Integration of a zero dead-volume PDMS rotary switch valve in a miniaturised (bio)electroanalytical system

Neus Godino,^a Francisco Javier del Campo,^a Francesc Xavier Muñoz,^a Mikkel Fougth Hansen,^b Jörg P. Kutter^b and Detlef Snakenborg^{*b}

Received 11th February 2010, Accepted 18th March 2010

First published as an Advance Article on the web 2010

DOI: 10.1039/c003000g

This work features the design, fabrication and characterisation of a miniaturised electroanalytical lab on a chip that allows the performance of a complete bioassay, from the capture of magnetic particles through their functionalisation and sample incubation to the detection of electroactive reaction products. The system is built using mainly polymeric materials such as PMMA and PDMS and fast prototyping techniques such as milling and moulding. The system also includes a set of microelectrodes, photo-lithographed on a silicon chip. The novelty lies in the design of the rotary microvalve, which contains a microreactor so that various reaction and incubation steps can be carried out in isolation from the detection event with zero dead volume. This avoids contamination and fouling of the electrodes by proteins or other organic matter, and extends the useful lifetime of the detector. The system operation is demonstrated by a model example, consisting in the functionalisation of streptavidin-coated magnetic particles with biotinylated β -galactosidase over periods ranging from 5 to 15 min, at which point the particles saturate. Although the system is intended for the development of enzyme-based electrochemical bioassays, the concept of its rotary microreactor can be applied more broadly.

Introduction

Miniaturised analytical systems represent an emerging and exciting field for multidisciplinary research.^{1,2} The development of these systems is a challenge of its own, as it requires the confluence of expertise from the physical sciences,^{3,4} typically biology or chemistry, and mechanical and electronics engineering. But in spite of the difficulties, blistering progress has been achieved over the past couple of years due to the appearance and refinement of new polymeric materials as well as to the adaptation of key fabrication techniques.^{5–7}

This article focuses on the use of two of those materials, namely poly(methyl-methacrylate), PMMA, and polydimethylsiloxane, PDMS, to develop a versatile miniaturised analytical system devised for the performance of electrochemical bioassays. This system builds on the architecture of a previous system, adding a rotary valve to it. The fact that the rotary valve also integrates a reaction chamber where magnetic particles can be captured represents a fabrication challenge particularly from the point of view of sealing moving parts.

Our previous work⁸ presented a versatile electroanalytical microsystem, easy to fabricate, use and re-configure. That system allowed the performance of a broad range of enzyme-based

bioassays. The microsystem permitted the capture of enzyme labelled magnetic beads, and their incubation with a relevant substrate, upstream from an electrochemical detector. After the reaction took place, the flow was restored and a current peak corresponding to the products was easily detected as in any other plug-flow system.

However, the simplicity in its design was both the main strength and the main pitfall of the system. The fact that there was a single channel meant that all the solutions used in the different operations—bead capture, sample and substrate injection, product detection, and washing—had to flow over the electrodes. The consequence was that part of the biological materials passing through the system adsorbed on the electrodes and caused gradual losses of sensitivity and reproducibility.

To preserve the electrodes longer, we want to minimize the contact of solutions containing biological material with them. Our approach consists of the integration of a microvalve in the flow path between the biochemical reaction chamber and the detector. This way we ensure that only the reaction products flow over the electrodes, while any other potentially fouling solutions are diverted through an alternative waste channel.

We have been able to integrate a microvalve into our original system due to its flexible architecture based on separate building blocks.⁸ This allows our system to accommodate new functionalities despite its small dimensions.

