Viscosity Effect on the Brownian Relaxation based Detection for Immunoassay Applications

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***Abstract*—Magnetic nanoparticles (MNPs) coated with Protein-G have been a model system to be used in different antibodies binding study. It is highly desirable to use a substrate-free biosensing system to detect antibodies binding in real-time. In this paper, we developed and applied a MNPs and search-coils integrated detection system, which is not only sensitive to the hydrodynamic volume of MNPs but also sensitive to the environment of MNPs, such as viscosity and temperature of the solution. We demonstrated that the viscosity effect influenced the amplitudes and phases of the 3rd (fH±2fL) and 5th (fH±4fL) harmonics for the mixed frequency testing scheme. The binding between antibodies and Protein-G on MNPs increased hydrodynamic volume of particles, as a result, it also changed the amplitudes and phases of harmonics, which are the object signals we need to analyze. We demonstrated that the viscosity of antibody solution is lower than that of MNP solution, and the antibody binding effect could be shielded by the viscosity effect to certain extent.**

I. INTRODUCTION

Magnetic nanoparticles (MNPs) have been utilized in biomedical detection systems because of its adjustable and comparable size to different molecules. Many research groups have pursued biochip based detection systems by utilizing the magnetic characteristic of MNPs. Ultra high sensitivity has been achieved for the detection of different molecules including antibodies. However, its limit is also obvious, the necessity of the to-be-detected molecules to bind onto the pre-coated substrate. Recently, the integration of MNPs and search-coils for a biosensing system has been quickly developed and adopted as a potential candidate for future substrate-free biosensing system [1-9]. One unclear point for this technique is the effect of viscosity of the solution, which will be investigated in this paper. Our experiment in this paper is based on the frequency mixing at the nonlinear region in the magnetization curve of superparamagnetic nanoparticles. Two sinusoidal magnetic fields with distinct frequencies are applied simultaneously to the sample: one with low frequency f_L(50Hz) but high amplitude A_L (100 Oe), written as $A_L \cos(2\pi f_L t)$, the other is high frequency f_H(35KHz) but low amplitude A_H (10 Oe), written as $A_H \cos(2\pi f_H t)$. This low frequency field drives MNPs into nonlinear region and the high frequency field is then applied to generate mixing-frequency signals, which work as marks of different MNPs [1,3,10]. The response signal that contains a linear combination mf_L+nf_H is then collected by a pair of balanced search coils. These harmonics are highly specific to the nonlinearity of the magnetization curve of the particles [1,3,10]. The relaxation mechanism for superparamagnetic nanoparticles is a joint effect of Néel and Brownian relaxation. For Fe_3O_4 particles with diameter smaller than 20nm, Néel relaxation dominates, and Brownian relaxation starts to dominate when its diameter is larger than 20nm [1]. It was demonstrated that antibodies bind to Protein-G layer on MNPs in real time, which changes the hydrodynamic volume of MNPs, leading to the change of the harmonics [3]. However, addition of antibody solution also changes environment of MNPs, such as the viscosity. In this study, the viscosity effect is analyzed and compared experimentally, and the immunoassay experiment is validated by considering the interference of the viscosity effect. We show experimentally that addition of antibody solution reduces the viscosity of MNPs solution, which increases the amplitude and decreases the phase of harmonics. And the binding of antibodies to MNPs increases the hydrodynamic volume, which decreases the amplitude and increases the phase of harmonics. We demonstrated that the viscosity factor attenuates the aimed signal in immunoassay application, which should be considered carefully for future search-coil based immunoassay applications.

II. METHOD

A. Néel relaxation and Brownian relaxation

As we apply the sinusoidal field H, the relaxation time τ governs a MNP's ability to follow changes in the applied field via two relaxation mechanisms, Néel and Brownian relaxation. Néel relaxation is the rotation of magnetization inside the magnetic core,

$$\tau_N = \tau_0 \exp\left(\frac{KV_m}{k_B T}\right) \quad (1),$$

where the time constant $\tau_0 = 10^{-9} s$, K is the anisotropy constant of MNP, V_m is the magnetic core

volume of MNP, k_B is Boltzmann constant, T is absolute temperature in Kelvin.

Brownian relaxation is the physical rotation of the hydrodynamic volume of MNP,

$$\tau_B = \frac{3\eta V_H}{k_B T} \quad (2),$$

where η is the viscosity of MNP solution, V_H is the hydrodynamic volume of MNP.

