Ultralow detection limit of giant magnetoresistance biosensor using Fe_3O_4–graphene composite nanoparticle label*

Jie Xu(徐洁)[1,2], Ji-qing Jiao(焦吉庆)[2], Qiang Li(李强)[1], and Shan-dong Li(李山东)[1,3,†]

[1] College of Physics, Key Laboratory of Photonics Materials and Technology in Universities of Shandong, and Laboratory of Fiber Materials and Modern Textile, the Growing Base for State Key Laboratory, Qingdao University, Qingdao 266071, China

[2] School of Chemical Science and Engineering, Qingdao University, Qingdao 266071, China

[3] National Laboratory of Solid State Microstructures, Nanjing University, Nanjing 210093, China

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A special Fe_3O_4 nanoparticles–graphene (Fe_3O_4–GN) composite as a magnetic label was employed for biodetection using giant magnetoresistance (GMR) sensors with a Wheatstone bridge. The Fe_3O_4–GN composite exhibits a strong ferromagnetic behavior with the saturation magnetization M_S of approximately 48 emu/g, coercivity H_C of 200 Oe, and remanence M_r of 8.3 emu/g, leading to a large magnetic fringing field. However, the Fe_3O_4 nanoparticles do not aggregate together, which can be attributed to the pinning and separating effects of graphene sheet to the magnetic particles. The Fe_3O_4–GN composite is especially suitable for biodetection as a promising magnetic label since it combines two advantages of large fringing field and no aggregation. As a result, the concentration x dependence of voltage difference $|\Delta V|$ between detecting and reference sensors undergoes the relationship of $|\Delta V| = 240.5 \lg x + 515.2$ with an ultralow detection limit of 10 ng/mL (very close to the calculated limit of 7 ng/mL) and a wide detection range of 4 orders.

Keywords: giant magnetoresistance biosensors, magnetic label, Fe_3O_4–graphene composite, lowest detection limit

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1. Introduction

Biomolecular identification has broad applications in biology and medicine such as gene expression monitoring, disease diagnosis, drug discovery, and so on. In 1998, Baselt *et al.* first demonstrated magnetic identification using giant magnetoresistance (GMR) sensors as biosensors.[1] The resistance of a GMR sensor changes with the magnetic field applied to the sensor, so a magnetically labeled biomolecule can induce a voltage signal. Obviously, the performances of the detection system, e.g., concentration detection range, sensitivity, etc., are mainly dominated by the sensitivity of sensors and the magnetic properties of magnetic labels, so researchers improved the performances of the system from both aspects above. Up to now, magnetic field sensitive sensors have been widely explored, such as superconducting quantum interference device (SQUID),[2,3] silicon Hall sensor,[4] magnetic planar Hall effect sensor,[5] giant magnetoresistive (GMR) sensor,[1,6–16] magnetic tunnel junction (MTJ) sensor,[17] and so on. Comparing with other sensors, GMR sensor is one of the prime candidates for magnetic biosensor because it shows some advantages, such as inexpensive, high sensitivity, easy to be integrated with Si-based integration circuits (IC). Therefore, it is easy to combine electronics and microfluidics, and to realize multichannel detection for many different analytes on a single chip at the same time.

As for the magnetic labels for bio-detection, the basic requirements are large magnetic fringing fields, monodispersion in size, and separating well from each other. Researchers have made a lot of effort to obtain the appropriate labels. In the early stage, the commercially available magnetic beads used by most researchers tend to have a mean diameter in the range from 0.1 μm to 3 μm, including the paramagnetic polystyrene beads and similarly sized ferromagnetic particles.[9] These labels have a narrow size dispersion, but are seriously mismatched in size with the typical analytes, such as DNA fragments or protein targets in the bio-detection, prejudicing the quantitative capabilities of the system. Wang *et al.* reported that the particles with $\sim$ 50 nm in diameter are the most suitable for magnetic bio-detection.[13] The decrease of particles size is beneficial to improve the detection sensitivity. For sensitive MR detection, metallic Fe, Co, Ni or their alloy nanaoparticles with high magnetic moments become good candidates.[18] These metal or alloy nanoparticles with a mean diameter in the range from nanometers to decades of nanometer exhibit a better magnetic performance and match DNA fragments or protein targets well. However, the aggregation among these metal or alloy nanoparticles limits the practical application. Superparamagnetic nanoparticles are an ideal

