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Feasibility of Integrating Microfluidics into Point-of-Care Bio-diagnostics

Swetha Manoharan

Feasibility of Integrating Microfluidics into Point-of-Care Bio-diagnostics

Swetha Manoharan

North Carolina Agricultural and Technical State University

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Swetha Manoharan

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Approved by:

Dr. Arvind Chandrasekaran Major Professor	Dr. Yeoheung Yun Committee Member
Dr. Stephen B Knisley Department Chair & Committee Member	Dr. Clay S. Gloster, Jr. Interim Dean, The Graduate College

Biographical Sketch

Swetha Manoharan completed her undergraduate education in India from the department of Biomedical Engineering at Anna University in 2016. She joined North Carolina Agricultural and Technical State University in 2018 for doing her Masters in Bioengineering. She learnt the basics of Microfluidics Engineering at the Bio-Inspired Microengineering (BIOME) laboratory, under the tutelage of Dr. Arvind Chandrasekaran. Upon graduation, she is planning to pursue a Doctoral degree in Computational Sciences and Engineering at North Carolina Agricultural and Technical State University.

Dedication

I dedicate this thesis to my parents, friends and professors who believed and helped me to achieve my goals throughout my graduate studies.

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Abstract

Potassium in the body needs to be maintained in proper levels for the normal functioning of heart and kidneys. The aim of this work is to develop a simple technique to measure the concentration of Potassium present in a bodily fluid sample, which can be used to assess the health condition of extremely sick patients, especially when the patient's condition needs to be diagnosed and results needs to be obtained rapidly under Point-of-Care settings.

Microfluidics is one of the platforms used in bio-diagnostics because of several advantages such as low sample volume requirement and integration into high-throughput screening. In this work, a polymer microfluidic device is designed and fabricated. Model test samples of varying Potassium concentrations are passed into the microfluidic channel. Colorimetric optical absorbance detection principle is used to measure the concentration of Potassium in the samples. The level of turbidity obtained when Potassium samples mix with chromogenic agent, indicates the concentration of Potassium present in the sample, which can be then detected by optical absorbance. The optical biosensing is done with the help of a handheld spectrometer, to measure the intensity of light passing through the samples, from which absorbance is calculated.

The results show that the proposed integrated microfluidic optical biosensing format can be potentially used in real-time Point-of-Care applications, for the measurement of the required analyte in test samples.

CHAPTER 1

Introduction

1.1 Introduction to Point-of-Care Diagnostics

Point-of-Care (POC) medical diagnostics involves testing of vital health parameters at the site of the patients, and providing rapid results (Shaw et al., 2016). The ability to perform chemical and biomedical diagnosis on critical patients right by their bedside is the need of the hour, because, target detections that result in delayed response could even cost their lives. Thus, POC testing calls for the development of a simple, easy to operate biosensor system capable of addressing the diagnostic requirements through rapid biosensing. The advantages of POC does not limit to conducting assays in proximity to patients without extensive sample preparation or elaborate sample handling, but also in faster processing times resulting in the generation of the test results rapidly (Luppa et al., 2011).

In some cases, POC can also be used for self-monitoring, as seen in the case of commercially available glucose monitoring or pregnancy detection kits. However, other potential applications of POC includes diagnosis carried out at home for prolonged health issues such as heart failures and diagnosis of infections in patients undergoing chemotherapy which avoids the need to go to a hospital, community pharmacy for health checkup, visiting a retail health when the clinics are closed, paramedical vehicle for pre-testing, urgent care centers when the patient's condition is not fatal, emergency room when immediate treatment is required, operating room to monitor the procedures in surgery and intensive care for monitoring the vital parameters to reduce deaths (St John et al., 2010). POC can also be used when there is a need for the detection of infectious diseases occurring in people in remote areas which lack infrastructure, and providing quality diagnostics, treatment is not possible and time is delayed POC can be used (Yager et al.,

2008; Peeling et al., 2010). The emergency department faces various issues associated with waiting time and overcrowding which delays patient care. Overcrowding further increases the time taken for diagnosis and treatments. In these situations, POC could play a major role in the timely discharge of patients from clinics as well as decrease the time of their hospital stay, which helps the overall maintenance of patient throughput (Rooney et al., 2014).

However, one of the main limitations of POC diagnostics is the requirement of unprocessed test samples drawn from patients to provide the desired sensitivity and specificity for conducting reliable assays in-situ. Although whole blood assays are generally considered as the gold standard for bioanalysis, such an approach may not be convenient to implement under all circumstances. Most of the currently validated clinical assays are carried out using plasma, prepared from whole blood samples inside sufficiently equipped laboratory conditions. In general, high-risk patients under intensive care or long-term hospitalization suffering from extremely sick conditions may not always be able to bleed enough quantity of blood. Possible nerve damage during venipuncture (Oven et al., 2017) could render the procedure extremely painful, traumatic and psychologically disturbing for the patients, such that even skilled phlebotomists could face limitations to extract enough quantity of blood under such circumstances.

One strategy to overcome the trauma associated with such painful venous blood draw procedures could be to adopt a technique that is less invasive and utilizes smaller volumes, possibly through a finger prick as implemented with some of the commercially available handheld blood glucose monitors. However, a limitation with this approach is that reduced sample volumes call for greater sensitivity of the measurement setup and may also not always contain the necessary distribution of the target molecules under all circumstances. This could lead to inconsistent readings and false negative results (Bond et al., 2015). Furthermore, common clinical problems

with whole blood assays such as hemolysis or coagulation could also result in an inaccurate representation of the analyte concentration (Ismail et al., 2005).

An alternate strategy would be to eliminate the necessity for whole blood and use other test samples for carrying out the required bioanalysis.

1.2 Introduction to Potassium Measurement

Potassium is the most abundant intracellular cation which is responsible for the cellular function of nerve and muscle tissue. The functioning of nerve and muscle depends on the levels of Potassium and Sodium to maintain the membrane potential. The normal kidney maintains homeostasis by the renal excretion of Potassium which depends on the ingested level (Palmer et al., 2016). Therefore, Potassium monitoring and level correction is a routine, but critical procedure carried out at the hospitals for patients undergoing cardiac, renal or neurological related diagnoses. Potassium disorders or abnormal Potassium levels in blood (Dyskalemias) happens often in clinical practice (Collins et al., 2017). People affected with diabetes mellitus have deficiency of insulin, due to which intracellular Potassium levels are not maintained appropriately (Dylewski et al., 2018).

