Lab on a Chip

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/loc

Lab on a Chip

RSCPublishing

PAPER

Cite this: DOI: 10.1039/xoxxoooooox

Electrochemical pesticide detection with AutoDip – A portable platform for automation of crude sample analyses

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Lisa Drechsel,[a] Martin Schulz,[a] Felix von Stetten,[ab] Carmen Moldovan,[c] Roland Zengerle[abd] and Nils Paust[ab]

Lab-on-a-chip devices promise automation of complex workflows from sample to answer with minimal consumption of reagents in portable devices. However, complex, inhomogeneous samples as they occur in environmental or food analysis may block microchannels and thus often cause malfunction of the system. Here we present the novel AutoDip platform which is based on the movement of a solid phase through the reagents and sample instead of transporting a sequence of reagents through a fixed solid phase. A ball-pen mechanism operated by an external actuator automates unit operations such as incubation and washing by consecutively dipping the solid phase into the corresponding liquids. The platform is applied to electrochemical detection of organophosphorous pesticides in real food samples using an acetylcholinesterase (AChE) biosensor. Minimal sample preparation and an integrated reagent pre-storage module promise easy handling of the assay. Detection of the pesticide chlorpyrifos-oxon (CPO) spiked into apple samples at concentrations 10^{-7} M has been demonstrated. This concentration is below the maximum residue level for chlorpyrifos in apples defined by the European Commission.

Introduction

The current trend in automated biochemical analyses systems employs microfluidics towards fully integrated lab-on-a-chip devices. Compared to conventional pipetting robots, which represent the actual "gold standard" in laboratory automation, the novel, portable tools promise highly sensitive, cheap and quick analyses, if required at the point of care.[1] A microfluidic platform approach, including defined fluidic unit operations, generic design, consistent concepts for integration and automation of a biochemical process as well as a suitable fabrication technology is necessary to meet the requirements of a broad market.[1]

A challenge for the application of microfluidic systems is the handling of "real world" samples as they occur in clinical diagnostics, environmental- and food analysis.[2] Components of inhomogeneous or viscous samples as well as precipitates or simply gas bubbles can easily block microchannels.[3] Notably, in comparison to other application fields, only low numbers of publications were reported in food microfluidics.[3,4] A main reason for this is the difficulty to handle complex sample matrices.[4,3]

Besides identification of toxins, vitamins, pathogens or heavy metals, identification of pesticides is one example of essential analyses adopted to real food samples. Current methods of pesticide detection in fruits or vegetables consist of a sample preparation step with organic solvents[5] and analysis by gas or liquid chromatography coupled to mass spectrometry for detection. These conventional multiresidue methods are expensive, time consuming, require specialized personnel and complex laboratory equipment.[3,6] Therefore, a lot of effort is put into the development of enzyme-based electrochemical biosensor systems as a quick and simple alternative, even

[a]*HSG-IMIT - Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany. E-Mail: Lisa.Drechsel@hsg-imit.de, Nils.Paust@hsg-imit.de*
[b]*Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany*
[c]*National Institute for R& D in Microtechnologies, Bucharest, Romania*
[d]*BIOSS - Centre for Biological Signalling Studies, University of Freiburg, Schänzlestr. 18, 79104 Freiburg, Germany*
Electronic supplementary information (ESI) available : See DOI:

capable of on-site pesticide monitoring.[6] Applying this approach, the most widely used class of pesticides, organophosphorous pesticides (OPPs),[7] can be detected by their inhibitory effect on the enzyme acetylcholinesterase (AChE).[8,6]

Next to portability of the system, automation of biosensor-based analysis is a prerequisite for simple and reliable on-site testing, reducing the probability of errors by mishandling.[9] The application of flow-based biosensor systems in electrochemical pesticide analysis has been extensively reviewed.[10,11] Multi-commuted flow analysis (MCFA), carried out in a flow system driven by a peristaltic pump and operated by a software switching solenoid valves seems to be a promising tool for automation of AChE-inhibition based assays.[12,11]

However, due to the use of small channels and flow cells, this approach suffers from the major problem of food microfluidics – the difficulty to process complex sample matrices – and is therefore limited to liquid samples (e.g. lake or river water[13,14], milk[15]). For solid or inhomogeneous food samples, an elaborated sample preparation procedure, usually based on solvent extraction, is required.[16,17] As reviewed by McGrath and colleagues, it is crucial to develop minimal sample preparation techniques for biosensor systems which still allow the detection of the analyte.[9]

As an alternative to an elaborated extraction procedure, detection of pesticides by incubation of biosensors in crude samples (infant food[18], broccoli chopped pulp[19]) was described. Caetano *et al.* even demonstrated the detection of carbaryl pesticide by immersion of a biosensor directly into a tomato.[20] In all these reports, the sensor had to be transferred manually from the sample to the measurement device (electrochemical cell). To our knowledge, there is no automation solution available for pesticide detection by biosensor incubation in crude, inhomogeneous food samples.

