FEASIBILITY OF SCREEN PRINTING CARBON ELECTRODE TO DETECT ASCORBIC ACID

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UNIVERSITI SAINS MALAYSIA
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FEASIBILITY OF SCREEN PRINTING CARBON ELECTRODE TO DETECT ASCORBIC ACID

by
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LIST OF SYMBOLS

E_p	Potential peak
I_p	Current peak
R^2	Correlation coefficient

LIST OF ABBREVIATIONS

AA	Ascorbic Acid
CA	Citric Acid
CV	Cyclic Voltammetry
SAMs	Self-assembled monolayers
SPEs	Screen printed carbon electrode
PANI	polyanaline
DA	Dopamine
UA	Uric Acid
MWCNT	Multi-walled carbon nanotube
LOD	Limit of detection
LOQ	Limit of quantitation

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KEBOLEHAN ELEKTROD KARBON CETAK LAYAR UNTUK MENGESAN ASID ASKORBIK

ABSTRAK

Elektrod karbon bercetak skrin (SPCE) yang digunakan untuk pengesanan elektrokimia terdiri daripada tiga elektrod: elektrod tambahan, kerja, dan rujukan. Elektrod kerja adalah elektrod utama di mana tindak balas elektrokimia dilakukan, sementara elektrod rujukan dan tambahan digunakan untuk melengkapkan litar elektronik. Kesan perubahan ketebalan dakwat konduktif elektrod kerja (2, 3, 4, 5, dan 7 lapisan SPCE) terhadap aktiviti elektrokimia terakhir disiasat dalam kajian ini. Kedalaman SPCE terendam dalam 20 mL analit ditetapkan pada 1.5 cm kerana panjang pasti tidak diketahui. Ini menunjukkan bahawa dengan meningkatkan lapisan ketebalan dakwat konduktif, puncak pengoksidaan juga meningkat. SPCE empat lapisan memberikan hasil yang optimum untuk mengesan AA pada kepekatan 3mM hingga 10mM dengan nilai R2 hampir 0,99. Selain itu, laporan ini juga menyelidiki pemilihan asid askorbik (AA) dari asid sitrik (CA) dengan menggunakan SPCE empat lapisan.

FEASIBILITY OF SCREEN PRINTING CARBON ELECTRODE TO DETECT ASCORBIC ACID

ABSTRACT

Screen-printed carbon electrodes (SPCE) used for electrochemical detection consist of three electrodes: auxiliary, working, and reference electrode. The working electrode is the principal electrode on which electrochemical reactions are performed, while the reference and auxiliary electrodes are used to complete the electronic circuit. The effect of varying the working electrode conductive ink thickness (2, 3, 4, 5, and 7 layers of SPCE) on the final electrochemical activity was investigated in this study. The depth of SPCE been submerged in 20mL of analyte is fixed at 1.5 cm since the exact length is unknown. It shows that by increasing the layer of conductive ink thickness, the oxidation peak is increasing too. Four-layer SPCE gives the optimum results for detection of AA at a concentration of 3mM to 10mM with the R^2 value is almost 0.99. Besides, this report also investigates the selection of ascorbic acid (AA) from citric acid (CA) by using the four-layer SPCE.

CHAPTER 1
INTRODUCTION

Chapter 1 introduces the overview of this research and the feasibility of screen printing carbon electrodes to detect ascorbic acid. In general, this chapter summarizes the research background of screen printing carbon electrodes and the viability to detect ascorbic acid as well as the problem statement and the objectives of this final year project.

1.1 Research Background

Ascorbic acid (AA) is an organic molecule that plays an important role in biological development, particularly in the development of human metabolism. As an antioxidant, AA is beneficial to the biological system as well as the pharmaceutical, cosmetic, chemical, and food industries (Yilmaz et al., 2008). AA has also been used to prevent and treat a variety of diseases and illnesses, including the common cold, infertility, cancer, and mental illness (Vinoth et al., 2015). Therefore, it is interesting and very important to study the electrochemical behaviour of ascorbic acid. To study voltammetry of ascorbic acid, various approaches have be made such as a cyclic voltammetric study of ascorbic acid using polyglycine modified carbon paste electrode, and poly(glutamic acid) modified carbon paste electrode, and graphene oxide/gold nanoparticle but with little explanation on the voltammetric study of ascorbic acid (Farida et al., 2019).

Bulky electrochemical electrodes and beaker-type cells have proven to be very useful in electro-analytical chemistry, but they fall short in many areas, including cost, portability, usability, and on-site analysis. Screen printing on planar substrates with appropriate inks forced through a patterned stencil or mask is an adaptive technique for overcoming these drawbacks. The resulting screen-printed electrodes (SPEs) have already been used in a wide range of real applications. This is largely driven by a billion-dollar-per-year glucose sensing market (Cui, 2017). Individuals now can measure their blood glucose levels at home thanks to the incorporation of SPEs. In addition to commercially available electrochemical glucose sensors, these SPEs have been frequently used for other biomedical applications like fructose determination in beverages (Nicholas et al., 2018), glucose determination in fermentation processes, and diagnosis of infectious disease, and virus. It has also been suggested that SPEs could be used to detect coronaviruses (Sarmphim et al., 2021).

Screen-printed electrodes are usually made of carbon, gold, and platinum. Carbon is commonly used because it is less expensive than gold and platinum, and relatively inert as well as has a broad potential operating window (Martin & Grgicak, 2014). For sensing applications, carbon electrodes are very appealing. These materials are not just chemically inert and but also offer a wide range of anodic operating potentials with minimal electrical resistance. They also have a crystal structure that is exceedingly clean, resulting in low residual currents and a good signal-to-noise ratio (Zhang et al., 2000).

1.2 Problem Statement

There are not many articles that can be used as a reference to conduct this experiment. Some of the problems are like what is the optimum depth for the SPCEs to detect the ascorbic acid. Which is a better way to do the next layer of the SPCE either by letting the previous screen-printed electrode dry at room temperature or use the oven. Besides that, what are the best choice of the scan rate and the number of the cycle for the cyclic voltammetry. For the preparation of AA solution, although it is stored in refrigerator, will it affect the result if it is stored for a long time (before it turns yellowish or oxidized).

1.3 Objective

The objective of this study is:

1. Screen printing of carbon conductive ink with different carbon layer.
2. Assess electric resistivity and characterize of screen-printed electrode.
3. Performance evaluation of carbon electrode in electrochemical sensing application to detect ascorbic acid.

CHAPTER 2
LITERATURE REVIEW

2.1 Screen Printing Electrode Principles

Screen printing, as a traditional and ancient technique, was introduced to the world over a thousand years ago and is still used today in fields such as art, textiles, and advertising. Researchers used screen printing to create electrode devices on various substrates to meet the demand for reproducible, stable, and disposable devices suitable for mass production. As a result, electrodes made using the screen-printing technique have sparked widespread interest, and the related SPEs fabrications have been evaluated (Metters et al., 2011). Furthermore, the wide range of SPE modifications opens a variety of new fields of application in environmental analysis.

