การพัฒนาสเปกโทรอิเล็กโทรเคมิคัลโฟลว์เซลล์สำหรับการตรวจวัดปริมาณคาเฟอีนในเครื่องดื่ม

นางสาวสุทัตตา เซ็นโส

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DEVELOPMENT OF SPECTROELECTROCHEMICAL FLOW-CELL FOR DETERMINATION OF CAFFEINE CONTENT IN BEVERAGES

Miss Sutatta Zenso

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
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Thesis Title	DEVELOPMENT OF SPECTROELECTROCHEMICAL FLOW-CELL FOR DETERMINATION OF CAFFEINE CONTENT IN BEVERAGES
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Field of Study	Chemistry
Thesis Advisor	Passapol Ngamukot, Ph.D.

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สุทัตตา เซ็นโส : การพัฒนาสเปกโทรอิเล็กโทรเคมิคัลโฟลว์เซลล์สำหรับการตรวจวัดปริมาณคาเฟอีนในเครื่องดื่ม (DEVELOPMENT OF SPECTROELECTROCHEMICAL FLOW-CELL FOR DETERMINATION OF CAFFEINE CONTENT IN BEVERAGES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร.ภัสสร์พล งามอุโฆษ, 61 หน้า.

งานวิจัยนี้ได้พัฒนาสเปกโทรอิเล็กโทรเคมิคัลโฟลว์เซลล์ขึ้นสำหรับการตรวจวัดหาปริมาณของคาเฟอีนในเครื่องดื่ม โดยโฟลว์เซลล์ที่พัฒนาขึ้นร่วมกับเทคนิคระบบการวิเคราะห์แบบไหลสามารถใช้ในการศึกษาทั้งด้านเทคนิคเชิงแสงและด้านเคมีไฟฟ้าได้ในอุปกรณ์ตรวจวัดเดียวกัน วัสดุที่ใช้ในการทำโฟลว์เซลล์เป็นพลาสติกประเภทอะคริลิคซึ่งเป็นวัสดุที่มีน้ำหนักเบา แข็งแรง และทนทานต่อสารเคมี โดยมีขนาด 34 × 50 × 15 มิลลิเมตร สำหรับตัวโฟลว์เซลล์ที่พัฒนาขึ้นประกอบด้วยเส้นใยแก้วนำแสง 2 เส้นที่ประกอบเข้ากับโฟลว์เซลล์โดยมีระยะห่างกัน 10 มิลลิเมตรเพื่อใช้ในการตรวจวัดหาปริมาณของคาเฟอีนโดยเทคนิคเชิงแสงที่ความยาวคลื่น 273 นาโนเมตร ซึ่งเป็นความยาวคลื่นที่ให้ค่าการดูดกลืนแสงที่สูงที่สุด สำหรับการตรวจวัดด้านเคมีไฟฟ้าใช้ขั้วไฟฟ้าใช้งานประเภทขั้วคาร์บอนในการวิเคราะห์หาปริมาณของคาเฟอีนโดยเทคนิคสแควร์เวฟโวลแทมเมทรีที่ความต่างศักย์ 1.4 โวลต์ เมื่อเทียบกับขั้วไฟฟ้าอ้างอิงประเภทซิลเวอร์ซิลเวอร์คลอไรด์ในระบบที่มีฟอสเฟตบัฟเฟอร์เป็นตัวทำละลาย จากการวิเคราะห์ได้ช่วงความเป็นเส้นตรงสำหรับการตรวจวัดคาเฟอีนเท่ากับ 0.01 ถึง 0.12 มิลลิโมลาร์ และ 0.05 ถึง 0.5 มิลลิโมลาร์ ซึ่งมีขีดจำกัดต่ำสุดในการตรวจวัดเท่ากับ 1.00 ไมโครโมลาร์ และ 3.10 ไมโครโมลาร์ สำหรับการวิเคราะห์ด้านเทคนิคเชิงแสงและด้านเคมีไฟฟ้าตามลำดับ นอกจากนี้ในการศึกษาความสามารถในการทำซ้ำสำหรับเทคนิคเชิงแสงและเคมีไฟฟ้าพบว่า ค่าเปอร์เซ็นต์ความเบี่ยงเบนมาตรฐานสัมพัทธ์มีค่าเท่ากับ 3.21 และ 4.98 ตามลำดับ สำหรับโฟลว์เซลล์ที่พัฒนาขึ้นเป็นโฟลว์เซลล์ที่มีขนาดเล็ก ราคาถูก สามารถเชื่อมต่อกับเทคนิคระบบการวิเคราะห์แบบไหลได้ง่าย รวมทั้งสามารถใช้ในการวิเคราะห์หาปริมาณของคาเฟอีนในตัวอย่างเครื่องดื่มได้ โดยมีผลการวิเคราะห์ที่มีค่าใกล้เคียงกับวิธีมาตรฐาน

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SUTATTA ZENSO: DEVELOPMENT OF SPECTROELECTROCHEMICAL FLOW-CELL FOR DETERMINATION OF CAFFEINE CONTENT IN BEVERAGES. ADVISOR: PASSAPOL NGAMUKOT, Ph.D., 61 pp.

A cost-effective spectroelectrochemical flow-cell was developed for determination of caffeine content in beverages. The developed flow-cell which coupled with sequential injection analysis (SIA) system can be applied for spectrophotometric and electrochemical studied in the same device. The flow-cell body was made of acrylic plastic material that is lightweight, hard and chemically inert. Its dimension was 34mm × 50mm × 15mm. The flow-cell consisted of 2 optical fiber probes with a path length of 10 mm for spectrophotometric investigated at the wavelength of 273 nm. Glassy carbon was chosen as a working electrode for caffeine oxidation studied by square wave voltammetry technique at the potential of 1.4 V vs Ag/AgCl in phosphate buffer solution. The linear ranges of caffeine determination were 0.01-0.12 mM and 0.05-0.5 mM with detection limit of 1.00 μM and 3.10 μM for spectrophotometric and electrochemical method, respectively. The reproducibility was studied and the relative standard deviation percentages (%RSD) were 3.21% and 4.98% for spectrophotometric and electrochemical measurements, respectively. This developed flow-cell is small, cost-effective and easy to connect with sequential injection analysis system. Moreover, the developed spectroelectrochemical flow-cell was successfully applied for the determination of caffeine in beverages samples and the results were insignificantly different when compare with a standard method.

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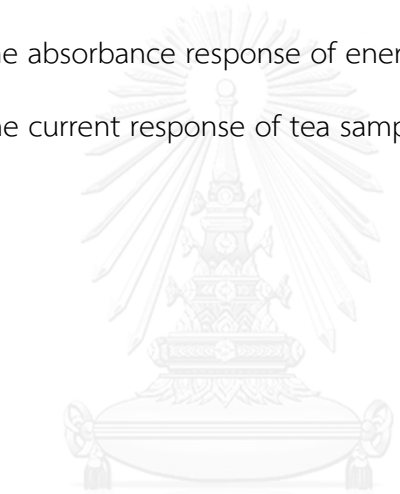

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LIST OF ABBREVIATIONS

A	ampere
CAF	caffeine
CCE	carbon-ceramic electrode
E	potential
FEB	fluorinated ethylene propylene
FIA	flow injection analysis
GCE	glassy carbon electrode
Gr	graphene
g	gram
ITO	indium tin oxide
IR	infrared
L	liter
LOD	limit of detection
LOQ	limit of quantification
MWCNT	multi-walled carbon nanotubes
M	molar
mM	millimolar
mm	millimeter
mL	milliliter
mg	milligram
nm	nanometer
OD	outer diameter

PBS	phosphate buffer solution
PEEK	Polyether ether ketone
RSD	relative standard deviation
SWV	square wave voltammetry
SS	stainless steel
SWCNT	single-walled carbon nanotubes
SEC	spectroelectrochemical method
SIA	sequential injection analysis
SD	standard deviation
UV	ultraviolet
UV-Vis	ultraviolet-visible
V	volt

CHAPTER I
INTRODUCTION

1.1 Introduction

Caffeine or 1, 3, 7-trimethylpurine-2, 6-dione is a crystalline methyl xanthine alkaloid. It is found in foods and beverages such as chocolate, coffee, tea, soft drinks and energy drinks. Caffeine is a stimulant of metabolic and central nervous system. It can increase an attention, alertness and body metabolic rate after caffeine using. However, there are health effects of caffeine both positive and negative effects depending on the amount of caffeine consumption, age range and body weight of the consumers. Caffeine intoxication or overdose of caffeine is the condition of central nervous system over-stimulation that occurs when the ingestion of caffeine is more than 400-500 mg at a time. The consumer can have insomnia, headache, confusion, rapid breathing, frequent urination and irregular heartbeat. In addition, the massive overdose can result in death [1]. Therefore, the studies of caffeine in foods and beverages are very important for the consumers [2].

Several techniques can be used for caffeine determination. The gas chromatography-mass spectrometry [3-7] and liquid chromatography-mass spectrometry [8-11] were widely used for determination of caffeine because they provide low detection limit, high selectivity and sensitivity. However, the chromatography that coupled with mass spectrometry methods are expensive and complicated instruments. Therefore, the spectrophotometry and electrochemical method were applied to determine the caffeine content because of their cost-effective and simple instruments. In addition, these techniques are easy to couple with flow-based analysis system. The advantages of this system are convenient, easy to use, spend less time and use small volume of chemicals. The spectrophotometric response of caffeine was investigated at the wavelength of 273 nm, which is the wavelength that gives the highest signal of caffeine. The glassy carbon electrode or

modified glassy carbon electrode are suitable to use as the working electrode for electrochemical investigation of caffeine oxidation at the surface of glassy carbon electrode and solution.

Spectroelectrochemical method (SEC) [12] is a technique that combines the spectrophotometry and electrochemical method. The spectroelectrochemical flow-cell was coupled with flow-based analysis system for studying of spectrophotometric and electrochemical properties at the same detection device. Therefore, the electro-generated and dual response mode can be applied for spectrophotometric and electrochemical measurements depending on the properties of chemicals. The electro-generated mode can be used for the chemicals which have the optical properties. It is the spectrophotometric measurement when the chemicals structure is changed by applying of potential. Moreover, the chemicals that have both spectrophotometric and electrochemical properties can use dual response mode for the analyte determination. It provides the results of both analytical techniques to confirm the accuracy of developed methods.

In this work, the spectroelectrochemical flow-cell was developed and coupled with sequential injection analysis (SIA). The portable spectrophotometer and optical fiber probes were chosen for studying of caffeine spectrophotometric properties and glassy carbon was chosen as the working electrode for caffeine electrochemical measurement in the dual response mode at the same developed flow-cell device.

1.2 Objective of the research

1. To develop the spectroelectrochemical flow-cell which can use for spectrophotometric and electrochemical detection of caffeine.

2. Determine the amount of caffeine in beverages by using the developed spectroelectrochemical flow-cell.

CHAPTER II
THEORY AND LITERATURE SURVEY

This chapter is the explanation about the caffeine that included the structure of caffeine, positive effects and negative effects for human health. The theory of spectrophotometry, electrochemical method and spectroelectrochemical method are described. In addition, the literature survey about the method and development of spectroelectrochemical flow-cell are presented in this chapter.

2.1 Caffeine

Caffeine or 1, 3, 7-trimethylpurine-2, 6-dione structure (Figure 2.1) can be classified as a xanthine alkaloid. It is a heterocyclic compound which the chemical formula is $C_8H_{10}N_4O_2$ and the molar mass is 194.19 grams. It is soluble in water and some organic solvent. Caffeine is the most popular stimulant of the world because it can increase the heart rate and breathing rate. However, it has the negative effects when the amount of caffeine consumption is overdose. Caffeine intoxication can cause insomnia, headaches and lead to migraines. In addition, massive overdose of caffeine can result in death.

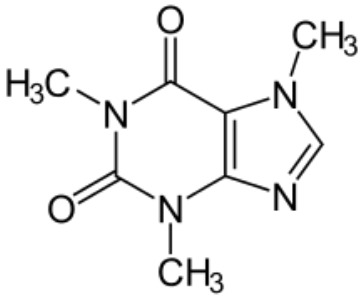

Figure 2.1 Chemical structure of caffeine

2.2 Spectrophotometry

Spectrophotometry is the quantitative technique that investigation of light reflection, transmission or absorption properties of chemicals. The light wavelength range of spectrophotometry is around 200-2500 nm and the spectrophotometry can be divided by the wavelength range such as UV-Vis spectrophotometry and IR spectrophotometry.

2.2.1 UV-visible spectrophotometry

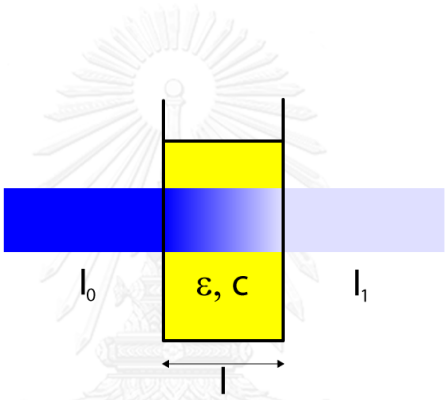

Figure 2.2 Schematic diagram of absorption measurement

The wavelength of UV-visible spectrophotometry is 200-800 nm. The UV region is 200-400 nm and the visible region is 400-800 nm that can be used for colorimetric measurement. The light source of UV-visible spectrophotometry is deuterium-halogen light source, which covers 190-1100 nm range. This technique is the studying of absorption property (Figure 2.2) that is proportional to the concentration of chemicals according to Beer-Lambert Law.

$$A = \log_{10} I_0/I_1 = \varepsilon lc$$

Where: A is absorbance

I_0 is initial intensity

I_1 is intensity of transmission light

ε is molar absorptivity (L/mol cm)

l is path length (cm)

C is concentration (mol/L)

In this work, the spectrophotometric was investigated at the wavelength of 273 nm [13-17] which is the wavelength that provides the highest absorbance signal for the determination of caffeine.

