aqueous dispersions, respectively (Table 1). The fluidMAG particles were supplied as aqueous solutions at 100 mg/mL, with this value including a 20 wt % coating of either dextran (fluidMAG D) or polyacrylamide (fluidMAG PAD) according to the manufacturer. Discounting the NP coatings, iron oxide concentrations were determined by Atomic Absorption Spectroscopy (AA); the AA values are reported for all NP concentrations. The iron oxide concentration determined by AA contrasts with the

nominal concentration for the fluidMAG particles provided by the manufacturer, as the fluidMAG NPs contained a lower percentage of iron oxide than anticipated (Table 1). The TDMNP particles synthesized in house had an oleic acid coating, allowing the particles to be dispersed in hexane. The commercial maghemite nanopowder was partially dispersible in water, using the method described above, so particle sizes and magnetic properties were determined using only the dispersible fraction.

TEM images of the NPs show a representative sample of each type of NP investigated (Figure 2). The sizes for iron oxide NP diameter represent the mean size found for at least 250 NPs using the Image J software area measurement tool; the reported error represents one standard deviation for the distribution of particle sizes. The dispersible fraction of maghemite nanopowder and TDMNP18 NPs were the largest of those evaluated with mean sizes of 16 nm and 18 nm, respectively, with the commercial maghemite nanopowder having the widest distribution of particle sizes. Commercially-available fluidMAG D and fluidMAG PAD had smaller iron oxide NP sizes of 8 and 9 nm, respectively.

Although fluidMAG D and fluidMAG PAD are sold as having hydrodynamic diameters of 50 nm each, we found that the number average hydrodynamic size was considerably smaller, at 19 nm and 29 nm, respectively for the fluidMAG D and fluidMAG PAD supplied (Table 1). The increase in diameter compared to the TEM data is explained by the coating of hydrophilic polymers. The hydrodynamic diameter of the aqueous maghemite nanopowder dispersion was found to be 35 nm, which is significantly larger than the 16 nm mean NP diameter determined by TEM (Table 1). Since the maghemite nanopowder particles have no coating, this result indicates that there is a small degree of aggregation in this aqueous dispersion. The Z-average particle size obtained from the DLS Zetasizer software is also reported, with values ranging from 54 to 60 nm. From these data, we judge that fluidMAG and maghemite aqueous dispersions did not form substantially large aggregates. The custom-synthesized NPs do not have reported hydrodynamic sizes as hexane dispersions were not measured by DLS.

XPS spectra (Figure 3) were used to determine that the fluidMAG and TDMNP NPs synthesized in our laboratory were magnetite as they did not display a satellite peak which would indicate maghemite [37]. However, the satellite peak at 719 eV confirms the γ -Fe₂O₃ crystalline structure of the maghemite nanopowder.

Saturation magnetization values and the magnetic susceptibility of the NPs were determined by VSM (Table 1). All the magnetite particles showed hysteresis curves with similar shapes. The remanent magnetization was zero and at the high applied fields, up to 10,000 Oe, the curves did not saturate, suggesting the particles were superparamagnetic. When the data were fit to the Langevin function, which describes the magnetization curve for superparamagnetic particles, the data deviated from a simple Langevin function. For nanoscale magnetic particles, particles at the low end of the distribution will have different magnetic properties from the particles at the high end of the size distribution. The magnetite samples can best be described as a mixture of superparamagnetic particles having a distribution of magnetic moments. Since the magnetization curves did not saturate, the values of specific magnetization were reported for an applied field of 10,000 Oe. For the

maghemite nanopowder, the hysteresis curve was that of a hard ferromagnet with a small coercivity of 150 Oe and a saturation magnetization of 57 emu/g. This was expected, since maghemite has a modest value of magnetocrystalline anisotropy energy density ($K_u \sim 10^4$ erg/cc [38]).

