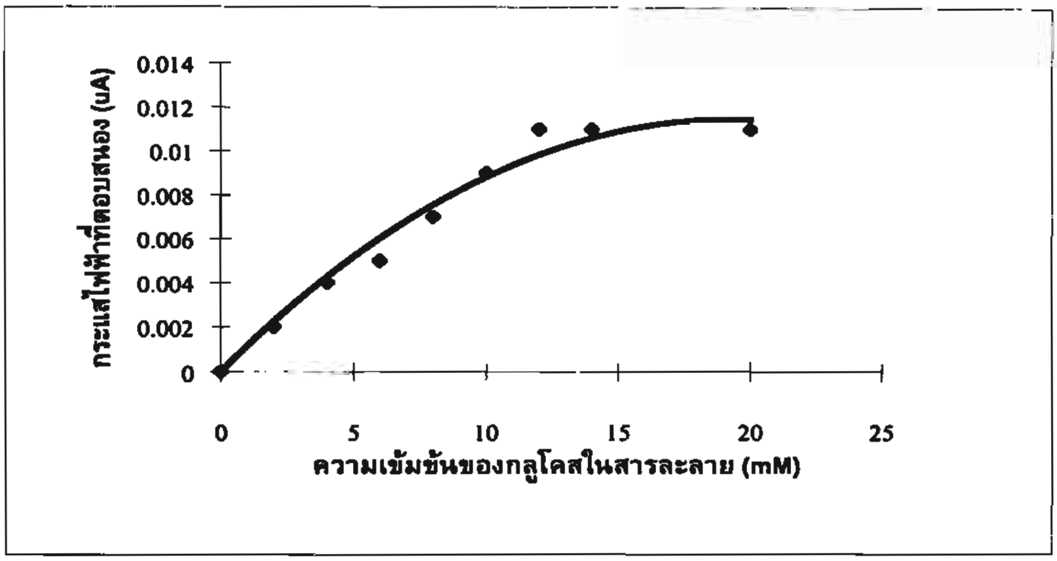
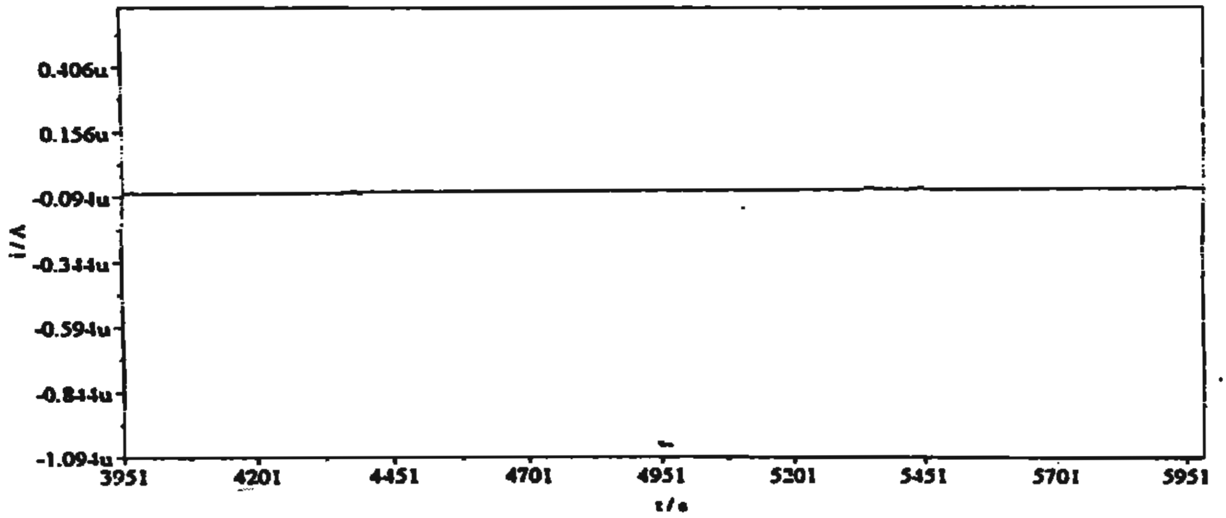


รูปที่ 37 แสดงความสัมพันธ์ระหว่างความเข้มข้นของน้ำตาลกลูโคสมาตรฐานและกระแสไฟฟ้าที่ตอบสนอง ที่ศักย์ไฟฟ้า 0.4 V

ถ้านำอิเล็กโทรดที่ไม่มี $Ru(NH_3)_6Cl$ ตรึงอยู่ในฟิล์มพอลิไพโรลมาทำการวัดความเข้มข้นของน้ำตาลกลูโคสมาตรฐานที่ศักย์ไฟฟ้า 0.0 V ไม่สามารถวัดค่ากระแสไฟฟ้าที่เกิดขึ้นได้ เนื่องจากไม่มีตัวกลางส่งผ่านอิเล็กตรอนที่มาทำหน้าที่แทนออกซิเจนซึ่งเป็นตัวกลางส่งผ่านอิเล็กตรอนในธรรมชาติ

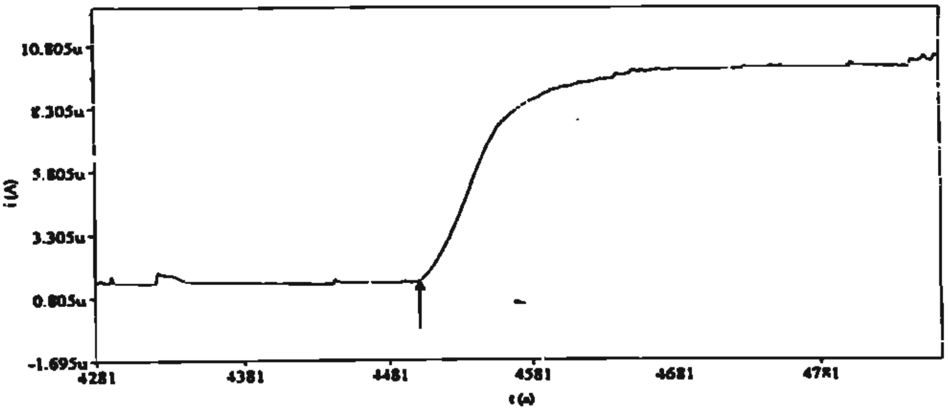


รูปที่ 38 แสดงความสัมพันธ์ระหว่างกระแสไฟฟ้าที่ตอบสนองกับเวลาในการตรวจวัดน้ำตาลกลูโคสมาตรฐานที่ศักย์ไฟฟ้า 0.0 V โดยใช้อิเล็กโทรดที่ไม่มีการตรึง $Ru(NH_3)_6Cl_3$

การทดสอบคุณภาพและความสามารถของอิเล็กโทรด

เวลาในการตอบสนองของกระแสสัญญาณ (response time)

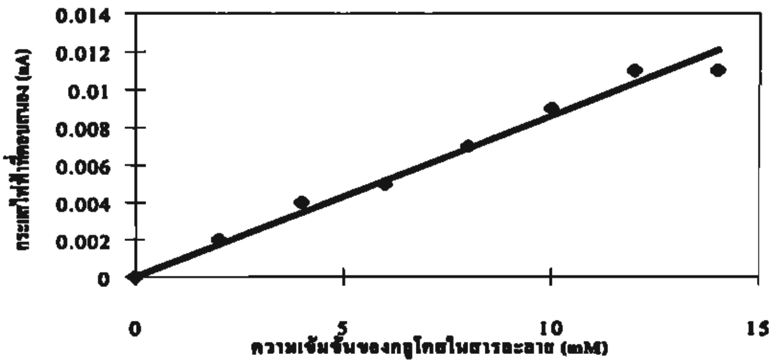
จากการศึกษาเวลาในการตอบสนองของกระแสสัญญาณของอิเล็กโทรดที่ทำการเตรียมฟิล์มพอลิไพโรลและมีการตรึงกลูโคสออกซิเดส โดยมี $Ru(NH_3)_6Cl$ เป็นตัวกลางส่งผ่านอิเล็กตรอน ทำการวัดปริมาณกลูโคสที่ศักย์ไฟฟ้า 0.4 V พบว่าเวลาในการตอบสนองของกระแสสัญญาณ คือ 5 วินาที ซึ่งแสดงดังรูปที่ 39



รูปที่ 39 แสดงเวลาในการตอบสนองของกระแสสัญญาณ เมื่อทำการตรวจวัดน้ำตาลกลูโคสมาตรฐาน ที่ศักย์ไฟฟ้า 0.4 V

ความไว (sensitivity)

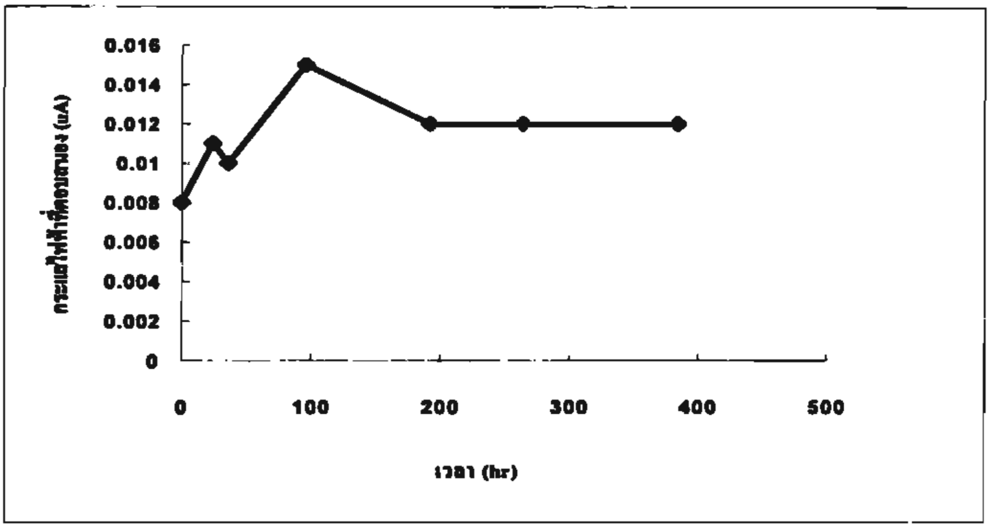
จาก calibration curve ของสารละลายมาตรฐานกลูโคส เมื่อทำการวัดปริมาณกลูโคสที่ศักย์ไฟฟ้า 0.4 V สามารถคำนวณความไวได้เท่ากับ 5.5 x 10^{-9} A/mM ดังรูปที่ 40



รูปที่ 40 แสดง ความสัมพันธ์ระหว่างความเข้มข้นของน้ำตาลกลูโคสมาตรฐานและกระแสไฟฟ้าที่ตอบสนองที่ศักย์ไฟฟ้า 0.4 V

ความเสถียร (stability)

ทำการตรวจวัดปริมาณกลูโคสในสารละลายมาตรฐาน ความเข้มข้น 12 mM ที่ค่าศักย์ไฟฟ้า 0.4 V ภายหลังทำการพัฒนาอิเล็กโทรดโดยเตรียมฟิล์มพอลิไพโรลที่มีการตรึงกลูโคสออกซิเดส และ Ru$(NH_3)_6$Cl ในช่วงเวลาต่างๆ ดังแสดงในรูปที่ 41



รูปที่ 41 แสดง ความสัมพันธ์ระหว่างระยะเวลาและกระแสไฟฟ้าที่ตอบสนองของสารละลายกลูโคสมาตรฐาน ที่ศักย์ไฟฟ้า 0.4 V

สรุป

งานวิจัยนี้สามารถสร้างแอมเพอโรเมทริกไบโอเซนเซอร์สำหรับวัดน้ำตาลกลูโคสได้ โดยการเตรียมแผ่นฟิล์มพอลิไพโรลที่มีการตรึง $Ru(NH_3)_6Cl_3$ และเอนไซม์กลูโคสออกซิเดส ด้วยกระบวนการอิเล็กโตรพอลิเมอไรเซชัน พบว่าภาวะที่เหมาะสมที่ใช้ในการเตรียมอิเล็กโทรดนี้มีดังนี้

- การทำอิเล็กโตรพอลิเมอไรเซชันของเอนไซม์บนอิเล็กโทรด :
  - อิเล็กโทรดขั้วทำงาน: ขั้วทองอิเล็กโทรด
  - ขั้วอ้างอิง: Ag/AgCl อิเล็กโทรด
  - ขั้วเคาน์เตอร์: อิเล็กโทรดแพลตินัม