In this context, we developed a novel, portable lab-on-a-chip platform which automates consecutive dipping of a biosensor into reagents and sample. To keep the platform compact and simple, the sequential dipping is realized by a rotational movement of the biosensor according to a ball-pen mechanism, operated by a simple external actuator. Since the solid phase is transported instead of the liquids, no

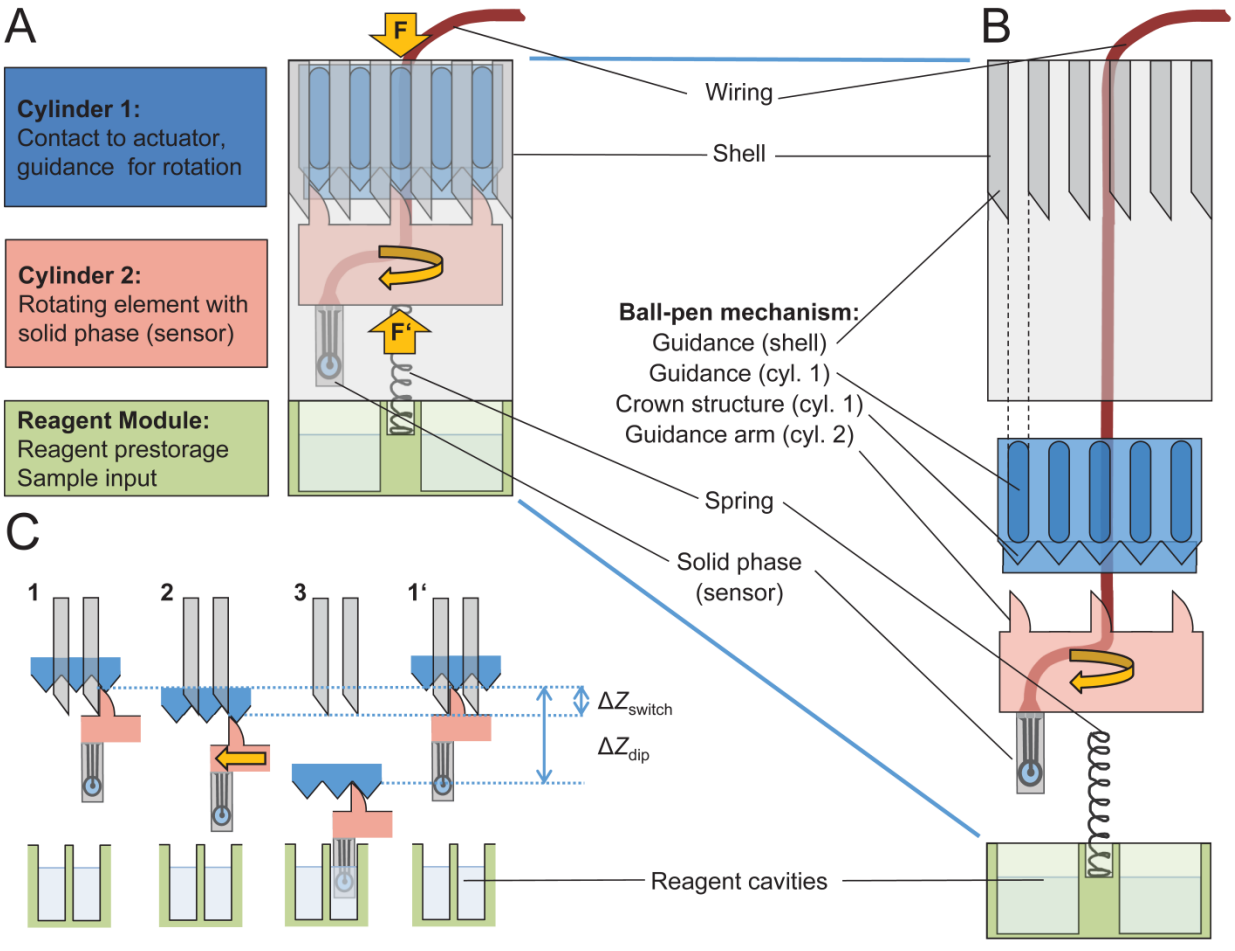

Fig. 1: Schematic drawing (A, B) and functional principle (C) of the AutoDip cartridge. (A) A static shell encloses two vertically movable cylinders. The rotation of cylinder 2, positioning the solid phase, is controlled by pen mechanics. Vertical movement and thereby operation of the ball-pen mechanism is directed by the interplay of a mechanical force (F) applied to cylinder 1 by an external actuator, and a counter acting spring force (F'). (B) Explosion view of the cartridge. (C) Rolled up view of the cartridge to illustrate the principle of rotational transport of the solid phase from one reagent cavity to another. The displacement that must be provided by the actuator for switching and dipping is illustrated by ΔZ_{switch} and ΔZ_{dip}, respectively. See text for detailed description.

microchannels, valves, or interfaces to external pumps are required. This allows the analysis of inhomogeneous samples with minimal sample pretreatment off-chip. As a proof of concept, we applied the AutoDip platform to pesticide analysis in real food samples.

