Magnetic fractionation and characterization of magnetic nanoparticles for magnetic particle imaging

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We performed magnetic fractionation and characterization of Resovist® magnetic nanoparticles (MNPs) for use in magnetic particle imaging (MPI). We showed that the core size of the fractionated MNPs can be controlled by adjusting the separation field. We also showed that particles with core size of approximately 27 nm are optimal for MPI applications. To obtain MNPs of an appropriate core size and a narrow size distribution, we performed combined positive and negative fractionation. In the obtained fractionated MNPs, the third harmonic magnetization, which is used as the MPI signal, was 2.75 times as large as that of the original sample.

Magnetic particle imaging (MPI) is a new modality for imaging the spatial distribution of magnetic nanoparticles (MNPs), used particularly for in-vivo diagnostics.1–5 In MPI, harmonic signals caused by the nonlinear magnetization properties of MNPs under an alternating current (ac) excitation field are used to generate an image. Hence, the performance of MPI strongly depends on the magnetic properties of the MNPs, such as core size, relaxation time, and their distributions. Hence, many studies have been performed to develop MNPs suitable for applications as an MPI tracer.

The commercially available MNP sample, Resovist®, (Fujifilm RI Pharma), is an attractive candidate MPI tracer. This product has already been safely used inside the human body as a contrast agent in magnetic resonance imaging (MRI). However, it has been shown that the MNP core size in Resovist® samples features a wide distribution. Thus, a portion of the MNPs in a Resovist® sample do not contribute to the harmonic signals.6–8 If we could extract the particles responsible for the rich harmonic signals from the original sample, we might expect an improvement in the performance of MPI. To this end, magnetic and/or hydrodynamic fractionations have been used to obtain MNPs of an appropriate core size.8–10 In Ref. 8, we showed that third harmonic magnetization of the fractionated MNP sample was 2.5 times as great as that of the original sample.

We also note that the extent of the distribution of the core sizes, as well as the average core size, affected the sensitivity of MPI. These results were attributed to harmonic magnetization from the MNPs sample depending on the extent of the core size distribution, as shown in Ref. 11. Therefore, it is important to select an MNP sample of an appropriate core size with a narrow size distribution.

In this study, we performed magnetic fractionation of a Resovist® MNP sample and characterized the fractionated samples for MPI applications. Magnetic fractionation was performed with the use of a direct current (dc) magnetic field and a separation column, as schematically shown in Fig. 1.12,13 An electromagnet (GMW 3470), was used to generate the dc magnetic field. The separation column (Miltenyi Biotec MS column), was filled with soft magnetic iron spheres to increase the magnetic field gradient inside the column. As shown in Fig. 1, the original MNP sample was divided into two fractions by a single magnetic fractionation step. Here, the fraction that was captured on the separation column is denoted as the positive fraction and the fraction that was not captured on the separation column is denoted as the negative fraction.

We prepared one original Resovist® sample and four fractionated samples (S1–S4) and investigated their magnetic properties. Each fractionated sample was a positive fraction from the original Resovist® MNPs obtained by single fractionation at a certain dc magnetic separation field, \( H_{dc} \). The strengths of the dc magnetic separation field are listed in Table I. Original Resovist® and S1–S4 samples were suspended in water and the concentrations were adjusted to be 1 mg-Fe/mL.

Figure 2 shows the static \( M-H \) curves of original Resovist® and S1 samples. As can be seen, the magnetization of S1 increased sharply with \( H \), compared with that of the original sample. This result indicates that the nonlinearity in the magnetization of the S1 sample was much greater than that of the original sample.

We used a Langevin function \( L \), to determine the static \( M-H \) curve as

\[
M = \int n_m(m) mL \left( \frac{\mu_0 m H}{k_B T} \right) dm.
\]
where $M$ is the mass magnetization in units of A·m$^2$/kg-Fe, $n_m(m)$ is the number density of MNPs with a magnetic moment $m$ per unit of iron mass, $k_B$ is the Boltzmann constant, and $T$ is the absolute temperature. By comparing the measured static $M$–$H$ curve with Eq. (1), we estimated the magnetic moment distribution in the sample, i.e., $n_m(m)$ vs $m$ curve.\(^{15-17}\) For this estimation, we used a non-negative least square (NNLS) method.\(^{16}\) On the basis of the following relationship between $n_m(m)$ and the number density of MNPs with a core size of $d_c$ per unit of iron mass, $n_d(d_c)$, we estimated the core size distribution of each sample as follows:

$$n_d(d_c) = n_m(m)M_s \frac{\pi}{6} d_c^3,$$

where $M_s$ is the saturation magnetization in units of A/m.