Total relaxation time τ is a linear combination of τ_N and τ_B:

$$\tau = \frac{\tau_N \cdot \tau_B}{\tau_N + \tau_B} \quad (3)$$

B. Magnetization theory

Under two sinusoidal fields, the total field is

$$H = A_L \cos(2\pi f_L t) + A_H \cos(2\pi f_H t) \quad (4)$$

Magnetization of MNPs can be approximated by the static Lagnevin function [9]:

$$\frac{M}{M_s} = L(\frac{m_0 \mu_0 H}{k_B T}) \quad (5),$$

where M_s is the saturation magnetization of the MNP, and M is the magnetization of MNP at field H, m_0 is the magnetic moment of one particle, H is the total field as shown in formula (4). Taylor Expansion near zero magnetization shows the major mixing frequency components are as follows:

$$\begin{aligned}
&\frac{M}{M_s} = L(\frac{m_0 \mu_0 H}{k_B T}) \quad (6)\\
&= \frac{1}{3}(\frac{m_0 \mu_0}{k_B T})H - \frac{1}{45}(\frac{m_0 \mu_0}{k_B T})^3 H^3 + \frac{2}{945}(\frac{m_0 \mu_0}{k_B T})^5 H^5 + \dots \\
&= \dots + \left[-\frac{1}{60} A_H A_L^2 \left(\frac{m_0 \mu_0}{k_B T} \right)^3 + \frac{1}{252} A_H^3 A_L^2 \left(\frac{m_0 \mu_0}{k_B T} \right)^5 \right. \\
&\left. + \frac{1}{378} A_H A_L^4 \left(\frac{m_0 \mu_0}{k_B T} \right)^5 + \dots \right] \cdot \cos\left[2\pi \left(f_H \pm 2 f_L \right) t \right] \\
&+ \left[\frac{1}{1512} A_H A_L^4 \left(\frac{m_0 \mu_0}{k_B T} \right)^5 + \dots \right] \cdot \cos\left[2\pi \left(f_H \pm 4 f_L \right) t \right] + \dots
\end{aligned}$$

Assuming a constant phase lag ϕ and no rotational motion of the carrier medium, we can obtain the actual phase lag ϕ and the magnetization amplitude M_0 shown as follows [11]:

$$\phi = \arctan(\omega \tau) \quad (7)$$

$$M_0 = M \cdot \cos(\phi) \quad (8)$$

where ω is angular frequency, τ is total relaxation time.

According to Lenz law, the induced voltage in search coil is proportional to

$$V \propto \frac{dM_0}{dt} \cdot v_{voulumeofparticle} \quad (9)$$

Thus, the collected signal in search coil is specific to characteristics of MNPs, such as relaxation time. The 3rd and 5th harmonics are among the best candidates to intuitively show these characteristics.

C. Experimental setup

We firstly investigate the viscosity effect on the collected signal. A high frequency of 35 KHz, 100Oe field and a low frequency of 50Hz, 10Oe field are applied simultaneously. Then we put 30uL MNP solution into the search-coil system (shown in Figure 1) and monitor the real-time phase and amplitude in 3rd harmonic (at 35.1KHz) and 5th (at 35.2KHz) harmonic. After 50~80 seconds, we added four solutions with the same volume of 50uL but different viscosities listed in Table 1. The signals were collected in real-time after the addition of solution with different viscosity for 150 seconds. Also, we studied the antibody binding effect under the same applied field. We put 50uL MNP solution, and prepared and test six different antibody samples listed in Table 2. We collected the signals before and after adding the different antibody samples.

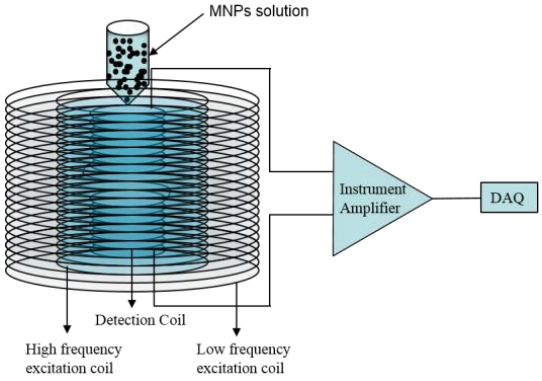


Figure 1. Search-coil based detection system.

