Homogenous magnetic markers immunoassay measurements by SV-GMR needle probe

R. G. Haraszczuk[1,2], S. Yamada[1], M. Kakikawa[1], T. Ueno[1], and A. Nafalski[3]
Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa, 920-1192, Japan, hrysiek@gmail.com
Institute of Computer Science, Lublin University of Technology, Nadbystrzycka 36B, Lublin, 20-618 Poland[2]
School of Electrical and Information Engineering, University of South Australia, Mawson Lakes SA 5095, Australia[3]

The aim is to present the methodology for magnetic markers immunoassay measurements by spin valve giant magnetoresistive SV-GMR needle probe, and compare results with an analytical model. Extremely small size and specific construction of this type probe 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. SV-GMR sensing element has dimensions 40 µm per 75 µm. Needle probe has sensitivity equal to 11µV/µT. This article presents setup feasible to perform spectroscopic magnetic markers measurement. It includes data connected with signal changes caused by magnetic markers dilution. It shows influence of particle size changes (120 nm, 1 µm, 3.5 µm, 6.5 µm) on real and imaginary part of the complex susceptibility of the specimen. While performing measurements, probe sensing elements were placed inside the sample.

I. Methodology for Immunoassay Studies

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 characteristic. When they are combined with target (e.g. bacteria, red blood cells) by using a specific anti-body they cause changes in the complex susceptibility of a specimen. We present measurement method allowing to detect changes caused by various amount of magnetic markers, and a possibility to detect changes caused by targets of different sizes. The measurement method includes the placement of the ferrofluid in an external magnetic field; the sample magnetization causes the 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. The Wheatstone bridge structure of the needle probe allows to measure simultaneously by GMR 1 signal change caused by the sample and to eliminate the influence of external field by using GMR 2 located two centimeters above the sample. Such configuration allows obtaining the changes of the flux density inside a sample (B_1) and applied field (B_0) as shown in Fig. 1.

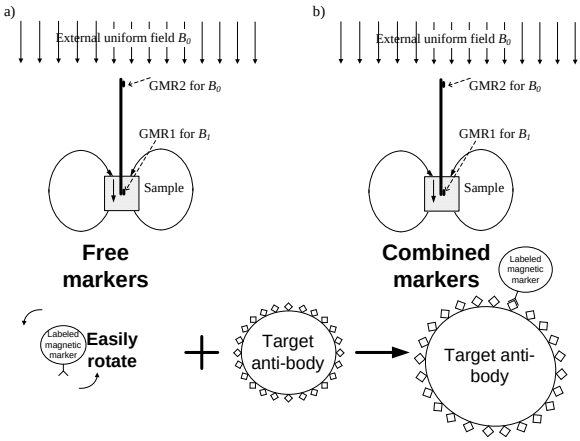

Fig. 1. Homogenous immunoassay principle

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

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

where N is the sample's demagnetizing factor and μ^* is close to unity. When sample dimensions are known the value N is given and flux density changes are proportional to the relative susceptibility of the specimen. In the 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 (2), (3) [1], [2].

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

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

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

$$\tau_B = \frac{3\eta V}{k_B T} \quad (4)$$

where V is the particle volume, η is the carrier's liquid viscosity, T is the temperature in Kelvins, k_B is the Boltzmann constant [3]. The relaxation time and complex susceptibility are directly proportional to the particle volume.

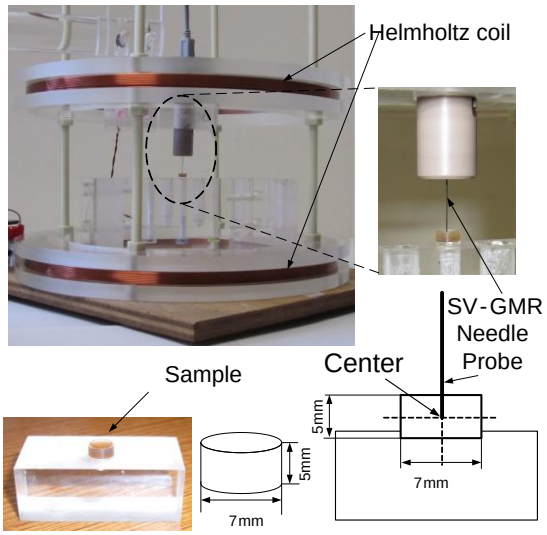

Fig. 2. Sample under examination

II. Results

The construction of a setup feasible to detect minute changes of complex susceptibility in liquid phase is presented in Fig. 2. As the uniform magnetic field generator a Helmholtz coil was applied. The Helmholtz coil has two coils with radius equal to 0.1 m and 100 turns each. Distance between coils is equal to the coil radius. The AC magnetic field frequency range was changed from 5 to 1000 Hz. The needle type SV-GMR probe, of which the main part is a needle consisting of four GMR sensing elements connected in a Wheatstone bridge. Each sensing element has dimensions of 40 µm per 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 x 250 µm. The needle probe has the sensitivity of 11 µV/µT. The sensor measurement range is within tens of nT to few mT.