A woven mesh supports an ink-blocking stencil, and a roller or squeegee is moved across the screen stencil to force or pump ink or other printable materials past the threads of the woven mesh in the open areas. In general, a series of woven meshes is prepared to print the various electrode parts. To print two ink layers on a substrate, the ink must first be solidified using thermal treatment. Finally, a protective ink coating is applied to the conductive track to protect it from the electrodes.

2.2 Printing materials and pretreatment

Silver ink and carbon ink are the most used pastes in the printing of SPEs. A conductive track is printed with silver ink, while the working electrodes are typically printed with carbon ink or gold ink. Carbon paste is a popular material because it is inexpensive, simple to modify, and chemically inert. The gold paste is also used in SPEs, but lesser than carbon due to its higher cost. However, the generation of self-assembled monolayers (SAMs) via strong Au S bonds is increasing interest in gold's use in SPEs. The screen-printed gold electrodes are applied as electrochemical biosensors, immune-, including enzymatic or genosensors (Serafín et al., 2011).

Mineral binders or insulating polymers are used in SPE printing inks to improve adhesion to the substrate. The presence of polymers in working electrode inks may protect the electrochemically active carbon particles and increase electron transfer resistance, resulting in slower kinetics of the heterogeneous reaction and the occurrence of quasi-reversible or irreversible redox processes at the SPEs. To improve the slow electron transfer of bare SPEs, certain materials can be added to the printing paste. Noble metals currently have the best overall catalytic performance and play a dominant role as electrocatalysts. Their high cost, however, is a significant barrier to commercial applications, so less expensive materials, such as manganese oxide (MnO_x), are used to replace the noble metal materials. Before screen printing, the commercial manganese oxide particles were thoroughly mixed into the carbon ink formulation (Šljukić et al., 2011). As a result, the prepared MnO_xSPCE performed well as an electro-catalyst for sensing nitrite ions and ascorbic acid with low detection limits. Bismuth oxide (Kadara et al., 2009) and nanoparticles of bismuth (Rico et al., 2009) have also been successfully mixed with carbon paste to prepare screen-printed bismuth electrode (SPBEs).

Another method for improving the electrochemical properties of SPEs is pretreatment. The primary goal of SPE pretreatment is to remove organic ink constituents and contaminants while increasing surface roughness and functionalities. As a result, several researchers have proposed a short electrochemical-anodization method, a potential cycling method, and a two-step pretreatment approach that combines chemical (soaked in a high-concentration NaOH solution) and electrochemical treatments (anodization in a mild NaOH solution) (Su et al., 2011). In addition, plasma treatment has been used to clean and modify the surface of SPCEs. Plasmas such as Ar, O2, SF6, and CHF3 have been used to improve the sensitivity and reproducibility of SPCEs (Ghamouss et al., 2007).

2.3 Novel Designs

The forms of SPEs can vary and can be designed based on the demands for analysis, with common forms including a disc, a ring, and a band. When compared to common SPEs, SPEs in the micrometer-scale range can exhibit advantages attributed to microelectrodes, such as improved signal-to-noise ratios, independence from forced convection, a low ohmic drop, and a faster mass-transport rate. Micrometer-scale SPEs, on the other hand, are considered difficult to produce and not very reproducible. To get around this limitation, some researchers print normal-sized working electrodes on polymer substrates (Xie et al., 2005). The formed transversal surface could function as a microelectrode after the plate has been cut perpendicular to the conductive track with a scalpel (Zaouak et al., 2009). Furthermore, screen printing was used to create a carbon-ink-based micro-tubular band electrode inside a well. The well's purpose is to provide a known volume for enzymatic reactions and other interesting reactions. To make the well-like electrodes, holes were punched or laser-drilled through the four-carbon pads, resulting in four well electrodes on one strip (Li et al., 2012). The fabrication process is shown in Figure 2.1.

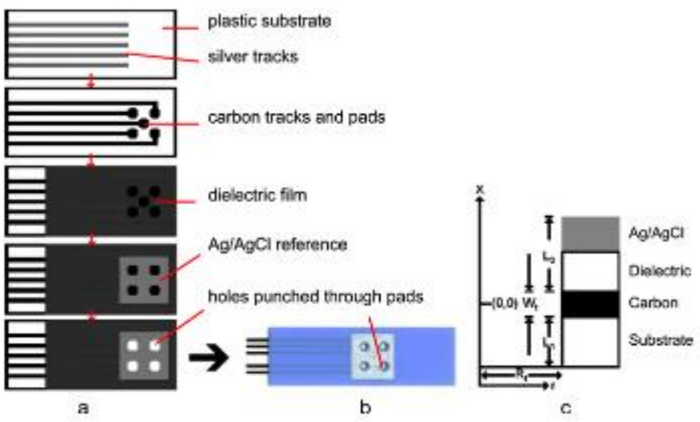


Figure 2.1 Schemes of (a) side view illustrating the method for manufacturing SPEs, (b) image of a good electrode strip, and (c) a vertical section cut through the centre of the well containing the coordinate system used for the good electrode simulation.

2.4 Application in electrochemical sensing

Vitamins are a diverse group of compounds with interesting electrochemical properties due to their complex chemical structures. Because most vitamins are naturally electroactive or become electroactive under certain conditions, their properties are still being used in electrochemical techniques. Most of the attention was given to screen-printed sensors developed for the analysis of L-ascorbic acid (vitamin C). Table 2.1 summarizes the performance characteristics of a variety of screen-printed devices developed over the last decade for the analysis of L-ascorbic acid. The following section goes into greater detail about the fabrication and subsequent application.

2.5 Mediated electron transfer

Conducting polymers, on their own, frequently lack the required mechanical and conductive properties exhibited by carbon; however, their catalytic properties make them useful materials in the modification of SPCEs (Elyashevich et al., 2006). Ambrosi et al. (2008) used a simple drop-coating procedure to deposit polyaniline (PANI) nanoparticles onto the surface of an SPCE; it was discovered that adding dodecylbenzene sulphonic acid (DBSA) during the conducting polymer synthesis resulted in the best catalytic response. The resulting nano-PANI-SPCE sensor had the broadest linear range of any sensor reported in this review (Table 2.1). The optimum operating pH was 6.8 and an applied potential of 0 V was feasible. The best operating pH was 6.8 and an applied potential of 0 V as possible. This is the lowest reported operating potential for a conducting polymer-based L-ascorbic acid sensor; under these conditions, other species present in pharmaceutical formulations are less likely to produce an interfering response. Figure 2.2 summarizes the sequence of reactions involved in the operation of the PANI-based sensor.