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†Corresponding author. E-mail: lishd@qdu.edu.cn

candidate for bio-detection because the aggregation is easy to avoid and the grain size is adjustable,[10,19–21] but it is a great challenge to detect such tiny magnetic nanoparticles because their magnetic moments are relatively low due to their limited grain sizes.

Magnetite Fe_3O_4 nanoparticles have attracted worldwide attention because of not only their unique size- and morphology-sensitive physical and chemical properties, but also their biocompatibility and remarkable magnetic properties.[22] Graphene possesses a large surface area, open porous structure, flexibility, chemical stability, and biocompatibility, suggesting a good matrix for metal oxides grown on it.[23] The process of Fe_3O_4–graphene composite is also controllable and relatively simple. On the other hand, the 2D graphene sheets act as a framework to pin and separate Fe_3O_4 nanoparticles, avoiding the aggregation among magnetic particles. Therefore, in this study, we fabricated biocompatible Fe_3O_4–GN composite as a special magnetic label used in the bio-detection. For the Fe_3O_4–GN composite particles, the Fe_3O_4 nanoparticles show a rod-like shape, and provide a large fringing field due to their high saturation magnetization (M_S) and remanence (M_r). However, the magnetic nanoparticles do not aggregate due to the supporting of graphene. As a result, an ultralow detectable concentration limit of 10 ng/mL for Fe_3O_4–GN solution is achieved in our experimental setup.

2. Experimental procedure

The exchange-biased GMR sensor (GF708, manufactured by Sensitec GmbH, Germany) was adopted in the detection system. In GF708 GMR sensor, four identical spin-valves (SVs) were connected forming a Wheatstone bridge to detect the voltage difference between detecting and reference SVs. The magnetic external field was exerted on the GMR sensor and the magnetic nanoparticles dispersed on the surface of the GMR sensor. The magnetic fringing field would be induced by the magnetic nanoparticles which in turn exerted on the free layer of the GMR sensor along the opposite direction of the external field. Thus, the effective magnetic field applied on the free layer would be reduced and the resistance of the detecting SVs in GMR sensors would be reduced accordingly, which resulted in an imbalance of Wheatstone bridge and got an output voltage V_{out}. The detailed detection procedure has been described in our previous work[24] and other reports.[12,14]

The direct current (DC) in-plane measuring method was employed using a home-made detection device in this study.[24] A Keithley 2400 source meter provided a constant current for the sensor as the detecting current, and a Keithley 2182 digital nanovoltmeter measured the V_{out} of the Wheatstone bridge. Helmholtz coils provided a steady-state in-plane magnetic field. In all measurement, a DC current of 10 μA 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.[12] Moreover, it can avoid the heating effect in sensors as well.

Fe_3O_4–GN composite nanoparticles were synthesized by a modified solvothermal method. 0.170-g $NaNO_3$ (0.050 M), 0.324-g $FeCl_3$ (0.10 M), and 0.011-g NaF (0.07 M) were dissolved in 20-mL deionized water with stirring. The red solution was transferred into the PTFE reactor. Graphene (GN) sheets were put into the prepared solution. The PTFE reactor was put into the stainless steel autoclave, which was then sealed. The reaction was maintained at 95 °C for 6 h. The precursor was subsequently washed with deionized water, then annealed at 550 °C for 2 h under N_2 atmosphere. Figure 1(a) shows the TEM images of the as-prepared Fe_3O_4–GN composite particles. Rod-like Fe_3O_4 nanoparticles with approximately 30 nm in diameter and 80 nm in length were grown on GN sheets with homogeneous dispersion. Five kinds of concentrations (0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, 10 μg/mL, and 100 μg/mL) of alcohol-soluble magnetic Fe_3O_4–GN composites were used to verify the sensitivity of the biodetection device. The dependence of V_{out} on the external magnetic field and detection time was then recorded by computer via GPIB connection for further analysis.

