Graphene Modified Screen Printed Carbon Electrode for Voltammetric Detection of Glutathione as Oxidative Stress Biomarker

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Abstract. Glutathione is a natural antioxidant in human body and the depletion of its content commonly used as a marker for oxidative stress condition. In this study graphene modified screen printed carbon electrode (SPCE-G) was developed as working electrode for voltammetric detection of glutathione. Graphene was synthesized using microwave with nitronium ion, while deposition of graphene on to SPCE was performed by electrodeposition methods. Glutathione measurement was carried out by cyclic voltammetry using 1 mM NaBr solution in 0.2 M NaClO\textsubscript{4} as electrolyte. The result indicated that graphene was successfully synthesized and deposited on to SPCE. The voltammetric measurement of glutathione based on oxidation of bromide in NaClO\textsubscript{4} at SPCE-G provided a linear response with a detection limit (LOD) of 8.01 \textmu M and %RSD of 2.95%. Despite further optimization is required to improve its analytical performances, graphene modified SPCE seems to be potential for voltammetric detection of glutathione.

Keywords: Glutathione, graphene, oxidative stress, screen printed carbon electrode, voltammetric

1. Introduction
Glutathione (GSH) is an important antioxidant which plays a role in redox equilibrium [1, 2]. The depletion of GSH level in human blood associated with oxidative stress, a condition that triggers various diseases such as cancer and cardiovascular disease [3,4]. Detection of GSH level is important to monitor the early stage of oxidative stress. Several techniques for GSH detection were already reported, such as high performance liquid chromatography [5], fluorescence [6,7], and spectrophotometric [8]. Those techniques are sensitive, otherwise it needs several types of chemicals, sophisticated instrument, time consuming preparation step, and highly skilled analyst.

Electrochemical detections of GSH are potential to be developed since it provides low detection limit, simple preparation, and rapid analysis time [9]. Otherwise, GSH has a weak electrochemical signal. Therefore a working electrode with high sensitivity is required for GSH detection. Ru et al. (2013) developed ubiquinone modified nanocarbon electrode for GSH detection [10], Valero-Ruiz et al. (2015) conducted indirect GSH detection based on oxidation peak of bromide and iodide at
platinum electrode [11], and Pasakon et al. (2013) developed measurement of GSH using graphene-poly(3,4-ethylenedioxythiophene) poly(styrene-sulfonate) (GPPEDOT:PSS) modified screen printed carbon paste electrode [12].

Carbon-based electrodes are widely used as working electrode in electrochemical sensors due to low background current, high electrical conductivity, and wide potential range [13]. The carbon-based material commonly used as working electrode in electrochemical measurement include glassy carbon [14], carbon nanotubes, nanofibers, nanowires, and nanocoils [15], diamond [16], graphite pastes [13], and graphene [17]. Among those carbon-based materials, graphene is a promising material for electrode development due to its superior properties.

In this report, graphene modified screen printed carbon electrode (SPCE) was fabricated and applied in electrochemical measurement of glutathione. Graphene was expected to provide higher sensitivity in electrochemical detection of glutathione since it provides high current density, high charge carrier mobility, very low resistivity, inert surface, and high thermal conductivity [18].

2. Experimental

2.1 Materials

Graphite (~20 µm in size) for graphene synthesis was obtained from Sigma-Aldrich, reduced glutathione was purchased from Sigma-Aldrich, while other chemicals were obtained from Merck. Electrochemical measurements were conducted using EDAQ potentiostat (Australia, New South Wales) with graphene modified Screen Printed Carbon Electrode (SPCE, Dropsens DRP C110) as electrode. All solutions were prepared with ultrapure water with resistivity of 18.2 Mohm cm.

2.2 Methods

2.2.1. Synthesis of Graphene. The synthesis of graphene from graphite was carried out using nitronium ion and microwave heating as reported by Chiu et al. (2012) [19]. In this report we performed some modification to Chiu et al. (2012) method. In brief, the amount of 20 mg of synthetic graphite powder was added and mixed into a solution of sulfuric acid and nitric acid (ratio of 1:1 with a total volume of 10 mL). The mixture was exposed to 30-60 s of 300 W microwave irradiation. The solution then neutralized in an ice-bath using 20 mL of deionized water follow with 4 M KOH until pH values were close to 7. A colloidal graphene solution was separated from precipitate impurities. Colloidal graphene was then washed using deionized water for 3 times and separated using centrifugation at 4000 rpm for 20 minutes to remove any unexfoliated graphite powder.

2.2.2. Graphene Modified SPCE Fabrication. Modification of SPCE using graphene was conducted using electrodeposition of graphene from 1 mg/10 mL graphene solution in 0.1 M phosphate buffer (PB) saline pH 7. Electrodeposition was carried out by cyclic voltammetry at potential range of -1.5 to 0.1 V vs Ag/AgCl and scan rate of 100 mV/s for 16 cycles. Unmodified SPCE and graphene modified SPCE were characterized by scanning electron microscopy, SEM (ZEISS EVO MA10, Germany).