2.2.2 Optical fiber

Optical fiber is made of glass (silica) or plastic. Its diameter is slightly thick. The light can transmit between the two ends of the fiber. The optical fiber consists of two parts. The main part is core at the middle and another is cladding that is around the core. The light can pass in the optical fiber when the refractive index of cladding is lower than the refractive index of core. This phenomenon is called the total internal reflection (Figure 2.3).

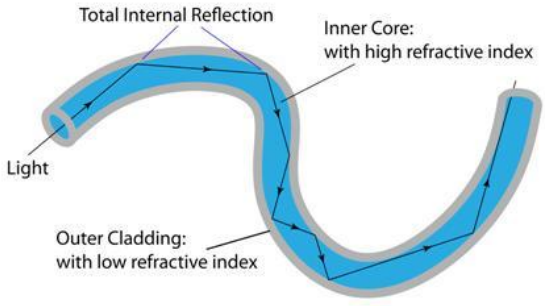

Figure 2.3 Total internal reflection phenomenon in optical fiber

2.3 Electrochemical method

Electrochemistry is the investigation of interaction between chemical change and electrical energy. It studies about electrical that related with chemical reaction at the surface between the electrode and the electrolyte. In general, electrochemical reaction at the interface of electrode and solution is redox reaction. It is the electrochemical process which is the transfer of electrons [18].

The mechanism of mass transport can be divided into three types that are diffusion, migration and convection. Diffusion is the movement by concentration gradient that is used for investigation of electrochemical properties. Migration is the movement of charge in electric field which can be eliminated by using supporting electrolyte and convection can occur with hydrodynamic or velocity force that can be controlled. Therefore, the current can be classified as diffusion controlled.

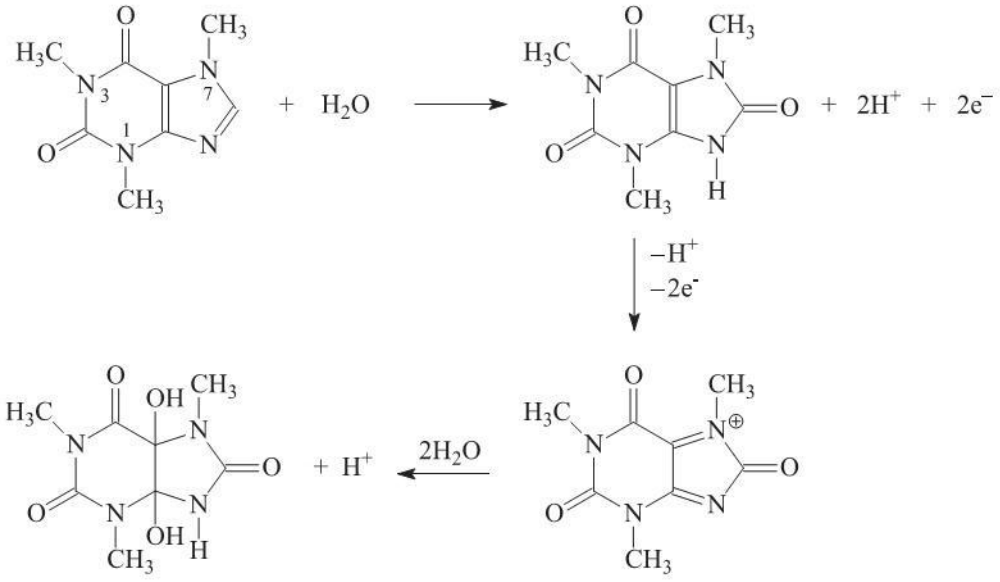

Figure 2.4 Mechanism of caffeine oxidation

The oxidation mechanism of caffeine (Figure 2.4) at the surface of a glassy carbon electrode has 2 steps of 2e- and 2H+ oxidation. The first step is C8 to N9 double-bond oxidation that is performed to give the substituted uric acid and another is 4, 5 diol oxidation [19].

2.3.1 Voltammetry

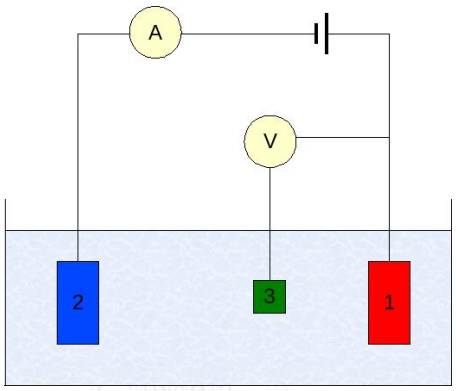

Figure 2.5 Three electrodes set up: (1) working electrode; (2) counter electrode; (3) reference electrode

Voltammetry is electrochemical method that measures the current when the potential is controlled. There are three electrodes (Figure 2.5) that are working electrode, reference electrode and counter electrode. The current is measured at the surface of working electrode while applied the potential that refer to the reference electrode and the counter electrode is used for balance the reaction that occurs from the working electrode. Generally, the working electrode should be placed next to the reference electrode for reducing of IR drop. The counter electrode often has the higher surface area than the working electrode to ensure that another reaction at the counter electrode can occur. Moreover, the electrochemical flow-cell should place the counter electrode at the downstream of flow system to ensure that the products from the counter electrode do not affect the current measurement from the working electrode.

The current is proportional to the concentration of analyte according to Cottrell equation.

$$i = \frac{nFAc^{0}(D)^{1/2}}{(\pi t)^{1/2}}$$

Where : i is current (A)

n is the number of electron

F is faraday constant (96,485 C/mol)

A is the area of planar electrode (cm^2)

C^0 is initial concentration of analyte (mol/cm^3)

D is diffusion coefficient of analyte (cm^2/s)

t is time (s)

2.3.1.1 Square wave voltammetry

The potential waveform (Figure 2.6) of square wave voltammetry or SWV is a square wave of constant amplitude on a staircase wave form. The important parameters of square wave voltammetry are potential amplitude, potential step and frequency. The current is measured by subtraction between the current at forward sample period (i_f) and reverse sample period (i_r). Therefore, this technique provides peak shape voltammogram.

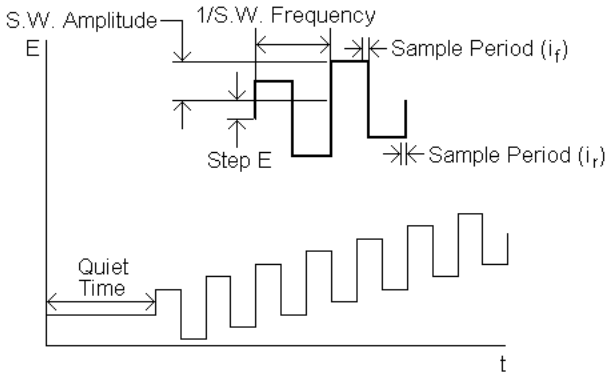

Figure 2.6 Potential waveform of SWV

2.3.2 Electrodes

Several electrodes can used for determination of caffeine such as boron-doped diamond [20] and carbon-based electrode such as carbon-paste electrode [21, 22] and glassy carbon electrode but the most common type of carbon-based working electrode is glassy carbon electrode (Figure 2.7) and modified glassy carbon electrode [23-29]. It can be used for wide working potential range. The properties of glassy carbon electrode are good electrical conductivity, high strength, highly corrosion resistance and low thermal expansion. In this research, the glassy carbon disc of 3 mm diameter, which has the surface area diameter of 1mm was chosen as the working electrode.

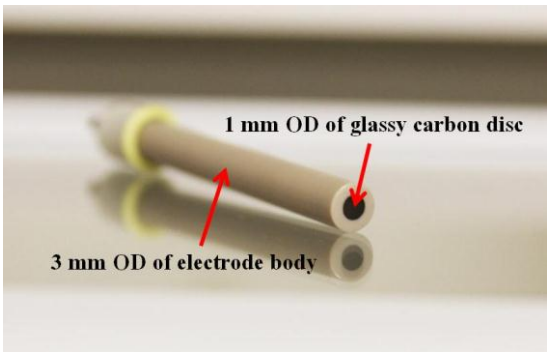

Figure 2.7 The glassy carbon electrode

2.4 Spectroelectrochemical method

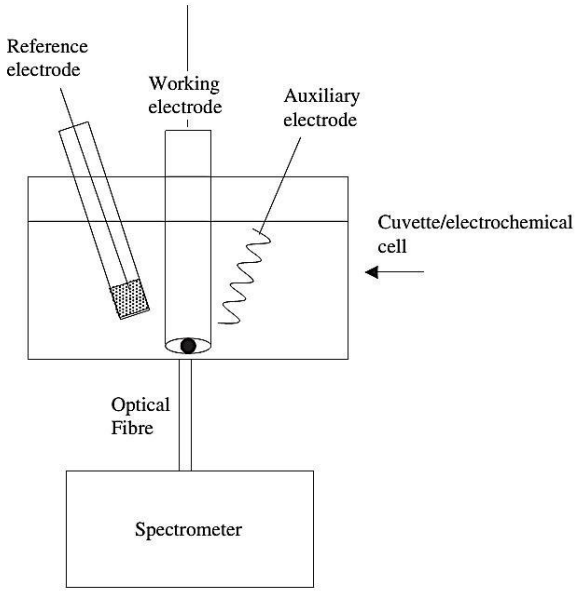

Figure 2.8 The spectroelectrochemical cell

The spectroelectrochemical (SEC) method is the technique that combines the spectrophotometry with the electrochemical method. The spectroelectrochemical cell (Figure 2.8) consists of optical measurement part and electrochemical part in the cell. This method is the investigation of optical properties at the same time of electrochemical change which is the electrons transfer at the interface of electrode surface and electrolyte. There are two modes of spectroelectrochemical method that are electro-generated mode and dual response mode. The electro-generated mode is the spectroscopic measurement when chemical is changed by applying of potential. Furthermore, the dual response is the investigation of spectroscopic and electrochemical properties of chemicals at the same time. The dual response can increase the choices of measurement in spectroscopic measurement, electrochemical measurement and spectroelectrochemical measurement.

2.5 Flow-based analysis system

The advantages of flow-based analysis system are rapid, reducing of human error, increasing of precision and minimize the chemicals. Therefore, flow-based system is used for routine analysis. The most common flow-based analysis systems are flow injection analysis (FIA) [30] system and sequential injection analysis (SIA) system.

2.5.1 Sequential injection analysis (SIA)

Sequential injection analysis or SIA (Figure 2.9) is an automated analysis system. SIA system consists of pump, selection valve, detector and software. The solutions are chosen by selection valve and the zones of solution are flown into the holding coil by pump. After that, the solutions flow to the detector for measurement. The advantages of SIA are increasing of precision, convenient automated system and the sequence of solutions was taken into the holding coil and it is force to the detector [31-33].

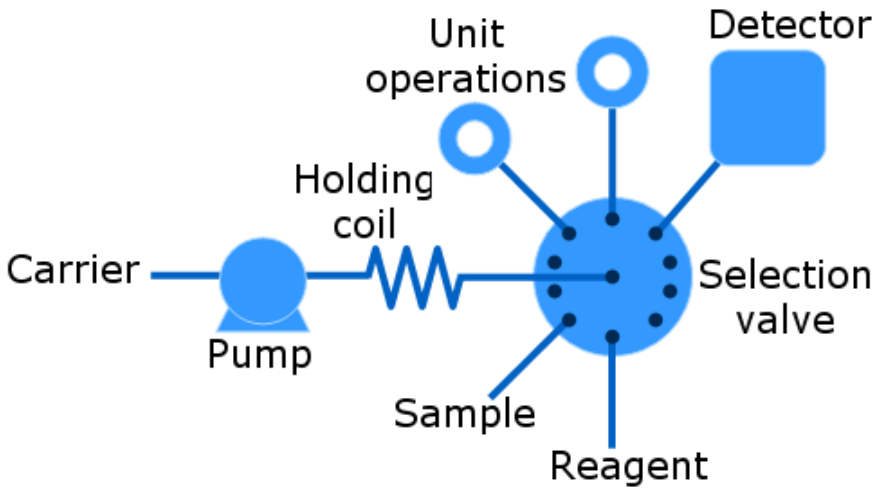

Figure 2.9 Schematic diagram of common SIA system

2.5.2 Spectroelectrochemical flow-cell

The spectroelectrochemical flow-cell can coupled to the sequential injection analysis system and connected with spectrophotometer and potentiostat (Figure 2.10) for optical and electrochemical studied. There are two configurations of flow-cell. First is wall-jet flow-cell, which is the solutions flow into the working electrode in vertical direction. Another is thin-layer or channel flow-cell that the flow path is parallel to the working electrode for electrochemical studied [34-38].

