Research Article
ISSN:2249-1236
CODEN: IJRRBI
Shereen Abu Zaid *et al.*, *Int*. J. Res. Rev. Pharm. Appl. Sci.,*(2021) 11(1) 056-067*

International Journal of Research and Reviews in Pharmacy and Applied sciences

Microfluidic paper-based Biosensors

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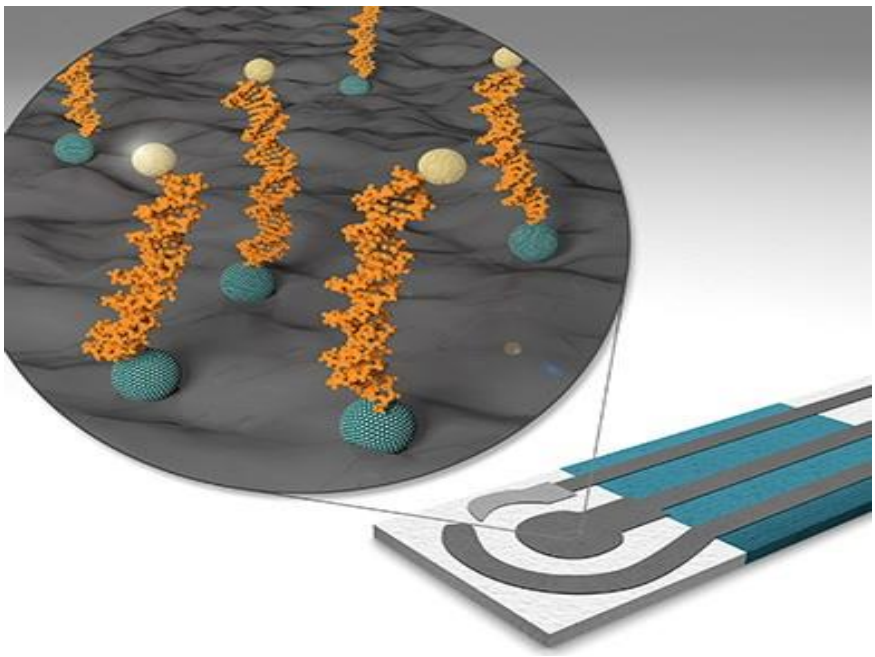

Abstract:
Nowadays, the healthcare systems face many challenges such as the increase in the number of patients, the increase in the hospitalizations rate, and raising the cost of healthcare services. Therefore, several technologies were developed to provide effective solutions for these problems. The microfluidics paper-based analytical devices (µPADs) have a significant role in improving healthcare services. In general, µPADs are one of the points of care solutions that provide fast, reliable, and cost-effective solutions for diagnosing several types of diseases. In this essay, the role of µPADs in the healthcare system will be considered. Also, the fabrication techniques and the detection mechanisms that are used in µPADs will be addressed. Finally, the challenges and prospects for µPADs will be discussed.

Keywords: Point of care, µPADs, colourimetric mechanism, electrochemical mechanism, antigen-antibody mechanism.

1. Introduction:

These days, several challenges increased the pressure for improving and developing the healthcare systems. In general, the high cost that is associated with the medical services, and the high rate of hospitalizations are one of the main factors that increased the need to improve the healthcare systems[1]. Moreover, the rapid increase in the number of chronic diseases is one of the parameters that raised the importance of developing rapid, low cost, and effective solutions to manage and diagnose these diseases[2].

In addition, most analytical tests require a specialized person to recognize the results of the tests. Moreover, in developing countries, this factor increases the need to develop new analytical procedures that are simple and easy to use by the patient himself[3]. According to the World Health Organization, the design and development of diagnostics devices for developing countries shall be based on the ASSURED criteria. In general, ASSURED criteria mean that the devices shall be affordable, sensitive, specific, user-friendly (simple to perform in simple procedure without professional training), rapid and robust (can be stored at room temperature and provides rapid results in less than 30 min), equipment-free (minimal equipment that can be solar-powered), and deliverable to end-users[4].

