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Optimization of synthesis and peptization steps to obtain iron oxide nanoparticles with high energy dissipation rates

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Abstract

Magnetic Fluid Hyperthermia (MFH) uses heat generated by magnetic nanoparticles exposed to alternating magnetic fields to cause a temperature increase in tumors to the hyperthermia range (43–47 °C), inducing apoptotic cancer cell death. As with all cancer nanomedicines, one of the most significant challenges with MFH is achieving high nanoparticle accumulation at the tumor site. This motivates development of synthesis strategies that maximize the rate of energy dissipation of iron oxide magnetic nanoparticles, preferable due to their intrinsic biocompatibility. This has led to development of synthesis strategies that, although attractive from the point of view of chemical elegance, may not be suitable for scale-up to quantities necessary for clinical use. On the other hand, to date the aqueous co-precipitation synthesis, which readily yields gram quantities of nanoparticles, has only been reported to yield sufficiently high specific absorption rates after laborious size selective fractionation. This work focuses on improvements to the aqueous co-precipitation of iron oxide nanoparticles to increase the specific absorption rate (SAR), by optimizing synthesis conditions and the subsequent peptization step. Heating efficiencies up to 1,048 W/g_{Fe} (36.5 kA/m, 341 kHz; ILP = 2.3 nH·m²·kg⁻¹) were obtained, which represent one of the highest values reported for iron oxide particles synthesized by co-precipitation without size-selective fractionation. Furthermore, particles reached SAR values of up to 719 W/g_{Fe} (36.5 kA/m, 341 kHz; ILP = 1.6 nH·m²·kg⁻¹) when in a solid matrix, demonstrating they were capable of significant rates of energy dissipation even when restricted from physical rotation. Reduction in

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Disclosure

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energy dissipation rate due to immobilization has been identified as an obstacle to clinical translation of MFH. Hence, particles obtained with the conditions reported here have great potential for application in nanoscale thermal cancer therapy.

Keywords

Specific absorption rate; magnetic nanoparticles; co-precipitation; peptization; ultra-sonication; tetramethylammonium hydroxide; magnetic fluid hyperthermia; optimization

1. Introduction

Magnetic Fluid Hyperthermia (MFH) as a cancer treatment uses the heat that results from exposing magnetic nanoparticles to alternating magnetic fields. The objective in this approach is to achieve a temperature rise to 43 – 47°C in small regions affected by cancer where nanoparticles have been deposited [1]. As a cancer therapy, MFH takes advantage of the increased sensitivity of cancer cells to heat, compared to their healthy counterparts [2]. Recent studies explain how vascularization of tumors can be stimulated by heat, improving the efficiency of chemotherapy and radiotherapy [3]. Although promising, there are still significant challenges to achieving the potential of MFH in treating cancer, of which the most pressing appear to be improving nanoparticle accumulation in tumor sites, achieving controlled and uniform temperature rise throughout the tumor tissue, and obtaining nanoparticles with predictable performance in the tumor environment [1,4]. Recently, various groups have reported efforts to improve the heat dissipation rates of magnetic nanoparticles, in an effort to reduce the amount of particles needed to achieve a desired temperature rise in tumors *in vivo*. In these studies, the energy dissipation rate performance of the nanoparticles is typically reported as the specific absorption rate (SAR), which depends on the amplitude and frequency of the magnetic field used during actuation. In order to avoid ambiguity, the SAR value can be normalized with respect to the frequency and intensity of the applied magnetic field, facilitating comparison of measurements carried out under different field conditions. This normalized energy dissipation rate is called the intrinsic loss power (ILP) [5]. There have been many recent excellent studies aiming to obtain magnetic nanoparticles with high SAR and ILP values, through control of particle clustering, shape, and/or internal exchange interactions. Typical ILP values for iron oxide nanoparticles are usually below 5 and 12 nH·m²·kg⁻¹ for commercial and synthetic preparations, respectively [5]. For example, superparamagnetic iron oxide nanocubes with a wide range of sizes have been reported with SAR values of up to 2,277 W/g_{Fe} (700 kHz, 24 kA/m, ILP = 5.7 nH·m²·kg⁻¹). However, despite their high SAR the authors state that the synthesis of nanocubes in a wide size range is not straightforward [6], which could limit their biomedical potential. Improvements in both heating power and MRI relaxivity have been achieved by formation of clusters or aggregates of single core cube-shaped nanoparticles [7]. In some cases ferromagnetic nanocube aggregates have been encapsulated in biocompatible polymers, achieving SAR values up to 2,614 W/g_{Fe} (1,000 kHz, 0.66 kA/m, ILP = 6,000 nH·m²·kg⁻¹), but aggregation is a major obstacle to their use *in vivo* [8,9]. However, according to the authors, this exceptionally high SAR was achieved using an alternating magnetic field with frequency of 1,000 kHz and amplitude of 0.66 kA/m,

