Wheatstone bridge-giant magnetoresistance (GMR) sensors based on Co/Cu multilayers for bio-detection applications

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Abstract. A Wheatstone bridge-giant magnetoresistance (GMR) sensor was successfully developed for a potential biomaterial detection. In order to achieve this, a giant magnetoresistive [Co(1.5nm/Cu(1.0nm))]20 multilayer structures have been fabricated by DC magnetron sputtering method, showing a magnetoresistance (MR) of 2.7%. The X-Ray diffraction (XRD) patterns showed that Co/Cu film multilayer has a high degree of crystallinity with a single peak corresponding to face-centered cubic (111) structure at \(2\theta = 44.1^\circ\). Co/Cu multilayers exhibit a soft magnetic behavior with the saturation magnetization (\(M_s\)) of 148.9 emu/cc and the coercivity (\(H_c\)) of 11.2 Oe. The magnetite \(\text{Fe}_3\text{O}_4\) nanoparticles used as a bimolecular labels (nanotags) were synthesized via co-precipitation method, exhibiting a soft magnetic behavior with \(M_s\) of 77.16 emu/g and \(H_c\) of 49 Oe. XRD patterns and transmission electron microscopy (TEM) images showed that \(\text{Fe}_3\text{O}_4\) was well crystallized and it grew in their inverse spinel structure with an average size of around 10 nm. The GMR sensor design was used to detect a biomolecules of streptavidin magnetic particles with concentration 10, 20, 30, and 40 \(\mu\)l/ml and \(\alpha\)-amylase enzyme with concentration 10, 20, 30, and 40 \(\mu\)l/ml captured using polyethylene glycol (PEG)/ \(\text{Fe}_3\text{O}_4\) nanoparticles. Various applied magnetic fields of 0-650 Gauss have been performed using electromagnetic with the various currents of 0-5 A. Here, the final value of the output voltage signals for the streptavidin magnetic particles concentration is 1.2 mV (10 \(\mu\)l/ml). The output voltage changes with the increase of concentration. It was reported that the output voltage signal of the Wheatstone bridge exhibits log-linear function in real time measurement of the concentration of streptavidin magnetic particles and \(\alpha\)-amylase enzyme respectively, making the sensor suitable for use as a biomolecule concentration detector. Thus, the combination of Co/Cu multilayer, Wheatstone bridge, magnetite and PEG polymer has potential application to be used in bio-detection applications where ultra-small bio-labels are needed.

1. Introduction

Magnetic bio-detection based on magnetic particles has been extensively studied nowadays. The basic principle is first labeling the targeting biomolecules with magnetic particles, and then these attached magnetic particles are captured by target-probe bio-molecular recognition and measured by magnetic sensors. There are remarkable advantages to use magnetic particles in the detection of biomolecules [1]. The detection of biological agents using magnetic sensors and labels, such as giant magnetoresistance (GMR) biosensor, Hall phenomena, magnetic tunnel
junction (MTJ) sensor, and superconducting quantum interference device (SQUID) [2], etc., draws an increasing attention.

One of the most attracting bio-detection sensors to be studied due to its wide application is GMR. GMR is the change in electrical resistance of some materials in response to an external magnetic field. Among the numerous magnetic sensors, the GMR sensors are the most powerful tools for bio-detection due to their advantages, for instance their low-cost, portability, high sensitivity, real-time electronics readout, low-power consumption, and less complex instruments [3]. GMR effects can be observed by using thin film multilayers. Some thin film multilayers which can be used as GMR sensors are Co/Cu multilayer [4], Ag/Co multilayer [5], Fe/Cr multilayer [6], and NiFe/Ag multilayer [7].

GMR sensors can be used in detecting the presence of biomolecule which is called biosensor. The working principles of biosensor based on GMR sensor are: the labeling of biomolecule with nano-tags since not all biomolecules employ magnetic characteristic and the detection of GMR sensor which is indicated by the change of magnetoresistance (MR). Magnetic nanoparticles (MNPs), especially the iron oxide magnetite (Fe3O4) have gained considerable attention because of its characteristics, such as high magnetic saturation (Ms), biomolecule-disperse, biomolecule-responsive, and superparamagnetic. However, the use of MNPs may accompany risks and deleterious effects associated with their increased usage, particularly when they are used without appropriate characterization as biological agents. For these reasons, MNPs are typically surface-modified or coated with biocompatible polymer molecules. One of the most commonly used polymers is polyethylene glycols (PEGs) which can improve their dispersibility, and is biocompatible, nontoxic, and low-costed.

