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Monitoring Minute Changes of Magnetic Markers' Susceptibility by SV-GMR Needle-Type Probe

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The aim of this study is to present the novel methodology for magnetic markers immunoassay measurements by spin valve giant magnetoresistive (SV-GMR) needle probe. Extremely small size and specific construction of this probe type allow detecting the changes of complex susceptibility directly from the sample. The needle-type probe consists of four SV-GMR elements connected in a Wheatstone bridge and it has sensitivity of 11 μV/μT. The SV-GMR sensing element has dimensions of 40 μm $\times$ 75 μm. This paper presents a setup feasible to perform spectroscopic magnetic markers measurement. It includes data related to signal changes caused by the magnetic markers dilution. It shows the influence of particle size changes (120 nm, 1 μm, 3.5 μm, 6.5 μm) on real and imaginary parts of the complex susceptibility of the specimen. While performing measurements, probe sensing elements were placed inside the sample. Monitoring changes of samples susceptibility allows performing homogenous immunoassay measurements in a liquid phase by SV-GMR probe. Based on the measurement results, analytical model of changes of complex susceptibility is derived and described.

***Index Terms*—Biomarkers, giant magnetoresistance, magnetic susceptibility, nanoparticles, spin valves (SVs).**

I. Introduction

MAGNETIC ferrofluids have been successfully used in various applications such as hyperthermia treatment, magnetic drug delivery, the contrast enhancement of magnetic resonance imaging, immunoassay, cell separation, etc. [1]–[7]. The magnetic ferrofluid particles labeled with antigen (e.g., avidin) are able to combine with a specific antibody (e.g., biotin). Free labeled markers have specific complex susceptibility characteristics. When they are combined with a target (e.g., bacteria and red blood cells) using a specific antibody, they cause changes in the complex susceptibility of a specimen. This paper presents a measurement method that detects complex susceptibility changes caused by various amount of magnetic markers and identifies changes caused by biological targets of assorted sizes. One of the advantages of this method is the capability to perform a direct examination of samples without additional washing of superfluous targets.

II. Methodology for Magnetic Markers Studies

The measurement method includes the placement of the ferrofluid in an external ac magnetic field; the sample magnetization causes a change of its magnetic flux density. With the amplitude of the external field high enough, this change can be detected by the sensing element of a needle probe, as shown in Fig. 1(a). The Wheatstone bridge structure of the needle probe allows to simultaneously measure by giant magnetoresistive (GMR) 1 a signal change caused by the sample and to eliminate the influence of the external field by using GMR 2 located 2 cm above the sample. Due to this configuration, obtaining the changes of flux density inside a sample (B_1) and of the applied field (B_0), as shown in Fig. 1, is possible.

The signal change caused by the sample measured inside it is practically independent from the sample size as it was presented in the previous study [4]. The relationship between the measured value $(B_1 - B_0)$ and the complex susceptibility $\chi* = \chi' + i\chi''$ can be easily derived from (1) [4]

$$\frac{B_1 - B_0}{B_0} = \frac{(\mu^* - 1)(1 - N)}{\mu^*} \approx (1 - N)\chi^* \tag{1}$$

where N is the samples demagnetizing coefficient and μ^* is the air magnetic permeability close to unity. When the sample dimensions are known, the value N is defined and the flux density changes are proportional to the relative susceptibility of the specimen. In case of diluted ferrofluids, where their susceptibility is dominated by Brownian rotation time of particles, the real and imaginary part of susceptibility can be given by [1] and [2]

$$\chi'(\omega) = \chi_\infty + \frac{\chi_1}{1 + (\omega\tau_B)^2} \tag{2}$$

$$\chi'(\omega) = \frac{\omega\tau_B\chi_1}{1 + (\omega\tau_B)^2} \tag{3}$$

where χ_1 is the constant susceptibility, χ_∞ is the susceptibility at infinite frequency, $\omega = 2\pi f$, and τ_B is the relaxation time given by [3]

$$\tau_B = \frac{3\eta V}{k_B T} \tag{4}$$

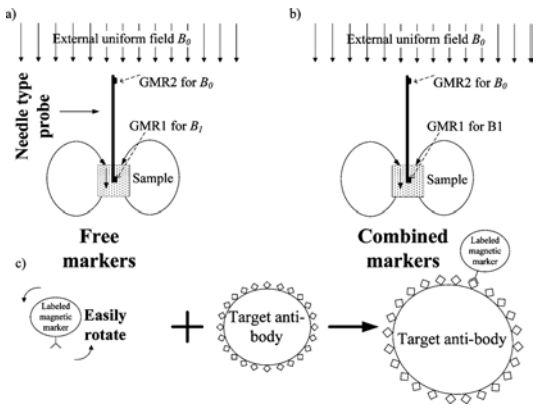

Fig. 1. Principle of homogenous immunoassay studies. (a) First, spectroscopy characteristic of free magnetic markers is studied; biological targets are added. (b) Frequency response of combined magnetic markers and biological target is examined. (c) Binding of free marker and biological target.

