



Uric Acid Sensor Based on PEDOT:PSS Modified Screen-Printed Carbon Electrode Fabricated with a Simple Painting Technique

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Abstract

A screen-printed carbon electrode is a suitable electrode for electrochemical sensors due to its simplicity and portability. This study aimed to fabricate a screen-printed carbon electrode modified with poly (3,4-ethylenedioxythiophene) polystyrene sulfonate (SPCE-PEDOT:PSS) to improve the electrochemical performance for uric acid detection. The SPCE was fabricated using a layer-by-layer painting process of conductive ink consisting of graphite as a conductive material, polystyrene as a polymeric binder, and dichloromethane solvent on a polyvinyl chloride paper substrate. The fabricated SPCE was then modified with PEDOT:PSS by a drop-casting method. The characterization of SPCE-PEDOT:PSS surface morphology was performed using the scanning electron microscopy technique. The SPCE-PEDOT:PSS provided an acceptable linearity ($R^2 = 0.9985, 0.9993, 0.9985$), sensitivity ($0.070, 0.015, 0.024 \mu\text{A}/\mu\text{M}$), precision (%RSD = 2.70%, 2.89%, 2.40%), limit of detection ($1.61 \mu\text{M}, 1.14 \mu\text{M}, 1.62 \mu\text{M}$), and limit of quantitation ($5.37 \mu\text{M}, 3.81 \mu\text{M}$ to $5.39 \mu\text{M}$) in measurement of uric acid standard solution using cyclic voltammetry, amperometry, and differential pulse voltammetry techniques, respectively. The studies using SPCE-PEDOT:PSS indicated that the electrode could be applied in the electrochemical measurement of uric acid in the human urine sample.

1. Introduction

A simple technique to fabricate a screen-printed electrode (SPE) offers advantages in being versatile and economical for developing point-of-care and real-time sensing devices [1]. An SPE can be used for routine electroanalysis as a disposable electrode since this type of electrode can prevent cross-contamination from previous measurements [2]. SPE is a planar strip consisting of three electrodes, namely working, reference, and auxiliary electrode. This SPE is convenient to be used as an electrochemical sensing device due to its simplicity and portability in measuring the concentration of electroactive species [3].

SPE could be fabricated using various types of conductive materials such as carbon-based materials (graphene, graphene oxide, reduced graphene oxide,

carbon nanotubes, and graphite) and metals (gold, silver, etc.) [4, 5, 6]. The most widely used carbon-based material in SPE fabrication is graphite. However, graphite's conductivity is lower than the other carbon allotrope and thus needs to be modified to improve its electroanalytical performance. One way to improve the electroanalytical performance of carbon-based material is using modifiers such as conductive polymer in the screen-printed carbon electrode (SPCE) fabrication. Although this modifier polymer's conductivity is lower compared to inorganic material, in terms of its elasticity and plasticity is superior. Therefore, the conductive polymer's utilization as a modifier for carbon-based material is an interesting idea to be investigated in the SPCE fabrication.

Screen printing and inkjet printing are frequently employed in the SPCE fabrication process [7, 8]. Both techniques offer a highly reproducible and low cost in the SPCE fabrication process. In addition to these techniques, an alternative method can be used to fabricate an SPCE unit, for example, the painting technique. This painting technique offers a convenient and straightforward process that also could be performed without sophisticated equipment in the SPCE fabrication [9].

Our previous work has investigated uric acid sensors' performance based on screen-printed carbon electrodes, but its sensitivity needs to be improved [10]. Therefore, this study aimed to fabricate a screen-printed carbon electrode by a simple painting technique and modify the electrodes using a conductive polymer as a modifier to enhance its electrochemical performance for the uric acid sensor. The poly (3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) was employed as a polymer modifier since this conductive polymer is widely used as electrode modifier [11, 12, 13, 14, 15, 16, 17, 18]. The resulting electrode (SPCE-PEDOT:PSS) was then characterized using scanning electron microscopy to obtain the electrode area's surface morphology. Several electroanalytical techniques such as cyclic voltammetry, amperometry, and differential pulse voltammetry were conducted using this modified electrode to evaluate their electroanalytical performance in uric acid solution. The reason for choosing uric acid as a sample in this analysis is that the high level of uric acid concentration in the human body leads to several diseases and decreases life quality. Therefore, the regular monitoring of uric acid concentration is necessary to prevent adverse effects on the human body's metabolism process. It is expected that the investigation of this fabricated SPCE-PEDOT:PSS has the potency to be applied as a future electrochemical sensor for uric acid monitoring.

