

POLYANILINE-INVERTASE-GOLD NANOPARTICLES MODIFIED GOLD ELECTRODE FOR SUCROSE DETECTION

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ABSTRACT

Sucrose sensor has been made by deposited the active materials on the surface of gold electrode. The active materials, i.e. polyaniline (PANI), invertase and gold nanoparticles, were deposited step by step. Aniline polymerization were conducted electrochemically at potential -500 to 1000 mV using voltammetry method with sweep rate 50 mV/s for 20 cycles in HCl solution pH 1.5. The modified electrode obtained was immersed in invertase 1 M phosphate buffer solution pH 6. The invertase trapping in polyaniline was performed using the same condition as aniline polymerization. Then, gold nanoparticles were deposited on the polyaniline-invertase modified gold electrode using Layer by Layer (LbL) technique. The polyaniline-invertase-gold nanoparticles modified gold electrode obtained was used to measure sucrose solution. Electrochemical signal of polyaniline (PANI)-invertase-gold nanoparticles modified gold electrode is increase with sucrose concentration. The sensitivity and detection limit of the electrode are $0.4657 \mu\text{A mm}^{-2} \text{mM}^{-1}$ and $9 \mu\text{M}$, respectively. No electrochemical interference signals from fructose and glucose have been observed in the sucrose measurement.

Keywords: sucrose; sensor; invertase; polyaniline; gold nanoparticles

ABSTRAK

Sensor sukrosa telah dibuat dengan cara melapiskan bahan-bahan aktif ke permukaan elektroda emas. Bahan-bahan aktif yaitu polianilin (PANI), invertase dan nanopartikel emas dilapiskan secara bertahap. Polimerisasi anilin dilakukan secara elektrokimia pada potensial -500 sampai 1000 mV menggunakan metoda voltametri dengan laju sapuan 50 mV/s sebanyak 20 siklik dalam larutan HCl pH 1,5. Elektroda termodifikasi yang diperoleh di rendam ke dalam larutan invertase dalam buffer fosfat 1 M pada pH 6. Penjebakan invertase ke dalam polianilin dilakukan menggunakan kondisi yang sama seperti saat polimerisasi anilin. Setelah itu, nanopartikel emas di endapkan ke permukaan elektroda emas yang termodifikasi polianilin-invertase menggunakan teknik lapis demi lapis (LdL). Elektroda emas termodifikasi polianilin-invertase-nanopartikel emas yang diperoleh digunakan untuk mengukur larutan sukrosa. Sinyal elektrokimia dari Elektroda emas termodifikasi polianilin-invertase-nanopartikel emas meningkat seiring dengan meningkatnya konsentrasi sukrosa. Sensitivitas dan batas deteksi elektroda masing-masing adalah sebesar $0,4657 \mu\text{A mm}^{-2} \text{mM}^{-1}$ and $9 \mu\text{M}$. Tidak teramati adanya gangguan sinyal elektrokimia dari pengukuran fruktosa dan glukosa selama pengukuran sukrosa.

Kata Kunci: sukrosa; sensor; invertase; polianilin; nanopartikel emas

INTRODUCTION

Sucrose is the common sugar which is used as a sweetener in various foods and beverages. Sucrose has the chemical formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ often found in the form of white crystal and soluble in water. Generally sucrose is produced from sugar cane or sugar beet. Production of sugar in Indonesia reaches 2.54 million tons which is processed from 35.4 tons sugar cane in 2013 (Data obtains from Central Bureau of Statistics). The sugar

production process involves separation of sucrose from sugar cane, and then continued with its crystallization. So the amount of sugar obtained depends on the amount of sucrose in the sugar cane. In turn, quality of the sugar cane gives significant influence on the production of sugar aside from production process efficiency of the factory itself. Until now the analysis of sugar cane quality is conducted only by the factory because of the complexity of the analysis operation and procedures. Therefore, the development of

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analytical methods to determine sucrose content in sugar cane that are rapid, accurate, inexpensive, selective, sensitive and simple still continued until now. This also is necessary for sugar cane farmer to determine sugar cane quality by themselves. The farmer and the factory can measure the quality sugar cane together to appraise the price. The method which considered potential with the above criteria is electrochemical sensor [1-2].