- มอโนเมอร์ ไพโรล: ความเข้มข้น 0.1 mM
- เอนไซม์: กลูโคสออกซิเดส (<u>Aspergillus niger</u>) 50 units/mL
- สารละลายบัฟเฟอร์และค่า pH: ฟอสเฟสบัฟเฟอร์ ความเข้มข้นประมาณ 0.1 M pH = 7
- ค่าศักย์ไฟฟ้าที่ใช้ในการพอลิเมอไรเซชัน 0.7 V เทียบกับ Ag/AgCl อิเล็กโทรด
- ปริมาณไฟฟ้าที่ใช้ในการพอลิเมอไรเซชัน 45 mC/cm^2
- การทำอิเล็กโตรสแตติกไบดิง
  - ใช้ปริมาณ nafion 1 μL เวลาในการรอให้ nafion แห้ง 10 นาที
  - สารละลาย $Ru(NH_3)_6Cl_3$ 10 mM จุ่มเป็นเวลา 10 นาที
- ค่าศักย์ไฟฟ้าที่ใช้ในการวัดกลูโคส 0.4 V เทียบกับ Ag/AgCl อิเล็กโทรด
- ภาวะที่ใช้ในการวัด
  - 0.1 M ฟอสเฟสบัฟเฟอร์ ค่า pH = 7 ที่ 25 °C
- sensitivity 9.0×10^{-10} A/mM
- เวลาตอบสนอง 80 วินาที
- storage stability มากกว่า 16 วัน
- ความเข้มข้นต่ำสุดที่วัดได้ 1.5 mM

Output

ผลงานวิจัยที่ตีพิมพ์

- Chailapakul, O.; Promnil. J.; Somasandrum, M. and Tanticharoen, M. "Immobilized $K_3F_e(CN)_6$ and Glucose Oxidase in polypyrrole on gold microelectrode and its application as glucose sensors" *J. Sci. Res. Chula. Univ.* (In press. June 2000 Issue)
- Ngamukot, P.; Chailapakul, O. and Tanticharoen, M. " Optimal condition for Preparation of Polypyrrole Films with Immobilization of Glucose Oxidase and $Ru(NH_3)_6Cl_3$ " *Analytical Sciences* , (Submitted 2000)

การไปเสนอผลงาน

- Chailapakul, O.; Promnil. J.; Somasandrum, M. and Tanticharoen, M. "Immobilized $K_3F_e(CN)_6$ and Glucose Oxidase in polypyrrole on gold microelectrode " ISE Sponsored Pre-Satellite Meeting of the 49th Annual Meeting of the ISE, Seoul, September 9-12. 1998
- Ngamukot, P.; Chailapakul, O. and Tanticharoen, M. " Optimal condition for Preparation of Polypyrrole Films with Immobilization of Glucose Oxidase and $Ru(NH_3)_6Cl_3$ ", *25th Congress on Science and Technology of Thailand*, pisanulok, October 24-26, 1999.
- Ngamukot,P Chailapakul, O. and Tanticharoen, M. "Amperometric Glucose Sensor Using $Ru(NH_3)_6Cl_6$-Nafion Coated Gold Electrode for Flow-Injection Analysis "The fourth Princess Chulabhorn International Science Congress: Chemicals in the 21st Century, Bangkok, December 28 1999–January 2 2000.
- Chailapakul, O.; Promnil. J.; Somasandrum, M. and Tanticharoen, M. "Immobilized $K_3F_e(CN)_6$ and Glucose Oxidase into polypyrrole on gold microelectrode and Its Application as Glucose Sensors" การประชุมเพื่อเสนอผลงานวิจัย โครงการทุนวิจัยหลังปริญญาเอก ครั้งที่ 1 โรงแรมเฟริกซ์ม กาจณบุรี 28-30 มกราคม 2543

ภาคผนวก

Optimal Condition for Preparation of Polypyrrole Film with Immobilization of Glucose Oxidase and $Ru(NH_3)_6Cl_3$

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Abstract

Preliminary studies of a new preparation procedure for an amperometric glucose sensor were investigated. The enzyme glucose oxidase was immobilized on the surface of a nafion coated gold electrode by entrapment in an electropolymerized film of pyrrole. Glucose responses were measured amperometrically via a $Ru(NH_3)_6Cl_3$ mediator. The optimal conditions for preparation of the sensor, the effects of applied potential, and the capability of the sensor were investigated. With the optimized glucose sensor, the detection limit was 1.5 mM and sensitivity was 9.0×10^{-10} A/mM. The response time was 80 seconds and the linearity of the sensor response ranged from 2 to 12 mM glucose solutions. The long-term stability of the sensor was improved by the electrostatic interaction between $[Ru(NH_3)_6]^{3+}$ and immobilized sulfonate groups of nafion. The storage and operational stability of the optimized glucose sensor exceeded 16 days and 9 times, respectively.

Introduction

Today electrochemical detection has clearly proved the favored method for use in biosensors. Electrochemical sensors may be divided into two categories: potentiometric devices that monitor the potential changes and amperometric devices that record the current flows when the potential is held at a constant value [1]. The first category includes pH meters, ion-selective electrodes and ion-selective field effect transistors. These instruments suffer from the inherent characteristics of potentiometry, i.e., rather slow response to solutions at low concentrations of analyte and high detection limits. Amperometric determination offers better sensitivity, lower detection limits and relatively low cost.

A further classification of amperometric biosensors may be made into direct and indirect electron transfer systems (as shown in Fig 1) [2]. For a direct amperometric biosensor, electron transfer occurs freely between the redox center of a catalytic protein and the electrode [3]. A cyanometalate-modified electrode based on direct electron transfer has been reported [4]. A more practical way is the use of electron mediators that shuttle electrons between the protein and the electrode. A mediator becomes important in attempts to eliminate oxygen depletion effects on signals of oxidoreductase sensors. Consider the following problem in the case of amperometric glucose sensors.

$$\text{Glucose} + \text{GOx} \longrightarrow \text{GOx}_{red} + \text{Gluconolactone}$$

$$\text{GOx}_{red} + O_2 \longrightarrow \text{GOx} + H_2O_2$$

$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} H_2O_2 + \text{Gluconolactone} \qquad \text{overall}$$

Glucose oxidase is a flavoprotein, which catalyzes the oxidation of glucose to gluconolactone. The natural electron mediator is oxygen, which is reduced to hydrogen peroxide. The operating potential for this sensor is +0.7 V (vs Ag/AgCl). At this potential, ascorbic acid and uric acid are very important interferences. The linearity of the calibration curve is affected by the depletion of oxygen in the sample. This mediated approach is used to remove oxygen dependence from the sensor's function and avoid the interferences. Several mediators have been reported. Compounds such as ferrocene have been shown to be well suited to this purpose [5,6].

Systems with the application of conducting polymer-modified electrodes have been developed. The optimal conditions for the preparations of polypyrrole films on platinum electrodes have been reported [7,8]. The electropolymerization of polypyrrole, polythiophene and polyaniline on the nafion-modified electrode have been investigated [9].

This paper reports a preliminary investigation of a new preparation procedure for an amperometric glucose sensor. Glucose oxidase and $Ru(NH_3)_6Cl_3$ were immobilized on a gold disk electrode, pre-coated with nafion, by entrapment in an electropolymerized film of pyrrole.

Experimental Section

Materials and Equipment.

The enzyme glucose oxidase (GOx) from *Aspergillus niger* was purchased from Sigma. Anhydrous dipotassium hydrogen orthophosphate was purchased from BDH. Potassium chloride, potassium ferricyanide and pyrrole were all from Merck. Nafion 117 solution was purchased from Fluka. Hexaammine ruthenium (III) chloride was purchased from Aldrich and glucose anhydrous from CARLO-ERBA. All electrochemical experiments were conducted with the PGSTAT10 potentiostat with the operating software (GPES version 4.4). The electrode polishing kit and Ag/AgCl reference electrode were purchased from BAS (Bioanalytical Systems, Inc.). The homemade gold disk electrode (6 mm diameter) and platinum gouge electrode were used as working and counter electrode, respectively. The three-electrode system was used in all experiments.

Surface Preparation of Gold Disk Electrode.

In order to prepare the gold disk electrode for determination of the electrode area or enzyme modification, the electrode surface must be rinsed with distilled water followed by methanol to flush away any encrusted material on the surface. Then, a few drops of the 1 µm diamond slurry were applied on the white nylon-polishing pad. The electrode was placed face down on the pad and polished using a smooth figure-eight motion with even pressure. After 1-2 minutes, the electrode was removed and all remaining polishing grit was rinsed away with distilled water. Next, the microcloth-polishing pad was wetted with distilled water and several drops of polishing alumina were added. The electrode was polished in the same manner as in the previous step. Then, the electrode surface was immersed in shallow distilled water in a small beaker and placed in an ultrasonic cleaner for no more than 5 minutes. It was rinsed again with distilled water and the remaining water was shaken off. The electrode was then ready for the experiments.

Electrochemical Cleaning of Gold Disk Electrode.

Electrochemical cleaning was performed using cyclic voltammetry. The gold disk electrode was immerged in 0.1 M sulfuric acid and the potential was swept between –0.2 to 1.6 V (vs. Ag/AgCl reference electrode) with a scan rate 0.1 V/s (about 10 cycles). Then all the electrodes were removed, rinsed well with distilled water and wiped dry.

Determination of Electrode Area.

The electrochemical area of the bare electrode was determined using cyclic voltammetry and the Randles – Sevcik equation [10] for a reversible redox couple, which is

$$\boxed{I_P = (2.69 \times 10^5)\, n^{3/2} A D^{1/2} C_\infty v^{1/2}}$$

When I_P is a peak current (Ampere), n is the number of electrons transferred, A is the electrode area (cm^3), D is the diffusion coefficient of the electroactive species (cm^3 s^{-1}), C_∞ is the bulk concentration of the same species (mol cm^{-3}), and v is the scan rate (V s^{-1}). The peaks currents obtained from each known concentration of electroactive species are plotted versus the concentration. If a reversible electroactive species which has a known diffusion coefficient is used, the electrode area can be calculated from the slope. In this research, the determination of the electrode area were performed in potassium ferricyanide, concentration range from 0.1 – 0.5 mmol cm^{-3} (Potassium ferricyanide has a diffusion coefficient 7.6×10^{-6} cm^2s^{-1} at 25°C).

Surface Pre-coating with Nafion.

The nafion solution was applied onto the cleaned gold disk electrode and left to air-dry. The applied volume of nafion ranged from 0.5 – 3.0 μL and the duration times waiting for air-drying were 10, 15 and 20 minutes. The dried nafion electrode was immersed in 10 mM hexaammine ruthenium (III) chloride solution for 10 minutes and left to air-dry. The peak current from each batch was obtained from a cyclic voltammogram in 0.1 M phosphate buffer solution (pH 7.0). Potentials were swept between –0.7 to 0.7 V (vs. Ag/AgCl reference electrode) with a scan rate 0.1 V/s (2 cycles).

Surface Pre-coating with $Ru(NH_4)_6Cl_3$.

First, 0.1 μL of nafion solution was applied onto the cleaned gold disk electrode and left to air-dry for 10 minutes. Next, the dried nafion electrode was immersed in hexaammine ruthenium (III) chloride solution (concentration range from 5 –25 mM) and dipping period range from 5 – 25 minutes. The electrode was left to air-dry. The peak current from each batch was obtained from a cyclic voltammogram in 0.1 M phosphate buffer solution (pH 7.0). Potentials were swept between –0.7 to 0.7 V (vs. Ag/AgCl reference electrode) with a scan rate of 0.1 V/s (2 cycles).

Preparation of Polypyrrole Film.

First, 0.1 μL of nafion solution was applied onto the cleaned gold disk electrode. The electrode was left to air-dry for 10 minutes. Next, the dried nafion electrode was immersed in 10 mM hexaammine ruthenium (III) chloride solution for 10 minutes and left to air-dry again. The electropolymerization of the polypyrrole film was carried out by chronocoulometry at 0.7 V (vs. Ag/AgCl reference electrode) and the concentration of pyrrole solution ranged from 0.1 – 0.3 M. The peak current from each batch was obtained from a cyclic voltammogram in 0.1 M phosphate buffer solution (pH 7.0). Potentials were swept between –0.7 to 0.7 V (vs. Ag/AgCl reference electrode) with a scan rate 0.1 V/s (2 cycles).

Immobilization of Glucose Oxidase.

The optimal conditions obtained from the three previous experiments were used in this step. After the electrostatic binding process between nafion and $[Ru(NH_3)_6]^{3+}$, the electrode was placed in pyrrole solution containing 50 units/mL of glucose oxidase. The electropolymerization of polypyrrole film was carried out by chronocoulometry at 0.7 V (vs.Ag/AgCl reference electrode) to immobilized the enzyme on the electrode surface.

Results and Discussion

Optimal Condition for Preparation of Glucose Sensor.

The optimal volume and duration time for air-drying of nafion at a fixed concentration of $Ru(NH_3)_6Cl_3$ were studied. The results are shown in table 1. The highest current response was found for 2.5 μL of nafion with 20 minutes duration time. But, when this condition was used to prepare the sensor, we found that it did not respond to standard glucose solution. Peeling of the polypyrrole film on the electrode surface was found. This was because the film too thick. Thus another set of conditions was used. It was found that 1.0 µL of nafion with a 10 minutes duration time was optimal.

The concentration and duration time for $Ru(NH_3)_6Cl_3$ at the optimal conditions for nafion were studied by varying the concentration of $Ru(NH_3)_6Cl_3$ solution from 5–25 mM with different dipping periods. The highest current response was found for 10 mM $Ru(NH_3)_6Cl_3$ solution and a dipping period of 10 minutes (as shown in table 2.).

