Performance of SGPN-NPs paste electrode as medical biosensor

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Abstract. SGPN-NPs paste electrode as biosensor have been successfully fabricated. The electrode was made by modification material such as silica gel, solid paraffin, liquid paraffin and nickel nanoparticles. All modification material would be mixed into paste and then modified on the silver electrode which covered by glass tube. Comparison result will be studied by silver electrode with and without modification. The performance of the biosensor was investigated in medical sample, i.e. insulin. The electrochemical response from the biosensor was performed by cyclic voltammetry (CV) in phosphate buffer solution (pH = 7.0) at room temperature. The best response of the biosensor was achieved by silver electrode with modification. This SGPN-NPs paste electrode can detect insulin at potential of -0.667 V. The obtained calibration curve followed a linear equation, Ipc = -5.1355 - 330.2550, with R² = 0.97564. The limit of detection (LOD) and sensitivity of the biosensor for insulin detection were 4.9225 nM and 1.6353 μA.nM⁻¹.mm², respectively. The result confirm that SGPN-NPs paste electrode is favorable for detecting medical sample, especially insulin.

1. Introduction

In the last few years, the medical world was faced by the emergence of various diseases that could bother the stability of human life. One of the diseases is diabetes. This diseases was caused by the increasing of glucose concentration in blood [1–3]. When this condition occur, the pancreas directly produces insulin as a response to the amount of glucose. Insulin is a peptide hormone controlling fat and carbohydrate metabolic process. The normal fasting insulin level in the blood is under 25 mIU/L or 0.86 ng/mL [2,4–8]. The dysfunction of insulin secretion in human’s body leads to poor glucose metabolism. Insulin also plays an important role in controlling the blood glucose levels within normal range, i.e. 3–8 mmol/L [4,9]. Early diagnosis of insulin will be consistent with the discovery of diabetes. If this can be done, it will help to prevent for more complication diseases. Thus, the accurate and selective analysis of insulin has attracted much attention for scientist.

For several years, capillary electrophoresis (CE) have been developed for determining insulin. This method has high separation efficiency, fast analysis time, and a simple setting system but less sensitive [10]. In the other hand, High Performance Liquid Chromatography (HPLC) was used to determine insulin level. HPLC requires a long detection process and low sensitivity in biological samples. This method requires an expensive equipment and skilled person [5]. Therefore, the fast, simple and low-cost method necessary for development. Lastly, Electrochemistry is an analytical method applying in widely purpose such as biosensor [9,11–14]. Therefore, electrochemical biosensor has gained attention for decades due to its practically, sensitivity, simplicity, stability, low cost and environmentally safeness. Many investigations have been reported electrochemical biosensor such as determination of urea [15], dopamine [16–18], sugars [19], glucose [20], sucrose [21], uric acid [11,22–24] and also insulin [2,6–9,12,25] was reported using modified electrode with incorporating nanoparticles.
One of nanoparticles is nickel nanoparticles. This nanoparticles widely used as modification material on several electrode to increase the intensity of the signal of insulin \cite{7,9,25,26}. Nickel nanoparticles shows high catalytic activity in the oxidation of organic compounds. Nickel nanoparticles is a semiconductor compound with an energy band gap of 3.6-4.0 eV \cite{7,27}. This material has been widely used in a number of applications such as catalysts, electrode materials for lithium ion batteries, photovoltaic devices, fuel electrodes, electrochemical super capacitors, magnetic materials and gas sensors \cite{25,28–30}. Due to its unique properties, nickel nanoparticles can be used as an incorporating material. Nickel nanoparticles is expected to be able providing a good signal in insulin determination which used as reactive modification material.

In the present study, we fabricate SGPN-NPs paste electrode as medical biosensor. The biosensor was made from several modification material which modified on silver electrode. The performance of biosensor was investigated in medical samples, i.e. insulin. Electrochemical performance would be conducted by cyclic voltammetry method. The optimal conditions, repeatability and reproducibility of the biosensor were also studied.

2. Experiments

2.1. Chemical and Materials

Trisodium citrate dihydrate ($C_6H_5Na_3O_7\cdot2H_2O$, 99.5\%) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Solid paraffin, liquid paraffin and silica gel kiesel H 60 were bought from Merck KGaA (Darmstadt, Germany). Insulin (100 IU/mL) was purchased from R. Lantus. All chemicals were used without any purification. Phosphate buffer (PB) with pH = 7.0 was made from Sodium dihydrogen phosphate dihydrate (NaH$_2$PO$_4\cdot2H_2O$, Merck, Germany) and sodium hydrogen phosphate dihydrate (Na$_2$HPO$_4\cdot2H_2O$, Merck, Germany). Silver wire (99.999\%, with diameter 1 mm and length of 7 cm) and glass tubes (diameter and length of 0.2 cm and 5 cm, respectively) were purchased from a local market. Nickel metal sheet (99.999\%) with thickness of 1 mm was obtained from PT. INCO Indonesia. Demineralized water was used for chemical preparation and cleaning.

