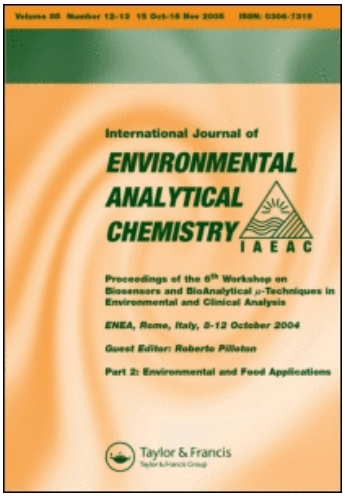

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Engineering a continuous flow electrochemical micro-cell for biosensor applications: new achievements

Mihaela Ilie[ab]; Remo Dejana[a]; Vittorio Foglietti[a]; Roberto Renda[c]; Luigi Nardi[d]; Amedeo Masci[d]; Bruno Lanza[e]; Maria Rita Montereali[d]; Livia Della Seta[d]; Walter Vastarella[d]; Roberto Pilloton[d]
[a] CNR/IFN, Rome, Italy [b] Univ. Politehnica Bucuresti, 161071 Bucharest, Romania [c] Università degli Studi di Torino, 10124 Torino, Italy [d] ENEA, Rome, Italy [e] ENEA, Portici (Napoli), Italy

Online publication date: 09 December 2009

To cite this Article Ilie, Mihaela , Dejana, Remo , Foglietti, Vittorio , Renda, Roberto , Nardi, Luigi , Masci, Amedeo , Lanza, Bruno , Montereali, Maria Rita , Della Seta, Livia , Vastarella, Walter and Pilloton, Roberto(2010) 'Engineering a continuous flow electrochemical micro-cell for biosensor applications: new achievements', International Journal of Environmental Analytical Chemistry, 90: 1, 31 — 39
To link to this Article: DOI: 10.1080/03067310902795951
URL: http://dx.doi.org/10.1080/03067310902795951

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 1, 15 January 2010, 31–39

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Engineering a continuous flow electrochemical micro-cell for biosensor applications: new achievements

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[a]*CNR/IFN, Via Cineto Romano 42, Rome, Italy;* [b]*Univ. Politehnica Bucuresti, LAPI, Bulevard Iuliu Maniu 1-3, 161071 Bucharest, Romania;* [c]*Università degli Studi di Torino, Via Verdi 8, 10124 Torino, Italy;* [d]*ENEA, SP 061, C.R. Casaccia, Via Anguillarese 301, 00060 Santa Maria di Galeria, Rome, Italy;* [e]*ENEA, C.R. Portici, Via Vecchio Macello 80055, Portici (Napoli), Italy*

(*Received 1 December 2008; final version received 3 February 2009*)

The functional tests of a previously obtained continuous flow micro-cell revealed a rather low value of the sensitivity of the chronoamperometric measurements as well as a poor reliability of both fluidic and electric connections. The engineering of the micro-cell regards the improvement of the following characteristics: (1) integrating the reference electrode on the chip, (2) designing a specific pattern of the electrodes and (3) serially distributing them along the pipeline reservoir. Thus, a plug-flow device is obtained and the preliminary tests confirm its functionality together with an improved sensitivity. The use of photosensitive glass (Foturan®) for obtaining the reservoir and its vertical openings allowed an improvement of the transversal shape and thus of the fluid flow. Better connections are achieved by the development of more reliable and rigid glass fluidic connections as well as of flexible elastomer-based solderless electric connections combined with both a standard DIL40 socket and standard flat cable connectors.