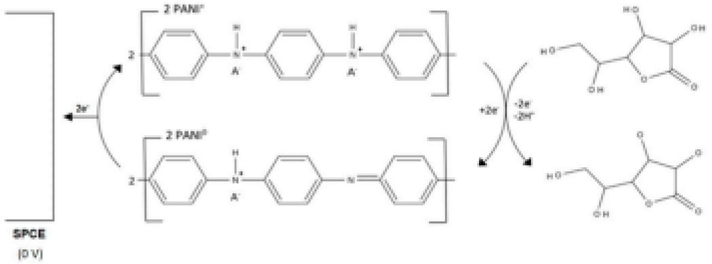


Figure 2.2 The reaction scheme of L-ascorbic acid at polyaniline (PANI)-SPCE

Milakin et al. (2013) also reported a PANI modified SPCE for the determination of L-ascorbic acid. Similarly, the authors used a drop-coating procedure to deposit an aniline and ammonium persulfate mixture in phosphate buffer; after deposition, the aniline polymerizes and the PANI binds to the carbon black surface. This PANI-SPC working electrode was successfully used in conjunction with conventional counter and reference electrodes to determine L-ascorbic acid in grapefruit juice. This device's detection limit was the lowest reported in the last decade for SPCE-based systems.

Inkjet printing in conjunction with screen printing procedures is an alternative method for preparing PANI-based sensors for L-ascorbic acid determination. The authors chose a filter paper substrate for the three-electrode sensor to create a more environmentally friendly device. The response characteristics were inferior when compared to other PANI-based sensors (Table 2.1); however, the authors suggest that the DBSA doping procedure described by Ambrosi et al. (2008) could improve their device characteristics if used in the conducting polymer synthesis steps (Kit-Anan et al., 2012).

For the development of an L-ascorbic acid sensor, a different mediator, quinoeimine, was used. The electrochemical reduction of the in situ generated o-aminophenol diazonium salt was used in this case as part of the fabrication procedure (Nassef et al., 2008). A two-step process is used to generate the signal. The L-ascorbic acid chemically reduces the o-quinoeimine form in the first step, which is followed by electrochemical oxidation of the reduced form of the mediator. The comparison of performance characteristics places this sensor slightly below some of the mediator based SPCEs (Table 2.1), but the selectivity achieved allowed for the analysis of a variety of fruits and juices with minimal sample preparation (Civit et al., 2008).

2.6 Unmediated electron transfer

There is publication which reported an mM detection limit, while almost other publications reporting the use of SPCEs for the determination of L-ascorbic acid achieved a detection limit in the µM range. This sensor made use of a commercially available screen-printed carbon working electrode with no surface modifications reported (Wonsawat, 2014). Ambrosi et al. (2008), on the other hand, reported a simple ink modification that resulted in a thousand-fold increase in the limit of detection over Wonsawat (2006). A chemically reduced graphene oxide powder and accompanying ionic liquid were added to the screen-printing ink (n-butylpyridinium hexafluorophosphate). The screen-printed graphene electrode (SPGNE) demonstrated improved peak potential separation of L-ascorbic acid (AA) in the presence of two other important biological compounds, dopamine (DA) and uric acid (UA), in addition to improved current response to L-ascorbic acid. These three compounds typically oxidize at similar potentials when using traditional electrode materials.

Carbon nanotubes are another popular form of carbon for electrode modification due to their unique electrochemical properties. Moving closer to miniaturization, (Crevillén et al., 2008) created a multi-walled carbon nanotube (MWCNT) SPCE coupled with an amperometric capillary electrophoresis microchip device. Signal-to-noise ratios, LODs, and LOQs all show improved response characteristics as a result of an MWCNT modification. The surface area of the nano-carbon structures was measured, and it increased significantly from 0.0075 cm3 for bare SPEs to 2.1 cm^3 for MWCNT-SPCEs. Although this device did not outperform some of the sensors in Table 2.1, it did successfully determine multiple water-soluble vitamins, including L-ascorbic acid, pyridoxine, and folic acid. A similar sensor setup was used in a previous publication by the same group to successfully determine a wide range of compounds important in food analysis, including polyphenols, vanilla flavours, and isoflavones (Hughes et al., 2016).

Table 2.1 Reports of screen-printed sensors for vitamin C (L-ascorbic acid).

Electrode Components	Supporting Electrolyte	Measurement Technique	Detection Limit (µM)	Linear Range (µM)	Sample/s	Modification Method
W: Nano-PANI SPCE R: Ag/AgCl C: Pt mesh	PBS pH 6.8	Amperometry 0 V	8.3	500–8000	Tablet pharmaceutical	Drop coating
W: PANI-SPCE	0.05 M Phosphate	Cyclic Voltammetry	0.1	1.00–80.00	Grapefruit juice	Oxidative chemical

R: Ag/AgCl C: GCE	buffer pH 7.0 & 0.5 M NaCl					polymerisation
W: PANI-SPCE R: Carbon C: Carbon	0.1 M Acetate buffer pH 5.0	Chronoamperometry 0.4 V	30	30.00–270.00	None reported	Inkjet printed Paper based design
W: o-AP-SPCE R: Ag C: Carbon	0.1 M Phosphate buffer pH 7.2	Amperometry 0.2 V	0.86	2.00–20.00	Apple, Kiwi, Lemon, Orange, Pineapple, Strawberry, Tomato	Electrografted film
W: SPCE R: Ag/AgCl C: Carbon	0.1 M Phosphate buffer pH 2.0	DPV 0.0 V > −1.2 V	1360	1000–10,000	Orange juice	Unmodified
W: SPGNE R: Ag/AgCl C: Pt	0.1 M Phosphate buffer pH 7.0	DPV −0.2 V > +0.6 V Ep = −0.5 V	0.95	4.00–4500.00	Injection formula	Graphene ink formulation
W: MWCNT-SPCE R: Ag/AgCl wire C: Pt wire	0.01 M Phosphate buffer pH 7.0	Amperometry −1.2 V	11	50.00–400.00	Tablet pharmaceutical Capsule pharmaceutical	Drop coating
W: N6-NFM-SPCE R: Ag C: Graphite	Buffer citrate pH 4	Amperometry 0.35 V	Nor reported	56.78–7381.33	Tangerine, Apple, Pear, Kiwi, Lemon, Strawberry	Electrospun membrane

W: Working Electrode. R: Reference Electrode. C: Counter Electrode. PANI-SPCE: Polyaniline screen-printed carbon electrode. o-AP-SPCE: o-Aminophenol film screen-printed carbon electrode. SPGNE: Screen-printed graphene electrode. MWCNT-SPCE: Multi-walled carbon nanotube screen-printed carbon electrode. N6-NFM-SPCE: Nylon 6 nano fibrous membrane screen-printed carbon electrode.

