Microfluidic paper-based analytical devices

Mehrdad Lotfi Choobbari[1], Mohammad Behnam rad[2], Amir Jahanshahi[1*], Hedayatollah ghourchian[2*]

[1]Semiconductors Research Lab, Department of electrical engineering, Amirkabir University of Technology (Tehran Polytechnique), Tehran, Iran
[2] Laboratory of Microanalysis, Institute of Biochemistry & Biophysics, Tehran University, Tehran, Iran *Corresponding Authors:
[*] Amir.Jahanshahi@aut.ac.ir
[*] ghourchian@ut.ac.ir

1. Introduction

Micro paper-based analytical devices (µPADs) have received extensive attention immediately after their emergence. The first report of paper microfluidics appeared in literature by George M. Whitesides and coworkers in 2007 [1]. There are several reasons for the received popularity and attention including: the low cost of cellulose paper utilized in the structure of µPADs which makes them economically viable; the light weight and the flexibility of the paper which eases the handling and the transportation process; the relatively simple and quick procedure of the fabrication of µPADs; the capillary flow in µPADs eliminating the need for external pumping and the porosity and biocompatibility of paper [2]. These advantages make µPADs a promising technology in clinical diagnostics and point of care testing (POCT) devices. The first presented approach for the fabrication of paper based microfluidic devices was based on the thick resist photolithography (such as SU-8).

Figure 1 shows a schematic of the fabrication process for this method. In this method the remained photoresist (as a hydrophobic barrier) plays the role of the wall of the microchannels. The region confined between two barriers forms the hydrophilic channels in which samples can flow via capillary forces. It should be noted that a plasma ashing step is required after the development, to remove the residuals of the patterned photoresist and increase the hydrophilicity of the microchannels.

Apart from this fabrication technique, other fabrication methods have been pursued in the literature too, such as solid ink wax printing, screen printing, plasma treatment, laser cutting, knife plotting, flexographic and toner or inkjet printing [3,4]. Despite the advantages and disadvantages of the mentioned methods, wax printing has been mainly adopted in the literature. We have also used this method for the fabrication of µPADs.

In parallel with investigation for the fabrication of µPADs, various detection techniques have been applied on µPADs for rapid diagnostics,

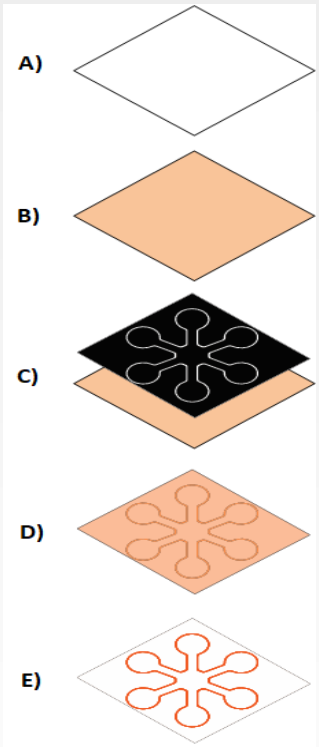

Figure 1. The procedure of photolithography technique: (A) the paper substrate (B) immersed in photoresist, (c) prebake and mask alignment, (D) postbake after exposure, (E) development.

such as colorimetry, electrochemistry, chemiluminescence (CL), fluorescence, mass spectrum (MS) and surface-enhanced Raman spectroscopy (SERS) [5].

In this work, we have adopted the colorimetric detection method to demonstrate a typical glucose assay. The principle of this assay is

based on an enzymatic reaction which provides a semi quantitative result. A color indicator is used to generate a visible color proportional to the product of this enzymatic reaction. The enzymes used in this work for the detection of glucose are glucose oxidase (GO_x), horseradish peroxidase (HRP), and also the potassium iodide (KI) as the color indicator. We present the difference between color intensity induced by various concentrations of glucose in the sample.

Keywords: microfluidic paper-based analytical device (µPAD); wax printer; colorimetric assay

2. Operation Principle

As mentioned earlier, wax printing method is used in this work for the fabrication of µPADs. The printer used in this method is a commercially available wax printer known as solid ink wax printer. The solid ink used by this printer melts when the printing procedure starts covering the paper partially according to the given design. The desired property of the wax printer that makes it suitable for the fabrication of µPADs is the hydrophobicity of its printed ink.

The hydrophobic printed pattern by this printer after being absorbed by the paper, can be considered as a hydrophobic barrier forming the walls of the microchannels. Thus the capillary flow in the paper will be directed between the walls of the microchannel [4].