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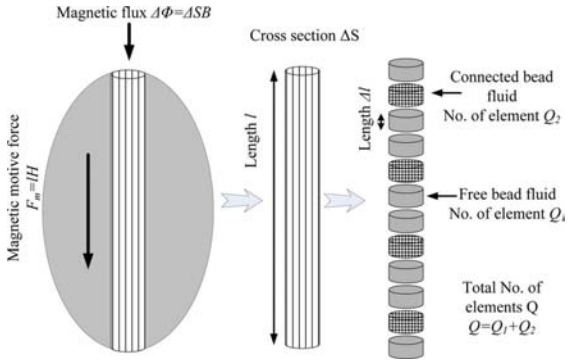

Fig. 2. Magnetic path and permeability of mixed fluids.

where V is the particle volume, η is the viscosity of the liquid carrier, T is the temperature in kelvins, k_B is the Boltzmann constant. The relaxation time and the complex susceptibility are directly proportional to the particle volume.

III. Susceptibility of Mixed Magnetic Fluid

The susceptibility of the mixed magnetic fluid that includes both free magnetic bead and combined magnetic beads with polymer beads is estimated according to the microscopic concept. Let us assume that such mixture has density of particles Q (1/m^3) consisting of Q_f (1/m^3) of monodispersed free markers and Q_c (1/m^3) of combined particles. According to the previous study [4], [5], the relative permeability and susceptibility are given by

$$\mu_f^* = 1 + C_d D_{vf} \qquad \mu_c^* = 1 + C_d D_{vc} \tag{5}$$

where C_d =3–4 is the experimental coefficient and Dv is the fluid volume density. Therefore, two fluids have relative permeability μ_1^* and μ_2^* and these permeabilities (and susceptibilities) are proportional coefficients to magnetic field strength H (or magnetomotive force F_m) and magnetic flux density B (or magnetic flux Φ). When the uniform magnetic field is applied to ellipsoidal cavity with mixed fluids, the magnetic flux density is uniform and its vector has direction, as shown in Fig. 2. Let us divide the ellipsoidal body into a number of columns composed of small cylindrical elements. Each element corresponds to one type of fluid (free or combined). The number of each type of column elements is proportional to the volume ratio of fluids. According to these assumptions, the total magnetic motive force F_m can be given by

$$\begin{aligned} F_m = (\phi R_m) &= \Delta\phi \left(\frac{\Delta l Q_1}{\Delta S \mu_1^*} + \frac{\Delta l Q_2}{\Delta S \mu_2^*} \right) \\ &= \frac{\Delta\phi l}{\Delta S} \left(\frac{\Delta l Q_1}{l \mu_1^*} + \frac{\Delta l Q_2}{l \mu_2^*} \right) \end{aligned} \tag{6}$$

where the total number of small elements is $Q = Q_1 + Q_2$. The ratios of volume of each fluid are α_1 and α_2. As the magnetic field strength H is F_m/l and the magnetic flux density B is $\Delta\Phi/\Delta S$, (6) changes to

$$\mu^* = \frac{B}{H} = \left(\frac{\Delta\phi}{\Delta S} \right) \Big/ \left(\frac{F_m}{l} \right) = 1 \Big/ \left(\frac{\alpha_1}{\mu_1^*} + \frac{\alpha_2}{\mu_2^*} \right) \tag{7}$$

