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A Simple Approach to Fabricate a Screen-Printed Electrode and Its Application for Uric Acid Detection

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In this work, we present the development of a simple technique for the easy fabrication of a homemade screen-printed carbon electrode (SPCE) and its analytical application for uric acid detection. The homemade SPCE was fabricated using the painting technique on a polyvinyl chloride (PVC) paper substrate. The conductive ink for the homemade SPCE fabrication was prepared from a combination of graphite to polystyrene (at a 10:2 w/w ratio) for the working and counter electrodes, while the reference electrode consisted of silver and polystyrene (at a 10:2 w/w ratio). The field-emission scanning electron microscope image of the homemade SPCE shows that it has a rough surface, with the flake-like graphite perfectly covering the substrate of the PVC paper. The homemade SPCE provides a signal higher than the commercially available SPCE for electrochemical characterization using $K_3Fe(CN)_6$ and a uric acid solution. The application of the homemade SPCE for uric acid measurements exhibited a detection limit of 1.94 μM and a quantitation limit of 6.46 μM, with an excellent reproducibility (% relative standard deviation) of 3.06% and a sensitivity of 5 nA μM^{-1} ($R^2 = 0.997$) in the dynamic range of 10–80 μM. The performance of the homemade SPCE also showed good selectivity using ascorbic acid as an interference when its concentration was one-tenth that of uric acid and was evaluated using the amperometry technique. The uric acid in human urine was detected successfully using the homemade SPCE by the standard addition technique, indicating that the SPCE fabricated showed a potency that could be further developed for the electrochemical sensor.

Keyword: Electrode, fabrication, graphite, sensor, uric acid

1. INTRODUCTION

In contrast with many sophisticated instruments, electrochemical techniques have attracted a great deal of interest for the quantitative detection of various analytes. Therefore, detection using electrochemical techniques has offered several advantages during analysis, such as a quick response time, simplicity, a lower cost than other analytical methods, and excellent potential for miniaturization for portable applications [1]. Among electrochemical detection techniques, sensing tools using screen-printed electrodes (SPEs) are preferable over other types of electrodes because of the ease of fabrication and operation methods, simplicity of preparation, and capability of being fabricated from various materials. These conditions offer the possibility of the mass production of SPEs at low fabrication costs [2]. Additionally, SPEs are, in fact, the most extensive electrochemical sensors to be used for in-situ analysis because of their rapid and linear response, high sensitivity, low power consumption, and ability to work properly at room temperature [3]. These benefits make it possible for them to be used as disposable electrodes.

A planar SPE device consists typically of three electrodes: the working electrode (WE), the reference electrode (RE), and the counter or auxiliary electrode (CE/AE). SPE is printed on a solid substrate by stepwise ink deposition using screen-printing technology [4] or other processes, such as painting and drop casting. Various types of substrates, such as alumina, ceramic, glass, plastic, or polyethylene terephthalate, have been used for ink deposition [5]. Meanwhile, the selection of the ink composition for SPE fabrication depends on its final application. The ink for SPE fabrication consists mainly of a conductive material (e.g., graphite, graphene, metal), a polymer binder (e.g., cellulose acetate, polyaniline, polyvinyl chloride, polyvinyl ferrocene acrylamide, polystyrene, or a silicon binder) to improve its adhesion to the substrate, and solvents [6]. An increasing concentration of the polymeric binder at a certain point can lower the electron transfer process; this can be resolved with the incorporation of electroactive materials, such as noble metals or metal oxides [7]. The most popular ink materials are carbon-based materials, silver, and gold, of which carbon has better options, such as its low cost and easy modification, chemical inertness, and low background current [6]. The common carbon-based materials used as WEs are graphite [5], graphene [8], and carbon nanotubes [9]. The SPEs in which a carbon-based material is used as the WE are known as screen-printed carbon electrodes (SPCEs). While the CE may be produced using a material similar to the WE, the RE is composed mostly of silver [4]. The SPE fabrication technique offers a low-cost process and is suitable for large-scale production [10].

In addition to the substrate type and ink composition, the fabrication process includes printing and drying, followed by curing; these are all critical steps in the fabrication of SPEs, with a significant impact on the electrochemical behavior of the final electrodes. The universal form of SPE consists of a circular area represented by the WE, and in between, the area of the CE and the RE. In the SPE, the area of the CE is typically larger than that of the WE, with the smallest area being the RE, and the size of RE does not depend on the other electrodes [11].

In this paper, we introduce a simple technique by which to fabricate SPCEs and then investigate the electrode performances using the techniques of cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The homemade SPCE was also investigated using a uric acid standard solution and uric acid in a urine matrix as a preliminary study for future sensing applications. From these experiments, the homemade electrode showed a linear output for uric acid at the micromolar concentration scale and has the potential to be further improved as a simple technique to fabricate the SPCE.

2. EXPERIMENTAL

Materials and Instrumentation

All chemicals of pro analysis grade were used as received without purification, including graphite powder (particle size <20 µm) and dichloromethane (DCM) from Merck. The silver particles, polystyrene, $K_3Fe(CN)_6$, ascorbic acid, and uric acid were obtained from Sigma-Aldrich, deionized water, the paraffin block, polyvinyl chloride (PVC) paper, and the pattern sticker (Camel Glossy) for the SPCE template were obtained from the commercial SPCE from DropSens (ref. 110, DS SPCE). Instrumentations were used involving the potentiostat Palmsens Emstat3 (ES316U669) and the field emission scanning electron microscope, Thermo Scientific Quattro S.