Chromatography and filter papers are among the most common types of papers used in the literature for paper microfluidics applications [3]. These papers provide the desired hydrophilicity of the microchannels. Therefore, the liquids can flow through the micro channels via capillary forces without any external pumping equipment.

3. Fabrication

To fabricate µPADs by wax printing method, we used the Whatman No. 1 filter paper and the solid ink wax printer "Xerox ColorQube 8580DN". We initially designed a flower like pattern of the channels using a 2D vector graphics software (AutoCAD or CorelDRAW) as shown in figure 2. The line width, channel width and the diameter of the circles are 0.25mm, 1.4mm and 5mm, respectively.

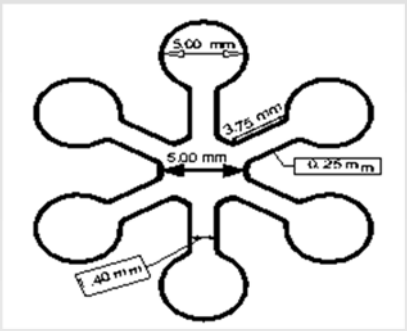

Figure 2. Designed pattern in software(AutoCAD)

As it is shown in figures 3 and 4, then the printed paper was placed on a hotplate at 120˚C for 30s. In this step the printed ink was melted once again and spreaded throughout the thickness of the paper. An oven also can be used for this step. Now, the micro channels are formed on the paper. It should be noted that the resolution of the printed pattern is extremely affected by the time and temperature in which the paper is placed onto the hotplate. It is experimentally observed that the ink after placing on hotplate spreads laterally as well as vertically through the thickness of the paper. The final dimensions and the resolution of the pattern and the different factors affecting these parameters have been investigated by carrilho et al [4].

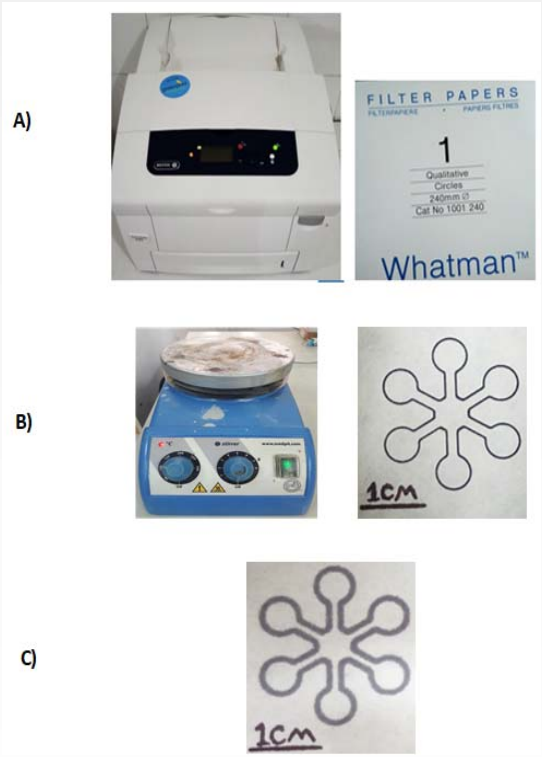

Figure 3. Fabrication process of µPADs based on the wax printing: (A) a filter paper (A5 size) is placed into the printer, (B) the printed paper is placed on a hotplate, (C) the fabricated µPAD.

info@microfluidics-conference.ir
http://microfluidics-conference.ir
نشانی دبیرخانه: تهران، دانشگاه صنعتی شریف، دانشکده مکانیک

4. Results and Discussion

As well as evolving of the fabrication techniques, detection mechanisms of µPADs have progressed immensely. Colorimetric detection method is one of the most widely used approaches for biological assays on paper substrates. This method is widely applied on µPADs in various applications such as clinical diagnostics, food safety, environmental monitoring, bioterrorism, point of care testing, etc [5]. Electrochemical µPAD (EµPAD) is another widely used paper-based device for the analytical assays on µPADs. EµPADs favorably enhance the sensitivity and selectivity of the assays. The goal of this method is to fabricate the electrochemical and electrical components on paper substrates, still benefitting from the relatively low cost and simplicity [2].

On the other hand, the colorimetric detection method provides a semi-quantitative measurement of analyte concentration in the sample. The colorimetric detection results can be interpreted by naked eye, digital camera, scanner or a relatively simple diode-photodiode setup. A calibration curve is usually suggested to improve the accuracy of this method [3]. The analysis of glucose, nitrite, lactate, uric acid, proteins and other analytes have been conducted on µPADs based on the colorimetric detection [1,2,4]. For example, detection of the glucose concentration in human blood, urine or other biological samples is one of the typical and important colorimetric assays performed on µPADs, presented in this work as well.

