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An analytical Task: A miniaturized and Portable μConductometer as a Tool for Detection of Pesticides

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1. Introduction

Biological background: Chloride ion sensing is important in many fields such as clinical diagnosis (Huber et al., 1998; Jiang et al., 1998) environmental monitoring (Huber et al., 2000; Martin & Narayanaswamy, 1997; Montemor et al., 2006) and various industrial applications (Babu et al., 2008; Badr et al., 1999). Considering the fact that chloride channels play crucial role in physiological processes it is not surprising that missregulation of chloride ion transport by these channels can cause serious disorders. Cystic fibrosis is a disease in which gene encoding of a protein, called cystic fibrosis transmembrane regulator, which functions as a chloride channel in epithelial membranes, is mutated and thus its function is altered (Ratjen & Doring, 2003). Besides the importance of monitoring chloride ions in patients with this disease, monitoring chloride ion in the environment is needed.
Building industry issue: Chloride ions content in concrete plays important role in the quality of reinforced concrete, as these ions induce depassivation of the steel rebar and initiation of the corrosion process leading to degradation of the structure. Chloride ions in concrete come from cement, aggregate materials and water used for creating concrete, or by diffusion of chloride ions from outside of the structure through water pores in the concrete. Determination of chloride ions in materials for concrete is thus necessary (Junsomboon & Jakmunee, 2008; Montemor et al., 2006). The content of chloride ions in waters is also monitored well (Hahn, 2005; Sebkova, 2003).
Environmental issue: Besides, according to proverb "God created 90 elements, man around 17, but Devil only one – chlorine", it is obvious that this element is also a component of substances very harmful to the environment, which are called pesticides. Organophosphate, carbamate and organochlorinated compounds belong to the most widely used organic pesticides. Principal attention of many scientists has been paid to organochlorinated hydrocarbons, which affect the nervous and respiratory system and, unfortunately, persist in the environment. In developed countries their use is strictly regulated, whereas new

technologies to remove their residues are searched for (Francova et al., 2004; Chroma et al., 2002; Chroma et al., 2003; Kucerova et al., 1999; Kucerova et al., 2001; Leigh et al., 2006; Sudova et al., 2007; Villacieros et al., 2005). Nevertheless, in developing countries there is no restriction on the use of these substances. Therefore monitoring of environment pollution by pesticides is still concern in both developed and developing countries (Fig. 1).

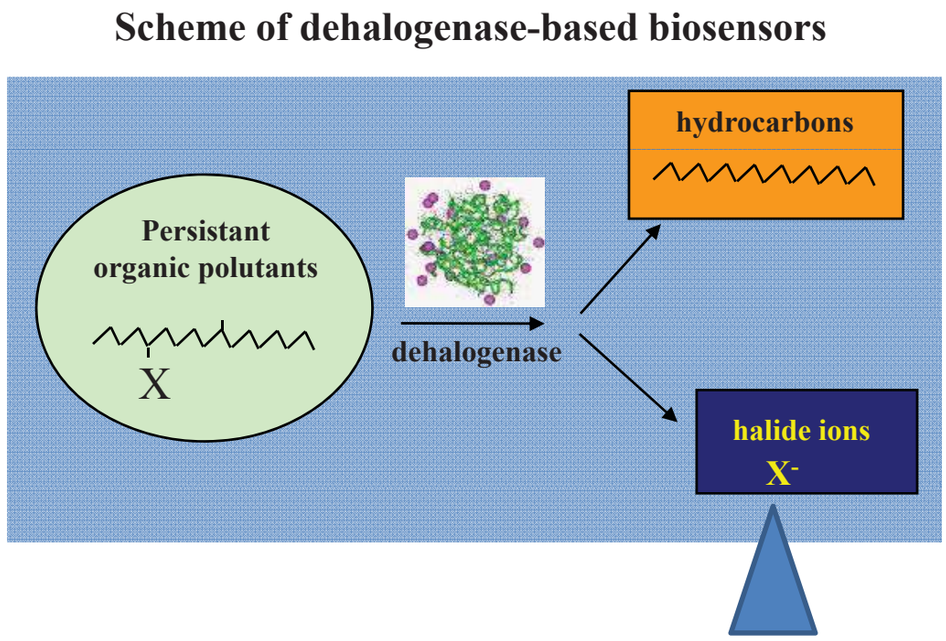


Fig. 1. Scheme of dehalogenase-based biosensors. Dehalogenase is able to cleave persistent organic pollutant (POP) into hydrocarbon and halide. The halide ion is subsequently detected by the appropriate detector.