The effect of the variation of pyrrole concentration with the optimal conditions for nafion and $Ru(NH_3)_6Cl_3$ was investigated. The electropolymerization of polypyrrole film was carried out by chronocoulometry at 0.7 V (vs. Ag/AgCl reference electrode). The film thickness was estimated by assuming that 45 mC/cm^2 corresponds to a film thickness of 0.1 µm [11]. Because the electrode area calculated by Randles – Sevcik equation was 0.2827 cm^2, thus the charge passed to grow the film (0.1 µm) for this electrode was 17.4 mC. The highest current response was found for 0.3 M pyrrole solution (as shown in table 3.). However a rugged film was found at this concentration, while 0.1 M pyrrole solution that gives a uniform film was the better choice.

Using to the optimal conditions described above, the surface of gold disk electrode was coated with nafion and $Ru(NH_3)_6Cl_3$. In the presence of nafion, which is a cation exchanger, the immobilized sulfonate groups serve as a charge compensator to $[Ru(NH_3)_6]^{3+}$. In this case, $[Ru(NH_3)_6]^{3+}$ was used as an electron mediator instead of oxygen. The enzyme, glucose oxidase, was immobilized by electropolymerization of a polypyrrole film. The schematic profile of the optimized sensor is shown in figure2. The electropolymerization using chronocoulometry was a reproducible method to produce the polymer film. The film thickness can be controlled by the charge passed into the solution.

Amperometric Detection of Glucose.

The capability of the sensor was demonstrated by the injection of a standard glucose solution into the electrochemical cell containing 5 mL of 0.1 M phosphate buffer. The current response of glucose was obtained using chronoamperometry at different operating potentials (under N_2 atmosphere). Operating potentials of 0.0, 0.2, 0.3, 0.4 and 0.7 V (vs. Ag/AgCl reference electrode) were investigated. For comparison, the sensor without an electron mediator was studied at the same potential. It was found that the sensor without an electron mediator gives a current response only at 0.7 V, but the sensor with electron mediator, $Ru(NH_3)_6Cl_3$, can give the current response at a potential range from 0.0 - 0.7 V. The results show that the sensor can be operated at the potential below 0.7 V to avoid the interference. The optimal potential for the detection of glucose was 0.4 V (vs. Ag/AgCl reference electrode) because a linear calibration curve could be obtained at this potential. Figure3. shows the amperometric response of the sensor to standard glucose solution.

Analytical Performance of Glucose Sensor.

The response time of the sensor for glucose analysis was studied using chronoamperometry at 0.4 V (vs. Ag/AgCl reference electrode). A 50 μL of 0.2 M standard glucose solution was injected into the electrochemical cell containing 5 mL of 0.1 M phosphate buffer (the final concentration of glucose was 2 mM). In the studied solution, it took about 80 second for the current response to reach its maximum.

The dynamic range of the sensor was investigated by monitoring the standard glucose solution in the concentration range from 2 – 14 mM. Figure4. shows a plot of the current response of standard glucose solutions. Good linearity was found in the low concentration range of the calibration curve. When the glucose concentration was larger than 10 mM, a curvature on the calibration curve appeared. The sensitivity of the sensor, obtained from the slope of the calibration curve, was 9.0 x 10^{-10} A/mM. The detection limit of the sensor was estimated to be about 1.5 mM.

The long-term stability of the sensor was investigated by monitoring its current response to standard glucose solutions at different times. In this research, $Ru(NH_3)_6Cl_3$ was used as the electron mediator. The sensor's life time depended on the remaining amount of $[Ru(NH_3)_6]^{3+}$ on the electrode surface that can be monitored by cyclic voltammetry. The long-term stability was divided in two categories, operational and storage stability. For the operational stability, the sensor was operated continuously to investigate the change of current response. The storage stability was studied by storing the sensor for a few days and recording the current response repeatedly. Operational stability for more than 9 times and more than 16 days of storage stability of the optimized sensor were found.

Conclusion

This paper illustrates a preliminary investigation into the preparation of an amperometric glucose sensor with $Ru(NH_3)_6Cl_3$ as electron mediator. The optimized sensor can be operated at the potential of 0.4 V (vs. Ag/AgCl reference electrode). Interfering species having an oxidation potential close to 0.7 V can be eliminated effectively. The electrostatic interaction between nafion and $Ru(NH_3)_6Cl_3$ offers good storage stability of the sensor. The electropolymerization using chronocoulometry improved the reproducibility for the preparation of polypyrrole film in the enzyme immobilization process. The detection limit was estimated to be about 1.5 mM with sensitivity of 9.0 x 10^{-10} A/mM.

However, further work is required, with regard to understanding the parameters that influence the reproducibility of the immobilization method, how to control the amount of enzyme and $Ru(NH_3)_6Cl_3$ that are immobilized, to develop a reproducible method to control the film thickness of nafion in the surface pre-coating step, for example. Moreover, the application of the amperometric glucose sensor as the detector in flow injection analysis (FIA) system is very interesting [12]. This further work is the subject of current study in our laboratory.

References

(1) Turner, A. P. F.; Karube, I.; Wilson, G. S. *Biosensors: Fundamentals and Applications*; Oxford University Press: Oxford, 1987.

(2) Cunningham, A. J. *Introduction to Bioanalytical Sensors*; Wiley: New York, 1998.

(3) Mosbach, K. *Immobilized Enyzmes and Cells: Part D*; Academic Press: San Diego, 1988.

(4) Du, G.; Lin, C.; Bocarsly, A. B. *Anal. Chem.* **1996,** *68*, 796-806.

(5) Bartlett, P. N.; Ali, Z.; Eastwick-field, V. *J. Chem. Soc. Faraday Trans.* **1992,** *88*, 2677-2683.

(6) Foulds, N. C.; Lowe, C. R. *Anal. Chem.* **1988,** *60*, 2473-2478.

(7) Fortier, G.; Brassard, E.; Belanger, D. *Biosensor & Bioelectronics* **1990,** *5*, 473-490.

(8) Gros, P.; Bergel, A. *J. Electroanal. Chem.* **1995,** *386*, 65-73.

(9) Imisides, M. D.; Jonh, R.; Wallace, G. G. *CHEMTECH* **1996,** *26*, 19-25.

(10) Gooding, J. J.; Praig, V. G.; Hall, E. A. H. *Anal. Chem.* **1998,** *70*, 2396-2402.

(11) Holdcroft, S.; Funt, B. L. *J. Electroanal. Chem.* **1988,** *240*, 89-103.

(12) Ruzicka, J.; Hansen, E. H. *Flow-Injection Analysis*, 2 ed.; Wiley: New York, 1988.

Table

Table 1. Current response of the gold disk electrode prepared by applying different amounts of nafion with different duration times for air-drying in surface pre-coating process (with fixed conditions for $Ru(NH_3)_6Cl_3$)

Amount of nafion applied (µL)	Current response (µA) at the various duration time waiting for air-dried		
	10 min.	15 min.	20 min.
0.5	151	229	237
1.0	167	0.05	157
1.5	209	217	215
2.0	210	202	201
2.5	142	248	253
3.0	202	237	148

Table 2. Current response of the gold disk electrode prepared by varying concentration of $Ru(NH_3)_6Cl_3$ solution with different dipping periods in surface pre-coating process (with fixed conditions for nafion)

Concentration of $Ru(NH_3)_6Cl_3$ dipping solution	Current response (µA) at the various dipping period				
	5 min.	10 min.	15 min.	20 min.	25 min.
5 mM	0.3	60	297	209	102
10 mM	187	298	254	231	263
15 mM	253	209	208	155	168
20 mM	265	236	182	164	168
25 mM	281	238	279	294	262

Table 3. Current response of the gold disk electrode prepared by varying concentrations of pyrrole solution in the electropolymerization process (with fixed conditions for nafion and $Ru(NH_3)_6Cl_3$)

Conentration of pyrrole solution	Current response (mA)
0.1 M	25.00
0.3 M	27.87

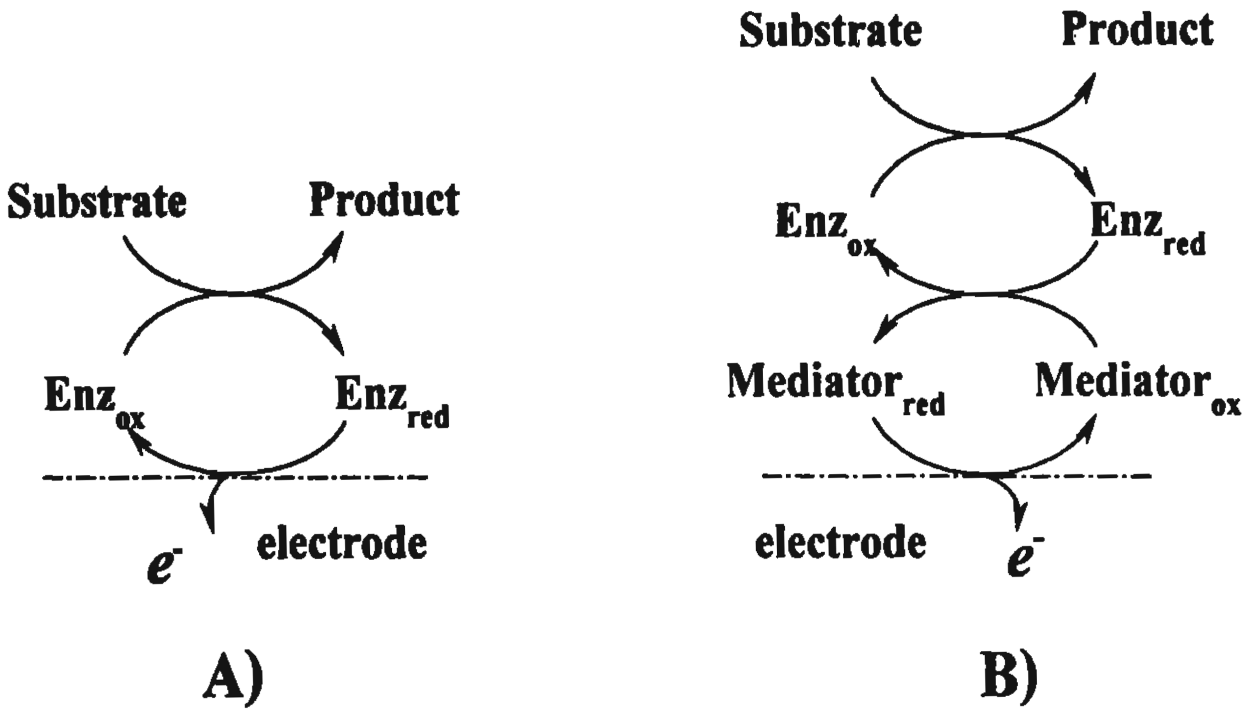
Figure Captions

Fig. 1. Schematic diagram of the direct electron transfer system (A) and the indirect electron transfer system (B).

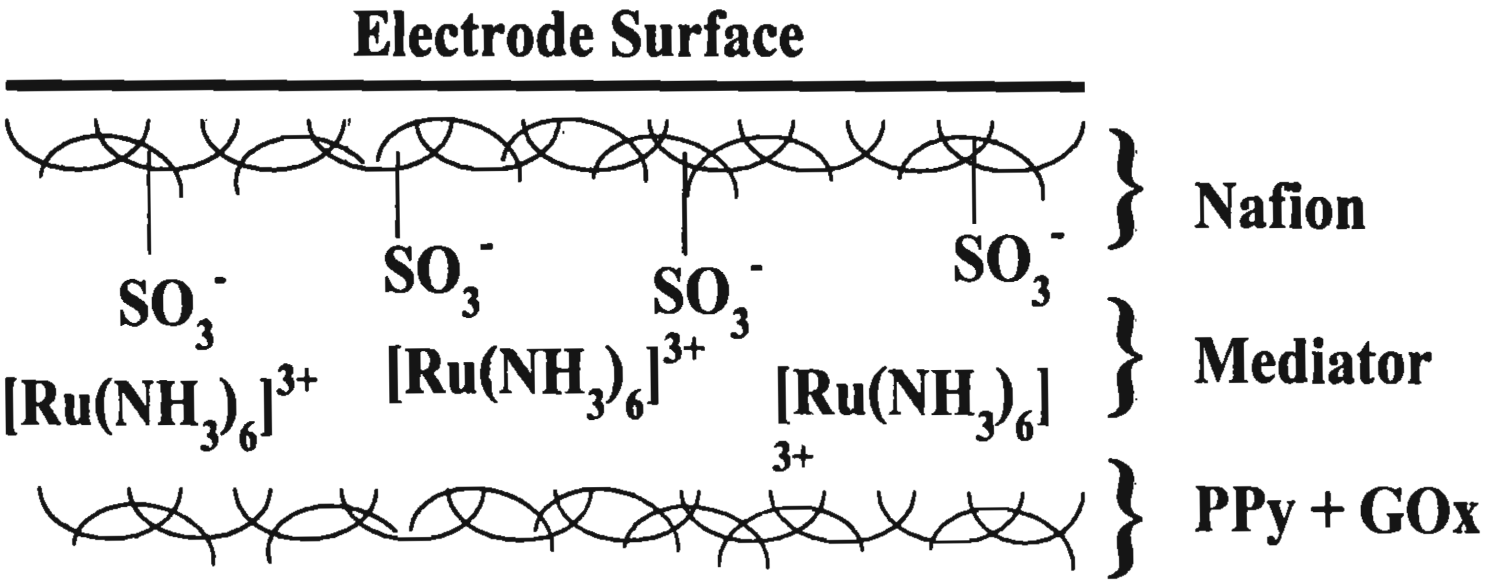
Fig. 2. Schematic profile of the optimized glucose sensor (PPy = Polypyrrole, GOx = Glucose Oxidase).