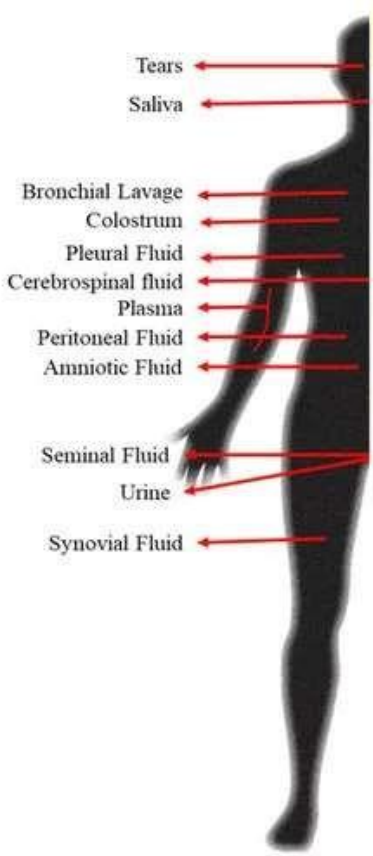
Hypokalemia and hyperkalemia are important electrolyte abnormalities which have the risk of development into serious or life-threatening cardiac arrhythmias and death, mainly among patients with cardiovascular or renal disease (Collins et al 2017). Hyperkalemia is mainly caused due to extracellular Potassium shift and the failure of the kidneys to excrete the Potassium. The symptoms are weakness, fatigue, nausea, vomiting, intestinal colic and diarrhea (Mushiyakh et al., 2012). Increased Potassium excretion through urine or gastrointestinal tract and intracellular shift leads to hypokalemia whereas low Potassium intake may also be a minor cause of hypokalemia

(Kardalas et al., 2018). Altered Potassium levels in the body under hyperkalemia or hypokalemia may cause arrhythmia, abnormal blood pressure, cramping, twitching or paralysis of muscles leading to the development of abnormal cardiac rhythms. If detected early, cardiac arrhythmias and muscle paralysis resulting from abnormal Potassium levels in the body can be prevented (Mushiyakh et al., 2012).

The feasibility of measuring Potassium concentration through non-invasive methods using saliva as the test fluid is an age-old concept that has been thought of as early as in 1956 (Burgen., 1956). However, the application of salivary analysis in the diagnosis of different biomedical conditions, is still evolving. In a work published by Davidovich et al. 2010, saliva obtained from patients has been used to compare the concentration of Potassium present in children with Down's syndrome and healthy children. In another study performed for detecting the concentration of saliva, comparison was made between diabetic and non-diabetic controls using an atomic absorbance spectrophotometer technique. The results showed that healthy patients had more Potassium whereas the diabetic patients had lower levels of Potassium. (Shirzaiy et al., 2015). More recently, salivary Potassium was compared between hemodialysis patients and healthy individuals by using colorimetric method. After deproteinization saliva was mixed with reagents such as tetraphenylborate and sodium hydroxide. The absorbance readings were taken at a wavelength of 580 nm using principle of colorimetry which indicates the concentration of Potassium in saliva (Rodrigues et al., 2016). Thus, these studies show that salivary analysis of Potassium could be used for a wide range of bio-diagnostic applications, motivating further research in this direction towards real-time and rapid Point-of-Care testing.

1.3 Rationale of the Thesis

Figure 1.1 presents a list of commonly available bodily fluids, their nature of extraction from the body and the general feasibility of Potassium detection for different biomedical applications.



Biofluids	Extraction procedure	Potassium detection
Tears	Non-invasive	✓
Saliva	Non-invasive	✓
Colostrum	Non-invasive	✓
Bronchial lavage	Invasive	✗
Pleural fluid	Invasive	✓
Breast milk	Non-invasive	✓
Cerebrospinal fluid	Invasive	✓
Plasma	Invasive	✓
Serum	Invasive	✓
Peritoneal fluid	Invasive	✗
Follicular fluid	Non-invasive	✓
Amniotic fluid	Invasive	✓
Seminal fluid	Non-invasive	✓
Synovial fluid	Invasive	✓
Urine	Non-invasive	✓

Figure 1.1: List of commonly available bodily fluids showing their nature of the extraction procedure and general feasibility of Potassium detection from those fluids.

Whole blood assays currently adopted in hospitals for Potassium monitoring is painful and sometimes unsuccessful, diagnostics of Potassium using non-invasively obtained bodily fluids could be far less traumatic and could generate rapid results when performed in a point-of-care setting. Among the bodily fluids used in clinical assays (shown in Figure 1), those commonly

available through non-invasive extraction procedures include urine (Mente et al., 2009), saliva (Hashemi et al., 2017) and sweat (Koh et al., 2016). While inducing sweat out of hospitalized patients is practically difficult, urinary extraction may also necessitate additional diuretics which would directly interfere with the measurands, aside from inducing other significant side effects (Bel'skaya et al., 2017). This research has been carried out with the objective of developing a simple biosensor platform that could be reliably used for instantaneous detection of target molecules from non-invasively obtained bodily fluid samples, and saliva presents a viable alternative to the traditional biofluids for biochemical analyses.

1.4 Aims and Scope of the Thesis

The aim of this research is to develop a Point-of-Care biosensor that could be used for real-time detection of Potassium using non-invasively extracted bodily fluids such as saliva. The feasibility of implementing this biosensor for real-time Point-of-Care testing applications will be verified by using the proposed biosensor format to detect Potassium in calibrated test samples. This research will be performed for achieving the following specific aims:

- Developing a polymer microfluidic device.
- Developing a portable biosensing technique that can be used to replace conventional biosensing methods.
- Integrating microfluidics with the optical biosensing system for Point-of-Care applications.

This thesis will provide a preliminary understanding of the following research areas:

- Design and development of microfluidics.
- Optical biosensing through colorimetric absorbance measurements.

CHAPTER 2

Literature Review

2.1 Introduction to Microfluidics

Microfluidics is the study of systems that can process small quantities of fluids by using tiny channels having dimensions in microscale typically 10 to 100 μm (Becker et al., 2009). The integration of microfluidics with traditional assay platforms has had a positive influence in the areas of biological and biomedical engineering (Coluccio et al., 2019).

There are several advantages of microfluidics making them useful in POC devices. Small reaction volume within the microfluidic channels leads to usage of small quantity of the sample. The components of a microfluidic system and their dimensions render clear advantages for using them to conduct analyses which are not possible in large scale (such as experiments involving rare clinical/biological samples obtained in small volumes). The feasibility of multiplexing the microfluidic channels enables high-throughput analysis, which could save time and resources due to the processing of several samples simultaneously on a single chip (Streets et al., 2013).

Although microfluidics was developed for more general applications, the implementation of microfluidics in the field of biomedical research has been steadily increasing. This is because, microfluidics presents the perfect platform for handling rare clinical substrates, given the low samples volume requirements. Due to all these advantages, the integration of microfluidics and biosensing can be a potential future platform for Lab-on-a-Chip devices, for a wide range of POC applications that require portability, disposability/reusability, real-time detection, high levels of accuracy and high-throughput analysis (Luka et al., 2015).

However, one of the main limitations of microfluidics comes from the laminarity of flow within the channels. Due to the physics behind laminar flow, certain fluidic functionalities generally observed with macro-scale fluidics such as particle manipulation could be restricted. One such example of a restricted fluid functionality would be the ability to achieve homogeneous mixing of fluids, which in general require more turbulence. Mixing is a crucial step in Lab-on-a-Chip, to perform Point-of-Care biological and chemical assays that needs robust sample preparation. Mixing efficiency in microfluidic systems can be improved by following certain procedures which involve integration of active external energy sources, and by designing the microfluidic system with more complex geometries that enable mixing two fluids. (Nguyen et al., 2017). Therefore, even though the field of microfluidics has developed considerably since the late 90's after it was introduced (Reyes et al., 2002), is clear that research is being conducted actively world-wide to improve the implementation of microfluidics in Point-of-Care applications.