III. VISCOSITY EXPERIMENT

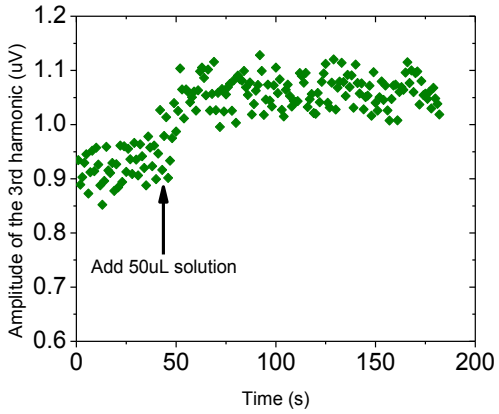
In this paper, we use IPG-25 nanoparticles (purchased from Ocean NanoTech, iron oxide core MNPs of 25nm diameter conjugated with around 10nm Protein G layer, 1mg/mL). For nanoparticles in this size, Brownian relaxation dominates [1].

Table 1 Viscosity effect on the collected signals

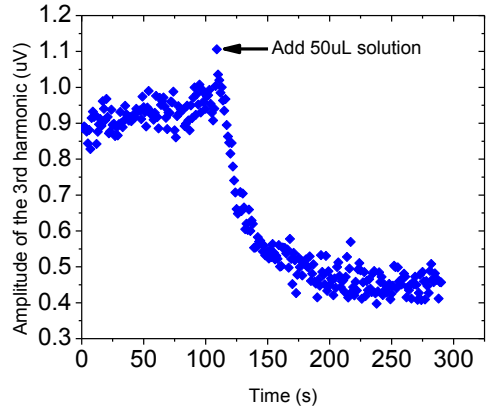
Solution (Room temperature)	Amplitude of the 3rd harmonic	Phase of the 3rd harmonic	Amplitude of the 5th harmonic	Phase of the 5th harmonic
Water(0.95cp)	+0.193uV	-2.549°	+0.023uV	+3.678°
PBS(unknown viscosity)	+0.178uV	-1.996°	+0.012uV	+2.827°
25%volume glycerol mixed with water(viscosity 1.18cp)	+0.144uV	-0.924°	+0.041uV	-0.285°
50% volume glycerol mixed with water(viscosity 1.59cp)	-0.356uV	+29.032°	-0.14uV	+12.284°

('+' indicates increase of amount, '-' indicates decrease of amount)

According to formula (2) and (3), the relaxation time is expressed as $\tau \approx \tau_B = \frac{3\eta V_H}{k_B T}$ .If the viscosity of MNP solution decreases, then the relaxation time τ decreases, the phase lag ϕ decreases and the amplitude increases. If the viscosity increases, τ increases, the phase lag ϕ increases and the amplitude decreases. We added 50uL solutions with different viscosities into 30uL IPG-25 solution, respectively. Data are shown in table 1 and plotted in Fig. 2. Viscosities are calculated according to [12].



(a)



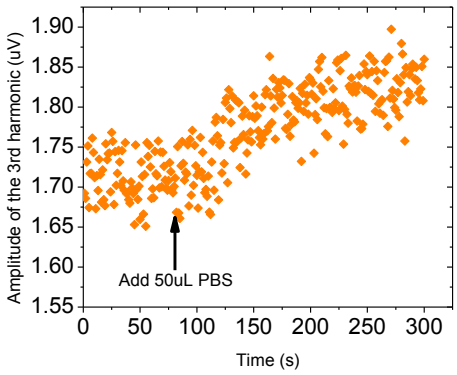
(b)

Figure 2. Viscosity effect on the amplitude of the 3rd harmonic (a) Add 25%volume glycerol mixed with water; (b) Add 50%volume glycerol mixed with water.

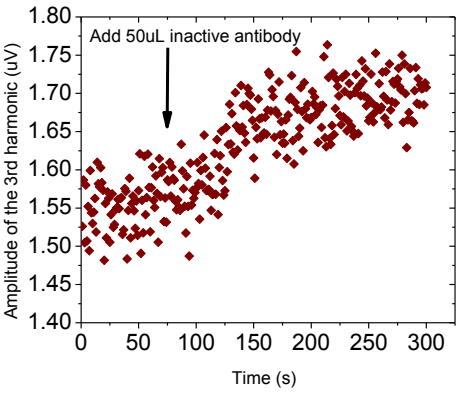
As shown in Table 1 and Fig 2, we can deduce that the viscosity of PBS is a little bit higher than water, it's between 0.95cp and 1.18cp. And the viscosity of IPG-25 solution is between 1.18cp and 1.59cp. The amplitude of the 5th harmonic increases or decreases as amplitude of the 3rd harmonic changes. The phase of the 5th harmonic may not change as the expected trend. That may be resulted from its relatively lower signal to noise ratio (SNR) compared to 3rd harmonic.

