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ABSTRACT

Liquid-phase magnetic immunoassay (MIA) using magnetic nano-particles (MNPs) has been studied as a more rapid method compared to optical methods for inspecting proteins and viruses. MIA can estimate the number of conjugated antibodies without being washed differently from conventional optical immunoassay. However, in the case of the liquid phase, it is considered that the magnetic properties of MNPs are affected by physical properties such as viscosity and impurity substances such as biological substances contained in the blood. In this study, the effect of sodium chloride (NaCl) in buffer and serum solution was evaluated to reveal the effect of serum because the sodium (Na^+) and chloride (Cl^-) ions in the serum dominate ion balance of blood. The measurement results of AC magnetic susceptibility and a dynamic light scattering (DLS) showed that the aggregation of MNPs was largely affected by the concentration of NaCl. This effect of the NaCl could be explained by shielding of the surface charge of MNPs by ions in the solution. Although the concentrations of NaCl in the buffer and serum solution were almost same, we found that MNPs were aggregated more in their size for those in the serum solution because of other impurities, such as proteins. These results suggest evaluation of effects of the contaminants in serum and optimization of polymer coatings of MNPs could be important factors to realize measurements of magnetic immunoassay with high accuracy.

I. INTRODUCTION

Magnetic nano-particles (MNPs) have been studied for medical diagnostic purposes—as the imaging agents of magnetic resonance imaging (MRI), hyperthermia, and drag delivery.[1–3] Among them, liquid-phase magnetic immunoassay (MIA) using MNPs has been studied as a more rapid method compared to optical methods for inspecting such as proteins and viruses.[4] The MIA generally uses polymer-coated MNP beads, on the surface of which antibodies are immobilized to detect conjugation with antigens. Conventional optical immunoassays like Enzyme-Linked Immunosorbent Assay[5] need to be washed to remove unbounded antibodies from solutions; however, the MIA can estimate the number of conjugated antibodies without being washed because the alternating (AC) magnetic properties of MNP beads vary when the antibodies are bounded with antigens. Thus, we studied the magnetic properties of MNPs under various conditions and substances using a developed MNP measurement system.[6–8] AC magnetic signal changes by volume change due to the antigen - antibody binding reaction, because the AC magnetic signal depends on rotation of magnetic moment within the particle; therefore, the viscosity of the sample solution is considered to affect the AC magnetic properties.[9,10] Our study revealed that the MNP beads in the serum solution exhibited AC magnetic properties different from those of MNP beads in the buffer solution though the viscosity of the solution was similar.[11] The sodium (Na^+) and chloride (Cl^-) ions in the serum dominate the ion balance of the serum, and it is generally known that electrolytes affect colloidal solutions like salting out. Here, to investigate the influence of serum, which

includes Na^+ and Cl^- ions to AC magnetic properties of MIA, the concentration dependence of properties of sodium chloride in the solutions was measured.

II. EXPERIMENTAL

A. Sample

The magnetic properties were evaluated using Resovist (Fujifilm Toyama Chemical Company, Ltd., Tokyo) as the MNPs, which is generally used as a MRI contrast agent for a liver. Resovist is a hydrophilic colloid solution, consisting of superparamagnetic iron oxide nano-particles coated with carboxydextran. Overall hydrodynamic diameter of Resovist is approximately 62 nm and iron oxide core is 4.2 nm in average.[1]

Sample solution was prepared by diluting Resovist with 4- (2-hydroxyethyl) -1-piperazineethanesulfonic acid (HEPES) buffer. The concentration of NaCl in the sample solution was adjusted in the rage of between 0 and 0.9 weight per volume percent (w/v%) since the NaCl concertation of normal saline solution is 0.9 w/v%.

Fig. 1(a) and (b) shows viscosities of the sample solutions as functions of NaCl and serum concentration, respectively. The viscosity of NaCl and serum solutions were measured using tuning fork vibration viscometer. The viscosity meter equipped vibrating tuning folk inserted in a sample solution. Because viscosity depends on the electric power supplied to keep vibration of a tuning folk at 30 Hz with a constant amplitude, the viscosity meter can measure the viscosity of the sample solution by measuring the electric power. The room temperature was kept to be approximately 25 °C though the series of measurements, so the effect of temperature on the signals could be reduced.

The measurement temperature of the NaCl solution was 24 °C and the serum solvent was 23 °C. These results clearly show that the viscosity was independent of both of NaCl and serum concentration.

