MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (µPAD) FOR RAPID DETECTION OF CADMIUM IN RIVER WATER

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MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (μPAD) FOR RAPID DETECTION OF CADMIUM IN RIVER WATER

by

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CERTIFICATE

This is to certify that the dissertation entitled **Microfluidic Paper Based Analytical Device (µPAD) For Rapid Detection of Cadmium In River Water Sample** is the bona fide record of research work done by Nor Izati Binti Che Ab Aziz 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 and partial fulfilment for the degree of Master of Science (Forensic Science).

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Date: 10/9/2020

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.

....

(Nor Izati Binti Che Ab Aziz)

Date: 10/9/2020

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

Cd	Cadmium
Pb	Lead
Hg	Mercury
Fe	Iron
TMOS	Trimethoxyoctadecylsilane
NaOH	Sodium hydroxide
PDMS	Polydimethylsiloxane
SDDTC	Sodium diethyldithiocarbamate
H_2DZ	Dithizone
CTAB	Cetyltrimethylammonium bromide
HNO_3	Nitric acid
˚C	Degree celcius
nm	Nanometer
λ	Wavelength
mL	Millilitre
L	Litre
M	Molarity

LIST OF ABBREVIATIONS

μPAD	Microfluidic paper-based analytical device
LOP	Lab on paper
LOC	Laboratory on chip
HMI	Heavy metal ion
ASV	Anodic stripping electrode
WHO	World Health Organization
EPA	Environmental Protection Agency
USEPA	United State Environmental Protection Agency
POCT	Point of care testing
LOD	Limit of detection
LOQ	Limit of quantification
RSD	Relative standard deviation
ICP-MS	Inductively combined plasma mass spectrometry
AAS	Atomic absorption spectroscopy
AES	Atomic emission spectroscopy
AFS	Atomic fluorescence spectroscopy
FI	Flow injection
LFA	Lateral flow immunoassays

PERANTI ANALITIK BERASASKAN KERTAS MIKROFLUIDIK (μPAD) BAGI PENGESANAN PANTAS KADMIUM DALAM AIR SUNGAI

ABSTRAK

Dalam beberapa tahun kebelakangan ini, isu pencemaran air dengan ion logam berat tidak pernah berakhir sehingga mendapat perhatian yang luas dari seluruh negara. Logam berat ini melebihi had yang ditetapkan adalah toksik bagi manusia dan juga alam sekitar. Oleh itu, kaedah yang sangat mudah digunakan, mesra alam dan mudah alih menggunakan μPAD dibuat untuk menentukan cadmium (Cd) pada tahap ultra-jejak menggunakan 1,5-diphenylthiocarbazone (dithizone) sebagai reagent kolorimetrik ($\lambda = 500$ nm) dalam cecair. Campuran bertukar warna koko/merah kepada oren dengan kehadiran Cd. Lilin paraffin dituangkan ke atas kertas penapis untuk membuat penghalang hidrofobik untuk saluran cecair. μPAD dibuat dalam masa 10 minit dan memberi kebolehalangan dan kestabilan yang tinggi. Penggunaan lilin paraffin dengan cap getah pegangan ditunjukkan untuk pengesanan Cd menggunakan pengesanan kolorimetrik. Pendekatan pengecatan lilin yang diberikan memberikan kaedah fabrikasi yang ringkas, pantas dan menjimatkan kos untuk fabrikasi μPAD. Pekali linear (R^2) adalah 0.9538 dan had pengesanan ion Cd menggunakan μPAD adalah 3.87 ppm. Hasil kajian menunjukkan bahawa had pengesanan yang diperoleh untuk kedua-dua kaedah tersebut lebih tinggi daripada had Cd yang dibenarkan iaitu 0.005 ppm. Kaedah ini dilaporkan kurang sensitif untuk logam ultra-jejak seperti Cd. Namun, untuk analisis sampel sebenar, pemulihan sampel air diukur sebanyak 82.5% menggunakan μPAD. Ia mempunyai nilai pemulihan yang baik. Sebagai kesimpulan, μPAD digunakan sebagai ujian saringan sebagai langkah paling penting dalam ujian berdasarkan kertas untuk mengenal pasti kehadiran analit tetapi langkah pengesahan diperlukan untuk analisis lebih lanjut menggunakan instrumen yang canggih.

MICROFLUIDIC PAPER-BASED ANALYTICAL DEVICE (μPAD) FOR RAPID DETECTION OF CADMIUM IN RIVER WATER

ABSTRACT

In recent years, the issue of contamination of water with heavy metal ion is never-ending, thus seeking extensive attention from all over the world. Heavy metal above a threshold limit is toxic to humans as well as the environment. Therefore, a very promising method using μPAD is presented for the rapid determination of Cd at an ultra-trace level using 1,5-diphenylthiocarbazone (dithizone) as colorimetric reagent (λ=500 nm) in aqueous solution. The mixture turns from brownish-red to orange colour in the presence of Cd. The use of paraffin wax with the handheld rubber stamp was demonstrated for the detection of Cd using colorimetric detection. The paraffin wax was used onto the filter paper to create a hydrophobic barrier for fluidic channels. The μPAD was fabricated within 10 min and provided high reproducibility and stability. The rubber stamping method provides a simple, rapid and cost-effective in fabrication of uPAD. The calibration curves were constructed for developed method of μPAD. The linear coefficient (R^2) was 0.9538 and the detection limits of Cd ion using μPAD was 3.87 ppm. The results demonstrate that the detection limits obtained for both methods were higher than the permissible limit of Cd which is 0.005 ppm. These methods were reported to be less sensitive for ultra-trace metal such as Cd. However, for real sample analysis, the recovery of Cd in the water samples was measured as 82.5% using μPAD. It has good recovery value. To conclude, the fabricated μPAD can be used as screening test which one of the most vital steps in paper-based assays to identify the presence of analyte but confirmation step is required for further analysis using the sophisticated instruments.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The issue of heavy metals has pursued and received significant attention from all over the world from the last few years (Liu *et al*., 2017; Halkare *et al*., 2019; Tsai *et al*., 2018; Czolk *et al*., 1992). Population growth, urbanization and industrialization have a deleterious impact on the quality of water in most countries (Li *et al*., 2013). Water shortages are a major threat to human health in developing countries, and are frequently taken for granted and haphazardly consumed (Lin *et al*., 2016; Almeida *et al*., 2018). Due to the toxicity, non-degradability and bioaccumulation of heavy metal ions (Halkare *et al*., 2019; Liu *et al*., 2019; Zhao *et al*., 2020; Lin *et al*., 2016; Kim *et al*., 2012; Benounis *et al*., 2006), pollution of water with heavy metal ions has been a major concern in the world of industrial development. Heavy metals can simply be described as the metals that are relatively abundant in the earth's crust, undergo multiple processes, and are used in measurable amounts but highly toxic to human health and the environment (Jain *et al*., 2019; Hormozi-Nezhad and Abbasi-Moayed, 2014; Vaughan and Narayanaswamy, 1998).

