Development of graphene-chitosan composite modified screen-printed carbon electrode and its application for detection of rutin

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Abstract. Graphene is a prospective material for sensing devices due to its excellent electronic, thermal, and mechanical properties. In this study, low oxygen graphene was synthesized from graphite and nitronium ion using a microwave-assisted method. Graphene was dispersed into 0.05% chitosan solution and deposited by drop casting method on to screen-printed carbon electrode (SPCE) to form graphene-chitosan composite modified SPCE (SPCE-G-chit). The electrochemical performance of SPCE-G-chit was evaluated using flavonoid 3',4',5,7-tetrahydroxyflavone-3β-D-rutinoside (rutin). SPCE-G-chit provides linearity with $R^2$ of 0.9944 for rutin measurement in the concentration range of $5 \times 10^{-7}$ to $1 \times 10^{-5}$ M. Meanwhile, the linearity of rutin at SPCE was lower ($R^2$ 0.9396). Limit of detection (LOD) and limit of quantitation (LOQ) of rutin measurement at SPCE-G-chit was lower (0.23 µM and 0.58 µM, respectively) compare to SPCE (LOD = 0.69 µM and LOQ = 1.75 µM, consecutively). The accuracy of rutin measurement at SPCE-G-chit was 99.45% while the accuracy of rutin measurement at SPCE was 72.18%. The result indicated that SPCE-G-chit provides superior analytical performance than SPCE and potential for electrochemical sensor development.

1. Introduction
Graphene is two-dimensional sheets of sp2 hybridized carbon atom packed in a hexagonal lattice. This material currently grabs high interest due to its extraordinary thermal and electrical properties [1,2]. The synthesis of graphene could be performed through a top-down or bottom-up method. The graphene synthesis which involves the structural breakdown of graphite followed by the interlayer separation into graphene sheets is known as the top-down approach. Meanwhile, the bottom-up technique utilizes carbon source gas to synthesize graphene on a substrate. The Top-down approach is widely used in graphene synthesis. One of the promising top-down methods for graphene synthesis was reported by Chiu et al. [3]. This work reported a top-down method for highly conductive low-oxygen graphene. This technique provides advantages since it prevents excessive cutting of the graphene sheets into small pieces and prevents the generation of holes and vacancies in the basal plane.

Rutin (3',4',5,7-tetrahydroxyflavone-3β-D-rutinoside) is flavonoid compound with a wide range of physiological activities such as anti-oxidant [4], anti-inflammatory [5, 6], antidiabetic [7], antihypertension [8], and antiviral [9]. Measurement of rutin has been conducted by using spectrophotometry [10], high-performance liquid chromatography [11, 12], and capillary electrophoresis [13]. Those techniques provide a low limit of detection. However, the methods need long and complicated sample preparation, many chemical reagents, sophisticated instrumentation, and
high skilled analyst. The electrochemical technique could be an alternative method for rutin measurement since it could provide fast response, high sensitivity and selectivity, and satisfactory reliability. This technique also uses inexpensive, low energy consumption, and simple operation instruments.

This study developed the voltammetric method for rutin measurement at graphene-chitosan composite modified screen-printed carbon electrode (SPCE). Graphene was used to provide higher conductivity to the electrode. Meanwhile, chitosan expected to promote absorption enrichment of rutin to the electrode surface due to its hydroxyl active group. On the other hand, graphene has the strong molecular inter-atomic force and van der Waals force between its layers that makes graphene easy to agglomerate during storage. Composite of chitosan and graphene expected to prevent graphene agglomeration and could improve its performance in rutin measurement.

2. Experimental

2.1. Materials
Graphite (~20 μm in size, Sigma-Aldrich) was used for graphene synthesis. All the chemicals used in this work were in analytical grade, purchased from Merck. Electrochemical measurements were conducted using EDAQ potentiostat (Australia, New South Wales) equipped with graphene-chitosan modified Screen-Printed Carbon Electrode (SPCE, Dropsens DRP C110), the Dropsens SPCE consist of carbon as working and counter electrode and silver as a reference electrode. All solutions were prepared using ultrapure water with the resistivity of 18.2 Mohm.

2.2. Methods

2.2.1. Synthesis of Graphene. The synthesis of graphene was carried out using microwave heating as reported previously by Chiu et al. (2012) [3]. The amount of 20 mg of graphite powder was mixed with 10 mL of sulfuric acid: nitric acid solution (in a ratio of 1:1). The mixture was exposed with 300 W microwave irradiation for 30-60 s. The solution then neutralized to pH 7 using 20 mL of deionized water follow with 4 M KOH in an ice-bath. A colloidal graphene solution was separated from precipitated impurities and unexfoliated graphite powder using centrifugation at 4000 rpm (rotor radius of 10 cm) for 20 minutes. Then the colloidal graphene was washed using deionized water three times and dried prior to use.