The most common electrochemical biosensors for sucrose detection involve several types of enzymes [3-4]. The benefit of using enzyme is its specificity reaction in analysis, so it can be used in a complex matrix sample [5]. Even though the enzyme can be used repeatedly for small volume of sample but the enzyme activity will decrease significantly with the time. In the development of sucrose sensor, three enzymes were used to get better signal. Multiple reactions involves during the sucrose detection. Invertase, mutarotase and glucose oxidase was immobilized together on the electrode surface. In this case, there are several problems that arise in the fabrication of the electrode which uses three types of enzymes together. First, each enzyme is well known to have an optimal pH operation, i.e. invertase at pH 4.5, glucose oxidase at pH 5.5 and mutarotase at pH 7.4 [6]. Because of the optimal working pH of that three enzyme are different, the best measurement will be in the middle of pH range of that the three enzymes (i.e. pH 6) in order to assure that all enzymes can work in the same time. Then, in this circumstance only one enzyme will work optimal in that pH but not for the others. Second, the invertase will hydrolyze sucrose to α -D-glucose and β -D-fructose, and then mutarotase will convert α -D-glucose to β -D-glucose until equilibrium is established. But the presence of hydrogen peroxide produced from glucose hydrolysis by glucose oxidase lead to the decrease of mutarotase activity (mutarotase oxidation by peroxide). Third, the addition of glucose oxidase as a component of the electrode gave greater electrochemical signal to glucose than to sucrose at equal concentration. So the electrode has poor selectivity. Besides enzymes, nanoparticles also have important role in sensor. It can enhance electrocatalytic [7-9] or optical properties [10-11] of materials.

In this work, modification of the electrode is performed only with one enzyme, i.e. invertase, for sucrose sensor. The gold nanoparticles was added to increase the performance of the sensor. The advantage, the electrode is more simple in fabrication and operational than the electrode which uses three type of enzyme.

EXPERIMENTAL SECTION

Materials

Sucrose, fructose, and glucose were purchased from Merck, Germany. Aniline, sodium citrate, Na_2HPO_4 , NaH_2PO_4 , NaHCO_3 and HAuCl_4 was purchased from sigma. Aniline was purified prior to use by distillation technique. Yeast, sand paper grade 1500, cotton gauze, aluminum foil and shrinkage cable were bought from local market. Gold 24K was purchased from Antam, Indonesia. All chemicals were used as purchased without further purification unless mentioned. Deionized water was used for all dilution.

Instrumentation

Heat gun was used to heat the shrinkage cable for fabrication of gold electrode. Ultrasonic dialysis machine and centrifuge were used for preparing invertase. Optical microscopy (Olympus BX60) was used to observe the surface of the electrode. pH of the solution was adjusted using bench top pH meter. All the electrochemical experiments were performed using potentiostat 161 system from eDAQ which equipped with Echem software. Three-electrode cell system with platinum as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference electrode (RE) and gold electrode as working electrode (WE) was used using voltammetry technique. General laboratory glasswares were used during all chemicals preparation.

Procedure

Preparation of gold electrode

99.99% gold bullion was forged and formed into a gold wire with a diameter 1 millimeter. The gold wire obtained was cut into 5 cm in length, then washed and dried using acetone, ethanol and deionized water respectively. The clean gold wire was inserted in the cable shrinkage and then heated using heat gun in order to attach tightly the insulator layer around the surface of the gold wire with the both ends of the gold wire in open state. The surface of one end of the gold wire surface was polished with sandpaper grade 1500 to get a smooth surface prior to further modification.

Synthesis of gold nanoparticles

Synthesis of gold nanoparticles was carried out according to Kurniawan et al. [12]. Briefly, 10 mL of 1 mM HAuCl_4 solution was heated to boils, and then

1 mL of 1% sodium citrate was added while stirring using a magnetic stirrer. Color of the solution changed from pale yellow to red wine. The reaction was stopped by cooling the solution at room temperature.

Isolation of invertase

Invertase was isolated from yeast described as the following. 50 g yeast was placed in beaker glass, and added 100 mL 0.1 M NaHCO₃. The mixture was stirred slowly until become mush, and continued at 7000 rpm for 5 min using homogenizer. The slurry obtained was poured into Erlenmeyer; closed using cotton plug wrapped with cotton gauze and then covered using aluminum foil. The slurry was incubated at room temperature for 24 h and then autolyzed using ultrasonic dialysis machine at 16 rms for 20 min. The autolyzed yeast obtained was centrifuged at 3500 rpm for 10 min; in turn it formed two separated layers (i.e. supernatant and biomass). The supernatant was collected by decantation for further experiment, and the biomass was discarded.

Preparation of polyaniline-invertase-gold nanoparticles modified gold electrode

Electropolymerization on the surface of gold electrode was done at potential -500 mV to +1000 mV using scan rate 50 mV/s for 20 cycles in 0.1 M aniline in 0.5 M HCl as electrolyte solution. The pH was adjusted at 1.5 using NaOH solution before electropolymerization performed. The polyaniline modified gold electrode obtained was soaked in 1 M phosphate buffer pH 6 solution. Invertase as described above was added with concentration 230 μ L in 25 mL. Trapping of invertase in polyaniline on the surface of polyaniline modified gold electrode was performed using the same technique and condition as the aniline polymerization that was described in the procedure above. The surface of polyaniline-invertase modified gold electrode obtained was monitored using optical microscopy. Deposition of gold nanoparticles on the surface of polyaniline-invertase modified gold electrode was done using Layer by Layer (LbL) deposition technique [13]. Briefly a Polyaniline-invertase modified gold electrode was immersed into gold nanoparticles solution which was prepared above for 15 min, then dried at room temperature. This treatment was repeated once again, and then it was soaked again into gold nanoparticles solution for 24 h. The surface of polyaniline-invertase-gold nanoparticles modified gold electrode obtained was also monitored using optical microscopy.