A heavy metal such as cadmium (Cd) can be easily found in soil, water and air due to increased industrial and agricultural activities as well as inappropriate discharges of heavy metal ions from wastewaters or domestic effluents without proper treatment (Zhang *et al*., 2018; Radhakrishnan *et al*., 2020). Heavy metal above a threshold level is harmful to human health and causes many life-threatening diseases (Lin *et al*., 2016; Momidi *et al*., 2017; Priyadarshini and Pradhan, 2017; Wei *et al*., 2012). The World Health Organization (WHO) and the Environmental Protection

Agency (EPA) have specifically specified the concentration limit for the identification of heavy metals in drinking water and food to ensure that their existence is within reasonable limits. The allowable exposure levels for Cd by the WHO and the United States Environmental Protection Agency (USEPA) are 5 ppb, respectively (Halkare *et al*., 2019).

Cadmium is chosen because of various advantages such as simple detection without sophisticated instruments, high sensitivity and selectivity over colour shift for different species (Kaur *et al*., 2018). But, it is a carcinogenic, poisonous and non-degradable heavy metal (Figure 1.1) (Momidi *et al*., 2017; Priyadarshini and Pradhan, 2017; Rasheed *et al*., 2018; Ebralidze *et al*., 2019; Li *et al*., 2013). Therefore, they are potentially toxic when it is ingested by a person through inhalation, ingestion or absorption by skin. As a consequence, acute or chronic intoxication can lead to severe, or even worse it can cause diseases such as cancer, cardiovascular disease, brain damage and kidney failure (Idros and Chu, 2018; Lin *et al*., 2016; Tsai *et al*., 2018; Verma and Gupta, 2015; Radhakrishnan *et al*., 2020; Azmi and Low, 2017; Kim *et al*., 2012). Hence, to overcome the problems that arose and the growing interest in environmental analysis, different techniques have been employed by many researchers.

Recently, some physical, chemical, and biological methods have been used to detect polluted toxic metals (Priyadarshini & Pradhan, 2017). Many highly sophisticated techniques show excellent and reliable results for the monitoring and detection of heavy metals such as inductively combined plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and atomic fluorescence spectroscopy (AFS) (Devadhasan and Kim; 2018; Liu *et al*., 2017). For instance, these instruments have many advantages mainly highly selective and extremely sensitive with detection limits of up to one in a billion (Li *et al*., 2013). However, they are bulky, complex pre-treatment procedures, time-consuming sample preparation and need expensive tools and cannot be used for field monitoring which creates significant disadvantages for these tools (Liu *et al*., 2017; Idros and Chu, 2018; Śliwińska *et al*., 2019; Zhou *et al*., 2019; Guo *et al*., 2019; Zhang *et al*., 2020; Lin *et al*., 2016). Therefore, a promising and highly desirable technique has been developed to counteract previous methods by using the microfluidic paper-based analytical device (μPAD) to detect heavy metal which is easy to prepare and can perform rapid inspection at the site (Guo *et al*., 2019). This method can be divided into three distinct techniques namely colorimetric, fluorescence and electrochemical (Lin *et al*., 2016; Busa *et al*., 2016; Liu *et al*., 2016; Almeida *et al*., 2018; Rasheed *et al*., 2018; Ajay Piriya *et al*., 2017; Kim *et al*., 2012).

Colorimetry is selected and commonly used due to its specificity, simplicity and compatibility with relatively low cost reporting systems, including smartphones and scanners and the most preferable detection technique to be combined with μPAD (Wu *et al*., 2019; Busa *et al*., 2016; Zhou *et al*., 2019; Morbioli *et al*., 2017; Kaur *et al*., 2018; Murdock, 2015). Colorimetric techniques also offered some advantages, such as high sensitivity, selective to different analytes, without complex spectroscopic instruments, rapid response and are particularly promising and non-destructive (Ajay Piriya *et al*., 2017; Zhou *et al*., 2019; Momidi *et al*., 2017; Kaur *et al*., 2018). It is an analytical method involving a colour change reaction which the naked eye can observe (Lin *et al*., 2016). This also defines the colour intensity of any interest-based compound based on the absorption of a given wavelength of light (Wu *et al*., 2019).

The intensity of the colour change is analysed using colour analysis software such as ImageJ (Sadollahkhani *et al*., 2014; Busa *et al*., 2016; Pathirannahel, 2018). Colorimetric sensors display excellent on-site detection capability of heavy metals (Idros and Chu, 2018). Besides, colorimetric readings are the most frequent-used method of detection in microfluidic devices which enable multiple analytes to be analyzed qualitatively, semi-quantitatively, and completely quantitatively (Morbioli *et al*., 2017). Many changes have been made to improve the efficacy of the method by combining with paper-based analytical devices (PADs) in many applications, particularly forensic detection, drug screening, water analysis, cell biology, food analysis and environmental monitoring (Ghosh *et al*., 2019; Busa *et al*., 2016; Wu *et al*., 2019). Besides, detection is one of the most vital steps in paper-based assays to identify the presence of analyte (Morbioli *et al*., 2017).

Thus, microfluidic paper-based analytical device (uPAD) shows excellent features such as highly portable, disposable, fast, sensitive, cheap, environmentally friendly, highly desirable performance, simple, long shelf life and performing on-site detection of heavy metals in river water (Devadhasan and Kim, 2018; Ghosh *et al*., 2019; Teepoo *et al*., 2019; Liu *et al*., 2017). In addition, microfluidic is the science and technology of devices that use fluid channels with dimensions ranging from tens to hundreds of micrometers to move and control tiny quantities of fluid. Microfluidics has undergone rapid growth with major impacts on analytical chemistry due to certain strengths including the ability to use small quantities of samples and reagents to perform separation and detection with high resolution and sensitivity (Busa *et al*., 2016).

