DEVELOPMENT OF A LOW ELECTRIC FIELD DNA ELECTROPHORESIS MICROCHIP FEATURING AMPEROMETRIC DETECTION

by

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DEDICATION

To the spirits of my parents; Hilmi Ghanim and Faridah Ghanim … who used to call me "Dr. Motasem" when I was a kid … but unfortunately, faith did not grant me the opportunity to live and celebrate these moments together with them … may Allah SWT grant both of them Jannatul Firdaus by his mercy…

To my second mother Saedah Ghanim for her prayers and tremendous sacrifices for me …

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To all my brothers, sisters and beloved family …

To my other half … To my lovely wife, Eman, who offered me unconditional love and support throughout the course of this thesis … To all my kids; Awais, Arees, Ubai, and Talha, who were suffering of being confined at house most of the time …

… I dedicate this work.

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LIST OF ABBREVIATIONS

AD	Amperometric Detection
BCT	Body Centered Tetragonal
bp	base pair
CA	Catechol
CAD	Computer-Aided Design
cDNA	complementary DNA
CE	Capillary Electrophoresis
CE	Counter Electrode
CMOS	Complementary Metal–Oxide–Semiconductor
CNT	Carbon Nanotubes
CV	Cyclic Voltammetry
CZE	Capillary Zone Electrophoresis
DA	Dopamine
dATP	Deoxyadenosine
dGTP	Deoxyguanosine Triphosphate
DI	Deionized
DNA	Deoxyribonucleic Acid
dsDNA	Double-Stranded DNA
EC	Electrochemical
ECD	Electrochemical Detection

EOF	Electro Osmatic Flow
EP	Epinephrine
FASS	Field-Amplified Sample Stacking
FCC	Face Cubic Centered
GBIP	General Purpose Interface
HCl	Hydrochloric Acid
HF	Hydrofluoric Acid
HNO3	Nitric Acid
HVPS	High Voltage Power Supply
ITO	Indium Tin Oxide
KCL	Potassium Chloride
LBL	Layer-by-Layer
LIF	Laser Induced Fluorescent
LOC	Lab-on-Chip
LOD	Limit of Detection
MB	Methylene Blue
MC	Microchip
MCE	Microchip Capillary Electrophoresis
MCE-AD	Microchip Capillary Electrophoresis with Amperometric Detection
µTAS	Micro Total-Analysis-System
MD	Microdialysis
ME	Microchip Electrophoresis
NaOH	Sodium Hydrochloride

PCB	Printed Circuit Boards
PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
PET	Polyethylene Terephthalate
PMMA	Polymethylmethacrylate
RE	Reference Electrode
RNA	Ribonucleic Acid
SAM	Self-Assembled Monolayer
SE	Separation Electrodes
SMU	Source Measure Unit
ssDNA	Single-Stranded DNA
Topas	Thermoplastic Olefin Polymer of Amorphous Structure
USB	Universal Serial Bus
UV	Ultra Violet
WE	Working Electrode

PEMBANGUNAN MIKROCIP ELEKTROFORESIS DNA MEDAN ELEKTRIK RENDAH MENAMPILKAN PENGESANAN AMPEROMETRIK

ABSTRAK

Setiap hari penciptaan dalam mikroelektronik dan peranti mikro sedang menuju ke arah penukaran peranti Mikrocip Elektroforesis Kapilari (MCE) kepada peranti microsystem sebenar Sistem Analisis Jumlah Mikro (μTAS) atau Makmal-Atas-Cip (LOC) yang berupaya melakukan kesemua prosedur analitikal in-situ. Pengesanan elektrokimia adalah salah satu cara terbaik bagi merekabentuk sesebuah sistem LOC atau μTAS terutamanya untuk pengasingan dan pengesanan analit elektroaktif, seperti dalam bioperubatan dan diagnosis dalam vitro. Salah satu sasaran electroactive analytes yang paling popular adalah asid nucleik. Penjenisan gen DNA atau pencapjarian telah di iktiraf sebagai salah satu kegunaan terpenting peranti-peranti mikro ini. Pencapjarian DNA biasanya di aplikasikan dalam elektroforesis kimia analisis seperti penjujukan dan pengesanan DNA, pengenalpastian mikroorganisma patogenik seperti kulat mikrob, dan diagnosis penyakit. Walaubagaimanapun, terdapat beberapa cabaran besar dalam merealisasikan sistem MCE miniatur yang boleh dibuat secara besar-besaran, seperti kos fabrikasi yang tinggi, serta saiz unit bekalan kuasa yang besar menyebabkan sistem keseluruhan menjadi tidak mudah alih. Dalam projek ini, MCE dengan arus yang sangat rendah dan menggunakan skim pengesanan amperometrik telah direka dan dibuat untuk penjujukan dan pengesanan DNA. Peranti ini dibuat daripada ukiran saluran mikro pada hibrid kaca/polydimethylsiloxane (PDMS), dengan elektrod platinum direnjis ke substrat

