Low-cost screen-printed and embossed LAMP micro-reactors

D. Gosselin[1,2,3]*, M. Gougis[1,2], M. Baque[1,2], P. Mailley[1,2], A. G. Bourdat[1,2], V. Maffini-Alvaro[1,2], F. Revol-Cavalier[1,2], S. Vignoud[1,2], F. Navarro, [1,2] M. N. Belgacem[3] , D. Chaussy[3], J. Berthier[1,2]

[1] Univ. Grenoble Alpes F-38000 Grenoble, France
[2] CEA, LETI, MINATEC Campus, F-38054, Grenoble, France.
[3]Univ. Grenoble Alpes, CNRS, Grenoble INP, LGP2, F-38000 Grenoble, France
*Corresponding author: david.gosselin@cea.fr

ABSTRACT

Accurate and sensitive diagnostics are of utmost importance in the fight against infectious diseases and epidemics. Indeed, without such diagnostics broad-spectrum antibiotics are generally used, resuting in an increased antimicrobial resistance [1,2]. Because laboratories and medical infrastructures are not always available, in particular in developping countries, there is a high need of Point-of-Care (PoC) diagnostic devices. Among other criteria [3], such devices must be low-cost, portable, and automonous. Besides they must work with a small amount of energy, available from a smartphone, a computer or batteries. In order to comply with these criteria, it is shown in this work that the combination of screen-printing and hot embossing technologies provides a convenient way to embed functions such as heating and electrochemical detection within a LAMP (Loop mediated amplification) micro-reactor.

Keywords: Screen-printing, Hot embossing, LAMP, Integration, PolyAniline.

1 INTRODUCTION

Diagnostic is a important step in the fight against infectious diseases and epidemics. While it is necessary to give a treatment to diseased people, it is just as important to give the appropriate one, avoiding the use of broad-spectrum antibiotics which may result in an increased antimicrobial resistance [1,2]. Besides because laboratories are not always within reach, in particular in developing countries, the development of Point-of-Care (PoC) devices is of utmost importance. According to the World Health Organization (WHO) [3] such a device needs to be, among other criteria autonomous, portable, and should not rely on bulky equipments.

Tests based on DNA amplifcation are a promising way to achieve the sensitivity and specificity required for these diagnostics. However the portability of such devices remains challenging. Progress has been made with the use of isothermal DNA amplifications [4,5]. Indeed because there is no need for temperature cycling, the integration of the temperature management is easier. Besides colorimetric detection have been reported for the LAMP (Loop mediated amplification) amplification [6]. This type of detection relies on the use of a pH sensitive dye. Indeed when the DNA polymerase adds a dNTP (desoxyribonucleotides tri-phosphate) while synthetizing a new DNA strand, a hydronium ion is released [7]. Thus the DNA amplifiation results in a decrease of the pH of the solution. However, these colorimetric assays only provide a Yes/No answer. To achieve a quantitative detection, a real-time monitoring of the amplification reaction is needed.

Real-time monitoring of pH during a DNA amplification reaction has been reported with the use of an ISFET [8]. However these systems are complexe to produce and are not suitable for Point-of-Care testing. This work reports the use of screen-printed electrodes allowing a continouous electrochemical measurement of the pH during a LAMP reaction. In addition, because they rely on the potentiometric response of PolyAniline [9,10], such measurements are performed with small energy consumption.

Screen-printing technology has also been found successful in printing micro-heaters tailored for a LAMP reaction [11]. The presented study will demonstrate that both of these functions can be successfully stacked to perform and monitor a LAMP reaction. In addition, the combined use of screen-printing and hot embossing technologies for the fabrication of integrated micro-heater is presented.

2 MATERIALS & METHODS

2.1 Screen-printed functions

For this study, a 175µm-thick foil of PolyCarbonate is used as a substrate for the screen-printed functions. This choice have been made to allow for the embossing of the reaction chamber. All the screen-printing steps, except the deposition of the PolyAniline, are performed by Séribase Industrie (France).

