國立中山大學機械與機電工程研究所

碩士論文

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多重酵素修飾線微流體系統於全血中尿素氮及血糖偵測

Multiple enzyme-doped thread-based microfluidic system for blood urea nitrogen and glucose detection in human whole blood

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於中華民國 103 年 6 月 26 日經本委員會審查並舉行口試，符合碩士學位論文標準。

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中文摘要

在現今的生醫晶片系統中，量測人體全血中的尿素和血糖是健康相當重要的指標。血尿素氮 (BUN) 的形成主要是來自蛋白質代謝的最終產物，因此血中尿素氮的濃度，也可以用來做評估腎功能的指標，所以當血液中的尿素氮值升高，超過標準值時表示腎臟過濾功能可能出現問題，這是一個重要的警訊。然而，隨著現今全球文明化和飲食文化的改變，許多的文明病便相伴而至例如糖尿病，所以血糖指數的多寡是相當重要的，當血糖值過高表示胰島素的缺乏是糖尿病的高危險群，因此開發一個生醫晶片整和電化學電泳 (CE-EC)去量測生醫樣品，以期能運用在醫療檢驗上做為疾病的預防和檢測機制是相當重要的。

本研究提出了一種新的方法來檢測人體全血中的尿素和葡萄糖，利用多種不同反應的酶滴在線微流體上使其和檢測樣品做反應以利偵測，再者使用 PVC 膜包覆在線體的外部形成一個密閉式管道。滴在線上的酶可以直接和樣品反應無須經過任何的表面改質和加工，而流經過的生物樣品經由酶的轉化，最後再由下端的電極做電化學檢測。所以利用以上的方法結合 CE -EC 檢測生物樣品，可以得到很好的量測結果。本研究進一步做人體的全血量測，首先利用 RBC 裂解液滴在多出的注入端部分而後在加入約 2-μL 全血混和後，利用正負高電壓驅動線微流體上的電滲流流動，可以裂解血液中的紅細胞和同時過濾掉其他雜質，做為一個過濾器使用，使其提高偵測性能和對阻塞管道的影響降低。由於焦耳熱效應關係，管道上緩衝液和樣品是很容易蒸發，而且溫度上也不耐高，容易造成線體的損壞，所以塗佈 PVC 膜可以改善以上的情形。此外，PVC 塗佈的線體可以在更高的分離電場 500 V/cm 進行操作。

一般尿素和飯前血糖的正常範圍值分別是 1.78~7.12 Mm 和 3.89~6.11 mM ，結果表示，此研發晶片系統在量測表準樣品尿素和葡萄糖中擁有良好的線性結果，尿素濃度範圍在 0.1 mM – 10.0 mM (R^2=0.9850) 間；葡萄糖濃度範圍在 0.1 mM – 13.0 mM (R^2=0.9668) 間，擁有良好的靈敏度。在全血的檢測中也成功分離出血

液中的離子，並測得尿素和血糖濃度分別為 3.98 mM 和 4.94 mM。本研究開發的線微流體系統提供了一個低成本，高性能且創新的方法應用在人體血液中的檢驗。

關鍵字: 線微流體，電泳，尿素氮，葡萄糖，PVC 膜

Abstract

In biological chip systems, urea and glucose are important indicators for detection in human blood. Urea forms in the liver as a waste product of protein metabolism then collects in the bloodstream. The concentration of blood urea nitrogen (BUN) is an important biological indicator used for monitoring the functionality of the kidney. In general, patients with kidney failure have high BUN levels. However, with changing food culture, glucose has also become a identify control factor for diabetes mellitus. Excessive glucose displays a deficiency of insulin. Therefore, developing a microfluidic device for capillary electrophoresis electrochemical (CE-EC) detection of bio-samples is a necessary.

This research presents a novel technique for detection in human whole blood which utilizes a novel enzyme-doped thread with a PVC (polyvinylchloride) membrane coating for on-site urea and glucose detection on a thread-based microfluidic device. The enzyme can be directly applied to the thread without delicate pretreatment or a surface modification process. The passing biomolecules are digested by the enzymes and then electrochemically detected downstream. With this approach, CE-EC detection with on-site bio-reaction can be simply achieved. The whole blood sample is first mixed with a RBC lysis buffer to prevent blood coagulation. The lysed RBC and other solid pieces are simultaneously filtered away while electrokinetically flowing through the thread-based microfluidic system. A thin layer of PVC membrane is coated on the enzyme-doped thread to further fix the applied enzyme and to prevent rapid evaporation of the running buffer due to the Joule heating effect. In addition, the PVC coated thread can be operated at a higher separation electric field of 500 V/cm due to the reduction of buffer evaporation.

Results also indicate that the developed system exhibits a good linear dynamic

range for detecting urea and glucose in concentrations from 0.1 mM – 10.0 mM (R^2=0.9850) and 0.1 mM – 13.0 mM (R^2=0.9668), which is suitable for adoption in detecting the BUN concentration in serum (1.78~7.12 mM) and the glucose fasting measuring range (3.89~6.11 mM). The whole blood detection shows that the developed thread-based microfluidic system can successfully separate the ions, BUN and glucose in blood. The calculated concentrations for BUN and GLU-AC in the whole blood sample are 3.98 mM and 4.94 mM, respectively.

Keywords: Thread-based microfluidic system, Electrophoresis, Blood urea nitrogen, Glucose, PVC membrane,

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Nomenclature

Ca_2^+ : calcium ion

Cl^- : chloride ion

dl : deciliter

E : electric field intensity (V/cm)

F^- : fluoride ion

GLU-AC : fasting blood glucose

H_2O_2 : hydrogen peroxide

K^+ : potassium ion

Mg^{2+} : magnesian ion

mM : millimolarity

ml : milliliter

mg : milligram

Na^+ : natrium ion

NH_4^+ : ammonium ion

n : electronic exchange of the number of molecules and the electrode

O_2 : oxygen

OH^- : hydroxide ion

pL : picolitre

S/N : signal-to-noise ratio

V : voltage

W : wattage

ν_{e0} : migration rate (cm/min)

μ_{e0} : electrophoretic mobility ($cm^2/V \cdot min$)

μm : micrometer

μL : microliter

μA : microampere

μL : microliter

ε *:* permittivity of the electrolyte solution (C/mv)

ζ *:* potential of interface (V)

η : viscosity of solution (Ns/m^2)

Abbreviations

AA : ascorbic acid

Bio-MEMS : biological micro electro mechanical system

BUN : blood urea nitrogen

CE-EC : capillary electrophoresis electrochemical

CNC : Computer numerical control

CV : cyclic voltammetry

CA : catechol

DA : dopamine

EP : electrophoresis

EOF : electroosmosis flow

EDL : electric double layer

GOD : glucose oxidase

GLU : glucose

GNEE : gold nano-electrode

HPCE : high performance capillary electrophoresis

HRP : horseradish peroxidase

ISE : ion-selective electrode

Mred / Mox : redox mediator

MES : 2-morpholinoethanesulfonic acid

PVC : **p**oly **V**inyl **C**hloride

PDMS : polydimethylsiloxane

PMMA : poly methyl methacrylate

RBL : red blood cell lysing buffer

μTAS : micro total analysis systems

3D-μPAD : three-dimensional microfluidic paper-based analytical device

2D : flat chip

Chapter 1 Introduction

1.1 Background

In recent years, advances in the technology industry have relied on depleting natural resources and have introduced pollution to the environment, such that the global industry production modes are committed to change. One emerging change is seeking simple and miniaturized product design in micro-electro-mechanical systems (MEMS), an industrial technology integrating microelectronics technology and mechanical engineering in the micrometer operating range. Due to the reduced size of the chip allowing for mass production and lower costs, there are a number of prospective areas of study.

Biomedical micro-electro-mechanical systems (bio-MEMS) utilizes MEMS technology for the production of biomedical detection devices, including biomedical microfluidic chips, biomedical micro-sensors and biomedical microfluidic chip which can be applied to capillary electrophoresis detection. These devices are inexpensive, simple to operate, require a short analysis time and small sample and are suitable for a single use. If this device can be used in a home medical detection system, it will likely be part of an important future trend of biomedical testing.

1.2 Capillary electrophoresis

1.2.1 The basic principle of electrophoresis

Electrophoresis (EP) means the different charged particles subjected to an externally applied electric field in the medium after go on the scenario of different positive and negative directions mobility. Because the charged / size ratio in the electrolytic solution will produce a different rate of migration for charge particles

which become the theoretical basis of the electrophoresis [1]. Electrophoresis is a separation technique by widely used that can used to separate variety of different materials, including DNA, bio-sample and different metal ions [2-4]. In 1937, Tiselius [5] proposed the first instrument of electrophoresis to separation human serum α1-, α2-, β-and γ-globulin.

Early conventional electrophoresis causes changes in the density of the buffer that effects heat convection by generating Joule heat when current flows in the medium, resulting in high voltage which causes a reduction in resolution. In order to overcome this problem, most research has proposed adding a solid material to the medium such as agarose and cellulose acetate membrane. This method not only produces a lower current with an increase in the resistance value of the electrophoretic medium, but also suppresses Joule heat in thermal convection. Although this method can provide an adequate solution, there is still the problem of an acting force between the sample and the solid medium, so researchers have begun to search for other solutions. In 1974, Virtanen [6] utilize glass tubes of 200 ~ 500 μm inner diameter to separate potassium, sodium, calcium and magnesium other metal ions and found that the smaller inner diameter of the separation column can reduce the cross-sectional area or increase the resistance value of the electrophoretic medium such that the thinning effect of the wall allows for better radiation. When thermal convection is smaller, Joule heat is reduced and resolution is significantly improved. In 1981, Jorgenson and Lukacs [7, 8] proposed a theory about high performance capillary electrophoresis (HPCE) by utilizing a 75 μm inner diameter capillary to separate amino acids and peptides. This illustrates a potential method deserving of more attention and research.

Capillary electrophoresis uses a relatively small diameter capillary conduit as a

transfer and separation device, with high voltage applied to the outside. This separation technique has different migration speeds, resulting in a sample with a different type of ion as the charge density, volume, mass and friction change. Migration velocity and direction of flow is affected by both the effects of electrophoresis and electro-osmosis flow (EOF) [9]. A diagram of such a device using this system is provided as Figure 1-1. A high voltage is applied to the capillary through a buffer solution that produces the electrophoresis and EOF. The ions are detected in the arrival terminal, achieving separation.

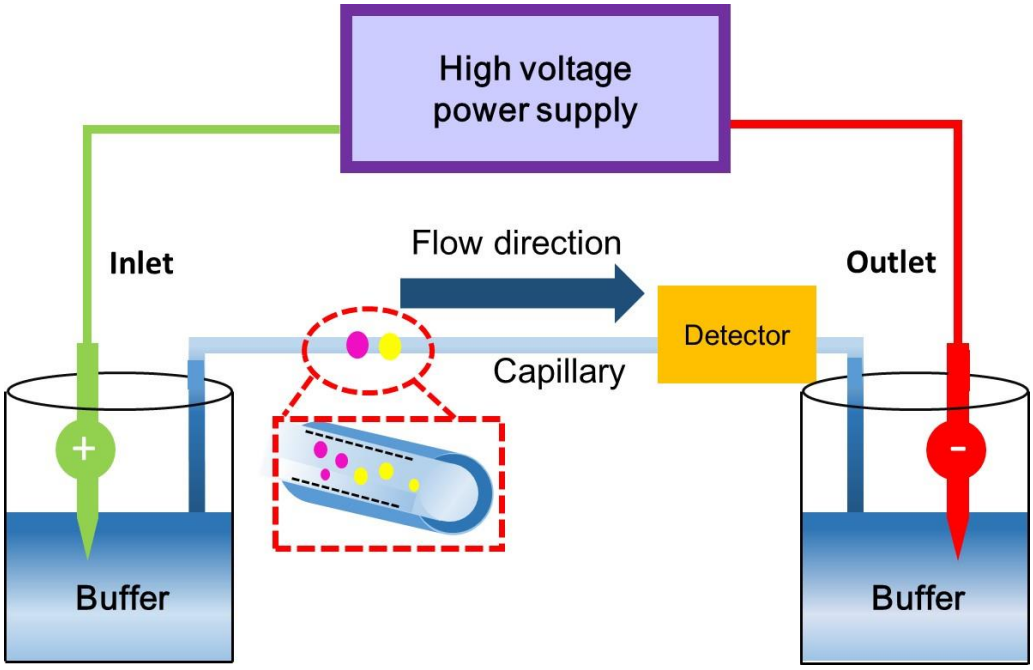

Figure 1-1. Schematic diagram of the capillary electrophoresis.

Factors of EOF for the glass capillary are determined by the amount of silica comprising the capillary tube, and therefore the Si-OH of the capillary surface causes some pH-dependent dissociation in the solution. Si-OH becomes the negatively charged Si-O- ions in the medium at pH values greater than 3. An electric double layer (EDL) is formed in Figure 1-2 (A) because the negatively charged capillary wall surface will attract cations of positive charge [10, 11].

The electric double layer is divided into two layers when the cation solution adsorbs on the solid wall surface, and the electric double layer forms the first layer, called the stern layer. As the distance from the wall gradually increases, the cation attraction gradually decreases, and the charge density decreases exponentially with the distance from the wall and easily diffuses in the solution. This layer is called the diffusion layer, and the size of the charge distribution density with thickness is termed the debye length. In the electric field, the electric double layer caused by the buffer with the wall produces the zeta potential. The cations in the diffusion layer, when affected by the coulomb electrostatic force, move to the negative and drag the hydrated molecules in the solution to produce EOF in the tube. Considering the capillary in cross-section, velocity will generally exhibit a parabolic curve. With EOF, however, the velocity will be constant and the curve flattens. Under EOF, an electric field in the diffusion layer of positively charged ions moves toward the cathode with the flow, as in Figure 1-2 (B) [12].

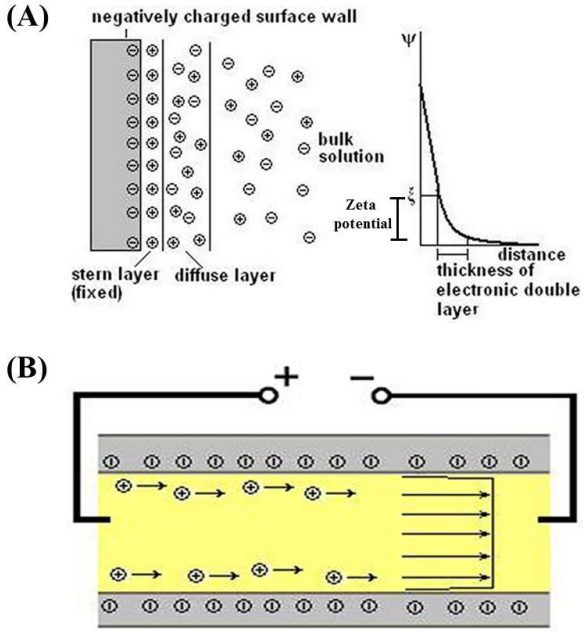

Figure 1-2. Illustrates the diagram of electric double layer (A) and cross-sectional view of the electroosmosis flow (B) [12].

