AUTOMATED ON-SITE DETECTION OF ORGANOPHOSPHOROUS PESTICIDES IN REAL FOOD SAMPLES

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ABSTRACT

A new dipping platform for automated electrochemical detection of organophosphorous pesticides (OPPs) in food samples is presented. In contrast to transporting liquids in microfluidic systems, we use a ball-pen mechanism to transport the sensor that consecutively dips into the sample, washing buffers and reagents. Thus, clogging and sticking problems caused by inhomogeneous samples are avoided and real food samples can be processed. To keep the system cost effective and simple, all reagents are pre-stored in a disposable module and the pen mechanism is driven by a single axis coordinator, only. Using our ball pen-like cartridge, we demonstrate reliable detection of the pesticide chlorpyrifos-oxon spiked into cucumber samples with concentrations down to the range of European maximum residue levels(10^{-7} M).

KEYWORDS: Pesticide detection, electrochemistry, food analysis, sensor, dipping, ball-pen mechanism, Lab-on-a-Chip

INTRODUCTION

Due to extensive use of pesticides in agriculture, strict control of pesticide levels in food is required. Current methods for pesticide detection involve gas- or liquid chromatography and mass spectrometry, which require complex sample preparation and large, expensive laboratory equipment [1]. OPPs are the most widely used class of pesticides and can be sensed by electrochemical enzyme sensors based on their inhibitory activity towards acetylcholinesterase (AChE). Although extensive research is carried out [2], no fully automated platform for the detection of OPPs in real fruit or vegetable samples is available so far.

THEORY

The enzyme AChE is immobilized on the working electrode of a three-electrode sensor, allowing amperometric measurements of enzyme activity. In presence of the enzyme substrate acetylthiocholine (ATCh), an electric current is generated between the working and the auxiliary electrode of the sensor. Due to the inhibitory effect of OPPs on AChE, a lower signal is expected with contaminated samples. For the evaluation of pesticide contamination, it is reasonable to generate signal peaks by alternating application of the substrate ATCh and buffer to the sensor electrodes at a defined time frame. The height of these peaks should be reproducible and decrease when the sensor is in contact with pesticides. However, electrochemical measurements are very sensitive, and the sample matrix may disturb the signal generation. Since AChE is inhibited irreversibly by OPPs, it is possible to incubate the sensor with the immobilized enzyme in the sample first and to remove disturbing matrix components by washing the sensor [3]. Regeneration of AChE activity by incubating the sensor in pralidoxime (2-PAM) demonstrates specificity of inhibition by the group of OPPs and excludes unspecific inhibitory effects by other matrix components.

For on-site detection of pesticides, sample preparation must be quick and simple (e.g. just milling), resulting in inhomogenous samples which would immediately block any microfluidic system. Therefore, a transport of the sensor to the different cavities containing substrate, sample and washing buffers was intended. For this purpose, we developed a cartridge with a ball-pen-like mechanism which is driven by a single-axis coordinator only, to keep the system as small and simple as possible.

The cartridge for pesticide detection consists of four parts and a spring as depicted in figure 1. Guidance structures of the shell and cylinder 1 are in contact with the tips of cylinder 2. By vertical actuation, cylinder 2 is rotating and the electrochemical sensor is dipped into the cavities of the reagent module, consecutively. The sensor can simply be plugged into the contacts of cylinder 2 and the wiring continues through the middle of cylinder 1 to the measurement device outside the cartridge. The reagent module shown in figure 2 is a disposable containing all required reagents and the sample, and can be sealed by a foil. In the future, the sensor could be pre-stored in the reagent module, too.

EXPERIMENTAL

A prototype of the cartridge was fabricated in stereolithography, and connected with the EmStat^2 potentiostat (PalmSens) as depicted in figure 3. A commercially available AChE sensor was used for the measurements (BVT Technologies). For sample preparation, 100 g of a cucumber (organic) were homogenized with 100 ml of buffer (0.04 M MOPS, pH 7.0, 100 mM NaCl) and the pH of the mixture was adjusted to pH 7.0. For proof of principle, pesticide (chlorpyrifos-oxon, 10^{-6} M) was spiked into the sample. The reagent module was pre-filled with buffer, substrate ATCh (1.5 mM in buffer), regeneration reagent pralidoxime (2-PAM, 1 mM in buffer) and the sample as depicted in figure 4.

The cartridge was applied to guide the sensor through the liquids, thereby dipping five times into a liquid before incubation for a distinct time. Amperometric measurements were performed to quantify enzyme activity by applying a voltage of 300 mV between reference electrode and working electrode and quantifying the current between working electrode and auxiliary electrode (see figure 5).

