Irregularly Shaped Iron Nitride Nanoparticles as a Potential Candidate for Biomedical Applications: From Synthesis to Characterization

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ABSTRACT: Magnetic nanoparticles (MNPs) have been extensively used in drug/gene delivery, hyperthermia therapy, magnetic particle imaging (MPI), magnetic resonance imaging (MRI), magnetic bioassays, and so forth. With proper surface chemical modifications, physicochemically stable and nontoxic MNPs are emerging contrast agents and tracers for in vivo MRI and MPI applications. Herein, we report the high magnetic moment, irregularly shaped γ′-Fe₄N nanoparticles for enhanced hyperthermia therapy and T₂ contrast agent for MRI application. The static and dynamic magnetic properties of γ′-Fe₄N nanoparticles are characterized by a vibrating sample magnetometer (VSM) and a magnetic particle spectroscopy (MPS) system, respectively. Compared to the γ-Fe₂O₃ nanoparticles, γ′-Fe₄N nanoparticles show at least three times higher saturation magnetization, which, as a result, gives rise to the stronger dynamic magnetic responses as proved in the MPS measurement results. In addition, γ′-Fe₄N nanoparticles are functionalized with an oleic acid layer by a wet mechanical milling process. The morphologies of as-milled nanoparticles are characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), and nanoparticle tracking analyzer (NTA). We report that with proper surface chemical modification and tuning on morphologies, γ′-Fe₄N nanoparticles could be used as tiny heating sources for hyperthermia and contrast agents for MRI applications with minimum dose.

1. INTRODUCTION

Nowadays, magnetic nanoparticles (MNPs), with proper surface chemical modifications, are emerging nanomaterials that have been exploited in the areas of magnetic resonance imaging (MRI) and magnetic particle imaging (MPI), drug/gene delivery,¹−¹⁰ hyperthermia,¹¹−¹⁶ bioassays,¹⁷−²⁸ cell sorting and separation,²⁹−³⁸ and so forth. For different applications, high magnetic moment MNPs are demanded for large magnetic torques in drug/gene delivery and cell sorting and separation applications, for high sensitivity magnetic bioassays, as well as for efficient and minimum dose usage in MRI, MPI, and hyperthermia applications. In view of this demand, γ′-Fe₄N nanoparticles are magnetically soft,³⁹−⁴⁰ chemically stable,⁴ⁱ−⁴⁵ cheap, and possess high saturation magnetization (182 emu/g).³⁸⁰⁵ Since year 2000, many groups have reported the facile synthesis of high purity γ′-Fe₄N nanoparticles.³⁸⁰⁵ Based on the Fe−N phase diagram, the γ′-Fe₄N phase forms at the temperature range from 200 to 680 °C.⁴¹ As proposed by the Lehrer diagram, the most stable iron nitride phase could be modified by tuning the nitriding potential that is controlled by the partial pressure of hydrogen and ammonia gas and the nitridation temperature.⁴² Thus, different iron nitride phases are obtained using the gas nitridation method to synthesize γ′-Fe₄N nanoparticles. During the nitridation process, ammonia gas provides nitrogen atoms and hydrogen gas is applied to tune the nitriding potential to obtain γ′-Fe₄N nanoparticles. Figure 1 shows the experimental setup for preparing γ′-Fe₄N nanoparticles. Hydrogen gas is used for reducing the starting materials, γ′-Fe₂O₃ nanoparticles, in a tube furnace. Then, the nitridation is proceeded in the same furnace under a mixture of ammonia and hydrogen gas to obtain γ′-Fe₄N nanoparticles, as shown in Figure 1a. The reduction and nitridation reactions are illustrated in Figure 1b.

After the synthesis of γ′-Fe₄N nanoparticles from γ′-Fe₂O₃ nanoparticles, the crystalline structures of both nanoparticles are characterized by X-ray diffraction (XRD), and the high purity γ′-Fe₄N phase is confirmed from our synthesized γ′-Fe₄N nanoparticles. The γ′-Fe₂O₃ and synthesized γ′-Fe₄N powders are wet mechanically milled with oleic acid (OA) to
of the starting material matches the γ-Fe2O3 nanoparticles (red solid line) and nitride nanoparticles (black solid line). The powder diffraction files (PDFs) are also plotted, as shown in the panels (i) γ′-Fe4N and (ii) γ-Fe2O3. The XRD pattern of the starting material, iron oxide nanoparticles, matches well with the γ-Fe2O3 (PDF card no. 00-004-0755). Based on the PDF of γ′-Fe4N (PDF card no. 00-006-0027), the main phase of the synthesized nitride nanoparticles is γ′-Fe4N. A tiny iron oxide peak around 36° is observed from the synthesized nitride nanoparticle sample. The oxidation might happen during the sample transfer process such as from the tube to the glove box. (b,c) Crystal structures of γ′-Fe4N and γ-Fe2O3 are revealed.

2. RESULTS AND DISCUSSION

2.1. XRD of γ-Fe2O3 and Synthesized γ′-Fe4N MNPs. The structure of γ-Fe2O3 and synthesized γ′-Fe4N are investigated by XRD. As shown in Figure 2a, the XRD pattern of the starting material matches the γ-Fe2O3 phase. After the hydrogen reduction and gas nitridation, nanoparticles show that γ′-Fe4N is the main phase. The γ′-Fe4N nanoparticles are successfully synthesized by the gas nitridation method. There is also a diffraction peak at around 2θ = 36° that is from iron oxide, which might be due to the oxidation when transferring the powder sample from the tube furnace to a glove box. The crystal structures of γ′-Fe4N and γ-Fe2O3 are also plotted in Figure 2b,c, respectively.

2.2. Morphology Characterization on γ-Fe2O3 and γ′-Fe4N Nanoparticles. The morphologies of γ-Fe2O3 and γ′-Fe4N nanoparticles are obtained using TEM. From each nanoparticle sample, three different samples are prepared for the TEM characterization: the wet mechanical milled γ-Fe2O3 and γ′-Fe4N nanoparticles in OA, named γ-Fe2O3@BM and γ′-Fe4N@BM, respectively; the supernatant of these nanoparticle suspensions after a ultra-centrifugation step (10,000 rpm for 20 min), named γ-Fe2O3@Ultra and γ′-Fe4N@Ultra; the supernatant from these nanoparticle suspension after keeping at room temperature for 24 h, named as γ-Fe2O3@Sup and γ′-Fe4N@Sup. The TEM images, illustration of each TEM sample preparation process, and schematic drawings of different shape nanoparticles are summarized in Figure 3a,b. Both γ-Fe2O3@Ultra and γ′-Fe4N@Ultra show well dispersed nanoparticles with diameters below 20 nm. Most of these nanoparticles have irregular shapes. TEM images of the as-milled nanoparticles, γ-Fe2O3@BM and γ′-Fe4N@BM, show that nanoparticles tend to aggregate together to minimize their surface energy. Around 60% of the nanoparticles from the γ′-Fe4N@BM sample are sintered bodies and aggregate together, which might happen during the reduction and nitridation which are handled at a relatively high temperature (400°C). These sintered bodies are also observed from the γ′-Fe4N@Sup sample. The γ-Fe2O3@Sup sample does not have any sintered bodies, but aggregations are still observed. Various shapes of nanoparticles are highlighted in the TEM images shown in Figure 3 by dashed lines. The corresponding schematic shapes of single nanoparticles and sintered bodies are drawn in Figure 3(i)–(viii). Micromagnetic simulations on the static magnetic responses of these γ-Fe2O3 and γ′-Fe4N nanoparticles (sintered bodies) are given in Figures 7 and 8.