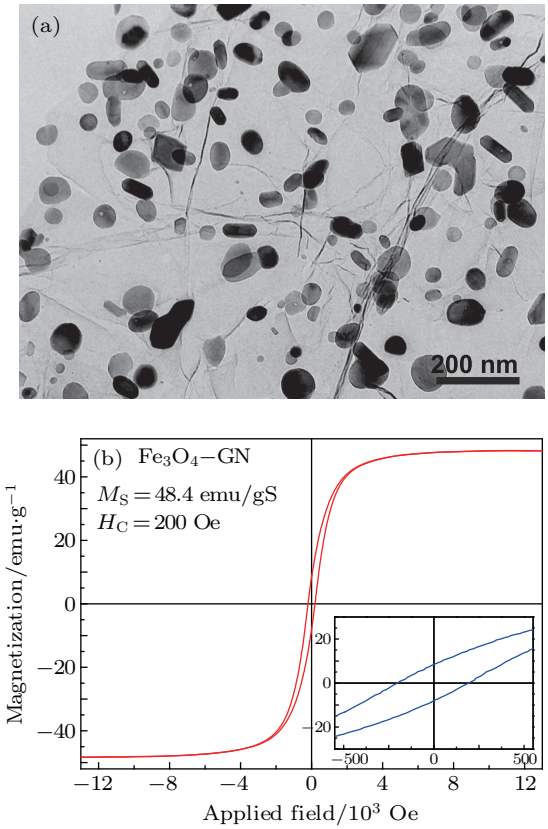

Fig. 1. (color online) (a) The TEM image of the Fe_3O_4–GN magnetic label, (b) the magnetic hysteresis loop of Fe_3O_4–GN composite at room temperature. The inset in panel (b) shows the enlargement of loop around 0 Oe.

Magnetic properties of Fe_3O_4–GN composite were measured using an alternating gradient magnetometer (AGM), the microstructure was observed by transmission electron microscopy (TEM).

3. Results and discussion

Figure 1(a) shows the TEM image of the Fe_3O_4–GN magnetic composites. As illustrated, the Fe_3O_4 nanoparticles exhibit a rod-like shape with a diameter of approximately 30 nm and a length of approximately 80 nm. The Fe_3O_4 nanoparticles were grown on the graphene sheets. It is interesting to note that the Fe_3O_4 nanoparticles separate well from each other, instead of aggregating like conventional nanoparticle systems,[9,25] which can be attributed to the supporting and separating effects from graphene sheets.

The magnetic hysteresis loop of Fe_3O_4–GN magnetic composites at room temperature is shown in Fig. 1(b). A large M_S of 48.4 emu/g, H_C of 200 Oe, and M_r of 8.3 emu/g were observed, which indicate that the sample has a relatively strong room temperature ferromagnetism. The observed saturation magnetization is comparable to that of bulk.[26] The inset of Fig. 1(b) shows clear coercivity and remanence confirming ferromagnetism for Fe_3O_4 nanoparticles instead of superparamagnetism.