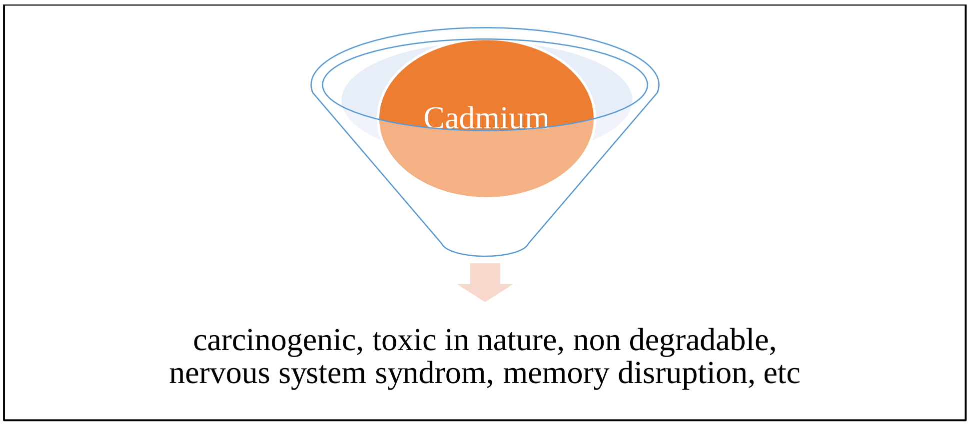


Figure 1.1: The effects of Cd on human health (Idros and Chu, 2018; Rasheed *et al*., 2018; Priyadarshini and Pradhan, 2017; Momidi *et al*., 2017).

1.2 Objectives

The general objective of this study was:

To fabricate microfluidic paper-based analytical device (µPAD) for rapid detection of cadmium (Cd) in a water sample.

Specific objectives of this study were:

1. To develop an optimized µPAD for measuring cadmium (Cd) concentration in the water sample.
2. To determine the analytical performance of the developed µPAD.
3. To validate the effectiveness of the developed µPAD for real sample analysis in comparison to the standard method.

1.3 Problem Statement

The adverse effects on human health and environmental pollution was caused by heavy metal contamination in the water. A heavy metal such as cadmium (Cd) is extremely toxic even at low concentration, thus causing severe diseases to humans and could endanger long-term exposure to life (Hormozi-Nezhad and Abbasi-Moayed, 2014; Lin *et al*., 2016; Rasheed *et al*., 2018; Priyadarshini and Pradhan, 2017; Ebralidze *et al*., 2019). This pollution is caused by the high metal ion content, which exhibits toxicity on accumulation. This heavy metal available to humans and the atmosphere by the burning of fossil fuels and other methods of combustion, the disposal of toxic waste, the leaching into natural water of metal ions due to acid rain, mining and agricultural activities (Jain *et al*., 2019). Therefore, many researchers have developed several techniques to detect the heavy metals, such as inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and atomic fluorescence spectroscopy (AFS) (Devadhasan and Kim, 2018; Śliwińska *et al*., 2019; Guo *et al*., 2019; Almeida *et al*., 2018; Hofstetter *et al*., 2018). However, these current methods had several disadvantages that required expensive instruments, skilled operators, laborious operation and time-consuming (Liu *et al*., 2017; Zhou *et al*., 2019; Zhang *et al*., 2020; Lin *et al*., 2016). In addition, metal poisoning is a serious problem in the forensic field which could lead to death (Verma, 2018). Therefore, rapid identification of heavy metals is one of the most important characteristics to measure and classify the presence of the analyte of interest in paper-based assays (Morbioli *et al*., 2017).

1.4 Significance of the Study

Many studies have been conducted by researchers to detect heavy metals. The established methods such as inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), atomic emission spectroscopy (AES) and atomic fluorescence spectroscopy (AFS) (Liu *et al*., 2017; Śliwińska *et al*., 2019; Almeida *et al*., 2018; Hofstetter *et al*., 2018; Priyadarshini and Pradhan, 2017; Li *et al*., 2013) possessed several disadvantages such as requires expensive instrument, have limits of hiring skilled operator, complex apparatus, high operating expenditures, sample preparation process become hard for real-time evaluations and it is time-consuming (Idros and Chu, 2018; Zhou *et al*., 2019; Guo *et al*., 2019; Zhang *et al*., 2020; Lin *et al*., 2016). To overcome these issues, efficient sensors are preferred to develop rapid, portable, low-cost, environmentally-friendly techniques is highly demanded that can be used in the detection of heavy metal ions for environment, aquatic and biotic life (Rasheed *et al*., 2018). Therefore, microfluidic paper-based analytical device (µPAD) is proposed in this study as a powerful analytical device that can satisfy these requirements (Devadhasan and Kim, 2018; Xie *et al*., 2019; Ghosh *et al*., 2019; Teepoo *et al*., 2019; Dindorkar *et al*., 2019; Yetisen *et al*., 2013).

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy metal occurrence

According to previous research, a toxic element such as cadmium (Cd) is not needed for the normal functioning of living processes and its presence in the human body has not revealed any beneficial health effects (Nardi *et al*., 2009; Alam *et al*., 2011; Liu *et al*., 2017; Fowler, 2009). Cd is most widely used in agriculture (phosphate fertilizers), metallurgy, plastics pigment, electroplating, etc. Subsequently, Cd accumulates easily in plants going through the food chain (Turdean, 2011; Lin *et al*., 2016; Ebralidze *et al*., 2019; Priyadarshini and Pradhan, 2017).

Many researchers have reported that this heavy metal has several disadvantages to human health and is regarded as pollutant to the environment (Rasheed *et al*., 2018; Verma and Gupta, 2015). For example, Cd accumulates in the kidney and liver for more than 10 years and affects a human body's physiological functions (Kim *et al*., 2012; Lin *et al*., 2016). The washed away fertilizers flow into the stream of the river and are quickly taken in by people as they drink the water. It is highly toxic and considered cancerous (Satarug *et al*., 2003; Zalups and Ahmad, 2003; Ozcan and Juhaimi, 2012). Cd is non-biodegradable and once consumed by humans, it will enter the human body and accumulate in the organs of the body, causing serious human health problems (Liu *et al*., 2017; McLaughlin *et al*., 2007).