Analytical task: Development of low cost and sensitive methods for the determination of pesticides in the environment is needed (Andrade-Eiroa et al., 2002; Heinisch et al., 2005; Heinisch et al., 2004; Taylor & Grate, 1995; Wegner et al., 2005). For direct detection of these molecules hyphenated techniques such gas or liquid chromatography with mass spectrometry (Alder et al., 2006; Hercegova et al., 2007; Hernandez et al., 2005; Lacorte & Barcelo, 1996; Lambropoulou & Albanis, 2007; Popp et al., 1997; Rodriguez-Mozaz et al., 2007; Sancho et al., 2006), capillary electrophoresis (Boyce, 2007; Ravelo-Perez et al., 2006), thin layer chromatography (Sherma, 2007) coupled with various detectors are used. These methods are often time-consuming, demanding on high-cost instrumentation and most of all, lack any no possibility of miniaturization. However, recently discovered enzymes called haloalkane dehalogenases (Fig. 1) are involved in biochemical pathways enabling bacteria to utilize halogenated compounds via releasing halogen ion from the molecule of halogenated hydrocarbon (Bosma et al., 2002; Damborsky & Brezovsky, 2009; Jesenska et al., 2005; Nagata et al., 1997; Nagata et al., 2005; Pavlova et al., 2009; Prokop et al., 2003). Haloalkane dehalogenases are key enzymes in the degradation of synthetic haloalkanes that occur as soil pollutants (Janssen, 2004). The reaction mechanism of haloalkane dehalogenases was initially clarified by detailed crystallographic and site-directed mutagenesis analyses of DhlA from *Xanthobacter autotrophicus* GJ10 (Janssen, 2004). A catalytic triad (i.e., nucleophile-

histidine-acid) is essential for the reactions catalyzed by members of the α/β-hydrolase family. Amino acid residues for the catalytic triad of LinB were proposed to be D108, H272, and E132 on the basis of a site-directed mutagenesis analysis (Hynkova et al., 1999). A catalytic pentad is composed of a catalytic triad and two hydrogen bond-donating residues providing halide stabilization (Janssen, 2004). One out of the two residues involved in the halide binding, i.e., tryptophan localized directly next to the nucleophilic aspartate, is invariable. The second halide-stabilizing residue is represented by a tryptophan residue in DhlA (W175) or an asparagine residue in DhaA (N41) and LinB (Bohac et al., 2002; Nagata et al., 2007). This feature can be used for suggesting appropriate biosensors which detect released chloride, as shown by Mikelova et al. (Mikelova et al., 2008) and Murthy et al. (Murthy et al., 2010). Numerous analytical methods for chloride ions in a variety of samples have been developed, such as ion chromatography (Jeyakumar et al., 2008; Pereira et al., 2008), near-infrared spectrometry (Wu & Shao, 2006), spectroscopy (Philippi et al., 2007), light scattering (Cao & Dong, 2008), ion-selective electrode method (Babu et al., 2008; Junsomboon & Jakmunee, 2008; Kumar et al., 2006; Shishkanova et al., 2007), turbidimetric method (Mesquita et al., 2002) and flow based methods coupled with different detectors (Bonifacio et al., 2007; Junsomboon & Jakmunee, 2008; Pimenta et al., 2004; Trnkova et al., 2008). The main aim of this paper was to propose, fabricate and test new conductometer for the fast and sensitive detection of chloride ions. Moreover, we coupled the conductometer with haloalkane dehalogenase, enzyme able to cleave chlorinated chemicals, in order to detect pesticides (Fig. 2).

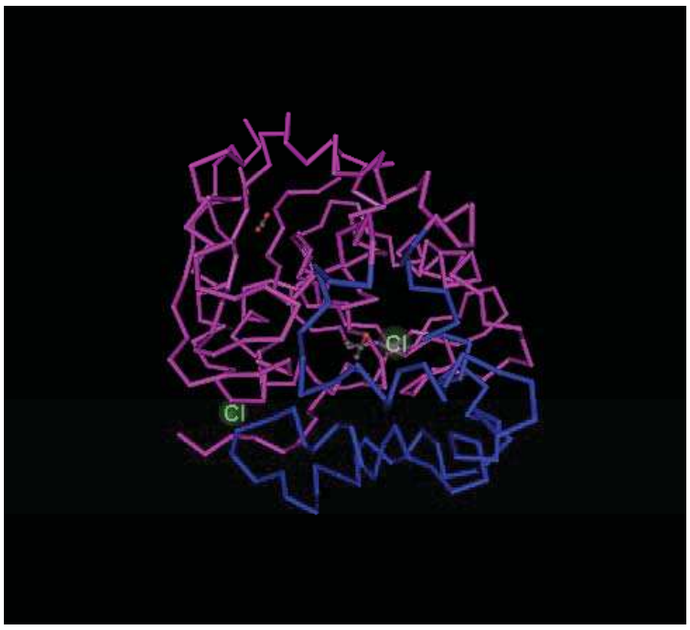


Fig. 2. Structure of haloalkane dehalogenase mutant Dha15 (I135fC176Y) from *Rhodococcus rhodochrous;* PDB ID: 3FWH, Gavira JA, Stsiapanava A, Kuty M, Dohnalek J, Lapkouski M, Kut I, Smatanova, 2009/1/18 . The figure prepared using the program Cn3D of the National Center for Biotechnology Information.

2. Experimental section

2.1 Chemicals, material and pH measurements

The chemicals used were purchased from Sigma Aldrich Chemical Corp. (USA) in ACS purity unless noted otherwise. The stock standard solutions were prepared with ACS water (Sigma-Aldrich, USA) and stored in the dark at -4 °C. Working standard solutions were prepared daily by dilution of the stock solutions. The pH value was measured using WTW inoLab Level 3 with terminal Level 3 (Weilheim, Germany), controlled by a personal computer program (MultiLab Pilot; Weilheim, Germany). The pH-electrode (SenTix-H, pH 0–14/3M KCl) was regularly calibrated by set of WTW buffers (Weilheim, Germany). Deionised water underwent demineralization by reverse osmosis using the instruments Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) and then it was subsequently purified using Millipore RG (Millipore Corp., USA, 18 MΩ) – MiliQ water.