kaca. Kajian ini telah membuktikan bahawa penggunaan gel Agarose sebagai bahan pengasingan dapat mengurangkan medan elektrik serendah 12 V/cm; sesuatu yang tidak dapat dicapai sebelum ini. Pada sudut berbeza, kos pembuatan telah dikurangkan secara dramatis melalui penggantian bahan-bahan nobel seperti emas, platinum dan lain-lain, dengan substrat papan litar tercetak yang terdapat di pasaran. Rekabentuk baru ini akan membantu dalam pengeluaran peranti LOC berkos rendah secara besar-besaran untuk kegunaan diagnosis DNA atau diagnosis elektroaktif yang lain. Eksperimen-eksperimen telah dilakukan untuk mengkaji sifat dan keupayaan chip mikro yang telah dibangunkan. Bahan ujikaji yang digunakan dalam eksperimen adalah tangga DNA komersial dan juga cap jari DNA sebenar yang dibuat melalui proses tindakbalas berantai polimerasa (PCR). Peranti yang dicadangkan ini telah berjaya mengasingkan sejumlah besar cebisan DNA bersaiz 200 pasangan tapak (bp) hingga 20,000 bp dengan kepekaan arus yang setiasa melebihi 100 nA.

DEVELOPMENT OF A LOW ELECTRIC FIELD DNA ELECTROPHORESIS MICROCHIP FEATURING AMPEROMETRIC DETECTION

ABSTRACT

Everyday inventions of microelectronics and micro-devices are advancing towards converting the Microchip Capillary Electrophoresis (MCE) devices into a true Micro Total Analysis System (μTAS) or Lab-On-a-Chip (LOC) microsystem that can perform the whole analytical procedures in-situ. Electrochemical detection (ECD) is one of the best means for designing a LOC or μTAS systems especially for separation and detection of electroactive analytes, as in biomedical and in vitro diagnostics. The most widely targeted electroactive analytes is nucleic acid. DNA genotyping or fingerprinting has been recognized as one of the most important applications of these micro devices. DNA fingerprinting is normally used in electrophoretic applications of analytical chemistry such as DNA sequencing and detection, identification of pathogenic microorganism like microbe fungus, and disease diagnostics. However, there are some major obstacles in realizing miniaturized and mass-producible MCE systems, such as high manufacturing cost, and the bulky power supply unit which make the whole system none-portable. In this project, a low cost as well as low electric field MCE utilizing an amperometric detection scheme was designed and fabricated for DNA sequencing and detection. The device was fabricated from a glass/polydimethylsiloxane (PDMS) hybrid engraved microchannel with platinum electrodes sputtered onto a glass substrate. It has been established that, the use of Agarose gel as separation material could reduce the electric field to as low as 12 V/cm; this has not been achieved previously. On the other side, the cost of fabrication was

dramatically reduced by replacing the noble materials such as gold, platinum etc, by commercially available printed circuit board (PCB) substrates. The new design would help in mass production of low cost LOC devices for DNA or other electroactive diagnostic purposes. Experiments were performed to explore the properties and performance of the fabricated microchips. The testing samples used in experiments were the commercial DNA ladders as well as real DNA fingerprints produced by polymerase chain reaction (PCR). The proposed device has successfully separated a wide range of DNA sizes 200 base pair (bp) to 20,000 bp with current sensitivity consistently higher than 100 nA.

CHAPTER 1

INTRODUCTION

1.0 Introduction

With the increase of bacterial and viral diseases, there is a growing demand for robust Deoxyribonucleic acid (DNA) based bio-analytical pathogenic detection systems. Preferably, such systems should employ powerful and indispensable designs and techniques for forensic human identification due to its ability to produce highly distinctive profiles from minute amounts of DNA. However, current genotyping processes and instruments are labor-intensive, demanding, and one must wait one or more days for results. Accelerating the instrument-dependent processes in casework analysis will ultimately assist in reducing DNA backlogs. Recently, researchers are focusing on producing new generation analytical devices that can be integrated in tiny and portable chips. These chips are expected to possess properties that fulfill the commercial needs such as portability, accuracy, affordability and simplicity; all these properties are desirable for a user friendly device. In fact, each property has been considered as a case study and being treated independently in this research. The most prominent researches and achievements in this area will be discussed critically.

1.1 Amperometric Detection

Among all electrochemical (EC) detection techniques, amperometric detection was the most widely used in MCE devices. Amperometric detection system is a three-electrode electrochemical detection system which utilizes potentiostat and three different electrodes. It is a chemical titration by which the redox current is measured between two electrodes under a fixed potential difference. Amperometry initializes three electrodes to achieve the redox current measurement, namely the working electrode where the redox occurs, the reference electrode which is used as the other part of the cell to keep the potential difference fixed, and the counter (auxiliary) electrode to keep the current balanced in the electrochemical cell by providing current in the direction opposite to the EC reaction (oxidation/reduction) on the surface of the working electrode. Figure 1.2 shows the integration of EC detection electrodes in MCE. Redox reactions and the generated signal strength vary depending on several factors such as electrode's material, analytes, and electrodes setup in the MCE. All will be addressed and discussed thoroughly in Chapter Two.