with additional transformation, (7) is expressed by

$$\chi^* = \alpha_1 \chi_1^* + \alpha_2 \chi_2^*. \tag{8}$$

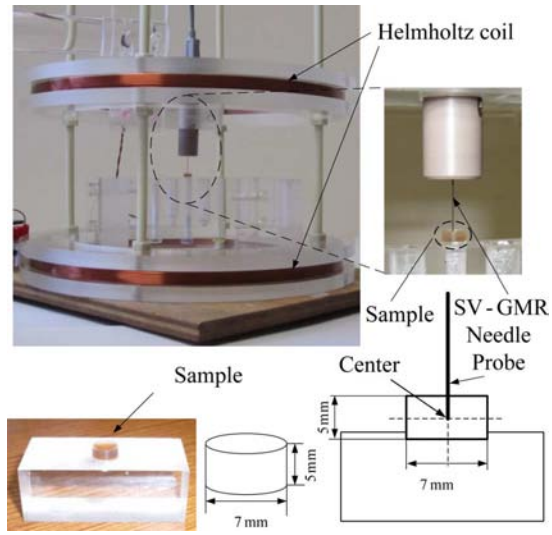

Fig. 3. Measuring setup.

Equation (8) states that the susceptibility of mixed fluids is proportional to the sum of products of the susceptibility and the volume ratio of each fluid. According to Debye's theory [8], the complex susceptibility is assumed to have the frequency characteristic given by

$$\chi^* = \frac{\alpha_1 \chi_1^*}{(1 + j\omega\tau_1)} + \frac{\alpha_2 \chi_2^*}{(1 + j\omega\tau_2)} \tag{9}$$

where τ_1 and τ_2 are the relaxation times of each fluid.

IV. Employed Setup

The construction of a setup feasible to detect minute changes of complex susceptibility in a liquid phase is presented in Fig. 3. The setup consists of two parts: the spin valve (SV) GMR needle-type probe and a field generator. Helmholtz coil was applied as the uniform magnetic field generator. It has two coils with radius equal to 0.11 m and 106 turns each. The distance between coils is equal to the coil radius. The ac magnetic field frequency was changed between 5 and 1000 Hz. The needle-type SV-GMR consists of four GMR sensing elements connected in a Wheatstone bridge configuration. Each sensing element has dimensions of 40 μm $\times$ 75 μm. One sensing element is placed at the tip of the needle and three other are located close to the bonding pads. The length of the needle is equal to 2 cm and its cross section is 250 $\times$ 250 μm. The needle probe has the sensitivity of 11 μV/μT. The sensor's measurement range is between tens of nanotesla and few millitesla. Details of the needle tip and the SV-GMR probe are depicted in Fig. 4.

V. Concentration, Target Size, and Particle Binding Number Dependence

In order to perform immunoassay measurements, combinations of four commercially available substances were investigated. One was streptavidin ferrofluid produced by R&D systems, and the other were three types of molecules consisting of biotin-coated polymer beads produced by Spherotech©. During the measurements, the sensor was placed centrally in the sample of a small amount of mixture (70 μl) located inside a container

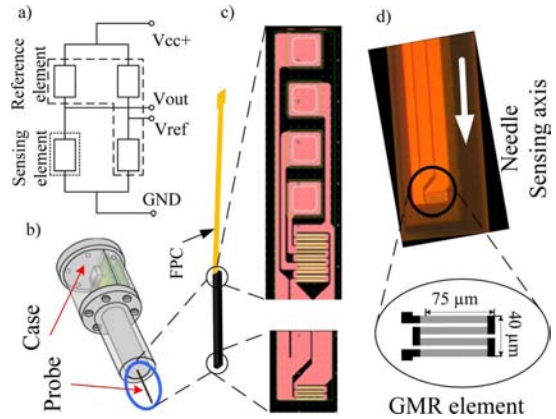

Fig. 4. SV-GMR probe. (a) Electrical connections of Wheatstone bridge. (b) Needle-type probe with case. (c) Needle with flexible printed circuit with enlarged needle tip and bonding pad. (d) Microscope picture of needle tip with marked sensing axis of SV-GMR element.

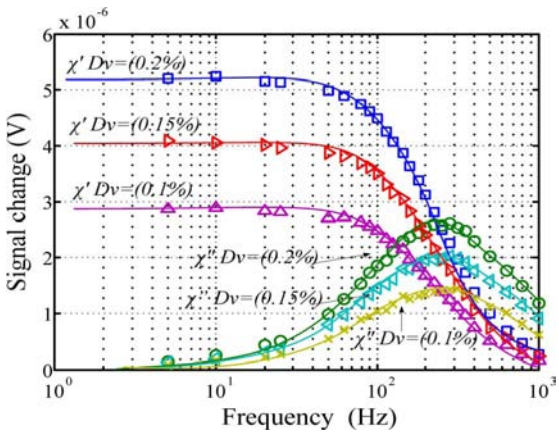

Fig. 5. Various ferrofluid volume density *Dv* influence on real part and imaginary part of the signal.