Electrochemical measurement

All electrochemical measurements were performed using three-electrode cell system which platinum as counter electrode (CE), Ag/AgCl (KCl 3 M) as reference

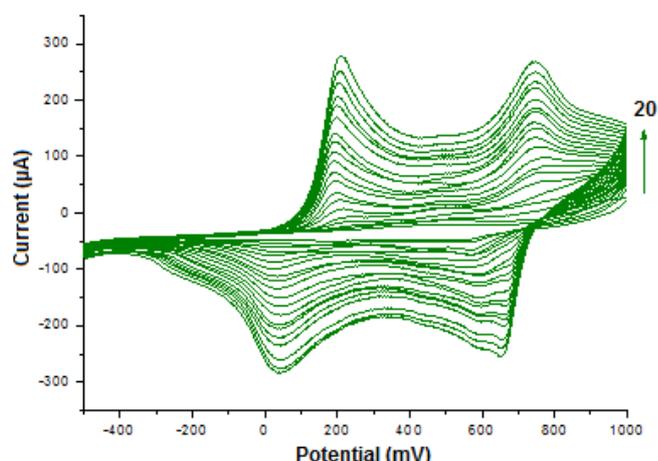


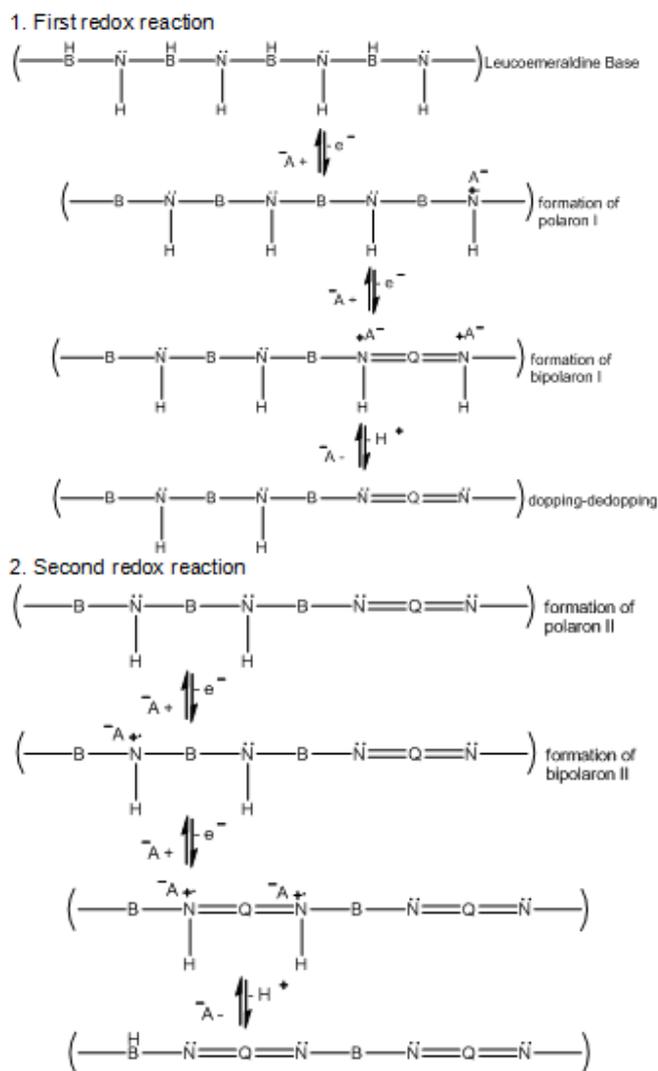
Fig 1. Voltammogram of aniline electropolymerization on the surface of gold electrode

electrode (RE) and a gold electrode, polyaniline modified gold electrode, polyaniline-invertase modified gold electrodes or polyaniline-invertase-gold nanoparticles modified electrodes as working electrodes (WE). Voltammetry technique was done at potential range -1000 mV to +1000 mV using scan rate 100 mV/s for 5 cycles to all samples. The sucrose standard solution (1–50 mM) was made by dissolving sucrose in phosphate buffer solution. Sucrose, fructose and glucose were measured to analyze the selectivity of the electrode obtained. Sensitivity of the electrode is calculated from the gradient plot of electrochemical signal (at maximum potential) vs. sucrose concentration. The limit of detection is calculated using 3σ of blank electrochemical signal. All measurements were done in 1 M phosphate buffer pH 6.78 unless mentioned.