B. SQUID magnetic immunoassay system

The harmonic AC magnetic characteristics of the prepared solutions were evaluated using a superconducting quantum interference device (SQUID) magnetic immunoassay system. The detail of the system was reported in Ref. 12. The AC magnetic field with the frequency of 1.06 kHz and the peak-to-peak amplitude of 8 $mT_{p\text{-}p}$ was applied to the sample by an excitation coil and the secondary magnetic field generated from the sample was detected by the gradiometer pickup coil, which was coaxially aligned with the excitation coil. The sample solution moved through the inside of the pickup coil along the center axis of the coils, so that the magnetic signal with positive and negative peaks can be observed along the axis. Thus, the baseline drift of the signals could be reduced by evaluating the peak to peak amplitude of the measured signals. The detected signal was transmitted to the SQUID[13] by an input coil on the SQUID, which was connected with the pickup coil in series. The third harmonic frequency component signals was lock-in detected after though a flux locked loop circuit[14] for the SQUID. Ten set of signals along the axis was measured for each samples and averaged. The measurement time was 50 seconds per one sample.

The detected signal of the sample includes the signal of solution as well as the signal of MNPs. The MNPs used here showed superparamagnetic, therefore, they showed nonlinear magnetization without hysteresis as a function of applied magnetic field. The detected signal includes not only a fundamental signal but also the harmonics signal because of nonlinearity of the magnetization. On the other hand, magnetic properties of diamagnetic materials such as sample solution itself have liner properties to applied field, so that obtained signals don't contain harmonic frequency components. Therefore, the harmonic frequency measurement can reduce effect of the magnetic signals from the solution, so that the measurements of the magnetic properties with higher accuracy could be possible. In this study, the third harmonic signal was measured since the signal intensity was highest among harmonic frequency signals. The detail of the harmonics was reported in Ref. 15.

C. Frequency characteristic evaluation device

AC magnetic properties in a wide frequency range were measured using a frequency characteristic evaluation system developed by our group. An AC voltage with an amplitude of 0.1 V and a frequency of 5 Hz to 5 kHz was applied to the sample using a solenoid type excitation coil with a mean diameter of 43 mm and 4000 turns, and the secondary magnetic field generated from the sample was detected by a gradiometer pick-up coil with a mean diameter of 16 mm and 1800 turns, which was coaxially mounted with the excitation coil. The detected signal was amplified 100 times by an amplifier and transmitted to a gain phase analyzer. The gain phase analyzer can measure the real and imaginary part of the signal to the applied magnetic field. The total gain of the system was calculated from the intensity of the current from a coil mounted inside the pick-up coil. The sample signal was subtracted from the reference signal, which was obtained from an empty sample case. Thirty sets of data were measured at 200 different frequencies between 5 Hz to 5 kHz and averaged.

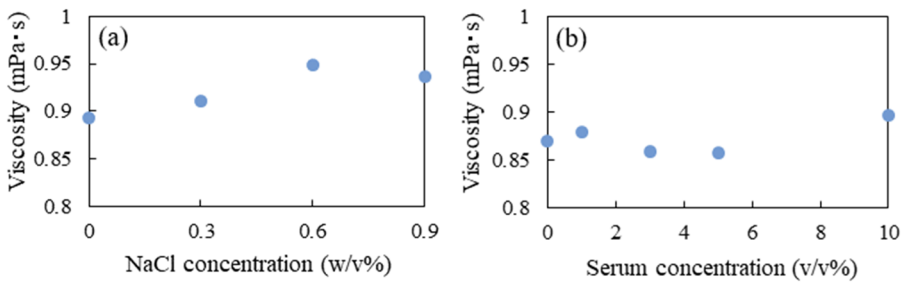

FIG. 1. Viscosities of the sample solutions with mixtures of (a) NaCl and (b) serum, respectively.

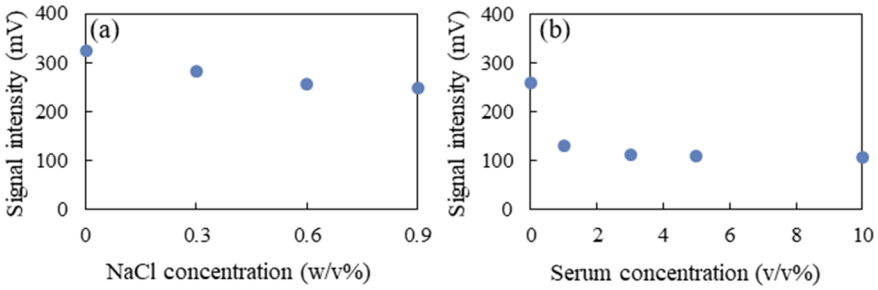

FIG. 2. Third harmonic characteristics of Resovist in (a) NaCl solutions and (b) serum solutions, respectively.

