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Evaluation of Serum Influence on Magnetic Immunoassay using Magnetic Nanoparticles

Shun Takeuchi, Tetsuro Hirata, RyotaIsshiki, Yuta Nakamura, Kayo Fujimoto, Kenji Sakai, Toshihiko Kiwa, KeijiTsukada
Graduate School of Natural Science and Technology, Okayama Univ., Japan

Abstract*—Immunoassays are widely used as a clinical test method for examining proteins in the blood. A reduction in measurement time is desired. Our group is developing a magnetic immunoassay method using magnetic nano-particles (MNPs). MNPs, modified with antibodies, are added to the specimen to cause an antigen-antibody reaction; the amount is measured from the change in magnetization characteristics before and after the reaction. Magnetization of MNPs is affected by the surrounding environment. Therefore, we investigated changes in the magnetization characteristics of MNPs in serum, assuming measurement of blood components. We measured the magnetization characteristics with a hybrid magnetometer using a high-temperature superconducting quantum interference device (HTS-SQUID). We measured the 3rd harmonic characteristics in a high-frequency magnetic field with AC magnetic susceptibility meter. The magnetic moment, obtained by magnetization characteristics, increased in the serum compared to the buffer solution. This was caused by the aggregation of MNPs in the serum. Moreover, the intensity of the 3rd harmonic signal decreased in the serum due to the AC magnetic field. This is compared to the buffer solution that decreased due to a higher viscosity of the serum.

***Index Terms*— Magnetic susceptibility, MNPs, Serum, SQUID.**

I. INTRODUCTION

Immunological tests are primarily used to detect antibody concentrations in specimens, to include urine and blood, as well as detect value supports diagnosis of diseases. Presently, immunological methods such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and chemiluminescence immunoassay (CLIA) are widely used as immunoassay methods [1]. Easier operation and decreased measurement time are desired for clinical diagnosis. Recently, magnetic nano-particles (MNPs) have been applied to immunological tests [2]—[4]. Our group has developed a magnetic immunoassay method to measure antigen-antibody reactions using MNPs with high-sensitivity magnetic sensor. In the conventional method, disadvantages include a long reaction time, a washing step, and a limitation of the sample presented by the transmission of light. Conversely, magnetic immunoassays are advantageous in that they can be applied to opaque samples as well as non-pretreated samples. Therefore, the total measurement time is shorter than the conventional immunoassay. Based on these advantages, we developed a magnetic property evaluation device using a high-temperature superconducting quantum interference device (HTS-SQUID) with ultrahigh sensitivity magnetic sensors and, subsequently, measured the basic function of MNPs for magnetic immunoassay. MNPs are superparamagnetic materials. These materials are magnetically saturated in highly magnetic fields and do not have hysteresis due to the relaxation phenomena. Néel relaxation is caused by the rotation of magnetic moment of the particles, and Brown relaxation is attributed to particle rotation [5], [6]. In particular, the Brown relaxation alters the magnetization depending on the viscosity of the solution. Therefore, the influence of the solvent for MNPs is considered to be critical. The magnetization characteristics of MNPs, in the magnetic field, are expressed by the following:

$$M(H) = \int \mu\rho(\mu) L\left(\frac{\mu H}{k_B T}\right) d\mu \qquad (1)$$

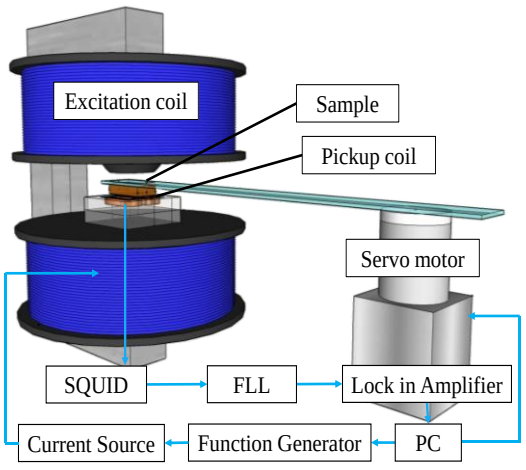
L is the Langevin function and is denoted as $L(x) = coth(x) - 1/x$. μ, $\rho(\mu)$, T, $k_B$are the value of the magnetic moment of one particle, distribution of the magnetic moment, temperature of the MNPs, and Boltzmann coefficient, respectively.

For accurate measurements, the magnetic properties of MNPs, and the dynamics of MNPs in blood components, such as serum, are important. In this study, the influence of serum on magnetic measurement was investigated.

II. METHODS

A. *Hybrid magnetic susceptibility meter*

The magnetization characteristic (M-H curve) was measured by the hybrid magnetometer as illustrated in Fig.1. This system consists of HTS-SQUID, electromagnets, servo motors, pickup coils, input coils, current source, a function generator, FLL circuits, lock-in amplifiers, and a PC.