The ideal magnetic label should possess various advantages simultaneously, including a large magnetic fringing field, a sufficiently fine grain size, a monodispersed grain size, and good dispersion. However, the large magnetic fringing field means relatively strong ferromagnetic properties in the magnetic label. It will result in an aggregation of magnetic particles, leading to a statistical concentration deviation of biomarkers. One effective solution is using superparamagnetic nanoparticles as the magnetic label for avoiding aggregation. In this case, a large transverse magnetic field ($H_T > 2$ kOe) has to be adopted to induce a fringing field.[12,14,16] However, the large H_T leads to not only the complexity of the measurement system, but also interferes with the normal work of GMR sensors. The sensitivity of the whole measurement system will deteriorate. Actually, it is highly expected that the magnetic label possesses strong magnetic properties, but does not aggregate together. In this study, a special Fe_3O_4–GN composite magnetic label was prepared, which is especially suitable for biodetection as a magnetic label because of its two advantages: one is its nanocrystalline structure with a rod-like shape, leading to a relatively strong magnetic fringing field; the other is that it does not aggregate due to the supporting of graphene sheets. As a result, an ultralow detectable limit and accuracy of biomarkers will be obtained using Fe_3O_4–GN magnetic label.

The external magnetic field dependence of V_{out} for a bare GMR sensor is measured. As illustrated in Fig. 2, a quasi step-like transfer curve is observed. The V_{out} increases rapidly and linearly with the increase of the magnetic field from -1 Oe to 5 Oe, where the sensor has the maximum sensitivity to the external field. In order to determine the most sensitive operating magnetic field, the first-order differential was taken for the transfer curve, and the extreme value appears at a bias field around 1 Oe.

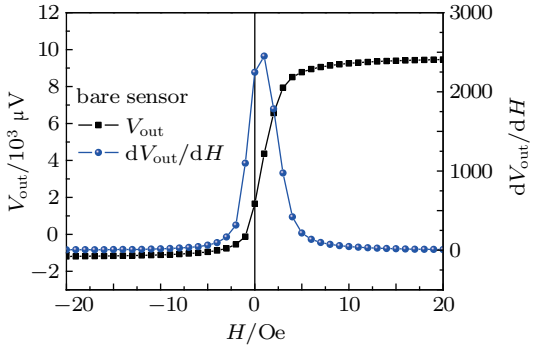

Fig. 2. (color online) The magnetic field dependence of the output voltage V_{out} and its derivative for bare GMR sensor.

The Fe_3O_4–GN composite was dissolved in the alcohol solution and dropped on the surface of the GMR sensor to detect V_{out}. The magnetic field operating range changed from $+95$ Oe to -95 Oe gradually. At first, the sensors are at the high resistance state, the resistance of the detecting SVs decreases because of the magnetic fringing field of the magnetic particles, and the V_{out} of Wheatstone bridge decreases consequently. When the magnetic field is reversed, the sensor is at the low resistance state, the resistance of the detecting SVs increases due to the magnetic fringing field of the magnetic particles, and V_{out} of the Wheatstone bridge increases. As shown in Fig. 3(a), the output voltage values of the sensor dropping with the magnetic nanoparticles (the red line) are smaller than those of the bare sensor (the black line) for the high-resistance state. On the contrary, the output voltage values of the sensor dropping with the magnetic nanoparticles are larger than those of the bare sensor for the low-resistance state. The different value between two V_{out} is defined as $\Delta V = V - V_0$, where V and V_0 are V_{out} of the GMR sensor with and without magnetic nanoparticles, respectively. In the magnetic field range between -1 Oe and 5 Oe, the ΔV can be observed clearly. However, for the magnetic field outside this range, the ΔV decreases rapidly and is unsuitable for detection, because the magnetic field is out of the sensitive range. The Fe_3O_4–GN composite alcohol solution was dropped on the GMR sensor and then the measurement data were recorded. The magnetic field H dependence of ΔV is summarized in Fig. 3(b). The ΔV increases with the particle concentration, which can be attributed to the increase of the induced magnetic fringing field with the increase of the magnetic particle concentration.