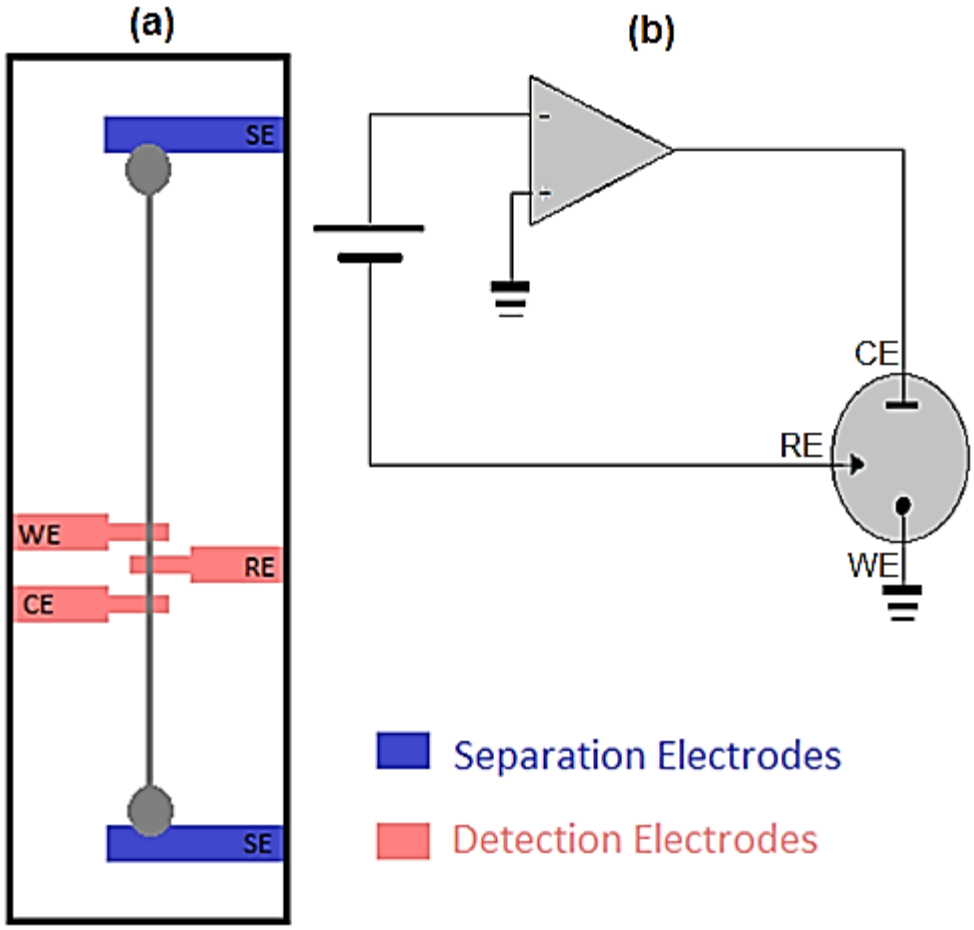

Figure 1.1: The amperometric detection electrodes integrated with: (a) MCE, and (b) a schematic of amperometric detection. Working electrode (WE), reference electrode (RE), and counter electrode (CE).

1.2 DNA Biosensors General Types

Commonly, DNA analysis devices can have three major functions; DNA fragments separation, DNA fragments detection, or both separation and detection. Separation only devices rely on bulky and external optical detection systems like UV or LASER detection systems, while detection only devices cannot be used in DNA fingerprinting (genotyping) since DNA fragments separation is the key factor in

fingerprinting analysis. However, analysis devices that integrate both detection and separation operations in a single device can be a successful DNA profiling or genetic fingerprinting device. Recently, researchers have been focusing on producing cheap and portable DNA analysis systems. Therefore, cost and portability became the major issues for realizing true LOC or μTAS devices for DNA analysis. One such device is the commercial DNA analysis LOC system (Figure 1.2) which facilitates in-situ detection and analysis of DNA fragments, without the assistance of any additional device.

Figure 1.2: DNA analysis LOC system from DNA Electronics.

1.3 Biosensors with Separation Function

As in conventional gel electrophoresis or capillary electrophoresis, DNA biosensors with built-in separation capability use the influence of the electric field to separate the different sizes of negatively charged DNA fragments. DNA biosensors apply the separation electric field in a microfluidic micro-channel between two electrodes called separation electrodes, located in each of the reservoirs as shown in Figure 1.1. However, the required separation electric field is one of the main obstacles of microchip portability in this type of DNA biosensors, since it is too high to be supplied by portable batteries. Stronger electric field will lead to faster separation process. Generally, DNA fragments separation occurs prior to the electrochemical detection. The detection can be in-channel, end-channel, or off-channel, which will be addressed in the next chapter.

1.4 Fabrication Materials and Cost

Typically, the most costly part in the fabrication of biochips is the use of noble metals such as gold and platinum as electrode material and the very expensive tools and equipment used in depositing them on the surface of glass substrates. Noble metals are generally used due to their inherent redox capability and conductivity properties. Although copper is cheaper as electrode material, and has shown very good performance in similar applications [1, 2], the machines for depositing copper film on glass substrates to form the electrodes, and the procedures involved, are still very expensive. Figure 1.3 shows the standard electrodes coating (deposition) system which can cost hundreds of thousands of US dollars; it is worth noting that, apart from this system there are other systems as well,

which are essential for fabricating the microchip substrates, as will be shown in Chapter 3. Therefore, there is urgent need for an affordable, easy to fabricate, and disposable DNA biosensor that is capable of performing a full in-situ DNA fingerprinting analysis.