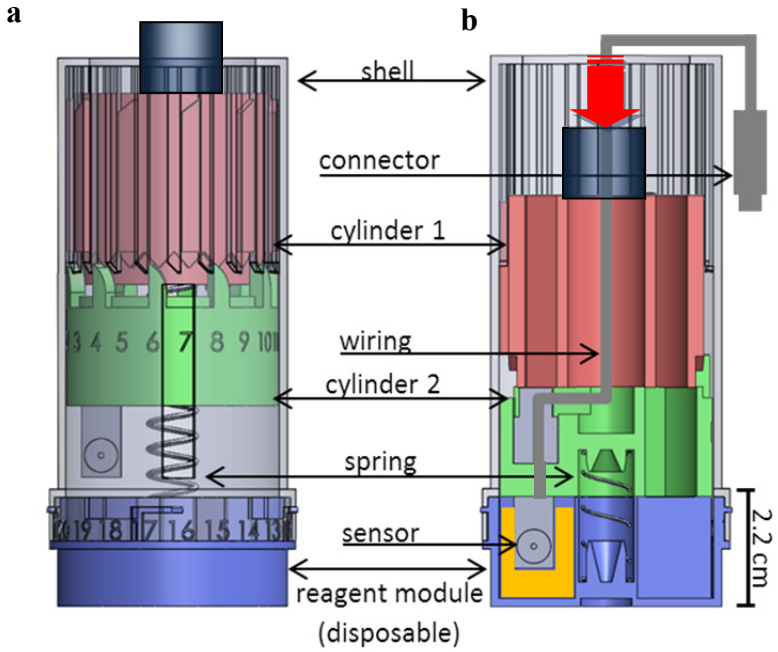

Figure 1: Cartridge for automated pesticide detection. The ball pen mechanism for rotating and dipping the sensor is realized by a shell, two cylindrical elements and a spring. a) Switching position: The spring is not compressed and the sensor does not contact reagents. B) Incubation position: The Spring is compressed, activated manually or by a single axis coordinator and the sensor is dipped into a reagent/sample for incubation.

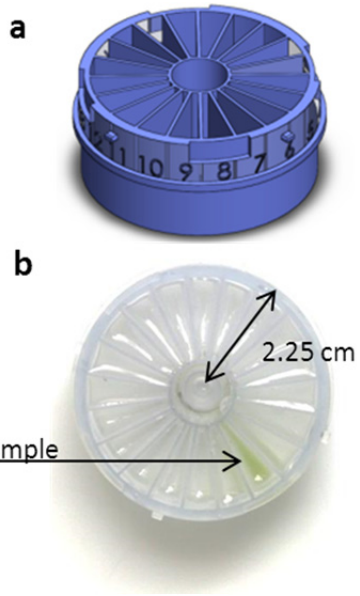

Figure 2: Reagent module as 3D-drawing (a) and as a prototype (b) filled with reagents and sample. For pre-storage of reagents, the module can be sealed by an easily removable foil like a yoghurt cup.

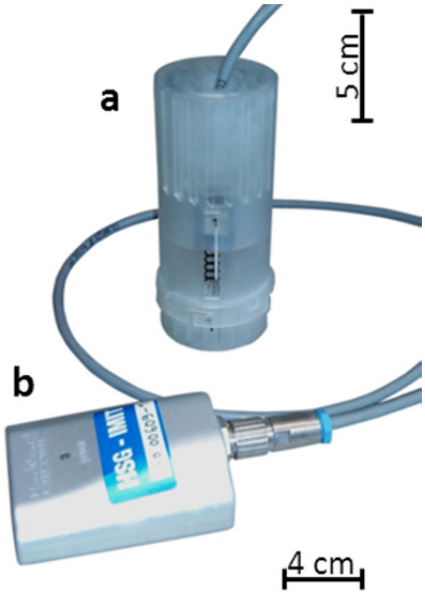

Figure 3: Prototype of assembled cartridge produced by stereolithography (a) and the potentiostat EmStat[2] (b).