Method

Formulation of Graphite Ink

The ink formula of the working electrode was made from a mixture of graphite powder and polystyrene at two different ratios of 10:2 and 10:4 (w/w). DCM was added to the mixture, with stirring for 2 min, to obtain a consistent graphite ink. The graphite ink was prepared at a concentration of 20% (w/w).

Formulation of Silver Ink (Ag)

The silver ink was formulated from a mixture of silver powder and polystyrene at a composition ratio of 10:2 (w/w). Then, DCM was added to the mixture, with stirring for 2 min, to obtain a consistent ink. The silver ink was obtained at a concentration of 20% (w/w).

Fabrication of the Homemade SPCE

The template for the homemade SPCE was designed using CorelDraw X7 software, with reference to the template of the commercially available SPCE from Methrohm (Figure 1). The template pattern was printed using a printer with a Camel glossy sticker paper produced by the cutting sticker technique.

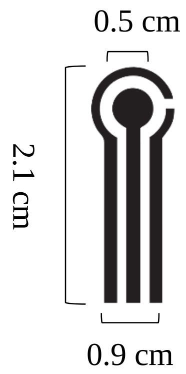


Figure 1. Pattern of the screen-printed carbon electrode on the sticker paper

The conductive ink was applied on the PVC paper substrate using a paintbrush. Initially, the PVC paper was cleaned with DCM prior to use. Then, the template of the SPCE was attached on top of the PVC paper substrate. Graphite ink was first applied onto the PVC paper substrate with a brush following the template of the SPCE. This step was repeated twice to obtain a homogeneous surface. Then, the SPCE was heated in the oven at 50°C for 5 min to obtain good adhesion between the graphite ink and the PVC substrate. Next, silver ink was applied to form the RE and dried for 5 min. Then, the template was peeled off the PVC paper substrate to obtain the SPCE. Paraffin wax was then used as an insulating layer on top of the homemade SPCE to give a fixed area of the electrodes. Finally, copper foil was attached to the RE, WE, and CE connection paths of the homemade SPCE.

Surface and Electrochemical Characterization of the Homemade SPCE

The homemade SPCE was characterized using a scanning electron microscope (SEM) to obtain the surface morphology characteristics. The electrochemical characterization of the homemade SPCE was performed using 1 mM $K_3Fe(CN)_6$ in the electrolyte of 0.1 M KCl using the CV technique; the potential range used was +0.8 V to –0.4 V, at a scan rate of 100 mV s^{-1}. The electrochemical performance of the homemade SPCE was then compared with that of the commercial SPCE. The stability of the SPCE was evaluated using the current signal from 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl using the CV technique. The measurement was performed in the potential range of +0.8 V to –0.4 V, at a scan rate of 100 mV s^{-1} for 50 cycles. Measurement of the $K_3Fe(CN)_6$ solution at different scan rates was also carried out with the homemade SPCE.

The performance of the homemade SPCE in electroanalysis was also evaluated for several parameters, including the linearity, precision, limit of detection (LOD), and limit of quantitation (LOQ) in the $K_3Fe(CN)_6$ solution. The linearity was evaluated using a calibration curve of the $K_3Fe(CN)_6$ concentration as the *x*-axis, with the current signal as the *y*-axis. The $K_3Fe(CN)_6$ solution was prepared at different concentrations of 20, 30, 40, 60, 80, and 100 µM in 0.1 M KCl. The measurement was repeated seven times. Precision was defined as a percentage of the relative standard deviation (% RSD) and calculated using the following equation:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(xi-\bar{x})}{n-1}} \qquad \ldots\ldots (1)$$

$\% \, RSD = \frac{SD}{\bar{x}} \; x \; 100\%$….. (2)

SD = standard deviation

RSD = relative standard deviation

xi = concentration of $K_3Fe(CN)_6$

$\bar{x}$ = the average of $K_3Fe(CN)_6$ concentration

n = number of repetitions (n = 7)

The LOD and LOQ were determined with reference to the ISO/IEC 17025. The calibration curve was determined from the oxidation and reduction currents of each cycle. The slope and standard deviation of the intercept obtained were then used to determine the LOD and LOQ limits. The equation was used as follows:

$$S(y/x) = \sqrt{\frac{(y-yi)2}{n-2}} \quad \text{….. (3)}$$

$$LoD = \frac{3 \; x \; S(y/x)}{Slope} \quad \text{….. (4)}$$

$$LoQ = \frac{10 \; x \; S(y/x)}{Slope} \quad \text{….. (5)}$$

S(y/x) = standard deviation

LoD = detection limit

LoQ = quantitation limit

Application of the Homemade SPCE for Uric Acid Detection

A 0.1-mM amount of uric acid solution in 0.1 M KCl was used to evaluate the homemade SPCE using the CV technique. The measurement was carried out in a potential window of +0.2 to +0.7 V, at a scan rate of 100 mV s^{-1} using the homemade SPCE. The measurement result was then compared with that of the commercial SPCE.