We developed an externally-actuated rotary valve, made in the same materials as the rest of the device. An important advantage of this new feature is that it minimises the dead volume between the reaction chamber and the detector, so the configuration also reduces peak broadening due to smearing/diffusion of detectable products inside the detector channel. A similar approach was

^aInstituto de Microelectrónica de Barcelona, IMB-CNM (CSIC), Esfera UAB, Campus Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. E-mail: franciscojavier.delcampo@imb-cnm.csic.es; Fax: +34 93 580 1496; Tel: +34 93 594 7700

^bDepartment of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Building 345 East, DK-2800 Kongens Lyngby, Denmark. E-mail: Detlef.Snakenborg@nanotech.dtu.dk; Fax: +45 45887762; Tel: +45 4525 5785

presented in 2005 by Yin *et al.*,⁹ who integrated a packed column within a small valve in a fully automated chromatographic system for peptide analysis. However, the microsystem we present here is much simpler and, more importantly, the valve is based on sealing achieved by contact with 2 surfaces. This allows the definition of microchannels in the rotary part for direct use on silicon or glass chips. This is in contrast to most systems, based on single sealing surfaces, which require the channels to be defined both in the rotary part and in the chip, complicating the chip layout and its fabrication. In addition, while our rotary system can be connected through simple holes punched in the PDMS, conventional rotary valves need to consider precise channel alignment.

Our system behaves like a revolving microreactor because it is actuated by an external moving part.¹⁰ Its working principle is similar to the pulsed/stopped flow also known as lab-on-valve (LOV) described by Kalberg,¹¹ which consists of stopping the flow while a reaction proceeds, and eventually starts a pump to push the reaction products towards the electrodes. As demonstrated in our previous work,⁸ simply stopping the flow for a few minutes increases the detection current several times. The system presented in this manuscript builds on that approach and improves its performance because (i) the capture and the detection chambers are independent, and (ii) the beads are captured upstream from the detector and this makes it possible to vary the flow rate to amplify the generated current.

The reason for using magnetic beads is that they are becoming increasingly popular in lab-on-a-chip devices for the practice of bioassays.^{12–19} They can be easily captured and moved around with the aid of a magnet, and virtually any biomolecule can be immobilised on their surface following conventional chemical approaches. Suitably modified magnetic particles can be used to pre-concentrate a sample, they can then be driven towards the detector, where the assay may be finished after performing any necessary intermediate steps. Once the assay is complete, they are released in a very simple way: blocking the magnetic field sliding a thin soft-iron piece between the magnets and the chip, and flushing a buffer solution through the system.

This almost continuous mode of operation is not possible with immunosensors where the transducer is directly functionalised, because the sensing surface must be regenerated after each assay.²⁰ The problem is, particularly in electrochemical systems, that this necessary regeneration of the transducer shortens the electrode lifetime, and increases important parameters such as cost, time and user effort.

In this work, we first describe the design and fabrication of a novel miniaturised analytical system. We then show that the microvalve can cope with flow rates well above the usual ones applied in this type of analytical system, and finally we demonstrate the operation of the system loading magnetic particles with an enzyme whose product is subsequently detected.

Experimental methods

This section describes the fabrication of our microsystem. It is divided in the design and fabrication of the microrotary valve and its packaging.

Microdevice

From the fabrication point of view, our microsystem consists of three main parts: a rotary valve assembly, a silicon microchip featuring a series of microelectrodes, and a holder, where both, rotary valve and silicon microchip, are fitted.

The rotary valve assembly

The rotary valve consists of two main functional elements (see Fig. 1): a cylindrical part that acts as a rotor, and a stator part that contains a cylindrical opening to house the rotor. While the stator facilitates connection to inlet and outlet channels, the rotor also includes a reaction chamber (see Fig. 1a). Turning the rotor allows the operator to connect the outlet of the reaction chamber either to the waste or the detection channel. The valve assembly is positioned so that the detection channel is positioned on top of the electrodes on the microchip (see Fig. 1b).

Both rotor and stator are fabricated in PDMS using casting methods.^{21,22} This configuration allows establishing a proper seal (i) between rotor and stator as well as (ii) between the rotor–stator assembly and a planar surface, *e.g.* a silicon or glass chip (as depicted in Fig. 1b). In the latter case, sealing is achieved by contact, simply by clamping chip and assembly together. In the case of sealing rotor and stator to each other, the vertical clamping force acting on the PDMS structures results in a lateral deformation that, in turn, generates a lateral stress that provides sufficient sealing between both elements.

