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Evaluation of AC magnetic characteristics of magnetic nanoparticles using biotin-avidin binding reaction in serum medium

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Abstract*— For magnetic immunoassay (MIA), a frequency characteristic evaluation device was developed to assess the alternating (AC) magnetic characteristics of magnetic nanoparticles (MNPs). The changes in the AC magnetic characteristics of the MNPs upon variation of the solvent medium were successfully monitored by using the frequency characteristic evaluating device. The changes in the alternating magnetic characteristics of the MNPs due to the biotin-avidin binding reaction in serum medium were comprehensively analyzed using the frequency characteristic evaluation device and superconducting quantum interference device (SQUID) for magnetic immunoassay. It was confirmed that agglutination of serum tended to occur when the concentration of the MNPs was lower. The biotin-avidin binding reaction could be confirmed from the change in the frequency characteristics by using the frequency characteristic evaluation device. The results of harmonic measurement showed that MIA with high sensitivity is possible even in a serum medium by using the SQUID magnetic immunoassay device.

***Index Terms*— Magnetic nanoparticles, Magnetic immunoassay, Biotin-Avidin, Serum.**

I. INTRODUCTION

Magnetic nanoparticles (MNPs) have shown great promise for application in magnetic immunoassays (MIAs) [1]. Conventional immunoassays are generally based on an optical method; the best example of the optical method is the enzyme-linked immune sorbent assay (ELISA) [2]. Compared to conventional optical methods, the analysis time required for MIAs is short, and such techniques can also be used to analyze specimens that do not transmit light. However, blood samples used in immunoassays have different viscosities owing to the influence of serum and other factors, and the viscosity affects the magnetization characteristics of MNPs [3]. In this study, we developed a device that can be used to evaluate the magnetic properties of MNPs under the influence of an alternating magnetic field and evaluate the alternating magnetic properties when the viscosity of the medium containing the MNPs is changed. We comprehensively evaluated the changes in the alternating magnetic characteristics of MNPs during the biotin-avidin binding reaction, which is widely used for fundamental immunoassay measurements, by using a newly developed superconducting quantum interference device (SQUID) magnetic immunoassay [4] system that has the functions as an immunoassay and frequency characteristic evaluation device.

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II. METHODS

A. Samples

To assess the frequency characteristic evaluation device, MNPs were suspended in glycerol to give different viscosities. Nanomag®-D (COREFRONT) MNPs with a particle size of 130 nm was used herein. A buffer solution was prepared by adding polyoxyethylene sorbitan monolaurate (Tween 20) to 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). The concentration of the MNPs was 1.2 mg·mL^{-1}, and the concentrations of glycerol were 0, 10, 20, 30, and 40%.

For analysis of the biotin-avidin binding reaction, iron oxide nanoparticles with biotin (Nanotech Ocean), prepared by adding biotin to the MNPs, and streptavidin-coated particles (Spherotech Inc.), prepared by adding avidin to polystyrene, and were used. The reaction model for binding the biotin beads and avidin polymer is shown in Fig. 1. Biotin is a water-soluble vitamin B, and avidin is a biotin-binding protein. The biotin-avidin binding reaction forms one of the strongest non-covalent bonds [5]. Therefore, as basic research, the influence of this bonding process on the magnetic characteristics of the MNPs was evaluated. The sample was diluted with phosphate buffered saline containing Tween 20 (PBST) to achieve serum concentrations of 0, 20, and 40%.

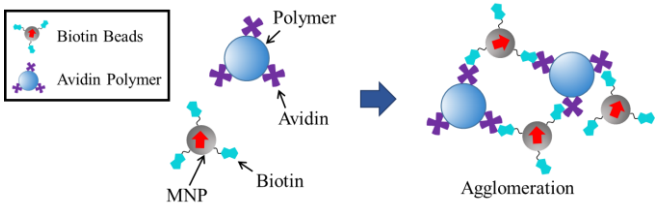

Fig. 1. Model of binding reaction between biotin beads and avidin polymer.