Fig. 3. The current response to standard glucose solution at 0.4V (vs Ag/AgCl reference electrode) by chronoamperometry.

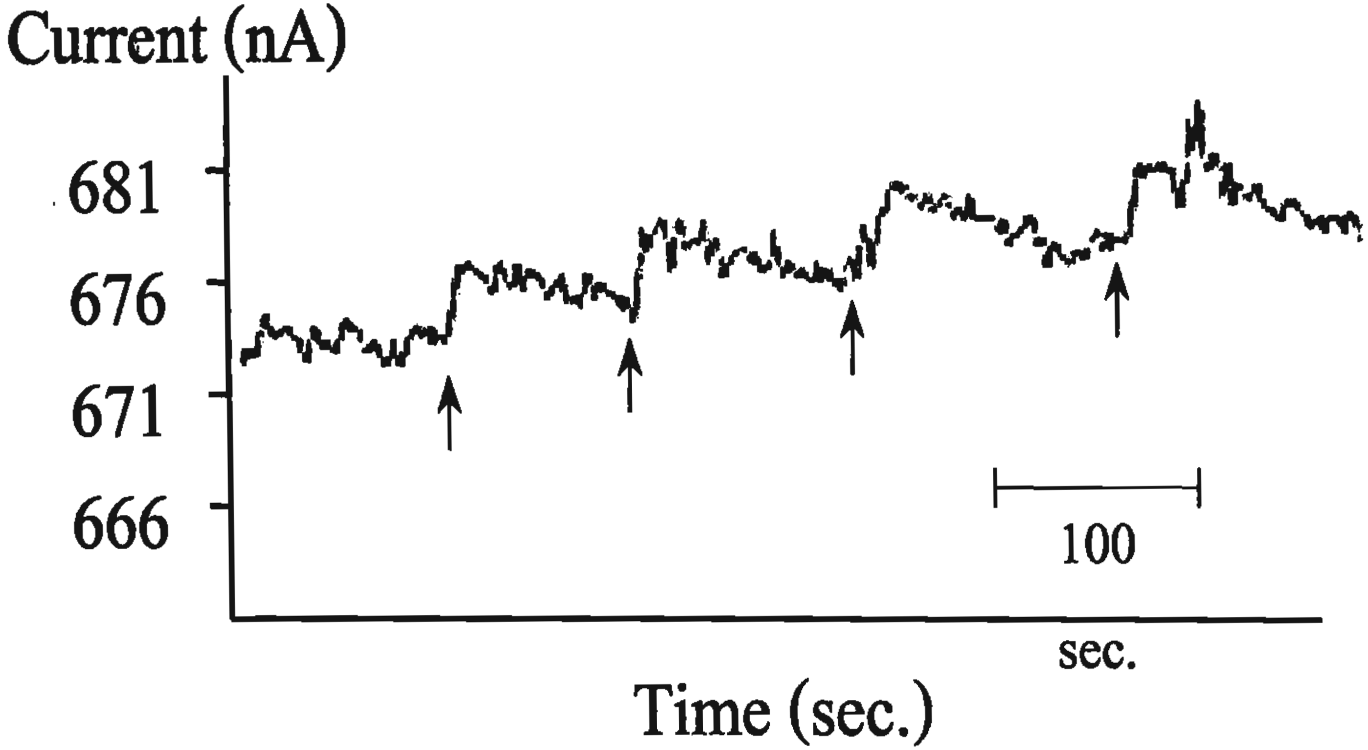
Fig. 4. Calibration curve obtained for the optimized sensor in glucose concentration range from 2 mM to 14 mM.



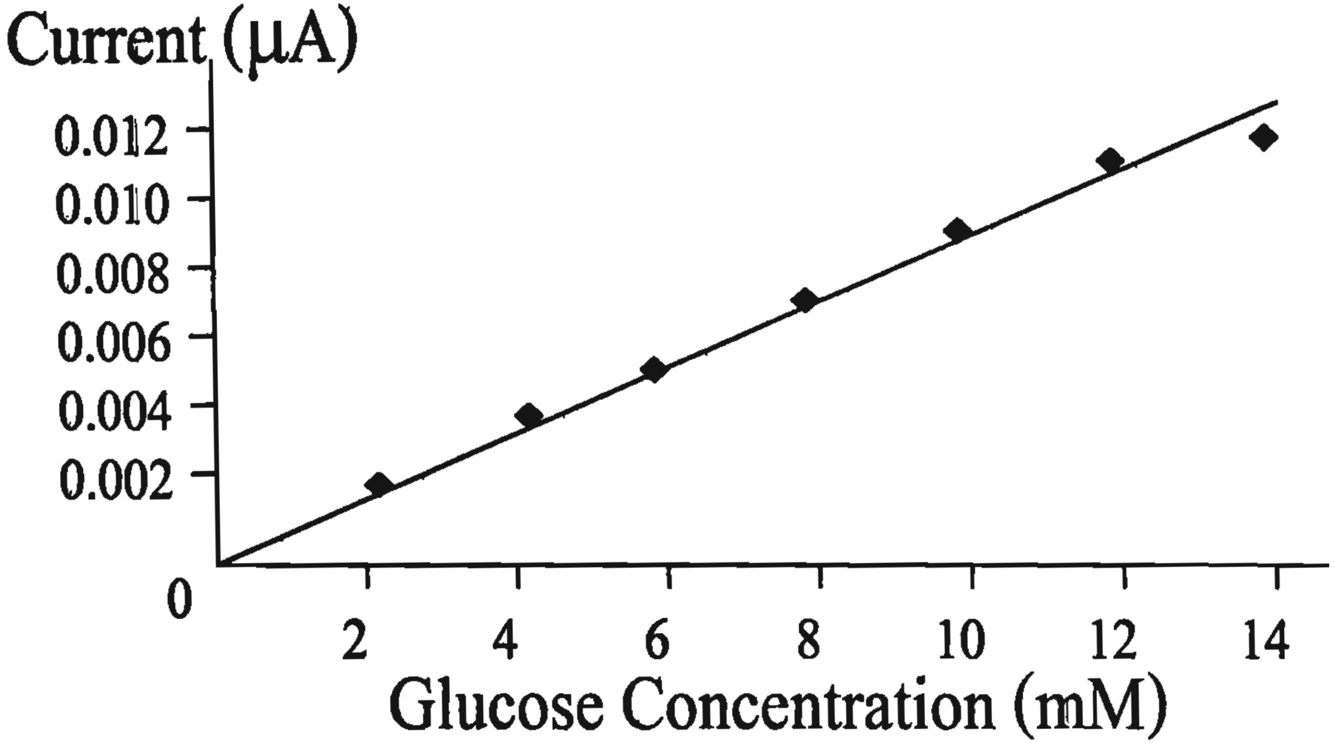
Ngamukot et al. Fig. 1



Ngamukot et al. Fig. 2



Ngamukot et al. Fig. 3



Ngamukot et al. Fig. 4

Immobilized $K_3Fe(CN)_6$ and Glucose Oxidase in Polypyrrole on Gold Microelectrode and Its Application as Glucose Sensors

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Abstract

The purpose of this research is to prepare a glucose sensor using electrochemical polymerization of pyrrole onto a gold microelectrode in the presence of the enzyme glucose oxidase and ferricyanide at a potential of 0.70 V *versus* Ag/AgCl. The enzyme glucose oxidase and ferricyanide are co-immobilized into the polypyrrole films during the electrochemical polymerization process. The two species compete for incorporation into the films so that on increasing the concentration of enzyme in the growth solution less ferricyanide is incorporated into the film. The ferricyanide entrapped within the film acts as a redox mediator for the oxidation of the enzyme at 0.45 V *versus* Ag/AgCl. The effects of variation in charge passed during polymerization, pyrrole concentration and ferricyanide loading are studied. Moreover, the determination of standard glucose and the stability of glucose sensor were investigated.

KEYWORD: POLYPYRROLE, GLUCOSE SENSOR, FERRICYANIDE, AMPEROMETRY

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1. Introduction

Electropolymerization is an adaptable and easily controlled method for the immobilization of enzymes at an electrode surface. This makes electropolymerization an interesting method for the immobilization of enzymes on microelectrodes, for applications such as glucose sensors.

An amperometric sensor is a device for measuring the current that is generated by an electrochemical reaction at a fixed potential. There are some advantages of the amperometrical sensors that make them very useful in bioprocess control. Amperometric sensors are inexpensive, simple to make and operate, and adaptable to process control instrumentation. The sensors can be often operated in a wide concentration range. However, they can sometimes lack selectivity[1] and can suffer from fouling when used in complex matrices[2]. For an amperometric glucose sensor, the reaction of glucose and oxygen in the presence of enzyme glucose oxidase (GOD) will generate hydrogen peroxide as shown below:

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2 \quad .....(1)$$

This is an electroactive species, which is oxidizable at an electrode surface as described by an electrochemical half reaction as follows.

$$H_2O_2 \xrightarrow{0.7\text{ V } \textit{vursus} \text{ SCE}} 2H^+ + O_2 + 2e^- \quad .....(2)$$

The response to hydrogen peroxide will be proportional to the concentration of glucose. This reaction is monitored amperometrically by measuring the current generated by the increase of hydrogen peroxide.

$$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2 \qquad \ldots\ldots\ldots\ldots (3)$$

as oxygen is consumed by the enzyme.

The limited solubility of oxygen in solution creates a problem at high substrate concentrations often encountered in bioprocessees, because it limits the linear range of the sensor. Oxygen can be generated from hydrogen peroxide at the electrode surface, when it is at a positive potential. However, this regenerated oxygen is sometimes not enough to meet the demands of the enzymatic reaction at high substrate concentrations due to loss of oxygen from diffusion.

In this study, preparation of a glucose sensor by immobilization of glucose oxidase and ferricyanide (Ferri) into polypyrrole (PPy) by electrochemical polymerization on gold (Au) microelectrode was investigated. The glucose sensor is expected tc determine glucose concentration at low potentials (lower than 0.7 V *vursus* SCE) in order to avoid interference.

Electron transfer mediators, such as ferri/ferrocyanide (E° = 0.34 V *vursus* SCE), ferrocene and benzoquinone can sometimes take the place of oxygen. This can sometimes overcome the problem of limited availability of oxygen[1]. Also, the focus of recent research is the coupling of an electron mediator with a species that electrochemically polymerizes to form a film over the electrode. The film can mediate electron transfer, serve to screen out interference and prevent electrode fouling[3]. Polypyrrole has been a particular choice for entrapment of protein molecules because films can be grown from aqueous solutions compatible with most biological elements[4].

2. Experimental

2.1 Chemicals

Glucose oxidase type II derived from *Aspergillus Niger* 10,000 unit from Sigma Chemical Company. 96% Pyrrole was received from Fluka Co. Ltd., and freshly distilled before use. β–D-Glucose purchased from BDH Ltd. was used to prepare the standard solutions of glucose. These solutions was prepared 24 hr before use to ensure mutarotation between the α and β forms was complete. Other reagents were analytical grade.

2.2 Equipment

A Potentiostat/gavanostat (Autolab, model PGSTAT10) was used for the electrochemical polymerization, and current measurement. Output from the scanning potentiostat/galvanostat was connected to a personal computer and HP Laser jet 4L printer, supported by GPES 4.4 operating software. The conventional three-electrode system consisted of gold microelectrode of diameter 180 μm (this number is obtained from electrochemical experiments as explained in topic 2.3.1), silver/silver chloride reference electrode, and platinum wire counter electrode. Electrochemical measurements were made using a Faraday cage for noise prevention and under N_2 gas.

2.3 Procedures

2.3.1. Preparation Gold Microelectrodes

The gold microelectrodes were prepared by sealing fine gold wire into capillary glass rods of diameter of 0.6 mm. (Figure 1). The gold microelectrodes were cleaned in H_2O_2 :

H_2SO_4, 3:1 solution, then they were polished with emery paper and then with aluminium oxide (0.3 μm particle size). Before use, the microelectrodes were pretreated by electrochemical cleaning in 0.1 M H_2SO_4 by cyclic voltammetry from 0.2 to −1.6 V *versus* silver/silver chloride, at scan rate of 0.1 V/sec. The radii were determined from the equation (4)[5] by measuring the diffusion controlled current for the oxidation of $K_3Fe(CN)_6$ in 0.1 M KCl.

$$i_p = (2.69 \times 10^{-5}) n^{3/2} A D_0^{1/2} v^{1/2} C_0^{*} \qquad \text{................(4)}$$

Where i_p = peak current, n = number of electron transfer, A = area of electrode [cm^2], D_0 = diffusion coefficient of ferricyanide in 0.1 M KCl (0.673×10^{-5} [cm^2/sec])[6], v = scan rate [V/sec], and C_0^{*} = concentration of ferricyanide [mol/cm^3].