Fig. 1 (a) Bottom view of the rotary valve: switching between two positions allows passing liquid from the reaction chamber either to waste or to the detection channel. (b) The valve assembly is aligned so that the electrodes are positioned below the detection channel. Pressing the PDMS rotor and stator against a microchip provides sufficient sealing to prevent leaks. (c) Three elements (top, middle and bottom part) were connected to provide a packaging housing the valve components as well as the microchip. Microchannels in combination with glued-in metal tubes and spring loaded pins allowed easy fluidic and electrical interconnection. Permanent magnets provided a magnetic field that could be shielded from the chip, by sliding in a metal plate.

Four brass pins connect the rotor to a shaft (see Fig. 1b). This allows the operator to apply the necessary clamping force for sealing and to rotate the valve.

The rotor has a diameter of 4 mm and a height of 2 mm. The four bottom flat holes that were cast into the rotor have a diameter of 0.8 mm and a depth of 1.5 mm. The reaction chamber has an ID of 3.2 mm and a height of 200 μm , the inlet and outlet are 400 μm wide. The stator (13 mm long, 5 mm wide, 2 mm high) contains the opening for the rotor (ID 4 mm) and the inlet and outlet channels (200 μm $\times$ 400 μm).

Although the elasticity of the PDMS is essential for the function of the microvalve, it increases the risk of collapsing the reaction chamber under excessive clamping force. Therefore, the softness of the silicone is an important parameter for the design of a working rotary microvalve. In this work we used R21-2615 (Nusil Silicone Technology) with a hardness value of 75 (shore A) for the rotor and Sylgard $\text{\textcircled{R}}$ 184 (Dow Corning) with a hardness of 50 (shore A) for the stator.

While the shaft is fabricated in polycarbonate, PC, to strengthen the system, the moulds to cast the PDMS components were made in PMMA because this material yields smoother surfaces. In both cases, micromilling was the fabrication method.

The silicon microchip

The silicon-based electrode chip (5 mm $\times$ 17 mm) featured four microbands (three bands of 500 μm width and 1 band of 1 mm width, respectively) each separated by 100 μm gaps and fabricated as described earlier.⁸ The chips were fabricated on 4-inch silicon wafers with a thermal oxide. Then the microelectrodes were fabricated by lift-off of a thin Ti/Au bilayer deposited by evaporation.

The holder

The holder was designed to house and align the valve components as well as the chip and magnets. The top and middle parts (20 $\times$ 40 $\times$ 3 mm PMMA) were bonded together using UV assisted bonding (2 min UV exposure and subsequent clamping at 80 $^{\circ}\text{C}$). Integrated microchannels (500 μm $\times$ 500 μm cross-section) with glued-in metal tubing (OD 500 μm) acted as fluidic inlet and outlet and allowed for easy interconnection. Different pockets housed the rotary valve components while spring-loaded pins connected the microchip electrode pads. Through-holes allowed attaching the bottom part to the top-middle assembly with an adjustable clamping force using screws. While this clamping force ensured the sealing of the stator to the chip as well as to the interconnection channels of the holder, an aluminium brace was fixed to the top part allowing the application of an adjustable pressure onto the shaft of the rotary valve assembly. Fixing the brace to the top part ensured that the same pressure was applied after disassembling and subsequent re-assembly of the bottom part (e.g. to replace the chip).

The sealing pressure was adjusted by assembling the holder on a transparent dummy chip. In contrast to silicon chips, this transparency permitted visual inspection and helped to avoid collapsing the reaction chamber under excessive pressure. Alignment pins (see Fig. 1c) inserted in the holder's top part allowed turning the shaft only over a certain range of degrees,

which ensured reproducible alignment of rotor and stator channels when switching between the two positions.

In addition, the bottom part also hosted two permanent magnets (Nd-Fe-B, Lika, Denmark, 2 $\times$ 2 $\times$ 2 mm³), a pocket for the silicon chip and a second pocket for insertion of an iron slab (20 $\times$ 5 $\times$ 0.5 mm) to shield the chip from the magnetic field of the magnets (see Fig. 1c). The two magnets are placed with opposite vertical polarisation to maximise the magnetic force on the beads in the channel.²³ On the other hand, this configuration of the magnets allows blocking the magnetic field and flushing the beads out of the microchamber by just inserting the iron slab between the chip and the magnets.⁸

Micromilling and -drilling were used to fabricate the holder components.