Figure 3(a) shows the estimated volume-weighted core size distributions, i.e., $n_d V_c$, for Resovist\(^\circledR\) and the S1 sample. Two peaks were found at $d_c = 7$ and 22 nm for the Resovist\(^\circledR\) sample. However, the peak at $d_c = 7$ nm almost disappeared in the $n_d V_c$ curve of the S1 sample. Hence, the MNPs with a smaller core size (i.e., those MNPs with a smaller magnetic moment) could be removed from the original Resovist\(^\circledR\) sample by fractionation.

In the same manner, we estimated the core size distributions of the S2–S4 samples, as shown in Fig. 3(b). Here, we denote the typical value of $d_c$ that gives the maximum value in $n_d V_c$ as $d_{c,typ}$. As shown in Fig. 3(b), the value of $d_{c,typ}$ was largest for S4, followed by S3, S2, and S1: the values of $d_{c,typ}$ are listed in Table I. The value of $d_{c,typ}$ increased as the strength of the dc magnetic separation field, $H_{dc}$, was decreased. This result indicates that the core size of the MNP sample can be controlled by adjusting the $H_{dc}$.

The distributions of the hydrodynamic size, as defined by $d_{HL}$, were measured by a commercially available DLS setup (Malvern Instruments Zetasizer Nano ZS). As shown in Fig. 4(a), the typical hydrodynamic size $d_{HL,typ}$ in the DLS measurements showed a maximum value for the S4 sample. In Table I, the values of $d_{HL,typ}$ are listed for all MNP samples. We observed a correlation between $d_{HL,typ}$ and $d_{c,typ}$ values, namely, both values increased in the order: S4 > S3 > S2 > S1. We also estimated the extent of the hydrodynamic size

Table I. Parameters of fractionated Resovist\(^\circledR\) MNPs.

| Sample       | Separation field $\mu_0 H_{dc}$ (T) | Positive/Negative | $d_{c,typ}$ (nm) | $d_{HL,typ}$ (nm) | PDI | $M_s/M_3$ (Resovist) |
|--------------|----------------------------------|-------------------|-----------------|-----------------|-----|-------------------|
| Original Resovist\(^\circledR\) | —                                | —                | 22.4            | 62.5            | 0.207 | 1                 |
| S1           | 0.67                             | Positive          | 21.9            | 63.3            | 0.218 | 1.48              |
| S2           | 0.34                             | Positive          | 27.5            | 67.8            | 0.193 | 2.42              |
| S3           | 0.17                             | Positive          | 32.1            | 77.7            | 0.172 | 2.38              |
| S4           | 0.07                             | Positive          | 37.4            | 101.6           | 0.127 | 1.96              |
| S5           | 0.39                             | Positive          | 27.5            | 60.5            | 0.168 | 2.75              |

Fig. 2. (Color online) Static $M$–$H$ curves of original Resovist\(^\circledR\) and S1 samples.

Fig. 3. (Color online) Estimated core size distribution. (a) Comparison between original Resovist\(^\circledR\) and S1 samples. (b) Comparison between positive fractionated samples (S1–S4). (c) Comparison between S2 and S5 samples.

Fig. 4(a).
distribution using software equipped with the DLS setup. The polydispersity index (PDI) value, which represents the extent of the distribution, is listed in Table I. The S4 sample featured the smallest PDI value, which increased for S3, S2, and S1, in that order. Hence, the S4 sample had the narrowest hydrodynamic size distribution.

Figure 5 shows the spectral amplitudes of the harmonic magnetization when an ac excitation field with an amplitude $\mu_0 H_{ac} = 20 \text{ mT}$ and frequency $f = 20 \text{ kHz}$ was applied. The measurements were performed with a homemade magnetic particle spectroscopy (MPS) setup.\(^{18}\) The S2 sample showed the greatest harmonic spectrum among the five samples, i.e., original Resovist\(^{®}\) and S1–S4 samples. In Table I, the amplitude of the third harmonic magnetization, $M_3$, is listed and that of the S2 sample was 2.42 times as great as that of the original Resovist\(^{®}\) sample. Note that $M_3$ is related to the sensitivity in MPI application.

The relationships between the $M_3$ value and typical core size, $d_{c,typ}$, of four fractionated samples (S1–S4) are shown in Fig. 6. The MNPs with a core size of approximately 27–30 nm showed the largest $M_3$ value. To obtain a fractionated sample with a $d_c$ of approximately 27–30 nm and a narrow size distribution, we performed a combined positive and negative fractionation. First, the original Resovist\(^{®}\) sample was poured onto the magnetic column under a dc separation field of $\mu_0 H_{dc} = 0.39 \text{ T}$. After eliminating the negative fraction under 0.39 T, the $\mu_0 H_{dc}$ was changed to 0.13 T. Then, purified water was poured onto the magnetic column and the negative fraction under 0.13 T was collected as S5.

