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FABRICATION OF PAPER-BASED DEVICES BY SPRAYING WITH LACQUER FOR THE DETERMINATION OF NICKEL

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ธารา นุรักษ์ : การประดิษฐ์อุปกรณ์ปฏิบัติการฐานกระดาษโดยการสเปรย์ด้วยแลกเกอร์สำหรับการตรวจวัดนิกเกิล. (FABRICATION OF PAPER-BASED DEVICES BY SPRAYING WITH LACQUER FOR THE DETERMINATION OF NICKEL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.ดร. อรวรรณ ชัยลภากุล, 68 หน้า

วิธีการสเปรย์ด้วยแลกเกอร์ถูกพัฒนาขึ้น เพื่อใช้ในการประดิษฐ์อุปกรณ์ปฏิบัติการฐานกระดาษ เริ่มต้นจากการนำหน้ากากเหล็กที่ออกแบบไว้วางลงบนกระดาษกรอง และยึดติดกันด้วยแผ่นแม่เหล็กที่วางไว้ด้านตรงกันข้าม หลังจากนั้นสเปรย์อะคริลิคแลกเกอร์ ลงบนกระดาษกรอง เพื่อสร้างพื้นผิวที่ไม่ชอบน้ำ ในขณะที่พื้นผิวที่ชอบน้ำถูกปกปิดด้วยหน้ากากเหล็ก ได้มีการศึกษาภาวะที่เหมาะสมสำหรับการประดิษฐ์อุปกรณ์ได้แก่ สเปรย์แลกเกอร์ชนิดเงาและประสิทธิภาพการเก็บรักษาอนุภาคของกระดาษกรองหมายเลข 4 ได้รับเลือกให้ใช้เป็นชนิดของแลกเกอร์และชนิดของกระดาษกรองที่เหมาะสม ตามลำดับ ในการประเมินประสิทธิภาพของอุปกรณ์ที่พัฒนาขึ้น อุปกรณ์ปฏิบัติการฐานกระดาษถูกนำมาใช้เพื่อการตรวจวิเคราะห์นิกเกิล โดยใช้เทคนิคการตรวจวัดทางเคมีไฟฟ้า ภาวะความเข้มข้นที่เหมาะสมของสารละลายคอปเปอร์ที่ 4.5 ส่วนในล้านส่วน ถูกใช้ในการเพิ่มสภาพไวในการตรวจวิเคราะห์นิกเกิล ภายใต้ภาวะที่เหมาะสมความสัมพันธ์เป็นเส้นตรงอยู่ในช่วง 1 ถึง 50 มิลลิกรัมต่อลิตร ซึ่งให้ค่าสัมประสิทธิ์สหสัมพันธ์ เท่ากับ 0.9971 ขีดจำกัดต่ำสุดของการวิเคราะห์ และขีดจำกัดต่ำสุดของการหาปริมาณ อยู่ที่ 0.5 และ 1.97 มิลลิกรัมต่อลิตร ตามลำดับ นอกจากนี้อุปกรณ์ปฏิบัติการฐานกระดาษร่วมกับการตรวจวัดทางเคมีไฟฟ้า ถูกนำไปใช้ในการตรวจวิเคราะห์นิกเกิลในตัวอย่างน้ำทิ้งจากโรงงานอุตสาหกรรมเครื่องประดับ และเปรียบเทียบค่าที่ได้ด้วยวิธีมาตรฐานอินดักทีฟลีคอปเปิลพลาสมาออปติคอลอิมิสชันสเปกโตรเมทรี ผลที่ได้พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญ ระหว่างวิธีการที่ถูกเสนอขึ้น (4.15±0.043 มิลลิกรัมต่อลิตร) และวิธีการมาตรฐาน (4.06±0.013 มิลลิกรัมต่อลิตร) ดังนั้นวิธีการสเปรย์เป็นทางเลือกที่ดีสำหรับการประดิษฐ์อุปกรณ์ปฏิบัติการฐานกระดาษ เนื่องจากวิธีดังกล่าวมีความง่ายต่อการใช้งาน ราคาถูก และรวดเร็ว

ภาควิชา........เคมี................................ลายมือชื่อนิสิต..

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ปีการศึกษา....2555................................

THARA NURAK: FABRICATION OF PAPER-BASED DEVICES BY
SPRAYING WITH LACQUER FOR THE DETERMINATION OF NICKEL.
ADVISOR : PROF., ORAWON CHAILAPAKUL, Ph.D., 68 pp.

A spraying method with lacquer was developed for the fabrication of paper-based devices. A patterned iron mask was initially placed on a filter paper and held tightly by a magnetic plate placed on the opposite side. After that, acrylic lacquer was sprayed on the filter paper to create hydrophobic area while the hydrophilic area was protected with the iron mask. The optimal conditions for the fabrication of this device were studied including lacquer type and particle retention efficiency of filter paper. Gloss spray lacquer and filter paper No.4 were chosen as optimal lacquer type and particle retention efficiency of filter paper, respectively. To evaluate its efficiency, the paper-based devices were used to determine nickel using electrochemical detection. Cu-enhancer solution was employed to increase sensitivity of nickel determination with the optimal concentration of 4.5 ppm. Under the optimal conditions, linear range was observed in the range of 1 to 50 ppm with a correlation coefficient of 0.9971. The limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 0.5 and 1.97 ppm, respectively. Moreover, these paper-based devices coupled with electrochemical detection were applied to determine nickel in waste water from jewelry factory and compared to those obtained with inductively coupled plasma optical emission spectrometry (ICP-OES). The results indicated that there were no significant variations between this proposed method (4.15±0.043 ppm) and ICP-OES method (4.06±0.013 ppm). Therefore, this spraying method was found to be an excellent alternative for the fabrication of paper-based devices due to its ease of use, low cost and rapidness.

Department : __Chemistry_____   Student's Signature_____

Field of Study : _Chemistry_____   Advisor's Signature_____

Academic Year : 2012_____

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LIST OF ABBREVIATIONS

PDMS	polydimethylsiloxane
ICP-OES	inductively coupled plasma optical emission spectrometry
LOC	Lab-on-a-chip
POC	point-of-care
ng	nanogram
ppb	part per billion
ppm	part per million
IARC	the international agency for research on cancer
AAS	atomic absorption spectrometry
FAAS	flame atomic absorption spectrometry
ETAAS	electrothermal atomic absorption spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
SV	stripping voltammetry
HMDE	hanging mercury drop electrode
SPEs	screen-printed electrode
$C_O{}^0$, $C_R{}^0$	the redox species at the electrode surface
k^o	the rate of the reaction
R	the molar gas constant (8.3144 $J.mol^{-1}.k^{-1}$)
T	the absolute temperature
K	degree Kevin
N	the number of electrons transfer
F	faraday constant (96,485 C/equiv)
E^o	the standard reduction potential for redox couple

O	reduced
R	oxidized
DPV	differential pulse voltammetry
NPV	normal pulse voltammetry
ASV	anodic stripping voltammetry
DMG	dimethylglyoxime
DPASV	differential pulse anodic strippimg voltemmetry
PMMA	polymethylmetacrylate
UV	ultraviolet
NC	Nitrocellulose
AgNPls	silver nanoplates
ELISA	enzyme-linked immunosorbent assay
hCG	human chorionic gohadotropin
WE	working electrode
RE	reference electrode
CE	counter electrode
$m\Omega$	milliohm
M	molar
g	gram
mL	milliliter
mg/L	milligram per liter
V	volt
LOD	limit of detection
LOQ	limit of quantitation
μL	microliter
mV	millivolt

cm	centimeter
s	second
^{o}C	degree Celsius
min	minute
SEM	scanning electron microscope
R^{2}	coefficient
SD	standard deviation
RSD	Relative standard deviation

CHAPTER I

INTRODUCTION

1.1 Introduction

Paper-based device have the potential to be good alternative analytical devices for healthcare related applications because they are portable and easy to use [1]. Moreover, they require small volume of sample and provide rapid analysis [2, 3]. Recently, Whitesides's group has developed paper-based devices from series of operations on a chip (Lab-on-a-chip) [4] and the screening assays (Test kit) [5, 6]. Paper-based device is a device made of paper which the main component is cellulose fiber. Paper is a low-cost material that can be purchased easily. Therefore, paper-based devices are widely popular and have many potential benefits in fields as diverse as environmental monitoring and clinical research. Currently, paper-based devices have become an interesting technology for research units, resulting in the development of method for the fabrication of paper-based devices. A lot of methods for fabricating the pattern on paper have been proposed, including photolithography [7], polydimethylsiloxane (PDMS) plotting [8], inkjet printing [9], cutting [10], plasma etching [11], wax printing [12], wax screen-printing [13], and wax dipping [14]. Each fabrication method has its own advantages and limitations that are similar and/or different. The first reported method was based on photolithography. This method can provide approximately 200 μm of teeny barrier line width between hydrophilic and hydrophobic areas [6]. However, photolithography method concerned the use of organic solvents, expensive photoresists and photolithography apparatus. Moreover, the fabrication process involves many complicated process. The PDMS plotting method also override the problem of physical inflexibility of devices using photolithography. This method does not use expensive photoresists, organic solvent, and photolithography instrument. Unfortunately, this method needs a customized plotter. The inkjet printing method involves the use of organic solvent to print onto the polymer-soaked paper by inkjet printer for creating hydrophilic area on the paper [15]. Plasma etching is a method to detract sizing agent [16] coating on the paper

using plasma treatment [17]. However, paper-based devices created by photolithography, inkjet printing and plasma etching methods still need organic solvents and polymers to create hydrophilic areas. In cutting method, a knife plotter is used to cut paper for creating pattern of microfluidic channels. However, this method has to use tape to cleave the paper pattern and it is difficult in implementation. Wax printing method utilizing a commercially available wax printer for the fabrication of paper-based devices [12]. This method is easy and fast to generate the patterned paper by wax printer. However, this method has lower barrier than photolithography (~850 μm of minimal barrier) [6] because the spread of the wax is difficult to handle when a wax was melted on hotplate. So, careful infiltration of wax must be regarded before creation of the pattern in this method. Wax screen-printing method is similar to wax printing method in the use of a commercial wax for the fabrication of paper-based devices, but wax screen-printing does not require wax printer for fabrication. This method can create a pattern using screen-printed block instead of commercial printer. The advantages of wax screen-printing method are low-cost, simple, and rapid. Nevertheless, wax screen-printing is difficult to produce the exact designed pattern with high barrier due to the spread of the wax as a wax printing. Recently, a new fabrication method for creating paper-based devices has been reported as wax dipping. Wax dipping does not require expensive equipments and organic solvents. However, the hydrophobic areas generated by the wax printing, wax screen-printing and wax dipping methods still use the hot plate for melting wax [14]. Moreover, limitations of these previous methods by wax are to consider wax spreading before creating the pattern and to require trained personnel for using and maintaining instruments. To overcome these limitations, a simple, rapid and low-cost fabrication method, that also provides several advantages, needs to be developed. Therefore, spraying method with lacquer was firstly developed for the fabrication of paper-based devices.

Acrylic lacquer is made of acrylic resin. One of the main characteristic features of acrylic resin is high transparency. Acrylic lacquer is one of polymer (resin) generated through chemical reaction by applying polymerization. The advantages of acrylic resin are water resistance, good adhesion and fast dry.

1.2 Objective of the research

The three main goals of this work are as follows:

1. To develop an easy, affordable, and simple method for fabrication of paper-based devices by spraying with lacquer.

2. To optimize experimental conditions for the determination of nickel using paper-based devices coupled with electrochemical detection.

3. To apply paper-based devices for the determination of nickel in real sample.

1.3 Scope of the research

To achieve the objective of this work, spraying method with lacquer was used to fabricate the pattern of paper-based devices. Three electrodes for electrochemical detection were screened on paper-based devices by screen-printing method. To optimize type of lacquer, three types of acrylic lacquer were investigated, including paint lacquer, flat spray lacquer and gloss spray lacquer. The particle retention efficiency of filter paper was studied using whatman filter paper No. 1 and No. 4. The influences of amount of graphite, supporting electrolyte, deposition potential, deposition time, modulation amplitude, modulation time, potential step, and concentration of Cu-enhancer solution were examined to obtain the optimal conditions of this experiment. To evaluate efficiency of this developed method, paper-based devices coupled with electrochemical detection were used to determine nickel in waste water from jewelry factory sample. The determination of nickel in real sample was carried out using the optimal conditions. In addition, the results of this proposed method was validated by ICP-OES method.

CHAPTER II

THEORY AND LITERATURE SURVEY

2.1   Lab-on-paper or paper-based devices

Lab-on-a-chip (LOC) has been developed as an analytical device which is a device made of a silicon, glass, or plastics and the fabrication method involves many complicated process as lithography based clean-room substructure [18]. However, it is more simplified than conventional analytical equipments.

Currently, lab-on-paper has becomes an alternative device technology for research unit. It is widely used in clinical laboratories as called paper tests or strip tests [19] and diabetes strip test [20] (Figure. 2.1). In 2007, Whitesides's group has invented paper-based analytical device [4]. that have attractive character including low cost, ease of use, low handing of reagent, portability, and disposability [5]. The main concept of lab-on-paper is a device made of paper which the main component is cellulose fiber. This device provides several advantages [21] as follows ;

- Inexpensive and many benefits
- Easy to chemically modified
- flammable and biodegradable
- flexible and compatible

Figure 2.1 Paper strip tests (a) pregnancy strip test [22] (b) diabetes strip test [23]

Due to these attractive and useful properties, lab-on-paper device can be considered as disposable diagnostic tests which are simple and fast. It is suitable for portable point-of-care (POC) diagnostics and on-site detection.