System operation

Fig. 2a schematically shows the system operation steps. There are two positions corresponding to the two different outlets: waste and detection. When the waste channel is open there is no contact between the solution and the electrodes as will be demonstrated in the following section. In this position, it is possible to inject biological solutions without the risk of fouling the electrodes and decreasing sensitivity. Moreover, once the magnetic beads (one micron in diameter, Dynal MyOne Streptavidin T1, Invitrogen Spain) are captured in the capture/reaction chamber, further biochemical reactions can be carried out without contaminating the detector. This allows for the performance of a wide range of electroanalytical experiments, making our system very flexible and attractive.

To demonstrate such versatility and flexibility, we used a model system based on the enzymatic loading of magnetic particles performing some enzymatic assays. Enzyme-based detection is very frequent in electrochemical immunoassay.^{24–26} In these indirect measurements, the target concentration is

Fig. 2 (a) Schematic diagram of the working system. Position A opens the waste outlet. This is the position for the injection and capture of the magnetic beads and all the biological reactions—processes not directly related to the detection. The microvalve is turned to position B only for the purposes of detection, to avoiding contact of the electrodes with other solutions. (b) Photographs of the valve in its two positions: the reaction chamber is connected to the waste (Position A, top) or to the detection channel (Position B, bottom). The contours of the PDMS parts are highlighted to show more clearly the different pieces and positions.

proportional to the enzyme concentration used as a label. Electrochemical detection requires that the enzyme reaction products are electroactive in the working potential window allowed by the experimental configuration.

Three common steps in enzymatic bioassays were tested. They consisted of: (i) linking biotinylated enzyme over streptavidin-coated magnetic beads, (ii) enzymatic reaction between the capture enzyme and its substrate and, (iii) detection of the enzymatic product at the electrodes. These steps are all carried out on the chip, the first two in the capture/reaction microchamber and the last one in the detection channel. As the capture/reaction chamber is integrated in the PDMS rotor, when the reaction finishes the valve is turned so that the products can be detected at the electrodes. The enzymatic system, chosen for demonstration purposes, was the well-known β -galactosidase using 4-aminophenyl- β -D-galactopyranoside (PAPG) as its substrate. The same enzymatic system was used in a previous work,⁸ where it was described in more detail. The enzymatic reaction produces *p*-aminophenol (PAP), which is electroactive and can be detected amperometrically at a mild oxidation potential. The oxidation potential of PAP using the same electrode configuration was also studied before.⁸ The oxidation potential of the substrate is around 0.3 V and for PAP it is around 0.1 V.

The main difference between both works is the integration of the rotary microvalve, which allows performing more complex biological processes on the chip without negatively affecting the detection. The working principle of this new system is very straightforward: in a first step, the system is filled with buffer to remove gas bubbles, both in the detector and waste channels. Then the valve is opened to the waste channel, and we can inject the bead suspension. After the magnets have captured a set of particles inside the reaction microchamber, an enzyme solution is introduced until it effectively displaces the buffer filling the microchamber. Then the flow is stopped for a few minutes during which the particles become loaded with enzyme through streptavidin–biotin interactions. The non-specifically bound enzyme is removed by passing a buffer. Next, the enzyme substrate is pumped into the chamber and the flow is stopped to allow the enzymatic reaction to generate a detectable amount of product.

All these steps are conducted with the microvalve in waste position, switching to the detection position immediately before the detection is going to take place. This ensures that only the enzymatic product (PAP) is allowed to flow over the electrodes, and only for a few seconds per measurement. We showed previously⁸ that this procedure can easily amplify the signal by at least one order of magnitude.

Note, that it is possible to perform sequential measurements as the captured beads are released using an iron slab to turn off the magnetic field⁸ resulting in an increase of the reproducibility and reusability of the system.

