An Electrochemical Sensor for Glucose Detection

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บทคัดย่อ

วิทยานิพนธ์นี้พัฒนาเซนเซอร์ทางเคมีไฟฟ้าแบบไม่ใช้เอนไซม์สำหรับการตรวจวัดกลูโคสร่วมกับระบบโฟลอินเจคชันแอมเพอโรเมตรี โดยการปรับปรุงผิวหน้าขั้วไฟฟ้าคาร์บอนพิมพ์สกรีนด้วยกราฟีนออกไซด์-กรดพอลีอะครีลิค-อนุภาคนาโนพาลาเดียม (GO-PAA-PdNPs/SPCE) ซึ่งสามารถเตรียมอนุภาคนาโนพาลาเดียมบนกราฟีนออกไซด์-กรดพอลีอะคลิลิค โดยวิธีการเกาะติดแบบไม่ใช้ไฟฟ้า จากผลการทดลองพบว่าขั้วไฟฟ้า GO-PAA-PdNPs/SPCE มีประสิทธิภาพการเร่งการเกิดปฏิกิริยาออกซิเดชันของกลูโคสที่ดีเยี่ยม ได้ศึกษาลักษณะสัณฐานวิทยาและพฤติกรรมทางเคมีไฟฟ้าของขั้วไฟฟ้าที่พัฒนาขึ้นด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่าน ฟลูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปคโทรเมตรี ไซคลิกโวลแทมเมตรี และเทคนิคแอมเพอโรเมตรี เพื่อเพิ่มประสิทธิภาพของการเร่งปฏิกิริยาออกซิเดชันของกลูโคส ได้ศึกษาปัจจัยต่างๆ ที่มีผลต่อสัญญาณการตรวจวัด ได้แก่ ปริมาณของ GO-PAA-PdNPs บนผิวหน้าขั้วไฟฟ้า ศักย์ไฟฟ้าที่ใช้ในการตรวจวัด อัตราการไหลและปริมาตรของตัวอย่าง ภายใต้สภาวะที่เหมาะสมเซนเซอร์ที่พัฒนาขึ้นมีขีดจำกัดในการตรวจวัด 22 ไมโครโมลาร์ ขีดจำกัดในการตรวจวัดเชิงปริมาณ 76 ไมโครโมลาร์ และให้ช่วงความเป็นเส้นตรงสองช่วงคือ 50 ถึง 15,000 ไมโครโมลาร์ และ 15,000 ถึง 60,000 ไมโครโมลาร์ มีความไววิเคราะห์ที่ดีเยี่ยม เสถียรภาพที่ดี และมีความจำเพาะสูง นอกจากนี้เซนเซอร์พัฒนาขึ้นสามารถนำไปประยุกต์ใช้ตรวจวัดกลูโคสในตัวอย่างทางชีวภาพซึ่งให้ผลการตรวจวัดสอดคล้องกับวิธีมาตรฐานเฮกโซไคเนสสเปกโทรโฟโตเมทรี

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Abstract

This thesis presents a report on a non-enzymatic electrochemical sensor consisting of a screen-printed carbon electrode (SPCE) modified with graphene oxide-poly(acrylic acid)-palladium nanoparticles (GO-PAA-PdNPs). The sensor was used in a system to determine glucose concentration by flow injection amperometry (FI-Amp). PdNPs were synthesized on GO-PAA by electroless deposition. The GO-PAA-PdNP-modified SPCE showed excellent electrocatalytic performance for the oxidation of glucose. The surface morphology of the modified electrode was characterized by transmission electron microscopy (TEM) and Fourier transform infrared (FT-IR) spectroscopy. The electrochemical behaviors of glucose on the different modified surfaces were also studied by cyclic voltammetry and amperometry. To achieve the optimal electrocatalytic oxidation of glucose at the GO-PAA-PdNPs/SPCE, amount of GO-PAA-PdNPs, the applied potential, sample volume and flow rate of the FI-Amp system were optimized. Under the optimal conditions, the current response when the glucose standard was injected provided a detection limit of 22 μmol L^{-1}, a limit of quantitation of 76 μmol L^{-1} and two linear ranges: 50 to 15,000 μmol L^{-1} and 15,000 to 60,000 μmol L^{-1}. This sensor had excellent sensitivity, good repeatability and high selectivity. The fabricated sensor was applied to detect glucose in biological fluid samples, and the results were in good agreement with those obtained from the standard hexokinase-spectrophotometric clinical method.

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Kritsada Samosorn

The Relevance of the Research Work to Thailand

The subject of this Master of Science Thesis in Chemistry is a non-enzymatic glucose sensor, based on the dispersion of graphene oxide-poly(acrylic acid)-palladium nanoparticles on a screen-printed carbon electrode. The sensor was incorporated into a flow injection-based amperometric (FI-Amp) detection system. This method will be useful in the detection of glucose in biological fluid samples. The fabricated GO-PAA-PdNPs modified SPCE can be applied to detect other analytes.

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List of Abbreviations

Amp	Amperometry
AOAC	Association of Official Analytical Chemists
CV	Cyclic voltammetry
EIS	Electrochemical impedance spectroscopy
FT-IR	Fourier transform infrared spectroscope
FI-Amp	Flow injection amperometry
GO	Graphene oxide
LOD	Limit of detection
LOQ	Limit of quantification
PAA	Poly (acrylic acid)
PdNPs	Palladium nanoparticles
RSD	Relative standard deviation
SPCE	Screen printed carbon electrode
TEM	Transmission electron microscope

CHARPTER 1

Introduction

1.1 Background and rationale

Diabetes mellitus is a chronic disease and is one of the leading causes of morbidity, death and major health problems worldwide [1]. It is caused by deficient insulin secretion or the inefficient response of the produced insulin, or both [2]. The International Diabetes Federation (IDF) predicts that the number of diabetic patients in the world will increase to 642 million by 2040 [3]. For these patients, the daily determination of blood glucose levels is necessary to ensure diagnosis and treatment [4]. Normally, the concentration of glucose in human blood is 4.1 to 5.6 mM [5]. An abnormally high glucose level in the body can create risks to organs, nerves and blood vessels which lead to comorbidities such as renal failure, loss of vision, retinopathy, high blood pressure, heart attack and strokes [6-9]. Thus, fast, accurate, and highly sensitive sensors are vital for effective glucose monitoring [10]. Methods developed for the detection and analysis of glucose include chromatography [11], fluorescence sensing [12], Raman spectroscopy [13] and electrochemical sensing [14, 15]. Among these methods, electrochemical sensing is receiving increased attention due to its simplicity, high sensitivity, excellent selectivity, fast response and ease of operation [16, 17]. Flow injection amperometry (FI-Amp) is an interesting approach to detect and analyse with high throughput, good precision, fast analysis and high reproducibility [18, 19]. FI-Amp systems also reduce the adsorption effect from sample contamination due to the reduced contact time between the electrode surface and the sample [20]. For these reasons, FI-Amp was chosen as the basis of the monitoring system in this work.

Electrochemical glucose sensors can be classified as enzymatic or non-enzymatic. Enzymatic sensors show good selectivity, high sensitivity, low detection limits, and are successfully applied in clinical areas [21, 22]. However, a simple, stable, reliable, and sensitive sensor for the measurement of glucose levels in blood samples is still to be developed. Over the past decade, the development of non-enzymatic glucose sensors has increased at a considerable rate. Several transition metal nanoparticles (MNPs), have been fabricated, using gold (Au) [23, 24], platinum (Pt) [25, 26], and palladium (Pd) [27, 28], and applied as non-enzymatic glucose sensors. These metals have a unique electronic structure consisting of unfilled d-orbitals and unpaired d-electrons which can be used to detect electrocatalytic activity toward glucose [29]. Pd is particularly attractive due to its excellent electrocatalytic activity and good stability [30-32].

To improve the electrocatalytic performance of MNPs, carbon-based nanomaterials such as carbon nanotubes (CNTs) [33], ordered mesoporous carbon (OMC) [4] and graphene oxide (GO) [21] have been proposed as ideal supports for the immobilization of MNPs. In particular, GO serves as a good supporting material due to its large surface area, low cost, high conductivity and ease of functionalization [34, 35]. However, the synthesis of MNPs on a carbon support still has serious problems of aggregation and low dispersion, which result in poor electrocatalytic performance and stability [36]. To improve stability, polymers such as polyaniline [37, 38], poly(vinyl alcohol) [39, 40], poly(ethylene glycol) [41, 42], poly(diallyldimethylammonium chloride) [31, 43] and poly(acrylic acid) (PAA) [44, 45] have been employed as stabilizing materials for MNP synthesis. Among these compounds, PAA is interesting because, in aqueous solutions, before reduction to form PdNPs, it produces carboxylate anions that bind with palladium ions (Pd^{2+}) via coordinate covalent bonds. This behavior reduces aggregation and increases dispersion of PdNPs on the GO surface [44, 46, 47]. As for the electrode, a portable, disposable and economical screen-printed carbon electrode (SPCE), which comprises a working electrode, a reference electrode and an auxiliary electrode in one piece, is an attractive choice [48]. Compared to the conventional 3-electrode system, SPCEs use smaller amounts of reagents and samples

[49, 50]. Therefore, modification of an SPCE with the prepared GO-PAA-PdNPs may present new opportunities for the fabrication of high-performance electrochemical sensors.

This thesis is the first report of the modification of a screen-printed carbon electrode (SPCE) with graphene oxide-poly (acrylic acid)-palladium nanoparticles (GO-PAA-PdNPs) for the determination of glucose in a flow injection amperometric (FI-Amp) system.

1.2 Physical and chemical properties of glucose

Glucose is a simple sugar (monosaccharide) in the class of carbohydrates (Figure 1.1). The molecular formula of glucose is $C_6H_{12}O_6$. In powder form, glucose is easily soluble in water. The structure of glucose consists of six carbon atoms and functional groups of alcohol, aldehyde (in the linear form) and ester (in the ring form). Glucose can be classified as an aldose, a word describing sugars that have an aldehyde group in the molecule. The suffix –ose denotes a sugar and the stem ald– signifies an aldehyde group. Some physical and chemical properties of glucose are listed in Table 1.1. The glucose molecule can have an open-chain structure and a ring structure that is formed by an intramolecular reaction between the C-5 hydroxyl group and the aldehyde C atom. Altogether, five different isomers of glucose are possible (see Figure 1.2.).

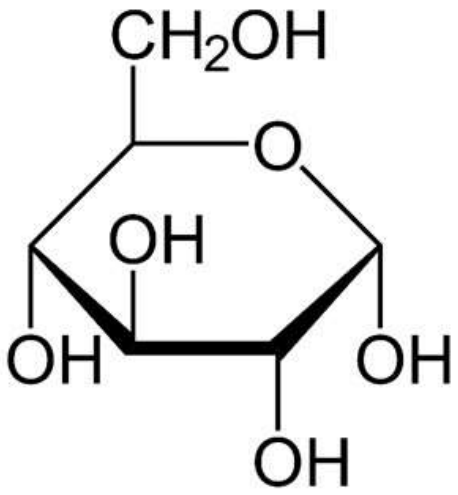


Figure 1.1 The chemical structure of glucose

Table 1.1 Physical and chemical properties of glucose [51]

Property	**Description**
Appearance	White, crystalline
Melting point	150 °C
Density	1.5620 g cm^{-3} (at 18 °C)
Molecular weight	180.16 g mol^{-1}
Solubility in:	
Water	Very soluble
Ethyl ether	Insoluble
Ethanol	Slightly soluble
Pyrimidine	Soluble

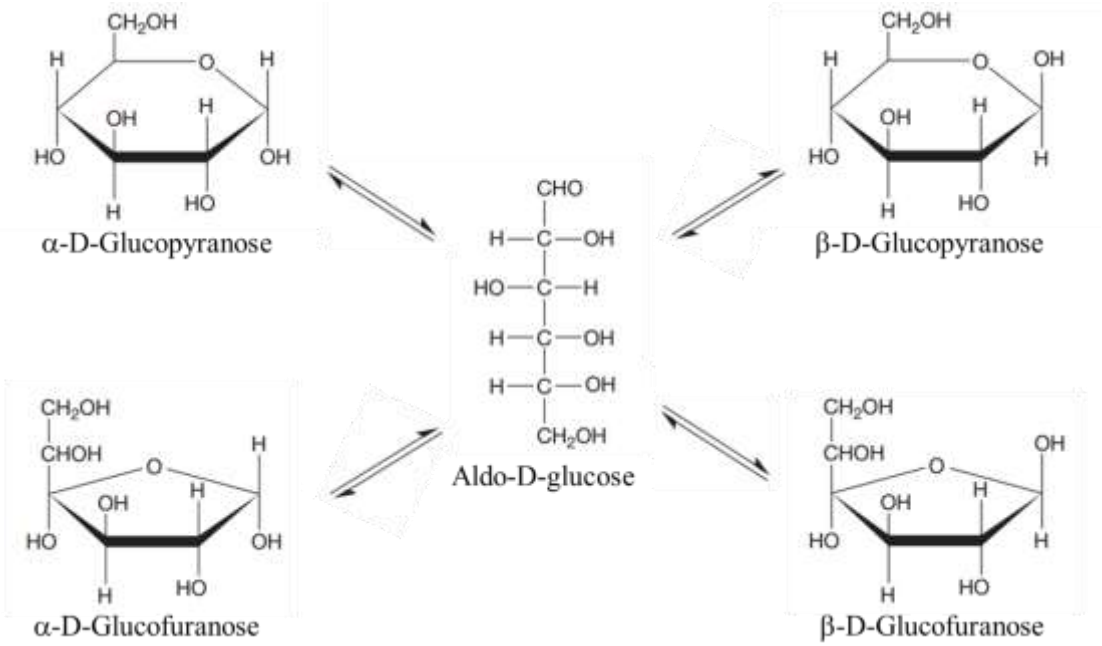


Figure 1.2 Five different isomers of glucose [51].

1.3 Glucose and diabetes mellitus

Glucose is found in honey and fruits and is the main sugar in the body of higher animals [51]. Normally, the concentration of glucose in human blood is 4.1 to 5.6 mM [5]. Abnormally high blood glucose levels over a prolonged period can cause diabetes mellitus. Increased glucose levels in the blood can be caused by the insufficient production of insulin (Type 1 diabetes) or the ineffectiveness of produced insulin (Type 2 diabetes), or both [2]. Insulin is a peptide hormone that regulates blood glucose levels [52]. For both type of diabetes, the daily determination of blood glucose levels is necessary to ensure diagnosis and treatment by injection of the insulin essential to the body [4, 6]. Lack of insulin can increase the glucose level in the blood, which can damage kidneys, eyes, nerves and heart, leading to comorbidities such as renal failure, vision loss, retinopathy, high blood pressure, heart attack and strokes [6-8]. The International Diabetes Federation (IDF) predicts that the number of diabetic patients in the world will increase to 642 million by 2040 and that the mortality rate of diabetic patients will be the seventh highest [3, 53]. However, many reports have demonstrated that rigorous control of blood glucose levels can improve the survival rate of diabetic patients [54-58].

1.4 Analytical methods for glucose detection

1.4.1 Conventional techniques

Several analytical approaches are used for the laboratory determination of glucose. Typical conventional techniques include gas chromatography coupled with thermal conductivity detection [59], anion exchange chromatography coupled with pulsed amperometric detection [11], high-performance liquid chromatography (HPLC) coupled with circular dichroism (CD) detection [60], gas chromatography combined with mass spectrometry [61, 62], isotope-dilution liquid chromatography-tandem mass spectrometry[63], Fourier transform infrared spectroscopy (FT-IR) [64], nuclear magnetic resonance [65], fluorescence [66-68], chemiluminescence [69] and UV–visible spectroscopy [70]. Capillary electrophoresis with diode array detection [71] has also been developed. However, these techniques suffer from limitations such as complicated pretreatment steps and high costs incurred for equipment and laboratory time [1, 59, 72]. Therefore, an effective analytical technique should eliminate these problems.