Micro-heaters

In some experiments of this work, screen-printed micro-heaters are used to heat up the reaction chamber to 65°C, temperature required for the LAMP reaction to occur. These micro-heaters consist of a screen-printed layer of resistive carbon paste. It has been shown that such micro-heaters are able to provide a stable temperature of 65°C for 1 hour with low voltage [11]. In this work the micro-heaters

have a rectangle shape, a length of 5mm, and a width of 13mm.

To avoid any interference when these microheaters are used in the reaction chamber a passivation layer is screen-printed on top of it. This layer will also make possible the stacking of the screen-printed electrodes on the micro-heater (Figure 1). Indeed it prevents any electrical contact between the electrodes and the micro-heater.

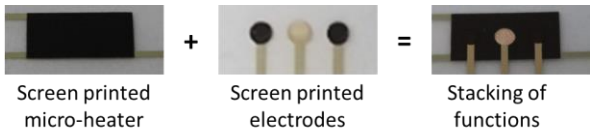

Figure 1 : Stacking of the two screen-printed components.

Electrodes

The potentiometric data relies on the measurement of the potential difference between a reference electrode and a working electrode. On the one hand, the reference electrode is made with a silver/silver chloride paste, DuPont 5874 (DuPont, USA). Its potential does not depend on the pH on the solution. On the other hand, the working electrode is coated with polyaniline, which degree of oxidation depends on the pH [9,10]. Just like for the micro-heaters a passivation layer is used to avoid any short-circuit - due to the presence of the liquid - between the electrodes. This layer covers the integrity of the screen-printed surface but the electrodes, which must be in contact with the liquid to allow a measurement.

The PolyAniline coating is drop cast. This is done by depositing a droplet of 300nL of PolyAniline, purchased at Rescoll (France), on the working electrode and then allowing it to dry at 60°C for 10 minutes.

Both the reference and the working electrodes have a disk shape of radius of 1mm. In our design, two working electrodes are located at each side of the reference electrode (Figure 2).

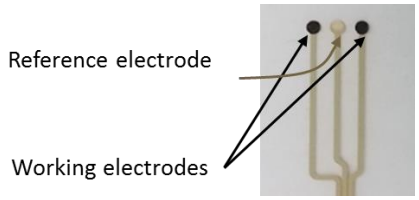

Figure 2 : Screen-printed PolyAniline-based electrodes.

2.2 Microfluidic integration

In this study the microfluidic integration will be done in two ways. Firstly the screen-printed elements can be used as a cover for a micromachined microfluidic chamber. Secondly, the screen-printed elements can be hot embossed to design the microfluidic chamber directly on the polycarbonate foil.

Milled microfluidic chamber

In the first case a 500µm-deep microfluidic chamber is in a 1.2mm-height PMMA sheet with a Datron M7HP equipment (DATRON, Germany). The chamber has a length of 10mm and a width of 4mm. Double-sided tape is then used to bond this milled chamber to the screen-printed PC foil. Note that this tape has been previously cutted in order to allow a direct contact between the electrodes and the liquid

Hot embossing

In the second case, hot embossing is used to directly form the microfluidic system on the PC foil. The mold exhibits a microfluidic chamber with the same dimensions as that described previously. The hot embossing is performed by Séribase Industrie. A picture of embossed electrodes is shown in figure 3. To cover these embossed systems double sided tape is used to bond a 1.2mm-height PMMA sheet.

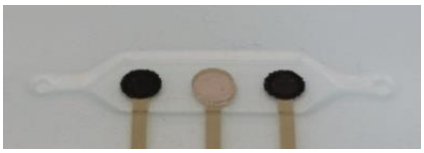

Figure 3 : Embossed electrodes

2.3 Reagents for the LAMP reaction

The LAMP reactions are performed using the Bst 2.0 DNA Polymerase purchased at NEB (New England Biolabs, USA). Bacillus thuringiensis (Bt) is used as DNA sample with a concentration of 10^5 copies/µL. The associated primers were purchased at Eurofins Genomics (Eurofins Scientific Group, Germany).