Results and discussion

Validation of the rotary microvalve

Fig. 2b shows pictures of the two different positions of the microvalve. Position (a), connecting reaction chamber to the waste channel, is used for most processes in this work including

capturing magnetic beads in the microchamber, cleaning or functionalization. In position (b), the liquid can flow from the reaction chamber into the detection channel instead being exposed to the electrodes. Switching between the two positions avoids direct contact between chemical or biological reagents and the electrodes, and only the target species required for detection gains access to the micro-electrochemical detection cell. Switching the configuration is done manually by turning the shaft. Reproducibility is achieved by the above mentioned alignment features of shaft and holder.

To evaluate the sealing of the rotary valve, the holder was assembled using a transparent chip. Subsequently, black ink was injected to provide better contrast and the assembly was visually inspected from the bottom using a microscope (see Fig. 2b).

Once the system was properly closed and adjusted by an adequate clamping force, no leakage could be observed. The valve could be switched repeatedly without degrading its performance. Although tests of the mean lifetime of the PDMS rotor were not performed, we did not observe degradation of its performance after repeated switching over a period of several weeks of continuous measurements.

One of the most important drawbacks of using PDMS to fabricate the rotor is that while turning the valve, a “twisting effect” can be observed resulting in different turning angles between top and bottom of the PDMS rotor. As a result, perfect alignment of rotor and stator inlets and outlets is hampered. This problem was minimised using a harder PDMS and inserting four pins symmetrically distributed to strengthen the rotor structure and distribute the stress more evenly. As the rotor is more rigid, the turning angles between top and bottom part of the rotor are smaller and alignment of rotor and stator becomes possible.

One of the most important features of the system is its simplicity. It is easy to open and close, to clean or replace its parts, *e.g.*, the chip or the PDMS rotor. However, due to PDMS elasticity, it is necessary to ensure that the variability of the dimensions of the detection channel is kept within certain levels after opening and closing the valve in order not to upset the measurement conditions. For a microband electrode inside a microfluidic channel, the relation between the stationary current and the dimensions of the channel was described by Compton *et al.*²⁷ as

$$I = 0.925nFcw(x_eD)^{2/3} \left(\frac{Q}{h^2d} \right)^{1/3} \quad (1)$$

where n is the number of exchanged electrons, F is the Faraday constant, c and D are the concentration and the diffusion coefficient of the electroactive species, w and x_e are the width and the length of the microband electrode, h and d are half the microchannel height and its width, and Q is the (volumetric) flow rate. As in our previous work,⁸ the electrochemical characterization consists of cyclic voltammetry (CV) at 50 mV s⁻¹ for different flow rates using an equimolar solution of ferro/ferricyanide (1 mM) in 0.5 M KNO₃.

Fig. 3 shows mean values of the stationary current after opening and closing the holder several times. As the sealing of the system is based on the compression of the PDMS gasket and rotor, the height of the channel in the PDMS stator is the most affected parameter. The thick line corresponds to the theoretical value of the stationary current for a height of 80 μ m and dotted

Fig. 3 Stationary current for 1 mM equimolar solution of ferro/ferricyanide in 0.5 M KNO_3 at different flow rates: experimental (filled square) and theoretical response for 80 μm channel height (thick line) and for deviations of $\pm 5 \mu\text{m}$ (dotted lines). Error bars are based on 3 independent tests, opening and closing the holder every time. Every test consists of 3 measurements. As a gold pseudo-reference is used, the redox potential of the couple is centered at 0 V, so measurements were performed at 0.2 V.⁸ A diffusion coefficient value of $6.5 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ was used for ferrocyanide.²⁸

lines for 85 and 75 μm , respectively. This means around a 6% change in the measured current. Another way to calculate the height of the channel consists of adjusting a linear fit between the mean current values and $Q^{1/3}$. The channel height can then be calculated from the slope of this graph as: $2h = 84 \pm 4 \mu\text{m}$. This implies a height compression of more than 50% as the channel nominal height value was 200 μm .