Figure 1.3: Edwards Auto 306 vacuum coating system.

In addition to the electrode material, other microchip fabrication materials can also influence the performance as well as the cost of the microchip; for instance, the microchannel substrate. Several studies have shown a wide range of potential materials in fabricating the substrate where the separation microchannel is engraved. Each of them has

its own properties that can influence the performance, life and the cost of the biosensor. A detailed study of these materials is presented and discussed in Chapter Two.

1.5 Portability

From the above discussion, it is obvious that there are two main factors influencing the portability of DNA biosensors; they are, the detection device or sensor and the power supply. Fortunately, researchers have integrated amperometric detection circuits and devices in small electronic circuits designed for ECD [3, 4]; this means that the problem associated with the detection device has been addressed. However, the power supply required for providing the necessary separation electric filed is still a challenging issue that hinders achieving portability.

As mentioned earlier, power supply portability is an issue for DNA biosensors dedicated for DNA fragments separation and/or detection rather than detection only biosensors. This is because, normally separation is achieved under the influence of high electric field strength, and consequently the portability of the power supply becomes more complicated since the minimal separation voltage achieved so far is 20 V/cm in a 5 cm long separation channel (total separation voltage is 100 V) [5]. As a matter of fact, integrating a portable power supply capable to provide 100 V in in-situ devices has not been accomplished so far. Accordingly, several recent studies have demanded reduction in the separation voltage in order to facilitate integration of power supply in in-situ devices. In general, voltage reduction can be achieved through two main approaches; first,

by optimizing a separation material with suitable porous medium, as porous medium permeability is a major parameter in the determination of separation voltage and separation speed, and second, by reducing the distance between the separation electrodes. The relationship between the distance between the separation electrodes and the electric field strength is governed by Equation 1.1.

$$E_{ave} = \frac{V_2 - V_1}{\Delta x} \qquad 1\text{-}1$$

Where E_{ave} is the average electric field, V_1 and V_2 are the low and the high voltages respectively, and Δx is the distance between the electrodes.

Many studies have proposed (by simulation) strategies for decreasing the distance between separation electrodes, and subsequently reducing the required voltage to levels affordable for portable batteries [6, 7]. However, so far, none of these strategies has been implemented or fabricated practically. This is because the circuitry of control system of the simulated design was complex and bulky.

1.6 Problem Statement

The existing DNA fingerprinting devices lack in the very important features such as portability and affordability. Portability is very vital in producing biosensors that can

tackle real-time forensic analysis applications, whereas affordability is required to make the research–dedicated biosensors available for customers in a user-friendly manner with affordable cost. The major challenge in portability is making the high voltage power supply (HVPS) unit portable. HVPS generates the required high electric field for samples separation. As high electric field can yield faster separation, reducing the voltage can affect the separation time. Therefore there is crucial need for a careful attempt to reduce the separation voltage that can be supplied by a portable power source, without sacrificing the separation time. The main affordability challenge is to find out alternatives to replace the costly noble electrode metals and the associated expensive tools. Hence in the DNA pathogenic biosensors industry, the growing development and demand of portable, affordable and disposable DNA analytical biochips necessitate efficient design and fabrication of LOC or μTAS devices. These devices should be able to conduct rapid, easy and portable DNA fingerprinting analyses for general foodborne organisms in order to facilitate the disease treatment. Real-time analysis biochips are highly advantageous for crime scene investigation processes. Such biochips are also very important for samples that change their biological properties in time; for instance, non-poisonous food can become poisonous after few hours due to the acceleration of bacterial replications. It is also required to explore and optimize the fabrication materials that are capable to be integrated in a microchip design that should be promising in terms of disposability, portability and affordability. Such a design is also essential in the perspective of in-situ analysis as evidenced by its crucial need in the market. Thus, developing fully functional in-situ devices that fit the commercial and industrial needs has become more vital than ever before.

After identifying the aforementioned gaps through a rigorous literature review, the current study is planned to achieve remarkable improvements in portability, disposability and affordability of DNA fingerprinting biosensors, by exploring different designs and fabrication materials. In order to achieve these tasks, the following research objectives have been spelled out.

1.7 Research Objectives

The current study is aimed to achieve the following two primary objectives:

1. To decrease the separation electric field to very low level that allows producing a very low power DNA fingerprinting system utilizing amperometric detection.
2. To investigate the use of affordable materials as alternatives of gold or platinum, thereby reducing the overall cost.

The following secondary objectives are set to realize the primary objectives:

1. To study the effect of separation electric field strength on the speed of DNA fragment migration.
2. To fabricate a DNA biosensor microchip that can tackle wider range of DNA fragment sizes and hence more DNA genotyping applications.