Other than this, Cd can be introduced into the human body by smoking and breathing in the environment contaminated with cadmium-dust (Kim *et al*., 2012). The effects of Cd can damage the liver, the bones, the kidneys and can lead to diabetes, cancer and heart disease (Harris *et al*., 2003; Fowler, 2009; Ozcan and Juhaimi, 2012).

Therefore, Cd is a highly toxic metal that is used in manufacturing workplaces. The permissible Cd exposure limit is very low (Rasheed *et al*., 2018). As a result, long exposure to Cd fumes can lead fever and muscle ache and inhalation of Cd contributes to respiratory, kidney and liver disorders (Rasheed *et al*., 2018). Cd-containing compounds are also carcinogenic and harmful (Harris *et al*., 2003; Kim *et al*., 2012). Therefore, identification of heavy metals in drinking water and food is important to ensure that their existence falls within reasonable limits. The allowable exposure limit for Cd by the WHO and the United States Environmental Protection Agency (USEPA) is 5 ppb, respectively (Halkare *et al*., 2019).

2.2 Introduction to colorimetric sensors

Colorimetric sensing is one of the most frequently used approaches for laboratory testing and industrial applications such as heavy metal detection in wastewater (Lin *et al*., 2016; Kim *et al*., 2012). Sensing future is based on factors such as simplicity, cost-effectiveness and rapid response (Ajay Piriya *et al*., 2017; Kim *et al*., 2012). Colorimetric approach-based sensors are important when evaluating the ideal characteristics. A sensor is a device which converts information about a system's chemical or physical property into an analytically useful signal (Ebralidze *et al*., 2019). Previous sensors are used to be bulky and complex, requiring various functional tools such as transducer, processing unit, detection unit, resulting in a delayed sensor response (Ajay Piriya *et al*., 2017).

Colorimetric sensors may be classified as chemical or biomolecules for types of molecules interactions, and are classified as chemical sensors and biosensors, respectively (Ajay Piriya *et al*., 2017). Colorimetric sensors are an important part of optical sensors that display distinguishable change in colour when reacted with the analyte (Narayanaswamy, 1993). It is used for instant analyte detection, which displays a change in colour that can be visually observed by the naked eye (Busa *et al*., 2016; Ajay Piriya *et al*., 2017; Momidi *et al*., 2017; Lin *et al*., 2016). Colour analysis software such as ImageJ (Ebralidze *et al*., 2019) can generally be used to determine the change in intensity at a certain wavelength within visible (400–800 nm) range.

2.2.1 Colorimetric techniques

Heavy metal Cd ion poses a significant risk and violently harmful effect on the human health and environment, even at the level of trace elements, and identification in low concentration environmental samples is crucial (Turdean, 2011; Knecht and Sethi, 2009; Guo *et al*., 2019). Several heavy metal detection techniques have been used such as colorimetric, luminescence, and electrochemical (Idros and Chu, 2018; Lin *et al*., 2016; Busa *et al*., 2016; Liu *et al*., 2016; Rasheed *et al*., 2018). Current colorimetry-based technology is all about decreasing size, low cost, in-situ and without any additional tools (Ajay Piriya *et al*., 2017). In addition, the colorimetric reaction is the most widely used technique in μPAD due to its ease of use, high sensitivity, non-destructive and clear signal readout (Momidi *et al*., 2017; Xia *et al*., 2016; Liu *et al*., 2016). For instance, to detect the analyte, a colorimetric sensor is used and shows a colour change that can be visually detected (Lin *et al*., 2016; Momidi *et al*., 2017; Ajay Piriya *et al*., 2017; Kim *et al*., 2012).

The development of an effective sensor presents many challenges. An ideal sensor should satisfy certain characteristics such as sensitivity, simplicity, robustness, accuracy, precision, minimal error, reproducibility and linearity (Ajay Piriya *et al*., 2017). Laboratory on chip (LOC) is therefore one of the well-known platforms on which sensor technology is implied with high success (Whitesides, 2006). It involves simple and portable devices made of polydimethylsiloxane (PDMS) that are used by flowing liquid samples within a microchannel to detect analytes (Busa *et al*., 2016).

Due to its low footprint and lesser user of analyte-containing reagents, microfluidics has gained broad acceptance in sensor technologies. LOC technology using paper such as lab-on-paper (LOP) has become famous for its low-cost, rapid detection, and self-sustainability (Ajay Piriya *et al*., 2017). LOP uses cellulose paper to trap the molecules in a targeted site and colorimetric method is used to detect them. Microarray with LOP can detect various samples at the same time (Whitesides, 2006).

The hydrophobic region, detection zone and sample zone are three important regions / zones (Figure 2.1) (Idros and Chu, 2018; Pathirannahel, 2018). It involves the passive movement of the analyte solution (metal ions) to the detection zone under the capillary action effects by reacting to colour change with loaded reagents (Xie *et al*., 2019; Lin *et al*., 2016; Fu and Wang, 2018). Colour intensity can be recorded through a scanner or camera that transmits off-site digitized readings for quantitative analysis (Wu *et al*., 2019; Morbioli *et al*., 2017; Xia *et al*., 2016). The change of colour is due to a chemical reaction. When the analytes are lowered into the μPAD sample zone, the liquid flows towards the detection zone due to filter paper capillarity and barriers created using various techniques (Idros and Chu, 2018; Lin *et al*., 2016). The smartphone-installed apps can quickly detect the uniform and stable colour when the μPAD is dry (Busa *et al*., 2016; Murdock, 2015). Thus, multiplexed detection of heavy metals can be performed in one single experiment using a single μPAD without the need for external processing elements (Xia *et al*., 2016).