In general, the microfluidics field is one of the areas which has a significant effect on the healthcare systems. Microfluidics is a system that use microchannels to process a small number of fluids. Moreover, the development and growth of the microfluidic field are related to several factors such as the need to develop high resolution and high sensitivity molecular analysis methods, the military interest to develop solutions to manage and diagnose the chemical and biological threats, and the development in the associated technologies such as the photolithography technology[5].

Furthermore, µPADs are one of the effective diagnostic devices that can be used in developing countries. The development of the µPADs that are designed from the patterned paper is based on the capabilities of the conventional microfluidic technologies and the specifications of the diagnostic strip. Moreover, the µPADs have a significant effect on the improvement of the bioanalysis field through increasing the speed of the tests, reducing the cost, reducing the amount of sample, and providing high resolution and sensitivity tests. In addition, the need for external supporting reagents or devices is reduced in the µPADs because the µPADs depend on the capillaries' specifications and evaporation mechanism to control the flow of reagents[3].
Additionally, the papers were used to design microanalytical devices for several reasons. Firstly, the availability of paper at a low cost is one of the main properties of the papers that support the use of papers in microanalytical devices. Also, the lightweight of the papers made them an effective solution to design analytical devices that are easy to transport and store. Moreover, the photometric specifications of the papers, such as the scattering of the light which causes the white colour of papers, make the papers an effective medium to detect the coloured reagents. Secondly, the chemical properties of the papers and the ability to modify the chemical structure of the papers improve the ability of the papers to detect the several substrates and to produce biocompatible materials with the biological samples. Furthermore, the flexibility of the papers improves the ability to fabricate the papers in different forms and makes them compatible with a wide range of fabrication techniques. Also, the ability to

dispose of the paper after use in a safe and easy method is one of the remarkable factors which improves the use of papers in design microanalytical devices[3].

Finally, the results of the earlier uses of the papers in the analytical chemistry are one of the motivating parameters that have a significant effect on the development of the µPADs[3]. For example, paper-based analytical methods were used to detect the concentration of coloured compounds in solutions such as the detection uric acid. Furthermore, paper-based analytical methods were used to detect several elements such as the sugar in urine and cadmium[6]. In this review, the fabrications methods of µPADs and the detection mechanisms that are used in µPADs will be considered. Also, the challenges and future aspects that face the µPADs will be discussed.

2.Methods and Materials

2.1.Fabrication of microfluidic paper-based analytical devices

The design and development of µPADs are based on several factors. Firstly, the identification of the suitable biological component which interacts with or recognizes the analyte is an important factor in the design of the µPADs. Secondly, the selection of the transducer, which is used to convert one form of energy to another form, is a critical element in the design and development stage. Finally, the identification of the fluid transportation mechanism is a key player in the design phase[6]. In general, µPADs consists of hydrophilic channels from pattering sheets of paper, and hydrophobic borders which separate the hydrophilic channels to control the direction of the fluid flow. Furthermore, the channels may be isolated from the external environment by an external sheet of polymer or can be left open to the external environment[3].

Additionally, the design approach of the µPADs is based on controlling the direction of the flow, improving the chemical reaction in certain areas, and enabling multiple reactions in the same paper strip[6]. In general, several fabrication methods are used in design the µPADs such as the wax printing technology. The wax printing technology is based on several steps as shown in figure 1. Firstly, the pattern is designed using computer software. Secondly, the printing step which includes the printing of the wax microstructure onto a hydrophilic membrane such as nitrocellulose membrane (NC membrane). Thirdly, the baking phase includes the melting of the wax-printed membrane using an oven to ensure that the melted wax is penetrated through the hydrophilic membrane. Furthermore, wax is a hydrophobic material that forms the walls that control the flow of fluid. Finally, the printed pattern is cooled to room temperature. In general, wax printing technology is considered one of the fast fabrication methods. In addition, this technology is considered one of the low-cost methods due to the use of papers and wax in this method. Also, the use of paper and wax made of this technology an environmentally safe because the papers and wax can be disposed of by burning[7], [8].

