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ELECTROCHEMICAL BEHAVIOR OF INSULIN AT SILICA GEL/CHITOSAN/NICKEL NANOPARTICLES PASTE ELECTRODE

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Abstract

The electrochemical properties of silica gel/chitosan/nickel hydroxide nanoparticles paste electrode to detect insulin in phosphate buffer at pH 7.4 (physiological pH) were investigated by cyclic voltammetry. Nickel hydroxide nanoparticles, silica gel, chitosan, and paraffin were heated and stirred to obtain a homogen mixture. The mixture then mounted to the surface of silver disk electrode which was covered by glass tube. The composition variation of the mounted paste was investigated. Composition of silica gel in the mixture was varied at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. The result indicated that silica gel of 40% has the highest response signal. At the similar condition the composition of chitosan was varied at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. The highest signal was obtained at 60% chitosan. The amount of nickel hydroxide nanoparticles added was investigated at 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL. The highest signal was obtained by the addition of 2 mL nickel hydroxide nanoparticles. The variation of paraffin as a binder was also investigated at 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, and 35%. The best performance obtained by 15% paraffin. The insulin electro oxidation at silica gel/chitosan/nickel hydroxide nanoparticles paste electrode show the peak of anodic potential at 0.8 V.

Key words: Insulin, Silica gel, Nickel hydroxide nanoparticles, Chitosan

INTRODUCTION

Insulin is an important hormone which produced in pancreas. It has an important role in control the level of glucose in the blood. The amount of the insulin produced is based on to the level of glucose in the blood, so that the glucose concentration in the blood remains constant [1]. The inability of pancreas to produce insulin which is needed by the body called diabetes. The glucose concentration in the blood will be high if not enough insulin to neutralize it. There are 2 types of diabetes. When the human body can not produce insulin, it is called diabetes type I. Diabetes type 2 is when the body can produce insulin but the amount of insulin is not enough to control glucose levels in the blood.[2]. Therefore, determining concentration of insulin in the blood is an important supplementary information besides information of blood sugar level in diabetes patients.

Recently, high performance liquid chromatography (HPLC) was used to determine the insulin concentration in a sample for routine analysis. HPLC method requires complicated sample preparation prior to insulin analysis such as sample must be extracted in dichloromethane and then the water phase was re-extracted by hydrochloric acid [3]. All

process, i.e. the sample preparation and measurement, for insulin analysis by HPLC method consume time about 1 hour. So that several researches try to develop more simple method to determine insulin such as using the electrochemical method which in nature this method consume faster time of analysis. In general, the electrochemical method can directly detect a sample less than 10 minutes [4]. Many investigations have been reported on electrochemical sensors in the medicinal application. For examples in detection of glucose [5] [6], dopamine [7], urea [8], and also insulin [9] was reported using simple modified electrode with incorporating nanoparticles [10] [11] [12] [13].

Several articles about determination of insulin using modified electrode have been reported. Silica gel modified carbon paste electrode gave limit detection 36 pM at pH 2.0 with sensitivity 107.3 pA/pM [14]. Silica nanoparticles-nafion modified glassy carbon electrode has limit detection of 30 nM at pH 7.35 with sensitivity 300 pA/nM [15]. Nickel oxide nanoparticles modified carbon nanotube electrode produce limit detection of 6,1 nM and sensitivity 1.8 µA/ µM [16]. Nickel oxide nanoparticles (NiOx) and glassy carbon modified guanine electrode with limit detection 22 pM at pH 7.4, and sensitivity 100.9 pA/pM. The guanine has amine ($-NH_2$) functional group, so it has a good electro catalytic which contribute for improvement of the sensitivity and limit detection of the electrode for insulin determination [17]. Other material has similar functional group (amine) to guanine is chitosan. This amine group can catalyze the reduction and oxidation process which it has been proved in glucose sensor [18], ammonia sensor [19], and uric acid sensor [20].

Nickel nanoparticles also have big contribution in insulin sensor properties, because it has important role in electron transfer for reduction and oxidation of insulin. Another form of nickel compound is nickel hydroxide ($Ni(OH)_2$) which has almost similar properties with nickel oxide nanoparticles [21], but it can synthesized in more simple procedure was synthesized only by electro analysis of nickel with high voltage method [22]. $Ni(OH)_2$ was applied for glucose sensor [23] [24], and Vitamin D sensor [25]. In the present work, we report composition optimization of paraffin/silica gel/chitosan/$Ni(OH)_2$ paste electrode for detecting of insulin in phosphate buffer solution at pH 7.4 by cyclic voltammetry.