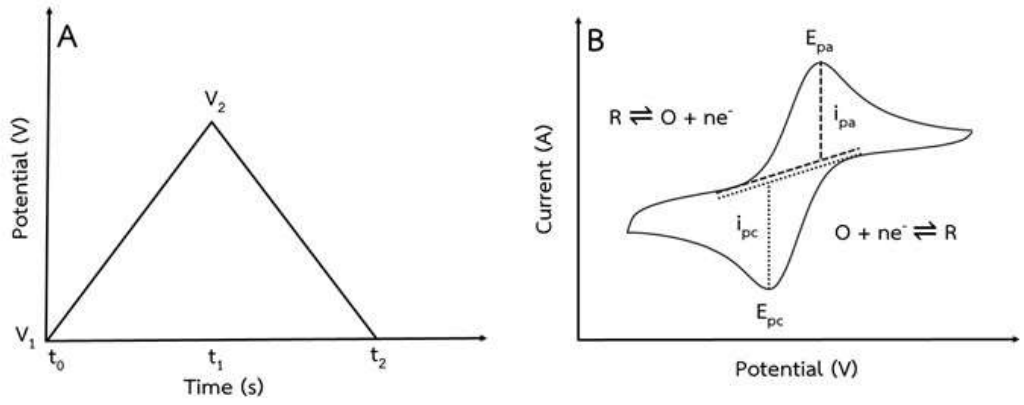
1.4.2 Electrochemical technique

The electrochemical method is an interesting analytical approach for the determination of glucose. The method supports high throughputs with good precision, high sensitivity, fast analysis and good reproducibility [18, 19]. Electrochemical sensors for the detection of glucose can be enzymatic or non-enzymatic. Enzymatic sensors have shown good selectivity and high sensitivity [21, 22] but they have some limitations, such as the high cost of enzymes, complicated immobilization process and poor reproducibility [73]. Moreover, enzymatic modified electrodes easily lost catalytic activity as a result of environmental conditions of temperature, pH value and humidity [74, 75]. To overcome the above problems, various transition metal nanoparticles (MNPs) have been applied as non-enzymatic glucose sensors.

Transition metals have special electronic structures of unpaired d-electrons and unfilled d-tracks which enhance electrocatalytic activity toward glucose [29]. Electrode modifications with gold included nitrogen doped graphenecarbon nanotubes decorated with gold nanoparticles [23], chitosan cryogel with embedded gold nanoparticles decorated multiwalled carbon nanotubes [15] and gold nanocages [76]. Modifications with platinum included platinum nanoflowers supported on a graphene oxide modified glassy carbon electrode [77], a graphene and platinum nanoclusters modified glassy carbon disc electrode [26] and a glassy carbon disc electrode modified with platinum nanoparticles decorated on carbon nano-onions [78]. Modifications with palladium metal included palladium nanoparticles supported on a MWCNTs modified electrode [33], a glassy carbon electrode modified with palladium nanoparticles supported on functionalized carbon nanotubes [32] and a glassy carbon electrode modified with ionic liquid-derived fibrillated mesoporous carbon decorated with palladium nanoparticles [4]. Despite this wide array of modifications, the performance of non-enzymatic glucose sensors can still be improved.

1.4.2.1 Cyclic voltammetry

Cyclic voltammetry (CV) is one of the most widely used methods of investigating the electrochemical properties of electroactive species on the surface of an electrode. CV is performed by sweeping the voltage of a working electrode forward from the initial potential V_1 to potential V_2 and back to V_1 (Figure 1.3A). A cyclic voltammogram is achieved by plotting the current response versus the applied potential (Figure 1.3B). This technique was employed in this work to study the electrochemical properties of glucose at SPCEs modified in different ways [79].



Excitation waveform **Response waveform**

Figure 1.3 The typical potential-excitation waveform for analog cyclic voltammetry (A): The typical current response of cyclic voltammogram for a reversible case (B) (Modified from Allen and co-worker [80])

1.4.2.2 Amperometry

Amperometry is an electrochemical technique commonly used for the determination of an analyte. It is based on the measurement of the current response as a function of time by applying a fixed potential to a working electrode (Figure 1.4). In this thesis, the technique was applied for the detection of glucose.

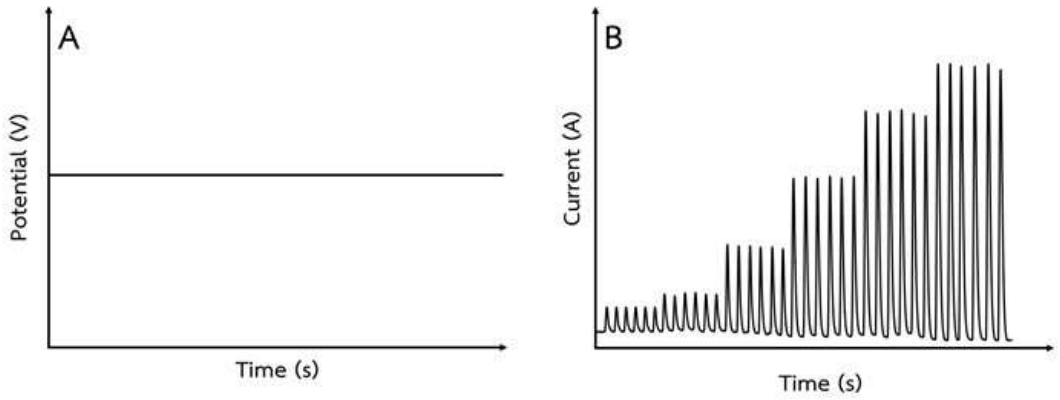


Figure 1.4 The typical excitation waveform for amperometry (A): amperogram of a typical current response as a function of time of flow injection (B) (Modified from Allen and co-worker [80])

1.4.2.3 Flow injection amperometry (FI-Amp)

The advantages of a flow injection system coupled with amperometric detection are its high throughput, good precision, fast analysis and high reproducibility [18, 19]. Moreover, the reduced contact time between the electrode surface and the sample in an FI-Amp system can reduce the adsorption effect from sample contamination [20]. FI-Amp has been employed for the analysis of compounds such as formalin [81], carbofuran [82], oxcarbazepine [83], nitrite [50], sulfite [84], chlorine [85], cotinine [86] and hydrazine [87]. In this work, the technique was used for the determination of glucose (Figure 1.5).

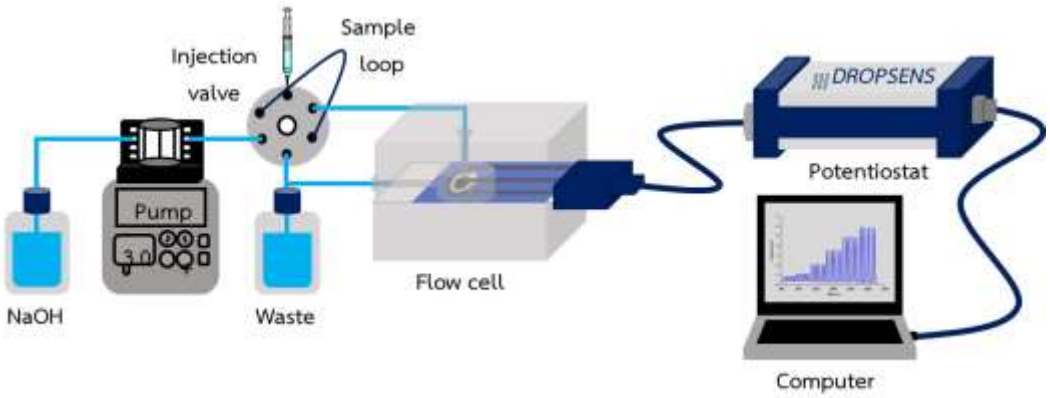


Figure 1.5 The flow injection ampermetry system (FI-Amp)

1.5 The improvement of electrochemical sensing

1.5.2 Metal nanoparticles

The various transition metal nanoparticles (MNPs) applied as non-enzymatic glucose sensors include gold (Au) [23, 24], platinum (Pt) [25, 26], and palladium (Pd) [27, 28]. These metals have exclusive electronic structures of unfilled d-tracks and unpaired d-electrons which enhanced electrocatalytic activity toward glucose [29]. Pd and Pt showed particularly good electrocatalylic properties for non-enzymatic glucose detection but Pd was more attractive than Pt since it was less expensive and showed higher electrocatalytic activity for glucose oxidation in alkaline media [88]. Several research works have used palladium-modified electrodes for the determination of glucose. Lu *et al.* (2011) synthesized palladium nanoparticle–graphene nanohybrids to modify a glassy carbon electrode for the amperometric determination of glucose in human serum samples [21]. Chen *et al*. (2010) prepared a functionalized carbon nanotube-palladium nanoparticles nanocomposite to modify a glassy carbon electrode for quantification of glucose in urine samples by amperometry [32]. Ye et al. (2015) developed a non-enzymatic sensor for the measurement of glucose by an amperometric technique using Pd nanocubes on the surface of a glassy carbon electrode [28]. Also, Haghighi *et al.* (2015) modified a glassy carbon electrode with ionic liquid-derived fibrillated mesoporous carbon-palladium nanoparticles for the detection of glucose by amperometry [4]. The electrocatalytic oxidation of glucose in the presence of Pd can proceed as described below in reactions (1), (2) and (3) and in Figure 1.6 [2, 28].

$$Pd + \text{glucose} \longrightarrow Pd\text{-glucose}_{ads} + H^+ + e^- \quad (1)$$

$$Pd(OH)_2 + \text{glucose} \longrightarrow Pd + \text{gluconolactone} + H_2O \quad (2)$$

$$Pd + 2OH^- \longrightarrow Pd(OH)_2 + 2e^- \quad (3)$$

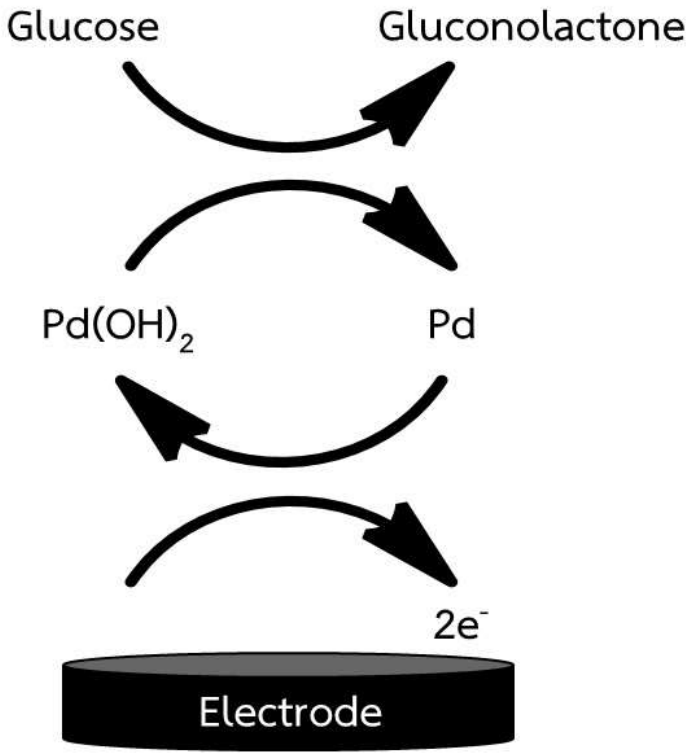


Figure 1.6 The behavior of electrocatalytic oxidation of glucose at a metallic palladium-modified electrode

1.5.3 Graphene oxide

The carbon nanomaterial graphene oxide (GO) is a single-layer sheet of graphite oxide. Structurally, GO consists of different oxygenated functional groups of epoxy, hydroxyl, quinone, lactone, phenol, carbonyl and carboxyl (Figure 1.7) [89]. Due to its large surface area, low cost, high conductivity and ease of functionalization, it was a good supporting material for the immobilization of MNPs [34, 35]. Several research groups used GO as a supporting material for MNPs. Hoa *et al*. (2015) prepared graphene oxide hydrogel and platinum nanoparticles to modify the surface of a glassy carbon electrode for the determination of glucose [90]. Wu *et al.* (2013) developed a non-enzymatic glucose sensor using graphene oxide-platinum nanoflowers on a glassy carbon electrode. Wu *et al*. (2012) also modified a glassy carbon electrode with synthesized graphene oxide-palladium nanoparticles for the detection of ascorbic acid [91] and Kokulnathan *et al.* (2018) developed an electrochemical chloramphenicol sensor using graphene oxide with palladium nanoparticles on a glassy carbon electrode [92]. However, the synthesis of MNPs on a GO support still has problems of aggregation and low dispersion due to restacking interactions and van der Waals forces between GO sheets. These interactions caused poor electrocatalytic performance and low stability [36, 93]. To overcome the above problems, it is extremely important to functionalize GO with a stabilizing material.

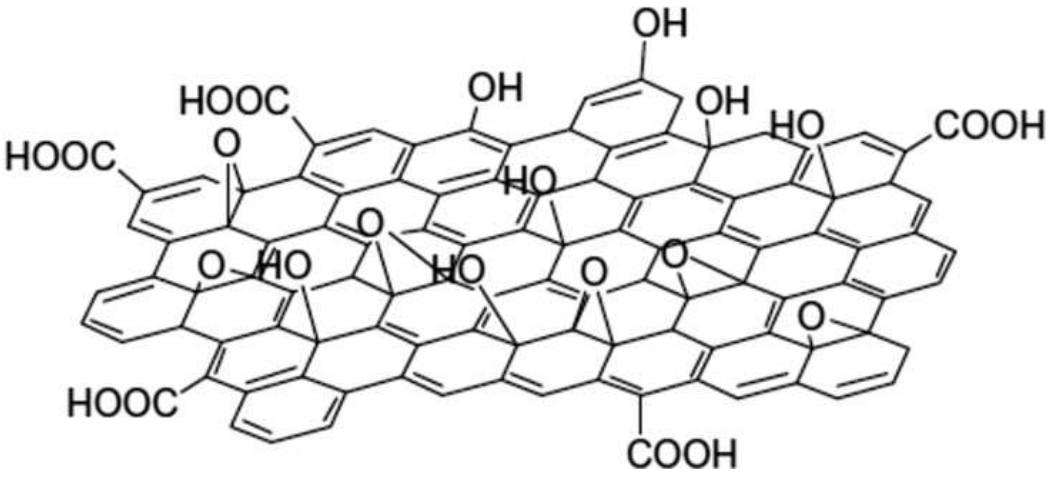


Figure 1.7 The structure of graphene oxide

1.5.4 Poly (acrylic acid)

Poly (acrylic acid) (PAA) is an anionic polyelectrolyte that has a lot of carboxylic groups in single side chains (Figure 1.8). It is an interesting polymer for stabilizing MNPs since, in aqueous solution, it can produce carboxylate anions that bind with metallic cations via coordinate covalent bonding. This behavior reduced aggregation and increased dispersion when MNPs were reduced with the reducing agent (Figure 19) [44, 46, 47]. Therefore, PAA is an interesting stabilizer if the surface of GO is to be employed as an ideal support for functionalization.

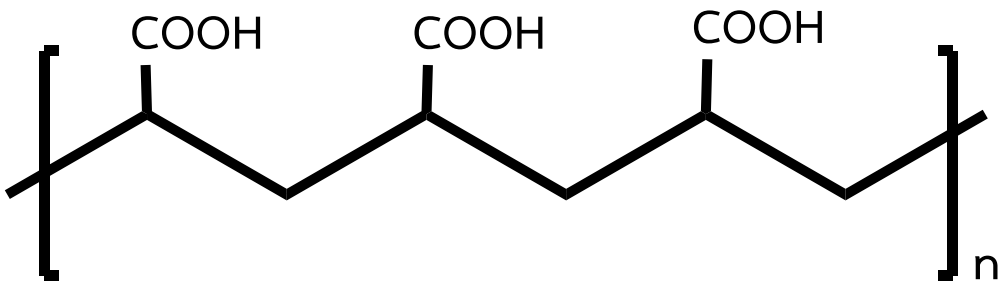


Figure 1.8 The chemical structure of poly (acrylic acid)

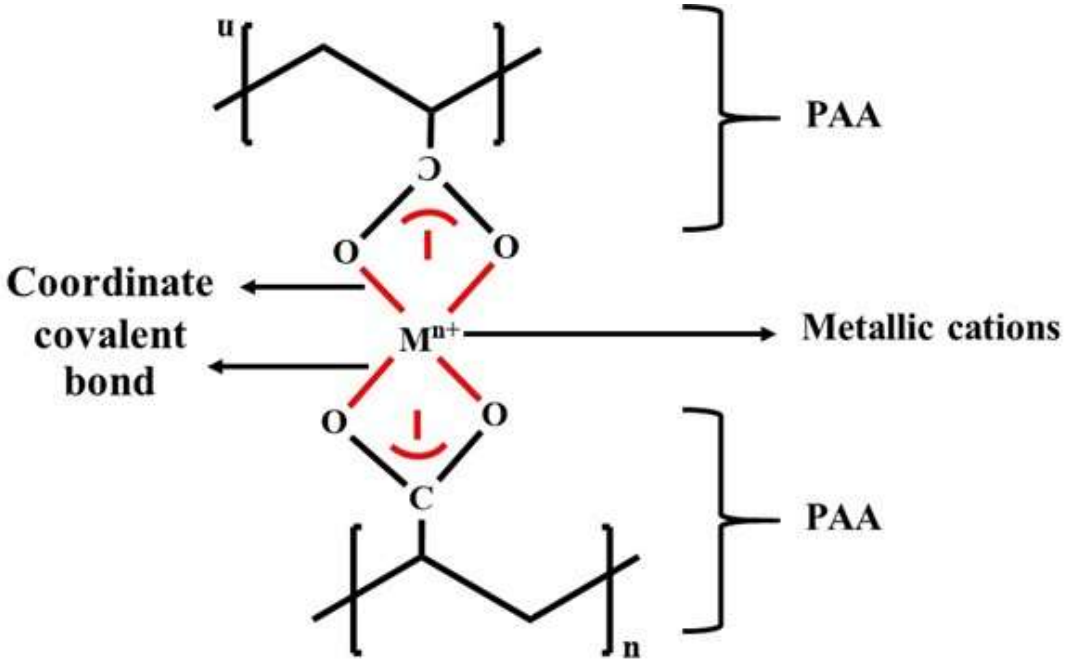


Figure 1.9 Coordinate covalent bond between metal ion (M^{n+}) and poly (acrylic acid)

1.6 Objective of the research

To develop a non-enzymatic glucose sensor coupled with a flow-injection amperometric system based on a GO-PAA-PdNPs-modified screen-printed carbon electrode.