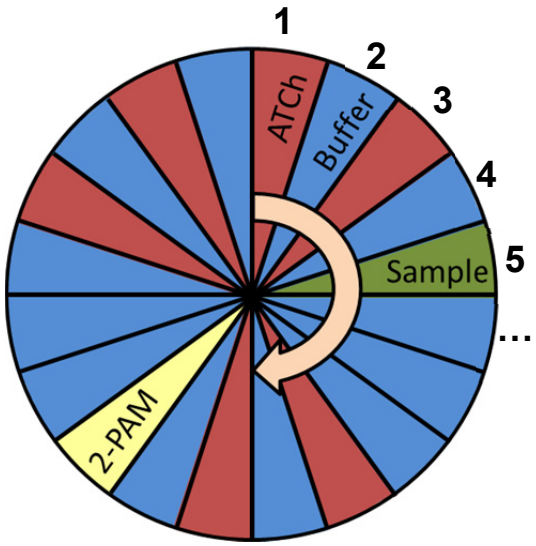

Figure 4: Order of reagents for one assay in the prefilled reagent module. The arrow indicates the start point and the rotational direction of the sensor. ATCH (red): Acetylthiocholine, substrate for the enzyme reaction; Buffer (blue) for washing the sensor; Sample (green): Milled cucumber; 2-PAM (yellow) praldidoxime, regeneration reagent. The numbers show the process steps for the ball-pen mechanism.

RESULTS AND DISCUSSION

The result of the ball pen mechanic actuated dipping assay for pesticide detection is plotted in figure 5. When the sensor is dipped into substrate, a current signal can be observed, which decreases when the sensor is dipped into buffer again, resulting in a current peak. A repetition of this sequence at each condition proofs the reproducibility of the signal.

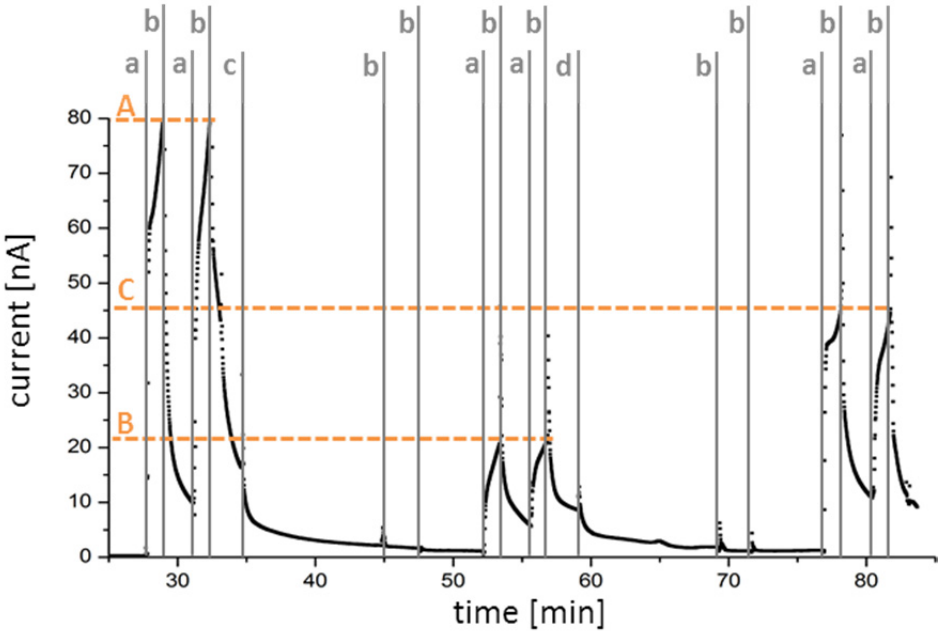

Figure 5: Results of a complete assay performed in a cartridge with cucumber sample. The current signal reflects the enzyme acticity of AChE before inhibition with pesticide (A), after incubation in a real food sample containing pesticide (B), and after regeneration with pralidoxime (C). Activation (a), washing (b), incubation in sample (c) and incubation in pralidoxime (d) were performed by manual actuation of the ball pen mechanism for a proof of concept. The incubation was realized by fixing the incubation position with a small stick.

After 10 minutes of sensor incubation in milled cucumber with pesticide chlorpyrifos-oxon (10^{-6} M) and three washing steps, the peak height decreases down to 26 % of initial activity, indicating the presence of pesticides. Consecutive regeneration of the enzyme for 10 minutes in pralidoxime followed by another three washings of the sensor restore the enzyme activity to 56 % of the activity before inhibition. This provides evidence that the inhibition was specifically caused by OPP. However, a part of the inhibition seems to be unspecific, since the signal does not reach the initial activity again. Nevertheless, detection of pesticide is possible under these conditions. Improving the washing or using another sensor could result in improved performance of the system. A dependence of matrix effects on the kind of fruit or vegetable is assumed and will be further investigated.

CONCLUSION

Our new dipping platform is applied to automated pesticide analysis from real food samples. A robust mechanical operation including 20 dipping steps was demonstrated. Since no microfluidic channels are included, any blocking is avoided and only minor sample preparation is required (homogenizing, setting of pH) [3]. Our new platform can also be used for further biological analysis in all assays that require the transfer of a solid phase into different liquid phases. Potentially, immunoassays, DNA extractions, and applications on magnetic beads can be implemented.

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