There are several methods for fabricating paper-based devices including, photolithography, polydimethylsiloxane (PDMS) plotting, inkjet printing , cutting, plasma etching, wax printing, wax screen-printing, and wax dipping. Each fabrication method has its own advantages and limitations that are similar and/or difference shown in Table 2.1

2.2 Fabrication method of paper-based device

Table 2.1 Methods for patterning paper [21]

Method	Advantages	Disadvantages
Photolithography	A wide variety of papers pattern up to 360 μm in width.	Emergence of polymers and solvents in hydrophilic areas

Method	Advantages	Disadvantages
PDMS plotting	Flexible hydrophobic barrier	Requirement of customized plotter
Ink-jet printing	Reagents can be printed directly into the test zones using the printer.	Requirement of customized inkjet printers ; Emergence of polymers and solvents in hydrophilic areas
Cutting	Hydrophilic channels are not exposed to polymers or solvents.	Devices must be encased in tape.
Plasma treatment (Etching)	Useful for laboratories equipped with a plasma cleaner	Emergence of polymers and solvents in hydrophilic areas
Wax printing, wax screen-printing and wax dipping	Simple and low cost	Spread of the wax in the paper ; requirement of commercial available hot plate

2.3 History of lacquer in Asia [24]

Lacquer is a natural polymer collected from lacquer trees. It has durability and beauty and has been used in Asia for a thousand years. Lacquer was first used as an adhesive for fixing gold foil, chipped porcelain, or attaching arrowheads to the wooden shaft. Then, lacquer was applied to bamboo, wood, and other furniture. It is still used in daily life for crafts and industrial equipment. Collecting lacquer sap is like collecting gum from a rubber tree. The sap of lacquer is collected from the 5 to

10-year-old lacquer trees between June and October. The sap is an emulsion of water in oil, which is called raw lacquer. After stirring to homogenize, it was heated to evaporate the water. Fine lacquer, is also called *kurome* lacquer in Japanese, is produced. In Asian countries, lacquer sap is mainly obtained from the lacquer trees of *Rhus vernicifera* in China, Japan, and Korea, *Rhus succedanea* in Vietnam and Taiwan, and *Melanorrhoea usitata* in Myanmar and Thailand. The differences of constituents and properties of the lacquers are not only with the species but also with the age of trees, place grown, and season of collection. The constituents are lipid components (60-70%), water (20-30%), plant gum (4-10%), the enzyme laccase (1.5-2%), and water-insoluble glycoprotein (3-5%).

2.4 Nickel Allergy [25]

Nickel allergy in the form of contact dermatitis is the most common reaction. Although the accumulation of nickel in the body through chronic exposure can lead to lung fibrosis, cardiovascular, and kidney diseases, the most serious concerns relate to nickel's carcinogenic activity which is reviewed below for more detail in regard to its human epidemiology, experimental animal models, and postulated molecular mechanisms. Allergic contact dermatitis caused by contacting with lacquer sap and lacquerware can affect the welfare of lacquer workers and the lacquerware industry. Many studies of the mechanism of lacquer allergy, including animal models, have been carried out and have established several hypotheses.

2.5 Nickel

2.5.1 Chemistry of nickel [26]

Nickel was discovered in 1751. It is found in the first transition series group VIII of the periodic table. There are five natural isotopes, and ^{58}Ni (68.27%) and 60Ni (26.10%) are the most abundant. An important isotope for biophysical studies is 61Ni which has a nuclear spin of 3/2. 61Ni labeling has been used to determine the presence of nickel in the active sites of several microbial enzymes. In addition, 63Ni, a beta emitter with a half-life of 100.1 years, has been used in

biological tracer studies. Nickel is found in one of several oxidation states, ranging from −1 to +4. However, the +2 oxidation state is the most predominant form of nickel in biosystems. Solubilized Ni^{2+} ions in aqueous media at neutral pH are hydrated to the greenish hexahydrated $[Ni(H_2O)_6]^{2+}$. The classical test for the detection of nickel is the reaction with alcoholic dimethyl glyoxime solution that can cause the formation of a scarlet chelate.

2.5.2 Environmental exposure [27, 28]

Because of unique chemical properties, metallic nickel and its compounds are important in modern industry and are widely used in many products as electroplating, electroforming. Moreover, they was used for production of nickel-Cadmium batteries and electronic equipment. Nickel alloys, like stainless steel, are mostly used in the production of tool, machinery, armament, and appliances. They are also used to cast coin, and to produce jewelry. The sources of environmental nickel contamination include the production and processing of nickel. Nickel compounds are also found in soils and are present in both insoluble forms, such as sulfides and silicates, and in a number of soluble forms. Nickel is also present in the atmosphere, and the species of nickel present depend on the source of contamination. From anthropogenic sources, nickel is emitted as oxides, sulfides, silicates, soluble compounds, and metallic nickel. Combustion of fossil fuels produces the greatest contribution of nickel compounds in ambient air. Therefore, the atmospheric concentration of nickel in industrialized areas has been estimated to be in the range of 120–170 ng/m^3, and 6–17 ng/m^3 in suburban areas. Direct leaching from rocks and sediments can produce significant concentrations of nickel in water where it is presented in dissolved forms as well as suspended insoluble particles. Nickel concentration in deep-sea water is usually in range from 0.1 to 0.5 ppb Ni, whereas surface water contains 15–20 ppb Ni.

2.5.3 Carcinogenic effects of nickel in humans [28, 28, 30]

The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of nickel in 1990. All nickel compounds except for metallic nickel are classified as carcinogenic to humans. The high consumption of nickel-containing products inevitably leads to environmental pollution by nickel and its by-products at all stages of production, recycling and disposal. Human exposure to nickel primarily occurs via inhalation and ingestion. Significant amounts of nickel in different forms may be deposited in the human body through occupational exposure and diet over a lifetime. Since nickel has not been recognized as an essential element in humans, it is not clear how nickel compounds are metabolized. However, that exposure to nickel compounds can have adverse effects on human health. Nickel allergy in the form of contact dermatitis is the most common and well-known reaction. Although the accumulation of nickel in the body through chronic exposure can lead to lung fibrosis, cardiovascular and kidney diseases, the most serious concerns relate to nickel's carcinogenic activity. The International Committee on Nickel Carcinogenesis in Man suggested that respiratory cancer risks are primarily related to exposure to soluble nickel concentrations above 1 mg/m^3 and to exposure to less soluble forms at concentrations above 10 mg/m^3. The committee was unable, however, the determination with confidence the level at which nickel exposure becomes a substantial hazard. Approximately 2% of the work force in nickel-related industries is exposed to airborne nickel-containing particles in concentrations ranging from 0.1 to 1 mg/m^3.

2.5.4 Conventional method for the determination of nickel [31]

The most common methods used to determine nickel in food, biological, and environmental matrixes are atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and voltammetry. All these methods require suitable sample pre-treatment to destroy the organic molecules that cause interferences. Particularly, flame atomic absorption spectrometry (FAAS) is subjected to less interferences respect to electrothermal

atomic absorption spectrometry (ETAAS) or inductively coupled plasma mass spectrometry (ICP-MS), but the use of a preconcentration step is required to reach an appropriate level of sensitivity. ICP-MS shows very low limits of detection but this method is very expensive for instrumentation costs and its maintenance. Moreover, several stripping voltammetry (SV) methods for trace nickel determination in various matrixes have been proposed using their organometallic complexes adsorbed onto the hanging mercury drop electrode (HMDE).

2.6 Electroanalytical method [32]

Electrochemistry is a branch of chemistry concerned with the interrelation of electrical and chemical effects. A large part of this field deals with the study of chemical changes caused by the passage of an electric current and the production of electrical energy by chemical reactions. In fact, the field of electrochemistry encompasses a huge array of different phenomena (e.g., electrophoresis and corrosion), devices (electrochromic displays, electro analytical sensors, batteries, and fuel cells), and technologies (the electroplating of metals and the large-scale production of aluminum and chlorine).

Scientists make electrochemical measurements on chemical systems for a variety of reasons. They may be interested in obtaining thermodynamic data about a reaction. They may want to generate an unstable intermediate such as a radical ion and study its rate of decay or its spectroscopic properties. They may seek to analyze a solution for trace amounts of metal ions or organic species. In these examples, electrochemical methods are employed as tools in the study of chemical systems in just the way that spectroscopic methods are frequently applied. There are also investigations in which the electrochemical properties of the systems themselves are of primary interest, for example, in the design of a new power source or for the electrosynthesis of some product. Many electrochemical methods have been devised. Their application requires an understanding of the fundamental principles of electrode reactions and the electrical properties of electrode-solution interfaces.

2.6.1 The three electrode system [33]

The three electrode system is important in voltammetry. The three electrode system consists of the working electrode, reference electrode, and the counter electrode. All three of these electrodes serve a unique roll in the three electrode system.

2.6.1.1 Working electrode

The working electrode is the electrode that occurs the interesting reaction. In the voltammetric method, the small surface area of the working electrode is used for enhancing polarization and minimizing depletion of the analyte. Common working electrodes can consist of inert metals such as gold; silver or platinum inert carbon such as glassy carbon or pyrolytic carbon, and mercury drop and film electrodes.

Carbon electrodes allow to scan more negative potential than platinum or gold electrode in the anodic potential because the rate of the electron transfer on the carbon surface electrode is slower than on the metal electrode, and type of carbon electrode such as glassy carbon, carbon fiber, screen-printed carbon, carbon film, and carbon paste electrode have been proposed to increase the electron transfer rate.

2.6.1.2 Reference electrode

Reference electrode is an electrode which has a stable and well-known electrode potential. The potential applied to the working electrode is measured within the context of a known potential, which is in turn obtained from the reference electrode. The high stability of the electrode potential is usually reached by employing a redox system with constant (buffered or saturated) concentrations of each participants of the redox reaction. The most widely used is the silver/silver chloride reference electrode because it is simple, low-cost, stable and non-toxic

2.6.1.3 Counter electrode

Counter electrode, is an electrode used in electrochemical detection. In a two-electrode system, either a known current or potential is applied between the working and counter electrodes and the other variable may be measured. When a three electrode cell is used to perform electroanalytical chemistry, the counter electrode, along with the working electrode, provides circuit over which current is either applied or measured. In this place, the potential of the auxiliary electrode is usually not measured and is adjusted to so as to balance the reaction occurring at the working electrode. The counter electrode often has a surface area much larger than the working electrode to ensure that the half-reaction at the counter electrode can occur fast enough so as not to limit the process at the working electrode. Counter electrodes are often fabricated from electrochemically inert materials such as gold, platinum, or carbon.

2.7 Screen-printed electrodes [34]

In recent years, a lot of the methods developed with have been based on the use of many electrodes due to their high sensitivity and selectivity, portable field-based size and low-cost. Since the 1990s, screen-printing technology is a technique which holds great promise for on-site monitoring. Therefore, the use of screen-printing technology in the serial production of disposable low-cost electrodes for the electrochemical determination of a wide range of substances is currently undergoing wide spread growth. Screen-printed electrodes (SPEs) are devices that are produced by printing different inks on various types of plastic or ceramic substrates. Polyester screens are generally used for printing with patterns designed by the analyst in accordance with the analytical purpose in mind. The composition of the various inks used for printing on the electrodes is determined by the selectivity and sensitivity required for each analysis. Alternatively, a wide variety of devices of this type are commercially available. The great versatility presented by the SPEs lies in the wide range of ways in which the electrodes may be modified. The composition of the printing inks may be altered by the addition of very different substances such as metals, enzymes, polymers, complexing agents, etc. On the other hand, the possibility

also exists of modifying the manufactured electrodes by means of depositing various substances on the surface of the electrodes such as metal films, polymers, enzymes, etc.

2.8 Voltammatric method [35]

Electroanalytical methods, particularly stripping analysis, are the most widely used that compete with atomic spectroscopy or other techniques, as far as trace analytes determination is concerned. In voltammetry, the effects of the applied potential and the behavior of the redox current are described by several well-known laws. The applied potential controls the concentrations of the redox species at the electrode surface ($C_O{}^0$ and $C_R{}^0$) and the rate of the reaction (k^0), as described by the Nernst or Butler– Volmer equations, respectively. In the cases, where diffusion plays a controlling part, the current resulting from the redox process as call as the faradaic current is related to the material flux at the electrode–solution interface and is described by Fick's law. The interplay between these processes is responsible for the characteristic features observed in the voltammograms of the various techniques. For a reversible electrochemical reaction which can be described by $\mathbf{O} + n\mathrm{e}^-$ $\mathbf{R}$, the application of a potential E forces the respective concentrations of $\mathbf{O}$ and $\mathbf{R}$ at the surface of the electrode (that is, $c_O{}^0$ and $c_R{}^0$) to a ratio in compliance with the Nernst equation:

$$E = E^0 - \frac{RT}{nF} \ln \frac{c_R^0}{c_O^0}$$

where R is the molar gas constant (8.3144 $J.mol^{-1}.K^{-1}$), T is the absolute temperature (K), n is the number of electrons transferred, F = Faraday constant (96,485 C/equiv), and E^0 is the standard reduction potential for the redox couple. If the potential applied to the electrode is changed, the ratio $c_R{}^0/c_O{}^0$ the surface will also change so as to satisfy Equation. If the potential is made more negative, the ratio

becomes larger (**O** is reduced) and, conversely, if the potential is made more positive, the ratio becomes smaller (**R** is oxidized).

2.9 Differential pulse [36]

Differential Pulse Voltammetry (DPV) is an extremely useful technique for measuring trace levels of organic and inorganic species. This technique is comparable to normal pulse voltammetry in that the potential is also scanned with a series of pulses. However, it differs from NPV because each potential pulse is fixed of small amplitude (10 to 100 mV), and is superimposed on a slowly changing base potential. Current is measured at two points for each pulse, the first point (1) just before the application of the pulse and the second (2) at the end of the pulse. These sampling points are selected to allow for the decay of the nonfaradaic (charging) current. The difference between current measurements at these points for each pulse is determined and plotted against the base potential. The potential wave form for differential pulse voltammetry is shown in Figure 2.2. The important parameters for differential pulse voltammetry are as follows. The sensitivities of differential pulse voltammetry are better than those of normal pulse voltammetry. Pulse amplitude is the height of the potential pulse, which may or may not be constant depending upon the technique.

(i) Pulse width is the duration of the potential pulse.

