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GMR sensors and magnetic nanoparticles for immuno-chromatographic assays

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ABSTRACT

Conventional tests based on immunorecognition and on the use of coloured colloidal particles have still some drawbacks that limit their use: they do not provide a quantitative determination of the analyte, and their sensitivity is limited. Our strategy to overcome these disadvantages consists in the use of superparamagnetic core-shell nanoparticles to tag the analyte. The use of these magnetic labels allows us to quantify the amount of analyte present in our sample with a very high sensitivity, detecting their magnetic response by means of the suitable magnetic sensor. Our method is based on measuring the magnetoresistive response of a spin-valve giant magnetoresistive (GMR) sensor placed in proximity to the magnetic nanoparticles present in the lateral flow strip. Here, a brief description of our prototype and of the measurement procedure will be presented, as well as preliminary assays using our biosensor to detect the hCG pregnancy hormone in a solution. A crucial aspect to take into account in order to increase the sensitivity is the proper functionalisation of the nanoparticle shell, in order to achieve an oriented immobilisation of the antibodies to be used in the immunorecognition process. Several strategies to further increase the sensor sensitivity are suggested.

1. Introduction

A great effort is currently being made in the research on lateral flow tests due to their wide use nowadays in Life Sciences. Their simplicity, low cost and the large variety of analytes that can be detected by means of this technique make them suitable for a large number of applications. Basically, they consist of a nitrocellulose membrane with micrometric pores allowing the flow of liquid via capillarity. In this membrane, a biological recognition agent is previously deposited in the test line and traps the targeted analyte if present in the flowing liquid. In standard immuno-chromatographic assays, the biological recognition events are labelled with colloidal particles giving identifiable colour to the test line when the test is positive. The test is considered positive when the amount of analyte is large enough so that the labelled colloidal particles can be detected by eye [1]. However, this test is not quantitative. In the last years there has been an increasing interest in developing quantitative high-sensitive lateral-flow assays for detection of antibodies, enzymes, virus, etc. with applications in clinical diagnosis, food industry and environment control.

Magnetic nanoparticles (MNPs) are currently being used in quantitative and highly-sensitive biosensors (magnetic biosensing) [2,3]. Inductive techniques have been used to detect and quantify the magnetic particles tagging biological tests [4–8]. Also Giant magnetoresistance (GMR) sensors have been used in different configurations for successful biosensing through the effect of the stray fields arising from the magnetic particles [9–18]. Quantitative protein detection assays with resolution to attomolar concentrations have been reported using arrays of GMR sensors [19,20].

In the particular case of lateral-flow assays, previous work [8,21,22] has focused on inductive detection of the magnetic labels, using an a.c. magnetic field. Such studies are promising but there is a lack of specific results on real biological tests. Even though several studies on the potential of MNPs for application in biosensing have been reported, only a small number of articles have provided results in real biological tests [17].

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The sensitivity of a biosensing experiment depends on the sensitivity of the sensor used to detect the biorecognition event, as well as on the sensitivity of the biochemical test itself. Recently, the importance of using a suitable nanoparticle functionalization strategy to immobilize the biological recognition agent on the surface of the magnetic labels has been demonstrated [23]. In particular, it has been shown that the sensitivity of a lateral-flow immunoassay for the detection of human chorionic gonadotropin (hCG) increases in one order of magnitude when the polysaccharide moieties of the anti-hCG antibody used for the immunorecognition have been used to immobilize the antibody on the MNP. This method improves the biological recognition with respect to the situation in which the amino groups of the antibody are used to link it to the magnetic label. Also a proper functionalization of the nanoparticle surface in order to make it fully inert for preventing unspecific adsorptions is crucial to improve the test sensitivity [23].

In the present article, we describe our sensor prototype, the measurements procedure, as well as preliminary calibration results, to determine the device sensitivity. We show as well the successful quantitative detection of MNPs in real biological lateral-flow tests performed following the protocols reported in [23], carried out with our prototype.

2. Sensor prototype and measurements method

The prototype can be seen in Fig. 1 [24]. Briefly, once the standard immuno-chromatographic test is performed, the nitrocellulose strip (with the MNPs immobilised on the test line, in the case the biological recognition event has taken place) is glued in a reverse way on the surface of a plastic wheel. The strip is positioned in the proximity of the magnetoresistive sensor by a mechanical system that allows the measurements of the sensor resistance (1) in soft contact between strip and sensor, and (2) with strip and sensor separated by 2 mm distance along the z direction (see Fig. 1). The measurements in contact and proximity in the z direction are done moving the tests line longitudinally along the sensor plane (x direction), in 0.1 mm steps (using a step motor). Thus, when the test line and the active part of the sensor coincide in the x position, a measurable output voltage/magnetoresistance is produced. At each x position, the difference between the sensor resistance in soft contact and 2 mm away along the z direction is explained by the presence of stray magnetic fields from the particles. Obviously, this signal is different from zero only when the x positions of the test line and the active part of the sensor are in proximity, which occurs in an interval of ≈ 1.5 mm corresponding to the test line width. Our differential measurement cancels out any influence of homogeneous external magnetic fields such as the earth magnetic field. The measurement takes a total time of 5 min which is very competitive with similar techniques.