IV. IMMUNOASSAY EXPERIMENT

Active antibodies can bind to Protein-G on MNPs, which increases the hydrodynamic volume of MNPs. According to formula (2) and (3), as the hydrodynamic volume of MNP increases, relaxation time τ increases, phase lag ϕ increases and the amplitude decreases. We used 50uL IPG-25 MNPs, added antibodies with different concentrations. Two control groups are tested with 50uL PBS solution and 50uL inactive antibody. Testing summary refers to Table 2.



(a)



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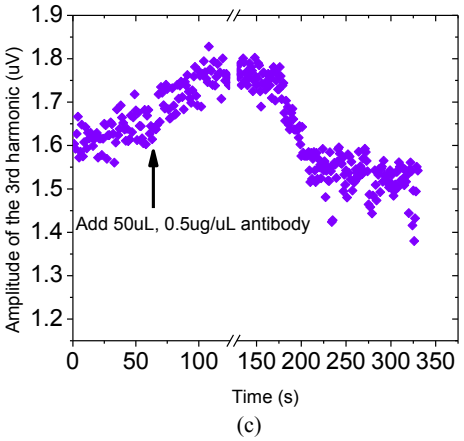

(c)

Figure 3. Antibody binding effect mixed with the viscosity effect on the amplitude of the 3rd harmonic (a) add 50uL PBS; (b) add 50uL inactive antibody; (c) add 50uL, 0.5ug/uL antibody.

Table 2 Antibody binding effect vs. viscosity effect

Sample	1	2	3	4	5	6
Solution (In room temperature)	Antibody 0.5ug/uL 50uL	Antibody 0.5ug/uL100uL	Antibody 0.05ug/uL,50uL	Antibody 0.025ug/uL, 50uL	Inactive Antibody 0.5ug/uL，50uL	PBS, 50uL
Amplitude, 3rd harmonic	-0.130uV	-0.078uV	-0.025uV	+0.015uV	+0.134uV	+0.133uV
Phase, 3rd harmonic	+3.161°	+0.539°	+0.862°	-0.909°	-2.512°	-2.225°

('+' indicates increase of amount, '-' indicates decrease of amount)

As shown in Table 2 and Fig 3, the viscosity of the antibody solution is close to that of PBS. From the collected data in sample 2, we added more antibody solution, and the antibody binding is expected to increase the average hydrodynamic volume in MNPs, thus the amplitude is supposed to reduce more than 0.13uV compared to sample 1. However, we get a 0.078uV reduction in amplitude, which indicates that the addition of the antibody solution decreases the viscosity of MNP solution, thus increases the amplitude to some extent. In the antibody addition experiment, we added the antibody solution first, and found an immediate increase of the amplitude, then we waited for about 5 minutes after we saw a gentle drop in the collected signal, which indicates that upon the addition of antibody solution, the viscosity effect appears first, and after about 5 minutes' reaction, the antibody binding effect appears gradually.

V. CONCLUSIONS

In this paper, we first investigated the viscosity effect on the collected signals for a MNPs and search-coil integrated immunoassay detection system. We found that the addition of water, PBS, and 25%volume glycerol mixed with water (viscosity 1.18cp) led to the increase of the amplitude and the decrease of the phase for the real-time signals. The Addition of 50% volume glycerol mixed with water (viscosity 1.59cp) led to the decrease of the amplitude and the increase of the phase. We could infer that the viscosity of MNP is between 1.18 cp and 1.59 cp. The viscosity of human blood is 4~5 cp, so the addition of blood could lead to the decrease of the amplitude, and the effect of antibody binding could be easily sheltered by this viscosity effect. Secondly, we studied the antibody binding effect, when we add 50uL antibody (0.5ug/uL) the amplitude decreased as we expected. But when adding 100uL antibody (0.5ug/uL), the amplitude decreased less than that 50uL case. Furthermore, when we used 50uL antibody with a concentration of 0.025ug/uL, the amplitude was basically no change. This demonstrated in our previous experimental result that the viscosity of antibody solution is smaller than MNP solution, this will lead to increase of amplitude. But the antibody binding with MNPs could lead to the decrease of the amplitude. In summary we found that the binding effect and the viscosity effect work against each other. For future immunoassay applications, control experiments are even more critical and should be paid special attention.

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