2. Methodology

2.1. Equipment and Material

Graphite powder (particle size 20 μm) and dichloromethane (CAS No. 75-09-2) were received from Merck, Darmstadt, Germany). Silver powder, polystyrene, PEDOT:PSS, uric acid, KCl were purchased from Sigma Aldrich and used without further purification. The commercial SPCE was obtained from DropSens (ref. 110, DS SPCE). Paraffin wax, pattern sticker of SPCE template (Camel Glossy), PVC paper sheet, deionized water was used during the entire experiment. The urine sample for analysis was collected from a healthy volunteer at 23 years old (pH of urine was 6.1).

Electrochemical experiments were carried out on the PalmSens Emstat 3 potentiostat (ES316U669). Scanning electron microscope (SEM) images were obtained from JEOL JSM 6510-LA. The standard apparatus in the laboratory such as Pyrex glassware, analytical balance (Ohaus Instrument Co. Ltd.), magnetic stirrer, micropipette (Eppendorf), and oven (Mettler GmbH) were used during the experiments.

2.2. The fabrication of SPCE and SPCE-PEDOT:PSS

The ink formula of the screen-printed electrode is a mixture of graphite or silver powder with polystyrene in a ratio of 10:2 (w/w) in dichloromethane (DCM) resulted in 20% (w/w) graphite ink. The mixture was stirred for 2 minutes using a magnetic stirrer to obtain a consistent ink. The SPCE pattern template was printed with a printer on a glossy camel sticker's paper surface, as shown in Figure 1.

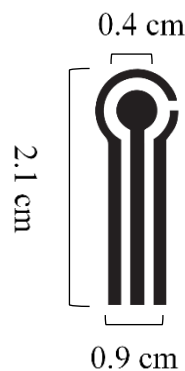


Figure 1. A template design for Screen-Printed Carbon in the sticker paper

For SPCE fabrication, the PVC paper sheet as a substrate was cleaned with dichloromethane before use. SPCE pattern in the sticker paper was attached to the surface of the substrate. Next, graphite ink was layer-by-layer painted to the PVC paper's surface using a brush and smoothed following a pattern of SPCE. PVC sheet with a pattern template was then heated in the oven at 50°C for 5 minutes to obtain a dried and attached ink to the substrate. Silver ink was then applied on top of the graphite layer to create the reference electrode and air-dried for 5 minutes. After 5 minutes, the sticker paper with a pattern was peeled off from the substrate. The Paraffin wax as an insulation layer was then applied beyond the electrode area of fabricated SPCE to give a hydrophobic barrier. At last, the copper foil was applied to the connection path of fabricated SPCE.

The modification of fabricated SPCE was performed with the beads of PEDOT:PSS dissolved in the mixture of deionized water and methanol (1:1). Approximately 0.6 mg PEDOT:PSS was dissolved in the 1.5 mL deionized water and then added with methanol until a total volume of 3 mL with a concentration of 0.2 mg/mL. The working electrode of fabricated SPCE was modified using 5 μL of PEDOT:PSS with a drop-casting technique. PEDOT:PSS modified SPCE (SPCE-PEDOT:PSS) was heated in the oven at 35°C during 30 minutes. To obtain the surface morphology of SPCE and SPCE-PEDOT:PSS, both electrode surfaces were characterized by SEM.

2.3. Evaluation of the electrochemical performance of fabricated SPCE and SPCE-PEDOT:PSS

The electrochemical measurement of fabricated SPCE and SPCE modified with PEDOT:PSS was performed using 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in the 0.1 M KCl using cyclic voltammetry technique. The investigation was performed in the potential range of +0.8 to -0.4V vs. pseudo-Ag, the

scan rate of 100 mV s⁻¹. The measurement was then continued with different scan rates of 50, 75, 100, 150, 200, and 250 mV s⁻¹. The electrochemical behavior from both fabricated SPCE and SPCE-PEDOT:PSS compared with commercial SPCE.

2.4. Uric acid measurement using fabricated SPCE and SPCE-PEDOT:PSS

Evaluation of uric acid measurement was performed using cyclic voltammetry technique with 0.1 mM uric acid in 0.1 M KCl. The measurement was performed in the potential window of +0.2 V to +0.7 V with a scan rate of 100 mV s⁻¹. The result from uric acid measurement was then compared with commercial SPCE.