CHAPTER 3
METHODOLOGY

3.1 Overview of Research Methodology

Overall, this final year project focus on the feasibility of screen-printing carbon electrode to detect ascorbic acid. Figure 3.1 shows the overview of the activity of this research.

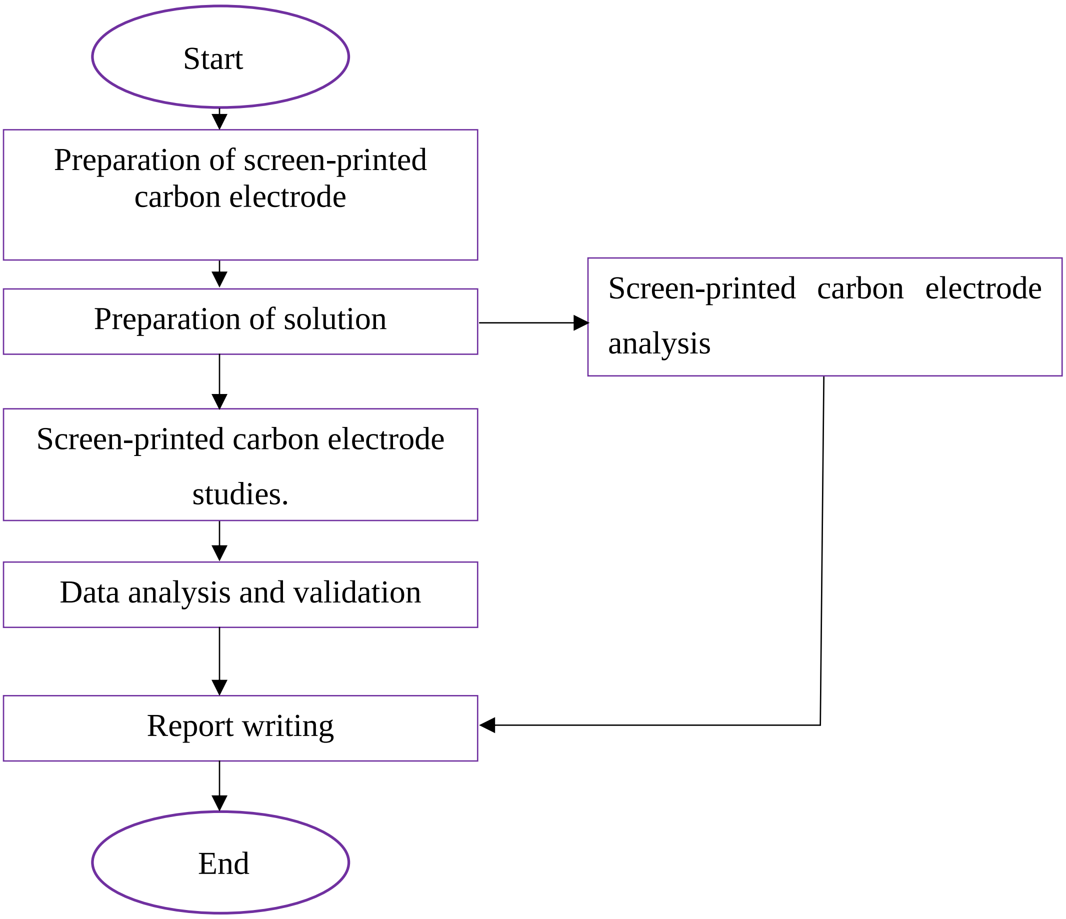


Figure 3.1 Flow diagram of research project on feasibility of screen printing carbon electrode to detect ascorbic acid.

3.2 Preparation of Screen-Printed Carbon Electrode

The technique of screen-printing is used to produce the carbon electrode. The tools needed are feeler gauge, cutting knife, glossy inkjet photo paper, squeegee, membrane thickness gauge, and substrate to print upon. The first step is to place the printing media, which in this study, the commercial carbon paste was used. The carbon paste was printed onto the glossy inkjet photo paper, which has been molded with a dimension of 4 cm long and 0.5 cm wide. With a feeler gauge, each layer of the screen-printed carbon electrode is set to 0.1 mm and placed between the substrate and the molded glossy inkjet photo paper. The constant movement of the printing media towards the substrate at a sufficient speed and pressure during a screen-print cycle is a critical factor, which is accomplished by the use of a squeegee. It must be done in one strike without stopping in the middle of the screen print.

3.3 Effects of printing parameter

In this work, the carbon electrodes were printed with multiple layers (2, 3, 4, 5, and 7) to determine its ability to transfer electron. In this case, the direction of the screen print should be consistent. After a layer of carbon has been screen printed, it will be let dry at room temperature for 3-4 hours to ensure complete drying. Before applying another layer of carbon paste, the dry screen-printed carbon electrode was smoothened with sandpaper. The thickness of the printed electrode was measured with a micron thickness gauge to around $100\mu m$. For the subsequent layer, the same process was repeated. The final screen-printed carbon electrode was the stack up multi-carbon layer with the same dimensions.

3.4 Preparation of buffer and testing solutions

1L of Phosphate buffer solution (PBS) with 0.05 M and pH 7.0 was prepared. The composition of PBS buffer used comprises of sodium phosphate monobasic monohydrate (MW: 137.99 g/mol) and sodium phosphate dibasic heptahydrate (MW: 268.07 g/mol). The amount of sodium phosphate monobasic monohydrate needed is

2.9135 g and sodium phosphate dibasic heptahydrate is 7.7435 g. Then, it was dissolved with distilled water and the pH of PBS was controlled with either hydrochloric acid (HCl) or sodium phosphate (NaOH) depends on the PBS pH after being tested with a pH meter.

Next is the ascorbic acid (AA) and citric acid (CA) stock solution. The stock solution molarity was prepared at 50mM and needs to be stored in a refrigerator to avoid it from oxidizing. The molarity for both AA and CA that be used for testing with the prepared screen-printed carbon electrode were 3mM, 5mM, 10mM, 15mM, and 20mM with the volume of 20 ml. It was prepared using the stock solution by dilution with the use of equation 3.1.

$$M_1V_1 = M_2V_2 \qquad \ldots \text{(eq 3.1)}$$

Where, M_1 = stock solution concentration

V_1 = volume of stock solution needed for dilution

M_2 = the required AA/CA molarity

V_2 = the volume of the diluted AA/CA

3.5 Electrochemical testing

The experiment is conducted with several different criteria. First, each of the prepared screen-printed carbon electrodes were tested with each of the different concentrations of ascorbic acid by using a potentiostat. The detection condition was 0.05 V/s for the scan rate from -0.8 V to 1.2 V with 10 cycle scans. Following the collection of all cyclic voltammetry (CV) data, several graphs were plotted, including (i) the highest oxidation vs the number of layer of the screen-printed carbon electrode (SPCE) and (ii) the oxidation peak at specific potential peak (Ep) vs the number of layer of the SPCE. The results were then analyzed, where the SPCE with the best performance was chosen to be tested with CA. Then, the selected SPCE was tested for AA/CA at various volume ratios (V/V). In this work, the amount of analyte used is 20ml, so after obtaining the result on which layer of SPCE and concentration of AA gives the optimum results, the concentration is used to prepare for CA. with the same concentration of AA and CA, for preparation of 100% AA or 100% of CA the total volume for each is 20 ml, if the volume ratio of AA/CA is 25%/75% the volume of AA will be 5 ml and volume of CA will be 15 ml and vice versa. If the ratio is 50%/50% the volume for AA and CA will be 10 m each.