RESULT AND DISCUSSION

Preparation of the Electrodes

Voltammogram of aniline electropolymerization for 20 cycles are shown at Fig. 1. The current is increased gradually for further cycles. It means the polyaniline layers have formed on the surface of gold electrode successfully. There are two peaks observed in both anodic and cathodic sweep. Anodic peak observed at +197 mV with current responses from 6 to 276.87 μ A, and at +743 mV with current responses from 52 to 269.2 μ A. The cathodic peak was found at 44 mV and 654 mV with current responses from -54,825 to -284 μ A, and -36,715 to -255,132 μ A respectively. Peak at +197 mV is the oxidation peak of leucoemeraldine (Polyaniline in fully reduced state) which is oxidized into emeraldine (Polyaniline in semi-oxidized state), while +743 mV is the oxidation peak of



Scheme 1

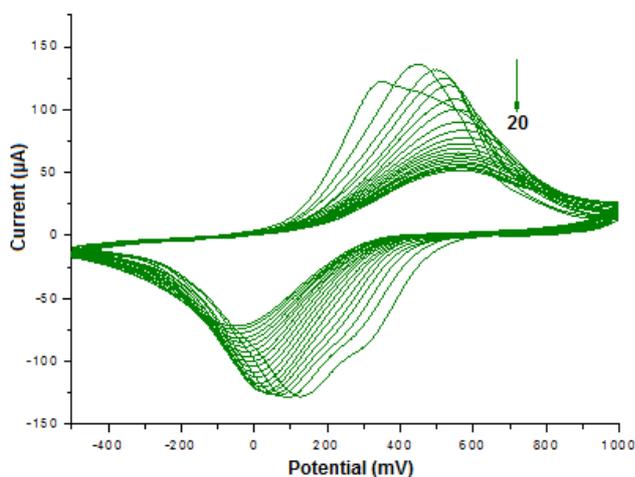


Fig 2. Voltammogram of polyaniline modified gold electrode in the presence of invertase in buffer phosphate solution

emeraldine which is oxidized into pernigraniline (full oxidized form of polyaniline) [14].

Electropolymerization was carried out at pH 1.5 in order to obtain conductive polymers. Polyaniline has to form head to tail coupling, where this is only occurred in acidic conditions. In more basic condition head to head coupling will take place, and as the result will form non-conjugated polymer which has nonconductive properties [15]. Doping-dedoping of polyaniline during electropolymerization reaction are shown at scheme 1.

Modification was continued by trapping of invertase on the surface of polyaniline modified gold electrode. Trapping was done in invertase solution which is described in experimental section. A small amount of aniline which remains in the surface of polyaniline modified gold electrode from previous polymerization process will be polymerized. Voltammogram of polyaniline modified gold electrode in the presence of invertase in 1M phosphate buffer solution pH 6 is shown at Fig. 2.

Voltammogram shows the current decrease for further cycles. Electropolymerization in a small amount aniline aims to trap invertase only on the surface of polyaniline modified gold electrode. Condition of the polymerization is similar to condition polymerization of aniline but at different pH. pH 6 was chosen according to pH stability of invertase [16], because in the low pH invertase will be denaturated. Even though the polyaniline formed at pH 6 is non-conductive layer [15], but we already have a conductive layer from previous aniline polymerization. If we keep non-conductive layer as thin as possible, the electrode should work as sucrose sensor. The voltammogram shows that the current is decreased gradually for further cycles because no more aniline available in the solution can be polymerized. The current is decreased from 132 to 49.6 μA after 20 cycles. The polymer-invertase modified gold electrode obtained was cleaned by immersing it to fresh buffer phosphate pH 6.

Last step, polyaniline-invertase modified gold electrode was immersed in gold nanoparticles solution which was described at experimental section. Gold nanoparticles were deposited automatically during the immersion. The immersion was performed only three times (i.e. the first for 15 min, the second for 15 min, and third for 24 h. The electrode was dried at room temperature before the next immersion to keep stability of deposited gold nanoparticles in the surface of polyaniline-invertase modified gold electrode. More immersion will give thicker gold nanoparticles, but the stability of the gold nanoparticle layer will be decrease. As the result, the gold nanoparticles layer will peel off from the surface of polyaniline-invertase modified gold electrode [12]. The surface images of gold electrode, polyaniline-invertase modified gold electrode, and

