Characterization of Magnetic Markers for Biosensing Application

Bhuiya, Anwarul Kabir
Department of Electrical and Electronic Engineering, Graduate Student

Yoshida, Takeshi
Department of Electrical Engineering

Enpuku, Keiji
Department of Electrical Engineering

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1. Introduction

In recent years an increasing interest in using magnetic nanoparticles for biological and medical applications developed. The size of the particles can range from a few nanometers to several micrometers and thus is compatible with biological entities ranging from proteins (a few nm) to cells and bacteria (several µm). Generally the magnetic particles are coated with a suitable ligand, which allows chemically binding of the particles to different biological systems.

One of the biological applications of the magnetic markers is the magnetic immunoassay. In this application, biological targets such as disease related proteins in blood or body fluid were magnetically detected. The magnetic signal generated from the bound markers was detected with sensitive magnetometers. A substantial advantage of the magnetic immunoassay is that it can perform the detection in liquid phase, i.e., without using the washing process called Bound/Free (BF) separation. We can magnetically distinguish between bound and unbound magnetic labels.

In the magnetic method, magnetic properties of the marker in solution, such as magnetic relaxation and AC susceptibility, have been used to perform the liquid phase immunoassay. It is known that these magnetic properties are caused by the Brownian rotation of the marker in solution, and are strongly affected by the parameters of the marker, such as distribution of marker size and magnetic moment.\textsuperscript{5-10} Because these distributions are not small in practical markers, detailed characterization of the magnetic markers is highly desirable for developing their medical applications. It is very important to know not only the average particle size and magnetic moment, but also the corresponding distributions.

In the present report, we show characterization of magnetic markers in solution. First, we show the measurements of AC susceptibility and magnetization of the markers. The effect of the viscosity of the carrier liquid on the AC susceptibility was also clarified. The experimental results were analyzed by the singular value decomposition (SVD) method in order to estimate the distributions of size $d$ and magnetic moment $m$ of the markers. From the frequency dependence of the susceptibility, we obtained that the marker size distributed from 40 nm to 80 nm with mean diameter of $d=60$ nm. From the magnetization curve, we estimated that the magnetic moment distributed from $5\times10^{-25}$ to $5\times10^{-24}$ Wbm with mean value of $m=3\times10^{-24}$ Wbm. Combining the above two results, we also obtained the dependence of $m$ on $d$.

2. Experimental Results
2.1 Experimental setup and sample

In this experiment, we used commercial magnetic markers supplied from Ocean Nanotech Company, USA. The marker composed of polymer coated Fe₃O₄ particles, whose specific diameter was 50 nm, and was dispersed in solution with concentration of 5 mg/ml. The 2 µl of the marker solution was diluted by 73 µl of the solution that consisted of the mixture of water and glycerol. The concentration of the glycerol was changed from 0% to 75% in order to change the viscosity of the solution. In Table 1, 6 different concentration solutions were listed.

In Fig. 1, an experimental setup is schematically shown. The excitation field of $H = H_{0}\sin 2\pi ft$ was applied by an excitation coil. A disk-shaped sample plate which contained 60 µl of the marker solution in its well was used. The size of the well was 5 mm in diameter. The sample plate was rotated by an ultrasonic motor and was positioned under the excitation coil. In this case, the markers were magnetized and had a magnetic moment $m$. The signal field $B_s$ generated by $m$ was detected by a magneto-resistive (MR) sensor (Honeywell, USA) that was installed 2 mm under the sample plate. The output signal of the MR sensor was connected to the lock-in amplifier in order to obtain both the real and imaginary parts of the signal.

Table 1 Samples with different concentration of glycerol. Viscosity of the mixed solution was normalized by that of pure water (sample 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td>100</td>
<td>85</td>
<td>70</td>
<td>55</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Glycerol (%)</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>75</td>
</tr>
<tr>
<td>$f_p$ (Hz)</td>
<td>2500</td>
<td>1750</td>
<td>1000</td>
<td>390</td>
<td>120</td>
<td>70</td>
</tr>
<tr>
<td>Viscosity</td>
<td>1</td>
<td>1.4</td>
<td>2.5</td>
<td>6.4</td>
<td>21</td>
<td>36</td>
</tr>
</tbody>
</table>

Fig. 1 Experimental set up.