2.2 Types of Microfluidic Systems

Several novel microfluidic designs and formats have been implemented for various applications. Different types of microfluidic systems are available based on different applications as described in the sub-sections below.

2.2.1 Microfluidic Classifications Based on Fluid Actuation Methods

Based on the handling of liquids, microfluidics can be classified as continuous flow microfluidics and digital microfluidics (Nguyen et al., 2017). Continuous flow microfluidic systems are used with external pumping mechanisms such as peristaltic pumps or syringe pumps driving the fluid flow within the microfluidic channels. These continuous flow microfluidic systems are most commonly used in applications that require mixing or particle separation. This is

discussed in more detail in Sections 2.2.2 and 2.2.3. Digital microfluidic systems generally comprise of a fluidic substrate with integrated electrodes, creating a setup with a dielectric layer and hydrophobic layer (Wang et al., 2017) wherein electric potential is applied for fluid actuation. This system is mainly used to merge, mix, split and dispense droplets. They can be integrated with analytical techniques serving as an advantage to digital microfluidics (Choi et al., 2012). Digital microfluidics does not require pumps and valves to control the liquid and clogging does not affect the liquid flow. However, given that the volume of liquid used in digital microfluidics is low evaporation is possible. The motion of the liquid is affected when the interaction of surface and droplet takes place which is a major difficulty while working with digital microfluidics (Freire et al., 2016).

2.2.2 Microfluidic Mixing Techniques

In certain applications involving biological or chemical processes such as biochemical analysis, drug delivery requires rapid mixing, which is not possible completely using microfluidics. Based on the type of mixing, microfluidic mixing can be classified as active and passive mixing. Passive micromixing does not need any external force to drive the process. They have components which are very easy to use in combination with other devices (easy to integrate) (Ward et al., 2015). Diffusion based mixing is one type of passive micromixing where fluids move from a region of higher concentration to a region of lower concentration until the concentration becomes uniform. In this way, homogenous mixing is obtained but this process could take a long time to complete. Geometric design of the microfluidic channels could also assist with passive mixing (Ward and Fan., 2015). It has also been shown that having an inlet with slanted wells increases the transport of fluids into the microchannels, thereby causing rapid mixing (Johnson et al., 2002).

On the other hand, active micromixing involves the use of external force to initiate the mixing process. Mixing takes lesser time when compared to passive mixing and can be done in a controlled manner (Hessel et al., 2005). However, active micromixers require additional components that are more complex to integrate with the microfluidic devices. In general, the active micromixers have certain disadvantages such as difficulty in fabrication, controlling the fluid flow is expensive and power consumption is more (Wang et al., 2003). Electro-kinetics based mixing draws advantage from the manipulation of the motion of particles or liquid under the influence of electric field. Electro-osmosis, electrophoresis, dielectrophoresis and electrowetting are all included under electrokinetic mixing (Chang et al., 2007). Acoustic mixing is an active micromixing process wherein fluidic mixing which happens due to acoustically generated gas bubbles (Wang et al., 2009, Wang et al., 2012, Ozcelik et al., 2014) using a piezoelectric transducer oscillated generally within the frequency range of 0.5-10 KHz. Acoustic waves can be generated in two formats, namely Bulk Acoustic Waves (BAW) (Cui et al., 2016) and Surface Acoustic Waves (SAW) (Ding

et al., 2013).

2.2.3 Particle Separation in Microfluidic Systems

Microfluidic systems can also be classified based on particle separation methods. Magneto-fluidic separation is used in applications where sample needs to maintain the temperature or pH without altering the viability of cells present (Karle et al., 2016). Inertial microfluidics play a major role in cellular sample processing due to cost effectiveness and high throughput. (Zhang et al., 2016). Acoustofluidic separation have also been implemented to isolate biological targets from complex fluids. Acoustofluidic separation is used in POC for separating submicron particles (Wu et al., 2019). Dielectrophoresis is a known particle manipulation technique used in microfluidic

separation (Gascoyne et al., 2002). Optofluidic separation can be used in biomedical applications because of the ability of a single device to separate high-resolution particles in nanoscale as well as in microscale (Shi et al., 2017).

2.3 Commonly Used Microfluidic Material Platforms

One of the critical parameters that needs to be taken into consideration for microfluidics is the process of manufacturing these devices, called microfabrication. There are several properties that must be considered for materials used in fabrication of microfluidic devices such as machinability, surface charges, water adsorption, surface properties, optical properties, ability to modify surfaces, and easiness of bonding/assembly to the traditional silicon platform (Shadpour et al., 2006).

Wide range of materials have been used for fabrication of microfluidic devices. Silicon that were traditionally used for applications in microelectronics were one of the initial materials for the fabrication of microfluidic devices (Terry et al., 1979). The cost for production for these materials is low. But the disadvantage with silicon is that the material is not transparent thereby limiting its biological applications which require visualization of the test samples (Wang et al., 2018).

Chemical resistance, optical transparency, biocompatibility and electrical insulation are some of the advantages of glass-based microfluidics (Wang et al., 2018). However, micromachining of glass channels is difficult and are generally more expensive. Thus, this leads to the development of plastic microfluidic devices (Ren et al., 2013). Commonly used plastics for fabricating microfluidics includes Polymethylmethacrylate (PMMA) (Sun et al., 2007), Polycarbonate (PC) (Selzer et al., 2017), Polystyrene (PS) (Pentecost et al., 2015), Polyethylene terephthalate (PET) (Hu et al., 2018) and Polyvinyl chloride (PVC) (Zhang et al., 2017).

2.3.1 Introduction to Poly Dimethyl Siloxane (PDMS) Based Microfluidics

Poly-Dimethyl Siloxane (henceforth called as PDMS) started to be used as a substrate in the field of microfluidics in the late 1990's. PDMS has favorable properties making them a suitable material platform to be used in a microfluidic system. PDMS has been used generally by microfluidic researchers because they are easy to fabricate, permeable to gases, optically transparent, flexible and can reversibly or irreversibly adhere to many substrates based on the requirements for specific applications (Regehr et al., 2009).

However, the disadvantages of PDMS includes absorption of small hydrophobic molecules (Wang et al., 2012), poor compatibility of PDMS with organic solvents (Lee et al., 2003) and swelling of the substrates which could be detrimental for bio-microfluidic applications. Diffusion could also be a major issue after oxygen plasma treatment, since the hydrophobic property is regained due to changes in hydroxyl groups produced by the polymer chains present in PDMS and leaching of oligomers from PDMS into the solution used.

