Development of nitric-oxide-sensor-based gold (Au) and platinum (Pt) metals on screen-printed electrode modified graphene

S Nurkhasanah, S Setiasih and R Wibowo

Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia

Corresponding author’s e-mail: rwibowo73@gmail.com

Abstract. Nitric oxide (NO) plays an important role in physiological processes. NO measurement accuracy is important to understand the essential functions of NO but attaining these levels of accuracy is difficult due to NO’s short half-life. NO concentrations can be determined using an analytical method that has fast response and high sensitivity and is simple and practical. In this study, gold and platinum metals are deposited onto a screen-printed electrode (SPE) surface in a solution mixture of HAuCl$_4$ in 0.05 M HSO$_4$ and KPtCl in 0.05 M HSO$_4$, which has been modified with graphene to detect NO. Experiments that varied the deposition concentrations of the Au/Pt onto the G/SPE are conducted to obtain an optimal deposition concentration ratio between Au/Pt and G/SPE. NO detection tests are conducted at +0.878 V against Ag/AgCl. Deposits of AuPt/G/SPE that have 1:1 concentrations form high-performance sensors due to its high sensitivity at 23029.92 µA mM$^{-1}$ cm$^{-2}$, a low detection limit of 2.2 x 10$^{-7}$ mM, and the best linearity, R = 0.9943. No is detected using the Griess Saltzman method. Based on the linearity results from the NO detection measurements, we observe that the electrochemical method provides significant improvements over the Griess Saltzman method.

1. Introduction

Nitric oxide (NO) is known to play a role in vasodilatation processes and is a factor in endothelium cell relaxation [1]. NO also plays an important role as a communication agent between peripheral nerve system cells and the central nerves in eukaryotic cells. This molecule also acts as both a platelet and cytotoxic agent.

Accurate measurements of NO are key to understanding the essential functions of NO during physiological processes. One of the drawbacks of studying and analyzing NO concentrations is that the half-life of NO is very short (approximately 6 seconds) [2]. Oxygen quickly and easily oxidizes NO to form nitrite ($\text{NO}_2^-$) and nitrate ($\text{NO}_3^-$), and thus, a fast, accurate, and undemanding analytical method is required to determine NO concentrations [3]. In the past, the colorimetric method has been widely used to determine NO levels. This method analyzes NO indirectly, where the molecule is converted to a more stable molecular form, such as $\text{NO}_3^-$ or $\text{NO}_2^-$. This method, however, requires long preparation times, many chemical reagents, and is expensive thereby rendering it disadvantageous [4].

In this study, we construct nitric oxide sensors using the electrochemical method. This method is sensitive and only requires simple equipment. The electrochemical method also has the advantage of being able to be used for ionic charge separation and detection [5]. Previous studies have developed nitric oxide sensors via the modification and installation of metals such as Au and Pt because electrode modification with a single, pure metal (Au or Pt) yields increased sensitivity and decreased selectivity. Platinum’s high electrocatalytic activity and sensitivity increases by combining it with Au [6]. Electrochemical sensor fabrication with AuPt/Graphene components was directly deposited onto the surface of a screen-printed electrode (SPE). Graphene was used to improve electron transfer. We
investigated the AuPt compositional effects on NO catalytic oxidation and the electrochemical method was compared with the Griess Saltzman method for NO analytical detection.

2. Experimental

2.1. Chemical reagents
All chemicals used are of analytical grade quality. Graphite powder, KMnO₄, NaNO₂, and H₂SO₄ were 98% pure, H₂O, and HCl were 5% and hydrazine was 85%, which were all purchased from Merck for graphene synthesis. Gold(III) chloride trihydrate (HAuCl₄) and Chloro-platinic(IV) potassium (K₂PtCl₆) were purchased from Sigma–Aldrich. Phosphate buffer saline (PBS) solutions (0.1M) were prepared with 0.1M Na₂HPO₄, NaH₂PO₄, and 0.1M NaCl. To synthesize NO gas we used FeSO₄ and H₂SO₄ from Merck and NaNO₂ from Sigma-Aldrich. Reagent grade sodium nitrite, N-1-naphthylethylenediamine (NEDD), and sulphanilamide (SULF) were obtained from Sigma–Aldrich and were used to make the NO stock concentrations.