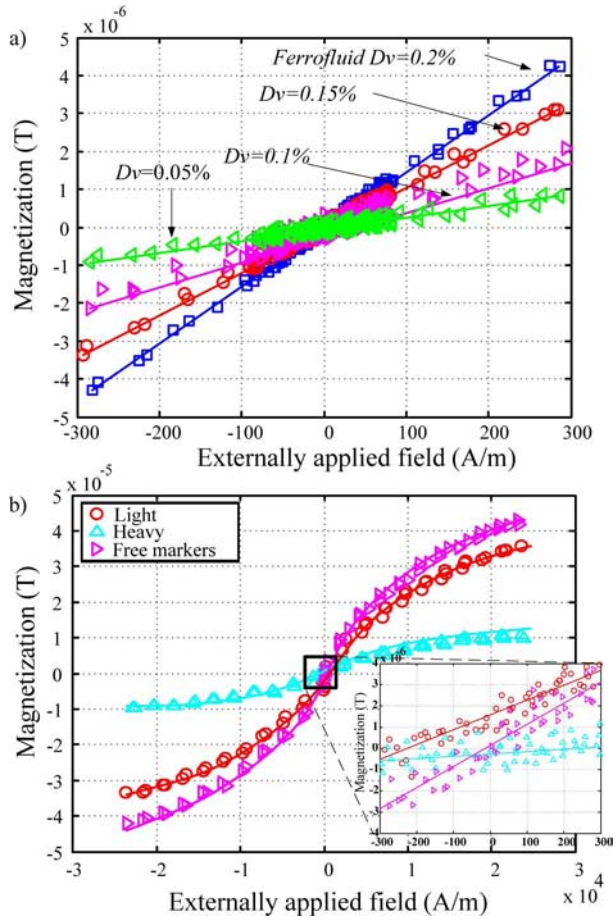

Fig. 6. VSM measurement. (a) Dilution influence. (b) Characteristics of centrifuged magnetic markers combined with polymer beads and free markers.

in the middle of the Helmholtz coil. While performing the experiments, temperature inside the setup was monitored and variations of temperature did not exceed 0.5 K. The average amplitude of the probe offset voltage (measured in the air) was subtracted from the sample's signal changes.

A. Ferrofluid Particle Concentration Influence

The ferrofluid dilution influences the imaginary part of the measured signal as presented in Fig. 5. Decrease in the amount of magnetic fluid causes the decrease in the real-part signal's amplitude and lowers the peak of the signal's imaginary part. The peaks in the imaginary part of the susceptibility occurred at frequency of 225 Hz. The sample's magnetization was measured by vibrating sample magnetometer (VSM), as shown in Fig. 6(a). Free markers have "S" magnetization shape in the field range of ± 23 kA/m. They do not display a hysteretic behavior with coercivity and remanence. They have a linear magnetization pattern in the range ± 200 A/m that corresponds to the amplitude of the applied external field in the Helmholtz coil. Analytical calculations performed on the data from VSM showed that a possible frequency peak of the signal's imaginary part should appear at 250 Hz. Analytical and measurement results are close enough to conclude that dilution does not cause a change in the relaxation time of ferrofluid particles. The complex susceptibility of the mixture is dominated mainly by free markers. The magnetic marker's real part of susceptibility is nonzero for frequencies higher than 1 kHz what can be seen in (2) (χ_∞). Subtracting sensor's offset causes that χ_∞ is not presented in graphs.

B. Binding Target Size Relationship

Another possible factor that can influence the characteristic of a ferrofluid is the particle size. In the case of this study, the particle size change is caused by a combination of polymer beads and ferrofluid particles. Three sizes of polymer with mean diameter 1.0, 3.5, and 6.5 μm were combined with ferrofluid particles. Achieved results of magnetization by binding ferrofluid with target particles of various sizes are presented in Fig. 7. When the size of the particle is increasing, the peak of the imaginary parts is shifting to the left side of the graph. The graphs have similar shapes as those in the results obtained by Enpuku *et al.* [3].