B. Frequency characteristic evaluation device

A frequency characteristic evaluation device was developed herein, as shown in Fig. 2, and used to evaluate the frequency characteristics of MNPs in solution. An AC voltage with amplitude of 0.1 V and a frequency in the range of 5 Hz to 5 kHz was applied to the excitation coil, and the secondary magnetic field generated due to the sample was detected via a pick-up coil. The excitation coil was made by using Cu wire having a diameter of 0.3 mm. The length, internal diameter, external diameter, and the number of turns of the excitation coil were 60 mm, 37 mm, 49 mm, and 4000 turns, respectively. The pick-up coil was made by using Cu

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wire having a diameter of 0.2 mm. The length, internal diameter, external diameter, and number of turns of the pick-up coil were 38 mm, 11.8 mm, 19.8 mm, and 1800 turns, respectively. A gradiometer was used as the pick-up coil to reduce environmental noise. The signal detected by the coil was transmitted to the gain phase analyzer (NF Corp.) through the amplifier circuit (NF Corp). The gain was calculated from the intensity of the current from the applied coil, which was read from the shunt resistance of CH1 and the detection signal of CH2. By subtracting the case signal from the sample signal, the sensitivity of the pick-up coil was corrected.

C. SQUID magnetic immunoassay device

The harmonic alternating magnetic characteristics of the biotin-avidin binding reaction were evaluated by using the SQUID magnetic immunoassay device. The configuration of the SQUID magnetic immunoassay device is shown in Fig. 3. An alternating magnetic field of 1.06 kHz, 8 mT_{p-p} was applied to an excitation coil and the secondary magnetic field generated from the sample was detected by a gradiometer pick-up coil. The sample volume was 50 µL. The signal detected by the coil was transmitted to the SQUID (SUSTERA) [6], and was lock-in detected by the multifunction circuit device via the flux locked loop circuit. The signal of the MNP solution was influenced by diamagnetic substances such as water; thus, the third harmonic, which can remove the influence of these substances, was used for the evaluation.

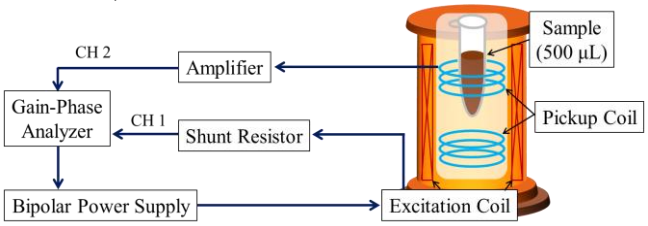


Fig. 2. Configuration of frequency characteristic evaluation device system

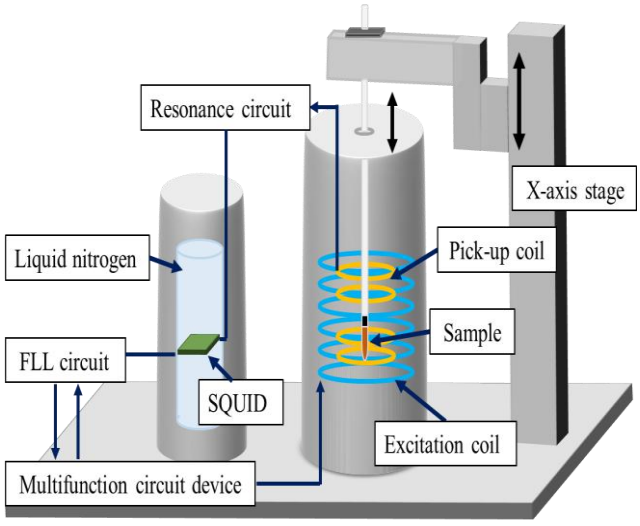


Fig. 3. Configuration of SQUID magnetic immunoassay device system.

