

# FERROMAGNETIC PARTICLES FOR AN IMPROVED HETEROGENEOUS BIOASSAY PERFORMANCE ON A DIGITAL LAB-ON-A-CHIP

Steven Vermeir<sup>1</sup>, Daan Witters<sup>1</sup>, Nicolas Vergauwe<sup>1</sup>, Karel Knez<sup>1</sup>, Martin Gijs<sup>2</sup>, Robert Puers<sup>3</sup>, Jeroen Lammertyn<sup>1</sup>

<sup>1</sup>MeBioS-BIOSYST KU Leuven-University of Leuven, Belgium, <sup>2</sup>Laboratory of Microsystems, Ecole Polytechnique Federale de Lausanne, Switzerland, <sup>3</sup>ESAT-MICAS KU Leuven-University of Leuven, Belgium

## ABSTRACT

In this work, we report on a novel strategy to implement and to optimize heterogeneous bioassays on an electrowetting-on-dielectric (EWOD) digital lab-on-a-chip analysis platform, namely the use of ferromagnetic particles as functional carriers for biomolecules in combination with a highly efficient extraction protocol. This extraction protocol serves as an extension to the common operations of the 'digital droplet toolkit', consisting of droplet dispensing, movement, merging and splitting. This new droplet-based function allows the introduction of a very efficient washing step, extremely important in immunoassays. In addition, the magnetic particles enhance the mixing process inside the droplet when a rotating magnetic field is applied. The strength of both droplet manipulations with respect to heterogeneous assays is proven by the integration of two different bioassays (an IgG-immunoassay and an enzymatic assay for glucose) on the digital lab-on-a-chip platform.

## KEYWORDS

Digital lab-on-a-chip, heterogeneous bioassays, ferromagnetic particles.

## INTRODUCTION

Until now, only one specific protocol, involving magnetic particles on a digital lab-on-a-chip platform, the so called 'particle enrichment protocol' [1,2], has been studied extensively and used in the framework of on-chip bioassays. More specifically, this protocol induces the magnetic collection and separation of particles, followed by a splitting step of the droplet. This results in an almost complete isolation of the magnetic particles in a smaller droplet. Although this approach has been demonstrated for the execution of immunoassays [3] or cell based assays [4], there are some drawbacks, related to this approach. The most important one is the presence of a significant amount of liquid surrounding the particles after droplet splitting. Hence, in case several unwanted components need to be removed from the particles, a large number of serial dilution steps need to be executed with this protocol, resulting in a decreased assay performance. A strategy to overcome this problem has been suggested by Fouillet et al. [5] who describe the extraction of a pellet of magnetic particles on an open EWOD configuration by removing all the supernatant.

In this work, we specifically aim to implement and to optimize this protocol on a closed EWOD configuration, allowing the execution of heterogeneous bioassays on the digital lab-on-a-chip analysis platform. In addition, the presence of the magnetic particles in combination with an external magnetic stirrer, permits the introduction of an additional mixing process inside the droplet. Both processes enhance the bioassay performance significantly, as shown by an immunoassay against IgG and an enzymatic glucose assay.

## EXPERIMENTAL

A detailed description of chip fabrication and interface set-up can be found in Witters et al. [6]. In this work, two different types of ferromagnetic particles were used. For establishing the operating diagram and the IgG-immunoassay, particles were taken from the MagNA Pure LC DNA Isolation kit I (Roche Diagnostics, Basel, Switzerland) whereas for the enzymatic assay, carboxyl-functionalized magnetic particles (1  $\mu\text{m}$ ) were purchased at Chemicell (Berlin, Germany). To biofunctionalize the first type of particles, a biotin-streptavidin immobilization protocol was followed, consisting of three steps. First, a layer of PLL-g-PEG-biotin solution is adsorbed to the particles, followed by a streptavidin binding step. Finally, biotin-labelled anti-rabbit IgG antibody was added to the particles. The binding of the enzymes (Hexokinase and D-glucose-6-phosphate dehydrogenase) on the carboxyl-functionalized particles was achieved by an one step protocol by means of the zero length linker 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Individual droplets of approximately 170 nL were used, surrounded by a thin shell of silicon oil.