In addition to the ion charge density greater than the small such as F^-, Cl^- and OH^- and the EOF greater than other electrophoretic velocity of other ions. The power of both the added EOF into the electrophoresis will be brought to the negative terminal of the voltage. The formula of EOF from (1) and (2) shows the EOF is proportional to the strength of electric field and the joule heat in the channel will cause the tube to the temperature of solution will be increases make the viscosity of the liquid changes or the electric field strength increases the greater effect of joule heat. EOF will affect the temperature distribution and heat transfer and joule heating will effect contain , so when the electric field is changed, the EOF does not increase or decrease was linearly proportional [13, 14].

$$\nu_{e0} = L/t \quad \text{.....(1)}$$

$$\mu_{e0} = \varepsilon\zeta/4\pi\eta \ = {}^{\nu_{e0}}/_{E} \quad \text{.....(2)}$$

ν_{e0} Migration rate (cm/min)

L Length of channel (cm)

t Time of current change (sec)

E Electric field intensity (V/cm)

μ_{e0} Electrophoretic mobility ($cm^2/V{\cdot}min$)

ε Permittivity of the electrolyte solution (C/mv)

ζ Potential of interface (V)

n Electronic exchange of the number of molecules and the electrode

η Viscosity of solution (Ns/m^2)

1.2.2 Substrate and production methods of electrophoresis chip

The chip-based capillary electrophoresis (CE) system is an important technique for separating such biological samples as ions, protein and DNA [15]. In addition,

these microchip devices are usually produced in materials such as glass, silicon, polymer, and even paper [16-20] using various microfabrication technologies. Glass-based microfluidic chips have several advantages, including excellent mechanical and chemical properties and optical transparency. Though a rapid fabrication process has been developed to produce a microfluidic system in a low-cost soda-lime glass [21], the fabrication processes for glass-based microfluidic devices is usually time consuming and delicate [22-24].

Alternatively, polymer materials have been adopted in microfluidic systems, which can be produced with a faster and cheaper fabrication process. These typical polymeric microfluidic devices have been produced with hot embossing, injection molding, laser ablation, and elastomer casting [25, 26]. For example, Ruecha et al. [27] used the polydimethylsiloxane (PDMS) elastomer to produce a capillary electrophoresis chip. The microfluidic device was sealed with an irreversible cross-linking process with the assist of the oxygen plasma activation. Pumera et al. also reported a low-cost and disposable PMMA chip integrated with contactless conductivity detectors for CEEC detection [28]. For example, PMMA-based capillary electrophoresis chip embedded with the gold nano-electrode (GNEE) was reported for CE-EC of ammonia concentration of urine. Some other CE-chips also have also been used for simultaneously measuring hydrogen peroxide, ascorbic acid and uric acid in glass-based microfluidic device [29]. The sensing performance of the in-column enzymatic reactions has also been experimentally investigated [30]. Recently, the use of the polyester thread as the microfluidic channel has shown a great potential for rapid and low-cost detection of biosamples [31]. Biomolecules and ions could be transported and electrochemically detected on a thread such that time-consuming fabrication processes for producing sealed microfluidic channel can be excluded. The

cost for producing microfluidic devices using this method was significantly reduced.

1.2.3 Detection methods of electrophoresis chip

The feasibility of integrating multiple microfluidic devices on a single chip in order to create micro total analysis systems (μTAS) has attracted considerable interest in recent years. The literature contains many proposals for capillary electrophoresis (CE) microchips designed to carry out the electrophoretic separation of various biochemical and chemical analytes [32-34]. Due to the micro-chip diameter of channel, only a small amount of sample (about μL-pL) in a considerable of volume is required to conduct micro-analyte detection, and the sensitivity of such detection systems are becoming paramount. Existing electrophoresis chip detection methods include laser induced fluorescence (LIF) [34, 35], fluorescence detection [36], mass spectrometry detection [37] and electrochemical detection (EC) [38].

I. Laser induced fluorescence

Laser-induced fluorescence detection principles using laser as an excitation light source through a lens focused on the capillary, with the molecule detected by absorbing energy after being excited to high energy bands. This laser light source is different from others in that laser has a high degree of coherence and its energy does not diverge, but rather can accurately be focused on narrow pipes without significant refraction and scattering. In addition, it can significantly enhance its sensitivity and selectivity when its power light and wavelength are adjusted to the most appropriate intensity of incident light, a subject of study in recent years [39]. The bulky laser gas detection equipment, however, cannot be miniaturized. Although suitable for miniaturization of light, the solutions of solid-state lasers and semiconductor lasers

are expensive.

II. Fluorescence detection

Another means of detection comes in the form of fluorescence detection. The sensitivity of fluorescence detection method and UV / visible absorption high detection method are similar, but the fluorescence detection method requires pretreatment in order to obtain high selectivity of measurement. In addition, the intensity of fluorescence radiation is directly proportional to the excitation light source and the current multi-use laser light as the excitation source [40, 41].

III. Mass spectrometry detection

Mass spectrometry can provide a large amount of chemical analyte information that other detection methods cannot achieve, not only detecting the molecular weight of the analytes, but also enabling the ability to determine their structures [42, 43]. The ionization methods most commonly used are to inject the sample into the capillary electrophoresis system with electrospray ionization (ESI) [44, 45] and fast atom bombardment (FAB) [46, 47]. With the electrospray ionization method, mass spectrometry can analyze more complex biochemical molecules of large molecular weight and proteins carrying multiple charges. Therefore, capillary electrophoresis mass spectrometry has become quite an important the detection method. However, due to the high cost of mass spectrometric equipment, it is not easy to implement.

IV. Electrochemical detection

Electrochemical reaction occurs between two different interfaces and is accompanied by a charge transfer. The charge transfer can be promoted by controlling

the energy of the electrons to the electrode. For example, when the electrode potential is changed to positive potential from negative, the electronic energy will be improved enough across the discontinuous interface between the electrode and the buffer, named the reduction reaction. Conversely, if the change is from negative to positive potential in the electrode, then the electron energy will decrease such that the electrons of the reactants will have enough energy to move across the interface to the electrode, named the oxidation reaction. The detection methods are conductivity detection, potentiometric detection and ampere detection methods [48, 49].

1.3 Electrochemical

1.3.1 The system of electrochemical electrode

Electrochemical measurement of the main electrodes are the reference electrode, counter electrode and working electrode which is a redox reaction while the system maintaining electrical neutrality of the reaction order to utilize the counter electrode. Means that, when the reaction at the working electrode for the oxidation reaction, the reduction reaction will be occur on the counter electrode and vice versa. In addition, reference electrode plays an important role which is used to describe compromise the potential [50]. The diagram shows on Figure 1-3.

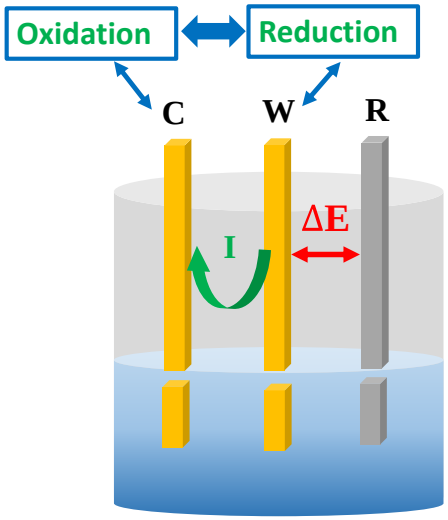

Figure 1-3. Schematic representation of the electrochemical three system used in this study.

Generally common electrode materials are divided carbon electrodes and metal electrodes. Carbon electrode types such as carbon ink, carbon paste and carbon fiber [51, 52] in a wide range of electrochemical detection applications. Although carbon electrode is low cost and compared with no damage to the electrode surface problem, but there are still defects such as electron transfer rate slower than the metal electrodes or not long-term exposure to organic solvents ... etc. Common metals are gold electrodes [53], platinum [54], argentum and palladium electrodes [52, 55], and preparation methods of metal electrodes are generally begin with evaporation, sputtering by chromium and titanium as adhesion layer was deposited on a glass substrate.

1.3.2 Detection methods of electrochemical

The main method used in capillary electrophoresis electrochemical detection of conductivity, potential and amperometric detection.

I. Conductivity detection

To analyze ion conductivity compared to the background detection signal, the electrolyte solution then the component ions must be known. First used in capillary zone electrophoresis by Mikkers *et al.* [56], a small electric current is fixed to a capillary of diameter 200 μm into two slots in the detection electrode. This method can measure conductivity differences between the analyte and the buffer solution. The ability to move relative to the detection signal is detected, and then calibrated. The conductivity is only associated with the number of charged particles, making it difficult to determine the type of particles and selectivity. Because this method interferes with the measurement of electrophoretic currents, it is rarely used in capillary electrophoresis [57, 58].

II. Potential detection

The potential detection method measures the activity of different ion solutions flowing through an ion-selective electrode (ISE) [59]. Specific ions are dissolved in a solution and these ions exchange within the film, causing changes in potential across the ion selective film. In addition, the electrode potential difference between the two is determined by the concentration of ions in solution. The basic structure of ion-selective electrodes is shown in Figure 1-4. The reference electrode was Ag / AgCl in which the ion-selective membrane is fixed with PVC soaked in buffer solution in order to maintain the stability of the film and the reference electrode potential [60, 61]. Selective membranes, however, have some disadvantages. They are vulnerable to interference by the separation voltage of electrophoresis, they not easy to manufacture, and different ions must use different membrane electrodes such that it cannot detect a variety of mixtures. Therefore the method is limited within

electrophoresis.

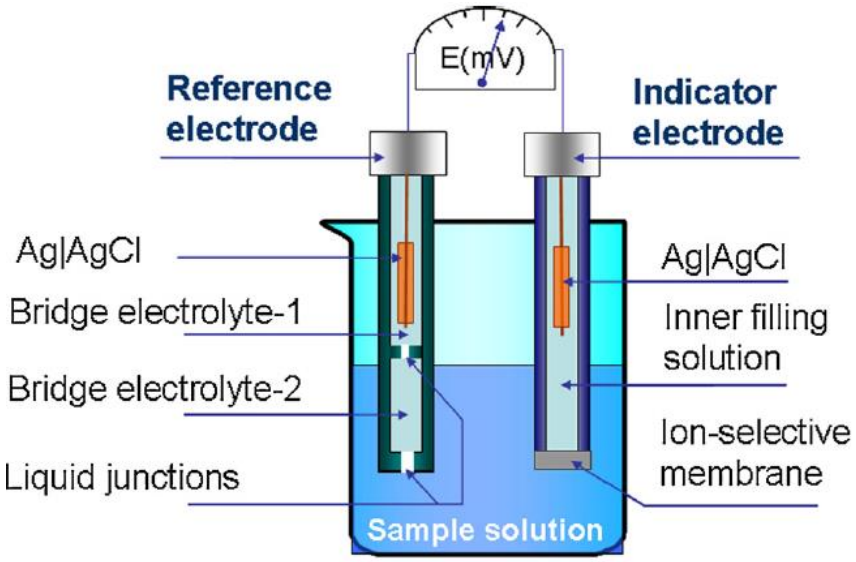

Figure 1-4. Schematic diagram of ISE [62].

III. Amperometric detection

Amperometric detection is most commonly used in electrophoresis detection methods because of its high sensitivity and selectivity. The working principle is that the analyte affects the redox reaction on the surface of the electrode, which can be detected in the current response after applying potential. The analyte reaches the electrode surface by mass transfer, whose three methods are migration, diffusion and convection. When the analyte is close to the electrode surface, chemical reactions (e.g., protonation, dimerization, catalytic decomposition) or physical reactions (e.g., adsorption, desorption) may occur [63]. Finally, the analyte reacts to current with the redox reaction before reaching the electrode surface, as shown in Figure 1-5.

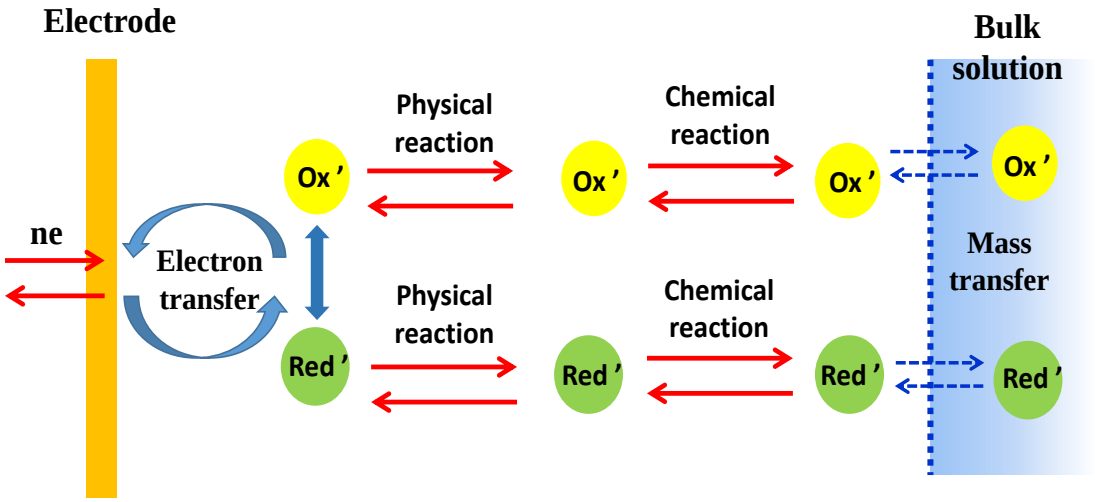

Figure 1-5. Schematic diagram of redox reaction on electrode surface.

Ampere detection method is according the placement location of electrode has off-column (electrode at the end of the channel) end-column (electrode outlet of the channel outside). In 1987 Wallingford and Ewing [52] successfully separate electrophoresis by utilizing end-column. But applying the separate potential will produce current which affect detection. There are three kinds of methods have been developed to improve namely end-channel, in-channel and off-channel detection mode [64].

1.4 Paper microfluidic chip

Recently, an even lower cost microfluidic system comprising hydrophilic paper bounded by a hydrophobic polymer was first reported by Ballerini [65]. The developed microfluidic device suggested a path for developing simple, inexpensive, and portable diagnostic assays for detecting disease and monitoring health. Martinez et al. [66] developed three-dimensional microfluidic paper-based analytical devices (3-D μPADs) shows in Figure 1-6 (A) which used SU-8 to pattern the microfluidic

channels on the paper substrate for biological assay tests. Liu et al. [67] also reported a three-dimensional (3D) microfluidic paper-based analytical device (3D-μPAD) for performing enzyme-linked immunosorbent assays (ELISA).

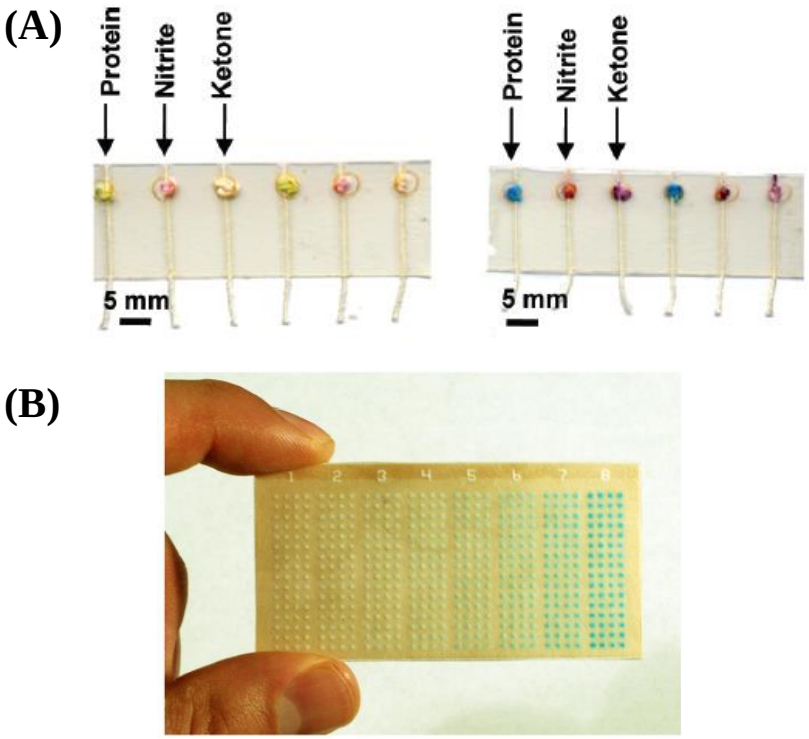

Figure 1-6. Colorimetric assays performed using the (A) woven array device and photograph of (B) the bottom of the 3D-μPAD, 1 min after adding the samples to the input wells. [68, 69]

Paper-based microfluidic systems have a number of advantages during practical applications, including high speed and low cost production processes, compatibility with commercial printing technology, and no requirement for an external driving force [70, 71]. Typically, it is necessary to define the hydrophilic and hydrophobic regions on the paper substrate of a paper-based microfluidic device, where the hydrophilic region is typically used as the liquid route for sample delivery. Li et al. [72] utilized alkyl ketene dimer (AKD) as the cellulose hydrophobization agent to turn the paper

hydrophobic, after which the paper was again treated with vacuum plasma to define the hydrophilic region where the chemical reactions occur. In recent year, even use paper describes the use of red blood cell (RBC) agglutination for separating plasma from finger-prick volumes of whole blood directly in paper, and demonstrates the utility of this approach by integrating plasma separation and a colorimetric assay in a single μPAD [73]. Recently, paper-based microfluidics has emerged as a multiplexable point-of-care platform which might transcend the capabilities of existing assays in resource-limited settings. Currently, in its early development stages, paper-based microfluidics is considered a low-cost, lightweight, and disposable technology [74]. But it still has issues including difficult to define hydrophilic / hydrophobic channel and the material easily damaged. Alternatively, Bhandari et al. [75] developed a scalable microfluidic platform by weaving wetting and non-wetting yarns. A similar approach was also adopted for developing diagnostic and immunoassays [68] shows in Figure 1-6 (B).