3 RESULTS AND DISCUSSIONS

The aim of this work is to demonstrate the ability of screen-printing and embossing technologies to integrate micro-heaters and electrochemical detection in a LAMP reaction chamber. Firstly the response of the PolyAniline to pH is studied and the ability of such electrodes to monitor a LAMP reaction is established. Secondly, the possibility of stacking the micro-heater and the electrochemical detection will be shown. Finally the use of hot embossing to form a microfluidic device with embedded screen-printed components will be adressed.

3.1 Potentiometric measurment of pH

First of all the potentiometric response of the PolyAniline electrodes was studied. This was done by using the screen-printed electrodes as a cover for a milled microfluidic chamber. Phosphate Buffer Solution (PBS) with various pH level have been used for this purpose. Figure 4 shows the reponse of the working electrodes to pH level variation.

The calibration of the system is carried out by experiments with different electrodes. A linear relationship between the pH level and the measured potential is obtained

(Figure 5). The sensitivity of the screen-printed electrode is found to be about 80mV by pH unit.

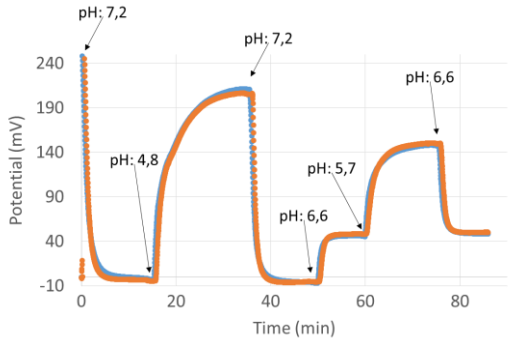

Figure 4 : Response of the PolyAniline-based electrodes to pH change.

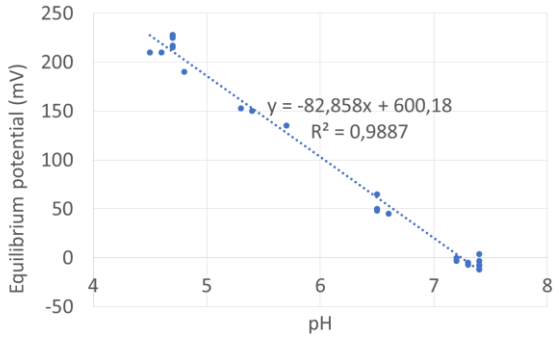

Figure 5 : Calibration of the electrodes (at 25°C)

3.2 Monitoring a LAMP reaction with the screen-printed electrodes

Since the screen-printed electrodes showed to be sensitive to pH variation, they have been used to monitor in real-time a LAMP reaction. Figure 6 depicts the response of such electrodes during a positive (with DNA sample) and a negative (without DNA sample) LAMP reaction. While a acidification of the solution is detected for the positive test, no pH variation is measured for the negative one. Thus the measured pH variation results from the DNA amplification.

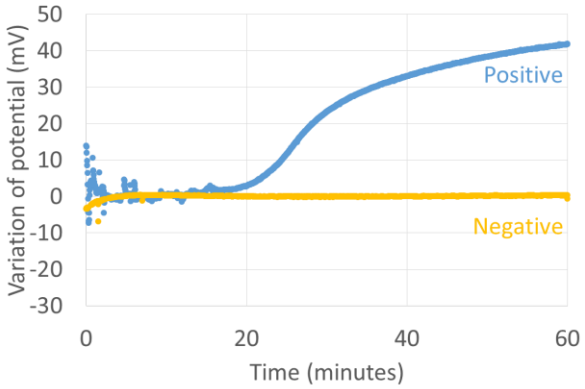

Figure 6 : Potentiometric monitoring of LAMP reactions.