Keywords: micro-cell; integrated metallic electrodes; flexible connections; continuous flow

1. Introduction

Biosensors, as functional analogues of chemoreceptors, are based on the direct spatial coupling of immobilised biologically active compounds, acting as a chemical recognition system, with a signal transducer and an electronic amplifier [1]. They use biological systems at different levels of integration to specifically recognise the substance to be determined. The first step of this recognition is the specific complex formation by interaction of the immobilised biologically active substance with the analyte. The physicochemical changes caused either by the complex formation or by the chemical conversion of the analyte (e.g. owing to enzymes) might be of electrical nature (charge or enzyme activity) and therefore they can be detected by means of

*Corresponding author. Email: mihaela.ilie@ifn.cnr.it

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902795951
http://www.informaworld.com

transducers, based on potentiometric or amperometric electrodes, that perform the second step of the recognition: transduction of the physicochemical effect into an electrical signal. In this way species as H^+, OH^-, CO_2, NH_3, H_2O_2 can be identified in a so-called electrochemical biosensor. Further on, the electric signal has to be amplified and processed. The electrochemical indication prevails over all other methods of transduction, due to their specificity, sensitivity, portability, speed, low cost and inherent miniaturisation [2,3]. Indeed, for nearly fifty years, potentiometric and amperometric enzyme electrodes are at the leading edge of biosensor technology [4,5]. Screen-printed electrodes have been widely used as transducers in electrochemical biosensors and their configuration applied in a number of applications [3–9]. The miniaturisation of the electrodes using techniques borrowed from the semiconductor industry and their integration in a micro-cell opens wide and interesting perspectives for the development of biosensors, offering the possibility to increase both the speed and the sensitivity of detection and to allow *in-situ* analysis and on-line monitoring [9,10]. Such a micro-cell, that works in continuous flow, has been previously developed by the authors [11]. Its functional tests have revealed some characteristics that should be improved: (a) sensitivity, (b) the reliability of both electric and fluidic connections. The causes of these drawbacks have been analysed and new technical solutions have been adopted having as a result a new engineered device.

2. Experimental

The electrochemical biosensor micro-cell consists of a set of working electrodes (WE), a counter-electrode (CE), and a reference electrode (RE) placed inside a reservoir provided with inlet and outlet openings. Electric connections allow the incoming and outgoing electric signals to be transmitted between the micro-electrodes and the external control unit. Fluidic connections allow the analyte solution to flow through the reservoir placed on the suction side of an external peristaltic pump. The improvements of each constructive part are presented below.

3. The micro-electrodes

The chrono-amperometric measurements have shown that the signal from each WE was influenced from the flow dispersion over the wide area (about 3 sq. mm) of the electrodes array. In order to improve the selectivity, the configuration of the WEs is now changed from an array to a line. This configuration of the WEs, that should be much better than before from the hydrodynamic point of view, allows the analyte to reach the electrodes one after the other, in a serial manner, minimising the effect of flow dispersion and increasing the height of the peaks obtained by chronoamperometry. The current measured on one electrode of the precedent micro-cell was about 10 nA and rather noisy; the size of a WE was of $0.005\,mm^2$ and that of one CE was of $0.02\,mm^2$, with a ratio of 1:16 between the surfaces of WE and CE. In order to improve the sensitivity and the signal-to-noise ratio, the area of CE and that of each WE are newly designed so that the WE/CE surface ratio reaches 1 : 50 (with a WE area of $0.010\,mm^2$). The reference Ag/AgCl electrode, with an active surface of $0.116\,mm^2$, is integrated on the chip. The patterns of the electrodes are presented in Figure 1a, b and c.