III. RESULTS AND DISCUSSION

A. Verification of frequency characteristic evaluation device

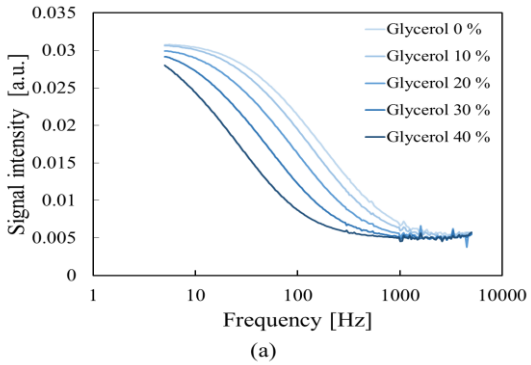
The frequency characteristics of the MNPs in glycerol solution are shown in Fig. 4. It was confirmed that the position of the peaks for the imaginary parts of the frequency shifted to the lower frequency region as the viscosity of the medium increased. MNPs are known to exhibit the Brownian relaxation phenomenon in which the MNP rotates in a liquid [7]. The Brownian relaxation time (τ_B) is expressed by Equation (1).

$$\tau_B = \frac{3\eta V}{\kappa_B T} \quad (1)$$

From Equation (1), it is expected that the solvent viscosity (η) should affect the relaxation time of the magnetic moment. The results shown in Fig. 1 are considered to be due to the slow movement of the MNPs in the solution with an increase in the concentration of glycerol, where the peak position shifted to the lower frequency region as the frequency at which the MNPs can respond to the alternating magnetic field decreased. It was confirmed that the imaginary part of the frequency of the Nanomag®-D (particle size 130 nm) solution had a maximum of approximately 180 Hz and that of the MNP solution with 40 % glycerol shifted to approximately 25 Hz. These results indicate that the Brownian relaxation phenomenon of a solution of MNPs can be evaluated from the frequency characteristics by using the developed frequency characteristic evaluation device.

B. Frequency characteristics of binding reaction

The frequency characteristics of non-binding biotin beads in serum medium are shown in Fig. 5. The concentration of the biotin beads was 500 $\mu g \cdot mL^{-1}$. From Fig. 5 (a), it was confirmed that the signal intensity of the real part of the frequency decreased with an increase in the serum concentration. This is considered to be that the MNPs aggregated in the solution due to the serum. Figure 5 (b) confirms that with an increase in the concentration of the serum, the signal intensity of the imaginary part increased and the position of the peak shifted slightly to the lower frequency region. This is because as the solvent viscosity increased with increasing serum concentration, the alternating magnetic response of the MNPs was delayed, and the frequency at which the MNPs could respond to the alternating magnetic field was lowered.



(a)

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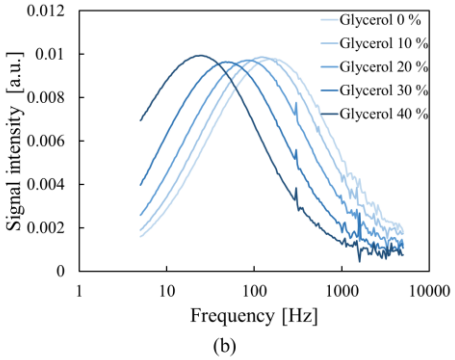
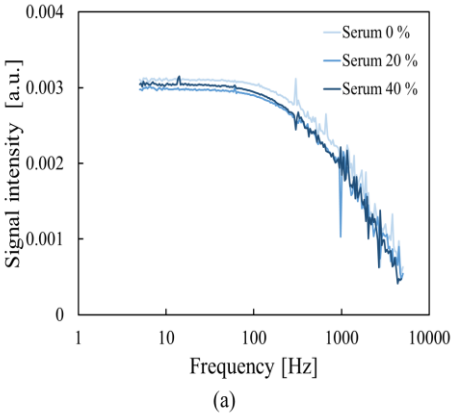


Fig. 4. Frequency characteristics of MNPs in glycerol solution (a) real part and (b) imaginary part.