2.2 Magnetic Heating of NPs

Magnetic heating experiments were conducted on each of the nanoparticle types investigated (Table 1) over a range of magnetic field frequencies (123 – 430 kHz) and intensities (15.1 – 47.7 kA/m). Here, the temperature rise ($T - T_0$), which is the difference between the measured and initial temperatures of each sample, is shown to increase over a period of ten minutes. The samples were well insulated and the nonspecific heating due to heat transfer from the coil surface to the solvents used for NP dispersion (which was less than 0.6 °C for water and 1.5 °C for hexane over a time period of ten minutes for all coil settings) was subtracted from the data, so that results represent heat generated by the NPs only (Figure 3). To test the effect of frequency and field strength independently, other experimental parameters were held constant. For example, fluidMAG D NP solutions were heated using field strengths of 15.1 to 47.7 kA/m at a constant 194 kHz frequency, and also tested over a range of frequencies from 123 to 430 kHz using the frequency modulator coil while holding the field strength constant at 38.2 kA/m. In addition to field intensity and frequency, the effect of NP concentration, NP size and type, and NP dispersion viscosity on magnetic heating were investigated independently.

2.2.1 Effect of Magnetic Field Intensity—Magnetic heating was evaluated for 20 mg/mL fluidMAG D NP dispersions that contained 8.6 mg/mL Fe_3O_4 . These NPs were exposed to AC magnetic field strengths ranging from 15.1 to 47.7 kA/m at a fixed frequency of 194 kHz (Figure 3). Thermal output increased with higher magnetic field intensities, with a nearly linear temperature rise over a period of 10 minutes. Similar results obtained by other research groups have shown that heating rates and SAR values increase with an increase in magnetic field strength [13, 17, 23, 24, 27]. Thus, heating can be maximized by using higher magnetic field intensities. However, for medical applications such as hyperthermia therapy, a patients' tolerance limit of the product of field strength (H) and frequency (f) has been reported by Brezovich to be 4.85×10^8 A/m-s [39].

The temperature-time data were converted to SAR values to normalize results for the various NP dispersions tested. The initial slopes of the temperature-time graph (in the linear region) were used to calculate SAR values (W/ g of iron content in NPs), with data normalized by the iron (Fe) mass in each experiment, as determined by AA (Table 1). As field strength was increased from 15.1 to 47.7 kA/m, the SAR for heating fluidMAG particles at 194 kHz increased from 3.7 to 43.3 W/g Fe (Table 2). These data support the dependence of SAR on the square of the field intensity, as is discussed in more detail later.

2.2.2 Effect of Magnetic Field Frequency—To investigate the effect of frequency independent of the field intensity, the coil was set at field strength of 38.2 kA/m while the frequency was varied from 123 to 430 kHz for heating experiments (Figure 4). Because the frequency depends strongly on coil design (number of turns, diameter, etc.), the frequencies

tested were set at values that could be obtained using the frequency modulator coil. The same fluidMAG D dispersions used for evaluating the effect of field intensity (8.6 mg/mL) were used for these experiments. The heating rate increased as the frequency increased, with a temperature rise of over 35 °C in five minutes at 430 kHz, the highest frequency setting tested. Interestingly, at 430 kHz and 38.2 kA/m (Figure 4), the five-minute temperature rise is much higher than at a lower frequency of 194 kHz but higher field strength of 47.7 kA/m (Figure 3). This demonstrates the significant role that AC field frequency has in achieving high heating rates and SAR values. The heating trend is born out in the SAR values for fluidMAG D (Table 2), with a greater than linear relationship between SAR and frequency. This is discussed later, as the data are fit to the Rosensweig equation.

2.2.3 Effect of NP Concentration—Concentration is one of the simplest variables to alter when assessing the therapeutic potential of NP dispersions for MFH. SAR values for iron oxide concentrations of 4.3 mg/mL, 6.4 mg/mL, and 8.6 mg/mL were tested for fluidMAG D NPs. This concentration range was selected so that reasonable heating could be achieved in a 10 minute time frame, without approaching the boiling point of water. Although the more concentrated NP dispersion heated faster, when normalized for comparison of SAR values, the values obtained were not statistically different (Table 3). This result is consistent with the concept of SAR, as it is normalized by the mass of NPs, and allows comparison between magnetic heating experiments of NPs with different concentrations. Similar results were obtained by de la Presa et al., who showed that SAR did not vary appreciably with concentration in the range of 6 to 300 mg/mL of iron, although there was a slight decrease in SAR when the concentration dropped below 5 mg/mL [18, 33]. Murase et al., have shown that the temperature rise for Resovist® solutions containing 45.5 mM to 115.4 mM Fe (corresponding to approximately 3.5 to 9.2 mg/mL iron oxide) follows a linear trend when exposed to 2.9 kA/m at 600 kHz [23]; this is also consistent with the SAR being independent of concentration. Thus, more concentrated NP solutions (if achievable) can deliver heat effectively, while minimizing the need for higher magnetic field strengths and frequencies. One study at low NP concentrations (below 1.2 mg/mL) showed a variance in SAR with concentration, but we discount these results due to lack of analysis for reproducibility [32].

