

Electronic Supplementary Information (ESI)

Micro-flow-injection (μ FIA) immunoanalysis of herbicide residue 2,6- dichlorobenzamide - toward automated at-line monitoring using modular microfluidics

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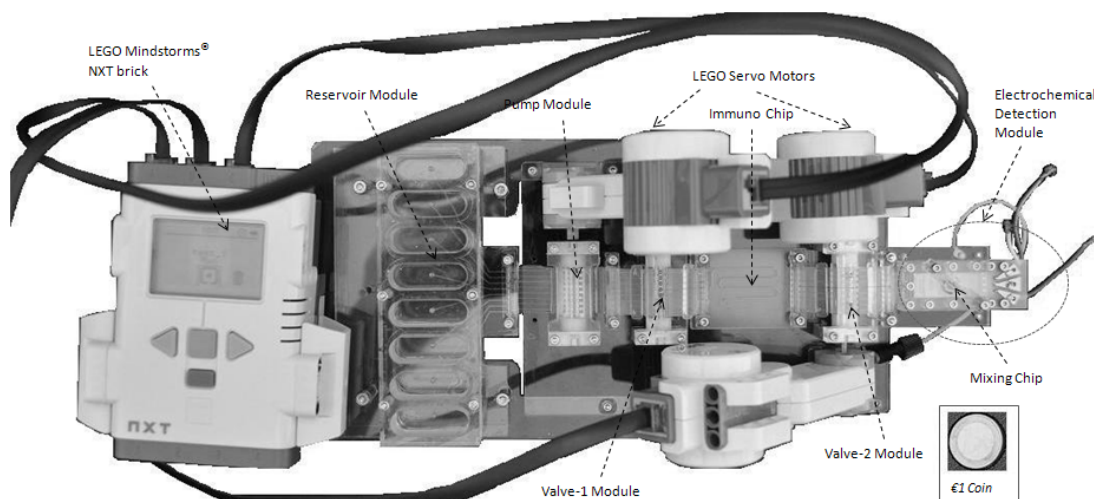


Figure S-1: A completely integrated amperometric BAM immunosensor prototype

S-1. Automated amperometric BAM immunosensor

S-2. Design and fabrication of various modules of amperometric BAM immunosensor

S-2-1. Immunochip

The most important module of the BAM immunosensor, the immunochip consists of two simple microchannels that were micromilled on a 3 mm thick PMMA substrate. This channel plate was bonded to a 1.5 mm thick lid made of PMMA with inlet and outlet holes in a pattern that complements the channel plate. Figure S-2(a) shows a bonded immunochip. The curved channel in which the immunosurface is created by immobilizing BAM hapten (immunoreaction channel - IRC) has the dimensions of 150 mm x 0.5 mm x 0.4 mm (L x W x D) with a volume capacity of 30 μL . The immunoreaction channel (IRC) is connected to the seven of the eight inlets allowing the sequential loading of different reagents (controlled by a miniaturized valve module) required for the BAM immunoassay. The other channel with the same width and depth is connected to the remaining inlet to have an independent flow of baseline buffer to the detection module. Two of the three outlets are interconnected and used to dispose the flow from the IRC either to the waste or to the detector, whereas the third outlet carries the baseline buffer flow to the detector directly by using another miniaturized valve module.

S-2-2. Mixing chip (MC)

The mixing chip (MC) (a part of the electrochemical detection module), fabricated in the same way as the immunochip, has a different channel shape as shown in Figure S-2(b). It has three active inlets and receives the flow from the immunoreaction chip. The baseline buffer flow enters into one