Nevertheless, PDMS has become popular among researchers mainly because of the favorable properties for prototypes fabrication within an academic laboratory setting. The material is relatively inexpensive (Ng et al., 2002). Optical clarity and transparency of PDMS to visible light makes it compatible with optical detection systems including regular microscopes. The -molding procedure is safe and easy to learn; and its flexibility allows the integration of elastomeric actuators and optical elements into devices. Additionally, PDMS has excellent sealing properties, making it very suitable for microfluidics, and can be easily bonded to itself, thereby allowing the fabrication of multilayer structures.

2.4 Microfluidics for Salivary Diagnostics

The incorporation of microfluidic methods in bioassays of saliva has further reduced sample and reagent consumption and has decreased the overall assay times (Javaid et al., 2016) due to transport of molecules from serum to saliva. Research studies in salivary diagnostics involving microfluidics include measurement of C-reactive protein using a fluorometric immunoassay (Christodoulides et al., 2005), on-chip polymerase chain reaction (PCR) system for rapid fluorometric detection of genetic deletion (Lien et al., 2009), paper-based device for quantification of the nitrate concentration (Bhakta et al., 2014) indicated in Figure 2.1 (A), detection of thiocyanate using droplet microfluidic device shown in Figure 2.1 (B) through Surface-Enhanced Raman scattering (SERS) (Wu et al., 2014), and spectrometric absorbance detection of NH3 and CO2 in saliva as a biomarker for stomach cancer (Zilberman et al., 2015) using the microfluidic device and setup is represented in Figure 2.1(C). In another work, on–chip simple polymerase chain reaction (PCR) system has been used for detection of multiple bacteria in saliva by including different primers and probes. Real-time PCR was integrated into the system as it enables rapid fluorescence detection of bacteria (Oblath et al., 2013).

2.5 Biosensing Approaches for Salivary Diagnostics

Several biosensing methods have been used for salivary diagnostics such as electrochemical, chemiluminescent, immunoelectrochemical and piezoelectric sensing. Electrochemical sensing has been used for detecting biomarkers which includes glucose detection using enzyme amperometric glucose sensor to detect salivary glucose (Yamaguchi et al., 1998), lactate in saliva using silicon sensor (Schabmueller et al., 2006), salivary phosphate using amperometric sensor made of pyruvate oxidase (Kwan et al., 2005), amperometric glucose oxidase based sensor for detecting salivary alpha amylase (Yamaguchi et al., 2003), Immunoglobulin A

(IgA) using peptide based sensor (McQuistan et al., 2014) and cancer-antigens using IL-8 mRNA and IL-8 protein multiplexed biomarkers detection (Wei et al., 2009). Alpha-amylase, cortisol and streptococcus pyogenes are some of the biomarkers detected by immunoelectrochemical sensing (Aluoch et al., 2005, Yamaguchi et al., 2013, Ahmed et al., 2013). Piezoelectric sensing has also been used for sensing IgA in saliva (Tajima et al., 1998). However, most of the reported microfluidic systems for salivary diagnostics included integrated optical sensing for their bio-detection scheme (Kumar et al., 2013).

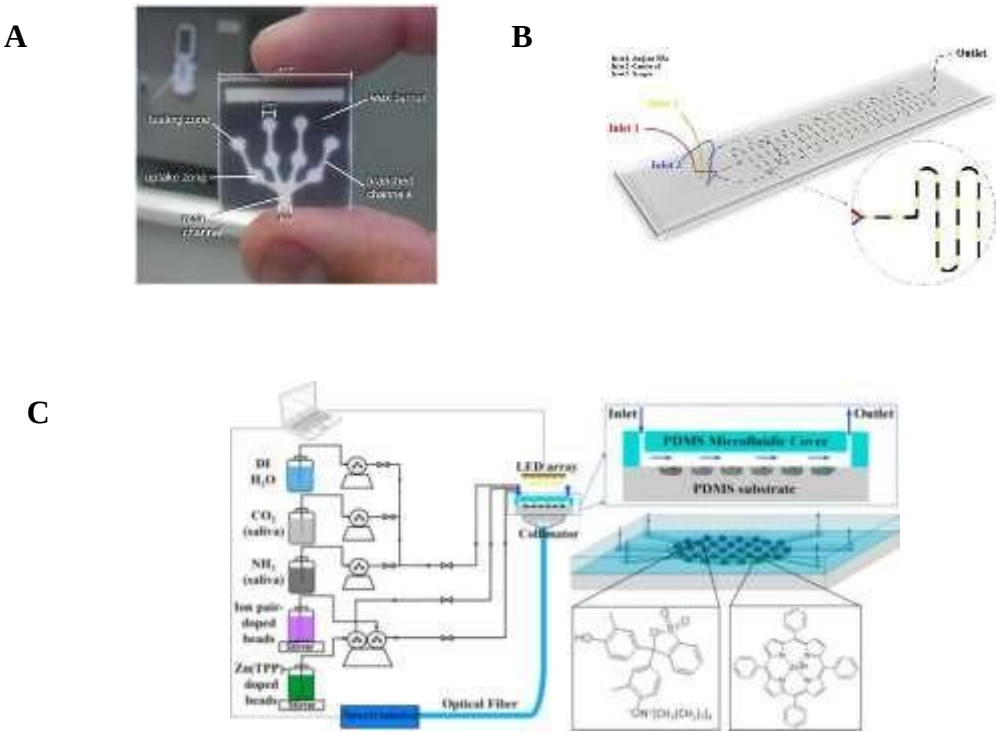


Figure 2.1: Microfluidic devices used in salivary diagnostics (A) Paper-based microfluidic device (μPAD) for nitrite detection in saliva (Bhakta, 2014) (B) Droplet microfluidic device for thiocyanate detection (Wu, 2014) (C) Representation of the setup and microfluidic PDMS based device for detection of NH_3 and CO_2 in saliva for diagnosis of stomach cancer (Zilberman, 2015)

2.5.1 Introduction to Optical Biosensing for Salivary Diagnostics

A basic optical biosensor consists of the biorecognition element and optical transducer. The main aim of sensing is to produce an optical signal which represents the concentration of the substance or the analyte present. In general, the optical sensing can be classified as label-based or label free. In label-based optical sensing, such as colorimetry, fluorescence or luminescence-based detection technique, a certain biomarker or an optical label is used to obtain the optical signal. In label-free optical sensing mode, direct detection occurs by the presence of the recognition element which detects the light transduced (Damborsky et al., 2016). Irrespective of the type of optical sensing, there are several advantages of this biosensing approach compared to the commonly used analytical techniques, such as high specificity, sensitivity, feasibility of miniaturization and low cost, among others (Dey et al., 2011).

Several optical biosensors have been researched upon, such as Surface Plasmon Resonance (SPR) based biosensors, Evanescent wave sensors, Fluorescence sensors, bioluminescent optical fibre based biosensors, Interferometric, and Surface-Enhanced Raman Scattering (SERS) biosensors.