The linearity parameter was evaluated using the uric acid solution at concentration orders as follows: 10, 20, 30, 40, 60, and 80 µM in 0.1 M KCl. Each concentration was measured using the DPV technique in the potential window of +0.2 to +0.7 V, at a scan rate 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 measurement was repeated seven times, with its precision being defined as the % RSD. The LOD and LOQ were calculated with reference to the ISO/IEC 17025.

The performance of the homemade SPCE in measuring uric acid in the presence of ascorbic acid as an interferent was also evaluated. An amount of 0.2 µmol of uric acid was added to 20 mL of 0.1 M KCl, every 50 s. Ascorbic acid, at a concentration of 0.02 µmol, was added to the solution to evaluate the selectivity of the measurement. The measurement was carried out using the amperometry technique at a potential of 0.5 V *vs*. the pseudo Ag RE.

The homemade SPCE was applied to detect uric acid in the urine sample. The urine sample was diluted 100 times; then, 200 and 400 µL of the 0.1 mM uric acid solution were added to the urine sample solution. The sample solution and the standard addition sample were then analyzed using the DPV technique in the potential range of +0.2 to +0.7 V, at a scan rate 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 concentration of the uric acid in the urine sample was then calculated using the following equation [12]:

$$\frac{S_{samp}}{C_A} = \frac{S_{spike}}{C_A\left({V_0}/_{(V_0+V_S)}\right)+C_S\left({V_S}/_{(V_0+V_S)}\right)} \text{ ….. (6)}$$

where S_{samp} is the signal of the sample, S_{spike} is the signal of the spike, C_A is the analyte concentration in th
C_S and V_S are the concentration and volume of the standard, respectively, and V_0 is the volume of the sampl

3. RESULTS AND DISCUSSION

Conductive Ink Formula

The ink formula for the SPCE used in this research consisted of graphite as a conductive material, with a particle size of less than 20 µm, polystyrene as a binder, and DCM as a solvent. The composition ratio between the binder and the solvent must be optimized as polystyrene is a nonconductive material. Based on previous research, the conductive ink for the homemade SPCE was fabricated using two different ratio compositions of graphite and polystyrene: 10:4 and 10:2 (w/w). Also, the conductive ink was suspended with DCM as a solvent, resulting in a 20% w/w conductive ink. The ink obtained from both compositions was a black colloidal solution, and it was stirred constantly to ensure its homogeneity before it was used in the fabrication of the homemade SPCE. As it evaporates easily, DCM was chosen as a solvent and was able to dissolve the polystyrene to form a homogeneous suspension with the graphite ink.

Fabrication of the Homemade SPCE

The homemade SPCE was fabricated by applying formulated carbon and silver ink on a PVC paper substrate. The conductive ink was applied on the PVC paper with a painting technique using a small brush and a template, as shown in Figure 1. The painting of the conductive ink onto the PVC paper was a crucial step and determined the quality of the homemade SPCE [11]. The template on top of the PVC paper substrate needed to be fully cast homogeneously with the conductive ink to ensure the reproducibility of its fabrication. Carneiro and co-workers [11] showed that increasing the number of the casting process from one to two layers of conductive ink in the fabrication of the homemade SPCE yielded a significant increase of the current intensity. This resulted in the Δ*Ep* of the oxidation and reduction peaks in the cyclic voltammogram becoming narrower. In this work, the number of conductive layers in the SPCE fabrication was a two-layer deposition.

The scheme of the homemade SPCE fabrication process is illustrated in Figure 2. Based on this figure, the conductive ink was applied smoothly to the surface of the PVC paper substrate using a brush. The quality of the homemade SPCE could be checked when the fabricated SPCE was immersed or dripped with the sample solution. If the conductive ink did not dissolve in the solution, then the homemade SPCE could be used for electrochemical measurements. In addition, a nonpolar component (polystyrene) in the conductive ink helped to maintain the adhesion of the ink onto the PVC paper, and prevented the graphite and silver ink from dissolving in the water-based solution.

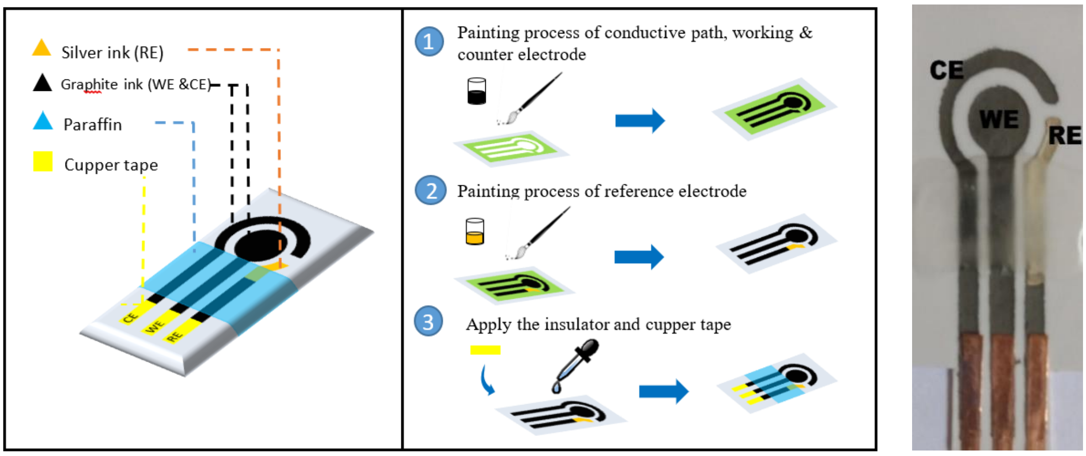


Figure 2. (A) The fabrication process of the homemade SPCE with a painting technique; (B) the result of the homemade SPCE with a painting technique.