1.8 Thesis Outline

This dissertation is organized in five main chapters. Chapter 1 addresses the fundamentals and the application of MCE, the problem statement and research objectives. In Chapter 2, a comprehensive review of experimental achievements so far in terms of design, materials and minimizing the required electric field, is provided. Chapter 3 gives a detailed account of the theories, materials and methods employed in the current research. A deep analysis and discussion on the experimental results of electric field as well as the use PCB substrates in microchip fabrication are presented in Chapter 4. This chapter is followed by the conclusion and the suggestions for future work in Chapter 5. The dissertation ends up with the references and appendices.

CHAPTER 2

LITERATURE REVIEW

2.0 Overview

This chapter focuses on reviewing the general Microchip Capillary Electrophoresis with Amperometric Detection (MCE-AD) microchips, which is the most widely employed ECD method for DNA and electroactive assays. A comprehensive survey on the recent developments of the MCE-AD, microchip design, electrodes configuration, separation media, and microchip and electrode materials, is presented. Furthermore, the latest attempts and achievements of reducing the strength of the separation electric field are critically argued. Finally, the use of printed circuit board (PCB) substrates to fabricate DNA separation microchips is reviewed.

2.1 Introduction

Electrophoresis separation is the traditional DNA analysis system by which DNA fragments are sequenced to form bands which are then visually detected. Electrophoresis is also known as electro kinetic-phenomenon which is the motion of dispersed particles relative to a fluid under the influence of an electric field that is space uniform. This phenomenon was discovered by Reuss in 1807 [8], who noticed that clay particles dispersed in water would migrate if an electric field was applied. Later in 1937, Tiselius had successfully separated a protein's mixture, demonstrating the electrophoresis

technique for the first time [9]. Two main types of electrophoresis for separating DNA fragments are available; the oldest and the most commonly used is Gel electrophoresis, and the second type is capillary electrophoresis (CE), also known as capillary zone electrophoresis (CZE), which mostly focuses on size miniaturization and decreasing cost, in addition to tools mobility. The Gel electrophoresis employs a gel in order to separate ionic species (DNA, ribonucleic acid (RNA) or protein molecules) by the influence of an electric field applied to the gel matrix, while most of the CE devices use micro capillaries.

The use of gel in electrophoresis applications was first reported in 1930s when sucrose gel was introduced. This technique had undergone several quality improvements until Agarose gel was introduced in 1970s [8]. Generally, the gel electrophoresis system consists of many parts and equipments such as electrophoresis unit, power supply, fluorescence and illumination system like Ultra Violet (UV) light emitter and/or a computer with camera for capturing DNA bands. All these units make it a bulky, complex, stationary and expensive device. Moreover, the experiments needed to perform the analysis are time-consuming, and can only be achieved in a bio lab or any similar lab that facilitates the required tools and materials. Generally, the analysis system requires 4 to 8 hours to complete excluding the time of transferring the testing samples [10]. As the bacteria and viruses duplicate themselves in short periodical cycles, there is a strong likelihood that the testing samples become contaminated within the period of 4 to 8 hours. This indicates that time is a crucial factor in DNA genotyping and fingerprinting analysis.

Because of the aforementioned reasons, the need for a portable DNA analytical device that can perform in-situ analysis is quite obvious. Accordingly, CE was introduced to tackle size and portability issues. CE is a technique which uses electric field to separate ionic species by their charge and frictional forces of the charged macro-molecules such as DNA and proteins by using microchannels rather than gel. CE was proposed not only to offer solutions to some of the current issues of the conventional bulky gel electrophoresis detection systems but also to enhance the system performance; CE has increased the results' sensitivity and system mobility, and decreased the sample volume. Although some improvements were achieved by using CE system it is not completely portable and still not affordable. Other drawbacks include high electric field, fluorescence and visualization, and the use of some chemicals which are not environmentally friendly such as loading dye and ethidium bromide. As a promising breakthrough in overcoming these drawbacks, the idea of using capillaries for the separation of bio samples has been introduced, resulting in micro sized separation and/or detection systems called Microchip Capillary Electrophoresis (MCE). MCE has solved many major issues of the current bulky conventional gel electrophoresis system, and also the problems of CE itself.

2.1.1 Historical Background of CE System

CE was introduced in 1981 by Jorgenson and Lukacs who had demonstrated the first high performance CE separation with 75 μm inside diameter capillary [11, 12]. Their main contribution was replacing the gel matrix in the conventional gel electrophoresis by micro capillaries for separating the electroactive samples such as DNA, RNA, carbohydrates or proteins. This improvement was one step further toward the whole

system miniaturization and portability. One of the very significant improvements of CE system is the extremely small sample volume in each experiment (1–50 nL per sample) compared to the conventional gel electrophoresis detection and analysis system (10 µL) [13]. Schematic of the CE system is shown in Figure 2.1. The system is composed of two buffer containers and a micro capillary connecting the containers, and a detector inside the capillary which is connected to a PC.