1.7 Benefits

It is expected that a non-enzymatic glucose sensor can be designed and produced that is rapid, low cost, easy to use, highly sensitive and stable and demonstrates good repeatability. The fabricated sensor can be used for the determination of glucose in human serum samples.

CHARPTER 2

Experiments

2.1. Materials

Sodium hydroxide and sodium chloride were from Merck KGaA (Darmstadt, Germany). Dopamine (DA, ≥98.0%) and uric acid (UA, ≥98.0%) were from Fluka (Buchs, Switzerland). Ascorbic acid (AA) was from Ajax Finechem. D-(+)-glucose reagent grade (≥99.5%), poly-(acrylic acid) (PAA, MW = 1,800), $PdCl_2$ and graphene nanoplatelets were from Sigma-Aldrich (St. Louis, USA). Before use, the nanoplatelets were treated with concentrated H_2SO_4 and HNO_3 solution at a ratio of 3:1 (v/v), filtered and dried overnight at 60 °C to form graphene oxide (GO).

2.2 Apparatus

Cyclic voltammetry and amperometric experiments were performed using the BiPotentiostat/Galvanostat μStat 400 (DropSens., Asturias, Spain) controlled by DropView 8400 software. Electrochemical impedance spectroscopy (EIS) was performed using an AUTOLAB (Metrohm Autolab B.V., Utrecht, Netherlands) potentiostat-galvanostat with Nova 1.11 software. Surface morphology of the modified electrodes was investigated by transmission electron microscopy (TEM) (JEM-2010, JEOL, USA) and Fourier transform infrared spectroscopy (FT-IR) (Lumos, Bruker, UK).

2.3 Preparation of standard solution

The stock of glucose standard solution 100.0 mmol L^{-1} was prepared by dissolving glucose in 0.1 mmol L^{-1} NaOH solution.

2.4 Preparation of GO-PAA-PdNPs and electrode modification

Thirty milliliters of GO suspension (1.0 mg mL^{-1} in water) was mixed with 20 mL of PAA (15 wt%) using an ultrasonic bath for 2 h and kept overnight under stirring at room temperature. The mixture was then filtered and dried overnight at 60 °C to obtain GO-PAA. To synthesize GO-PAA-PdNPs, 50 mL of 0.20 mg mL^{-1} GO-PAA dispersion was mixed with 2.0 mL of 0.020 mol L^{-1} $PdCl_2$ solution and adjusted to pH 10 under stirring. Then, 4.0 g of sodium borohydride were slowly added into the mixture under stirring at room temperature. The obtained GO-PAA-PdNPs were filtered and washed with ethanol and double-deionized water. The solid was then dried at 60 °C for 12 h. To modify the SPCE (Zensor TE100 electrodes, graphite working electrode, Ag/AgCl pseudo-reference electrode, and graphite auxiliary electrode), 8.0 µL of 10 µg $µL^{-1}$ GO-PAA-PdNPs containing 0.25 % Nafion, were drop casted onto the working electrode and dried at 60 °C (Figure 2.1).

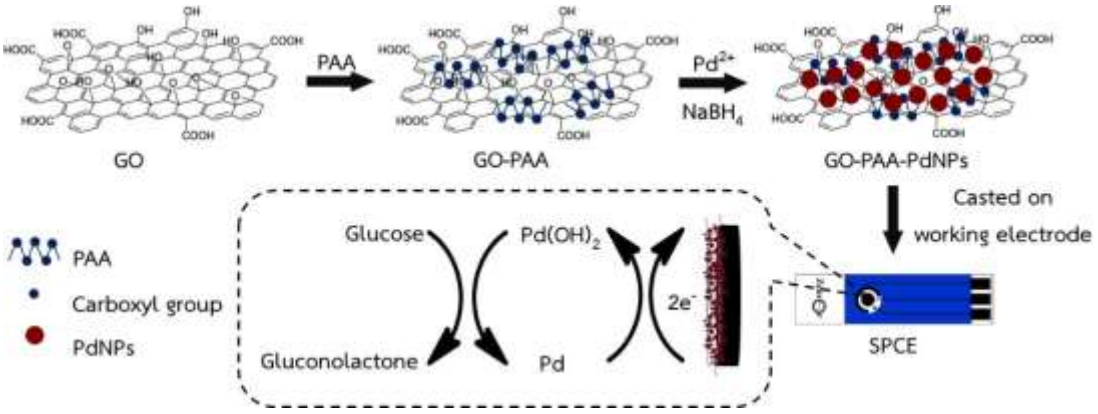


Figure 2.1 The scheme of the preparation of the GO-PAA-PdNPs-modified SPCE

2.5 Electrochemical measurements

The electrochemical properties of a bare SPCE, a GO/SPCE, a GO-PAA/SPCE, a GO-PdNPs/SPCE and a GO-PAA-PdNPs/SPCE were characterized by cyclic voltammetry (CV) from -0.70 to 0.60 V at a scan rate of 50 mV s^{-1} in 0.10 mol L^{-1} NaOH (Figure 2.2A). The electrochemical cell was filled with 5.0 mL of 0.10 mol L^{-1} NaOH and a few microliters of glucose standard solution were dropped in. The oxidation current response of glucose was measured and analyzed. The FI-Amp system consisted of a six-port injection valve (Valco Instrument, USA), a peristaltic pump (Miniplus 3, Gilson, France) and a lab-built flow cell containing the GO-PAA-PdNPs-modified SPCE. The electrode was connected to the DropSens μStat 400 potentiostat. Glucose standard solutions were prepared in 0.10 mol L^{-1} NaOH (the carrier electrolyte) and injected via the six-port injection valve. The amperograms were recorded with DropView 8400 software (Figure 2.2B).

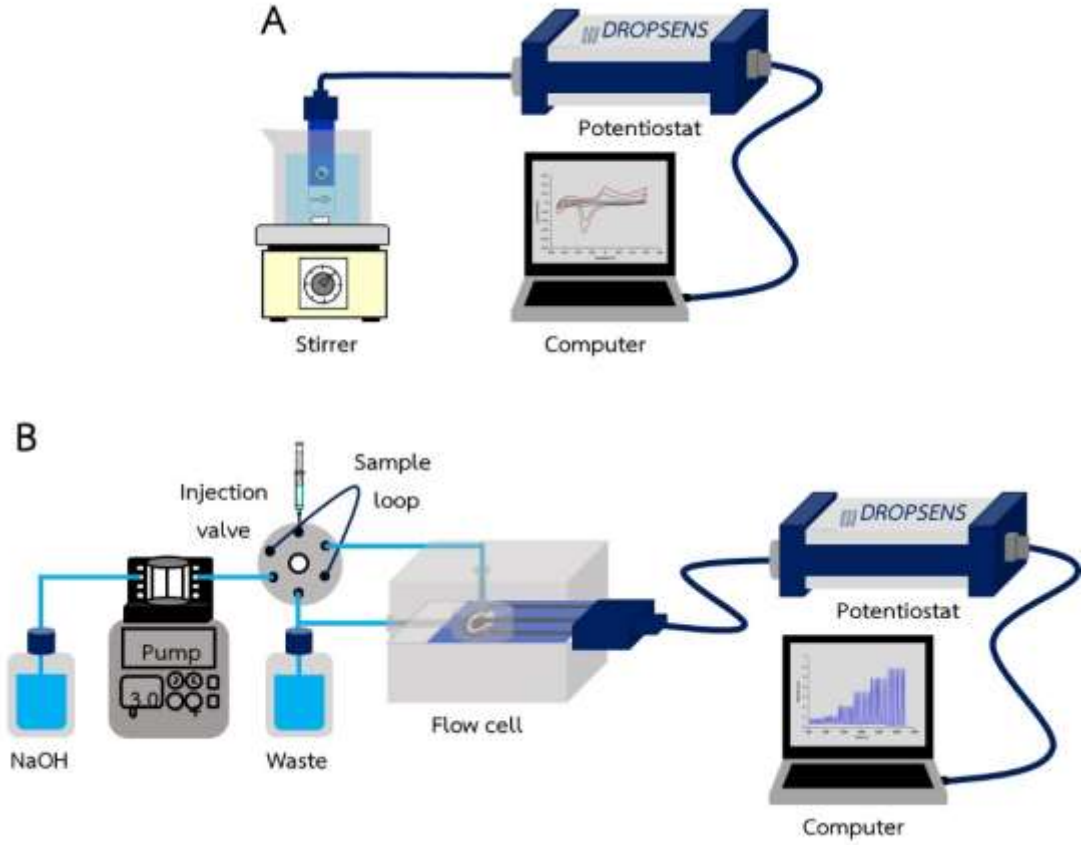


Figure 2.2 The scheme of batch system (A) and the flow injection amperometric (FI-Amp) measurement (B) for glucose determination

2.6 Optimization of the system

To obtain good electrocatalytic performance during glucose detection at the GO-PAA-PdNPs/SPCE, the operational conditions of the flow injection system were optimized. The operational parameters were the amount of PdNPs-PAA-GO applied to modify the electrode, the applied potential, the flow rate and the sample volume. Each parameter was optimized using 0.1 mol L^{-1} NaOH solutions containing 0.10, 0.25, 0.50, 0.75 and 1.00 mmol L^{-1} of glucose. The optimized conditions that provided the highest sensitivity were considered optimal.

2.6.1 Amount of GO-PAA-PdNPs on SPCE

The amount of GO-PAA-PdNPs drop casted on the SPCE was studied in the range of 20.0 to 120.0 µg (20, 40, 60, 80, 100 and 120 µg). The amount of GO-PAA-PdNPs that gave the highest sensitivity was chosen as the optimal condition for the next parameter.

2.6.2 Glucose oxidation potential

For electrocatalytic detection of glucose, the oxidation potential is an important factor in the system's performance. The influence of the applied potential covering the oxidation peak was investigated in the range of -0.30, -0.20, -0.10, 0.00, 0.10 and 0.2 V. For further experiments, the applied potential that provided the highest sensitivity was selected as the optimal condition.

2.6.3 Flow rate and sample volume

The performance of the flow-based sensor was evaluated at various flow rates (0.25, 0.50, 0.75, 1.00, and 1.25 mL min^{-1}) and sample volumes (50, 100, 200, 300 and 400 µL). These parameters were optimized together by injecting 0.9 mmol L^{-1} glucose at different flow rates and sample volumes. The combined flow rate and sample volume that provided the highest current response was considered the optimal condition.

2.7 Analytical performances

The analytical performances of the GO-PAA-PdNPs modified SPCE in the FI-Amp system were studied under the optimal conditions for glucose determination as follows.

2.7.1 Linearity

The linearity was determined by using glucose standard solutions between 0.05 and 60.00 mmol L^{-1} in the FI-Amp system. The linear dynamic range was determined by plotting the current responses and concentrations of glucose. The correlation coefficient (r) of the linear dynamic range should be greater than 0.99 [94].

2.7.2 Limit of detection and quantification

The limit of detection (LOD) is the lowest concentration of an analyte that can be determined. The detection limit can be calculated from the equation

$$LOD = 3S_a/b.$$

where S_a is the standard deviation of intercept and b is the slope of the calibration curve [95].

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be quantitatively estimated and is calculated from the equation

$$LOQ = 10S_a/b$$

2.7.3 Selectivity

The selectivity of a non-enzymatic glucose sensor is vital in the diagnosis and management of diabetic patients. Therefore, the influence on the determination of glucose from interfering compounds, such as ascorbic acid (AA), dopamine (DA), uric acid (UA) and chloride ion (Cl^-) which commonly co-exist with glucose in blood samples, was studied under optimal conditions. To test these interferences, a series of solutions containing 5.0 mmol L^{-1} of glucose was mixed with 0.100 mmol L^{-1} AA, 0.50 mmol L^{-1} UA, 12.0 nmol L^{-1} DA and 100 mmol L^{-1} Cl^-. These concentrations are higher than the normal levels of these interferents in blood [5]. Each mixture was diluted 10 times before measurement with the proposed glucose sensor. The current responses to 5.0 mmol L^{-1} glucose with and without interfering compounds were compared and analyzed.

2.7.4 Repeatability

Repeatability implies the acceptance between successive determinations of the same analyte. It was studied by measuring fifteen replications of each of three concentrations in the range of the lower linearity, i.e. 0.50, 1.00 and 2.50 mmol L^{-1}. Acceptability was estimated from the relative standard deviations (RSDs).

2.7.5 Reproducibility

Reproducibility explains the closeness of acceptance between the obtained responses from the same process under different conditions (different modified electrodes). Six sets of the fabricated electrode were prepared on different days for measuring each concentration of glucose (0.10, 0.25, 0.50, 0.75 and 1.00 mM).

2.7.6 Stability

Under the optimal conditions, the GO-PAA-PdNPs modified SPCE was assessed for operational stability by repeatedly measuring the current response of one fabricated electrode to consecutive injections of 0.25 mmol L^{-1} glucose. Each current response was converted to a percentage of the initial current response. The electrode was considered stable for as long as the current response remained within ±10% of the first injection.

2.8 Real sample analysis

Blood serum samples from Hatyai Hospital, Hat Yai, Songkhla, Thailand, were spiked with known amounts of glucose and then diluted with 0.10 mol L^{-1} NaOH before analysis (final concentration of glucose at 0.10, 0.25, 0.50, 0.75 and 1.00 mmol L^{-1}). The sensitivities of the standard curve and that of spiked serum samples were compared by two-way ANOVA to determine any matrix effect. Samples at dilutions that showed no matrix effect were then tested using the non-enzymatic sensor. Results from the sensor and the standard hexokinase-spectrophotometric method were statistically compared by the Wilcoxon signed rank test.

Recovery describes the closeness of acceptance between the true value and the found value. It expresses the accuracy of an analytical method. The accuracy was studied by adding different concentrations of glucose into blood serum samples (final concentration of glucose at 0.10, 0.25, 0.50, 0.75 and 1.00 mmol L^{-1}). In this work, the % recovery was calculated from the equation

$$\text{Recovery (\%)} = ((C_{spiked} - C_{control\ sample}) \times 100)/C_{concentration\ to\ know}$$

in which C_{spiked} is the concentration of a fortified sample, $C_{control\ sample}$ is the concentration of an unfortified sample and $C_{concentration\ to\ know}$ is the concentration of an analyte added to the test sample.

The percentage of recovery depends on the concentration of the analyte added to the sample and should be determined for at least three different concentrations covering the analytical range. The values should be consistent with acceptable percentages of recovery. In this thesis, the acceptable value were those prescribed in AOAC guidelines [94].

CHARPTER 3

Result and discussion

3.1 Characterization

3.1.1 Electrodes characterization

A TEM image of the GO-PdNPs without PAA shows aggregated PdNPs on the GO support (Figure 3.1A), while the one with PAA (Figure 3.1B) had a more uniform distribution of PdNPs. This latter feature increases the surface area and hence the effective active sites. The FT-IR characterization shows the GO spectrum (Figure 3.1C) with an OH stretching band at 3452 cm^{-1}, symmetric and antisymmetric CH_2 stretching vibrations at 2940 and 2868 cm^{-1}, a carbonyl group (C=O) band at 1640 cm^{-1} and a carboxyl group (–COOH) stretching band at 1,384 cm^{-1} [96, 97]. When GO was functionalized with PAA, the bands at 3450, 1,638 and 1384 cm^{-1} corresponding to the hydroxyl group (–OH), carbonyl group (C=O) and carboxyl group (–COOH) increased in intensity (Figure 3.1D) [44, 50, 98], that is, the PAA covered the GO surface. When Pd^{2+} was deposited on the GO-PAA to generate GO-PAA-PdNPs (Figure 3.1E), the OH stretching band at 3452 cm^{-1} and the carbonyl group (C=O) band at 1640 cm^{-1} significantly decreased. The disappearance of the carboxyl group (–COOH) stretching band at 1,384 cm^{-1} in this case may be caused by a new interaction between PdNPs and the carboxyl groups of PAA-GO, which can be described by a two-step procedure. First, the carboxylate ion of PAA-GO interacted with the Pd^{2+} through a coordinate covalent bond. Second, Pd^{2+} was reduced by sodium borohydride and converted into PdNPs on GO-PAA [50, 99, 100].