(ii) Sample period is the time at the end of the pulse during which the current is measured.
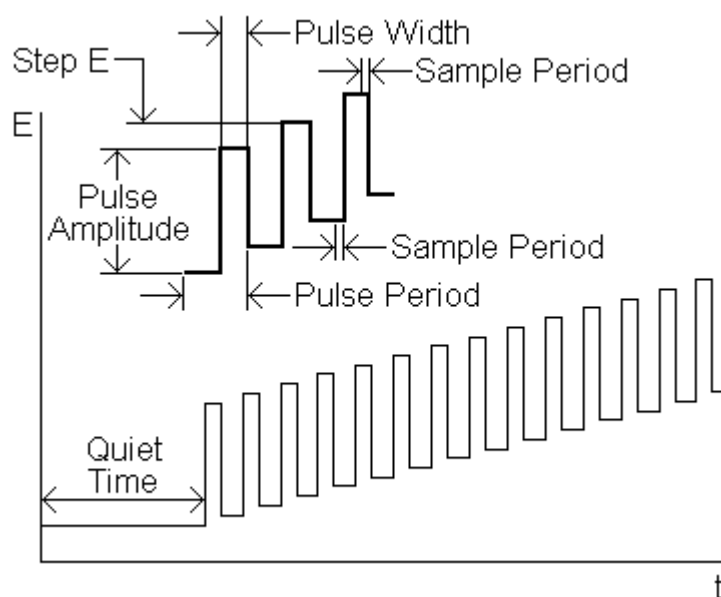
Figure 2.2 Potential for differential wave form pulse voltammetry [37]**.**

In differential pulse voltammetry, fixed magnitude pulses are applied to the working electrode at a time just before the end of the drop, as shown in Figure 2.3. The current is sampled twice, just before the pulse application and again late in the pulse life. The first current is instrumentally subtracted from the second and this current difference is plotted against the applied potential. The resulting differential pulse voltammogram is current peaks, the height of which is directly proportional to the concentration of the corresponding analytes.
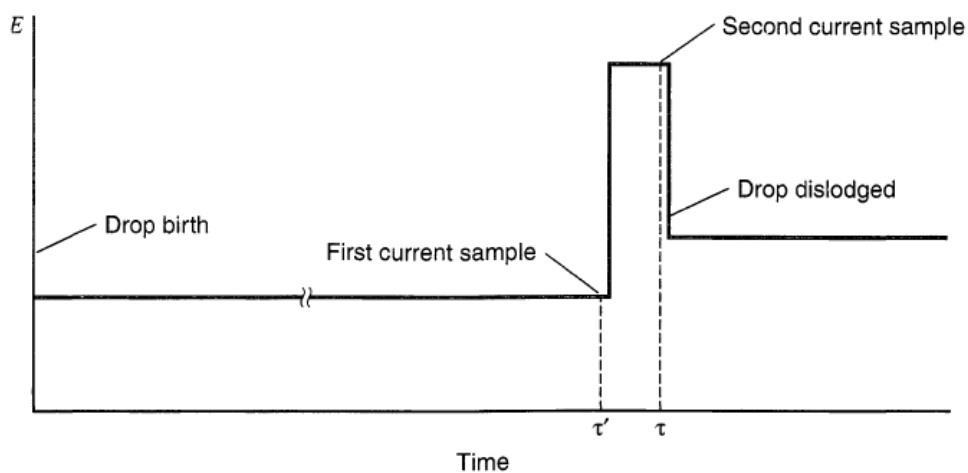
Figure 2.3 A single drop of a differential pulse voltammetry [37].

The peak-shaped response of differential pulse measurements, which are shown in Figure 2.3, also results in improvement of resolution between two species with similar redox potentials. Such quantitation depends not only on the corresponding peak potentials but also on the widths of the peak.
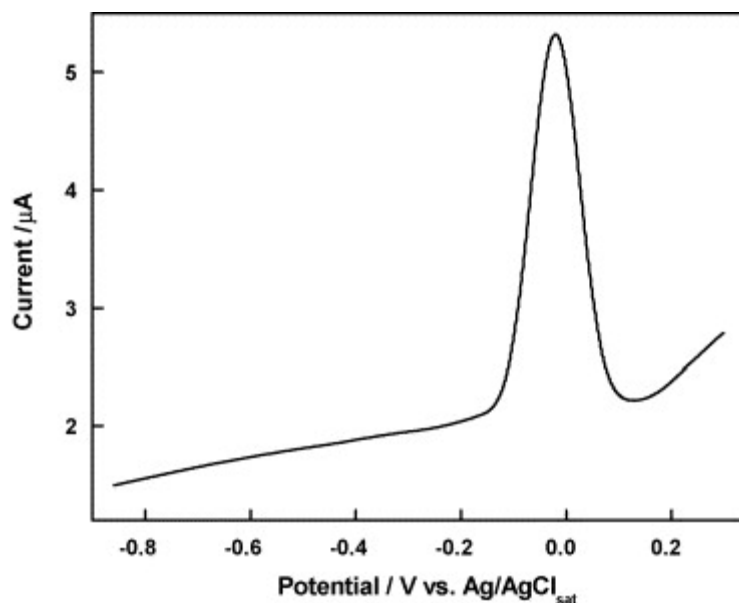
Figure 2.4 Differential pulse voltammogram [36]

The selection of the pulse amplitude and potential scan rate usually requires a tradeoff among sensitivity, resolution and speed. For example, larger pulse amplitudes result in larger and broader peaks. Irreversible redox systems result in lower and broader current peaks compared with those predicted for reversible systems. In addition, the technique can provide information about the chemical form for appearing analyte to improvements in sensitivity and resolution.

2.10 Anodic stripping voltammatry [37]

The preconcentration techniques have the lowest limits of detection of the commonly used electroanalytical techniques. The one most commonly used is anodic stripping voltammetry (ASV). ASV is most widely used for trace metal determination and has a practical detection limit in the partper- trillion range. ASV have their own

unique features, all have two or into a working electrode. It is crucial preconcentration step that results in the exceptional sensitivity that can be achieved. During the second step, the preconcentrated analyte is measured or stripped from the electrode by the application of a potential scan.

2.11 Literature surveys

2.11.1 Electrochemical detection for the determination of nickel

In 2000, Ghoneim *et al*. [38] demonstrated the determination of nickel in water samples by differential pulse adsorptive stripping voltammetry after adding dimethyl glyoxime (DMG) using a hanging mercury drop electrode. It was found that the detection limit of nickel is very low compared to that of most of the other analytical methods.

In 2007, Christidis *et al*. [39] presented a portable electrochemical instrument for detecting and identifying nickel in soil. The system used differential pulse anodic stripping voltammetry. The sensor used disposables screen-printed electrodes, carbon working electrode, Ag/AgCl reference electrode, and carbon counter electrode. The supporting electrolyte was 0.1 M sodium chloride (NaCl). The instrument provided those facilities found in a traditional laboratory based instruments, but in a portable design which does not require the support of a personal computer.

In 2009, Khadro *et al*. [40] developed a new miniaturized system for nickel detection by differential pulse anodic stripping voltammetry (DPASV). The miniaturized system consisted of a screen-printed microelectrode chip, a carbon working electrode, an integrated Ag/AgCl reference electrode, a platinum counter electrode, and 20 µL of polymethylmetacrylate (PMMA) microcell. The system was compared to the measurement using a glassy carbon electrode. The results was found that there are several advantages comparing to a classical electrochemical system.

2.11.2 The fabrication method for paper-based devices

In 2007, Martinez *et al*. [7] introduced the first prototype of a paper-based device. Photolithography is the method which is used for fabricating hydrophilic and hydrophobic areas on the paper. SU-8 negative photoresist is coated on the paper and polymerized by exposure to UV light through a transparency film with a patterned on paper. The unexposed photoresist was removed from the paper after development. This method can provide approximately 200 μm of teeny barrier line width between hydrophilic and hydrophobic areas.

In 2008, Derek *et al*. [8] demonstrated a new method for fabricating patterns on the paper by printing poly(dimethysiloxane) or PDMS to define microchannels in paper. This method was called plotting method. The patterns of PDMS plotting on the paper were defined by a modified desktop plotter which requires a computers to control it. This method also requires a customized plotter and the expensive instruments.

In 2008, Koj *et al*. [9] developed an inkjet printing method for the fabrication of microfluidic multianalyte chemical sensing devices made from paper. The inkjet printing method involves removing a hydrophobic polymer as poly(styrene) from the paper by an inkjet printer. Organic solvent was printed onto the paper for creating a hydrophilic area on the paper. Furthermore, this method does require the specialist to operate and does not need for curing polymer or developing of photoresists.

In 2008, Andres *et al*. [10] reported the cutting method which was the easiest method for fabricating paper-based devices. A knife plotter was used to cut the paper into the patterns of hydrophilic channels. However, this method has to use tape to cleave the paper pattern; Therefore, it is difficult in implementation.

In same year, Xu *et al*. [11] demonstrated the plasma etching which was the method to detract sizing agent or polymer coating on paper using plasma oxidation through a metal mask for lab-on-paper.

In 2009, Emanuel *et al*. [12] developed the wax printing for the fabrication of paper-based devices which the procedure presently seems the best for large-volume. This method used a commercial available wax printer for the

fabrication of paper-based devices. For the advantage of this method, the patterned paper can be fed directly into an inkjet printer for delivery of reagents into the test zones of the devices.

In 2009, Dungchai *et al*. [13] reported the wax screen-printing method for creating microfluidic channels on paper. This method is similar to wax printing method in using a commercial wax for the fabrication of paper-based devices. This method can created a pattern using screen-printed block instead of commercial printer. The advantages of wax screen-printing method are low-cost, simplcity, and rapidness. However, this method is difficult to handle when wax was melted on hotplate.

In 2010, Songjaroen *et al*. [14] presented the new fabrication method based on wax dipping. Wax dipping method used iron mould to created hydrophilic channel using permanent magnet to attach the mould and glass slide to supported the paper. After that, the assembly mould was dipped into the melted wax for generating of pattern on the paper. This method does not require expensive equipments and organic solvents. However, these previous methods by wax need to consider wax spreading before creating the pattern and to require trained personal for performing.

2.11.3 Applications of paper-based devices

In 2009, Dungchai *et al*. [41] reported the first demonstration of electrochemical detection for paper-based microfluidic devices (lab-on-paper) coupled with screen-printed electrodes on the patterned paper. The paper-based devices were demonstrated for the determination of glucose, lactate and uric acid in biological samples using chronoamperometry. This study showed the successful of paper-based microfludics and electrochemical detection as sample, low-cost, and portable alternative for point of care monitoring.

In 2010, Dungchai *et al*. [42] demonstrated the devices which were designed to simultaneously quantify analytes using multiple indicators for each analyte. Different indicator colors were generated at different analyte concentration ranges as well as increasing the ability to better visually discriminate colors. Glucose, lactate, and uric acid in the control serum and urine samples were determined by this

device to demonstrate the applicability. The results for the multi-indicator and single indicator system were compared using untrained readers to demonstrate the improvements in accuracy achieved with the new system.

In 2010, Apilux *et al*. [43] reported a novel lab-on-paper device coupled with electrochemical and colorimetric determination for the detection of Au (III). Square wave voltammetry was used to determine Au (III). The device was able to detect Au (III) concentrations as low as 1 ppm. The interference of Au (III) determination was screened using a colorimetric method. Moreover, the procedure was successfully applied to determination of Au (III) in real sample.

In the same year, Lu *et al*. [44] introduced the fabrication of paper based devices (lab-on-paper) in nitrocellulose (NC) membrane by a wax-printing method for the determination of protein immobilization. The fabrication parameters such as the printing mode and the baking time, and the performances of the wax-patterned NC membrane such as printing resolution, protein immobilization, and sample purification capabilities were studied. The fabrication process could be achieved without the use of organic solvents, and the wax-patterned NC membrane could be produced on a large scale. This developed device would introduce new possibilities and enhance broad applications in clinical diagnosis, food safety, and environmental screening.

In 2012, Apilux *et al*. [45] demonstrated the paper-based devices (lab-on-paper) methodology with nanoparticle to develop a new tool for the determination of Hg (II). The paper-based device was constracted using wax screen printing, and Hg (II) could be detected with the naked eye via the changting color of the AgNPls and was analysis using Adobe Photoshop software. The method was presented good sensitivity for Hg (II) and the signal could be enhanced with addition of Cu (II). In addition, this method developed was successfully used to determine Hg (II) levels in real water samples.

In 2013, Apilux *et al*. [46] presented the new of knowledge which was the first report on paper-based devices for automating the sequential multistep procedures of a sandwich-type enzyme-linked immunosorbent assay (ELISA). The devices required only a single-step application of the sample. The devices were based on nitrocellulose (NC) paper and the inkjet printing method was used to create the

flow channel and device barrier patterns. The developed devices provided a simple procedure for the sandwich ELISA and reduced assay time and reagent consumption. The colorimetric results were successfully used to determine the levels of human chorionic gohadotropin (hCG) by ELISA.

With the obvious advantages of paper-based devices such as low cost, easy to use, requirement of small volume of the sample, easy to modify chemically, and rapid analysis, they are remarkably attractive analytical devices for healthcare clinical diagnosis, and point-of-care monitoring in environmental.

CHAPTER III

EXPERIMENTAL

This chapter has given the information of instruments and equipments, chemical, electrode preparation, sample preparation and methodology used in this work.

3.1 Preparation of paper-based device pattern

3.1.1 Instruments and equipments

The instruments and equipments for the preparation of paper-based devices are listed in Table 3.1

Table 3.1 List of instruments and equipments for the preparation of paper-based devices

Instruments/equipments	Suppliers
Magnetic plate	Local area shop, Bangkok, Thailand
Iron mask	Laser cutting shop, Bangkok, Thailand
Scanning electron microscope JSM-6400	JEOL Ltd., Japan
Olympus microscope (Olympus BX50)	Tokyo, Japan
Filter paper, grade 1 circle, 11 μm	Whatman, UK
Filter paper, grade 4 circle, 25 μm	Whatman, UK

3.1.2 Chemicals

The chemicals for the preparation of paper-based devices are listed in Table 3.2

Table 3.2 List of chemicals for the preparation of paper-based devices.