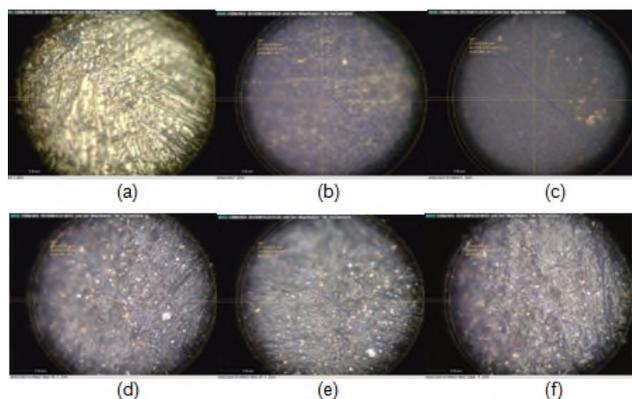


Fig 3. Surface images of (a) gold electrode (b) polyaniline modified gold electrode (c) polyaniline-invertase modified gold electrode (d) (e) (f) polyaniline-invertase-gold nanoparticles modified gold electrode after immersion in gold nanoparticles solution for 15 min, 30 min, and 24 h, respectively

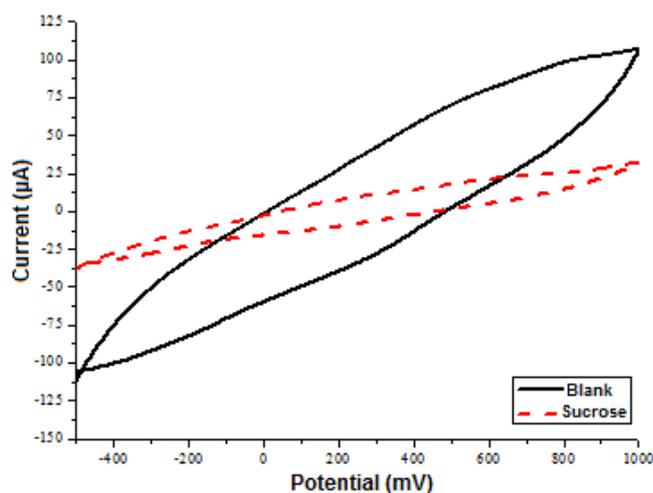


Fig 5. Voltammogram of polyaniline modified gold electrode in the presence (red) and in the absence (black) of 100 mM sucrose

polyaniline-invertase-gold nanoparticles modified gold electrode obtained were shown at Fig. 3.

Color change on the surface of the electrode can be monitored for each modification step. Electropolymerization of aniline changes the surface color of the electrode from yellow (Fig. 3a) to black (Fig. 3b). Trapping invertase in polyaniline, that also involves electropolymerization of aniline, changes to darker color (Fig. 3c) than in previous condition. Fig. 3(d), (e) and (f) show gradual change to brighter color that indicates the more immersion in gold nanoparticles solution, the more gold nanoparticles will be deposited on the surface of polyaniline-invertase modified gold electrode.

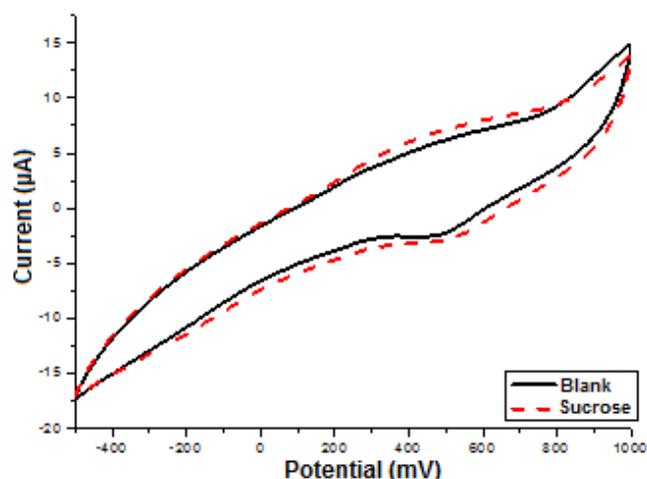


Fig 4. Voltammogram of gold electrode in the present (red) and in the absence (black) of 100 mM sucrose

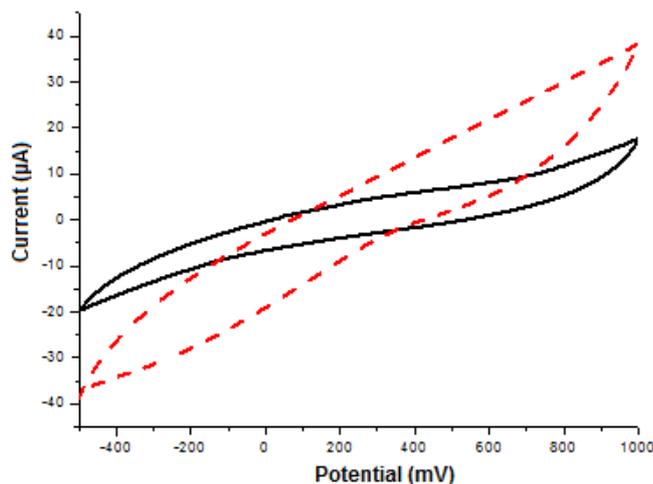


Fig 6. Voltammogram of polyaniline-invertase modified gold electrode in the present (red) and in the absence (black) of 100 mM sucrose