C. Binding Target Number Relationship to Complex Susceptibility

Various numbers of targets (polymer beads) were added to the mixture and signal changes were monitored. Magnetic marker signal changes are linearly proportional to the number of binding particles, as shown in Fig. 8. The same dependence

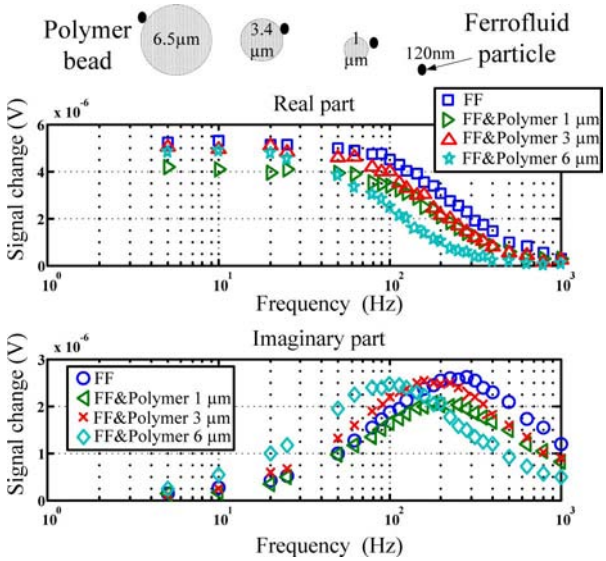

Fig. 7. Particle size influence. (a) Real part. (b) Imaginary part of susceptibility.

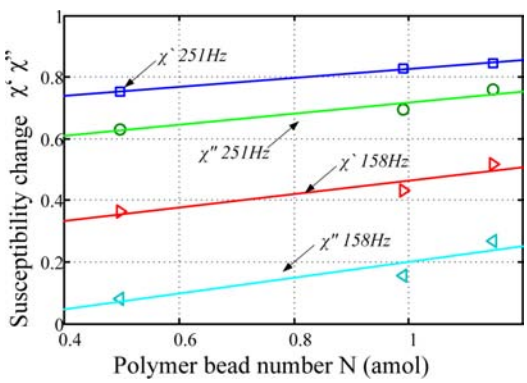

Fig. 8. Various amount of 6 μm polymer beads combining ferrofluid particles. Linear dependence validates that immunoassay has been performed properly.

is achieved for target beads with sizes equal to 1 and 3.5 μm. Furthermore, examination of separated combined markers and free markers by VSM was conveyed. Mixture (70 μl) consisting of magnetic markers and 6 μm size polymer beads was separated in a centrifuge. The resultant heavier (markers combined with polymer beads) particles were extracted at first from the mixture. The lighter free markers were condensed with the aid of a bar magnet and extracted. The lighter and heavier particles were examined in VSM. Fig. 6(b) presents the shape of the magnetization curve of free markers and heavier particles (magnetic markers combined with polymer beads) and lighter particles (free markers extracted from solution). Extracted free markers still demonstrate superparamagnetic behavior, whereas combined with polymer beads markers behave like paramagnetic materials.

VI. Sensitivity of Presented Setup

The SV-GMR needle-type probe is a gradient meter. It measures the differences between the applied field and changes of the magnetic field caused by the sample. The differential amplitude between the free markers (real part) and that of the sensor $(V_{\rm out} - V_{\rm ref})$ was equal to 9 μV. This signal amplitude is achieved with 0.3 μg of ferrofluid. With the current setup in a nonshielded environment, it is possible to perform the measurement of very small magnetic flux density changes as small as 50 nT. That time differential amplitude of the probe is equal to 300 nV. Experimentally, it is possible to measure 30 ng of ferrofluids. It can be assumed that 30 ng of ferrofluid corresponds to 6.5×10^7 of markers in 70 μl of solution. Assuming that one marker can bind exactly one biological target, it is possible to estimate molecular sensitivity of the system as 2.4×10^{-16} mol/ml (or 0.24 fmol/ml). Setup sensitivity estimation does not include false binding and partial binding of markers. Therefore, the estimated value can decrease in a real reaction unless one marker always binds with only one polymer bead.

VII. Conclusion

Presented measurement technique utilizes the SV-GMR needle-type probe to estimate the complex susceptibility of magnetic markers. In order to increase the setup's sensitivity, a higher excitation field and frequency are required. Currently, the excitation field is equal to 160 A/m inside the Helmholtz coil. Homogenous magnetic immunoassay studies were successfully conducted with the presented setup that allows performing direct examination of the sample, where additional washing of superfluous targets is unnecessary. In the near future, this novel technique can be extended to estimate the quantities of bacteria, viruses, and cells.

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