Surface Characterization and Electrochemical Performance of the Homemade SPCE

To investigate the surface morphology, a SEM was used to obtain a photomicrograph of the homemade SPCE (graphite and polystyrene at a ratio of 10:2 w/w) and compared with the commercially available SPCE (Figures 3A and 3B). From Figure 3 shows clearly that the surface morphology of the homemade SPCE has a flake-like shape, with a particle size less than 20 µm. The homemade SPCE exhibited a rough surface and was expected to provide a higher actual electrochemically active area for the electron transfer process. Presumably, the electrochemically active area of the homemade SPCE is higher than that of the commercial SPCE and, thus, will enhance the electrochemical response during measurement.

Before being used in the electrochemical investigation, the homemade SPCE was treated to improve its electrochemical performance by yielding reproducible and reliable measurements. This surface pretreatment is fundamental to improving the activity of the homemade SPCE by increasing the roughness of the morphology and removing the contaminants at the electrode’s surface. There are various steps to improve the WE’s activity, such as mechanical, chemical, and physical treatment. However, in this research, we performed the pretreatment steps using an electrochemical method by applying 30 cycles of CV using 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl. In the first cycle, as shown in Figure 3C, line a, two small peaks corresponding to the contaminants (indicated by arrows) were initially detected; after 30 cycles, these peaks disappeared (Figure 3C, line b). In addition, after 30 cycles of the pretreatment process, the peak intensity of the oxidation and reduction currents increased than those of the first cycle in the cyclic voltammogram. According to Carneiro and co-workers [11], an increase in the number of pretreatment cycles leads to an increase in the current signals for both oxidation and reduction, as evidenced by the cyclic voltammogram of the homemade SPCE in Figure 3C.

