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A Novel Selective and Sensitive Electrochemical Sensor for Insulin Detection

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Abstract

A novel selective and sensitive electrochemical sensor for insulin detection has been fabricated and investigated. The electrochemical sensor was made from a mixture of silica gel, chitosan and nickel hydroxide ($Ni(OH)_2$) nanoparticles, which was mounted on a silver wire and covered by a glass tube (silica gel/chitosan/$Ni(OH)_2$ nanoparticles paste electrode). The sensor was characterized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The anodic and cathodic currents of the silica gel/chitosan paste electrode with $Ni(OH)_2$ are 580 μA and -750 μA, respectively. Without $Ni(OH)_2$, however, the currents are 150 μA and -250 μA, respectively. The sensitivity and limit of detection of the silica gel/chitosan/$Ni(OH)_2$ nanoparticle paste electrode for insulin detection are 5573 × 10^5 pA/pMcm^2 and 0.25 pM, respectively. The sensor also shows good reproducibility of measurement for 35 days with an RSD of 0.29%. The fabricated electrodes also show good reproducibility, with an RSD of 1.39%.

Keywords*: insulin, silica gel, chitosan, nickel hydroxide nanoparticles*

1. Introduction

The increase in the glucose concentration in the blood is influenced by the amount of carbohydrate that is consumed by a person. When the level of glucose in the blood increases, the pancreas produces insulin as a response to the amount of glucose to control the level of glucose in the blood [1]. Insulin is an important hormone that consists of polypeptide chains A and B, which are composed of 21 and 30 amino acids, respectively [2]. The inability of the pancreas to produce enough insulin to control glucose concentrations in the blood is called diabetes. According to the International Diabetes Federation (IDF), in 2014, there were 387 million cases of diabetes in the world, of which 9 million were in Indonesia. In 2012, the World Health Organization (WHO) estimated that approximately 1.5 million people died from diabetes. There are two types of diabetes. When the pancreas in the human body cannot produce insulin at all, it is called diabetes type 1. The second type is diabetes type 2, which occurs when the pancreas can still produce insulin but the amount is not enough to control glucose in the blood at a normal concentration [3–5]. Therefore, to obtain information related to the type of diabetes from the patient, it is important to have a method or instrument to measure the insulin in the blood directly before attempting medical treatment for the diabetes patient. The determination of insulin concentrations has been investigated intensively because it can be used as a tool to help treat diabetes patients.

The detection of a definite compound, e.g., insulin [6], glucose [7–9], dopamine [10], and urea [11], using a modified electrode with an electrochemical method has been reported. The electrochemical method has several advantages: the sample can be measured directly without complicated sample preparation, and the analysis process takes less than 5 minutes and does not use chemical reagents, so it is more effective and efficient [12]. Other methods such as high-performance liquid chromatography (HPLC) and immunoassay methods require complicated sample preparation, are time consuming, and require reagents such as HCl and dichloromethane [13] or polyethyleneimine and 3-amino propyltriethoxysilane [14] for detection, which are relatively expensive. Several modified electrodes for insulin determination have been

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reported. A silica gel/carbon paste electrode was used to measure insulin in phosphate buffer. The sensitivity and limit of detection (LOD) of the electrode were 1511 pA/pMcm^2 and 36 pM, respectively [15]. Conversely, the sensitivity and LOD of a nickel nanoparticle (NiOx)/glassy carbon/guanine electrode were 803 pA/pMcm^2 and 22 pM, respectively [16]. Besides for insulin sensor, the carbon material was applied for gas sensor [17]. Salimi et al.'s electrode had a better LOD than Jaafariasl et al.'s electrode but worse sensitivity. In the present work, we fabricate a silica gel/chitosan/$Ni(OH)_2$ paste electrode to measure insulin in 0.1 M phosphate buffer (pH 7.4) that demonstrates better performance in terms of sensitivity and LOD than both Salimi et al.'s and Jaafariasl et al.'s electrodes.