2.2 AC susceptibility

2.2.1 Frequency dependence

Measurements of the frequency dependence of the AC susceptibility are shown in Fig. 2. The real part $\chi'$ and the imaginary part $\chi''$ are shown in Figs. 2(a) and 2(b), respectively. As shown, the real part $\chi'$ decreased monotonically with frequency. On the other hand, the imaginary part $\chi''$ had a peak value at some frequency: we define the frequency which gives the maximum value of $\chi''$ by $f_p$. It is well-known that this peak frequency is related to the Brownian relaxation time $\tau_B$ of the marker as $f_p = 1/(2\pi\tau_B)$.

The peak frequencies $f_p$ significantly changed among samples. They were approximately 2500, 1750, 1000, 390, 120 and 70 Hz for the samples 1 to 6, respectively. This large change of $f_p$ was due to the difference in the viscosity of the carrier liquid, as will be shown below.

2.2.2 Effect of viscosity of liquid

As shown in Fig. 2(b), the peak frequency became lower from sample 1 to 6. As listed in Table 1, this corresponds to the increase of the glycerol concentration in the solution. Since the viscosity of the glycerol is much higher than that of the water, the viscosity of the mixed solution becomes higher with the increase of the glycerol concentration.

The effective viscosity of the mixed solution can be obtained as follows. It is well known that the Brownian relaxation time is given by $\tau_B = 3\eta V_h/k_BT$, where $\eta$ is the viscosity of the liquid, $V_h$ is the hydrodynamic volume of the particle. Therefore, the peak frequency $f_p$ is inversely proportional to the viscosity $\eta$. Using the measured value of $f_p$ listed in Table 1, we can estimate the viscosity of each sample.

In Fig. 3, viscosity of samples, which was normalized by that of pure water (sample 1), was shown as a function of the concentration ratio of glycerol. As shown, viscosity of the mixed solution became 1.4, 2.5, 6.4, 21 and 36 times larger than that of water (sample 1) for samples 2 to 6.

Using the measured peak frequency $f_p$, we re-plot the frequency dependences of the susceptibility shown in Figs. 2(a) and 2(b). The results are shown in Figs. 4(a) and 4(b). Here, the horizontal axis represents the frequency normalized by the peak frequency $f_p$ for each sample, while the vertical axis represents the susceptibility normalized by the peak values of $\chi'' (f_p)$ for each sample.

As shown, the frequency dependencies became the
same for all samples. This indicates that the difference in the viscosity of the carrier liquid caused the change in the frequency dependence of the susceptibility among samples.

![Fig. 2 Frequency dependence of the AC susceptibility for 6 samples listed in Table 1, (a) Real part \( \chi' \), and (b) imaginary part \( \chi'' \).](image)

**Fig. 2** Frequency dependence of the AC susceptibility for 6 samples listed in Table 1, (a) Real part \( \chi' \), and (b) imaginary part \( \chi'' \).

![Fig. 3 Viscosity of the water/glycerol mixed solution as a function of glycerol concentration.](image)

**Fig. 3** Viscosity of the water/glycerol mixed solution as a function of glycerol concentration.

### 2.3 M-H curve

We measured the magnetization of the markers in solution. In Fig. 5, the circles show the experimental results. Here, vertical axis represents the value of magnetization \( M \) at \( f = 0 \) Hz, \( M(0) \), while the horizontal axis represents the amplitude \( H_a \) of the excitation field. In the experiment, the value of \( M(0) \) was obtained from the value of \( M(90 \text{ Hz}) \) since \( M \) became constant independent of the frequency at low frequencies.

![Fig. 4 Normalized frequency dependence of AC susceptibility. (a) real and (b) imaginary part for the different concentration.](image)

**Fig. 4** Normalized frequency dependence of AC susceptibility. (a) real and (b) imaginary part for the different concentration.

As shown, \( M \) increased linearly at small values of \( H_a \), while it began to saturate at higher \( H_a \). It is known that this saturation of \( M \) was caused by the nonlinear effect of Brownian relaxation in high excitation fields.