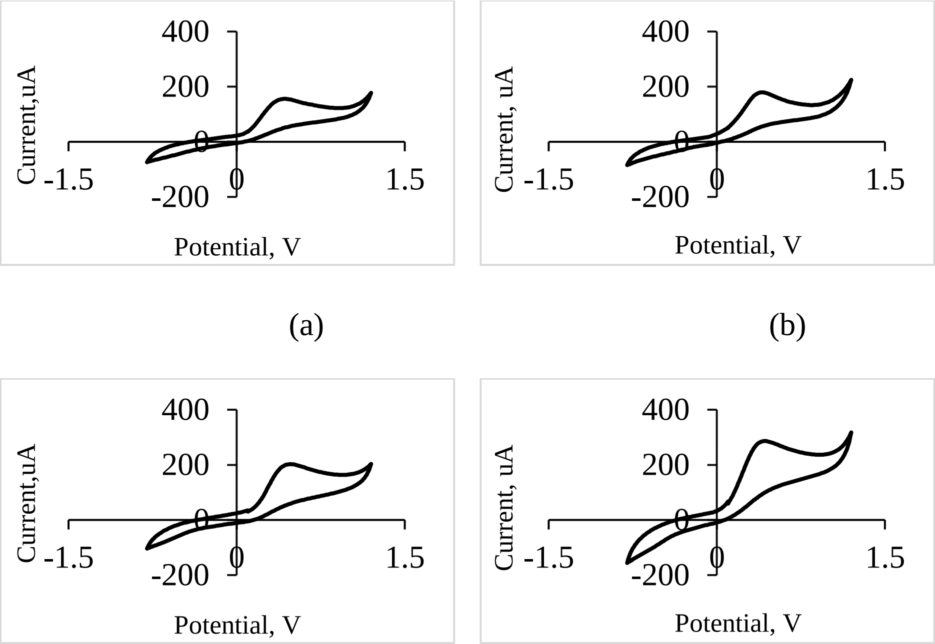
3.1.1 Thesis and report writing

The data and results were presented. In the report writing, the CV of the detection of the AA with different concentrations with a different layer of SPCE was evaluated and discussed. The SPCE that gave the optimum performance was also presented accordingly. Conclusion and suggestions were made based on the results obtained.

CHAPTER 4
RESULTS AND DISCUSSION

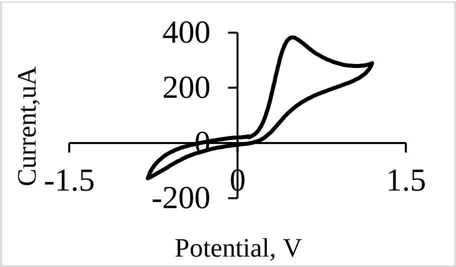
4.1 The influence of SPCEs cast layers on cyclic voltammetry

Electrochemical oxidation of ascorbic acid (AA) at the screen-printing carbon electrode (SPCEs) with different printed layers were studied by cyclic voltammetry (CV) with the same scan rate 0.05 V/s. In this work, data was taken from the 6th cycle from the total number of 10 scans, since the scan cycle of 5th, 6th, and 7th cycle were almost overlapping. In Figure 4.1, for the testing of 3mM concentration of AA, the two layers of SPCE have shown the lowest current peak I_p or oxidation peak which was at 155.7 μA (Figure 4.1a). Meanwhile, the seven layers of SPCE (Figure 4.1e) have displayed the highest I_p or oxidation peak at 382.7 μA. It was noticeable that the anodic peak increasing when the layer of SPCEs increases within the same concentration of AA. This is because the thicker the SPCE the more contact area of the SPCE has contact with the analyte. When a higher volume of AA is detected the higher the current value.



(a) (b)

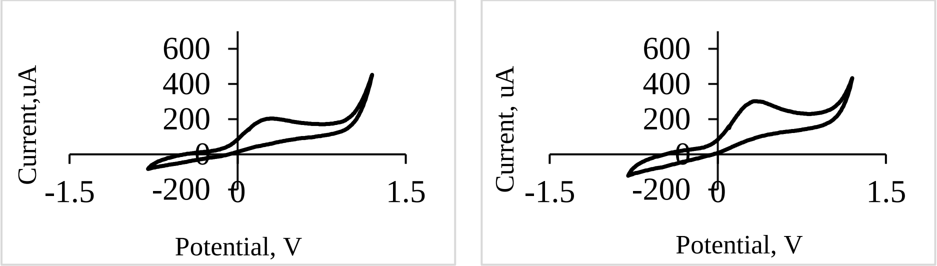
(c) (d)



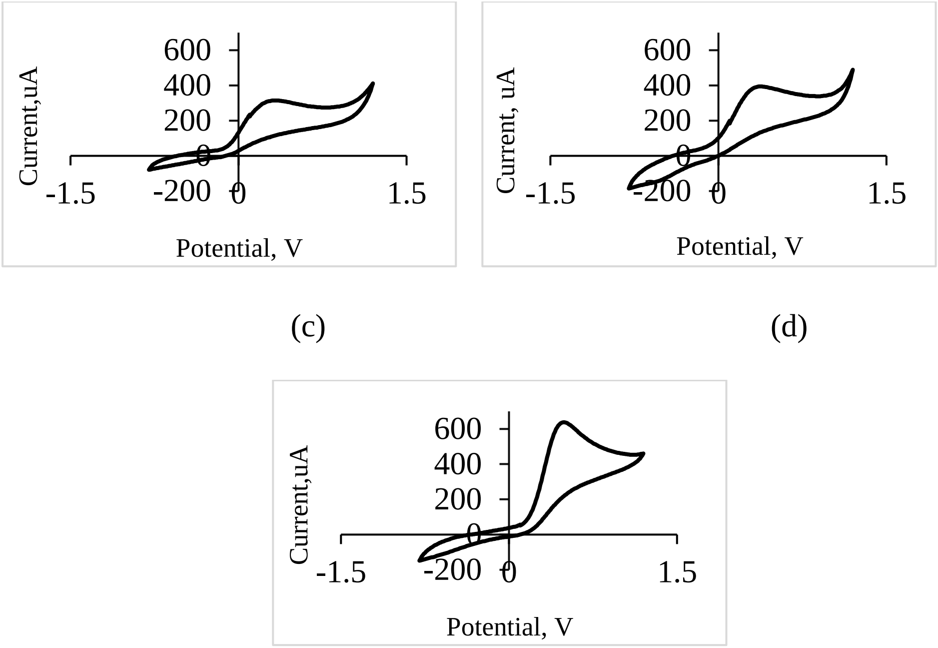
(e)

Figure 4.1 Cyclic voltammetry at 6th cycle of SPCE comprised of: (a) two (b) three (c) four (d) five and (e) seven layers in the presence of ascorbic acid of 3mM at 7.0 pH PBS with scan rate of 0.05 V/s.