2.2. AuPt/G/SPE fabrication
Graphene was prepared from natural graphite powder using a modified Hummers method. Typically, 3 g of graphite was added to 46 mL of 98% H₂SO₄ and 3 g of NaNO₂. We then added 10 mL of 30% H₂O₂ to the mixture to prevent graphite oxidation reactions. The unused graphite, in the resulting mixture, was removed by centrifugation. This yielded a brown graphene oxide solid product. NO was dissolved in 50 mL of deionized water, stirred for 1 hour, and then added to 1 mL of 85% hydrazine to form a black graphene solid. The bare SPE was washed using deionized water. At a rate of 1 mg mL⁻¹, graphene was placed on the SPE and air dried to form the “G/SPE.” The G/SPE was washed with deionized water. We electrosynthesized the AuPt onto the G/SPE by applying +650 mV and +180 mV for Au and Pt, respectively, with a deposition time of 90 s in an aqueous solution of 0.5M H₂SO₄, containing different Au/Pt molar ratios (we maintained the total concentration of HAuCl₄ and K₂PtCl₆ at 2 mM). The resulting electrode (denoted as AuPt/G/SPE) was subsequently rinsed with water and used for further measurements. Electrochemical experiments were performed using a CHI 660B computer-controlled potentiostat (CH Instrument Inc., USA).

2.3. Nitric oxide gas preparation
Nitric oxide was chemically produced by mixing 10 mL of 1M NaNO₂, 10 mL of FeSO₄, and 10 mL of 2M H₂SO₄ in a glass tube. Before and during generation, the entire apparatus was purged with N₂ to remove O₂. Generated NO was dissolved in 20 mL of 1M PBS (pH = 7.4). The saturated NO concentrations and NO detection were measured using both the spectrophotometric and electrochemical method. The detection of NO proceeded by diluting the stock solution with 0.1M PBS. All NO standard solutions were prepared daily by appropriately diluting the stock solution.

3. Results and discussion

3.1. Synthesized graphene
Graphene was synthesized using the Hummer method [1], where synthesis is divided into two stages, i.e., graphite oxide and graphene synthesis. In the graphite oxide synthesis process, a graphite intercalation compound forms from graphite, allowing more efficient oxidization. During this process, several functional groups, such as the hydroxy, epoxy, ketone, carboxyl, and carbonyl groups formed on carbon [2]. These functional groups give the material highly hydrophilic characteristics and allow easy exfoliation into the graphite oxide. The addition of H₂O inhibits the oxidation process. The purification process is carried out to neutralize the pH and reduce the remaining SO₄²⁻. During the purification process, the exfoliation of graphite into the graphene oxide occurs. The addition of hydrazine and HCl reduces the graphene oxide.

From figure 1, we observe that the X-ray diffraction (XRD) graphene has a peak at 2θ = 22.4094° with a d spacing of 3.96418 Å. Graphene peaks have low intensity, which indicates that reduction processes have eliminated the oxygen and water functional groups. This yields a graphene structure that contains only carbon atoms that tend to have amorphous crystal structures. A second peak exists at 2θ = 25.2973° with a d spacing of 3.51780 Å, which we identify as the graphite peak. The presence of graphite indicates the existence of graphite that has not been fully oxidized.
3.2. Electrochemical method
To enable the accurate calibration of NO sensor we first established a preparation method to consistently reproduce the NO stock solutions. NO gas is synthesized according to the following reaction (equation (1)):

\[
2 \text{NaNO}_2 (aq) + 2 \text{FeSO}_4 (aq) + 3 \text{H}_2\text{SO}_4 (aq) \rightarrow 2 \text{NO (g)} + \text{Fe}_2(\text{SO}_4)_3 (aq) + \text{H}_2\text{O (aq)}
\]  

(1)

We use a concentrated H_2SO_4 solution to perform disproportionation with a NaNO_2 solution as the source of NO_2^- [3]. We varied the bubble time to determine the NO saturation concentration (1.8 mM).