However, the weaving procedures used in these methods are relatively skillful and time consuming techniques such that it is challenging to weave different substrates of identical properties. In this regard, a new microfluidic system utilizing polyester threads as fluidic channels was reported [76, 77]. Successful separation and electrochemical detection of the ion samples was achieved without using the sealed microfluidic channels which require this time-consuming fabrication process. The concept of using polyester threads as microfluidic channels has been demonstrated. However, the sensing performance of such a developed microfluidic system is an issue for practical bio-detection applications since a planar electrode system is adopted for EC detection.

1.5 Motivation and objective

MEMS technology has been under development for many years, with traditional structures for capillary electrophoresis being glass, polymer substrates and PDMS. Capillary electrophoresis integrated with biomedical detection has become increasingly high-profile. Microfluidic chip miniaturization can significantly reduce testing costs, and has therefore become the focus of recent development. Although capillary electrophoresis chips have been successfully miniaturized, miniaturized electrophoresis relies mainly on glass, acrylic and polymer materials as the substrate to produce a closed micro-channel. More importantly, using a closed micro-channel faces some challenges regarding bonding, its high cost and time-consuming production. Current detection chip systems are not fully universal and production costs are high. Therefore, developing a low-cost, rapid and accurate miniaturized medical chip detection system is very important.

This study develops a novel thread-based microfluidic system with 3D detecting electrodes and variable volume injection capabilities. Due to the greater injected volume for the samples and higher contact surface area for electrochemical detection of passing biosamples, the current response for the sample peaks can be greatly enhanced with this simple approach. The enhanced peak S/N ratio for different injection volumes is systematically investigated by CE-EC detection of biosamples of dopamine and ascorbic acid. In order to solve the shortcomings of an open channel, a novel technique was developed to form a PVC coated thread doped with various enzymes of urease, glucose oxidase and horseradish peroxidase for on-site bio-sample separation, bio-catalytic reaction and electrochemical detection. Enzymes modified on a polyester thread coated with a thin layer of PVC, and this was used as the liquid route and the reactor for converting urea and glucose into ammonium ions and

hydrogen peroxide. Performance of the developed thread-based CEEC system is evaluated by detection of human whole blood. The developed microfluidic device demonstrates potential for developing low-cost yet high performance biosensors for clinical applications. Compare to previous works, surprisingly, the proposed assay provided a simultaneously multiple detection and high performance in Table 1-1.

Table 1-1. Compare of data for electrophoresis determination.

Detection mode	Analytes	Concentration	Material of chip	Response	Reference
Amperometric Detection	Uric acid Dopamine	15 – 110 µm 1 – 165 µm	PDMS/Glass	3 – 60 nA	[78]
Amperometric Detection	Glucose	0.5 mM	Glass	0.2 – 0.4 µA	[30]
Amperometric Detection	Dopamine Catechol	0.1 - 1 mM	PDMS/Glass	0.5 – 5 nA 0.1 – 1 nA	[3]
Amperometric Detection	Urea	5 mM	PMMA	10 nA	[79]
Amperometric Detection	Dopamine Catechol	0.1 -10 mM 1 mM	PMMA	1 – 13 nA 0.1 - 0.5 nA	[16]
Amperometric Detection	Cholesterol	0.1 – 7 mM	PDMS/Glass	0.25 – 4 µA	[27]
Amperometric Detection	Glucose	1 – 40 mM	PDMS/Glass	0.1 – 45 nA	[80]

1.6 Thesis organization

I. Chapter 1. Introduction

This chapter introduces the principle and development of the capillary electrophoresis chip with detailed descriptions of the electrochemical detection and fabrication. The study explains the motivation and purpose of this article in its

attempts to solve the shortcomings of traditional capillary electrophoresis chips.

II. Chapter 2. Theory and Design

The chapter introduces the characteristics and fabrication of the thread and explores 2D and 3D electrodes. In addition, it emphasizes the importance of the biomicrofluid theory.

III. Chapter 3. Methods and Materials

This chapter details the thread microfluidic device developed in this study, including design concepts, sample preparation, production methods, experimental architecture, surface modification techniques and experiments required. In the experimental process architecture, the experimental architecture detail of the thread microfluidic system is presented and an explanation is provided on how to detect the biosample with electrophoresis electrochemical detection.

IV. Chapter 4. Results and Discussion

First, we explain how to improve the hydrophilic and variable volume which can enhance the signal in the microfluidic thread system. In addition, we explain how to solve open channel interference by utilizing the PVC membrane to create a closed channel which can detect urea and glucose on an enzyme-doped thread. At last we successfully apply the clinical human whole blood in our system.

V. Chapter 5. Conclusions and Future Work

This chapter outlines the major conclusions from the results discussed in the previous chapters, and sets a target for future improvement and development.

Chapter 2 Theory and Design

2.1 Characteristics of thread

2.1.1 Hydrophilic and capillary force

Cohesive and adsorption force is caused by capillary competition. Adsorption of the liquid on the surface is due to the formation of hydrogen bonds between water molecules and the solid, resulting in the adsorption force being greater than the cohesive force. In contrast, the cohesion phenomenon causes water molecules to be unable to form hydrogen bonds with a solid, resulting in cohesive force being greater than adsorption force. However, active capillary force has the advantage of moving along the porous or permeable material to the end of transmission. A staggered thread fiber structure like paper or cloth will cause the liquid to move, having a surface diffusion, because tiny pores will connect to each other to form an elongated channel, and the liquid adhesive can be spread to both sides the channel that is capable of absorbing the liquid base material. Porous materials can effectively enhance the absorption, such as sponges will have many small pores which are equivalent to many capillaries such that a large number of sponges can absorb the liquid.

The thread used here has bundled hollow porous fibers to absorb liquid efficiently depending on the groove depth and shape of the fiber. In addition, this study utilizes a polymer of mixed hydrophilic groups with synthetic fibers or a grafted copolymerization method of chemical modification to increase the hydrophilic groups.

2.1.2 Characteristics of polyester thread

Polyester is one of the most amazing fibers for threads which can be made to look like silk and can also be made to look like cotton. Polyester thread looks, feels, and performs like nylon thread. But, there is one important difference—polyester thread has superior resistance to sunlight (UV), mildew, and abrasion. This makes polyester thread the first choice for sewing, binding or wrapping anything that is consistently used outdoors or in moist areas. It is one of the strongest thread fibers and is usually colorfast. The fibers recover quickly after extension and absorb very little moisture. It is also resistant to chemicals and can be washed or dry-cleaned with most common cleaning solvents. That is made by spinning or twisting together shorter lengths of polyester fibers. This is similar to the way cotton threads are made. These are then twisted together to produce a thread of the desired size. Spun polyester thread appears like cotton thread but provides superior strength and durability.

Polyester fiber is manufactured by polycondensation of an organic dicarboxylic acid and a dihydric alcohol to manufacture polyethylene terephthalate. The current generation of polyester fiber has a melting point of is 260℃, a softening point of 210℃, a specific gravity of 1.38 g / cm and moisture is 0.4%. Polyester has some advantages, such as a high degree of crystallinity, careful construction, and good resistance to chemicals [81]. Polyester fibers lack reactive hydrophilic groups, which means that it is not easy for hydrophilic molecules to enter the fibers. Clothing with wicking capabilities uses a special chemical graft bond to improve the hydrophilic properties, but such chemicals pollute the environment. The use of plasma surface modification of polyester fibers [82] provides better hydrophilic properties.

2.2 Explore 2D and 3D electrodes

Alternatively, a three-dimensional electrode provides a higher effective area for electric signal transductions such that the performance of a microdevice with 3D electrode is a significant improvement [83]. Enhanced electrochemical reactions on an enzyme immune-assay using a microchip device with micro-fabricated 3D electrodes has also been demonstrated [84]. Experimental investigation into the current responses of using 2D and 3D electrodes were reported. Results showed that the measured electrochemical responses had around a 35-fold incensement while using the 3D sensing electrodes. Application on biosample utilizing 2D and 3D nanoelectrodes (NEEs) to detect DNA which can comprehend current ratio of 85% for 3D and 45% for 2D NEEs [85]. Furthermore, capillary electrophoresis with contactless conductivity detection was used to determine concentrations of ions in human serum and urine, and determined potassium, sodium, calcium, magnesium cations, and chloride anions in human serum samples, with additional ions found in urine [86]. The photo shows on Figure 2-1.

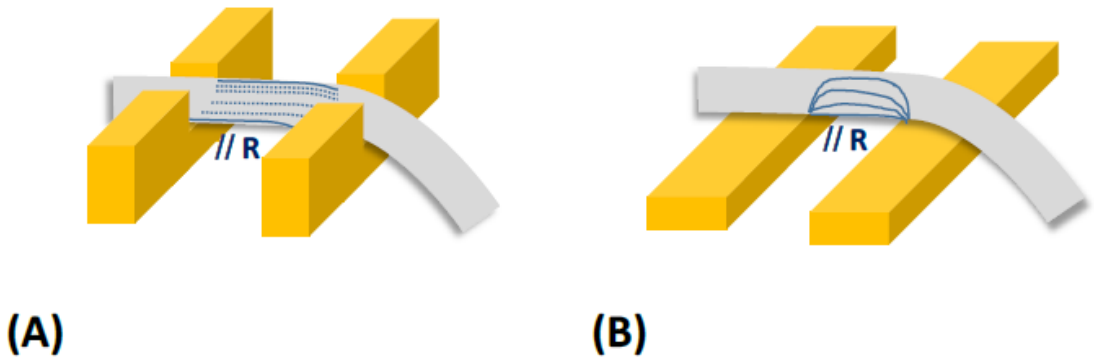

Figure 2-1. Schematic illustration of the contact and the parallel resistance of the (A) 3D contact electrode and (B) 2D flat electrode.

2.3 Biomicrofluidic chip

Monitoring blood urea nitrogen (BUN) and blood glucose are important for patients with diabetic or kidney diseases. A microfluidic system can be integrated with a solid-state sensor incorporated with enzyme-carrying alginate microbeads for on-site analyze of urea, glucose and creatinine in the human serums [87]. Nevertheless, the operation procedure of this device is comparatively delicate. In general, urea and glucose are unstable molecules such that they are difficult to detect with typical electrochemical procedures without the assistance of an enzymatic reaction. In general, urea can be rapidly converted into ammonium ion in the presence of urease and glucose can be converted into hydrogen peroxide (H_2O_2) using glucose oxidase (GOD). The sensor principle of the redox biosensor is utilizing a redox enzyme to proceed to the catalytic reaction. Thereby generated redox current changes are directly proportional to signal current variation. The amperometric mechanism uses a method to generate current to measure glucose and urea concentration.

I. Glucose detection

The three general glucose measurement methods are reduction, condensation and enzymatic methods. The enzyme method commonly uses glucose oxidase (GOD), hexokinase (HK) and glucose dehydrogenase. Due to poor reduction and condensation accuracy, the most commonly used method to detect glucose is the enzymatic method. Their reaction formulas are shown in (3) (4) [80, 88].

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \quad \text{.....(3)}$$

$$H_2O_2 \xrightarrow{0.6\sim0.8\ \text{V}} O_2 + 2H^+ + 2e^- \quad \text{.....(4)}$$

When high-potential is applied to blood, other substances will also undergo the

redox reaction at the same time, and introduce electronic transmission interference [89]. For improve problems of oxidation consumption and hydrogen peroxide dissociation, redox mediators have been developed for combination with horseradish peroxidase (HRP) [90, 91]. These mediators have the common characteristics of small molecular weight, lower redox potential and reversibility. Because it has these characteristics, it can replace other method to provide more stable and high sensitivity. Reaction (5) ~ (8) and Figure 2-2 describe how glucose can be reduced to generate an electronic conduction mediator.

$H_2O_2 + HRP_{red} \rightarrow H_2O + HRP_{ox}$ ……(5)

$HRP_{ox} + M_{red} \rightarrow HRP_{red} + M_{ox}$ ……..(6)

$M_{ox} + 2H^+ + 2e^- \rightarrow M_{red}$ ……..(7)

Net reaction: $H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$ ……...(8)

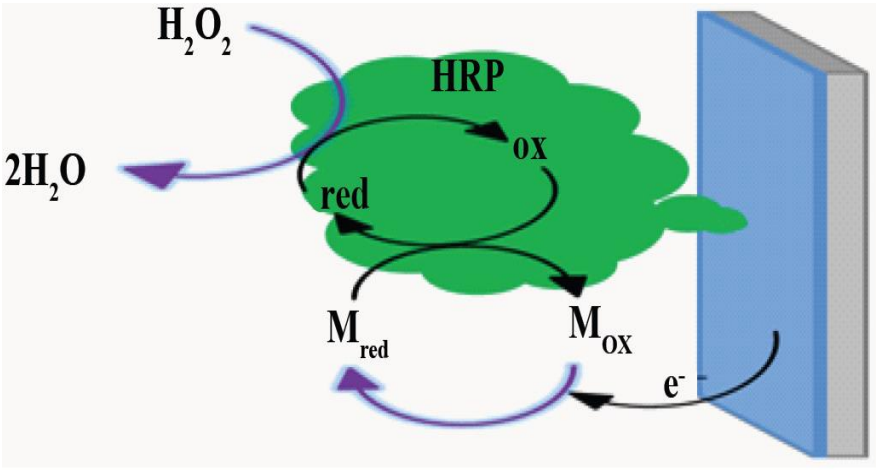

Figure 2-2. Schematic diagram of the reaction sequence within the enzyme electrode. [92]

II. Urea detection

In biological systems, urea forms in the liver as a waste product of protein metabolism then collects in the bloodstream. Urea is a by-product, which is often monitored in blood to obtain information on kidney disease. It is generally accepted

that mixing with urease is the best marker for evaluating the level of uremic toxins. Further, the concentration of blood urea nitrogen (BUN) is an important biological indicator used for monitoring the functionality of the kidney. In general, patients with kidney failure have high BUN levels. Recent research has also reported that the BUN level in serum might be correlated with heart failure [93, 94]. The standard method for determining the concentration of BUN is the enzymatic UV absorbance method. This method is straightforward but is limited by the large sample required, a limited detectable linear range and batch process issues. Alternatively, microfluidic systems integrated with an electrical detection scheme have potential for detecting BUN in a simple and efficient method. However, direct detection of whole blood samples using microfluidic devices is still challenging due to the interference from the existence of other blood compositions [30, 95]. Urease promotes the reaction like formula (9) involving the decomposition of urea to give ammonia and carbon dioxide.

$$NH_2CONH_2 + 3H_2O \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- + OH^- \quad \ldots\ldots\ldots\ldots (9)$$

In practice, ammonium ion is a cation which has a reduction potential of around -0.2 V ~ -0.3 V and can be easily detected using a negative potential state. Alternatively, glucose can be converted into hydrogen peroxide (H_2O_2) using the glucose oxidase (GOD). However, the H_2O_2 is an oxidation regent and has a standard reduction potential of around 1.776 V. Therefore, it is difficult to simultaneously detect these two products with a single set of electrochemical detecting electrodes. It is essential to apply the secondary enzyme of horseradish peroxidase (HRP) to further react with hydrogen peroxide to form the reduction products at a low negative voltage [96].

