Integration of amperometric sensors for microchip capillary electrophoresis application

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Abstract. Capillary electrophoresis is a technique for the separation and analysis of chemical compounds. Techniques adopted from the microchip technology knowledge have led to recent developments of electrophoresis system with integration on microchip. Microchip Capillary Electrophoresis (µCE) systems offer a series of advantages as easy integration for Lab-on-a-chip applications, high performance, portability, speed, minimal solvent and sample requirements. A new technological challenge aims at the development of an economic modular microchip capillary electrophoresis systems using separable and independent units concerning the sensor. In this project we worked on the development of an interchangeable amperometric sensor in order to provide a solution to such electrode passivation and facilitating the use of tailored sensors for specific analyte detection besides. Fluidic chips have been machined from cyclic olefin polymer pallets (Zeonor®) using a micro-injection molding machine.

1. Introduction

Recently particular attention has been given to the development of microchip capillary electrophoresis systems (µCE) because of their fast and efficient separation capabilities [1]. While microchip technology has improved over the last decade, the techniques of detection have lagged behind. Although preferred techniques in the past such as laser-induced fluorescence (LIF) and mass spectroscopy (MS) showed high sensitivity of detection, they often require off-chip control instrumentations limiting the mobility and the portability of the system. Electrochemical detection (EC) seems to be the meeting point between the concept of portability (low volume of the control instrumentation) and the need for increased sensitivity and resolution [2,3]. Furthermore electrochemistry is also highly compatible with integration processes and microfabrication technologies. The electrochemical detection modes used for microchip capillary electrophoresis systems are: amperometric detection, potentiometric detection and conductivity detection. The amperometric detection is generally considered the most powerful method in term of resolution, but it is limited to the electroactive compounds. For this reason, the other two methods are seen as complementary methods for the non-electroactive compounds [4].

A general limitation for the amperometric detection method lies in the fact that the detector electrodes are in direct contact with the solution and may therefore deteriorate due to corrosion processes. Here we present a new modular approach to the development of a microchip capillary electrophoresis device. Fluidic chips have been designed and developed in our facilities using a micro-injection
molding machine. We have also developed a range of micro-electrodes made of indium tin oxide (ITO), silver and platinum by means of patterning, etching and deposition techniques using the internal fab of the Tyndall National Institute. A three electrodes configuration has been used for sensing: ITO working electrode, AgCl reference electrode and Pt counter electrode. Primary importance has been given on the improvement of an oxidative technique to form an AgCl electrode on-chip. The stability of the sensor has been investigated using cyclic voltammetry and potentiometry with excellent outcomes. The Electroosmotic flow (EOF) has been tested into the Zeonor® using fluorescein dye in 20 mM sodium borate solution. Amperometric detection test of the single sensors showed significant response with homovanillic acid, 4-hydroxy-3-methoxymandelic acid, L-Tryptophan and 3-Indoxyl sulfate.

2. Experimental

2.1. Design

The fluidic chip is composed of two layers in Zeonor®. The design has been ideated in order to have a modular sensor as well as a modular separation channels (bottom layer). In this case bottom layers with different designs for the separation channel (serpentine or squared) could be used with the same top layer. The chip sizes are of 60mm x 15mm x 1mm (LWH) for the top layer and 60mm x 15mm x 2mm (LWH) for the bottom layer. The channels have a squared cross-section with dimensions 50µm x 50µm. The three amperometric electrodes have been evaporated on a pyrex substrate of the following sizes: 28mm x 3mm x 0,5mm (LWH).

![Figure 1. Exploded view of the system in a CAD application](image1)

![Figure 2. Amperometric sensors in five different designs](image2)

2.2. Fabrication of the fluidic chip

The chip layers have been accomplished using the Babyplast® 6/10P microinjection moulding machine available in the Life Sciences Interface lab (Fig. 3 and Fig.4). A solvent vapour bonding technique has been used to attach the mating layers. This technique consists in the exposition of one layer to cyclohexane vapour into a chamber [5]. Over the last years the most popular bonding technique used has been the thermal fusion. In the specific case of microfluidic devices a series of disadvantages have been encountered using this approach. Since the chip is heated slightly above the glass transition temperature of the material, residual internal stress can lead to bulk material reflow with results of deforming small features or clogging the channels. The solvent vapour bonding takes over this issue increasing the polymer chain mobility of the exposed surfaces so facilitating the bonding process [5]. Optimization of parameters as temperature and exposure time have been done running a series of tests.
We found necessary to remove any kind of protrusions which made uneven the surface of the layers probably due to the extraction of the sprue from the mould of the Babyplast® machine. Protrusion were frequently present at the edge of the channels or reservoirs and were removed using a fine sand paper. Cleaning with 2-propanol and deionized water was then necessary to remove any kind of burrs present on the surface. An adequate chamber created with a petri dish and a glass lid was placed on a hot-plate in a range of temperature between 30°C and 40°C, estimated and controlled using a digital thermometer. After the sufficient amount of time to get temperature equilibrium between the solution and the hot plate, the bottom layer was exposed into the chamber for approximately 20 s. The bottom layer was then removed from the vapour and inserted with the top layer in a hand-made aligner in order to facilitate the alignment between the detection and the injection channel with the separation channel. The aligner with the fluidic layers were then pressed in a clamp to evenly distribute the pressure. To improve the bond strength, a treatment with UV light (UV/Ozone Procleaner Plus, Bioforce Nanosciences) for 20 mins was performed. In fact Mair et al. [5] observed a positive shift of about 50% in burst pressure using a UV treatment (13.5 J/cm² at 260 nm). Presumably chain scissions are induced by UV light and cross-linking reactions occurs at the bonded interface.