Figure 2 shows that the bubbling time linearly increases with both the current and peak NO oxidation. Longer bubble times produce elevated NO oxidation currents and peaks. At 60 minutes there is a significant increase in the current and oxidation peak. At 70 and 80 minutes, the increase remains but is not significant. There is also a base measurement (PBS without NO) where no NO oxidation peaks form. The existence of the base serves as a comparison for solutions containing NO.

3.3. Griess Saltzman method
NO reacts with molecular oxygen and its characteristics of high permeability [4] indicate that verification of the stock concentration is important. One of these verification methods is the Griess Saltzman method. In this method, nitric oxide levels are indirectly measured by analyzing nitrite, NO_2^-, which is a product of NO oxidation, in which molecules are more stable. The gas formed from synthesis products was dissolved in 0.1M PBS and a Griess reagent, composed of SULF and NEDD under acidic conditions [5]. The addition of the NO solution yielded absorbance values shown in figure 3, where there is a maximum absorption spectral peak at \( \lambda = 527 \text{ nm} \).
A primary arylnitrosamine is produced from the nitrosation of SULF nitrosation produces a primary arylnitrosamine via an intermediary generated by the following NO/O₂ reaction:

\[ 2\text{NO} + \text{O}_2 \rightarrow \text{NO}_x \]

Arylnitrosamine decomposes rapidly into diazonium ions, which then react with NEDD to form azo compounds [6]. Inset figure 3 indicates that, from minute 5 until minute 40, the absorbance increases, with the highest absorbance at 60 minutes. These results indicate that NO that has been dissolved in 0.1M PBS have higher concentrations. We then increased the bubble time variation observing that between minute 70 and 80 absorbance continued to increase but the increase was not significant. This is due to the fact that the NO became oversaturated.

3.4. Nitric oxide voltammetry

We observed the electrochemical nitric oxide by analyzing the AuPt/G/SPE electrode voltammograms. The electrochemical NO reaction along the metal surface occurs at a positive potential (typically 0.6–0.9 V vs. Ag/AgCl) [7]. NO oxidation mechanisms occur through the following three processes:

\[ \text{NO} \rightarrow \text{NO}^- + \text{e}^- \]
\[ \text{NO}^- + \text{OH} \rightarrow \text{HNO}_2 \]
\[ \text{HNO}_2 + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + 2\text{e}^- + 3\text{H}^- \]

We observed AuPt/G/SPE electrocatalytic activity from Au-Pt concentration variations to NO oxidation. Figure 4a shows the LSV response for the modified G/SPE with a Au-Pt molar ratio in the 1.8 mM NO (red line) and, for comparison, we measured the LSV response without NO (black line). No obvious changes in the voltammetric shape were observed by changing the Au/Pt molar ratios. The different effects of the Au/Pt molar ratio within the NO oxidation potential on the AuPt/G/SPE can be observed in figure 4b. The current response increased with an increasing Au/Pt molar ratio, i.e., from 0:100 to 50:50. However, the current response decreased when the amount of Au was increased, indicating that we attained the highest current response with an Au/Pt ratio of 50:50. The peak potential for NO oxidation on the AuPt/G/SPE slightly decreased from +0.930 to +0.870 V when increasing the Au/Pt ratio from 0–100%. In voltammetry, the electrode potential provides thermodynamic information about the electrochemical reaction, whereas the current gives the reaction rate [8].