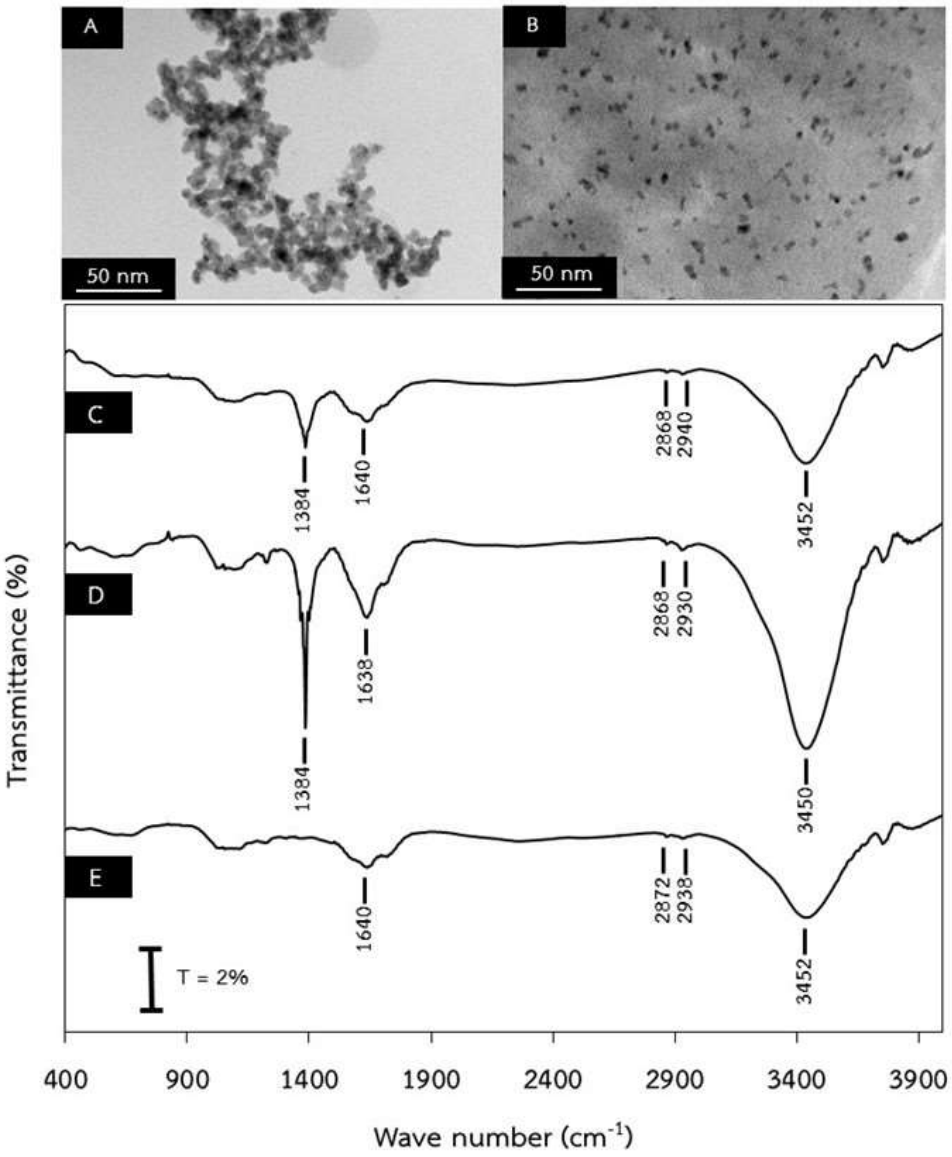


Figure 3.1 TEM images of (A) GO-PdNPs, (B) GO-PAA-PdNPs and FT-IR spectra of (C) GO, (D) GO-PAA and (E) GO-PAA-PdNPs.

Electrochemical characterization of the modified electrode by CV with 5.0 mmol L^{-1} $[Fe(CN)_6]^{4-/3-}$ in a 0.10 mol L^{-1} KCl solution is shown in Figure 3.2. With the SPCE (trace a), well-defined redox peaks of the marker were observed. The GO-modified SPCE (trace b) showed an increased peak current due to its good conductivity. When the GO-PAA/SPCE was tested (trace c), the nonconductive properties of PAA reduced the peak current. Meanwhile, the GO modified with the conductive PdNPs (trace d) showed a marked increase in the current response. In the case of GO-PAA-PdNPs/SPCE, the redox peak currents (trace e) were more than double those of the GO-PdNPs/SPCE, indicating an effective enhancement of the current response by the combined properties of the GO, PdNPs and PAA.

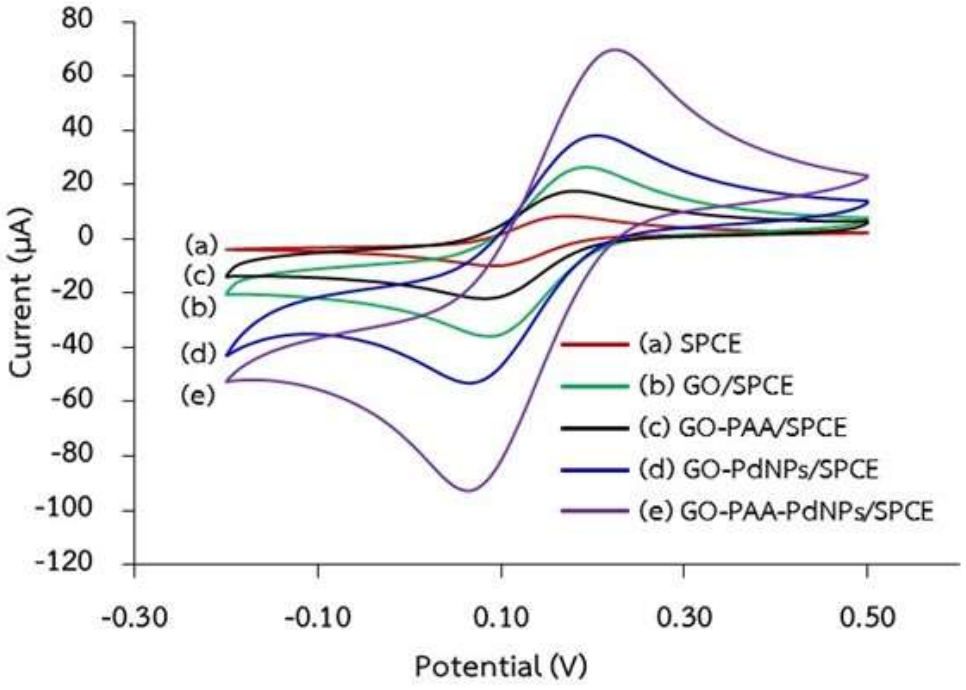


Figure 3.2 Cyclic voltammograms obtained from different modified SPCEs in 5.0 mmol L^{-1} $[Fe(CN)_6]^{4-/3-}$ containing 0.10 mol L^{-1} KCl.

Electrochemical impedance spectroscopy (EIS) was used to study the electron transfer between the electrolyte and the electrode surface. The electron transfer resistance (R_{et}) can be estimated from the semicircle diameter of the Nyquist plot impedance spectrum. The Nyquist plot can be measured by fitting it to an equivalent electrical circuit (Randles circuit). EIS was measured under the following conditions: frequency between 100 kHz and 0.01 Hz, frequency number of 50 and an amplitude of 0.25 V. Figure 3.3 shows the plots of the different electrodes in 5.0 mmol L^{-1} $[Fe(CN)_6]^{4-/3-}$ containing 0.10 mol L^{-1} KCl. The semicircle diameter of the GO/SPCE (trace b, R_{et} = 1,192 Ω) decreased dramatically compared with that of the bare SPCE (trace a, R_{et} = 3,139 Ω) due to the high surface area and good conductivity of GO. After GO-PAA was modified on the SPCE, the nonconductivity of PAA caused the R_{et} to become larger (trace c, Ret = 2,356 Ω). In contrast, the GO-PdNPs/SPCE (trace d), with the PdNPs having good conductivity, enhanced the electron transfer at the surface of the electrode, which was reflected by the very small R_{et} of 115 Ω. Meanwhile, the GO-PAA-PdNP-modified SPCE (trace e) demonstrated linearity with a very small semicircle (R_{et} = 33 Ω). These results showed that the GO-PAA-PdNPs can improve the electron transfer and conductivity of the electrode.

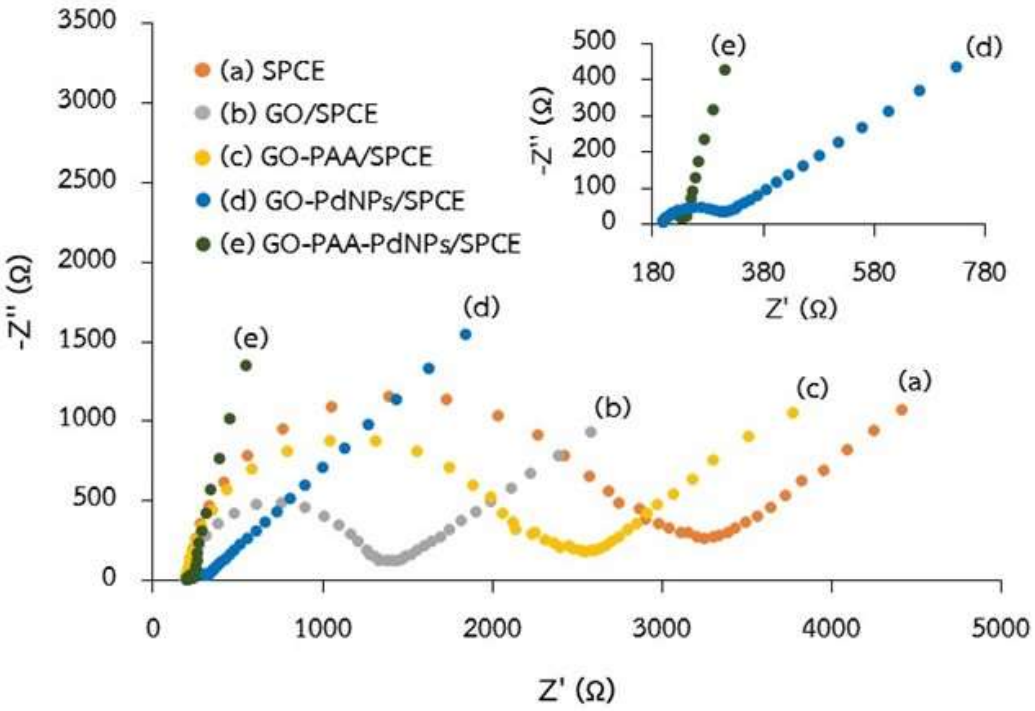


Figure 3.3 Nyquist plot of the bare SPCE (a), GO/SPCE (b),GO-PAA/SPCE (c), GO-PdNPs/SPCE (d) and GO-PAA-PdNPs/SPCE (e) in 5.0 mmol L^{-1} $([Fe(CN)_6]^{4-/3-}$ containing 0.10 mol L^{-1} KCl.

3.1.2 Electrochemical behaviors

The CVs of a bare SPCE (Figure 3.4A (trace a)), GO/SPCE (Figure 3.4A (trace b)) and GO-PAA/SPCE (Figure 3.4A (trace c)) in 0.10 mol L^{-1} NaOH showed no current peaks. For the ones modified with GO-PdNPs (Figure 3.4A (trace d)) and GO-PAA-PdNPs (Figure 3.4A (trace e)), three current peaks were observed [28]. In the anodic sweep, a small peak at a potential of approximately -0.40 V (peak I) was observed due to the desorption of hydrogen atoms, which diffuse from the inside of the Pd lattice to the surface. A potential peak due to oxidation on the Pd surface and the formation of Pd oxide is also observed at approximately −0.20 V to 0.25 V (peak II, reactions 1, 2 and 3). In the cathodic sweep, the reduction of Pd oxide occurred at a potential of −0.32 V (peak II, reaction 4).

$$Pd + OH^- \longrightarrow Pd\text{-}OH_{ads} + H_2O + e^- \quad (1)$$

$$Pd\text{-}OH_{ads} + OH^- \longrightarrow Pd\text{-}O\ (Pd\ oxide) + H_2O + e^- \quad (2)$$

$$Pd\text{-}OH_{ads} + Pd\text{-}OH_{ads} \longrightarrow Pd\text{-}O\ (Pd\ oxide) + H_2O \quad (3)$$

$$Pd\text{-}O\ (Pd\ oxide) + H_2O + 2e^- \longrightarrow Pd + 2OH^- \quad (4)$$

Furthermore, the cathodic peak attributed to the reduction of Pd oxide can be used to calculate the surface coverage (Γ, mol cm^{-2}) of the catalytic activity by the equation $\Gamma = Q/nFA$, where Q is the charge from the integration of the cathodic peak of Pd oxide, n is the number of electrons in the reaction (n = 2), F is Faraday's constant (96,487 C) and A is the geometric area of the SPCE (0.070 cm^2). The surface coverages of the catalytic activity of the GO-PAA-PdNPs/SPCE and GO-PdNPs/SPCE (n = 3) were $(8.56 \pm 0.05) \times 10^{-8}$ and $(4.19 \pm 0.07) \times 10^{-8}$ mol cm^{-2}, respectively. The 2-fold higher surface coverage of the GO-PAA-PdNP/SPCE was due to the presence of PAA, which acted as a stabilizing agent and increased the dispersion of PdNPs on the surface of GO.

The addition of 1.0 mmol L^{-1} glucose into 0.10 mol L^{-1} NaOH showed no significant change in the current peak for the SPCE (Figure 3.4B (trace a)), GO/SPCE (Figure 3.4B (trace b)) or GO-PAA/SPCE (Figure 3.4B (trace c)), indicating no catalytic activity of glucose. For the GO-PAA-PdNPs/SPCE (Figure 3.4B (trace d)) and GO-PdNPs/SPCE (Figure 3.4B (trace e)), the anodic current peak of glucose at a potential of approximately 0.0 V was clearly observed. The electrocatalytic oxidation of glucose in the presence of Pd can be described as follows [2, 28]. At a potential of approximately -0.50 V, glucose adsorbs and accumulates on the Pd surface to form an adsorbed intermediate and then releases one proton per glucose molecule (peak IV, reaction 5). The hydroxyl ion adsorbed on the surface of Pd catalyzes the oxidation of the adsorbed intermediate, which leads to a broad peak at this potential.

$$Pd + glucose \longrightarrow Pd\text{-}glucose_{ads} + H^+ + e^- \quad (5)$$

Palladium hydroxide ($Pd(OH)_2$) can oxidize glucose to gluconolactone and itself generates Pd at a potential of approximately 0 V (peak V, reaction 6).

$$Pd(OH)_2 + glucose \longrightarrow Pd + gluconolactone + H_2O \quad (6)$$

Then, Pd undergoes a two-electron oxidation to form $Pd(OH)_2$ (reaction 7).

$$Pd + 2OH^- \longrightarrow Pd(OH)_2 + 2e^- \quad (7)$$

Between GO-PAA-PdNPs and GO-PdNPs, the electrocatalytic oxidation of glucose by the GO-PAA-PdNPs/SPCE provided a better signal. The sensitivities (standard glucose at 0.10, 0.25, 0.50, 0.75 and 1.0 mmol L^{-1}) of both electrodes also confirmed the aforementioned results, where the GO-PAA-PdNPs/SPCE exhibited a 2-fold higher sensitivity than the GO-PdNPs/SPCE (Table 3.1 and Figure 3.5C). Therefore, the GO-PAA-PdNPs/SPCE provided good electrocatalytic performance for the determination of glucose.