2. Research Method

2.1. Chemicals and Materials

Silica gel G.60, K_2HPO_4, and KH_2PO_4 were purchased from Merck and used without any purification. Insulin (100 IU/mL) was purchased from R. Lantus. Chitosan was prepared by Instrumentation and Analytical Sciences Laboratory, Institut Teknologi Sepuluh Nopember. Nickel hydroxide ($Ni(OH)_2$) nanoparticles were prepared according to the procedure developed by Budipramana et al. Briefly, nickel metal was electrolysed in sodium citrate solution at 100 °C. The potential of electrolysis was kept constant at 55 V via stirring for 30 minutes. The $Ni(OH)_2$ nanoparticle solution obtained was used in all experiments [18–20]. Solid paraffin, glass tubes (inner diameter, 0.2 cm; length, 5 cm), silver wire (outer diameter, 0.8 mm; length, 7 cm), and demineralized water were purchased from a local market.

2.2. Instrumentation

All electrochemical measurements were carried out using a potentiostat/galvanostat from eDAQ (potentiostat E161 and e-corder 410, which is equipped with e-chem software vs 2.0.1). A three-electrode cell system was used in all measurements unless otherwise described, with platinum as the counter electrode (CE), Ag/AgCl (KCl 3 M) as the reference electrode (RE), and a modified electrode as the working electrode (WE).

2.3. Fabrication of the Silica Gel/Chitosan Paste Electrode with $Ni(OH)_2$

The silica gel/chitosan/$Ni(OH)_2$ paste electrode was fabricated according to the following procedure. Silica gel and chitosan were mixed in a 4:6 weight ratio and thoroughly hand-mixed with an agate mortar and pestle to produce a homogeneous mixture. The mixture was heated to 65°C, and $Ni(OH)_2$ solution (2 mL/100 mg of the mixture) and paraffin (15 % from the mixture weight) were then added. The hand-mixing was continued until the mixture became slightly solid. The resulting mixture was inserted in the bottom of the tube glass (geometrical surface area: 0.5 cm^2) and connected to silver wire from the top of the tube glass. The surface of the paste electrode was then polished using abrasion paper grade 2000. The silica gel/chitosan/$Ni(OH)_2$ paste electrode was used to measure insulin at concentrations of 0.0, 0.1, 0.3, 0.5, and 0.7 pM in 0.1 M phosphate buffer (pH 7.4) with cyclic voltammetry in the potential range of -1.0 to 1.0 V and a scan rate of 100 mV/s. The limit of detection (LOD) was calculated according to three times the standard deviation of the cyclic voltammetry response. The silica gel/chitosan/$Ni(OH)_2$ paste electrode was used to measure 1.0 μM glucose, 1.0 μM urea, 1.0 μM ascorbic acid, and 1.0 μM insulin. All of the foreign substances and insulin were dissolved in 0.1 M phosphate buffer at pH 7.4. Five silica gel/chitosan/$Ni(OH)_2$ paste electrodes were fabricated and used to measure 1.0 μM insulin. One of the silica gel/chitosan/$Ni(OH)_2$ electrodes was used to measure 1.0 M insulin every day for 7 days and then on the 14th, 21st, 28th, and 35th days.

3. Results and Analysis

3.1. Electrochemical Characterization of the Silica Gel/Chitosan Paste Electrode

The preliminary experiment was carried out to observe the influence of $Ni(OH)_2$ on the silica gel/chitosan paste electrode in the response of 1.0 μM insulin. The cyclic voltammograms of the silica gel/chitosan paste electrode with (c, d) and without (a, b) $Ni(OH)_2$ are shown in Figure 1. From the Figure 1 we know that the peak anodic current (i_{pa}) on the silica gel/chitosan paste electrode with $Ni(OH)_2$ (d) (580 μA) and without $Ni(OH)_2$ (b) (150 μA). The i_{pa} value

indicates the peak of oxidation to insulin at a peak anodic potential (E_{pa}) of 0.8 V. Conversely, there is also a reduction of insulin indicated by the value of the peak cathodic current (i_{pc}) with $Ni(OH)_2$ (d) (-750 μA) and without $Ni(OH)_2$ (-250 μA) that occurred at the peak cathodic potential (E_{pc}) of -0.5 V.