III. RESULTS AND DISCUSSION

A. Harmonic frequency measurements

The AC magnetic properties of Resovist in different solutions were evaluated by third harmonic frequency signals measured by the SQUID magnetic immunoassay system. The measurement results of Resovist in the NaCl and serum solutions are shown in Fig. 2. The concentration of Resovist in the NaCl solution was 270 μg/mL and in the serum solution was 200 μg/mL. The volume of each sample was 50 μL. The signal intensity of NaCl and serum solutions deceased with increasing the concentrations of NaCl and serum. Magnetization of MNPs are generally related to both of Brownian relaxation time τ_B; rotation of whole particles in liquid and Neel relaxation time τ_N; rotation of the magnetic moment within the particles.[4,11,16] According to Ref. 4, Neel relaxation time of Resovist was out of our measurement range, so the magnetic signal by Neel relaxation time could be independent of the measurement frequency range we used. Therefore that change in the signal intensity could be attributed to change in the τ_B, which can be expressed as follow,

$$\tau_B = \frac{3\eta V}{k_B T}, \tag{1}$$

wherein η is the solvent viscosity and V is hydrodynamic particle volume. k_B and T are Boltzmann constant and the temperature of the liquid.

According to Eq. (1), the relaxation time of the magnetic moment are affected by both of the solvent viscosity and the particle volume. For our samples, the solvent viscosity was almost same for each sample as shown in Fig. 1. Therefore change in the signal intensity; so that change in the τ_B, can be related to change in the particle volume by the aggregation of MNPs.

However, the signal change of serum solution was bigger than NaCl solution although the NaCl concentration is almost the same. Serum contains electrolytes such as Na^+ and Cl^- as well as contaminants such as proteins, carbohydrates, and lipids. Therefore, the signal change is thought to have increased due to the influence of other contaminants. From the measurement results, the concentration dependence of NaCl in the solution was confirmed.

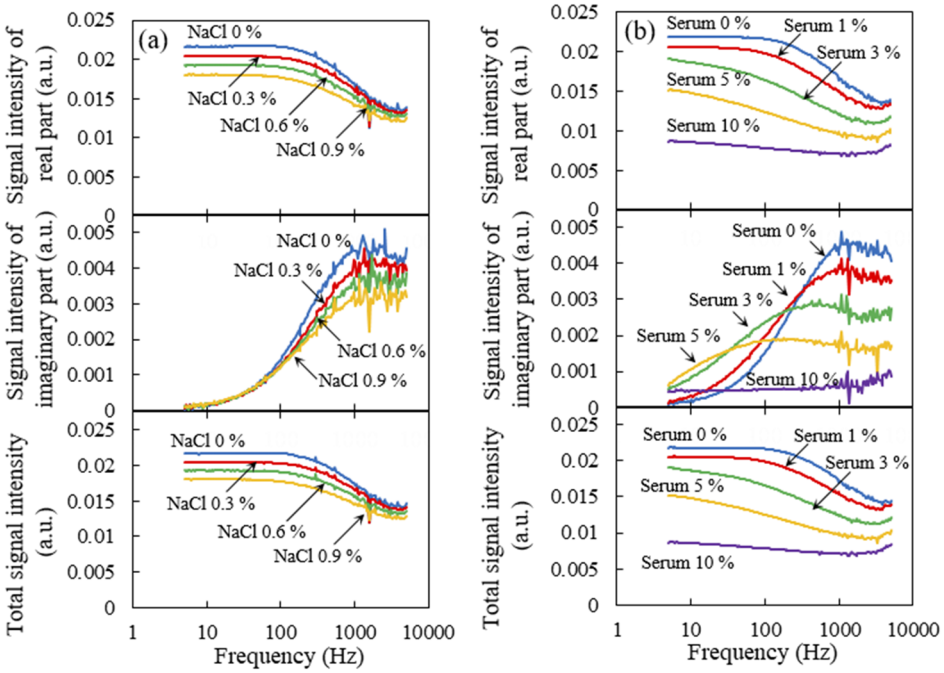

FIG. 3. Frequency dependence of real part, imaginary, and total signal intensity, respectively of Resovist in (a) NaCl solutions and (b) serum solutions, respectively.