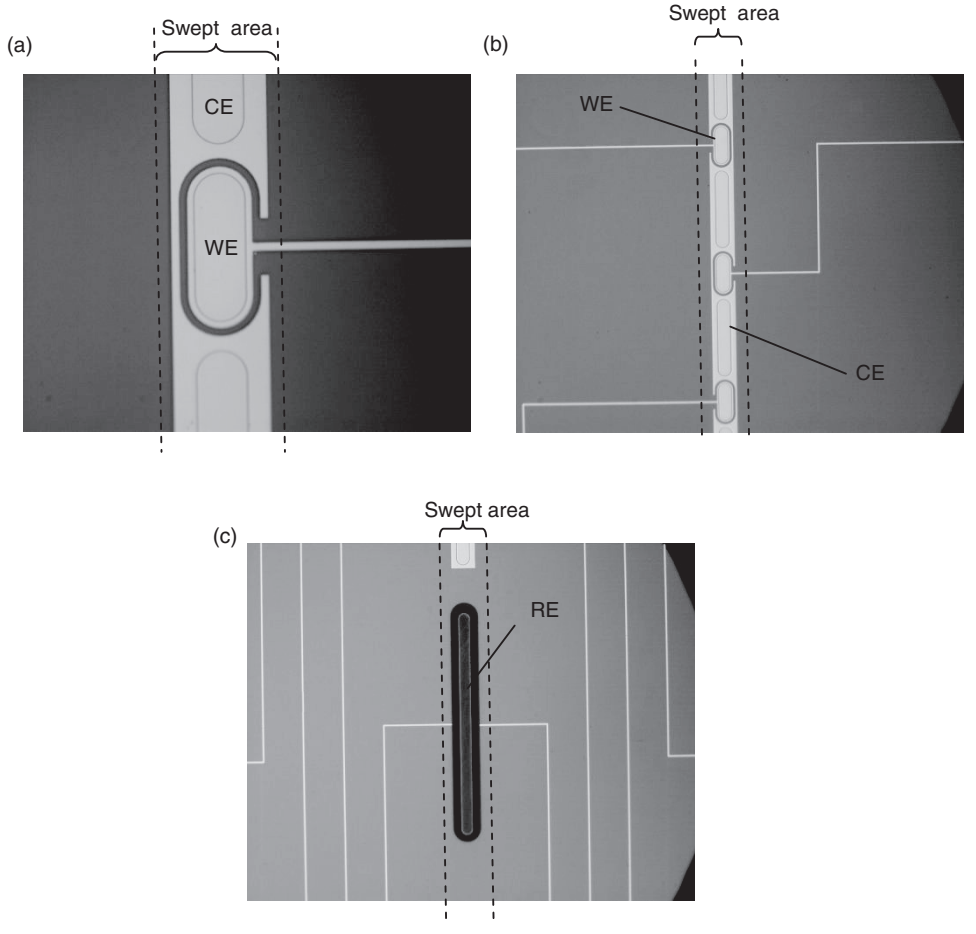


Figure 1. Design of the integrated electrodes. (a) Working electrode (WE). (b) Part of the counter electrode (CE) – connections not shown. (c) Reference electrode (RE). Optical micrograph; minimum line width 0.010 mm.

4. The reservoir

The serial configuration of the electrodes requires a pipeline-shaped reservoir to determine an improved plug-flow of the analyte liquid carrier, restricting the stream lines to a straight area. The previous reservoir had been obtained by isotropic double side etching of Borofloat® type glass [12]. That is why the profile of the opening had a variable diameter along its length with a minimum value in the middle, as one can see in Figure 2 (a = sketch, b = top view). A smoother profile requires an anisotropic etching process, as the wet etching of irradiated photosensitive glass is. We choose the photosensitive glass Foturan® [12] with which we obtained an aspect ratio of 1 : 30 when etching the irradiated glass in 10% HF solution. Due to glass transparency the UV exposure radiation reaches also the back side of the glass plate so that etched structures deeper than 0.25 mm cannot be obtained without penetrating the 0.5 mm plate. We adopted a sandwich solution for the reservoir and its openings. UV-exposure, thermal development and wet etching have been

