MAGNETIC FLUIDIZED BED IN MICROFLUIDICS: HYDRODYNAMIC CHARACTERIZATION AND VALIDATION TO IMMUNOCAPTURE

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ABSTRACT

Fluidized beds, in which a solid/fluid mixture behaves as a fluid, are widely used in chemical engineering due to the enhanced solid/liquid contact. Its integration in a microfluidic chip is achieved here by the implementation of a magnetically controlled setup. Beads distribution and recirculation flows were studied by particle image velocimetry (PIV). This system potentially opens the way to a wide range of applications in bioanalytical chemistry and biology. It was applied here to the immunocapture of a model protein biomarker. The specificity of this immunoextraction was demonstrated and preconcentration factors above 400 and sensitivities of 150 fM were achieved.

KEYWORDS: Fluidized bed, magnetic beads, immunocapture, particle image velocimetry, biomarkers, preconcentration

INTRODUCTION

A fluidized bed is formed when particles are suspended in a steady-state dynamic regime under a balance of viscous drag and another counterbalancing force (usually gravity). This configuration provides an efficient stirring and enhances the heat and mass transfer between the solid and liquid phases as compared to a packed bed. Fluidized beds have been widely used in chemical engineering for diverse applications ranging from heterogeneous catalysis to recombinant protein purification or combustion. Hence, fluidized beds are an appealing strategy to enhance the kinetics of liquid-solid processes in microfluidics, but until recently their implementation was hindered by the weakness of gravitational forces at the microscale.

We recently introduced the concept of magnetically-driven fluidized beds to solve this problem [1]. In this approach superparamagnetic beads are confined in a microfluidic device by the use of a permanent magnet. The balance between the magnetic and drag forces applied on them, was optimized in order to compensate for the decaying intensity of the magnetic field (Fig. 1).The design of the microfluidic device was also customized to ensure the bed stability while providing a uniform and constant bead recirculation. The performances of this integrated microreactor were investigated for the capture and preconcentration of biomarkers.

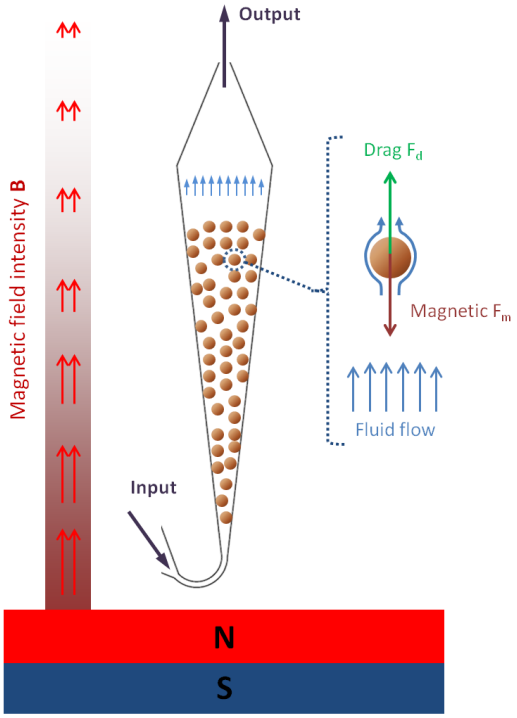

Figure 1: Working principle of the microfluidic fluidized bed: beads are in equilibrium inside the chamber due to the balance between drag and magnetic forces.

EXPERIMENTAL

The design of the microfluidic device is shown in Fig1. With the purpose of compensating for the decaying intensity of the magnetic field when moving away from the magnet, a V-shaped chamber with decreasing flow velocity has been designed to ensure the bed stability over a large flow rate range. The chamber dimensions are 10 mm in length and a width that linearly increases from 100 µm at its entrance to a maximum of 1.8 mm. This chamber was integrated in a PDMS chip with three inlets (not shown) and a 50µm-width channel connecting the chamber to the output channel serving as an internal resistance. External valves were used for the control of the reagent and sample injection. Flow rates and pressure were simultaneously recorded. The system can be operated in constant flow rate or constant pressure mode using an MFCS/Fluiwell (Fluigent). Depending on the pressure applied, two regimes can be obtained: a packed bed at low pressure and a fluidized bed for higher pressure. The resulting system can be seen in Fig. 2 when the bed is in the closed and fluidized state (Fig. 2a). In the fluidized regime, high flow rates of up to 3 µL/min can be imposed without noticing the loss of magnetic beads. They allow the analysis of large volumes of samples for a microfluidic device.

