MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (µPAD) FOR RAPID DETECTION OF IRON IN AGRICULTURAL SOIL

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SCHOOL OF HEALTH SCIENCES
UNIVERSITI SAINS MALAYSIA

2020

MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (µPAD) FOR RAPID DETECTION OF IRON IN AGRICULTURAL SOIL

By

NUR FATIN NAJIHAH BINTI MARZUKI

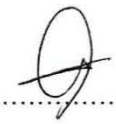
Thesis submitted in partial fulfilment of the requirements
for the degree of
Master of Science (Forensic Science)

September 2020

CERTIFICATE

This is to certify that the dissertation entitled **"MICROFLUIDIC PAPER BASED ANALYTICAL DEVICE (μPAD) FOR RAPID DETECTION OF IRON IN AGRICULTURAL SOIL"** is the bona fide record of research work done by NUR FATIN NAJIHAH BT MARZUKI, matric number P-SKM0024/19 during the period from February 2020 to September 2020 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Master of Science (Forensic Science).

Supervisor,



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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledge. I also declare that it has not been previously for concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

(Nur Fatin Najihah Binti Marzuki)

Date: 10/9/2020

ACKNOWLEDGEMENTS

The completion of this thesis could been impossible done without participation and assistance of so many people. Their contributions are sincerely admired and gratefully acknowledged.

Principally, I would like to thank the supreme power the Almighty Allah who is obviously the one has always guiding me to work on the right path of life. Next it is great pleasure to acknowledge my deepest thanks and gratitude to my supervisor, Dr Nik Fakhuruddin Nik Hassan for willing to help me solve the problems occurred during my lab works and thesis writing. I am very appreciated the efforts and guidance given by him.

Besides, I would also like to pass recognition to all lab assistants in Science Forensic, Analytical and Bio Medicine Laboratories for teaching and guarding in lab works also providing the chemicals and apparatus needed during the lab work beginning.

Furthermore, I would like to give appreciation to my lab mate, Nor Izati Che Ab Aziz for big helps given to me in order to complete the research project. Last but not least, I would like to thanks my beloved family as well as my other friends who have gave me moral supports along the semester.

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LIST OF SYMBOLS

℃	Degree Celcius
λ	lambda
Fe	Iron
Mg	Magnesium
Ca	Calcium
F	Fluoride
As	Arsenic
Ag	Silver
Ni	Nickel
Cu	Copper
Cr	Chromium
Al	Aluminium
Zn	Zinc

LIST OF ABBREVIATIONS

µPAD	Microfluidic Paper Based Analytical Device
ICP AES	Inductive Coupled Plasma Atomic Emission Spectroscopy
AAS	Atomic Absorption Spectroscopy
UV Vis	Ultraviolet Visible
nm	Nanometer
µm	Micrometer
µL	Microliter
mm	milimeter
g	gram
RGB	Red Green Blue
HSV	Hue Saturation Value
2D	Two Dimensional
3D	Three Dimensional
PDMS	Polydimethysiloxane
DOD	Drop - on - Demand
SU-8	Epoxy-Based Negative Photoresist

PERANTI ANALITIK BERASASKAN KERTAS MIKROFLUIDIK (µPAD) UNTUK PENGESANAN PANTAS BESI DALAM TANAH PERTANIAN

ABSTRAK

Pengumpulan logam berat di alam sekitar menjadi ancaman kepada kesihatan kerana, ketahanan, bioakumulasi dan toksisiti kepada tumbuhan, haiwan dan manusia. Penggunaan kertas semakin diakreditasi sebagai substrat yang mesra pengguna dan lazim untuk pembinaan peranti mikrofluidik. Peranti analitik berasaskan kertas mikrofluidik (µPAD) menyediakan teknologi pengganti untuk pengembangan alat diagnostik yang ekonomik, mudah alih, pakai buang dan berkos rendah untuk meningkatkan ujian titik penjagaan (POCT). Oleh itu, peranti analitik berasaskan kertas mikrofluidik ini (µpad) untuk mengesan logam besi dengan cepat dalam tanah pertanian telah melaporkan LOD dan LOQ serendah 5 ppm dan 15 ppm untuk analisis µpad dan 0.2 ppm dan 0.6 ppm untuk instrument spektroskopi ultraungu/tampak. dengan julat linear 0.5 hingga 8 ppm untuk instrumen spektroskopi µpad dan ultraungu/tampak masing-masing memperoleh R^2= 0.9248 dan R^2 = 0.9998. Pembentukan µpad menggunakan lilin parafin pada kertas saringan kualitatif mengambil masa tidak sampai 10 minit. Pengesanan besi dalam sampel tanah pertanian menghasilkan warna kompleks oren-merah dengan penggunaan reagen kromogenik iaitu 1,10-phenanthroline. Kelebihan yang ditawarkan oleh teknik µPAD ini yang dicadangkan di sini berkaitan dengan penambahbaikan dan kos rendah untuk pengesanan besi untuk pra-rawatan sampel dengan pantas, ringkas dan sederhana di lokasi persampelan.

MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (µPAD) FOR RAPID DETECTION OF IRON IN AGRICULTURAL SOIL

ABSTRACT

Accumulation of heavy metal in the environment becomes a health hazard because of their persistence, bioaccumulation and toxicity to plants, animals and human beings. Paper is increasingly accredited as a user-friendly and prevalent substrate for construction of microfluidic devices. Microfluidic paper-based analytical devices (µPADs) provide a substitute technology for development of economical, movable, disposable and low-cost diagnostic tools for improving point of care tests (POCTs). Hence, this microfluidic paper-based analytical devices (µpads) for rapid detection of iron metal in agricultural soil has reported LOD and LOQ as low as 5 ppm and 15 ppm for µpads analysis and 0.2 ppm and 0.6 ppm for UV/Visible spectroscopy. Calibration curve with linear range of 0.5 to 8 ppm for µpad and UV/Visible spectroscopy obtained R^2=0.9248 and R^2=0.9998 respectively. The fabrication of µpad using the paraffin wax on the qualitative filter paper was less than 10 minutes. The detection of iron in agricultural soil samples produced orange-red complex colour with the application of chromogenic reagent, 1, 10-phenanthroline. The advantages offered by this µPAD technique proposed herein in association with the improvements and low cost for iron detections for rapid, simple sample pretreatment and on-site measurement.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Soil is one of the basic natural components and exceptionally imperative because it is portion of a medium for plant development where it can reuse and recycle the resources and nutrients required by plant. The heavy metals may be added and present within the soil due to agricultural activities such as utilization of fertilizers and pesticides, soil revisions, and in waste materials recycled to the soil (Rahman and Zaim, 2015). Within the Southeast Asia section particularly in Malaysia, one of the foremost imperative crops developed in this nation is paddy plantation. Sort of fertilizers and pesticides have been utilized to extend the crop yield.

Different sorts of herbicides, fungicides and bug sprays moreover have been connected as trim control measures to secure the crop from infections, bugs and weeds. In any case, these sorts of activities lead the commitment of inebriated metal contamination in numerous agricultural sectors and may moreover cause chemical degradation of the soil due to the aggregation of a few poisonous components at unsatisfactory levels. This can be since most fertilizers utilized by farmers particularly are not enough decontaminated or purified amid the method of manufacturing process and they commonly contain a few contaminants and among them are destructive metals that can cause hurt to human and environment (Khairiah *et al*., 2012). In truth, agreeing to Gimeno-Garcia and the researchers in 1996, intoxicated metals such as heavy metals regularly compose the dynamic ingredients of pesticides. The intoxicated

or inebriated metals such as overwhelming metal can affect human wellbeing when it enters the food chain.

Apart from Asia region, copper (Cu), arsenic (As) and lead (Pb) are available in agricultural soils in Australia and New Zealand as a result of long-lasting uncontrollable utilization of pesticides (Yap *et al.,* 2009). Collection of heavy metal within the environment gets to be a wellbeing danger since of their perseverance, bioaccumulation and harmfulness to plants, creatures, animals and human creatures (Rahman and Zain, 2015). Heavy metals that are connected to soil water and soil particles will be ingested by plant roots and assembled in vegetables (Aweng *et al*., 2011). The nutrients that plants require in bigger sums are called macronutrients which are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur.

Plant micronutrients are also fundamental in much littler amounts to the wellbeing of the soil and plants as well. Nickel is the basic micronutrient required for the development of higher plants (Brown *et al*., 1987) and required to complete the total the life cycle of the plant and attainable seed. The phytoavailability is the method which is to control the exchange of follow components from soil to plant has been related with free nickel particle action in soil arrangement and emulsion (Massoura *et al*., 2006; Rooney *et al*., 2007; Ge *et al*., 2000).

Another pivotal micronutrient is iron metal. In plants, press is included in chlorophyll synthesis, and it is imperative for the conservation and preservation of chloroplast structure and function. In high-impact of aerobic soils, iron is

predominately found within the Fe^{3+} form (Rout and Sahoo, 2015). Meanwhile, the chromium (Cr) is not the basic component for plants, so its retention does not happen through particular components or process. The harmfulness of Cr depends on its speciation, which determines metal take-up, transport, and aggregation. The foremost steady shapes of this metal within the environment and organic frameworks are the trivalent Cr(III) and hexavalent Cr(VI) (Becquer *et al*. 2003).

One of the foremost multiskilled and reliable techniques of detection of heavy metals is ICP-MS (Inductively Coupled Plasma – Mass Spectrometry). It has been created since the 1980s (Bertin *et al*. 2016; Houk, 1986). Besides, an ICP-AES (Inductively Coupled Plasma – Nuclear Emanation Spectrometry) based technique has been built up to distinguish heavy metal toxins or pollutant in wastewater (Isai and Shrivastava, 2015). Another common location technique is AAS (Atomic Absorption Spectrometry), which is based on optical retention. These days, marine contamination has gotten to be a widespread issue and marine lives safety has played a vital part in human wellbeing (Höfer, 1998). Fatema and the team in 2015 have applied AAS instrument technique to measure the concentrations of Pb, Cd, As, Cr, and Hg in shrimps. In general, these current instrumental techniques have points of interest within the discovery and detection of heavy metals as they are enough sensitive, particular and accurate even for the determination at trace levels (Neves *et al*., 2009; Saad e*t al*., 2015). In any case, all of them require costly and bulky hardware, well-trained staff, and difficulty in operation and time-consuming. Hence, the researchers and analysts have been endeavouring to create cheap, basic, sensitive, particular, precise, user-friendly, and environmental-friendly discovery gadgets moreover on-site

monitoring device. The microfluidic paper-based analytical device (μPAD) have developed as one of the foremost promising devices.