2.2 Designing the μconductometer

During the design process of the microchip, attention was paid to characteristics of the measurement method and the goal was to design such a microsystem structure as would use the advantages of this method. A block scheme is shown in Fig. 3A. The comb-like electrodes on the sensor are connected to a bridge of switches which allow to change polarity of the bias current flowing through the sensor controlled by CLK phase. Four subranges of the sensor currents were designed to induce sensor voltage drop on the sensor from 10 mV to 100 mV. Te offset compensation designed allows to compensate not just the opamp (operational amplifier) offset but also to eliminate the parasitic voltage drop at sensor switches.

2.3 Fabrication of the new μconductometer and sensors

The screen-printed comb-like Pt electrodes were fabricated as sensing part of the sensor (Fig. 3B). The screen-printed working electrode was fabricated using standard thick-film cermet paste on an alumina substrate with dimension 25.4 × 7.2 mm. The paste used for leads and contact pads was AgPdPt based paste type ESL 9562-G (ESL Electroscience, UK). The working electrode was fabricated from carbon paste BQ-221 (DuPont, USA). Isolation was made from dielectric paste ESL 4913-G (ESL Electroscience, UK). Prior to measurement with electrically modified sensors, an optical analysis of platinum layer on the sensor was carried out. The measurements were performed on a confocal laser microscope LEXT (Olympus, Germany). The electrode was connected to ASIC microchip performing bipolar pulse technique, which was implemented into new conductometric instrument controlled by microcontroller including A/D and D/A converters and UART circuits assuring USB data loading (Fig. 3C). Moreover, impedance precise analyzer Agilent 8492A (EIS, USA) controlled by LabView program was utilized for Electrochemical Impedance Spectroscopy and calibration of the fabricated instrumentation.

2.4 Enzyme

Haloalkane dehalogenase (E.C. 3.8.1.5.) was donated by Prof. Jiri Damborsky from Loschmidt Laboratories, Faculty of Science, Masaryk University, Brno, Czech Republic. Briefly about these enzymes: haloalkane dehalogenases make up an important class of enzymes that are able to cleave carbon-halogen bonds in halogenated aliphatic compounds. There is a growing interest in these enzymes because of their potential use in

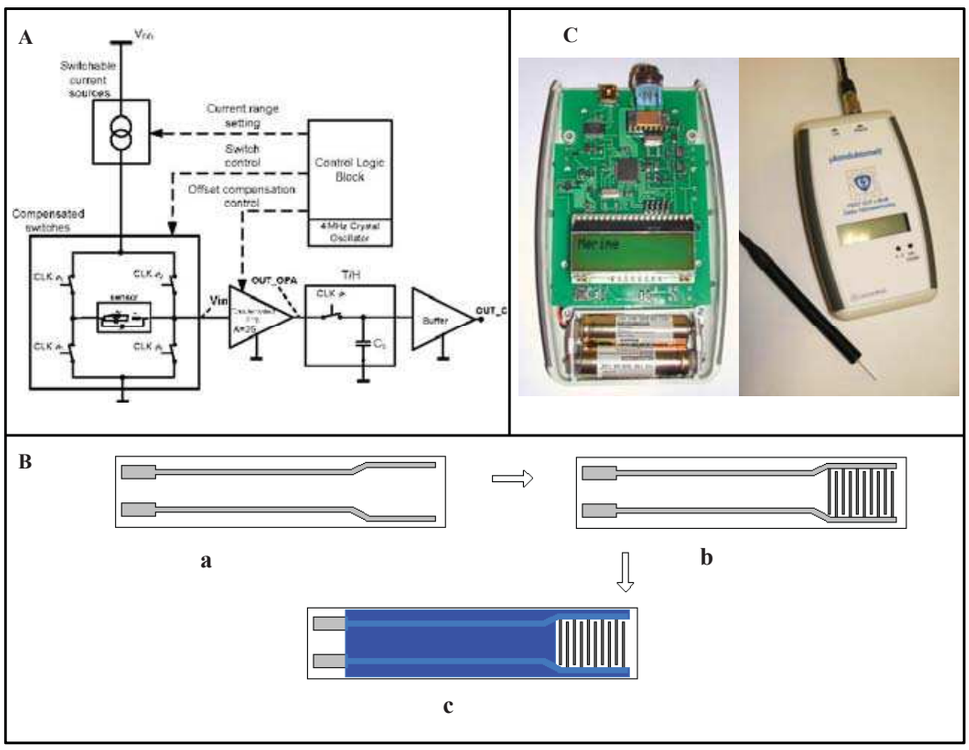


Fig. 3. Home made μconductometer. (A) A block scheme of the bipolar technique implemented into the microchip. (**B**) Fabrication of the sensor: a: conductive layer, b: electrodes, c: double covering layer. (**C**) Hand-held instrumentation with the chip designed (ASIC).