2.3. Hydrodynamic Size of γ-Fe2O3 and γ′-Fe4N Nanoparticles. Figure 4a shows the hydrodynamic size distribution of the γ′-Fe4N@BM sample measured by the DLS, where the size distribution peaks around 25 nm, and this too correlates with the TEM image Figure 4(i) added in the
Isopar G have formed well-dispersed nanoparticle sample solution in sample shows far less aggregations, and this suggests that they distribution of Fe2O3 nanoparticle samples. Three different samples are used for TEM measurements. (a) TEM characterization on milled nanoparticles in OA are named BM; original nanoparticle suspensions ultra-centrifuged at 10,000 rpm for 20 min are named Ultra; original nanoparticle suspensions suspended for 24 h are named Sup. A drop of each suspension is obtained from supernatant and dropped onto TEM grid (copper meshes with amorphous carbon coating). The TEM grids are air-dried at room temperature, which will be used for TEM measurements. (a) TEM characterization on γ-Fe2O3 nanoparticle samples. Three different samples are characterized: γ-Fe2O3@Ultra, γ-Fe2O3@BM, and γ-Fe2O3@Sup. The corresponding TEM images are shown on the right panel. Most of the nanoparticles show irregular shapes with some spherical nanoparticles, as shown in (i–iv). Less aggregations of nanoparticles are observed from the supernatant of the sample γ-Fe2O3@Ultra. (b) TEM characterization on γ-Fe2O3 nanoparticle samples. Three different samples are characterized: γ-Fe2O3@Ultra, γ-Fe2O3@BM, and γ-Fe2O3@Sup. Well-dispersed nanoparticles are observed from the γ-Fe2O3@Ultra sample. For the samples γ-Fe2O3@BM and γ-Fe2O3@Sup, most of the nanoparticles show irregular shapes and the size of the nanoparticles are larger than that of the starting material, γ-Fe2O3 nanoparticles, as shown in (v–viii). More aggregations are observed which is due to the sintering of nanoparticles during the reduction and nitridation processes as well as the non-super-paramagnetic properties of these sintered bodies.

Subset. In comparison to γ′-Fe4N@BM, the γ′-Fe2O3@BM sample shows far less aggregations, and this suggests that they have formed well-dispersed nanoparticle sample solution in Isopar G fluid. Figure 4b shows the hydrodynamic size distribution of γ′-Fe4N@BM sample where the peak is around 100 nm, agreeing with the TEM image Figure 4(ii) added in the subset. The DLS result of the γ′-Fe4N@BM show another peak at around 1000 nm, this finding suggests that the γ′-Fe4N MNP are aggregated into clusters. A photograph of γ′-Fe4N@BM sample is given in the glass bottle, where the uppermost part is the supernatant, while the sediments are nanoparticle clusters. Another interesting point to be noted is that the hydrodynamic size distribution in the DLS results appear slightly larger than the observed TEM images. The reason behind this being that the γ′-Fe2O3@BM and γ′-Fe4N@BM nanoparticles are formed as a result of wet ball milling in OA.

Figure 4. Hydrodynamic size distribution of (a) γ′-Fe2O3@BM and (b) γ′′-Fe4N@BM samples measured by DLS. γ′′-Fe2O3@BM shows a peak of 25 nm which corroborates with the TEM image in the subset. In addition, it also shows very small peaks at >100 nm suggesting a very small amount of clustering of the nanoparticles in the sample. γ′′-Fe4N@BM peaks around 100 nm implying significant sintering of the nanoparticles, and the peaks near 1000 nm signifies the clustering of these sintered bodies. (c) A schematic drawing of OA surfactant conjugation on the nanoparticles. The thickness of the OA layer is around 2 nm, which increased the hydrodynamic size of nanoparticles by 4 nm.

Thus, these nanoparticles are coated with a thin layer of OA (around 2 nm thick), and this makes the hydrodynamic size distribution from DLS appears to be slightly larger than that observed from the TEM images. The schematic drawing of OA coated MNP is given in Figure 4c.

2.4. Hydrodynamic Size and Concentration of the γ′-Fe4N@BM Nanoparticles in Isopar G Fluid. The DLS results give a slight discrepancy with that of the TEM images, although we justified the discrepancy by two explanations in the last section: first, as the samples were ball milled, they added an extra 2 nm OA coating around the nanoparticles and made the hydrodynamic size distribution slightly larger than observed from the TEM images; second, the hydrodynamic peaks around 100 and 1000 nm from the γ′′-Fe4N@BM sample helped us infer that the particles might have sintered and aggregated. Thus, in order to further characterize the size of the synthesized γ′-Fe4N nanoparticles, another competent optical characterization method, the NTA (details regarding the techniques and models have been mentioned in the Materials and Methods section), is used. An added advantage of characterizing the nanoparticles by the NTA over the DLS is that they also give an information about the concentration of the particles from the solution. Figure 5 shows that the γ′′-Fe4N@BM sample has a concentration of the order of 10^7 particles/mL. Five independent NTA measurements, each having a time span of 60 s, are carried out and labeled as curves I–V in Figure 5. The nanoparticle concentration is averaged over five measurements and represented by the shadowed area in Figure 5. For each measurement, a 1 min video is recorded using the camera of the NTA (videos are provided in the Videos S1–S5). Snapshots at the 15th, 30th, 45th, and 60th seconds are summarized on the right panel of Figure 5. The NTA results also confirm that the synthesized γ′-Fe4N nanoparticles show both sintering (peaks at 46, 136 and 187 nm) and clustering (peaks at 609 nm and over 1000 nm) as
Fe₄N Nanoparticles Measured using a Vibrating Sample Magnetometer, which explains the severe aggregations from the sample. This argument has been addressed in the Materials and Methods Section of this paper.

The static (dc) hysteresis loops of γ-Fe₂O₃ and γ'-Fe₄N nanoparticles show a saturation magnetization $M_s$ of 51 emu/g and coercivity of 22 Oe. The γ-Fe₂O₃ nanoparticles saturate at 2000 Oe with a short axis of 10 nm. The γ'-Fe₄N nanoparticles saturate at 4000 Oe with a short axis of 50 nm. Bright spots from the snapshots are nanoparticles with larger spots representing clustered nanoparticles. The NTA size distribution corroborates well with DLS results of the γ'-Fe₄N@BM sample, as discussed in Figure 4b.

was concluded from the DLS results. It is to be noted here that, the characterization of γ'-Fe₄N@BM samples by NTA have not been reported and the same by γ-Fe₂O₃@BM have not been made. This argument has been addressed in the Materials and Methods Section of this paper.