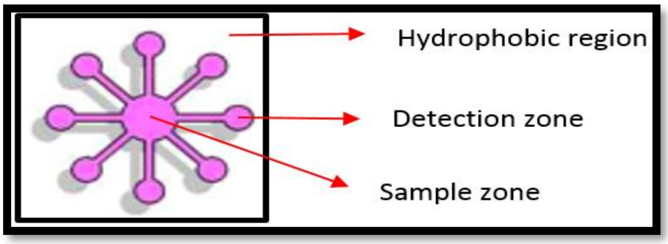
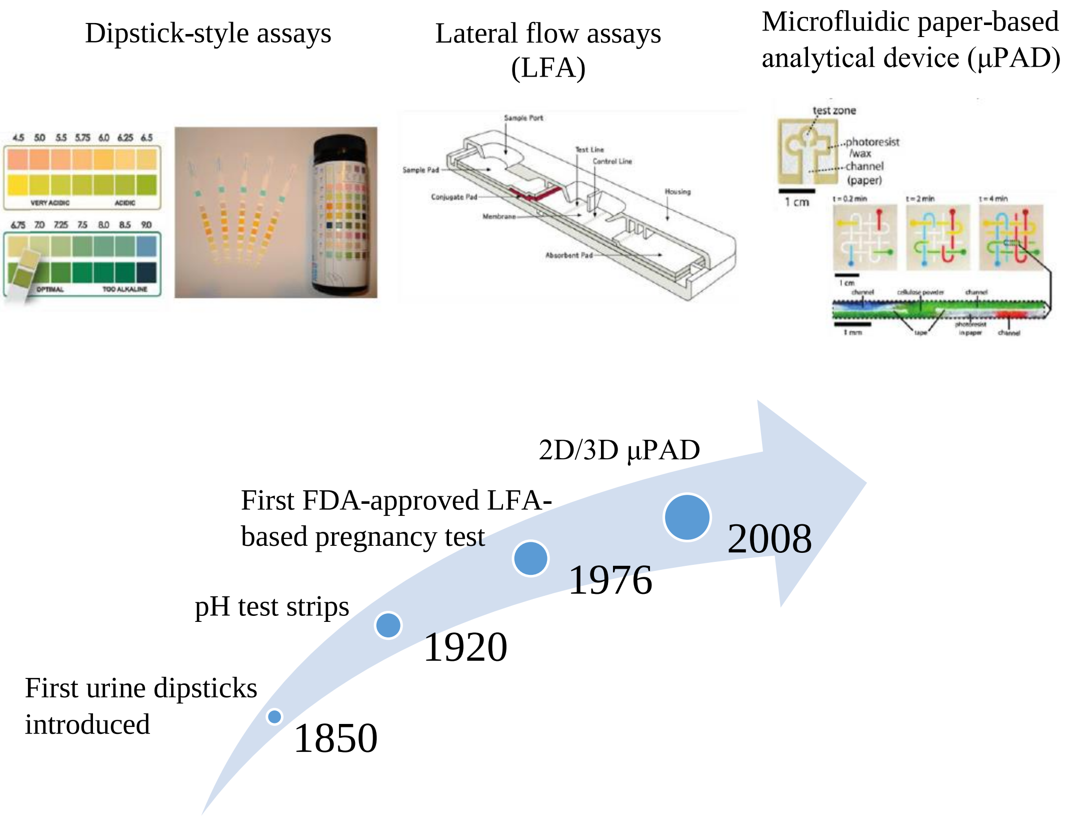


Figure 2.1: Three distinct regions/zones on μPAD (Idros and Chu, 2018; Pathirannahel, 2018).

2.3 Historical timeline of µPAD

Paper-based materials have been incorporated into rapid diagnostic assays for a wide range of point-of-care (POC) applications (Lepowsky *et al*., 2017; Wang *et al*., 2012; Wu *et al*., 2019; Martinez *et al*., 2010; Xia *et al*., 2016; Murdock, 2015) and in different forms including dipsticks, lateral flow assays (LFAs) and microfluidic paper-based analytical devices (µPAD) (Yetisen *et al*., 2013; Parolo and Merkoci, 2013). Paper has been used as a substrate for diagnostics for quite some time with urine dipsticks being introduced in 1850, followed by pH test strips in the 1920s, the first FDA-approved LFA-based based pregnancy test in 1976 (Murdock, 2015) and the introduction of 2-dimensional (2D) and 3-dimensional (3D) µPAD in 2008 (Whitesides, 2013) (Figure 2.2).



Dipstick-style assays

Lateral flow assays (LFA)

Microfluidic paper-based analytical device (µPAD)

Figure 2.2: Evolution of paper-based assays (Murdock, 2015).

The first paper-based diabetes dipstick test to quantify glucose in urine was proposed in the 1950s, followed by its commercial introduction to consumer markets in the 1960s (Yetisen *et al*., 2013). Dipstick assays were typically used for quick, one-step reagent assays in which the analyte reacts directly to the substrate, such as pH detection, water chemical level detection or urinalysis (Yetisen *et al*., 2013; Murdock, 2015). In the case of pH detection or other reagents, strips of either filter or chromatography paper are coated in pH indicator solutions. The strips are then dried and either used in a multiplexed assay, or mixed with multiple reagents on a single plastic strip (Murdock, 2015). Urinalysis test strips incorporate multiple analyte identification on one stripe, identifying as many as 10-12 different substances such as glucose, insulin, ketones, and bilirubin (Murdock, 2015; Roberts, 2007).

Lateral flow immunoassays (LFAs) may be subdivided into two major types that are direct (double antibody sandwich assays) and competitive (inhibitive) formats (Murdock, 2015). LFAs are used if more bioassays are needed, such as when attempting to determine the presence of specific antigens or proteins in a sample, qualitatively or quantitatively (Murdock, 2015; Millipore, 2009). LFAs typically have five main components: a sample pad, a conjugate pad, a nitrocellulose membrane, a wicking pad and a plastic backrest (Millipore, 2009). These types of molecules may not react directly with a substrate and may require specific antibodies to act as capture molecules to trap them from the sample onto the surface of the paper-based diagnosis using several type assays (Murdock, 2015).

According to their compactness, portability and simple analysis without external instrumentation, dipstick and lateral-flow formats have dominated rapid diagnostics over the last three decades. The lack of measurement quantitation has, however, questioned the creation of μPAD (Yetisen *et al*., 2013). μPAD has recently emerged as a multiplexible point-of-care platform that could surpass current assay capabilities in resource-limited settings (Yetisen *et al*., 2013). However, µPAD may allow for fluid handling and quantitative analysis for potential applications in health care, veterinary medicine, environmental monitoring, drug screening, cell biology, food analysis, and water analysis (Wu *et al*., 2019; Xie *et al*., 2019; Ghosh *et al*., 2019; Teepoo *et al*., 2019; Almeida *et al*., 2018; Busa *et al*., 2016). The WHO has set seven diagnostic guidelines in resource-poor settings. These tests must be: (i) inexpensive, (ii) adaptive, (iii) accurate, (iv) user-friendly, (v) fast and reliable, (vi) equipment-free, and (vii) provided to those who need it (Yetisen *et al*., 2013). Therefore, µPAD is the best analytical tool that satisfies those requirements needed.