Platform design and functional principle

The core of the novel AutoDip platform is a cartridge consisting of five parts as depicted in figure 1: cylinder 1, cylinder 2, shell, reagent module and the spring. Vertical movement is automated by an external actuator. The functional solid phase (e.g. a biosensor) is attached to cylinder 2. Transformation of vertical into rotational movement of cylinder 2, that allows the consecutive dipping of the solid phase into the 20 chambers of the reagent module, is realized by integration of a ball-pen mechanism as recently communicated.[21] Cylinder 1 serves as contact point for the external actuator which controls the vertical movement within the shell. It comprises a crown structure for rotation of cylinder 2. The shell encases cylinders 1 and 2 and also contains guidance structures for rotation. The wiring of the biosensor runs through centered holes of cylinders 2 and 1 to an external potentiostat.

In figure 1C, the rotational movement of the solid phase from one reagent cavity to another is depicted schematically. In the initial state (1), guidance arms of cylinder 2 are in contact with guidance structures in the shell and the crown structure of cylinder 1. Upon actuation, the guidance arms are pressed below this holding (displacement of ΔZ_{switch}, state 2), cylinder 2 rotates and the guidance arms are fixed in the crown structure of cylinder 1. In that radial position, the sensor is dipped into the sample and reagent cavities (displacement of ΔZ_{dip}, state 3). After dipping and, if applicable, incubation, the external actuator is released and cylinder 1 and 2 are raised by the spring force. Simultaneously, cylinder 2 rotates further as the guidance arms get in contact with the guidance structures of the shell again, finally reaching the initial position again, but having rotated 1/20 of a complete circle (1').

As recently reviewed by Hitzbleck and Delamarche, integration and release of reagents is still a challenge in microfluidic devices.[22] In the AutoDip platform, the reagent pre-storage is solved by the integration of a disposable reagent module. This module can be sealed by a foil which is removed by the user like the cover of a yoghurt cup or can be lanced by the sensor during the analysis procedure.

A detailed 3D model of the cartridge is depicted in figure 2A. Figure 2B shows the prototype of the reagent module manufactured by stereolithography with partially removed sealing. A picture of the reagent module pre-filled with the reagents required for one complete assay and a homogenized food sample is provided below (fig. 2C). In the current design, the reagent module contains 20 reagent cavities which are filled with 700 µl of reagent, each. The external actuator is fixed on top of the cartridge (fig. 2D). Portable external control modules for programming the actuator and a small potentiostat for data acquisition complete the platform.

The application of a ball-pen mechanism for automation of biochemical assays was already demonstrated with our LabTube platform.[23] The LabTube is operated in a standard

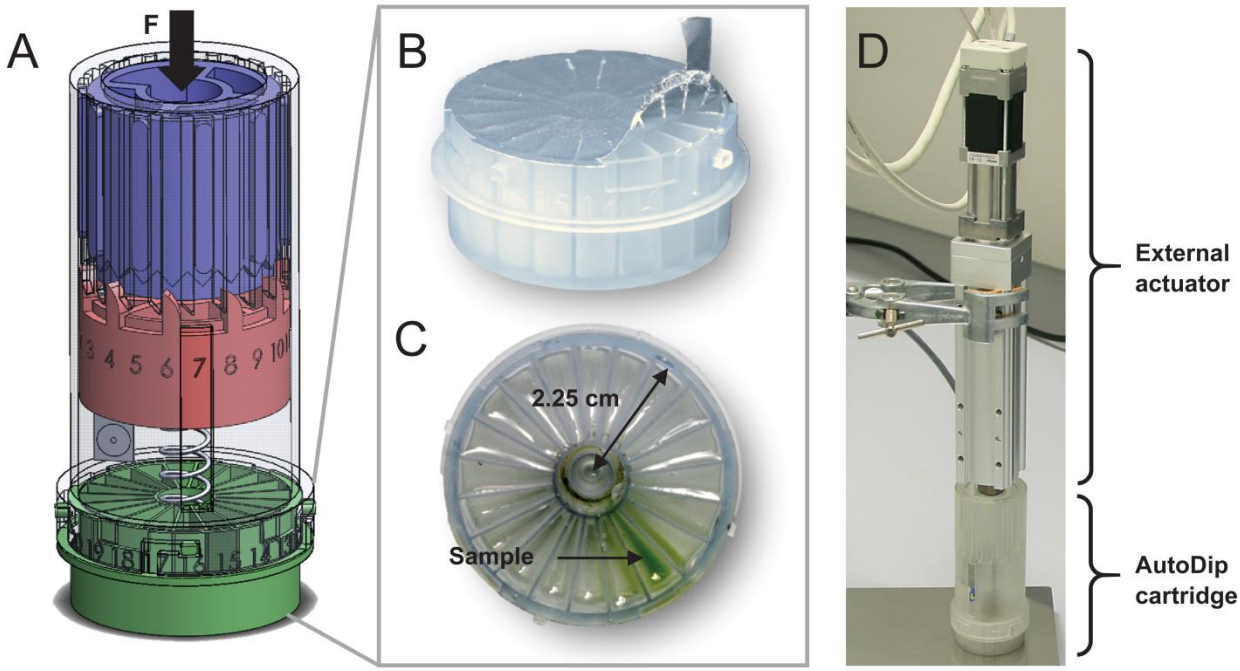

Fig. 2: Detailed AutoDip design and evaluation model. (A) 3D-drawing of the AutoDip cartridge. (B) Image of the reagent module fabricated in stereolithography and sealed by a peelable foil. (C) Top view of reagent module pre-filled with reagents and a homogenized food sample (green). (D) Prototype of the AutoDip cartridge with external vertical actuator.

laboratory centrifuge and the ball-pen mechanism is actuated by changes in centrifugal acceleration. In contrast to a centrifuge, the AutoDip actuator can easily be transported by one single person, rendering the platform suitable for on-site or in-field analyses.