For the next concentration of AA at 5mM (Figure 4.2), for the least number of SPCE layer which is two layers, the highest oxidation peak current was 196.8 μA and the highest oxidation current output of 638.3 μA when SPCE of seven-layers was tested. This trend was similar to Figure 4.1, but at higher magnitude of current output. In fact, this result is predictable since the concentration of the AA used in Figure 4.2 was more than the previous one in Figure 4.1. But, one interesting observation is that the increment of the value of the highest oxidation value for each SPCE was not the same. For example, the two-layers SPCE showed an increment of about 26% when increased the AA concentration from 3mM to 5mM. But, the seven-layers SPCE showed the increment of about 66%.



(a) (b)

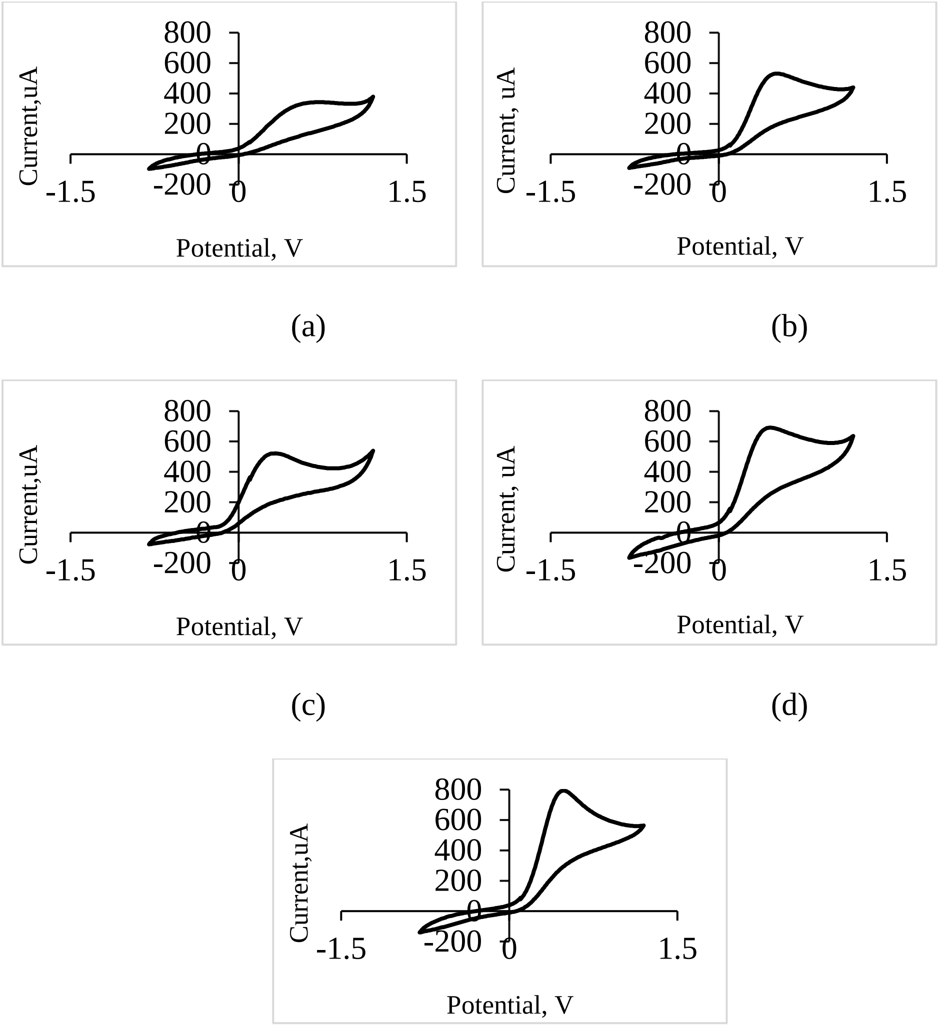


(c) (d)

(e)

Figure 4.2 Cyclic voltammetry at 6th cycle of SPCE comprised of: (a) two (b) three (c) four (d) five and (e) seven layers in the presence of ascorbic acid of 5mM at 7.0 pH PBS with scan rate of 0.05 V/s

Then, a 10mM concentration of AA was used and results were shown in Figure 4.3. Again, the trend of current output versus the number of SPCE layers were the same compared to Figure 4.1 and Figure 4.2. By using 10mM concentration of AA, the highest oxidation value for two-layers of SPCE was 342.5 μA and the highest oxidation current value for seven-layers of SPCE was 793.6 μA. The increment of current was vice versa compared to the one in 5mM. The two-layers SPCE showed the increment of about 76% while for the seven-layers SPCE, the increment was about 24%. Based on the research observation, it was found that when the number of SPCE layer was lower or thickness of the SPCE was lower, the detection of AA will be more sensitive when the concentration of AA was higher. In contrast, for the higher number of SPCE layers or thicker thickness of SPCE, the AA solution didn't penetrate all over the SPCE. Therefore, the increment of electricity output will be slower.



