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Combining Microfluidics and Electrochemical Detection

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Abstract—**This paper describes two configurations that integrate electrochemical detection into microfluidic devices. The first configuration is a low-cost approach based on the use of PCB technology. This device was applied to electrochemiluminescence detection. The second configuration was used to carry out amperometric quantification of electroactive species using a serial dilution microfluidic system.**

I. Introduction

With main trends of instrumental developments towards Micro Total Analytical Systems (μTAS) or Lab-on-a-Chips[1], there have been strong needs for integrating sensitive detection schemes in microfluidic devices. Indeed, such systems require only very small volumes of samples or reactants, while improving throughput to achieve multi-channel and multi-specific analyses. To complete this description, it is worth noticing that these systems also allow portability and single-use that are key aspects when developing tools for environmental or medical monitoring.

The implementation of electrochemical detection approaches (amperometry, impedance, potentiometry, …) into μTAS or Lab-on-a-Chip devices have raised strong interest since the start of the microfluidic field. Indeed, such methods can be easily miniaturized and require only low power while being able to reach low limits of detection[2], [3]. In addition, electrochemical detection presents a unique advantage compared to optical techniques (UV detection) that is the possibility of downsizing the detection window without loss of performance. However, electrochemical approaches presents also drawbacks that are inherent to the technique such as the fouling of the electrodes and the need to have electroactive analytes. Nonetheless, thanks to electrochemical tagging and indirect detection (for example by using metallic nanoparticles), it is possible to extend the range of detectable species to non electroactive analytes[4], [5].

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In this paper, we review two configurations that were developed in our group to implement electrochemical detection into microfluidic devices. In the first configuration (**Fig. 1**), we used a low-cost approach based on Printed Circuit Board technology to design the electrodes used to carry out electrochemiluminescence detection of H_2O_2 in microfluidic format[6]. Indeed, PCB technology offers interesting advantages in terms of cost, design facilities, fabrication service for large scale production and electronic integration. In addition, electrodes obtained using PCB approach are thicker than the electrodes obtained using thin-film approach and can therefore withstand larger currents.

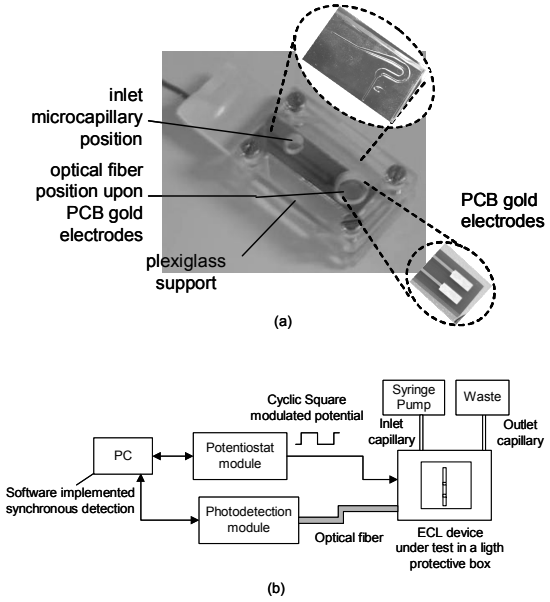

Fig. 1. Configuration used to carry out electrochemiluminescence experiments with a PDMS microfluidic device coupled to PCB electrodes. (a) photographs and (b) schematic representation of the experimental set up.

In the second configuration (**Fig. 2**), we used serial dilution microfluidic systems combined to electrode networks to perform amperometric quantification using an integrated calibration mode [7]. Quantification using calibration curves is widely used in analytical chemistry but suffers from two main drawbacks: (i) it is a time-consuming process and (ii) periodic re-evaluation should be performed. With the device developed in our group, the calibration curve is generated simultaneously to the sample analysis and the dilution necessary to its generation are obtained automatically due to the microchannel configuration. Therefore, our approach circumvent the two main drawbacks listed previously.

II. EXPERIMENTAL

A. Electrode Fabrication

In this work, we used two approaches to obtain metallic electrodes integrated into a microfluidic device:

(i) the usual thin-film technique (evaporation of a thin (~200 nm) metallic layer on a patterned insulating substrate, followed by a lift-off step) was used to obtain a complex network of Au electrodes that was coupled to the serial dilution device,

(ii) the PCB electrodes were obtained from Cirly S. A. (Lyon, France). They were manufactured on an insulating substrate, typically glass/cloth epoxy (laminated FR4) and they were made of electrodeposited copper (35 µm thick) coated by electroless 80 nm thick gold layer.