2.5. Static Magnetization Curves of γ-Fe₂O₃ and γ'-Fe₄N Nanoparticles Measured using a Vibrating Sample Magnetometer. The static (dc) hysteresis loops of γ-Fe₂O₃@BM and γ'-Fe₄N@BM samples are measured at room temperature using a VSM system, as plotted in Figure 6. The magnetic field is swept from −5000 to +5000 Oe with a step width of 5 Oe (or −2000 to +2000 Oe with a step width of 2 Oe), and the averaging time for each data point is 100 ms so that the magnetizations of nanoparticles are able to relax and align to the field direction. The γ-Fe₂O₃@BM nanoparticles show a saturation magnetization $M_s$ of 51 emu/g and superparamagnetic property with negligible coercivity of 22 Oe. On the other hand, the γ'-Fe₄N@BM nanoparticles show a hysteresis loop with a coercivity of 166 Oe and a saturation magnetization $M_s$ of 164 emu/g. This large coercivity explains the sintered bodies (sizes around 100 nm) from the TEM, DLS, and NTA results in Figures 3b, 4b, and 5, respectively. At zero field, the remanent magnetization is around 26% of $M_s$, which explains the severe aggregations from the γ'-Fe₄N@BM sample in Figures 4b and 5. The larger coercivity and higher saturation magnetization $M_s$ of γ'-Fe₄N over γ-Fe₂O₃ suggest that γ'-Fe₄N could be a potential candidate for magnetic hyperthermia. Because of this non-superparamagnetic property and sintered bodies of synthesized γ'-Fe₄N nanoparticles, the stability of γ'-Fe₄N nanoparticles in OA is examined, and given in Supporting Information S5, where the γ'-Fe₄N@BM, γ-Fe₂O₃@BM, γ'-Fe₄N@Ultra, and γ-Fe₂O₃@Ultra samples are placed at room temperature for 7 days of continuous observations.

2.6. Mumax³ Simulation and Magnetic Properties Analysis. Micromagnetic simulations conforming the different nanoparticle shapes observed in Figure 3(i)–(viii) are carried out on Mumax³. The simulation models shown in Figure 7a–d are the evenly dispersed γ-Fe₂O₃ nanoparticles of different shapes and sizes. Figure 7e,f shows the γ'-Fe₄N sintered body models. Figure 7g is the γ'-Fe₄N nanoparticle cluster model. The simulation results show that all the γ-Fe₂O₃ nanoparticles modeled in this work are superparamagnetic and their magnetic moments align to the external dc field as macro-spins (Figure 8a–d). On the other hand, the 100 nm γ'-Fe₄N nanoparticles show domain walls, and their remanent magnetization is non-negligible (see the magnetization in Figure 8e at 0 Oe). In addition, the nanoparticle clusters are also simulated in this work (γ'-Fe₄N nanoparticles with average size of above...
It was found that the remanent magnetization is very significant, which contributes to the hysteresis loop in the VSM result. At zero external field (0 Oe), the $5 \times 5$ spherical $\gamma'$-Fe$_4$N nanoparticle clusters show very strange domain patterns because of the following: (1) each $\gamma'$-Fe$_4$N nanoparticle shows domain walls similar to the situation in Figure 8e; (2) the interparticle interactions are negligible because of the clustering. The collective magnetic responses of $\gamma'$-Fe$_4$N nanoparticle clusters result in non-zero remanent magnetization observed in Figure 6.

### 2.7. Dynamic Magnetic Responses of $\gamma$-Fe$_2$O$_3$ and $\gamma'$-Fe$_4$N Nanoparticles in Aqueous Solutions

The dynamic magnetic responses are characterized by a homebuilt MPS system. Where the $\gamma$-Fe$_2$O$_3$ and $\gamma'$-Fe$_4$N nanoparticles suspended in OA solution (overall volume of 200 μL, concentration of 67 mg/mL) are characterized and recorded by this MPS system. This MPS system generates an alternating current (ac) magnetic field to magnetize MNPs, and as a result,
the magnetic moments of nanoparticles relax to align with the external driving field through the Néel- or Brownian-relaxation dominated process or through the joint Néel–Brownian relaxation process (Supporting Information S1).26,48,55,56 In this work, the magnetic moments of both γ-Fe2O3 and γ′-Fe4N nanoparticles in OA relax along the ac magnetic field through a Néel relaxation-dominated process. Theoretical analysis can be found from Supporting Information S2. The ac magnetic driving field can be tuned with varying frequencies f from 50 to 2850 Hz, and the amplitude of the driving field is set at 170 Oe. The relaxation of magnetic moments of MNPs subjected to ac field is dynamic magnetic responses. This time-varying magnetic moment causes electromotive force (EMF) in a pair of differently wound pick-up coils (Faraday’s law of induction). As a result, the dynamic magnetic responses of γ-Fe2O3 and γ′-Fe4N nanoparticles are recorded (Supporting Information S1) as real-time voltage. Because of the nonlinear magnetic responses of nanoparticles under driving field f, higher odd harmonics at 3f, 5f, 7f, and so forth are found from the frequency domain of the collected voltage signal.10,49,51,57 Figure 9a–c summarizes the amplitudes of the 3rd, the 5th, and the 7th harmonic voltage signals denoted in red, green, and blue respectively for γ-Fe2O3@BM and γ′-Fe4N@BM samples under the same external ac fields. The calculated harmonic amplitudes as the driving field frequency varies from 50 to 2850 Hz. The red and black stars mark the critical frequencies \( f_{\text{crit}} \) where γ-Fe2O3 and γ′-Fe4N nanoparticles show highest dynamic magnetic responses. (d) In \( f \)-dominant region, the magnetic moments of nanoparticles are almost synchronized with the ac magnetic field, and the detected harmonic amplitude increases as the driving field frequency \( f \) increases. (e) In \( f-\phi \) co-led region, the dynamic magnetic responses reach to maxima, where the enhancement effect of \( f \) and the attenuation effect of \( \phi \) are equally important. (f) In \( \phi \)-dominant region, the magnetic moments of nanoparticles cannot synchronize with the fast-changing ac magnetic field; thus, a phase lag \( \phi \) between the magnetic moments and field causes attenuated harmonic amplitudes detected by the pick-up coils (discussed in Supporting Information S3). Figure 9 summarizes the amplitudes of the 3rd, the 5th, and the 7th harmonics are replotted in Figure 10a–l. Figure 10a–f and g–l corresponds to the dynamic magnetic responses of γ′-Fe4N and γ-Fe2O3 nanoparticles, respectively. Each time window records the voltage signals within one period of ac driving field (i.e., 1/f second). The first to the sixth rows correspond to the scenarios, where driving field frequency \( f = 350, 650, 950, 1250, 1500, \) and 2450 Hz, respectively. Distortions in the voltage signal are observed from both γ-Fe2O3@BM and γ′-Fe4N@BM samples (highlighted in grey in Figure 10), where the voltage signal from γ′-Fe4N@BM sample shows severer distortions over the γ-Fe2O3@BM sample under the same driving field condition. These distortions are caused by the periodically synchronized higher odd harmonics (the 3f, 5f, and 7f harmonic voltage signals denoted in red, green, and blue solid curves). Whenever the crests and troughs of higher odd harmonics are synchronized, the cumulative effect causes small convex and concave in the total signal curve, respectively, as highlighted in Figure 10a–l.