Chapter 3 Methods and Materials

3.1 Chips design

3.1.1 3D detecting electrode chip

Figure 3-1 illustrates the design concept of the developed thread-based microfluidic system with 3D detection electrodes and variable injection volume capability. Two polyester threads are wound and used as the fluidic channels, one for sample injection and one for separation. With this approach, a delicate fabrication process of the sample delivery channels can be avoided. The microfluidic routes are easy to renew simply by replacing the polyester threads. In addition, higher injection volumes can be achieved by winding the injection thread multiple turns around the separation thread. The greater injection volume can significantly enhance the detection signal and lower the limit of detection for this developed thread-based microfluidic system. Since the surface property of the polyester thread was natively hydrophobic, the polyester thread was first treated with oxygen plasma and then soaked with the running buffer for capillary electrophoresis operations prior to the test. The separated bio-samples were electrochemically detected using the EC electrodes downstream. Note that the diameter for the adopted polyester thread was 200 μm and the lengths for the injection and separation threads were 20 mm and 60 mm, respectively. Each EC electrode was 300 μm in width and they were placed 50 mm downstream from the knot. In order to further improve the EC detection performance, ten 200 μm x 200 μm notch structures were produced on the EC electrodes for better electric contact with the polyester thread. Alternatively, the standard 2D electrode was the same EC electrodes without the notch structures. The 3D contact electrodes reduced the contact resistance and the parallel resistance between the two neighboring

electrodes such that the measured sensing response greatly increased with this approach. Note that the CE-EC tests were started with the most outer notch of the electrodes. Once the adopted notch was corroded or failed, the inner notches could be also used for EC detection such that the lifetime of the electrode was greatly extended.

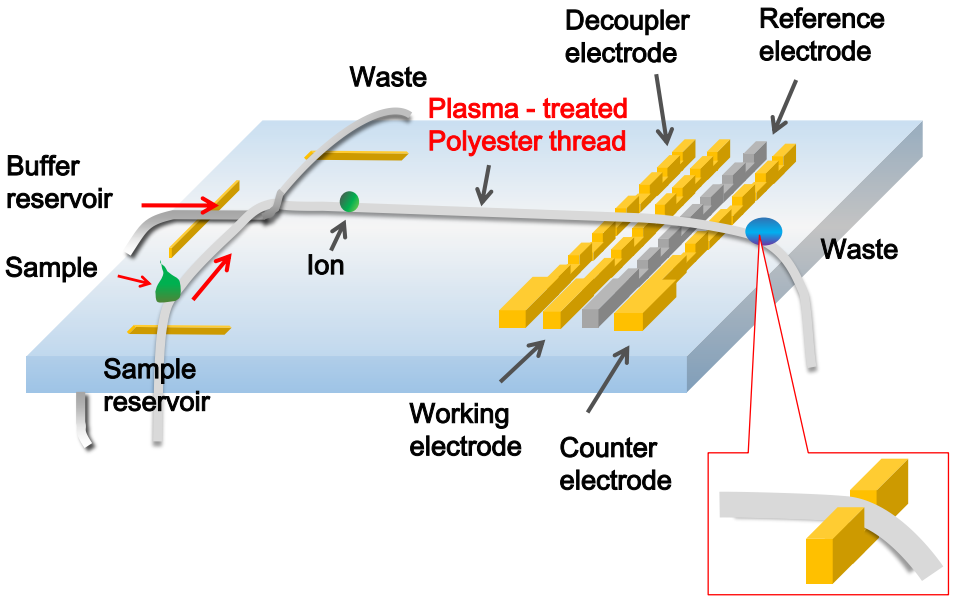

Figure 3-1. Schematic of the proposed thread-based microfluidic system with variable injection volume and 3D electrochemical detection electrode.

3.1.2 Enzyme - doped thread coated with PVC membrane chip

This study proposes a novel PVC coated membrane of the thread-based microfluidic system, with a variable volume injection capability and 3-dimensional (3D) electrodes for the CE-EC detection of urea and glucose. The developed thread-based CE-EC system is capable for on-site detection of urea and glucose on a single thread. Figure 3-2 presents the schematic illustration of the working principle of the developed CE-EC micro-device. Since enzymes can be attached on the polyester thread simply by direct applying a drop of enzyme solution on the thread,

the thread can be doped with various enzymes for different sample detections. Excess thread to be a filter which can react with RBC lysis buffer and blood that can solve block of RBC. The passing samples in the thread will be digested with the immobilized enzymes on the thread and electrochemically detected. A thin layer of PVC solution is then applied on the wetted thread. Prior to the application of PVC solution, the enzyme-doped thread is immersed in buffer solution to fully soak the buffer solution on the thread to prevent form the infusion of PVC solution into the fiber bundle of the thread. Due to the immiscible property between the PVC solution and the running buffer, a thin PVC layer on the thread can be formed after drying the organic solvent. The formed PVC membrane not only prevents the buffer solution from rapid evaporation during CE test but also confines the fiber bundle of the polyester thread. Therefore, the applied electric field can be increased for sample separation, which would enhance the quality for the electrochemical signals of the samples.

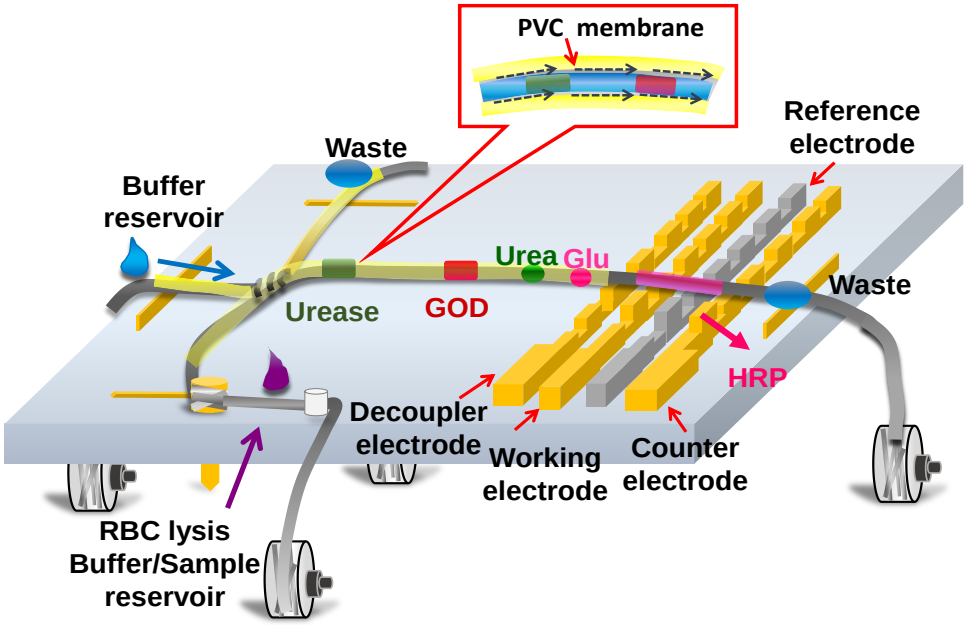

Figure 3-2. Schematic of the enzyme-doped thread coated with PVC membrane for high-performance CE-EC detection.

3.2 Biochips fabrication

3.2.1 3D electrodes fabrication

Figure 3-3 presents the simplified fabrication process of the platform for the thread based microfluidic system with 3D EC electrodes and multiple injection volume capability. Basically, the platform was produced in PMMA substrate with hot embossing and a metal plating procedure. First, a concave master mold was fabricated in a 2-mm thick 6060 aluminum alloy using a micro CNC machine (Model SD3025, SUDA Instrument Company, China) (Fig. 3-3 (A)). Prior to the hot embossing process, a piece of thin aluminum foil was placed between the master mold and the cleaned PMMA substrate as a sandwich structure (Fig. 3-3 (B)). The aluminum foil was used as a 50 μm thick demolding layer that acted as a mask for metal sputtering. The hot embossing process was operated at 140^{o}C for 30 minutes at an applied pressure of 6.4 MPa. The protruding EC electrodes were covered with foil after the hot embossing process. The aluminum foil on the third protruded EC electrode (reference electrode) was then peeled off and sputtered with Cr/Pt layers at a thickness of 175 nm and 660 nm, respectively (Fig. 3-3 (C)). The sputtered reference electrode was then covered with an electrostatic tape and the other EC electrodes (decoupler, working and counter electrodes) and contact electrodes were again sputtered with Cr/Au layers (Fig. 3-3 (D)). The residual aluminum foil was then stripped from the PMMA substrate after the metal deposition processes (Fig. 3-3 (E)). Polyester threads with a plasma treatment were finally assembled on the PMMA substrate for fluid transportation. (Fig. 3-3 (F)) In order to inject the sample at various volumes into the separation thread for CE-EC detection, the injection thread was wound around the separation thread from one to five times. Variable volume injection can be achieved with this simple approach

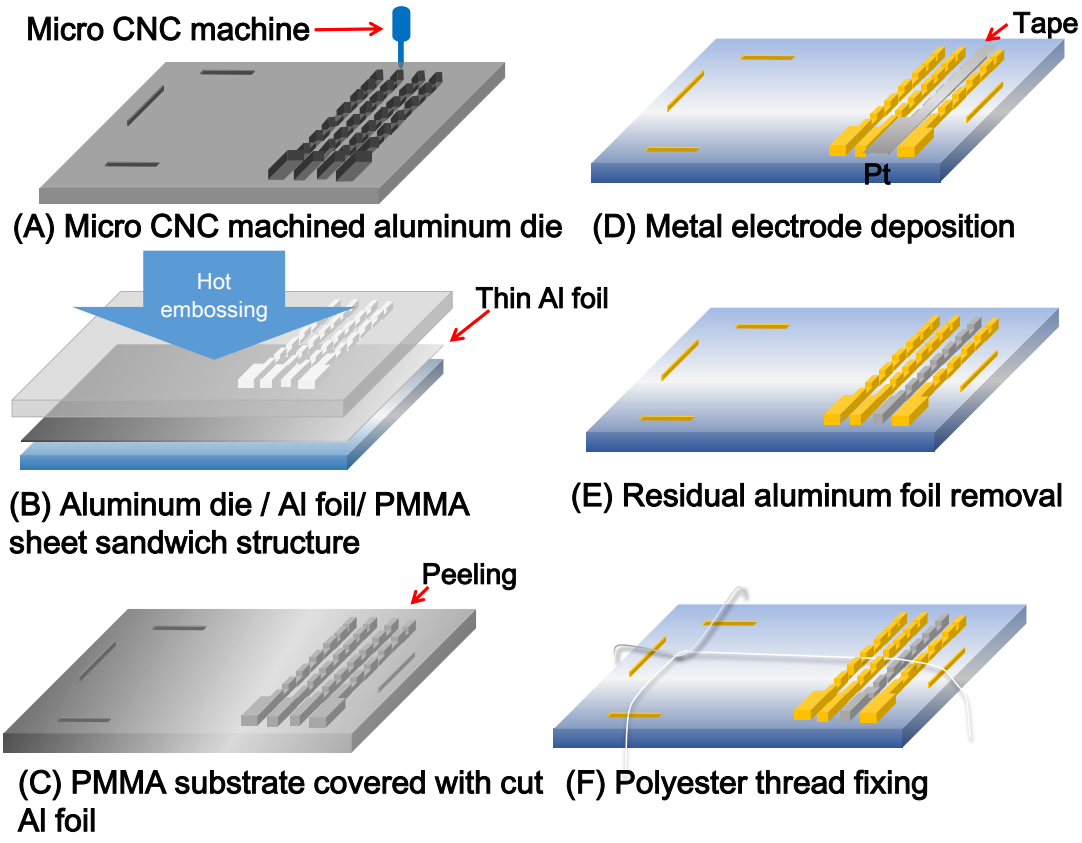

Figure 3-3. Schematic for the simplified fabrication process for the thread-based microfluidic device.

3.2.2 Enzyme - doped thread coated with PVC membrane fabrication

Figure 3-4 shows the simplified fabrication process for producing the proposed thread-based microfluidic system. The details for the fabrication process can be found in the previous report. A concave master mold was first fabricated on a 2-mm thick aluminum plate using a micro CNC machine. The convex electrode structures were then produced on a PMMA substrate by hot embossing (Fig. 3-4 (A)). The working and counter electrodes for electrochemical detection were then produced by sputtered Cr/Au layers and the reference electrode was with Cr/Pt layers (Fig. 3-4 (B)). Polyester threads of around 200-μm in diameter were fixed on the PMMA substrate as the liquid routes for CE operation (Fig.3-4 (C)). Specific enzymes including urease,

glucose oxidase (GOD), horseradish peroxidase (HRP) and catechol (mediator) were then directly applied on different sites of the thread with a 2-μL pipette (Fig. 3-4 (D)). A thin PVC solution was sprayed on the desired region with the assistance of a plastic mask (Fig. 3-4 (E)). The PVC solution would form a thin membrane covering the thread after evaporating the solvent. Enzyme-doped polyester thread coated with PVC membrane was finally produced for simultaneously EC detection of urea and glucose. (Fig. 3-4 (F))

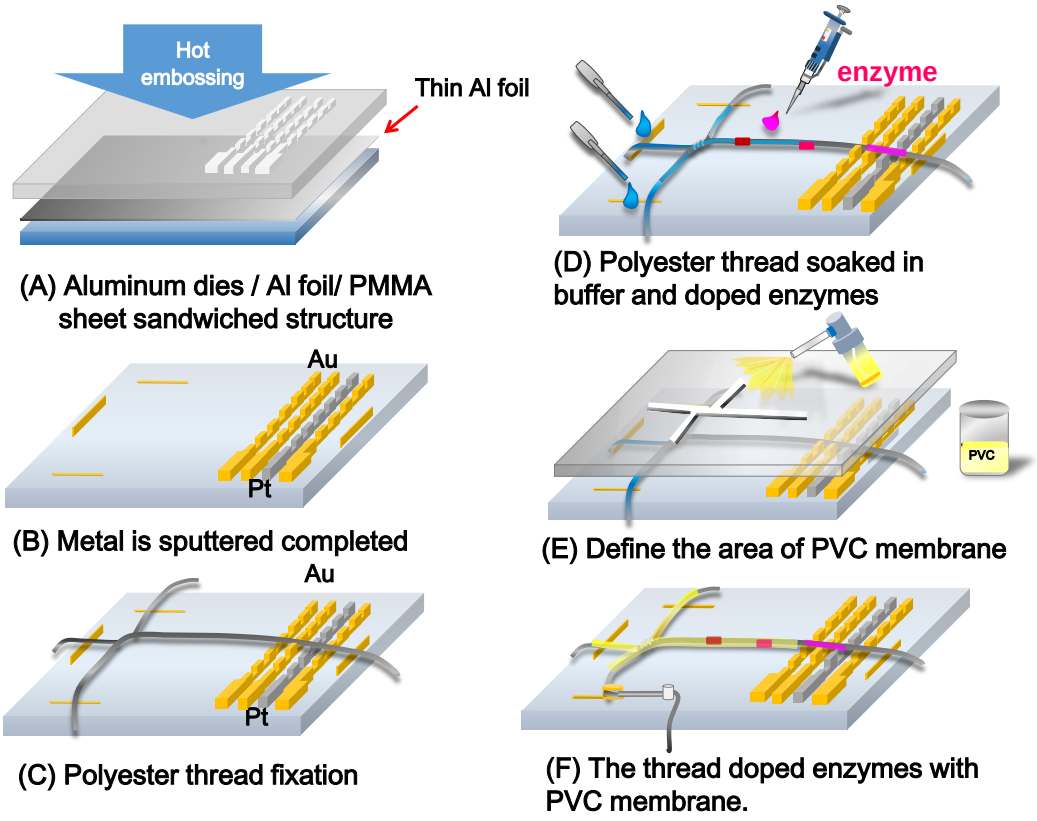

Figure 3-4. Schematic for the simplified fabrication process for the thread-based microfluidic device.