2.2.3. Electrochemical Measurement of Glutathione. Stock solution of GSH with concentration of 1 x 10^{-3} M was prepared in 1 mM NaBr solution in 0.2 M NaClO_{4}. GSH solutions in various concentrations (1 to 100 µM) were prepared from each stock solution using 1 mM NaBr in 0.2 M NaClO_{4} as electrolyte. Each solution was measured using cyclic voltammetry at graphene modified SPCE (SPCE-G). Analytical performance of SPCE-G in GSH measurement was also evaluated, including precision, limit of detection and limit of quantitation.
3. Result and Discussion
3.1 Microwave-Enabled Low-Oxygen Graphene

In this work, synthesis of graphene was conducted by microwave assisted process. During the graphene synthesis, nitronium ion (generated from the reaction of H_2SO_4 with HNO_3) was interacted with graphene surface and formed multiple aromatic radical ion pairs. At high temperatures under microwave irradiation, multiple hydroxyl and/or epoxy groups can be formed across the graphene surface following oxygen transfer to the aromatic radicals. The process leads to the exfoliation of graphite to form graphene oxide. Synthesis of graphene using microwave in short duration (30 to 60 second) could reduce the cutting of the graphene sheet significantly and generated graphene sheets will contain a smaller number of hydroxyl groups and / or epoxides. In addition, heat from microwaves can also increase the dispersion of graphene in the solution [19].

The color of graphene colloidal solution obtained from microwave assisted synthesis was dark gray. Qualitative test using 0.05 M KMnO_4 was performed on a small portion of the graphene colloidal solution. Alteration of KMnO_4 color from purple to brown indicated the presence of graphene in colloidal solution (Figure 1a). Furthermore, UV-Vis spectrum of colloidal solution exhibited λ maximum at 273.5 nm (Figure 1b). This result confirmed that graphene has successfully synthesized. Unfortunately, the yield of graphene synthesis was low, i.e. 15%.

Graphene obtained from the synthesis in previous step was further used to modify screen printed carbon electrode (SPCE). This modification was carried out to enhance sensitivity of the electrode in GSH detection. Graphene is a material consisting of a single sheet of carbon from a graphite structure with a sp2 bond. All carbon atoms in graphene have a hexagonal shape in the planar ring system. Graphene was reported for its superior physical properties including high electron mobility (~ 10000 cm^2 / Vs), good optical transparency, large specific surface area (2.630 m^2 / g), high Young modulus (~ 1 TPa), and high thermal conductivity ~ 3000 W / mK) [20].

![Graphene synthesis images](image1.png)

**Figure 1** Positive result for qualitative analysis of graphene (a) λ maximum (273.5 nm) of UV spectrophotometry indicated the presence of graphene (b), hydroxyl or epoxy reduction peak during deposition of graphene (c), SEM image of deposited graphene on SPCE (d).
3.2 Graphene Modified Screen Printed Carbon Electrode and Its Morphological profile

Immobilization of graphene at SPCE surface was carried out by electrodeposition in graphene solution for 16 times cycles. The reduction peak at potential of -0.7 V to -0.8 V was observed in voltammogram of electrodeposition process (Figure 1c). It was predicted to be correlated with hydroxyl or epoxy reduction during deposition process. Lee et al. (2016) stated that synthesized graphene may contain oxygen functional group such as hydroxyl and epoxy groups [21]. Those functional group gives oxidation peak at a potential of 0.5 V and two reduction peaks at potential of -0.8 V and -1.15 V vs Ag/AgCl in phosphate buffer solution. Figure 1c revealed that peaks current intensity was decrease as the number of cycles increase. The decrease in peak intensity is related to the electrodeposition process. Graphene with oxygen groups is electrochemically reduced through electrodeposition process to produce reduced graphene with better conductivity [22, 23]. SEM profile of graphene modified SPCE (Figure 1d) indicated that graphene was deposited on the surface of SPCE, but it was not completely covered the whole part of SPCE.