In the recent work, Suharyadi et al. had investigated the detection of Fe3O4 magnetic nanoparticles using GMR sensors based on Co/Cu multilayer by four-point probe system (FPPS). However, that experiment shows that signal output of GMR sensor was resistance [8]. Even though a unique resistance can be used as sensing element, a Wheatstone bridge setup is always a good recommendation as the starting step in the design of resistive sensors [9]. Thus, Nurpriyanti et al. investigated the detection of Fe3O4 magnetic nanoparticles using GMR sensors based on Co/Cu multilayer by Wheatstone bridge circuit to provide output signal of voltage [10]. However, this sensor was not portable and real-time. As a solution to these problems, auto-balancing bridge-based topologies were set up in order to improve the Wheatstone bridge features. Hence, the devices are driven by a control signal which depends on the system status that makes the devices portable and real-time.

In this paper, the possibility of detection streptavidin magnetic particles and α-amylase enzyme utilizing GMR sensors in Wheatstone bridge is investigated using Co/Cu multilayer films. Furthermore, Fe3O4 magnetic nanoparticles are used to be more comparable for conjugating biomolecules. In addition, developing Wheatstone bridge-GMR biosensor more portable and able to read signal output in real-time for various biomolecules is also studied.

2. Experiment details

A multilayer GMR film structure was designed as [Co(1.5nm)/Cu(1.0nm)]20 fabricated by DC magnetron sputtering under pressure was about 3x10⁻⁵ Pa on silicon substrates. The magnetic property measurement of the Co/Cu multilayer was carried out at room temperature by using vibrating sample magnetometer (Riken Denshi Co Ltd, VSM). The GMR sensor was connected to a printed circuit board (PCB) with two part probe and was wire-bounded directly to the pads on the PCB. The Keithley 2401 source meter provides constant current for the GMR sensor and measures the V_out of the Wheatstone bridge in magnetic fields using electromagnetics between 0 and 650 Gauss in the field in-plane geometry at room temperature. In all measurement, a DC current of 10 mA was applied to the Wheatstone bridge for the measurement of V_out. For a small variation of resistance in sensors, the constant-current mode can offer more linear response and
higher sensitivity than the constant-voltage mode [11]. Moreover, it can avoid the heating effect in the sensor as well.

The Fe₃O₄ magnetic nanoparticles were synthesized by chemical co-precipitation method [8,10]. Room temperature VSM measurements have been conducted by using Riken Denshi Co Ltd vibrating sample magnetometer (VSM). Transmission electron microscopy (TEM) analysis was performed using a JEOL JEM-1400. The streptavidin magnetic particles (Sigma-Aldrich) were dispersed in ethanol by sonication for 5 min and vortexed for 15 s. Four different concentrations (10, 20, 30, and 40 μl/ml) were prepared. Fe₃O₄/PEG was synthesized following a simple two steps co-precipitation approach with polyethylene glycole 4000 (PEG-4000) with concentration ratio of 0.5 mg: 0.5 mg respectively for Fe₃O₄/PEG. For labeling process, 0.1 gram of Fe₃O₄/PEG was stirred and added with enzyme α-amylase (Sigma-Aldrich) with concentrations 10, 20, 30, and 40 μl/ml. Furthermore, the mixture dissolved in 1 ml of distilled water by means of sonicated for 30 min at room temperature.

Fourier Transform Infrared (FTIR) spectra of the Fe₃O₄ and Fe₃O₄/PEG were measured with FTIR spectroscopy. Transmission Electro Microscope (TEM) JEOL JEM-1400 was used for characterizing the microstructure of the Fe₃O₄ and Fe₃O₄/PEG. The characterization of Fe₃O₄/PEG and the addition of α-amylase enzyme were only done for observing the functional groups by using FTIR spectroscopy.

3. Result and discussion

The Fe₃O₄ have exhibited good magnetic response and are easily attracted to a magnet placed beside with superparamagnetic behavior. The saturation magnetic moments, coercivity field and remanent magnetization obtained are 77.16 emu/gram, 49 Oe and 7.68 emu/gram respectively. TEM performed for both Fe₃O₄ and Fe₃O₄/PEG. The low-resolution image shows the existence of spherical Fe₃O₄ of about 10 nm with uniform grain size and good dispersibility although in some place still agglomerate. The addition of PEG can decrease the agglomeration and reduce the particle size. In addition, ring diffraction patterns for both of them, are closely related to XRD analysis, which corresponding to the crystal plane (220), (311), (400), (440), and (511) [8,10].

A structure of the Co/Cu multilayer thin films for the GMR sensor was applied for the detection of biomolecules for streptavidin magnetic particles and α-amylase enzyme. The magnetoresistance (MR) of the sensor changes linearly with the logarithm of streptavidin magnetic particles and α-amylase enzyme concentration as shown in Fig. 1(a) and (b), respectively.

**Figure 1.** MR ratio as a function of (a) streptavidin magnetic particles concentration and (b) α-amylase enzyme concentration.

The direction of Co/Cu multilayer is in-plane to electromagnetics strips. In the absence of an external magnetic field, GMR sensors have median magnetoresistance (MR) and they work at the most sensitive region. When magnetic nanoparticle labels are bound to the sensor surface, they can be magnetized as dipoles, and they introduced stray fields may reduce the magnetic field. The
change in magnetic field leads to a change in MR of the sensor, which can be measured and read out primary detection signal using the Wheatstone bridge.