The μPAD as the promising and capable platform has appeared awesome potential within the development and improvement of POCTs (point-of-care tests) (Parolo and Merkuri, 2013; Tokel *et al*., 2014; Sun *et al*., 2014; Hsieh *et al*., 2015; Yetisen *et al*., 2013). This concept was to begin with proposed by the Whitesides and other researchers in 2007 (Martinez *et al*., 2010) and the photoresist-patterned paper was utilized to manufacture the microfluidic gadgets so that the fluid might transport through capillary force within the non-attendance of external equipment. Since at that point, μPADs have been well known in an assortment of applications, such as clinical diagnostics diagnostics (Martinez *et al*., 2007; Aragay *et al*., 2011; Yang *et al*., 2013; Gao *et al*., 2013; Zhang *et al*., 2014; Mu *et al*., 2015; Chapman *et al*., 2015), food safety (Aid *et al.,* 2015; Tram *et al*., 2014), environmental monitoring (Sanjay *et al*., 2015; Semeenoi *et al*., 2013; Cate *et al*., 2013) and and bioterrorism (Zhang *et al*., 2013; Zhao *et al*., 2012; Dou *et al*., 2015; Li *et al*., 2014; Taudte *et al*., 2013) due to the preferences of movability, straightforwardness, economic reasonableness and in minimum sample amount and chemical substances consumption.

Paper substrate is hydrophilic by nature. Hence, to manufacture the μPAD, hydrophobic barriers are as designed and created to restrict and confine the liquid stream inside the specified area or coordinate the fluidics take after designated trails. A few fabrication methods, including photolithography (He *et al*., 2013; Klasner *et al*., 2010; Yu *et al*., 2015; Park *et al*., 2014; Martinez *et al*., 2008), wax printing (Lu *et al*., 2009; Carilho *et al*., 2009; Renault *et al*., 2014; Chaiyo *et al*., 2015), screen-printing

(Dungchai *et al*., 2011; Nie *et al*., 2010), plasma treating (Li *et al*., 2008; Li *et al*., 2010), flexography (Maattanen *et al*., 2011; Olkkonen *et al*., 2010) and laser treating (Chitnis *et al*., 2011) have been created.

Agreeing to Ebralidze and the team in 2019, a sensor may be a device that transform information containing chemical or physical property of the system into an analytically useful signal. Interactions between the medium and the sensor driving to change in optical properties or driving to optical signals that can be examined and translated are regularly utilized for the creation of optical sensors. Based on IUPAC (International Union of Pure and Applied Chemistry) classification, optical sensors can be assist subdivided agreeing to the sort of optical properties, which have been connected for sensing absorbance which is measured in a straightforward medium, caused by the absorptivity of the analyte itself or by a response with a few reasonable indicator or chromogenic reagent. Colorimetric sensors are the vital portion of optical sensors that represent to distinct colour change upon response with the analyte. This colour change can frequently be identified and visualized by the naked eye. Commonly, the change in intensity can be often detected at a certain wavelength within visible range of (400–800 nm). It also can be detected by utilizing extraordinary instrumented techniques.

1.2 Research Objectives

General objective

The general objective of this study was:

i. To fabricate microfluidic paper analytical device (µPAD) for rapid and simultaneous detection of iron in paddy field and tapioca plantation soil samples.

Specific objectives

The specific objectives of this study were:

i. To develop an optimized µPAD for measuring iron concentrations paddy field and tapioca plantation in soil samples.

ii. To determine the analytical performance of the developed µPAD.

iii. To validate the effectiveness of the developed µPAD for real sample analysis in comparison to the established method.

1.3 Problem Statements

Different sorts of fertilizers have been utilized by most ranchers extraordinarily to modernize the crop yield. Amid the process of manufacture, not adequately filtered or purified fertilizer take place due to financial reasons, in this manner they have undesirable substances, impurities and harmful heavy metals. Numerous sorts of herbicides, bug sprays, fungicides have too been connected as the control measures to ensure the crop yield or plantation from illnesses, bugs and weeds. These activities have been detailed as the contributors of heavy metal such as iron contamination in numerous rural districts and may moreover advance the chemical degradation of soil

coming about in accumulation of undesirable components. The excessive Fe^{2+}causes free radical production that harms cellular structure irreversibly and harm membrane or layers, DNA and proteins in plants due to its take-up by roots and transportation to leaves and via transpiration stream (Arora *et al.*, 2002; de Dorlodot *et al.*, 2005). The iron within the crop may enter the food chain and afterward posture a hazard to human wellbeing. The presence of an abundance or immoderate of iron admissions surpassed the allowable limit is unsafe since they tend to bioaccumulate within the human body. Producing harmful subsidiaries from the change of iron metal to ferric hydroxide precipitate that will lead to disease, arthropathy, and different endocrine disorder in human (Bordoloi *et al*., 2011).

The environment has ended up growingly contaminated with heavy metals in line with innovation has progressed that are greatly destructive to human wellbeing, and subsequently the issue of developing instruments methods able of identifying analyte based on concentrations in numerous sample matrices on the scale of parts per million (ppm), or indeed parts per billion (ppb), has drawn great interest in later times. To reach needs of the monitoring community for high sensitivity and low location limits, mostly analytical instrumented methods rely on costly instrument, which needs a high level of training to function reliably. The existing instruments such as AAS, AES and ICP are costly, difficulty in operation, tedious sample preparation and time-consuming.

1.4 Significances of the Study

The handheld device for on-site examination of forensic interest samples have been rising up rapidly within the last a long time, resulting in the improvement of

screening tests to test samples. The marvel of metal components within the soil is one of the imperative trace evidence in forensic science. There is a developing require for effective or low-cost advanced technologies to identify and monitor the contaminant concentrations rapidly, directly and on-site to supply more timely data or information with respect to the degree and magnitude of contamination. Microfluidic paper-based analytical devices (μPADs) offer an opportunity to convey this demand by expanding the geographic and recurrence scope of environmental monitoring, whereas at the same time decreasing the analytic costs and trouble of the measurement process. This μPAD platform empower the realization of low-cost, flexible, basic and portable analytical devices. The μPADs ordinarily comprise of a little piece of designed paper with a 2D or 3D structure able of testing a few tens of microliters of the fluid test inside a generally brief time. The μPADs, created by papers are reasonable, user-friendly, widespread and don't require external system or equipment and complex fabrication processes compared to conventional microfluidic analytical devices gadgets which are manufactured by silicon, glass and super polymer as their substrates. Rapid and on-site quantitative estimation is required for detection the level of iron metal in agricultural soil (Xia *et al*., 2016).