bioremediation, as industrial biocatalysts, or as biosensors. Structurally, haloalkane dehalogenases belong to the α/β-hydrolase fold superfamily (Ollis et al., 1992). Without exception, haloalkane dehalogenases contain a nucleophile elbow (Damborsky, 1998), which is the most conserved structural feature within the α/β-hydrolase fold. The other highly conserved region in haloalkane dehalogenases is the central β-sheet. Its strands, flanked on both sides by α-helices, form the hydrophobic core of the main domain that carries the catalytic triad Asp-His-(Asp/Glu). The second domain, consisting solely of α-helices, lies like a cap on top of the main domain. Residues at the interface of the two domains form the active site. Whereas there is significant similarity in the catalytic core, the sequence and structure of the cap domain diverge considerably among different dehalogenases. The cap domain is proposed to play a prominent role in determining substrate specificity (Kmunicek et al., 2001; Pries et al., 1994b). A reaction mechanism for haloalkane dehalogenase has been proposed on the basis of x-ray crystallographic (Verschueren et al., 1993), site-directed mutagenesis (Pries et al., 1995; Pries et al., 1994a), and kinetic (Schanstra & Janssen, 1996; Schanstra et al., 1996) studies with the haloalkane dehalogenase of Xantobacter autotrophicus GJ10 (DhlA). Catalysis proceeds by the nucleophilic attack of the carboxylate oxygen of an aspartate group on the carbon atom of the substrate, yielding displacement of the halogen as halide and formation of a covalent alkyl-enzyme intermediate. The alkyl-enzyme intermediate is subsequently hydrolyzed by a water molecule activated by the histidine. A catalytic acid (Asp or Glu) stabilizes the charge developed on the imidazole ring of the histidine during the hydrolytic half-reaction.

2.5 Enzymatic experiment

Enzyme haloalkane dehalogenases were dissolved (7.6 g/mL) in phosphate buffer 0.1 mM with pH 7.6. the Dissolved 1-chloro-hexane (as pesticide substitute) in the phosphate buffer was added to enzyme solution and tempered 1 hour at 38°C according to (Mikelova et al., 2008), which ensured all hexane molecules to enter into reaction with enzyme. Samples of this analyte were dropped into buffer with 5 min intervals.

3. Results and discussion

Primarily, a attempt to test our hypothesis on suggesting conductivity-based biosensor for detection of pesticides was made. In this experiment, we used WTW conductometer (Wissenschaftlich-Technische Werkstätten - WTW, Weilheim, Germany). The measurements were carried out with InoLab Cond 730 coupled with conductivity cell TetraCon 325. The instrument was firstly used for detection of various concentrations of chloride ions. The logarithmic calibration curve is shown in Fig. 4A. The dependence was linear with R^2 above 0.9. Further, we were interested in the issue whether the instrument could be able to detect changes in resistance depending on addition of haloalkane dehalogenase (500 nM) and its substrate as 1-chlorohexane (H_3C-$(CH_2)_5$-Cl) (Fig. 4B). It clearly follows from the results obtained that the additions of the substrate with different volumes have detectable influence on the resistance. Moreover, we investigated dependence of the resistance on time of the reaction. The results obtained are shown in Fig. 4C. Almost all substrate was cleaved up to 30 min. It can be concluded that coupling of conductometer and haloalkane dehalogenase can be used for detection of pesticides.

3.1 µConductometer

In the following experiments, we aimed at suggesting, fabricating and testing such a home-made conductometer for similar purposes, as would have better parameters than InoLab Cond 730 coupled with conductivity cell TetraCon 325. The bipolar pulse technique was originally described in 1970 for measurements with conventional electrodes (Johnson & Enke, 1970). Nowadays, the technique is applied with microelectrode systems (Pumera et al., 2006). The first integration of the technique into microchip came in 2002 in our laboratories using AMIS technology. A new microchip with improved parameters was designed and fabricated in 2006. To detect chloride ions we used newly designed instrument carrying out bipolar pulses (Fig. 3). The method of bipolar pulse consists of applying two consecutive voltage pulses of equal amplitude and pulse width, but of opposite polarity, to a cell and then measuring the cell current precisely at the end of the second pulse. The method directly measures conductance as in the AC technique of measurements. With this measurement we can reach 0.01% accuracy if the pulse widths of both pulses are equal to within 1% and the double layer voltage is kept to less than 1% of the applied voltage (Johnson & Enke, 1970). Another advantage of bipolar pulse measurements is the elimination of capacitance as the double layer capacitance and the faradaic capacitance. The width of pulses should be determined to minimise the error caused by non-capacitive part of the impedance. The instrument measurement and calibration using resistor was performed with United Kingdom Accreditation Service calibration certificate. The error at range 3 and 4 is below 1 %, at range 2 below 1.4 % (Fig. 5). The lowest range error is much higher because of a circuit fault on the microchip. This lowest range is not necessary for our measurements.