2.4 The disadvantages of the existing method

Usually, the presence of trace amounts of heavy metal ions (HMI) in environmental samples is determined by spectrophotometric techniques (Zhou *et al*., 2019; Śliwińska *et al*., 2019; Guo *et al*., 2019; Hofstetter *et al*., 2018; Lin *et al*., 2016). However, the direct analysis of some complex samples like seawater presents some difficulties due to the high salt content, causing matrix interference and insufficient precision. In such cases, a typical dilution of the sample may be necessary before the analysis, which can create the problem and leads to poor results (Barton *et al*., 2015).

Therefore, there are many techniques employed in metal determination such as electrochemical techniques, atomic absorption spectrometry, atomic emission spectrometry with inductively coupled plasma excitation, X-ray fluorescence, optical sensors (Devadhasan and Kim, 2018; Liu *et al*., 2017; Hormozi-Nezhad and Abbasi-Moayed, 2014; Idros and Chu, 2018; Priyadarshini and Pradhan, 2017; Lin *et al*., 2016; Li *et al*., 2013). However, they possessed several disadvantages.

2.4.1 Comparison between existing methods in different sample

The determination of heavy metal in airborne particulate matter is studied, sensitive and accurate methods are required for the analysis, as well as suitable pretreatment methods for the sample (Ochsenkühn-Petropoulou and Ochsenkühn, 2001). Some of the pretreatment methods are very time-consuming. Most of the analytical multielement techniques used are inductively coupled plasma-atomic emission spectrometry (ICP-AES) and anodic stripping voltammetry (ASV). The capability of two analytical techniques which are ICP-AES and ASV have been compared for the determination of Cd in airborne particulate matter, collected on cellulose filters, from the atmosphere (Ochsenkühn-Petropoulou and Ochsenkühn, 2001). Two methods were tested for the analysis of filters loaded with airborne particulates. ICP-EAS and ASV need leaching or digestion to transfer the elements of interest into the liquid form. As a result, the detection limits of Cd using ICP-AES and ASV have been compared (Table 2.1). The results revealed that ASV is to be preferable.

Table 2.1: Determined elements and detection limits for the ICP-AES and ASV (Ochsenkühn-Petropoulou and Ochsenkühn, 2001).

Determined element	**Cd**	
Methods	**ICP-AES**	**ASV**
Detection limits (ng/mL)	3.4	0.2

According to Manzoori and Bavili-Tabrizi, although atomic absorption spectrometric methods used either in the flame or graphite furnace mode (FAAS and ET-AAS) are a powerful analytical tool for the determination of trace elements in a great number of samples, preconcentration and separation of the metals with different chelating agents are still necessary. Many elements have been used for the removal and preconcentration of trace Cd from various samples prior to their determination by

FAAS. However, these techniques are rather time-consuming, tedious and require a large amount of samples (Manzoori and Bavili-Tabrizi, 2002).

The use of ICP-MS in food laboratory analysis is becoming more common nowadays compared to GFAAS or ICP-OES (Leblanc *et al*., 2005). This technique has some benefits including simultaneous measuring capacity of multielements, combined with very low detection limits (Parsons and Barbosa, 2007). In addition, it provides a wider linear dynamic range that enables the determination of major and trace elements at the same sample injection. Many researchers have reported that heavy metal such as Cd was detected using ICP-MS in various types of food samples. Results indicated that the detection limit (LOD) for Cd was 0.2 ng/g (Nardi *et al*., 2009). ICP-MS also provides simpler spectral interpretation and isotopic information compared to the ICP-OES. But ICP-MS has certain limitations. The high concentration of organic matrix also results in matrix interferences and/or spectral interference from polyatomic ions for the analysis of food samples (Nardi *et al*., 2009).

Besides, flow injection (FI) analysis system for on-line pre-concentration and determination of Cd in aqueous samples is described by ICP-AES with a charge-coupled detector. The use of ICP-AES for the identification of trace elements in actual samples in FI systems has many benefits, such as the ability to simultaneously detect multiple trace metal ions, low detection limit and high repeatability, and the detection limit of the proposed Cd process was 1.0 ng/L (Karami *et al*., 2004). For this study, however, a new preconcentration method for chelation with sodium diethyldithiocarbamate (SDDTC) was created, which means that this method required sample pretreatment steps before evaluating heavy metal ions (Karami *et al*., 2004).

In short, many existing methods such as ICP-MS, AES and AAS are not suitable to do on-site detection due to many disadvantages. They are bulky, complex pre-treatment procedures prior to analysis, time-consuming sample preparation, need very expensive instruments and cannot be used for field monitoring which creates the major drawbacks of these instruments (Zhang *et al*., 2020; Śliwińska *et al*., 2019; Zhou *et al*., 2019; Rasheed *et al*., 2018). There are many forms of processes of pretreatment which have been clarified specifically in several scholars. Therefore, these current methods are not the best, the need to establish the most promising and highly desirable technique to overcome previous methods by using the microfluidic paper-based analytical tool (μPAD) to detect heavy metals that are easy to prepare and can be easily inspected on-site (Guo *et al*., 2019).

2.5 The development of μPAD

μPAD is not a recent technology but has been implemented since 2007 (Busa *et al*., 2016; Xia *et al*., 2016). This analytical tool offers quantitative analysis in many fields, including medicine, education, and environmental monitoring (Wu *et al*., 2019; Xie *et al*., 2019; Ghosh *et al*., 2019; Teepoo *et al*., 2019; Almeida *et al*., 2018). μPAD has a special characteristic consisting of microchannel hydrophilic and hydrophobic networks that allow the fluid to flow (Xia *et al*., 2016). μPAD's ability to conduct micro-scale laboratory operations using small equipment that enhances capability as a multiplexible point of care testing (POCT) platform provides important features of μPAD in many fields of research studies due to its affordable, easy-to-use and specifically designed for use in developing countries (Lepowsky *et al*., 2017; Wang *et al*., 2012; Wu *et al*., 2019).