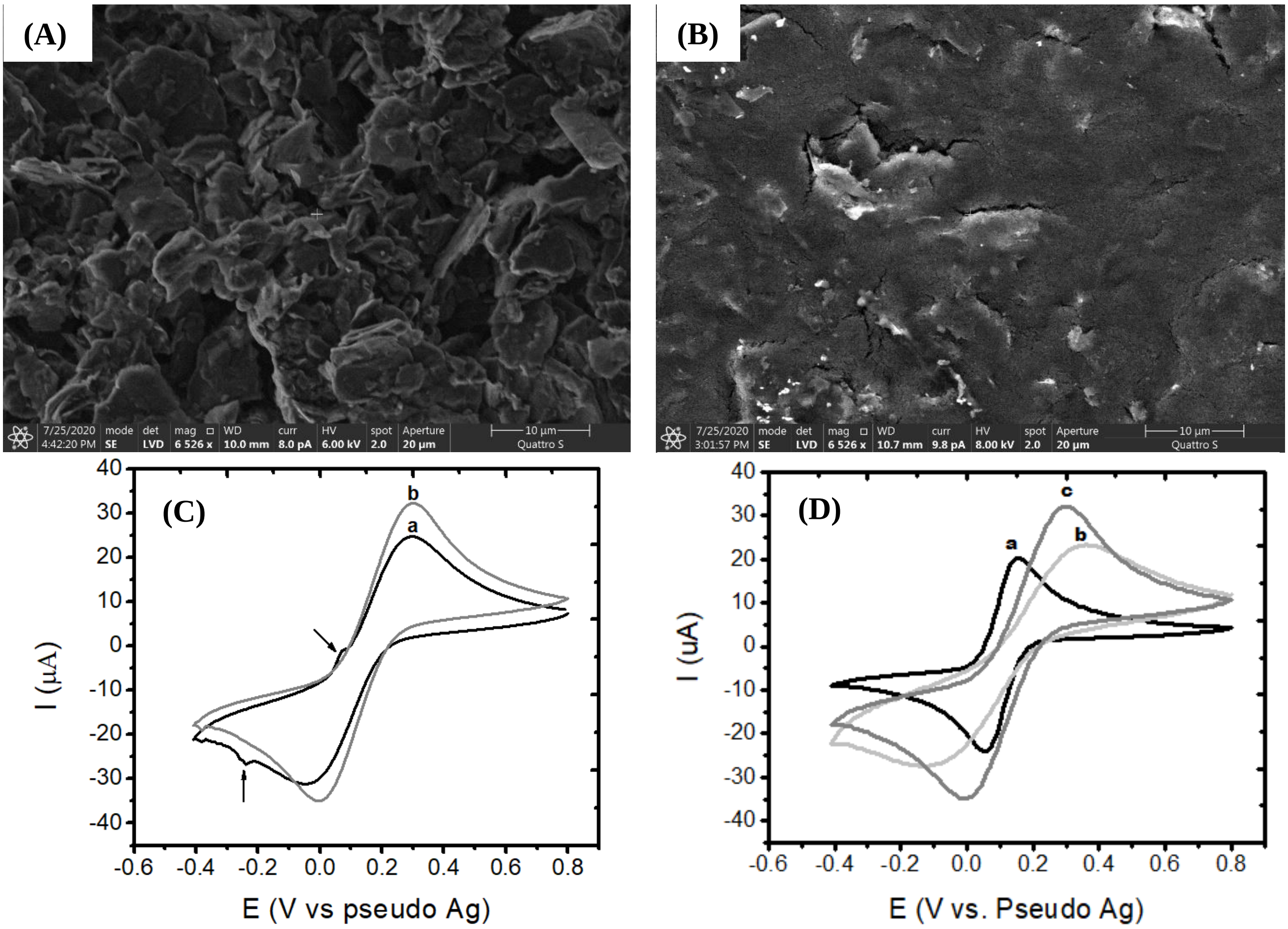


Figure 3. (A) SEM image of the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio; (B) SEM image of the commercial SPCE; (C) cyclic voltammogram of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl (scan rate, 100 mVs^{-1}) at the homemade SPCE before surface treatment (a) and after surface treatment (b); and (D) CV of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl (scan rate, 100 mVs^{-1}) at the commercial SPCE (a), the homemade SPCE with the 10:4 (w/w) graphite-to-polystyrene ratio (b), and the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio (c).

Figure 3D shows the comparison of the electrochemical response from 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl measured with the homemade SPCE and the commercial SPCE. The result shows that the homemade SPCE with the graphite-to-polystyrene ratio of 10:2 (w/w) gives a current signal higher than the ratio at 10:4 (w/w). This result indicates that the increasing amount of polystyrene as a nonconductive material in the ink composition leads to a decrease in electrode conductivity. Moreover, the SPCE with the graphite-to-polystyrene ratio of 10:2 (w/w) gives a higher intensity to the oxidation and reduction peak currents and a higher current density than the commercial SPCE. Presumably, this is due to the rough surface of the homemade SPCE affording a higher actual electroactive area, leading to a higher peak of the oxidation and reduction currents.

When investigating the redox system of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl using the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio, the ratio of the anodic to cathodic current (I_{pa}/I_{pc}) was almost 1. This indicates a faster electron transfer rate within the Fe^{2+}/Fe^{3+} system. By contrast, the homemade SPCE with the 10:4 (w/w) graphite-to-polystyrene ratio showed an I_{pa}/I_{pc} lower than 1, indicating a slower electron transfer process. Additionally, the final ΔE_p suggests the presence of a quasi-reversible system for both the homemade SPCE and the commercial SPCE as the values were higher than 59/n mV, which is considered to be the ΔE_p theoretical value for an ideal electron transfer.

Overall, the electrochemical performance of the three homemade SPCEs gave almost identical values in terms of E_{pa} and E_{pc} and I_{pa} and I_{pc}. The durability of the electrode was evaluated by applying 50 cycles of CV in 1 mM $K_3Fe(CN)_6$ solution. The result showed that the % RSD for the oxidation peak was 4.50% and for the reduction peak was 2.67%. This indicated that the homemade SPCE provided a durable electrochemical response.

Next, the scan-rate effect on the cyclic voltammogram of the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio in 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl is shown in Figure 4A. Clearly, when the scan rate was increased from 50 to 250 mV s^{-1}, both the I_{pa} and I_{pc} increased. Also, when the scan rate increased, the E_{pa} shifted to more positive potentials, while the E_{pc} moved to more negative potentials. This is an indication of quasi-reversible electrochemical reaction processes that are fast, reversible, and have a good Nernstian behavior [13]. Figure 4B also shows that both the anodic and cathodic peak currents increased linearly with the square root of the scan rate. This result indicated that the electrochemical reaction on the surface of the homemade SPCE was controlled by the diffusion process.

In a further study, CV was employed to plot the calibration curve from the measurement of the $K_3Fe(CN)_6$ solution in different concentrations at the homemade SPCE under the optimum conditions. The results in Figure 4C show that the oxidation and reduction peaks increased linearly with the increasing concentration of $K_3Fe(CN)_6$ from 20 to 100 µM. As shown in Figure 4D, two linear calibrations were derived from the anodic and cathodic peak currents with the $K_3Fe(CN)_6$ concentration (20–100 µM) as the equations of I_{pa} (µA) = 0.0248C – 0.3446 ($R^2 = 0.9962$) and I_{pc} (µA) = −0.0294C − 0.3546 ($R^2 = 0.9989$), respectively. Overall, this is a positive result regarding the homemade SPCE being well suited and able to produce a quantitative response at a certain concentration of analyte within µM.

Based on the results obtained from the variations in the scan rate in the measurement of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl, further electrochemical data can be extracted and used to predict the actual electrochemically active area of the homemade SPCE using Randles–Sevcik equation (7). From this equation, the electrochemically active area for the homemade SPCE can be calculated as 0.988 cm^2.

$$i_p = 0.4463nF(nF/RT)^{1/2}AD^{1/2}v^{1/2}C \qquad \ldots\ldots (7)$$

where i_p is the peak current (A), n (=1) is the number of transferred electrons, F is the Faraday constant, R is the gas constant, T is the absolute temperature, D is the diffusion coefficient of $[Fe(CN)6]^{3-}$ (7.60×10^{-6} cm^2s^{-1}), A is the effective area of the electrode (cm^2), v is the scan rate (Vs^{-1}), and C is the concentration of the solution (mol cm^{-3}).