Furthermore, inkjet printing technology is one of the printing technologies that is used widely used in industrial applications such as the manufacture of the light-emitting diode (LED). In general, the low cost, and the short cycle time are one of the main characteristics of this technology. In addition, the ability to create patterned substrates and control the distribution of chemical reagents make this technology an effective solution to fabricate the µPADs. Also,

inkjet technology is a non-contact method. therefore, it reduces the rate of contamination and protects the substrate from damaged compare with contact methods. In general, the fabrication process of the µPADs using inkjet printing technology includes several steps as shown in figure 2. The first step is substrate preparation which includes immersing a filter paper in a solution such as the dissolvent of Polystyrene in Toluene. The second step is the construction of the hydrophilic channels through adding a solvent droplet such as Toluene to the specific area in the paper to dissolve the hydrophobic material. Finally, the construction of the sensing areas through adding the chemical reagents droplet into these areas[9].

In addition, photolithography is one of the simple methods that is used to design µPADs. On the other hand, the main drawback of this technology is the long processing time, which is related to the use of post-processing steps, in comparison with other methods such as inkjet printing mechanisms. In general, photolithography includes several stages as described in figure 3. Firstly, the substrates preparation stage includes the soaking of the papers in a photoresist solution, then the paper is dried to eliminate the solvent regent. Furthermore, the top side of the paper-photoresist substrate is covered by a transparency film and the bottom side is covered by a black film. Secondly, the pattern transfer stage which includes the printing of the pattern on the top of the paper-photoresist substrate using a photocopying machine. Thirdly, the polymerization stage which includes the use of UV light to polymerize the photoresist material. Finally, the post-processing stage which consists of an additional curing stage and cleaning of unpolymerized photoresist material[6], [10].

Moreover, the flexographic printing method plays a significant role in the improvement of the µPADs as a kind of point of care solutions through its ability to design small fluidic channels which helps in reducing the amount of required sample for the test. Also, flexographic printing is considered an effective method for large-scale fabrication. As shown in figure 4, the flexographic printing technology consists of three rollers: the impression roller on which the paper substrate is fixed, the plate roller which is used to transfer the pattern onto the paper substrate, and the anilox roller which consists of small cells that determine the amount of ink which transfer into the plate roll. In general, the rotation speed of anilox roll is higher than the rotation speed of the impression and plate roll which helps in distribution the ink effectively[11].

In addition, the use of plasma treatment in the fabrication of µPADs has a significant effect on improving the ability to design functional parts such as filters. In general, the steps to design µPADs using plasma treatment includes the salinization of the paper substrate by using organofunctional molecule such as octadecyl trichlorosilane (OTS) which forms a hydrophobic layer on the paper substrate. Secondly, the plasma treatment is used to eliminate this hydrophobic material from specific locations. Then the regions that are exposed to plasma transfer into hydrophilic channels. Moreover, the intensity and time of treatment play an important role in determining the width of the channels. Therefore, the selection of these factors is an important parameter in the fabrication of the µPADs by plasma treatment[8].

Moreover, laser treatment is one of the methods that can be used for fabrication the of small microfluidic channels. In general, the laser printing system consists of several parts as shown in figure 5. Firstly, the laser source is used to transfer the pattern to a photopolymer substrate. Secondly, the galvanometer is used to control the magnitude of the laser beam. Then the

focused lens is used to control the distribution of the laser beam over the substrate. Finally, a post-processing thermal treatment is required to improve the structure of the micro channels[8], [12].

Furthermore, the wet etching mechanism is one of the fabrication methods of the µPADs which is based on the salinization principle that is used in plasma treatment. In the first step, a paper substrate is immersed in a hydrophobic solution such as trimethoxy octadecyl silane. Moreover, a paper mask is used to transfer the pattern into the paper and to form the hydrophilic channels. In addition, the screen-printing mechanism is one of the simple fabrication methods that can be used to design the µPADs. On the other hand, the poor resolution of the fabricated channels is the main drawback of this technology.