### 3. Analysis

#### 3.1 Expression for susceptibility and magnetization

We analyze the experimental results shown in Sec. 2 by taking account of the distribution of size \( d \) and magnetic moment \( m \) of the marker. In this case, we define the parameters as follow. The diameter of the \( i \)-th magnetic nanoparticle is \( d_{ni} \), thickness of the coating material is \( t \), and the hydrodynamic diameter of the marker is \( d_{hi} = d_{ni} + 2t \). Distribution function of the diameter is \( f(d) \).
Then, the number of \(i\)-th markers is \(n_i=\delta(d_h)\Delta d_h\), and the magnetic moment is given by \(m_i=\mu_0 M_s V_{mi}\) where \(V_{mi}=\left(\frac{\pi}{6}\right)d_{mi}^3\) is the volume of the magnetic nanoparticle and \(M_s\) is the saturation magnetization.

\[
\chi''(\omega) = \frac{1}{5\mu_0 k_BT} \sum_i \frac{n_{m_i}^2}{1+(\omega\tau_i)^2} \chi_0 \tag{1}
\]

\[
\chi'(\omega) = \frac{1}{5\mu_0 k_BT} \sum_i \frac{\omega\tau_i n_{m_i}^2}{1+(\omega\tau_i)^2} \chi_0 \tag{2}
\]

where \(k_B\) is the Boltzmann constant, \(V_T = \sum n_{m_i}^2\) is the total volume of the sample, and \(\chi_0\) represents the susceptibility at high frequency limit. The relaxation time of the Brownian rotation of the particle is given by

\[
\tau_i = 3\eta V_{mi} / k_BT \tag{3}
\]

Expression for the \(M_1-H\) curve is given by\(^{14,15}\)

\[
M_1(0) = \frac{H}{3\mu_0 k_BT} \sum \delta (d_h) \Delta d_h g(m_i) \left[\left(\frac{\chi'_1(0)}{\chi_0}\right) - 1\right] \Delta m_i \tag{4}
\]

where \(g(m)\) is the distribution function of \(m\), and \(\xi_i = m_iH / k_BT\) is the parameter representing the strength of the applied field. The relationship between the distribution function \(f(d_h)\) and \(g(m)\) is given as follows. Note that \(g\Delta m\) is the number of particles with the magnetic moment \(m\), while \(\delta(d_h)\) is the number of particles with diameter \(d_h\). Since these values should be the same, we obtain

\[
f(d)\Delta d = g(m)\Delta m \tag{5}
\]

### 3.2 SVD method

Since the frequency dependence of the susceptibility is theoretically given by Eqs. (1) and (2), we can estimate the size distribution of the markers by comparing the experimental results with the theoretical ones, as shown below. In the comparison, we first choose \(N\) sets of \(\tau_i (i=1,...,N)\) in Eqs. (1) and (2). Then we take \(n_{m_i^2}\) as unknown values, and determine the values of \(n_{m_i^2}\) so as to obtain the best fit between the experimental and theoretical results. With this technique we can obtain the \(\chi''\cdot n_{m_i^2}\) curve. This curve is transformed into the \(d_h\cdot n_{m_i^2}\) curve using Eq. (3), which gives the size distribution of the markers.

To determine the values of \(n_{m_i^2}\) in the presence of experimental error in the practical data, we use the mathematical technique known as SVD method. Details of the SVD methods are described in refs 9-11).

### 3.3 Distribution of particle size \(d\)

Using the SVD method, we estimate the size distribution of 6 samples listed in Table 1. Note that the same magnetic markers were used in these samples, while the viscosity of the carrier liquid were different among samples. In the analysis, we used the frequency dependence of the imaginary part of the susceptibility shown in Fig. 2(b), because the imaginary part is unaffected by the value of \(\chi_0\), as shown in Eq. (2). In applying SVD method, we took 3 largest singular values.

The estimated size distributions for different concentrations are shown in Fig. 6. Here, the horizontal axis is the hydrodynamic diameter \(d_h\), while the vertical axis represents the fraction of \(n_{m_i^2}\).