(a) (b)

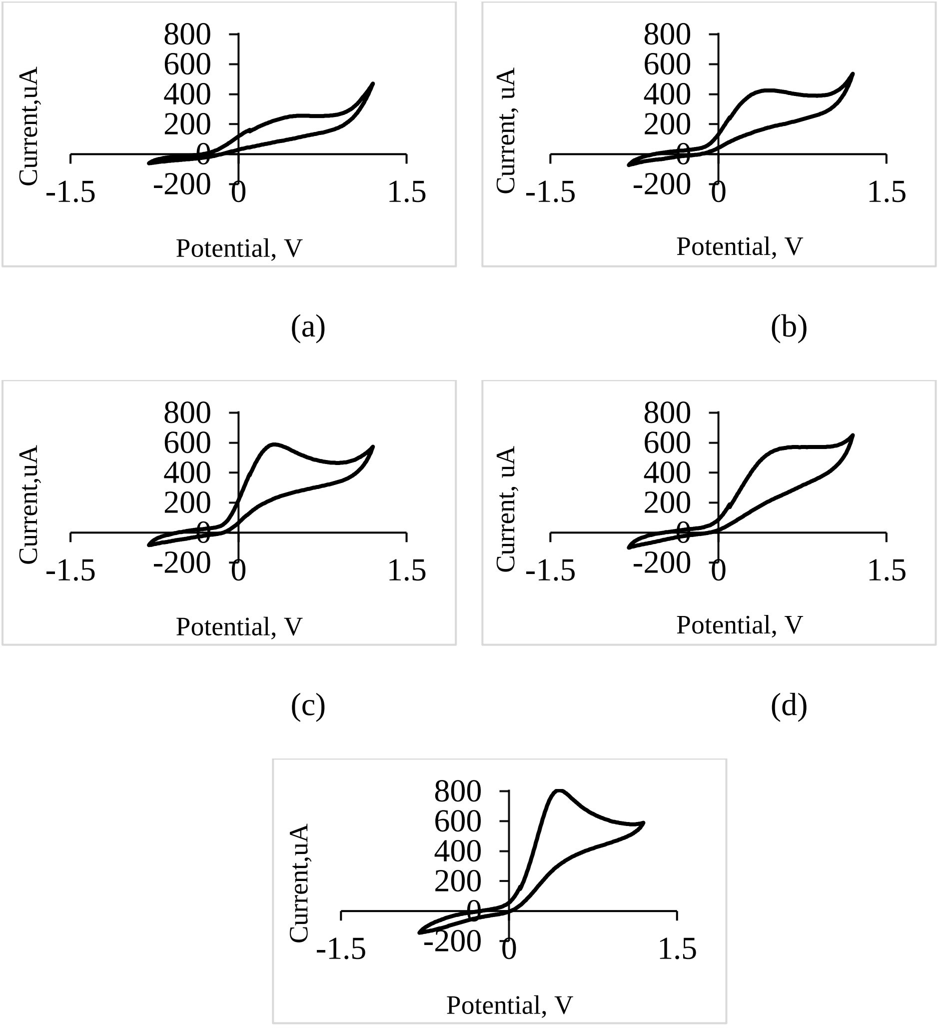
(c) (d)

(e)

Figure 4.3 Cyclic voltammetry at 6th cycle of SPCE comprised of: (a) two (b) three (c) four (d) five and (e) seven layers in the presence of ascorbic acid of 10mM at 7.0 pH PBS with scan rate of 0.05 V/s.

The experiment was then repeated with the second-highest concentration of AA, 15mM, and the results are shown in Figure 4.4. The trend is slightly different when using high concentration of AA. The highest oxidation output values of all SPCEs were shown a dropping trend except for the four-layer and seven-layer of SPCEs, compared to the previous concentration of 10mM of AA in Figure 4.3. For example, the highest oxidation current value for 10mM two-layer SPCE was 342.5

μA, but the oxidation current value was 256.8 μA when testing with 15mM of AA. For the three-layer SPCE, the previous highest oxidation value was 530.5 μA (10mM of AA in Figure 4.3b) and for 15mM was 426 μA (Figure 4.4b). Lastly, the five-layer SPCE showed the highest oxidation value at 691.4 μA for 10mM of AA and 570 μA for 15mM of AA. Ideally, this phenomenon should not occur. The higher concentration of AA should rendered with higher current output. This observed trend may due to the saturation of detection limit of SPCE or improper cleaning prior to performing the test.



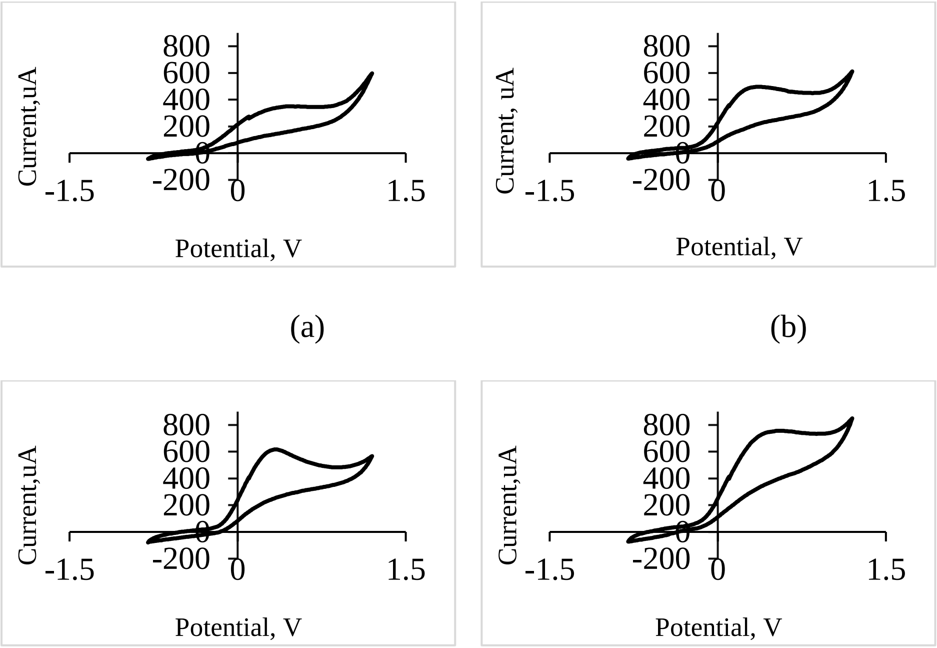
(a) (b)

(c) (d)

(e)

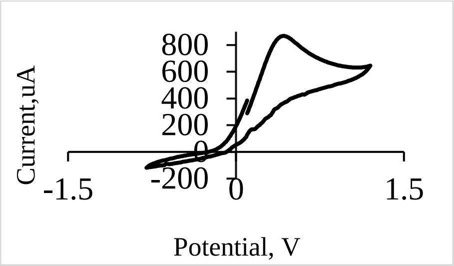
Figure 4.4 Cyclic voltammetry at 6th cycle of SPCE comprised of: (a) two (b) three (c) four (d) five and (e) seven layers in the presence of ascorbic acid of 15mM at 7.0 pH PBS with scan rate of 0.05 V/s.