Moreover, wax is one of the materials that are used in screen-printing technology to build the hydrophobic wall between the microchannels. The use of wax in screen printing reflects on the reduction of the cost of this technology. Also, wax is an environmentally safe material. In general, the fabrication process of the µPADs by wax-screen printing technology includes several steps as shown in figure 6. The screen printing uses wax to transfer the pattern into the surface of the paper. In the second step, a heat plate is used to melt the wax which forms the hydrophobic walls.

2.2 The detection mechanism of microfluidic paper-based analytical devices:

The µPADs are one of the point of care (POC) solutions that are used to provide rapid, inexpensive, simple, and environmentally safe methods to diagnose diseases in developing countries. Moreover, the biosensor consists of two main parts the biological component which interacts with the analyte and the transducer which is used to convert one form of energy to other forms. Furthermore, the interaction between the analyte and sensing material includes several changes such as visual changes, and electrical changes.

In general, several detection mechanisms are used in the µPADs. Firstly, the colourimetric mechanism is one of the simple, fast, and inexpensive detection methods. This principle-based on several interactions such as the enzymatic reactions, the change of PH, and the chemiluminescence activity include visual changes such as the change in the colour. Furthermore, the colourimetric mechanism is used in different applications such as the detection of nitrite, the detection of glucose, and the detection of the PH[6].

For example, in one of the applications to detect the PH and nitrite using a colourimetric principle, the built-in camera of a smartphone was used to capture an image to the µPAD which is used to detect the PH and nitrite as shown in figure 7. In addition, the µPAD consists of a central sample area connected with four PH sensing areas and three nitrite sensing areas through independent channels, and a separated reference area. Moreover, two types of PH sensing reagents are used for the detection of PH. In general, the sample flows from the central sample area into the seven sensing areas. The interaction between the sensing reagent and the sample changes the colour of the sensing area. The smartphone built-in camera was used to capture the image of the µPAD. Also, mobile software was used to process the captured image and to identify the PH and nitrite[13].

Additionally, the electrochemical mechanism is one of the effective and rapid quantitative methods that are used in µPADs. The electrochemical principle is based on using electrodes to measure the electrical activities such as the voltage and the current that is resulted from the chemical reactions. In general, the electrochemical µPAD system as shown in figure 8 consists of the sample area connected with the working electrode (WE), the reference electrode (RE), and the counter electrode (CE). The chemical interaction between the sample and sensing reagent includes the ejection of electrons (ionic current) that depend on the concentration of the molecules. Furthermore, the electrodes are used to convert this ionic current into an electrical current[6].

Moreover, the antigen-antibody mechanism is one of the detection methods that are widely used in detection the small molecules and proteins. In general, this principle is based on using a sensing area that contains a functional group that can covalently bond with a specific protein. Moreover, the changes that are resulted from this chemical bonding such as the electrical changes and colour changes can be measured using electrodes or a photodetection system[8].

In general, there is a wide range of detection principles that can be used in the µPADs. The selection of the mechanism depends on the changes that are resulted from the interaction between the sensing reagent and the sample. Moreover, the electrical and visual changes are the most effects that are associated with that interaction.

3.Results and Discussion

3.1.Challenges and Prospects:

In general, the development of the µPADs field is related to several factors. Firstly, the current trend of the healthcare system which includes the movement toward the point of care tests is one of the key players in the development of the µPADs. Also, the rapid growth in mobile health (mhealth) applications increases the importance of developing µPADs. Finally, the development of 3D printing technologies has a significant impact on the improvement of the design of the µPADs[6].