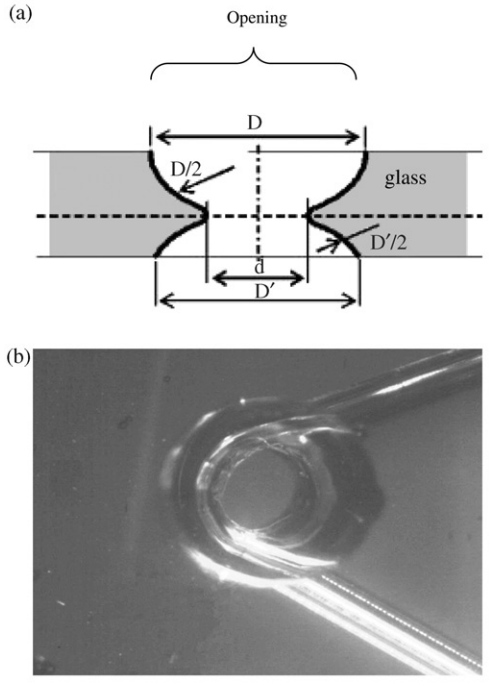


Figure 2. The profile of the opening obtained by means of isotropic wet etching of borofloat glass. (a) Sketch of the cross section. (b) Top view of the opening after etching.

performed on separate glass plates. The profile of the openings is definitely improved, as one can see in the cross section sketch presented in Figure 3a (1 – silicon chip with electrodes; 2 – reservoir; 3 – cover with openings) as well as in the top view (Figure 3b). The reservoir (4) and the cover (3) with openings are presented in the exploded view of etched opening (Figure 4a). The two parts have been glued over the chip (Figure 4b).

5. The fluidic connections

The previous fluidic multi-part adaptors allowed the capillaries to be attached to the openings by means of silicone and polyetilene-polypropilene parts inserted one into the other; the silicone polymer rubber adaptor was glued over the opening on the glass cover with room temperature vulcanising silicone; depending on the cutting conditions the surface of these adaptors achieved different types of channels (with either radial or helix shape) that facilitated the silicone glue to enter the opening and to create problems to the analyte stream lines [12]. Besides this, the glued connections were less reliable in time: with use, liquid leaks invaded the external electric connections on the chip and/or bubbles invaded the reservoir. In order to overcome these main disadvantages we replaced the multipart silicone adaptor with a single-part glass one, a mini-pedestal obtained from an ordinary glass micro-pipette by means of small scale glass blowing [12] (see Figure 4a–2). These home-made glass adaptors are glued with araldite onto the cover plate (Figure 4b–2). This strongly reduces the breakage risks related to the connection of the

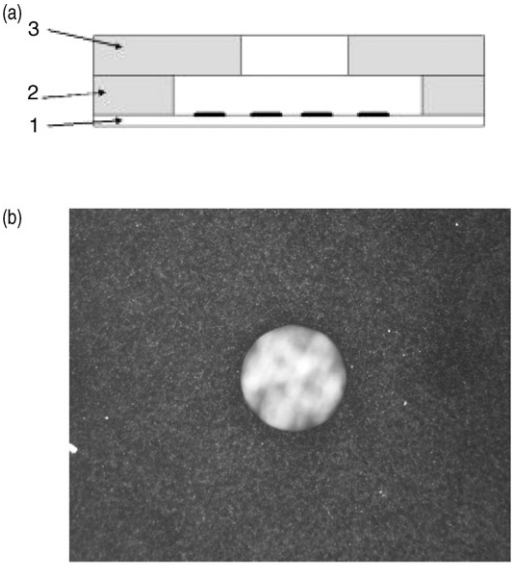


Figure 3. Profile of the opening obtained by means of anisotropic wet etching of photosensitive glass. (a) Sketch of the cross section, 1 – chip with electrodes, 2 – reservoir, 3 – cover with openings. (b) Top view of the opening after etching.