which yields a highly unlikely ILP value of $6,000 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$. Bacterial magnetosomes have been proposed as an alternative to the preparation of magnetic materials using chemical methods. For example, magnetosomes consisting of chain-like aggregates of iron oxide nanoparticles have resulted in SAR values of $875 \text{ W/g}_{\text{Fe}}$ (183 kHz, 32 kA/m, ILP = $4.7 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$). However, the isolation of individual magnetosomes from these chain-like aggregates remains a challenge to their clinical application [10]. An alternative approach is to tune heat dissipation rate by introducing exchange-coupling in nanoparticles with core-shell structure. Core-shell nanoparticles with a CoFe_2O_4 core and Fe_3O_4 shell were reported to have SAR values of up to $1,120 \text{ W/g}_{\text{Fe}}$ (500 kHz, 37.3 kA/m, ILP = $1.6 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$) [11]. However, to achieve this effect the biocompatibility of the nanoparticles is compromised by the introduction of cobalt into the structure. Other nanoparticle shapes have been reported to yield high energy dissipation rates. Maghemite nanoflowers have been reported with SAR values of $\sim 1,900 \text{ W/g}_{\text{Fe}}$ (700 kHz, 21.5 kA/m, ILP = $5.9 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$) using a modified thermal decomposition procedure [12]. Similarly to nanoflowers, maghemite assemblies with superparamagnetic behavior have been reported to have SAR values of $\sim 1,500 \text{ W/g}_{\text{Fe}}$ (520 kHz, 29 kA/m, ILP = $3.4 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$). The role and benefits of multi-core interactions can be explained in terms of reduced magnetocrystalline anisotropy and enhanced magnetic moment which helps preserve the superparamagnetic behavior of the clusters, potentiating thermal losses [13]. Finally, and perhaps of greatest relevance to the work reported here, Fortin *et al* reported maghemite nanoparticles obtained using a post synthesis size-sorting step which had SAR values of up to $1,650 \text{ W/g}$ (24.8 kA/m, 700 kHz, ILP = $3.8 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$). However, without the post-synthesis treatment, the study revealed that SAR dropped to 135 W/g (ILP = $0.98 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$) [14].

Despite the overall high heating efficiency provided by exchange-coupled, size-sorted and clusters of nanoparticles with different morphologies, the experimental protocols used to obtain these particles are often complex, time consuming, and can face challenges in scalability. In contrast, it is widely known that a simple, inexpensive, and efficient method for the synthesis of magnetic nanoparticles is the co-precipitation of iron salts in alkaline media under an inert atmosphere, attributed to Massart [15]. The main advantage of the co-precipitation method is that a single synthesis batch easily yields gram quantities of nanoparticles. The SAR of iron oxide nanoparticles obtained by co-precipitation fluctuates in a wide range of values, typically from $40 - 300 \text{ W/g}_{\text{Fe}}$ [16–18] for co-precipitation method without post-synthesis size-selective treatments, and about $10 - 100 \text{ W/g}_{\text{Fe}}$ for commercial nanoparticle preparations [5,19] This wide range of values and the variability in the co-precipitation synthesis represent major limitations to eventual clinical application if high energy dissipation rates cannot be consistently obtained.

There have been various studies aiming to optimize magnetic nanoparticle synthesis by the aqueous co-precipitation route, with the aim of controlling the physical size of the inorganic iron oxide cores. Among these studies, different parameters have been considered such as media composition, reactant injection rates, temperature, stirring rates, pH, molar ratios, etc. [20–22]. However, although energy dissipation rates are expected to be a function of the physical size and magnetic properties of the iron oxide nanoparticles, systematic

optimization studies of the co-precipitation synthesis to achieve high energy dissipation rates are still lacking.

Among the many examples of preparation of iron oxide nanoparticles by the co-precipitation method we found that to date the highest value obtained without any post-synthesis size-fractionation appears to be that reported by Kossatz *et al* [23], who reported SAR values of 900 W/g_{Fe} (435 kHz, 15.4 kA/m, ILP = 8.7 nH·m²·kg⁻¹) and 650 W/g_{Fe} (435 kHz, 15.4 kA/m, ILP = 6.3 nH·m²·kg⁻¹) for iron oxide nanoparticles suspended in water and agar, respectively, using a post-synthesis peptization step with kerosene at high temperature. These energy dissipation rates are higher than other commonly reported values in the range of 40 to 300 W/g_{Fe} (ILP ~ 1 to 3 nH·m²·kg⁻¹). Unfortunately, Kossatz *et al.* do not explain the reasons behind their enhanced energy dissipation rate, or what they did differently from other groups in terms of experimental protocols. In addition, the lack of magnetic characterizations makes it difficult to compare the magnetic behavior of their particles to those of others and to evaluate its impact on the observed SAR values.

Motivated by a need for improvement of the co-precipitation synthesis to obtain particles with high energy dissipation rates, the present work demonstrates that high energy dissipating nanoparticles can be reproducibly obtained by optimizing the co-precipitation synthesis conditions and the subsequent peptization step. Three parameters were studied for optimization purposes: temperature, iron ion precursor concentration, and sonication conditions during peptization. These parameters were chosen based on results from our own preliminary experiments and based on observations described elsewhere [24,25]. The preliminary experiments showed that by simultaneously increasing the synthesis temperature and using ultra-sonication during the peptization step at a fixed iron concentration, higher SAR values could be obtained. A series of nanoparticle synthesis batches were synthesized by varying the above parameters between pre-set levels, and the combination of these levels and their factors was studied using SAR as the response variable. Optimal results were obtained when the synthesis temperature was 85°C, total iron concentration was 0.30 M, and when ultra-sonication was used during the peptization step. As for the specific absorption rate, values of up to 1,048 W/g_{Fe} (341 kHz, 36.5 kA/m, ILP = 2.3 nH·m²·kg⁻¹) were obtained. When fixed in agarose these particles reached up to 719 W/g_{Fe} (341 kHz, 36.5 kA/m, ILP = 1.6 nH·m²·kg⁻¹), demonstrating that they can dissipate a high amount of energy even when they are restricted from physical rotation. These results were confirmed by conducting additional experiments under the optimal conditions, and high SAR values were consistently obtained.