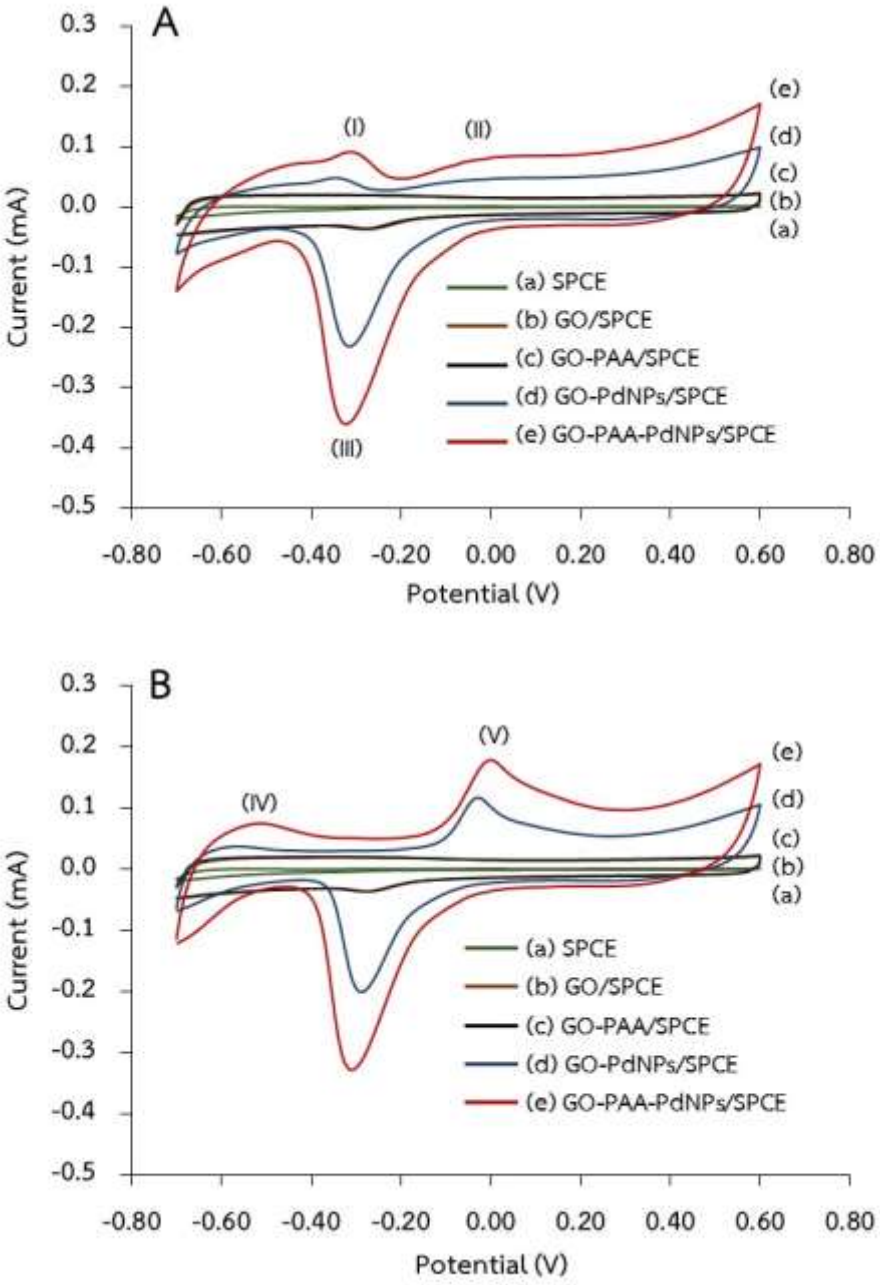


Figure 3.4 Cyclic voltammetry of bare SPCE, GO/SPCE, GO-PAA/SPCE, GO-PdNPs/SPCE and GO-PAA-PdNPs/SPCE at a scan rate of 0.050 V s^{-1} in 0.10 mol L^{-1} NaOH without glucose (A) and with 1.0 mmol L^{-1} glucose (B).

Table 3.1 The comparison of the regression equation between SPCE, GO/SPCE, GO-PAA/SPCE, GO-PdNPs/SPCE and GO-PAA-PdNPs/SPCE

Type of electrodes	Regression equation	r
SPCE	-	-
GO/SPCE	-	-
GO-PAA/SPCE	-	-
GO-PdNPs/SPCE	y = (2.80±0.02)x + (0.08±0.01)	0.9992
GO-PAA-PdNPs/SPCE	y = (5.46±0.05)x + (0.32±0.03)	0.9985

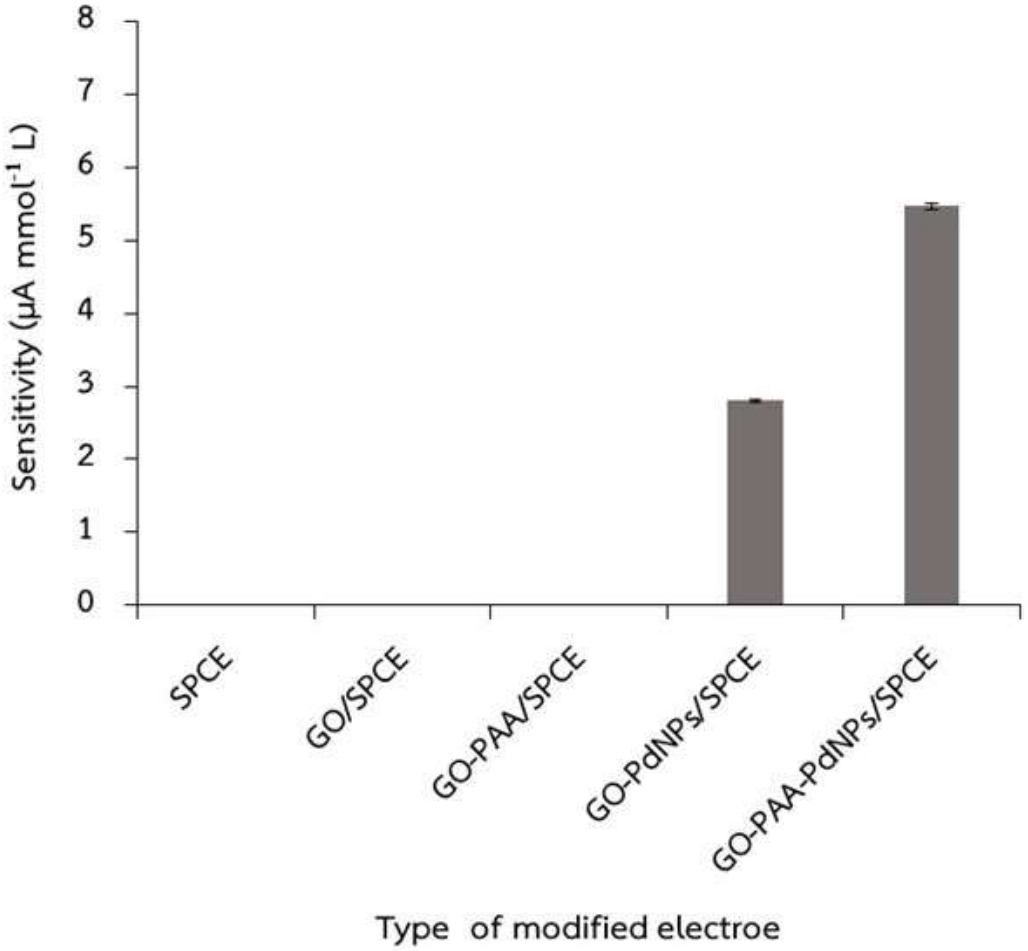


Figure 3.5 Comparison of the sensitivity between SPCE, GO/SPCE, GO-PAA/SPCE, GO-PdNPs/SPCE and GO-PAA-PdNPs/SPCE.

3.1.3 Effect of scan rate

The electrochemical behavior of the GO-PAA-PdNPs/SPCE at different scan rates from 20 to 80 mV s^{-1} for a solution of 0.50 mmol L^{-1} glucose in 0.10 mol L^{-1} NaOH was first studied by CV. The anodic peak current for the oxidation of glucose was found to be linearly correlated with the square root of the scan rate ($v^{1/2}$) and had a linear regression equation of I_{pa} (mA) = (0.0141±0.0002)$v^{1/2}$ $(mV/s)^{1/2}$ + (0.033±0.002) (mA) (r = 0.9989) (Figure 3.6). These results confirmed that the electrocatalytic oxidation of glucose at the GO-PAA-PdNPs/SPCE was controlled by the diffusion process [33, 101-103].

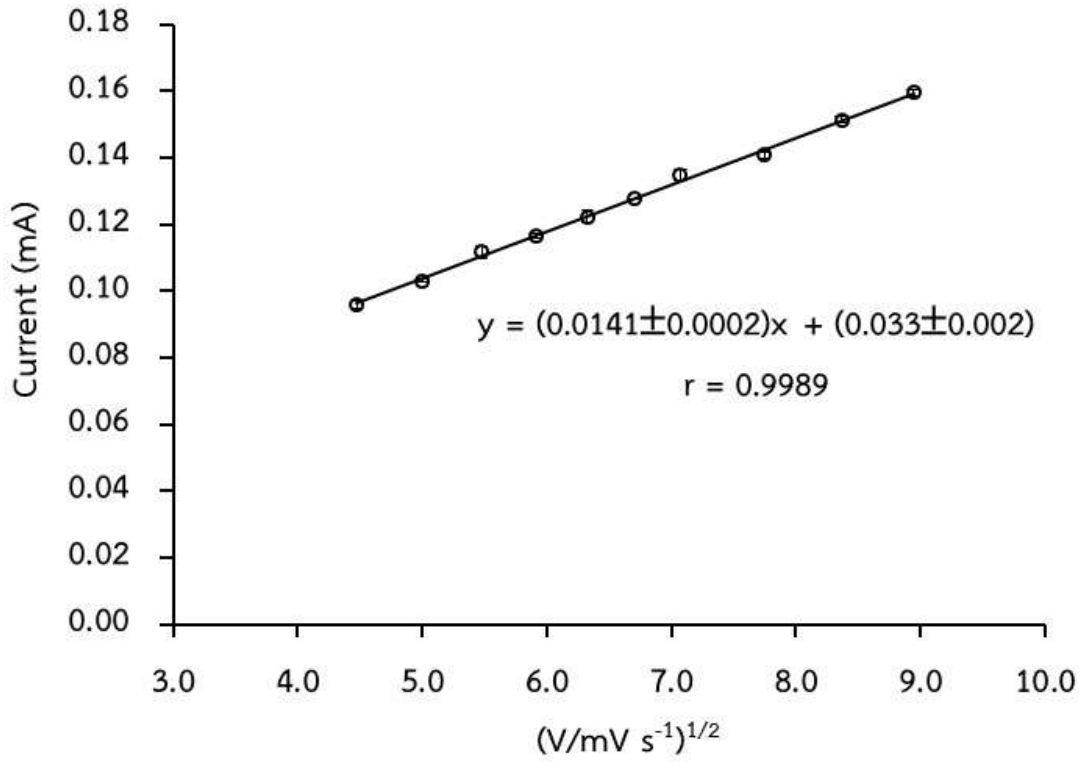


Figure 3.6 The calibration plot of the anodic peak currents versus the square root of scan rates.

3.2 Optimization of the flow injection system

To obtain good electrocatalytic performance of glucose detection at the GO-PAA-PdNPs/SPCE, the operational conditions of the flow injection system were optimized. The parameters were tested with 0.10, 0.25, 0.50, 0.75 and 1.0 mmol L^{-1} glucose in 0.10 mol L^{-1} NaOH solution. The optimal condition was the one that provided the highest sensitivity.

3.2.1 Amount of GO-PAA-PdNPs on SPCE

The effect of the amount of GO-PAA-PdNPs on the electrocatalytic performance of glucose was studied in the range of 20 to 120 µg (Table 3.2 and Figure 3.7). The sensitivity reached a maximum, i.e., the maximum electrocatalytic activity of GO-PAA-PdNPs for the oxidation of glucose, at 80 µg and then leveled off at higher amounts. A similar result was observed in a previous report [104]. Therefore, 80 µg of GO-PAA-PdNPs was used for further experiments.

Table 3.2 The effect of amount of GO-PAA-PdNPs modified on SPCE of glucose determination in concentration of 0.10 to 1.00 mmol L^{-1} (n=3)

Amount of GO-PAA-PdNPs (µg)	Regression equation	r
20	y = (1.55±0.01)x + (0.04±0.01)	0.9991
40	y = (3.58±0.03)x + (0.18±0.02)	0.9991
60	y = (3.95±0.02)x + (0.11±0.01)	0.9998
80	y = (4.64±0.02)x + (0.10±0.01)	0.9997
100	y = (4.68±0.02)x + (0.04±0.01)	0.9998
120	y = (4.64±0.02)x + (0.21±0.01)	0.9999

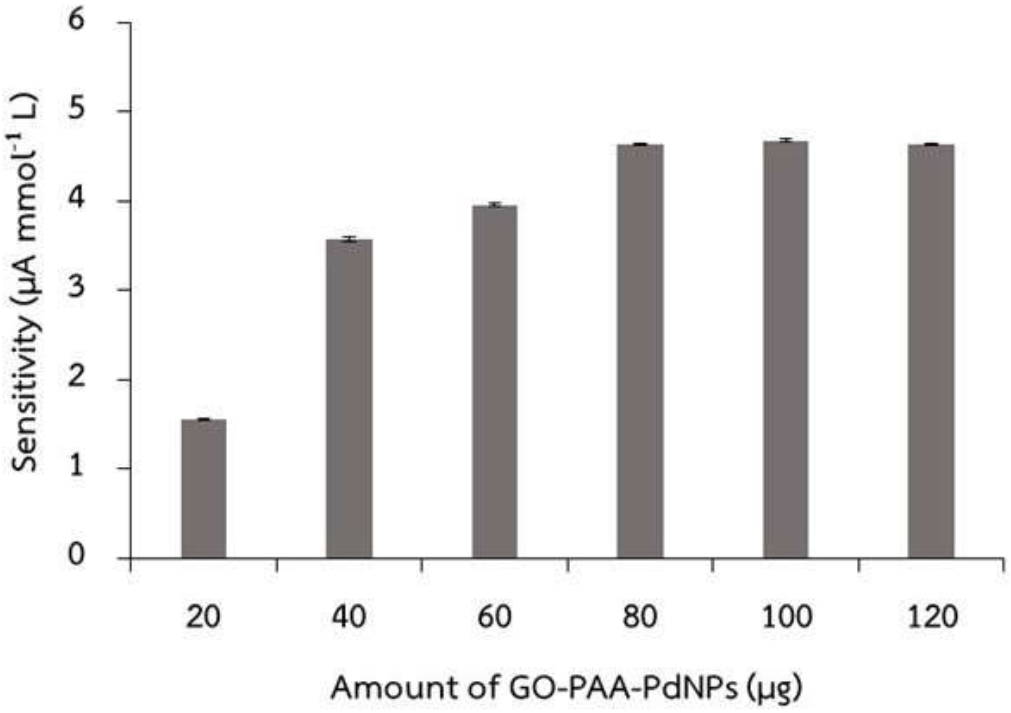


Figure 3.7 The sensitivity (0.10-1.00 mmol L^{-1} glucose) of the developed sensor to different amounts of GO-PAA-PdNPs (sample loop 200 μL; flow rate 0.75 mL min^{-1}; applied potential 0 V).

3.2.2 Glucose oxidation potential

For the electrocatalytic activity of glucose, the oxidation potential is an important factor in the system's performance. The influence of the applied potential covering the oxidation peak was investigated in the range from -0.30 to 0.20 V. The sensitivity of the responses increased as the applied potential increased from -0.30 to -0.10 V and then gradually decreased (Table 3.3 and Figure 3.8), corresponding well with the CV (Figure 3.4B). Therefore, for subsequent experiments, an applied potential of -0.10 V was chosen.

Table 3.3 The effect of applied potential of GO-PAA-PdNPs modified on SPCE of glucose determination in concentration of 0.10 to 1.00 mmol L^{-1} (n=3)

Applied potential (V)	Regression equation	r
-0.30	y = (2.48±0.21)x + (0.55±0.13)	0.9892
-0.20	y = (3.82±0.25)x + (0.57±0.15)	0.9935
-0.10	y = (5.46±0.05)x + (0.32±0.03)	0.9985
0.00	y = (4.64±0.02)x + (0.10±0.01)	0.9997
0.10	y = (3.24±0.01)x + (0.19±0.01)	0.9997
0.20	y = (1.79±0.02)x + (0.28±0.01)	0.9982

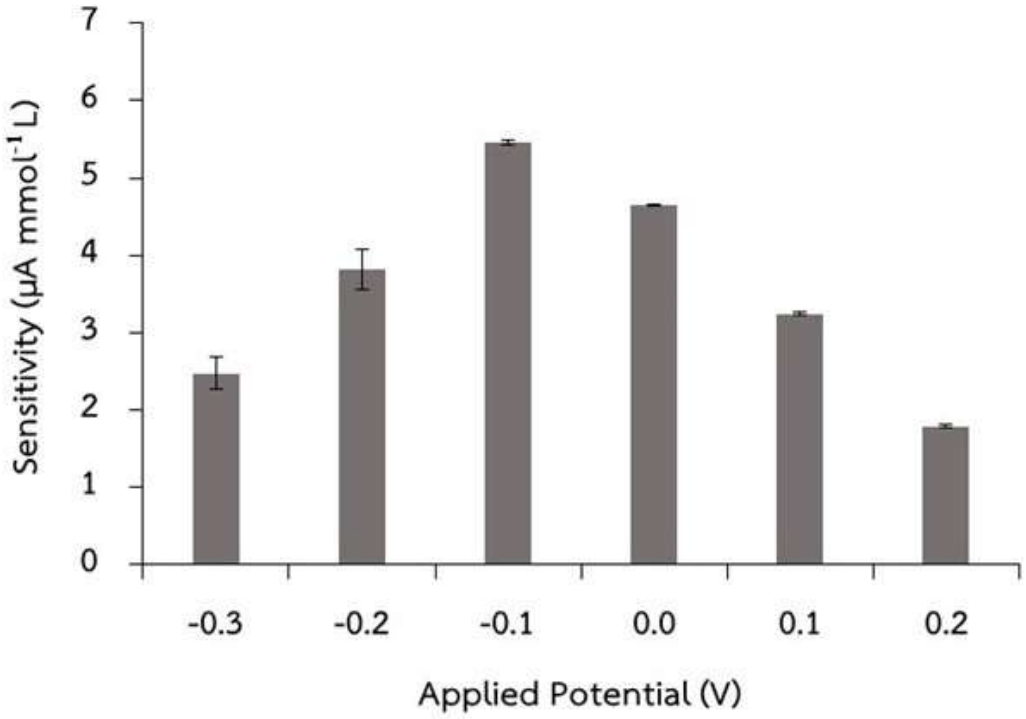


Figure 3.8 The effect of different applied potentials on the sensitivity (0.10-1.00 mmolL^{-1} glucose) of the fabricated sensor (amount of GO-PAA-PdNPs 80 μg; sample loop 200 μL; flow rate 0.75 mL min^{-1}).