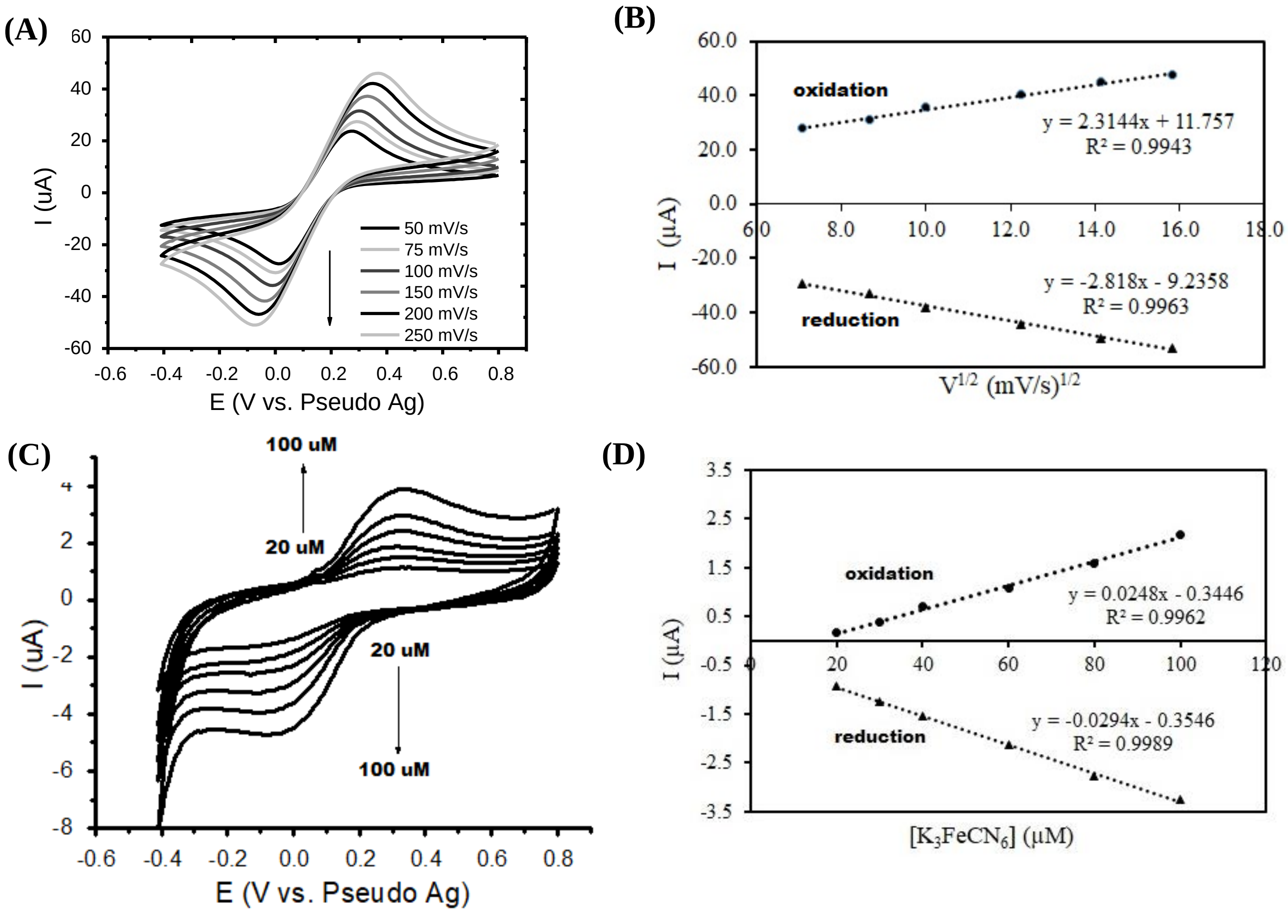
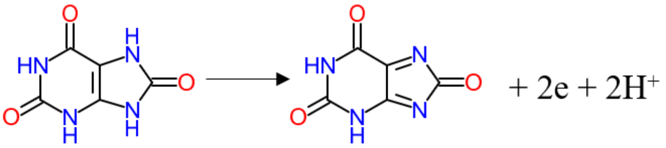


Figure 4. (A) Cyclic voltammogram of 1 mM $K_3Fe(CN)_6$ in 0.1 M KCl at the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio, measured at various scan rates; (B) linear correlation between the square root of the scan rate with the oxidation and reduction peak currents; (C) cyclic voltammogram of different concentrations of $K_3Fe(CN)_6$ in 0.1 M KCl (scan rate, 100 mV s^{-1}) at the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio; and (D) linear correlation between the concentration of the $K_3Fe(CN)_6$ solution in 0.1 M KCl with the oxidation and reduction peak currents.

Application of the Homemade SPCE for Uric Acid Detection

The usefulness of the electrochemical sensing platform based on the fabricated SPCE was tested by the determination of uric acid. Uric acid is one of the main side products in the human urine nucleoside metabolism. A high uric acid concentration in human fluid correlates with some diseases, such as metabolic syndrome and risk factors for cardiovascular diseases [14−16]. If the uric acid concentration in blood is ≥7 mg/dL for men and ≥6.0 mg/dL for women, this condition is described as hyperuricemia [17], whereas a uric acid concentration in urine exceeding 750 mg/day in women and 800 mg/day in men can cause hyperuricosuria [18]. Uric acid levels must be checked routinely to ensure that its concentration is within the normal levels.