RESULTS AND DISCUSSION

The dynamic behaviour of the fluidized bed has been analyzed through particle image velocimetry (PIV) as shown in Fig. 2 (b and c). In these experiments, 1% of the total number of magnetic particles were Cy5 labelled to allow their individual tracking. A high velocity at the centre of the bed is observed, followed by a perpendicular circulation to the borders of the chamber when arriving to the end of the bead ensemble. A backflow near the borders takes place, as a result of the minimum flow velocity in that region. Interestingly, a fraction of the beads are seen to recirculate backwards before reaching the end of the bed, further improving the stirring of the particles. No stagnation point is observed. These PIV results clearly demonstrate the possibility to achieve an efficient beads recirculation. The hydrodynamic characterization of the device confirmed a behaviour very similar to those of macroscopic fluidized beds.

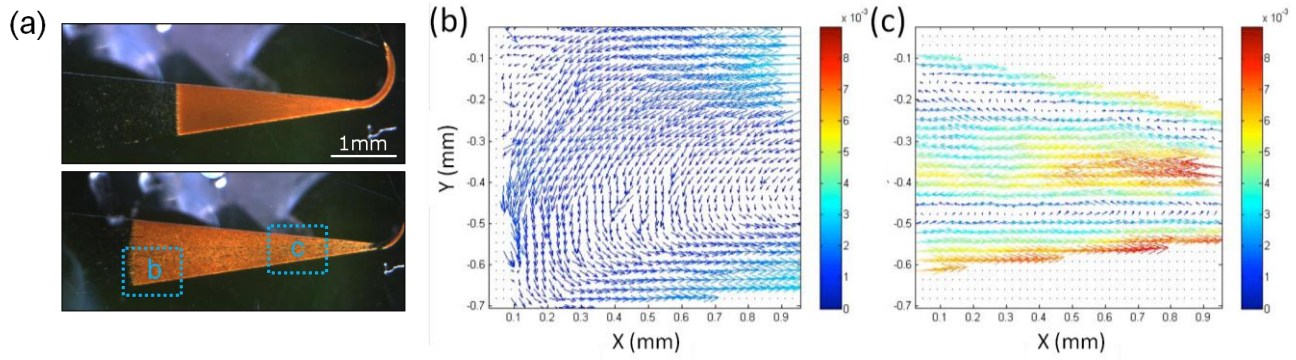

Figure 2. (a) Image of particle bed in the packed (no flow) and fluidized regimes (here 1µL/min). PIV profiles for both the end (b) and the entrance (c) of the fluidized bed (side bar units: mm/s).

Both the porosity and the internal bead distribution of the fluidized bed were also investigated. As shown in Fig. 3 the magnetic beads spontaneously self-organize into linear columns in the presence of a magnetic field. The porosity of the bed is thus the result of linear paths parallel to the field (Fig. 3). Whereas the porosity of the bed is constant in the packed regime (40%), the porosity of fluidized beds is a quasi-linear function of the fluid flow-rate that increases up to 80% when reaching the maximum operating flow rate. It can thus be easily tuned as a function of the flow rate.

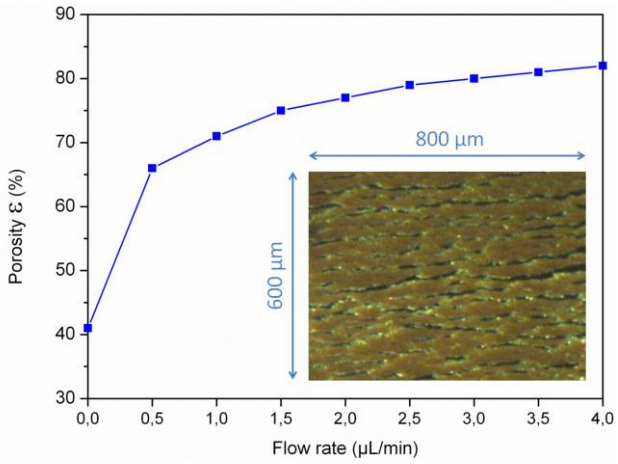

Figure 3. Bed porosity as a function of the applied flow rate. The inset shows the internal organization of the bed and the resulting porosity in the center of the bed for a flow rate of 1.0 µL/min.