(a) System diagram

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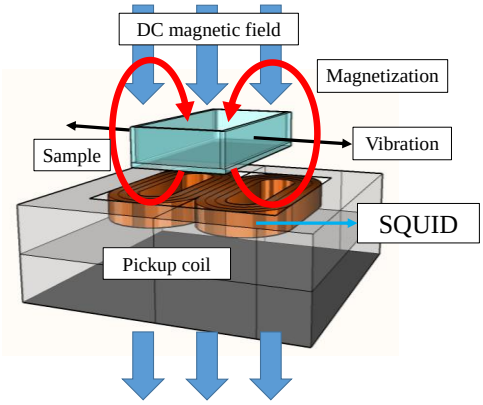

(b) Principle of measurement

Fig.1Schematic of a hybrid type magnetometer

HTS-SQUID, a FLL circuit, and input coils are installed in the magnetic shield. When the magnetic field is applied to a sample, using an electromagnet, the sample is magnetized, and a secondary magnetic field is generated from the sample. By vibrating this magnetized sample, the magnetic flux passing the pickup coil changes, and induced electromotive force is generated in the coil. A pickup coil uses a gradiometer. A current flows into the coil due to the induced electromotive force, and a signal is transmitted to the SQUID cooled in the dewar through the input coil. The signal, detected by the SQUID, is analyzed by a lock-in amplifier through an FLL circuit. The measurement of the M-H curve was performed at a frequency of 10 Hz in a DC magnetic field from−500 to 500 mT.

B. AC magnetic susceptibility meter

Third harmonic characteristics, in the high frequency magnetic field, were measured by the HTS-SQUID AC magnetic susceptibility meter developed by Mizoguchi et al [11]. Fig.**2** illustrates a schematic diagram of the AC susceptibility meter. For the measurement, we applied an alternating magnetic field with a frequency of 1 kHz and 8 mT_{pp} using the application coil and detected with a pickup coil. The pickup coil uses a gradiometer. The detected signal is transmitted to the SQUID, and lock-in detection is performed on the 3rd harmonic of 3 kHz. The reason for acquiring the 3rd harmonic is for high-sensitivity detection of low concentration MNPs.

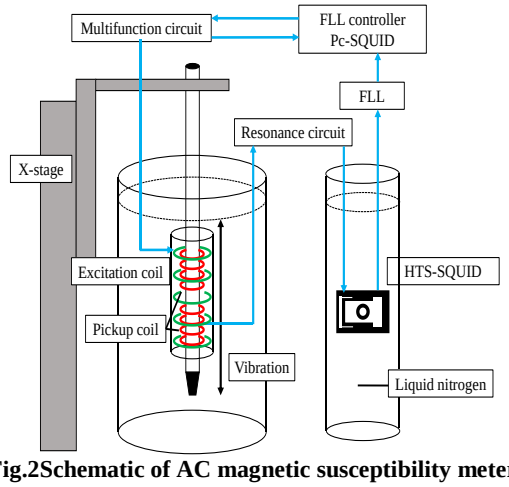

Fig.2Schematic of AC magnetic susceptibility meter

In the measurement of the MNPs in an aqueous solution, the super paramagnetism of the MNPs and the diamagnetic signals of the water of the solvent are included. MNPs show nonlinear and nonhysteretic*M-H* curves. Water demonstrates linear magnetization characteristics. When an alternating magnetic field is applied, harmonic signals are generated from the MNPs. The AC susceptibility meter detects the 3rd harmonic signal change of the immune reaction. In the alternating magnetic field of 1 kHz, the sample reciprocates, moving in the vertical direction by the motor, and the magnetic flux passing through the pickup coil is changed. Reciprocating frequency in the vertical direction is approximately 0.1 Hz. In this study, signals of MNPs in a buffer solution and serum concentration of 10% and 30% were measured.

III. RESULTS AND DISCUSSION

The M-H curves of MNPs, in a buffer solution and 10%, 30% serum, were measured with a hybrid magnetometer, as illustrated in Fig 3.

They were saturated at a high magnetic field, and there is no hysteresis caused by superparamagnetic characteristics. The magnetic moment of MNPs, in the buffer solution, increased as the serum concentration increased. This is assumed to be due to the aggregation in serum. Figure 4 shows the time waveforms of dispersing MNPs in buffer solution and in serum 10%, 30% solution measured by the AC magnetic susceptibility meter. Time waveform has two peaks. Because the peak stands while passing over the pickup coil, it occurs twice in one reciprocation. The signal is averaged over 10 iterations; sin3fand cos3frefer to the imaginary and real aspects of the 3rd harmonic signal, respectively. Peak-to-peak values of the time waveform in Fig.4 is outlined in Fig.5. The peak value decreases as the serum concentration increases. In addition, it is clear that there is a substantial decrease in the real part. The phase delay of the MNPs signal increases, attributed to the high viscosity of serum.