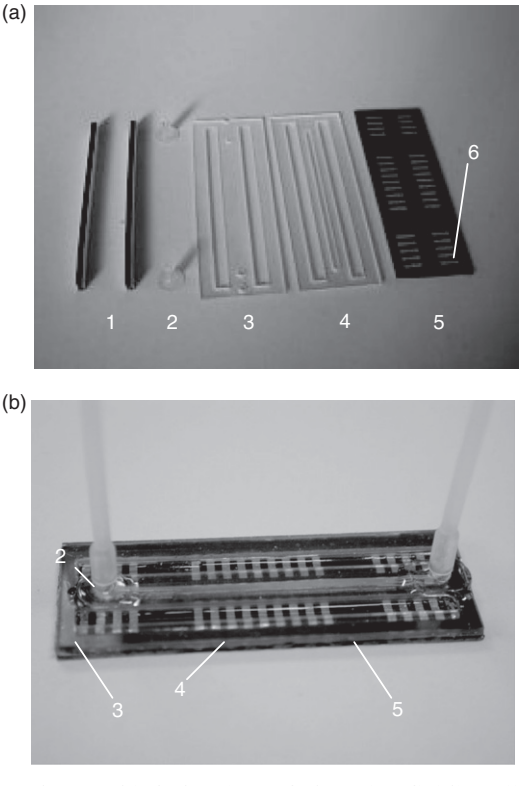


Figure 4. Exploded (a) and assembled (b) view of the microfluidic cell consisting of: 1-'zebra' connectors; 2-fluidic connectors; 3-cover with openings; 4-reservoir with channel; 5-chip with electrodes and pads-6.

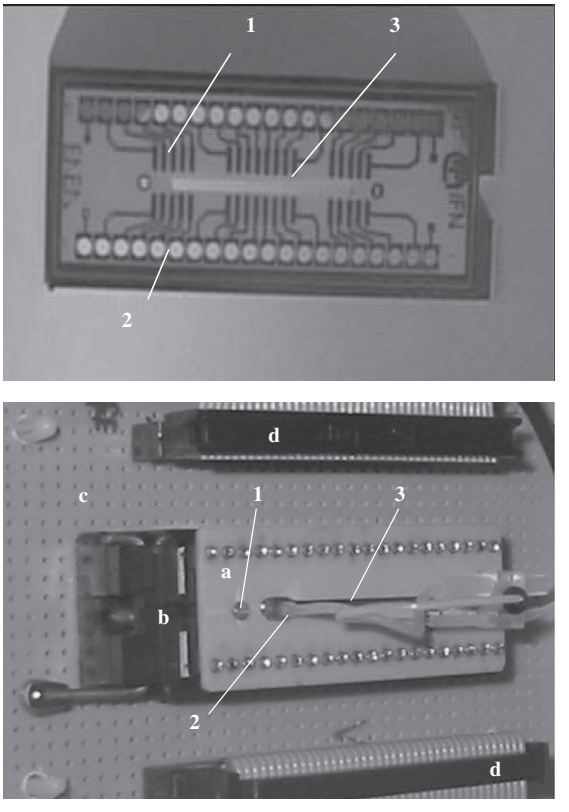


Figure 5. (a) The DB with pads-1, holes-2 for inserting the DIL40 socket pins, and slot-3 that allows the fluidic connections to pass through. (b) The connected engineered microcell consisting of: a-the upside down DB soldered over the DIL40 socket; b-ZIF socket; c-FB; d-flat cable standard connectors; 1-miniscrews; 2-fluidic connections; 3-slot.

flexible capillaries used as pipe-lines. So, the flexible multi-part adaptors have been replaced with simpler and more rigid parts.

6. Electric connections

Aiming a modular structure, the electric connections from the electrodes up to the external control unit consist mainly of two custom designed Printed Circuit Boards (PCBs) (Figure 5b): (a) the device board (DB) soldered on a DIL40 socket and (c) the family board (FB) provided with standard PCB-flat cable connectors. The standard DIL 40 socket and its corresponding ZIF (zero insertion force) socket (b) provide a plug-in connection between the two PCBs. Flat cables and standard connectors (d) lead to the external control unit.

The DB (Figure 5a) is provided with both pads (1) and small holes (2) to insure the connection with the chip-pads and respectively the insertion of the DIL40 socket pins.