In order to verify the saturated detection of SPCE, the CV analysis was carried out using 20mM of AA and results were shown in Figure 4.5. The trend of the highest layer of SPCE having the highest oxidation current output is observing. This means, the SPCE may yet to be saturated with 20mM of AA. However, the highest oxidation current value was not found to be highest when three-layer SPCE was tested. At 20mM of AA, the oxidation current output was found at 495.2 μA This current value was found lower when comparing to 10mM of AA (530.5 μA). It was suspected the defect of 3 layers SPCE when testing with 20mM of AA. The SPCE may have defect void space between the cast carbon layers. Therefore, reduce its efficiency to transfer electron during CV analysis. For the remaining of SPCEs at different layers, they showed normal trend where the highest oxidation current peak was observed when SPCE was tested for higher of AA. But, the increment rate was high. Therefore, the optimal concentration for AA detection of SPCE could be up to 10mM.



(a) (b)

(d) (d)



(e)

Figure 4.5 Cyclic voltammetry at 6th cycle of SPCE comprised of: (a) two (b) three (c) four (d) five and (e) seven layers in the presence of ascorbic acid of 20mM at 7.0 pH PBS with scan rate of 0.05 V/s.

In order have a clearer comparison on the performance of SPCE with different concentration of AA, the highest oxidation current output and oxidation peak for each SPCE were tabulated. Table 4.1 summarized the values of current output (μA) found at the highest oxidation peak. Table 4.2 tabulated the potential peak value (V) when the oxidation current peak was found. In general, the higher the testing concentration of AA or the higher cast-layer of SPCE, larger current output will be observed until it reached the saturation level. On the other hand, all SPCEs have shown stable CV performance (Table 4.2), where the potential peak (V) was found at the similar value from 0.43 to 0.55V, except two outlier data at 0.64V and 0.71V.

Table 4.1 Value of highest oxidation current output (μA) for each SPCE at different concentration of AA.

		AA concentration (mM)				
		3	**5**	**10**	**15**	**20**
SPCE	**2**	155.7	196.8	342.5	256.8	350.4
Layer	**3**	179.5	297.9	530.5	426	495.2

	4	202.4	310.5	512.3	574.4	605.9
	5	286.2	393.5	691.4	570	756
	7	382.7	638.3	793.6	806.5	868.5

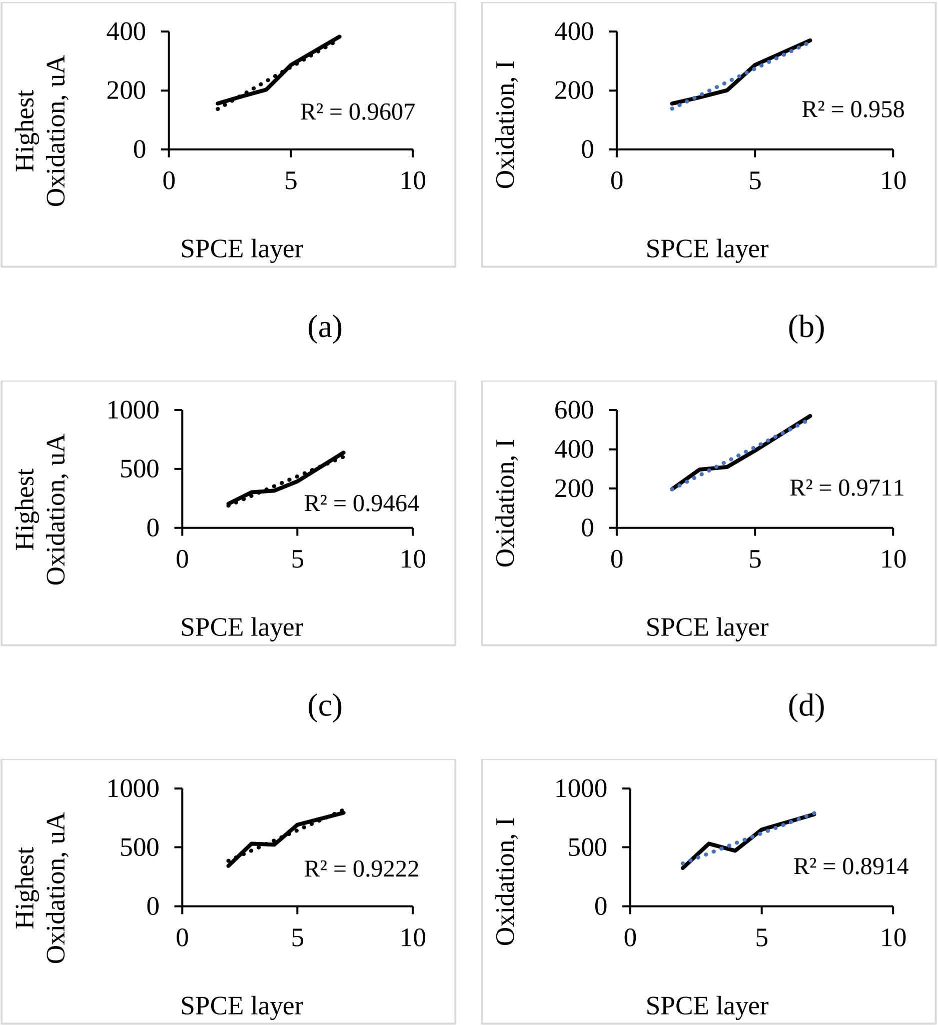
Table 4.2 Value of potential peak (V) for each SPCE at different concentration of AA.

		AA concentration (mM)				
		3	**5**	**10**	**15**	**20**
SPCE Layer	**2**	0.43	0.4	0.71	0.57	0.47
	3	0.4	0.4	0.52	0.46	0.4
	4	0.48	0.4	0.4	0.4	0.4
	5	0.43	0.4	0.46	0.64	0.54
	7	0.49	0.49	0.48	0.45	0.42

4.2 Relationship of oxidation current with different layer of SPCE

In the previous section, it shows that within the same concentration of AA, the increasing number of layers of SPCEs have the higher oxidation current output at similar potential peak, E_p. However, the potential peak was found in a range (Table 4.2) but not at the exact value. Therefore, in Figure 4.6, two types of comparisons were carried out. The first comparison was by plotting the highest oxidation current output against the different number of SPCE layers at different concentration of AA. While, the second comparison was by plotting the oxidation current output at a fixed potential peak against different number of SPCEs layers.

When comparing the first set of data, which was plotting the highest oxidation current output against the SPCE layers at different concentrations of AA, the value of R^2 for 3mM of AA concentration can achieved at 0.9607. This is the highest R^2 compared to other concentration of AA. For the second set of comparison, which was plotting the oxidation current output at a fixed potential peak, the best R^2 value was 0.9711, when 5mM of AA was tested. Overall, R^2 values for both set of comparisons also showed more than 0.9, except Figure 4.6(f) at 0.8914, for 15mM concentration of AA. From Figure 4.6, it can be concluded that the in-house produce SPCE showed a stable CV performance, in which the oxidation current output is linearly proportionate to the increasing layers of SPCE with stable potential value.



(a) (b)

(c) (d)