The linearity was investigated by performing the uric acid measurement in the series of concentrations of 20, 30, 40, 60, 80, and 100 μM in 0.1 M KCl. Each concentration was measured using cyclic voltammetry with a scan rate of 100 mV s⁻¹, a differential pulse voltammetry of 25 mV s⁻¹, a potential step of 5 mV, a potential pulse of 25 mV, and a pulse time of 0.1 s. The potential window was used in both measurements as +0.2 V until +0.7 V. The measurement of uric acid was also performed using chronoamperometry technique at fixed potential +0.48 V during 120 s. Each voltammetry and amperometry measurement with different concentrations of uric acid was repeated six times. This investigation can be calculated the relative standard deviation (%RSD), and the calculation of limit of detection and limit of quantification was referred to the ISO/IEC 17025.

2.5. The measurement of uric acid in the human urine

The measurement of uric acid in the human urine was performed with the standard addition technique. At first, fresh urine was diluted 100 times, and each 5 mL of fresh urine was added to the standard of uric acid in the series of 200, 400, 600, 1000, 1500, and 2000 μL. Each uric acid concentration was measured with cyclic voltammetry, differential pulse voltammetry, and chronoamperometry techniques with the same parameters stated in section 2.4. The concentration of uric acid in a sample was calculated based on x-intercept

of the standard addition calibration curve using the equation (1)

$$x - \text{intercept} = -\frac{C_A \times V_0}{C_S} \quad (1)$$

the x-intercept is x value when y is zero. C_A is analyte concentration in the sample, V₀ is an initial volume of sample, and C_S is the standard deviation.

3. Result and Discussion

3.1. Fabricated SPCE and SPCE-PEDOT:PSS

In this research, the SPCE fabrication was carried out using the painting technique, which is a good option due to its simple procedures and can be applied in a conventional laboratory condition. A polyvinyl chloride (PVC) paper used as a substrate for SPCE fabrication in this work since PVC is a durable polymer and expected not being easily damaged. To obtain a good SPCE unit fabricated with the painting technique, an optimum ink formula (20% w/w) was employed, a mixture of graphite powder and polystyrene as a binder polymer with dichloromethane as solvent. Although polystyrene is a non-conductive polymer and expected to reduce ink conductivity, this polymer is beneficial to use as a hydrophobic barrier that keeps the conductive ink sticking to the PVC surface. Meanwhile, dichloromethane is used as a solvent to prepare conductive inks because of its high volatility and dispersity. This is expected to facilitate rapid evaporation during SPCE fabrication. Another research has explored the SPCE fabrication using the painting technique [9, 10, 19].

The fabricated SPCE was further modified with PEDOT:PSS to increase the electrode conductivity. PEDOT:PSS is a conductive polymer widely used to modify screen-printed electrodes due to its high stability and conductivity [5, 6, 7, 8, 9, 10, 11, 12]. In this study, PEDOT:PSS was dissolved in a mixture of methanol and water at a concentration of 0.2 mg/mL. The modification process of SPCE was performed by a drop-casting technique using the solution of 5 μL PEDOT:PSS in a concentration of 0.2 mg/mL. The characteristics of surface morphology of the fabricated SPCE and SPCE-PEDOT:PSS obtained from electron microscope images were shown in Figure 2.