However, the frequency shift was smaller than that obtained with glycerol (see Fig. 4). This is attributed to the difference in the viscosity of serum and glycerol. The viscosity of the 40% glycerol solution was 4.31 mPa·s, whereas the viscosity of the 40% serum solution was 1.35 mPa·s [8]. The smaller frequency shift with serum is attributed to the lower viscosity of serum relative to that of glycerol.

The frequency characteristics of the biotin-avidin binding reaction in serum solvent are shown in Fig. 6. Each sample was diluted to a biotin-bead concentration of 500 $\mu g \cdot mL^{-1}$ and an avidin-polymer concentration of 1.5 $mg \cdot mL^{-1}$. From Fig. 6 (a), it was confirmed that the signal intensity of the real part increased as the serum concentration increased. This is because the extent of the biotin-avidin binding reaction decreased due to the influence of serum. As the serum concentration increased, more of the MNPs in the solvent remained unbound. Therefore, the signal became more intense. From Fig. 6 (b), it was confirmed that the peak shifted to the lower frequency region with an increase in the serum concentration. As also shown in Fig. 5 (b), this is because the frequency at which the MNPs can respond to the alternating magnetic field became lower as the solvent viscosity increased with increasing serum concentration.

In the case of Fig. 5 (unbound MNPs) and the case of Fig. 6 (bound MNPs), it was confirmed that the signal intensity decreased at all serum concentrations. This is because the particle size increased upon binding of the biotin beads with the avidin polymer and the particles became unable to respond to the alternating magnetic field.



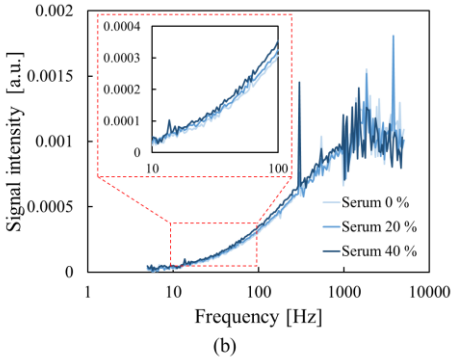


Fig. 5. Frequency characteristics of biotin beads in serum solution (a) real part and (b) imaginary part.

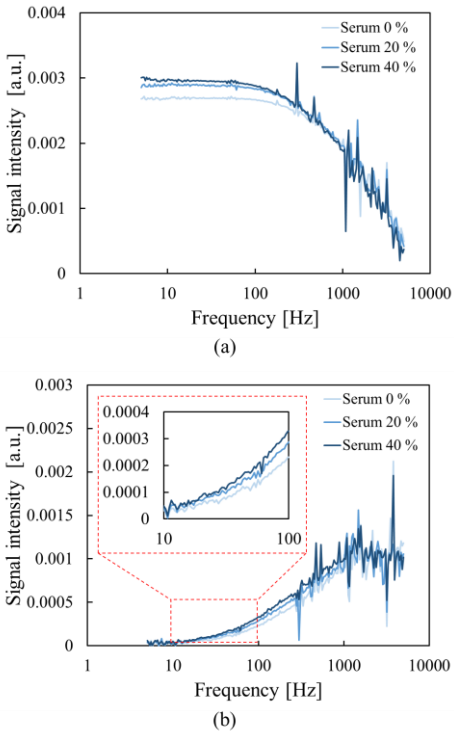


Fig. 6. Frequency characteristics of biotin-avidin binding reaction (a) real part and (b) imaginary part.