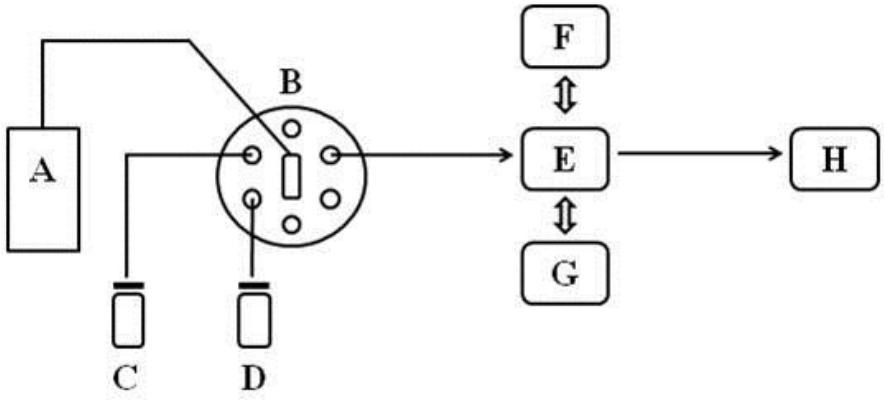

Figure 2.10 Schematic diagram of SIA system which is coupled with spectroelectrochemical flow-cell; (A) pump, (B) selection valve, (C,D) standards or samples, (E) spectroelectrochemical flow-cell, (F) spectrophotometer, (G) potentiostat and (H) waste

2.6 Literature reviews

In 2008, Belay et al. reported UV-Vis spectrometry for determination of caffeine in coffee beans. Water and dichloromethane were used as the solvents for liquid-liquid extraction and the responses were performed at the wavelength of 272 and 274.7 nm, respectively. The deviations of five independent measurements were 0.01 to 0.04 %w/w.

In 2014, Tautua et al. reported the determination of caffeine using UV-Vis spectrometry and investigated the absorbance at the wavelength of 270 nm. Carbon

tetrachloride was chosen as extracting solvent. The linear range was obtained at 10 to 60 ppm of caffeine and correlation factor was 0.996. The deviation of caffeine determination in carbonated soft drink and energy drink samples were 0.35 to 1.05 ppm.

In 2013, Gupta et al. reported the simultaneous of ascorbic acid (AA) and caffeine (CAF) using square wave voltammetry (SWV) technique with multiwall carbon nanotubes (MWCNT) modified glassy carbon electrode (GCE). The oxidation of ascorbic acid and caffeine occurred at -10 mV and 1103 mV. The linear range of ascorbic acid and caffeine were obtained at 10 to 500 μM and detection limit were 1×10^{-2} μM and 3.52×10^{-3} μM, respectively. This method was applied to determination of caffeine in tea leaves, coffee, cold drink and urine samples.

In 2011, Yong Sun et al. developed Nafion–Gr modified glassy carbon electrode (Nafion–Gr/GCE) for determination of caffeine. The electrochemical properties were investigated by cyclic voltammetry and differential pulse voltammetry. Detection limit of 1.2×10^{-7} M and relative standard deviation of 5.2 % were obtained. The obtained recovery of determination of caffeine in real samples was 98.6 to 102 %.

In 2014, Torres et al. reported the modifications of glassy carbon electrode (GCE) surface with poly(3,4-ethylenedioxythiophene), Nafion and multi-walled carbon nanotubes. The electrolyte solutions of phosphate buffer saline, sodium perchlorate and choline chloride with oxalic acid were tested and the results of phosphate buffer saline electrolyte provided the best response. The sensor was successfully applied to determine the caffeine content in beverages and drugs. The detection limit of this electrochemical sensor was 38.9 nM.

In 2011, Guo et al. developed poly(safranine T) film modified glassy carbon electrode (GCE). This voltammetric sensor was applied to determine caffeine content in tea samples. The linearity concentration of this work was 3×10^{-7} to 1×10^{-4} M and detection limit of this sensor was 1×10^{-7} M. The recovery of caffeine determination in tea samples was 96.6 to 106.4 %.

In 2013, León et al. developed cuvette-based spectroelectrochemical flow-cell. This work studied two difference working electrodes that were stainless steel pipe (SS) and indium tin oxide (ITO). The developed flow-cell was coupled with flow injection (FIA) analysis system. Ferrocyanide was chosen for electro-generated studied and investigation of spectrometric response at the wavelength of 420 nm. The best results were obtained by using the stainless steel electrode with a linear ranging from 1.2×10^{-4} to 5×10^{-3} M and the deviation was 5.4 % of RSD.

CHAPTER III

EXPERIMENTAL

This chapter describes about the instruments and apparatus, chemicals, chemical preparation, development of spectroelectrochemical flow-cell, optimize condition of spectrophotometry, optimize condition of electrochemical method and the determination of caffeine in real samples in this research.

3.1 Instruments and apparatus

3.1.1 Sequential injection analysis (SIA)

The instruments and apparatus of sequential injection analysis are shown in Table 3.1

Table 3.1 The instruments and apparatus for sequential injection analysis

Instruments and apparatus	Suppliers
Syringe pump	IDEX Health & Science, USA
Selection valve	IDEX Health & Science, USA
Tubing (FEP OD:1/16)	IDEX Health & Science, USA
Fittings (PEEK nut)	IDEX Health & Science, USA

3.1.2 Development of spectroelectrochemical flow-cell

The software, instrument and apparatus for spectroelectrochemical flow-cell development are shown in Table 3.2

Table 3.2 The software, instruments and apparatus for spectroelectrochemical flow-cell development

Instruments and apparatus	Suppliers
SolidWorks 2012 software	
Tubing (FEP OD:1/16)	IDEX Health & Science, USA
Fittings (Tefzel nut, PEEK ferrule and stainless steel ring)	IDEX Health & Science, USA
Acrylic plastic	Pan Asia, Thailand
Teflon plastic	

3.1.3 Chemical preparations

The instruments and apparatus for chemical preparations are shown in Table 3.3

Table 3.3 The instruments and apparatus for chemical preparations

Instruments and apparatus	Suppliers
pH meter (SP-2001)	Suntex, Taiwan
Micropipette and tips	Brand, Germany
DI water system	
Laboratory glasswares	

3.1.4 Spectrophotometric measurements of caffeine

The instruments and apparatus for spectrophotometric measurements of caffeine are shown in Table 3.4

Table 3.4 The instruments and apparatus for spectrophotometric measurements of caffeine

Instruments and apparatus	Suppliers
Spectrophotometer(AvaSpec-2048)	Avantes, USA
Light source (AvaLight-DHc)	Avantes, USA
Optical fiber probes (Micro Transmission Dip probe)	Avantes, USA
Laboratory glasswares	

3.1.5 Electrochemical measurements of caffeine

The instruments and apparatus for electrochemical measurements of caffeine are shown in Table 3.5

Table 3.5 The instruments and apparatus for electrochemical measurements of caffeine

Instruments and apparatus	Suppliers
Potentiostat (PGstat101)	BAS Inc., japan
Working electrode (glassy carbon OD:3mm ID:1mm)	BAS Inc., japan
Reference electrode screw type (Ag/AgCl)	BAS Inc., japan
Counter electrode (stainless steel pipe)	BAS Inc., japan
Electrical connector	
Laboratory glasswares	

3.2 Chemicals

3.2.1 Spectrophotometric measurements of caffeine

The chemicals that used for spectrophotometric measurements of caffeine are presented in Table 3.6

Table 3.6 The chemicals for spectrophotometric measurements of caffeine

Chemicals	Suppliers
Caffeine	Sigma-Aldrich, Germany
Sodium phosphate dibasic	Sigma-Aldrich, Germany
Sodium phosphate monobasic	Sigma-Aldrich, Germany
DI water	

3.2.2 Electrochemical measurements of caffeine

The chemicals that used for electrochemical measurements of caffeine are presented in Table 3.7

Table 3.7 The chemicals for electrochemical measurements of caffeine

Chemicals	Suppliers
Caffeine	Sigma-Aldrich, Germany
Sodium phosphate dibasic	Sigma-Aldrich, Germany
Sodium phosphate monobasic	Sigma-Aldrich, Germany
DI water	

3.2.3 Interference study

The chemicals that used for studying of interferences are presented in Table 3.8

Table 3.8 The chemicals for studying of interferences

Chemicals	Suppliers
Caffeine	Sigma-Aldrich, Germany
Glucose	Carlo Erba, France
Sucrose	Sigma-Aldrich, Germany
Fructose	Sigma-Aldrich, Germany
Citric acid	Fluka, Germany
Sodium phosphate dibasic	Sigma-Aldrich, Germany
Sodium phosphate monobasic	Sigma-Aldrich, Germany
DI water	

3.2.4 Sample preparations

The chemicals that used for sample preparations are presented in Table 3.9

Table 3.9 The chemicals for sample preparations

Chemicals	Suppliers
Sodium phosphate dibasic	Sigma-Aldrich, Germany
Sodium phosphate monobasic	Sigma-Aldrich, Germany
DI water	

3.3 Chemical preparations

3.3.1 Preparation of phosphate buffer solution (PBS)

3.3.1.1 The 0.05 M sodium phosphate dibasic solution

The solution of 0.05 M sodium phosphate dibasic was prepared by dissolving of 0.7098 g sodium phosphate dibasic in 100 mL of DI water.

3.3.1.2 The 0.05 M sodium phosphate monobasic solution

The solution of 0.05 M sodium phosphate monobasic was prepared by dissolving of 0.6000 g sodium phosphate monobasic in 100 mL of DI water.

3.3.1.3 Phosphate buffer solution (PBS)

The various pH values of phosphate buffer solutions were prepared by the mixing of 0.05 M sodium phosphate dibasic stock solution and 0.05 M sodium phosphate monobasic stock solution that according to Table 3.10 and adjust the volume to 100 mL by DI water.

Table 3.10 preparation of phosphate buffer solution at various pH

pH	Volume of 0.05 M sodium phosphate dibasic solution (mL)	Volume of 0.05 M sodium phosphate monobasic solution (mL)
5.8	0.4000	4.6000
6.4	1.3250	3.6750
7	3.0500	1.9500
7.6	4.3500	0.6500
8	4.7350	0.2650

3.3.2 Preparation of caffeine solution

3.3.2.1 The 10 mM caffeine stock solution

The 10 mM of caffeine stock solution was prepared by dissolving of 0.1942 g caffeine in 100 mL of DI water.

3.3.2.2 The standard caffeine solution

The different concentration of standard caffeine solutions were prepared by adding the various volumes of 10 mM caffeine stock solutions and dilute to 25 mL by phosphate buffer solution (PBS).

3.4 Development of spectroelectrochemical flow-cell

The spectroelectrochemical flow-cell was designed by SolidWorks 2012 software (Figure 3.1 and Figure 3.2) which is the 3D mechanical-engineering design software. The dimension of this flow-cell is 34 mm × 50 mm × 15 mm. The diameter of the solution flow is 1mm. There are six ports figuration that are five of 5 mm diameter ports and a 9 mm diameter ports. In addition, the path length of optical detection is 10 mm. The flow-cell body is made of poly(methylmethacrylate) or acrylic plastic material which is hard, chemically inert, lightweight, transparent and has good impact strength.

The design of flow-cell can be divided to 2 types of flow-cell configuration that relates with hydrodynamic of flow system. First is wall-jet arrangement, the stream flows perpendicular to the working electrode and the hydrodynamic of turbulent occurs when the stream contacts with the surface of working electrode. This configuration type can increase the sensitivity of measurement but the distance between the working electrode and stream jet must be studied for the optimum distance. Second is thin-layer which is developed in this work. The stream flows parallel to the working electrode and the hydrodynamic of this configuration is laminar flow. Thin-layer configuration can control the stream flow by flow-rate

controlled. In addition, the design of dual response configuration flow-cell, which can measure by spectrophotometry and electrochemical method at the same time in the same detection device can reduce the dead volume between 2 separated detectors. Therefore, this configuration can increase the sensitivity of measurement when compare with the 2 separated detectors configuration.