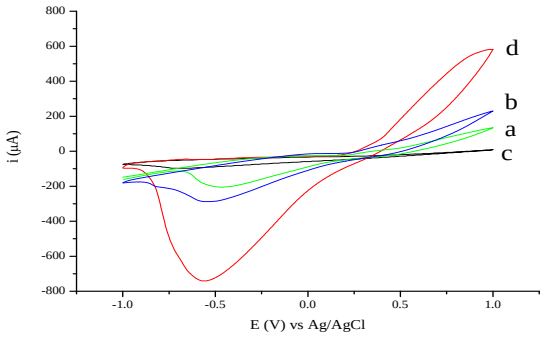

Figure 1. The Cyclic Voltammogram of Phosphate Buffer, pH 7.4, solution (a, c) and 1.0 μM insulin in phosphate buffer, pH 7.4, solution (b, d) for silica gel/chitosan (a, b) and silica gel/chitosan/$Ni(OH)_2$ paste electrodes (c, d). Scan rate, 100 mV/s

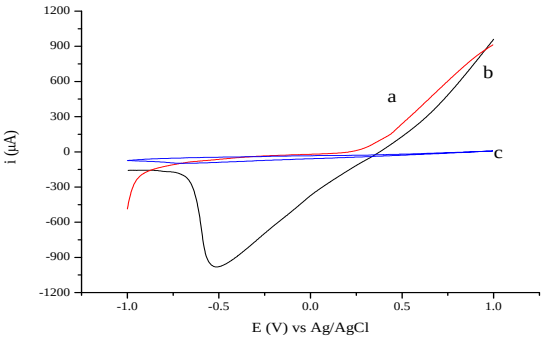

Figure 2. The Linear sweep voltammogram of insulin using silica gel/chitosan/$Ni(OH)_2$ nanoparticles paste electrode, (a) Anodic sweep, (b) Cathodic sweep, and (c) Anodic and Cathodic sweep of blank solutions

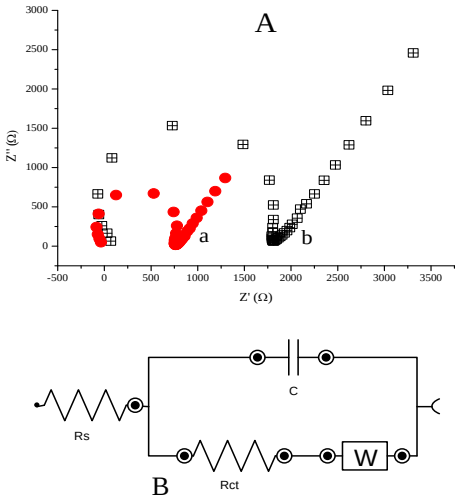

Figure 3. (A) Nyquist plot of silica gel/chitosan paste electrodes with (a) and without $Ni(OH)_2$ (b) in 1.0 μM insulin solution in 0.1 M phosphate buffer at pH 7.4. (B) EIS equivalent circuit used to fit EIS diagrams.

The presence of $Ni(OH)_2$ on a silica gel/chitosan paste electrode can be used as a good insulin sensor because $Ni(OH)_2$ has good electrocatalytic properties and can oxidize and reduce insulin on the electrode surface. The i_{pa} and i_{pc} values obtained upon blank measurement with the silica gel/chitosan paste electrode with (c) are much smaller than the i_{pa} and i_{pc} values obtained without $Ni(OH)_2$. This indicates that the silica gel/chitosan paste electrode with $Ni(OH)_2$ is more sensitive than that without $Ni(OH)_2$ because the $Ni(OH)_2$ has high reactivity properties so fast electron transfer occurs and is able to interact with insulin detection even at a low concentration. A possible reaction between $Ni(OH)_2$ and insulin (amino acid) is the following: [21].

$$Ni(OH)_2 \leftrightarrow NiOOH + e^- + H^+ \quad (1)$$

$$NiOOH + Insulin \rightarrow Ni(OH)_2 + Insulin_{(red)} \quad (2)$$

Figure 2 shows the Linear Sweep Voltammetry of the insulin with (a) for anodic sweep, (b) for cathodic sweep in phosphate buffer pH 7.4 using silica gel/chitosan/$Ni(OH)_2$ nanoparticles paste electrode. Anodic sweep does not have any peak of oxidation of insulin. Increasing of current in the end of the voltammogram is probably due to the oxidation of $Ni(OH)_2$. Conversely, the cathodic sweep show a reduction peak of insulin. This is indicated that the insulin only can be reduced by this electrode while in the other works reported oxidation of the insulin [16, 21]. The most probably reduction reaction is carboxylic acid group change to aldehyde group.