2. Materials and methods

2.1. Materials

Iron (II) chloride tetrahydrate (FeCl₂·4H₂O) 99%, iron (III) chloride hexahydrate (FeCl₃·6H₂O) 99%, tetramethylammonium hydroxide (TMAOH) 25% w/v, agarose high melting point, hydroxylamine hydrochloride, sodium acetate, 1,10-phenantroline monohydrate and iron standard (Cat. #56209) were purchased from Sigma-Aldrich. Ammonium hydroxide (NH₄OH) 29% v/v and nitric acid 70% v/v (Optima Grade) were purchased from Fisher Scientific. All reagents were used as received.

2.2. Synthesis of iron oxide nanoparticles

Iron oxide nanoparticles were synthesized through the co-precipitation method using a scheme similar to that described by Herrera *et al* [26]. The method was modified slightly to incorporate the effect of the studied factors. Briefly, deionized water was degassed with nitrogen for 30 minutes and used to prepare aqueous solutions with different concentrations of iron (II) and iron (III) salts. The total iron concentration ($\text{Fe}^{2+} + \text{Fe}^{3+}$) was varied between experiments, but the molar ratio $\text{Fe}^{3+}:\text{Fe}^{2+}$ was kept constant at 2:1. Iron solutions were sonicated in a bath for 20 min, degassed for 5 minutes, and mixed in a small 250 mL glass reactor. The solution was heated and then NH_4OH was added to the reactor, followed by one-hour reaction at the studied temperatures (80, 85 or 90°C). The pH was kept between 8.0 and 9 by adding small aliquots of NH_4OH throughout the reaction. The resultant iron oxide solution was cooled to room temperature, poured into conical tubes, centrifuged, and then magnetically decanted. All syntheses were run in duplicate.

2.3. Peptization of iron oxide nanoparticles

Three different schemes were used for peptization of nanoparticles, using tetramethylammonium hydroxide (TMAOH) as the peptizing agent. TMAOH was added to conical tubes containing the iron oxide (IO) nanoparticle precipitate using different IO/TMAOH volume ratios (0.5, 1 and 2). Tubes were homogenized by vortexing to disperse the iron oxide nanoparticles in the peptizing agent. Peptization was accompanied by sonication, using either a bath sonicator (FS110D, Fisher Scientific) or an ultrasound probe (XL2020, Misonix Inc.) during 0, 20 and 60 minutes as studied during preliminary experiments. Peptized nanoparticles were centrifuged and magnetically decanted, and then the obtained colloid was air-dried overnight. Afterwards, dried nanoparticles were suspended in water to yield aqueous nanoparticle solutions at a concentration of 100 mg/mL, which were used as stock solutions for further use. These suspensions were stored at 4°C.

2.4. Nanoparticle characterization

The stock solutions were used to prepare diluted nanoparticle suspensions for characterization purposes, using ultra-sonication for 20 minutes after the diluted suspension was prepared. Most of these characterizations were carried out with suspensions of 10 mg/mL, except for transmission electron microscopy (TEM) and dynamic light scattering (DLS), which used concentrations of 60 and 0.1 mg/mL, respectively. In addition, bath-sonication of samples was carried out for at least 20 minutes prior to each characterization technique.

2.4.1. DLS measurements—Hydrodynamic diameters were determined through room temperature dynamic light scattering (DLS), using a particle size analyzer (Zeta PALS, Brookhaven Instruments). Aqueous nanoparticle samples (500 μL) with concentration between 0.1 and 1 mg/mL were filtered using a 0.2 μm nylon syringe filter prior to measurements. The log-normal distribution of the volume-weighted measurements was used to obtain the mean hydrodynamic diameter and geometric deviation.

2.4.2. Dynamic magnetic susceptibility measurements—The mechanism of magnetic relaxation of the nanoparticles was evaluated via dynamic magnetic susceptibility

(DMS) measurements using a susceptometer (DynoMag, Imego), in a frequency range of 1 Hz to 160 kHz at an applied field amplitude of 0.5 mT (5 G). A 200 μ L aliquot of aqueous nanoparticle suspension was used for analysis of liquid samples; the same volume was used for nanoparticles fixed in a 1.5% w/v agarose solution to analyze particles with restricted physical rotation. The agarose solution was prepared by dissolving 3 mg of high-melting point agarose in 200 μ L of aqueous nanoparticle suspension (10 mg/mL) in a glass vial. The vial was placed in a bath-sonicator at 65°C for 1 hour, to allow complete dissolution of agarose. The vials were then placed in an ice bath for a few seconds and then left to reach room temperature to obtain a solid matrix.

2.4.3. Magnetic measurements—Equilibrium magnetic measurements were performed using a superconducting quantum interference device (SQUID) magnetometer (MPMS3, Quantum Design). One hundred microliters of sample were used to obtain magnetization curves at 300 K and magnetic field between -7 and 7 T. From these curves, the experimental saturation magnetization was obtained by averaging seven points in the maximum magnetization region. The magnetic diameter of the nanoparticles was obtained by fitting the experimental data to the Langevin model weighted using a lognormal size distribution. The blocking temperature of the samples was obtained from so-called zero-field cooled (ZFC) and field cooled (FC) curves of temperature dependence of the magnetization at 10 Oe, in the temperature range of 4 to 400 K. Nanoparticles in powder form (10 mg) were used for this purpose. The blocking temperature was determined from the maximum of the ZFC curve, obtained using a polynomial fit to the data in that region.