On the other hand, several factors have limited the applications of µPADs. Firstly, the sensitivity of the µPADs which is affected by the concentration of the sample is one of the main challenges that face the µPADs. Therefore, the development of the mechanism to control the sample concentration is an important factor in developing the field of µPADs. Secondly, the ability to develop multiple tests µPADs is one of the main factors to reduce the cost of the µPADs. In addition, the control of the physical and chemical properties of the papers is an important parameter to improve the µPADs. In general, the fabrication methods have a clear effect on the properties of the papers. Therefore, the development of the fabrication method is a critical point in the design of µPADs. Also, the development of engineered papers can help in improving the physical and chemical properties of the papers[6].

In addition, the stability of the biological components, such as natural enzymes, has a clear effect on the development of the µPADs. Therefore, the development of artificial components

can provide an alternative solution for biological components. Moreover, the development of the quantification µPADs is an important factor to improve the applications of these devices. Finally, the growth of the printing biosensors field such as the electrodes and biomolecules can improve the design and development of the µPADs[6].

2. CONCLUSION:

In general, the high cost of healthcare services and the rapid growth in the number of diseases are one of the main factors that increase the need for developing rapid and inexpensive diagnostics methods. Moreover, µPADs is one of the cost-effective, rapid, and environmentally safe devices. Also, the fabrication of the microchannels is an important step in the design and development of the µPADs. Moreover, several fabrications methods are used in design the µPADs such as wax printing, photolithography, and inkjet printing. The selection of the fabrication is an important factor in the design of the µPADs because each fabrication method has a different effect on the physical and chemical properties of the papers. In addition, different detection mechanisms can be used in µPADs. the selection of the detection mechanism depends on the changes that are resulted from the interaction between the sample and sensing reagent. Moreover, several factors have limited the applications of the µPADs such as the sensitivity of the devices, and the stability of the biological components. Finally, the development of the engineered papers, the field of printing biosensors, and mobile health applications can improve the design and development of the µPADs in the future.

Figures:

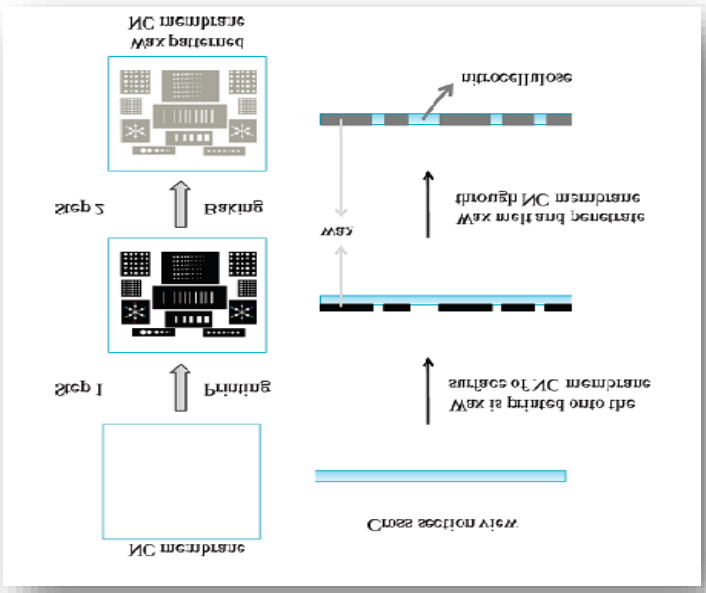

Figure 1: the process of fabrication of µPAD in nitrocellulose(NC) membrane by wax printing.[7]

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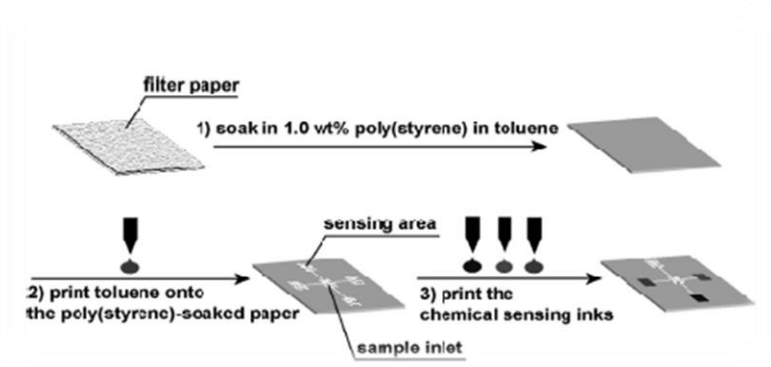