Chemicals	Suppliers
Paint lacquer (Miss new brand)	Local area shop, Bangkok, Thailand
Flat spray lacquer (Layland F-76)	Local area shop, Bangkok, Thailand
Gloss spray lacquer (layland C-75)	Local area shop, Bangkok, Thailand
Acetone	Merck, Germany

3.1.3 Methodology

The spraying method with lacquer was used to fabricate the pattern on the filter paper. The iron mask, which was designed by an Adobe Illustrator and manufactured using laser cutting technique, was used to protect hydrophilic area on paper (Figure 3.1). Hydrophobic area was created by spraying method. To fabricate paper-based devices (Figure 3.2), the paper was put on a magnetic plate. Next, the iron mask was placed on the paper and it was temporarily attached by means of magnetic force with the magnetic plate placed on the backside of the paper. Then, the paper was sprayed with lacquer. After that, paper was dried with air and the iron mask was removed from the paper. Finally, the image of hydrophobic and hydrophilic areas of the pattern on paper were taken using an optical microscope and scanning electron microscope .
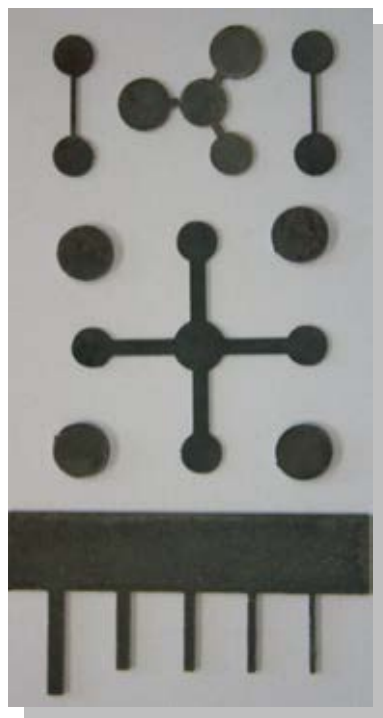
Figure 3.1 The images of the iron mask

Cross-section
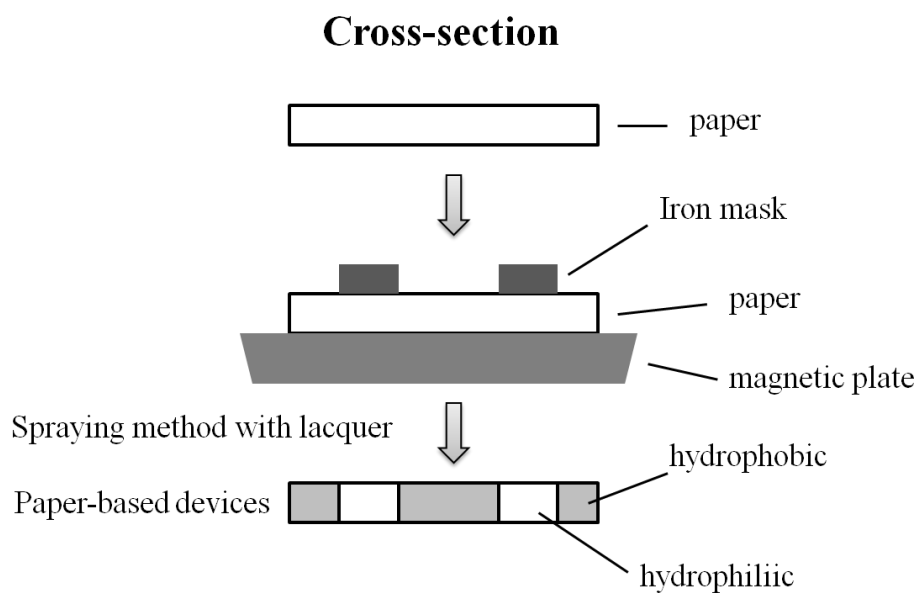
Figure 3.2 Schematic diagram for the fabrication of paper-based device
by spraying method with lacquer

For the fabrication of paper-based devices, the optimal factors including lacquer type and particle retention efficiency of filter paper were studied because the properties of different lacquer type and filter paper affect to fabricate hydrophilic and hydrophobic areas of paper-based devices.

3.1.3.1 The effect of lacquer type

To optimize type of lacquer, three types of acrylic lacquer were investigated, including paint lacquer, flat spray lacquer and gloss spray lacquer.

3.1.3.2 The effect of particle retention efficiency of filter paper

The particle retention efficiency of filter paper was studied using WhatmanTM filter paper No. 1 and No. 4. The particle retention efficiency of WhatmanTM filter paper No. 1 and No. 4 were 11μm and 20-25 μm, respectively.

3.2 Preparation of screen-printed electrodes

3.2.1 Instruments and equipments

The instruments and equipments for the preparation of screen-printed electrodes are listed in Table 3.3

Table 3.3 List of instruments and equipments for the preparation of screen-printed electrodes

Instruments/equipments	Suppliers
Screen-printed blocks	Chaiyaboon Co., Bangkok, Thailand
Hot air oven	Memmert, USA
Stop watch	Mini Timer, Japan

3.2.2 Chemicals

The chemicals for the preparation of screen-printed electrodes are listed in Table 3.4

Table 3.4 List of chemicals for the preparation of screen-printed electrodes.

Chemicals	Suppliers
Carbon ink	Electrodag PF-407C, Acheson, California, USA
Silver/silver chloride ink	Electrodag 7019, Acheson, California, USA
Graphite powder	SIGMA-ALDRICH, USA
Acetone	Merch, Germany

3.2.3 Methodology

The screen-printed electrodes were prepared in-house. The three electrodes were fabricated by screen-printing method. The carbon ink was used as the working electrode (WE) and the counter electrode (CE), and silver/silver chloride ink was used as the reference electrode (RE) and conductive pads. All electrodes were screened on the patterned paper and cured in the oven at 65 ^{o}C for 30 min. The paper-based devices coupled with electrochemical detection are shown in Figure 3.3.
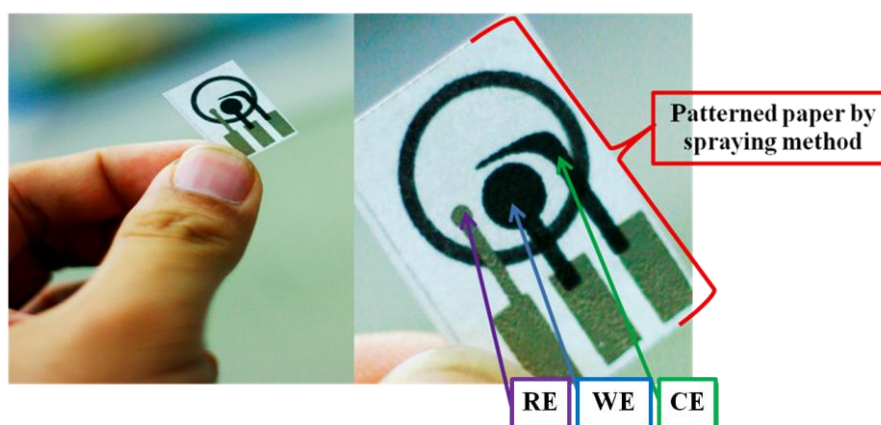
Figure 3.3. Paper-based devices coupled with electrochemical detection

3.3 Chemical preparation

3.3.1 Instruments and equipments

The instruments and equipments for the chemical preparation are listed in Table 3.5

Table 3.5 List of instruments and equipments for the chemical preparations.

Instruments/equipments	Suppliers
Milli Q Water System R ≥ 18.2 MΩ cm^{-1}	Millipore, USA
Analytical balance, Mettler Toledo AB204-S	Mettler, Switzerland
pH meter, 827 pH Lab	Metrohm, Switzerland
Autopipette and tips	Eppendorf, Germany

3.3.2 Chemicals

The chemicals for the chemical preparation are listed in Table 3.6. All reagents were analytical grade. The 18 MΩ cm^{-1} resistance water, obtained from a Millipore Milli-Q purification system, was used to prepare solution in this experiment.

Table 3.6 List of chemicals for the chemical preparations.

Chemicals	Suppliers
Sodium chloride	Merck, Germany
Cupper sulfate	VWR International Ltd., England
Nickel standard solution ICP CertiPUR$^{\circledR}$, 1000 mg/l Ni(NO$_3$)$_2$ in HNO$_3$ 2-3%	Merck, Germany

3.3.3 Methodology

3.3.3.1 Preparation of 0.1 M sodium chloride

0.1 M sodium chloride, which served as a supporting electrolyte, was prepared from sodium chloride (99.5%). 0.5844 g of sodium chloride was dissoloved in Milli-Q water to final volume of 100 ml.

3.3.3.2 Preparation of stock of copper solution

A stock solution of 100 mg/L copper solution was prepared by dissolving 0.2511 g of copper sulfate in 100 ml of Milli-Q water.

3.3.3.3 Preparation of mix solutions between nickel and copper solution

Mix solutions were prepared by mixing standard nickel solution and copper solution in appropriate ratio. The concentration of copper solution at 4.5 mg/L was prepared from a 100 mg/L of copper solution, and the concentrations of nickel were diluted with 0.1 M of sodium chloride to final concentration of nickel at 1, 5, 10, 20, 30, 40, and 50 mg/L.

3.4 The determination of nickel by paper-based devices coupled with electrochemical detection

3.4.1 Instruments and equipments

The instrument and equipment for the determination of nickel are listed in Table 3.7

Table 3.7 List of instruments and equipments for the determination of nickel.

Instruments/equipments	Suppliers
Potentiostat (Autolab PGSTAT 30)	Metrohm, Switzerland
Faraday cage	Copperplate, custom made

3.4.2 Methodology

A potentiostat (Autolab PGSTAT 30) was used to perform differential pulse anodic stripping voltammetric (DPASV) experiments. For differential pulse anodic stripping voltammetric experiments, DPASV was performed at deposition potential of -0.9 V, deposition time of 180 s, modulation amplitude of 0.1 V, modulation time of 0.07 s and potential step of 0.005 V in the potential range from -0.4 V to 0 V vs. Ag/AgCl.

Figure 3.4 A potentiostat (Autolab PGSTAT 30)

3.5 Analytical performance

    3.5.1 Linearity

        The standard solution of nickel in the concentration range of 1-50 ppm was analyzed by DPASV under the optimal conditions. The average of triplicate measurements were used to plot calibration curve between 1 to 50 ppm, which linear range can be obtained.

    3.5.2 Limit of detection (LOD)

        The limit of detection (LOD) was determined by statistical method from the calibration curve in the range of 1-50 ppm and calculated from $3SD_{blank}/S$. SD_{blank} is the standard deviation of blank measurement (n=3) and S is the slope of the linearity or the sensitivity of the method. In this work, the LOD, is the lowest concentration that the signal can be measured.

    3.5.3 Limit of quantitation (LOQ)

        The limit of quantitatation (LOQ) was determined by statistical method from the calibration curve in the range of 1-50 ppm and calculated from $10SD_{blank}/S$. SD_{blank} is the standard deviation of blank measurement (n=10) and S is the slope of the linearity or the sensitivity of the method.

3.6 The determination of nickel in real sample

3.6.1 Instruments and equipments

The instruments and equipments for the determination of nickel in real sample are listed in Table 3.8

Table 3.8 List of instruments and equipments for the determination of nickel in real sample.

Instruments/equipments	Suppliers
Milli Q Water System, R ≥ 18.2 MΩ cm^{-1}	Millipore, USA
Autopipette and tips	Eppendorf, Germany
pH meter, 827 pH Lab	Metrohm, Switzerland
Hot plate, C-MAG HS 10	IKA$^{®}$, Thailand
Ultrasonic steri-cleaner	Sturdy, Taiwan
ICP-OES (ICP 6500)	Thermo Scientific
Potentiostat (Autolab PGSTAT 30)	Metrohm, Switzerland
Faraday cage	Copperplate, custom made

3.6.2 Chemicals

The chemicals for the determination of nickel in real sample are listed in Table 3.9. All reagents are analytical grade. The 18 MΩ cm^{-1} resistance water, obtained from a Millipore Milli-Q purification system, was used to prepare solution in this experiment.

Table 3.9 List of chemicals for the determination of nickel in real sample.

Chemicals	Suppliers
Nitric acid 65%	Merck, Germany
Cupper sulfate	VWR International Ltd., England

3.6.3 Sample preparation

The waste water sample of a jewelry factory was received from The Gem and Jewelry Institute of Thailand (Public Organization). The jewelry waste sample was prepared by filtration of 20 mL of the waste water with whatman No. 1 filter paper. Then the sample was mixed with 20 mL of HNO_3 and the solution was heated at 200 ^{o}C to evaporate the solvent. After that, the sample was made up with 20 mL of MilliQ water and was heated at 100 ^{o}C until dryness (repeated 3 times). Next, the sample was rinsed with mixed solution between 20 mL of 0.1 M NaCl and Cu solution 4.5 ppm (pH 6.7). Finally, the sample was sonicated for 30 min and clear solution was ready for analysis.

3.6.4 Repeatability

The repeatability was studied by ten repeatable measurements of analyte solution. The repeatability was assessed in terms of the relative standard deviation (% RSD), using the following formula:

$$\% \, RSD = \frac{Standard \ deviation}{Mean} \ x \ 100$$

3.6.5 Validation of proposed method with ICP-OES

Under the optimal conditions, the proposed method was applied for the determination of nickel in waste water from jewelry factory sample. The results of this proposed method was validated by ICP-OES method.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the results and discussion obtained from the fabrication of paper-based devices by spraying with lacquer coupled with electrochemical detection for the determination of nickel were presented. The optimization of operational parameters was described. In the following sections, The proposed method was successfully applied to determine nickel in waste water from jewelry factory and compared to another technique.