The silica gel/chitosan paste electrodes with and without $Ni(OH)_2$ were also characterized by electrochemical impedance spectroscopy (EIS) in 0.1 M phosphate buffer at pH 7.4. Figure 3 (A) shows the Nyquist plot of the silica gel/chitosan paste electrode with (a) and without $Ni(OH)_2$ (b), and Figure 3 (B) shows the equivalent circuit. R_{ct}, R_s C, and W are the charge transfer resistance, solution resistance, capacitance, and Warburg impedance, respectively.

The R_{ct} values for the silica gel/chitosan paste electrode with and without $Ni(OH)_2$ are 767 Ω and 1400 Ω, respectively. This indicates that the silica gel/chitosan paste electrode with $Ni(OH)_2$ is approximately two times more conductive than that without $Ni(OH)_2$. Higher conductivity means better electron transfer during the oxidation and reduction reactions on the electrode surface, which in turn can increase the sensitivity and limit of detection of the electrode [22, 23].

The calculated C values of the silica gel/chitosan paste electrode with and without $Ni(OH)_2$ are 1.91 nF and 1.29 nF, respectively. This indicates that the ability of the silica gel/chitosan paste electrode with $Ni(OH)_2$ to store electrical energy is greater than that of the electrode without $Ni(OH)_2$. The W values of the silica gel/chitosan paste electrodes with and without $Ni(OH)_2$ are 187 μMho and 66.4 μMho, respectively. This indicates that the mass transport occurring at the silica gel/chitosan paste electrode with $Ni(OH)_2$ is twice that without $Ni(OH)_2$. When the mass transport increases, conductivity also increases and can increase the electrode sensitivity [21, 24]. A higher W value means better electrical potential on the electrode surface, and this can increase the electric induction that is proportional to the current flowing in the electrode [25].

3.2. Calibration Curve of Insulin by Silica Gel/Chitosan/$Ni(OH)_2$ Paste Electrode

The silica gel/chitosan/$Ni(OH)_2$ paste electrode was studied to measure insulin at different concentrations. Figure 4 shows (A) the cyclic voltammograms of insulin at various concentrations and (B) a calibration curve that is obtained using an i_{pc} value of -0.5 V. The silica gel/chitosan/$Ni(OH)_2$ paste electrode was studied to measure insulin at different concentrations. Figure 4 shows (A) the cyclic voltammograms of insulin at various concentrations and (B) a calibration curve that is obtained using an i_{pc} value of -0.5 V.

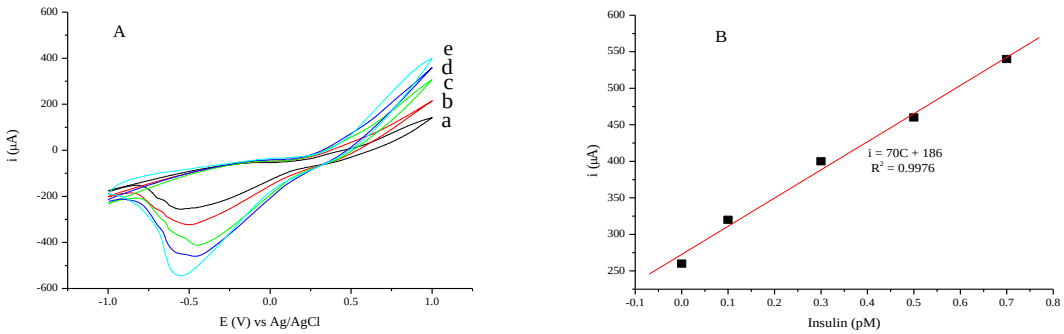

Figure 4. Cyclic voltammograms of various concentrations of insulin in 0.1 M phosphate buffer solution at pH 7.4 (A): 0 pM (a), 0.1 pM (b), 0.3 pM (c), 0.5 pM (d), 0.7 pM (e). Calibration curve of insulin (B)

The correlation coefficient (R^2), sensitivity and limit of detection (LOD) of the calibration curve are 0.9976, 5573 × 10^5 pA/pM cm^2 and 0.25 pM, respectively. The performance of several electrodes for insulin detection is shown at Table 1.