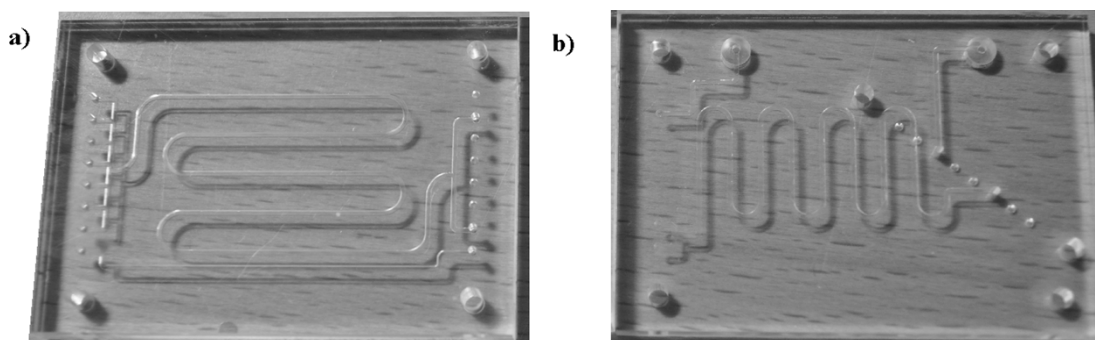


Figure S-2: Bonded a) immunochip and b) mixing chip.

of the inlets and continues to the detector. The other two inlets of MC receive the flow from IRC and direct it, either to the waste channel or to the detector.

S-2-3. Electrode chamber (EC)

The main part of the detection module is the electrode chamber (EC), which is fabricated of PMMA as two pieces as shown in Figure S-3. The lower half, made of five mm thick PMMA substrate, has a 1.5 mm deep rectangular ‘pocket’ to place the screen printed ceramic electrode (L: 34mm; W: 10 mm; T: 0.5 mm), which has a circular carbon working electrode (4 mm in diameter), carbon counter electrode and Ag/AgCl reference electrode. The upper part of the electrode unit has a 0.7 mm high counter-projection with a 0.8 mm deep circular chamber with 8.5 mm radius. When pressing the two parts together, a space is provided for the 0.5 mm thick electrode chip. While screwing the two halves together, a 0.5 mm thick PDMS gasket with a hole of diameter 8.5 mm (aligned over the circular chamber) is placed on top of the electrode in the lower part to ensure the assembly is perfectly leak-proof. The upper surface of the upper half has the provision to connect the MC with the assembled EC using a specially designed double-sided interconnection block with eight channels. Two diametrically opposite channels are activated as inlet and outlet for the circular chamber to minimize the chances of air bubbles. Three holes (2 mm in diameter) on the upper half provide the provision for three spring-loaded contacts (soldered onto a Printed Circuit Board (PCB)) to be aligned and connected with the electrode contact pads when the parts are screwed together.

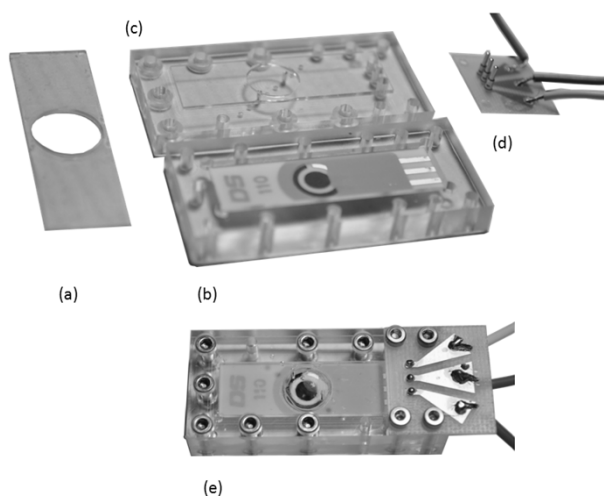


Figure S-3: The components of the electrochemical detection chamber: (a) a PDMS gasket used to tighten the two halves together, (b) a lower half with a screen printed carbon electrode in a 1.5 mm deep pocket, (c) an upper half with a 0.7 mm deep counter protrusion and a 0.8 mm deep circular well, (d) a printed circuit board with soldered spring loaded contact pins, and (e) an assembled electrochemical detection chamber.