In Fig. 3(c), the core size distribution of the S5 sample is compared with that of the S2 sample. As shown, MNPs with a large $d_c$ were eliminated in S5 and the $d_c$ distribution of S5 became narrower than that of S2. The typical core size of S5 was 27.0 nm, as listed in Table I. The hydrodynamic size distribution of S5 is shown in Fig. 4(b). As shown, the $d_H$ distribution also became narrower compared with that of the S2 sample. Corresponding to the narrow distribution, the PDI value of the S5 sample became smaller than that of S2, as listed in Table I.

The harmonic spectrum of S5 sample is shown in Figs. 5 and 6. As expected, S5 showed the largest harmonic magnetization among the four samples. The amplitude of the third harmonic magnetization of S5 sample was 2.75 times as great as that of the original Resovist\(^{®}\) sample as shown in Fig. 6. These results indicate that the S5 sample featured MNPs with an appropriate core size and a narrow size distribution for MPI applications.

We now discuss the reason for the fractionated sample with a $d_c$ of approximately 27–30 nm showing the highest $M_3$ value under an applied excitation field of $\mu_0 H_{ac} = 20 \text{ mT}$ and $f = 20 \text{ kHz}$. For this purpose, we estimated the magnetic moment $m$ and relaxation time of S1–S4 samples. With the use of the relation, $m = M_3 d_{c,typ}^2/6$, the values of $m$ for S1–S4 were calculated to be 1.79, 3.54, 5.64, and 8.92 aA m$^2$, respectively. The nonlinearity of magnetization becomes larger for larger $m$ values; hence, S4 sample with the largest $m$ value is preferable for obtaining a large harmonic signal.

However, we also have to take account of the relaxation time when we discuss dynamic behavior of MNPs. Dynamic magnetization occurs via two mechanisms, i.e., Brownian and Néel mechanisms. We calculated field-dependent Brownian relaxation times, $\tau_B$, from the typical hydrodynamic size $d_{H,typ}$ using the following equation:\(^{19}\)
Here, $\eta$ is the viscosity of the surrounding medium. With the use of the relation $f_B = 1/(2\pi\tau_N)$, the characteristic Brownian frequency, $f_B$, can be calculated to be 4.1, 6.2, 6.5, and 4.6 kHz for S1–S4, respectively.

On the other hand, the field-dependent Néel relaxation time, $\tau_N$, is given by the following Brown’s expression:

$$\tau_N = \frac{\sqrt{\pi}}{2\sqrt{\sigma}} \tau_{N0} \exp\left[\sigma(1 + h^2)\right] \left(1 - h^2\right)^{\cosh \xi - h \sinh \xi},$$

(4)

where $\tau_{N0} = 10^{-9}$, $h = \xi/2\sigma$, $\sigma = (K\mu_0 d_{\text{typ}}^3)/(6\kappa B T)$, $K$ is the anisotropy constant, and $\xi = (\mu_0 m H_\text{ac}/\sqrt{2})/(k_B T)$. Note that the effective value of the ac excitation field, $H_{\text{ac}}/\sqrt{2}$, is used for the calculation of $\xi$. If we assume $K = 6 \text{kJ/m}^3$, which is the typical value of Resovist\textsuperscript{®} particles,\textsuperscript{17,20,21,23} the characteristic Néel frequency, $f_N = 1/(2\pi\tau_N)$, can be calculated as 6287.0, 441.4, 15.6, and 0.07 kHz for S1–S4, respectively. Comparing the values of $f_B$ and $f_N$, we find that the Néel mechanism is dominant for S1–S3 samples and the Brownian mechanism is dominant for the S4 sample.

As shown in the above calculation, the characteristic Brownian frequency of the S4 sample ($f_B = 4.6$ kHz) is too low to respond to an excitation field with $f = 20$ kHz. As a result, third harmonic magnetization, $M_3$, is degraded despite the large $m$ value, as shown in Fig. 6. However, the characteristic Néel frequency of S2 sample ($f_N = 441.4$ kHz) is sufficiently high to respond to an excitation field of $f = 20$ kHz. As a result, the S2 sample showed a large $M_3$ value, as shown in Fig. 6.

This result indicates that a large third harmonic signal can be obtained with the use of MNPs having both a large $m$ value and a sufficiently large $f_N$ value compared with the frequency of the ac excitation field, i.e., 20 kHz. We note that the S5 sample can satisfy both these two requirements.

In conclusion, we performed magnetic fractionation of a Resovist\textsuperscript{®} sample, and characterized the fractionated samples for MPI applications. The core size of the fractionated MNP samples could be controlled by adjusting the magnetic separation field. To obtain MNPs suitable for MPI applications, we performed combined positive and negative fractionations under suitable magnetic separation fields. One fractionated MNP sample (S5) showed an appropriate core size and narrow size distribution. The third harmonic magnetization of the S5 sample was 2.75 times as great as that of the original Resovist\textsuperscript{®} sample.

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