2.2. Instrumentation

Electrochemical measurement were conducted using a potentiostat from eDAQ (potentiostat E161 and e-corder 410, equipped with e-chem software version 2.1.13).

2.3. Synthesis of nickel nanoparticles

Nickel nanoparticles was prepared at Laboratory of Instrumentation and Analytical Sciences, Chemistry Department, Faculty of Sciences, Institut Teknologi Sepuluh Nopember. The nanoparticles was
synthesized by electrochemical method as from our previous work [27]. Briefly, nickel metal sheet as anode and cathode were electrolyzed in sodium citrate solution at 100°C. The electrolysis process was carried out at 55 V under stirring condition (30 minute). The formation of nickel nanoparticles was shown by changes in color from colorless to light green and then getting thicker. The obtained nickel nanoparticles (N-NPs) was used to fabricate SGPN-NPs paste electrode. The nickel nanoparticles was characterized by JEOL JMI40m (HR-TEM) High Resolution Transmission Electron Microscope. The image of TEM is shown in Figure 1. We can found that nickel nanoparticles have a spherical form with average size of 75 nm.

2.4. Fabrication of paste electrodes

The paste electrodes was prepared by mixture several modification materials such as silica gel, solid paraffin, liquid paraffin and nickel nanoparticles in various mass. The mixture was formed into paste and then inserted it onto glass tubes which include silver electrode. The paste was inserted as much as 0.05 g to get the same mass in all electrodes. The surface of electrode was abraded using emery paper grades 1000.

2.5. Performance of SGPN-NPs paste electrode as medical biosensor

Electrochemical experiments were observed using the cyclic voltammetry method as our previous work [9,31]. All electrochemical experiments were carried out using three-electrode cell system. The system uses platinum wire as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference electrode (RE), and the paste electrodes as working electrode (WE). The potential was swept from -1.0 to +1.0 V vs. Ag/AgCl (KCl 3 M), with 20 seconds of rest time before being measured and scan rate of 100 mV/s. The testing solutions are PB solution (pH = 7.0) as blank and insulin in various concentration as analytes. All measurements were done at room temperature.

The optimum composition of modification material was obtained from comparison responses of the paste electrodes. The response of paste electrodes were investigated on 25 mL of various concentration of insulin in PB solution (pH = 7.0). For linear range was observed from current determination of insulin in concentration range 0-7 nM at PB solution (pH = 7.0). This experiments was carried out using SGPN-NPs paste electrode with best composition. The responses were plotted as current vs. insulin concentration. The linear regression of the responses would be used to calculate sensitivity and limit of detection (LOD) from the SGPN-NPs paste electrode.

Repeatability of the SGPN-NPs paste electrode was determined from the electrode current. Three electrodes were prepared as described in section 2.4. Each electrode was measured 10 times for 2 hours. The measurement was conducted in 25 mL of 7 nM insulin in PB solution (pH = 7.0). Reproducibility of the SGPN-NPs paste electrode was determined from one electrodes which prepared as described in section 2.4. The electrode was measured 10 times for 3 days consecutively. The experiments were investigated in 25 mL of 7 nM insulin in PB solution (pH = 7.0). The data obtained from repeatability and reproducibility were analyzed by t-test and F-test.

3. Result and Discussion

The effect of modification material on silver electrode as medical biosensor

The effect of modification material such as silica gel, solid paraffin, liquid paraffin and nickel nanoparticles on silver electrodes can be conducted by two different methods. The first method is comparing the response of silver electrode without modification toward blank (PB solution) and analytes (insulin). While the second method is comparing the response of silver electrodes with modification in blank and analytes [16,19,31]. The composition used in the silver modification was randomly chosen because the optimum composition of the electrode was unknown.
Figure 2. Cyclic voltammograms obtained for silver electrode without modification in PB solution at pH 7.0 (a) and 7 μM insulin in PB solution, pH 7.0 (b).

The result of the first method was shown in Figure 2. The response from unmodified silver electrode was produced a high current during insulin measurement compare blank solution. To ensure the performance of unmodified silver electrode, then the measurements were taken on various concentration of insulin. No significant changes for the electrode response in all insulin concentrations (Figure 3). It means that unmodified silver electrode not good to insulin detection.

