Biomolecule detection using wheatstone bridge giant magnetoresistance (GMR) sensors based on CoFeB spin-valve thin film

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Abstract. A potential wheatstone bridge giant magnetoresistance (GMR) biosensor have been successfully developed for biomolecule detection. [IrMn(10 nm)/CoFe(3 nm)/Cu(2.2 nm)/CoFeB(10 nm)] spin-valve structure has been chosen as the magnetic sensing surface, showing a magnetoresistance (MR) of 6% fabricated by DC magnetron sputtering method. The Fe₃O₄ magnetic nanoparticles used as biomolecular labels (nanotags) was synthesized by co-precipitation method, exhibiting soft magnetic behavior with saturation magnetization (Ms), remanent magnetization (Mr) and coercivity (Hc) is 77.2 emu/g, 7.8 emu/g and 51 Oe, respectively. The X-ray diffraction (XRD) patterns and transmission electron microscopy (TEM) images showed that Fe₃O₄ was well crystallized and grew in their inverse spinel structure, highly uniform morphology with an average grain size was about 20 nm. Fe₃O₄ was coated with polyethylene-glycol (PEG)-4000 for surface functionalization. Detection of biomolecule such as formalin, gelatin from bovine-skin and porcine-skin were dispersed in ethanol at room temperature. Induction would cause a shift in output voltage with a minimum delta output voltage (ΔV) 4.937 mV (10%) for formalin detection, 2.268 mV (7%) for bovine-skin gelatin and 2.943 mV (7%) for porcine-skin gelatin detection. The ΔV of the wheatstone bridge in real-time measurement decrease by increase in biomolecules concentration. The change of ΔV with various concentration of biomolecule indicates that the spin-valve thin film with wheatstone-bridge circuit is potential as a biosensor.

1. Introduction

Nowadays, technologies for biomolecular diagnostics have broad applications in biology and medicine. It is necessary to establish a reliable, sensitive and simple quantitative method to detect biomolecule. Magnetism provides great opportunities for researchers to remotely control and detect small biological samples. Magnetic immunoassay is one type of immunoassays that can quantitatively detect biomolecules.

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, giant magnetoresistance (GMR) sensor is one of magnetic sensor that can be the powerful tools for bio-detection due to their merits of low cost, portability, high sensitivity, and real-time electronic readout [1].

The mechanism of GMR biosensors is detecting the magnetic fringing fields of the particle labels after capture by target-probe biomolecular recognition. Wheatstone-bridge can be used in sensor system
because its high sensitivity that can read signal output in millivolts, so if there is small change in sensor resistance will change signal output voltage. Magnetic fringing field will be induced by the magnetic nanoparticles which in turn exerts on the free layer of the GMR sensor along the opposite direction of the external field. Thus, the resistance of the detecting spin-valve in GMR sensors will be reduced, which results in an unbalance of Wheatstone bridge and gets an output voltage.

To achieve the desired detection sensitivity and quantification, nanometer-sized magnetic particles should be used as the biomolecular labels because of their comparable size to biomolecules and monodispersity of dimensional and magnetic properties. One of magnetic nanoparticles (MNPs) which gained considerable attention as labels to the GMR sensors is Fe$_3$O$_4$ that have superparamagnetic characteristics and high magnetic saturation ($M_s$) [2]. Thus make the Fe$_3$O$_4$ MNPs responsive to the external magnetic field [3]. To capture biomolecules and achieved better dispersibility, the Fe$_3$O$_4$ MNPs are surface-modified or coated by biocompatible polymers. Polyethylene glycol (PEG) is a popular one since their long polymeric chains are highly soluble in water and nontoxic [4], biocompatible, and low-costed [5].

In recent work, Suharyadi et al. [6] had investigated the detection of Fe$_3$O$_4$ MNPs and formaldehyde using GMR sensor by four point probe system. However, that experiment shows that signal output of GMR sensor was resistance. 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 [7]. Hence, Nurpriyanti et al. [8] investigated the detection of Fe$_3$O$_4$ MNPs using wheatstone bridge GMR sensors. However, the sensors was not portable and real-time.

Therefore, the aim of the present work is to develop more portable wheatstone-bridge GMR biosensor and able to read signal output in real time for various concentration of formaldehyde, bovine and porcine skin gelatin with Fe$_3$O$_4$ nanoparticles as nanotags.