2.4.4. Iron quantification—The iron content in liquid suspensions of nanoparticles was determined using a colorimetric assay based on complexation of Fe^{2+} with 1,10-phenanthroline. Ten microliters of aqueous nanoparticle suspension (10 mg/mL) were digested overnight at 101°C with 1 mL of 70% nitric acid (Optima Grade, Fisher Scientific) using a dry block. Subsequently, ten microliters of digested sample were evaporated at 115°C for 30 minutes, followed by addition of 46 μ L of deionized water and 30 μ L of hydroxylamine hydrochloride (8.06M). The mixture was left to react for 1 hour to reduce Fe^{3+} to Fe^{2+} . After iron reduction, 49 μ L of sodium acetate (1.22 M) was added, followed by 75 μ L of 1,10-phenanthroline monohydrate (13 mM). One hundred microliters of each sample were run in a UV-Vis spectrophotometer (UV-2600, Shimadzu Scientific Instruments, Inc.) at 508 nm. The obtained absorbance values were used to determine the iron concentration, using a standard calibration curve. Samples and calibration standards were run in triplicate.

2.4.5. Energy Dissipation Rates and Specific Absorption Rate—The rate of energy dissipation of the nanoparticles (both in liquid and solid matrices) was obtained by exposing samples to an alternating magnetic field produced by the coil of an induction heater (EasyHeat 8310 LI, Ambrell). Liquid samples consisted of 200 μ L of aqueous nanoparticle suspension in a glass vial placed inside a glass test tube. The test tube was then positioned inside an eight-loop coil (1.2" diameter $\times$ 3.25" length). A cooling jacket was used to thermally isolate the sample from the coil and to control the starting temperature of the measurement. A fiber optic temperature probe was used to record the temperature rise of

the sample at 0.2 s intervals after the magnetic field was turned on. Another probe recorded the temperature of the coil, and during all experiments the coil temperature was lower than the sample and jacket temperature. All measurements were run in triplicate, at 36.5 kA/m and 341 kHz. Changes in temperature of the samples were recorded for a total of 120 seconds. Blanks were run to verify that there was no indirect heating of the samples. There is debate in the literature regarding the most appropriate method of quantifying the specific absorption rate (SAR) of magnetic nanoparticles. Some authors contend that measurements must be done under adiabatic conditions to ensure the measurement is accurate [27], whereas others have attempted to quantify the relative rates of energy dissipation by the particles and loss to the surroundings [28]. In our case we follow the recommendations of Fortin *et al* who indicated that because thermal exchange with the surroundings is negligible for a sample in thermal equilibrium the SAR can be readily obtained from the initial rate of temperature rise immediately after turning on the alternating magnetic field [14]. As such, we calculated SAR according to the relation

$$SAR = C_p \frac{m_s}{m_{Fe}} \left. \frac{\Delta T}{\Delta t} \right|_{t \rightarrow 0} \quad (1)$$

Where m_s is the mass of the sample, m_{Fe} is the mass of iron in the sample, C_p is the heat capacity of the solvent, which in the case of liquid samples was water ($C_p = 4.185 \text{ J/}^\circ\text{C}\cdot\text{g}$) and in the case of solid samples was agarose 1.5% w/v ($C_p = 3.90 \text{ J/}^\circ\text{C}\cdot\text{g}$) [29], and $\Delta T / \Delta t$ is the initial slope of the time-dependent heating curve. Solid samples were prepared as described in section 2.4.2, since prior to SAR determination, DMS measurements were carried out to confirm that most of the Brownian particles were restricted from physical rotation. The iron concentration was obtained through the colorimetric assay.

2.4.6. Transmission Electron Microscopy—The size and shape of the nanoparticle inorganic cores were determined via transmission electron microscopy (TEM), using nanoparticle suspensions with concentrations of 60 mg/mL. Carbon-coated copper grids were immersed in the nanoparticle suspensions and dried in an oven at 60°C for 15 minutes. Images were recorded using a transmission electron microscope (200CX, JEOL) and analyzed by counting at least 200 particles per image (ImageJ 1.48v, NIH). A log-normal size distribution was used to obtain the number-weighted mean core diameter and geometric deviation.

2.5 Experimental Design and Statistical Analysis

A full factorial experimental design with three factors and three center points was used to study the contribution of the studied factors. For this design, a total of 15 runs was considered for each combination of factors and their levels, and each run was conducted in duplicate (A and B). Factors and their levels are summarized in Table 1, and the complete set of experimental factor-level combinations is indicated in Table 2. Data were analyzed by means of the Surface Response Method (RSM) using the SAR value as response variable, and the objective of the optimization routine was to determine the optimal conditions to obtain SAR values as high as possible. R3.1.1 was used to process data and obtain all statistical information [30].