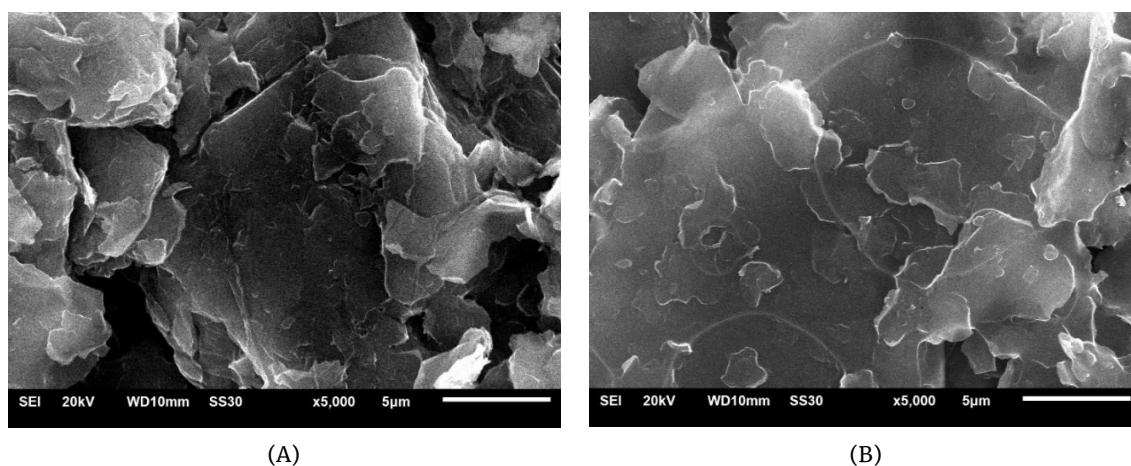


Figure 2. SEM image of (A) fabricated SPCE, (B) fabricated SPCE-PEDOT:PSS

In Figure 2A, the fabricated SPCE was observed having a surface covered with graphite flakes with diverse sizes in the range of $<20\ \mu\text{m}$. Among the graphite particles under a microscopic image, small cavities were observed, which provide a rough morphology with a large surface area. Figure 2B shows the surface morphology of SPCE modified with PEDOT:PSS, and this conductive polymer's presence leads to the formation of a thin film on the electrode surface. In our work, only $1\ \mu\text{g}$ of PEDOT:PSS was added onto the SPCE surface. Therefore, the thin film on the electrode surface is not clearly observed. However, another research has reported a conductive polymer's obvious thin film on their electrode surface [11, 20]. In addition to the analysis of surface morphology using SEM, Energy Dispersive X-Ray Spectroscopy (EDX) was also conducted to determine the element compositions in the surface of both SPCE and SPCE-PEDOT:PSS electrodes. However, the results did not show the presence of sulfur and oxygen from PEDOT:PSS. This is presumably due to the ratio of these elements is very small compared to carbon as the main constituent of the SPCE electrode. According to the literature, PEDOT:PSS consists of layers of flattened PEDOT-rich particles separated by quasi-continuous PSS lamella [21].

3.2. Electrochemical Performance of SPCE-PEDOT:PSS

The electrochemical performance of fabricated SPCE and SPCE-PEDOT:PSS was evaluated using $1\ \text{mM}$

$\text{K}_3\text{Fe}(\text{CN})_6$ solution in $0.1\ \text{M}$ KCl. Figure 3A shows the comparison of cyclic voltammograms of $1\ \text{mM}$ $\text{K}_3\text{Fe}(\text{CN})_6$ on commercial SPCE (i), fabricated SPCE (ii), and SPCE-PEDOT:PSS (iii). The oxidation and reduction currents of iron ions in $1\ \text{mM}$ $\text{K}_3\text{Fe}(\text{CN})_6$ solution at fabricated SPCE are 1.2 times higher than commercial SPCE. This is presumably due to the actual electroactive area of fabricated SPCE is higher than commercially available SPCE. Furthermore, the iron redox process's current intensity in $1\ \text{mM}$ $\text{K}_3\text{Fe}(\text{CN})_6$ solution at SPCE-PEDOT:PSS was 2.2 times greater than that of commercially available SPCE. PEDOT:PSS as a conductive polymer on the electrode surface increases the conductivity of the electrode. Modification of PEDOT:PSS towards SPCE was reported previously to increase the electrode's conductivity [5, 10]. The ratio of anodic and cathodic currents (I_{pa}/I_{pc}) in the SPCE-PEDOT:PSS measurement is close to 1, indicating a fast electron transfer rate between $\text{Fe}^{2+}/\text{Fe}^{3+}$ systems. Meanwhile, the ΔE_p obtained from an anodic and cathodic peak at commercial SPCE, fabricated SPCE, and SPCE-PEDOT:PSS implies that the presence of a quasi-reversible system since the value of ΔE_p are higher than $59/n\ \text{mV}$, which is considered as ΔE_p theoretical value for an ideal electron transfer.