2. Experimental Detail

The spin valve thin film as the magnetic sensing surface was fabricated by DC magnetron sputtering method. Spin valve structure consists of [Ta (2nm)/IrMn (10nm)/CoFe (3nm)/Cu (2,2nm)/CoFeB (10nm)/Ta (5nm)]. The magnetic properties were characterized by Vibrating sample magnetometer (VSM). Spin Valve structure was chosen as the sense element because of its high field sensitivity at room temperature [9].

The Fe$_3$O$_4$ magnetic nanoparticles were synthesized by chemical co-precipitation method. For pure Fe$_3$O$_4$ nanoparticles, FeSO$_4$, 7H$_2$O and FeCl$_3$,6H$_2$O (Merck Germany) as a source of Fe$^{3+}$ and Fe$^{2+}$ were dissolved in deionized water. The obtained dark orange solution was stirred at 60$^\circ$C in order to obtain homogeneously solution. An aqueous NH$_2$OH solution 10% then added dropwise to that mixture under constant magnetic stirring 450rpm for 90 min. After that, the Fe$_3$O$_4$ nanoparticles were separated and purified by magnetic separation and washed with deionized water to make them free of any residual salts, and then dried in furnace for 2 hours at 80$^\circ$C [6,8]. Finally, the black solid powder was obtained and characterized. The process was continued by synthesis Fe$_3$O$_4$ nanoparticles functionalized with polyethylene-glycol 4000 (PEG-4000). This functionalization was intended to capture biomolecules. The crystal structure and phase identification were confirmed by the X-ray diffractometer Shimadzu XD with Cu-K radiation ($\lambda=1.5406$ Å). The vibrational spectra of nanoparticles were recorded by using Fourier transform infrared spectroscope Prestige Shimadzu-21. The morphology and size distribution were studied by using transmission electron microscope (TEM) Jeol JEM-1400. The magnetic measurements of nanoparticles were carried out by using vibrating sample magnetometer (VSM) Riken Dentshi Co Ltd at room temperature.

For biomaterial detection, 1mg of PEG-coated Fe$_3$O$_4$ nanoparticles was added with various biomolecules concentration. The concentration of formalin were 2%, 4%, 6%, 8% and 10% and for bovine-skin and porcine-skin gelatin were 1%, 3%, 5% and 7%. Furthermore, the mixture dissolved in 1 ml of ethanol by means of sonicated for 30 min at room temperature. 10μl of the solution was dispersed on the surface of the GMR sensor under a bias magnetic field along the in-plane direction. 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 devices are driven by a control signal which depends on the system status that makes the devices portable and real-time. Various applied magnetic fields of 0-650 Gauss have been performed using electromagnetic. 10 mA constant DC current was applied to the wheatstone bridge for measure signal output voltage ($V_{out}$). The current was provided by Keithley-2401 which also functions as a voltmeter. 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 [10]. Moreover, it can avoid the heating effect in sensors as well [9].

3. Result and Discussion

The Spin valve [Ta(2nm)/IrMn(10nm)/CoFe(3nm)/Cu(2.2nm)/CoFeB(10nm)/Ta(5nm)] as magnetic sensing surface were deposited on silicon substrate by DC magnetron sputtering under pressure was about $3 \times 10^{-5}$ Pa. 8 Oe exchange bias field ($H_{ex}$ bias) was obtained due to the exchange coupling between ferromagnetic and antiferromagnetic layers. Moreover, the saturation magnetization ($M_s$) was about 1162.5 emu/cc [8]. The Magnetoresistance was measured as a function of the external magnetic field ($H$) showing magnetoresistance 6% [8]. MR curve has shown the GMR phenomenon as the change in resistance that influenced by magnetic field. It can be explained with origin of GMR by spin-dependent scattering. In the parallel magnetization case, scattering is strong for the electrons with spin anti-parallel (minority carries) to the magnetization of the ferromagnetic layers and weak for electrons with spin parallel (majority carries). The anti-parallel of alignment of the magnetic layers result in appreciable scattering from electrons of both spin directions. The lower amount of scattering events in the parallel case leads to a lower resistance state than in the anti-parallel case.