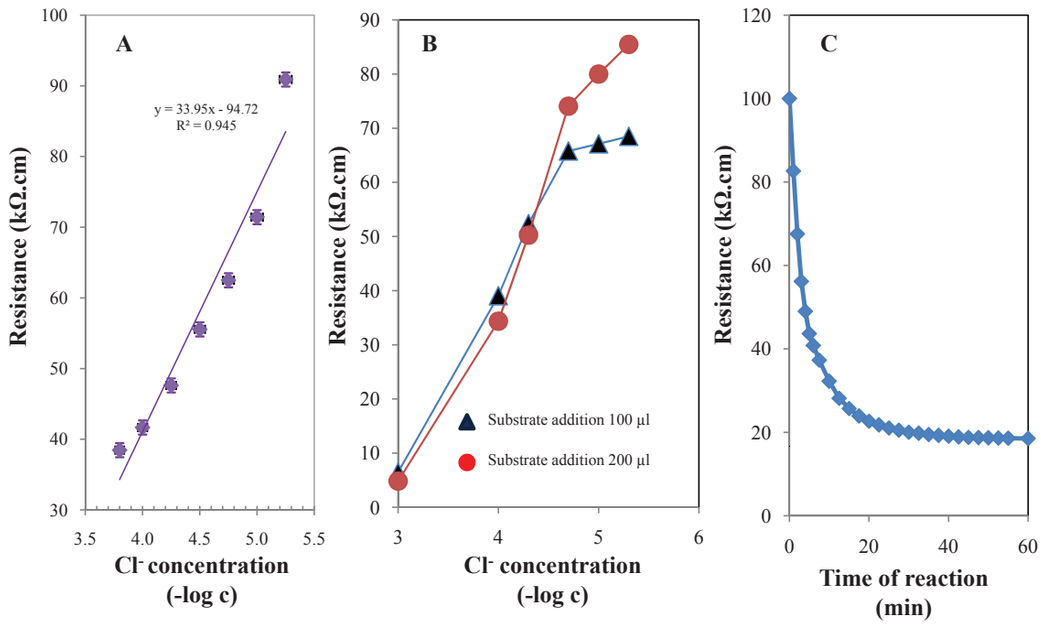


Fig. 4. WTW measurements. (**A**) Dependence of resistance on logarithmic concentration of chloride ions. (**B**) Dependence of resistance on two additions (100 and 200 µl) of the substrate (1-chlorohexane). (**C**) Dependence of resistance on time of reaction of haloalkane dehalogenase (500 nM) with 1-chlorohexane (5 µM).

3.2 Fabrication and characterization of sensors coupled to the µconductometer

We used screen-printed comb-like Pt electrodes as a sensor. The goal was to design the microsystem structure and during the design process of the microchip attention was paid to characteristics of the measurement method. The comb-like electrodes on the sensor are connected to a bridge of switches which allow to change polarity of the bias current flowing through the sensor. Four sub-ranges of the sensor currents were designed to induce sensor voltage drop on the sensor from 10 mV to 100 mV. The offset compensation designed allows to compensate not just the opamp (operational amplifier) offset but also to eliminate the parasitic voltage drop at sensor switches.

Prior to measurement with electrically modified sensors, an optical analysis of platinum layer on the sensor was carried out. The measurements were performed on a confocal laser microscope LEXT. It uses a laser for the screening of a surface point by point. Superimposing of measured data provides sharp images with magnification of up to 14 400 × with a resolution of 0.12 µm. In addition, this device allows easy measurement of surface relief. The anode of the electrode showed in Fig. 6A was galvanized (the comb at the top of the figure), but the cathode was not galvanized (the comb in the middle of the figure). the galvanized platinum was black, unlike the printed platinum, which had a silver-grey colour. To better assess the surface, measurements with higher magnification were needed. These measurements showed that the deposited layers were homogeneous and smooth (Fig. 6B). Under more careful treatment of the observed figures, it was possible to identify scales that originated on the surface. They hold tight, because the whole sensor was rinsed thoroughly with demineralized water after galvanization.

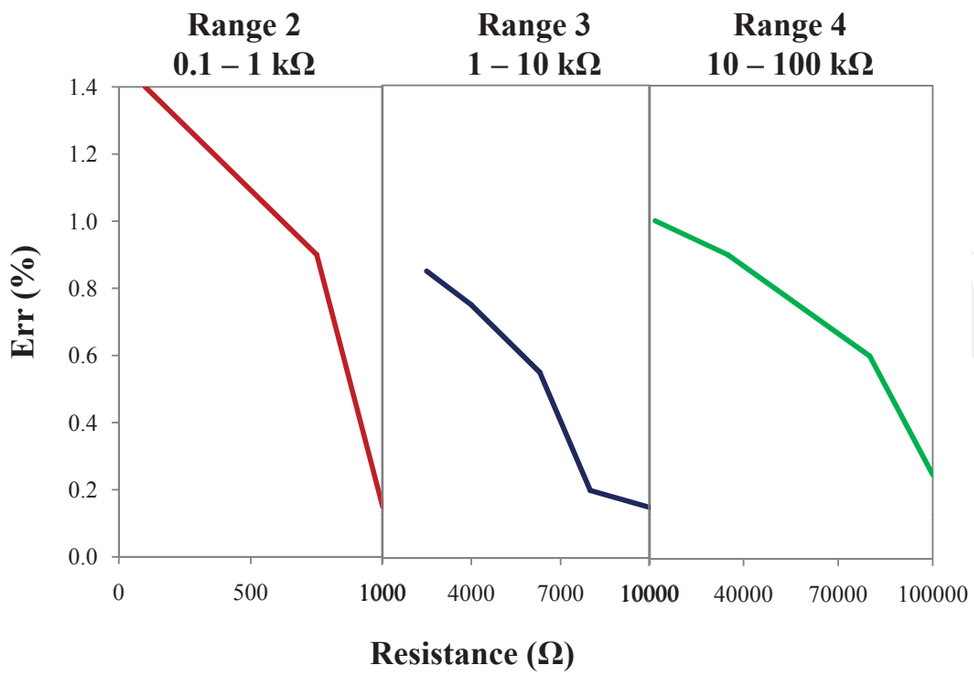


Fig. 5. Home made µconductometer. Errors of resistance measurements at three ranges measured by a µconductometer.