## RESULTS AND DISCUSSIONS

Figure 1 represents the different steps, involved in the particle extraction process. As shown by these images, particles are extracted at the boundary of the permanent magnet. The last images (line C) indicate also the complete removal of the supernatant from the extracted particles. This extraction protocol was studied more in detail by establishing an operating diagram as a function of the applied voltage and the concentration of the magnetic particles. Three different outcomes were possible ('blocked transport', 'droplet transport', and 'particle concentration') but by choosing the optimal parameter values, the supernatant was completely removed from these particles without particle loss (99% extraction efficiency). When particle concentration was below a critical value ( $< 4 \text{ mg mL}^{-1}$ ), droplet transport is possible, irrespective of the applied voltage (starting from 70 V). This indicates that in this

situation the magnetic force is subsidiary to the electrowetting force. At higher particle concentrations, two different outcomes are possible. When the applied voltages are equal or above a critical value (80 V), this particle extraction process occurs. Below this value, no droplet transport occurs because of the decreased electrowetting force and the increased magnetic force.

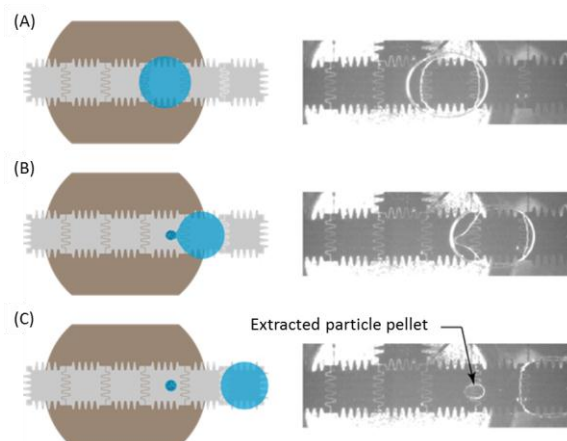


Figure 1. Overview of the particle extraction protocol. By transporting first the droplet once back and forth across a track of electrodes, above the magnet, the concentration of particles on one side increases. Without interruption of the droplet movement, the droplet is transported away from the magnet, resulting in the extraction of the particle pellet.

The efficiency of this process was demonstrated by the execution of an IgG-immunoassay. Hereto, different concentrations of rabbit IgG antigen (Ag) were loaded on the reservoirs of a chip, as shown in Figure 2. These Ags were labeled off-chip with Alexa Fluor 647 donkey anti-rabbit IgG as detection antibody (dAb). After Ag incubation with the capture antibody and binding, the supernatant containing the unbound species was removed, followed by three additional washing steps with phosphate buffered saline (PBS), residing in the washing buffer reservoir (WB). The fluorescence of the dAb was determined by inverted fluorescence microscopy, after particle isolation. A clear linear relationship was established for the different Ag concentrations (Figure 3(B), full line) and because of the very effective extraction protocol, the number of washing steps was reduced significantly, compared to the particle enrichment protocol [2], resulting in a very low averaged CV-value (<3%). A second benefit of this extraction protocol formed the reduced amount of washing buffer that was needed to complete the assay. Here, only three washing buffer droplets of 170 nL each were necessary, avoiding multiple loading steps onto the chip as in the case of the particle enrichment protocol.