2.3.2. Electrochemical Polymerization of Pyrrole and Immobilization of Glucose Oxidase and Ferricyanide

Electrochemical polymerization of pyrrole onto a gold microelectrode in the presence of the enzyme glucose oxidase and ferricyanide solution was performed at a potential of 0.70 V *versus* silver/silver chloride. Ferricyanide was also used as an electrolyte during polymerizing process.

2.3.3 Determination of Glucose

The amperometric response to glucose was evaluated using the three electrode system in 5 ml of 10 mM phosphate buffer pH 7.0. After applying an overpotential, the current background was left to stabilize and then glucose solution was added into the measuring cell.

The current-time response was recorded as the difference between the stable current (i) after substrate addition and the background current (i_0)

3. Results and Discussion

3.1 Cyclic Voltammetry of Ferricyanide Incorporated in the Polypyrrole Films

Experiments were performed to determine the optimal conditions of the PPy/Ferri/GOD film preparation. Cyclic voltammetry of ferricyanide and the response to glucose from Au/PPy/Ferri/GOD electrode were evaluated as a function of charge passed, pyrrole concentration and ferricyanide concentration.

Cyclic voltammetry of ferricyanide at PPy and PPy/Ferri modified electrodes at 100mV/s was performed in phosphate buffer solutions having pH 7.0. (pH 7.0 is used because it is similar to physiological conditions.) There was a significant difference between the two electrodes, as shown in Fig. 2. Characteristic voltammograms for the redox of ferricyanide in phosphate buffer solution solution were observed at the PPy/Ferricyanide electrode. At PPy modified electrodes, no evidence of electron transfer was observed.

3.2 Effect of Charge Passed during Polymerization on the Enzyme Electrodes

For these experiments, the concentration of the following compounds was kept constant: glucose oxidase at 25 unit/cm^3, pyrrole concentration was 40 mM and $K_3Fe(CN)_6$ concentration was 50 mM. Cyclic voltammetry was used to investigate the effect of charge passed during film growth on the amount of ferricyanide incorporated. In Fig 3, the charge passed is plotted as a function of anodic peak current (i_{pa}) measured from ferricyanide oxidation. It is found that the amount of ferricyanide incorporated increases linearly with the total charge passed during film growth. This suggests that film thickness increases linearly with charge passed over this range. Based on this information, the film thickness would be estimated by assuming a charge density of 1 mC cm^{-2}. This gives an apparent film thickness of 0.22×10^{-6} cm^{7}.

The quantity of charge passed should presumably be related to the quantity of enzyme entrapped in the same manner. The effect of charge passed on the amperometric response of an Au/ PPy/Ferri/GOD electrode is studied and the results is shown in Fig.4. The response to glucose at 0.45 V decreases on increasing the charge passed beyond 8 μC. These results can be explained as follows: The average distance, X_r travelled by a glucose molecule through the film before reacting is given by [8]

$$X_r = \sqrt{D_s K_m / K_{cat} e_T} \quad \text{...............(5)}$$

Where D_s is the diffusion coefficient, K_m is the Michaelis for the enzyme, K_{cat} is rate constant for the breakdown of the enz-substrate complex and e_T is the total enzyme concentration. When the length X_r is greater than the film thickness, much of the glucose will be lost to the bulk solution before it can react. Hence increasing the film thickness will increase the response, as this enables more glucose to be retained. However, if the film thickness becomes much longer than X_r, most of the glucose will react only at the outer edge of the film. This means the mediators have a long way to travel before reaching the electrode, and so some mediators are lost to the bulk solution. Hence now increasing the thickness causes the response to go down. The optimum response will occur when the film thickness and X_r are equal. Thus Fig 4, X_r can be estimated as approximately 0.1 micron .

Thus, for all subsequent experiments charge passed the electrodes during polymerization was 8 μC.

3.3 Effect of Pyrrole Concentration on the Response of the Enzyme Electrodes

We have also investigated the effect of variation of the pyrrole concentration at fixed concentrations of ferricyanide (50 mM) and enzyme (25 unit/cm^3), on the response to glucose at 0.45 V, Fig. 5. We find that, at a constant enzyme concentration, the amount of ferricyanide incorporated into the film, as determined by cyclic voltammetry, increases with increasing the pyrrole concentration in the growth solution, upto 40mM. Beyond this, the amount of entrapped ferricyanide decreases.

The effect of the pyrrole used to prepare the Au/PPy/Ferri/GOD electrodes is presented in Fig. 6. The total charge passed in modifying the electrodes is 42.5 mC/cm^2 and corresponds to a film thickness of about 0.095 μm, assuming 45 mC/cm^2 is equivalent to a thickness of 0.1 m [7]. The steady state current response to 20 mM glucose was recorded at 0.45 V. The optimal concentration of pyrrole was 40 mM as indicated by the maximum current response observed. Thus, for all subsequent experiments a concentration of 40 mM pyrrole was used.

3.4 Effect of Ferricyaide Concentration on the Response of the Enzyme Electrodes

Fig 7 shows the correlation between the ferricyanide concentration as a function of anodic peak current (i_{pa}) measured from ferricyanide oxidation. We find that the amount of ferricyanide incorporated increases linearly when the ferricyanide concentration increases. We don't obtain a maxima ferricyanide oxidation from Fig.7.

Glucose oxidase is negatively charged at a pH higher than 4.2 [9]. During electropolymerization at 0.7 V, therefore glucose oxidase can be incorporated into films as a counter ion of polypyrrole [10]. When comparing glucose oxidase to ferricyanide, which also has a negative charge, ferricyanide can diffuse easier and faster than glucose oxidase according to the size of ferricyanide which is smaller than glucose oxidase.

The effect of the ferricyanide concentration on the response of the modified electrode to 20 mM glucose was studied as depicted in Fig 8. We find that with increasing ferricyanide incorporation into the film, the current response of glucose increases until reaching an optimum at 25 mM ferricyanide. Beyond this concentration, the current response of glucose starts to decreases. Thus there appears to be a competition between the enzyme and the ferricyanide for incorporation into the film. This is because both are negative charged and are entrapped into the positively charged polymer by electrostatic attraction. Beyond 25 mM ferricyanide, the quantity of entrapped glucose oxidase decreases so the response to glucose starts to decrease. We can conclude that the polymerization solution should contain 25 mM ferricyanide.

Stability

Preliminary studies of the stability of the immobilized enzyme films were performed. We found that there was change in the response with repeated use, and that the responses decreased by more than 50% of the initial value after storage for 24 h at room temperature in glucose-free buffer solution. The stability information suggested that the decrease in the response, in part, due to the diffusion away of ferricyanide into the solution and in part due to loss of enzyme activity.

Sensitivity

The glucose sensitivity of this sensor which was optimized in this experiment was found to be ca 0.05 nA/mM with a linear response up to ca 5 mM as shown in Fig 9.

4. Conclusions

Both GOD and ferri/ferrocyanide can be entrapped within polypyrrole films. Using cyclic voltammetry we can recognize ferricyanide incorporated during film growth. Amperometric experiments to measure glucose suggest that the two species compete with each other for sites within the film. This is to be expected since they are both incorporated as counter ions during the deposition of the cationic polymer.

The optimum condition to prepare the glucose is use of electrochemical polymerization of 40 mM pyrrole onto a gold microelectrode in the presence of the 45 unit/cm^3 enzyme glucose oxidase and 25 mM ferricyanide at a potential of 0.70 V *versus* Ag/AgCl. The ferricyanide entrapped within the film is able to mediate the re-oxidation of the enzyme so that glucose can be detected amperometrically using these films at 0.45 V *vursus* Ag/Ag Cl. The optimized sensitivity was found to be ca 0.05 nA/mM.

Acknowledgments

We thank The Thailand Research Fund for financial support (PDF/85/2540).

References

1. Work, J.V.; Yacynych, A.M. *Sensors in Bioprocess control*; Work, J.V.; Yacynych, A.M.,Ed., 1990 ; Vol. 6.
2. Lowery, L.P.; Macateer, K.; Elatrash, S.S.;Duff, A.; O'Neill, R.D. *Anal Chem.* **1994**, 66, 1754
3. Cho, J.-H.; Shin, M-C.; Kim, H.-S. *Sensors and Actuators B* **1996**, 30, 137.
4. Asavapiriyanout, S. *J.Electroanal. Chem.* **1984**, 177, 229.
5. Bard, A.J.; Faulkner, L.R. *Electrochemical Methods*; John Wiley & Sons: Newyork, 1981
6. Adams, R. N. *Electrochemistry of Solid Electrodes.*; Marcel Dekker: New York, 1969.
7. Holdcroft, S.; Funt, B.L. *J. Electroanal Chem.* **1988**, 240, 89
8. Bartlett, P.N.; Whitaker, R.G. *J. Electroanal . Chem .* **1987**, 224, 27
9. Swoboda, B.E.P.; Massey, V *J.Biol. Chem .* **1961**, 241, 3409
10. Diaz, A.F.;J.I.Castillo; Logan, A.;Lee, W.-Y. *J.Electroanal. Chem.* **1981**, 129, 115

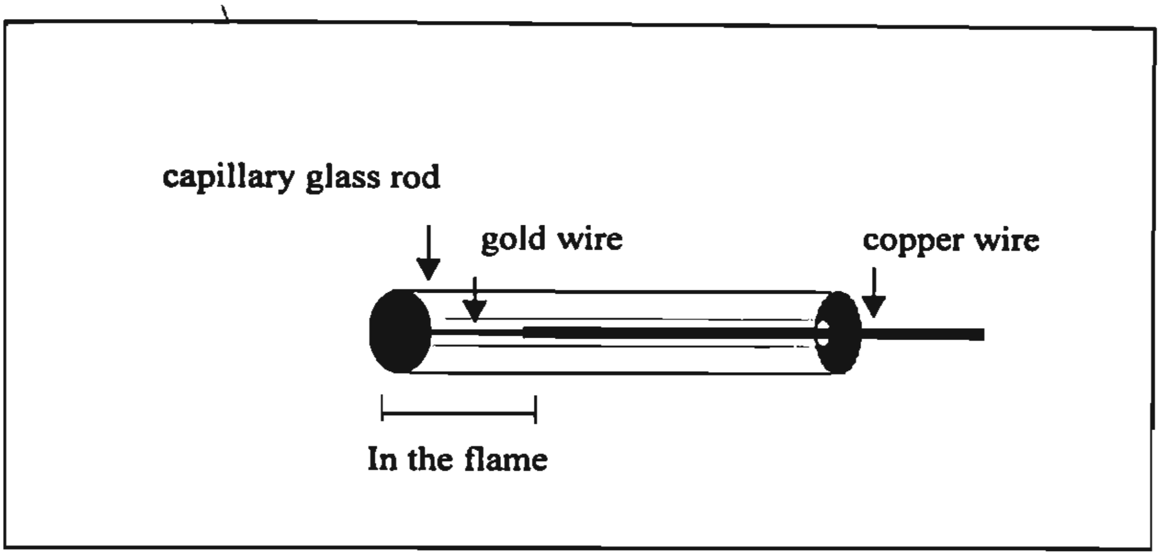

Fig. 1 preparation of gold microelectrode

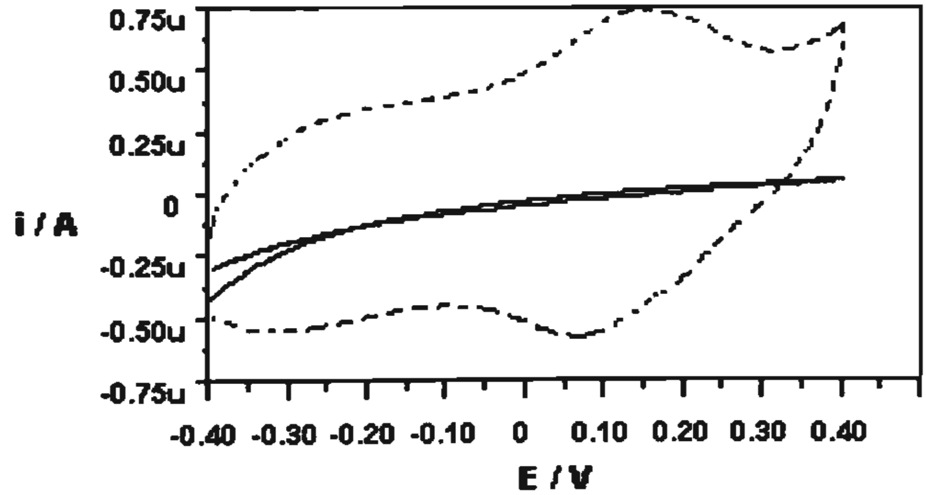

Fig. 2 shows cyclic voltammograms for——the Au/PPy/GOD electrode and -------- for Au/PPy/Ferri/GOD electrode obtained in phosphate buffer pH 7 after the electropolymerization. Scan rate 100 mV/s