Figure 2: The process of fabrication of µPAD by inkjet printing.[9]

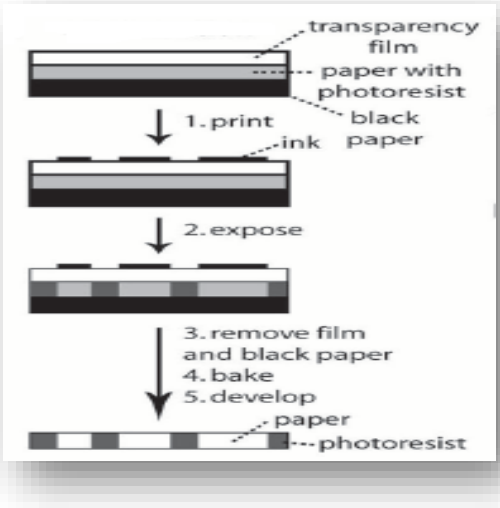

Figure 3: The process of fabrication of µPAD using photolithography.[10]

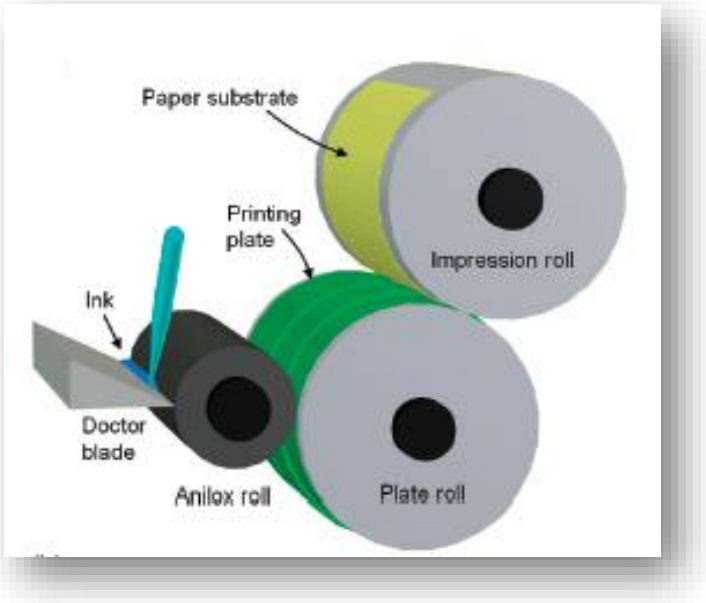

Figure 4: The process of fabrication of µPAD using Flexographic printing.[11]

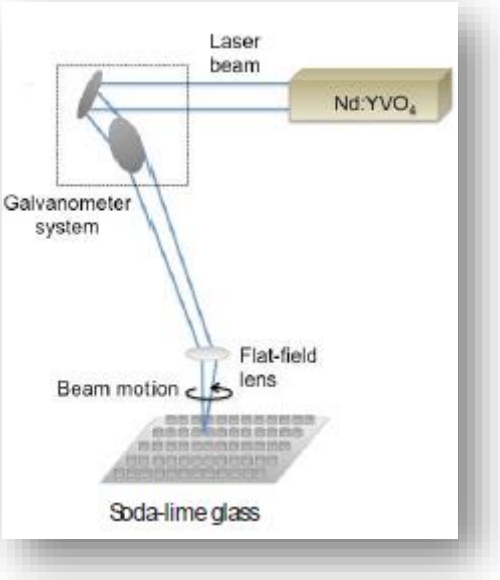

Figure 5: The process of fabrication of µPAD using a laser printing system.[12].