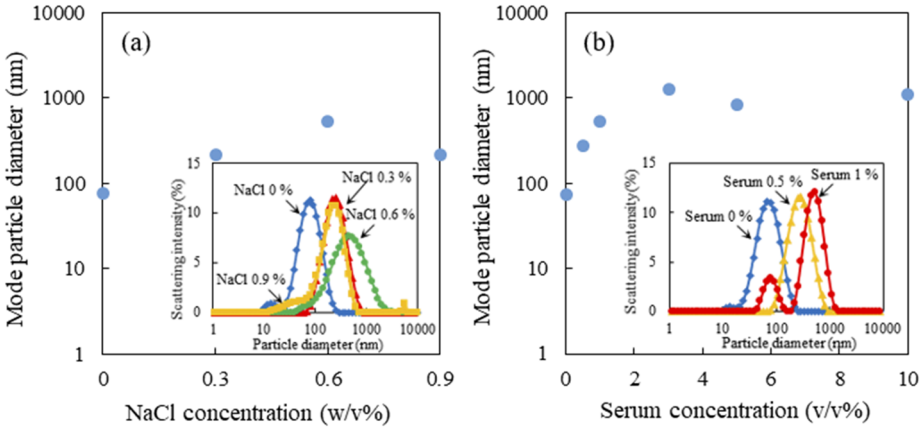

FIG. 4. Mode particle diameter of Resovist in (a) NaCl solutions and (b) serum solutions as a function of concentration. Particle diameter distribution for each sample is shown in inset.

B. Frequency dependence

The frequency characteristic evaluation system was used to evaluate the AC magnetic properties of Resovist in NaCl and serum solutions. The concentration of the Resovist was 50 mg/mL and the sample volume was 500 μL for this measurements. The real parts, imaginary parts and total signal intensity of the measured signals of the samples with NaCl and serum solutions are shown in Fig. 3, respectively. In the NaCl solution, both of real and imaginary parts of the signals decreased in their intensity with increasing the concentration of NaCl due to the aggregation of MNPs as the same reason with the results in Fig. 2(a). In the serum solution, The real and imaginary parts of the measured signals decreased as the concentration of serum increased. However, the peak frequency shown in the imaginary part of the measured signals shifted to a lower frequency as the concentration of the serum increased. The difference between the concentration dependence of the imaginary part of the signals for serum and that of the signals for NaCl can be explained by the difference in the magnitudes of particle volumes when the concentrations of NaCl and serum change; the change in volume for serum solution was larger than that for NaCl in our experimental range. For the sample with 10% - serum, the signal was independent of frequency, which also indicates that the size of aggregated particles for the serum sample was larger than that for the NaCl sample. Therefore, τ_B was too long to be detected in the measured frequency range. Some of the MNPs of Resovist have short a Neel relaxation time compared with Brownian relaxation; thus, the measured magnetic signal at 10% -serum concentration was caused by only these MNPs that have short Neel relaxation time.

These results suggest that effects of various components, such as proteins and ions, in the serum are influencing factors in practical measurements of biological samples. It is necessary to perform a signal analysis to consider the influence of these components for highly accurate MIA.

C. Particle size measurement

In order to support discussion above, the particle size distribution of Resovist was measured using a dynamic light scattering (DLS), and the aggregation of particles due to solvent change was evaluated. DLS could be one of good option to evaluate the size of MNPs in the solution. The sample volume was 1.0 mL and the concentration of Resovist was 200 μg/mL. The particle size distribution and the mode particle diameter of Resovist in solutions with NaCl and serum solutions are shown in Fig. 4(a) and (b), respectively. In the NaCl solution, the distribution of the particle size and the mode particle diameter increased by increasing the concentration of NaCl, which indicates the aggregation of Resovist due to the NaCl was promoted by shielding of the surface charge of the Resovist. As the NaCl concentration increased from 0% to 0.9%, the mode particle diameter of Resovist increased from 78 nm to 220 nm. In the serum solution, the aggregation of Resovist due to the serum was also observed by DLS measurement. The Resovist agglutinate rapidly at the serum concentrations from 0% to 3%, and the particle size is almost the same at the serum concentration larger than 3%. The mode particle diameter of Resovist in 10% serum was 1.1 μm. It is clear that Resovist were aggregated in the serum solution than the Resovist in NaCl solutions as discussed in Fig. 3 and 4.

IV. CONCLUSION

The effect of serum on AC magnetic properties for MIA was evaluated. The measurement results of AC magnetic susceptibility and DLS suggests that the sodium and chloride ions in the serum affects the aggregation of MNPs, which could be caused by shielding of the surface charge of the MNPs by ions. The serum solutions are more MNPs aggregated than NaCl solutions due to the influence of various components such as proteins different from ions.

The results suggest that understanding of properties of MNPs and optimization of parameters of the MIA systems depending on each sample conditions could be important for high-sensitive measurements when actual bio-samples, such as blood, are measured.

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