Institute the thickness of the coated electrode, refer to the previous average parameter literature. The type of metal deposition rates are different and the sputtering parameters used in the study are shown in Table 3-1 and the thickness of reference

electrode is 660 nm, further electrode thickness are 660 nm.

Table 3-1. Electrophoresis chip of each electrode parameters.

Electrode - metal	Sputtering power - time	Thickness
Reference - Pt	50 W-720 s	660 nm
Counter / Working- Au	50 W-720 s	660 nm
Deculoper - Au	50 W-720 s	660 nm

3.3 Explore the surface of plasma-treated

The surface property of typical polyester thread is natively hydrophobic and usually somewhat fluffy, making it difficult to use in microfluidic applications. Therefore, it is necessary to modify the surface of commercial polyester thread to improve the wettability and smooth the surface. Figure 3-5 presents the SEM images of a 200 μm thread before and after the plasma treatment. Note that the polyester thread was treated with a 100-W oxygen plasma at 10 mTorr for 10 minutes. Visual inspection confirms that the plasma treated polyester thread (Fig. 3-5(C)) is smoother than the non-treated one in Fig. 3-5(D). A solid bundle of polyester fiber without stray fibers was obtained after the plasma treatment. The Fig. 3-5(A) (B) shows the photo picture of the corresponding polyester thread soaked with a 10-μL colored droplet. Results show that the treatment both significantly increased the surface hydrophobicity and removed contamination on the thread surface for better fluidic operation.

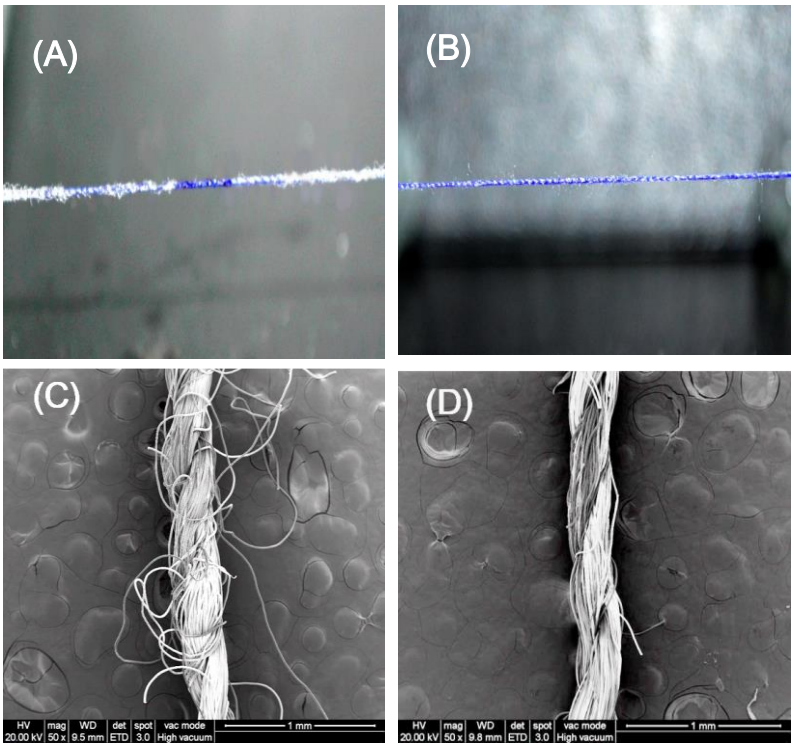

Figure 3-5. SEM images (50 x) show for the polyester threads (C) before and (D) after the plasma treatment. Note that (A) (B) are photos of the corresponding polyester thread soaked with a 10-μL colored droplet. (Power: 100 W, Time: 10 min)

3.4 Explore PVC membrane coated with thread

The simplified fabrication process emerges for establishing the proposed enzyme-doped thread-based microfluidic system with 3D EC electrodes and PVC membrane coating. Furthermore, the insoluble solvent of organic / aqueous phase was used to form a sealed microfluidic channel. Utilize PVC solution spray of quantitative on the thread to build this phenomenon. That is a rapid and easily method to formulate a closed channel for thread microfluidic system.

Show in Figure 3-6 presents the SEM images of the threads with and without PVC coating. It is clear that a PVC layer was successfully formed on the thread surface (Fig. 3-5(A)). From the cross-section view, the fiber bundle was well

constrained by the PVC membrane of around 50 μm in thickness (Fig. 3-5(B)). Alternatively, a rougher surface can be observed for the thread without PVC coating (Fig. 3-5(C)). The fiber bundle was also loose (Fig. 3-5(D)) such that the CE separation performance was hindered in compare with the thread with PVC coating.

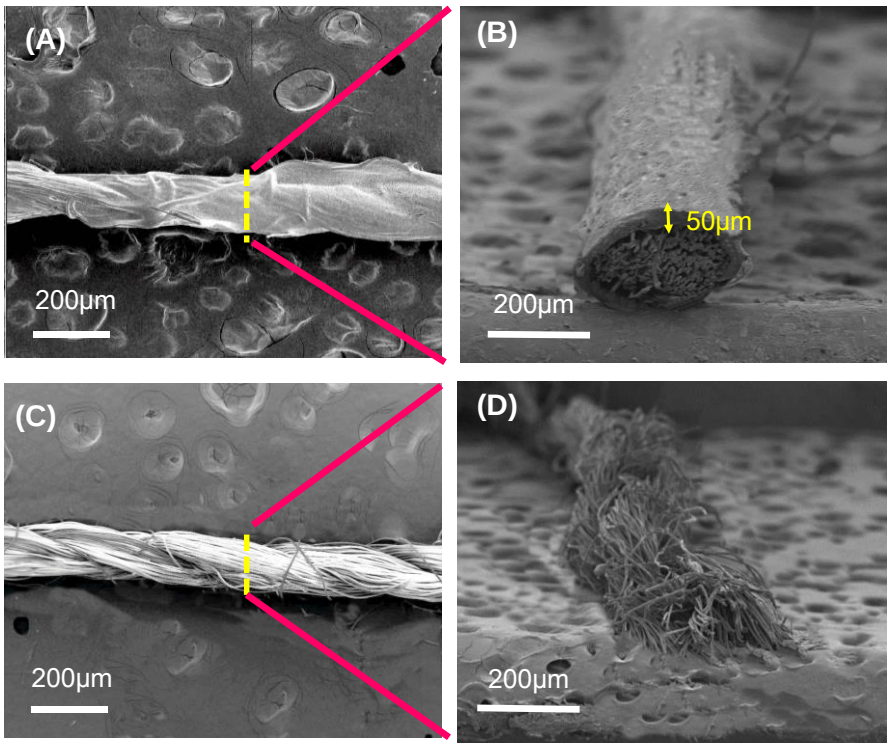

Figure 3-6. SEM images showing the polyester threads with (A)(B) and without PVC coating (C)(D). Note that (A)(C) are eagle view and (B)(D) are cross section view.

3.5 Variable sample volumes

In previous literature, a master microfluidic system was fabricated using standard photolithographic and chemical etching techniques, but proved time-consuming and costly. García and Backofen later [97, 98] proposed a double-T structure to improve the signal in electrophoresis bio-sample detection, and found the structure to be highly stable.

Overall, the developed thread-based microfluidic system is capable of injecting variable sample volumes into the separation thread simply by wrapping the injection thread different numbers of times around the separation thread. Utilized this way, the system provides a simple and low-cost method for thread-based CE-EC applications.

3.6 Reagent and apparatus

3.6.1 Reagent preparation

Experiments buffer is used in MES (2 - (N-morpholino) ethanesulfonic acid, Sigma-aldrich, USA), to configure with the Mini-Q deionized (DI) concentration were 0.1 mM and 1 mM. Then we use blue ink (PILOT, Taiwan) facilitate to observe the line microfluidic surface morphology under camera and measured the hygroscopic of amount. The reagent of measurement the injecting variable sample volumes including dopamine (DA) and L-ascorbic acid (AA) were purchased from Sigma-Alderich (USA). Detect the stander bio-sample and enzyme utilizing catechol (CA, Sigma-aldrich, USA), urease (Jack Beam Type III, U1500-20KU), glucose oxidase (Type VII, G2133-10KU), horseradish peroxidase (Type I, P8125-5KU), Urea (Carbamide, 99%) was purchased from Panreac Química (Spain) and glucose (J.T Baked) respectively. The human whole blood was obtained from a 24 year old female volunteer from a local hospital and red blood cell lysing buffer (RCB lysis) was purchased from Sigma-Alderich (USA). The PVC membrane was manufactured weight percentage by 33 wt% of PolyVinyl Chloride (PVC, Fluka, Switzerland) and 66 wt% of Dibutyl (sebacate, Fluka, Switzerland) dissolve in Tetrahydrofuran (THF, Alfa Aesar, Germany).

3.6.2 Experimental apparatus

The experimental setup using for CE-EC biomolecule detection by developed thread-based microfluidic system. A high voltage power supply (MP-3500-250P, Major Science, Taiwan) was used for sample injection and separation. The separated biomolecules were then electrochemically detected with a commercial electrochemical analyzer (Model CHI611C, CH instruments, U.S.A). A home-built circuit and four DC motors were used to control the tension of the polyester threads and automatically replace the polyester threads. In addition, to avoid the sample cross-contamination so replacement of the thread must be important to make the entire process more automated runner replacement. The DC motor module was powered by a 12 V DC power supply (DP-3630S, HILA, Taiwan). The complete apparatus shows in figure 3-7.

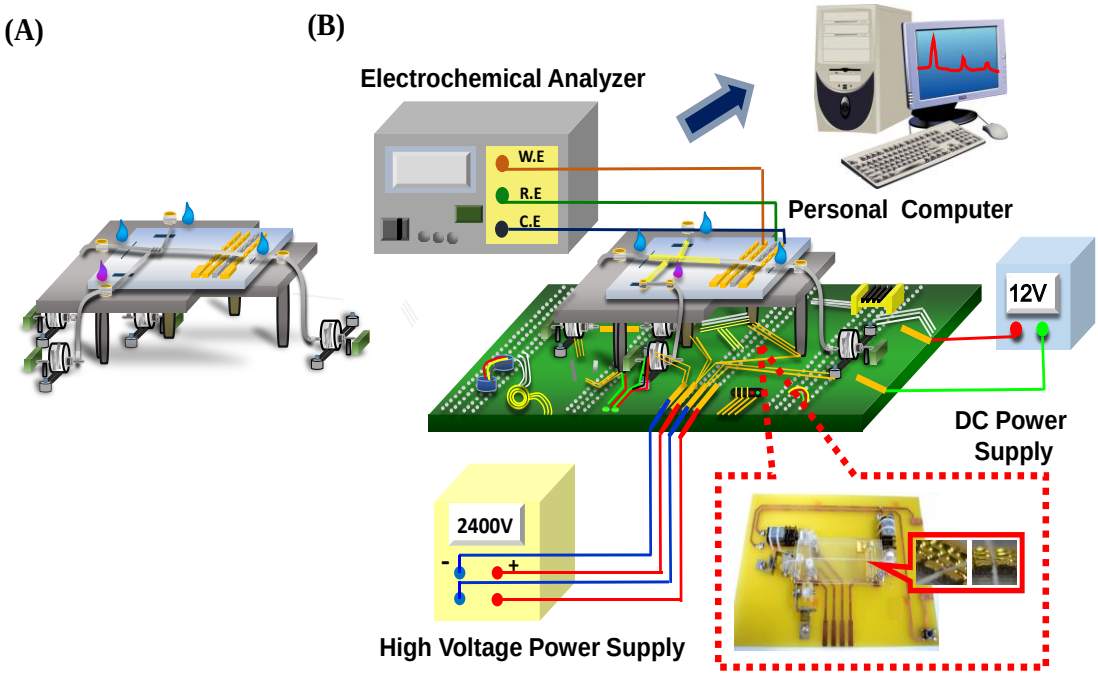

Figure 3-7. Experimental setup for detect thread-based CE-EC detection system on chip (A) and (B) with filter and PVC membrane on chip.

3.7 Experimental procedure

3.7.1 Fundamental measurements

In this study, the plasma system equipment was set up in our lab and application consisted of treatment with 100-W oxygen plasma at 10 mTorr for 10 minutes. During plasma processing, the thread was suspended in the vacuum chamber so that the entire surface of the thread received the plasma modification treatment.

Next, the EOF mobility of the threads coated with the PVC membrane was measured. First, the thread was immersed in the buffer microfluidic and the electrode was connected to the electrochemical analyzer, with the potential of the work electrode set at 0 V. After applying high voltage stability for 20 seconds, the thread is immersed in two concentrations of buffer: first 1 mM, and second 0.1 Mm. When the electric field drives the liquid, the resistance of flow will be changed so the current value becomes smaller. This reaction time is substituted into equation (1) and (2) to calculate the EOF for the current equilibrium reaction. In this regards, this study compared the electro-osmotic mobility for a conventional glass-based microfluidic channel and the polyester thread coated with the PVC membranes of different thicknesses.

After using the winding technique to produce different variable volume injection amounts, the CE-EC test was used to measure the corresponding signal for different injection volumes. In addition, the theoretical plate number and the S/N ratio were calculated for comparison with the detection. The sample used for CE-EC detection of a mixed sample was composed of 0.5 mM dopamine and 0.5 mM ascorbic acid. Note that the buffer solution used for this test was 1.0 mM MES buffer (pH=5.0).

Moreover, it is necessary to first test the enzyme concentration that will provide

the best results. Results indicated that the saturated concentrations for urease and glucose oxidase were 20 mg/ml and 10 mg/ml, respectively.

3.7.2 Biosample detection

The sensing performance of biosample detection were compared urea for the chip with 2D and 3D electrodes [76]. Accordingly, a PMMA substrate with concave 3D electrodes was produced with hot-embossing. This method was used to enhance the sensing performance of the CE-EC system. The microfluidic chip is detected with different concentrations of urea and produces the redox reaction with urease. Working, counter and reference electrodes are required to be connected to electrochemical equipment for cyclic voltammetry (CV) analysis. First the potential of the scan range was set at 0.6 V ~ -0.8 V with a scan rate of 0.1 V/s. Figure 3-8 shows the thread microfluidic detection plane and concave three-dimensional electrode structure setup used to investigate the plasma-treated thread microfluidics and electrochemical detection performance. The fixed potential is applied to an electrochemical analyzer, and this fixed potential being an oxidation-reduction detection potential. Due to the electric field, the ions will be detected by electrochemical equipment and its current signal will be measured using amperometric detection as well as 2D and 3D electrophoretic separation. Finally we used the thread doped enzymes and coated PVC membrane to test the urea and glucose.

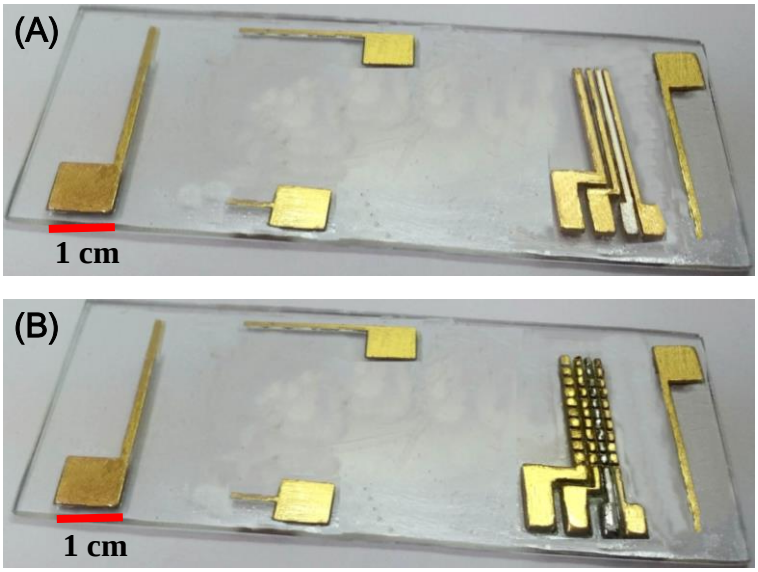

Figure 3-8. The photos show of 2D (A) and 3D (B) electrode.