3.3 Electrochemical Responses of Glutathione in 1 mM NaBr in 0.2 M NaClO₄ as Electrolyte at Graphene Modified SPCE

Glutathione measurements were carried out in 1 mM NaBr in 0.2 M NaClO₄ as electrolyte solution. Oxidation peak at potential of 0.5 V vs. Ag/AgCl was detected as illustrated at Fig. 2a. This peak predicted to be oxidation peak of bromide ion to form bromine (1). Valero-Ruiz et al. (2015) reported that the presence of glutathione could enhance the intensity of bromide oxidation peak. Sodium chlorate oxidized bromide to produce bromine (1) which acted as an oxidizer in the glutathione oxidation [11]. Furthermore, bromine oxidized glutathione to produce GSO₃H (2). As concentration of glutathione increase, more bromine was required to oxidize glutathione. This condition tends to enhance the oxidation of bromide that could increase the intensity of bromide oxidation peaks current in this measurement. The complete reaction can be seen in the following equation and scheme:

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\begin{align*}
\text{Oxidation:} & \quad 2\text{Br}^- \iff \text{Br}_2 + 2e^- \\
\text{Reduction:} & \quad 2\text{H}^+ + \text{ClO}_4^- + 2e^- \rightarrow \text{ClO}_3^- + \text{H}_2\text{O} \\
\text{Total reaction:} & \quad 2\text{H}^+ + \text{ClO}_4^- + 2\text{Br}^- \rightarrow \text{ClO}_3^- + \text{Br}_2 + \text{H}_2\text{O} \quad (1) \\
\text{Oxidation of glutathione:} & \quad \text{GSH} + 3\text{Br}_2 + 3\text{H}_2\text{O} \rightarrow \text{GSO}_3\text{H} + 6\text{HBr} \quad (2)
\end{align*}
\]

Scan rate of glutathione measurement was varied to evaluate the correlation between scan rate and peak current. Intensity of oxidation peak current was higher as scan rate increase. Correlation between square root of scan rate and peak current followed linear correlation, indicated that mass transfer to the electrode surface follow diffusion mode (Figure 2b).

Measurement of glutathione in the presence of NaBr in NaClO₄ electrolyte was carried out based on oxidation peak of bromide. This measurement was successfully performed at SPCE-G (Figure 2c). The regression equation from the measurement of glutathione using SPCE-G was \( \text{ipa (\mu A)} = 0.0321x + 2.7762 \quad (R^2 = 0.9900) \). Precision was evaluated from measurement of 5 series of glutathione concentrations and each measurement was performed in 6 replicates. Precision of glutathione measurement at SPCE-G was 2.95%. Furthermore, limit of detection and limit of quantitation of glutathione measurement at SPCE-G were 8.1 µM and 24.55 µM (Table 1).
Figure 2 Cyclic voltammogram of $10^{-4}$ M glutathione in 1 mM NaBr in 0.2 M NaClO₄ (a), voltammogram cyclic of $10^{-4}$ M glutathione in 1 mM NaBr in 0.2 M NaClO₄ in various scan rate (b), cyclic voltammogram at scan rate of 100 mV/s and its linear correlation graph of glutathione in 1 mM NaBr in 0.2 M NaClO₄ at SPCE-G (c).

Table 1 Resume of Analytical Performance of Glutathione Measurement at SPCE-G

| Parameters | NaClO₄ in the presence of NaBr as electrolyte | SPCE-G | Valero-Ruiz et al. 2015 (vs SCE) |
|------------|---------------------------------------------|--------|----------------------------------|
| Oxidation potential (V) (vs Ag/AgCl) | 0.60 | 0.95 |
| Linearity range (µM) | 1-100 | 19.9-300.7 |
| Slope (µA/µM) | 0.0321 | 0.019 |
| Intercept (µA) | 2.7762 | - |
| Determination coefficient ($R^2$) | 0.9900 | - |
| Limit of Detection (µM) | 8.10 | 22.8 |
| Limit of Quantitation (µM) | 24.55 | - |
| Precision (%RSD) | 2.95 | - |

This result indicated that SPCE-G was potential for glutathione measurement. Measurement of glutathione at SPCE-G provide better precision, limit of detection, and sensitivity compare to platinum electrode reported by Valero-Ruiz 2015. Otherwise, before application for measurement of glutathione concentration in human biological fluid, analytical performance (LOD and LOQ) of glutathione measurement at SPCE-G need to be improved. Normal glutathione concentration in biological fluid...
was in range of 2-12 µM and symptom of stress oxidative was indicated by the decline of glutathione concentration to lower than 2 µM.

4. Conclusion
Graphene modified screen printed carbon electrode (SPCE-G) seems to be potential for electrochemical detection of glutathione. Glutathione detection at SPCE-G provided comparable analytical performance to that conducted by platinum electrode as reported by previous research. Otherwise, further optimization need to be conducted to improve the analytical performance of SPCE-G in glutathione measurement.