S-2-4. Reservoir modules

The reservoir module also consists of two parts. The upper part is made of 10 mm thick PC and has eight elliptical holes (each having 3 mL volume capacity when closed). The lower part is essentially a microfluidic chip made of a channel plate bonded with a lid (both are made of 2 mm thick PMMA). The channel plate has eight individual micromilled channels of width 0.5 mm and depth 0.4 mm. The upper reservoir part is screwed onto the chip. In order to make the reservoir leak-proof, a PDMS gasket is inserted between the two parts. The upper surface of the chip lid has elliptical grooves to accommodate the PDMS gasket. These grooves and the gasket are of the same shape as the elliptical holes of the upper reservoir part. The eight channels end at a specific 8-hole interconnection junction. Figure S-4 shows an assembled reservoir module and its parts.

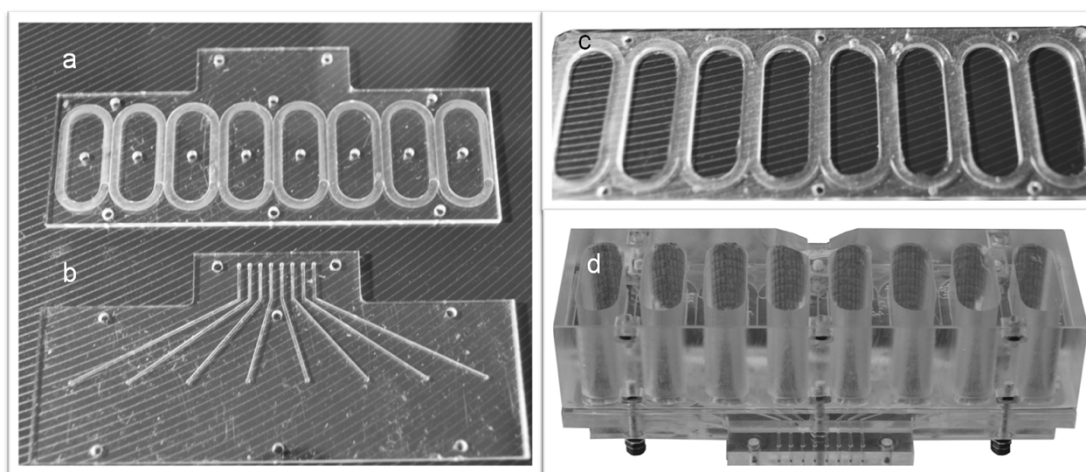


Figure S-4: Components of a reservoir module. a) A channel plate of the lower part, b) a lid of the channel plate with grooves to hold a PDMS gasket and the upper part, c) a PDMS gasket, and d) an assembled reservoir module

S-2-5. Miniaturized pump and valves

The microfluidic device described in this paper uses an 8-channel version of the miniaturized peristaltic pump reported by Skafte-Pedersen et al.¹ for the effective pumping of various reagents used in the BAM immunoassay. It comprises of three main components: i) a multi-roller, ii) a pumping inlay made of PDMS with embedded eight channels, and iii) a rigid rotor bed made of polycarbonate. The fabrication details of these components are given in the supplementary material of¹.

The sequential addition of different reagents into the IRC and the subsequent flow of these liquids into the MC are well controlled and guided by two miniaturized valve modules which was originally designed and published by Conde *et al.* ². The valve modules also consist of three components: i) a valve inlay made of PDMS, ii) two blocks of rigid supports, and iii) a brass shaft stacked with eight asymmetric discs which are aligned and screwed together. These valves can control the flow in 8 channels independently.

Figure S-1 shows the completely assembled electrochemical BAM immunosensor prototype. This was achieved by fixing the various modules described above onto a base plate using 2-mm screws and nuts. The modules are interconnected by using specially designed features called ball joint interconnection blocks (BJIBs) reported by Sabourin *et al.* ³ and complementary planar structures with eight holes of diameter of 0.8 mm (separated by a distance of 2.25 mm from centre to centre) which is flanked by two 2-mm holes (with a distance 25 mm from centre to centre).