The enzymatic oxidation of iodide to iodine is the main principle used in colorimetric detection of glucose. A change of color from transparent to brown is detected upon introducing the glucose. The intensity of the color is proportional to the concentration of glucose. In this reaction, glucose reacts with glucose oxidase (GO_x) in the presence of oxygen and water to produce hydrogen peroxidase (H_2O_2). Then the H_2O_2 is reduced to H_2O by horseradish peroxidase (HRP) while potassium iodide (KI) is oxidized to iodine, and consequently a brown color is generated [1,3]. To perform this assay on the µPAD, we first dropped a mixed solution (3µL) of GO_x and HRP on six detection zones (figure 3) and the reagents were allowed to dry in ambient condition. Next, we dropped the mixed solution (3µL) of potassium iodide (KI) and trehalose which acts as a stabilizer for enzymes, on each one of the detection zones and allowed the paper to be dried in ambient condition. The preloaded µPAD is ready now for performing the assay.

Figure 4. The logo of Amirkabir University of Tehran, fabricated by wax printing procedure and colored by edible color solution.

Unfortunately the µPAD is very sensitive now to the storage condition [1]. Samples of glucose in different concentrations (6.5mM, 5.2mM, 3.9mM, 2.6mM, 1.3mM, 0.63mM) were added to the six detection zones and after 10 minutes under ambient condition, the color change was observed (figure 5). As it can be seen in figure 5, the number written on each detection zone indicates the glucose concentration (mM/L) in the sample. The produced color intensity is proportional to the concentration of glucose in the sample (Figure 5), so the lowest concentration produced the lowest color intensity. The color gradient shown in figure 5 is the result of this criteria which is produced by decreasing the glucose concentration from 6.5mM to 0.63mM.

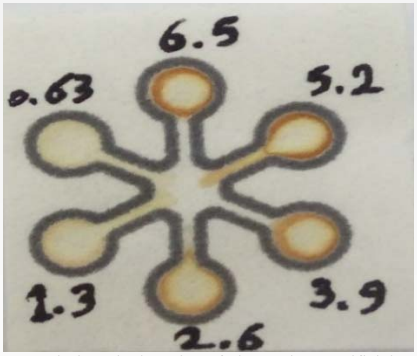

Figure 5. Colorimetric detection of glucose in an artificial sample on µPAD.

5. Conclusion

Performing biological assays for several times during a day and in a less expensive and relatively rapid manner can greatly affect the world healthcare. Microfluidic paper based analytical devices (µPADs) offers notable advantages such as low cost, simplicity and low

reagent consumption. There are various methods proposed for the fabrication of µPADs. Among them, the photolithography technique and the wax printer method have been widely adopted. The photolithography technique gives the minimum feature sizes among other methods, but it is expensive and time consuming. It is worth noting that the materials used in this method are bio-incompatible. The wax printer method is less favorable in terms of the minimum feature size. Although it is relatively rapid, inexpensive and uses biocompatible materials. By use of this approach, we accomplished the fabrication of µPADs in less than 5 minutes. The paper substrate used in this work was a filter paper Whatman No. 1 which makes the setup economically viable in disposable applications. Among various approaches for analytical assays on µPADs, colorimetric detection method was adopted in this paper to show a typical glucose assay. The results provided by this method are semi quantitative measurement of target analyte in the sample. If the determination of analyte has been done in more quantitative and precise way, a calibration curve should be used in combination with an external instrument, as a next step for this work.

Acknowledgements

This work was supported with an INSF grant number 95848969.

6. References

[1] Martinez, A. W., Phillips, S. T., Butte, M. J., & Whitesides, G. M. (2007). Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie International Edition*, *46*(8), 1318-1320.

[2] Maxwell, E. J., Mazzeo, A. D., & Whitesides, G. M. (2013). based electroanalytical devices for accessible diagnostic testing. MRS bulletin, 38(4), 309-314.

[3] Evans, E., Gabriel, E. F. M., Coltro, W. K. T., & Garcia, C. D. (2014). Rational selection of substrates to improve color intensity and uniformity on microfluidic paper-based analytical devices. Analyst, 139(9), 2127-2132.

[4] Carrilho, E., Martinez, A. W., & Whitesides, G. M. (2009). Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Analytical chemistry*, *81*(16), 7091-7095.

[5] Liu, S., Su, W., & Ding, X. (2016). A Review on Microfluidic Paper-Based Analytical Devices for Glucose Detection. *Sensors*, *16*(12), 2086.