3. Results and Discussion

3.1. Particle size

Nanoparticle core diameters ranging from 12 to 20 nm were obtained from different syntheses performed according to the experimental design (see Table SI-1 for a summary). Figure 1 illustrates TEM images and histograms for three representative runs, corresponding to nanoparticles with low, intermediate, and high energy dissipation rates. In the figure, D_p represents the number weighted mean diameter and $\ln\sigma$ is the geometric deviation of the lognormal distribution. As is usual with the co-precipitation synthesis, the nanoparticles were roughly spherical, somewhat polydisperse, and there was evidence of small aggregates. However, it was also evident that changing synthesis and peptization conditions led to changes in the primary particle size and the prevalence of aggregates in the samples. Moreover, when comparing the TEM images shown in Figure 1 to those obtained by our group in past syntheses which were conducted without the aforementioned changes, the reduction in the size of aggregates became evident, especially when strong sonication conditions were used in the peptization step.

DLS measurements indicated the nanoparticles had volume-weighted hydrodynamic diameters ranging between 29 and 58 nm (see Table SI-1) depending on synthesis and peptization conditions. We note that the diameters obtained from TEM are number-weighted, a factor that must be taken into account when comparing to the volume-weighted hydrodynamic diameters. As examples, for sample 5B the corresponding volume-weighted core diameter was 25 nm, which is smaller than the corresponding volume weighted hydrodynamic diameter of 38 nm. Similarly, for samples 11B and 15B the volume-weighted physical diameters were 17 nm and 32 nm, and the volume-weighted hydrodynamic diameters were 36 nm and 58 nm, respectively. Because the TMAOH treatment should not significantly contribute to the hydrodynamic diameter of the particles, we attribute these larger hydrodynamic diameters to the presence of small aggregates of two or three individual particles. Similar observations apply to samples obtained under other conditions.

The smallest hydrodynamic diameters were obtained for batches with low iron concentration, in agreement with previous observations by Forge *et al* [22]. Overall, hydrodynamic diameters were lower when compared to those reported for nanoparticles peptized with TMOAH without any polymer coating [24]. The reduction in hydrodynamic diameter confirms that the use of ultra-sonication during peptization was effective in breaking larger aggregates, improving dispersion of particles. TMAOH aqueous solutions are comprised by $N(CH_3)_4^+$ cations and OH^- anions; the later coat the surface of nanoparticles while attracting the $N(CH_3)_4^+$ cations by adsorption. This creates a diffuse shell around each particle, leading to a net electrostatic repulsion between adjacent particles which raise the energy required for the particles to agglomerate, yielding a stable colloid. As for the ultra-sonication times it was determined that 20 minutes reduced the hydrodynamic diameter to more than one-half the value obtained at 0 minutes (e.g. without ultra-sonication). By increasing the ultra-sonication time to 60 minutes, no significant changes in hydrodynamic diameter were observed when compared to that of 20 minutes (see Figure SI-1).

2.2 Magnetic behavior

Room temperature magnetic characterization revealed nanoparticles with saturation magnetizations that were close (>83%) to the bulk value for iron, which is approximately 124.4 emu/g_{Fe} [31]. This implies that the method used for iron quantification was accurate. Equilibrium magnetization curves for all samples, illustrated in Figure 2a for selected samples, indicated the particles were superparamagnetic at room temperature, as no remanent magnetization was observed. By fitting the experimental magnetization values to the Langevin model weighted using a lognormal size distribution [32], the magnetic diameter (D_m) was obtained, with values ranging from 9 – 14 nm (see Table SI-2). Saturation magnetization values decreased as particles became smaller, in agreement with previous reports [33,34]. One possible cause is increased contribution of canting of magnetic moments on the surface of the particles [35]. Figure 2b depicts the temperature dependent magnetization under zero field-cooled (ZFC) and field-cooled (FC) conditions between 4 and 400 K. In general terms, and as expected, an increase in blocking temperature (T_B) was observed as particle size increased. For example, the blocking temperature was 264K for particles with a physical diameter of 12 nm, and higher than 400K for particles with a physical diameter of 20 nm. An increase in blocking temperature is expected as particle size increases due to the exponential dependence on crystal volume of the blocking temperature [36].

It is interesting to note that although equilibrium magnetization measurements indicated all samples are superparamagnetic at room temperature, blocking temperature values for some samples were higher than 300 K. The blocking temperature is often interpreted as the temperature below which a collection of nanoparticles ceases to behave as a superparamagnetic material. The reason why these two techniques yield apparently conflicting results is due to the time scales involved in each measurement, with the time scale for the ZFC-FC measurement (14 s) being shorter than the time scale than the equilibrium magnetization measurement (23 s). Hence, the blocking temperatures imply that for some samples at room temperature there is a non-negligible fraction of nanoparticles that are thermally blocked (not superparamagnetic), and should therefore contribute a Brownian relaxation component to their response to alternating magnetic fields. This was confirmed through DMS measurements, which indicated that all synthesized particles had a Brownian relaxation peak of varying intensity. Representative results are shown in Figure 3, for two synthesis runs (9B and 12B). The presence of a peak in the out-of-phase (imaginary) component of the susceptibility at frequencies near 100 kHz (Brownian peak) was observed in all the runs, but with different peak susceptibility intensities. For particles synthesized at low iron concentration, the height of the Brownian peak decreased, while the in-phase (real) component of the susceptibility did not appear to change significantly. For run 9B, with hydrodynamic diameter of ~29 nm and total iron concentration of 0.08M, the flat-shaped Brownian peak rises barely above 2×10^{-4} units of magnetic susceptibility. However, for run 12B with total iron concentration of 1.0M and hydrodynamic diameter of ~49 nm, the Brownian peak is well defined and reaches more than 1×10^{-3} units of magnetic susceptibility. The distance between the curves of real and imaginary parts of the susceptibility was markedly smaller as the particle size increased, indicating a larger contribution of the Brownian relaxation mechanism.