The variation of potential observed during this reaction is 40mV. Nevertheless, because the reaction occurs at 65°C, pH quantification cannot be achieved using the calibration curve of figure 5 (obtained at 25°C). In fact, the change of temperature has an impact both on the pH level of an aqueous solution [12] and on the equilibrium potential of an electrode. The latter depends on the temperature of the system according to the Nernst law:

$$E = E^0 + \frac{RT}{nF}\ln\left(\frac{a(Ox)}{a(\mathrm{Re}\,d)}\right) \quad (1)$$

Where E^0 is the standard potential, R is the universal gas constant, T is the temperature, a is the chemical activity for the relevant species, F is the Faraday constant and n is the number of exchanged electrons.

However, such a pH quantification is not important for a diagnostic test. Indeed when performing a DNA amplification, the two useful informations are wether or not an amplification occurs and, if so, at what time it had occured. Because both of these data can be extracted from the experiment, it is demonstrated that the screen-printed PolyAniline based electrodes are successful in monitoring a LAMP reaction in real-time.

3.3 Performing and monitoring the LAMP reaction with stacked screen-printed components

For this experiment, the heating is achieved by Joule effect by applying a 8V voltage to the screen-printed micro-heater. The monitoring of the DNA amplification is done with the PolyAniline base elctrodes in the same way as for the previous experiments.

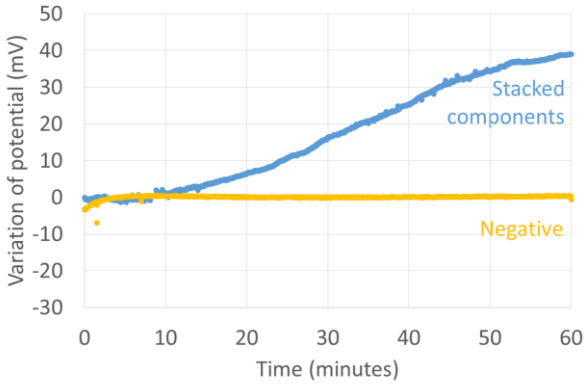

Figure 7: Performing and monitoring the LAMP reaction with stacking screen-printed components.

Figure 7 shows the variation of potential measured by the electrodes according to time. It is shown that a variation of the pH level is also recorded during this experiment. This demonstrates, on the one hand the ability of the screen-

printed micro-heater to provide an adequate heating, and on the other hand the possibility to stack the electrodes on the micro-heater without dysfunction.

3.4 Embossed micro-reactors with embedded electrodes

In this subsection, it will be shown that the combination of screen-printing and hot embossing technologies is a convenient way to integrate the PolyAniline based electrodes directly inside a microfluidic chamber. In fact by performing the hot embossing after the screen-printing step of the electrodes results in a microfluidic chamber with embedded electrochemical sensors (Figure 3).

It is first demonstrated that embossed electrodes provide an adequate response to pH. Figure 8 shows that the behaviour of such electrodes is the same as the not embossed ones.

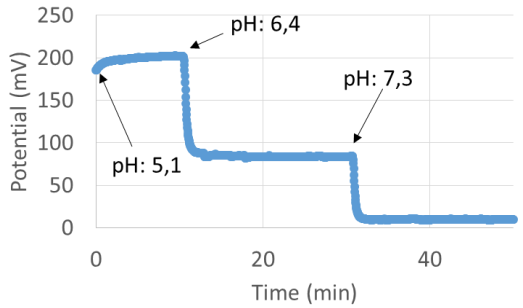

Figure 8 : Response of the embossed PolyAniline-based electrodes to pH change.

Based on these results, the embossed electrodes are used to monitor a LAMP reaction (Figure 9). For this experiment the heating is provided by a MJ Research PTC-200 thermocycler (GMI, USA). During the LAMP reaction a variation of pH is measured, further demonstrating the ability of the embossed electrodes to monitor the pH level of the solution.

