High frequency lateral flow affinity assay using superparamagnetic nanoparticles

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ABSTRACT

Lateral flow assay is one of the simplest and most extended techniques in medical diagnosis for point-of-care testing. Although it has been traditionally a positive/negative test, some work has been lately done to add quantitative abilities to lateral flow assay. One of the most successful strategies involves magnetic beads and magnetic sensors. Recently, a new technique of superparamagnetic nanoparticle detection has been reported, based on the increase of the impedance induced by the nanoparticles on a RF-current carrying copper conductor. This method requires no external magnetic field, which reduces the system complexity. In this work, nitrocellulose membranes have been installed on the sensor, and impedance measurements have been carried out during the sample diffusion by capillarity along the membrane. The impedance of the sensor changes because of the presence of magnetic nanoparticles. The results prove the potentiality of the method for point-of-care testing of biochemical substances and nanoparticle capillarity flow studies.

1. Introduction

Lateral Flow Assays (LFA) are simple devices used to detect the presence of an analyte. LFA are widely spread especially for the home pregnancy tests, but also used for several other qualitative ones. The amount of the analyte can be estimated by measuring the color intensity of the strip line. Magnetic Lateral Flow Assays (M-LFA) are intended to improve the sensitivity and reproducibility in the estimation of the analyte. M-LFA require a magnetic sensor and using magnetic labels instead of coloured particles. Most used magnetic labels are superparamagnetic nanoparticles (SPMN), that is, sufficiently small nanoparticles whose magnetization randomly flip direction under the influence of temperature.

Different magnetic recording techniques have been proposed for M-LFA, like resonant coil magnetometers [1], giant magnetoresistance based sensors [2,3], or planar coils [4]. Several works have been done in the detection of several biological entities, like allergens in food samples [5], tumor markers [6], human hormones [7], bacteria [8], parasites [9] or heart damage markers [10]. In the absence of an external magnetic field, the magnetization of SPMN appears to be zero in average, therefore all these methods require the application of an external magnetic field in order to magnetize the SPMN for the sensors to detect theirs to the applied field.

In this paper, the feasibility of a recently discovered superparamagnetic particles detection method [11] is analyzed for its use as a M-LFA. This method detects the SPMN due to the eddy-current induced by the vibrating particles in a conductor surface, through which a high frequency current is circulating. The main advantage of this Radio Frequency Lateral Flow Assays (RF-LFA) method over the previous M-LFA is that an external magnetic field is unnecessary. The RF-LFA sensor is analyzed for continuous flow measurements during the LFA using membranes with one capture line.

2. Materials and methods

2.1. Magnetic particles

Two different kinds of SPMN have been used. One kind is magnetite coated with poly(acrylic acid), dispersed in water (Nanogap SL FeO-2204W) at a nominal concentration of 2% w/w (20 μg/μL), and with a mean diameter of 10.0 nm ± 2.5 nm (TEM) and hydrodynamic diameter around 30 nm ± 10 nm. This product exhibits superparamag-

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netic behavior at room temperature, with a saturation magnetization of 50 emu/g. The other kind is fluidMAG-Streptavidin (Chemicell), a starch matrix containing magnetite nanoparticles, with hydrodynamic diameter around 100 nm, weight of volume 10 μg/μL, 40 emu/g of saturation magnetization, whose magnetic nanoparticles have 12.3 nm ± 20% mean diameter and its superparamagnetic state has been confirmed elsewhere [12]. Pattern samples with different concentration of both SPMN were prepared depositing and drying onto a 10 mm×2 mm blotting paper, 20 mm thick.

2.2. Nitrocellulose membranes

Nitrocellulose membranes (UniSart CN95) were purchased from Sartorius (Spain). Other materials used were: glass fiber sample pads (GFCP001000, Millipore, Germany) and absorbent pads (Whatman, USA). Biotin-conjugated bovine serum albumin (BSA) to form the detection line was purchased from Sigma-Aldrich (Spain). An IsoFlow reagent dispensing system (Imagene Technology, USA) was used to dispense the detection line. The sample buffer consisted of 9.55 mM PBS pH 7.4 (Amresco K812) with 2% Tween 20. The biotin-conjugated BSA at a concentration of 2 mg/mL in mQ water was spotted onto the Nitrocellulose Membrane (NM) to form the detection line using the IsoFlow reagent dispensing system, being the width of the capture line around 0.6 mm. The detection pad was dried for 1 h at 37 ℃, and the nitrocellulose membrane was cut into 25 mm long and 14 mm wide.

2.3. Impedance measurements

Impedance measurements were performed by means of a Precision Impedance Analyzer Agilent 4294A, using the 16048G test leads and customized printed circuit board. Impedance was continuously measured in single frequency mode as a function of time. Data acquisition and instrument control were done by a computer using National Instruments LabVIEW.