To evaluate its electrochemical performance for practical applications, the homemade SPCE was used to measure uric acid based on its oxidation reaction in 0.1 M KCl (pH ~ 6.5–6.9) as an electrolyte solution. Uric acid is a weak acid as pKa1 5.5 [19], with better solubility in neutral and alkaline conditions than in acidic conditions. As shown in Figure 5A, only an oxidation peak of uric acid was observed on both the homemade and commercial SPCE; the oxidation reaction of uric acid followed Equation 8. In addition, the oxidation current intensity of 0.1 mM uric acid in the KCl electrolyte was found to be higher on the homemade SPCE (Figure 5A, line c) than on the commercial SPCE (Figure 5A, line b). These results are also consistent with the $K_3Fe(CN)_6$ observations. This was predicted because the actual electrochemically active area in the homemade SPCE was higher than that in the commercial SPCE.



….. (8)

Uric acid at the fabricated SPCE was measured using DPV, as presented in Figure 5B. The goal of using the DPV technique is to increase the sensitivity and electrochemical response in the uric acid measurement. DPV experiments were performed using the homemade SPCE at the 10:2 (w/w) graphite-to-polystyrene ratio in 0.1 M KCl containing different individual concentrations of uric acid (Figure 5C). The results showed that the oxidation peak current of uric acid at the homemade SPCE increased linearly over the range of 10–80 µM, with a % RSD of 1.47–4.22% and a precision average of 3.06%. The LOD and LOQ values were 0.325 and 1.085 mg L^{-1} (1.94×10^{-6} and $6.46\ 10^{-6}$ mol L^{-1}), respectively. The results obtained from this study are comparable with those from previous reports, as listed in Table 1.

Table 1. The screen-printed electrode and its performance in uric acid measurements

Electrode	Linear Range	Detection Limit	Reference
Polyacrylic acid-modified multi-wall carbon nanotubes screen-printed carbon electrode	0–30 µM	0.458 µM	[20]
Polycarbonate-uricase-cellulose acetate-cobalt phthalocyanine-screen-printed carbon electrode	15–250 µM	15 µM	[21]
Screen-printed carbon electrode-graphene oxide nanoribbons+poly(3,4-ethylenedioxythiophene):polystyrene sulfonate	0.5–200 µM	11 nM	[22]
Reduced graphene-screen-printed carbon electrode	10–3000 µM	0.1 µM	[23]
Screen-printed carbon electrode based on multi-wall carbon nanotubes modified with uricase	5–1000 µM	0.33 µM	[24]
Screen-printed carbon electrode based on graphene quantum dots and ionic liquid	0.5–20 µM	0.03 µM	[25]
Uricase/Chitosan-graphene cryogel/Prusian blue/screen printed carbon electrode	2.5–400 µM	2.5 µM	[26]
Graphite modified by Fe_3O_4@Au-Cys/PANI for screen-printed electrode	20–1000 µM	1.80 µM	[27]