Finally, it is important to ensure that the valve does not leak. This was tested electrochemically as follows. A solution containing both ferrocyanide and ferricyanide was passed through the cell, with the electrodes polarised at 0.2 V vs. Au, while the valve was switched between its two positions. As Fig. 4a shows, a steady-state current is measured when the valve is open to the detectors, and no current is measured when the valve is turned the other way. The presence of leaks or valve malfunction would have been flagged by the measurement of non-zero currents while

Fig. 4 (a) Chronoamperometric results at 0.2 V versus Au in 1 mM of ferro-, 1 mM ferri-cyanide in 0.5 M KNO_3 at a flow rate of $50 \mu\text{L min}^{-1}$. The valve is switched between positions A and B several times. (b) Picture of the assembled system without the electrodes using a transparent dummy chip. In position A the reaction chamber is connected to the waste channel, while position B allows to flush the products from the reaction chamber to the electrodes (not shown). For visualization, fluorescence ink was injected.

the valve pointed away from the detector. This test was repeated at 50, 150, 200, 250, 300, 500 and $1000 \mu\text{L min}^{-1}$, and no leaks were observed. In all cases, the valve could be turned in excess of 15 times, even at the higher flowrates.

Fig. 4b shows a picture of the assembled system. For visualization, fluorescent ink was injected to facilitate the observation of both positions. The valve was switched manually turning the brass rod attached to the shaft.

Enzymatic assay

The main motivation to integrate the microvalve is to separate as many operations as possible from the detection, to avoid direct contact between the electrodes and the chemical and biological reagents and thus extend electrode lifetime. To demonstrate the correct function of our system, an enzymatic assay was performed. The first experiments demonstrate that in a single channel system contamination of the electrodes occurs naturally as biomolecules adsorb on them over time of operation. To show the necessity of the microvalve integration contamination experiments were carried out allowing direct contact between the enzyme and the electrodes. Finally, an enzymatic assay is performed, in which a certain amount of magnetic beads are injected, captured and functionalized inside the capture/reaction chamber.

Reduction of contamination

Functionalisation is often achieved by physisorption,²⁹ which means direct incubation of antibodies or enzymes with a given surface for a controlled period of time. To demonstrate that non-specific adsorption of the enzyme over the electrodes may affect flow measurements, a solution of $10 \mu\text{g mL}^{-1}$ concentration of β -galactosidase was incubated for 10 min in the detection chamber, and then flushed with a buffer. Fig. 5a reveals the presence of trace enzyme left in the system, adsorbed on the electrodes. The figure shows the current measured when a solution of 1 mM of substrate (PAPG) was injected at $10 \mu\text{L min}^{-1}$ before and after the enzyme had been in the detector chamber. There are two limiting cases: (i) when the electrodes are clean and (ii) when they are contaminated by adsorbed enzyme. Before injecting the enzyme, the stationary current was around 3.5 nA, which corresponds to the typical blank signal level. However, after exposing the electrodes to an enzyme solution for 10 min, the current increased to 8 nA. This shows that a short contact time is enough to contaminate the electrodes and affect the measurements; *p*-aminophenol is produced as soon as the enzyme substrate is introduced in the system and the background signal increases, which may lead to erroneous data interpretation. Therefore, using a microvalve, the introduction of positive errors during sequential measurements is drastically reduced.

Bead functionalisation

The reaction chamber of the microvalve is connected to the waste outlet during the process of functionalisation of the beads (Fig. 2). This prevents the reagents from adsorbing and fouling the electrodes while the magnetic beads are functionalised inside the capture/reaction chamber. This represents a number of

Fig. 5 (a) Results for the non-specific adsorption of the enzyme on the electrode surface. When the electrode is clean (grey filled square) the stationary current measured at 0.25 V versus an Au pseudo-reference is 3.5 nA for a solution of 1 mM of substrate (PAPG) injected at $10 \mu\text{L min}^{-1}$. 10 min after exposing the electrodes to the enzyme, the current increases to 8 nA (black filled squares). (b) Stationary current at 0.25 V versus an Au pseudo-reference for a solution of 1 mM of substrate (PAPG) injected at $10 \mu\text{L min}^{-1}$ for different incubation times and always using the same concentration of magnetic beads and enzyme.

advantages such as volume reduction of sample and reagents, better reproducibility and possibility of automation.