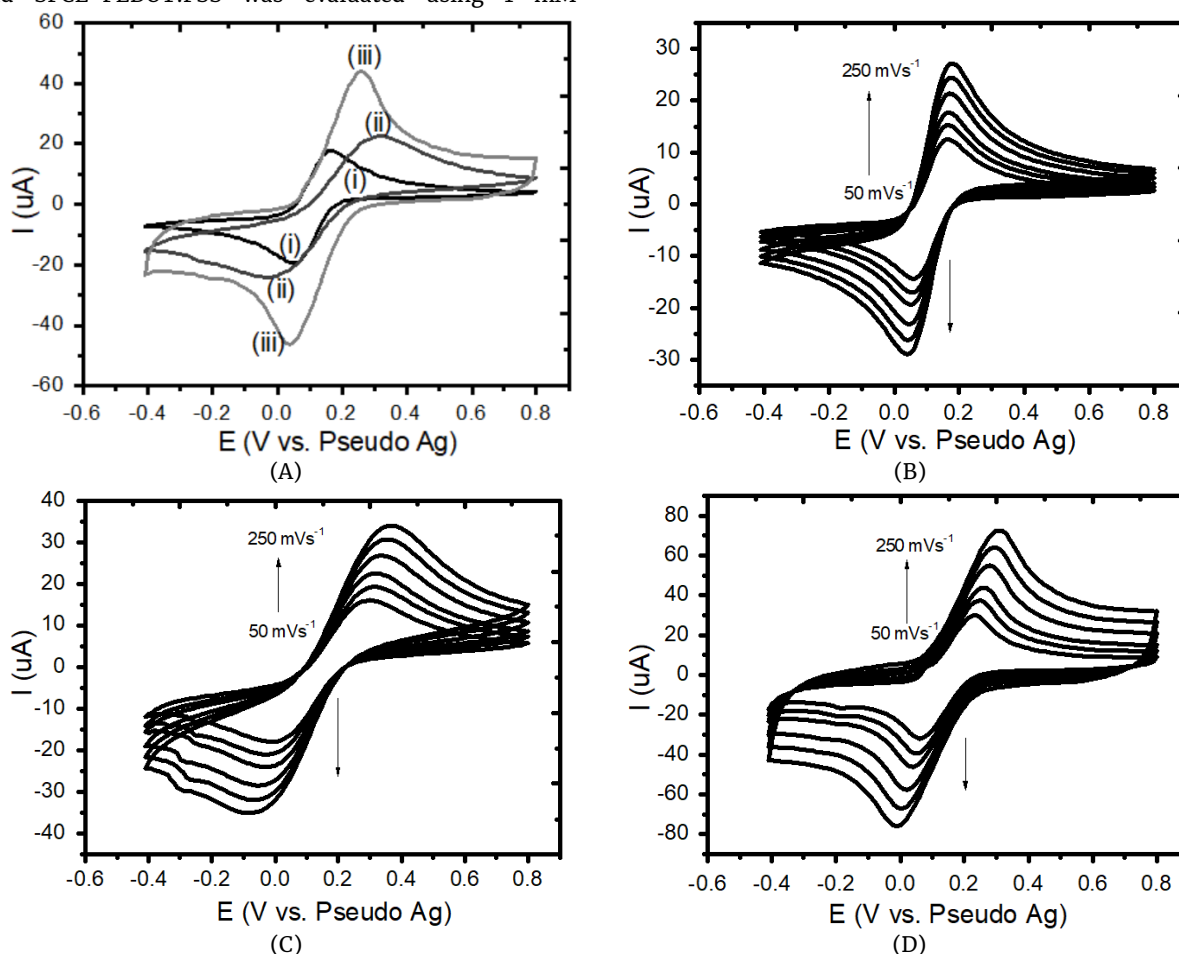


Figure 3. (A) Cyclic voltammogram of $\text{K}_3\text{Fe}(\text{CN})_6$ $1\ \text{mM}$ in KCl $0.1\ \text{M}$ at commercial SPCE (i), fabricated SPCE (ii), and fabricated SPCE+PEDOT:PSS (iii), Cyclic voltammogram of $\text{K}_3\text{Fe}(\text{CN})_6$ $1\ \text{mM}$ in KCl $0.1\ \text{M}$ at scan rate of $50, 75, 100, 150, 200,$ and $250\ \text{mVs}^{-1}$ using (B) commercial available SPCE, (C) fabricated SPCE, (D) fabricated SPCE-PEDOT:PSS