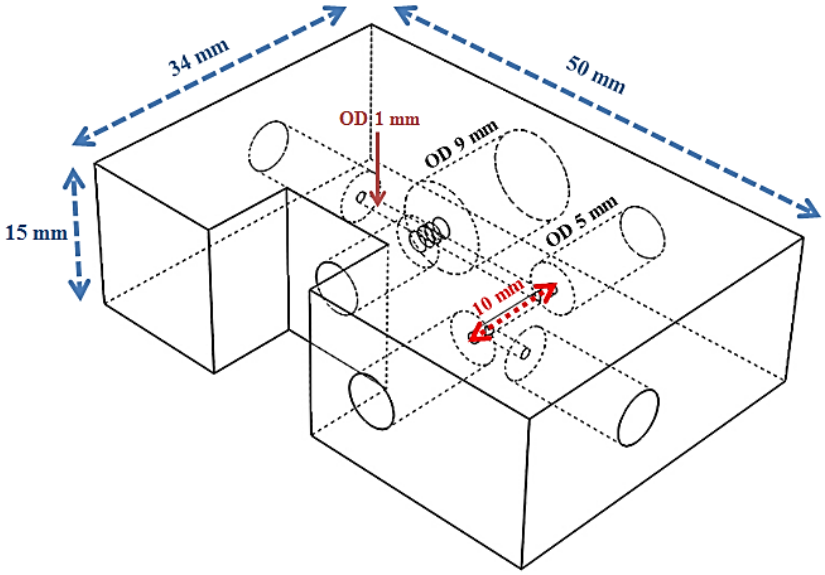

Figure 3.1 The 3D design of spectroelectrochemical flow-cell

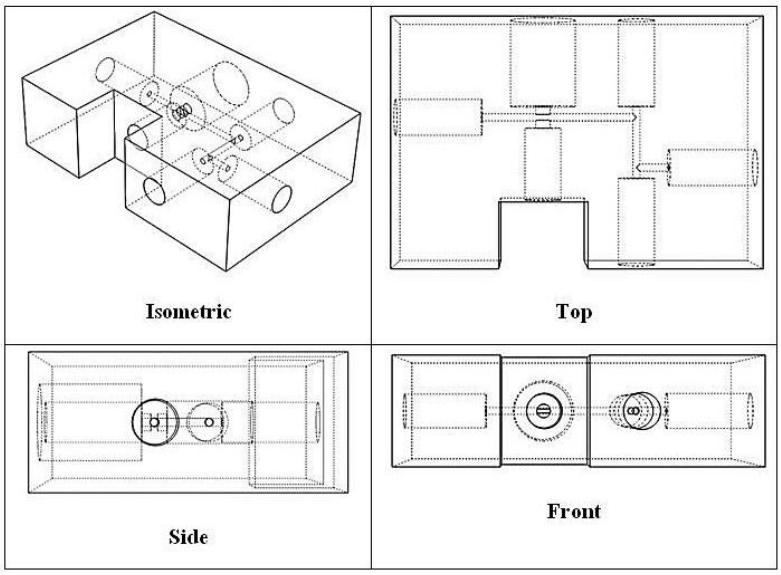

Figure 3.2 The top view, side view and Front view of flow-cell design

The developed spectroelectrochemical flow-cell (Figure 3.3) consists of inlet, outlet, spectrophotometric detection part and electrochemical detection part. There are five 1/4-28 flat-bottom screw ports for a 1/16 inch OD of inlet tubing, two 1.6 mm OD optical fiber probes, a 1.6 mm OD of stainless steel pipe counter electrode which act as the outlet of flow system. They were fit with super flangeless fitting. Another is 1/4-28 screw of Ag/AgCl reference electrode. Moreover, there is a M10 flat-bottom screw port for 3 mm OD which has the surface area diameter of 1 mm glassy carbon working electrode that was fit with PTFE or Teflon nut and 2 mm thickness of silicone seal for preventing of liquid leakage.

The position of the working electrode is next to the reference electrode to reduce IR drop which affects to the potential of electrochemical measurement and the counter electrode is placed on downstream of flow system to ensure that the products from the counter electrode do not interfere the current measurement at the working electrode surface.

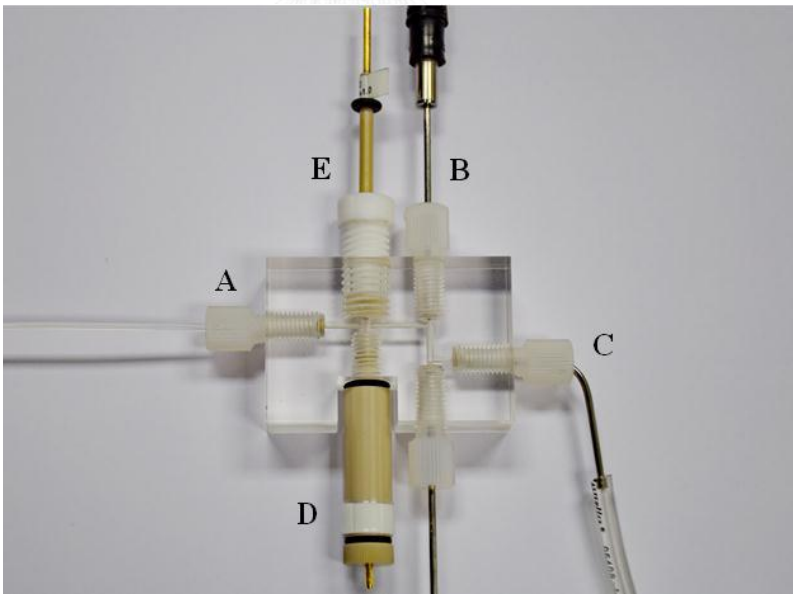

Figure 3.3 Developed spectroelectrochemical flow-cell. (A) inlet, (B) optical fiber probes, (C) outlet/counter electrode, (D) reference electrode and (E) working electrode

3.5 Sequential injection analysis (SIA)

Sequential injection analysis system for spectroelectrochemical analysis is shown in Figure 3.4. There is six ports selection valve that choose the phosphate buffer solution (PBS) carrier or standards and samples to the holding coil by syringe pump force. The solutions were dispensed to the spectroelectrochemical flow-cell which coupled with spectrophotometer and potentiostat for investigation of spectrophotometric and electrochemical response.

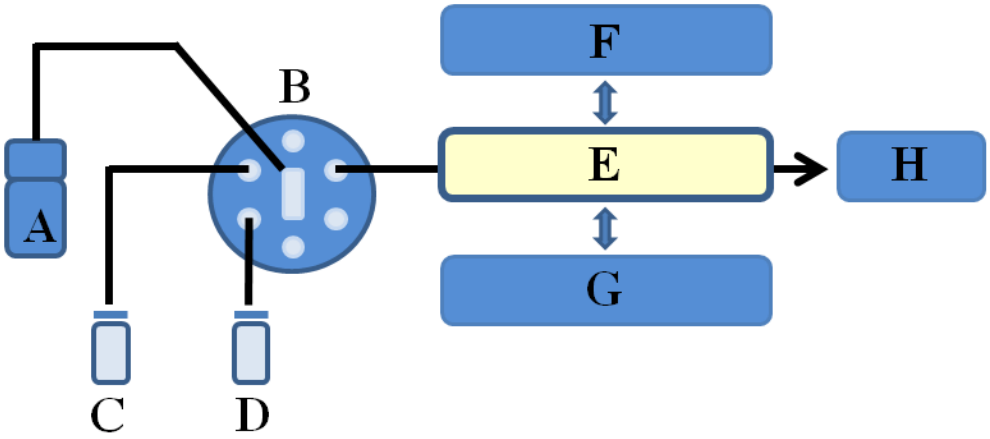

Figure 3.4 Schematic diagram of SIA system which is coupled with spectroelectrochemical flow-cell; (A) syringe pump, (B) selection valve, (C) phosphate buffer solution carrier, (D) standards or samples, (E) spectroelectrochemical flow-cell, (F) spectrophotometer, (G) potentiostat and (H) waste

3.6 Spectrophotometric experiments

The developed spectroelectrochemical flow-cell was coupled with sequential injection analysis (SIA) system and applied to determine the caffeine content by spectrophotometric measurement. The AvaSoft application software was used to control the spectrophotometric measurements. The carrier of 2 mL phosphate buffer solution (PBS) and 0.5 mL of caffeine standards or samples were aspirated to the holding coil, respectively. After that the solutions were dispensed to the developed

flow-cell. The light from AvaLight-DHc light source transmits between the end of two optical fiber probes with the path length of 10 mm and channel diameter of 1mm. The absorption responses of caffeine were measured at the wavelength of 273 nm which provides the highest absorbance signal and recorded by AvaSpec-2048 spectrophotometer.

3.6.1 Optimization of spectrophotometric measurements

3.6.1.1 The pH of phosphate buffer solution (PBS)

The pH of phosphate buffer solution effect was studied for spectrophotometric measurements because the pH can affect the structure of caffeine and the wavelength of caffeine spectrum will shift. The absorbance response of various pH values phosphate buffer solutions at 5.8, 6.4, 7, 7.6 and 8 were investigated.

3.6.1.2 The volume of caffeine

The flow rate of 5 mL/min and the phosphate buffer solution (PBS) volume of 2 mL were fixed in this work. The caffeine volumes of 0.1, 0.25, 0.5, 0.75 and 1 mL were studied. The optimum caffeine volume can provide the highest absorbance results.

3.7 Electrochemical experiments

The electrochemical part in developed flow-cell consists of the 3 mm with 1 mm diameter of glassy carbon working electrode, screwed type Ag/AgCl reference electrode and stainless steel pipe counter electrode. The Nova software was used to control the electrochemical measurements. The carrier of 2 mL phosphate buffer solution (PBS) and 0.5 mL of caffeine standards or samples were aspirated to the holding coil, respectively and the solutions were dispensed to the developed flow-cell. The square wave voltammetry (SWV) technique was chosen for determination

of caffeine and the phosphate buffer solution was used as the supporting electrolyte. In addition, the optimization conditions of square wave voltammetry parameters were studies in this research. The current which occurred at the glassy carbon electrode was measured at 1.4 V vs. Ag/AgCl by PGstat101 Potentiostat.

3.7.1 Optimization of electrochemical measurements

3.7.1.1 The pH of phosphate buffer solution (PBS)

The pH of phosphate buffer solution was studied for electrochemical measurements because the pH can affect the caffeine structure and the number of electron for caffeine oxidation will decrease. The current and peak potential of various pH values of phosphate buffer solutions at 5.8, 6.4, 7, 7.6 and 8 were studied.

3.7.1.2 The volume of caffeine

The flow rate of 5 mL/min and the phosphate buffer solution (PBS) volume of 2 mL were fixed in this work. The current and peak potential that use the various caffeine volumes of 0.25, 0.5, 0.75 and 1 mL were studied. The optimum caffeine volume can provide the highest current results.

3.7.2 Optimization of square wave voltammetry (SWV) parameters

The optimized parameters of square wave voltammetry were studied in this work. The important parameters of square wave voltammetry, which affect the electrochemical response and the caffeine peak potential are step potential and amplitude (Table 3.11).

Table 3.11 The optimized parameters of square wave voltammetry

SWV parameters	Examined values
Step potential	0.004 to 0.01 V
Amplitude	0.01 to 0.06 V

3.8 Analytical performance

3.8.1 Linearity, LOD and LOQ

The linear range for determination of caffeine using spectrophotometry and electrochemical method were investigated. The concentrations of caffeine were varied. The averages of triplicate measurements were used and the calibration curve of each technique was obtained.

The limit of detection (LOD) and the limit of quantification (LOQ) of caffeine determination for spectrophotometry and electrochemical method were calculated from 3SD and 10SD, respectively when the sample blank does not provide the response and SD is the standard deviation from triplicate of low concentration standard caffeine.

3.8.2 Reproducibility

The reproducibility of caffeine determination from the developed flow-cell was studied. The seven replicates of 0.1 mM standard caffeine was used for the reproducibility studied in dual response mode. The reproducibility was reported in the term of relative standard deviation percentage (%RSD).

$$\%RSD = \frac{\text{Standard deviation (SD)}}{\text{Mean}} \times 100$$

3.8.3 Interference study

The main interference species in beverages samples that are glucose, sucrose, fructose and citric acid were tested. The responses of 0.1 mM caffeine and each interference were investigated in dual response mode. The highest concentration of each interference, which provides less than 5% relative error of mixing response and pure caffeine response was obtained.

$$\%\text{Relative error} = \frac{X_i - X_c}{X_c} \times 100$$

Where : X_i is signal of caffeine and interference mixing

X_c is signal of caffeine

3.9 Real sample analysis

3.9.1 Determination of caffeine in energy drinks samples

The samples were prepared by 1 mL of energy drinks were dissolved in 25 mL of phosphate buffer solution (PBS). The carrier of 2 mL phosphate buffer solution (PBS) and 0.5 mL of samples were aspirated to the holding coil, respectively. The solutions were dispensed to the developed flow-cell. The signals of caffeine in samples were obtained.

3.9.2 Determination of caffeine in tea samples

The samples were prepared by 2.5 mL of tea samples were dissolve in 25 mL of phosphate buffer solution (PBS). The carrier of 2 mL phosphate buffer solution (PBS) and 0.5 mL of samples were used for flow-based analysis and the signals of caffeine in samples were obtained.

The caffeine signals of samples from developed flow-cell were compared with cuvette-based configuration standard method and VA stand standard method for spectrophotometry and electrochemical method, respectively. The accuracy was reported in the term of %relative error.

$$\%\text{Relative error} = \frac{X_d - X_s}{X_s} \times 100$$

Where : X_d is the caffeine content of developed flow-cell analysis

X_s is the caffeine content of standard method analysis

The t-test of paired two sample for means was tested in this work to confirm that the amount of caffeine from the developed method analysis was insignificantly different when compare with a standard method. The results of sample analysis from developed flow-cell were compared with cuvette-based configuration standard method and VA stand standard method for spectrophotometry and electrochemical method, respectively.

CHAPTER IV
RESULTS AND DISCUSSION

This chapter presents the results of this research that consists of the optimization parameters, the analytical performance and the real sample analysis.

4.1 Optimization of spectrophotometric measurements

4.1.1 The pH of phosphate buffer solution (PBS)

The 5.8 to 8 pH range of phosphate buffer solution which used as the solvent of caffeine solution was tested in this work. The caffeine concentration of 0.05 mM and caffeine volume of 500 mL were used to studying of pH effect. The spectrophotometric response at various pH was shown in Figure 4.1. The pH can affect the caffeine structure. Therefore, the wavelength of caffeine spectrum was shifted and the absorbance was decreased when the wavelength of caffeine measurement was fixed at 273 nm. The results showed that the absorbance signal was increased when the pH was increased and the absorbance signal was decreased after the pH of 7. Therefore, the pH of 7 provided the highest signal for spectrophotometric measurements.