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References
[1] Huber WW, Parzefall W 2007 Thiols and the chemoprevention of cancer Curr. Opin. Pharmacol 7 404-409.
[2] Ortega AL, Mena S, Estrela JM 2011 Glutathione in Cancer Cell Death Cancers 3 1285-1310.
[3] Aoyama K, Nakaki T 2015 Molecular and Genetic Basis of Neurological and Psychiatric Disease Fifth Edition.
[4] Landriscina M, Maddalena F, Laudiero G, Esposito F 2009 Adaptation to oxidative stress, chemoresistance, and cell survival Antiox. Redox Signal 11 (11) 2701-2716.
[5] Garcia SC, Schoot K, Charao M, Moro A, Bulcao R et al 2008 Quantification of reduced glutathione by HPLC-UV in erythrocytes of hemodialysis patients Biomed. Chromatogr 22 460-468.
[6] Yin J, Kwon Y, Kim D, Lee D, Kim G et al 2014 Cyanine-Based Fluorescent Probe for Highly Selective Detection of Glutathione in Cell Cultures and Live Mouse Tissues J Am. Chem. Soc 136 5351-5358.
[7] Wang HB, Chen Y, Li Y, Liu YM et al 2016 A sensitive fluorescence sensor for glutathione detection based on MnO2 nanosheets-copper nanoclusters composite RSC Adv 6 79526-79532.
[8] Hormozi-Nezhad MR, Seyedhossein E 2012 Spectrophotometric determination of glutathione and cysteine based on aggregation of colloidal gold nanoparticles Sci. Iran 19 (3) 958-963.
[9] Herfield JC, McAuley B, Compton RG 2012 Electrochemical determination of glutathione: a review Analyst 137 (10) 2285-2296.
[10] Ru J, Qin DD, Huang BM, Xue ZH, Zhou XB et al 2013 An electrochemical glutathione biosensor: Ubiqunine as a transduser Talanta 110 15-20.
[11] Valero-Ruiz E, Gonzalez-Sanchez MI, Batchelor-McAuley C, Compton RG 2015 Halogen mediated voltammetric oxidation of biological thiols and disulfides Analyst-RSC.
[12] Pasakon P, Karuwan C, Sriprachabwong C, Wisitsoraat A 2013 Electrochemical detection of glutathione based on inkjet-printed graphene modified screen printed carbon paste electrode Sensors Letters 11 2218–2226.
[13] Svancara I, Vytras K, Kalcher K, Walcairus A, Joseph Wang 2009 Carbon paste Electrodes in Facts, Numbers, and Notes: A review on the Occasion of the 50-Years Jubilee of Carbon Paste in Electrochemistry and Electroanalysis Electroanalysis 21(1) 7 - 28.
[14] Amare M, Admassie S 2012 Polymer modified glassy carbon electrode for the electrochemical determination of caffeine Talanta 15 (93) 122-128.
[15] Zhao W, Elias AL, Rajukumar LP, Kim HI, O’brien DJ et al 2016 Carbon Nanotubes: Controllable and Predictable Viscoelastic Behavior of 3D Boron-Doped Multiwalled Carbon
Nanotube Sponges  Particle & Practicle System Characterization 33(1)
DOI: 10.1002/ppsc.201670001

[16] Fujishima A, Einaga Y, Rao TN, Tryk DA 2005 Diamond Electrochemistry. Tokyo: BKC. Inc.

[17] Choi W, Lahiri I, Seelaboyina R, Kang YS 2010 Synthesis of graphene and its applications: A Review Critic. Rev. Solid. Stat. Mater. Sci 35 52-71.

[18] Fuhrer MS, Lau CN, MacDonald H 2010 Graphene: Materially better carbon MRS bulletin 35 290 – 295.

[19] Chiu PL, Mastrogiavanni DDT, Wei D, Louis C, Jeong M, Yu G, Saad P, Flach CR, Mendelsohn, Garfunkel E, He H 2012 Microwave- and nitronium ion-enabled rapid and direct production of highly conductive low-oxygen graphene Journal of the American Chemistry Society 134 5850-5856.

[20] Basua S, Bhattacharyya P 2012 Recent developments on graphene and graphene oxide based solid state gas sensors Sensor and Actuators B Chemical 173 1-21.

[21] Lee S, Park SK, Choi E, Piao Y 2016 Voltammetric detection of trace heavy metals using an electrochemically deposited graphene/bismuth nanocomposite film-modified glassy carbon electrode Journal of Electroanalytical Chemistry 2016 1-17

[22] Yuan B, Zeng X, Xu C, Liu L, Ma Y, Zhang D, Fan Y 2013 Electrochemical modification of graphene oxide bearing different types of oxygen functional spesies for electro-catalytic oxidation of reduced glutathione Sensors and Actuators B: Chemical 2013 1-29

[23] Yuan B, Xu C, Liu L, Zhang Q, Ji S, Pi L, Zhang D, Huo Q 2013 Cu2O/NiOx/graphene oxide modified glassy carbon electrode for the enhanced electrochemical oxidation of reduced glutathione and nanoenzyme glucosa sensor Electrochimica Acta 13 1-26.