The effect of scan rate on cyclic voltammogram of commercial SPCE fabricated SPCE and SPCE-PEDOT:PSS was also evaluated using 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl. Both the I_{pa} and I_{pc} are increasing as the scan rate was increased from 50 to 250 $mV s^{-1}$. Also, the E_{pa} shifted to more positive potentials while the E_{pc} moved to more negative potentials when the scan rate increased both of commercial SPCE and modified SPCE (Figure 3B-3D). This potential shift was more significant in fabricated SPCE than commercial SPCE. This is an indication of quasi-reversible electrochemical reaction processes. In a further study, the electrochemical data was extracted from the linear correlation of the square root of scan rate (x-axis) and current (y-axis). The linear regression equation is employed to calculate the actual electrochemically active area of the SPCE using Randles-Sevcik equation (2). F is the Faraday constant, R is the gas constant, A is the effective area of the electrode (cm^2), T is the absolute temperature, i_p is the peak current (A), D is the diffusion coefficient of $[Fe(CN)_6]^{3-}$ ($7.60 \times 10^{-6} cm^2 s^{-1}$), n is the number of transferred electrons in a redox reaction, ν is the scan rate ($V s^{-1}$), and C is the concentration of the solution ($mol cm^{-3}$). Based on the equation, we calculated that electrochemically active areas for commercial SPCE, fabricated SPCE, and SPCE-PEDOT:PSS are 0.764 cm^2 , 0.778 cm^2 , 1.82 cm^2 , respectively.

$$i_p = 0.4463nF(nF/RT)^{1/2}AD^{1/2}\nu^{1/2}C \quad (2)$$

3.3. Electrochemical Performance of SPCE-PEDOT:PSS for Uric Acid Measurement

The electrochemical response of fabricated SPCE and SPCE-PEDOT:PSS in uric acid measurement was evaluated by measuring uric acid solution. Uric acid is a weak acid as pK_{a1} 5.5 and pK_{a2} 10.3 [22]. The pK_{a1} is responsible for converting uric acid to the more soluble anionic salt in human urine which its pH average is 5.9. Figure 4 shows a cyclic voltammogram of 0.1 mM uric acid at commercial SPCE (line a), fabricated SPCE (line b), and fabricated SPCE-PEDOT:PSS (line c). As shown in Figure 4, only an oxidation peak of uric acid was observed (following equation 3) at all working electrodes. This phenomenon indicated that uric acid's possible reaction going irreversible at the potential window applied in this study.

Figure 4 noted that the oxidation current intensity of 0.1 mM uric acid in the KCl electrolyte was higher on the fabricated electrodes (both fabricated SPCE and SPCE-PEDOT:PSS) than on the commercial SPCE. These results agree with the $K_3Fe(CN)_6$ observations (Figure 3A). A cyclic voltammogram of uric acid at SPCE-PEDOT:PSS was broad and revealed a high capacitive current produced at the working electrode's surface during the measurement. This was predicted due to the capacitive double layer created at the layer between SPCE and PEDOT:PSS film. The enhancement of the peak current during uric acid measurements is likely to be linked with an increase of surface area and roughness of porous PEDOT:PSS film on the electrode surface [23, 24]. This broad capacitive current was observed on oxidation and

reduction peaks of uric acid investigated with SPCE-PEDOT:PSS in Figure 4c. The impact of charging current could be eliminated by using differential pulse voltammetry technique which measures current twice during measurement, which leads to the elimination of charging current.

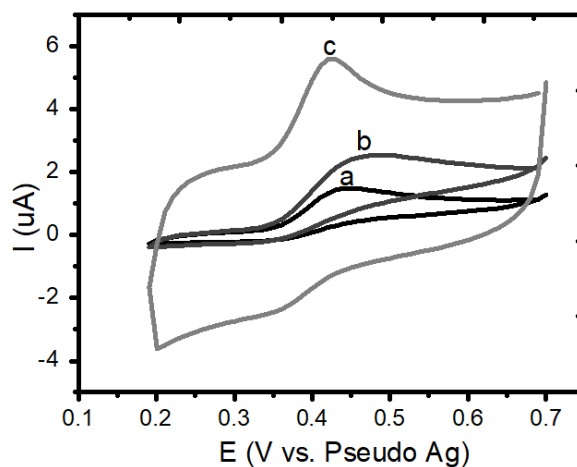
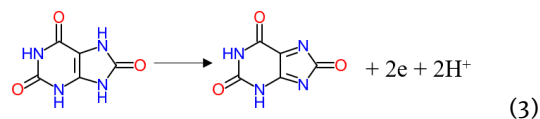


Figure 4. Cyclic voltammogram of 0.1 mM uric acid in KCl 0.1 M at commercial SPCE (a), fabricated SPCE (b) and fabricated SPCE+PEDOT:PSS (c)



The performance of SPCE-PEDOT:PSS in uric acid measurement at a concentration range of 20–100 μM was also evaluated using cyclic voltammetry, differential pulse voltammetry, and amperometry. Cyclic voltammetry is infrequently used for quantitative analysis, and hence this technique is commonly employed for understanding redox processes. However, in this study, it was applied for uric acid measurement and compared to other voltammetric techniques. Differential pulse voltammetry was used in this electrochemical study since this technique provides higher sensitivity and superior in capacitive or background current elimination. This can be achieved by taking the current twice during measurement, before applying the pulse and at the end of the pulse during the potential scanning process. Meanwhile, this study's amperometry technique at +0.48 V vs. Ag/AgCl was expected to provide selectivity for uric acid measurement [25].