As shown in Fig. 6, the size distribution became almost the same among samples. Peak values of \(n_{m_i^2}\) were obtained for the diameter \(d_h = 56, 55, 55, 55, 54, 56\) nm
from the sample 1 to 6 respectively. Note that the size distribution should be the same among samples, since the same markers were used. Therefore, the agreement of the size distribution shown in Fig. 6 indicates the validity of the size estimation using the SVD method.

Using these size distributions shown in Fig. 6, we reconstructed the frequency dependence of the susceptibility from Eqs. (1) and (2). The results are shown in Fig. 7(a), 7(b) and 7(c) for the sample 1, 4 and 6, respectively. The solid lines are the calculated results, while the symbols are the measured ones. In the calculation, we set $\chi_{\infty} = 0$ in obtaining the real part of the susceptibility.

As shown, good agreement was obtained between the calculated and experimental results for different solutions. This agreement shows the validity of the present estimation using SVD technique.

3.4 Distribution of magnetic moment $m$

Next, we estimate the distribution of magnetic moment $m$ from the magnetization curve shown in Fig. 5. For this purpose, we compare the experimental results with Eq. (4). In this case, we first choose $N$ sets of $m_i$ ($i = 1, \ldots, N$) in Eq. (4). Then we take $g_i$ as unknown values, and determine the values so as to obtain the best fit between the experimental and theoretical results by using the SVD method.

In Fig 8(a), the estimated distribution of $m$, i.e., $g_i m_i$ curve is shown. As shown, the value of $m$ distributed from $5\times10^{-25}$ to $5\times10^{-24}$ Wbm with mean value of $m=3\times10^{-24}$ Wbm. For the following discussion, we also show in Fig. 8(b) the $g_i m_i^2$ curve.

Substituting the distribution of $m$ shown in Fig. 8(a) into Eq. (4), we could reconstruct the $M/H$ curve. In Fig. 5, the solid line shows the reconstructed results. As shown, good agreement was obtained between the experimental and reconstructed results. This agreement indicates the validity of the estimation of $m$ distribution.
3.5 Relationship between $m$ and $d$

We now discuss the relationship between the magnetic moment $m$ and size $d$. In the case of single domain particles, it is well known that $m$ is proportional to the volume of the particle, i.e., $m$ is proportional to $d^3$. In practical markers, however, aggregation of particles occurs in making markers. Therefore, it is expected that the magnetic marker consisted of agglomerate of particles. In this case, the magnetic moments of individual particles within the agglomerate should not align in the same direction, i.e., agglomerate of particles will behave like multi-domain particles. As a result, simple relationship that $m$ is proportional to $d^3$ should be modified in this case.

The relationship between $m$ and $d$ can be obtained by combining the $nm^2$-$ds$ curve shown in Fig. 6 and the $g_m$-$m^2$-$m$ curve shown in Fig. 8(b). In Fig. 9, circles represent the estimated dependence of $m$ on $d$. As shown, $m$ increased in proportion to the $d^3$ for the case of $d<40$ nm. This relationship is consistent with the result of single domain particles. When $d$ becomes larger, on the other hand, dependence of $m$ on $d$ changed: $m$ becomes proportional to $d^2$, and then proportional to $d$ with the increase of $d$.

It must be noted that Fe$_3$O$_4$ is expected to form the single domain particle when the diameter $d$ is less than 40 nm, while it forms the multi-domain particle for the case of $d>40$ nm. The result shown in Fig. 9 is consistent with this prediction.

4. Conclusion

We characterized the magnetic properties of magnetic markers in solution for biosensor application. Frequency dependence of the AC susceptibility and the magnetization curve, which were dominated by the Brownian rotation of the marker, were measured. The effect of the viscosity of the carrier liquid on the AC susceptibility was also clarified. The experimental results were analyzed by the singular value decomposition (SVD) method. The distribution of marker size $d$ was obtained from the frequency dependence of the susceptibility. The distribution of magnetic moment $m$ was obtained from the magnetization curve. The relationship between $m$ and $d$ was also discussed. The present estimation method using SVD technique will be useful to obtain the distribution of $d$ and $m$, which are the important parameters of the magnetic marker for biosensor application.
References