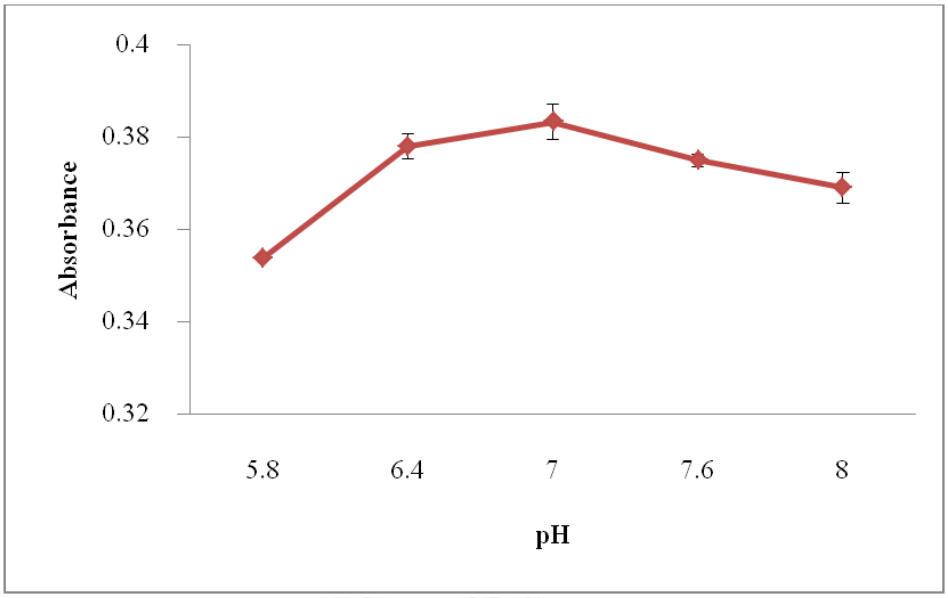

Figure 4.1 The absorbance response effect at various pH of phosphate buffer solution

4.1.2 The volume of caffeine

The flow rate of flow-based system is the volume of the solution per unit time. The flow rate studied is related to the solution volume studied. Therefore, the caffeine volume of 0.1-1 mL was investigated when the flow rate of 5 mL/min and the excess phosphate buffer solution of 2 mL were fixed in this experiment. The caffeine concentration of 0.05 mM at pH 7 were used for caffeine volume studied. The absorbance response of various caffeine volumes were shown in Figure 4.2. The Absorbance of caffeine was increased while the caffeine volume was increased but the peak shape of 0.5 mL was better than the peak shape of 1 and 0.75 mL because the volume of 1 and 0.75 mL were excess and the peak shapes were broad. Therefore, the caffeine volume of 0.5 mL was chosen as the optimized caffeine volume condition for spectrophotometric measurements because the caffeine volume of 0.5 mL provided good peak shape and high absorbance.

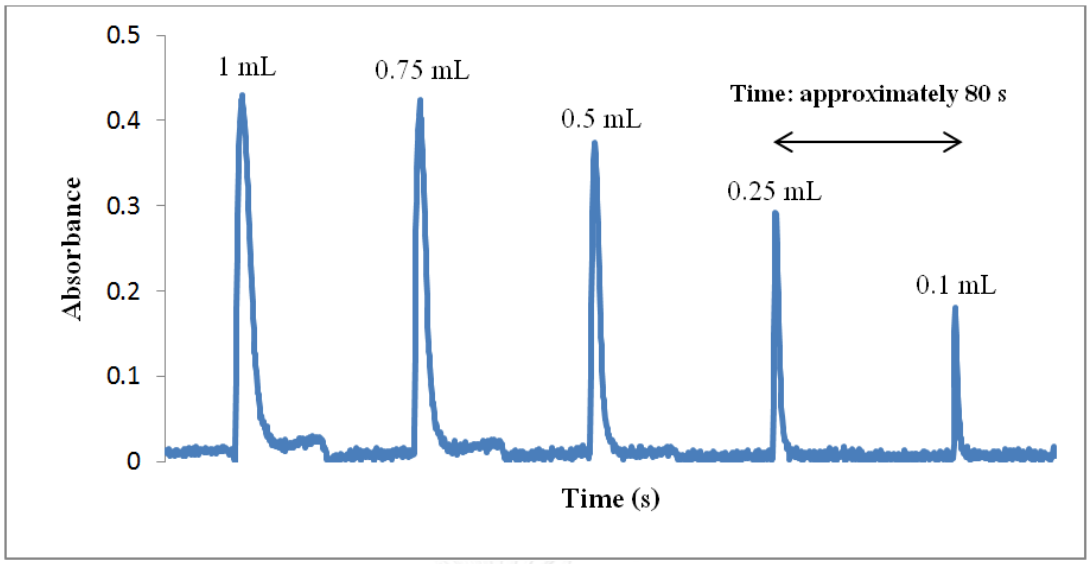

Figure 4.2 The absorbance response at various volumes of caffeine

4.2 Optimization of electrochemical measurements

4.2.1 The pH of phosphate buffer solution (PBS)

The pH of 5.8 to 8 of phosphate buffer solution, which were used as the supporting electrolyte of electrochemical analysis was studied in this experiment. The caffeine concentration of 0.5 mM and caffeine volume of 500 mL were used for pH studied in electrochemical measurements. The electrochemical responses at various pH were shown in Figure 4.3. The pH can affect the caffeine structure and the number of electron for caffeine oxidation was decreased. Therefore, the current was decreased when the number of electron was decreased. The result showed that the current signal was increased when the pH was increased and the current signal was decreased after the pH of 7. Therefore, the pH of 7 provided the highest response for electrochemical measurements.

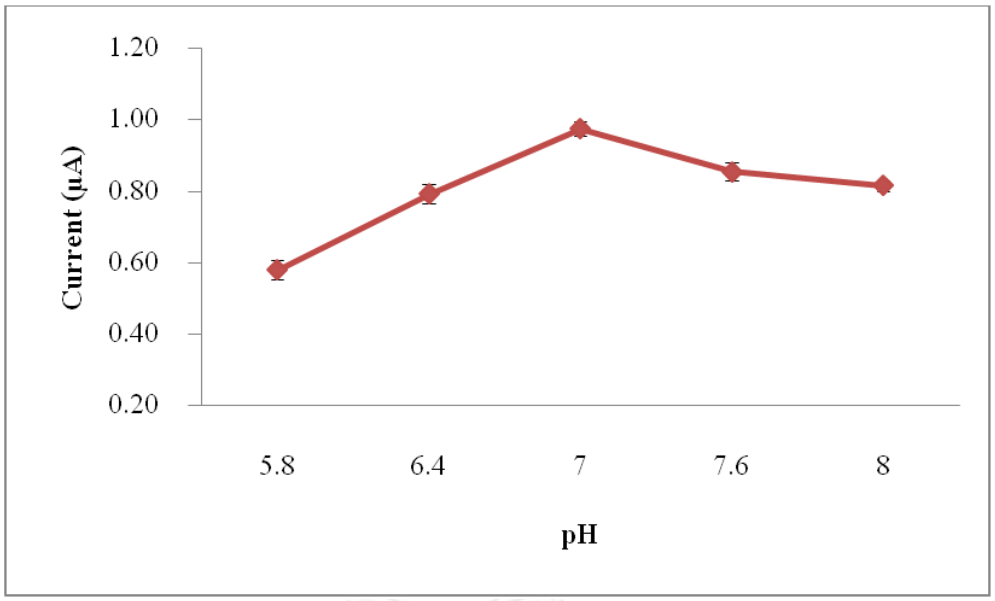

Figure 4.3 The current response effect at various pH of phosphate buffer solution

4.2.2 The volume of caffeine

The caffeine concentration of 0.5 mM and the pH of 7 were used for caffeine volume studied. The caffeine volumes of 0.25-1 mL were investigated when the flow rate of 5 mL/min and the excess phosphate buffer solution of 2 mL were fixed in this work. The current response of various caffeine volumes were shown in Figure 4.4. The current signal was proportional to the volume of caffeine. The current was increased when the caffeine volume was increased but the peak potential of 1 and 0.75 mL shifted to higher potential than the peak potential of 0.5 mL. Therefore, the caffeine volume of 0.5 mL was chosen as the optimized caffeine volume condition for electrochemical measurements because the caffeine volume of 0.5 mL provided the lower peak potential and good peak shape. Moreover, it still provided high electrochemical signal.

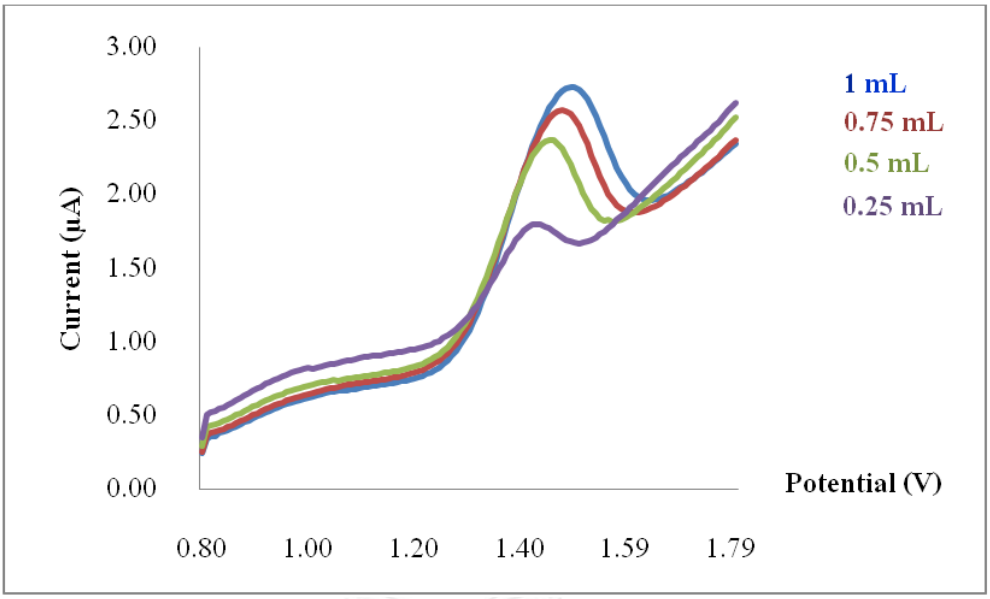

Figure 4.4 The voltammogram of various volume of caffeine

4.3 Optimization of square wave voltammetry (SWV) parameters

4.3.1 Step potential

The step potential parameter of 0.004 V to 0.01 V was investigated for square wave voltammetry (SWV). The caffeine concentration of 0.5 mM, the caffeine volume of 0.5 mL and the pH of 7 were used for step potential studied. The results of electrochemical response were obtained and shown in Figure 4.5 and Figure 4.6. The step potential can affect the current response and the peak potential of caffeine. The results showed that the current was increased when the step potential was increased to the step potential of 0.008 V and the current was decreased after the step potential of 0.008 V. Therefore, the step potential of 0.008 V was chosen for the optimized step potential of square wave voltammetry because the step potential of 0.008 V gave the highest current signal for electrochemical measurements.

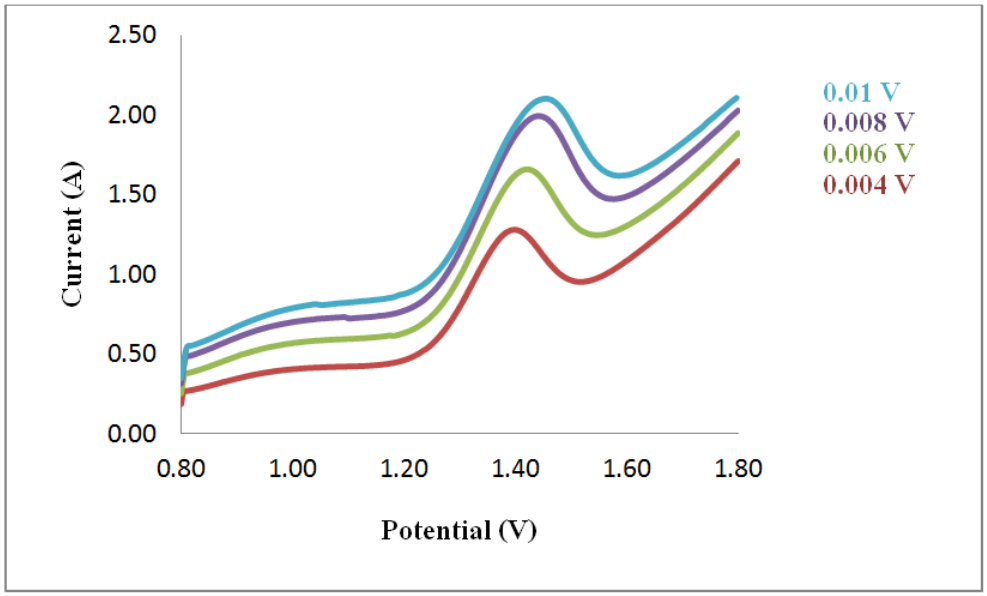

Figure 4.5 The voltammogram of various step potential of square wave voltammetry

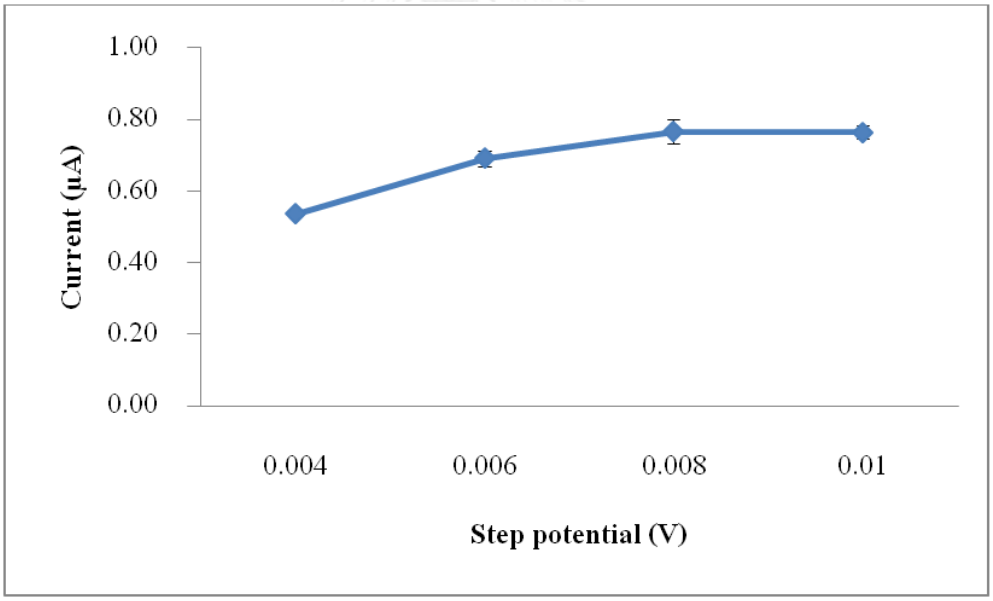

Figure 4.6 The current response effect at various step potential of square wave voltammetry