Characterization of the Electrodes

Voltammogram of gold electrode in the present and in the absence of 100 mM sucrose in phosphate buffer solution pH 6.78 using scan rate 100 mV/s can be seen at Fig. 4. The electrochemical signal obtained is relatively low, and also there is no significant difference of electrochemical signal in the presence and in the absence of sucrose. It means that the gold electrode does not sensitive to sucrose.

Fig. 5 is voltammogram of polyaniline modified gold electrode in the present and in the absence of 100 mM sucrose in phosphate buffer solution pH 6.78 using

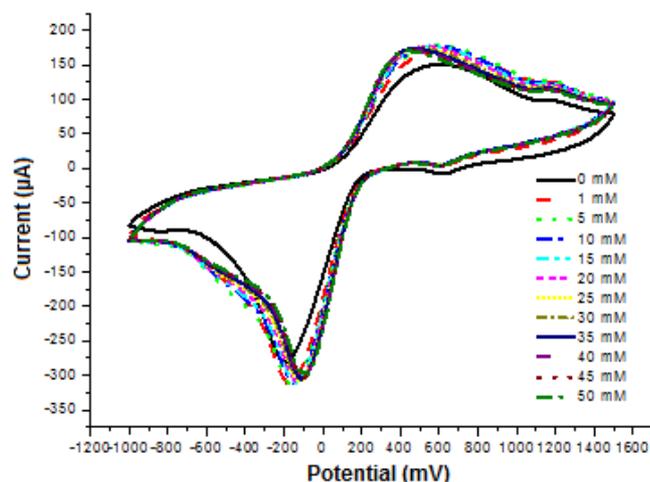


Fig 7. Voltammogram of polyaniline-invertase-gold nanoparticles modified gold electrode at various sucrose concentration

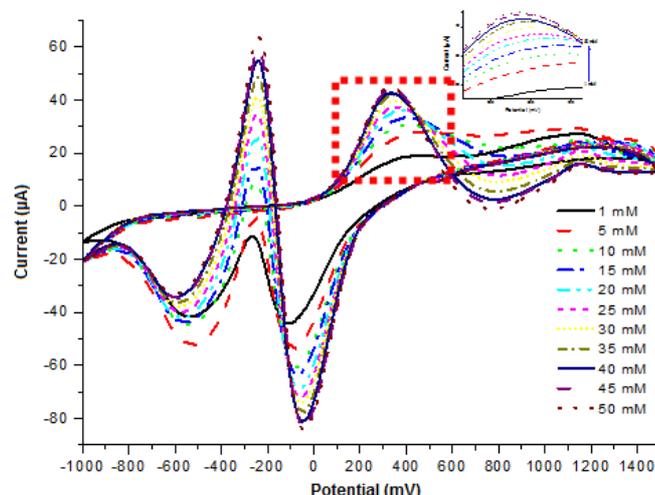


Fig 8. Voltammogram of polyaniline-invertase gold nanoparticles modified gold electrode at various sucrose concentration after corrected by the blank

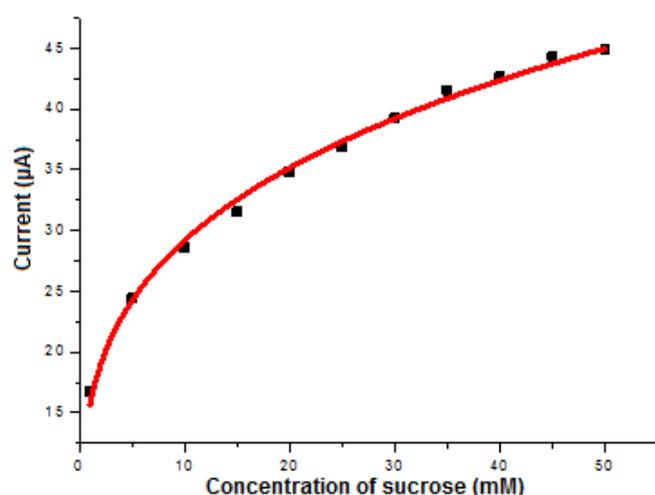


Fig 9. Dependence current measured at various sucrose concentrations at potential +328 mV