C. Harmonic characteristics of binding reaction

The dependence of the signal intensity on the concentration of the biotin beads, evaluated using the SQUID magnetic immunoassay device, is shown in Fig. 7. It was confirmed that the signal intensity increased with an increase in the number of biotin beads at all serum concentrations. The dependence of the signal on the concentration of the biotin beads could be confirmed using SQUID magnetic immunoassay. It was also confirmed that the signal intensity decreased due to the influence of serum. The signal decayed by 12% with 500 $\mu g \cdot mL^{-1}$ of biotin beads and by 28% with 2.5 $\mu g \cdot mL^{-1}$ biotin beads when the serum concentration was changed from 0 to 40%. Based on this result, it is proposed that the lower the concentration of biotin beads, the greater the agglutination of the MNPs due to the influence of serum. For the data presented in Fig. 5, the concentration of biotin beads (500 $\mu g \cdot mL^{-1}$) was high; thus, the change in the signal

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intensity is considered to be small.

Figure 8 shows the dependence of the signal of the biotin-avidin binding reaction on the avidin concentration. The biotin bead concentration was 2.5 $\mu g \cdot mL^{-1}$. It was confirmed that at all serum concentrations, the signal intensity decreased with an increase in the avidin polymer concentration. This is because the number of unbound MNPs in the solution decreased due to the biotin-avidin binding reaction. The dependence of the signal intensity on the avidin-polymer concentration could be confirmed in all serum solutions. It was confirmed that as the serum concentration increased, the signal change decreased. Similar to the results in Fig. 7, this is attributed to aggregation of the MNPs by serum. Therefore, the initial signal is considered to have decreased. The detection limit for the avidin-polymer using the SQUID magnetic immunoassay device was 0.5 $\mu g \cdot mL^{-1}$. The results corresponding to the harmonic characteristics of the binding reaction showed that highly sensitive MIA is possible even in serum medium.

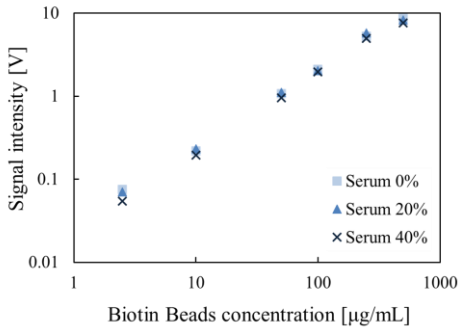

Fig. 7. Dependence of signal on biotin bead concentration in serum solution using third harmonic signal.

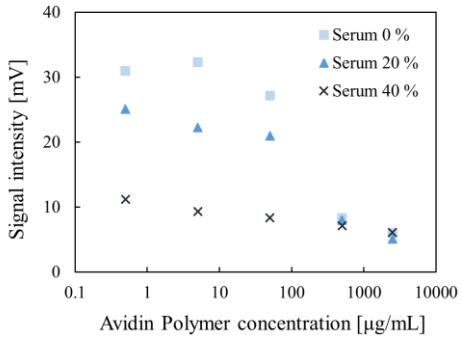

Fig. 8. Dependence of signal on avidin polymer concentration in serum solution using third harmonic signal.

IV. CONCLUSION

The AC magnetic response characteristics of MNPs in serum medium could be comprehensively evaluated by using the developed frequency characteristic evaluation device and SQUID magnetic immunoassay device. The detection limit of the avidin polymer using the SQUID magnetic immunoassay device was 0.5 $\mu g \cdot mL^{-1}$, which indicates that it is possible to perform sensitive MIA even in serum medium.

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Keiji Tsukada received Dr. Eng. and the Ph. Dr. degrees from Tsukuba University in 1990, and 2001, respectively. He joined the Central Research Laboratory, Hitachi Ltd. in 1982, where he was involved in the study of integrated solid-state chemical sensor for blood analyses. He was with the Superconducting Sensor Laboratory from 1991-1996. He was involved in the research and development of SQUID's and multichannel SQUID system. He was with the Central Research Laboratory, Hitachi Ltd. from 1996-2003. He was a Project Leader of the SQUID application research group. He is presently a Professor of Graduate School of Interdisciplinary Science and Engineering in Health Systems, Okayama University. He is involved in the research of chemical sensor, magnetic sensor and superconducting sensor devices, and their applications.