RESEARCH METHOD

Chemicals and materials

Silica gel kiesel G.60, K2HPO4, KH_2PO_4 were purchased from Merck. Insulin 100 IU/mL was purchased from R. Lantus. Chitosan was prepared by Instrumentation an Analytical Sciences Laboratory, ITS.Nickel hydroxide nanoparticles ($Ni(OH)_2$) was prepared using procedure by Budipramana et all. Briefly, nickel metal was electrolyzed in citric acid solution.at 100 ^{0}C. Then the electrolysis potential was kept constant at 55 V in stirring condition for 30 minutes. The $Ni(OH)_2$ nanoparticle solution obtained was used in the experiments.[22]. Paraffin solid, tube glass (diameter 0.5 cm and long 5 cm), silver wire (diameter 1 mm and length 7 cm), and aqua demineralization were bought from local market.

Instruments

Electrochemical experiments were performed using three-electrode cell system which platinum as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference electrode (RE), and modified electrode as working electrode (WE). All electrochemical measurements were carried out using potentiostat/galvanostat (eDaQ e-corder 410).

Fabrication of Paste Electrode

General procedure to fabricate the electrode is the following. Silica gel and chitosan was mixed together, and homogenized using magnetic stirrer. Then $Ni(OH)_2$ solution was added

and heated at 65^{o}C for 5 minutes before paraffin added. The stirring was continued stirred until the mixture becomes a solid. The homogenized mixture (10 mg) was inserted into tube glass while the silver wire was inserted and connected to the mixture from the other end of tube glass. Then the surface of paste electrode was polished using abrasion paper grade 2000. The influence of paraffin concentration in the paste electrode was studied (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, and 35% against total weight of silica gel and chitosan. The influence of silica gel and chitosan concentration also were studied (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%). Addition of $Ni(OH)_2$ solution at constant mixture of silica gel-chitosan-paraffin was varied at 0.0 mL, 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, and 5.0 mL.

RESULT AND DISCUSSION

Optimization of paraffin concentration in the fabrication of paste electrode

Optimization of paraffin concentration, which was used to bind all active material (silica gel and chitosan) for making paste electrode, was studied. 1.0 μM insulin solution in phosphate buffer at pH 7.4 was measured by paste electrode which has been made in various concentration of paraffin using cyclic voltammetry. The results can be seen in Table 1.

Table 1. Optimization of paraffin concentration in the fabrication of paste electrode

Paraffin	Silica gel + Chitosan (50%:50%)	Condition
1%	100%	Failed
2%	100%	failed
3%	100%	Failed
4%	100%	Failed
5%	100%	Failed
6%	100%	Failed
7%	100%	Failed
8%	100%	Failed
9%	100%	Failed
10%	100%	Failed
15%	100%	Current measured
20%	100%	Current unmeasured
25%	100%	Current unmeasured
30%	100%	Current unmeasured
35%	100%	Current unmeasured

Table 1 shows that the electrode with 1% - 10% paraffin can not be made since not enough binder so that when the electrode immersed in the sample solution, it was broken into pieces. In the other hand when the paraffin concentration was too high, for 20 – 35%, then the electrode became not conductive so no more current will be measured. In this work 15% paraffin gave the optimum response such as Fig.1.Fig 1 shows the cyclic voltammogram of the solution with (a) and without (b) 1.0 μM insulin in 0.1 M phosphate buffer at pH 7.4. No significant different can be seen at signal of blank and insulin sample.

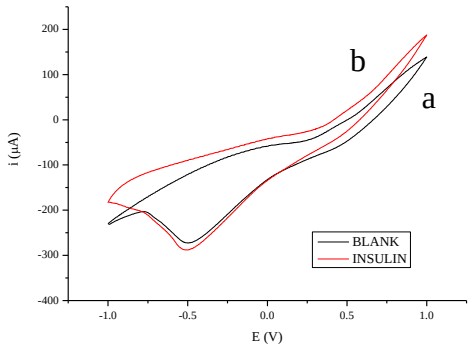

Fig. 1. Cyclic voltammogram of phosphate buffer at pH 7.4 (a) 1.0 µM insulin in phosphate buffer at pH 7.4. (b)

Optimization of silica gel and chitosan composition at paste electrode

Composition of silica gel and chitosan in the electrode has been investigated at varied concentration from 0%-100%. The electrodes were used to measure 1.0 µM insulin solution and the results were shown in Table 2.