Material and Methods

Cartridge design and fabrication

Design of cartridge components was performed using the CAD Software Solidworks 2013. Evaluation models were fabricated by stereolithography using WaterShed XC 11122 (DSM-Somos) material (TEUFEL Prototypen GmbH, Germany). Spring D-143 H was provided by Gutekunst Federn, Germany. Evaluation models were greased with Glisseal HV (Borer Chemie, Germany) to ensure smooth movement of revolvers inside the shell and reliable switching of the ball-pen mechanism.

Platform assembly

A simple external actuator was constructed to automate the vertical movement of cylinder 1 and thereby the switching of the ball-pen mechanism. In the prototype arrangement, this programmable electrical cylinder is mounted to a standard laboratory stand, and the AutoDip cartridge is placed below (see fig. 2D). Detailed composition of the external actuator is described in the electronic supplementary information.

The electrochemical biosensor was plugged into a multipole connector (DF 2 MK 9 Z, Fischer Elektronik, Germany) attached to cylinder 2 and connected to the external potentiostat (EmStat2, Palmsens, Netherlands).

Assay design

For AChE-inhibition based pesticide detection, a commercially available acetylcholinesterase biosensor (AC1.AChE, BVT Technologies, Czech Republic) was applied. This biosensor for amperometric determination of AChE activity contains three electrodes: A working electrode with immobilized AChE enzyme, a counter electrode and a reference electrode (silver). Acetylthiocholine (ATCh) was used as substrate which is hydrolyzed by AChE to thiocholine and acetate. In a second reaction step, thiocholine is anodically oxidized to generate dithio-bis-choline. Two electrons are involved in this process, resulting in a current output of the biosensor in the nA range,[24] reflecting enzyme activity.

A measurement protocol that most likely avoids electrochemical and enzymatic interferences was chosen.[25] Thereby, the initial enzyme activity is recorded, the biosensor is incubated in the insecticide solution for inhibition, rinsed, and the residual signal after insecticide treatment is determined. Due to the inhibitory effect of OPPs on AChE, the current is reduced after contact with pesticide.

In continuous flow systems, the response of a biosensor to repeated injections of enzyme substrate ATCh into a continuous buffer stream is recorded.[26,15] The amplitude of the resulting current peaks represents the relative enzyme activity. We developed an alternative method to record current peaks by dipping the sensor into the reagents. The biosensor is first incubated in ATCh solution for a defined time to evoke a current response, and then introduced into buffer solution to stop the reaction. Measurement of at least two of these signal peaks demonstrates reproducibility of peak heights (fig. 3). If OPPs are present, the height of the peaks is reduced after incubating the sensor in the sample. This residual signal is recorded after washing the sensor in buffer to avoid interferences of the sample matrix with the measurement (fig. 3).

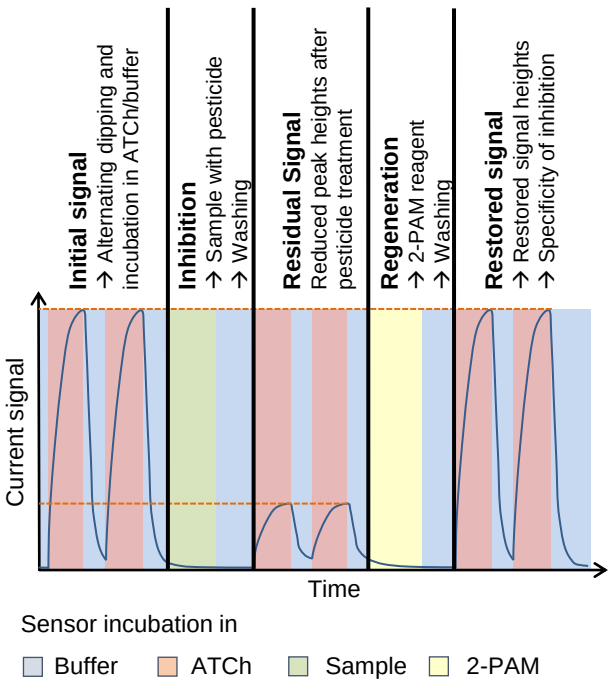

Fig. 3: Schmematic of current profile of the electrochemical pesticide detection assay with an acetylcholinesterase biosensor. ATCh (red): Acetylthiocholine, substrate for the enzyme reaction; Buffer (blue) for equilibrating and washing the sensor; Sample with pesticide (green); 2-PAM (yellow) pralidoxime, regeneration reagent. Current peaks are generated by incubation of the sensor in ATCh for a defined time and subsequent dipping of the sensor into buffer. A repetition of this sequence demonstrates reproducibility of the signal.