Among these different types of optical biosensors, SPR is widely used (Damborsky et al., 2016). Evanescent wave sensors are used where background noise needs to be reduced to the minimum (Xhoxhi et al., 2015). In microfluidic systems, Laser induced fluorescence (LIF) is commonly used because of their ease of implementation with different biological systems that respond to fluorescence based biosensing (Marques da Silva et al, 2018). SERS when combined with microfluidics could shrink the size of systems because of their ability of detection even when sample volume present is low (Wang et al, 2014).

Different types of optical biosensing have also been demonstrated specifically for microfluidics based salivary diagnostics. A portable optoelectronic microfluidic sensor for direct detection of ammonia (NH3) and carbon dioxide (CO2) in saliva which are by product of gram-negative bacterium Helicobacter pylori stomach cancer infection which converts urea into CO2 and NH3 using the enzyme urease secreted. This optoelectronic microfluidic device is composed of organic dyes encapsulated in ion exchange resin microbeads paired to a quaternary ammonium ion and Zinc tetra phenyl porphyrin which are sensitive to NH_3 and CO_2 respectively that responds to Optical sensors (Spectrometer) at a single wavelength or a narrow range. Whose absorbance gives a direct information of NH3 and CO2 concentration enabling the aspect of early detection of stomach cancer (Zilberman et al., 2015).

The optical microfluidic biosensor can detect protein biomarkers such as IL-8, IL-1B and MMP might be useful to diagnose oral cancer at the early stages. Organic photodetectors (OPD) are made of polythiophene C70 whereas the interficial layer of the PMMA device is polyethylenimine. After surface biofunctionalization of PMMA, Immunogold silver assay was performed and the resultant concentration of biomarkers obtained matched the results obtained from ELISA proving that this optical biosensor can be a promising application in diagnosis of various diseases at point of care (Dong et al., 2017).

2.6 Conclusion

As mentioned in the previous section, most of the reported microfluidic systems for salivary diagnostics included integrated optical sensing for their bio-detection scheme (Kumar et al, 2013). Integrated optical microfluidic biosensing could therefore be potentially beneficial for Point-of-Care applications.

CHAPTER 3

Methodology

3.1 Development of Microfluidic System

It has been established that the use of microfluidic based biosensors enables rapid detection in patients at emergency conditions, as well as allows for high-throughput analysis of the results. Additionally, microfluidics requires lower sample volume for operation which therefore enables easier collection of biofluids in smaller volumes. Therefore, for the present application proposed in this thesis, a microfluidic device was fabricated and integrated with optical absorption detection system to create a prototype of a simple absorption-based biosensor. Given the advantages and simplicity of fabricating prototype microfluidic models on PDMS as listed in Chapter 2, the microfluidic device herein was fabricated using PDMS and bonded with a glass slide, such that the optical transparency of the device enables free-space optics overcoming the necessity of integrating waveguides for handling the optical detection.

3.2 Design of the Microfluidic Device

The design of the microfluidic device is schematically shown in Figure 3.2. The device consists of the inlet, outlet and bio-detection zone. Potassium sample containing chromogenic reagent is passed through the inlet which has a diameter of 2mm because the diameter of the micropipette used is 1mm. The designed bio-detection zone has a diameter of 6mm to provide clearance for allowing the optical source to pass through them.

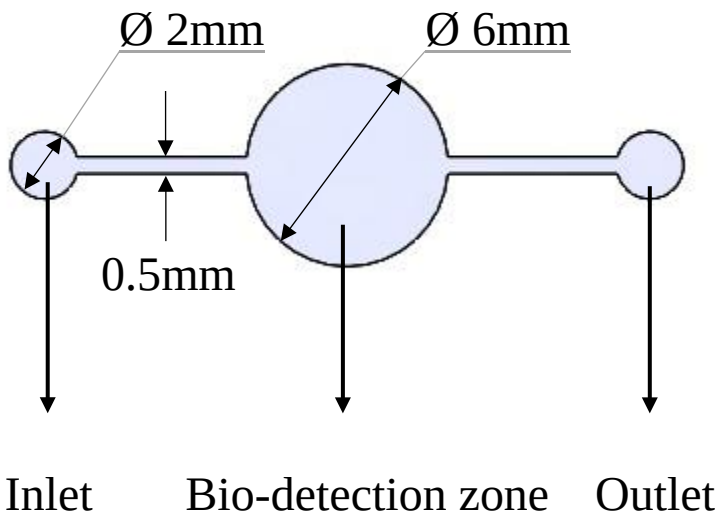


Figure 3.1 Microfluidic device design (Ø represents the diameter of the circle)

Inlet diameter: 2mm

Outlet diameter: 2 mm

Channel width: 0.5mm

Bio-detection zone diameter: 6 mm

3.3 Fabrication

The process flow for the fabrication of the PDMS microfluidic device is schematically shown in Figure 3.2.

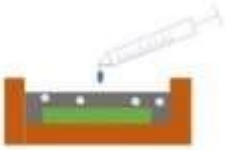
SUB- PROCESS	PROCESS DESCRIPTION	SCHEMATIC DIAGRAM
1	3 D printed mold	
2	Casting PDMS	
3	Degassing	
4	Curing	
5	PDMS peeled from surface	
6	PDMS after punching holes	
7	Bonded with glass slide	

Figure 3.2: Steps involved in PDMS based microfluidic device fabrication

3.3.1 Fabrication of Mold

The mold for PDMS fabrication was designed in the Solid Works using the dimensions given in Figure 3.1. Part design was used to make a design instead of assembly because the part design is easy and saves time. A center rectangle was used to draw a square and dimensions were adjusted using smart dimension and extruded downwards using boss extrude. The channel is then designed on the surface of square and extruded up to a height of 500 um. Thereafter, a secondary square is constructed inside the original square and extruded upwards to create the side walls of the part, as shown in Figure 3.3.

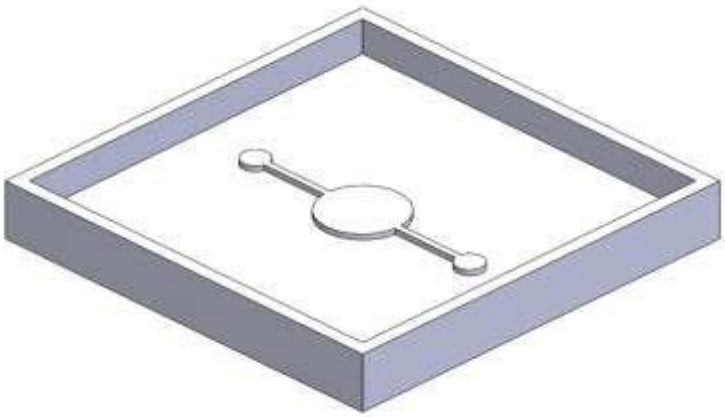


Figure 3.3: Mold designed using Solidworks for fabrication of PDMS

The mold was fabricated using 3D printing (Amin et al., 2016). The 3D printer setup (Form Lab 2, USA) shown in Figure 3.4 consists of the cartridge, build platform, resin tank, wiper, and levelling tool. Before printing, the resin tank was positioned and attached, with respect to the alignment marks. The wiper, which is used for removing excess ink after printing every layer, was aligned and pushed forward firmly in place. To replace the resin in the system, the orange plastic valve from the bottom of any new cartridge is removed before installation onto the 3D printer. The new cartridge must be aligned properly at the back of the printer and pushed down appropriately.