Hydrodynamic diameters were also determined by fitting the Debye model to DMS measurements, using the in-phase (χ') and out-of-phase (χ'') components of the dynamic susceptibility. Good agreement was observed when comparing hydrodynamic diameters obtained from DLS and DMS measurements, as depicted in Figure 4. Data points positioned along the 45° diagonal indicate quantitative agreement between hydrodynamic diameters determined from DLS and DMS measurements. It is important to note that hydrodynamic diameters obtained from χ' and χ'' were not always the same even when the same model was applied to fit experimental data. These differences occur because the mathematical expression for χ'' has a numerator that is directly proportional to the cube of the hydrodynamic diameter, thus making χ'' more sensitive to changes in values of the hydrodynamic diameter relative to χ' [37].

3.3 SAR measurements in liquid suspensions

The SAR was calculated using the initial slope of the temperature profiles obtained under the application of the alternating magnetic field, and then normalized by iron content, according to Eq. (1). All measurements were carried out at a field amplitude of $H_0 = 36.5$ kA/m and frequency of $f = 341$ kHz. Figure 5 shows representative heating ramps of the aqueous nanoparticle suspensions including the temperature profiles of the induction coil and blank (deionized water). The initial temperature for all measurements was 25 °C, which as shown in Figure 5a remained unaltered for the blank in all of the measurements, indicating that the sample holder was thermally isolated from the coil. The initial slope is depicted as a thick red line and was obtained by fitting a linear model to the experimental temperatures recorded during the first few seconds of heat generation. As shown in Figure 5b, temperature increments of up to 65 °C (corresponding to a final temperature of 90°C) were recorded for samples with the highest initial rate of temperature rise (0.93°C/s), whereas temperature increments of less than 10°C were observed for other samples, for example those prepared with low iron concentration. As with many of the magnetic properties, the initial rate of temperature rise increased with particle size, with run 15B having the highest particle size, the highest saturation magnetization, and the steepest initial rate of temperature rise. This is in agreement with previous work where the proportionality between particle size and rate of heating has been reported [17].

A wide range of SAR values were obtained (Table SI-2), and depended on the combination of studied factors and their levels. Figure 6 shows SAR values for all synthesis runs (1 – 15), segregated into four different groups. These groups were studied independently in order to understand how SAR was affected by controlling synthesis and peptization conditions. The first group (blue bars) comprises SAR values obtained when working with a fixed initial iron concentration of 0.30 M. It can be noticed that SAR remains virtually unaltered by increasing the synthesis temperature from 80°C to 90°C while keeping the same type of sonication (runs 1 and 3), but there was an improvement when ultra-sonication was used during peptization (runs 2 and 4).. The use of stronger amplitudes during ultra-sonication along with higher amounts of peptizing agent, when compared to those achieved with bath-sonication, appeared to improve rupture of particle aggregates. From preliminary experiments we learned that when ultra-sonication was applied after the peptization step, there was an improvement in the rupture of particle aggregates, but less improvement on

SAR. We hypothesized that by using ultra-sonication during the peptization step, the rupture of large aggregates would take place simultaneously with the adsorption of tetramethylammonium cations to the surface of the nanoparticles. In this scenario, by having smaller aggregates of single cores the surface-to-volume ratio would increase, which would require more peptizing agent to be adsorbed to those aggregates, improving the stability of the particles. Therefore, more stable nanoparticle suspensions coupled to smaller hydrodynamic diameters led to an enhancement in heat dissipation rate.

Samples in Group 2 (brown bars) represent SAR values obtained by fixing the synthesis temperature at 85°C but varying the initial iron concentration from 0.08M to 1.0M, and using both bath-sonication and ultra-sonication during the peptization step. In this case, the SAR seems to be strongly affected by total iron concentration used during the reaction, while the use of ultra-sonication does not show an improvement in SAR for the same iron concentration. However, regardless of the type of sonication, there was an increase in SAR when using higher iron concentrations (runs 7 and 8), but the heat dissipation rates achieved were not as high as those achieved when using ultra-sonication along with 0.30 M of iron (i.e. run 8 vs run 4). According to our discussion above of the relationship between SAR and particle size, we can expect lower heat dissipation rates for smaller particles. This is confirmed by analyzing the hydrodynamic diameters of runs with low iron concentration (runs 5, 6, 9 and 10), which had the smallest hydrodynamic diameters among all the synthesis runs, and the lowest SAR values.

Group 3 (orange bars), comprises SAR values from syntheses with sonication at its medium level, and supports the observations made for groups 1 and 2. Even for nanoparticles with low initial iron concentration, an increase in the synthesis temperature leads the SAR to rise from 235 to 409 W/g_{Fe} (run 9 and 10). A similar tendency was observed for runs with high iron concentration (runs 11 and 12), though the increase in SAR is slightly less for the same temperature increase. However, by increasing simultaneously the temperature and iron concentration during synthesis, SAR was increased by almost a factor of three, relative to the value when both factors were at their low levels (run 9 vs run 12).