Strong nucleophiles like pralidoxime (2-PAM) can specifically restore activity of biosensors after AChE inhibition with organophosphorous pesticides.[27,18] A successful regeneration by pralidoxime treatment leads to the conclusion that the inhibition was caused by OPPs. In case it is not possible to restore the signal, components of the sample matrix may be involved in enzyme inhibition.[18,17] A regeneration step with sensor incubation in 2-PAM is therefore required for reliable pesticide detection. To remove residual regeneration reagent, the sensor is washed again before the restored signal is determined (fig. 3).

For details of the electrochemical measurement procedure and data analysis refer to the electronic supplementary information.

Reagents

The same buffer solution was used in all experiments (0.04 M MOPS, pH 7.0, 100 mM NaCl). A 100 mM stock solution of enzyme substrate acetylthiocholine chloride (ATCh, Sigma Aldrich) was prepared in DI water and stored at -20 °C. The working solution of 1.5 mM ATCh was generated by dilution of the stock solution in buffer for immediate use. A 1 mM solution of pyridine-2-aldoxime methochloride (2-PAM, Sigma-Aldrich) in buffer was prepared on the day of use. The pesticide chlorpyrifos-oxon (CPO) (Dr. Ehrenstorfer, Germany) was dissolved in 100 % ethanol (Carl Roth, Germany) to generate a 10^{-2} M stock solution which was stored at -20 °C. Dilutions of the stock solution in buffer or homogenized sample (spiking) were prepared and used the same day.

Sample preparation

Apples from organic agriculture were bought at local wholefood shop. The fruit was cut into large pieces and 100 g of the sample were homogenized with 100 ml of buffer using a standard kitchen blender. The pH was tested using pH test stripes (pH-Fix 6-10, Macherey-Nagel) and adjusted to pH 7 with NaOH. If applicable, the sample was spiked with pesticide CPO as described above.

Results and discussion

In the following section, we first characterize signal generation with our AutoDip platform in combination with the commercially available AChE biosensor. Afterwards, different concentrations of the pesticide CPO in buffer are analyzed. Finally, we demonstrate pesticide detection in a real food sample (apple).

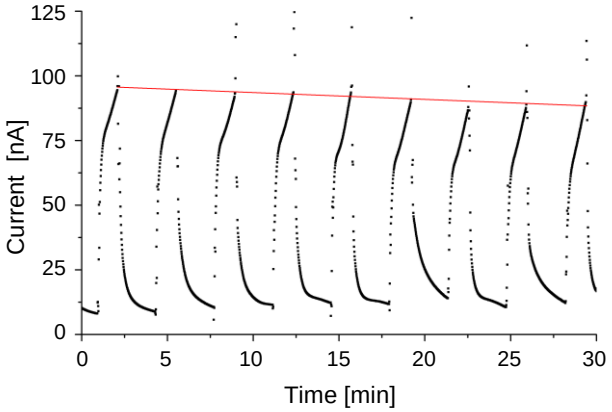

Fig. 4: Current profile of the AChE biosensor during alternating dipping into enzyme substrate acetylthiocholine (ATCh) and buffer using the AutoDip cartridge (sensor 2). The decline of the peak heights over time is visualized by the red line. Outliers appearing above the line were generated during switching and are not considered when determining peak heights.

Evaluation of the automated dipping protocol

We first recorded the current response of the biosensor to alternating dipping into enzyme substrate ATCh and buffer. While the sensor was exposed to ATCh, a current in the nA range could be measured which decreased immediately after switching the ball-pen mechanism to the next chamber and dipping the sensor into buffer (fig. 4). By measuring 9 consecutive current peaks, a decrease of the amplitude over time (sensor 1: 0.69 ± 0.12 %/min; sensor 2: 0.29 ± 0.04 %/min; sensor 3: 0.60 ± 0.16 %/min) was observed. Possible explanations for this behaviour could be a loss of immobilized AChE during the experiment or a decrease of enzyme activity due to degeneration processes. In comparison, Jeanty et al. reported a lower signal drift of ~ 9 % per hour (i.e.