By contrast, a non-galvanized electrode surface is completely different. There is a large grain size and porosity of the surface evident (Fig. 6C). After galvanizing the surface porosity is not seen. The flakes, which were seen in the previous figure, are probably only bright point on the surface, as can be seen from the centre of Fig. 6D. To improve the equality of electrode surface ultrasound was used during galvanizing. After starting the electrolysis at the plated surface hydrogen begins to develop and this hydrogen evolution hinders the whole process. Ultrasonic vibration releases gas bubbles from the surface and the deposited layers are cleaner and smoother. Therefore, we used this procedure for the preparation of electrodes in the following part of the experiment.

3.3 Characterization of the µconductometer and fabricated sensors

The characterization of screen-printed sensor has been done under chlorides ions measurements using electrochemical impedance spectroscopy and NaCl analyte (from 100 nM to 1 mM). It was found that electrodes with two fingers showed instability and very poor reproducibility with standard deviation of measurement higher than 45 %. It confirms the results described in (Jacobs et al., 1995), where the drawback causing this error is well explained. Multiple numbers of fingers are eligible for precise and reproducible measurement. The reproducibility was increased by galvanic plating of black platinum on the total sensing area of electrodes. The diffusion part (Warburg impedance) becomes significant with increasing concentration of conductive ions because of very small interelectrode distance of the electrode structure. The phosphate buffer with concentration of 1 mM was found as optimal for measurement according to measured conductivity (resistance) about 2 kΩ. The sensor sensitivity as rate R/c (c - concentration of NaCl) was

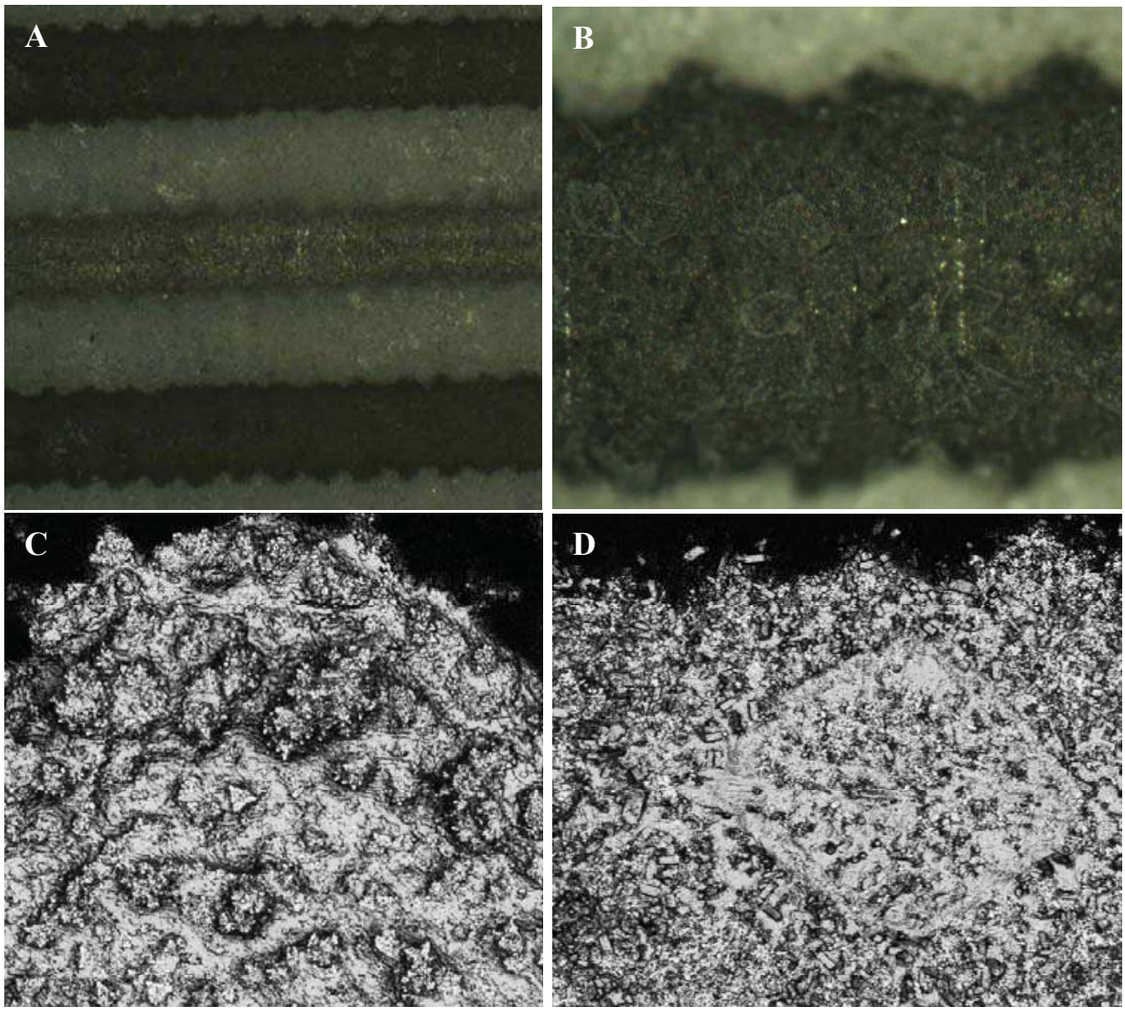


Fig. 6. Home made μconductometer. Comparison of surfaces of (A) galvanized and (B) non-galvanized electrodes. Detail galvanized electrode surface. Surface detail of (C) non-galvanized electrode and (D) galvanized electrode.