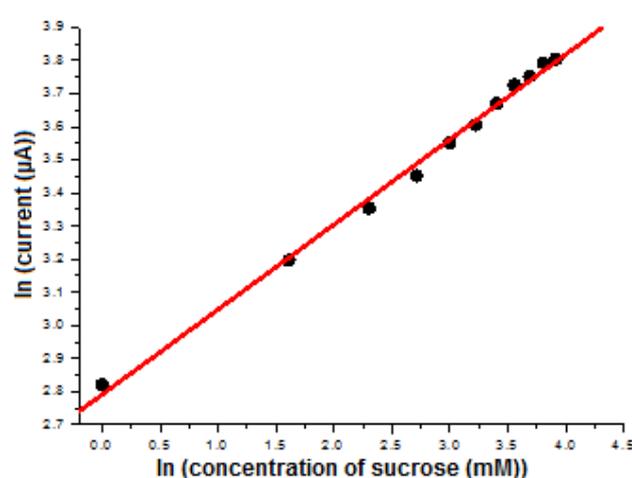


Fig 10. Linear calibration curve of simplified equation for sucrose detection using polyaniline-invertase-gold nanoparticles modified gold electrode

scan rate 100 mV/s. The electrochemical signal of polyaniline modified gold electrode in the absence of sucrose is higher than in the presence of sucrose. Addition of sucrose gives lower signal most probably because sucrose is non electroactive species. More sucrose added in the solution, lower electrochemical signal will be obtained. In this circumstance the polyaniline modified gold electrode also cannot be used for sucrose sensor.

The different circumstance is shown by voltammogram of polyaniline-invertase modified gold electrode in Fig. 6. The electrochemical signal of polyaniline-invertase modified gold electrode in the present of sucrose is higher than in the absence of sucrose. Addition of invertase in polyaniline has

converted sucrose to an electroactive species. Invertase breaks the bonding in sucrose to form glucose and fructose, and then the glucose can be oxidized and the oxidized product also can be reduced again. Even though the redox reaction can take place, but the voltammogram shows a sloping shape without peak that indicates a slow redox reaction rate. The electrochemical signal is also relatively low in comparison to voltammogram of polyaniline-invertase-gold nanoparticles modified gold electrode at Fig. 7.

Sucrose oxidation and reduction peak at polyaniline-invertase-gold nanoparticles modified gold electrode were observed at potential +433 mV and -141 mV respectively. The oxidation peak of polyaniline-invertase-gold nanoparticles modified gold

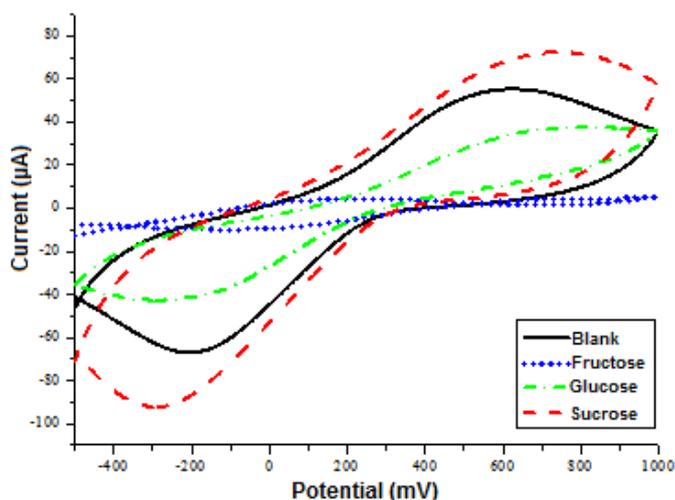


Fig 11. Voltammogram of polyaniline-invertase-gold nanoparticles modified gold electrode in the presence of 100 mM sucrose, fructose or glucose

electrode shifts to more positive and the reduction peak shifts to more negative with sucrose concentration. Fig. 8 is voltammogram of polyaniline-invertase-gold nanoparticles modified gold electrode in the presence of sucrose after corrected by the blank. The voltammogram shows clear increment with sucrose concentration. It means that polyaniline-invertase-gold nanoparticles modified gold electrode can be used for sucrose detection. A regular increase was shown at potential -266, -45, and 328 mV. Electrochemical signal at all these potential can be used for sucrose detection, but only one of them (at +328 mV) will be discussed in this paper. However, the same procedures and calculation also can be applied to two other potentials (-266 mV and -45 mV).

The points obtained at Fig. 9 are fitted using nonlinear curve fitting to get a calibration curve using origin software. The best fitting have been done using allometric equation (i.e. $a + bx^c$) that is drawn in red line. The equation obtained is as follows:

$$y = 7.8476 + 8.9968^{0.3659}x$$

The coefficient correlation (R^2) of the equation is 0.9983. This complicated equation can be simplified by converting it to a linear equation as the following:

$$\ln y = \ln 7.8476 + \ln 8.9968 + 0.3659 \ln x$$

$$\ln y = 4.2571 + 0.3659 \ln x$$

This equation can be redrawn as shown at Fig. 10. The coefficient correlation (R^2) of this equation is 0.9975.