4.3.2 Amplitude

The amplitude parameter of 0.01 to 0.06 V was investigated for square wave voltammetry (SWV). The caffeine concentration of 0.5 mM, the caffeine volume of 0.5 mL and the pH of 7 were used for amplitude studied. The results of electrochemical response were obtained and shown in Figure 4.7 and Figure 4.8. The amplitude can affect the current response and the baseline of voltammograms. The results showed that the current signal was proportional to the amplitude. The current response was increased when the amplitude was increased. However, the baseline current was proportional to the amplitude too. The current signal of 0.06 V was insignificant different when compared with the current signal of 0.05 V but the baseline current of 0.06 V was higher than the amplitude of 0.05 V. Therefore, the amplitude of 0.05 V was chosen for the optimized amplitude parameter.

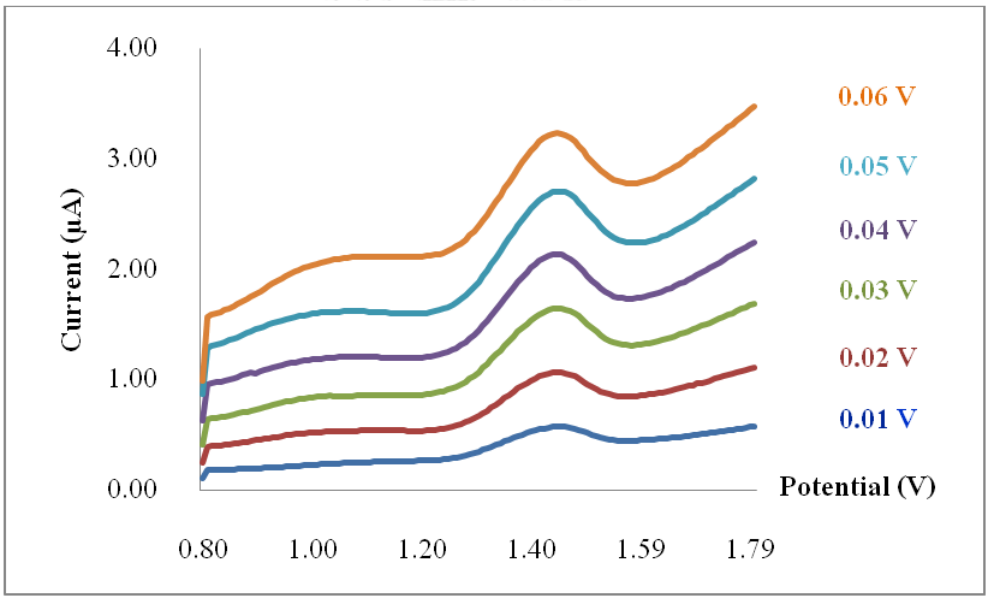

Figure 4.7 The voltammograms of various amplitudes of square wave voltammetry

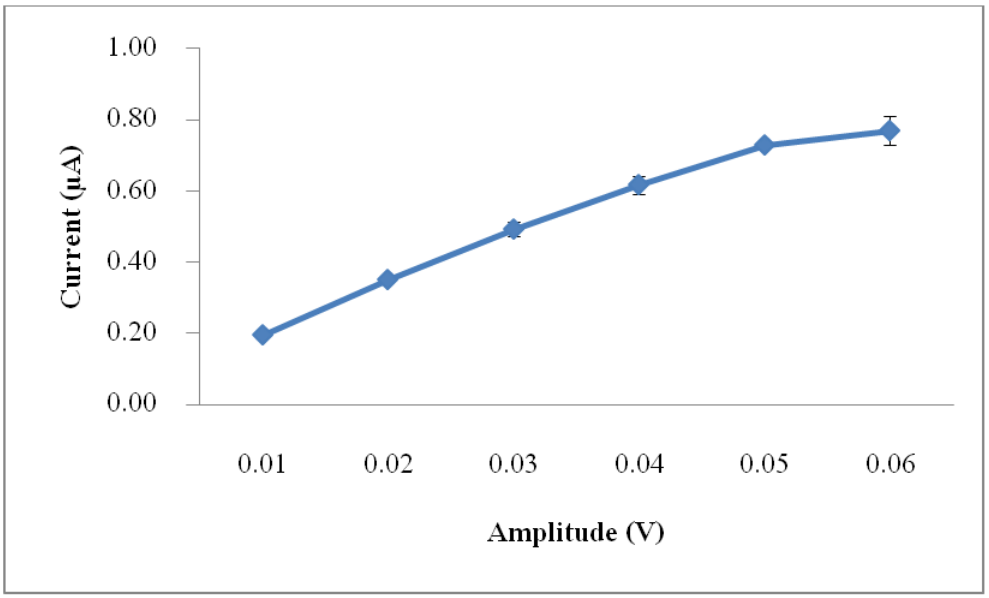

Figure 4.8 The current response effect at various amplitude of square wave voltammetry

4.4 Analytical performance

4.4.1 Linearity, LOD and LOQ

The linearity of caffeine for spectrophotometry was studied. The absorbance signals of various caffeine concentrations were investigated (Figure 4.9) and the calibration curve of caffeine was obtained in Figure 4.10.

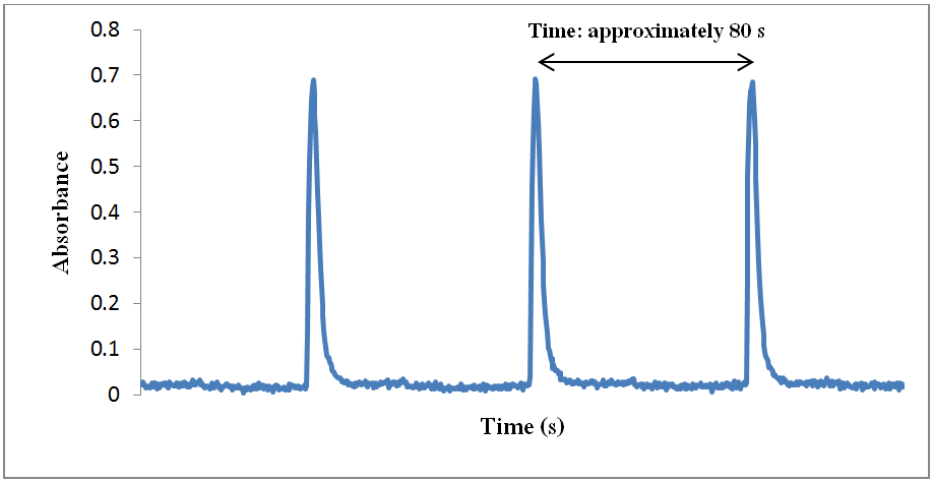

Figure 4.9 The absorbance response of 0.1 mM caffeine

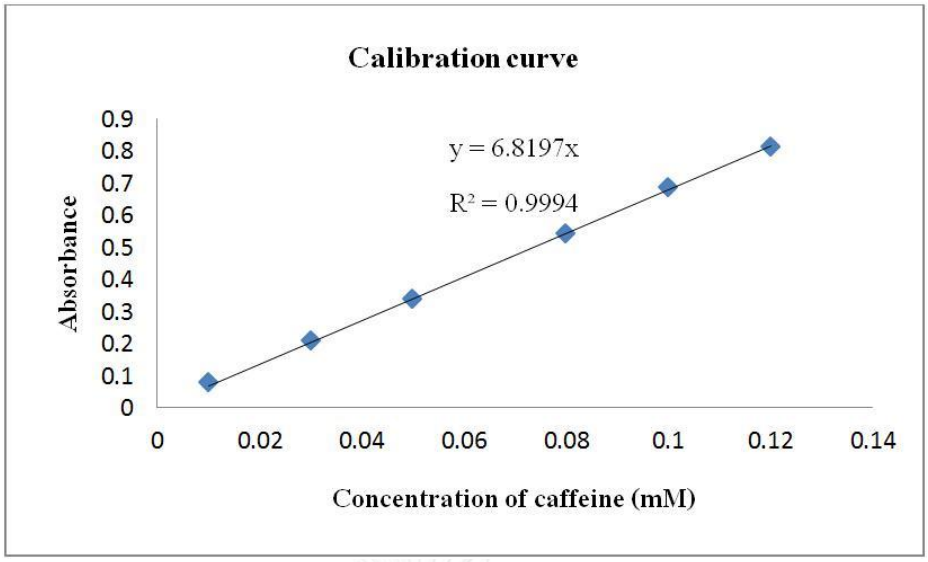

Figure 4.10 The calibration curve of caffeine for spectrophotometry from developed flow-cell

The linearity of caffeine for electrochemical method was studied. The current responses of various caffeine concentrations were investigated (Figure 4.11). The linear range of caffeine determination and the calibration curve of caffeine for electrochemical measurements were obtained in Figure 4.12.

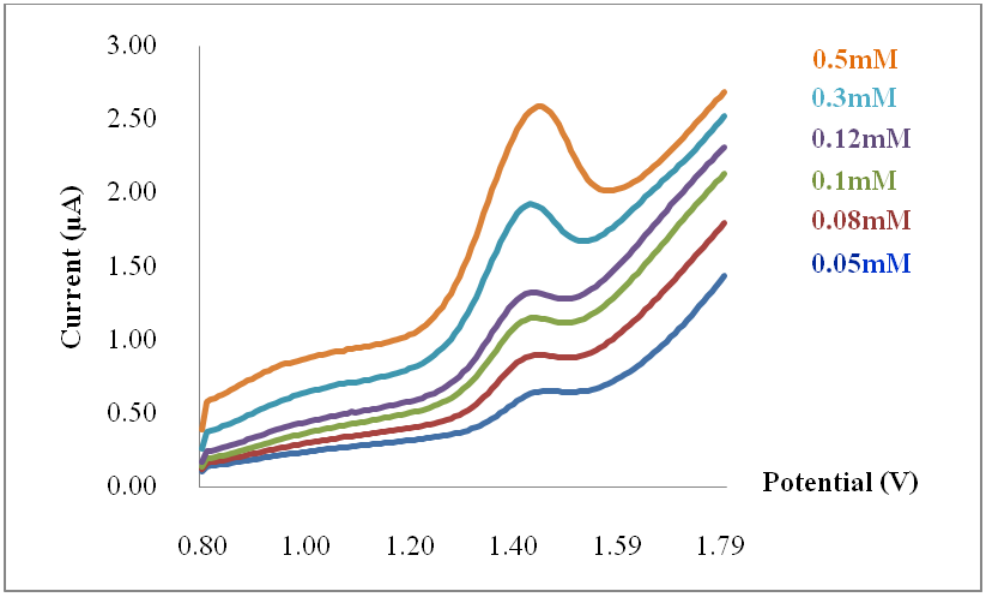

Figure 4.11 The voltammograms at various concentrations of caffeine

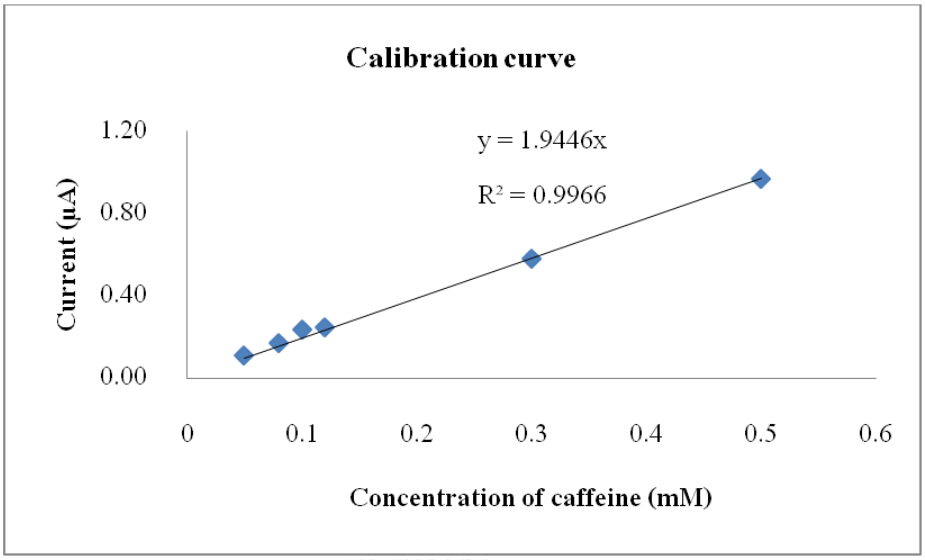

Figure 4.12 The calibration curve of caffeine for electrochemical method from developed flow-cell

The conclusion of validation parameters of spectrophotometry and electrochemical method for caffeine determination from developed spectroelectrochemical flow-cell are showed in Table 4.1.