Many earlier studies have shown the benefits of using paper as an analytical substrate (Pathirannahel, 2018; Xia *et al*., 2016; Guo *et al*., 2019). For example, the paper is readily available and cheap, using existing printing or cutting techniques, it can be easily designed into separate hydrophilic and hydrophobic zones. It is capable of wick fluids by capillary action without the need for external power sources, is lightweight and easy to transport, disposable and biodegradable (environmentally friendly) (Xia *et al*., 2016; Almeida *et al*., 2018). These characteristics of μPAD help to measure or identify the presence of the analyte in the sample (Morbioli *et al*., 2017).

2.5.1 The advantages of μPAD

A wide range of diagnostic tests are being performed for the construction of microfluidic devices using paper as a substrate. μPAD is built by patterning hydrophilic channels marked by hydrophobic barriers (Lin *et al*., 2016; Pathirannahel, 2018). Unlike regular dipstick assays μPAD has different sample and reaction zones areas. This allows simultaneous reaction of the samples in different reaction zones with different reagents. Additionally, reaction times can also be altered by adjusting the features on the μPAD (Pathirannahel, 2018). The capillary flow rate depends on the size of the pores in the substrates, and the range of substrates with the need size of pores should be considered meeting the requirements of different applications (Lin *et al*., 2016).

Furthermore, μPAD needs only small amounts of fluid and little to no external supporting equipment to strength, since the fluid movement in μPAD is largely regulated by capillarity and evaporation (Martinez *et al*., 2010). μPAD flow is a passive process which is governed by capillary fluid transport. Capillary action is the result of interaction between adhesive and cohesive forces and is driven by intermolecular forces at the liquid-air interface between the fluid particles (surface tension, cohesive force) and the liquid-porous fiber interface (van der Waals force, adhesive force) (Lepowsky *et al*., 2017). Therefore, the flow rate through a paper channel can be managed by controlling the cross-sectional area, paper permeability, channel length or viscosity of fluid (Lepowsky *et al.*, 2017). Accordingly, these devices are designed to achieve four simple capabilities in one analytical device (Xie *et al.*, 2019; Yetisen *et al.*, 2013). These capabilities are:

1. The distribution of a sample into multiple regions allowing for numerous analyses or replicating one analysis multiple times
2. The samples move through capillary action without the need of a pump or other external forces
3. The capability of analysing with small volumes
4. The minimal generation of hazardous waste

Further, developing μPAD does not necessarily require complex machinery. Therefore, the cost of developing μPAD is very minimal and the fabrication of this device is relatively simple (Pathirannahel, 2018). One of the greatest advantages of this device is the versatility of its potential applications. With simple modifications of the reagents and without any external modifications, μPAD can be utilized for a variety of purposes depending on the researches (Pathirannahel, 2018).

2.5.2 Selection of the paper as a substrate for μPAD

As the most abundant biopolymer on the Earth, the paper is recognized as user-friendly for the construction of microfluidic devices (Priyadarshini, 2017; Xia *et al.*, 2016). Paper has several additional advantages as a material for making diagnostic devices such as paper is thin, lightweight (~10 mg/cm^2), available in a wide range of thicknesses (0.07-1 mm) and easy to stack, store and transport (Wang *et al.*, 2012). Paper is usually white (because it scatters light) and is a good medium for colorimetric tests because it provides a strong contrast with a coloured substrate. Additionally, the paper is flammable, so μPAD can be disposed of by incineration easily and safely after use. It is flexible and compatible with a host of existing printing technologies that could be used to fabricate μPAD.

However, most importantly, the paper consists of cellulose fibers and cellulose can be extracted from a broad range of plants and animals and there is a wide range of cellulose particle types that are being studied for many purposes (Moon *et al*., 2011). Cellulose-based materials such as paper and nitrocellulose membranes are commonly used as the substrate for point-of-care diagnostic devices (Lepowsky *et al*., 2017). These cellulose fibers are hydrophilic and allow aqueous solutions to flow easily through capillary action (Xie *et al*., 2019; Busa *et al*., 2016), a high surface area to volume ratio that improves detection limit for colorimetric methods (Priyadarshini, 2017; Sahin and Arslan, 2008; Pathirannahel, 2018).

The high porosity (fibrous structure), negative surface charges and high specific surface area of cellulose are beneficial for adsorbing and gathering heavy metal ions (Zhou *et al*., 2019). This important concept has made paper as a substrate of interest in the field of microfluidics. Cellulose is made up of polymer of glucose which composed of hundreds to thousands of linearly arranged D-glucose units (Figure 2.2). The repeat unit, n is linked together through oxygen covalently bonded to C1 of one glucose ring and C4 of the adjoining ring (1 ⟶ 4 linkages) and so-called the β 1-4 glucosidic bond (Pathirannahel, 2018). The degree of polymerization depends on the extraction method and source of the material (Moon *et al*., 2011).

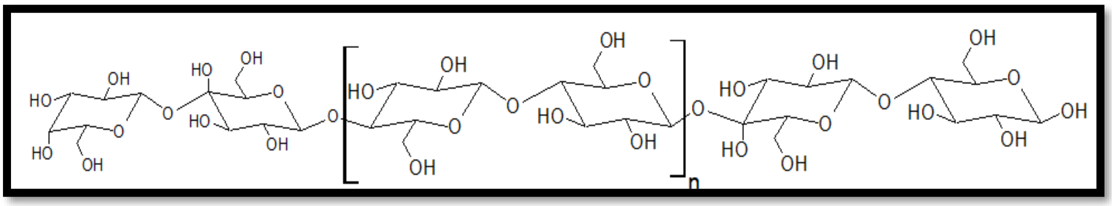


Figure 2.2: The structure of cellulose (n = degree of polymerization) (Moon *et al*., 2011; Pathirannahel, 2018).

For example, the degree of polymerisation of cotton may vary from 800 to 1000 monomer units depending on the treatment method (Klemm *et al.*, 2005). In paper-based products, the primary structural factor typically consists of 90-99% cellulose fibers (Sahin and Arslan, 2008; Pathirannahel, 2018). Cellulose fibers are hollow tubes consisting of approximately 1.5 mm, 2.0 μm and 2 μm in length, width and wall thickness (Pelton, 2009), respectively. Because of its polyfunctionality, cellulose is different from other polymers. Compared to other polymers, the long chains provide greater stiffness (Klemm *et al.*, 2005).