3.7.3 Human whole blood detection

For CE-EC detection of the BUN concentration with the developed system, one drop of blood sample 20 μl of whole blood mixed with 2 μl of urease (20 mg/ml) was applied to the sample reservoir of the injection thread. Note that all the threads were soaked with 0.1 mM of MES buffer for the capillary electrophoresis test. The typical applied electric strengths for CE injection and separation were 200 V/cm (20 s) and 300 V/cm (40 s), respectively.

In additional the enzyme and mediator (catechol) can be directly doped on the thread without delicate pretreatment or surface modification process. Then the insoluble solvent of organic (PVC) / aqueous phase was used to form a sealed microfluidic channel. The whole blood sample is firstly mixed with RBC lysis buffer on the excess thread to prevent blood coagulation and blockage of the channel. The lysed RBC and other solid pieces were simultaneously filtered away while electrokinetically flowing in the thread-based microfluidic system. Alternatively, the PVC coated injection thread was 200V/cm (60 s) and could sustain a higher

separation electric field up to 500 V/cm (80 s), resulting in a faster separation and higher signal response for detection of the same sample.

Chapter 4 Results and Discussion

4.1 EOF characteristics of the thread microfluidic system

Electroosmotic flow (EOF) mobility is a critical concern for evaluating the capability of an electrokinetically driving system such as the capillary electrophoresis. In past thread microfluidic is the open channel which affect the running buffer might evaporate during the electro-migration process. Then the Joule heat and the temperature will be increase bring the thread burnout (400 V/cm) [76]. It definitely coated membrane on the thread to prevent the problems. The CE system with a higher EOF mobility is able to provide a faster sample transportation rate. In this regards, this study compared the electroosmotic mobility for the conventional glass-based microfluidic channel and the polyester thread coated with the PVC membranes of different thicknesses. The PVC solutions for coating the threads were diluted with different amounts of tetrahydrofuran (THF). Figure 4-1 presents the measured EOF mobility of the threads with and without PVC coating and the conventional glass channel. Results show that the EOF mobility of the thread-based microfluidic system is about 4 times higher than that of conventional glass-based microfluidic system. Furthermore, The EOF will be affect when the excessive THF will too diluted which immersed in the thread. It is noted that the thread with PVC coating might have lower EOF mobility since the fibers in the bundle would be slightly coated 1 c.c THF with hydrophobic PVC due to the infusion of the PVC solution. The thin PVC membrane on the enzyme-doped thread first can further prevent from the rapid evaporation of the running buffer caused by joule heat during CE separation. Not only PVC can prevent higher joule heat but can form a closed channel which the surface is multiple holes has better radiating. Results show that the PVC coated thread can be operated at a

higher separation electric field of 500 V/cm. Second, it was a porous structure which have a good ability to heat radiation and can prevent external particle.

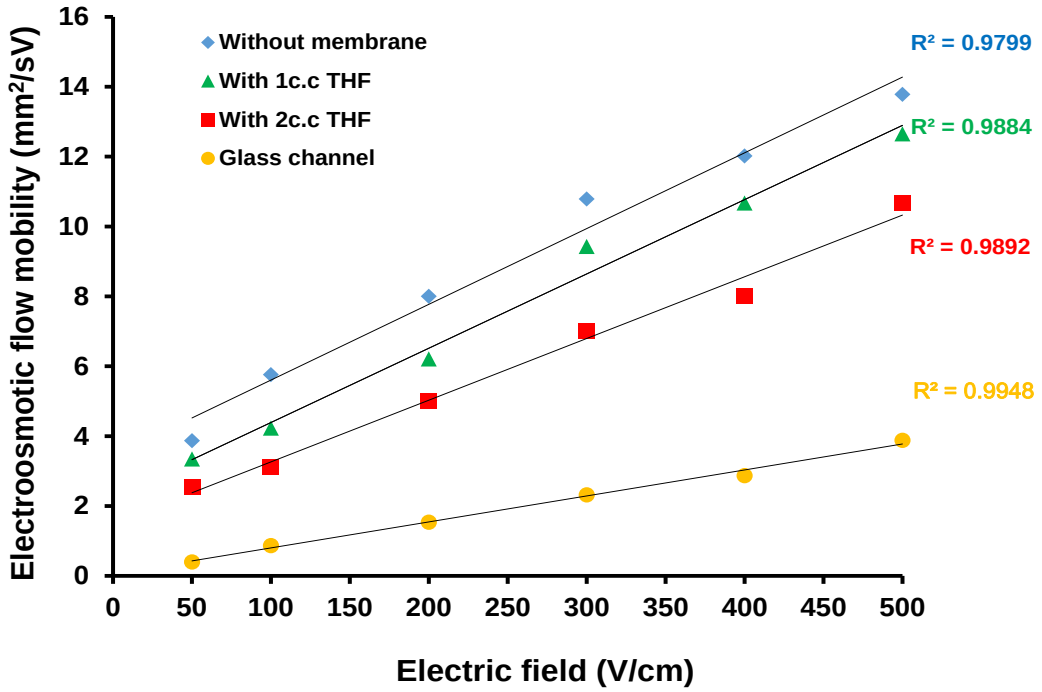

Figure 4-1. Measured EOF mobility of the threads with and without PVC coating and conventional glass channel.

4.2 Explore variable volumes on thread

In order to evaluate the detection performance of the variable volume injection technique, the CE-EC test was used to measure the corresponding signal with different injection volumes. Figure 4-2 presents the measured current responses for CE-EC detection of a mixed sample composed of 0.5 mM dopamine and 0.5 mM ascorbic acid. Note that the buffer solution used for this test was 1.0 mM MES buffer (pH=5.0). The number of times that the injection thread was wound around the separation thread for each case was from 1 to 5 turns. The measured current response

for dopamine using 1 to 5 turns was 1.12, 1.43, 2.11, 2.12, and 2.29 μA, respectively. The current response significantly increased for the 2-turn and 3-turn schemes then somewhat plateaued at higher turn numbers. Alternatively, the separation performance of the developed device was evaluated using a standard index of the theoretical plate number [99, 100]. The theoretical plate number (N) calculated on equation (10).

$$N = 5.54 \cdot \left[\frac{t}{W_{1/2}}\right]^2 \quad \text{.....(10)}$$

t Migration time of analyte

W Half peak of analyte width

The calculated plate numbers shows in figure 4-4 for the dopamine peaks for injection volumes of 1 to 5 turns also increased from 10862 to 27017. Results showed that the peak height and the calculated plate number increased with an increasing number of turns. The quantitative evaluation for the injection volume was further calculated by integrating the peak area of dopamine peak. Results showed that the injection volumes for 1 to 5 turns were 0.5126, 0.6276, 0.7212, 0.7633 and 0.7801, respectively. Different from the conventional microfluidic system with physical channels as the liquid routes; the thread-based microfluidic system used polyester thread as the liquid route for sample separation and detection. The injection volume was not simply defined by the area of the cross channel. Instead, a number of factors will all affect the injection volume including the hydrophilicity and contact area of the thread, the raw material of the thread and the tension of the thread. Therefore, the S/N ratio of the signal peak provides a better presentation on the performance improvement of the variable injection volume technique. Figure 4-3 shows the calculated S/N ratios for the DA peaks were 6.32, 12.08, 20.74, 22.37, and 23.04, respectively. Alternatively, higher injection volume might increase the more

background current when test more time during EC detection, reduce the signal-to-noise ratio of the detection peaks and unstable in the electropherogram. Even residual more volume on thread will impact detection. Results indicate that the calculated S/N ratio and above factors became stable at more than 3 turns, such that the turn number was fixed to 3 for further tests will be stable and best.

During thread-based CE separation, around 5-μL of buffer solution was applied on the thread outside each electric driving electrode. Although the running buffer might evaporate during the electro-migration process, the evaporated buffer solution would be simultaneously supplied utilizing capillary force on the hydrophilic threads from the buffer solution around the electric driving electrodes. In this regards, the concentration of the analyte did not significantly change during detection.

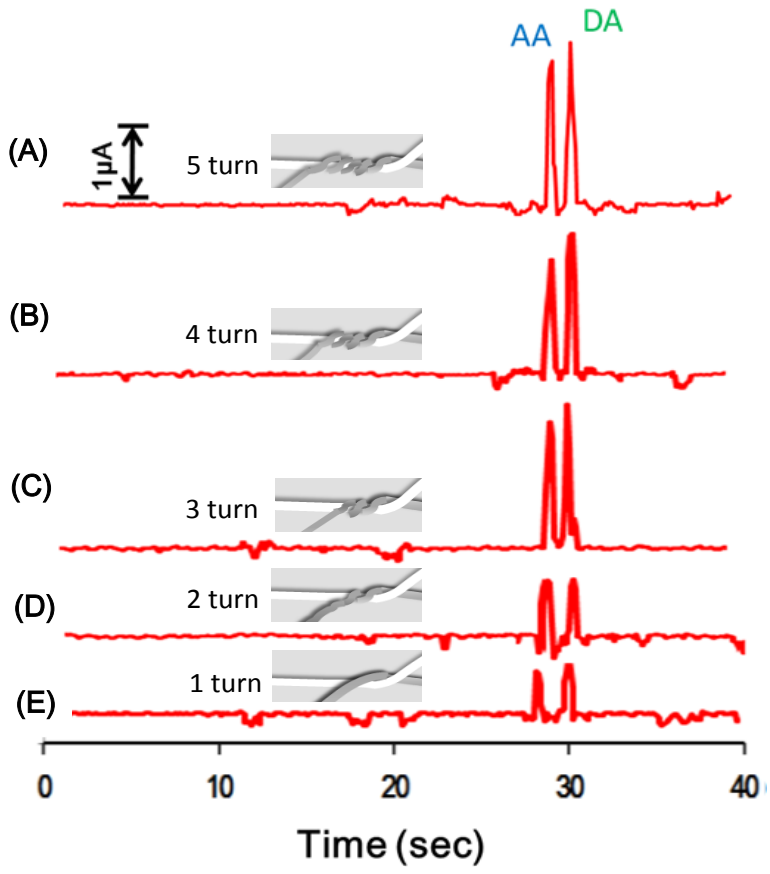

Figure 4-2. Electropherograms for detecting a mixed sample composed of 0.5 mM

each dopamine (DA) and ascorbic acid (AA) with different injection volumes by wrapping (A) 5 turns, (B) 4 turns, (C) 3 turns, (D) 2 turns, and (E) 1 turn. (Applied EC detection potential: +0.38 V)

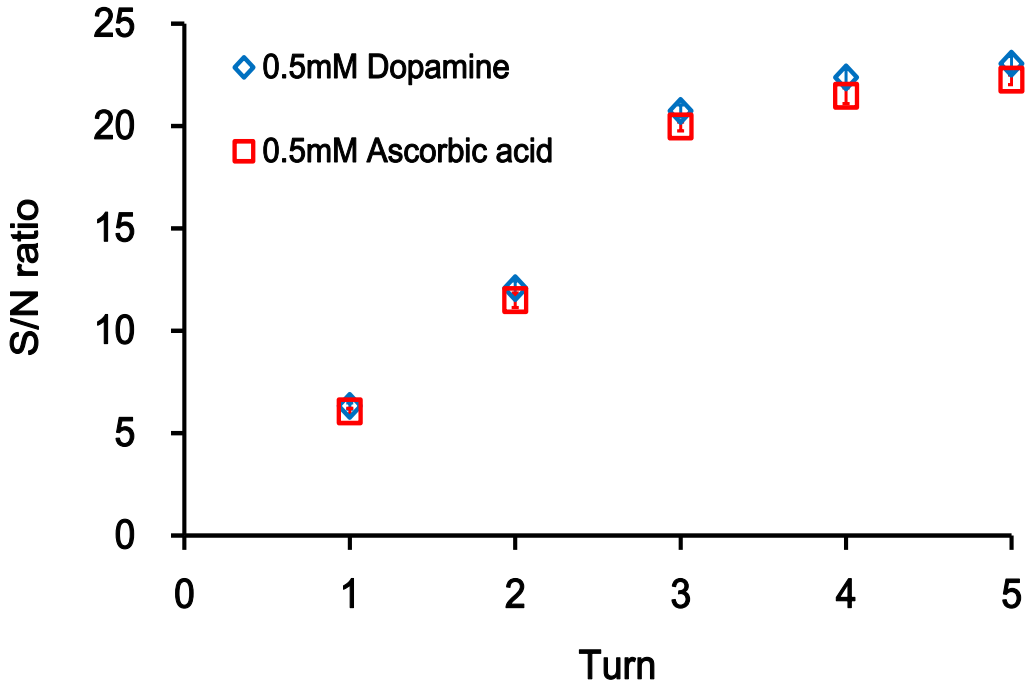

Figure 4-3. Calculated peak S/N ratio for detecting dopamine (DA) and ascorbic acid (AA) at different sample injection volumes. (Applied EC detection potential: +0.38 V)

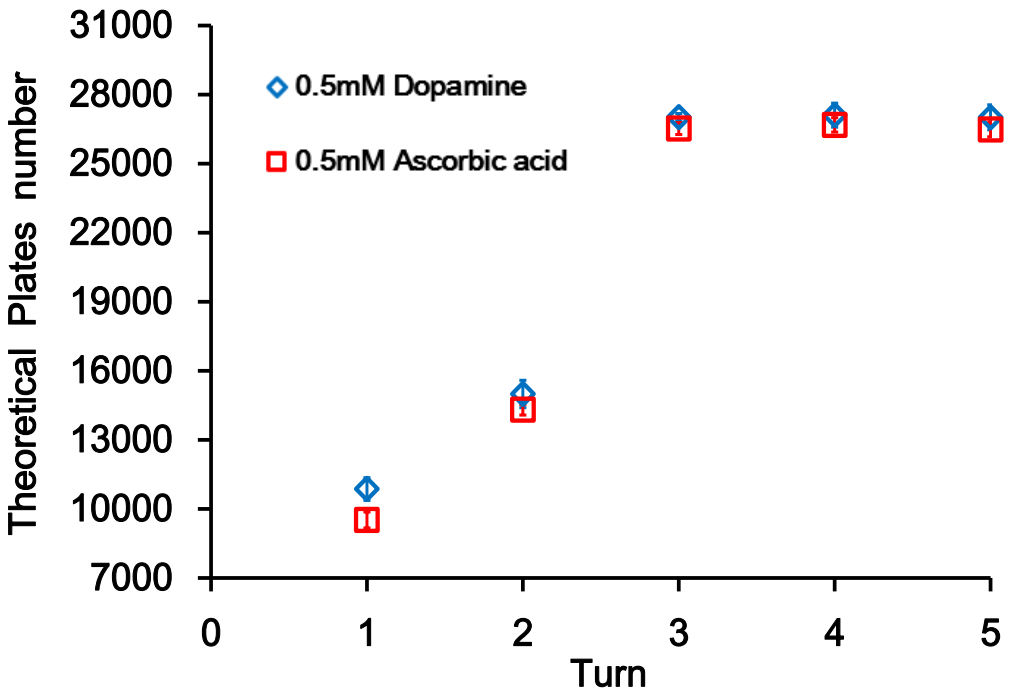

Figure 4-4. Calculated plates number for detecting dopamine (DA) and ascorbic acid (AA) at different sample injection volumes.