3.2.3 Flow rate and sample volume

To achieve good performance of the flow-based sensor, the flow rate (0.25-1.50 mL min^{-1}) and sample volume (50-400 µL) were optimized together by testing 0.90 mmol L^{-1} glucose. For each flow rate, the oxidation response of glucose increased with the sample volume and reached saturation at 200 µL (Table 3.4 and Figure 3.9). For each sample volume, the oxidation response of glucose increased up to 0.75 mL min^{-1} and remained almost constant at higher values. Although the sample volumes of 200, 300 and 400 µL at flow rates of 0.75, 1.00 and 1.25 mL min^{-1} provided similar current responses, the large sample volume caused a broad current response and a long analysis time, while the high flow rate was disturbed by back pressure and bubbles. To avoid the aforementioned problems, a flow rate and sample volume of 0.75 mL min^{-1} and 200 µL, respectively, were chosen as the optimal conditions.

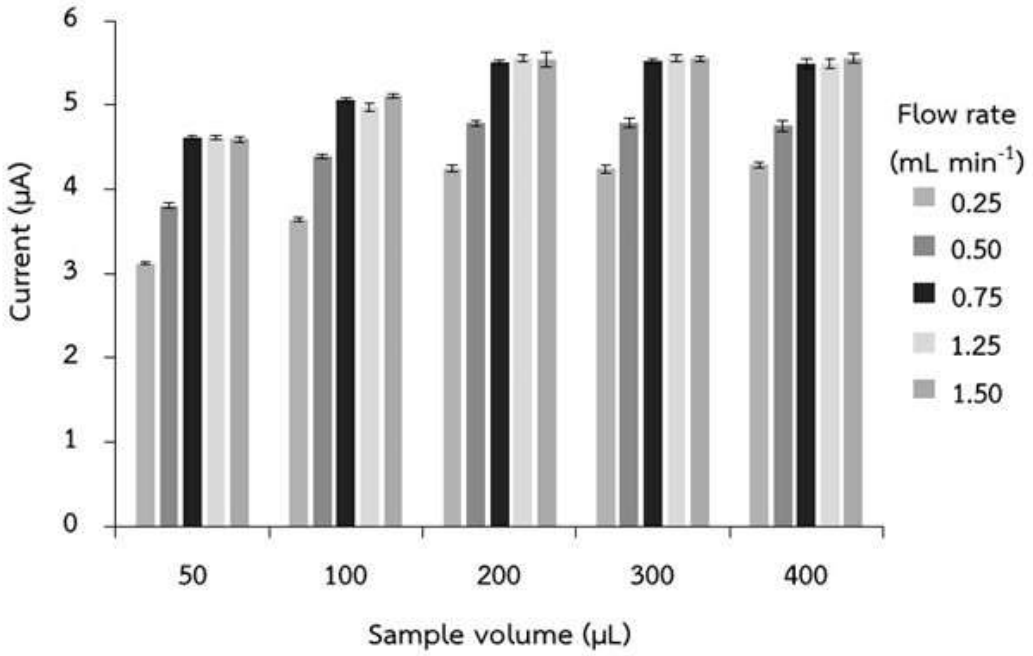


Figure 3.9 The effect of the flow rate and sample volume on the current response (0.90 mmol L^{-1} glucose) of GO-PAA-PdNPs/SPCE (amount of GO-PAA-PdNPs 80 µg; applied potential -0.10 V).

Table 3.4 Effect of flow rate and sample volume on the current response of GO-PAA-PdNPs modified on SPCE of glucose determination in concentration of 0.10 to 1.00 mmol L^{-1} (n=3)

Sample volume (µL) / Flow rate (mL min^{-1})	Current (µA)				
	50	100	200	300	400
0.25	3.12±0.02	3.64±0.02	4.25±0.04	4.24±0.05	4.29±0.03
0.50	3.80±0.03	4.39±0.03	4.79±0.03	4.79±0.05	4.75±0.07
0.75	4.61±0.02	5.07±0.03	5.50±0.03	5.5±0.03	5.**5**±**0**.06
1.00	4.61±0.02	4.98±0.05	5.56±0.04	5.55±0.04	5.49±0.06
1.25	4.59±0.03	5.10±0.03	5.54±0.09	5.55±0.04	5.55±0.06

3.3 Analytical performance

3.3.1 Linearity, limit of detection and limit of quantification

Under the optimal conditions, the analytical performance of the GO-PAA-PdNPs/SPCE for glucose determination was studied between 0.050 and 60 mmol L^{-1} in the FI-Amp system. Figure 3.10A shows an example of the amperometric responses of different concentrations of glucose. The current responses increased with increasing glucose concentrations. A sample throughput of 48 h^{-1} was achieved. The calibration plot between the current responses and glucose concentrations exhibited two linear ranges, i.e., from 0.050 to 15 mmol L^{-1} and from 15 to 60 mmol L^{-1}, with different sensitivities (Figure 3.10B). This is likely due to the different activities of the electrode surface with low and high concentrations of glucose. Due to a large number of active sites with respect to the total number of glucose molecules, the sensitivity is high at lower glucose concentrations. However, with higher concentration, there were relatively less active sites (mainly at the surface of the electrode), and thus, a decrease in the sensitivity occurred [105]. Another possible explanation is the transition from a monolayer of adsorbed glucose on the GO-PAA-PdNPs/SPCE surface into a more complex multilayer [106]. The detection and quantitation limits were calculated based on 3 and 10 standard deviations of the intercept divided by the slope of the calibration curve and were found to be 22 and 76 μmol L^{-1}, respectively.

The GO-PAA-PdNPs/SPCE was compared with other palladium-modified electrodes for the nonenzymatic determination of glucose (Table 3.5). The developed glucose sensor exhibited a wide linear range, the lowest detection limit and detection potential, and the highest sensitivity. These excellent figures of merit could be attributed to the combination of the unique properties of each material employed in the electrode modification. In particular, the PAA increases the distribution of PdNPs on the GO surfaces, leading to an excellent electrocatalytic performance for the determination of glucose.

Table 3.5 Comparison of the analytical performance of the developed nonenzymatic glucose sensor with other sensors for the determination of glucose

Modified electrode	Potential detection (V)	Detection limit (mmol L^{-1})	Linear range (mmol L^{-1})	Sensitivity (μA $mmol^{-1}$ L cm^{-2})	Reference
Pd nanocube/GCE	-0.05	-	1 - 10	34	[28]
PdNPs–CNTs/GCE	0.025	-	1-10	11	[107]
			11-20	6.3	
Pd/IFMC/GCE	0.40	200	1-55	-	[4]
Pd–CSP/C/GCE	-0.05	237	1-8	17.7	[108]
PdNPs-FCNTs/GCE	0.40	-	0 - 46	11.4	[32]
GO-PAA-PdNPs/SPCE	-0.10	22	0.05 - 15	75	This work
			15-60	37	

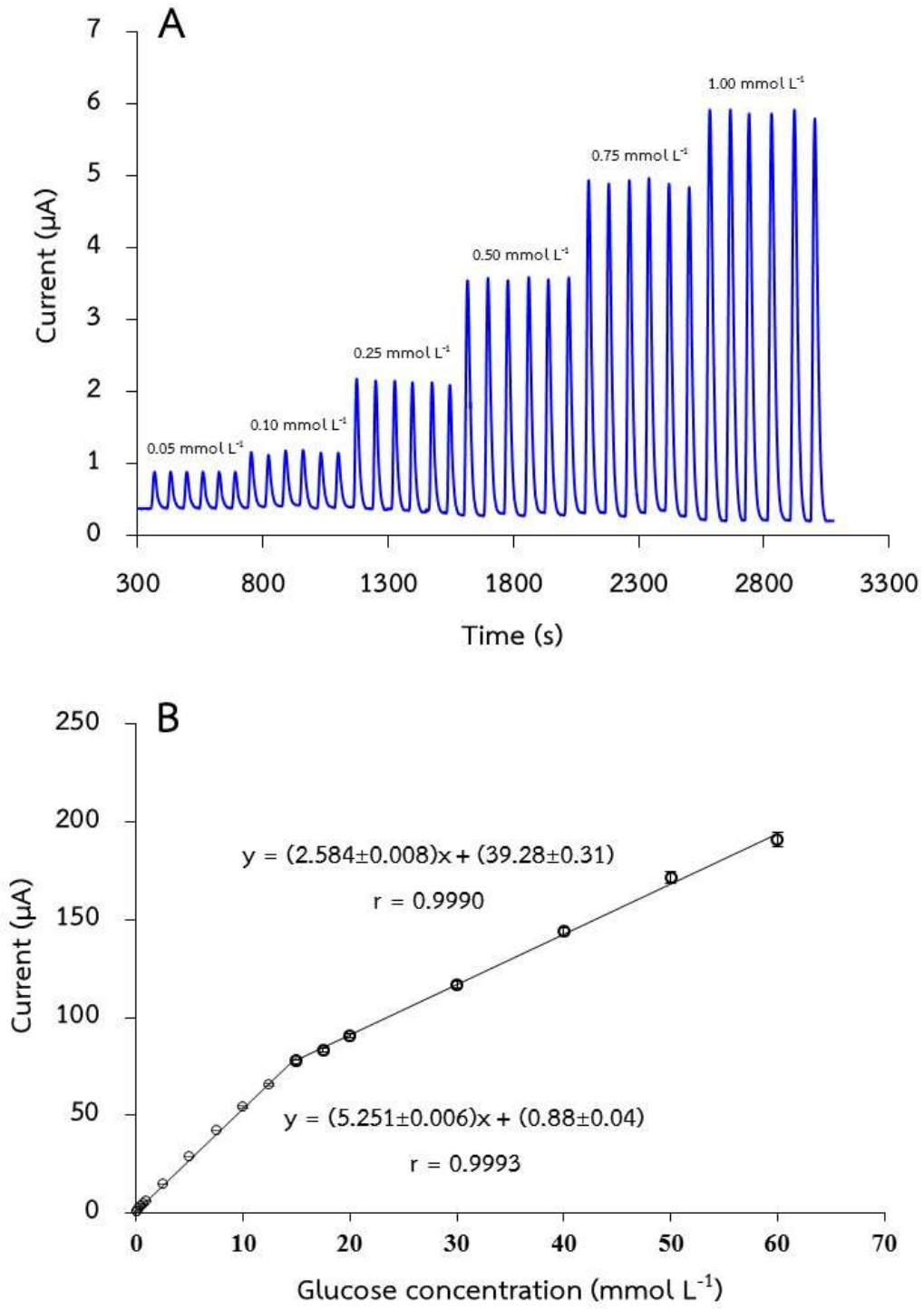


Figure 3.10 (A) FI- Amperogram of GO-PAA-PdNPs/SPCE at different concentrations of glucose. (B) The calibration curve of the current response of the glucose sensor.

3.3.2 Selectivity

The influence of possible interfering compounds that commonly coexist with glucose in blood samples was studied under the optimal conditions. A series of solutions containing 5.0 mmol L^{-1} glucose mixed with 0.10 mmol L^{-1} AA, 0.50 mmolL^{-1} UA, 12 nmol L^{-1} DA and 100 mmol L^{-1} Cl^- were tested. The concentrations of these interfering compounds were higher than their normal levels in blood [5]. Each mixture was diluted 10 times before measurement. The results showed no significant difference between the response of glucose alone and the glucose with the interfering compound(s) ($P > 0.05$) (Table 3.6 and Figure 3.11A), even at 10 times higher concentrations (Table 3.6 and Figure 3.11B). This glucose sensor showed excellent selectivity toward the detection of glucose in blood serum samples.

Table 3.6 The influence of interfering compounds on the current response of glucose determination at 5.00 mmol L^{-1} (n = 3).

Sample types	Current (μA)				SD
	1	2	3	Average	
Glu 5.0 mmol L^{-1}	3.24	3.22	3.09	3.18	0.08
Glu 5.0 mmol L^{-1} + AA 0.10 mmol L^{-1}	3.2	3.29	3.18	3.22	0.06
Glu 5.0 mmol L^{-1} + UA 0.50 mmol L^{-1}	3.13	3.25	3.2	3.19	0.06
Glu 5.0 mmol L^{-1} + DA 12.0 nmol L^{-1}	3.28	3.224	3.18	3.23	0.05
Glu 5.0 mmol L^{-1} + Cl^- 100 mmol L^{-1}	3.15	3.28	3.24	3.22	0.07
Glu 5.0 mmol L^{-1} + AA 1.0 mmol L^{-1}	3.14	3.17	3.28	3.20	0.07
Glu 5.0 mmol L^{-1} + UA 5.0 mmol L^{-1}	3.23	3.26	3.28	3.28	0.03
Glu 5.0 mmol L^{-1} + DA 120 nmol L^{-1}	3.11	3.27	3.24	3.21	0.09
Glu 5.0 mmol L^{-1} + Cl^- 500 mmol L^{-1}	3.2	3.27	3.14	3.20	0.07
Glu 5.0 mmol L^{-1} + Cl^- 1000 mmol L^{-1}	3.18	3.25	3.27	3.23	0.05

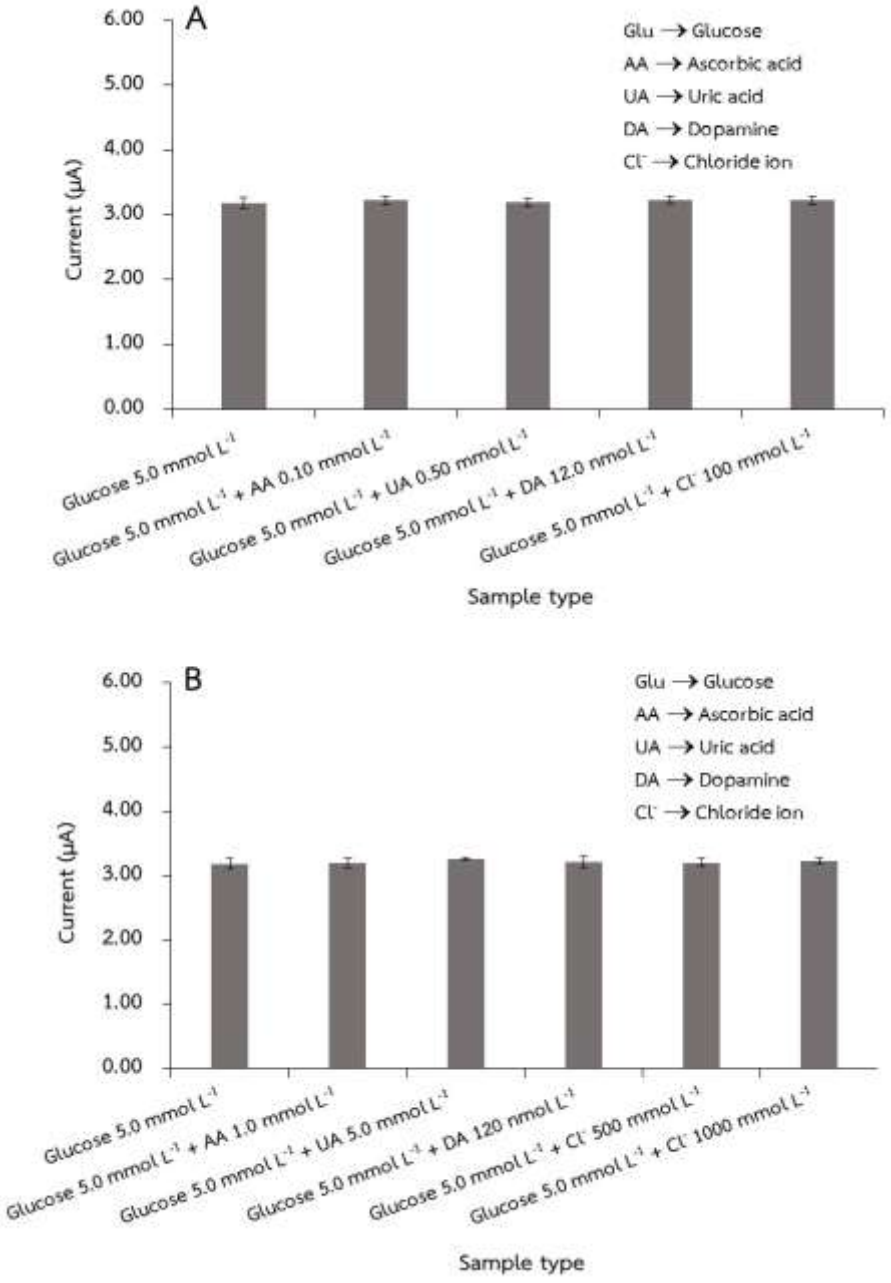


Figure 3.11 (A) The current responses of 5.0 mmol L^{-1} glucose with and without the interfering compounds: 0.10 mmol L^{-1} AA, 0.50 mmol L^{-1} UA, 12 nmol L^{-1} DA and 100 mmol L^{-1} Cl^- and (B) with 10 times concentrations of the interfering compounds.