Table. 2. Optimization of silica gel and chitosan compositon at paste electrode

Composition	Paraffin (%)	Silica Gel (%)	Chitosan (%)	i_{pa} (µA)	i_{pc} (µA)
1	15	0	100	20	-20
2	15	10	90	30	-30
3	15	20	80	40	-40
4	15	30	70	50	-50
5	15	40	60	71	-88
6	15	50	50	55	-55
7	15	60	40	50	-50
8	15	70	30	41	-45
9	15	80	20	31	-38
10	15	90	10	22	-24
11	15	100	0	14	-22

Table 2 show that the current observed was increase gradually from composition of number 1 to 6. Then the current obtaind decrease gradually from composition 6 to 11. The higher current obtained shows the higher electro catalytic process on insulin in the surface of the working electrode. Then the best composition of silica gel and chitosan for insulin determination was shown by silica gel 40% and chitosan 60% which gave signals of i_{pa} 71 µA and i_{pc} -88 µA. Cyclic voltammogram of this composition was shown in Fig. 2. Cyclic voltammogram of the solution with (a) and without (b) 1.0 µM insulin in 0.1 M phosphate buffer at pH 7.4 showed significant different in the current observed in comparison with Fig 1.

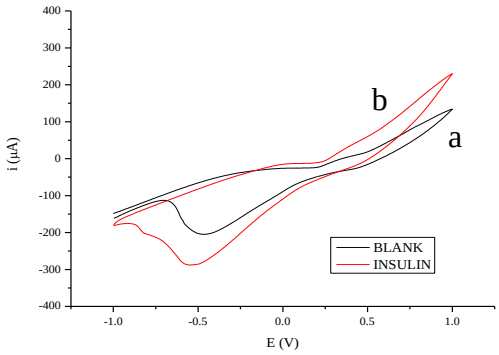

Fig. 2. Cyclic voltammogram of paste electrode compositon number 5 from Table 2 for determination absence (a) and 1.0 µM insulin (b) in phosphate buffer at pH 7.4.

Optimization of $Ni(OH)_2$ at paste electrode

The varied addition of $Ni(OH)_2$ solution in the electrode has been investigated from 0 mL – 5 mL. The electrodes, which have been made, were used for detection of 1.0 µM insulin solution in phosphate buffer at pH 7.4 and the results are shown in Table 3.

Table 3. Influence of $Ni(OH)_2$ concentration at paste electrode on current measured

Composition	Paraffin (%)	Silica gel (%)	Chitosan (%)	$Ni(OH)_2$ (mL)	i_{pa} (µA)	i_{pc} (µA)
1	15	40	60	0	71	-88
2	15	40	60	1	150	-175
3	15	40	60	2	320	-400
4	15	40	60	3	200	-350
5	15	40	60	4	80	-90
6	15	40	60	5	50	-60

Table 3 shows that the $Ni(OH)_2$ give a big influence on current measured. The current measured is increase gradually until 2 mL addition of $Ni(OH)_2$, then the current decrease for more addition of $Ni(OH)_2$. At optimum $Ni(OH)_2$ addition (2 ml), beside the current response is higher than the other, but the shape of voltammogram is quite different. The peak shift to the more negative value, and there are two peaks which are produced during anodic sweep. This difference is because of the presence of the insulin in solution.

The highest current in this works were achieved by the electrode with ratio silica gel: chitosan 4:6 with 15% paraffin as binder and addition 2 ml $Ni(OH)_2$ solution. The cyclic voltammogram of solutions in the absence (a) and in the presence (b) of 1.0 µM insulin in 0.1 M phosphate buffer at pH 7.4 can be seen at Fig 3. The i_{pa} and i_{pc} obtained are 320 µA and 400 µA respectively. It shows a significant increase of the current in comparison with Fig 1 and Fig 2.

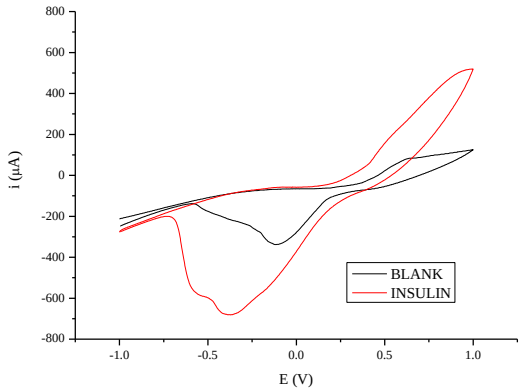

Fig. 3. Cyclic voltammogram of the solutions in the absence (a) and in the presence (b) of 1.0 μM insulin in 0.1 M phosphate buffer at pH 7.4 using the paste electrode composition number 3 from Table 3.

CONCLUSION AND SUGGESTION

Optimization of ratio paraffin, silica gel, chitosan, and $Ni(OH)_2$ for making insulin electrode have been done. The optimum composition is achieved by ratio silica gel:chitosan 4:6 with 15% paraffin as binder and addition 2 ml $Ni(OH)_2$ solution. Measurement was performed n in phosphate buffer at pH 7.4 by cyclic voltammetry. Cyclic voltammogram shows potential application of the sensor for insulin detection. According to these results, detail characterizations were needed such as and linier range of the measurement, Limit of Detection (LOD), sensitivity and selectivity of the electrode

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