CHAPTER 2

LITERATURE REVIEW

2.1 Principle of Microfluidics Paper Based Analytical Device (µ-PAD)

Cellulose fibres are the main component of paper and these fibres have hydrophilic properties in nature and permit aqueous solution to flow effectively through capillary action. Hence, due to these properties, the paper has ended up as a substrate of intrigued within the field of microfluidics (Li *et al*., 2010). The organize of cellulose in sheets of paper makes pores. These pores permit the fluids to enter the paper. Concurring to Moon and the co-workers in 2011, paper-based substrates appear hydrophilic properties that empower the capillary liquid flow. Typically, due to the totals of micro/nanometer-sized cellulose fibres setting off as a permeable or porous material (pore size between 0.45 and 11 µm). Hence, no external system or equipment of fluid is required. As a result, the flow behaviour of liquid on paper will depend on the composition of the paper type including filter, chromatographic, nitrocellulose paper, or nanopaper, also the measurements and geometry of the sketched out fluidic channel, and whether the flow runs horizontally or vertically. For instance, filter paper (Whatman 1) is adequate not as it were for sample loading and transporting but also for path network and additionally as absorbent pad or cushion (Parolo and Merkoçi, 2013).

These qualities make cellulose perfect for utilizing as the substrate in microfluidic paper-based analytical device (Pelton, 2009). Within the µPADs, the sample is presented into the centre of the sampling zone. The liquid or fluid started to

flow along the channels towards the response regions and after about two minutes, the whole sample from sampling zone reached to the detection zone where the reagents were confined. Ten minutes afterward, photos were taken employing a Smartphone camera and sent to a computer, first to obtain the corresponding values for each channel of the RGB colour space, and after that to process of data information (Vidal *et al*., 2018). Figure 2.1 illustrates the design of microfluidic paper based analytical device clarified the area of location of detection and target or sampling zones of (µPAD) (Idros and Chu, 2018).

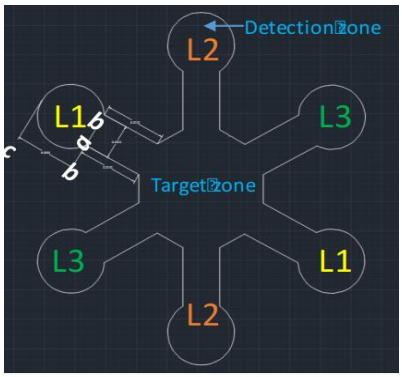

Figure 2.1: Pattern of a microfluidic paper based analytical device (µPAD) (Idros and Chu, 2018).

2.2 The Shaping of Microfluidic Paper Based Analytical Devices (µPADs)

2.2.1 Shaping of Two – Dimensional µPADs

The 2D (two dimensional) fabrication shaping of cellulose with decreased rates of fabrication errors has permitted prompt prototyping of these devices. This sort of shaping was first carried out by knife a cut plotter or by employing a laser treatment procedure by Fenton and other researchers in 2009 as appeared in Figure 2.2 and Chitnis and the team in 2011. The foremost well-known fabrication technique of µPAD set up in 2009 was wax printing method (Carrilho *et al*., 2009) in Figure 2.3. This effective cost method permits enormous and quick fabrication of devices by

following after two basic fabrication steps likes wax printing of patterns and heat treatment for penetration of the hydrophobic wax into the channel paper. Besides, wax printed gadgets are well suited with most of the aqueous solution except organic solution. Contradicting with filter paper, nitrocellulose (NC) uncovered great benefits such as high protein binding capabilities, sample purification and controlled liquid flow utilizing the wax printing fabrication method (Lu *et al*., 2010).

Figure 2.2: The 2D shaping via a knife plotter or by using a laser treatment technique (Fenton *et al*., 2009).

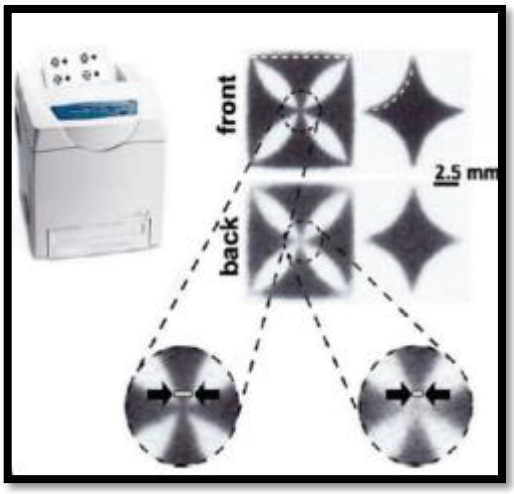

Figure 2.3: The 2D shaping via wax printing (Carrilho *et al*., 2009).

2.2.2 Shaping of Three – Dimensional µPADs

The fabrication of 3D (three dimensional) µPADs has developed as problem solving for different discovery and detection of analytes. Figure 2.4 shows the first 3D µPAD that has been established from stacking 2D layers of papers patterned by SU- 8

photolithography (Martinez *et al*., 2008). In this way, the double-sided tape was utilized to join and attach the 2D paper layers and cellulose powder to connect with the fluidic reservoirs for vertical flow of reagents. However, the utilize of adhesive tape was not only required special equipment for shaping reservoirs and channels precisely, but also could contaminate the sample permitting non-specific absorption. In differentiate, the fabrication method of 3D µPAD has included a spray to glue and stack different layers of 2D patterns (Lewis *et al*., 2012) was presented as appeared in Figure 2.5. This has come about in devices with less stacked layers encouraging a rapid vertical flow. The sprayed glue did not influence biocompatibility and hydrophilicity of the devices, but reduced the reduced the horizontal flow of fluids by 1.3 times.