4.1 The fabrication of paper-based devices by spraying with lacquer

4.1.1 The effect of lacquer type for the fabrication of paper-based devices

In this work, the effect of lacquer type was studied. Three types of acrylic lacquer were investigated, including paint lacquer, flat spray lacquer and gloss spray lacquer. For paint lacquer, the paper was painted with paint lacquer instead of spraying. The results indicated that paint lacquer was inappropriate for the fabrication of the pattern on the paper because the penetration of the lacquer into the filter paper and the diffusion under the iron mask could not be controlled (Figure 4.1a). To study flat spray lacquer and gloss spray lacquer, spraying method was used to fabricate paper-based devices. To use flat spray lacquer (Figure 4.1b), the hydrophobic and hydrophilic areas can be generated on the paper-based devices. However, the uniformity of pattern on paper was difficult to observe. For gloss spray lacquer, the results indicated that the hydrophobic and hydrophilic areas were clearly observed (Figure 4.1c). Therefore, gloss spray lacquer was chosen as the best type of lacquer for fabricating paper-based devices in this work.
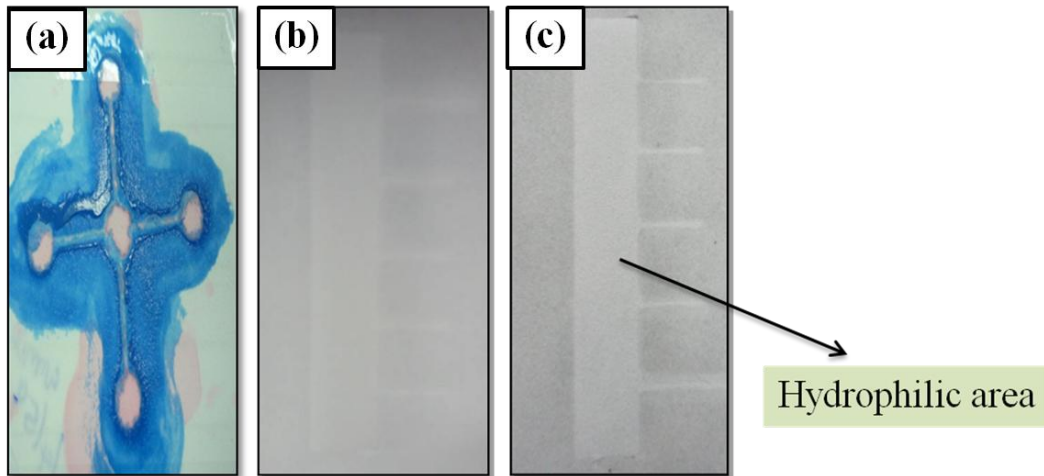
Figure 4.1 The effect of lacquer type for the fabrication of paper-based devices:
(a) paint lacquer (blue color) (b) flat spray lacquer (colorless) (c) gloss spray lacquer
(colorless)

4.1.2 The effect of particle retention efficiency of filter paper for the fabrication of paper-based devices

Since the distribution of the lacquer could not be controlled for penetration of the lacquer into the fiber of the paper, the effect of particle retention efficiency of filter paper was studied. The particle retention efficiency of a depth-type filter is expressed in terms of the particle size (in μm). A retention level of 98% of the total number of particles initially challenging the filter was obtained [20]. The whatman filter paper No. 1 and No. 4 were used to study in this work. The particle retention efficiency of whatmanTM filter paper No. 1 and No. 4 were 11 μm and 20-25 μm, respectively. The results indicated that whatman filter paper No. 4 gave better results than whatman filter paper No. 1 because filter paper No. 4 has larger porosity than filter paper No. 1 (Figure 4.2). Therefore, the lacquer was easily and rapidly penetrated into fiber of filter paper No. 4. In this work, whatman filter paper No. 4 was chosen to fabricate paper-based devices.
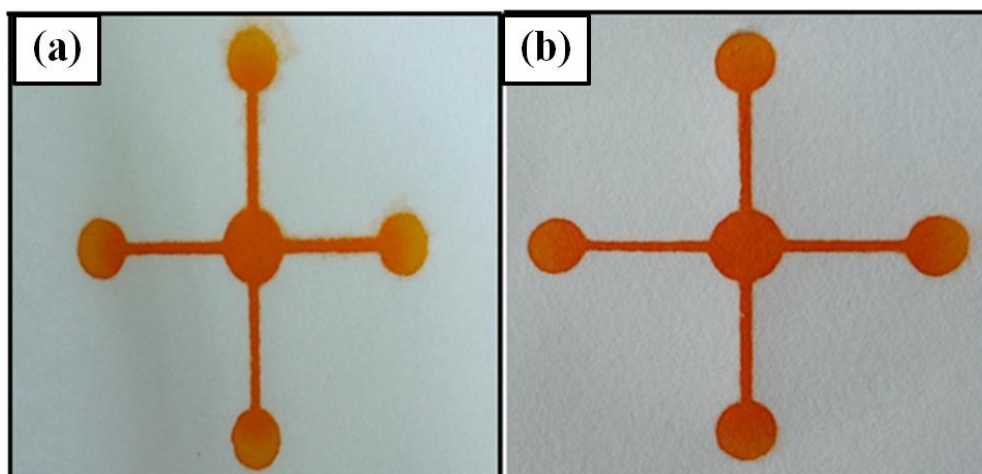
Fiqure 4.2 The effect of the particle retention efficiency of filter paper for the fabrication of paper-based devices (a) Whatman filter paper No. 1 with the porosity of 11µm (b) Whatman filter paper No. 4 with the porosity of 25 µm

4.2 The characterization of hydrophilic and hydrophobic areas on paper-based devices

The hydrophobic area was created by spraying lacquer on filter paper while the hydrophilic area was protected by an iron mask. The lacquer could not be absorbed into the hydrophilic area. Therefore, the pattern of hydrophobic and hydrophilic areas was generated on the paper. The fabricated pattern on the paper was observed by a optical microscope (Olympus CX31), as shown in Fig.4.3a and 4.3b. It was clearly observed that the surface of the paper was changed as a result of lacquer coating. From Fig. 4.3a, the left side of the paper was the native surface, whereas the right side was coated with lacquer and completely turned into a hydrophobic area. In Fig. 4.3b, the result indicated that colored food dye could not percolate into the hydrophobic area because of lacquer coating. Fig. 4.3c shows the hydrophobic property of our paper-based devices comparing to the hydrophilic zone. The droplet of colored food dye on hydrophobic area was apparently observed. It was not readily absorbed into the paper due to the hydrophobic surface of the paper. In addition, the patterned paper fabricated by spraying with lacquer was verified by scanning electron microscope (JSM-6400) as shown in Fig.4.4a and 4.4b. Fig. 4.4a shows the cross

section surface of the paper uncoated with the lacquer. For Fig 4.4b, the cross section surface of paper was changed because the porosity of the paper was infiltrated with lacquer. From the results, sample could flow along hydrophilic area and was not adsorbed into hydrophobic area. Therefore, this proposed method is efficient to fabricate paper-based devices.
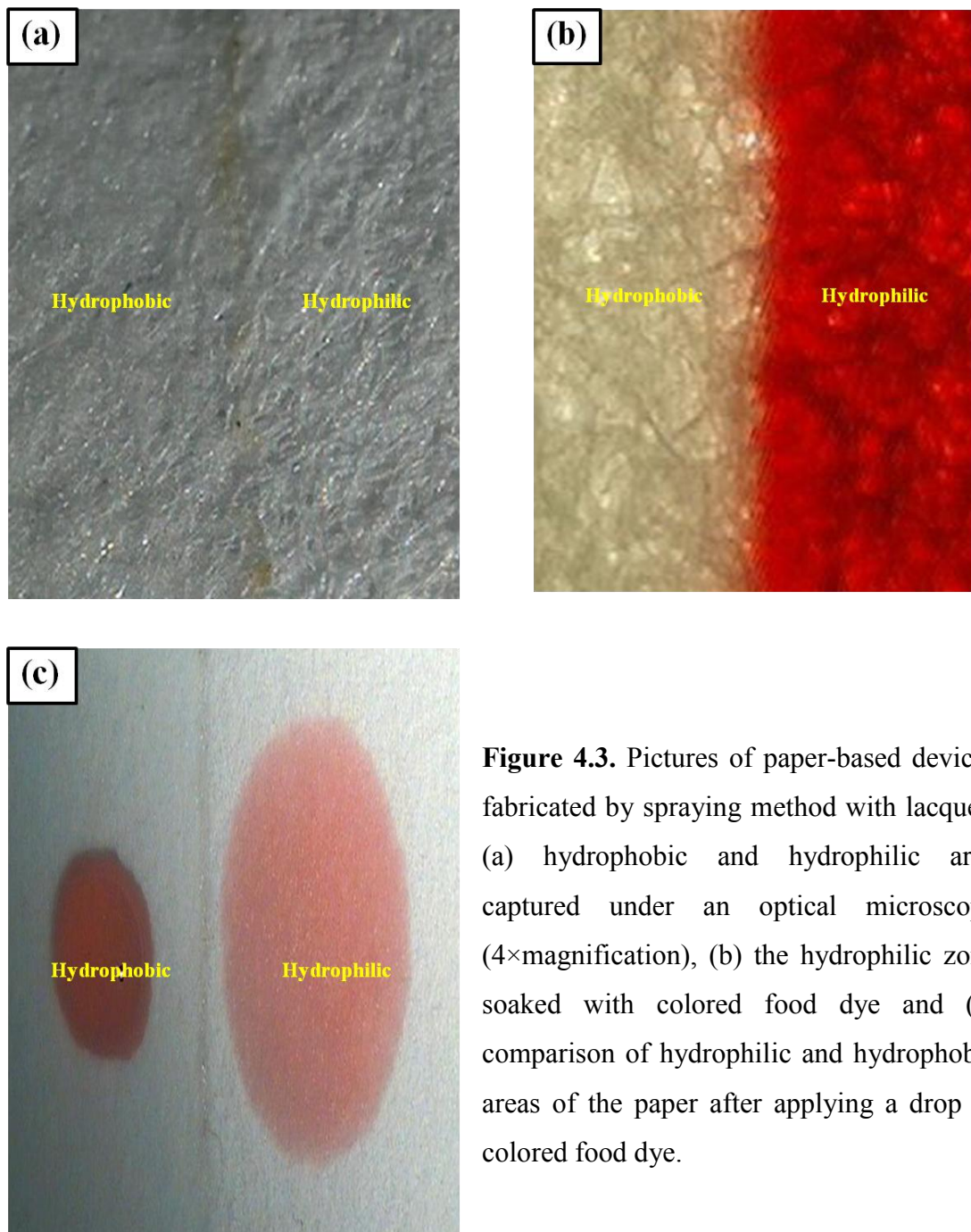
Figure 4.3. Pictures of paper-based devices fabricated by spraying method with lacquer: (a) hydrophobic and hydrophilic area captured under an optical microscope (4×magnification), (b) the hydrophilic zone soaked with colored food dye and (c) comparison of hydrophilic and hydrophobic areas of the paper after applying a drop of colored food dye.
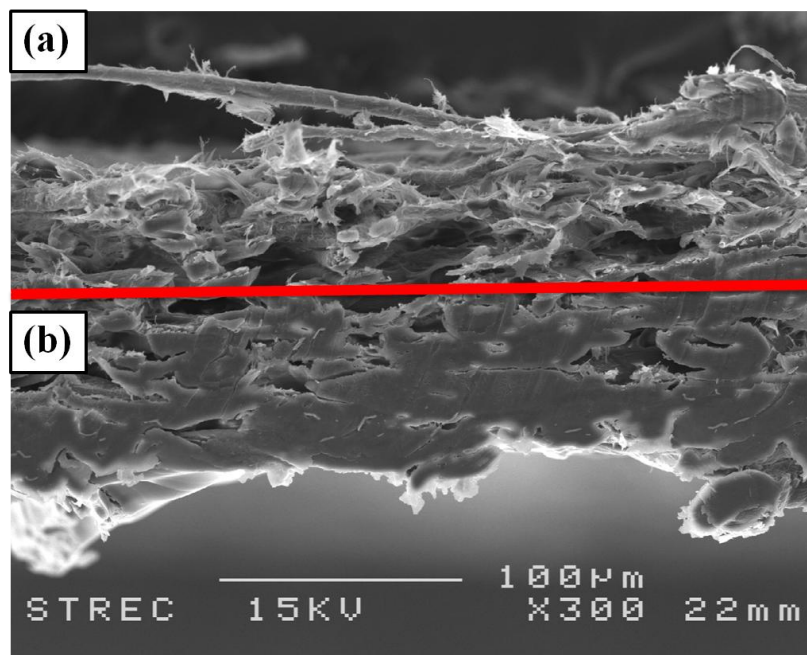
Figure 4.4 . SEM image presented the cross section surface of whatman filter paper No. 4 (a) uncoated with lacquer and (b) coated with lacquer

4.3 Optimization of the experimental conditions

4.3.1 The effect of amounts of graphite

For electrochemical detection, the amounts of graphite could affect to the electrochemical signal. Therefore, the optimization of amounts of graphite was studied for the electrochemical detection of nickel. The amounts of graphite on the oxidation current were examined in the range from 0.0 to 0.4 g of graphite as shown in Figure 4.5. It was found that the oxidation currents of nickel increased with the increase of amounts of graphite from 0.1 to 0.3 g. and then decreased at 0.4 g of graphite because a lot of graphite could affect to the surface of carbon electrode in the fabrication of electrode process. The results was found that the oxidation currents were insignificantly different at 0.2 and 0.3 g of graphite . Hence, the amount of graphite at 0.2 g was chosen as the suitable condition since it required less amount of graphite.
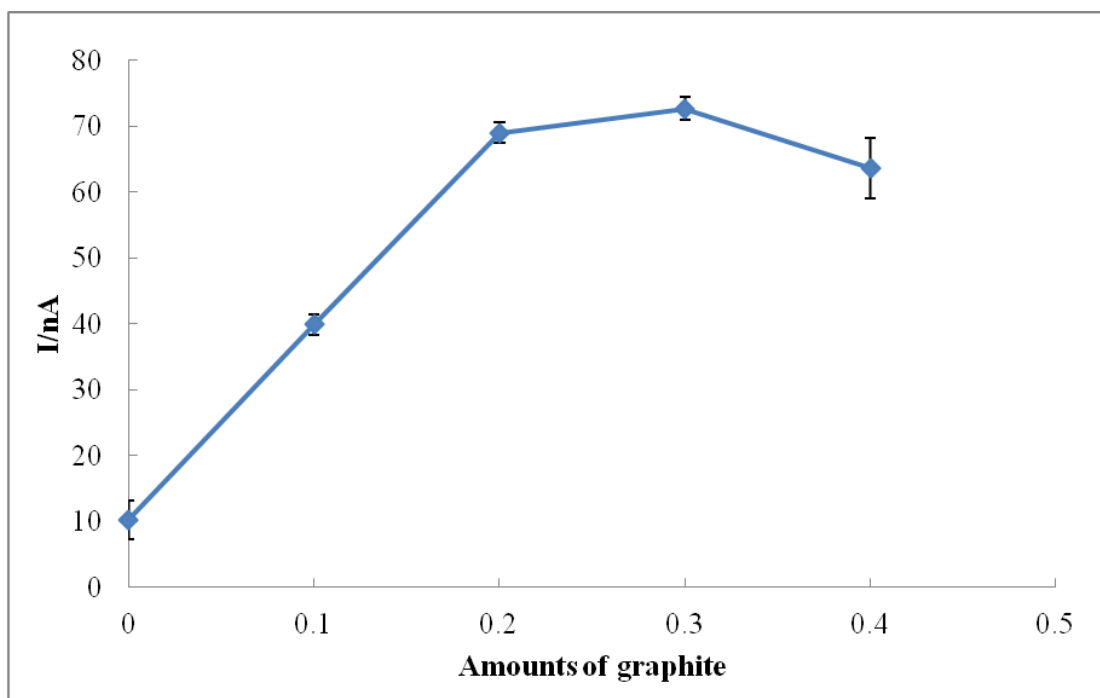
Figure 4.5 The effect of graphite amounts on DPASV peak currents for a solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the screen-printed carbon electrodes. Conditions: deposition time, 180 s; deposition potential, -0.9 V; step potential, 0.005 V.