The Fe$_3$O$_4$ nanoparticles exhibit superparamagnetic behaviour. $M_s$ decrease from 77.2 emu/g to 49.6 emu/g and $M_r$ decrease from 7.8 emu/gram to 6.4 emu/gram after Coated with PEG [6,8]. $H_c$ increase from 51.2 Oe for Fe$_3$O$_4$ nanoparticles to 61.5 Oe for PEG-Coated Fe$_3$O$_4$ nanoparticles [6,8]. The X-ray diffraction peaks corresponding to the (111), (220), (311), (400), (511) and (440) planes, which indicates the cubic spinel crystal structure of pure Fe$_3$O$_4$. The estimated average crystallite size of Fe$_3$O$_4$ was about 20.1 nm and increase to 22.3 nm after coated with PEG. TEM images in figure 1(a) and (b) shows that Fe$_3$O$_4$ nanoparticles and PEG-Coated Fe$_3$O$_4$ nanoparticles exhibit a spherical shapes with uniform grain size. Moreover, the addition of PEG can decrease the agglomeration. The SAED patterns for both of them, is closely related to XRD analysis [6,8].

![Figure 1](image_url)

**Figure 1.** TEM images and selected area electron diffraction (SAED) patterns of (a) Fe$_3$O$_4$ nanoparticles and (b) PEG-Coated Fe$_3$O$_4$ nanoparticles.

For biomolecule detection, 1μl solution of PEG-coated Fe$_3$O$_4$ nanoparticles and biomolecules were dropped on the surface of the GMR sensor to detect the output voltage ($V_{out}$). When the sensors are at the high resistance state, the $V_{out}$ of Wheatstone bridge will decrease because the magnetic fringing field of the magnetic particles exerting magnetic moments in the free layer of the spin valve is opposite to the external magnetic field, and vice versa. The different value of $V_{out}$ between without with and without 650 Gauss magnetic field is defined as $\Delta V=V(H)-V(0)$, where $V(H)$ and $V(0)$ refer to the $V_{out}$ of the
GMR sensor with and without 650 Gauss magnetic field, respectively. $\Delta V$ is proportional to the MR value where the greater the $\Delta V$, the magnetic moments on the free layer are easier to magnetize by the external magnetic field.

**Figure 2.** (a) The output voltage of wheatstone-bridge and (b) delta output voltage as function of formalin concentration.

**Figure 3.** (a) The output voltage of wheatstone-bridge and (b) delta output voltage as function of bovine-skin gelatin concentration.

**Figure 4.** (a) The output voltage of wheatstone-bridge and (b) delta output voltage as function of porcine-skin gelatin concentration.

Figure 2(a), 3(a) and 4(a) shows that sensor output voltage rising from $H=0$ for various biomolecule concentration and then tend to saturated at $H=650$ Gauss, implying that magnetic particles binding has reached equilibrium on sensor surface. Lower saturated signal level is detected for higher concentration of biomolecule. The changes of the output voltage are a result of changes in resistivity of the CoFeB spin-valve thin film after coated with various biomolecules concentration. The different value of $V_{out}$ between without with and without 650 Gauss magnetic field are plotted in figure 2(b), 3(b) and 4(b).
The delta output also decrease with the increase of biomolecule concentration. When the biomolecule concentration increase, PEG-coated Fe₃O₄ nanoparticles bond more biomolecules. Paramagnetic properties of the biomolecule made decrease in MNPs saturation magnetization. It caused a decrease in the effect of magnetic induction and made interaction between magnetic moments of nanoparticles and ferromagnetic layer weakened. This resulted magnetic moments of ferromagnetic layer was easier to magnetize by external magnetic field. All of this made magnetoresistance of thin film decrease with the increase of biomolecule concentration. The ΔV is proportional to the MR value. The schematic of biomolecule detection was shown in Figure 5.

**Figure 5.** Schematic of biomolecule detection and interaction between ferromagnetic layer and nanoparticles Fe₃O₄.

4. Conclusion

A wheatstone bridge giant magnetoresistance (GMR) biosensor was successfully developed for biomolecule detection. The delta output voltage decrease with the increase of biomolecule concentration. This shift in delta output voltage is a direct proof that detection of formaldehyde and gelatin in various concentrations has been achieved.

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