2.2.4 Effect of NP size—Magnetite NP dispersions of four different sizes (8, 11, 13 and 18 nm TDMNPs) were heated using a 47.7 kA/m magnetic field at a frequency of 194 kHz (Table 2). Under these conditions, SAR values increased from 30.1 ± 2.1 to 75.7 ± 2.3 W/g Fe as the average NP size increased. When heated using the same 47.7 kA/m, 194 kHz field, the commercial fluidMAG particles (which have 8-9 nm magnetite cores coated with polymer) were found to have SAR values of 41 - 43 W/g Fe, which lies in between the SAR values obtained for TDMNP8 and TDMNP13 NPs.

Larger NPs displayed higher SAR values, regardless of the NP source or composition. The experimental SAR values for the 16 nm maghemite nanopowder dispersions were 249.1 ± 4.7 W/g Fe while the similarly-sized TDMNP18 dispersions had an SAR of 325.9 ± 16.0 W/g Fe when both were subjected to magnetic heating using a 38.2 kA/m, 430 kHz field. Although 13.5 nm maghemite nanoparticles have been reported to have a somewhat higher

SAR than 12.8 nm magnetite NPs [40], the 16 nm maghemite nanopowder did not heat as well as the 18 nm TDMNP18 magnetite particles. Thus, the size disparity between the TDMNP18 and maghemite nanopowder dispersions is likely more responsible for differences in heat generation than the crystalline structure of the iron oxide NPs.

Nanoparticle size is one of the primary factors investigated for optimization of SAR. It is commonly observed that for larger sized particles (slightly greater than the transition range between particles with single and multiple crystal domains), magnetic heating is amplified by an increase in the ferromagnetic nature of the particles as well as contributions of hysteresis losses, although there is yet to be a consensus on particle size optimization. The larger particle heating is supported by Hergt et al., who determined that the contribution of hysteresis losses in larger particle sizes dominate over heat generation due to Néel relaxation [21]. Ma et al. also tested magnetite NPs covering a wide range of sizes (from 7.5 nm to 416 nm), but found a midrange size (46 nm) to yield the highest SAR when heated at 32.5 kA/m and 80 kHz [15]. Li et al. [41] have demonstrated a similar size optimum in magnetite NPs where they found that 24 nm particles had the highest SAR values when subjected to magnetic fields of 9.6 to 23.9 kA/m at 100 kHz (when compared to particles ranging in size from 8 nm to 103 nm) [41]. They attributed the heating of the 24 nm particles to a combination of relaxation and hysteresis losses [41].

Nanoparticle size impacts the types of relaxation processes that can give rise to magnetic heating. For superparamagnetic particles (typically of smaller sizes), the combination of Néel and Brownian relaxation cause heating to be optimized for a particular particle composition and size, with hysteresis losses dominating relaxation processes as particle sizes increase to become ferromagnetic [21, 36]. Gonzales-Weimuller et al. used mathematical models to predict SAR for magnetite NPs ranging up to 15 nm, with the optimal NPs size being 12.5 nm at field strength of 24.5 kA/m, and a frequency of 400 kHz [24]. According to the authors, reducing the polydispersity of NPs is essential to obtain higher heating rates, and accurate size-SAR trends [24]. A more recent paper from the same research group found that 16 nm monodispersed magnetite NPs give optimal heating for a field of 14 kA/m at 373 kHz [42].

Although the largest particles tested in our experiments (18 nm) displayed the highest SAR (Table 2), there are limitations on the maximum size of NPs that can be administered to humans. For intravenous administration, particles with hydrodynamic diameters larger than 100 nm are cleared by phagocytic uptake and the liver before a significant concentration can accumulate in a target region for therapy [43]. In addition, larger particles are generally infeasible for *in vivo* applications because they tend to have lower colloidal stability as surface coatings may not sufficiently prevent aggregation [44]. Smaller sized NPs (< 10 nm) are also quickly filtered out of the bloodstream by the kidneys [43], yielding a desirable range of particles sizes for intravenous administration of approximately 10-100 nm. This size corresponds to the hydrodynamic diameter, so the particles investigated here are likely candidates that would avoid first pass clearance and reach targeted cells and tissues.