4.3.2 The effect of the supporting electrolyte

The influence of the supporting electrolyte was also investigated. It was carried out by DPASV in the potential range from -0.5 to 0.0 V. To optimize type of supporting electrolyte, two types of supporting electrolytes were investigated, including 0.1 M HCl and 0.1 M NaCl. As shown in Figure 4.6, the relationship between the current and potential was investigated. The results showed that the use of 0.1 M NaCl gave higher current than 0.1 M HCl. Therefore, 0.1 M NaCl was chosen as the best type of supporting electrolyte.
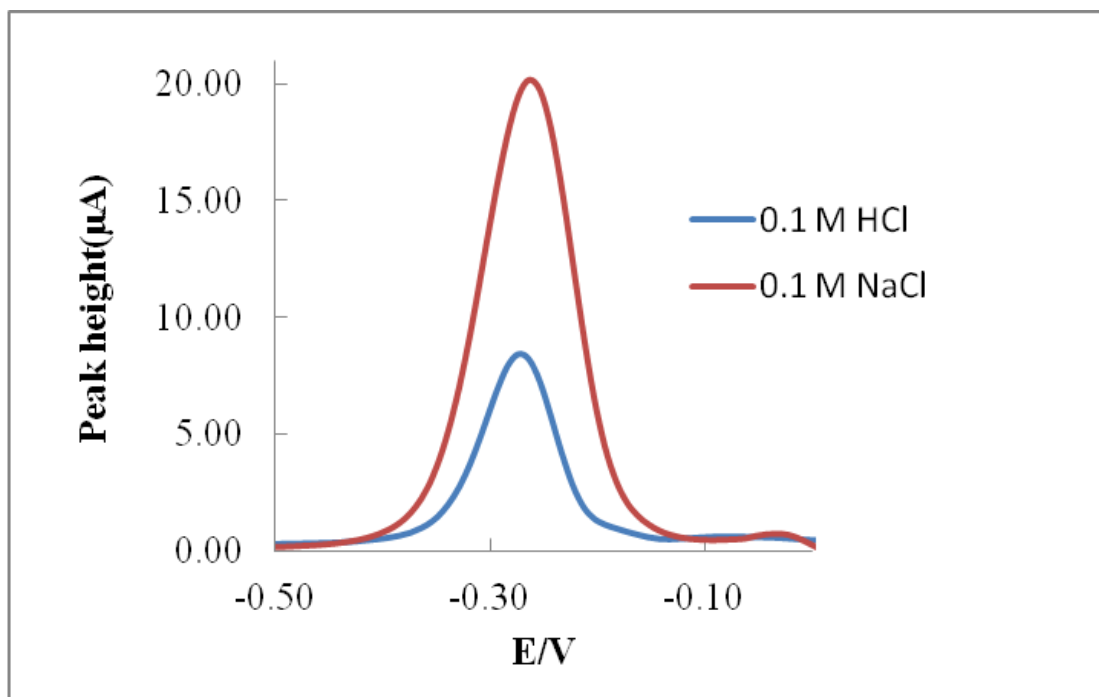
Figure 4.6 DPAS voltammograms of Ni (II) in two types of supporting electrolytes for a solution containing 50 μL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition time, 180 s; deposition potential, -0.9 V; step potential, 0.005 V

4.4 Study of the DPASV parameters

ASV method is based on electrochemical reduction of matal ions at working electrode to deposit the metals on the electrode surface and subsequent anodic stripping by scanning the potential to anodic aspect to allow electrooxidation of the deposited metals at a characteristic potential of each metal, as reported as a voltammogram in this step. Optimal conditions for deposition and stripping steps of DPASV of Ni^{2+} in paper-based devices coupled with screen-printed carbon electrode were investigated as the following.

4.4.1 The effect of the deposition potential

The effect of the deposition potential applied to the carbon electrode in paper-based devices on the DPASV peak currents of Ni^{2+} was shown in Figure 4.7.

It was found that the highest currents was obtained up to -0.9 V, and then the currents of Ni^{2+} decreased. Due to the high voltage will cause gas bubbles at the surface electrodes. Therefore, the deposition potential of -0.9 V was chosen for the subsequent experiment.
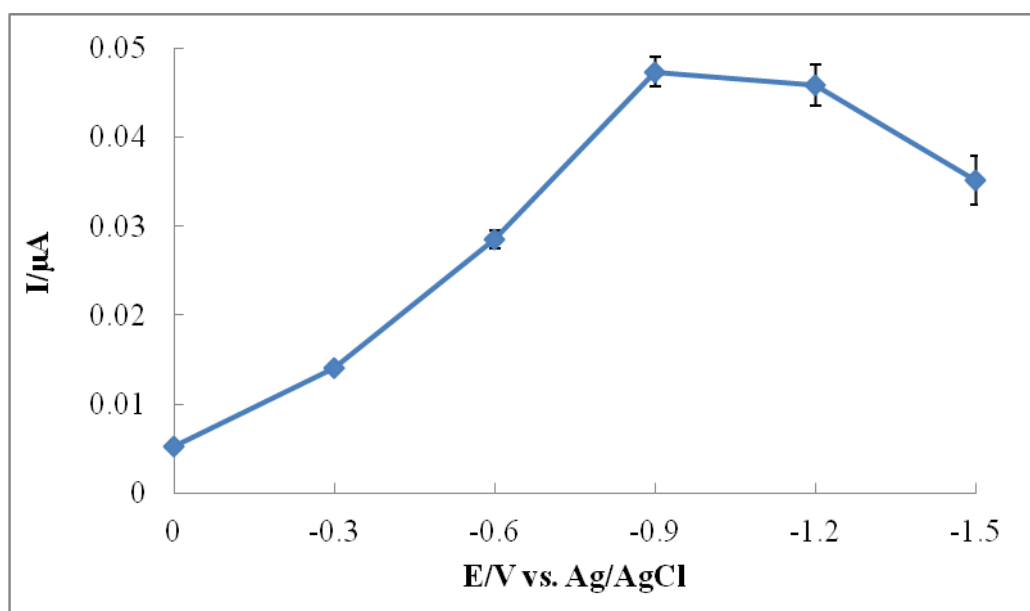
Figure 4.7 The effect of the deposition potential on the DPASV peak currents for solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition time, 180 s; step potential, 0.005 V

4.4.2 The effect of the deposition time

The effect of deposition time upon the stripping response of 50 ppm of Ni^{2+} was examined. As shown in Figure 4.8, the signal of Ni^{2+} increased with the increase of the time in the range of 60 to 180 s and then the signal slightly decreased with the time upper than 180 s. Therefore, the optimal deposition time of 180 s was selected for the following experiment.
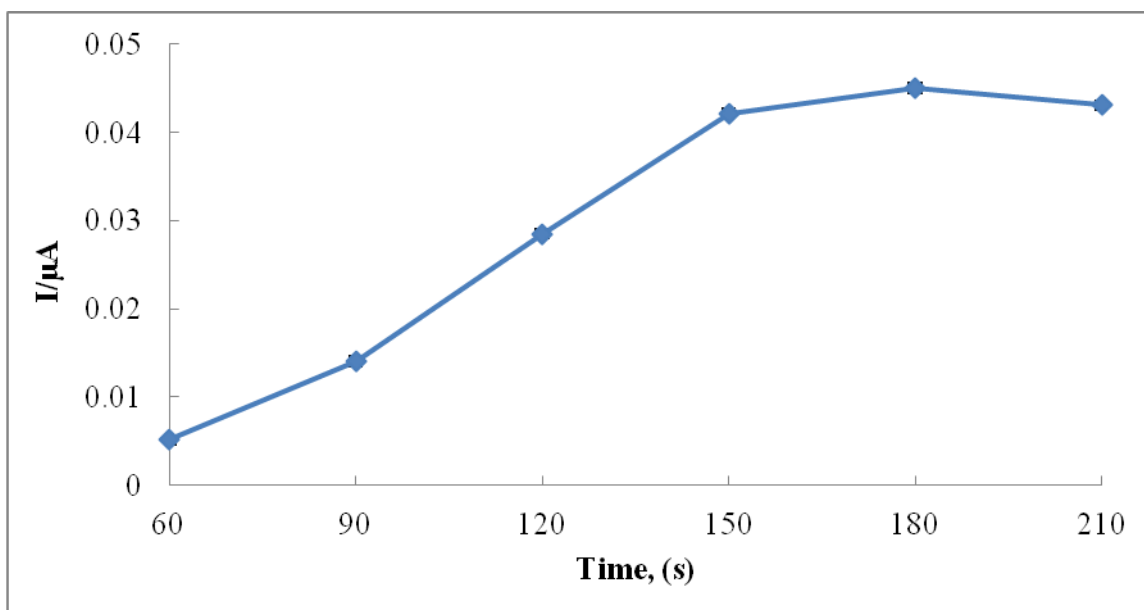
Figure 4.8 The effect of the deposition time on the DPASV peak currents for solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition potential, -0.9 V; step potential, 0.005 V

4.4.3 The effect of the potential step

Next, the effect of the step potential was studied in the range of 2 mV to 12 mV. From Figure 4.9, the peak current increased when the potential step increased until the potential step of 5 mV, and then the peak current decreased. Therefore, the optimal value was found to be 5 mV, due to the increment in the peak intensity caused by an increment of step potential up to 5 mV.
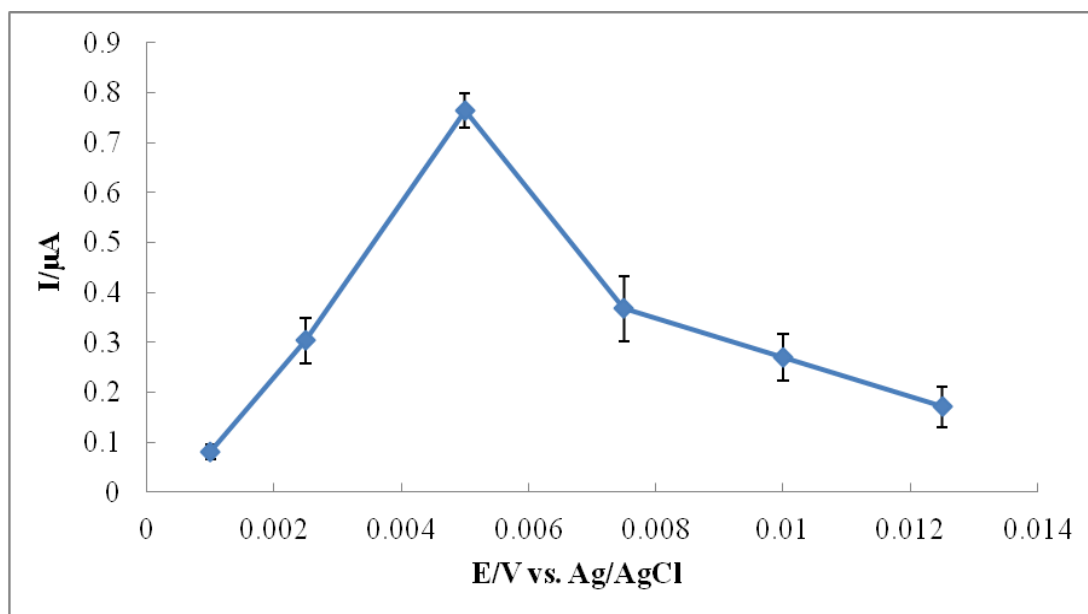
Figure 4.9 The effect of the potential step on the DPASV peak currents for solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition potential, -0.9 V; deposition time, 180 s.

4.4.4 The effect of the modulation amplitude

To obtain the optimal conditions for the quantitative of nickel, the effect of the modulation amplitude was examined. The pulse amplitude was varied from 10 mV to 125 mV. As shown in Figure 4.10, the peak currents of Ni^{2+} increased when modulation amplitude increased, and the peak currents decreased above 100 mV. Therefore, the modulation amplitude of 100 mV was chosen as optimal value.
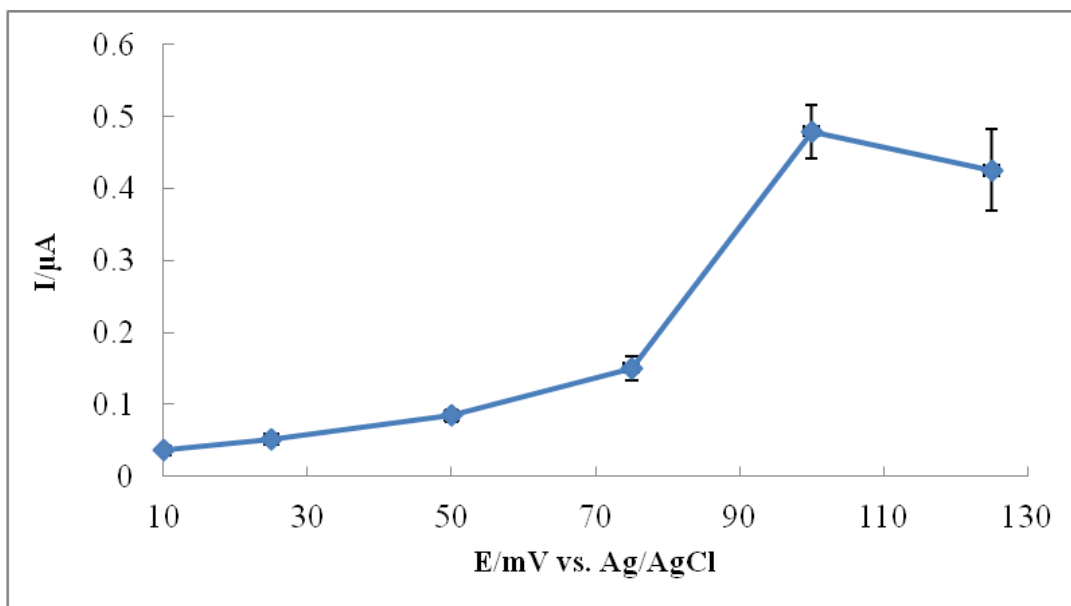
Figure 4.10 The effect of the modulation amplitude on the DPASV peak currents for solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition potential, -0.9 V; deposition time, 180 s; potential step. 0.005 V.