3.5. LSV detection of NO

The results of the AuPt modification on the G/SPE with molar ratio variation are used in the nitrogen monoxide detection tests as well as calibration curve construction to observe linearity. The LSV method was used to obtain the NO oxidation current with concentrations from 0.18–1.8 mM and a NO oxidation potential of +0.878 V.
Figure 4. (a) LSV responses to AuPt/G/SPE with different Au/Pt molar ratios and 1.8 mM NO in a 0.1M PBS solution, (b) Peak current dependence and corresponding peak potentials of the 1.8 mM NO for the different Au/Pt molar ratios in the AuPt–rGO modified electrodes.

Figure 5. (a) LSV responses for the AuPt/G/SPE modified electrodes to NO with different concentrations in a 0.1M PBS solution. (b) The corresponding calibration plot between LSV response and NO concentration

Figure 5 shows that the modified AuPt/G/SPE increases NO’s electrocatalytic activity. The peak current increases with elevated NO concentrations where the electrochemical reaction from NO to HNO occurs. The AuPt/G/SPE with a 50:50 molar concentration ratio has the best linearity, $R^2 = 0.9943$. This AuPt/G/SPE has excellent sensor performance with the lowest LOD value of 2.2 x 10⁻³ M and the highest sensitivity of 23029.92 μA μM⁻¹ cm⁻¹ compared with the other modified electrodes. For comparison, we also evaluated the electrochemical performance of the Au/G/SPE, Pt/G/SPE, AuPt/G/SPE 30:70, and the AuPt/G/SPE 70:30 with LSV under identical conditions. The corresponding sensitivities were determined: 12883.85, 13346.46, 20324.54, and 16445.55 μA μM⁻¹ cm⁻¹, respectively.

3.6. Detection of NO using the Griess Saltzman method

NO detection is also compared with the Griess Saltzman method. The main principle of this method is to create a reaction between the Griess reagent and the products of NO synthesis dissolved in 0.1M PBS with 1 hour of bubbling to form NO with saturated concentrations. Concentration variations were obtained from dilution using 0.1M PBS. The relation of absorbance and concentration is plotted in figure 6.
Figure 6. Absorbance versus concentration using Griess Saltzman method

Based on the two measurement methods, the capability to detect NO in the electrochemical method is significantly better than the Griess method. The linearity is greater in the electrochemical method, $R^2 = 0.9943$, compared with the spectrophotometric measurements, $R^2 = 0.9847$.

4. Conclusions
A new electrochemical sensor has been successfully fabricated by combining graphene and AuPt onto a SPE surface. Graphene was synthesized using the Hummer method by oxidizing graphite to form graphene oxide, which was then reduced by hydrazine. The measurement results have been validated by XRD characterization as well as JCPDS 05-0592. The AuPt/G/SPE were optimized and investigated for NO detection. Results indicate that the AuPt/G/SPE has high sensitivity (23029.92 $\mu$A $\mu$M cm$^{-1}$) and a low LOD (2.2 x 10$^{-3}$ M). Based on linearity results, NO detection using the electrochemical method is advantageous compared with the Griess Saltzman method.

Acknowledgements
The authors would like to thank Enago (www.enago.com) for the English language review.

References
[1] Hummers W S and Offeman R E 1958 J. Am. Chem. Soc. 80 1339
[2] Shao G, Lu Y, Wu F, Yang C, Zeng F and Wu Q 2012 J. Mater. Sci. 47 4400–09
[3] Zhang X, Wang H, Liang S C and Zhang H S 2002 Talanta 56 499–504
[4] Beckman J S, Wink D A and Crow J P 1996 Methods in Nitric Oxide Research, eds. M Feelisch and J S Stamler (New York: John Wiley & Sons)
[5] Promega 2009 Griess Reagent System: Instruction for Use of Product G2930 (New York: Promega Corporation) Technical Bulletin 229
[6] Brown F O, Finnerty N J, Bolger F B, Millar J and Lowry J P 2005 Anal. Bioanal. Chem. 381 964–71
[7] Privett B J, Shin J H and Schoenfisch M H 2010 Chem. Soc. Rev. 39 1925–35
[8] Liu Z, Forsyth H, Khaperb N and Chen A 2016 Analyst 141 4074–83