2.8. Real-Time Dynamic Magnetic Responses Recorded by MPS. The relaxation of magnetic moments of MNPs subjected to ac driving field causes detectable EMF in the pick-up coils, and this EMF is a time-varying voltage signal. This analog voltage signal collected from pick-up coils is sampled at a sampling rate of 500 kHz, and the higher odd harmonics (due to the nonlinear dynamic magnetic responses of γ-Fe2O3 and γ′-Fe4N nanoparticles) are extracted. The discrete-time total voltage signal, the 3rd, the 5th, and the 7th harmonics are re-plotted in Figure 10a–l. Figure 10a–f and g–l corresponds to the dynamic magnetic responses of γ′-Fe4N and γ-Fe2O3 nanoparticles, respectively. Each time window records the voltage signals within one period of ac driving field (i.e., 1/f second). The first to the sixth rows correspond to the scenarios, where driving field frequency \( f = 350, 650, 950, 1250, 1500, \) and 2450 Hz, respectively. Distortions in the voltage signal are observed from both γ-Fe2O3@BM and γ′-Fe4N@BM samples (highlighted in grey in Figure 10), where the voltage signal from γ′-Fe4N@BM sample shows severer distortions over the γ-Fe2O3@BM sample under the same driving field condition. These distortions are caused by the periodically synchronized higher odd harmonics (the 3f, 5f, and 7f harmonic voltage signals denoted in red, green, and blue solid curves). Whenever the crests and troughs of higher odd harmonics are synchronized, the cumulative effect causes small convex and concave in the total signal curve, respectively, as highlighted in Figure 10a–l.

2.9. Phase Lags Between Dynamic Magnetic Responses and ac Magnetic Fields. According to Faraday’s law of induction, the time-varying magnetic flux induces EMF in the pick-up coils. During one relaxation process of magnetic moments of nanoparticles to align with the direction of external ac field, the stray field causes the EMF in the coils, and there is a 90° phase shift between magnetic moment and detected voltage from pick-up coils. As shown in Figure 10m–r, the real-time voltages collected from γ-Fe2O3@BM and γ′-Fe4N@BM samples along with the ac fields are plotted during a time window of one period of ac driving field. On top of the 90° phase shift due to the law of induction, we observed phase differences of 51.9, 31.8, 19.5, 13.5, 8.6, and −4.4° between the voltage and field under the driving field frequencies of 350, 650, 950, 1250, 1850, and 2450 Hz, respectively. The detected voltages from γ-Fe2O3@BM and γ′-Fe4N@BM samples are quite synchronous, indicating the identical phase lags of both types of nanoparticles to the ac driving fields. The calculated
phase lags of magnetic moments of MNPs to different driving field frequencies are summarized in Figure 11e.

**2.10. Dynamic Magnetization Curves of γ-Fe₂O₃ and γ ′-Fe₄N Nanoparticles.** The dynamic magnetization responses of γ-Fe₂O₃@BM and γ ′-Fe₄N@BM samples are calculated using the real-time voltage signals from Figure 10. Figure 11a,b shows the normalized magnetization curves of γ-Fe₂O₃@BM and γ ′-Fe₄N@BM samples subjected to different driving field frequencies, respectively. At both low and high driving field frequencies, the γ ′-Fe₄N nanoparticles show higher dynamic magnetic responses than γ-Fe₂O₃. In addition, as we gradually increase the frequency of driving field, the dynamic hysteresis loops transform from long ellipses to flat ovals for both samples, as shown in Figure 11c,d. This is due to the fact that, as the ac field sweeps faster, both types of nanoparticles are unable to synchronize with the fast-changing ac fields; thus, a larger phase lag of magnetic moment to external driving field is induced.

For magnetic hyperthermia treatments, when MNPs are subjected to the ac field, the area of their magnetic hysteresis loop, \( A \), corresponds to the dissipated energy.\(^{58-60}\) The power generated by these MNPs or specific absorption rate (SAR), is evaluated by the equation, \( \text{SAR} = A \cdot f \). Because the maximum SAR achievable is directly proportional to the saturation magnetization of MNPs, γ ′-Fe₄N nanoparticles reported in this work can enhance the SAR and meanwhile minimize the dose.

As shown in Figure 11a,b, the dynamic magnetization curves of γ ′-Fe₄N@BM and γ-Fe₂O₃@BM are compared under different driving field conditions, γ ′-Fe₄N@BM shows a larger hysteresis loop area \( A \) over γ-Fe₂O₃@BM, indicating that γ ′-Fe₄N@BM could be potentially applied as high-performance heating sources in hyperthermia treatment with minimum dose.

**2.11. Normalized Harmonics and Harmonic Ratios.** Because the harmonic amplitudes resulted from the dynamic magnetic responses of nanoparticles are dependent on the quantity of nanoparticles from the testing sample and the pick-up coil design (winding number, width and diameter), as discussed in Supporting Information S3.\(^{10,49}\) Thus, the normalized magnetic responses and the harmonic ratios are used as nanoparticle quantity-independent metrics for characterizing the dynamic magnetic properties of nano-
Figure 12a–c shows the normalized 3rd, 5th, and 7th harmonics from $\gamma'$-Fe$_4$N and $\gamma$-Fe$_2$O$_3$ samples at varying driving field frequencies, corresponding to the recorded harmonic amplitudes in Figure 9a–c. The normalized harmonic curve of $\gamma$-Fe$_2$O$_3$ shows a sharper peak compared to $\gamma'$-Fe$_4$N. The harmonic ratios are summarized in Figure 12d–k under driving field frequencies of 150, 350, 650, 850, 1250, 1650, 2250, and 2650 Hz. The harmonics of the $\gamma'$-Fe$_4$N sample decays at a slower rate as the harmonic number increases (black lines in Figure 12d–k).