Group 4 (green bars) comprises SAR values obtained from synthesis runs with the three factors fixed at their medium levels, and they account for the center points of the experimental design. These runs are replicates of each other and they were obtained under a synthesis temperature of 85°C, 0.30M of initial iron concentration and peptization using both bath-sonication and ultra-sonication with Fe₃O₄/TMAOH in 1:1 volume ratio. The high SAR values observed for these samples were in fact, improvements of those obtained under similar conditions observed in other groups (i.e. runs 4 and 12), demonstrating that the use of ultra-sonication during the peptization of particles is key to improving heat dissipation rates. Moreover, it was observed that ultra-sonication increases SAR, but the simultaneous combination of iron concentration and temperature at the studied medium levels showed a synergistic effect that was not observed when these two factors had lower or higher values. Notably, this group yielded the highest SAR values for the entire set of synthesis runs, with energy dissipation rates of up to 1,048 W/g_{Fe} (run 15).

As noted before, the ILP value is a convenient metric of the energy dissipation rate of iron oxide nanoparticles that allows for comparisons between nanoparticles characterized under different magnetic field conditions. In this work, values of up to $2.3 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$ were obtained at 36.5 kA/m and 341 kHz. This value exceeds the values reported by others at similar field strengths, and frequencies around 500 kHz [14]. In fact, reported ILP values for heat dissipation measurements with frequencies around 500 kHz are typically below $1 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$ with some exceptions between 4 and $5 \text{ nH}\cdot\text{m}^2\cdot\text{kg}^{-1}$ [38], and one notable recent study reporting an ILP value of 8.7 [23]. It is important to note that even when some groups report exceptionally high ILP values, care must be taken in order to ensure that conversion of SAR into ILP is properly done. For this purpose, it is necessary to ensure that the magnetic response of the nanoparticles is linear with the applied magnetic field. A comparison can be done between the maximum applied magnetic field amplitude and the magnetic field at which the saturation magnetization was achieved; the first must be much smaller than the latter [23]. In this work, from SQUID measurements the saturation magnetization was reached above 4000 kA/m, and this value is 100 times higher than the field amplitude applied during measurements, meeting the corresponding requirements of magnetic linear response.

3.4 SAR measurements in solid matrices

Measurements of heat dissipation rates in solid matrixes were carried out by fixing the nanoparticles in agarose gel and applying a magnetic field with the same intensity and frequency as used for liquid suspensions. Confinement of nanoparticles in intracellular vesicles and in biological tissues may limit their free rotation, which has been reported to cause a reduction in the total amount of heat dissipated [39]. Thus, SAR measurements in solid matrixes may provide an estimate of the reduction in energy dissipation rate in the complex biological environment. Immobilization of particles in agarose gel was confirmed using DMS measurements, as depicted in Figure 7, which presents a comparison between the magnetic susceptibility of the particles suspended in water and immobilized in agarose gel for the same synthesis run. The characteristic peak of the out-of-phase component of the susceptibility, namely the Brownian peak, shows a marked reduction when particles are fixed in the solid matrix. Similar observations indicated that most of the Brownian particles were restricted from physical rotation, showing a flat curve for the out-of-phase component.

The immobilization of particles revealed a decrease in SAR when compared to the values reached by liquid suspensions for the same synthesis run. This reduction was attributed to suppression of the Brownian relaxation mechanism. This situation is depicted in Figure 8a wherein the SAR values for both liquid and solid particle preparations are presented for all synthesis runs. It is important to note that even when the total amount of heat dissipated was reduced when fixing the particles in the agarose gel, the remaining fraction still resulted in excellent SAR values. Exceptional heat dissipation rates of up to $719 \text{ W/g}_{\text{Fe}}$ were achieved for the immobilized nanoparticles, indicating that they are promising candidates for *in vivo* MFH application. In fact, the maximum SAR values in solid matrices reported here were higher than those reported for liquid suspensions by other groups[5]. Figure 8b also compares the SAR and ILP values obtained under restricted nanoparticle rotation in our work with that of other representative publications.

3.5 Optimization and reproducibility

Optimal experimental conditions were identified to produce nanoparticles with high heat dissipation rates by using the Response Surface Method (RSM) [40]. As discussed before, optimal results were those obtained when the temperature, iron concentration and peptization were at their medium levels. These results were optimized, and they are depicted in the contour plot shown in Figure 9, which relates the different SAR values that can be obtained by moving along the different levels of temperature and iron concentration. The contour black lines represent the wide range of SAR values discussed earlier, and the red lines represent the hydrodynamic diameters at which SAR can be obtained. The purpose to incorporate particle sizes in the contour plot is to provide a practical way to relate a factor, which was not part of the experimental design, but is a parallel consequence of modifying the experimental conditions.

The contour plot shown in Figure 9 graphically illustrates the optimization routine that was assayed with the experimental data and SAR values, and it displays the regions where different SAR values can be obtained by tailoring the studied parameters. The plot depicts an optimal region which predicts that the highest SAR values can be obtained for temperatures of approximately 85°C, iron concentrations above 0.50 M, and ultra-sonication during peptization. However, the highest SAR values shown in Figure 6 were those obtained when working with an iron concentration of 0.30 M, even when high SAR values were also obtained with iron concentrations of 1.0 M. This is an indication that a more accurate appropriate medium level for the total iron concentration was, in fact, identified. In addition, even when a set of conditions was experimentally found to maximize SAR, there is an optimum region under which high SAR values can be consistently obtained within some limits, while reducing variability. This region was evaluated by conducting new synthesis runs. Figure 10 shows the SAR value versus synthesis runs under the optimized conditions. The eight syntheses included in Figure 10 are replicates of each other, and all of them were carried out using ultra-sonication during the peptization step. Another important aspect of the optimization is the establishment of the prediction interval; a predicted range for SAR values in subsequent experiments. The lower and upper limits of this interval are shown in Figure 10 as horizontal red lines. As shown in Figure 10, all SAR values obtained in this new series of experiments were inside the prediction interval, suggesting that the RSM successfully predicted the range of SAR values that can be obtained under optimal conditions.