Figure 3. Cyclic voltammograms obtained for silver electrode without modification at various concentration of insulin (1 [black], 2 [red], 3 [green], 4 [blue], 5 [light blue], 6 [magenta] and 7 [yellow] μM) in PB solution, pH 7.0.

In the other hand, the result of second method gives different response. We found that insulin give a cathodic peak, whereas no response in blank solution (Figure 4). The peak indicate that insulin undergoes a reduction reaction due to its interaction with a modified silver electrode. Figure 4 was also shown that the response of silver electrode with modification toward insulin has a higher cathodic current. For this method, we only used silica gel and nickel nanoparticles as modification material on silver electrode. According to literature, silica gel is semiconductor so that it can increase the current during insulin measurement [9]. Another modification material, i.e. nickel nanoparticles is also a semiconductor metal so that the response will be more increase [7,25,28]. Higher sensitivity was shown
with the present of nickel nanoparticles (Figure 4b). It is because the nickel nanoparticles has a high reactivity properties so that the electron transfer will be faster and can detect insulin even at low concentration \([2,11]\). A possible reaction between nickel nanoparticles and insulin is the following Eq. 1 and 2:

\[
\text{nickel nanoparticles } [\text{Ni(OH)}_2] \leftrightarrow \text{NiOOH} + e^- + H^+, (1)
\]

\[
\text{NiOOH} + \text{Insulin} \rightarrow \text{Ni(OH)}_2 + \text{Insulin}_{\text{red}}, (2)
\]

This result was proved that silica gel and nickel nanoparticles can be used as active modification material for insulin detection. For best composition will be discussed in the next section.

**Figure 4.** Cyclic voltammograms obtained for silver electrode with modification of silica gel and nickel nanoparticles in PB solution at pH 7.0 (a) and 7 μM insulin in PB solution, pH 7.0 (b).

**Optimization of modification material composition for SGPN-NPs paste electrode**

Determination of the best paste composition of modified silver electrodes was carried out in several modification material. The first modification is the using of solid paraffin and liquid paraffin. Paraffin is used as an adhesive to make paste electrode. In this study, we investigate the effect of liquid paraffin and solid paraffin for making a better paste. Figure 5a and 5b shows the effect of various mass addition from solid paraffin and liquid paraffin, respectively. The best result were obtained at the addition of 0.03 g solid paraffin and 0.04 g liquid paraffin.

Furthermore, the optimum of silica gel used was determined. The responses of the modified silver electrode at various silica gel is shown in Figure 5c. According to the voltammogram response, it can be seen that the mass of silica gel of 0.05 g has the highest current. The potential in varying silica gel was observed undergoes changes, it is likely that the mass of silica gel give an effect during insulin measurement. In the next study, the optimum composition of silica gel was used to determine the optimum composition of nickel nanoparticles. The results from silver electrode with modification material such as solid paraffin, liquid paraffin and silica gel in various mass of nickel nanoparticles is shown in Figure 5d. The best result was achieved at the addition of 0.03 g nickel nanoparticles.
Figure 5. Optimization composition of modification material, i.e. solid paraffin (a), liquid paraffin (b), silica gel (c) and NiO nanoparticles (d) at 7 μM insulin in PB solution, pH 7.0.

Performance of SGPN-NPs paste electrode as medical biosensor
The detection performance of the SGPN-NPs paste electrode was studied at different concentration of insulin. Cyclic voltammogram of insulin at various concentration is shown in Figure 6a, where the peak of the cathodic current increases with the increase of insulin concentrations. Figure 6b represent the calibration curve obtained using an Ipc value of -0.667 V. The equation of linear regression and the correlation coefficient (R²) are Ipc (μA) = -5.1350c (nM) - 330.2550 and 0.97564, respectively.

Sensitivity of the the SGPN-NPs paste electrode to identify insulin is 1.6353 μA.nM⁻¹.mm⁻². This value was calculated using the linear regression equation (Figure 6b). Limit of detection (LOD) belongs to the smallest concentration that can be detected by the electrode or biosensor [25,17,11]. This value can be determined using the following equation (Eq. 3):

\[ LOD = 3S_b/m, \]

where \( S_b \) is the standard deviation of the blank signal and \( m \) is the slope of the calibration curve. The LOD of the SGPN-NPs paste electrode for insulin detection has been found to be 4.92257 nM.
Figure 6. Cyclic voltammograms obtained for SGPN-NPs paste electrode at various concentration of insulin (1, 2, 3, 4, 5, 6, and 7 nM) in PB solution (pH = 7.0) (a). Calibration curve of insulin (b).