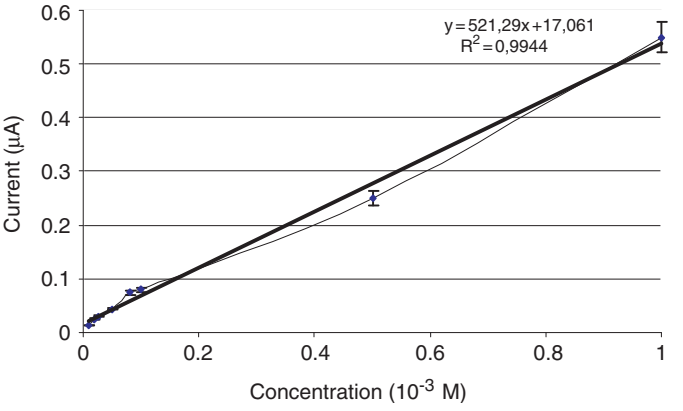


Figure 6. The calibration curve obtained from the signal provided by the WE no. 1 when injected hydrogen peroxide in the range 0.0001 M–0.001 M, with a carrier buffer solution of KCl with pH = 0.6 and a concentration of 0.1 M.

In order to allow the connection to the external control unit, on the chip, the miniaturised ($0.08 \times 0.12\,mm^2$) electrodes (Figures 1a, 1b, 1c) are linked to bigger pads ($3 \times 0.6\,mm^2$) situated outside the reservoir area (Figures 4a–6).

The pad-to-pad solderless connection between the chip and the DB is achieved by means of the so called 'zebra' connectors FUJIPOLY® Gold 8000. These connectors (Figure 4a–1) are based on flexible elastomeric elements consisting of low durometer silicone rubber cores alternating with flat metallic gold plated conductors vulcanised in a row and being parallel to each other. Thus, a contact resistance of about 200 Ω is achieved. The previous chip-to-DB electric connections, made of soldered Al wires, with a contact resistance of about 5 kOhm, have now been replaced by the solderless 'zebra' connectors.

7. Results

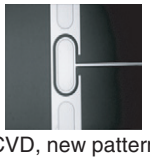
The resulting device (see Figure 5b), consists of the chip with electrodes, the glass reservoir with channel, glass cover with inlet/outlet openings and pedestals as fluidic connections. The solderless connections between the chip and the custom designed DB, the FB and standard PCB connectors allow the electric signals to and from the external control unit (a potentiostat Autolab PG Stat 10) to reach the electrodes.

As one can see in the Figure 5b, the DB turns upside down. An additional plate (not shown) fixed by means of two mini-screws (1) presses the 'zebra' connectors in between the chip and the DB. The reservoir and the openings plate, assembled onto the chip remain hidden below the DB while the fluidic connections (2) pass through the slot (3) above the DB allowing the connection of the pipe-line capillaries to the inlet and outlet of the reservoir.

The slot (3) on the DB allows also the inspection of the reservoir channel during the measurements.

The cell is very easy to plug in the measuring board (an in-house-built modular addressing system) that applies and receives the signals to and from the cell, by polarising the selected WE and by reading the current that passes through it.

Table 1. The sensitivity calculated for several gold electrodes, with different patterns and surfaces, obtained by Chemical Vapor Deposition or by Screen printing (* sensor AC1.W1.RS produced by BVT Technologies a.s. Czech Republic www.bvt.cz, by the courtesy of BVT Technologies a.s.).

Gold electrode	WE surface area A (mm^2)	Sensitivity S (μA/M)	Normalised sensitivity S/A (μA/M/mm^2)
 CVD, old pattern	0.003	1.7	600
 CVD, new pattern	0.010	50	5000
 Screen printed *	0.950	798	840

The calibration curve obtained from electrode no. 1 due to hydrogen peroxide injections in the range of 0.1 mM –1 mM is presented in Figure 6. The carrier was PBS pH = 7.00 (KCl 0.1 M). The sensitivity S has been calculated as the slope of the calibration curve, i.e. ($R_{ss} - R_{bl}$) *versus c*, where R_{ss} *is the steady state response*, R_{bl} *is the background signal* and *c* is the concentration of the analyte (H_2O_2) within the linear concentration range of the calibration curve [1].