3.3.3 Repeatability and reproducibility

The repeatability of the nonenzymatic sensor was studied by measuring fifteen replications for each of the three concentrations in the lower range of linearity, i.e., 0.50, 1.00 and 2.50 mmol L^{-1}. The results provided relative standard deviations (RSDs) of 0.52%, 1.51% and 1.28%, which are acceptable according to AOAC guidelines, i.e., <7.3%, <5.3% and <3.7%, respectively [94] (Table 3.7 and Figure 3.12). To evaluate the reproducibility, six GO-PAA-PdNPs/SPCEs were prepared on different days to monitor glucose (0.10, 0.25, 0.50, 0.75 and 1.00 mmol L^{-1}) (Table 3.8 and Figure 3.13A). The sensitivities of the six electrodes were 5.56±0.09, 5.63±0.08, 5.52±0.08, 5.58±0.08, 5.63±0.08 and 5.61±0.08 μA $mmol^{-1}$ L. The RSD of the average sensitivity for the six electrode preparations was 0.92% (Table 3.9 and Figure 3.13B). These results showed that the developed glucose sensors have good reproducibility.

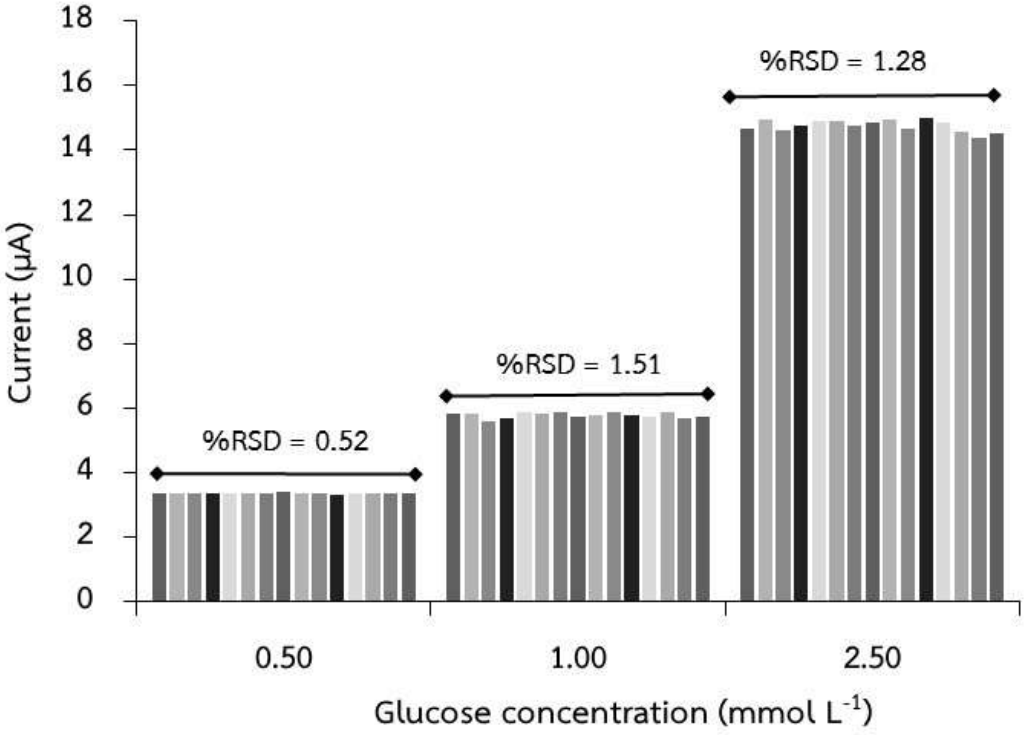


Figure 3.12 The current responses of the sensor obtained from fifteen measurements for each concentration (0.50, 1.00 and 2.50 mmol L^{-1}).

Table 3.7 The repeatability of the GO-PAA-PdNPs/SPCE on the current response of fifteen replications for each of three concentrations of 0.50, 1.00 and 2.50 mmol L^{-1} glucose.

Injection times		Concentration of glucose (mmol L^{-1})		
		0.50	**1.00**	**2.50**
Current Response (μA)	1	3.38	5.82	14.66
	2	3.38	5.85	14.95
	3	3.35	5.61	14.63
	4	3.37	5.67	14.75
	5	3.35	5.86	14.91
	6	3.38	5.84	14.88
	7	3.37	5.9	14.75
	8	3.4	5.72	14.85
	9	3.37	5.8	14.96
	10	3.35	5.86	14.68
	11	3.33	5.77	15
	12	3.35	5.75	14.83
	13	3.38	5.9	14.54
	14	3.37	5.69	14.35
	15	3.37	5.75	14.5
Average		3.37	5.79	14.75
SD		0.02	0.09	0.19
%RSD		0.52	1.51	1.28

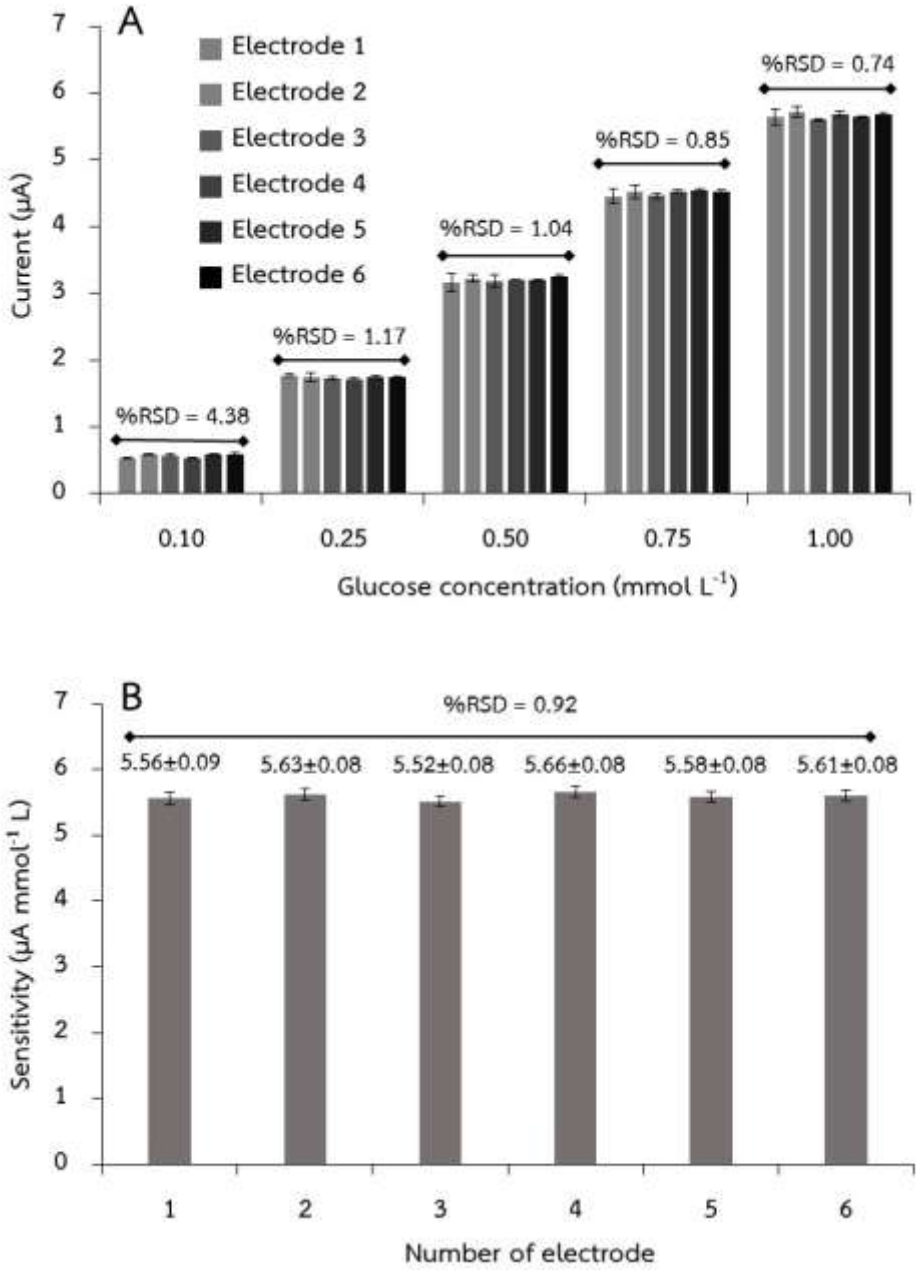


Figure 3.13 (A) The current responses from six GO-PAA-PdNPs/SPCE and (B) the sensitivity of six fabricated electrodes for the detection of glucose (0.10-1.00 mmol L^{-1}).

Table 3.8 The reproducibility of the GO-PAA-PdNPs/SPCE on the current response from the six electrodes.

Number of electrode		Concentration of glucose (mmol L^{-1})				
		0.1	**0.25**	**0.50**	**0.75**	**1.00**
Current Response (μA)	**E1**	0.53	1.77	3.16	4.45	5.64
	E2	0.59	1.75	3.22	4.52	5.71
	E3	0.58	1.72	3.19	4.46	5.60
	E4	0.53	1.72	3.21	4.53	5.69
	E5	0.58	1.75	3.19	4.54	5.64
	E6	0.58	1.74	3.26	4.53	5.68
Average		0.57	1.74	3.20	4.51	5.66
SD		0.02	0.02	0.03	0.04	0.04
%RSD		4.38	1.17	1.04	0.85	0.74

E : Electrode

Table 3.9 The reproducibility of the GO-PAA-PdNPs/SPCE on the Sensitivity from the six electrodes.

Number of electrode	Regression equation	r
E1	y = (5.56±0.09)x + (0.22±0.06)	0.9959
E2	y = (5.63±0.08)x + (0.23±0.05)	0.9967
E3	y = (5.52±0.08)x + (0.24±0.05)	0.9962
E4	y = (5.66±0.08)x + (0.19±0.05)	0.9962
E5	y = (5.58±0.08)x + (0.24±0.05)	0.9991
E6	y = (5.61±0.08)x + (0.24±0.05)	0.9998

3.3.4 Stability

Under the optimal conditions, a GO-PAA-PdNPs/SPCE was tested for operational stability by repeatedly measuring the current responses of continuous injections of 0.25 mmol L^{-1} glucose (Table 3.10 and Figure 3.14A). The electrode showed excellent stability for up to 450 injections, with an average relative response of 100.0±2.5% (RSD = 2.5%). After 450 injections, the current response gradually decreased to below 95%. The loss of the current response might be because of the loss of GO-PAA-PdNPs from the SPCE surface, which led to a decrease in the surface area available on the electrocatalyst for glucose. This phenomenon was confirmed by the decrease in the reduction peak of Pd after glucose measurements (Figure 3.14B).

Table 3.10 The stability of the GO-PAA-PdNPs/SPCE of glucose determination for 450 repeating injections of concentration of glucose at 0.25 mmol L^{-1}**.**

No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)
1	100	26	99	51	100	76	100	101	99
2	98	27	101	52	98	77	99	102	107
3	103	28	101	53	102	78	98	103	107
4	98	29	101	54	99	79	101	104	101
5	103	30	102	55	99	80	100	105	98
6	102	31	102	56	98	81	101	106	101
7	100	32	98	57	103	82	97	107	102
8	100	33	99	58	97	83	97	108	103
9	100	34	100	59	98	84	97	109	105
10	100	35	102	60	101	85	98	110	107
11	99	36	98	61	99	86	100	111	105
12	102	37	99	62	101	87	99	112	104
13	103	38	98	63	100	88	99	113	98
14	101	39	99	64	100	89	99	114	94
15	103	40	100	65	100	90	100	115	92
16	99	41	100	66	98	91	98	116	93
17	101	42	101	67	99	92	99	117	108
18	98	43	102	68	99	93	105	118	104
19	98	44	100	69	95	94	102	119	100
20	99	45	102	70	101	95	104	120	102
21	100	46	97	71	98	96	100	121	103
22	98	47	102	72	100	97	102	122	102
23	100	48	103	73	100	98	100	123	103
24	100	49	103	74	99	99	99	124	103
25	101	50	98	75	101	100	98	125	104

Table 3.10 The stability of the GO-PAA-PdNPs/SPCE of glucose determination for 450 repeating injections of concentration of glucose at 0.25 mmol L^{-1} (continuous).

No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)
126	104	151	102	176	98	201	100	226	106
127	100	152	98	177	101	202	95	227	97
128	100	153	100	178	95	203	96	228	102
129	102	154	104	179	99	204	100	229	97
130	97	155	100	180	102	205	102	230	100
131	102	156	97	181	100	206	101	231	101
132	103	157	98	182	98	207	105	232	101
133	106	158	94	183	100	208	101	233	101
134	102	159	101	184	99	209	98	234	100
135	99	160	103	185	101	210	103	235	102
136	98	161	99	186	103	211	103	236	102
137	99	162	98	187	102	212	101	237	103
138	99	163	98	188	97	213	100	238	106
139	98	164	98	189	102	214	98	239	103
140	100	165	100	190	100	215	99	240	102
141	101	166	100	191	101	216	98	241	104
142	101	167	103	192	98	217	104	242	104
143	100	168	99	193	100	218	99	243	98
144	97	169	100	194	99	219	96	244	101
145	104	170	95	195	102	220	100	245	98
146	101	171	98	196	98	221	94	246	105
147	102	172	103	197	96	222	95	247	98
148	97	173	98	198	102	223	105	248	105
149	97	174	103	199	99	224	101	249	98
150	97	175	100	200	94	225	99	250	104

Table 3.10 The stability of the GO-PAA-PdNPs/SPCE of glucose determination for 450 repeating injections of concentration of glucose at 0.25 mmol L^{-1} (continuous).

No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)	No.	relative current (%)
251	100	276	100	301	100	326	98	351	103
252	100	277	100	302	99	327	105	352	97
253	103	278	99	303	99	328	104	353	97
254	99	279	98	304	100	329	100	354	97
255	100	280	98	305	98	330	104	355	98
256	99	281	97	306	98	331	100	356	96
257	98	282	99	307	100	332	103	357	98
258	99	283	100	308	100	333	97	358	97
259	100	284	100	309	100	334	101	359	98
260	100	285	100	310	98	335	97	360	101
261	98	286	100	311	101	336	99	361	101
262	99	287	99	312	96	337	99	362	103
263	98	288	99	313	98	338	103	363	100
264	101	289	99	314	98	339	95	364	103
265	99	290	99	315	97	340	100	365	99
266	97	291	98	316	100	341	100	366	103
267	98	292	98	317	101	342	95	367	97
268	97	293	98	318	98	343	101	368	98
269	98	294	100	319	98	344	97	369	99
270	101	295	100	320	98	345	102	370	103
271	96	296	98	321	96	346	98	371	104
272	101	297	97	322	96	347	101	372	98
273	98	298	103	323	104	348	95	373	100
274	102	299	103	324	100	349	99	374	95
275	99	300	103	325	99	350	96	375	99

Table 3.10 The stability of the GO-PAA-PdNPs/SPCE of glucose determination for 450 repeating injections of concentration of glucose at 0.25 mmol L^{-1} (continuous).

No.	relative current (%)	No.	relative current (%)	No.	relative current (%)
376	97	401	98	426	101
377	103	402	102	427	97
378	98	403	98	428	99
379	99	404	98	429	102
380	99	405	96	430	99
381	101	406	99	431	100
382	101	407	98	432	101
383	101	408	100	433	97
384	99	409	100	434	98
385	101	410	98	435	97
386	101	411	96	436	101
387	101	412	99	437	102
388	100	413	100	438	98
389	99	414	96	439	98
390	100	415	97	440	97
391	102	416	99	441	97
392	101	417	101	442	97
393	100	418	97	443	97
394	98	419	96	444	98
395	99	420	102	445	97
396	101	421	102	446	97
397	98	422	101	447	97
398	99	423	99	448	95
399	99	424	102	449	98
400	100	425	98	450	95

Table 3.10 The stability of the GO-PAA-PdNPs/SPCE of glucose determination for 450 repeating injections of concentration of glucose at 0.25 mmol L^{-1} (continuous).