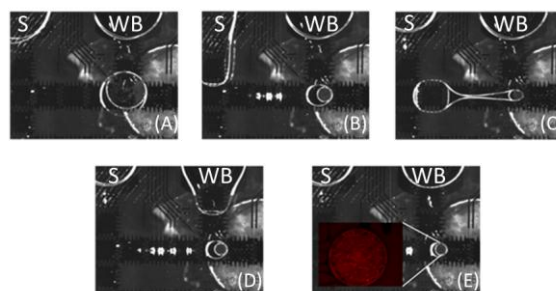


Figure 2. Overview of the on-chip immunoassay. In a first phase, a droplet from the sample (S) reservoir is directed to the pellet with the biofunctionalized particles (B-C), followed by three additional washing steps (D-E).

A second advantage of the integration of ferromagnetic particles is the enhanced mixing efficiency when chips were placed on top of a magnetic stirrer, driven by an electromotor. In the presence of a magnetic field, particles formed self-assembled chains along the field lines. By applying a magnetic torque, these chains rotated, increasing the mixing efficiency drastically inside the droplet (Figure 3 (A)). This was proven by repeating the previous immunoassay with an active mixing procedure. Compared to the passive mixing approach, higher sensitivity was obtained with as result an improvement of 90% of the limit of detection (Figure 3 (B)):  $12 \text{ ng mL}^{-1}$  vs.  $6.4 \text{ ng mL}^{-1}$ .

To investigate more in detail the effect of the magnetic particle-induced mixing process, a homogeneous enzymatic assay for glucose was compared with a heterogeneous assay, involving enzyme-conjugated magnetic particles. In the latter assay, enzymatic conversion was executed with the aid of the active mixing process. After 5 minutes of reaction, the supernatant was guided towards a detection spot on the chip. In addition to an increased assay sensitivity, also a drastic decrease in assay variability (6.1% to 3.2%) was observed in case of an active mixing process. The improved assay performance parameters could not be attributed to the amount of enzymes present in the assay, since the used concentrations for enzyme immobilization were lower than the concentrations used in the homogeneous enzymatic assay. In addition, the biofunctionalized particles could be reused several times. Finally, the

heterogeneous approach allowed the automated quantification of D-glucose in blood serum.

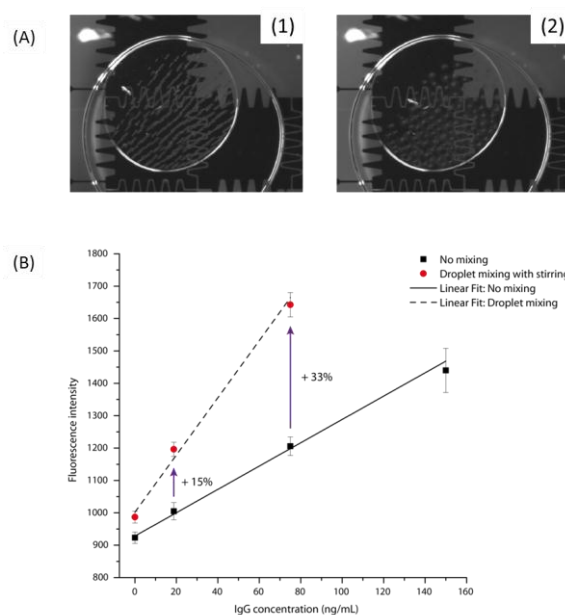


Figure 3. (A) Effect of particle stirring inside a droplet. In the presence of a magnetic field, particles start to form chains (1) and when the magnetic field rotates, chains break and start to rotate simultaneously with the rotating field (2). (B) Effect of an active mixing process on the calibration curve of the IgG-immunoassay.

## CONCLUSIONS

The aforementioned results indicate that the use of biofunctionalized ferromagnetic particles greatly enhance the possibilities of digital lab-on-a-chip technology with respect to the execution of heterogeneous bioassays. The impact of this approach on the assay performance factors is enormous, which could be important for some application areas, e.g. diagnostics, environmental monitoring, etc..

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## CONTACT

Jeroen Lammertyn 0032-16-321459 or [jeroen.lammertyn@biw.kuleuven.be](mailto:jeroen.lammertyn@biw.kuleuven.be)