2.2.5 Effect of NP Type—For the seven different nanoparticles tested, the highest SARs were found for the 18 nm magnetite NPs synthesized in-house and the maghemite

nanopowder, with values from 250 – 325 W/g Fe when tested at 430 kHz, the highest frequency achievable in our experimental setup (Table 2). For a set field strength and frequency fluidMAG NPs and TDMNP8 NPs (both approximately 8 nm) showed a difference in heat generation capabilities. At all tested field strengths and frequencies fluidMAG NPs have higher SAR than TDMNP 8 NPs (Table 2). Even the slightly larger sized 11 nm TDMNP11 NPs have lower SAR than fluidMAG NPs. fluidMAG D and fluidMAG PAD NPs that had similar size and composition, but different surface coatings produced similar SAR values. The larger maghemite and TDMNP18 NPs with a similar size range also have a difference in SAR values. However, this change observed in SAR values can be a result of difference in composition, polydispersity, and magnetic properties of these NPs.

2.2.6 Effect of Solution Viscosity on Heating—The effect of viscosity on SAR values was investigated by preparing NP dispersions in different viscosity alginate solutions, and also in cross-linked calcium alginate gel in its hydrated state (Figure 5). For both fluidMAG D particles and maghemite nanopowder, SAR values decreased with higher solution viscosities even when the NP concentration and magnetic field parameters were kept constant. The cross-linked alginate solution provided the most rigid environment surrounding the NPs and had the lowest SAR values. By maintaining the same NP concentration in aqueous and alginate solutions, SAR should only be impacted by changes in free particle rotation or a change in solution heat capacity (which was minimal due to the use of dilute alginate solutions). The trend of decreasing SAR values with higher viscosities points to a reduction in the Brownian relaxation component of heating as found by Chen et al. for iron oxide NPs in 0.9 cP to 43.2 cP glycerol solutions [35]. Because blood and cell proteins attach rapidly to foreign objects such as magnetic NP through opsonization [45], particles that heat primarily through Brownian relaxation are not likely to be candidates for MFH therapy.

2.3 Rosensweig Equation Validation with Experimental Data

The theoretical relationship between SAR and magnetic field properties was used to evaluate magnetic heating data [19]. Rosensweig's equation is valid for predicting magnetic heating as long as the particles remain in the linear portion of hysteresis loop when the magnetic field is applied. Here, we investigated fluidMAG D NPs, and at the high frequencies tested, we assume this to be true. Since this equation (Equation 2) shows SAR to be proportional to the energy generation term, P , and P is proportional to H^2 , a plot of SAR against the square of the field strength was fit to a linear equation (Figure 7A). The correlation coefficient, R^2 , of the curve fit was 0.96, confirming that our experimental data follow Rosensweig's theory.

As long as the applied frequency is below a threshold frequency, the Rosensweig equation predicts that P is proportional to the square of the applied field frequency, which is confirmed by our experimental results (Figure 7B). Kallumadil et al., have stated that when the imaginary component of magnetic susceptibility in the Rosensweig equation is frequency-independent, SAR is directly proportional to applied frequency [19, 20]. As a few other research groups have shown through simulation and experiments [23, 27], utilizing

higher frequencies can be a viable option for improving the heating efficiency of NPs for cancer treatment, again with the caveat that the product magnetic field strength and frequency ($H \cdot f$) remains at a tolerable level for patients.

To evaluate the frequency dependence of our experimental SAR values, the Rosensweig equation was rearranged to show a double inverse linear relationship between power, $1/P$, and the square of frequency, $1/f^2$. From equation (2), π , μ_o , χ_o , H , and τ can be assumed to be constant and independent of applied frequency. Substituting $\mathbf{A} = \pi\mu_o\chi_o H^2$ and $\mathbf{B} = 2\pi\tau$, in equation (2) we obtain:

$$P = A \frac{H f^2}{1 + (B f)^2} \quad \text{Eq. (3)}$$

Inverting equation (3) we get:

$$\frac{1}{P} = \frac{1 + B^2 f^2}{A B f^2} \quad \text{Eq. (4)}$$

which can then be rearranged to relate power and frequency as:

$$\frac{1}{P} = \frac{1}{A B f^2} + \frac{B}{A} \quad \text{Eq. (5)}$$

Fitting our data to this equation, we obtain a linear graph with a R^2 value of 0.97 (Figure 7B), confirming that the results are in agreement with Rosensweig's theory.