Table 1. Performance Comparison for Several Electrodes

Researcher	Electrode	LOD (pM)	Sensitivity (pA/pMcm)	pH
Salimi et al, 2008	NiOx/glassy carbon/ guanine	22	803	7.4
Jafariasl et al, 2011	Silica gel/carbon	36	1511	2.0
Rafiee et al, 2013	NiOx/carbon nanotube	6100	1800×10^3	7.4
This work, 2016	Silica gel/chitosan /$Ni(OH)_2$	0.25	5573×10^5	7.4

Table 1 shows that the silica gel/chitosan/$Ni(OH)_2$ paste electrode has a higher sensitivity and LOD compared to the other working electrode. Accordingly, $Ni(OH)_2$ has good electrocatalytic properties against insulin. Thus, the silica gel/chitosan/$Ni(OH)_2$ paste electrode can be promoted as an alternative method for insulin determination.

3.3. Interference Study

The response of the silica gel/chitosan/$Ni(OH)_2$ paste electrode was studied against glucose, urea, and ascorbic acid. Figure 5 shows voltammograms for (a) ascorbic acid, (b) urea, (c) glucose, and (d) insulin.

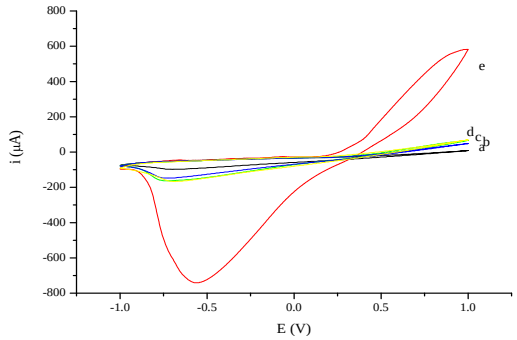

Figure 5. Cyclic voltammograms of 0.1 M phosphate buffer at pH 7.4 (a), 1.0 μM ascorbic acid in 0.1 M phosphate buffer at pH 7.4 (b), 1.0 μM urea in 0.1 M phosphate buffer at pH 7.4 (c), 1.0 μM glucose in 0.1 M phosphate buffer at pH 7.4 (d), and 1.0 μM insulin in 0.1 M phosphate buffer at pH 7.4 (e).

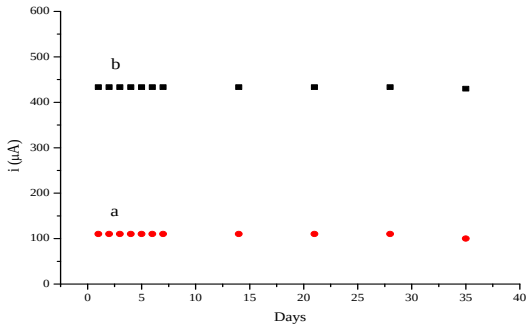

Figure 6. The anodic current (a) and cathodic current (b) of silica gel/chitosan/$(NiOH)_2$ paste electrode for the determination of 1.0 μM insulin in 0.1 M phosphate buffer at pH 7.4 for 35 days.

The i_{pa} and i_{pc} values of insulin on the silica gel/chitosan/$Ni(OH)_2$ paste electrode are 590 μA and -710 μA, respectively. These are higher than the i_{pa} and i_{pc} values of glucose, urea, or ascorbic acid. This indicates that the electrode has good selectivity to insulin.

3.4. Stability of Electrode

The stability of the silica gel/chitosan/$Ni(OH)_2$ paste electrode was evaluated for 35 days. The results obtained can be seen in Figure 6.

Five silica gel/chitosan/$Ni(OH)_2$ paste electrodes were used to measure insulin. The results show that the electrode has a relative standard deviation (RSD) of 1.39%, which means that the electrodes can be fabricated with good precision. The anodic and cathodic currents of insulin on the surface of the silica gel/chitosan/$Ni(OH)_2$ paste electrode remain constant for 35 days at 110 μA and -430 μA, respectively. This indicates that the silica gel/chitosan/$Ni(OH)_2$ paste electrode has a good reproducibility of measurement (RSD 0.29 %).

4. Conclusions

The silica gel/chitosan/$Ni(OH)_2$ paste electrode has shown good performance for insulin determination. The sensitivity and LOD of the electrode are 5573×10^5 pA/pM cm^2) (R^2 = 0.9976) and 0.25 pM, respectively. This electrode also shows good selectivity for insulin detection; the signals of the insulin are relatively high in comparison with those of glucose, ascorbate acid, and urea. The reproducibility of the five electrodes is relatively good, with an RSD of 1.39 %. The stability of the electrode for 35 days is also relatively good, with an RSD of 0.29 %.

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