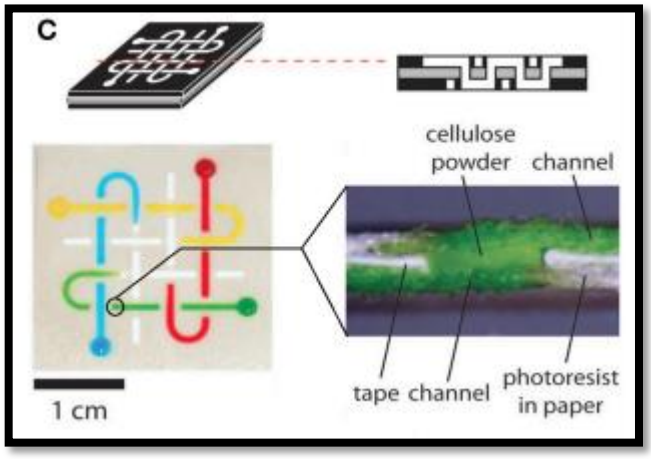

Figure 2.4: The 3D µPAD has been established from stacking 2D layers of papers patterned by SU- 8 photolithography (Martinez *et al*., 2008).

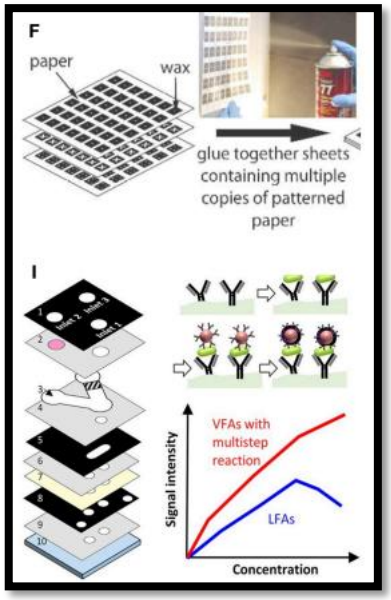

Figure 2.5: The 3D µPADs included a spray to glue and stack multiple layers of 2D designs (Lewis *et al*., 2012).

2.2.3 Shaping of Do – It – Yourself (DIY) µPADs

The reasonableness and transportability of microfluidic paper-based devices have opened the way for making a point of the µPAD to be fabricated beneath a Do-It-Yourself (DIY) format where the user can implement and execute a set of pre-fabricated microfluidic devices or fabricate the device on his/her own style. For occasion, Lu and the team in 2009 have created a wax pen where the design should be drawn on both sides of the paper. At that point, resolution refinement was accomplished by firstly outlining out the pattern using an inkjet printer and after that manually drawing the pattern utilizing the wax pen. This fabrication technique is basic, but expanding reproducibility mistakes from one user to another. In 2014, a handheld instrument empowering the rapid has been created to form the paper-based microfluidic devices by a stamping process (Garcia *et al*., 2014) as appeared in Figure 2.6. Paraffin is utilized to configure the hydrophobic boundaries that are transferred onto the paper in less than 2 minutes by heating the handheld tool. Figure 2.7 displays

an upgraded adaptation of the wax pen approach was presented for electrochemical detection of glucose (Li *et al*., 2017), whereby a customized wax pen and a conductive-ink write composed by graphite powder and protein ink are utilized to direct compose hydrophobic obstructions and conductive electrodes, respectively.

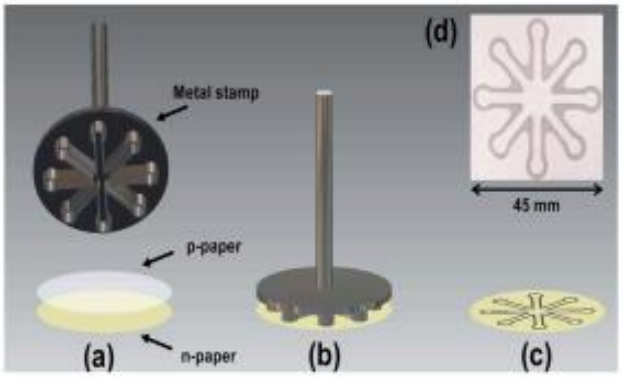

Figure 2.6: Handheld tool for the rapid creation of paper-based microfluidic devices via a stamping process (Garcia *et al.*, 2014).

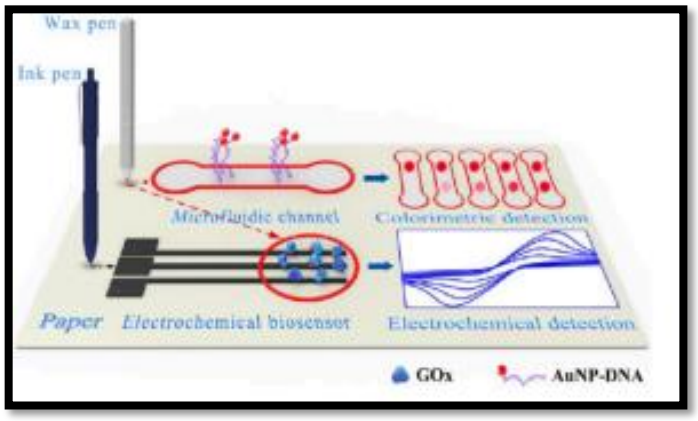

Figure 2.7: The wax pen approach for electrochemical detection of glucose (Li *et al*., 2017).