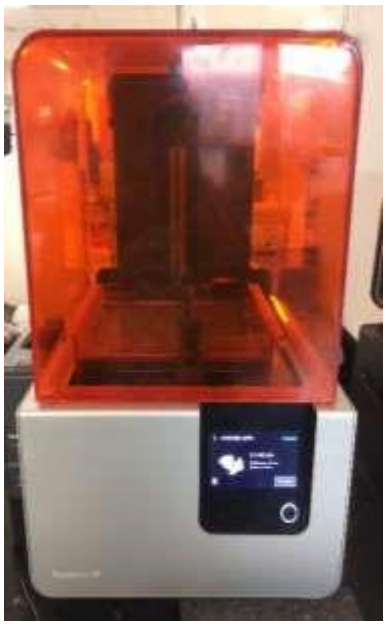


Figure 3.4: 3D Printer for preparing mold

The *.stl file to be printed is selected and the height of the layer is adjusted using print setup before the print job is executed. Opening the vent cap on top of the cartridge allows the resin to be filled up in the resin tank and heat up to 35 °C prior to the start of the print. After printing, the build platform was removed carefully, and the 3D printed part is detached and cleaned thoroughly using 90% Isopropyl Alcohol (Sigma Aldrich, USA) to remove any uncured resin. For this process, the mold was transferred onto a petri dish and IPA was sprayed to clean the part. The part was thereafter cured for 30 minutes inside the convection oven at 60 C. A finished mold is as shown in Figure 3.5 shows a finished part after cleaning.

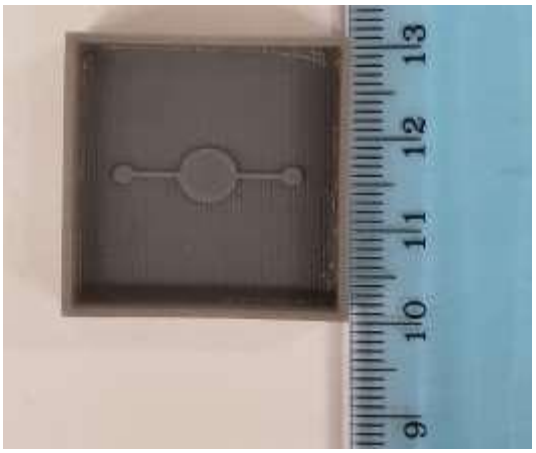


Figure 3.5: Final finished mold after cleaning

3.3.2 Fabrication of Microfluidic Device

For the preparation of the device, PDMS pre-polymer (Sylgard 184, Dow Corning, USA) is mixed with the curing agent in the ratio of 10:1 by volume and poured onto the mold (Fig 3.6 A). Air bubbles were removed by placing the setup inside a vacuum chamber (Fig. 3.6B). The setup was then placed inside an oven at 70 ◦C for 4 h. Upon curing, the PDMS was thereafter peeled from the mold, and cut appropriately (Fig 3.6C). Holes were made on the device using biopsy punches in order to create inlet and outlet for fluid flow (Fig 3.6D).

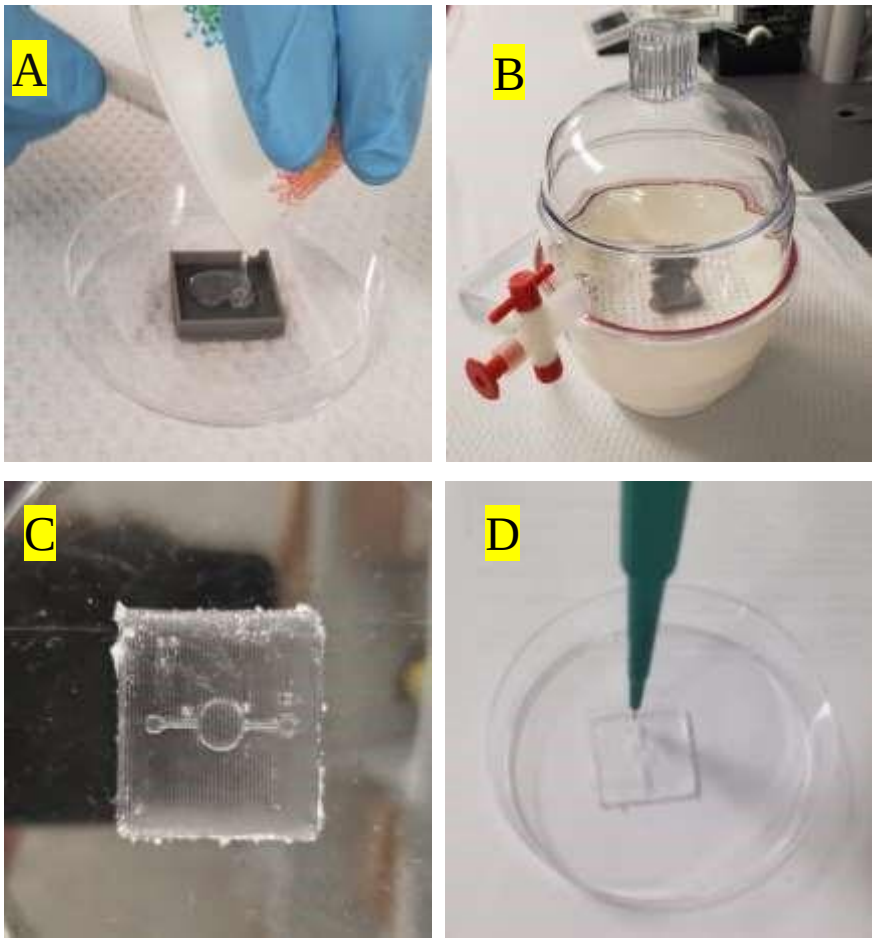


Figure 3.6: Preparation of PDMS microfluidic chip (A) PDMS poured onto the mold (B) Degassing process using vacuum desiccator (C) PDMS peeled from the mold (d) Punching holes on the inlet and outlet of the device

3.4 Packaging

Irreversible bonding of PDMS with another PDMS or glass substrate is carried out by applying semisolid PDMS to the base of the microfluidic device without touching the channel and bonding the device on the glass slide followed by placing the device into the oven and left undisturbed for 2 hours. The main reason for choosing materials such as PDMS or glass for

bonding is because of their optical transparency. The bonded microfluidic device is as shown in Figure 3.7.