The sensitivity is then normalised to the WE area in order to compare this result with those obtained with other types of gold electrodes having different shapes and areas: CVD (circular pattern) or printed electrode used in the sensor AC1.W1.RS produced by BVT Technologies, Czech Republic (www.bvt.cz). The comparative results are presented in the Table 1. One can note the highest normalised sensitivity obtained with the new designed electrodes. We presume the main role in the increasing of the sensitivity was played by the plug-flow characteristic of the new cell.

8. Discussion

Starting from the functional analysis of a previously obtained continuous flow micro-cell, new technical solutions have been approached in order to increase the reliability of both fluidic and electric connections as well as the characteristics of the chronoamperometric measurements. An engineered electrochemical micro-cell has been manufactured, with an optimised design: on chip integrated reference electrode, higher area of WE (0.010 mm^2) and higher ratio of CE/WE areas (50 : 1). Flexible electric connections, and glass fluidic connections have been promoted to improve the reliability. An electric plug-in and fluidic plug flow device has been obtained having a lower contact resistance (0.2 kOhm) than the

previous one (5–10 kOhm). First tests (hydrogen peroxide injection) have shown the functionality of the device and the calibration curve has been determined for one WE. The sensistivity has been calculated for the linear range of the calibration curve. In order to compare the obtained sensitivity with that one of others electrode with different shapes and areas the surface normalised sensitivity has been calculated. The highest sensitivity has been obtained with respect to the previously obtained gold electrodes and also to the BVT gold electrode, perhaps due to the plug flow of the analyte. Further efforts will be dedicated to deepen the functional characterisation of the device.

Acknowledgements

The authors acknowledge the Ministry for Foreign Affairs' Programm of Scientific and Technological Cooperation between Italy and Romania for the years 2006-2008 (Project: Bioelectronic micro devices for bioanalysis and diagnosis. Feasibility study for clinical applications) and the Project CoSMIC (ENEA target project on biosensors).

References

[1] D.R. Thévenot, K. Toth, R.A. Durst, and G.S. Wilson, Pure Appl. Chem. **71**, 2333 (1999).
[2] J. Wang, Biosens. Bioelectron. **21**, 1887 (2006).
[3] M. Ravera, G. Bagni, M. Mascini, D. Osellal, *Bioinorganic Chemistry and Applications*, Volume 2007, Article ID 91078, 11 pages, doi: 10.1155/2007/91078.
[4] M. Pohanka and P. Skládal, J. Appl. Biomed. **6**, 57 (2008).
[5] F. Scheller and F. Schubert (Eds.). *Biosensors*, *Techniques and Instrumentation in Analytical Chemistry*, Vol. 11, Elsevier, Amsterdam (1992).
[6] W. Vastarella, B. Lanza, A. Masci, and R. Pilloton, Proceedings of the 9th Italian Conference Sensors and Microsystems, Ferrara, Italy, 8–11 February 2004, pp. 19–24, Eds. C. Di Natale, a.o., World Scientific Publishing Co. Pte. Ltd., ISBN 981-256-386-5.
[7] W. Vastarella, V. Rosa, C. Cremisini, L. Della Seta, M.R. Montereali, and R. Pilloton, Intern. J. Environ. Anal. Chem. **87**, 689 (2007).
[8] M.R. Montereali, W. Vastarella, L. Della Seta, and R. Pilloton, Intern. J. Environ. Anal. Chem. **85**, 795 (2005).
[9] E. Magner, Analyst **123**, 1967 (1998).
[10] S.M. Jung, Y.D. Hywa, M. Nam-Ki, H. Suk-In, and J. Biosci, Bioeng. **103**, 32 (2007).
[11] J. Maly, M. Ilie, V. Foglietti, E. Cianci, A. Minotti, L. Nardi, A. Masci, W. Vastarella, and R. Pilloton, Sensor. Actuator. B Chem. **111–112**, 317 (2005).
[12] W. Vastarella, M. Ilie, E. Cianci, A. Coppa, V. Foglietti, L. Nardi, A. Masci, and R. Pilloton, Proceedings of the 11th Conference of AISEM, 8–10 February 2006, Lecce, Italy, in press.