In order to perform immunoassay measurements, combinations of three commercially available substances were investigated. One was streptavidin ferrofluid produced by R&D Systems, second and third were two types of molecules consisting of biotin coated polymer

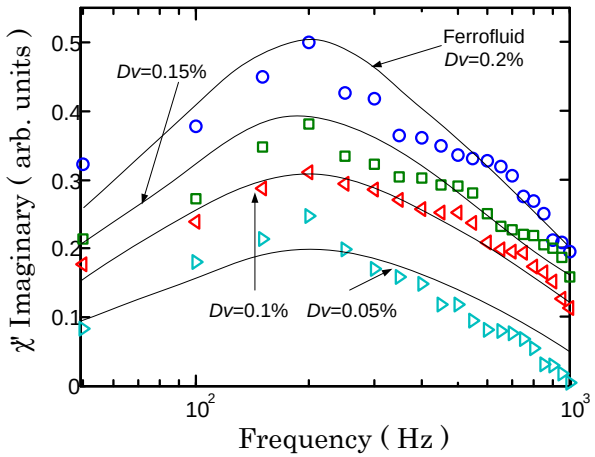

Fig. 3. Influence of ferrofluid's dilution on imaginary part of susceptibility

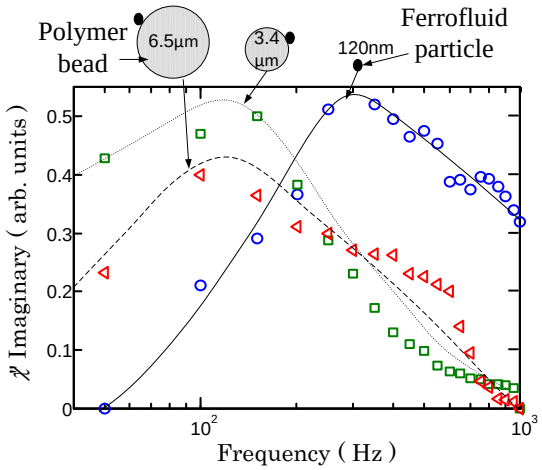

Fig. 4. Particle size influence imaginary part of susceptibility

beads produced by Spherotech. While performing the measurement, sensor was placed centrally in the sample. Small amount of mixture (70 µl) was placed inside container in the middle of the Helmholtz coil.

Results of dilution influence on the imaginary part of the measured signal are presented in Fig 3. 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. Peaks in the imaginary part of susceptibility occurred at frequency 200 Hz. Analytical calculations performed with data from vibrating sample magnetometer (VSM) showed that possible frequency peak should appear at 250 Hz. It can be noticed that a peak occurs at the same frequency in examined samples, what confirms that dilution does not cause a change in the relaxation time of ferrofluid particles. Theoretical analysis results are close to the measured values. Another possible factor which can influence the characteristic of ferrofluid is the particle size. In case of this study, a particle size change is caused by a combination of polymer beads and ferrofluid particles. Three types of polymer with mean diameter 1.0, 3.5 and 6.5 µm were combined with ferrofluid particles. Achieved results of binding ferrofluid with target particles of various size are presented in Fig. 4. When the size of the particle is increasing, a peak of the imaginary parts is shifting to the left side of the graph. The graphs have similar shapes as those in results obtained by K. Enpuku and et al. [3].

III. References

[1] M. K. Krishnan, "Biomedical Nanomagnetics: A spin through possibilities in imaging, diagnostics, and therapy", IEEE Transactions on Magnetics, vol.46, no.7, pp.2523-2558, 2010.

[2] P.C. Fannin, B.K.P. Scaife, S.W. Charles, "A study of the complex ac susceptibility of magnetic fluids subjected to a constant polarizing magnetic field", Journal of Magnetism and Magnetic Materials, Vol. 85, pp. 54-56, 1990.

[3] K. Enpuku, Y. Tamai, T. Mitake, M. Matsuo, A. Tsukamoto, T. Mizoguchi and A. Kandori, "Liquid phase immunoassay using AC susceptibility measurement of magnetic markers", Appl. Phys. Express 2, 2009.

[4] C.P. Gooneratne, M. Kakikawa, M. Iwahara, and S. Yamada, "GMR sensor application in detecting and estimating magnetic fluid weight density inside various size tumors", J. Mag. Soc. Jpn., 33, 175, 2009.