2.3 Principle of Colorimetric Detection

The detection on a microfluidic paper based analytical devices (μPADs) can be implemented visually by comparing the colour intensity in each reaction or detection zone. Different devices such as cameras (Apilux *et al*., 2012), scanners (Satarpai *et al*., 2016) and smartphones (Bas, 2017) can be utilized to get colorimetric data apart from

the human eyes (Li *et al*., 2015). By employing a reasonable computer program or software application, it is possible to obtain the parameters of any colour space from a computerized picture of μPADs. These parameters can be utilized to get the colour intensity vs. concentration regression line. The RGB (Red Green Blue) colour spaces are the parameters that has been connected to advanced digital iamges that allow three channels of data that can be utilized to produce a calibration curve (Meelapsom *et al*., 2016). The device was captured under the following optimized conditions. Utilizing ImageJ software, the pictures were processed to get the values of the three distinctive RGB colour spaces channel.

2.4 Toxicity and Legislation of Iron to Human and Environment

2.4.1 Occurrence of Iron

Iron metal commonly existing as metallic component, containing 4.6 % of the volcanic rocks and 4.4% of sedimentary rocks (Morel and Hering, 1993). Native iron concentrations are region-specific and can contrast altogether even within localized zones due to soil type and the presence of other sources. Sandy soils have the most reduced in general of iron content in the interim, the clayey soils have the most noteworthy iron content (McGovern, 1987). Iron can happen in either the divalent Fe^{2+}or trivalent Fe^{3+}forms. However, iron occur dominantly as Fe^{3+}oxides in soils (Bodek *et al*., 1988).

2.4.2 Iron Contents in Fertilizers and Pesticides

Fertilizers as the sources of plant nutrients. The components of H, C, O, are major basic components gained from air and water through photosynthesis. The other

14 fundamental mineral components provided by the soil, of which 12 are ordinarily managed in agricultural soil through the utilize of fertilizers. These comprises of the macro-nutrients (N, P, K, Mg, Ca, S), and the micro-nutrients (B, Cu, Zn, Fe, Mn, Mo), whereas the other 2 are Cl and Ni. Fertilizers exist as solid, liquid or vaporous states containing one or more plant nutrients. They are either connected to the soil, specifically on the plant (foliage) or included to aqueous solution (fertigation) to preserve soil richness or fertility and revamp the crop advancement and yield. Natural or organic fertilizers are secured from organic matter -animal matter, human squander or vegetable matter likes manure or compost. Inorganic fertilizers are those made from material that does not originated from plants or animals, and thus disposing of the carbon-containing materials except ureas (Heng, 2016).

Pesticides play a vital part in agricultural soil since of their capacity to kill assortment of pests. Nevertheless, little sums of pesticide residue may retain within the food chain and trigger a potential hazard for human wellbeing due to their capacity to cause subacute and persistent toxicities. The foremost broadly utilized pesticides are the organophosphorus and carbamate pesticides, which have been detailed to have nearly totally supplanted the organochlorine pesticides that comprising DDT substance. The DDT substance in pesticides was cancelled by EPA in 1972. (Porrini *et al*., 2003). Mineral substance from pesticides such as Na, K, Ca, Fe, Mg and Zn were examined in Malaysian kinds of nectar by Moniruzzaman and other analysts in 2014.

2.4.3 Iron Uptake by Plants

Plants may also conciliate the reduction of Fe^{3+} to Fe^{2+} to control uptake by plants. Plants utilize two diverse techniques to solubilize and assimilate or absorb iron

from the soil. Firstly, plants diminish Fe (III)- complexes at the root surface and assimilate the Fe^{2+}ions delivered through this root-associated reduction and the second technique is to discharge particular Fe (III)-binding with low atomic weight, organic polydentate ligands which known as phytosiderophores to solubilize Fe^{3+} particles and make them accessible for plant absorption (Römheld and Marschner, 1986).

2.4.3 Accumulation and Acceptable Limit of Iron in Soil

Iron is absorbed by plants as the ferrous ion (Fe^{2+}), with concentrations of total iron in plants commonly extending from 4 to greater than 16 ppm. These concentrations are approximately equal to boron, manganese, and zinc substances of plants. Numerous plants contain less than 16 ppm of iron, but not sufficient for iron to be considered a macronutrient (Thompson and Troeh, 1973). Agreeing to the World Health Organization (WHO) in 2000, the permissible limit of iron metal content in soil is 150 ppm (Kacholi and Sahu, 2018).

2.4.4 Effects of Iron on Plants

Iron is considered a plant micronutrient and exists within the oxidized, ferric (Fe^{3+}) form. When the reduced ferrous oxide comes into contact with air, it oxidized to the ferric state that is insoluble in water, resulting in precipitate. The precipitate at that point can accumulate on soils in regions where groundwater releases to the surface. To be absorbed, iron must be in the ferrous Fe^{2+} state which is in a soluble form (Ems and Huecker, 2019). Iron is absorbed by plants as the ferrous particle Fe^{2+}which is significant for the formation of chlorophyll and functioning in a few of

the enzymes of the plant's respiratory system. Much of the iron in well-drained soils is in the ferric Fe^{3+} form, which is not reasonable to be uptake by plants.

Iron deficiencies have resulted about from an excess content of manganese and conceivably copper whereby these heavy metals are oxidizing agents that change over ferrous irons to the more insoluble ferric form. Other than that, iron deficiencies moreover caused by manganese toxicity happen in acidic soils that otherwise would supply adequate iron for plant development. Fossil remnants of a few primitive soils contained sufficient iron to serve as iron ore. These compounds, however, are as well insoluble to reach plant needs, indeed as a micronutrient (Thompson and Troeh, 1973). The mechanisms of iron uptake and transport by plants have gotten much study about and consideration since they are the key forms within the supply of iron to plants.