Figure 5 shows the voltammogram obtained from cyclic voltammetry, amperometry, and differential pulse voltammetry techniques, accompanied by a linear calibration curve of uric acid measurement from each technique. The result denoted that each technique provides a comparable result in uric acid measurement. The linearity of uric acid measurement is acceptable for all techniques. The precision of uric acid measurement is expressed as a percentage of relative standard deviation, which is less than 3%, sensitivity in the range of 0.015 to 0.07 $\mu A/\mu M$. Simultaneously, the limit of detection and limit of quantitation are in the range of 1.14 to 1.16 μM and 3.81 to 5.38 μM , respectively (Table 1).

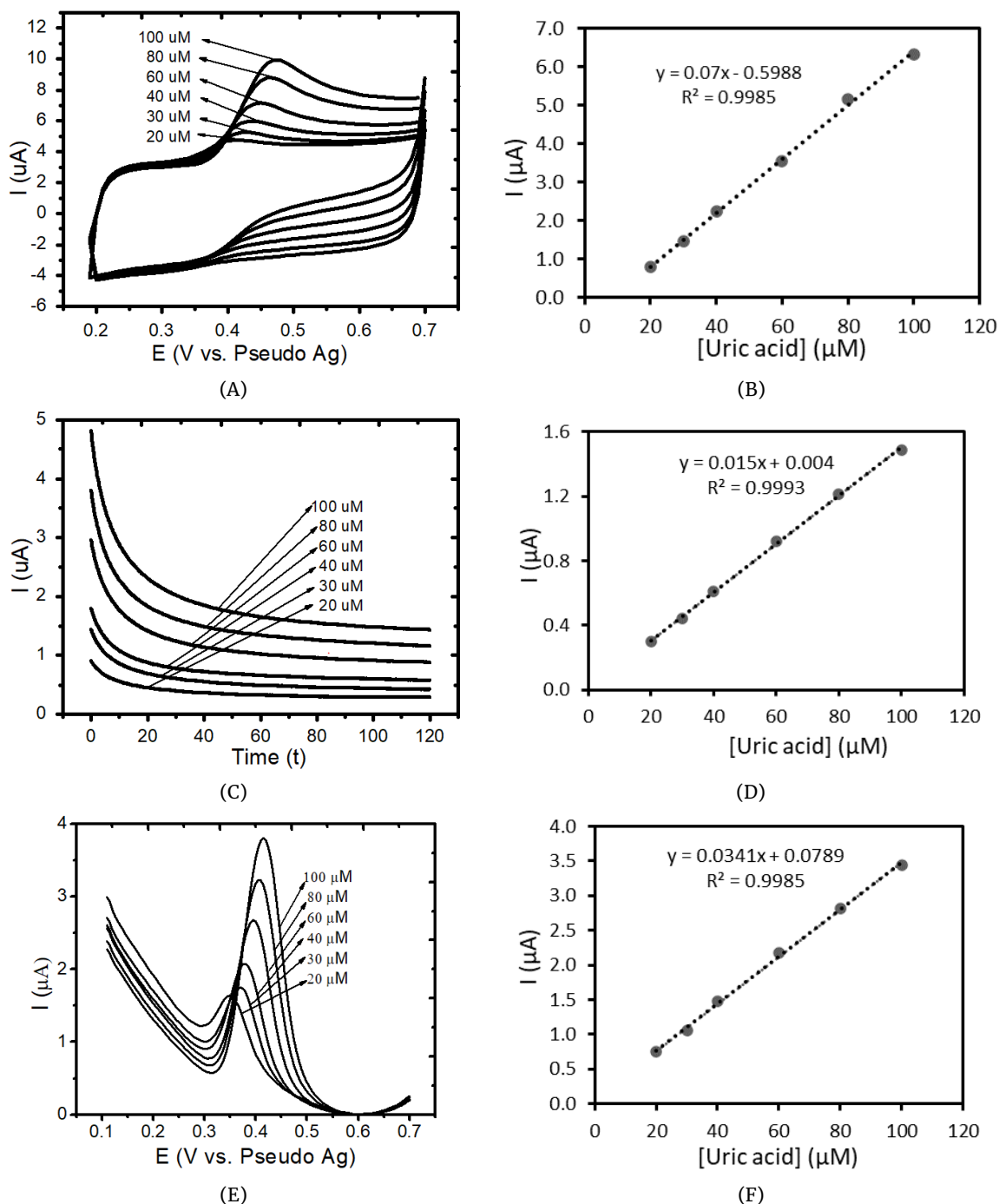


Figure 5. Response from a measurement of various concentration of uric acid in KCl 0.1 M at fabricated SPCE+PEDOT:PSS using different measurement techniques (A) cyclic voltammetry, (C) amperometry, and (E) differential pulse voltammetry and linear correlation between uric acid concentration and oxidation current of uric acid at fabricated SPCE+PEDOT:PSS using different measurement techniques (B) cyclic voltammetry, (D) amperometry, and (F) differential pulse voltammetry

As a comparison, the SPCE fabricated using a similar technique provides a detection limit of 1.94 μM in uric acid detection [10]. SPCE incorporated the catalyst cobalt phthalocyanine provides a detection limit of 15 μM [25]. Other reports by Jirakunakorn *et al.* [26] have developed uricase on porous cryogel (cry) platform of graphene-incorporated chitosan (Chi) on top of a Prussian blue layer (PB) electrodeposited on a screen-printed carbon electrode (Uricase/Chi-Gr cry/PB/SPCE)SPCE and obtained detection limit of 2.5 μM .

3.4. Performance of SPCE-PEDOT:PSS for Uric Acid Measurement in Human Urine

The performance of SPCE-PEDOT:PSS for uric acid measurement in a real sample was evaluated using a human urine sample. The evaluation was conducted using the multiple point standard addition method. The standard addition method was carried out by adding various volumes of 0.1 mM uric acid into 5 mL of a urine sample with 100 times dilution. Based on the calibration curve constructed from a volume of standard (x-axis) and current (y-axis), the concentration of an analyte in a urine sample was calculated following equation (1). The