3. STABILITY AND BIOCOMPATIBILITY

The stability of OA-coated $\gamma$-Fe$_2$O$_3$ and $\gamma'$-Fe$_4$N nanoparticles are investigated in Supporting Information S5. With smaller sizes and less aggregations, $\gamma$-Fe$_2$O$_3$ nanoparticles show better stability. In this work, OA is used as surfactant on $\gamma'$-Fe$_4$N nanoparticles, these lipophilic MNPs show very good dissolvability in polar liquids such as oil. In addition, it is reported that OA can form a dense protective monolayer that binds firmly to the MNP surfaces with enhanced colloidal stability. However, for biomedical applications, the lipophilic substances (i.e., OA-coated MNPs) are not good candidates, and thus, the practical use of these MNPs are limited. In the future, we can further functionalize OA-coated MNPs with trialkoxysilanes. Thus, the functionalized nanoparticles can be dispersed in various aqueous solutions such as human serum and plasma. Another option is to improve the biocompatibility of $\gamma'$-Fe$_4$N nanoparticles such as the synthesize of core@shell nanoparticles where the high $M_s$ $\gamma'$-Fe$_4$N material could be the core and biocompatible materials such as Au, Ag, and SiO$_2$ and so forth serve as the shells. Thus, the core@shell nanoparticles would have both high magnetic moments and biocompatibility. In addition, the biocompatibility and colloidal stability of $\gamma'$-Fe$_4$N nanoparticles can be further enhanced by conjugating chemical compounds such as chitosan, polyethylene glycol, amino acids, citric acid, and so forth, so that the water solubility of MNPs can increase significantly.

4. CONCLUSIONS

In this paper, we reported high magnetic moment, irregularly shaped $\gamma'$-Fe$_4$N nanoparticles with OA surfactant. The crystalline structure, static (dc) and dynamic (ac) magnetic properties are characterized by VSM and MPS systems and compared to the $\gamma$-Fe$_2$O$_3$ nanoparticles. Our $\gamma'$-Fe$_4$N nanoparticles show superior magnetic properties with more than 3 times higher saturation magnetization when compared to $\gamma$-Fe$_2$O$_3$, which makes them promising candidates for applications that require high magnetic moment per particle such as in vitro magnetic biosensing, magnetic separation, drug delivery, and so forth. The larger coercivity and higher saturation magnetization $M_s$ of $\gamma'$-Fe$_4$N compared to $\gamma$-Fe$_2$O$_3$ suggest that $\gamma'$-Fe$_4$N could be a potential candidate for magnetic hyperthermia. In addition, because of the sintering in the nitradation process, the synthesized $\gamma'$-Fe$_4$N nanoparticles, with irregular shapes, hold great promise for enhancing $T_2$ relaxivity as contrast agents in MRI applications. Nanoparticles with a larger hydrodynamic size distribution generate greater magnetic fields coupling induced inhomogeneous magnetic field distribution and artificially enhanced magnetic field inhomogeneity. This inhomogeneity is extremely advantageous.
for MRI applications as they pave the way for varied relaxation rates ($R_1$ and $R_2$) and hence varied relaxation times ($R_1 = 1/T_1$ and $R_2 = 1/T_2$) over a specific area of tissue to be imaged. This phenomenon enhances the contrast efficiency between adjacent tissues for MRI applications.

5. MATERIALS AND METHODS

5.1. Chemicals. $\gamma$-Fe$_2$O$_3$ powder (purity 99.5%, size 20 nm) is purchased from MTI Corporation, Richmond, CA. OA is purchased from Fisher Scientific, Hampton, NH. Isopar G fluid is purchased from ExxonMobil, Irving, TX.

5.2. Wet Mechanical Milling. Four hundred milligram of $\gamma$-Fe$_2$O$_3$ and $\gamma$-Fe$_4$N powders are prepared for wet mechanical milling process, respectively. In the case of wet milling, 400 mg powder is dispersed in 6 mL of OA (CH$_3$(CH$_2$)$_7$CH==CH(CH$_2$)$_7$COOH) under an argon atmosphere in a glove box. The milling conditions are 14 mm ball diameter and a vial rotation of 8000 rpm for 8 h.

5.3. XRD Characterization. Samples for the XRD measurements are prepared in the glove box. Certain amounts of $\gamma$-Fe$_2$O$_3$ and $\gamma$-Fe$_4$N nanoparticles are put on a piece of glass and sealed by epoxy to avoid oxidation when the samples are taken out of the glove box for the XRD measurement. XRD pattern are measured using a Bruker D8 discover 2D diffractometer (40 kV and 35 mA). A cobalt radiation source (wavelength $\approx$ 1.79 Å) is used to get better signal. The XRD patterns are converted to copper radiation for a convenient comparison.

5.4. Sample Preparation and TEM Characterization. The as-milled $\gamma$-Fe$_2$O$_3$ and $\gamma$-Fe$_4$N nanoparticles in OA (labeled as $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM in this paper, with a concentration of 67 mg/mL) are ultra-sonicated for 1 h to make the nanoparticles evenly dispersed. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension (concentration of 0.67 mg/mL) is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids. The $\gamma$-Fe$_2$O$_3@$BM and $\gamma$-Fe$_4$N@BM suspensions are diluted by 100 times in OA, and then, 10 μL of each suspension is dropped onto TEM grids.

5.5. DLS and NTA Characterization. Wet mechanically milled $\gamma$-Fe$_2$O$_3$ (transparent, refractive index, r.i = 2.91) and $\gamma$-Fe$_4$N (absorbing, irregularly shaped) (50 μL) in OA suspensions are dropped on a filter paper and air-dried and then fit into a 5 mm diameter 12 mm long gelatin capsule (gelcap). During the VSM measurement, the gelcap is inserted into a sample tube and affixed to the sample-rod. The magnetic field is swept from $-$5000 to +5000 Oe with a step width of 5 Oe (or $-$2000 to $+$2000 Oe with a step width of 2 Oe), and the averaging time for each measurement is 100 ms.

5.6. VSM Characterization. $\gamma$-Fe$_2$O$_3$ and $\gamma$-Fe$_4$N nanoparticles (25 μL) in OA suspensions are dropped on a filter paper and air-dried and then fit into a 5 mm diameter 12 mm long gelatin capsule (gelcap). During the VSM measurement, the gelcap is inserted into a sample tube and affixed to the sample-rod. The magnetic field is swept from $-$5000 to +5000 Oe with a step width of 5 Oe (or $-$2000 to $+$2000 Oe with a step width of 2 Oe), and the averaging time for each measurement is 100 ms.

5.7. Mumax3 Simulation. The TEM images and DLS and NTA characterizations give us a legitimate knowledge on the idea about the shapes and sizes of $\gamma$-Fe$_2$O$_3$ and synthesized $\gamma$-Fe$_4$N nanoparticles. We simulate the shapes of the nanoparticles using micromagnetic framework, Mumax3, and observe the magnetization distribution within the $\gamma$-Fe$_2$O$_3$ and $\gamma$-Fe$_4$N nanoparticles. The magnetic properties of the nanoparticles were obtained from our experimental and previously reported results listed in Table 1 and 2. A total of seven different nanoparticle shapes are modeled and plotted in Figure 7. Four different shapes of $\gamma$-Fe$_2$O$_3$ nanoparticles are modeled: spherical with a diameter of 15 nm (Figures 7a and 8a), spherical with a diameter of 25 nm (Figures 7b and 8b), cubic with a side length of 15 nm (Figures 7c and 8c), and ellipsoid with a long axis of 30 nm and a short axis of 10 nm (Figures 7d and 8d). Three different shapes of $\gamma$-Fe$_4$N nanoparticles are modeled: sintered body, spherical with a diameter of 100 nm (Figures 7e and 8e), ellipsoid with a long axis of 200 nm and a short axis of 50 nm (Figures 7f and 8f), a 5 × 5 array of 100 nm spherical $\gamma$-Fe$_4$N nanoparticles clustered together (Figures 7g and 8g). The uniaxial anisotropy $\gamma$-Fe$_4$N nanoparticles with easy axis align along [1 1 1] and cubic anisotropy $\gamma$-Fe$_4$N nanoparticles with easy axis align along [0 0 1] are assumed, and external magnetic field is applied along [1 1 0] direction (see Figure 7). The mathematical models for uniaxial and cubic anisotropy energy distributions are given in Supporting Information S4.