Table 4.1 The validation parameters of spectrophotometry and electrochemical method from developed flow-cell

Validation parameters	Spectrophotometry	Electrochemical method
Linear range	0.01-0.12 mM	0.05-0.5 mM
R^2	0.9994	0.9966
%RSD	0.42-2.53 %	0.82-3.49 %
LOD	1.00 µM	3.10 µM
LOQ	3.20 µM	10.40 µM

4.4.2 The validation parameters comparison of developed flow-cell and standard method

The calibration curve of cuvette-based configuration flow-cell for spectrophotometry was shown in Figure 4.13 and the validation parameters comparison of developed flow-cell and cuvette-based configuration flow-cell for spectrophotometry was shown in Table 4.2.

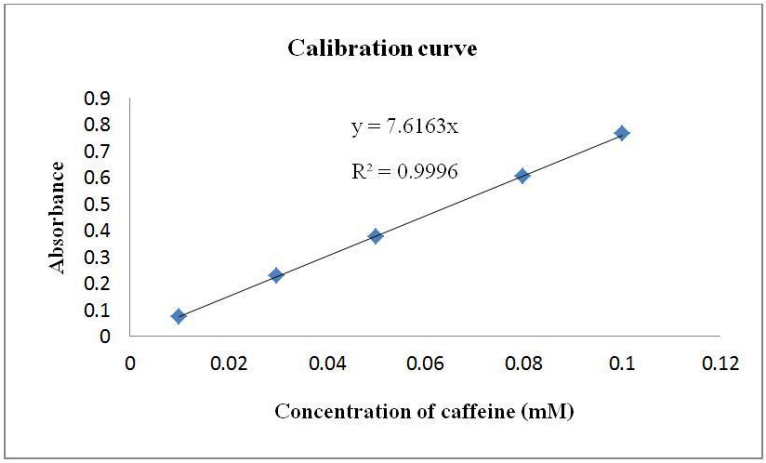

Figure 4.13 The calibration curve of caffeine for spectrophotometry from cuvette-based configuration flow-cell

Table 4.2 The validation parameters comparison of developed flow-cell and cuvette-based configuration flow-cell for spectrophotometry

Validation parameters	Developed flow-cell	Cuvette-based configuration flow-cell
Linear range	0.01-0.12 mM	0.01-0.1 mM
R^2	0.9994	0.9996
%RSD	0.42-2.53 %	0.62-1.37 %
LOD	1.00 µM	0.20 µM
LOQ	3.20 µM	0.70 µM

The calibration curve of VA stand for electrochemical method was shown in Figure 4.14 and the validation parameters comparison of developed flow-cell and VA stand for electrochemical method was shown in Table 4.3.

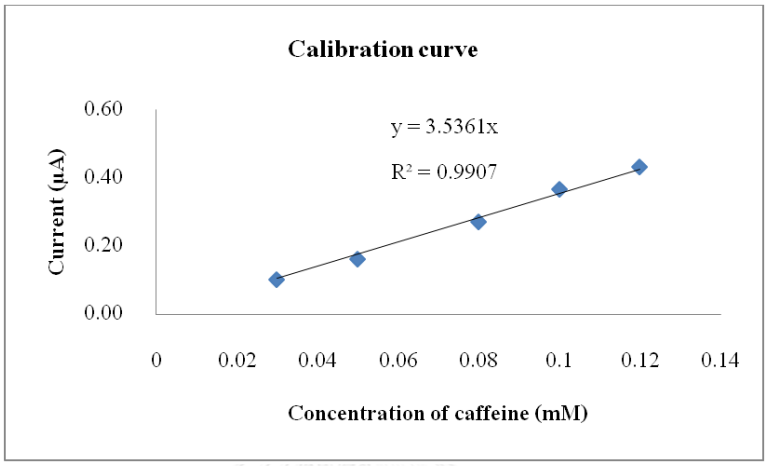

Figure 4.14 The calibration curve of caffeine for electrochemical method from VA stand

Table 4.3 The validation parameters comparison of developed flow-cell and VA stand for electrochemical method

Validation parameters	Developed flow-cell	VA stand
Linear range	0.05-0.5 mM	0.03-0.12 mM
R^2	0.9966	0.9907
%RSD	0.82-3.49 %	1.49-8.57 %
LOD	3.10 µM	3.80 µM
LOQ	10.40 µM	12.60 µM

4.4.3 Reproducibility

The seven replicate of 0.1 mM standard caffeine was used for the reproducibility studied in dual response mode. The caffeine volume of 0.5 mL and pH of 7 were used for reproducibility studied. The reproducibility was reported in the term of relative standard deviation percentage (%RSD). The results showed that the reproducibility of seven replicates (n=7) for spectrophotometry and electrochemical method were 3.21 and 4.98 %, respectively.

4.4.4 Interference study

The responses of 0.1 mM caffeine and each interfere mixing were investigated in dual response mode. The interferences that studied in this work were glucose, sucrose, fructose and citric acid. The highest concentration of each interference which provides less than 5% relative error of mixing response and pure caffeine response was obtained and reported in Table 4.4 and Table 4.5 for spectrophotometry and electrochemical method, respectively.

Table 4.4 The interference study for spectrophotometry

Interferences	Concentration of interferences (mM)	%Relative error
Glucose	30 mM (300 folds)	0.69 %
Sucrose	10 mM (100 folds)	0.64 %
Fructose	10 mM (100 folds)	-2.28 %
Citric acid	10 mM (100 folds)	1.81 %

Table 4.5 The interference study for electrochemical method

Interferences	Concentration of interferences (mM)	%Relative error
Glucose	30 mM (300 folds)	1.27 %
Sucrose	10 mM (100 folds)	2.87 %
Fructose	10 mM (100 folds)	-3.36 %
Citric acid	10 mM (100 folds)	0.37 %

4.5 Real sample analysis

4.5.1 Determination of caffeine in energy drinks samples

The sample 1, sample2 and sample 3 are energy drinks. The electrochemical method could not determine the amount of caffeine in energy drinks because of the interferences matrix in energy drinks. The current signal of caffeine in energy drinks cannot be detected in both developed flow-cell and VA stand analysis. The caffeine in energy drinks was determined by spectrophotometry that compared with cuvette-based configuration flow-cell. The results of caffeine amount in 150 mL of energy drinks were reported in Table 4.6.

Table 4.6 The determination of caffeine in energy drinks by spectrophotometry

	Labeled (mg)	Amount of caffeine (mg) (developed flow-cell)	Amount of caffeine (mg) (cuvette-base flow-cell)	%Relative error
Sample 1	50.00	69.84	71.90	-2.87
Sample 2	50.00	67.15	68.97	-2.64
Sample 3	50.00	67.82	67.68	0.21

4.5.2 Determination of caffeine in tea samples

The sample 4 and sample 5 are tea samples. The spectrophotometry could not determine the amount of caffeine in tea samples because of the interferences matrix in tea samples which provided the overlap spectrophotometric signal with caffeine. The absorbance signals of caffeine in tea samples were more than 1 of absorbance unit both developed flow-cell and cuvette-based configuration analysis. The caffeine in tea samples was determined by electrochemical method that compared with VA stand. The results of caffeine amount in 100 mL of tea samples were reported in Table 4.7.

Table 4.7 The determination of caffeine in tea samples by electrochemical method

	Labeled (mg)	**Amount of caffeine (mg) (developed flow-cell)**	**Amount of caffeine (mg) (VA stand)**	**%Relative error**
Sample 4	12.70	13.87	12.65	9.64
Sample 5	10.30	11.21	11.14	0.63

The t-test of paired two sample for means, which compared between the developed flow-cell and a standard method was tested in this work. The results of samples analysis for spectrophotometry and electrochemical method showed that the t stat was lower than the t critical (two-tail). Therefore, the amount of caffeine in beverages samples results from the developed flow-cell were insignificantly different when compare with a standard method. The results of t-test for spectrophotometry and electrochemical method were shown in Figure 4.15 and Figure 4.16, respectively.

t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.0955667	0.0944556
Variance	1.133E-05	7.353E-06
Observations	9	9
Pearson Correlation	0.7182907	
Hypothesized Mean Difference	0	
df	8	
t Stat	1.4125548	
P(T<=t) one-tail	0.097743	
t Critical one-tail	1.859548	
P(T<=t) two-tail	0.195486	
t Critical two-tail	2.3060041	

Figure 4.15 The t-test result of spectrophotometry

t-Test: Paired Two Sample for Means		
	Variable 1	*Variable 2*
Mean	0.0613	0.0645667
Variance	2.336E-05	5.861E-05
Observations	6	6
Pearson Correlation	0.8663771	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.8934591	
P(T<=t) one-tail	0.05842	
t Critical one-tail	2.0150484	
P(T<=t) two-tail	0.1168401	
t Critical two-tail	2.5705818	

Figure 4.16 The t-test result of electrochemical method

CHAPTER V
CONCLUSIONS AND SUGGESTION FOR FUTURE WORK

5.1 Conclusions

The spectroelectrochemical flow-cell was developed for spectrophotometric and electrochemical properties studied of caffeine in this work. The developed flow-cell is cost-effective, small, chemically inert, lightweight and easy to couple with sequential injection analysis (SIA) system. This combination required a small injection volume for both sample and reagents. It also offered an automation system with a rapid measurement which is very easy to operate. Moreover it is easy to assemble and disassemble the electrodes and optical fiber probes with a standard thread (1/4-28 UNF). The results showed that the developed flow-cell can be used for determination of caffeine for both spectrophotometric and electrochemical measurements in the same detection device. In addition, the developed flow-cell provided a good linearity of calibration curve, a low detection limit and a good reproducibility for both spectrophotometric and electrochemical analysis. The validation parameters comparison of developed flow-cell was approximate with cuvette-based configuration analysis and VA stand for spectrophotometry and electrochemical method, respectively. The developed spectroelectrochemical flow-cell was successfully applied for the determination of caffeine in beverages samples. The concentration range of 0.01-0.12 mM for spectrophotometry and 0.05-0.5 mM for electrochemical method can be used for the determination of caffeine in real samples and the results were insignificantly different when compare with a standard method.

5.2 Suggestion for future work

A chronoamperometry is another interesting electrochemical technique for the determination of caffeine. The combination of this technique with the flow-based system such as SIA is easy and the combined system can be automatically operated. The signal currents are recorded as a time-domain response data plot (chronoamperomertic response) at a constant operating potential throughout the experiment. Because the operating potential has to be kept constant throughout the experiment, so, the bulky and expensive potentiostat can be replaced with a smaller, cheaper electronics regulator or a car battery. In this approach, the whole system can be miniaturized.

In this research, the peak potential of caffeine can be fixed and the current of caffeine measurements were recorded. The chronoamperomertic response of various concentrations caffeine was shown in Figure 5.1. The chronoamperometric response of 0.1 mM caffeine and the calibration curve of caffeine by chronoamperometry were shown in Figure 5.2 and Figure 5.3, respectively.