4.4.5 The effect of the modulation time

The modulation time was studied in the range of 0.03 to 0.15 s. The investigative currents were plotted against the modulation time as shown in Figure 4.11. The peak current increased when the modulation time increased until the modulation time of 0.07 mV, and then the peak current decreased. Therefore, the optimized modulation time of 0.07 s was obtained for the determination of Ni^{2+}
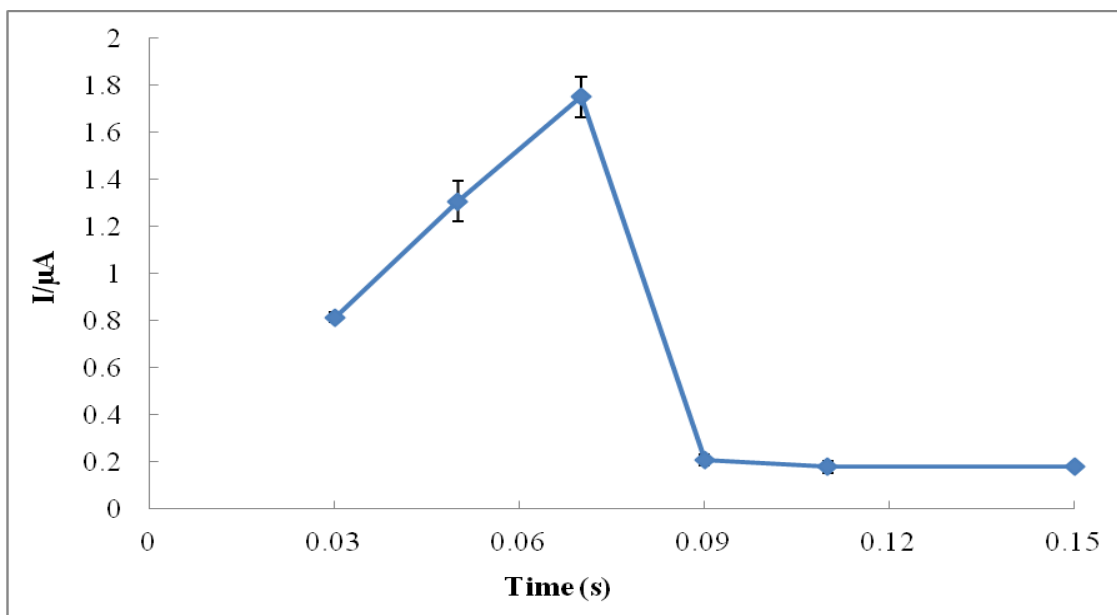
Figure 4.11 The effect of the modulation time on the DPASV peak currents for solution containing 50 µL of 50 ppm of Ni^{2+} in 0.1 M NaCl using the carbon electrodes. Conditions: deposition potential, -0.9 V; deposition time, 180 s; potential step. 0.005 V.

4.5 Study of the concentration of Cu^{2+} solution for enhancing the sensitivity of Ni^{2+}

4.5.1 The effect of Cu-enhancer solution for the determination of nickel

To determine nickel, Cu-enhancer was used to increase sensitivity. The effect of the concentration of copper was studied in range from 0.5 to 7.5 ppm. For, figure 4.12, the relationship between current and concentration of copper was shown with the concentration of nickel of 0.5, 5, and 50 ppm, respectively. The oxidation peak currents increased when the concentration of copper increased due to the increment in the current caused by an increment of concentration of Cu-enhancer solution. The peak current increased until the concentration of copper of 4.5 ppm and then decreased because unsuitable concentration of copper may be disturbance the signal of nickel. Therefore, the optimum concentration of copper at 4.5 ppm was

chosen. Figure 4.13 showed the comparison signal of nickel in each concentration with Cu^{2+} 4.5 ppm.
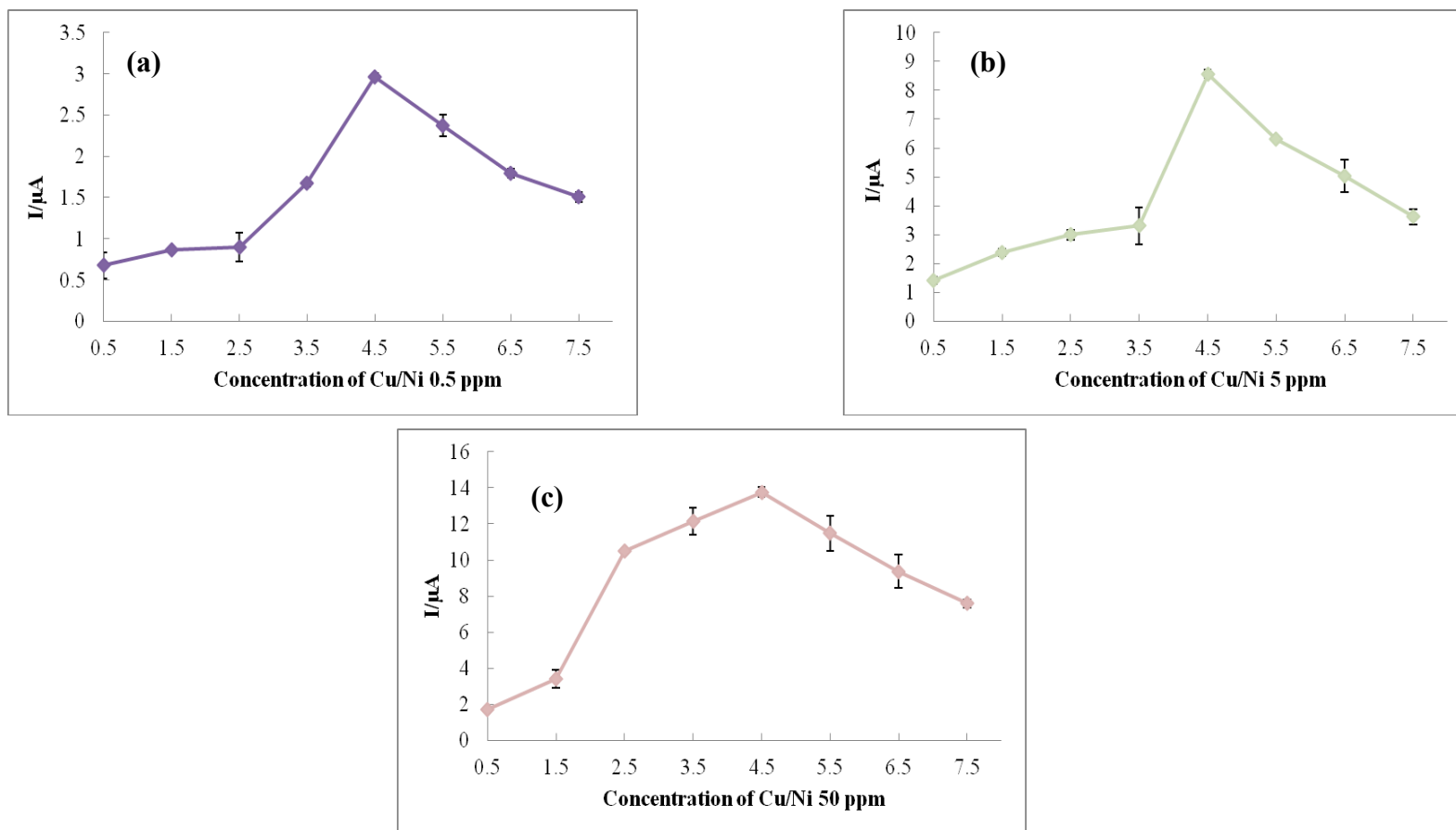
Figure 4.12 The effect of Cu (II) concentrations for enhancing the signal of nickel , solution containing mixed solutions between Ni (II) (a.) 0.5, (b.) 5, (c.) 50 ppm and Cu (II) 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 ppm in 0.1 M NaCl using the screen-printed carbon electrode on the paper
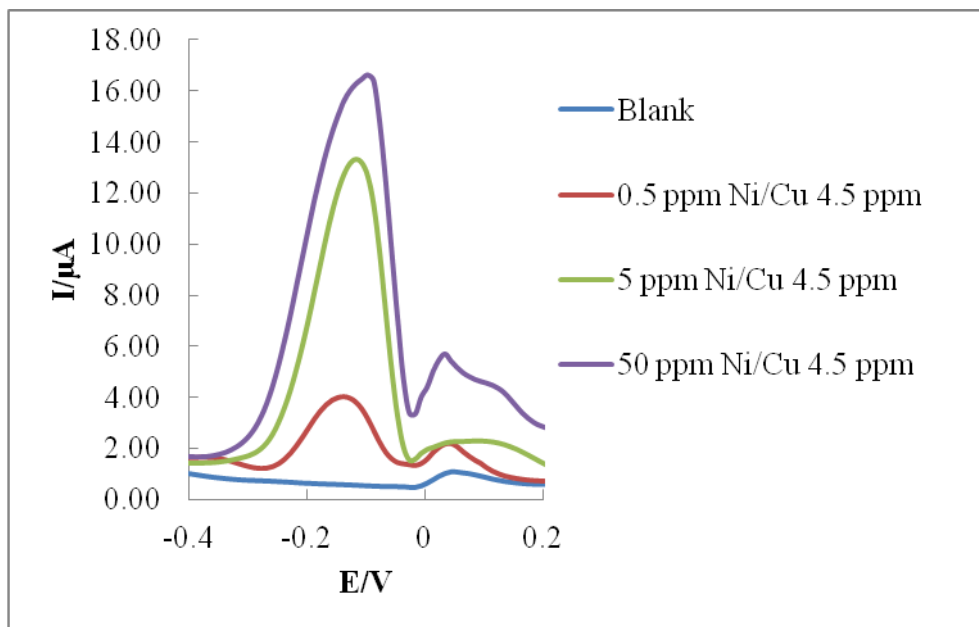
Figure 4.13 Comparison mixed solutions between Ni (II) 0.5, 5 and 50 ppm at Cu
(II) 4.5 ppm using the screen-printed electrode on the paper

4.6 Analytical performance

Differential pulse anodic stripping voltammetry (DPASV) is the typical technique used to achieve lower limit of detection . Therefore, the differential pulse anodic stripping voltammetry was selected to determine nickel. For figure 4.14, it was found that differential pulse anodic stripping voltammogram of nickel was clearly observed.
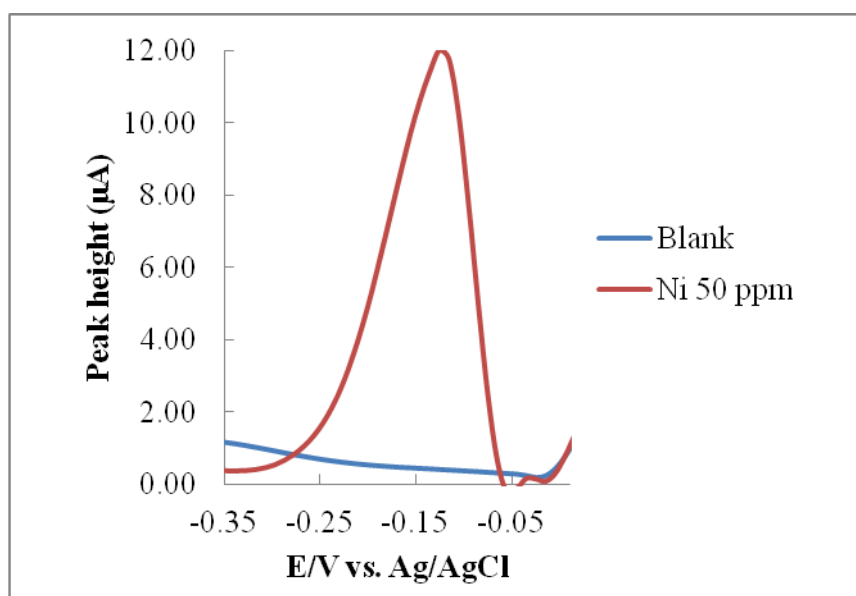
Figure 4.14 Differential pulse anodic stripping voltammograms of 50 ppm of nickel in 0.1 M NaCl (pH 6.7).