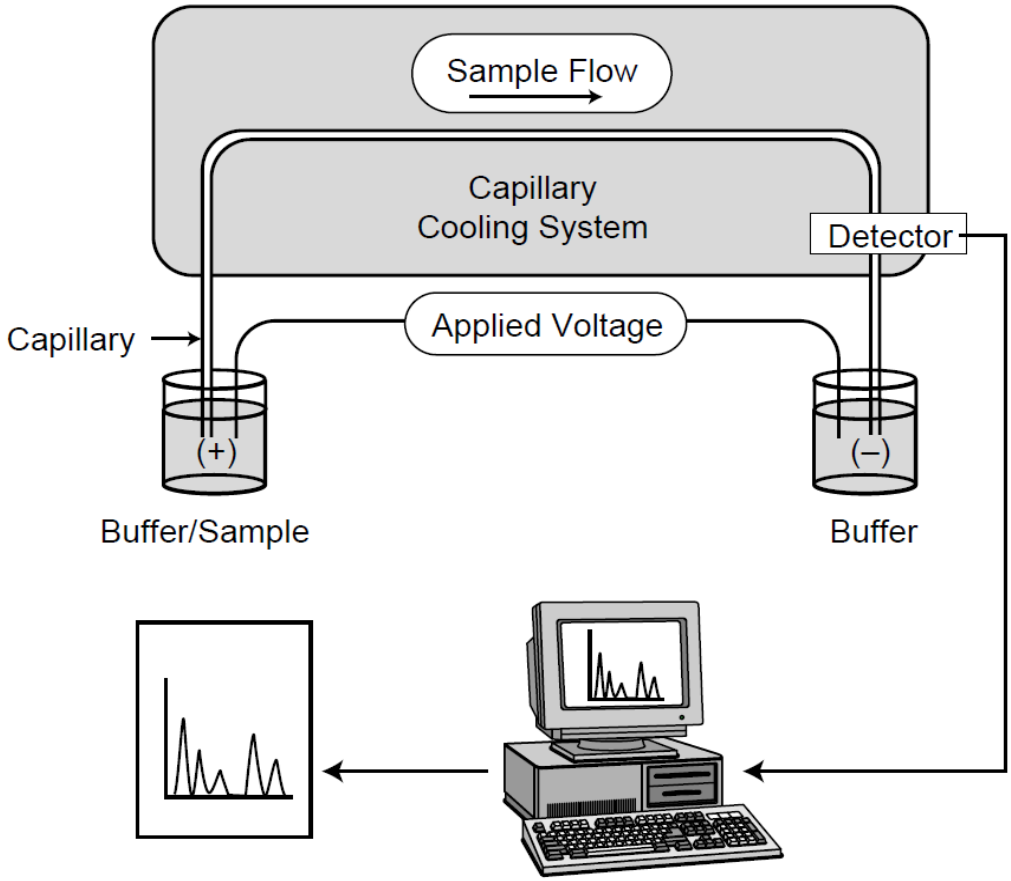

Figure 2.1: Schematic of a CE instrument with normal polarity (anode at injection side) [14]

Since the requirement for a true portable detection system such as the so called micro total-analysis-system (μTAS) or Lab-on-Chip (LOC) is increasing day by day, researchers have investigated the important features of the CE system in designing and fabricating smaller and more portable micro systems. The first microfabricated CE device was introduced more than two decades ago [15]. This new microchip CE device has shortened the separation time significantly, which in turn could reduce the separation voltage. On the other hand, this new microfabricated system still lacks portability because it uses the old fluorescence and visualization detection system. The tools used in the detection system are the same as those in the old CE detection system. Several improvements in the MCE system were achieved in the first decade, in terms of detection methods and techniques, fabrication materials, and miniaturization. However the achievements during this period were not successful to produce a micro-fabricated and fully portable DNA analysis device, because miniaturization and portability were yet to be accomplished. The subsequent improvements of integrating the bio-analytical devices on a single chip would depend on the integration of miniaturized tools of electrophoresis, separation, and detection systems.

In fact, DNA bands detection system is one of the main factors that have a direct positive impact on the system miniaturization. Despite the good performance of general optical detectors like fluorescence and ultraviolet (UV) DNA detection systems [16], they were not easily miniaturized and integrated with MCE devices. Therefore, researchers have investigated electrochemical detection (ECD) as an alternative to the optical systems. EC detection is achieved by detecting the charged particles like DNA molecules. The first

EC biosensor microchip was introduced by Gavin et al. [17]. EC detection has attractive features such as sensitivity, portability, low cost, low-power requirements and increased compatibility with microfabrication technologies [18]. EC detection falls under three main categories namely, voltammetry, coulometry and potentiometry [19]. As the most widely used detection method, voltammetry, especially the simplest technique amperometry, exhibits a remarkable performance improvement when electrode sizes are reduced into a low micrometer range.

Usually, the electrochemical detection signals of DNA sensing devices are obtained either directly from the oxidation of nucleobases (eg. Adenine or Guanine), or indirectly using DNA redox-active materials, or enzymes immobilized upon DNA hybridization [20]. Among all the aforementioned detection signals, direct detection of DNA nucleobases is the simplest, the fastest and the most portable. This is due to the fact that it does not require additional chemicals to enable DNA detection compared to other methods; significant time is required for those additional chemicals to interact with DNA molecules. Similarly, the DNA hybridization detection method also takes hours because of the time required for immobilization [21]. Moreover, direct detection is more portable because it reduces the number of chemicals required for running DNA analysis experiments and makes it more user-friendly.