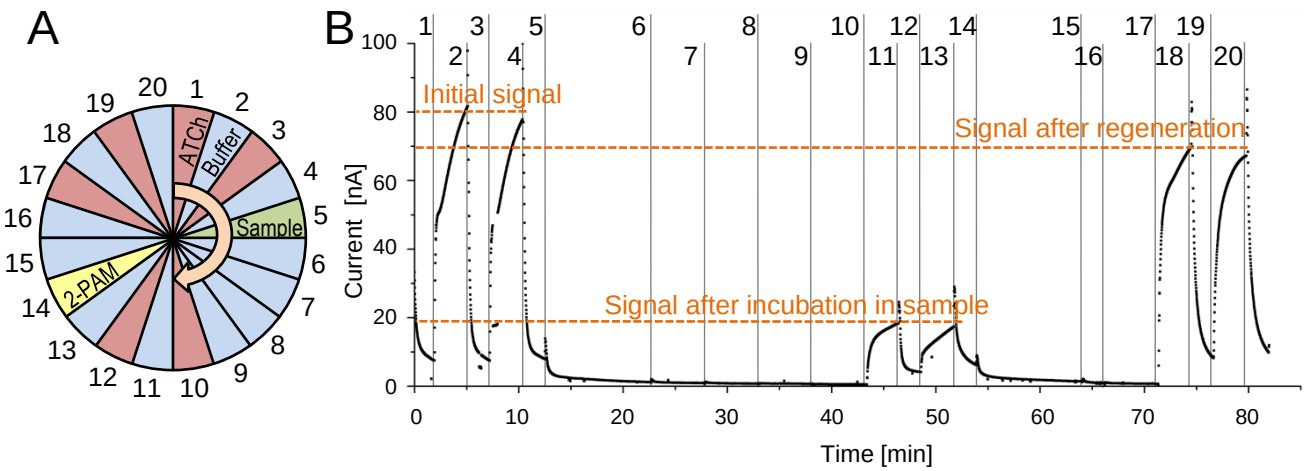

Fig. 5: Reagent scheme and according current profile of a complete pesticide assay in the AutoDip cartridge. (A) Loading scheme of prefilled reagent module. The arrow indicates the starting point and the rotational direction of the sensor. ATCh (red), buffer (blue), sample (green); 2-PAM (yellow). The numbers show the process steps for the ball-pen mechanism. (B) Complete pesticide assay with 10-6 M CPO in buffer as a sample. The numbers refer to the reagent cavities in (A). Reduction of the signal after incubation in sample and regeneration after incubation in 2-PAM indicate the presence of CPO.

0.15 %/min) using a flow injection system and a flow cell to generate the signal peaks.[26] As discussed in the following section, reliable pesticide detection is possible despite this considerable reduction of signal over time. Furthermore, the results demonstrate a reproducible current peak response with the developed dipping protocol.

Pesticide detection in buffer

To evaluate pesticide detection with our AutoDip platform, we performed a number of experiments with three different concentrations (10^{-7}, 10^{-6} M, 10^{-5} M) of the pesticide CPO in buffer. The reagent module was prefilled as depicted in fig. 5A. A representative result of a pesticide assay (10^{-6} M CPO) is shown in figure 5B.

The initial signal of the biosensor was determined by measuring the amplitude of two consecutive current peaks (1-4) and calculating the mean value. After incubation of the AChE sensor in sample with pesticide (5) and four washing steps (6-9), a residual signal with decreased amplitude (26 ± 1 % of the initial signal) was acquired (10-13). Regeneration of the AChE sensor in pralidoxime (14) and washing in buffer (15, 16), resulted in a restored signal (86 ± 2 % of the initial activity. 17-20). Considering the assay time of 90 minutes, this is in the range of the signal loss over time determined in the previous experiment (fig. 4).

Data from all experiments with different CPO concentrations were collected and plotted in figure 6. Inhibition of AChE-based biosensor activity clearly depended on the amount of pesticide in the sample chamber. While 10^{-7} M CPO reduced the peak amplitude to 58 ± 11 % of the initial signal, higher concentrations of 10^{-6} M and 10^{-5} M CPO resulted in average decreased responses of 22 ± 3 % and 11 ± 3 %, respectively. As revealed by control experiments, reduction of the signal after sensor incubation in buffer without pesticide is in accordance with the signal drift as discussed in context with fig. 4. The same is applicable for the activities measured after the regeneration process, where the sensors displayed average restored signal heights around 60 % to 80 % (fig. 6). Furthermore, using a comparable AChE biosensor with enzyme from the same species (electric eel) in a continuous flow system, Jeanty et al. reported similar inhibition rates with CPO.[13]

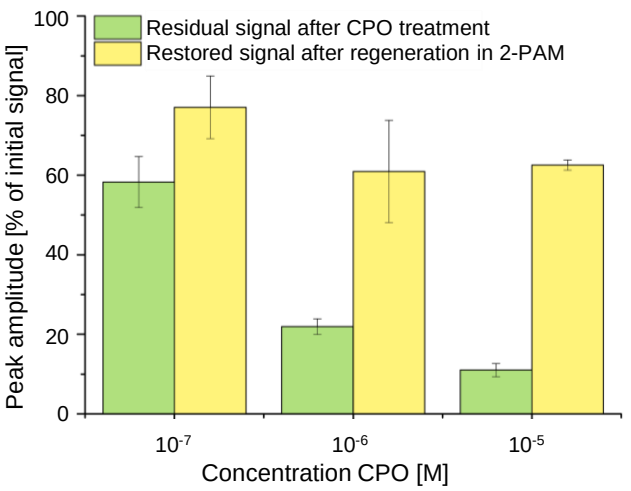

Fig. 6: Pesticide detection in buffer. Residual signals after incubation of the biosensor in buffer with pesticide CPO in three different concentrations (green). Restored signal after regeneration of the biosensor in 2-PAM (yellow). Each column represents a mean value of three independent experiments. Error bars indicate standard deviations.