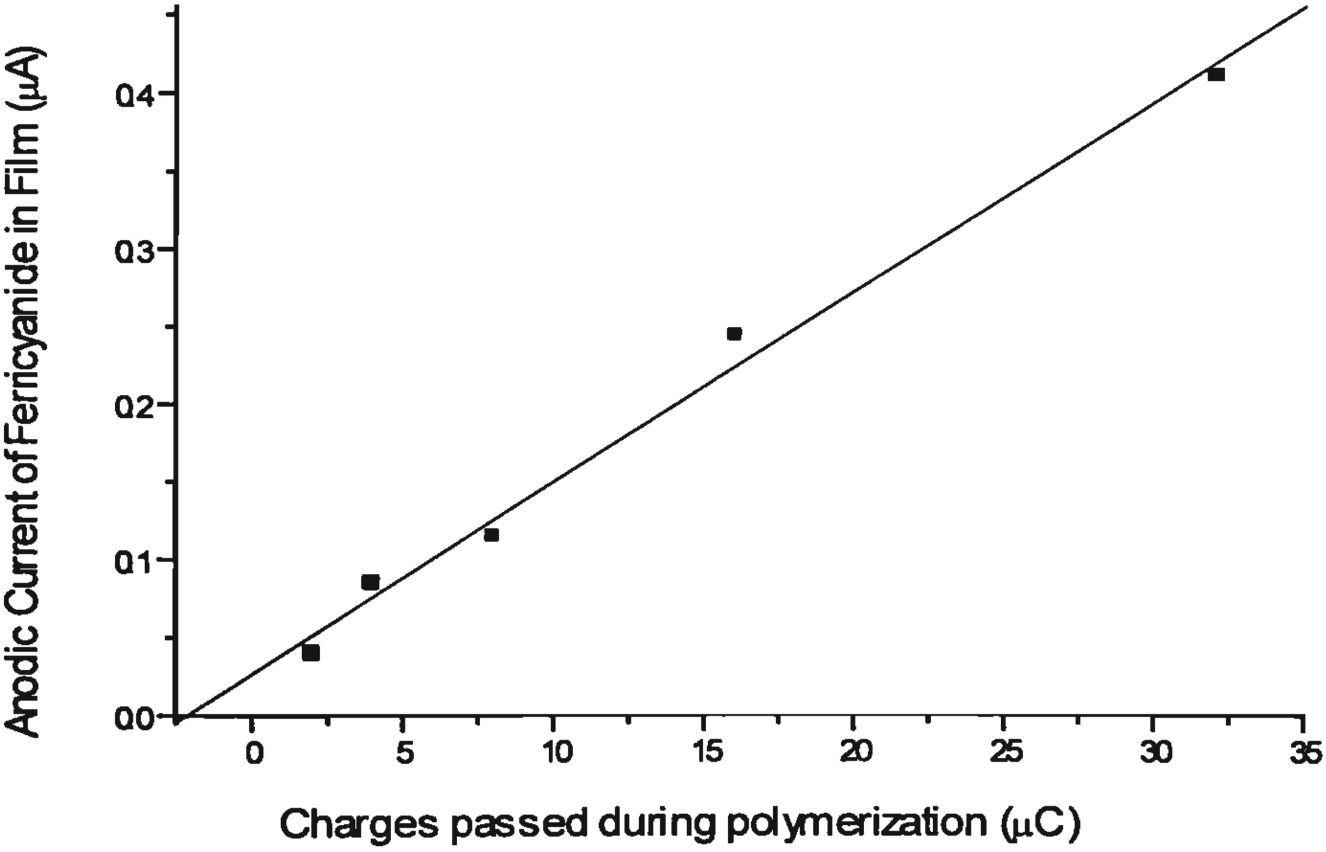

Fig. 3 shows the relationship between charge passed Au/PPy/Ferri/GOD electrode and i_{pa} of ferricyanide incorporated into the polypyrrole films.

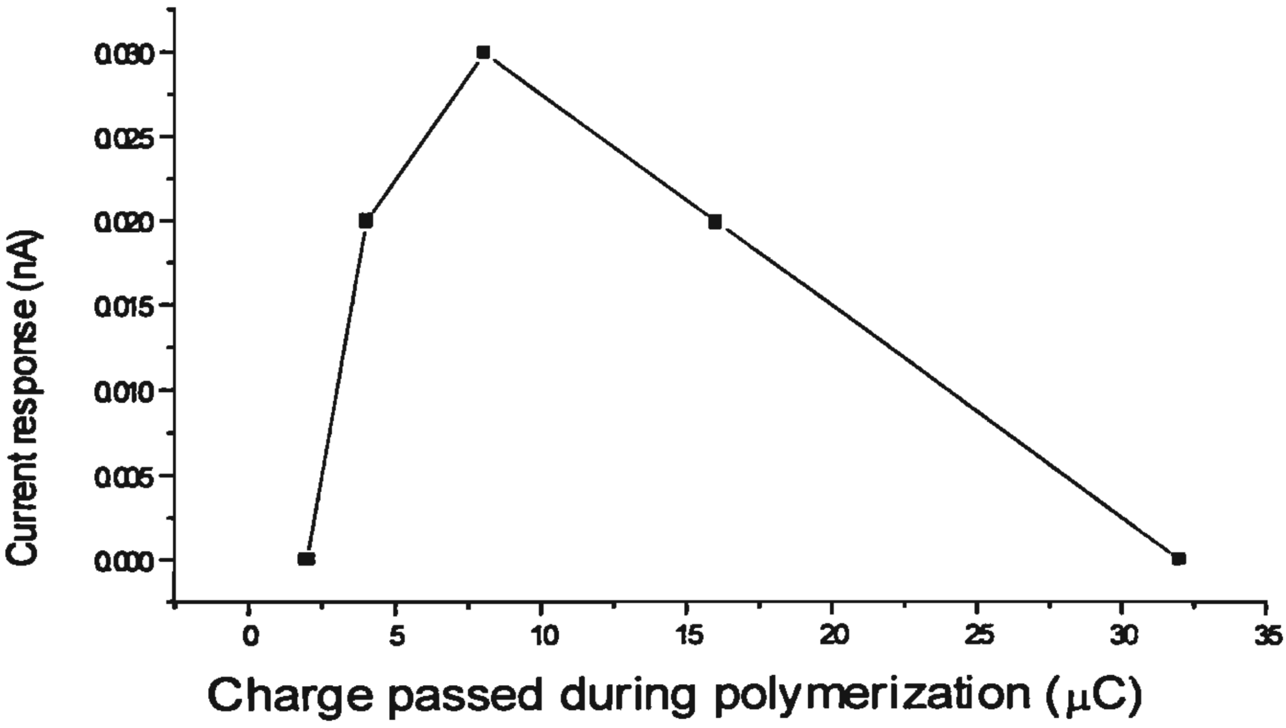

Fig. 4 shows the relationship between the current response to 100 mM glucose solution and charge passed into the 40 mM pyrrole, 25 unit/cm^3 GOD and 50 mM ferricyanide solution.

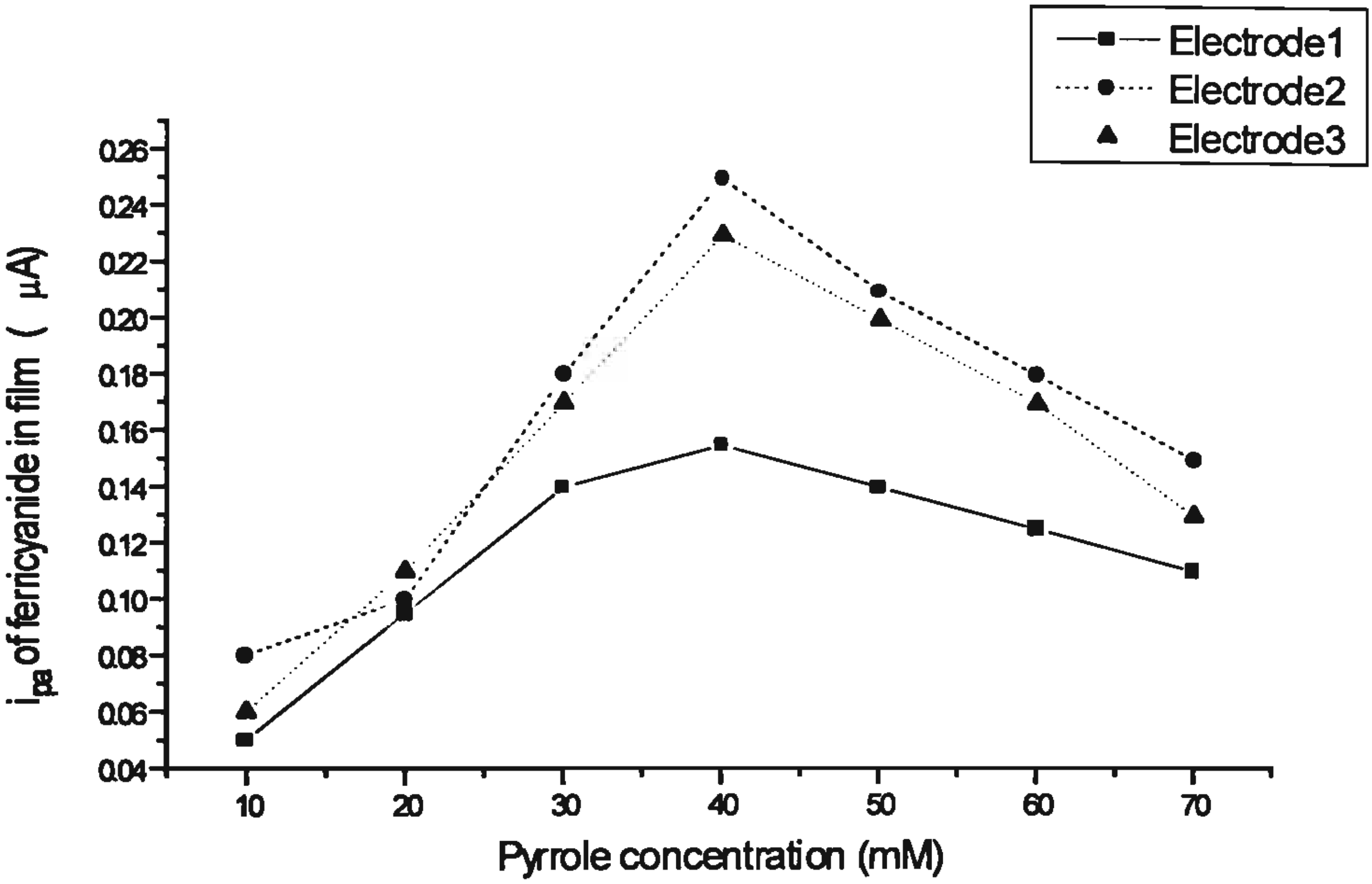

Fig.5 shows the effect of pyrrole concentration function with i_{pa} of ferricyanide incorporated in films.

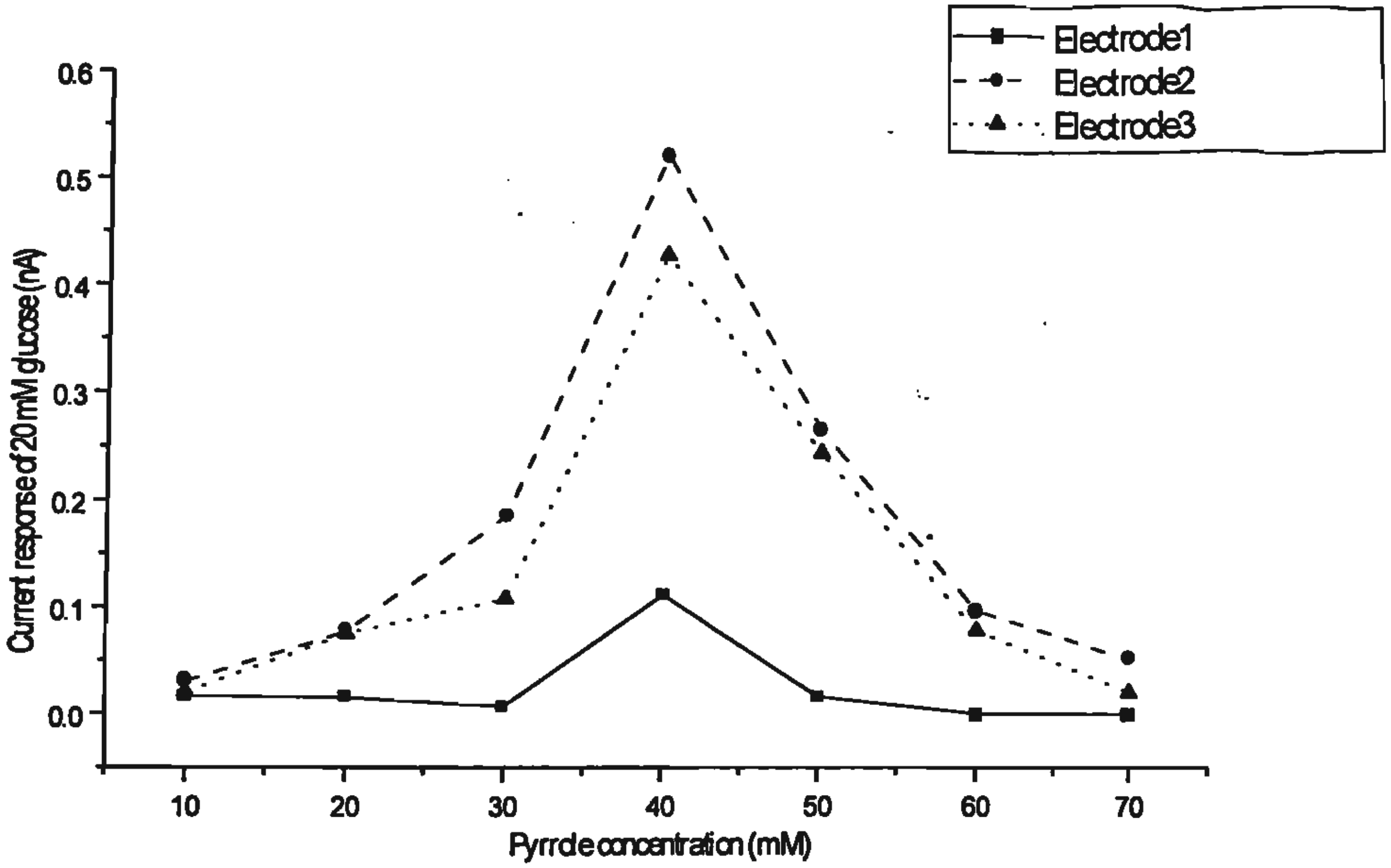

Fig. 6 shows the relationship between pyrrole concentration and current response to 20 mM Glucose. The films were grown at 0.7 V from aqueous buffered solutions containing 50 mM ferricyanide and 25 unit/cm3 GOD in the variation of pyrrole concentration.