4. Conclusions

Superparamagnetic iron oxide nanoparticles with exceptionally high heat dissipation rates were obtained by using a simple, cost-effective and straightforward co-precipitation method, along with an ultrasonic-assisted and enhanced peptization step. No post-synthesis treatments, size-selective separations, toxic metals or organic solvents were employed throughout the course of the experimental work, demonstrating that the simple co-precipitation protocol can be improved by tailoring key synthesis parameters. The use of a high energy ultrasound probe during peptization of the nanoparticles allowed for improvements in particle dispersion, breaking the larger clusters to yield small particle

aggregates which were stabilized by adsorption of tetramethylammonium hydroxide cations. Experimental observations were used to determine an optimal region under which the values of SAR can be successfully predicted, and subsequent experimentation demonstrated that SAR values were obtained as predicted and in a reproducible fashion. The achieved high heat dissipation rates were observed even when particles were fixed in agarose gel, chosen to mimic biological environments such as intracellular organelles and body tissues. In such rotation-restricted matrices, the best-performing nanoparticles dissipated up to 719 W/g_{Fe}, a high value in comparison to other studies of heating with particles restricted from physical rotation. Overall, the reported SAR values, both in liquid suspensions and in agarose gels, exceed most values published so far for similar co-precipitation synthesis approaches to produce superparamagnetic iron oxide nanoparticles.

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Highlights

- ☐ Optimization of synthesis and peptization of magnetic nanoparticles is achieved.
- ☐ A simple co-precipitation method yielding high SAR values is presented.
- ☐ Ultra-sonication during the peptization step improves particle dispersion.
- ☐ High SAR values without post-synthesis fractionations are consistently achieved.
- ☐ SAR remained high even for samples with restricted particle rotation.

Figure 1.

TEM images and size distributions of representative runs: a) 5B, $D_p = 15 \pm 0.41$ nm; b) 11B, $D_p = 12 \pm 0.35$ nm; c) 15B, $D_p = 20 \text{ nm} \pm 0.40$ nm.

Figure 2.

Magnetic characterization. a) Equilibrium magnetization curves showing field dependence at 300K with M_s values of 109, 104 and 144 emu/g_{Fe} for runs 5B, 11B and 15B, respectively. b) ZFC/FC magnetization curves from 4 to 400 K with T_B values of 264 K for run 5B and higher than 400K for runs 11B and 15B.

Figure 3.
DMS measurements for two different initial iron concentration. a) Run 9B with iron concentration of 0.08M and $D_h=32$ nm b) Run 12B with iron concentration of 1.0M and $D_h=50$ nm. Solid symbols are experimental values and continuous lines are fittings to the Langevin-Chantrell model.

Figure 4. Parity plot for volume-weighted hydrodynamic diameters obtained from DLS and DMS measurements.

Figure 5. Temperature rise profiles for representative synthesis runs exhibiting different heating slopes. Measurements were performed at an applied magnetic field of $H_o = 36.5$ kA/m and $f = 341$ kHz.

Figure 6.

Comparison of SAR values according to the effect of the studied factors. Blue bars: Temperature and sonication at fixed iron concentration. Brown bars: Iron concentration and sonication at fixed temperature. Orange bars: Temperature and iron concentration at fixed sonication type. Green bars: Temperature, iron concentration and sonication at their medium levels.

Figure 7.

DMS plot for run 15B comparing data from liquid suspensions (blue markers) and data from particles fixed in agarose gel (brown markers).

Figure 8.

SAR values for particles immobilized in solid matrices. a) Comparison between suspended (blue markers) and agarose-fixed (maroon markers) nanoparticles for the different synthesis runs. b) Comparison of SAR and ILP values between our immobilized particles and those reported by other research groups [14,17,23]

Figure 9.
Contour plot of the response surface for SAR.

Figure 10.

Evaluation of the predicted behavior of SAR under optimized conditions by means of additional synthesis runs.

Table 1

Factors and levels for the optimization study.

Factor	Low level	Mid-level	High level
Temperature (°C)	80	85	90
Total Iron Concentration (M)	0.08	0.30	1.00
Sonication	Bath-Sonication	Bath and Ultra-Sonication	Ultra-sonication

Table 2

Experimental design for the optimization of SAR

Experimental run	Factor-level combinations		
	Temperature	Iron concentration	Sonication
1	Low	Medium	Low
2	Low	Medium	High
3	High	Medium	Low
4	High	Medium	High
5	Medium	Low	Low
6	Medium	Low	High
7	Medium	High	Low
8	Medium	High	High
9	Low	Low	Medium
10	High	Low	Medium
11	Low	High	Medium
12	High	High	Medium
13	Medium	Medium	Medium
14	Medium	Medium	Medium
15	Medium	Medium	Medium