| parameters       | description          | values          |
|------------------|----------------------|-----------------|
| MNP dimension    | spherical diameter   | (a) 15 nm       |
|                  | cubic dimensions     | (b) 25 nm       |
|                  | ellipsoid dimensions | (c) 15 nm × 15 nm × 15 nm |
|                  |                      | (d) 30 nm × 10 nm × 10 nm |
| cell size        | length × width × thickness | 1 nm × 1 nm × 1 nm |
| $\mathbf{A}$    | Gilbert damping factor | 0.2            |
| $\mathbf{A}$    | exchange constant    | 10$^{-11}$ J/m  |
| $M_s$            | saturation magnetiztion | 280 kA/m       |
| $K_u$            | uniaxial anisotropy  | 4.6 kJ/m$^3$   |
500 kHz. The analogue voltage signal is sampled at a sampling rate of
information S1) and real time voltage signal is collected for 10
nanoparticles is inserted into the pick-up coils (see Supporting
magnetic
mL) are sealed in a plastic vial for MPS measurements. The ac
nanoparticles in OA suspensions (concentration of 67 mg/
5.8. MPS Measurement. 200 μL γ-Fe₂O₃ and γ-Fe₄N nanoparticles in OA suspensions (concentration of 67 mg/mL) are sealed in a plastic vial for MPS measurements. The ac magnetic field frequency f is varied from 50 to 2850 Hz, with amplitude set at 170 Oe. For each run, the vial containing nanoparticles is inserted into the pick-up coils (see Supporting Information S1) and real time voltage signal is collected for 10 s. The analogue voltage signal is sampled at a sampling rate of 500 kHz.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01130.

MPS system setups and magnetic relaxation mechanisms of MNPs under ac driving fields, Néel relaxation-dominated γ-Fe₂O₃@BM and γ-Fe₄N@BM MNPs in OA, models of dynamic magnetic responses, magneto-crystalline anisotropy energy of γ-Fe₂O₃ and γ-Fe₄N, and stability of γ-Fe₂O₃ and γ-Fe₄N nanoparticles in OA (PDF)

γ-Fe₄N NTA curves I–V (ZIP)

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REFERENCES
(1) Adolphi, N. L.; Huber, D. L.; Bryant, H. C.; Monson, T. C.; Fegan, D. L.; Lim, J.; Trujillo, J. E.; Tessier, T. E.; Lovato, D. M.; Butler, K. S.; Provencio, P. P.; Hathaway, H. J.; Majetich, S. A.; Larson, R. S.; Flynn, E. R. Characterization of Single-Core Magnetite Nanoparticles for Magnetic Imaging by SQUID Relaxometry. Phys. Med. Biol. 2010, 55, 5985.
(2) Arami, H.; Khandhar, A. P.; Tomitaka, A.; Yu, E.; Goodwill, P. W.; Conolly, S. M.; Krishnan, K. M. In vivo multimodal magnetic particle imaging (MPI) with tailored magneto/optical contrast agents. Biomaterials 2015, 52, 251–261.
(3) Bauer, L. M.; Situ, S. F.; Griswold, M. A.; Samia, A. C. S. Magnetic Particle Imaging Tracers: State-of-the-Art and Future Directions. J. Phys. Chem. Lett. 2015, 6, 2509–2517.
(4) Chieh, J.-J.; Huang, K.-W.; Lee, Y.-Y.; Wei, W.-C. Dual-Imaging Model of SQUID Biosusceptometry for Locating Tumors Targeted Using Magnetic Nanoparticles. J. Nanobiotech. 2015, 13, 11.
(5) Du, Y.; Lai, P.; Leung, C.; Pong, P. Design of Superparamagnetic Nanoparticles for Magnetic Particle Imaging (MPI). Int. J. Mol. Sci. 2013, 14, 18682–18710.
(6) Iqbal, M. Z.; Ma, X.; Chen, T.; Zhang, L. E.; Ren, W.; Xiang, L.; Wu, A. Silica-coated super-paramagnetic iron oxide nanoparticles (SPIONPs): a new type contrast agent of T1 magnetic resonance imaging (MRI). J. Mater. Chem. B 2015, 3, 5172–5181.
(7) Mishra, S. K.; Kumar, B.; Khush, S.; Tripathi, R. P.; Gangenahalli, G. Increased Transverse Relaxivity in Ultrasmall Superparamagnetic Iron Oxide Nanoparticles Used as MRI Contrast Agent for Biomedical Imaging. Contrast Media Mol. Imaging 2016, 11, 350.
Radiother. particle-Based Hyperthermia for Cancer Treatment. Langmuir Nanoclustering and Dipolar Interactions in Heat Generation for P.; Craievich, A.; Sanchez, F. H.; Ferna
1419. ACS Omega http://pubs.acs.org/journal/acsodf
Future Directions. Haegele, J. Magnetic Particle Imaging: Current Developments and 322
Predicting the Therapeutic Effect of Magnetic Hyperthermia. 14 J. Magnetic Nanoparticles for Drug Delivery. 52 Advances, and Future Opportunities. Particle Spectroscopy-Based Bioassays: Methods, Applications, 60 Dalton Trans.
Nanoscale Metal Organic Frameworks for Potential Targeted 48 Compounds? A Virus. Biomaterials 2020, 5, 11756–11767.

(8) Panagiotopoulos, N.; Vogt, F.; Barkhausen, J.; Buzug, T. M.; Duschka, R. L.; Lüdtke-Buzug, K.; Ahlborg, M.; Bringout, G.; Debbeler, C.; Graser, M.; Kaether, C.; Stelzner, J.; Medimagh, H.; Haegel, J. Magnetic Particle Imaging: Current Developments and Future Directions. Int. J. Nanomed. 2015, 10, 3097.

(9) Shen, Z.; Wu, A.; Chen, X. Iron Oxide Nanoparticle Based Contrast Agents for Magnetic Resonance Imaging. Mol. Pharm. 2016, 14, 1352–1364.

(10) Wu, K.; Su, D.; Saha, R.; Wong, D.; Wang, J.-P. Magnetic Particle Spectroscopy-Based Bioassays: Methods, Applications, Advances, and Future Opportunities. J. Phys. D: Appl. Phys. 2019, 52, 173001.