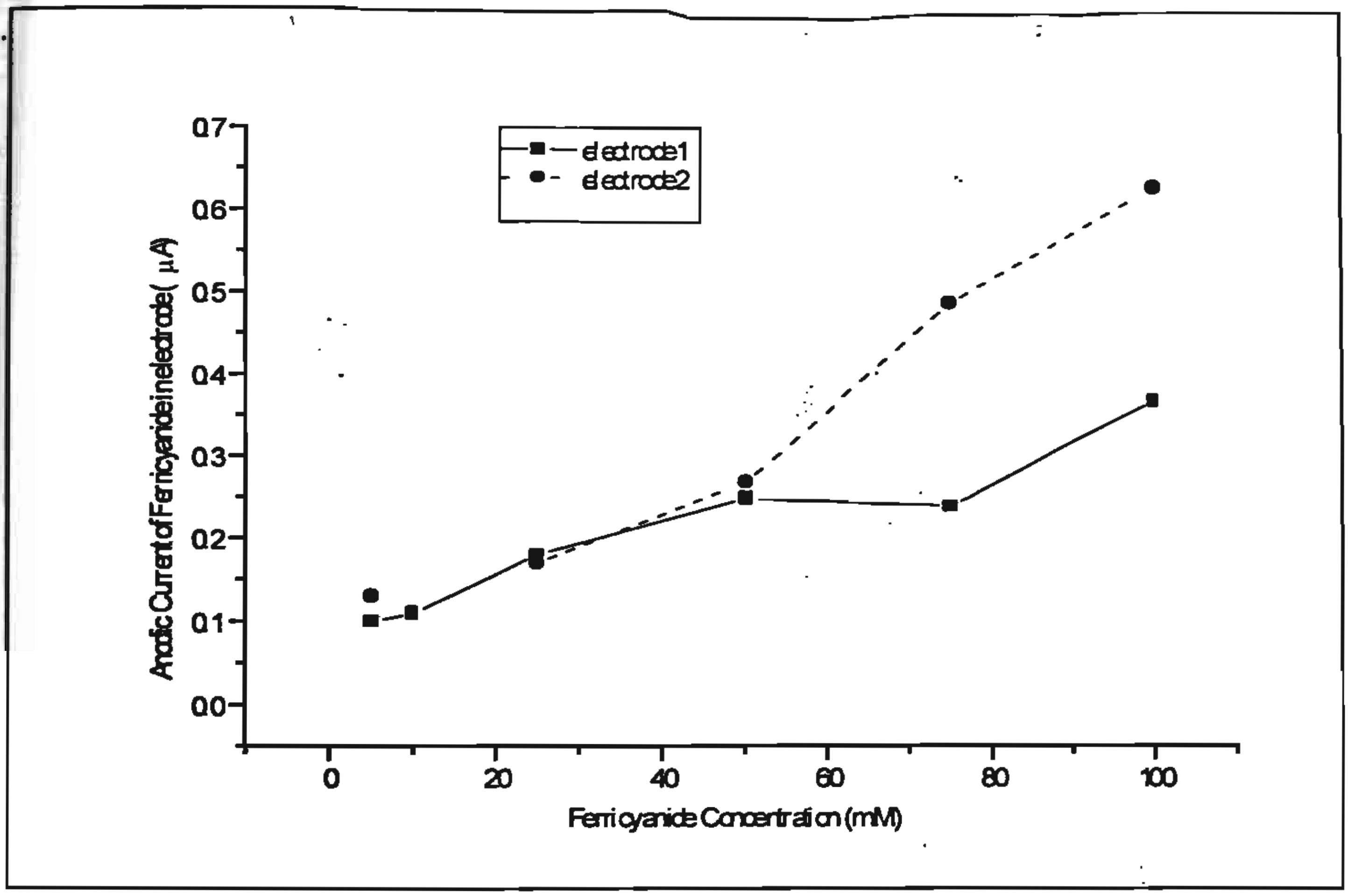

Fig. 7 shows the effect of ferricyanide concentration function with i_{pa} of ferricyanide incorporated films.

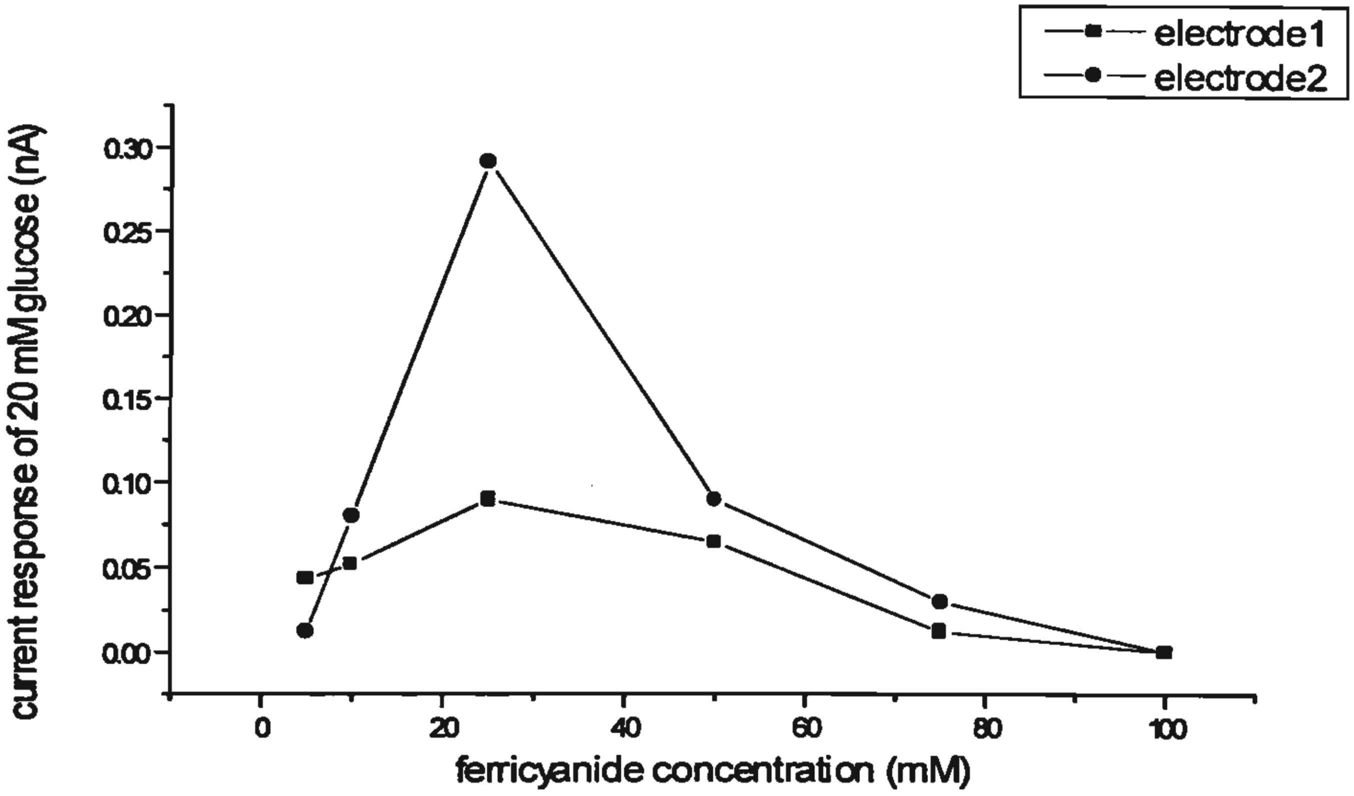

Fig. 8 shows the relationship between ferricyanide concentration and current response to 20 mM Glucose. The films were grown at 0.7 V from aqueous buffered solutions containing 50 mM pyrrole and 25 unit/cm^3 GOD in the variation of ferricyanide concentration.

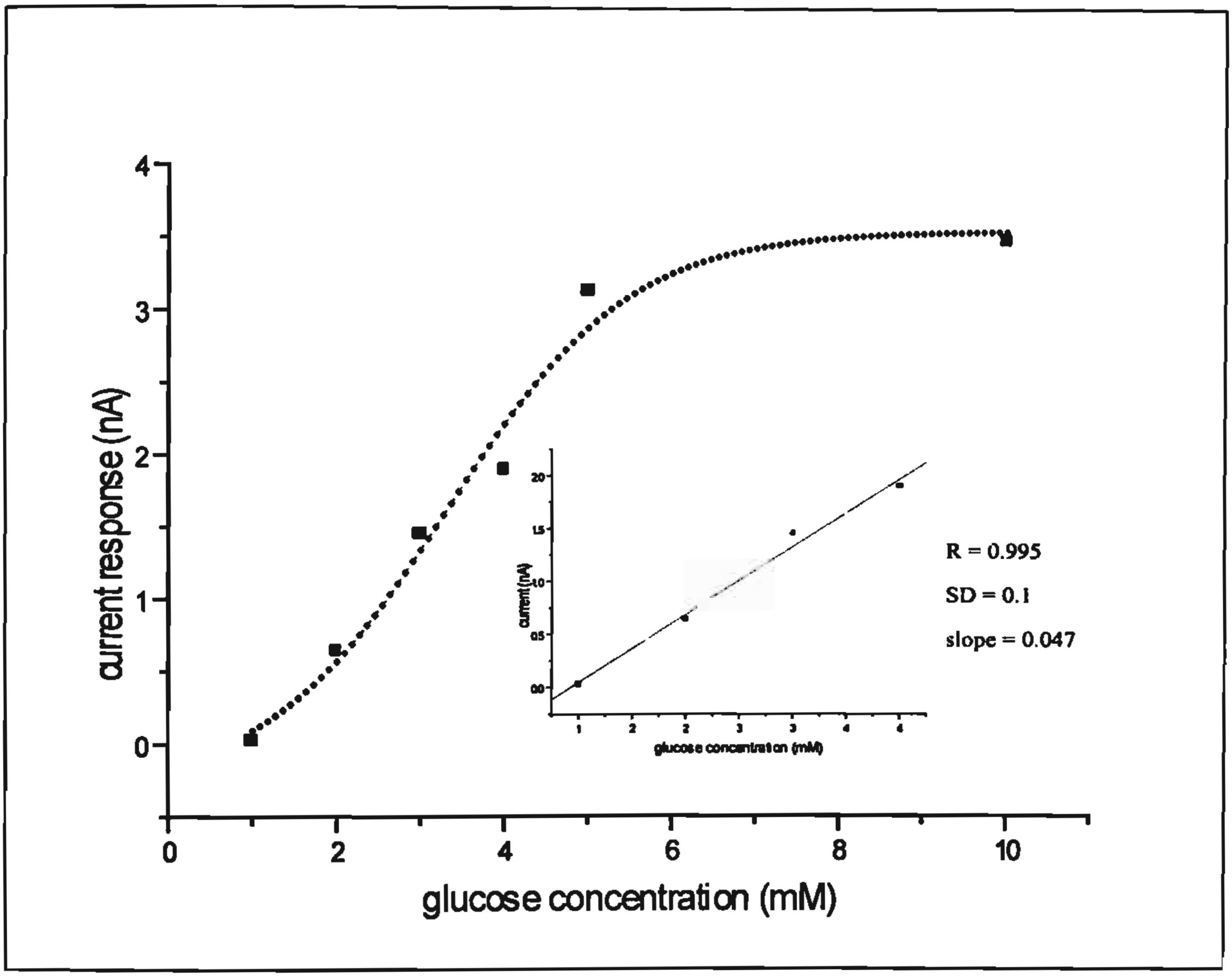

Fig. 9 Calibration curve of glucose sensor at 0.45 V using ferricyanide as mediator

Abstract Book

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Orawon Chailapakul

IMMOBILIZATION OF GLUCOSE OXIDASE AND $K_3Fe(CN)_6$ INTO POLYPYRROLE ON Au MICROELECTRODE

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The co-immobilization of enzymes with mediator into conducting polymers has gained interest recently(1,2,3). We have fabricated glucose sensors by immobilization of glucose oxidase into polypyrrole films on 155 μm diameter Au electrodes. $Fe(CN)_6^{-3}$ was co-immobilized as electron mediator between the enzymes and the electrode. After polymerization from the pyrrole/glucose oxidase/ $Fe(CN)_6^{-3}$ solution, investigation of $Fe(CN)_6^{-3}$ embedded in conducting polymer films was carried on in 0.1 M phosphate buffer pH 7, as shown in Figure. 1.