Range of injections	Average relative current	SD	%RSD
1-100	99.9	1.8	1.8
101-200	100.2	3.1	3.1
201-300	100.0	2.5	2.5
301-400	99.4	2.3	2.3
401-450	98.6	2.0	2.0
1-450	100.0	2.5	2.5

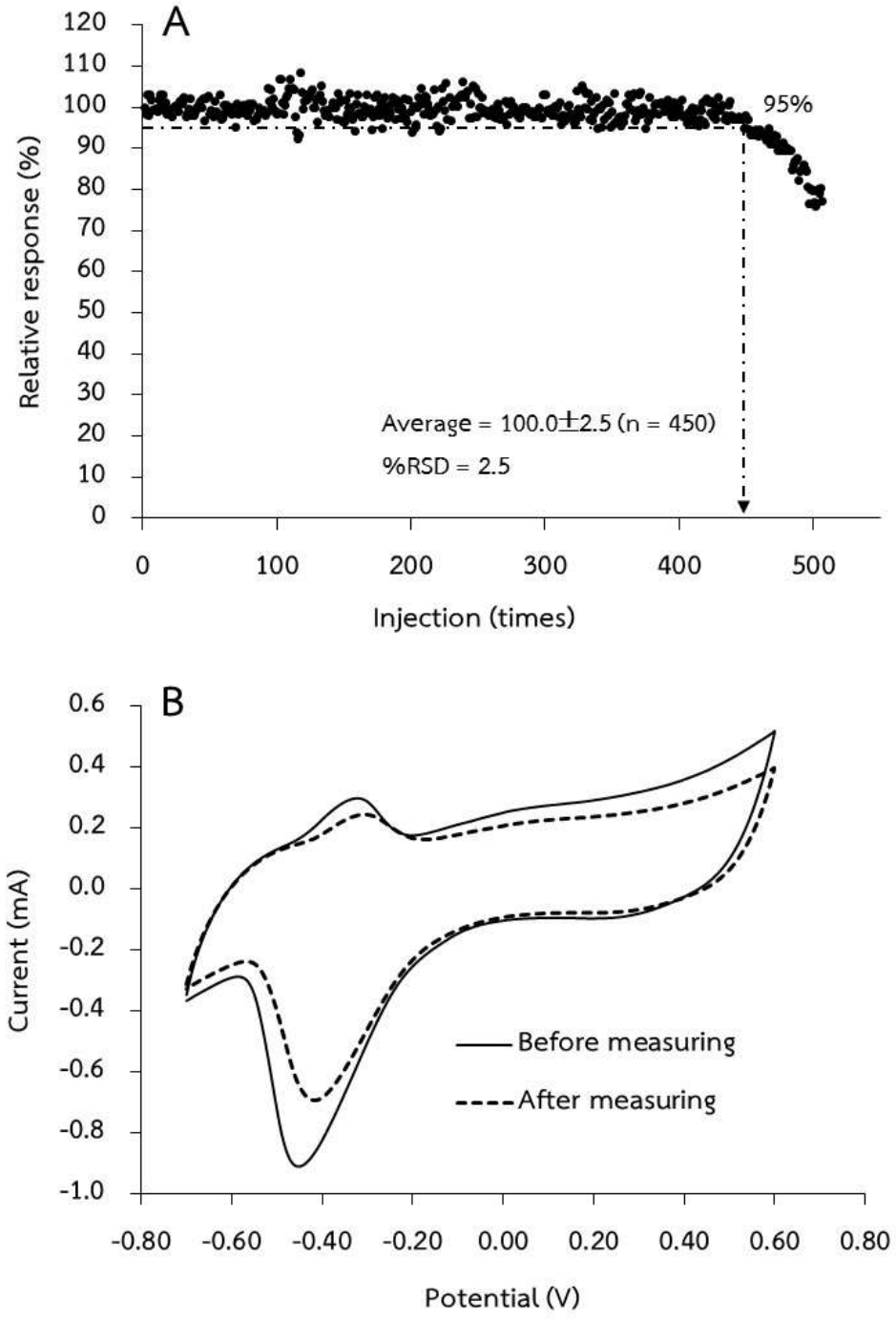


Figure 3.14 (A) Operational stability of the fabricated sensor; (B) cyclic voltammogram of the GO-PAA-PdNPs/SPCE in 0.1 mol L^{-1} NaOH before and after measuring glucose.

3.3.5 Analysis of blood serum samples

To examine the performance in practical applications, the developed sensor was used to detect glucose in blood serum samples. The recoveries were studied by spiking standard glucose into blood serum samples and then diluting the samples 10 times with 0.10 mmol L^{-1} NaOH before each measurement. The slopes of the calibration curves obtained for the standard glucose and spiked samples were compared by two-way ANOVA. There was no significant difference between the two slopes ($P > 0.05$); therefore, there was no matrix effect. The calibration curve for the glucose standard can be used to calculate the concentration of glucose in blood serum samples. The results of twenty samples are listed in Table 3.11. The concentrations of glucose obtained from the developed sensor when compared to those from the standard hexokinase-spectrophotometric method in Hatyai hospital showed no significant differences ($P > 0.05$). The recoveries obtained from glucose detection ranged from 85 ± 2 to 106 ± 3% (Table 3.11). These recoveries were well within the acceptable limits according to AOAC guidelines. Therefore, the nonenzymatic sensor could be applied to measure glucose in blood serum samples.

Table 3.11 The determination of glucose in blood serum samples (n = 3) and the recovery test of glucose from blood serum samples

Sample	Hospital method (mmol L^{-1})	Fabricated sensor (mmol L^{-1}) (n = 3)	%Recovery of fabricated sensor (n = 3) Concentration of spiking (mmol L^{-1})				
			0.10	0.25	0.50	0.75	1.00
1	7.67	7.61 ± 0.09	85 ± 2	104 ± 3	100 ± 3	101 ± 1	98 ± 1
2	4.47	4.5 ± 0.1	91 ± 3	103 ± 4	96.0 ± 0.5	100.0 ± 0.7	100 ± 1
3	5.66	5.70 ± 0.07	87 ± 2	102 ± 4	106.0 ± 0.9	100 ±	100.0 ± 0.9
4	4.76	4.73 ± 0.04	92 ± 2	106 ± 3	103 ± 1	103 ± 1	100.0 ± 0.2
5	5.45	5.47 ± 0.05	94 ± 1	96 ± 3	101 ± 1	100 ± 1	99.0 ± 0.2
6	8.08	8.0 ± 0.1	87 ± 3	106 ± 3	100 ± 2	102 ± 2	98 ± 1
7	6.74	6.76 ± 0.04	102 ± 1	99 ± 2	102 ± 2	98.0 ± 0.4	101.0 ± 0.5
8	4.66	4.63 ± 0.07	100 ± 3	103 ± 3	99 ± 3	102.0 ± 0.4	99.0 ± 0.9
9	7.40	7.3 ± 0.2	97 ± 3	102 ± 1	99.0 ± 0.7	101 ± 2	101.0 ± 0.6
10	5.61	5.52 ± 0.08	102 ± 4	94 ± 3	99 ± 2	97 ± 2	101.0 ± 0.4
11	8.05	8.2 ± 0.2	100 ± 4	108 ± 4	91 ± 0	100 ± 1	101.0 ± 0.4
12	7.44	7.5 ± 0.2	95 ± 4	94 ± 1	104 ± 2	100 ± 1	98 ± 1
13	5.66	5.7 ± 0.1	96 ± 0	94 ± 3	101 ± 2	102 ± 2	99 ± 1
14	7.47	7.4 ± 0.1	96 ± 0	105 ± 4	98 ± 2	102 ± 0	100 ± 2
15	5.55	5.6 ± 0.1	98 ± 4	94 ± 0	99 ± 2	97 ± 1	99.0 ± 0.9
16	9.46	9.5 ± 0.1	101 ± 3	98 ± 3	100 ± 2	98 ± 1	101.0 ± 0.5
17	10.75	10.8 ± 0.2	93 ± 3	102 ± 3	97 ± 1	101 ± 1	100.0 ± 0.7
18	8.38	8.4 ± 0.1	95 ± 3	105 ± 3	98 ± 2	97 ± 1	102.0 ± 0.9
19	5.21	5.2 ± 0.1	95 ± 3	93 ± 5	106 ± 1	102.0 ± 0.4	98.0 ± 0.6
20	9.16	9.4 ± 0.3	94 ± 4	101 ± 1	105 ± 2	100 ± 1	100.0 ± 0.4

CHARPTER 4

Conclusions

In this thesis, GO-PAA-PdNPs were successfully prepared by electroless deposition and were used to modify the SPCE working electrode for the determination of glucose by FI-Amp. The combination of the unique properties of these materials greatly improved the sensor performance. In particular, PAA increased the distribution of PdNPs on the GO surface, thus increasing the surface area for the excellent electrocatalytic oxidation of glucose. Under the optimal conditions, this nonenzymatic glucose sensor provided two wide linear ranges, high sensitivity (75 μA $mmol^{-1}$ L cm^{-2}), a low limit of detection (22 μmol L^{-1}), a low limit of quantification (76 μmol L^{-1}), good operational stability (450 injections), good repeatability (%RSD $<$ 1.51%), and good recovery (85 ± 2 to 106 ± 3%). This strategy opens new opportunities for the determination of glucose in biological fluid samples.

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Appendix

PACCON
PURE AND APPLIED CHEMISTRY INTERNATIONAL CONFERENCE 2018

CHEMISTRY TOWARD A SUSTAINABLE FUTURE

FEBRUARY 7–9, 2018

ICC Hat Yai in PSU, Hat Yai, Songkhla, THAILAND

Organizers : Faculty of Science, Prince of Songkla University (PSU)
in conjunction with the Chemical Society of Thailand (CST) under the patronage of
Professor Dr. Her Royal Highness Princess Chulabhorn Mahidol

PLENARY SPEAKERS

Prof. Dr. Sir J. Fraser Stoddart
Nobel Laureate in Chemistry, 2016
Northwestern University, USA

Prof. Dr. David W. C. MacMillan
Princeton University, USA

Prof. Dr. Eric Bakker
University of Geneva, SWITZERLAND

SCIENTIFIC SESSIONS

1. Analytical Chemistry
2. Environmental Chemistry and Renewable Energy
3. Food and Agricultural Chemistry
4. Inorganic Chemistry
5. Material Chemistry and Nanotechnology
6. Natural Products, Biological Chemistry and Chemical Biology
7. Organic and Medicinal Chemistry
8. Physical and Theoretical Chemistry
9. Polymer Chemistry

SPECIAL SESSIONS

1. Introduction to metabolite profiling and metabolomics for natural product research, organized by Prof. Dr. Jean-Luc Wolfender, University of Geneva, SWITZERLAND
2. Industrial Chemistry and Innovation, organized by PACCON 2018
3. Spa and Cosmetics, organized by PACCON 2018

IMPORTANT DATES

REGISTRATION :
Registration and payment deadline for presenters : December 12, 2017
Early registration : July 15 - December 25, 2017
Late registration : After December 25, 2017
On-site registration : February 7-9, 2018

SUBMISSION :
Abstract submission opens : July 15, 2017
Abstract submission deadline : October 15, 2017
Notification of abstract acceptance : November 30, 2017
Full paper submission deadline : January 15, 2018
Notification of full paper acceptance : March 15, 2018

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Non-enzymatic electrochemical glucose sensor based on palladium nanoparticles-graphene oxide (PdNPs-GO) modified electrode

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- PdNPs-GO modified on glassy carbon electrode (GCE) for glucose detection.
- PdNPs-GO/GCE exhibits excellent repeatability.
- PdNPs-GO/GCE has potential for glucose detection in biological fluid samples.

Non-enzymatic electrochemical glucose sensor based on palladium nanoparticles-graphene oxide (PdNPs-GO) modified electrode

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Introduction

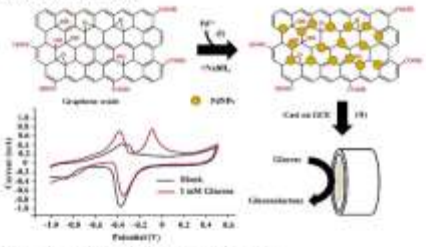
Diabetes mellitus is a chronic disease related to glucose digestion. It is one of the leading causes of morbidness, death and a significant health problem in the worldwide. Therefore the detection of glucose is necessary. A non-enzymatic electrochemical sensor is an interesting to be developed as a glucose sensor. It is easy to use, rapid and has high sensitivity. In this work, a non-enzymatic electrochemical sensor using a glassy carbon electrode (GCE) modified with palladium nanoparticles-graphene oxide (PdNPs-GO/GCE) has been developed for the determination of glucose by flow injection amperometry (FI-Amp). PdNPs provided an excellent electrocatalytic activity toward the oxidation of glucose whereas GO acted as a supporting material for the immobilization of PdNPs.

Objective

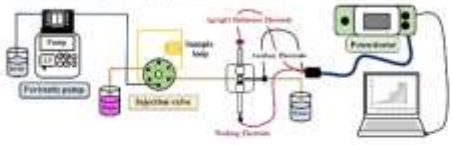
To develop a non-enzymatic electrochemical sensor for determination of glucose based on the PdNPs-GO modified GCE in a flow injection system.

Methods

Synthesis of PdNPs-GO



Measurement by FI amperometric detection



Conclusion

A non-enzymatic electrochemical sensor using a glassy carbon electrode (GCE) modified with palladium nanoparticles-graphene oxide (PdNPs-GO/GCE) was successfully developed for the determination of glucose by flow injection amperometry (FI-Amp). This system provided high sensitivity, wide linear range, low detection limit, and excellent repeatability. It is possible to apply this sensor to detect glucose in biological fluid samples.

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- Faculty of Science Research Fund, Prince of Songkla University Contract No.1-2558-02-001
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- Trace Analysis and Biosensor Research Center (TAB-RC)
- Department of Chemistry, Applied Science and Physics, Faculty of Science, Graduated School , Prince of Songkla University

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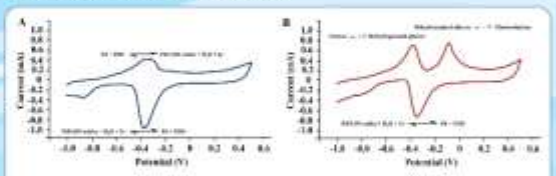
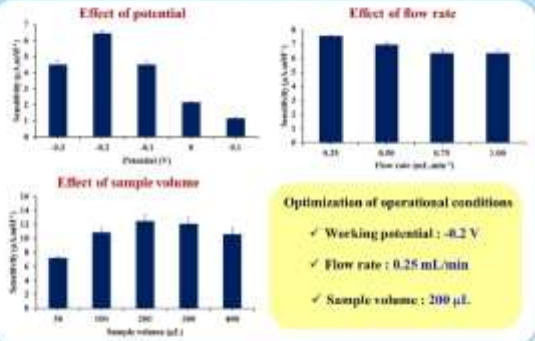
Results

Electrochemical characterization



Cyclic voltammograms of PdNPs-GO modified GCE without glucose (A), with 1.0 mM glucose (B)
Conditions: 0.1 M KOH electrolyte, scan rate 50 mV/s

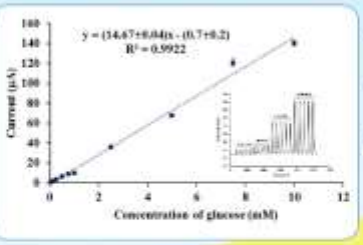
Optimization of the FI-Amp system



Optimization of operational conditions

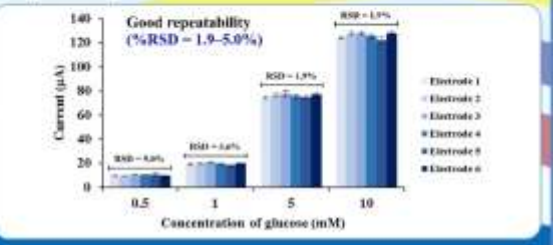
- ✓ Working potential : -0.2 V
- ✓ Flow rate : 0.25 mL/min
- ✓ Sample volume : 200 µL

Performance of PdNPs-GO/GCE by FI-Amp system



- ✓ Linear range : 0.05 to 10 mM
- ✓ Detection limit: 0.03 mM
- ✓ Quantification limit: 0.1 mM

Repeatability of the sensors



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Scholarship Awards during Enrolment

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List of Publication and Proceedings

Poster presentation

Samoson, K., Kanatharana, P., Thavarungkul, P., and Limbut, W. "Non-enzymatic electrochemical glucose sensor based on palladium nanoparticles-graphene oxide (PdNPs-GO) modified electrode". PACCON 2018, PSU International Convention Center - Thanon Poonnakan, Tambon Kho Hong, Amphoe Hat Yai, Chang Wat Songkhla 90110, Thailand, February, 2018.