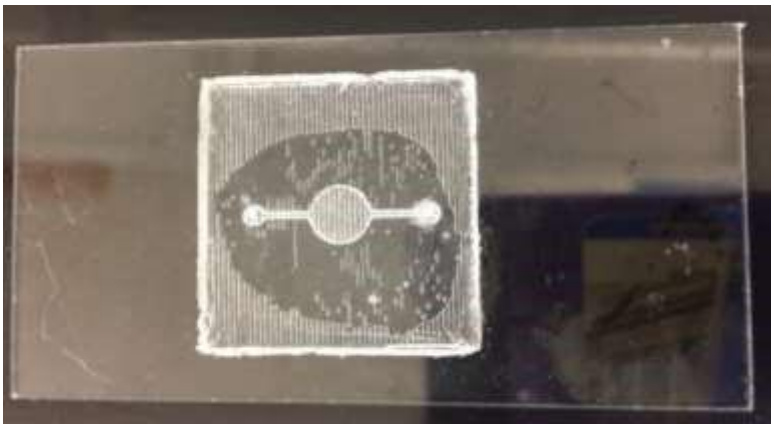


Figure 3.7: Final bonded microfluidic device

3.4.1 Flow Characterization

To test the flow of fluids into the microfluidic channel, water was mixed with food coloring dye, and pipetted through the inlet. Leak proof flow of liquid inside the microfluidic channel is as shown in Figure 3.8.

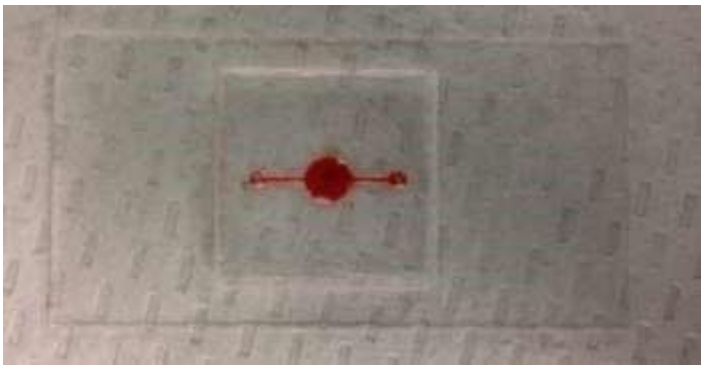


Figure 3.8: Device testing using colored dyes

3.5 Conclusion

The simple microfluidic design presented here takes into consideration that the test sample mixed externally with appropriate reagents, could be used with the microfluidic device for biosensing. Given the difficulty in achieving active mixing inside microfluidic devices due to the laminarity of flow, a more advanced setup (as presented by Surendran et al., 2019) could incorporate on-chip mechanisms that allow sample mixing before detections.

CHAPTER 4

Integrated Optical-Microfluidic Testing

4.1 Design of the Hybrid Integrated Absorption Detection Setup

The proposed hybrid integrated test setup for the colorimetric absorption measurements is schematically shown in Figure 4.1. The setup is designed in such a way that light passes through the sample present in the biodetection zone of the microfluidic device. Samples flow into the inlet of the device, through the channel, exit the device through the fluid outlet. The microfluidic device is clamped firmly by attaching to an optical positioner. The optical attenuation due to colorimetric absorption is measured using a handheld UV-visible spectrometer and is indicative of the concentration of Potassium present in the test sample.

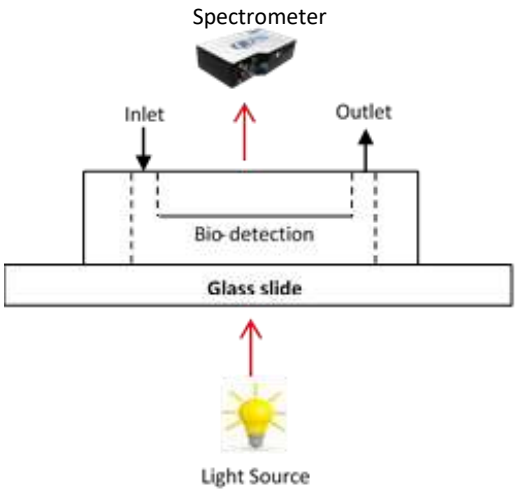


Figure 4.1: Schematic representation of optical detection setup

The broadband light source (DH-2000) output ranges from 215 to 2500 nm and the spectrometer (QE Pro-FL, Ocean Optics, USA) can detect wavelengths in the range of 185-1100 nm. The main advantage of using broadband source is to collectively measure the intensities of

light passing through the sample at various wavelengths, from which the wavelength of interest can be chosen.

The data get stored in the computer with the ocean-view software which can be extracted and plotted subsequently. After assembly, the experimental setup, shown in Figure 4.2, is covered using a black box for preventing disturbance from external light.

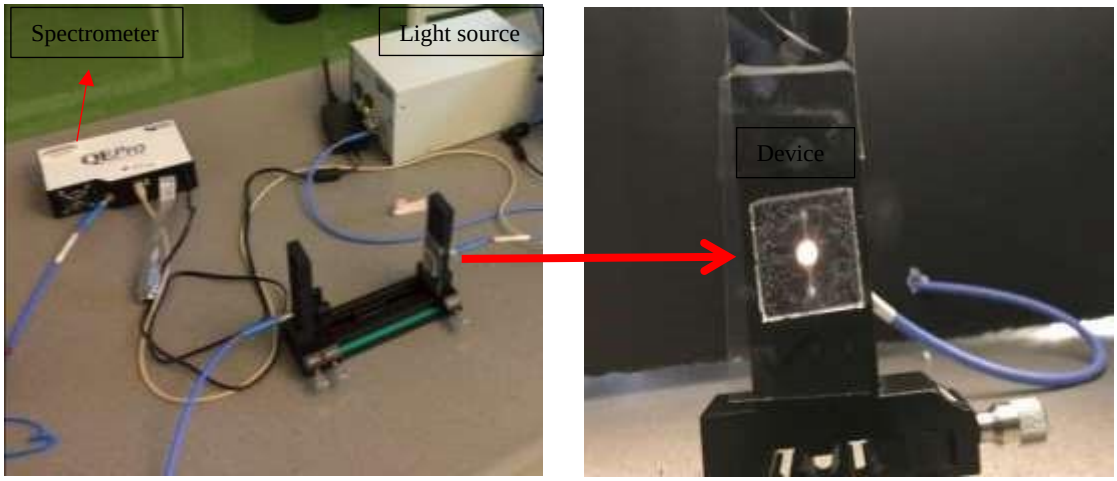


Figure 4.2: Optical detection setup showing the positioning of the microfluidic device with light passing through the bio-detection zone.

4.2 Preparation of Test Chemicals

For the experiments, a standard Potassium solution of known concentration was used as a model fluid to mimic the Potassium present in biological samples. The Potassium colorimetric assay kit (E-BC-K279) was obtained from Cedarlane Labs (Burlington, NC, USA). The standard 1mmol/L Potassium solution present in the kit was diluted with deionized water to concentrations of 0.75, 0.50 and 0.25 mmol/liter in separate microcentrifuge tubes. Then 800 ul of chromogenic agent is mixed with 200 µl of the diluted samples and mixed uniformly using vortex mixer.