4.3 Evaluate concentration of enzyme

Figure 4-5 shows the measured current responses for 10 mM urea sample using the thread modified with different concentrations of urease. The optimized enzyme concentrations on the thread were determined while the current responses reached saturation. Results indicated that the saturated concentrations for urease was 20 mg/ml. Figure 4-6 represents the measured current responses for measuring 10 mM glucose samples using a thread modified with different concentrations of GOD. Note that the secondary enzyme of horseradish peroxidase (HRP) was at a concentration of 5 mg/ml and 0.5 mM catechol (mediator). The optimized enzyme concentrations on the thread were determined while the current responses reached saturation. Results indicated that the saturated concentrations for GOD were 10 mg/ml.

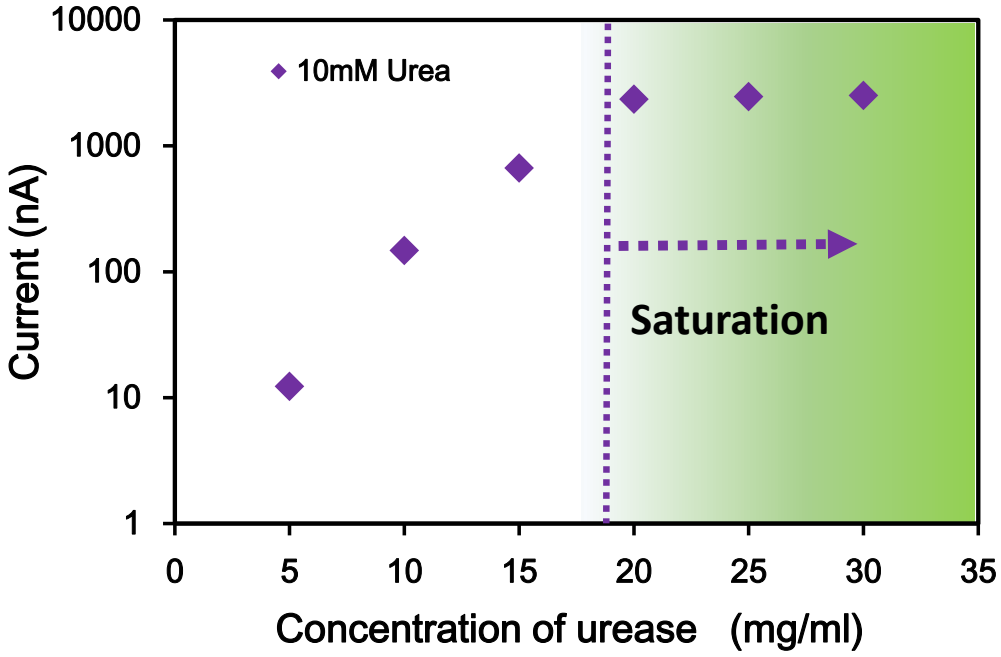

Figure 4-5. Measured current responses for detecting urea with the threads mixed with different concentrations of urease.

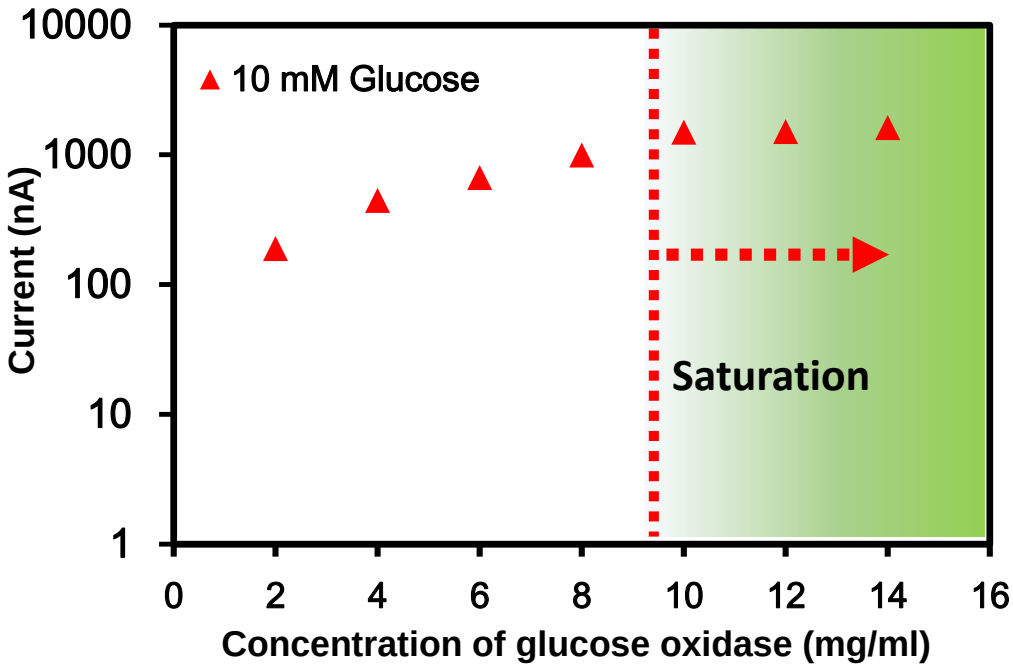

Figure 4-6. Measured current responses for detecting glucose with the threads coated with different concentrations of GOD.

4.4 Performance evaluation on the 2D and 3D electrodes

Figure 4-7 presents the voltammograms obtained when using plasma-treated polyester threads setup on 2D flat sensing electrode and concave-shaped 3D sensing electrode, respectively. (Note that the plasma-treated thread diameter is 200 μm in both cases and sample is 5 mM urea.) It can be seen that the concave-shaped 3D sensing electrode yields a significantly higher current response and is thus better suited to EC detection applications than the 2D flat sensing electrode. It is seen that the current increases approximately linearly with an increasing concentration in both devices. However, for a given 5 mM urea concentration, the measured current in the concave-shaped 3D sensing electrode is around 1.75 times higher than that in the 2D flat sensing electrode. The nice linearity confirmed that high performance electrochemical detection by concave-shaped 3D sensing electrode.

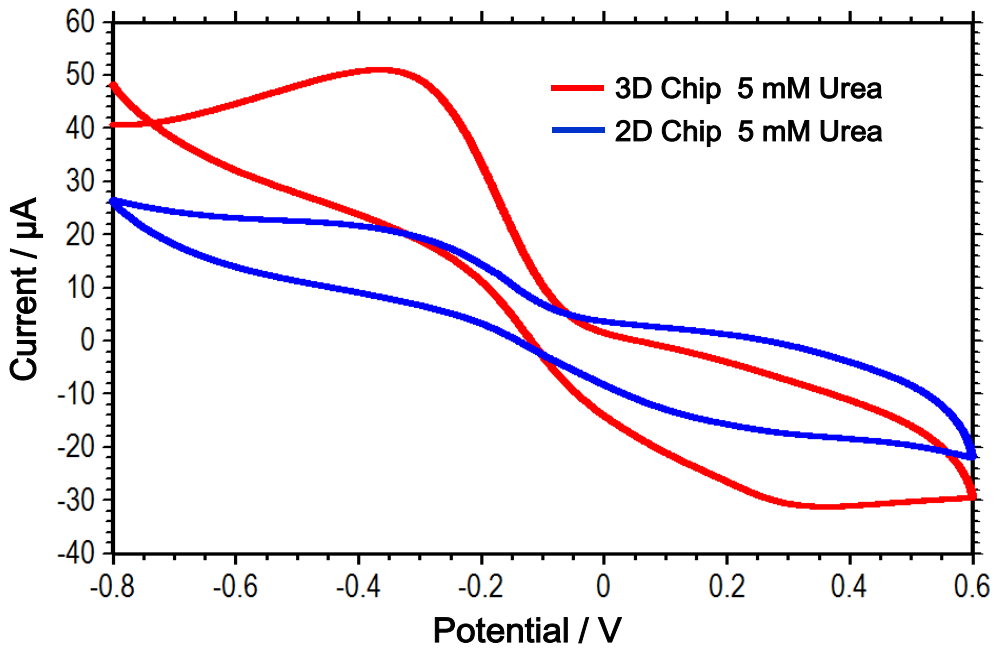

Figure 4-7. The cyclic voltammetry measures urea with different electrode.

Figure 4-8 illustrates the measured current responses for CE-EC detection of urea samples in different concentrations in the thread-based microfluidic system. Urea samples with concentrations from 0.1 to10.0 mM mixed with urease (20 mg/ml) were placed in a 0.1 mM MES buffer. In general, urea is electrochemically inert and is difficult to detect with a standard electrochemical procedure. However, urea can be rapidly converted into ammonium ion with the assistance of urease. The reaction product of ammonium ion is electrochemically active and is easy to detect.

Experimental results confirmed that the developed thread-based microfluidic system exhibited good linear response for detecting urea in the concentration range of 0.1 – 10.0 mM, a linear range sufficient to examine the concentration of blood urea nitrogen in clinical samples (1.78~7.12 mM). That we can get the good R square on 3D (R=0.9848) and 2D (R= 0.9764) respectively.

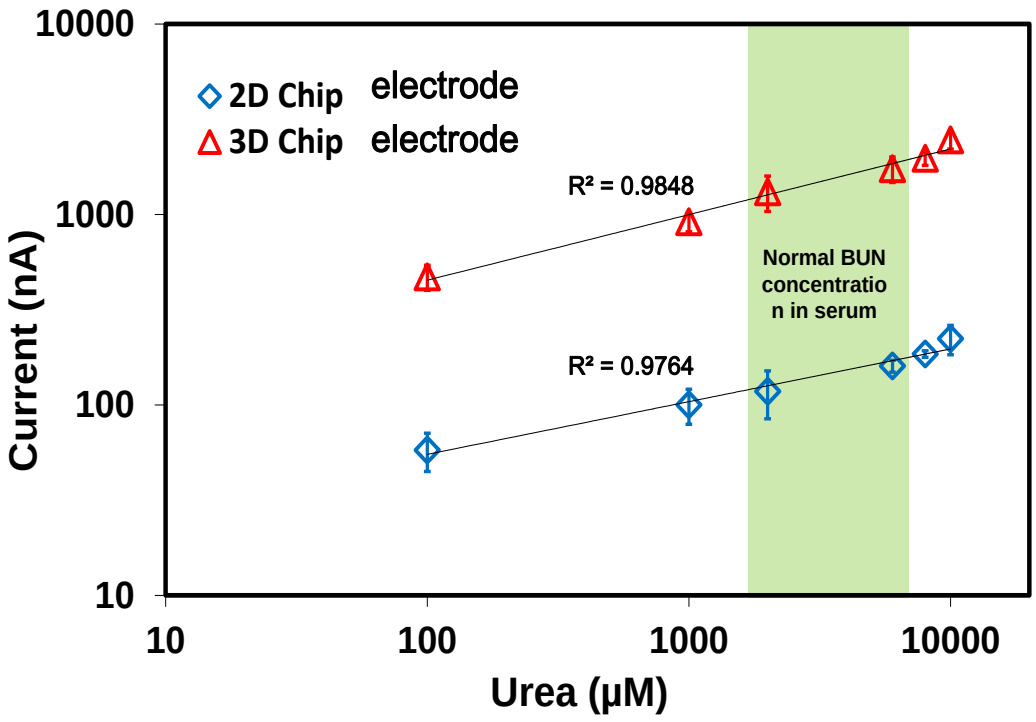

Figure 4-8. Measured electrophoresis for detecting urea in various concentrations

using 2D and 3D detection electrodes. Note that the normal BUN concentration for humans is in the range of 1.78~7.12 mM. (Applied EC detection potential: -0.23 V)

4.5 Electrophoresis of enzyme doped thread coated with PVC membrane

In general, the normal concentrations for blood urea nitrogen (BUN) and blood glucose (GLU) in serum are 1.78~7.12 mM and 3.89~6.11 mM, respectively. In order to evaluate the sensing performance of the system, mixed samples composed of urea and glucose with thee concentrations of 0.1 mM~10.0 mM and 0.1 mM~13.0 mM, respectively, were measured using the developed thread-based microfluidic system. Note that the enzyme concentrations for urease and GOD applied on the thread were 20 mg/mL and 10 mg/mL, respectively. As described above, 5 mg/mL of HRP and 0.5 mM catechol (mediator) were applied on the region above the EC electrodes for further reducing the detection potential of hydrogen peroxide. Figure 4-9 presents the measured current responses for EC detecting different concentrations of urea and glucose using the thread-based microfluidic system. Results show that the developed system exhibited nice sensing performance for simultaneously detecting these two biomolecules. The reaction products of urea and glucose were successfully electrochemically detected with the developed system.

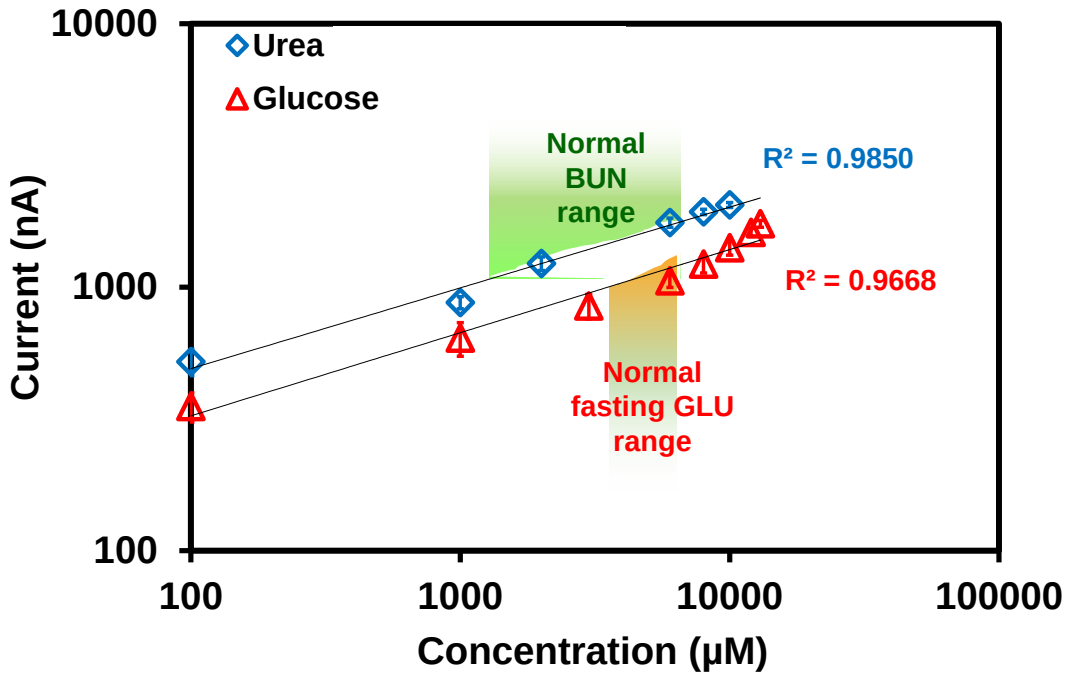

Figure 4-9. Measured current responses for detecting various concentrations of urea and glucose solutions.

4.6 Detection of human whole blood on thread microfluidic system

The sensing performance of the developed thread-based microfluidic system was finally evaluated by detecting the BUN concentration in human whole blood. Prior to the CEEC test, the BUN level of the whole blood sample was measured with standard detection protocol in a medical examination laboratory. The inspected BUN concentration in the blood sample was 11.2 mg/dl, which is equivalent to 3.98 mM of blood urea. Note that 1 mg/dL of detected urea in-vitro is equivalent to 2.14 mg/dL of BUN in serum. For CE-EC detection of the BUN concentration with the developed system, one drop of blood sample 20 μl of whole blood mixed with 2 μl of urease (20 mg/ml) was applied to the sample reservoir of the injection thread. Note that all the threads were soaked with 0.1 mM of MES buffer (pH=7.4) for the capillary

electrophoresis test. Figure 4-10 shows the electropherogram for CE-EC detection of the whole blood sample. It is noted that the flow speed of the electro-osmosis flow (EOF) of the buffer was faster than the migration speed of the ion samples such all the ions could reach the detection region for potential state measurement. The separated cations in the human blood were reduced with an applied potential of −0.23 V on the sensing electrodes and induced the current responses. Successfully separating and detecting cations including K^+, Na^+, Ca_2^+, Mg_2^+ and NH_4^+ in the whole blood was demonstrated. Note that the normal concentration for these ions in normal human serum are in in the range of 3.8~5.2 mM, 136~146 mM, 2.3~2.7 mM, 0.8~1.2 mM and 1.78~7.12 mM, respectively. Ion concentration will change depending on physical condition, especially that of sodium. The current response for the peak corresponding to the ammonium ion was 16.5 μA. The whole blood test confirmed can be detected but it has some defects comprising the higher concentration will effect more unstable and noise, the poor reproducibility and the RBC will block the channel. However, we need to dilution and improve that.