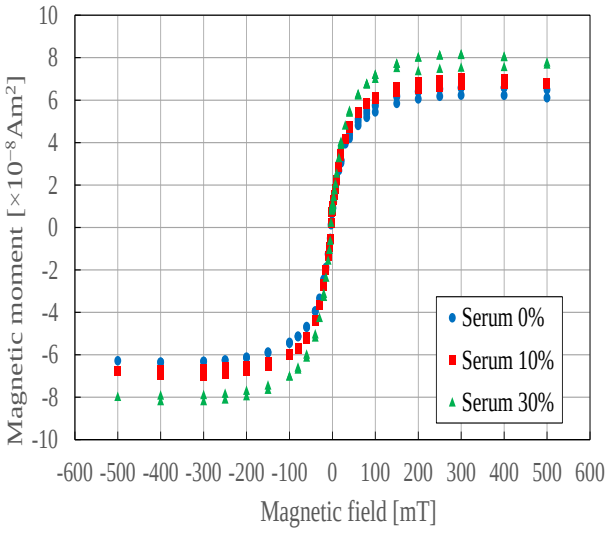

Fig.3 Magnetization curve with change in the serum

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concentration

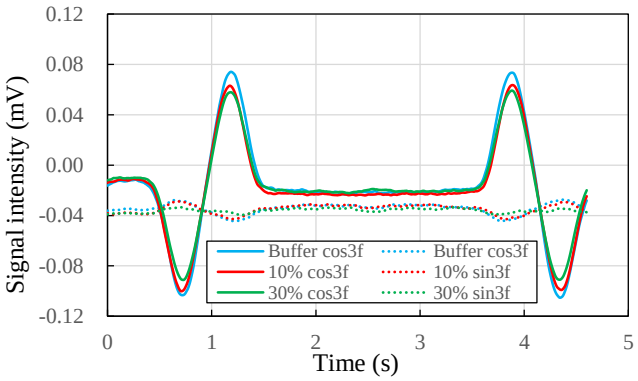

Fig.4 Signal waveform of MNPs with change in the serum concentration

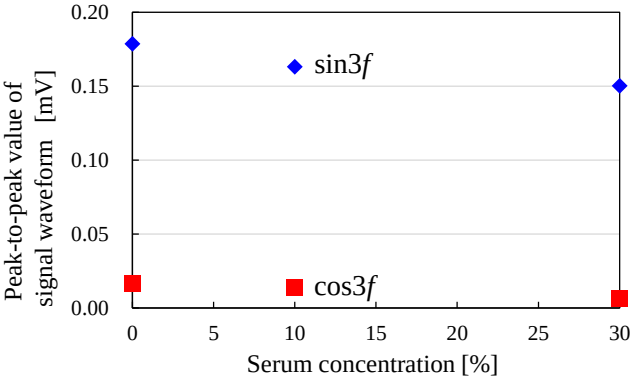

Fig.5 Peak-to-peak MNPs signal with change in the serum concentration

IV. CONCLUSION

The influence of serum on MNPs was measured using the developed magnetic susceptibility meter. In the magnetization curve measurement, the magnetic moment increased as the serum concentration increased. This is due to the aggregation of MNPs by the serum. In the harmonic signal measurement, the peak-to-peak values of magnetic signal decreased as the serum concentration increased. This is due to the phase delay caused by the viscosity of the serum. In the future, the influences of other blood components and whole blood on MNPs should be evaluated using the developed magnetometer to realize a quantitative measurement of magnetic immunoassay.

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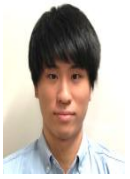
AUTHOR BIOGRAPHY

Shun Takeuchireceived the B.S. degree from Okayama University in 2017. He is a currently graduate student in the Graduate School of Natural Science and Technology, Okayama University; Japan.His subject includes magnetic measurement system.



Tetsuro Hirata received the B.S. degree from Okayama University in 2017. He is a currently graduate student in the Graduate School of Natural Science and Technology, Okayama University, Japan. His subject includes magnetic measurement system.

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RyotaIsshiki received the B.S. degree from Okayama University in 2016. He is a currently graduate student in the Graduate School of Natural Science and Technology, Okayama University, Japan. His subject includes magnetic measurement system.

Yuta Nakamurareceived the B.S. degree from Okayama University in 2016. He is a currently graduate student in the Graduate School of Natural Science and Technology, Okayama University, Japan. His subject includes magnetic measurement system.

Kayo Fujimoto is a currently research assistant in the Graduate School of Natural Science and Technology, Okayama University, Japan.

Kenji Sakai received Dr. Eng. degree from Doshisha University in 2010. After that, he was a Research Fellow of the JSPS at Doshisha University. He is currently an assistant professor of Okayama University. He is now involved in the research of magnetic sensor and SQUID devices and their applications, non-destructive evaluation system, and gas sensor.

Toshihiko Kiwa received Dr. Eng. degree from Osaka University in 2003. After that he worked for one year as a JSPS fellow at the Research Center for Superconductor Photonics, Osaka University, where he was involved in the development of terahertz and superconductor devices. Currently, he is an associate professor of Graduate school of natural science and technology, Okayama University. His research interests include chemical sensors, magnetometric sensors, and terahertz devices.

KeijiTsukada 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 Department of Electrical and Electronic Engineering, Okayama University. He is involved in the research of chemical sensor, magnetic sensor and superconducting sensor devices, and their applications.