Table 1. Performance comparison for several electrodes.

| Electrode                        | Linear range   | LOD     | Ref  |
|----------------------------------|----------------|---------|------|
| CNTs                             | 20-400 ng.mL⁻¹ | 7.75 ng.mL⁻¹ | [20] |
| MWCNTs/DMF modified electrode    | 250 nmol.L⁻¹ to 1.6 mol.L⁻¹ | 250 nmol.L⁻¹ | [17] |
| Ni(OH)₂-GN/GCE                    | 800-6400 nM    | 200 nM  | [27] |
| NiONPs/Nafion-MWCNTs/SPCE        | 20-260 nM      | 6.1 nM  | [11] |
| SGPN-NPs paste electrode         | 1-7 nM         | 4.92257 nM | This work |

The performance of the SGPN-NPs paste electrode for insulin detection was compared to several electrodes. The comparison data was summarized in Table 1. The result indicates that the SGPN-NPs paste electrode has a better performance. Thus, the SGPN-NPs paste electrode has demonstrated as an alternative biosensor for insulin determination.

Repeatability and Reproducibility of SGPN-NPs paste electrode as medical biosensor

Repeatability of measurement for SGPN-NPs paste electrode was conducted to examine the electrode stability when being tested repeatedly [19]. The repeatability data of three SGPN-NPs paste electrode for 2 hours is shown in Table 2. The cathodic current of reduction peak from the measurement has been analyzed by F-test and t-test with 95% confidence interval. Table 3 indicate that the SGPN-NPs paste electrode has a good repeatability for insulin detection.

Table 2. Repeatability of three SGPN-NPs paste electrode at 7 nM insulin in PB solution, pH = 7.0.

| Electrode | Measurement of cathodic current (μA) |
|-----------|-------------------------------------|
|           | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      |
| A         | 230.66  | 229.52  | 229.06  | 227.52  | 225.76  | 221.84  | 205.21  | 200.90  | 198.03  | 195.66  |
| B         | 220.90  | 210.66  | 200.84  | 197.74  | 198.61  | 196.32  | 194.15  | 195.18  | 194.93  | 190.07  |
| C         | 218.80  | 217.46  | 215.35  | 210.15  | 205.23  | 203.23  | 200.48  | 199.07  | 195.19  | 191.25  |
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Table 3. F-test and t-test of repeatability data.

| Electrode | F-test | t-test |
|-----------|--------|--------|
|           | H      | H      |
| A vs. B   | Accepted | rejected |
| A vs. C   | Accepted | rejected |
| B vs. C   | Rejected | accepted |

Reproducibility testing of SGPN-NPs paste electrode toward insulin was performed to know accuracy and precision between measurements [3,9,22,32]. The data for 3 days was summarized in Table 4. F-test and t-test (Table 5) shows that the measurement data has a precision and accuracy with insignificant changes of 95% confidence interval.

Table 4. Reproducibility of SGPN-NPs paste electrode at 7 nM insulin in PB solution, pH = 7.0 for three days.

| Days | Measurement of cathodic current (μA) |
|------|-------------------------------------|
|      | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
| 1    | 228.28 | 225.35 | 223.87 | 220.85 | 215.60 | 212.89 | 210.04 | 205.33 | 203.49 | 200.76 |
| 2    | 225.77 | 220.02 | 216.55 | 214.55 | 212.55 | 210.09 | 208.88 | 207.55 | 205.97 | 203.21 |
| 3    | 223.24 | 219.44 | 217.00 | 215.99 | 210.01 | 207.11 | 205.07 | 200.99 | 199.01 | 197.55 |

Table 5. F-test and t-test of reproducibility data.

| Days | F-test | t-test |
|------|--------|--------|
|      | H      | H      |
| 1 vs. 2 | Accepted | rejected |
| 1 vs. 3 | Accepted | rejected |
| 2 vs. 3 | Rejected | accepted |

4. Summary
The SGPN-NPs paste electrode has shown good performance as medical biosensor, especially for insulin determination. The best response of the biosensor was achieved by silver electrode with modification in composition 0.03 g solid paraffin : 0.04 g liquid paraffin : 0.05 g silica gel : 0.03 g nickel nanoparticles. The sensitivity and LOD of the biosensor are 1.6353 μA.nM⁻¹.mm² and 4.92257 nM, respectively. This biosensor also shows good repeatability and reproducibility for insulin detection.

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