determined from calibrating curve for 20 kHz measuring sinusoidal signal and NaCl concentration changes in buffer. Determined sensitivity -2.2 $\Omega \cdot \mu M^{-1}$ (R^2=0.995) was obtained by recalculating to chloride ions.

3.4 Detection of pesticides

Many species of chemotrophic bacteria contain enzymes (dehalogenase) which are able to release halides from the molecule of halogenated hydrocarbon. Haloalkane dehalogenase (E.C. 3.8.1.5.) can be used to cleave hydrolytically the carbon-halogen bond forming corresponding alcohol, halide anion and a proton (Janssen et al., 1994). These enzymes can be utilized for detection of pesticides made of chlorinated hydrocarbons. The halide released by the enzyme can be detected by using an electrochemical detector, as it was shown above. It is known that the enzymatic activity can be affected by many factors like buffer composition, pH, ionic strength and temperature. To detect the halogenated hydrocarbons,

the haloalkane dehalogenase LinB from bacterium *Sphingobium japonicum* UT26 was used (Nagata et al., 1997). The experimental conditions for the highest activity of this enzyme described by Nagata et al. had to be modified in our experiments, because the presence of glycine buffer (pH 8.6) used for measurement of kinetic properties of LinB is not suitable for electrochemical analysis due to high background signal and reduced sensitivity of the electrochemical response. The glycine buffer was therefore replaced with for borate buffer pH 7.6. Under these experimental conditions, the enzymatic release of chloride from chlorinated hydrocarbon 1-chlorohexane (1 mM) was investigated. Detection of cleaved chloride ions was carried out with two instruments: i) a commercial R-C meter Agilent 4284A and ii) a home-made μconductometer as mentioned above.

To verify the correctness of theoretical assumptions on which the measurement is based, it was necessary to carry out specific measurements. The enzyme itself is very conductive and the addition of this biologically active compound to the buffer at a concentration, which ensures efficient progress of reaction, resulted in a decrease of resistance down to 150 Ω. The decrease in conductivity caused by the chlorid anions themselves, which are released from the pesticide, is not so great because a pesticide is supplied in solution in very small quantities. The measurability of the pesticide concentration in the solution would thus be easily questionable, as already mentioned increase in conductivity could be described as one caused only by the enzyme, rather than pesticides. Therefore, we carried out measurements in an environment of pure buffer to which the enzyme was added and, then, a substance representing the pesticide 1-chlorohexane.

The main obstacle consists in the measurement of enzyme activity with 1-chlorohexane. It is necessary to maintain a high temperature of the solution throughout the reaction for relative long time (about 50 minutes). Moreover, it was necessary to close the entire reaction system due to volatility of 1-chlorohexane. The possible release of 1-chlorohexane into the environment could significantly decrease its concentration in reaction solution. To test μconductometer, three concentrations of 1-chlorohexane, 500 nM, 5 and 50 μM to evaluate the detectable range and two concentrations of the enzyme (100 and 500 nM) to test the hypothesis of the enzyme as a catalyst depending on the quantity were chosen.

The measured course (marked with pink colour, Fig. 7) means a solution having a concentration of the enzyme (500 nM) and 1-chlorohexane (50 μM) and was supposed to verify the accuracy of measurements. It follows from the results obtained (part II) that the addition the enzyme caused a decrease of resistance of 161 Ω. Further decrease in resistance (39 Ω) in part III of the Figure was caused by chloride ions released from 1-chlorohexane. The second course (marked in violet, Fig. 7) is measured with the same concentration 1-chlorohexane, but with five fold lower concentration of added enzyme (100 nM). The purpose of the measurements was to demonstrate that the same amount 1-chlorohexane can be cleaved as a substantially smaller amount of the enzyme and thus the decrease in resistance by the enzyme itself can be avoided. The decrease in resistance caused by the enzyme is 33 Ω and the decrease in resistance caused by chlorine ions released from 1-chlorohexane was 17 Ω. The difference compared to the chlorine-based decrease in the first measurement with the higher concentration of the enzyme is probably caused by lower concentration of the enzyme. This presumption is also supported by lower rate of reaction because the curve is not as steep as in the previous case. The last two curves (marked in blue and red, Fig. 7) represent the situation when we added the enzyme as 500 nM, but concentrations of 1-chlorohexane were much lower (500 nM and 5 μM). The Decrease in the resistance of the solution after adding the enzyme was 149 Ω, after adding 1-chlorohexane was 8 and/or 10 Ω for 500 nM and 5 μM of 1-

chlorohexane, respectively. The results are in good agreement with those measured by WTW instrument (Fig. 4). Moreover, bipolar pulse measurements have a greater sensitivity compared to WTW instrument opening a window for other applications.