Sensitivity and limit of detection of polyaniline-invertase-gold nanoparticles modified gold electrode was determined using linear curve of simplified equation in the Fig. 10. The calculation shows that the sensitivity and limit detection of the polyaniline-invertase-gold

nanoparticles modified gold electrode are $0.4657 \text{ mm}^{-2} \text{ mM}^{-1}$ and $9 \text{ }\mu\text{M}$, respectively.

Selectivity of the polyaniline-invertase-gold nanoparticles modified gold electrode

Selectivity of polyaniline-invertase-gold nanoparticles modified gold electrode for sucrose detection was studied in the presence of glucose and fructose in. The voltammogram of polyaniline-invertase-gold nanoparticles modified gold electrode in the presence of 100 mM sucrose, glucose or fructose can be seen at Fig. 11. Electrochemical signal of sucrose and fructose solution was below the blank. Only the sucrose gives positive electrochemical signal. It indicates that polyaniline-invertase-gold nanoparticles modified gold electrode has good selectivity for sucrose detection.

CONCLUSION

Detection of sucrose using gold electrode and polyaniline modified gold electrode does not give electrochemical signal. Whilst the polyaniline-invertase modified gold electrode give low electrochemical signal and a slow redox reaction in the voltammogram profile. Deposition of gold nanoparticles on the surface of polyaniline-invertase modified gold electrode shows great improvement on electrochemical signal. It has been proved can be used for selective sucrose sensor in the presence of fructose and glucose because both of the compounds does not give electrochemical signal during detection. The sensitivity of the electrode is $0.4657 \text{ }\mu\text{A. mm}^{-2} \text{ mM}^{-1}$. Detection limit of the electrode is $9 \text{ }\mu\text{M}$.

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REFERENCES

1. Shekarchizadeh, H., Ensafi, A.A., and Kadivar, M., 2013, *Mater. Sci. Eng., C*, 33 (6), 3553–3561.
2. Aytaç, S., Kuralay, F., Boyacı, İ.H., and Unaleroglu, C., 2011, *Sens. Actuators, B*, 160 (1), 405–411.
3. Bisenberger, M., Bräuchle, C., and Hampp, N., 1995, *Sens. Actuators, B*, 28 (3), 181–189.
4. Gülce, H., Çelebi, S.S., Özyörük, H., and Yildiz, A., 1995, *J. Electroanal. Chem.*, 397 (1-2), 217–223.

5. Mohammadi, H., Amine, A., Cosnier, S., and Mousty, C., 2005, *Anal. Chim. Acta*, 543 (1-2), 143–149.
6. Soldatkin, O.O., Peshkova, V.M., Dzyadevych, S.V., Soldatkin, A.P., Jaffrezic-Renault, N., and El'skaya, A.V., 2008, *Mater. Sci. Eng., C*, 28 (5-6), 959–964.
7. Kurniawan, F., Tsakova, V., and Mirsky, V.M., 2009, *J. Nanosci. Nanotechnol.*, 9 (4), 2407–2412.
8. Budipramana, Y., Suprpto, S., Ersam, T., and Kurniawan, F., 2014, *ARPN J. Eng. Appl. Sci.*, 9 (11), 2074–2077.
9. Kurniawan, F., 2008, New Analytical Applications of Gold Nanoparticles, *Dissertation*, University of Regensburg, Germany.
10. Krishnamoorthy, S., 2015, *Curr. Opin. Biotechnol.*, 34, 118–124.
11. Bu, L., Shen, B., and Cheng, Z., 2014, *Adv. Drug Delivery Rev.*, 76, 21–38.
12. Kurniawan, F., Tsakova, V., and Mirsky, V.M., 2006, *Electroanalysis*, 18 (19-20), 1937–1942.
13. Dhand, C., Das, M., Datta, M., and Malhotra, B.D., 2011, *Biosens. Bioelectron.*, 26 (6), 2811–2821.
14. Modarresi-Alam, A.R., Amirazizi, H.A., Movahedifar, F., Farrokhzadeh, A., Asli, G.R., and Nahavandi, H., 2015, *J. Mol. Struct.*, 1083, 17–26.
15. Yu, E.H., and Sundmacher, K., 2007, *Proc. Saf. Environ. Prot.*, 85, 489–493.
16. Ivanov, S., Kurniawan, F., Tsakova, V., and Mirsky, V.M., 2009, *Macromol. Mater. Eng.*, 294 (6-7), 441–444.