Conclusions

A number of physical and chemical factors impact magnetic heating of iron oxide NPs. Magnetic fields with intensities ranging from 15 to 48 kA/m and covering a range from 123 to 430 kHz were investigated, along with seven different nanoparticle formulations representing a range of particle sizes (8 to 18 nm), coatings (oleic acid, dextran, polyacrylamide, or none), and compositions (Fe_2O_3 and Fe_3O_4). The NP concentration was not found to influence SAR in the concentration range studied. Thus, thermal power input can be maximized by localizing high concentrations of NPs. For magnetite NPs synthesized in the range of 8 to 18 nm, the largest particles displayed the highest SAR values. Comparing the different types of NPs it was seen that TDMNP18 magnetite NPs and maghemite nanopowder had the highest SAR values. Based on other reports in the literature, the optimal size for radio frequency heating of magnetite NPs is likely in the neighborhood of 16 nm [42]. Frequency tuning, whereby different magnetic field frequencies could be generated with the same field intensity, allowed testing of the same nanoparticle dispersions over a range of frequencies. Operating at higher frequencies achieved higher SAR values, as predicted by the Rosensweig equation. Even with the successful heating of magnetite and maghemite at 430 kHz and 38.2 kA/m, patient tolerance of magnetic fields will likely require NP optimization to achieve high SAR values while staying below an $H \cdot f$ product of 4.85×10^8 A/m-s, as described by Brezovich [39], or reaching high local NP concentrations.

Despite limitations due to patient tolerance, it has been suggested that this criterion can be exceeded for treating more severe types of cancer when high heating efficiency is needed [29]; there are also reports of commercial hyperthermia treatment equipment which operate beyond this range [46].

The viscosity of the solution surrounding NPs has a deleterious effect on magnetic heating. The SAR values for fluidMAG D NPs and maghemite nanopowder decreased when the NPs were dispersed in more viscous environments due to a reduction in the Brownian relaxation component of magnetic heating. The reduced heating in viscous solutions (which simulate the extracellular matrix) has implications for the use of magnetic NPs for hyperthermia therapy. SAR values were reduced to a fraction of the SARs observed in free solution (which is used by most researchers to evaluate magnetic hyperthermia). Evaluation of NP heating in viscosities matching the desired application is therefore highly recommended.

Acknowledgments

The authors gratefully acknowledge the support of National Cancer Institute under NIH grant R21CA141388, and The University of Alabama. They also thank Dr. Yuping Bao for use of dynamic light scattering equipment in her lab. This work used instruments in the Central Analytical facility, which is supported by The University of Alabama.

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Highlights of this paper

- investigates magnetic heating through the specific absorption rate for seven different iron oxide nanoparticles using field strengths ranging from 15-48 kA/m and frequencies from 123 to 430 kHz, with the capability of adjusting frequency independent of field strength;
- confirms an optimal size for nanoparticle heating that agrees with others in the literature;
- experimentally verifies Rosenweig's equation to predict the effect of field frequency on heating; and
- reports a reduction in heating when magnetic nanoparticles are in a high viscosity environment.

Figure 1.
Hyperthermia coil experimental set-up.

Figure 2.
Representative TEMs of particles investigated: A) fluidMAG D NPs, B) TDMNP11 NPs, C) TDMNP18 NPs, D) maghemite nanopowder.

Figure 3.
XPS spectra used to determine iron oxide structure of fluidMAG D, maghemite nanopowder, TDMNP 18, TDMNP 13, and TDMNP 11 NPs.

Figure 4.

Effect of magnetic field strength on temperature rise for fluidMAG D NPs ($f = 194$ kHz, $[NP] = 8.6$ mg/mL).

Figure 5.
Effect of magnetic field frequency on temperature rise for fluidMAG D NPs ($H = 38.2$ kA/m, $[NP] = 8.6$ mg/mL).

Figure 6.

Effect of viscosity on SAR values for fluidMAG D and maghemite nanopowder dispersed in water and various alginate solutions ($H = 38.2$ kA/m, $f = 430$ kHz, [fluidMAG D] = 8.6 mg/mL, [Maghemite Nanopowder] = 1.6 mg/mL). fluidMAG D was not tested at 481 cP.

7A)

7B)

Figure 7.