concentration of uric acid in urine samples obtained were 5.84 mM, 5.74 mM, and 12.74 mM, from cyclic voltammetry, amperometry, and differential pulse voltammetry technique, respectively. Meanwhile, uric acid was excreted in human urine is 250–750 mg/day or 1.48–4.46 mmol/day in normal condition. If it is assumed that the volume of urine secreted by a human is 800–2000 mL, then the concentration of uric acid in human urine is in the range of 0.77–5.58 mM.

Table 1. Analytical performance of uric acid measurement using three different techniques at SPCE-PEDOT:PSS and at SPCE without modification taken from [10]

Electroanalytical technique	Limit of Detection (μM)	Limit of Quantitation (μM)	Precision (%RSD, n=6)	Sensitivity ($\mu\text{A}/\mu\text{M}$)
Cyclic voltammetry	1.61	5.37	2.70	0.070
Amperometry	1.14	3.81	2.89	0.015
Differential Pulse Voltammetry	1.62	5.39	2.40	0.034
Fabricated SPCE [10]	1.94	6.46	3.06	0.005

4. Conclusion

This work reports the fabrication of poly (3,4-ethylenedioxythiophene) polystyrene sulfonate modified screen-printed carbon electrode (SPCE-PEDOT:PSS) and its performance in uric acid measurement. Modification of SPCE with PEDOT:PSS led to an increase in electroanalytical performance of the SPCE and was predicted to provide a higher capacitive or charging current to the electrode. The fabricated SPCE-PEDOT:PSS shows comparable electroanalytical performance with the commercial SPCE. The SPCE-PEDOT:PSS shows good linearity ($R^2 = 0.9985, 0.9993, 0.9985$), sensitivity (0.070, 0.015, 0.024 $\mu\text{A}/\mu\text{M}$), high precision (%RSD = 2.70%, 2.89%, 2.40%), and low LOD (1.61 μM , 1.14 μM , 1.62 μM) and LOQ (5.37 μM , 3.81 μM to 5.39 μM) when evaluated with a uric acid standard solution respectively for cyclic voltammetry, amperometry, and differential pulse voltammetry techniques. This work successfully introduced an electrochemical sensing device using the disposable electrode made from graphite ink modified with a conductive polymer, which holds great promise for uric acid measurement to be applied in routine analysis.

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