2.4.5 Toxicity of Iron

In waterlogged soils such as paddy field, the concentration of soluble iron may increase by a few systems of magnitude due to low redox potential (Schmidt, 1993). Subsequently, press may be taken up in over the top sums. In any case, it is possibly harmful and can advance the formation of reactive oxygen-based radicals, which can harm the imperative cellular constituents, for example, the membranes by lipid peroxidation. Acidity or blackening of the roots are side effects of plants uncovered to an immoderate content of iron levels (Laan *et al*., 1991). Iron for the most part exists as Fe^{3+} chelate form within the soil and plants sometimes and then cannot assimilate it under different physiological conditions such as in high soil pH in alkaline soils (Lindsay and Schwab, 1982).

One of the foremost noteworthy transformations in flooded soils is conversion or reduction of $Fe^{3+}$to Fe^{2+} form. This transformation may permit higher or overabundance uptake of Fe^{2+} by developing plants and may cause harmfulness to them. Iron poisonous is assigned to the low redox potential of flooded soils, low soil pH, low soil fertility as well as accumulation of harmful organic acids or hydrogen sulphides (Fageria *et al.*, 2008). The exchange of iron metal from plants to human fundamentally by means of the food chain and introduction such as inward breath from the air or dermal course. The iron created hydrogen free radicals assault DNA causing mutation, resulting in cellular damage and malignant change which is can cause a cluster of diseases (Grazuleviciene *et al.*, 2009).

2.5 Optimisation and Analytical Performance Required for Microfluidics Paper Based Analytical Devices (uPADs)

A few parameters should be optimized for the manufacture of uPADs including volume of indicator or chromogenic reagent and metal ion sample dropped onto the detection and sampling zones, retention colour quality due to interaction between reagents and targeted analyte of the sample conjointly microfluidic channel resolution considering the length, width, diameter and stability of the paper. The measured analytical performances that will be measured incorporate the linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability and recovery.

Ostad and the colleagues in 2017 have carried out the parameters for enhancement which are sorts of paper and volume of reagents added on detection zone. Based on their study, three sorts of Whatman® channel papers No. 41 which has the biggest pore size and in this way the most elevated flow rate has been utilized. The

Figure 2.8 demonstrates the microfluidic channel determination of paper fabrication, consequently 0.1 µL of reagents managed to be loaded on detection zones with standard micropipette and this volume has been chosen for further analysis. The limit of detection is 8.3 mg L^{-1} and 1.0 mg L^{-1} and the relative standard deviation is 8.3% and 5.9% for calcium and magnesium, respectively. A linear range from 10 to 100 mg $L^{-1}$for calcium and two linear ranges of 4–20 mg L^{-1} and 20–100 mg $L^{-1}$for magnesium were obtained in the paper based microfluidic device. The concentration of calcium and magnesium were effectively determined in tap, stream, mineral and household purifier water samples.

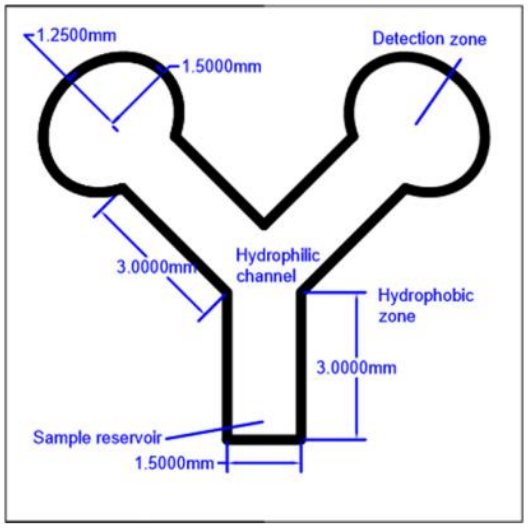

Figure 2.8: The microfluidic channel resolution of paper fabrication (Ostad *et al.*, 2017).

Vidal and the colleagues in 2018 have fabricated the design of the microfluidic channel resolution of paper as appeared in Figure 2.9. The ideal volume of reagents to be loaded on the response region was 0.5 µL to wet and form the standard distribution within the detection zone. Twenty µL of the test was the ideal volume to be stacked onto the examining zone and fill up a µPAD through the hydrophilic channels. The linear range was 0.23–2.26 mg $L^{-1}$for fluoride with a limit of detection (LOD) and

limit of quantification (LOQ) of 0.13 mg $L^{-1}$and 0.25 mg L^{-1}. The working range for nitrite was 0.05–10.0 mg L^{-1} with LOD of 0.03 mg $L^{-1}$and LOQ 0.13 mg L^{-1}.

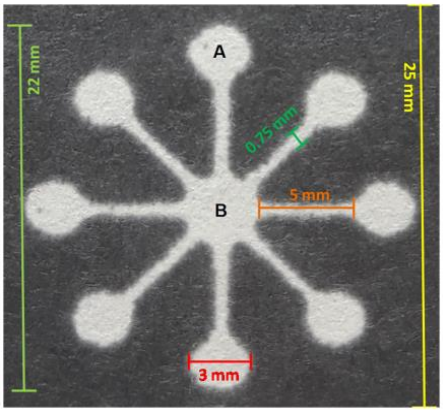

Figure 2.9: The design the microfluidic channel resolution of paper (Vidal *et al*., 2018).