For both configurations, Ag/AgCl reference electrodes were prepared by electrodeposition of a thin layer of Ag that was subsequently oxidized in presence of chloride ions.

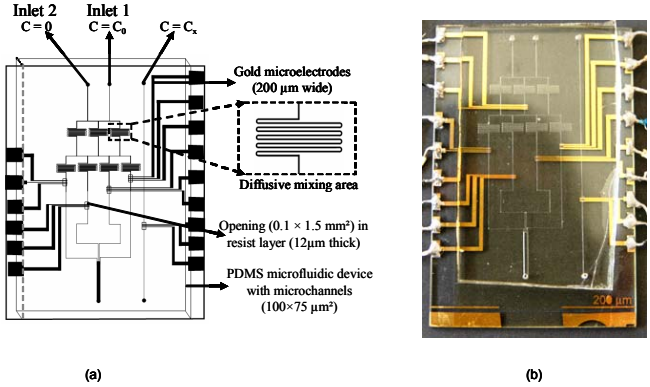

Fig. 2. Configuration used to carry out amperometric quantifications with an integrated calibration mode. (a) Schematic representation of the PDMS microfluidic structure associated to the Au electrode network, (b) photograph.

B. Microfluidic device fabrication

For both configurations, PDMS microfluidic structures were obtained by Soft-Lithography using a master with dry film photoresist microstructures(**Fig.3**). The method to obtain the master was already described elsewhere[8].

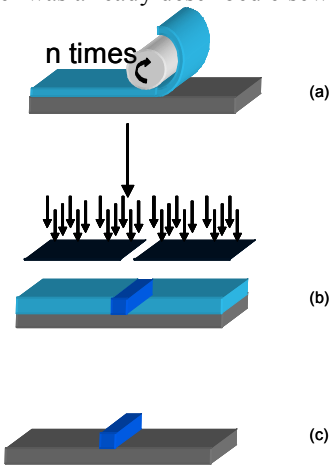

Fig. 3. Schematic representation of the fabrication steps of the master (a) dry film photoresist lamination, (b) irradiation and (c) development.

Briefly, dry film photoresist (Etertec HQ-6100) layers (number depending on the final thickness expected) were laminated on a glass slide. Then, these films were irradiated through a photomask using a mask aligner (irradiation time depends on the number of layer laminated). After development with an aqueous solution of potassium carbonate, the structure was hardened by a final irradiation. In this work, microchannels that were 70 µm thick and 100 µm wide were used to carry out amperometric quantification whereas microchannels 210 µm thick and 2 mm wide were employed for the electrochemiluminescence device.

C. Operation of the microfluidic devices and electrochemical measurements

The electrochemical measurements and control of the potential at electrodes were performed using a home made potentiostat. The electrochemiluminescence experiments were carried out using a liquid light guide (LLG214, from L.O.T. Oriel, Courtaboeuf, France) characterized by its large core diameter (5 mm), high transmittance (more than 80% at 425 nm) and its large acceptance cone (72°) optimizing the collecting efficiency. The collected signal is detected by a photomultiplier tube-based luminometer (Biolumat, Berthold, Pforzheim, Germany). *Via* an acquisition board, the data are transmitted to a PC for processing and monitoring.

III. RESULTS AND DISCUSSION

A. Electrochemiluminescence microfluidic device

Electrochemiluminescence (ECL) is a highly sensitive analytical technique for a variety of biochemical applications such as immunoassays and DNA probe assays. One advantage of this method is the small background signal during detection that allows reaching low detection limits. Here, we performed luminol ECL detection to quantify hydrogen peroxide (H_2O_2). However, luminol-based ECL analysis can be applied to quantification of a large range of species such as glucose, choline and metal ions. Oxidation of luminol can be obtained by applying a potential of 0.7 V/AgAgCl at Au electrodes, leading to ECL emission around 425 nm in presence of hydrogen peroxide (in absence of this species no light emission is observed). Therefore, ECL signal is proportional to H_2O_2 concentration. **Fig. 4** shows results obtained in a microfluidic format with integrated Au PCB electrodes. **Fig. 4a** shows typical waveform of modulated ECL signal in microfluidic conditions. Such modulated approach allows easy background substraction even in case of signal level shift. From **Fig. 4b**, we can observe a linear relationship between the detected ECL signal and hydrogen peroxide concentration in the range 50 nM to 0.1 mM.