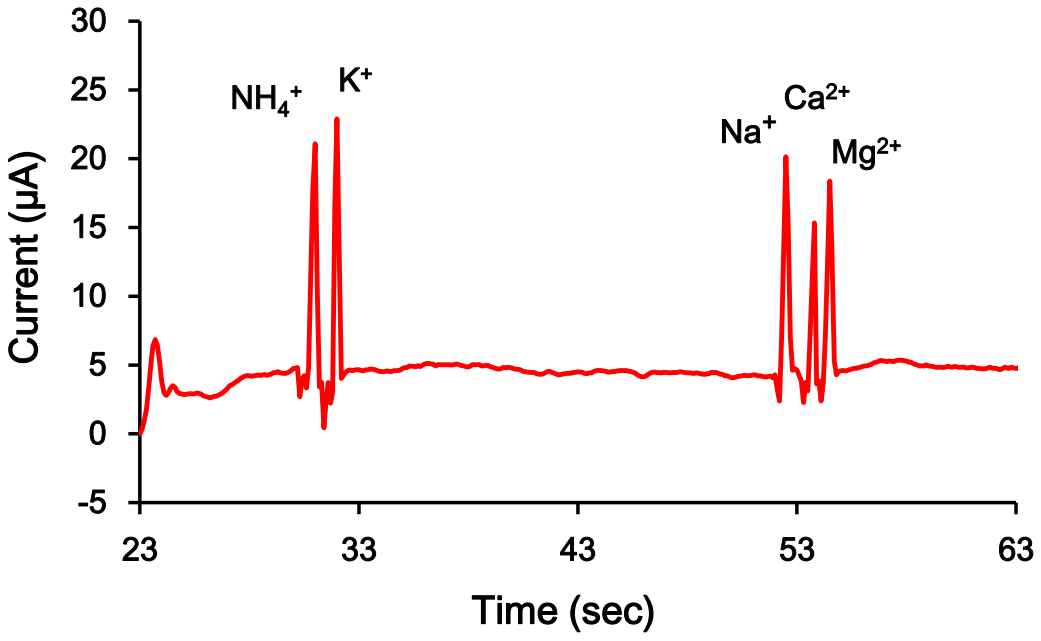

Figure 4-10. Electropherogram for detecting human whole blood with the developed

thread-based microfluidic device. The calculated BUN concentration for this case was 3.98 mM. (Buffer: 0.1 mM MES, injection: 200 V/cm for 20 s, separation: 300 V/cm for 40 s, Applied EC detection potential: -0.23 V)

4.7 Detection of human whole blood with enzyme doped thread coated with PVC membrane

The thread has multi-stranded holes in its structure which can filter the RBC, but the percentage of total blood which will block the channel is about 45%. In order to improve this problem, we utilize the 2-μL RBC lysis buffer drop on the excess injection thread to be a filter. An applied voltage of 300 V/cm continuing for 60s provided sufficient time to react with 2-μL injection of human whole blood. Afterwards, a separate voltage of 500 V/cm continuing for 80 s was used to drive the ions to their detection. Figure 4-11 (A) and (B) obviously shows difference images of whole blood without and with RBC lysis buffer on glass where make sure the reaction is effective. Figure 4-11 (C) and (D) illustrate the polyester threads with 2-μL whole blood with and without the RBC lysis buffer when it has experimented. It is clear that the blood coagulation will occur if no lysis buffer is applied.

Figure 4-12 presents the electropherogram for detecting human whole blood with the developed thread-based microfluidic device. The calculated BUN and GLU-AC in concentration were 3.87 mM and 4.94 mM, respectively. The separated cations in the human blood were reduced with an applied potential of -0.28 V on the sensing electrodes and induced the current responses. The current response for the peak corresponding to the ammonium ion and GLU were 2.02 and 1.41 μA, respectively. Compared with the conventional glass system, the enzyme-doped thread

coated with PVC membrane can supply more stability and repeatability. It obviously resolves blockage problems and can simultaneously detect different samples. Next we need to dilute the blood with RBC lysis, which can reduce the noise of the original high concentration and interference of RBC. The developed microchip device provides a low-cost and high performance method for blood sensor detection.

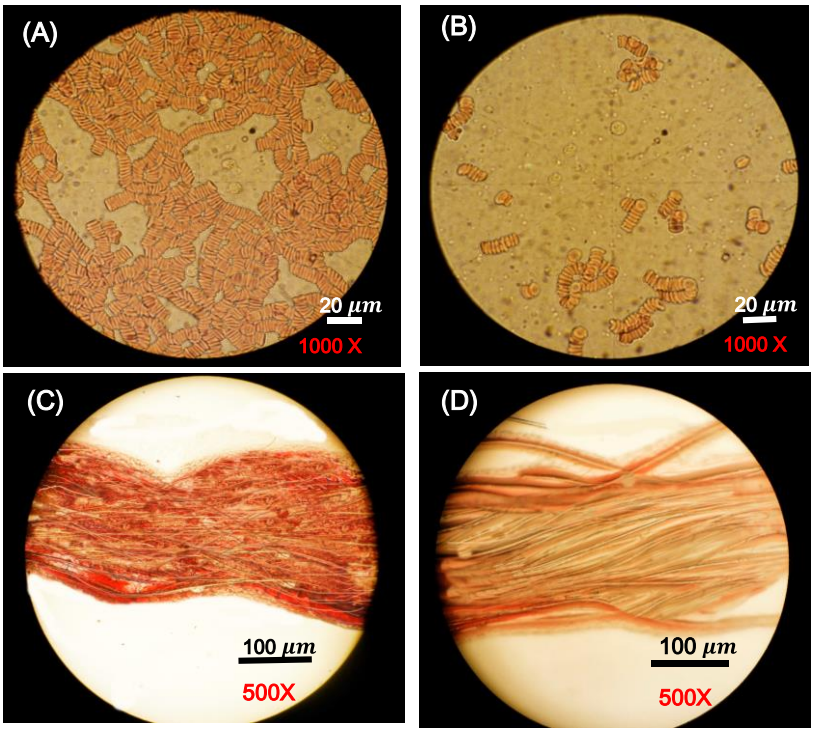

Figure 4-11. Drop the RBC lysis buffer mixed with blood on glass (A) and without (B). Photo images of the polyester threads with 2-μL whole blood without RBC lysis buffer (C) and with RBC lysis buffer on (D).

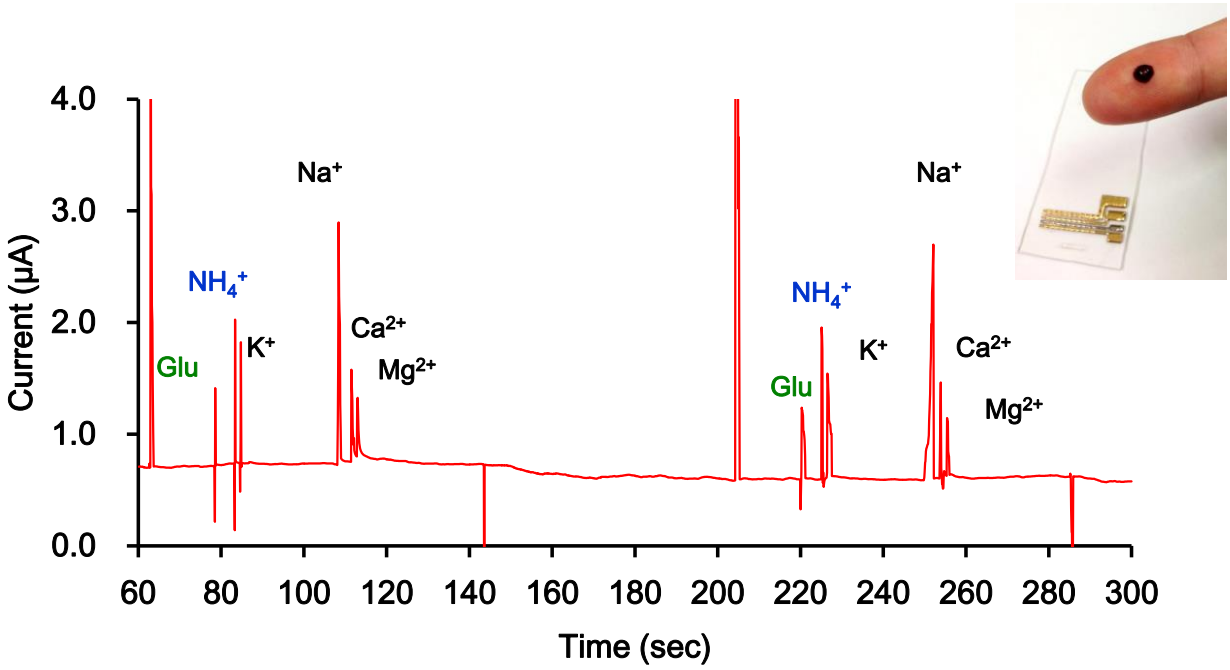

Figure 4-12. Electropherogram for detecting human whole blood with the developed thread-based microfluidic device. The calculated BUN and GLU-AC in concentration for 3.87 mM and 4.94 mM. (Buffer: 0.1 mM MES (pH=7.4), injection: 300 V/cm for 60 s, separation: 500 V/cm for 80 s, Applied EC detection potential: −0.28 V)

Chapter 5 Conclusions and Future Work

5.1 Conclusions

This study developed a novel technique to form a PVC coated thread doped with various enzymes of urease, glucose oxidase and horseradish peroxidase for on-site bio-sample separation, bio-catalytic reaction and electrochemical detection. Utilizing the technique of doping on the thread can provide several advantages, those of avoid dissociation of H_2O_2 before reaction with GLU, preventing interference of mixed samples, and providing sufficient transport to react urea. In addition, the enzyme can be directly applied to the thread without delicate pretreatment or a surface modification process. The whole blood sample is firstly mixed with an RBC lysis buffer to prevent blood coagulation. The lysed RBC and other solid pieces were simultaneously filtered away during electrokinetic flow in the thread-based microfluidic system. A thin layer of PVC membrane is coated on the enzyme-doped thread to further protect enzyme and to prevent the rapid evaporation of the running buffer due to the Joule heating effect. Enzyme-modified polyester thread coated with a thin layer of PVC was used as the liquid route and the reactor for converting urea and glucose into ammonium ions and hydrogen peroxide. The products were then separated and detected using the CE-EC detection scheme. With this approach, a sealed microfluidic channel embedded with various enzymes could be easily produced. Results showed that the thread with PVC coating exhibited higher electro-osmotic mobility in comparison with that of the conventional glass microfluidic channel. In addition, the surface of PVC coated thread could have porous structures which sustain good cooling effect that can provide a higher operation voltage than the uncoated one, resulting in a better detection performance. The novel device developed in the present

study provides a simple yet high performance method for rapid detection of bio-samples.

5.2 Future work

In this study, we utilize PVC to coat the channel, which not only provides better heat dissipation but also becomes a closed channel. Although the open channel system has some advantages, the channel still allows interfere from the external environment. However, our research proposes this novel system combining other material to improve and enhance more sensitivity, and is especially appropriate for biomedical applications.

Literature review has mentioned microfluidic systems used in paper colorimetry that can be implemented on our thread in order to react on separate different areas to detect specific samples. The color change could determine the results and could be used in various biomedical samples for detection of different functions such as protein and concentration detection. Compared with the easily damaged paper, the thread is very strong. Future trends suggest the multi-detection capabilities of this thread microfluidic system device. Besides testing multiple samples on the thread, combined electrochemical detection will be important, as in utilizing an antigen-antibody to detect different blood type or adding nanoparticles to separate and enlarge the signal of a DNA fragment to replace EtBr stained DNA electrophoresis analysis. In addition, another novel method can be the integration of immunomagnetic bead separation techniques mixed with fluorescent dye on thread by fixing with magnet. Utilizing the spectrum to read the specific sample by beads catching on thread is another means of easy and low cost detection. In addition we can test characteristic of different threads to detect more sample comprising silk, aluminum thread, cotton.

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Publication list

International journal:

1. **Y.-A. Yang**, C.-H. Lin, and Y.-C. Wei, "Thread-based microfluidic system for detection of rapid blood urea nitrogen in whole blood," Microfluidics and Nanofluidics, pp. 1-8, 2014. (SCI, Impact Factor = 3.371)
2. **Y.-A. Yang**, C.-H. Lin, "Detection of Whole Blood Urea Nitrogen and Glucose Utilizing Multiple Enzyme-doped Thread-based Microfluidic System," Biosensors and Bioelectronics. 2014 (SCI, Impact Factor = 5.437) / (submitted)

International conference:

1. **Yu-An Yang**, Wen-Cheng Kuo, and Che-Hsin Lin, "Enzyme-doped polyester thread coated with PVC membrane for on-site urea and glucose detection on a thread-based microfluidic system," in Micro Electro Mechanical Systems

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2. **Yu-An Yang**, Yi-Chi Wei, Che-Hsin Lin* "High Performance Thread-based CE-EC System with Variable Volume Injection Capability and 3D Detection Electrodes" in Nano/Micro Engineered And Molecular Systems (NEMS), 2014 IEEE 9th International Conference. USA, Hawaii, Apr 13-16
3. **Yu-An Yang,** Che-Hsin Lin* "Enzyme-doped Polyester Thread Coated With PVC Membrane For Blood Urea Nitrogen And Glucose Detection In Human Whole Blood" in Miniaturized Systems for Chemistry and Life Sciences (Micro TAS), 2014 IEEE 18th International Conference. USA, San Antonio, Oct 26-30.
4. **Yu-An Yang,** Che-Hsin Lin* "Multiple Enzyme-doped Thread-based Microfluidic System for Blood Urea nitrogen and Glucose Detection in Human Whole Blood" in Nano/Molecular Medicine and Engineering (NANOMED), 2014 IEEE 8th International Conference. Taiwan, Kaohsiung, Nov 09 – 12.

Conference in Taiwan:

1. Yi-Chi Wei, **Yu-An Yang**, Che-Hsin Lin* "Electrophoresis Separation and Electrochemical Detection on a Novel Line-Based Microfluidic Device" 2012 Chiness Society and of Mechanical Engineers, Kaohsiung, Taiwan, Dec 7-8, 2012
2. **楊諭安**、魏翊旂、林哲信*, "創新可變量線微流體系統於三維電極整合電化學電泳做尿素檢測" The 17th Nano and Microsystem Technique Conference, Taichung, Aug 22-23, 2013.
3. **Yu-An Yang**, Yi-Chi Wei, Che-Hsin Lin*," High Performance Thread-based CE-EC System with Variable Volume Injection Capillary 3D detection

Electrodes ", 2014 Conference on Precision Machinery and Manufacturing Technology, Kenting, Taiwan, May 23-25, 2014.

4. **Yu-An Yang**, Che-Hsin Lin*, "Multiple Enzyme doped thread-based microfluidic system for blood urea nitrogen and glucose detection in human whole blood" The 18th Nano and Microsystem Technique Conference, Taine, Aug 21-22, 2014.

Parent:

1. Che-Hsin Lin, Yi-chi Wei, Lung-min Fu, **Yu-an Yang**, Thread-based microfluidic guiding system 公開號: US20130299352 A1,美國專利申請中