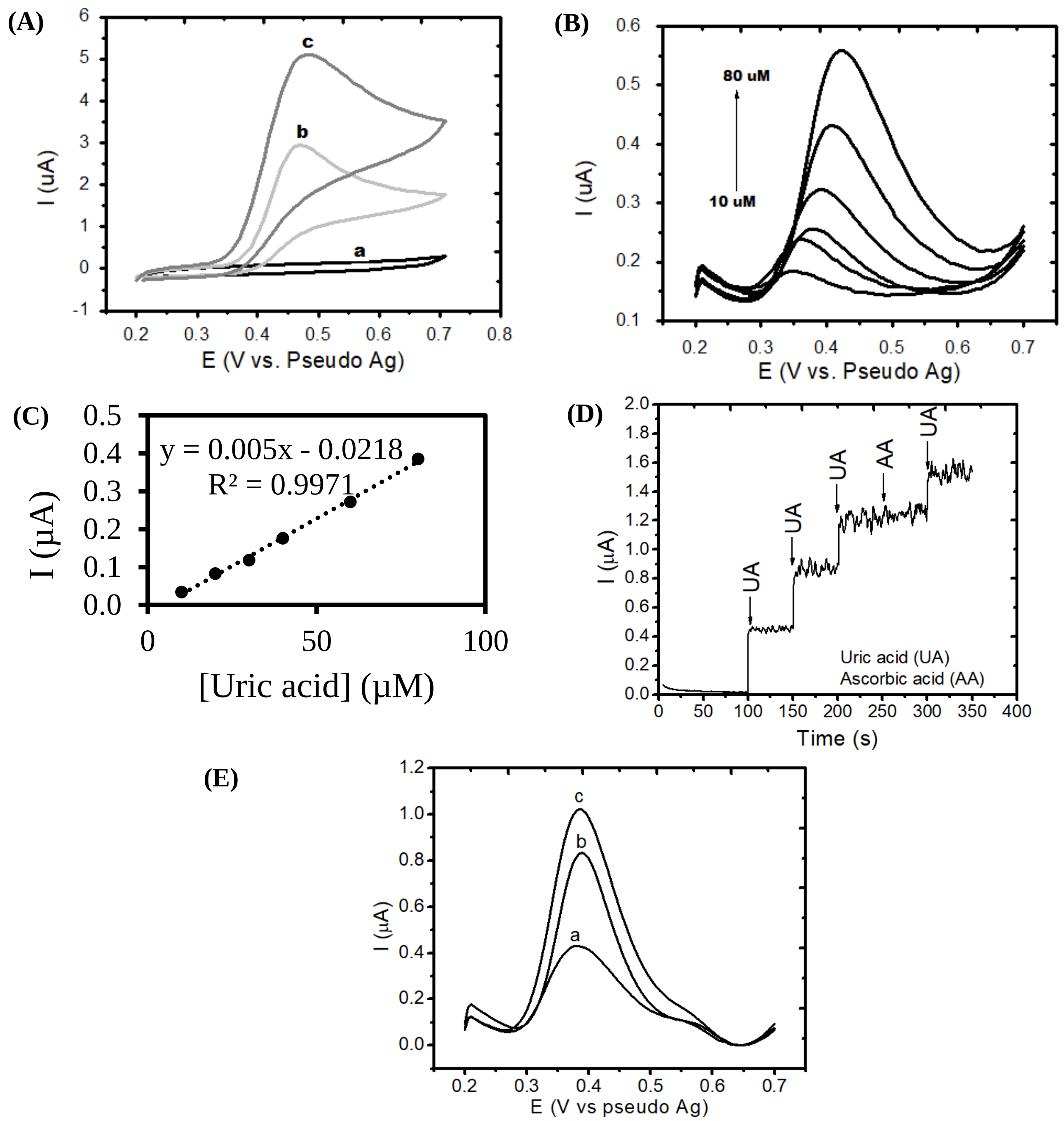


Figure 5. (A) Cyclic voltammogram at a scan rate of 100 mVs^{-1} of 0.1 M KCl at the homemade SPCE (a), 0.1 mM uric acid in 0.1 M KCl at the commercial SPCE (b), and 0.1 mM uric acid in 0.1 M KCl at the homemade SPCE with the 10:2 (w/w) graphite-to-polystyrene ratio (c); (B) differential pulse voltammogram of different concentrations of uric acid in 0.1 M KCl; (C) linear correlation between the concentration of uric acid in 0.1 M KCl with the oxidation peak current; (D) the amperometric response on the successive addition of 0.2 µmol of uric acid and 0.02 µmol of ascorbic acid into 20 mL of 0.1 M KCl; and (E) differential pulse voltammograms of urine diluted 100 times (a), sample (a) with the addition of 200 µL of 0.1 mM uric acid (b), and sample (a) with the addition of 400 µL of 0.1 mM uric acid (c).

The possible interference that may be present in a sample was investigated to evaluate the selectivity of the homemade SPCE in measuring uric acid. The coexistence of ascorbic acid and uric acid in a biological sample is common, and the oxidation potential of these overlaps greatly [28]. As shown in Figure 5D, the amperogram shows the determination of uric acid at a potential of 0.5 V *vs.* pseudo Ag with ascorbic acid as an interference. The results showed that the presence of ascorbic acid at one-tenth the concentration of uric acid did not cause significant interference during the uric acid determination.

Next, the homemade SPCE was used to measure the uric acid concentration in a human urine sample. To determine the uric acid concentration, the urine sample was diluted 100 times and the standard addition method was employed using 0.1 mM uric acid. Figure 5E shows the voltammograms from the urine sample with 100 times dilution (line a), the urine sample added to 200 µL of 0.1 mM uric acid (line b), and the urine sample added to 400 µL of 0.1 mM uric acid (line c). Based on the calculation using Equation 6, the uric acid concentrations in the human urine samples were obtained as 1.50 and 1.71 mM, respectively for standard addition of 200 µL and 400 µL of 0.1 mM uric acid. In the literature, the normal concentration of uric acid excreted in human urine is 250–750 mg/day. Assuming that the average volume of urine excreted by a human being is 800–2000 mL, then, the uric acid concentration in human urine was obtained as 0.744–5.58 mM. It can be summarized that the homemade SPCE developed in this research can be used to quantify the normal concentration of uric acid in human urine.

4. CONCLUSIONS

This work summarizes the procedure used to fabricate and characterize the easy preparation of a homemade SPCE, using simple and low-cost materials, to determine uric acid levels in synthetic and real samples. The homemade sensor shows a comparable performance with that of the commercial SPCE. This sensor also shows good linearity on the oxidation and reduction peak currents when they were evaluated with $K_3Fe(CN)_6$ in KCl 0.1 M. This sensor offers high stability over 50 consecutive measurements with % RSDs of 4.50% and 2.67% for the currents of both the oxidation and reduction peaks, respectively. In addition, the sensor has the advantages of easy preparation and can produce a quantitative response at a micromolar concentration range. The homemade sensor was used successfully to analyze uric acid with low LOD (1.94×10^{-6} mol L^{-1}) and LOQ ($6.46\ 10^{-6}$ mol L^{-1}) values, with excellent reproducibility and high sensitivity. Also, the homemade SPCE can be applied to quantify the uric acid concentration in real human urine with 100 times dilution, using ascorbic acid as an interferent. Therefore, the proposed technique to fabricate the homemade SPCE has the potential to be further improved for electrochemical sensors.

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