In this case the magnetic beads are coated with streptavidin, which easily links to the biotinylated β -galactosidase. The experiment consists of first introducing $50 \mu\text{L}$ of $53 \mu\text{g mL}^{-1}$ magnetic beads at $50 \mu\text{L min}^{-1}$ which are captured by the magnets under the microchamber. Next, $25 \mu\text{L}$ of $10 \mu\text{g mL}^{-1}$ concentration of β -galactosidase is introduced at $50 \mu\text{L min}^{-1}$ and the flow is stopped for a short period of time. After the incubation, buffer solution (Buffer Z) is introduced at the same rate to clean and flush out the excess enzyme. When the microchamber is cleaned, a small volume of substrate (1 mM) is injected and the flow is stopped for 10 min. This last step allows the enzyme to react with the substrate and build up a detectable concentration of the product. It also amplifies the detection, as previously described.⁸ After this, the detection channel is opened and the product of the enzymatic reaction is pushed towards the electrodes at $10 \mu\text{L min}^{-1}$ and detected by chronoamperometry. Fig. 5b shows the results for different incubation times. As expected, there is a sigmoidal increase of the current with incubation time before a saturation value is achieved. This saturation

means that no more biotinylated enzyme is available to link the streptavidin over the beads.

Conclusions

We have described the fabrication of a rotary microvalve in PDMS and its integration in an electrochemical miniaturised analytical system. This integration has been possible thanks to the flexible architecture of the original system, which allowed the capture of magnetic beads and the electrochemical detection of electroactive products. The new system represents an improvement over the original system because it allows the physical separation of the various reaction and incubation steps, (e.g. functionalisation) necessary in bioassays from the detection event. This avoids contamination and fouling of the electrodes by proteins or other organic matter, and extends the useful lifetime of the detector.

We have described the design and fabrication of all the parts comprising our electroanalytical microsystem. The system is built from readily accessible materials, mainly PMMA and PDMS, using standard fabrication techniques such as milling, moulding and photolithography.

The main innovation is the open structure of the rotary microvalve, which is sealed on the one hand by the walls of the PDMS stator surrounding it, and on the other by the chip on which it sits. This suppresses the need to define the channels on the chip, which facilitates chip design, simplifies fabrication and ultimately reduces overall cost. Despite the fact that this rotary valve is used in this work to preserve a set of working electrodes, the concept can find application in other fields beyond electroanalysis; we have shown that, despite its sophistication, the system is easy to operate, very reliable, and allows the performance of operations such as capture of magnetic particles, their functionalisation with a model enzyme, β -galactosidase, and reaction with a substrate, and the detection of the resulting electroactive products down to the low nanogram per litre range.

We believe that this concept system can be used to develop a broad range of bioassays, which is the subject of ongoing work.

Acknowledgements

This work was funded by the Spanish Ministry of Science and Innovation, MICINN, through the MICROBACTOMETER project (TEC2006-13109-C03-01/MIC). JdC acknowledges a Ramón y Cajal fellowship, also from the Spanish MICINN. Neus Godino was funded through an I3P scholarship.

Notes and references

- 1 A. G. Crevillén, M. Hervás, M. A. López, M. C. González and A. Escarpa, *Talanta*, 2007, **74**, 342–357.
- 2 J. West, M. Becker, S. Tombrink and A. Manz, *Anal. Chem.*, 2008, **80**, 4403–4419.
- 3 K.-i. Ohno, K. Tachikawa and A. Manz, *Electrophoresis*, 2008, **29**, 4443–4453.
- 4 E. Verpoorte, *Electrophoresis*, 2002, **23**, 677–712.
- 5 H. Becker and C. Gärtner, *Anal. Bioanal. Chem.*, 2008, **390**, 89–111.
- 6 H. Becker and L. E. Locascio, *Talanta*, 2002, **56**, 267–287.
- 7 G. M. Whitesides, *Nature*, 2006, **442**, 368–373.
- 8 N. Godino, D. Snakenborg, J. P. Kutter, J. Emnès, M. F. Hansen and d. C.F.J., *Microfluid. Nanofluid.*, 2010, **8**, 393–103.