During measurements, frequency and exciting voltage were fixed at 110 MHz and 500 mV respectively, as it was previously proved that the maximum sensitivity is obtained at such values [11]. In order to decrease the noise level, bandwidth was set at 5 (measurement accuracy maximized) and the point averaging factor to 16.

The sensing printed circuits have been manufactured on a copper-clad laminate FR-4 by machining using LPKF ProtoMat E33 circuit board plotter. In this paper, the sensing element is a meander-line structure with four parallel 12 mm long, 0.25 mm wide and 35 μm thick, conducting lines separated 0.25 mm (see Fig. 1). The total width of the sensing area is 2 mm, the same than the width of the dried samples and twice the width of the capture line of the lateral flow assay.

When measuring the pattern samples of SPMN a rectangular glass slot of 10.0 mm×0.3 mm is installed over the sensing area and the samples are horizontally inserted in the slot until the SPMN are located just over the sensing element [13].

We define *Signal* as the variation of the magnitude (|Z|) and the phase (φ) of the impedance under the presence of nanoparticles.

$$\Delta|Z| = |Z|_{NP} - |Z|_0 \qquad \Delta\phi = \phi_{NP} - \phi_0$$

We define *Resolution* as the minimum mass of nanoparticles detectable by our system, expressed by the following equation:

$$Resolution = \frac{m\sigma_{noise}}{\Delta A},$$

where ΔA is the signal produced by the nanoparticles when measuring variable A, which can be either |Z| or φ ; σ_{noise} is the standard deviation of the noise of such variable and m is the mass of nanoparticles in the sample.

2.4. Continuous flow measurement

When measuring during continuous flow, the NM is glued over the sensor, carefully positioning the yet invisible capture line over the sensing meander line. The plastic layer incorporated to the NM prevents the fluids to directly get in touch with the sensor. Finally, sample pad and absorbent pad are installed with an overlap between them and the NM of around 2 mm.

Ferrofluid FeO-2204W were diluted 1:2 in Phosphate Buffer Saline (PBS) in order to have both SPMN fluids at the same concentration (10 μg/μL). Samples were carefully deposited drop by drop. As the micropipettes have metallic components and can interfere with the measurements, the analyzer is temporally stopped for a short time and the measurement restarted using the LabView software.

Every experiment has been started depositing a drop of buffer to wet the membrane over the sensor. Later, SPMN were deposited on the sample pad close to the NM and more buffer was added on the opposite side of the sample pad, resulting in the SPMN being pushed forwards by the buffer. Impedance measurements were done from the first deposition until the uncaptured particles passed through the sensor, as shown in Fig. 2.

3. Results and discussion

Pattern samples were measured and data are shown in Table 1. First conclusion is that the signal of the magnitude of the impedance, $\Delta|Z|$, is better than the signal of the phase of the impedance, $\Delta\phi$. Smaller quantities of SPMN can be detected from the magnitude, therefore, only it will be considered in the following. Secondly, the FeO 2204-W nanoparticles present higher signal than the fluidMAG-Streptavidin.

The impedance of the sensing element depends not only on its geometry and dimensions but also on the dielectric constant of the materials around it [14]. When the air over the sensor is replaced with any other dielectric material ($\epsilon_r > \epsilon_0$), its impedance decreases, as Z is proportional to the inverse of the square root of the relative permittivity in any case.

$$Z \propto \frac{1}{\sqrt{\epsilon_r}}$$

When the NM is wet, the buffer saline displaces the air inside the membrane, so the relative permittivity notably increases producing a fall in the impedance value, as can be observed in Fig. 3. It is worth mentioning that when depositing the first drop, the impedance does not change appreciatively until the buffer wets the NM near the sensor line, and that the impedance does not change any further after the NM fraction over the sensing element is filled with buffer instead of air. As the buffer advances by capillarity inside the NM, it pushes away all the
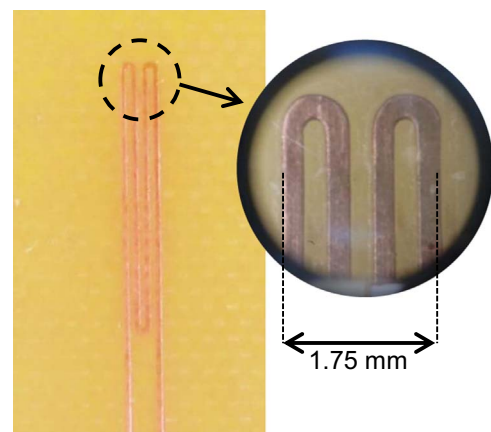

Fig. 1. Photographs of the sensing circuit.