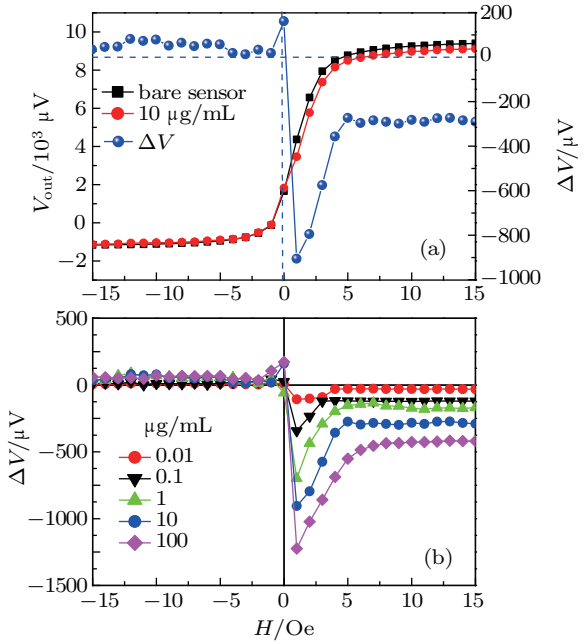

Fig. 3. (color online) (a) The comparison of output voltage V_{out} and ΔV for GMR sensor with/without magnetic label, (b) the concentration dependence of ΔV.

The GF708 is a magnetic field sensor with a Wheatstone bridge composed of four synthetic SVs. In which, two SVs are shielded as reference resistors and the resistance is constant, the other two SVs' resistance changes with the magnetic field and induces the change of the output voltage. If the four SVs are exactly the same, the output voltage transfer curve should have an antisymmetric distribution centered on zero voltage. After dropping the magnetic particles on the chip, the fringing field from the particles will induce a tiny change of the output voltage ΔV, which is also antisymmetric centered on zero voltage. However, the value of ΔV is so tiny that it is easily submerged in noise. In order to improve signal-to-noise ratio, the output voltage transfer curve of GF708 is designed asymmetric centered on zero voltage, i.e., the two antisymmetric peaks are combined into one negative peak. Thus, the signal-to-noise ratio is doubly enhanced to effectively avoid being submerged in noise. The transfer curve for the bare sensor in Fig. 3(b) shows an obviously asymmetric output voltage of +9398 μV and −1165 μV for high and low resistances, respectively, thus the output voltage difference ΔV shows a single peak around the bias field of 1 Oe, instead of antisymmetric double peaks.

In addition, the detection time dependence of V_{out} for the GMR sensor was also measured at its sensitive operating point of magnetic field $H = 1$ Oe to investigate the stability of the measurement system. As illustrated in Fig. 4(a), V_{out} for all the five kinds of particle concentrations (0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, 10 μg/mL, and 100 μg/mL) are 101 μV, 228 μV, 432 μV, 795 μV, and 1020 μV, respectively, which increases in turn with the increase of the particle concentration due to the increase of magnetic fringing field exerted on the free layer of the GMR sensors. The lowest detectable concentration limit of Fe_3O_4–GN composite solution is 0.01 μg/mL at this moment. Moreover, $|\Delta V|$ linearly increases with the logarithm of the particle concentrations, undergoing a relation of $|\Delta V| = 240.51\lg x + 515.2$ (Fig. 4(b)). It was reported that the $|\Delta V|$ of the GMR sensors exhibits a linear increase with the coverage area of the magnetic particles on the surface in the case of lower concentration.[8] This study revealed that the linear $|\Delta V|$–x relationship will be replaced by a nonlinear one in the case of higher concentration. Shen *et al.* explained the nonlinear relationship using a "coffee ring" effect for a macroscopic evaporating water droplet with suspended particles on a solid surface,[27] where the actual coverage area of particles is proportional to the "ring" size and the logarithm of the particle concentrations. Actually, beside the coffee ring effect, when the magnetic nanoparticle concentration reaches a certain value, the overlap among the particles is inevitable. At the same time, the magnetic dipole interaction enhances. These factors will lead to a reduction of magnetic fringing field, and $|\Delta V|$–x loses the linear relationship. The detailed theoretical analysis is ongoing. Nevertheless, the nonlinear $|\Delta V|$–x relationship provides a possibility to extend the detection range. For example, in this study, a large detection range covering 4 orders is available. On the other hand, an ultralow detection limit of 10 ng/mL can be obtained as well, which is very close to the theoretical detection concentration limit of 7.2 ng/mL for Fe_3O_4–graphene nanoparticles solution based on the fitting function. This detection limit of our system is consistent with the other reports based on the commercial GMR sensor (GF708) using 10-nm superparamagnetic Fe_3O_4 nanoparticles as the labels,[12] however, the bias field perpendicular to the sensor for magnetizing the superparamagnetic nanoparticles was discarded in our detection system because of the large remanence of the rod-like shape Fe_3O_4 nanoparticles.