The redox property of the DNA nucleotide bases (adenine and guanine) enables the electrochemical detection of DNA fragments. This is because reduction/oxidation reaction of the nucleotide bases occurs on the surface of the detection electrode under a

specific potential. This redox reaction generates an electric current which can give information about the detected DNA fragments. The information can be derived from the time of redox reaction and/or the strength of the detected signal which reflects the size of the DNA molecule due to the proportional relation of charge and mass to the migration speed of DNA molecules. The inherent ability of DNA fragments to produce redox reactions has accorded to the EC detection a very high importance in comparison to other counterparts like optical detection. This is because of the aforementioned features of EC detection techniques in addition to the inherent integration capability with MCE compared to the optical detection systems.

2.1.2 Introduction to MCE

Microchip capillary electrophoresis is increasingly becoming important tool for point-of-care, vitro diagnostics, and modern environmental and biomedical analyses. Recently, microchips meant for DNA study such as the integrated microfluidic devices, are gaining popularity among scientists and engineers, owing to their benefits in terms of improved speed, low-cost, high portability and stable performance. With the advent of microelectronic technology, DNA microchips with integrated analytical capability could affordably be fabricated so that most laboratory assays could be quickly and cheaply performed.

Currently, there are various methods and techniques in MCE for the sequencing and detection of DNA and electroactive analytes. In the literature, these types of DNA

MCEs are referred to as the DNA biosensors. Depending on the detection mechanism and sensing characteristics, the working principle of MCE can generally be categorized as: (i) optical detection, and (ii) electrochemical detection. In general, optical detectors use Laser Induced Fluorescent (LIF) [22-26] and UV light [27-30]. ECD can be further classified as (i) the amperometric, (ii) the potentiometric [31-33], (iii) the voltamperometric [33-37], and (iv) the impedimetric [38, 39]. Despite having good sensitivity and high specificity [16, 23, 40, 41] the optical techniques suffer from several major drawbacks such as, limit of detection (LOD), larger size, heavier in weight and higher power supply requirements. More importantly most of the optical-based assays require bio-markers like ethidium bromide, to enable fluorescence and viewing. This poses safety and health issues since this type of bio-markers is toxic and poisonous. Therefore, the latest development on MCE detection methods is gravitating towards ECD, especially when electroactive analytes are targeted. Moreover, ECD is approaching the fluorescence sensitivity, easy to fabricate and inexpensive.

Among the ECD methods, the amperometric detection (AD) is widely accepted as the best method for microchip integration. Generally, amperometric detection method is applicable to any electroactive analyte that can exchange charges through either oxidation or reduction over electrodes surfaces. Moreover, some other non electroactive analytes can be detected through intercalation technique. Since intercalation can convert non-electroactive analytes to electroactive, therefore, the detection can be electrically performed by the addition of intercalators like adding the electroactive dye to non-electroactive analyte [42, 43], and hence, amperometric detection can be applied to the

intercalated analytes [44, 45]. Intercalation could not only enhance the electrical properties of analytes, but also impart electrical properties to inorganic compounds [46, 47] . Thus with the aid of intercalation, MCE-AD could target a wide range of analytes. Moreover, these outstanding features make MCE-AD an attractive and effective tool for designing a truly μTAS.

Amperometric detection is achieved by measuring current while applying modest potential to the working electrode. This technique was reported for the first time by Woolley in 1998 [48], then found its way through many researches, and currently available as the most effective detection method, due to its superiority over other techniques. Amperometric detection is more suitable for electroactive analytes under modest potential. Being negatively charged, DNA is an electroactive analyte; when DNA comes in contact with the working electrode, it triggers either oxidation or reduction reaction, causing the formation of electrical charges. The nature of DNA redox reaction depends upon the working electrode surface material and the applied potential between the reference and the working electrode. Therefore, it is possible to design a detection circuit based on amperometric measurement, for all electroactive analytes including DNA [3, 4].

Recent developments in μTAS necessitate the discussion of some of the key aspects influencing the microchip performance and miniaturization. Few researchers have provided excellent reviews on μTAS. For instance, Xu et al. [19] outlined the progress in integration of electrochemical elements in μTAS, while Kailas and Kang [49] focused on

developments and applications of MCE for detection and separation of DNA fragments. Teles and Fonseca [50] reviewed various DNA immobilization techniques, as well as new micro and nanotechnological platforms for biosensing, and geno sensor transduction.

2.2 Microchip Design

Configurations and design of amperometric microchips play a major role in MCE characteristics. Among the various factors affecting the MCE characteristics, some of the important issues are, the performance, and the cost of fabrication. Each of these issues needs to be studied separately in order to understand the problems and technical limitations associated with the microchip design. Another influential factor is properties of the targeted analyte, since analytes have different electrical properties. For instance, the nucleic acids are negatively charged due to the phosphate group ions.