Potassium (K^+) sample when mixed with the chromogenic agent Sodium Tetraphenyl Borate ($(C_6H_5)_4B^-Na^+$) reacts to form Potassium Tetraphenyl Borate ($(C_6H_5)\ B^-K^+$) (Paul et al., 1959) as per the following chemical reaction:

$$(C_6H_5)_4B\text{-}Na^+ + K^+ \longrightarrow (C_6H_5)\ B\text{-}K^+ + Na^+ \ldots (1)$$

The resultant reaction mixture ends up as a turbid solution. The level of turbidity indicates the concentration of Potassium present which can be measured at 450 nm using handheld spectrometer.

4.3 Potassium Measurement from On-chip Optical Absorption

The optical density of the samples is measured at 450 nm for all the concentrations based on optical absorption. The optical absorbance is given by:-

$$A_\lambda = -\log_{10}\left(\frac{S^\lambda - D\lambda}{R\lambda - D\lambda}\right) \quad \ldots (2)$$

λ —Wavelength of light used

A_λ—Absorbance

S_λ—Intensity of light passing through the sample

D_λ—Dark intensity

R_λ—Intensity of light passing through a reference medium.

Dark intensity D_λ is found by measuring optical signal intensity by placing an obstacle on the path of light source. Reference intensity is found by using water as the reference medium that

is pipetted into the biodetection zone. The dark and the reference intensity waveforms are as shown in Figure

4.3.

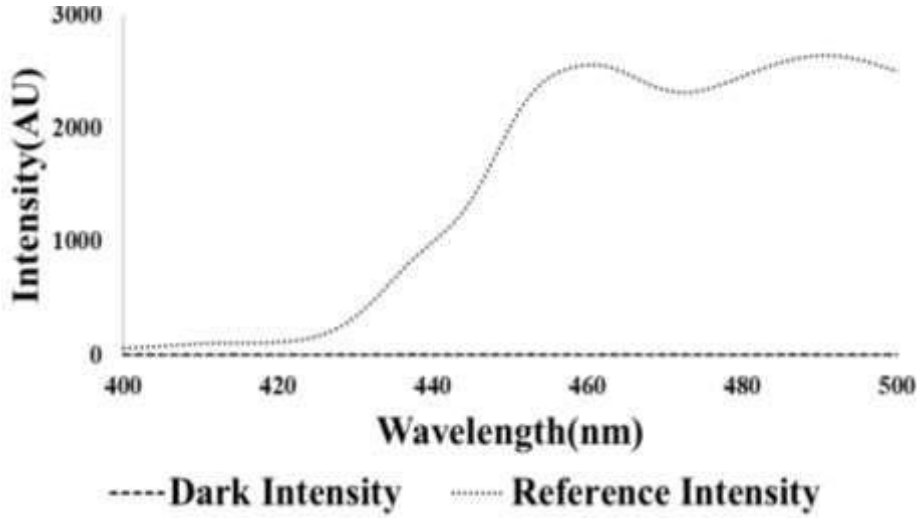


Figure 4.3: Comparison of dark intensity with reference intensity at 400-500 nm wavelength

The intensity of light passing through samples of each concentration were recorded. The absorbance was calculated using the optical intensity values obtained for the different samples at wavelength of 450 nm. The variation of absorbance values with different concentration of the samples is shown in Figure 4.4

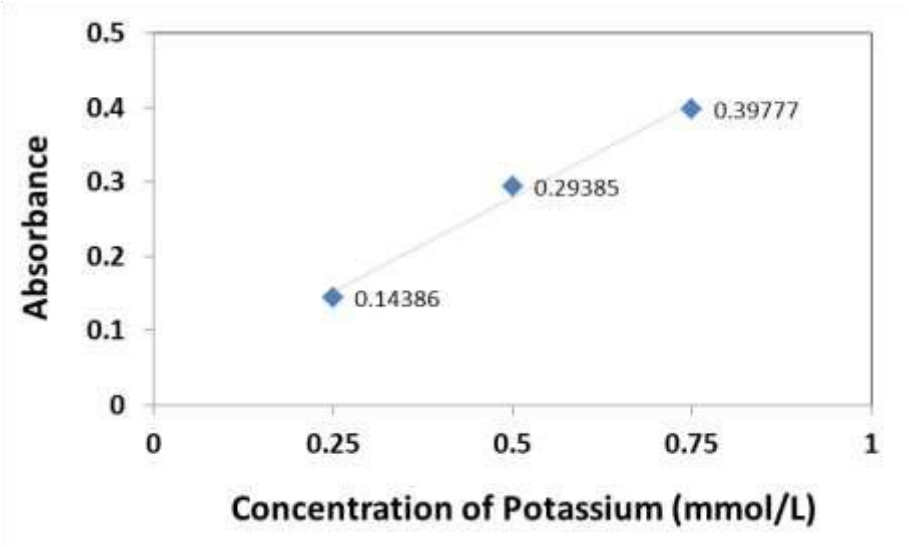


Figure 4.4: Results representing the increase in absorbance with respect to increasing concentration

4.4 Discussion

Higher intensity of light passing through turbid samples of lower Potassium concentration resulted in low colorimetric absorbance, corresponding to the concentration of Potassium present in the sample. From the graph shown in Figure 4.4, the absorbance (A) – concentration (C) relationship can be defined by the following expression:

$$A = 0.507C+0.0246 \ldots\ldots (3)$$

The variation of absorbance with concentration shows a linear trend as expected in accordance to the Beer-Lambert's law, which states that optical absorbance is directly proportional to the concentrations of the attenuating species in the material sample (Kim et al., 2016) as per the relation

$$A=\epsilon LC \ldots\ldots (4)$$

where ϵ is the absorptivity of the sample and L is the optical path length.

Therefore, the results obtained from the optical-microfluidic experiments validate the implementation of the integrated setup for colorimetric absorbance based biosensing, to detect the concentration of specific analytes present in test samples.

CHAPTER 5

Conclusion and Future Works

5.1 Conclusion from the Present Work

The main idea behind using microfluidics with a handheld spectrometer was to obtain rapid results using a minimal sample volume, and to ensure portability of the setup for application in Point-of-Care setting. The results of the present work show that integrated optical microfluidics can be potentially used as a biosensing method for real-time Point-of-Care bio-diagnostics.

One of the challenges with the present format of the device is to obtain consistency in the reading because of the noise associated with either mechanical vibrations or optical background interference. Both these problems could be addressed by a more robust integration of the microfluidic device with optical detection setup. If consistent results could be obtained through the proposed technique, this method can be used to replace the conventional methods for rapid biosensing.

5.2 Scope for Future Works

Saliva presents a viable alternative to the traditional biofluids used in biochemical analyses, as it is considered a filtrate of the blood representing the physiological state of the body. Salivary diagnostics is increasingly being recognized as an equivalent to serum analysis (Bagalad et al., 2017). Complicated pretreatment of the sample may also not be necessary with saliva (Mitchell et al., 2009). The present work provides confidence in using a Point-of-Care biodetection system that could be reliably used for the detection of Potassium from saliva. This would be extremely useful for Point-of-Care diagnosis in hospital settings for extremely sick patients.

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