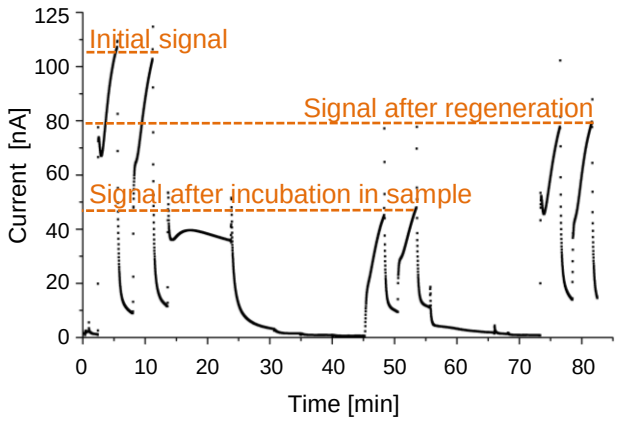

Fig. 7: Complete pesticide assay in a homogenized apple sample spiked with 10^{-7} M CPO. Analysis was performed as described in fig. 5. A representative experiment out of three replicates shown. Data described in the text represent mean values calculated from replicates

Pesticide detection in a real food sample (apple)

As a proof of principle, we performed a complete pesticide assay in the AutoDip cartridge analyzing a homogenized apple sample spiked with 10^{-7} M CPO. After measuring the initial signal, the sensor was dipped into the sample. Interestingly, a non-specific signal was observed during sensor incubation in the apple sample, which decreased immediately after washing the sensor (fig. 7). This underlines the importance of biosensor readout in absence of the sample matrix. Subsequently, a residual current signal of 47 ± 5 % was measured. Regeneration of the AChE enzyme activity in 2-PAM resulted in restored current peaks heights of 76 ± 4 % of the initial signal, demonstrating that the inhibition of AChE was specifically caused by CPO. Apart from the non-specific activation during sensor incubation in sample, the results of the experiments in apple samples were comparable to the measurements in buffer (fig. 6).

The restored signal peaks did not reach the height of the initial current signal again. Similar observations were made during the measurements in buffer and are dedicated to the signal drift. In context with real samples, also matrix effects may account for low regeneration rates. However, an experiment with pure homogenized apple without pesticide revealed almost no effects of the sample matrix. There was a considerable difference between the "blank" inhibition by apple pulp without pesticide and the signal reduction by apple with 10^{-7} M CPO. Notably, the maximum residue level (MRL) for chlorpyrifos in apple determined by the European Commission

is even higher than the pesticide concentration used here. In summary, it can be concluded that the AutoDip setup is able to detect CPO spiked into apple samples at concentrations of 10^{-7} M, even one magnitude below the official MRL for chlorpyrifos in apple.

Conclusion and outlook

The AutoDip platform is a portable tool for on-site analyses. Implementation of a ball-pen mechanism assures robust mechanical transfer of a solid phase from one reagent reservoir to another. The current design features 20 consecutive transfer steps. The mechanism is automated by a simple and portable external actuator. A disposable module for integrated pre-storage of up to 20 single use reagents promises easy handling by non-specialized personnel. Microfluidic systems are not suitable for processing complex and inhomogeneous matrices. In contrast, AutoDip automates transfer of a solid phase to the reagents instead vice versa and thus allows handling of crude real-world samples.

As a proof of principle, the platform was applied to pesticide analysis using an electrochemical biosensor as a solid phase. We demonstrated detection of the pesticide chlorpyrifos-oxon (CPO) spiked into crude apple samples in concentrations of 10^{-7} M, which is one magnitude below the official MRL.

The implemented AChE biosensor assay provides only black and white answers for unknown pesticide contaminants. An attempt to create a selectivity of AChE biosensors is the use of multisensor arrays with different enzymes or enzyme variations and artificial neural networks.[28,29,30] With regards to miniaturization of biosensors and flexibility of the AutoDip platform, it could be possible to insert a multibiosensor with different enzymes into the system, allowing identification and quantification of certain pesticides from real samples.

The platform is open to implementation of a large spectrum of biochemical analysis methods by integration of different solid phases and reagents. As future perspective, a solid phase with a functionalized surface (immobilized antibodies or antigens) would allow the implementation of an immunoassay. Inserting a magnet as solid phase would allow automated handling of magnetic beads and therefore generate a very flexible tool applicable for immunoassays, nucleic acid extractions or other affinity purifications, depending on the bead surface. The AutoDip platform could therefore potentially be used for handling and sorting of cells in small scale applications. Besides the electrochemical measurements, further read-out variants such as optical detection could be implemented. Importantly, the AutoDip cartridge can be fabricated as a closed, disposable system, allowing analysis of hazardous substances or infectious material without the risk of contamination after sample input. In addition, the platform consists of only four major parts that are amenable to large scale production. In sum, these advantages render the novel AutoDip platform an attractive candidate for on-site analysis.