On the other hand the amino acid at pH 7.4 is either negatively charged (e.g. aspartic acid and glutamic acid), or positively charged (e.g. arginine and lysine). Hence, the analyte's charge polarity can be crucial to the design of amperometric circuit and to the alignment of electrodes in microchips. Moreover, DNA fragments redox reactions can generate current peaks of several micro amperes, while other electroactive analytes are measured in pico amperes. Another critical factor is the alignment of working electrodes as different alignments give different configurations, and every configuration has its own features and performance characteristics. Since detecting analytes is the basic function of the working electrode, placement of this electrode/s in the separation channel is quite

sensitive and meaningful. Alignment of electrodes is also important to isolate them from being subjected to the high separation voltage during amperometric detection. In general, isolation is achieved by three basic approaches: (a) in-channel detection, (b) end-channel detection and (c) off-channel detection (a decoupler is employed here), as shown schematically in Figure 2.2.

Next, in-channel and off-channel configurations are discussed in more details, since they are the most preferred choices and so the most generally implemented in amperometric MCEs including DNA biosensors. These two configurations are introduced to minimize some of the problems associated with the end-channel detection, specifically, the decreased detector response due to diffusion, and band broadening, which will be elaborated in the ensuing section.

2.2.1 End-Channel Detection

This is the most popular configuration used in ECD. Here, the detection electrode is located inside the separation channel outlet (buffer waste/detection reservoir), generally, about 5 μm to 50μm away from the end of the separation channel. Lee and Chen [51] installed a copper wire as working electrode, at the end of 10cm long separation channel, for the detection of carbohydrates. The detection sensitivity was improved and band broadening was not a main issue; yet this lengthy separation channel required very high voltage (2000 V) and a relatively long separation time (400 s). The separation time is the

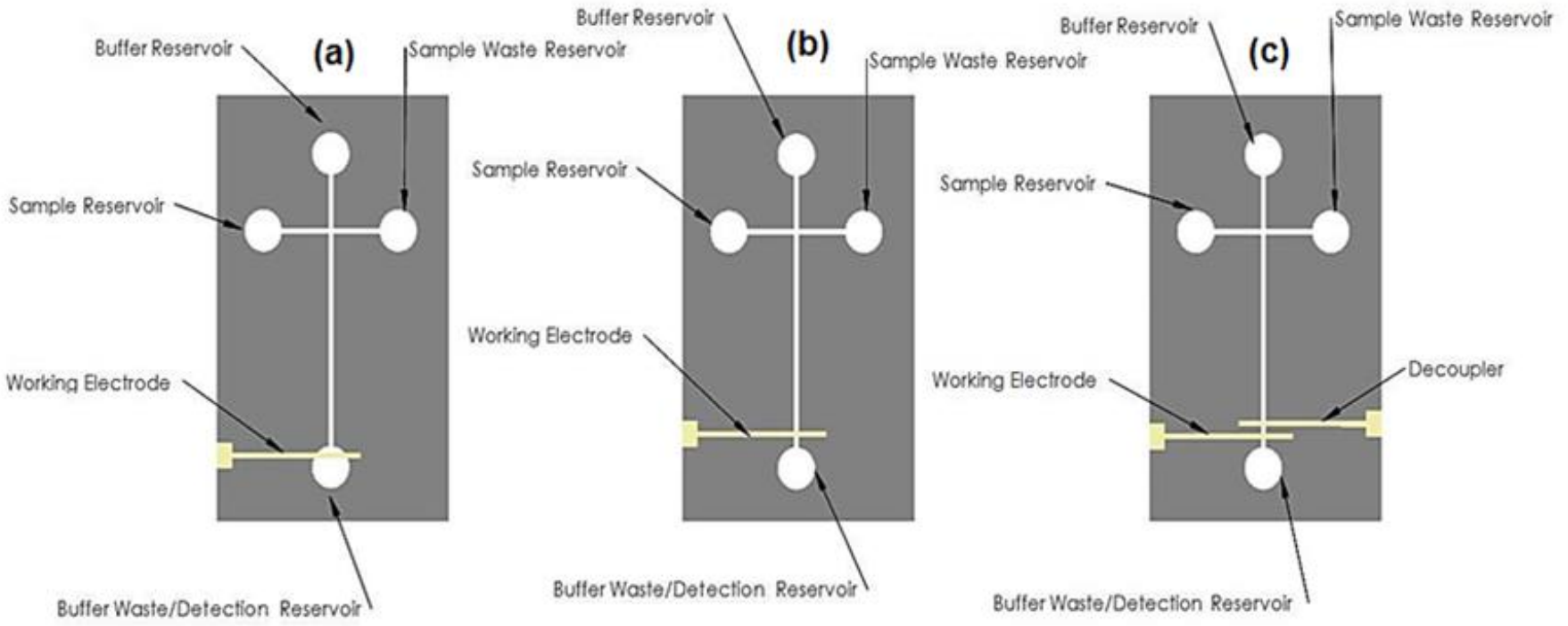

Figure 2.2: Schematics of electrode alignments for amperometric MCE detection: (a) end-channel electrode alignment, (b) in-channel electrode alignment, and (c) off-channel electrode alignment. In-channel uses are increasing in the recent researches.