An ultra-low detection limit was achieved in this study. Wang *et al.* investigated five different types of magnetic labels using a GMR sensor and found the detection limit of 0.1 nmol/mL, i.e., the number of particles 6.02×10^{13} per mL.[13] Li and Kosel demonstrated the capability of the magneto-resistive sensor made of an electromagnetic trap using the commercial magnetic microparticles with the size of 2.8 μm and found the detection limit of 1.29×10^8 bacteria/mL.[28] In this study, Fe_3O_4 nanoparticles with 30 nm in diameter and 80 nm in length were adopted, and the detection limit can be estimated as follows. The density of the Fe_3O_4 particles is about 5.0 g/cm^3, then the mass of a single microparticle is $m_{mass} = \rho V = 5.0 \times [\pi \times (1.5 \times 10^{-6})^2 \times 8 \times 10^{-6}] = 2.826 \times 10^{-16}$ g and 1-g sample contains particles of $n = 3.54 \times 10^{15}$, so the detection limit of 10 ng/mL corresponds to the number of particles 3.54×10^7 per mL, which is less than the reported results. This study suggested that, be-

sides the sensitivity of the sensors themselves, the particle size and magnetic properties of the magnetic labels are the critical factors for the sensitivity of the detection system.

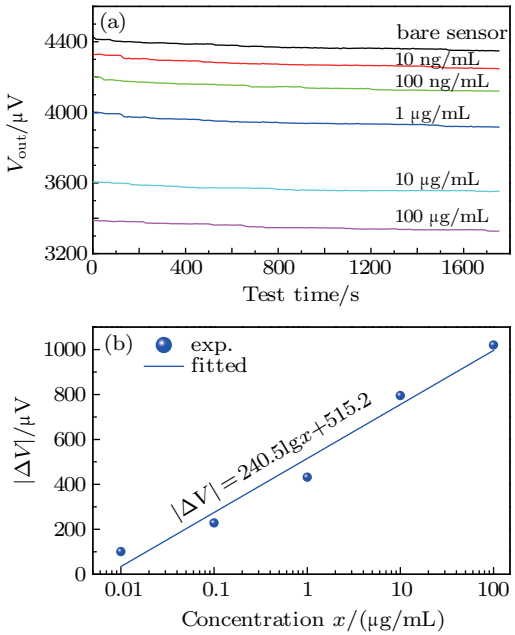

Fig. 4. (color online) (a) The detection time dependence of the output voltage at various concentrations, (b) the concentration dependence of $|\Delta V|$ and its fitted line.

4. Conclusion and perspectives

A special Fe_3O_4–GN composite magnetic label with a large fringing field but not aggregation was prepared for exploring the biodetection concentration limit using GMR sensors with a Wheatstone bridge. It was revealed that the voltage difference $|\Delta V|$ of V_{out} for sensors with and without magnetic particles is proportional to the logarithm of the particle concentrations, undergoing a relationship of $|\Delta V| = 240.5 \lg x + 515.2$, suggesting both a low concentration limit and a wide detection range of over 4 orders. The lowest detectable concentration limit of Fe_3O_4–GN composite solution is 10 ng/mL in our experimental setup, which is close to the theoretical limit of 7.2 ng/mL.

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