The typical real-time binding curves (Change of output voltage vs. external magnetic field) for magnetic streptavidin with different concentrations (10, 20, 30, and 40 μl/ml) on the GMR sensors in Wheatstone bridge are shown in Fig. 2a. The signal from the GMR sensors were treated with the same volume of ethanol without streptavidin magnetic particles served as the background reference. Beginning at $H=0$, the signal for various streptavidin magnetic particles concentration showed a rise, which reflects real-time binding of MNPs to sensor surface. These rising signals saturate within 650 Gauss, implying that magnetic particles binding has reached equilibrium on sensor surface. Higher saturated signal level is detected for higher concentration of streptavidin magnetic particles. Their final average signals for logarithm of streptavidin magnetic particles concentrations are plotted in Fig. 2b.

**Figure 2.** (a) Output voltage versus External magnetic field for the GMR sensor bio-detection of the magnetic streptavidin with different concentrations and (b) Plot of the sensor output signal versus the concentration of streptavidin magnetic particles.

Furthermore, we also demonstrate detection of $\alpha$-amylase enzyme with concentration 10, 20, 30, and 40 μl/ml. Similar binding curves are also observed for streptavidin magnetic particles. The reported signal values are shown in Fig 3. For GMR bio-detection technology; the final output signal has a relationship with the bound number of MNPs [12]. Consequently, for detecting a biological or chemical agent using GMR biosensor, it is critical to build a model that the number of bound MNPs is dependent on the added amount of the biological target.

**Figure 3.** (a) Output voltage versus external magnetic field for the GMR sensor bio-detection of the $\alpha$-amylase enzyme with different concentrations and (b) Plot of the sensor output signal versus the concentration of $\alpha$-amylase enzyme.
The increase of output voltage is caused by interaction between magnetic moments of MNPs and ferromagnetic strength. The magnetic moments of ferromagnetic layer were harder to magnetize by external magnetic fields. Thus, these cause an increase of the output voltage. The addition of streptavidin magnetic particles and $\alpha$-amylase enzyme respectively caused an increase in magnetic induction so the voltage increased with the increase of streptavidin magnetic particles and $\alpha$-amylase enzyme concentrations respectively.

4. Conclusion
The output voltage changes with the increase of concentration. It was reported that the output voltage signal of the Wheatstone bridge exhibits log-linear function in real-time measurement of the concentration of streptavidin magnetic particles and $\alpha$-amylase enzyme respectively, making the sensor suitable for use as a biomolecule concentration detector since it can detect the quantity of biomolecules. Thus, the combination of Co/Cu multilayer, Wheatstone bridge, magnetite and PEG polymer has potential application to be used in bio-detection applications where ultra-small bio-labels are needed.

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References
[1] Rife, J., Miller, M., Sheenan, P., Tamanaha, C., Tondra, M., and Whitman, L 2003 Sensors Actuators A: Physics 107 209-218
[2] Kotitz, R., Matz, H., Trahms, L., Koch, H., Weitschies, W., Rheinlander, T., Semmler, W., and Bunte, T 1997 IEEE Trans. Appl. Supercond 7 3678–3681
[3] Li, G., Sun, S., Wilson, R.J., White, R.L., Pourmand, N., and Wang, S.X 2006 Sensors Actuators A: Physics 126 98–106
[4] Rajasekaran, N., Mohan, N., Chelvane, J.A., and Jagannathan, R 2012 Journal of Magnetism and Magnetic Materials 324 2983-2988
[5] Angelakeris, M., Papaioannou, E.T., Poulopoulos, P., Valassiades, O., and Flevaris, N.K 2003 Sensors and Actuators A 106 91-95
[6] Baibich, M.N., Broto, J.M., Fert, A., Nguyen Van Dau, F., Petroff, F., Etienne, P., Creuzet, G., Friederich, A., and Chazelas, J 1988 Physical Review Letters 61 2472–2475
[7] Rochaz, L.V., Cuchet, R., and Vaudaine, M.H 2000 Sensors and Actuators 81 53-56
[8] Suharyadi, E., Pardede, I., and Hasibuan, F.A 2016 Proceeding Progress In Electromagnetic Research Symposium (PIERS) 566-571
[9] Reig, C., Cubells-Beltran, M.D., and Muñoz, D.R 2009 Sensors 9 7919–7942
[10] Nurpriyanti, I., Pardede, I., and Suharyadi, E 2016 Proceeding International Seminar on Sensors, Instrumentation, Measurement and Metrology (ISSIMM) 32-36
[11] Mathivannan 2007 N PC-based Instrumentation: Concepts and Practice Prentice-Hall Of India.
[12] Schotter, J., Kamp, P.B., Becker, A., Pühler, A., Reiss, G., and Brückl, H 2004 Biosensors Bioelectro 19 1149–1156