(11) Arruebo, M.; Fernández-Pacheco, R.; Ibarra, M. R.; Santamaría, J. Magnetic Nanoparticles for Drug Delivery. Nano Today 2007, 6, 22–32.

(12) Chen, Y.; Al, K.; Liu, J.; Sun, G.; Yin, Q.; Lu, M. Multifunctional Envelope-Type Mesoporous Silica Nanoparticles for PH-Responsive Drug Delivery and Magnetic Resonance Imaging. Biomaterials 2015, 60, 111–120.

(13) Chowdhuri, A. R.; Bhattacharya, D.; Sahu, S. K. Magnetic Nanoscale Metal Organic Frameworks for Potential Targeted Anticancer Drug Delivery. Imaging and as an MRI Contrast Agent. Dalton Trans. 2016, 45, 2963–2973.

(14) Ding, Y.; Shen, S. Z.; Sun, H.; Sun, K.; Liu, F.; Qi, Y.; Yan, J. Design and Construction of Polymerized-Chitosan Coated Fe3O4 Magnetic Nanoparticles and Its Application for Hydrophobic Drug Delivery. Mater. Sci. Eng. C 2015, 48, 487–498.

(15) Estelrich, J.; Escrivanio, E.; Queralt, J.; Busquets, M. A. Iron Oxide Nanoparticles for Magnetically-Guided and Magnetically-Responsive Drug Delivery. Int. J. Mol. Sci. 2015, 16, 8070–8101.

(16) Bao, G.; Mitragotri, S.; Tong, S. Multifunctional Nanoparticles for Drug Delivery and Molecular Imaging. Annu. Rev. Biomed. Eng. 2013, 15, 253–282.

(17) Yu, L.; Liu, J.; Wu, K.; Klein, T.; Jiang, Y.; Wang, J.-P. Evaluation of Hyperthermia of Magnetic Nanoparticles by Dehydrating DNA. Sci. Rep. 2015, 4, 7216.

(18) Andreu, I.; Natividad, E.; Solozábal, L.; Roubeau, 0. Nano-Objects for Addressing the Control of Nanoparticle Arrangement and Performance in Magnetic Hyperthermia. ACS Nano 2015, 9, 1408–1419.

(19) Bañoře-López, M.; Teijeiro, A.; Rivas, J. Magnetic Nanoparticle-Based Hyperthermia for Cancer Treatment. Rep. Pract. Oncol. Radiother. 2013, 18, 397–400.

(20) Blanco-Andujar, C.; Teran, F.; Ortega, D. Current Outlook and Perspectives on Nanoparticle-Mediated Magnetic Hyperthermia. Iron Oxide Nanoparticles for Biomedical Applications; Elsevier, 2018; pp 197–245.

(21) Carrey, J.; Mehdaoui, B.; Respaud, M. Simple Models for Dynamic Hysteresis Loop Calculations of Magnetic Single-Domain Nanoparticles: Application to Magnetic Hyperthermia Optimization. J. Appl. Phys. 2011, 109, 083921.

(22) Coral, D. F.; Mendoza Zélis, P.; Marcillo, M.; Morales, M. D. P.; Craievich, A.; Sanchez, F. H.; Fernández van Raap, M. B. Effect of Nitrogen Phase Diagram. J. Appl. Phys. 2014, 116, 12166–12164.

(23) Yu, M.; Xu, Y.; Mao, Q.; Li, F.; Wang, C. Electromagnetic and Absorption properties of nano-sized and micro-sized Fe 4 N particles. J. Alloys Compd. 2016, 656, 362–367.

(24) Van Voorthuyzen, E. H. D. M.; Boerman, D. O.; Chechenin, N. C. Low-Temperature Extension of the Lehrer Diagram and the Nitrogen Phase Diagram. Metall. Mater. Trans. A 2002, 33, 2593–2598.

(25) Kooi, B. J.; Somers, M. A. J.; Mettemeijer, E. J. An evaluation of the Fe-N phase diagram considering long-range order of N atoms in y′-Fe4N1-x and α-Fe4N. Metall. Mater. Trans. A 1996, 27, 1063–1071.

(26) Liu, J.; Guo, G.; Zhang, F.; Wu, Y.; Ma, B.; Wang, J.-P. Synthesis of α′-Fe6N2ribbons with a porous structure. Nanoscale 2019, 11, 5309–5311.

(27) Wu, K.; Klein, T.; Krishna, V. D.; Su, D.; Perez, A. M.; Wang, J.-P. Portable GMR Handheld Platform for the Detection of Influenza A Virus. ACS Sens. 2017, 2, 1594–1601.

(28) Choi, J.; Gani, A. W.; Bechtstein, D. J. B.; Lee, J.-R.; Utz, P. J.; S. X. Portable, One-Step, and Rapid GMR Biosensor Platform with Smartphone Interface. Biosens. Bioelectron. 2016, 85, 1–7.

(29) Klein, T.; Wang, Y.; Tu, L.; Yu, L.; Peng, Y.; Wang, W.; Wang, J.-P. Comparative Analysis of Several GMR Strip Sensor Configurations for Biological Applications. Sens. Actuators, A 2014, 216, 349–354.

(30) Tian, B.; Zárdán Gómez de la Torre, T.; Donolato, M.; Hansen, M. F.; Svedlindh, P.; Strömberg, M. Multi-Scale Magnetic Nanoparticle Based Optomagnetic Bioassay for Sensitive DNA and Bacteria Detection. Anal. Methods 2016, 8, 5009–5016.

(31) Su, D.; Wu, K.; Saha, R.; Peng, C.; Wang, J.-P. Advances in Magnetoresitive Biosensors. Micromachines 2019, 11, 34.

(32) Chen, Y.; Xiangyu, Y.; Wang, Y.; Zhang, X.; Cha, R.; Sun, J.; Jiang, X. One-Step Detection of Pathogens and Viruses: Combining Magnetic Relaxation Switching and Magnetic Separation. ACS Nano 2015, 9, 3184–3191.

(33) Herrmann, I. K.; Schlegel, A.; Graf, R.; Stark, W. J.; Beck-Schimmer, B. Magnetic Separation-Based Blood Puriﬁcation: A Promising New Approach for the Removal of Disease-Causing Compounds? J. Nanobiotechnol. 2015, 13, 49.

(34) Iramanesh, M.; Helliger, J. Magnetic Separation: Its Application in Mining, Waste Puriﬁcation, Medicine, Biochemistry and Chemistry. Chem. Soc. Rev. 2017, 46, 5925–5934.

(35) Zhang, X.; Wang, J.; Li, R.; Dai, Q.; Gao, R.; Liu, Q.; Zhang, M. Preparation of Fe3O4@C@Layered Double Hydroxide Composite for Magnetic Separation of Uranium. Ind. Eng. Chem. Res. 2015, 52, 10152–10159.

(36) Inglis, D. W.; Riehn, R.; Austin, R. H.; Sturm, J. C. Continuous Microfluidic ImmunoMagnetic Cell Separation. Appl. Phys. Lett. 2004, 85, 5093–5095.