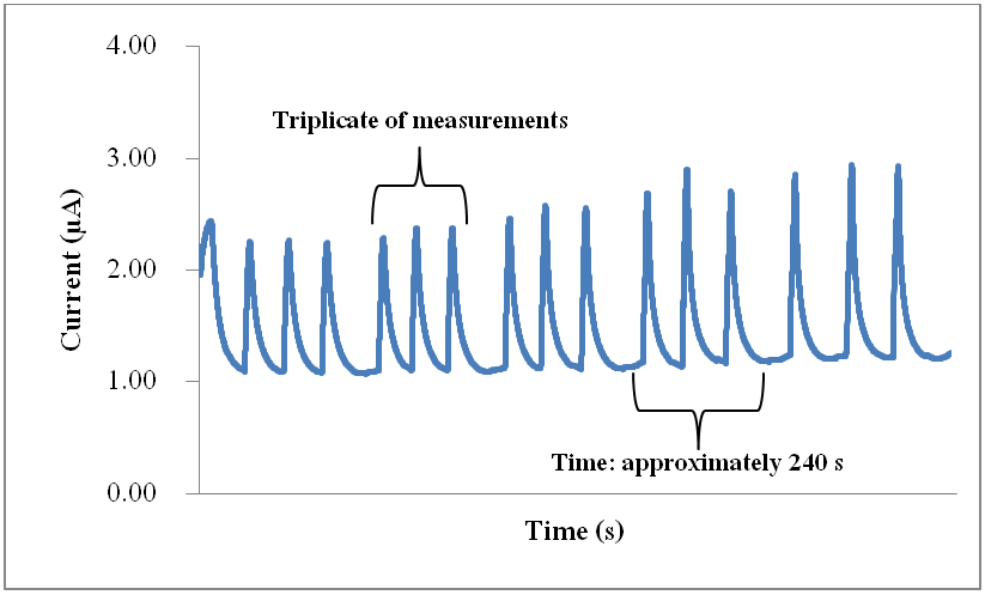

Figure 5.1 The chronoamperometric response of various concentrations caffeine

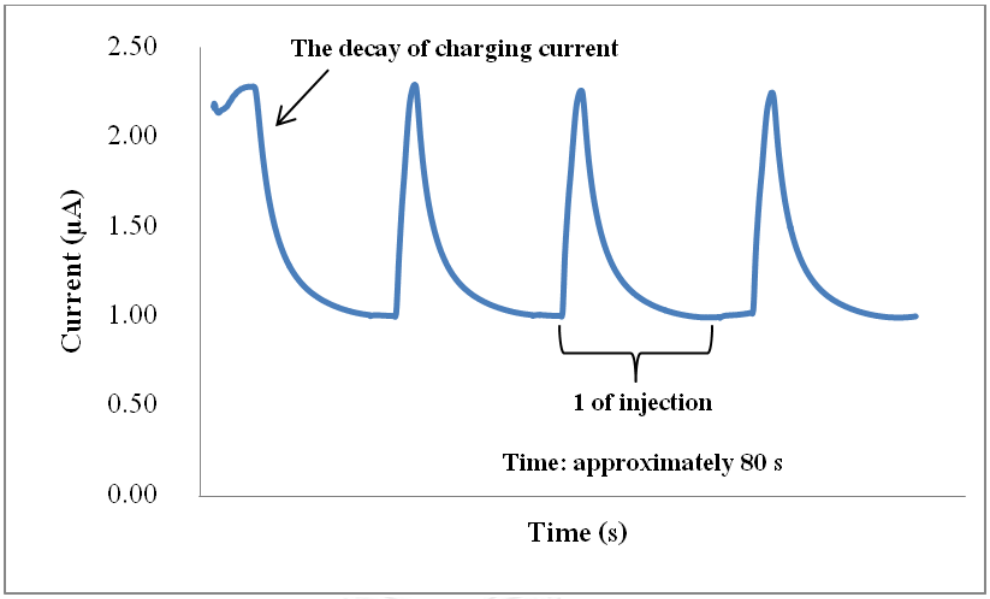

Figure 5.2 The chronoamperometric response of 0.1 mM caffeine

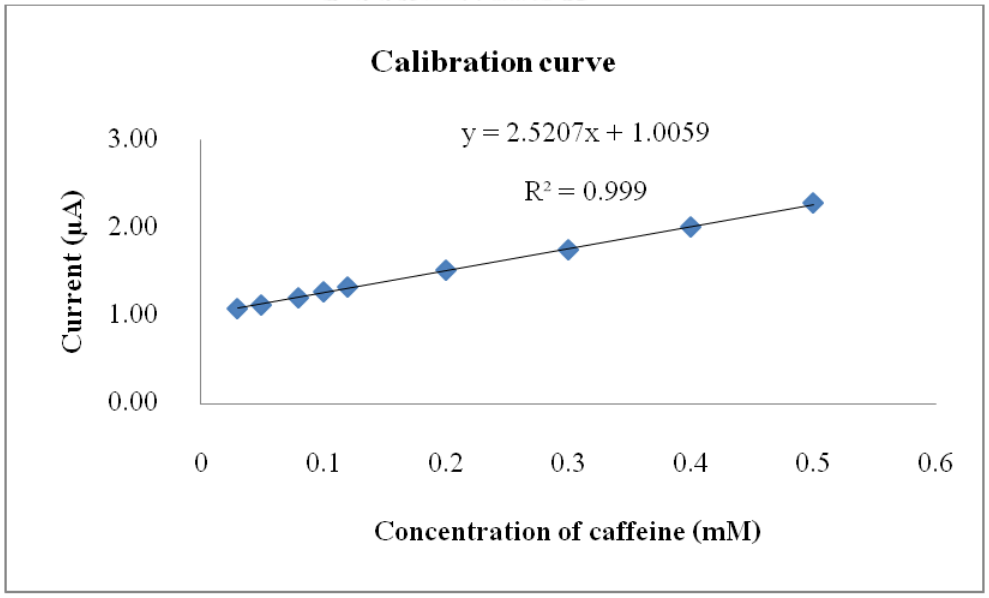

Figure 5.3 The calibration curve of caffeine by chronoamperometry

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APPENDIX

APPENDIX A

Optimization of spectrophotometric and electrochemical measurements

The pH of phosphate buffer solution (PBS)

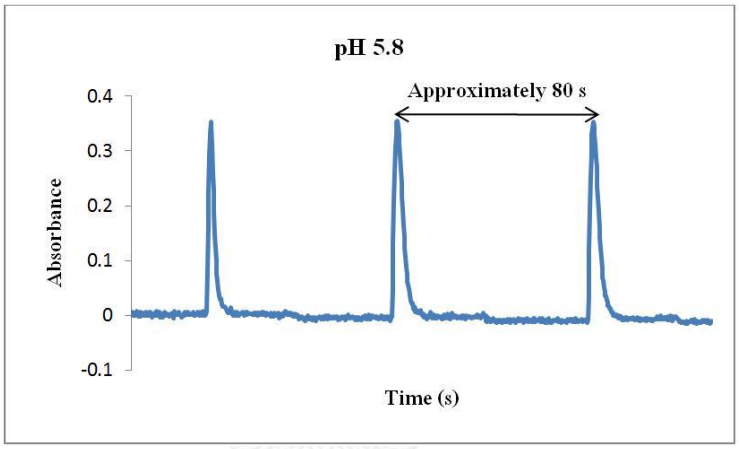

Figure A1 The spectrophotometric response of pH 5.8 at 0.05 mM caffeine

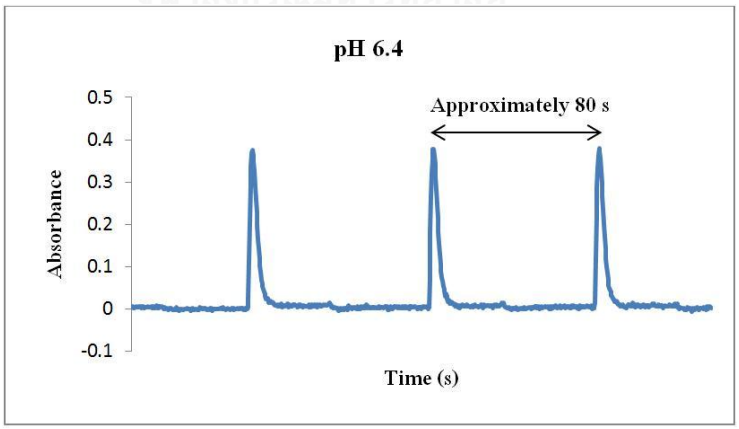

Figure A2 The spectrophotometric response of pH 6.4 at 0.05 mM caffeine

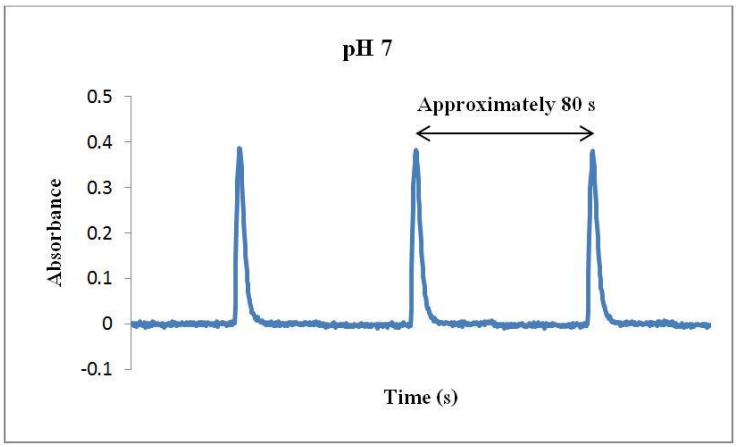

Figure A3 The spectrophotometric response of pH 7 at 0.05 mM caffeine

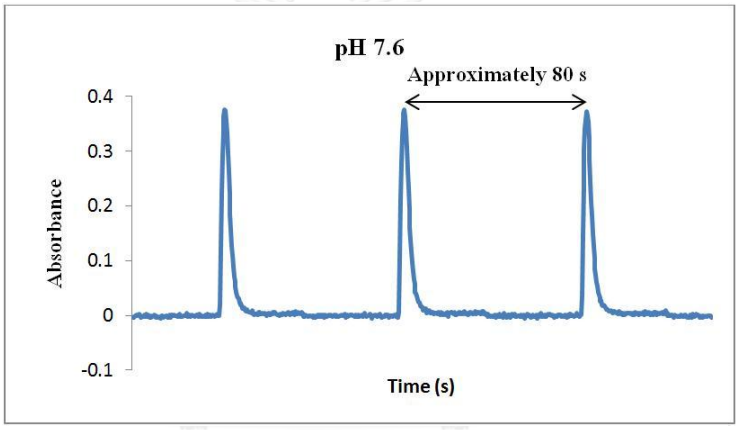

Figure A4 The spectrophotometric response of pH 7.6 at 0.05 mM caffeine

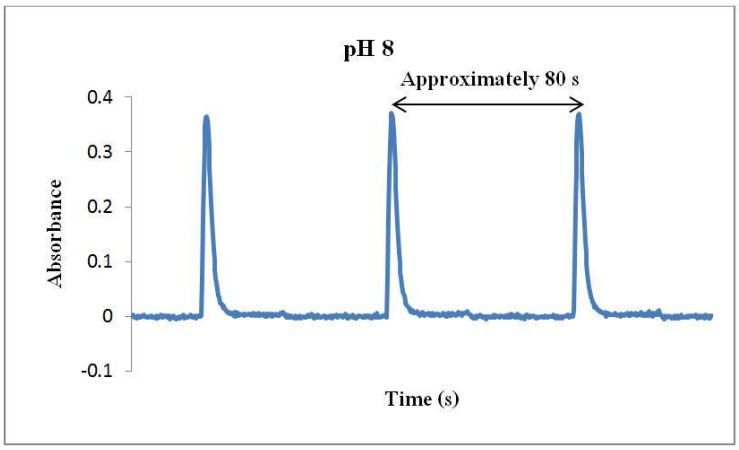

Figure A5 The spectrophotometric response of pH 8 at 0.05 mM caffeine

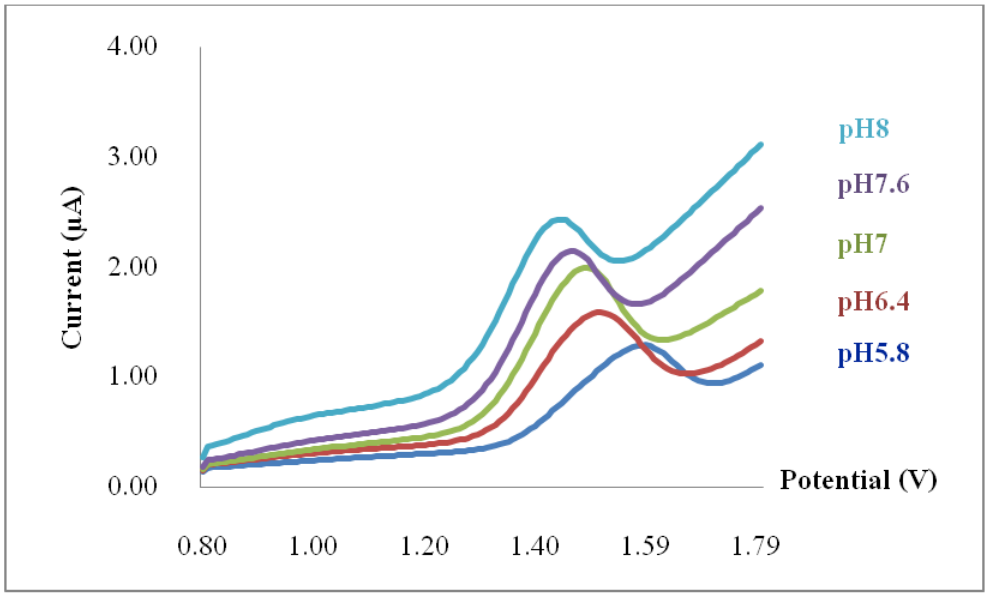

Figure A6 The voltammograms of various pH values at 0.5 mM caffeine

APPENDIX B

Analytical performance

Reproducibility

Table B1 The reproducibility of spectrophotometric measurement

Seven replicates	Average absorbance (n=3)
1	0.7922
2	0.8117
3	0.7489
4	0.7502
5	0.7543
6	0.7586
7	0.7548
Average	0.7671
SD	0.0251
%RSD (n=7)	3.21 %

Table B2 The reproducibility of electrochemical measurement

Seven replicates	**Average current μA (n=3)**
1	0.23
2	0.24
3	0.25
4	0.25
5	0.27
6	0.25
7	0.23
Average	0.24
SD	0.02
%RSD (n=7)	4.98 %

APPENDIX C

Real sample analysis

Table C1 The absorbance response of energy drinks samples

	Average absorbance	**%RSD (n=3)**
Sample 1	0.7406	1.37 %
Sample 2	0.6984	0.16 %
Sample 3	0.7051	1.06 %

Table C2 The current response of tea samples

	Average current (μA)	**%RSD (n=3)**
Sample 4	0.12	2.85 %
Sample 5	0.10	4.92 %

VITA

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Proceeding :

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