The magnetoresistive sensors in our device are spin-valve devices ($200 \times 3\ \mu m^2$). Two spin-valve structures (type I and type II) were used, showing a maximum magnetoresistance of $\approx 7\%$ and 9%, respectively, and linear response around zero magnetic field. At 350 Oe vertical field, a maximum sensitivity of 0.17%/Oe was calculated for both types of sensors. This sensitivity is comparable to that of the sensors used in [25]. The type-I spin-valve stack consists of (6.5 nm)NiFeCr/(4 nm)NiFe/(2 nm)$Co_{80}Fe_{20}$ as free electrode, (2.2 nm)Cu as non-magnetic spacing, and (3.5 nm)$Co_{80}Fe_{20}$/(30 nm)MnNi/(5 nm)NiFeCr as pinned top electrode. The type-II spin-valve stack is Si/(50 nm)Al_2O_3/(6.5 nm) NiFeCr/(4 nm)NiFe/(2 nm)$Co_{80}Fe_{20}$/(2.2 nm)Cu/(35 nm)$Co_{80}Fe_{20}$/(30 nm)MnNi/(5 nm)NiFeCr. Both, pinned and free layers have crossed anisotropies (free layer easy axis aligned with sensor length) to promote hysteresis-free response while detecting in-plane stray fields. When comparing to other sensors used for similar purposes [26], this is a fundamental advantage because it provides a unique sensitivity direction (that perpendicular to the free layer easy axis), and a narrow and steep linear range resulting from the shape anisotropy of the free layer. Furthermore, the small width of our sensing element (3 μm) provides unprecedented spatial resolution in lateral flow assays using magnetic labels.

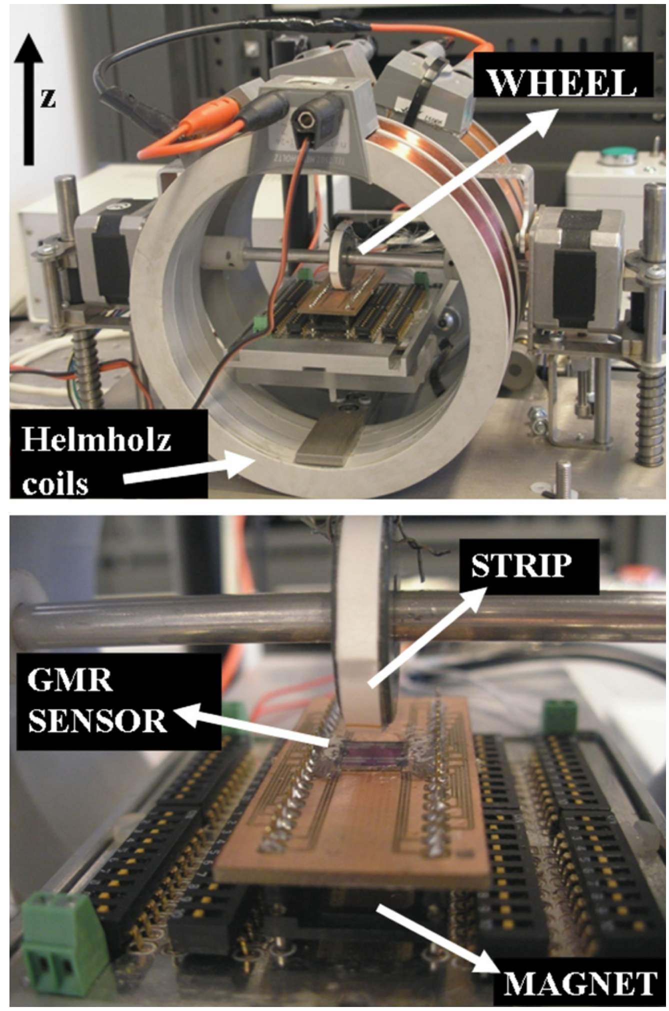

Fig. 1. (upper panel) General view of the prototype; (lower panel) detail showing the wheel and chip carrier with the GMR sensor (9×9 mm^2). Helmholtz coils' outer diameter is 150 mm.

These stacks were deposited by ion beam deposition [27] in a Nordiko 3600 system. In order to produce a sufficient magnetization in the MNPs, a perpendicular field was applied by means of a permanent magnet located just below the spin-valve sensor. The value of the perpendicular magnetic field acting on the particles was set between ≈ 300 and 500 Oe by modifying the magnet position along the vertical, in order to optimise the magnetic field arising from the particles' fringe and the signal. A d.c. current no larger than 30 μA was applied to the spin valves for resistance measurements, to avoid the sensor heating. The detection set-up is inside a pair of Helmholtz coils (see Fig. 1) providing a magnetic field along x which polarizes the spin valve at the operation point (i.e., at the maximum slope of the magnetoresistance curve, attained usually within a few tens of Oe around zero).