4 CONCLUSION

In conclusion it has been shown in this work that screen-printed PolyAniline-based electrodes produce a sensitive response to pH change within a solution. This system has been used to monitor in real-time a LAMP reaction through the pH variation induced by the DNA polymerisation.

The possibility of stacking two screen-printing functions, namely the micro-heater and the PolyAniline-based electrodes, has also been successfully demonstrated. This stacking have been further used to perfom and monitor a LAMP reaction. Such an integration of these two functions required for a DNA amplification based diagnostic is very promising for Point-of-Care device.

Finally it has been demonstrated that hot embossing technology is compatible with screen-printed functions and that it offers a convenient way to obtain a thermalmolded device with embedded screen printed electrodes.

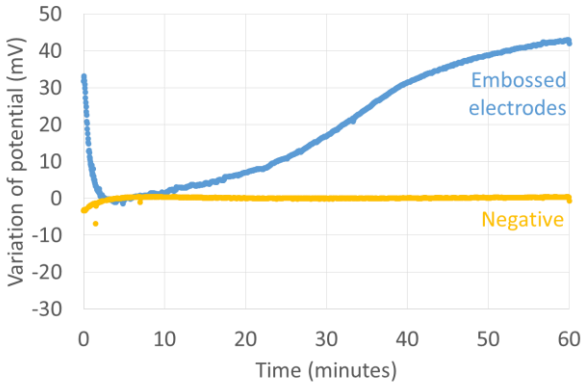

Figure 9 : Potentiometric monitoring of a LAMP reaction within an embossed chamber.

REFERENCES

[1] A. Tay, A. Pavesi, S. R. Yazdi, C. T. Lim, and M. E. Warkiani, Biotechnology Advances, 34(4), 404–421, 2016.

[2] R. W. Peeling, and D. Mabey, Clinical Microbiology and Infection, 16(8), 1062–1069, 2010.

[3] H. Kettler, K. White, and S. Hawkes, "Mapping the landscape of diagnostics for sexually transmitted infections", WHO/TDR, Geneva, 2004.

[4] Y. Zhao, F. Chen, Q. Li, L. Wang, and C. Fan, Chem. Rev., 115(22), 12491–12545, 2015.

[5] M. Safavieh, M. K. Kanakasabapathy, F. Tarlan, M. U. Ahmed, M. Zourob, W. Asghar, and H. Shafiee, ACS Biomaterials Science & Engineering, 2(3), 278–294, 2016.

[6] N. A. Tanner, Y. Zhang, and T. C. Evans, BioTechniques, 58(2), 2015.

[7] C.-Y. Chen, Frontiers in Microbiology, 5, 2014.

[8] C. Toumazou, L. M. Shepherd, S. C. Reed, G. I. Chen, A. Patel, D. M. Garner, C.-J. A. Wang, C.-P. Ou, K. Amin-Desai, P. Athanasiou, H. Bai, I. M. Q. Brizido, B. Caldwell, D. Coomber-Alford, P. Georgiou, K. S. Jordan, J. C. Joyce, M. La Mura, D. Morley, S. Sathyavruthan, S. Temelso, R. E. Thomas, and L. Zhang, Nat Meth, 10(7), 641–646, 2013.

[9] C. Ge, N. R. Armstrong, and S. S. Saavedra, Analytical Chemistry, 79(4), 1401–1410, 2007.

[10] A. A. Karyakin, O. A. Bobrova, L. V. Lukachova, and E. E. Karyakina, Sensors and Actuators B: Chemical, 33(1), 34–38, 1996.

[11] D. Gosselin, J. Berthier, A. Delon, D. Chaussy, N. Belgacem, and G. Delapierre, Nanotech'2016 Conference.

[12] W. L. Marshall and E. U. Franck, Journal of Physical and Chemical Reference Data, 10(2), 295–304, 1981.