(37) Tagawa, K.; Kita, E.; Tasaki, A. Synthesis of Fine Fe4N Powder and Its Magnetic Characteristics. Jpn. J. Appl. Phys. 1982, 21, 1596.

(38) Wu, X. L.; Zhong, W.; Jiang, H. Y.; Tang, N. J.; Zou, W. Q.; Du, Y. W. Magnetic properties and thermal stability of y′-Fe4N nanoparticles prepared by a combined method of reduction and nitriding. J. Magn. Magn. Mater. 2004, 281, 77–81.

(39) Dhanasekaran, P.; Salunke, H. G.; Gupta, N. M. Visible-Light-Induced Photosensitizing of Water over y′-Fe4N and y′Fe4N/α-Fe2O3 Nanocatalysts. J. Phys. Chem. C 2012, 116, 12156–12164.

(40) Yu, M.; Xu, Y.; Mao, Q.; Li, F.; Wang, C. Electromagnetic and Absorption properties of nano-sized and micro-sized Fe 4 N particles. J. Alloys Compd. 2016, 656, 362–367.

(41) Van Voorthuyzen, E. H. D. M.; Boerman, D. O.; Chechenin, N. C. Low-Temperature Extension of the Lehrer Diagram and the Nitrogen Phase Diagram. Metall. Mater. Trans. A 2002, 33, 2593–2598.
(47) Liu, J.; Guo, G.; Zhang, X.; Zhang, F.; Ma, B.; Wang, J.-P. Synthesis of α'-Fe16N2 foils with an ultralow temperature coefficient of coercivity for rare-earth-free magnets. *Acta Mater.* 2020, 184, 143–150.
(48) Tu, L.; Wu, K.; Klein, T.; Wang, J.-P. Magnetic Nanoparticles Colouration by a Mixing-Frequency Method. *J. Phys. D: Appl. Phys.* 2014, 47, 153001.
(49) Krause, H.-J.; Wolters, N.; Zhang, Y.; Offenhäuser, A.; Miethe, P.; Meyer, M. H. F.; Hartmann, M.; Keusgen, M. Magnetic Particle Detection by Frequency Mixing for Immunoassay Applications. *J. Magn. Magn. Mater.* 2007, 311, 436–444.
(50) Achtensicht, S.; Pourshahidi, A. M.; Offenhäuser, A.; Krause, H.-J. Multiplex Detection of Different Magnetic Beads Using Frequency Scanning in Magnetic Frequency Mixing Technique. *Sensors* 2019, 19, 2599.
(51) Zhang, X.; Reeves, D. B.; Perreard, I. M.; Kett, W. C.; Griswold, K. E.; Gimi, B.; Weaver, J. B. Molecular Sensing with Magnetic Nanoparticles Using Magnetic Spectroscopy of Nanoparticle Brownian Motion. *Biosens. Bioelectron.* 2013, 50, 441–446.
(52) Khurshid, H.; Shi, Y.; Berwin, B. L.; Weaver, J. B. Evaluating Blood Clot Progression Using Magnetic Particle Spectroscopy. *Med. Phys.* 2018, 45, 3258–3263.
(53) Vierreck, T.; Draack, S.; Schilling, M.; Ludwig, F. Multi-Spectral Magnetic Particle Spectroscopy for the Investigation of Particle Mixtures. *J. Magn. Magn. Mater.* 2019, 475, 647–651.
(54) Draack, S.; Lucht, N.; Remmer, H.; Martens, M.; Fischer, B.; Schilling, M.; Ludwig, F.; Vierreck, T. Multiparametric Magnetic Particle Spectroscopy of CoFe2O4Nanoparticles in Viscous Media. *J. Phys. Chem. C* 2019, 123, 6787–6801.
(55) Wu, K.; Liu, J.; Wang, Y.; Ye, C.; Feng, Y.; Wang, J.-P. Superparamagnetic Nanoparticle-Based Texture Test. *Appl. Phys. Lett.* 2015, 107, 053701.
(56) Wu, K.; Schliep, K.; Zhang, X.; Liu, J.; Ma, B.; Wang, J.-P. Characterizing Physical Properties of Superparamagnetic Nanoparticles in Liquid Phase Using Brownian Relaxation. *Small* 2017, 13, 1604135.
(57) Nikitin, P. I.; Vetoshko, P. M.; Ksenевич, T. I. New Type of Biosensor Based on Magnetic Nanoparticle Detection. *J. Magn. Magn. Mater.* 2007, 311, 445–449.
(58) Mehdouari, B.; Melfre, A.; Carrey, J.; Lachaize, S.; Lacroix, L.-M.; Gougone, M.; Chaudret, B.; Respaud, M. Optimal Size of Nanoparticles for Magnetic Hyperthermia: A Combined Theoretical and Experimental Study. *Adv. Funct. Mater.* 2011, 21, 4573–4581.
(59) He, S.; Zhang, H.; Liu, Y.; Sun, F.; Yu, X.; Li, X.; Zhang, L.; Wang, L.; Mao, K.; Wang, G.; Lin, Y.; Han, Z.; Sabirianov, R.; Zeng, H. Maximizing Specific Loss Power for Magnetic Hyperthermia by Hard-Soft Mixed Ferrites. *Small* 2018, 14, 1800135.
(60) Asensio, J. M.; Marbax, J.; Mille, N.; Lacroix, L.-M.; Soulantica, K.; Fazzini, P.-F.; Carrey, J.; Chaudret, B. To Heat or Not to Heat: A Study of the Performances of Iron Carbide Nanoparticles in Magnetic Heating. *Nanoscale* 2019, 11, 5402–5411.
(61) Yang, K.; Peng, H.; Wen, Y.; Li, N. Re-Examination of Characteristic FTIR Spectrum of Secondary Layer in Bilayer Oleic Acid-Coated Fe3O4 Nanoparticles. *Appl. Surf. Sci.* 2010, 256, 3093–3097.
(62) Shete, P. B.; Patil, R. M.; Tiwale, B. M.; Pawar, S. H. Water Dispersible Oleic Acid-Coated Fe3O4 Nanoparticles for Biomedical Applications. *J. Magn. Magn. Mater.* 2015, 377, 406–410.
(63) Marinka, T. F.; Chicinaș, H. F.; Neamțu, B. V.; Isnord, O.; Pascuta, P.; Lupu, N.; Stoian, G.; Chicinaș, I. Mechanosynthesis, Structural, Thermal and Magnetic Characteristics of Oleic Acid Coated Fe3O4 Nanoparticles. *Mater. Chem. Phys.* 2016, 171, 336–345.
(64) Bloemen, M.; Brullot, W.; Luong, T. T.; Geukens, N.; Gils, A.; Verbiest, T. Improved Functionalization of Oleic Acid-Coated Iron Oxide Nanoparticles for Biomedical Applications. *J. Nanopart. Res.* 2012, 14, 1100.
(65) Liu, J.; Su, D.; Wu, K.; Wang, J.-P. High-Moment Magnetic Nanoparticles. *J. Nanopart. Res.* 2020, 22, 66.