To validate the potential of this microfluidic fluidized bed, we investigated the possibility to use this system to immunoextract and preconcentrate a biomarker from a large sample volume. Experiments were carried out using antibody (IgG) coated beads and a model biomarker (Alexa Fluor 488 rabbit IgG) was used to validate the bioanalytical procedure. This consists of a four steps protocol (Fig. 4): (1) biomarker capture by flowing the sample through the bed at 1 µL/min, (2) buffer washing to remove interfering molecules at 1.5µL/min, (3) Sequential elution called stop and go mode (3a) incubation of the fluidized bed in a closed state in a citric acid solution (elution buffer) to break the Ag/Ab (no flow rate), and (3b) final elution and fluorescent detection of the analyte at a low flow rate (0.2 µL/min). A sharp gaussian fluorescence-intensity peak is obtained, with a height correlated to the final biomarker concentration. A second peak from a subsequent incubation and elution step serves to verify the efficiency of the first elution (a low second peak indicating a high efficiency for the first one).

To study the reproducibility of the system and the linearity of the correlation between the peak intensity and the biomarker initial concentration, a calibration curve was obtained from 20µL biomarker samples of different initial concentrations. In the studied concentration range no saturation was observed and the data could be correlated with a linear fit (ρ=0.98). For this sample volume a limit of detection of 0.2 ng/mL (1.5 pM) has been achieved. It is important to notice that the high flow rate that can be imposed in this fluidized bed opens the way to large sample analyses that can be especially relevant for the analysis of low level biomarkers. Processing a larger sample volume of 200 µL, preconcentration factors of 400 have been achieved, making possible the detection of the biomarker at concentrations as low as 150 fM. This is a x30 improvement as compared to the data presented in 2012. We have demonstrated the possibility to perform such immunocapture in microfluidic fluidized bed both in PBS buffer and in real serum.

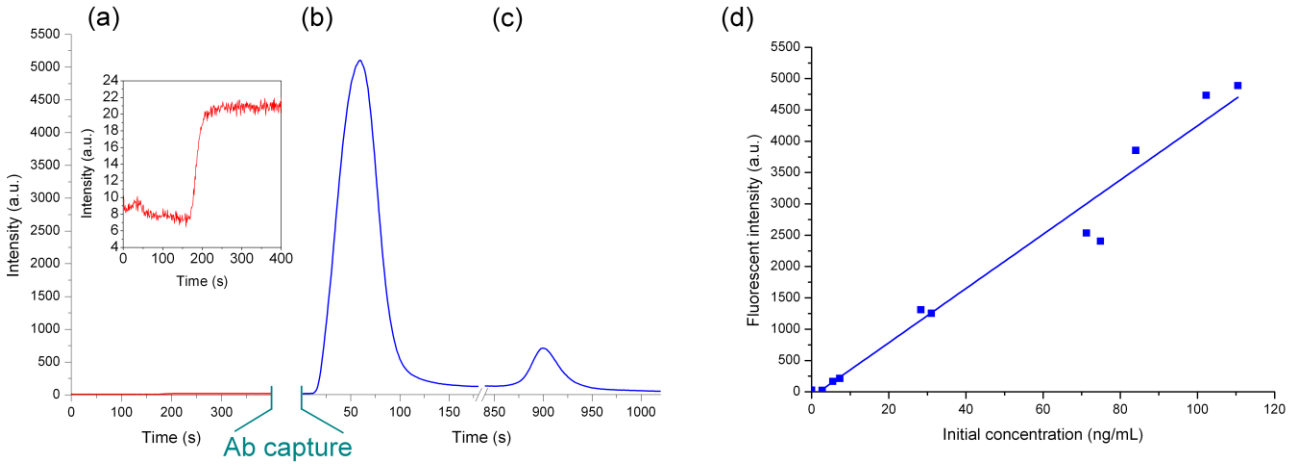

Figure 4. Evolution of a fluorescent antibody concentration during a typical stop flow experiment: (a) sample front before concentration, (b) concentrated peak after antibody capture and subsequent elution and (c) second elution to verify the efficiency of the first one (In this example the concentration factor is 350x). The right figure shows the calibration curve obtained for the detection rate of 20 µL samples (d).

CONCLUSION

This new microfluidic dynamic microcolumn combines a high density of magnetic particles, for high capacity, an active hydrodynamic stirring, for increased kinetics, and a high flow rate with low backpressure. Miniaturized fluidized beds appear to be efficient for immunocapture and pre-concentration of analytes from initial sample volumes in the mL range into µL or even nL scale extracts. Other applications, e.g. as microreactors, are also at hand. As compared to packed beds, these fluidized beds require low operation pressure, they are not prone to fouling (since large non affine particles are dragged across by the flow), and they can be regenerated easily by a simple flush. They will thus find numerous applications, either in direct detection mode, or as a sample preconditioning module upfront of further microfluidic components.

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