Acknowledgements

We gratefully acknowledge financial support from the Bundesministerium für Bildung und Forschung within the project Pestiplat (FKZ 16SV5520K) of the European MNT-ERA.NET program.

References

1 D. Mark, S. Haeberle, G. Roth, F. von Stetten and R. Zengerle, Chem Soc Rev, 2010, 39, 1153–1182.
2 A. G. Crevillén, M. Hervás, M. A. López, M. C. González and A. Escarpa, Talanta, 2007, 74, 342–357.
3 Y. T. Atalay, S. Vermeir, D. Witters, N. Vergauwe, B. Verbruggen, P. Verboven, B. M. Nicolaï and J. Lammertyn, Trends in Food Science & Technology, 2011, 22, 386–404.
4 A. Escarpa, Lab Chip, 2014.
5 A. Wilkowska and M. Biziuk, Food Chemistry, 2011, 125, 803–812.
6 C. S. Pundir and N. Chauhan, Anal. Biochem., 2012, 429, 19–31.
7 D. Sharma, A. Nagpal, Y. B. Pakade and J. K. Katnoria, Talanta, 2010, 82, 1077–1089, ISI:000282480500001.
8 T. R. FUKUTO, Environmental Health Perspectives, 1990, 87, 245–254.
9 T. F. McGrath, C. T. Elliott and T. L. Fodey, Anal Bioanal Chem, 2012, 403, 75–92.
10 B. Prieto-Simón, M. Campàs, S. Andreescu and J.-L. Marty, Sensors, 2006, 6, 1161–1186.
11 E. J. Llorent-Martinez, P. Ortega-Barrales, M. L. Fernandez-de Cordova and A. Ruiz-Medina, Analytica Chimica Acta, 2011, 684, 30–39, ISI:000286848400004.
12 G. A. Alonso, R. B. Dominguez, J. L. Marty and R. Munoz, Sensors (Basel), 2011, 11, 3791–3802, PM:22163822.
13 G. Jeanty, A. Wojciechowska, J. L. Marty and M. Trojanowicz, Analytical and Bioanalytical Chemistry, 2002, 373, 691–695, ISI:000177859200004.
14 B. Bucur, M. Dondoi, A. Danet and J.-L. Marty, Analytica Chimica Acta, 2005, 539, 195–201.
15 R. K. Mishra, R. B. Dominguez, S. Bhand, R. Munoz and J. L. Marty, Biosens.Bioelectron., 2012, 32, 56–61, PM:22221795.
16 I. Palchetti, A. Cagnini, M. Del Carlo, C. Coppi, M. Mascini and A. P. Turner, Analytica Chimica Acta, 1997, 337, 315–321.
17 H. Schulze, R. D. Schmid and T. T. Bachmann, Analytical and Bioanalytical Chemistry, 2002, 372, 268–272, ISI:000176102900008.
18 H. Schulze, E. Scherbaum, M. Anastassiades, S. Vorlova, R. D. Schmid and T. T. Bachmann, Biosens.Bioelectron., 2002, 17, 1095–1105, ISI:000178791900024.
19 I. Ion and A. C. Ion, MATERIALS SCIENCE & ENGINEERING C-MATERIALS FOR BIOLOGICAL APPLICATIONS, 2012, 32, 1001–1004.

20 J. Caetano and S. A. Machado, Sensors and Actuators B: Chemical, 2008, 129, 40–46.
21 L. Drechsel, M. Schulz, F. von Stetten, R. Zengerle and N. Paust, Proceedings of 17th International Conference on Miniaturized Systems for Chemistry, Freiburg, Germany, 2013.
22 M. Hitzbleck and E. Delamarche, Chem Soc Rev, 2013, 42, 8494–8516.
23 A. Kloke, A. R. Fiebach, S. Zhang, L. Drechsel, S. Niekrawietz, M. M. Hoehl, R. Kneusel, K. Panthel, J. Steigert, F. von Stetten, R. Zengerle and N. Paust, Lab Chip, 2014, 14, 1527–1537.
24 L. F. Sgobbi, C. A. Razzino, I. G. Rosset, A. C. Burtoloso and S. A. Machado, Electrochimica Acta, 2013, 112, 500–504.
25 F. Arduini, A. Amine, D. Moscone and G. Palleschi, Mikrochim.Acta, 2010, 170, 193–214, ISI:000281384000002.
26 G. Jeanty, C. Ghommidh and J. Marty, Analytica Chimica Acta, 2001, 436, 119–128.
27 S. Okazaki, H. Nakagawa, K. Fukuda, S. Asakura, H. Kiuchi, T. Shigemori and S. Takahashi, Sensors and Actuators B: Chemical, 2000, 66, 131–134.
28 T. T. Bachmann and R. D. Schmid, Analytica Chimica Acta, 1999, 401, 95–103.
29 T. T. Bachmann, B. Leca, F. Vilatte, J.-L. Marty, D. Fournier and R. D. Schmid, Biosensors and Bioelectronics, 2000, 15, 193–201.
30 C. B. Roepcke, S. B. Muench, H. Schulze, T. T. Bachmann, R. D. Schmid and B. Hauer, Food Control, 2011, 22, 1061–1071.