The cellulose network creates pores in paper. These pores allow for the penetration of liquids into the paper. Liquids move from large pores to smaller pores, depending on the capillary pressures (Sahin and Arslan, 2008). The special features of cellulose found in the paper make paper unique and suitable as the substrate in microfluidic paper-based analytical devices and it is used as an inexpensive, easily available, sustainable and recyclable tool because of certain qualities like paper. The paper is then quickly printed and coated, and is a successful filter. It is easy to store, hold, biodegradable and quickly burned. The porous structure allows lateral-flow assays and inexpensive microfluidic tools (Wang *et al.*, 2012; Guo *et al.*, 2019; Almeida *et al.*, 2018; Busa *et al.*, 2016; Leung, 2011; Martinez *et al.*, 2010).

Whatman filter paper grade 1, 3, 4 and nitrocellulose membranes are the commonly used papers for patterning the hydrophilic channels. Of all, Whatman filter paper grade 1 has demonstrated excellent colorimetric detection accuracy and sensitivity (Priyadarsini, 2017). It is also a smooth and uniform surface on both sides, with a medium flow rate and a thickness of 0.18 mm which allows printing in commercial machines (Idros and Chu 2018). Grade 1 paper consists of 98% α-cellulose with no additives used such as reinforcing agents, thus reducing the potential for intervention (Idros and Chu, 2018). Grade 2 cellulose chromatography paper has a slower flow rate but is ideally suited for higher resolution particularly when using optical scanning (Lepowsky *et al.*, 2017). But paper towels available for domestic use are also being experimented for use in μPAD but show lower dimensional accuracy (Priyadarsini, 2017).

2.5.3 Portability, user-friendliness and on-site analysis of μPAD

One of the main features of μPAD is portability. Since μPAD is considered portable to the field, the risk of contamination or degradation of the analyte is considerably reduced and sample preservation needs are avoided (Pathirannahel, 2018; Almeida *et al*., 2018). Thus on-site analysis allows for faster results response at a lower analytical cost (Almeida *et al*., 2018). Many of the methods developed for making μPAD user-friendly include smartphones, portable cameras and portable scanners (Yetisen *et al*., 2013; Lepowsky *et al*., 2017; Wu *et al*., 2019; Busa *et al*., 2016; Jayawardane *et al*., 2015; Murdock, 2015; Martinez *et al*., 2008).

When photographing a detection area of a μPAD using a cell phone camera, care must be taken to ensure appropriate light exposures. For example, place the phone (with the flash switched off) inside a wooden box containing two LEDs used to track light exposure (Ortiz-Gomez *et al*., 2016). It has been shown that the smartphone can be used with or without a flash and without light-tightened enclosure by using a control zone next to the detection zone while taking the image (Busa *et al*., 2016; Almeida *et al*., 2018; Lopez-Ruiz *et al*., 2014; Sicard *et al*., 2015; Murdock 2015).

However, the importance of a smartphone goes beyond the simple use of its camera to capture the image of a μPAD and calculate a concentration (Busa *et al*., 2016; Roda *et al*., 2016; Murdock, 2015), but it can also be used to collect, store and exchange data online in real time (Pathirannahel, 2018). It also created a QR (Quick Response) code with analyte information that enabled the smartphone to read μPAD and output data (Busa *et al.*, 2016; Santhiago *et al.*, 2014; Pathirannahel, 2018; Murdock, 2015). While μPADs are meant to be portable, very few μPAD-based studies have been recorded on field tests (Jayawardane *et al.*, 2014; Karita and Kaneta, 2014). In some cases, different ambient conditions (laboratory vs field) can affect the performance of the device.

For example, when the μPAD was tested outdoors for reactive phosphate determination, the reaction zone turned blue before the water sample was added due to the UV photo-reduction of molybdate at exposure to sunlight (Jayawardane *et al.*, 2014; Pathirannahel, 2018). A UV-resistant laminating pouch was used to shield the μPAD from severe light exposure and hence prevent this problem. In another case, a 3D printed support was required to ensure reproducible flow conditions for the detection of microorganisms in the field (Kim and Yeo, 2016). Such examples help to demonstrate the value of testing μPAD under both laboratory and field conditions. The storage stability of the proposed μPAD also needs to be applied outside the laboratory (Almeida *et al.*, 2018).

2.5.4 Using μPAD for water analysis

Microfluidic paper-based analytical devices (μPADs) are recognised as a potentially efficient analytical platform because of many advantages such as they are readily available and cheap, can be easily designed into discrete hydrophilic and hydrophobic zones using existing printing or cutting technologies, can wick fluids without external power sources by capillary action, is lightweight. Although most of the work on μPAD focussed on the point-of -care diagnostic method (Xie *et al.*, 2019), other applications including environmental analysis, drug screening, cell biology, food analysis, and water analysis are commonly used (Wu *et al*., 2019; Xie *et al*., 2019; Ghosh *et al*., 2019; Teepoo *et al*., 2019; Almeida *et al*., 2018; Busa *et al*., 2016; Yetisen *et al*., 2019).

Today, water pollution is a major environmental issue affecting millions of people, and regular monitoring of water quality is on the rise (Almeida *et al*., 2018). While living organisms need a few to trace amounts, at higher concentrations, they are toxic. Even they are not biodegradable and can persist in the aquatic environment (Almeida *et al*., 2018). Therefore, many studies have been developed and applied to water analysis, and rapid identification of heavy metals is important to identify and analyse the heavy metal materials. Thus, μPAD is recognised as a good analytical method capable of meeting these requirements (Guo *et al*., 2019; Pathirannahel, 2018; Xia *et al*., 2016; Leung, 2011; Pelton, 2009).

2.6 Fabrication methods of μPAD

The fabrication of μPAD is generally based on the creation of hydrophilic zones on paper, patterned by hydrophobic or physical barriers using various hydrophobic agents or cutting methods, respectively (Almeida *et al*., 2018). The μPAD can be fabricated by using two-dimensional (2D) or three-dimensional (3D) lateral-flow system (Yetisen *et al*., 2013; Lepowsky *et al*., 2017) to transport fluids in both horizontal and vertical dimensions depending on difficulties of the diagnostic application (Almeida *et al*., 2018; Xia *et al*., 2016). Previous studies have been discussed that there are numerous methods available for fabricating μPAD. However, each technique portrays its benefits and drawbacks.