Linear fit of SAR data to fit Rosensweig's equation. SAR values from magnetic heating data for fluidMAG D experiments conducted at $f = 194$ kHz were plotted as a function of H^2 in Figure 6A. In Figure 6B, heating data with variable frequency at a fixed $H = 38.2$ kA/m were plotted according to equation (5).

Table 1

Properties of NPs investigated for magnetic heating

Nanoparticles	Particle ID	Iron Oxide Concentrations a (mg/mL) ^a	Solvent	Iron Oxide Diameter TEM (nm) ^b	Hydrodynamic Diameter DLS (nm)	Magnetization at 10,000 Oe M (emu/g) ^e	Magnetic Susceptibility χ_o ^e
Magnetite coated with starch	fluidMAG D	4.3 ± 0.1	Water	8 ± 2	19 ± 7 ^c ; 60 ^d	80 ± 6	0.12 ± 0.03
		6.4 ± 0.1					
		8.6 ± 0.1					
Magnetite coated with polyacrylamide	fluidMAG PAD	8.3 ± 0.2	Water	9 ± 2	29 ± 9 ^c ; 55 ^d	46 ± 1	0.05 ± 0.01
Magnetite coated with oleic acid	TDMNP8	6.2 ± 0.1	Hexane	8 ± 2	<i>f</i>	<i>f</i>	<i>f</i>
	TDMNP11	10.8 ± 0.2		11 ± 2	<i>f</i>	50	0.04
	TDMNP13	6.4 ± 0.0		13 ± 4	<i>f</i>	<i>f</i>	<i>f</i>
	TDMNP18	8.1 ± 0.0		18 ± 4	<i>f</i>	67	0.13
Maghemite without coating	Maghemite nanopowder	1.6 ± 0.0	Water	16 ± 9	35 ± 10 ^c ; 54 ^d	57 ^g	0.11 ^g

^aConcentration and standard deviation determined by AA spectroscopy^bMean diameter ± one standard deviation for analysis of more than 100 particles using Image J software on TEM images (Figure 1)^cNumber average diameter ± one standard deviation measured by DLS^dZ-average diameter reported from DLS Zetasizer software^eDetermined using vibrating sample magnetometry^fSample not evaluated^gMeasured using non-dispersed dry maghemite nanopowder

Table 2

SAR values for Magnetic Heating of NPs at Fixed Field Strengths and Frequencies

Nanoparticle Type	Field Strength, H (kA/m)	Frequency, f (kHz)	SAR (W/g Fe)	SAR (W/g NP) ^a
fluidMAG D (8.6 mg/mL)	15.1	194	3.7 ± 1.2	2.7 ± 0.9
	22.8		15.3 ± 2.9	11.1 ± 2.1
	35.8		26.8 ± 2.9	19.4 ± 2.1
	38.2		29.9 ± 1.0	21.6 ± 0.7
	47.7		43.3 ± 4.4	31.3 ± 3.2
	38.2	123	15.1 ± 2.1	10.9 ± 1.5
		143	23.1 ± 2.8	16.7 ± 2.0
		194	29.9 ± 1.0	21.6 ± 0.7
		430	105.6 ± 5.0	76.4 ± 3.6
fluidMAG PAD	47.7	194	41.7 ± 2.3	30.2 ± 1.7
TDMNP8	22.8	194	6.5 ± 1.5	4.7 ± 1.1
	35.8		14.6 ± 1.5	10.6 ± 1.1
	47.7		30.1 ± 2.1	21.8 ± 1.5
TDMNP11	47.7	194	33.4 ± 1.2	24.2 ± 0.9
TDMNP13	47.7	194	52.8 ± 4.3	38.2 ± 3.1
TDMNP18	47.7	194	75.7 ± 2.3	54.8 ± 1.7
	38.2	430	325.9 ± 16.0	235.8 ± 11.6
Maghemite NP	38.2	430	249.1 ± 4.7	174.2 ± 3.3

^aIron oxide crystal structure determined by XPS for normalizing SAR values per g NP

Table 3

Effect on NP concentration on SAR from magnetic heating of fluidMAG D

Magnetite Concentration (mg/mL)	SAR (W/g Fe)	SAR (W/g NP) ^a
4.3	45.5 ± 6.4	32.9 ± 4.6
6.4	43.9 ± 3.2	31.8 ± 2.3
8.6	43.3 ± 4.4	31.3 ± 3.2

^a Iron oxide crystal structure determined by XPS for normalizing SAR values per g NP