2.6 The Advancements of past study on Microfluidics Paper Based Analytical Device (µPAD)

The paper-based microfluidic analytical devices (µPADs) or also known as point-of-care tests (POTs) are accomplishing wide acknowledgment that have critical purposes in numerous areas such as in food assay, diagnosis and environment. (Jokerst *et al*., 2012; Mentele *et al*., 2012; Yetisen *et al*., 2013). Filter paper that has been utilized to fabricate µPADs has hydrophilic nature, ease of forming on paper, high wicking properties, expandable, cheap, high porosity, replaceable, non-harmful and biodegradability. The white colour of filter paper and due to its hydrophilic properties of white colour of filter paper can be utilized to measure for rapid colorimetric assay within the absence of necessity of external powers to drive the analyte into the detection or discovery zone from the target or sampling zone.

Whitesides and the group in 2007 have presented and established the idea of fabricating microfluidic channels on paper for multiplex analyte detection. The detection zone is based on calorimetry which measuring the colour concentrated concerning the concentration of the analyte. Various creation strategies of µPADs based on colorimetric detection for different samples discovery have been created and indeed presently the advancement proceeding by the analysts (Sriram *et al*., 2017). The working rule behind µPADs is that hydrophobic boundary capacities as physical obstruction for sample distribution pathway in hydrophilic region.

The hydrophobic obstruction created on the channel paper is critical to design the hydrophilic channel comprising of detection and sampling zones for sample detection. The hydrophobic boundary helps to explore the movement of arrangement from sampling to detection zones. Different sort of platting strategies utilized to manufacture the µPAD depends on the accessibility of materials, machines and chromogenic reagents or indicators. The solution travel by passive capillary activity through a designated pathway of channel papers to the zone of detection for synchronous numerous analyses. The momentary of colour change happen whereby the reagent arrangement responds with target analyte in sample, hence this colour change picture can be effortlessly snapped by utilizing smartphone or any electronic gadget with high resolution (Martinez *et al*., 2008). Present fabrication methods as well as the challenges and the downsides of µPAD ought to be tended to along with future viewpoints.

2.6.1 Photolithography fabrication technique

In 2007, Whitesides and the group were the primary analysts who created µPADs utilizing photolithography as the standard method of printed circuit board and it employments light to form the conductive pathways on chips. They have demonstrated this capability by the synchronous detection of glucose and protein in 5 mL of urine. This strategy includes light exposure through a mask to venture the picture of a pattern that is more like a negative picture in standard photography. Photolithography could be a helpful, fast, and cheap strategy, and with this procedure, hydrophobic areas that compose the patterns which are made of polymeric boundaries (Xia *et al*., 2016). Figure 2.10 portrays the method for fabricating paper into millimetre-sized channels: a) Photolithography was used to fabricate SU-8 photoresist embedded into paper; b) the designed paper was modified for bioassays.

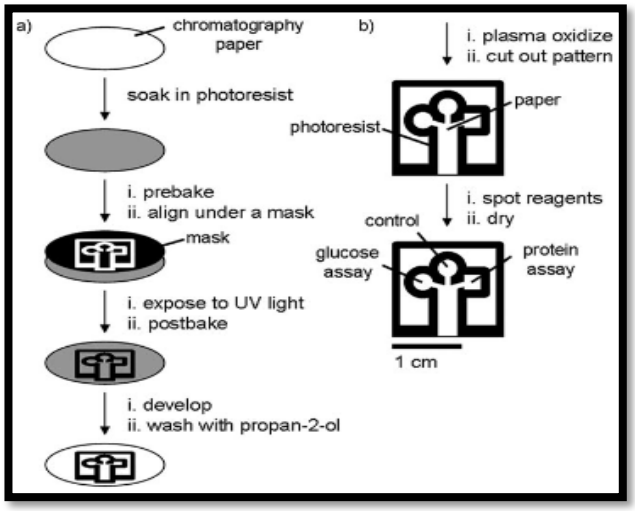

Figure 2.10: The method for fabricating paper into millimetre-sized channels:
a) Photolithography was used to fabricate SU-8 photoresist embedded into paper.
b) The patterned paper was modified for bioassays (Whitesides *et al*., 2007).

The low-priced, quick and taken a toll compelling fabrication method of desktop 3D printer as shown in Figure 2.11 was made to urge superior of lithographic method and this method created hydrophilic channel on hollow structured polymeric substrate. The pore measure of 74 – 125 µm channel paper in cellulose powder shape encourage to be transport medium through capillary activity making target test may move rapidly from sampling to detection zones. The controlling of channel profundity can be performed squarely and it produced high resolution features on the 3D printer's printing.

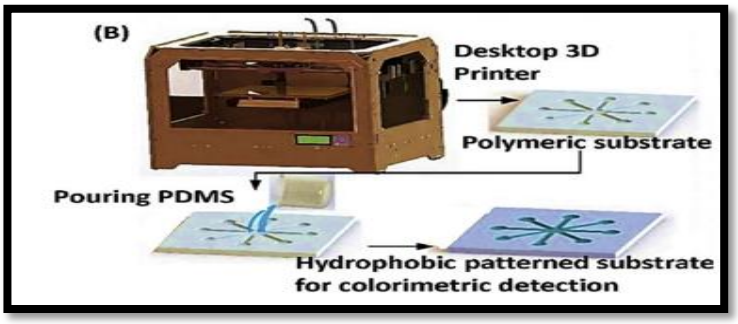

Figure 2.11: The fabrication process of 3D printer (He *at al*., 2016).

2.6.2 Wax printing

On the other hand, wax printing can be utilized to concoct a microfluidic channel for the coordinate investigation, for example in soil investigation for explosives, it is troublesome due to its complex matrix which contains numerous non-volatile and semi-volatile compounds which will compromise analyte detection. Humid acids present in soil meddled with colorimetric strategies of detection (Halasz *et al*., 2002) in this way, it has been common hone to apply a sample preparation step before investigation to avoid such interferences (Steinfeld and Wormhoudt, 1998). Different extraction techniques have been investigated for the expulsion of unsettling