2.3. Fabrication of the amperometric sensor

The amperometric sensor has a three electrodes configuration with an indium tin oxide (ITO) working electrode, a Pt counter electrode and Ag/AgCl reference electrode (Fig.5 and Fig.6). Pads and connections have been done in gold material. Five different designs of the sensor corresponding to five different dimensions of the electrodes have been developed. All of them have been fabricated in the internal fab facility of the Tyndall National Institute. After the deposition of the electrodes, a silicon nitride passivation layer has been grown on the wafer with openings on the electrodes area and pads. This is normally done to avoid such conduction between connections and letting the current flowing just in the electrodes area when dipped in the electrochemical solution. A further treatment with a ferrichloride solution (FeCl₃) of the wafer was performed to form an AgCl layer on top of the silver deposited in the fab for the reference electrode [6]. Then a necessary operation of dicing of the wafer was performed. The height of the electrodes is 500 µm for all the five designs to make the alignment easier in the channel 50 µm wide. The widths of the five sensors are reported below in the Table 1.
2.4. Integration

The integration of the sensor was carried out using an optical UV glue (NOA 68, Norland Optical Adhesives). The capillary forces drove the glue from the lateral access of the sensor until the sides of the channel. When the glue approached to the walls of the channel, a UV gun permitted the polymerization and hardening of the glue. Even an appropriate interface for the sensor has been built in order to avoid soldering operations of wires onto the pads allowing for easy connection to the electrochemical instrumentation (Fig. 7). The interface is composed of two units: one printed circuit board and a plastic cap designed using a CAD software and developed using a computer numerical control (CNC) machine available in the mechanical workshop of the Tyndall National Institute. The plastic cap was then fixed onto the printed circuit board using plastic screws.
2.5. **Electrochemical instrumentation**

Amperometric detection, potentiometry and cyclic voltammetry have been carried out using the battery-powered Palmsens® (Palmsens Instruments BV, Houten, Netherlands) (Fig. 8), a portable device which can be connected to a laptop or a pocket PC via cable or Bluetooth connection. The high-voltage power supply used for the electroosmotic flow test is the LabSmith High Voltage Sequencer HVS448 6000D (LabSmith, Livermore, CA, USA).

3. **Results and discussion**

3.1. **Characterization of the electrochemical cell using cyclic voltammetry**

All the five design of the sensors have been characterized using cyclic voltammetry in a solution of 15 mM K$_4$Fe(CN)$_6$·3H$_2$O in 1 M KNO$_3$. Five scans at 25mV/s have been delivered for each sensor to check the stability of the AgCl reference electrode and results then compared with a commercial external Ag|AgCl|1 M KCl reference electrode. The measurements were stable for all the five sensor with good and repeatable outcomes as shown in Fig.9. A potential shift has been noticed for the five designs after a comparison with the results obtained using the external commercial Ag|AgCl|1 M KCl reference electrode. The measurements resulted shifted for a value included in the interval 10 mV – 20 mV probably due to the oxidation technique to form the silver chloride on top of the reference electrode.
3.2. Characterization of the electrochemical cell using amperometric detection

Amperometric detection tests have been performed separately for each sensor with the aim of identifying the main compounds which could be detected using an indium tin oxide working electrode. Amperometric detection was started while the analyte sequentially introduced in a 10 mL beaker at 50 s intervals each. Analytes at issue were homovanillic acid, 4-hydroxy-3-methoxymandelic acid, L-tryptophan, 3-indoxyl sulfate respectively. The detection potential chosen was 800 mV at a 0.2 s scan rate.

![Electropherogram](image)

**Figure 10.** Electropherogram: E = 0.8 V, t_{run} = 250 s, scan rate = 0.2, analyte concentration = 25 μM.

3.3. Electroosmotic flow test

Cyclic olefin polymer is a pretty new material used in microfluidic and it is still not clear which is the charge state of the surface. For this reason an electroosmotic flow test was necessary to evaluate the presence of a specific charge on the channel surfaces which could support this kind of flow. The buffer solution used was sodium borate in a concentration of 20 mM. Once the system was filled of the solution and the channel confirmed bubble free, the sample reservoir was loaded with drops of fluorescein. The sequence of injection was then started and pictures taken from an Olympus IX2 optical microscope (Olympus Imaging & Audio Ltd, Southend-on-Sea, Essex, UK) (Fig. 11) with a motorized lens under the chip. Injection sequence is reported in Table 2.

![Sequential pictures](image)

**Figure 11.** Sequential pictures of the EOF at the intersection (from the left to the right) after 8s, 9s, 10s.
4. Conclusion

A first attempt of integration of an amperometric sensor using on-column configuration here is done. Zeonor® showed compatibility with the electroosmotic flow and a further step in microfluidic applications has been completed. Amperometric sensors with an on-chip Ag/AgCl reference electrode represent a possible application for microchip capillary electrophoresis systems since the stable and repeatable results obtained. A complete test of the fluidic and the sensors integrated is at the moment under investigation.

5. Acknowledgements

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6. References

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### Table 2. Injection sequence for the electroosmotic flow test

|                      | Step A | Step B | Step C |
|----------------------|--------|--------|--------|
| **Time (s)**         | 5      | 1      | 10     |
| **Sample reservoir (V)** | 100    | 40     | 80     |
| **Sample waste reservoir (V)** | -100   | 40     | 80     |
| **Inlet (V)**        | 50     | 200    | 200    |
| **Outlet (V)**       | 50     | -200   | -200   |