2.2.2. Preparation of Chitosan-Graphene Composite. Chitosan was dissolved in acidified deionized water (pH 3.5, obtained by addition of acetic acid into water). As the dissolved chitosan obtained, the solution was brought to a pH of 5 by the addition of 0.1 M NaOH. The amount of 1.5 mg graphene was dissolved in 1 mL of chitosan solution. The homogeneous graphene-chitosan composite was obtained by sonication of the mixture for 5 hours.

2.2.3. Graphene-Chitosan Modified SPCE Fabrication. The amount of 4 μL of the graphene-chitosan composite solution was dropped on the SPCE surface (SPCE was activated in 0.1 M NaOH solution prior to modification), the SPCE then dried at 60 °C. Graphene-Chitosan modified SPCE (SPCE-G-Chit) and SPCE were characterized by scanning electron microscopy, SEM (ZEISS EVO MA10, Germany).

2.2.4. Electrochemical Measurement of Rutin. The rutin solution in a concentration of 5 x 10^{-7} M to 1 x 10^{-5} M was prepared in phosphate buffer as an electrolyte. The optimum pH of the electrolyte was investigated between pH of 3-8. Each solution was measured using differential pulse voltammetry at graphene-chitosan modified SPCE (SPCE-G-Chit). Analytical performance of SPCE-G-Chit for rutin measurement was also evaluated, including linearity, the limit of detection and quantitation, accuracy, and electrode stability.
3. Results and Discussion

3.1. Graphene-chitosan composite modified SPCE

Synthesis of graphene was conducted using nitronium ion generated from the reaction of H$_2$SO$_4$ with HNO$_3$ under microwave irradiation. In this synthesis technique, graphite was oxidized follow by exfoliation of graphite oxide to form graphene oxide. This process occurs in short duration (30 to 60 second) that prevents excessive cutting of the graphene sheets into small pieces and prevent the generation of holes and vacancies in the basal plane. In addition, the dispersion of graphene in the solution also increases by the heat from microwaves [3]. This process also generated graphene sheets with a smaller number of hydroxyl or epoxides. A dark grey graphene colloidal solution was obtained from synthesis using microwave assisted technique. A qualitative test using 0.05 M KMnO$_4$ proved that the colloidal solution was graphene solution by color changing from purple to brown (Figure 1A). UV-Vis spectrum of colloidal solution exhibited a $\lambda$ maximum at 272.5 nm (Figure 1B), indicated that graphene has successfully synthesized.

Figure 1. (A) Result for qualitative analysis of graphene: graphene (i), graphene with KMnO$_4$ (ii), graphene with KMnO$_4$ after 30 min (iii), KMnO$_4$ after 30 min (iv), (B) $\lambda$ maximum (272.5 nm) of UV spectrum of graphene solution, (C) SEM image of SPCE, (D) SEM image of deposited graphene-chitosan composite on SPCE.

Graphene-chitosan composite was obtained by the dispersion of graphene powder into a chitosan solution. Yin et al. reported that the positive charge of chitosan in an acidic atmosphere would interact electrostatically with a negative charge of graphene [14]. This leads to the blocking of interaction between graphene and preventing graphene aggregation. In line with this explanation, Han et al. reported that in graphene-chitosan composite, chitosan would act as dispersants and stabilizers [15]. Furthermore, Sayyar et al. reported that there are interactions such as hydrogen bonds between chitosan with hydroxyl or epoxides group oxide in graphene [16].
Modification of SPCE using graphene-chitosan composite was carried out by the drop-casting method. Carbon electrode at SPCE was activated by cyclic voltammetric treatment of the electrode in 0.1 M NaOH before modification [17]. This treatment was expected to remove the organic binder at the SPCE surface which would increase the activated area and provide functionalization of the electrode surface [17]. This treatment was intended to increase the immobilization of graphene-chitosan composite on the electrode surface.

The scanning electron microscopy profile showed that the SPCE surface was covered by graphene-chitosan composite. The present of graphene-chitosan at the surface of SPCE indicated by the rough surface of SPCE-G-Chit (Figure 1C and D). Agglomerate on the surface of SPCE predicted as chitosan, while the graphene was dispersed inside the chitosan.