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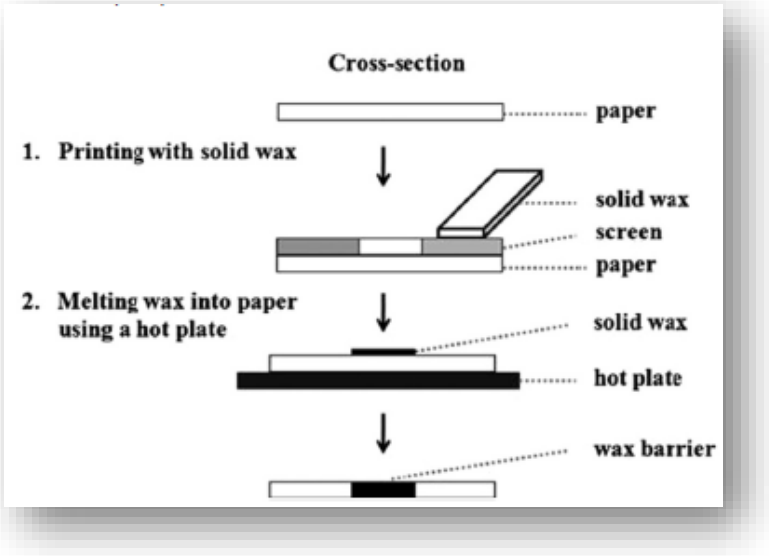

Figure 6: The process of fabrication of µPAD using wax-screen printing[8]

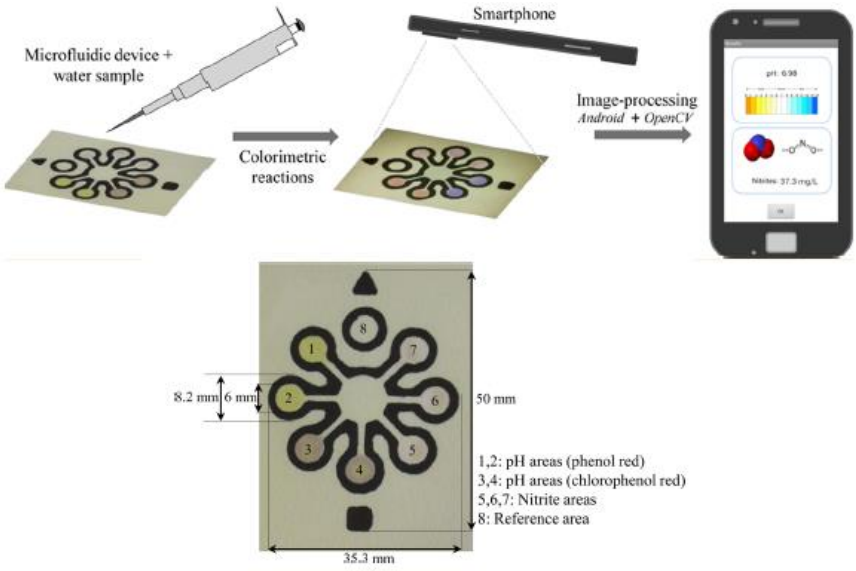

Figure 7: The detection of PH and nitrite using a colourimetric mechanism.[13]

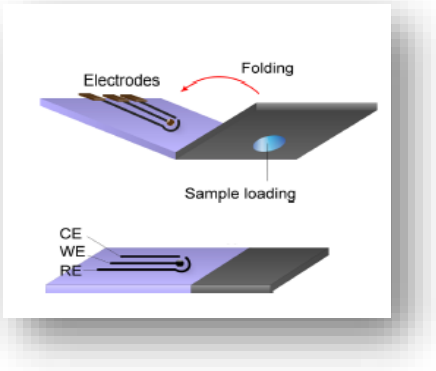

Figure 8: µPAD based on the electrochemical mechanism.[6]

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