1	9 H. Yin, K. Killeen, R. Brennen, D. Sobek, M. Werlich and T. Van De Goor, <i>Anal. Chem.</i> , 2005, 77 , 527–533.	1
	10 C. Zhang, D. Xing and Y. Li, <i>Biotechnol. Adv.</i> , 2007, 25 , 483–514.	
	11 P. S. Francis, S. W. Lewis, K. F. Lim, K. Carlsson and B. Karlberg, <i>Talanta</i> , 2002, 58 , 1029–1042.	
5	12 J. W. Choi, K. W. Oh, J. H. Thomas, W. R. Heineman, H. B. Halsall, J. H. Nevin, A. J. Helmicki, H. T. Henderson and C. H. Ahn, <i>Lab Chip</i> , 2002, 2 , 27–30.	5
	13 J. Do and C. H. Ahn, <i>Lab Chip</i> , 2008, 8 , 542–549.	
	14 S. Farrell, N. J. Ronkainen-Matsuno, H. B. Halsall and W. R. Heineman, <i>Anal. Bioanal. Chem.</i> , 2004, 379 , 358–367.	
10	15 M. Gabig-Ciminska, A. Holmgren, H. Andresen, K. Bundvig Barken, M. Wümpelmann, J. Albers, R. Hintsche, A. Breitenstein, P. Neubauer, M. Los, A. Czyz, G. Wegrzyn, G. Silfversparre, B. Jürgen, T. Schweder and S. O. Enfors, <i>Biosens. Bioelectron.</i> , 2004, 19 , 537–546.	10
	16 M. A. M. Gijs, <i>Microfluidic. Nanofluid.</i> , 2004, 1 , 22–40.	
15	17 V. N. Goral, N. V. Zaytseva and A. J. Baeumner, <i>Lab Chip</i> , 2006, 6 , 414–421.	15
	18 X. Llopis, M. Pumera, S. Alegret and A. Merkoçi, <i>Lab Chip</i> , 2009, 9 , 213–218.	
	19 E. Verpoorte, <i>Lab Chip</i> , 2003, 3 , 60N.	
20	20 X. Muñoz-Berbel, N. Godino, O. Laczka, E. Baldrich, F. X. Muñoz and F. J. del Campo, in <i>Impedance-Based Biosensors for Pathogen Detection</i> ed. S.N. York, 2008.	20
	21 D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, <i>Anal. Chem.</i> , 1998, 70 , 4974–4984.	
	22 J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. A. Schueller and G. M. Whitesides, <i>Electrophoresis</i> , 2000, 21 , 27–40.	5
	23 K. Smistrup, M. Bu, A. Wolff, H. Bruus and M. F. Hansen, <i>Microfluid. Nanofluid.</i> , 2008, 4 , 565–573.	
	24 J. Rossier, F. Reymond and P. E. Michel, <i>Electrophoresis</i> , 2002, 23 , 858–867.	
	25 J. S. Rossier and H. H. Girault, <i>Lab Chip</i> , 2001, 1 , 153–157.	10
	26 A. Warsinke, A. Benkert and F. W. Scheller, <i>Fresenius J. Anal. Chem.</i> , 2000, 366 , 622–634.	
	27 R. G. Compton, A. C. Fisher, R. G. Wellington, P. J. Dobson and P. A. Leigh, <i>J. Phys. Chem.</i> , 1993, 97 , 10410–10415.	
	28 A. J. Bard and L. R. Faulkner, <i>Electrochemical Methods. Fundamentals and Applications</i> , 2nd edn, John Wiley & Sons, New York, 2001.	15
	29 O. Laczka, E. Baldrich, F. J. Del Campo and F. X. Muñoz, <i>Anal. Bioanal. Chem.</i> , 2008, 391 , 2825–2835.	
25		25
30		30
35		35
40		40
45		45
50		50
55		55