3.2. Electrochemical response of rutin at SPCE-G-chit
The electrochemical measurement of rutin at SPCE and SPCE-G-Chit was carried out in phosphate buffer (PB) using differential pulse voltammetry technique. The electrochemical response of rutin in different pH of PB was investigated since its oxidation depends on the pH. Figure 2A showed that as pH of PB increase, the intensity of rutin peak current also increases and the oxidation potential tends to shift to a more negative potential. This result indicated that the oxidation of rutin tends to be more favorable at the alkaline condition. Oxidation of rutin is accompanied by the release of hydrogen ion (Figure 2B), this reaction expected to be more favorable in alkaline condition. However, in the alkaline condition, the amine functional group of chitosan will be deprotonated and loss of its positive charge. This condition lead to the release of graphene from the chitosan matrix and electrode surface. From this experiment, the author found that at pH 4 the electrochemical responses were more stable and the precision of measurement was higher. Based on that consideration, PB pH 4 was decided to use as an electrolyte in further measurements.

![Figure 2](image-url)

**Figure 2.** (A) Differential pulse voltammogram of rutin in phosphate buffer at different pH (3 to 8) with a scan rate of 100 mVs⁻¹, (B) Redox reaction of rutin [18].
3.3. Analytical performance of SPCE and SPCE-G-chit for rutin measurement

Measurement of rutin in different concentrations was conducted using PB pH 4 as the electrolyte. The peak current intensity of rutin oxidation was linearly increased as the concentration of rutin increased. Linear regression was obtained from the correlation between rutin concentration with the intensity of oxidation peak current, both at SPCE and SPCE-G-Chit. Figure 3 showed that the current intensity of rutin oxidation at SPCE-G-Chit was higher compared to the measurement at SPCE indicated that electron transfer at SPCE-G-chit was higher compared to SPCE. The determination coefficient and sensitivity of rutin measurement at SPCE-G-chit also higher compare to SPCE. The determination coefficient of rutin measurement at SPCE and SPCE-G-Chit respectively was 0.9396 and 0.9944. The result indicated that SPCE-G-chit provides better analytical performance than SPCE.

![Differential pulse voltammogram of rutin at different concentration at (A) SPCE, (B) SPCE-G-Chit (scan rate 100 mVs⁻¹).](image)

The detection limit (LOD) and the quantification limit (LOQ) of rutin measurement at SPCE were $5.8 \times 10^{-7}$ M and $17.5 \times 10^{-7}$ M, respectively. Meanwhile, LOD and LOQ of rutin measurement at SPCE-G-Chit consecutively were $2.3 \times 10^{-7}$ M and $6.9 \times 10^{-7}$ M. This result indicated that SPCE-G-Chit was more sensitive for rutin measurement. Other work by reported that LOD and LOQ for rutin measurement at SPCE were $1 \times 10^{-7}$ M and $3 \times 10^{-7}$ M, respectively [19].

Accuracy was investigated by measurements of $10^{-5}$ M rutin in 0.1 M PB pH 4, in 3 replicates both at SPCE and SPCE-G-Chit. The concentration of rutin then calculated based on peak current intensity and calibration curve. The accuracy was determined by comparing the real concentration and the concentration obtained from measurement. The % accuracy for SPCE and SPCE-G-kit respectively were 72.46% and 99.45%.

The stability of SPCE and SPCE-G-Chit was evaluated through measurements of 10-5 M rutin in 0.1 M PB pH 4. Measurements were conducted for 50 times consecutive measurement per day for a week. The result showed that both electrodes provide a stable response on the first day. Otherwise, the response was decrease and the electrode was damage after the second day, both for SPCE and SPCE-G-Chit. Measurement of rutin at SPCE provide the precision with % RSD of 3.38 after 40 measurements. While the SPCE-G-chit has moderate precision with %RSD of 4.17 after 34 measurements.

4. Conclusion

Low oxygen graphene was successfully synthesized from graphite and nitronium ion using microwave assisted technique. Dispersion of graphene in an acidic solution of chitosan produces a graphene-chitosan composite. The graphene-chitosan composite used for SPCE modification increase the electrochemical performance of the electrode in rutin measurement. The linearity and accuracy of rutin measurement at SPCE-G-Chit were higher compared to SPCE. Meanwhile, the detection limit (LOD) and the quantitation limit (LOQ) of rutin measurement at SPCE-G-chit was lower compare to SPCE.
The result indicated that in rutin measurement, SPCE-G-chit provides superior analytical performance compare to SPCE.

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