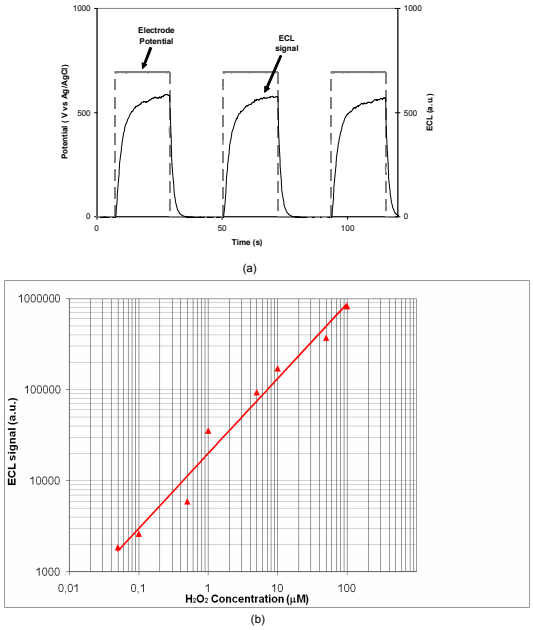

Fig. 4. (a) Waveforms of a modulated electrode potential and ECL intensity in microfluidic conditions, (b) ECL signal vs. hydrogen peroxide concentration obtained in microfluidics condition.

B. Amperometric quantification using a serial dilution microfluidic device

Using the microfluidic structure presented in **Fig. 2**, it is possible to prepare 4 solutions containing an active molecule at a concentration linearly decreasing (concentration 0, $C_0/3$, $2C_0/3$ and C_0) by just injecting 2 solutions (a buffer solution and a solution containing the active species at concentration C_0). Such capacity associated with a network of electrodes was exploited in order to be able to obtain a calibration curve simultaneously to the detection of the active species in a sample solution. **Fig 5** presents the results obtained when using a model electroactive species $Fe(CN)_6^{4-}$ and the microfluidic device described in **Fig. 2** using a flow rate of 0.125 mL h^{-1} for all inlets (larger flow rates were not appropriate as efficient mixing was not achieved). Due to the flow rate difference in the various microchannels of the device, the electrochemical signal I_{ss} was divided by $Q^{0.3315}$ in order to obtain a linear calibration curve. Using a representation as $I_{ss}/Q^{0.3315}$ vs. C, we found a value of 2.6 mM instead of 2.5 mM for the sample concentration C_x, resulting in a relative deviation between the theoretical concentration and the concentration estimated using the *on-chip* calibration better than 5% in this case. This deviation can be explained by electrode reproducibility and also by flow rate correction required with this approach. We can also notice that in Fig. 5, the calibration points were correctly aligned (r^2 = 0.999) as expected and it made this curve suitable for use as a calibration curve.

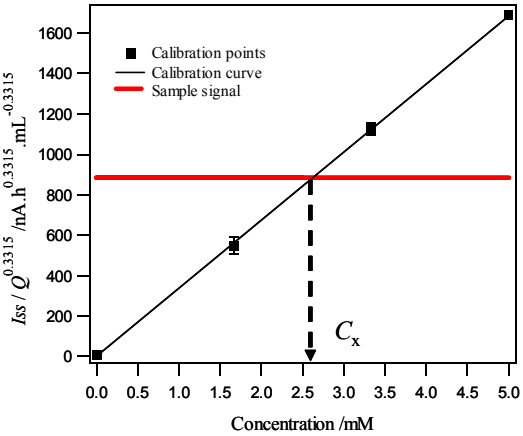

Fig. 5. Calibration curve obtained when flowing a 5 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl solution as a calibration solution, 2.5 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl for the sample solution and a 0.1 M KCl solution as a dilution solution.

CONCLUSION

We showed here two configurations to carry out electrochemical detection into microfluidic environment. In the first configuration, we showed that PCB based technology can be used to design electrodes to be implemented into microfluidic channels whereas the second configuration demonstrates simple quantification based on calibration curve. These technologies are presently under investigation in our group to be applied to biomedical detection.

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