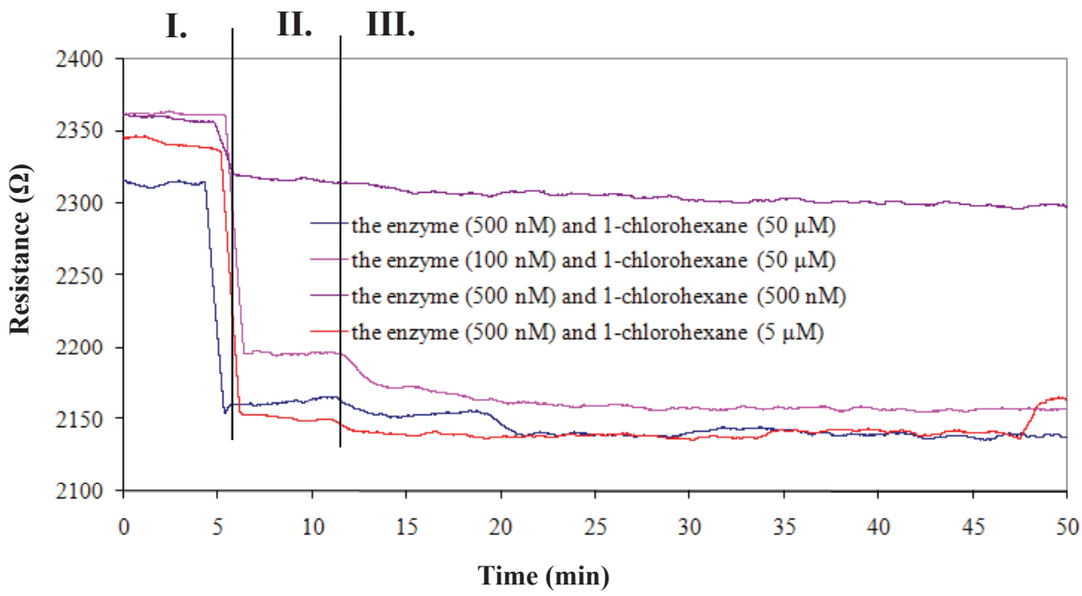


Fig. 7. Home made μconductometer. Dependence of resistance of various solutions consist of the enzyme and 1-chlorohexane on the time of the reaction.

Further, we calibrated the entire system. The measured calibration curve for the solution enzyme-1-chlorohexane with changing 1-chlorohexane concentration given on the x-axis has the following slope-intercept equation: y = 5.84 x (concentration of the enzyme – 100 nM; concentration of 1-chlorohexane – 500 nM, 1 μM, 5 μM, 10 μM and 50 μM). Subsequently, we plotted the dependence of resistance on the concentration of the enzyme itself with the following slope-intercept equation: y = 0.268 x (concentration of the enzyme – 10 nM, 100 nM, 500 nM and 1,000 nM). When comparing the difference between measurements with addition of pure enzyme and enzyme-1-chlorohexane, slopes of both curves were different. This means that despite the very high conductivity change caused by the enzyme itself we are able to detect reaction with 1-chlorohexane, because with the increasing concentration of cleaved 1-chlorohexane resistance decreases rapidly. Moreover, we compared the results obtained with those obtained with Agilent 8492A and WTW and we found that they instrument were in good agreement. Moreover we found a high positive correlation (r = 0.954) between results obtained.

4. Conclusion

A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. Simple biosensors are of great development and would be considered as analytical tools in 21st century, which could be used for both diagnostic and environmental monitoring purposes (Bidmanova et al., 2010; Cagnin et al., 2009; Caruana & Howorka, 2010; Ferreira et al., 2009; Gawel et al., 2010;

Kurzawa & Morris, 2010; Lee et al., 2010; Libertino et al., 2009; Murthy et al., 2010; Nayak et al., 2009; Ronkainen et al., 2010; Roy & Gao, 2009; Scarano et al., 2010; Vikesland & Wigginton, 2010; Yoo & Lee, 2010). The main advantage of these tools is their rapidity. This feature is very important for operational decisions in the event of natural or other emergencies. In this study, simple μconductometer was fabricated and tested. the results of comparison of the Agilent 8492A and WTW instrument with bipolar pulses technique confirm that this new instrumentation gives similar results. The resistance discrepancy for EIS was determined 2 Ω indicating detection limit about 1 μM of chloride ions. The new instrumentation reaches a discrepancy of 4 Ω determining detection limit about 2 μM of chloride ions released by reaction between enzyme and pesticide. Based on this, we were able to determine detection limit for pesticide as 100 nM.

5. Acknowledgements

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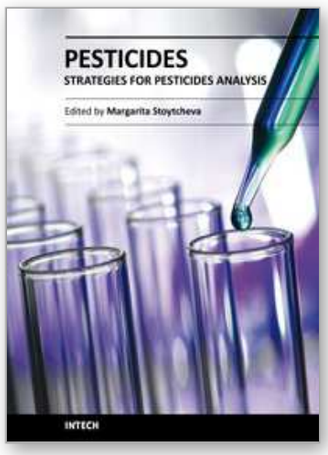
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This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

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