S-2-6. Pump characterization

Leak pressure testing of the ball joint interconnection blocks, flow pattern of the miniaturized peristaltic pump and validation of the miniaturized valve for mixing and routing are described elsewhere ¹⁻³. After assembling the different modules, except the detection chamber, onto a base plate, the miniaturized peristaltic pump was calibrated by flowing Milli Q[®] water through each channel. The Lego[®] servo motor that drives the pump was run for five minutes at four different speeds expressed in % power (40%, 50%, 60% and 75%). Flow rate of each channel was estimated in $\mu\text{L min}^{-1}$ with three repetitions. Figure S-5 shows the results of pump calibration with mean values of 8 channels. The flow rate of the channels increases linearly with the applied power to the LEGO servomotor. As the error bars (+/- standard error of mean) indicate, disparity in flow rates is slightly higher at higher applied power. Ideally, all the channels are expected to behave in the same manner at a particular level of motor speed. However, the middle channels are found to be more consistent in this regard compared to the channels at the sides of the PDMS inlays. In this project, the speed of the LEGO[®] motor was set to be 50%, at which the channels showed a flow rate of $31.06 (+/-0.24) \mu\text{L min}^{-1}$, which is the time needed to fill the IRC loop. At this rate the pump inlay also showed a better durability.

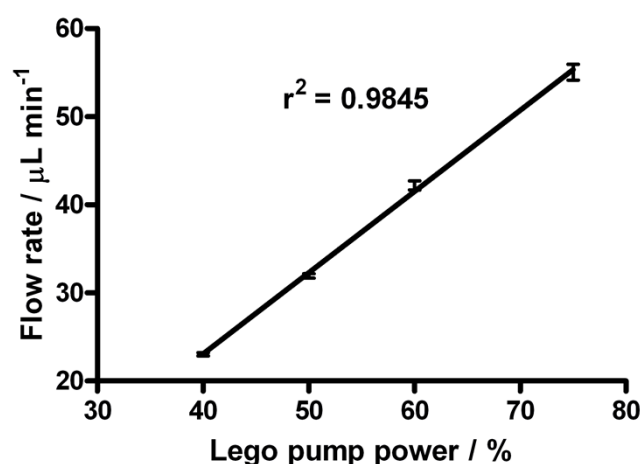


Figure S-5: Pump calibration - Flow rate dependence on the applied power to the LEGO servomotor.

S-3. The cascade of fluidic operations during immunosorbent regeneration and generation of the standard curve

- The IRC was filled with the mixture of HYB 273 working solution and BAM / Milli-Q® water and the flow was stopped for 15 min to allow the immuno reaction to take place.
- The IRC was washed with PBST under continuous flow for 3 min.
- The IRC was filled with the substrate (TMB) and the flow was stopped for 40 min to allow for quantitative completion of the enzymatic reaction.
- After the completed enzymatic reaction, the substrate in the IRC was fed to the MC and D by a stream of PBS and the current signal was recorded.
- The IRC was filled with the regeneration buffer and the flow was stopped for 5 min.
- The IRC was washed with PBST under continuous flow for 3 min.
- In the regeneration experiments, an additional substrate reaction and signal recording was conducted to evaluate the effectiveness of the regeneration step.

The above described cycle was repeated at least three times for each standard solution.

2. A. J. Conde, D. Sabourin, P. Skafte-Pedersen and M. Dufva, *15th International Conference on Miniaturized Systems for Chemistry and Life Sciences, October 2-6, 2011, Seattle, Washington, USA, 2011*, 978-970-9798064-9798064-9798065/ μ TAS 9792011/\$9798020©9798011CBMS-9790001.
3. D. Sabourin, D. Snakenborg and M. Dufva, *Microfluid Nanofluid*, 2010, **9**, 87 - 93.