4.6.1 Calibration curves

Under the optimal experimental conditions, the electrochemical performance of the DPASV for the determination of nickel by paper-based devices coupled with electrochemical detection was studied. To generate linear calibration curve for three electrodes including the working electrode (WE), the counter electrode (CE) and the reference electrode (RE). 50 µL of solution was dropped on the hydrophilic area of paper-based device. Peak potential of nickel was obtained in potential region between -0.20 to -0.10 V *versus* Ag/AgCl. The results showed that voltammograms provided a well-defined oxidation peak. Therefore, paper-based

devices were efficient to determine nickel. The relative standard deviation of all concentrations of nickel was 1.25% (n=3), demonstrating acceptable reproducibility for this device. Defined peaks with current proportional to the analyte concentration were observed as shown in Figure 4.15. Calibration curve of the anodic current against concentrations generated linear range between 1 to 50 ppm with a high coefficient of 0.9971 (Figure.4.16). The resulting calibration plots are linear with the sensitivity of 0.2075 µA/ppm.
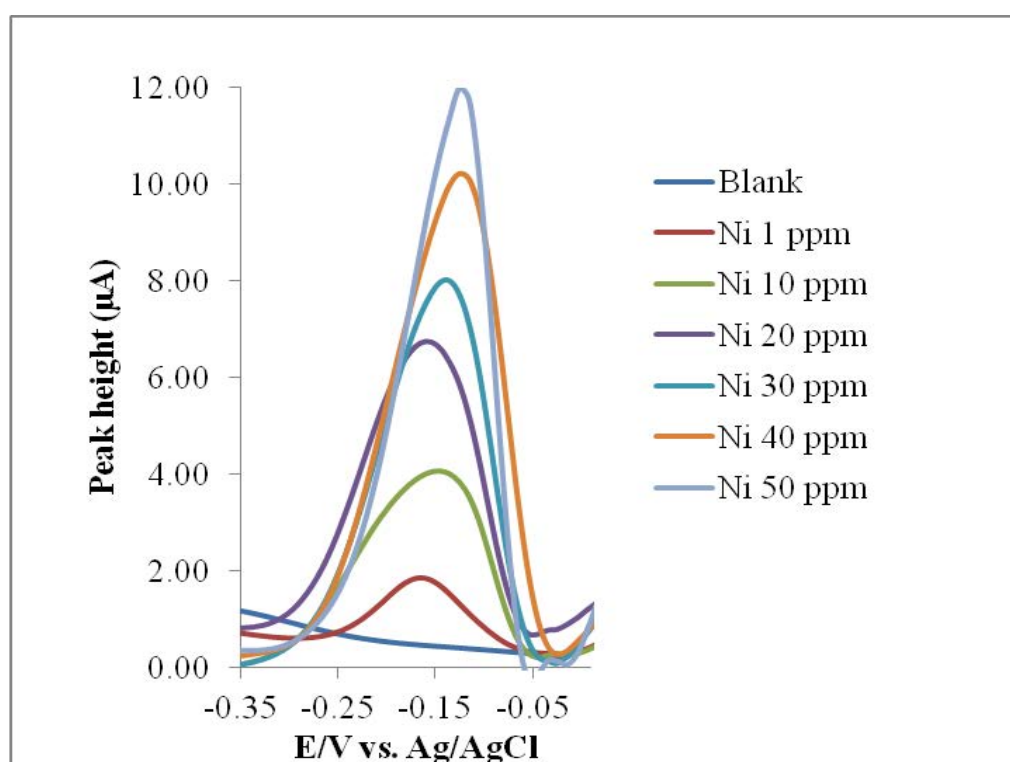
Figure 4.15 Differential pulse anodic stripping voltammograms of nickel (1, 10, 20, 30, 40, 50) in 0.1 M NaCl/Cu 4.5 ppm (pH 6.7), measured under the optimal experimental conditions.
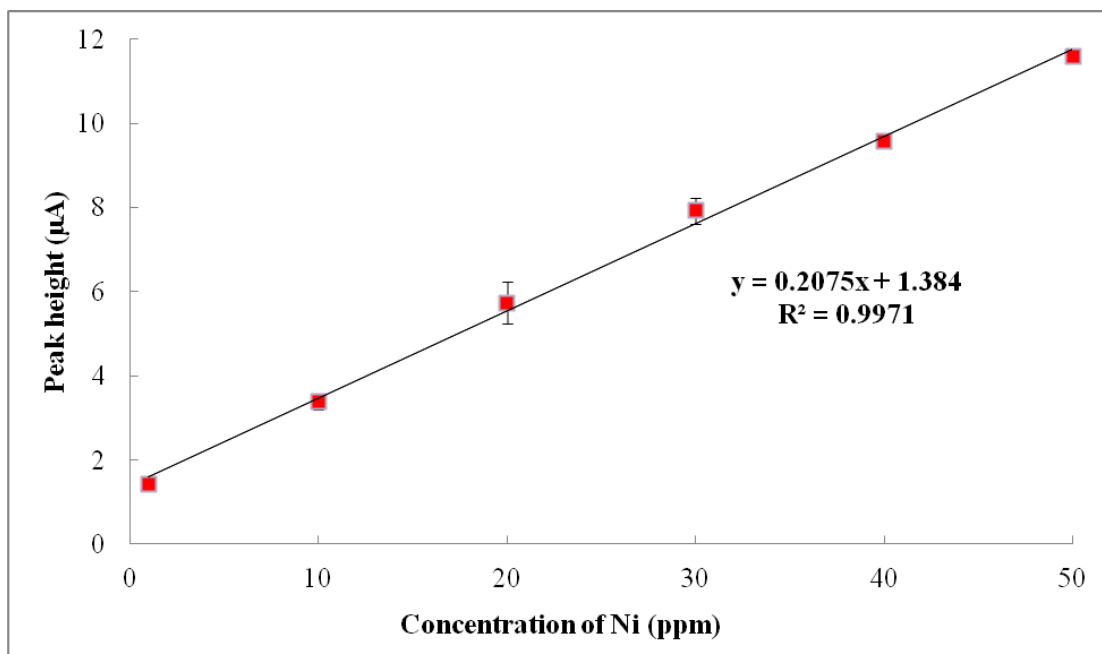
Figure 4.16 The calibration plot of anodic currents at -0.9 V of deposition potential for the determination of nickel are shown in the inset, *n* = 3.

4.6.2 The limit of detection (LOD) and the limit of quantitation (LOQ)

The limit of detection (LOD) was obtained at concentration as low as 0.5 ppm based on signal-to-noise ratio (S/N) of 3 and the limit of quantitation (LOQ) was also found to be 1.97 ppm based on signal-to-noise ratio (S/N) of 10.

4.6.3 Repeatability

In this work, the repeatability was studied by ten repeatable measurements of standard nickel solution. The paper-based device showed the high reproducibility in different concentrations (Table 4.1).

Table 4.1 The relative standard deviations of nickel (n=10)

Concentration (ppm)	SD	%RSD
1	0.06	4.74
10	0.11	3.13
20	0.25	4.56
30	0.37	3.67
40	0.35	3.83
50	0.38	3.78

4.7 Analytical application in a real sample

To evaluate efficiency of this developed method, paper-based devices with electrochemical detection was used to detect nickel in waste water from jewelry factory. The determination of nickel in real sample were carried out using the optimal conditions. The waste water from jewelry factory sample was determined using external standard. The results were found to be 4.15±0.043 ppm (n=3) for electrochemical detection.

4.8 Comparison the proposed method with ICP-OES

In addition, the results of this proposed method were validated by ICP-OES method. The results were found to be 4.15±0.043 ppm (n=3) for electrochemical detection and 4.06±0.013 ppm (n=3) for ICP-OES method. The paired t-test was used to validate our method versus the ICP-OES method. No significant difference was found at the 95% confidence level. Therefore, the analyzed values of nickel in waste water from jewelry factory can be accepted.

CHAPTER V

CONCLUSIONS AND FUTURE PERSPECTIVE

5.1 Conclusions

Spraying method with lacquer was successfully developed for the fabrication of paper-based devices. The iron mask was used to protect the surface of hydrophilic areas and hydrophobic areas were created by the spraying with lacquer. The spraying method does not suffer from problems of interference from residues remaining in the hydrophilic area after the fabrication. For electrochemical detection, the electrodes were screened on paper-based devices by screen-printing method. The paper-based devices were shown to be useful for electrochemical detection methods, and were applied for the determination of nickel in waste water from jewelry factory sample. Under optimal conditions, the differential pulse anodic stripping voltammogram provided a well-defined oxidation peak. Moreover, success in increasing the ability to detect signal of nickel using the Cu^{2+} enhancing solution with the appropriate concentration. The calibration curve of the anodic current against concentrations generated linear range between 1 to 50 ppm with a high coefficient of 0.9971. The limit of detection (LOD) and the limit of quantitation (LOQ) of the proposed method were found to be 0.5 and 1.97 ppm, respectively. Furthermore, this method was successfully used for the quantification of nickel in waste water from jewelry factory. For these reasons, the spraying method is an alternative method for the fabrication of paper-based devices due to its ease of use, affordability and simplicity. In addition, this method does not require complicated and expensive instruments or organic solvents. Therefore, the spraying method with lacquer is effective to fabricate paper-based devices. Moreover, the paper-based devices coupled with electrochemical detection are useful for the investigation of nickel and may have a potential for various kinds of real samples.

5.2 Future perspective

In the future, spraying method with lacquer for the fabrication of paper-based devices can create other pattern by designing various iron masks to generate hydrophilic pattern. Paper-based devices coupled with electrochemical detection can be applied for the determination of other metals. Moreover, paper-based devices could be used for improves sensitivity using micro and/or nano materials to modify the electrode surface or increase the electrode area.

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APPENDIX

Table A1   The t-test distribution.

Degrees of Freedom	Probability, p		
	0.1	**0.05**	**0.01**
1	6.31	12.71	63.66
2	2.92	4.30	9.93
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
7	1.89	2.37	3.50
8	1.86	2.31	3.36
9	1.83	2.26	3.25
10	1.81	2.23	3.17
11	1.80	2.20	3.11
12	1.78	2.18	3.06
13	1.77	2.16	3.01
14	1.76	2.14	2.98

Table A2   The oxidation peak current of nickel at different amount of graphite.

Amount of graphite (g)	Peak current (µA)			Average	SD
	1	**2**	**3**		
0	10.34	11.21	10.56	10.73	0.33
0.1	35.78	38.76	35.21	36.58	1.45
0.2	67.89	63.12	64.81	65.27	1.74
0.3	68.45	64.37	64.11	65.64	1.87
0.4	56.23	50.87	60.34	55.81	4.74

Table A3 The oxidation peak current of nickel at different deposition potential.

Deposition potential (V)	Peak currents (µA)			Average	SD
	1	2	3		
0	0.005	0.006	0.005	0.005	0.0003
-0.3	0.013	0.014	0.014	0.014	0.0002
-0.6	0.029	0.029	0.027	0.029	0.0011
-0.9	0.048	0.048	0.045	0.047	0.0017
-1.2	0.046	0.043	0.048	0.046	0.0023
-1.5	0.037	0.032	0.036	0.035	0.0028

Table A4 The oxidation peak current of nickel at different deposition time.

Deposition time (s)	Peak currents (µA)			Average	SD
	1	2	3		
60	0.005	0.006	0.005	0.005	0.0003
90	0.014	0.014	0.014	0.014	0.0002
120	0.029	0.029	0.027	0.029	0.0011
150	0.037	0.048	0.041	0.042	0.0054
180	0.046	0.043	0.046	0.045	0.0018
210	0.039	0.041	0.051	0.043	0.0063

Table A5 The oxidation peak current of nickel at different modulation amplitude.

Modulation amplitude (mV)	Peak currents (μA)			Average	SD
	1	2	3		
10	0.036	0.038	0.038	0.036	0.0004
25	0.051	0.053	0.051	0.052	0.0015
50	0.088	0.089	0.075	0.084	0.0077
75	0.162	0.158	0.131	0.151	0.0166
100	0.516	0.482	0.441	0.479	0.0374
125	0.384	0.401	0.491	0.425	0.0568

Table A6 The oxidation peak current of nickel at different modulation time.

Modulation time (s)	Peak currents (μA)			Average	SD
	1	2	3		
0.03	0.800	0.805	0.838	0.815	0.021
0.05	1.216	0.132	1.384	1.308	0.085
0.07	1.831	1.763	1.659	1.751	0.086
0.09	0.1945	0.234	0.195	0.208	0.023
0.11	0.1498	0.198	0.186	0.178	0.025
0.15	0.1708	0.191	0.177	0.179	0.010

Table A7   The oxidation peak current of nickel at different potential step.

Potential step (V)	Peak currents (µA)			Average	SD
	1	2	3		
0.0010	0.078	0.095	0.068	0.080	0.014
0.0025	0.323	0.252	0.336	0.304	0.045
0.0050	0.725	0.779	0.789	0.764	0.032
0.0075	0.309	0.356	0.438	0.368	0.065
0.0100	0.234	0.324	0.251	0.269	0.048
0.0125	0.126	0.207	0.179	0.170	0.041

Table A8   The oxidation peak current of nickel at 0.5 ppm.

Concentration of Cu (II) (ppm)	Peak currents (µA)			Average	SD
	1	2	3		
0.5	0.643	0.508	0.889	0.679	0.158
1.5	0.850	0.864	0.881	0.865	0.013
2.5	0.668	1.123	0.885	0.898	0.178
3.5	1.704	1.619	1.705	1.676	0.040
4.5	3.017	2.920	2.962	2.966	0.039
5.5	2.282	2.281	2.564	2.376	0.133
6.5	1.878	1.750	1.759	1.796	0.058
7.5	1.516	1.568	1.432	1.505	0.056

Table A9   The oxidation peak current of nickel at 5 ppm.

Concentration of Cu (II) (ppm)	Peak currents (µA)			Average	SD
	1	2	3		
0.5	1.574	1.395	1.272	1.414	0.124
1.5	2.472	2.460	2.223	2.385	0.115
2.5	3.144	3.090	2.750	2.995	0.174
3.5	3.780	3.730	2.399	3.303	0.639
4.5	8.705	8.624	8.376	8.568	0.139
5.5	6.350	6.234	6.399	6.326	0.071
6.5	5.049	5.718	4.351	5.039	0.558
7.5	3.982	3.521	3.360	3.621	0.263

Table A10 The oxidation peak current of nickel 50 ppm.

Concentration of Cu (II) (ppm)	Peak currents (µA)			Average	SD
	1	2	3		
0.5	1.717	1.878	1.545	1.713	0.136
1.5	3.805	3.754	2.692	3.417	0.513
2.5	10.430	10.510	10.620	10.520	0.078
3.5	11.110	12.367	12.954	12.144	0.769
4.5	13.940	13.990	13.360	13.763	0.286
5.5	10.210	12.160	12.170	11.480	0.969
6.5	8.173	9.565	10.390	9.376	0.915
7.5	7.696	7.804	7.248	7.583	0.241

Table A11 The peak currents at the different concentration of nickel for the calibration curve

Concentration of Ni (II) (ppm)	Peak currents (µA)			Average	SD
	1	2	3		
1	1.435	1.480	1.356	1.424	0.062
10	3.197	3.564	3.382	3.381	0.184
20	5.637	5.297	6.283	5.739	0.500
30	7.665	7.828	8.277	7.923	0.316
40	9.593	9.527	9.623	9.581	0.049
50	11.440	11.590	11.750	11.593	0.155

Table A12 T-test: paired two sample for means

	Variable 1	Variable 2
Mean	4.055969038	4.151815
Variance	0.000161749	0.001821744
Observations	3	3
Pearson Correlation	-0.997778261	
Hypothesized Mean Difference	0	
df	2	
t Stat	-2.997751886	
P(T<=t) one-tail	0.047794661	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.095589322	
t Critical two-tail	4.30265273	

VITA

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Research Interest:

2010-Present: Fabrication of paper-based devices by lacquer spraying method for the determination of nickel (II) ion in waste water

2009: Improvement of natural rubber properties with copolymer nanoparticles

The full paper (Proceeding) and publication:

2012: A simple and rapid method for fabrication of paper-based devices by
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2011: Preparation and characterization of nanocomposites of natural rubber
with polystyrene and styrene- methacrylic acid copolymer
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Awards

2012: Gold coin on badminton competition from Chulalongkorn university
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2010: The Chulalongkorn University's Graduate Scholarship on the auspicious
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