At a fixed potential, steady-state responses to glucose were records in the absence of stirring, due to radial diffusion of the analyte. In Figure.2, it was found that the optimal polymerization charge for creating the GOD/ferricyanide films should be approximately 42.5 $mC.cm^{-2}$, based on the steady-state response to 100 mM glucose

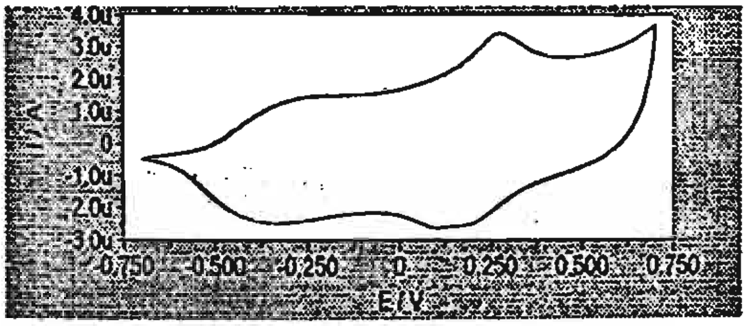

Fig. 1.Cyclic voltammogram of the Au/polypyrrole/GOD/Ferricyanide electrode obtained in 0.1 M phosphate buffer pH 7 at the potential between – 0.750 V to +0.750 V vs Ag/AgCl. The scan rate was 100 mV/s.

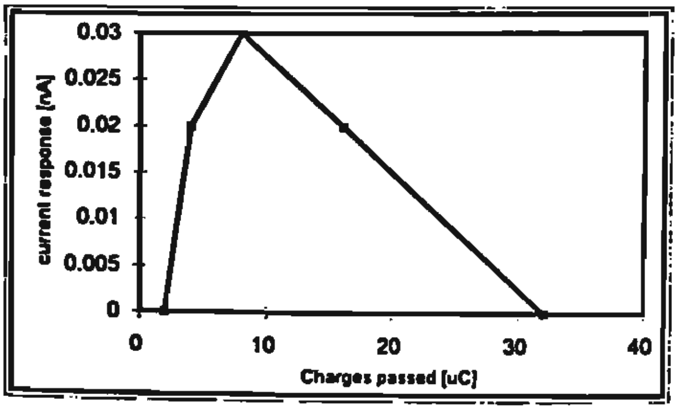

Fig. 2.The relationship between polymerization charge at 0.7 V vs Ag/AgCl used to prepare polypyrrole/GOD/Ferricyanide-films and current response obtained from determining 100 mM glucose at polymer-modified electrodes at 0.45 V vs Ag/AgCl.

In addition, the response time and stability of the sensors are studied, and the detection limit and sensitivity are determined. The kinetic parameters of the sensor are discussed

References

(1) Bartlett, P. N., Ali. Z.and Eastwick-Field, V., *J. Chem.Soc.,Faraday Trans.*, **88** (1992) 2677
(2) Ikeda. S, Yoshioka. T and Nankai. S., *Denkikagaku.*, 63 (1995) 1145
(3) Ge. F, Zhang XE, Zhang ZP, et al., *Anal Lett.,* 31 (1998) 383

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PROGRAMME AND ABSTRACTS

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Optimal Condition for Preparation of Polypyrrole Film with Immobilization of Glucose Oxidase and $Ru(NH_3)_6Cl_3$

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In this research, the specific glucose detection device, glucose sensor, was developed. Enzyme-modified gold disc electrode with $Ru(NH_3)_6Cl_3$, as an electron madiator instead of oxygen, were able to determine glucose at potential lower than +0.7 V to avoid the interference. The conducting polymer, polypyrrole, were used to entrap glucose oxidase at the surface of the electrode. It was found that the sensor can detect glucose at the potential of +0.4 V, the response time is 80 seconds with a sensitivity of 9.0×10^{-10} A/mM. The storage stability of the sensor exceeded 16 days and the operational stability is more than 9 times. In addition, it was found that the detection limit was 1.5 mM.

การศึกษาสภาวะที่เหมาะสมในการเตรียมฟิล์มพอลิไพโรลที่ใช้ตรึงกลูโคสออกซิเดสและ $Ru(NH_3)_6Cl_3$

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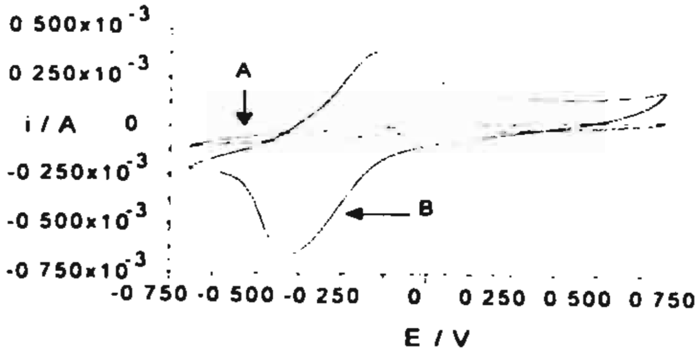
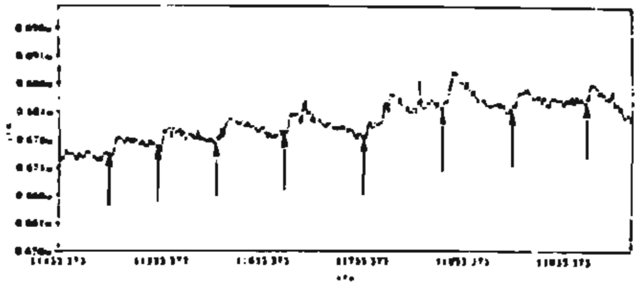
ปัจจุบันเทคนิคทางเคมีไฟฟ้าได้ถูกพัฒนาให้มีความจำเพาะกับการวิเคราะห์น้ำตาลกลูโคสในปริมาณน้อยๆได้ โดยอาศัยอุปกรณ์ที่เรียกว่า กลูโคสเซนเซอร์ งานวิจัยนี้ต้องการพัฒนากลูโคสเซนเซอร์ซึ่งสามารถวัดปริมาณกลูโคสได้ที่ศักย์ไฟฟ้าต่ำกว่า +0.7 V เพื่อเป็นการหลีกเลี่ยงตัวรบกวนต่างๆ ที่เจือปนอยู่ในเลือดโดยใช้ $Ru(NH_3)_6Cl_3$ เป็นตัวส่งผ่านอิเลกตรอนและใช้พอลิไพโรลซึ่งเป็น conducting polymer ทำหน้าที่ตรึงเอนไซม์กลูโคสออกซิเดสไว้ที่ผิวหน้าของขั้วไฟฟ้าทำงาน (gold disc electrode) นอกจากนี้ยังมีการศึกษาสภาวะที่เหมาะสมในการเตรียม รวมทั้งประสิทธิภาพและความเสถียรของกลูโคสเซนเซอร์ที่เตรียมได้ จากการศึกษาพบว่าสามารถใช้วัดปริมาณกลูโคสที่ศักย์ไฟฟ้า +0.4 V ใช้เวลาในการตอบสนอง 80 วินาที ความไว 9.0×10^{-10} A/mM มี operational stability มากกว่า 9 ครั้ง และมี storage stability มากกว่า16 วัน นอกจากนี้สามารถวัดกลูโคสที่มีความเข้มข้นต่ำถึง 1.5 mM

INDEX KEY WORDS: glucose sensor, polypyrrole, $Ru(NH_3)_6Cl_3$, Amperometry

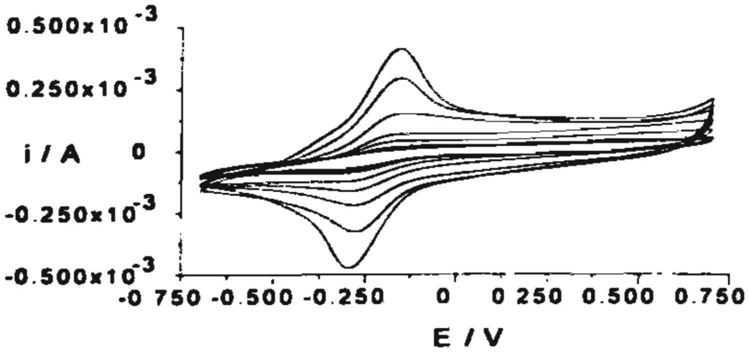
ชื่อเรื่อง (ไทย) การศึกษาสภาวะที่เหมาะสมในการเตรียมฟิล์มพอลิไพโรลที่ใช้ตรึงกลูโคสออกซิเดสและ $Ru(NH_3)_6Cl_3$



รูปที่ 1. แสดงไซคลิกโวลแทมโมแกรมในสารละลาย 0.1 M ฟอสเฟตบัฟเฟอร์ ก่อน (A) และหลัง (B) พอลิเมอไรเซชั่น



รูปที่ 2. แสดงกระแสที่ตอบสนองต่อสารละลายมาตรฐานกลูโคส 2 mM โดยเทคนิคแอมเปอโรเมทรี (หัวลูกศรแสดงตำแหน่งที่ฉีดสารละลาย)



รูปที่ 3. แสดงไซคลิกโวลแทมโมแกรมที่ได้จากการทำ operational stability test เมื่อนำเซนเซอร์ไปใช้งานติดต่อกัน 9 ครั้ง (ความสูงของกระแสที่ลดลง หมายถึง $Ru(NH_3)_6Cl_3$ ที่ตรึงไว้มีปริมาณลดลง)

เอกสารอ้างอิง

1. Imisides M.D., John R., Wallace G. G., *Chemtech*, 1996, *26(5)*, 19-25

2. Holecroft, S., Funt, B.L., *Journal of Electroanal. Chem*, 1988, *240*, 89-103

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Program - Abstracts

P-165

COMPARATIVE DETERMINATION OF ALUMINIUM (III) IN TAP WATER BY FLOW INJECTION SPECTROPHOTOMETRY USING PYRIDOXAL SALICYLOYLHYDRAZONE AND CHROME AZUROL S.

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Two different spectrophotometric flow injection (FI) systems were developed for trace aluminium determination, based on the reactions between aluminium (III) with two different selective the reagents, Pyridoxal Salicyloylhydrazone (PSH) and Chrome Azurol S. PSH reacts with aluminium (III) in an acid solution resulting in a yellow complex with a suitable absorption at 415 nm. The optimum conditions for trace aluminium (III) determination were investigated. A linear calibration graph over the concentration range 0.0 – 0.8 ppm of Al (III) was established. Another FI–colorimetric method was based on the reaction between aluminium (III) and Chrome Azurol S in an acetate buffer pH 4.6 solution, forming a water-soluble purple complex, giving a maximum absorption at 567.5 nm. The optimum conditions for this method were determined and a linear calibration graph over the concentration 0 – 0.4 ppm of Al (III) was obtained. Both recommended methods were found to be reproducible, accurate and sensitive which have been applied to the determination of aluminium (III) in tap water samples collected from Chiang Mai University campus, the aluminium contents in the samples were found to be in the range 0.08-0.30 ppm.

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AMPEROMETRIC GLUCOSE SENSOR USING $Ru(NH_3)_6Cl_3$ – NAFION COATED GOLD ELECTRODE FOR FLOW – INJECTION ANALYSIS

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[1]Department of Chemistry, Faculty of Science, Chulalongkorn University
[2]National Center for Genetic Engineering and Biotechnology

Enzyme-modified amperometric glucose sensor was adapted to flow-injection analysis (FIA) for the determination of glucose. The conducting polymer, polypyrrole, was used to entrap glucose oxidase at the surface of a gold disc electrode. Nafion precoating was applied to improve the long-term stability of the sensor.(1) The problem of interference signals can be overcome by taking advantage of $Ru(NH_3)_6Cl_3$, as an electron mediator instead of oxygen. It was found that the sensor can operate at a potential lower than + 0.7 V (*vs.* Ag/AgCl) to avoid interference. Glucose solution was injected into the carrier stream of phosphate buffer before passing through a homemade flow-cell connected with the amperometric glucose sensor. The combination of the amperometric method and flow-injection analysis(2) provides a high performance glucose determination method in term of specificity, reproducibility and good accuracy.

References:

(1) Imisides, M. D.; Jonh, R.; Wallace, G. G. *CHEMTECH* 1996, *26*, 19-25.
(2) Ruzicka, J.; Hansen, E. H. *Flow-Injection Analysis*, 2 ed.; Wiley: New York, 1988.

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การประชุมเพื่อเสนอผลงานวิจัย
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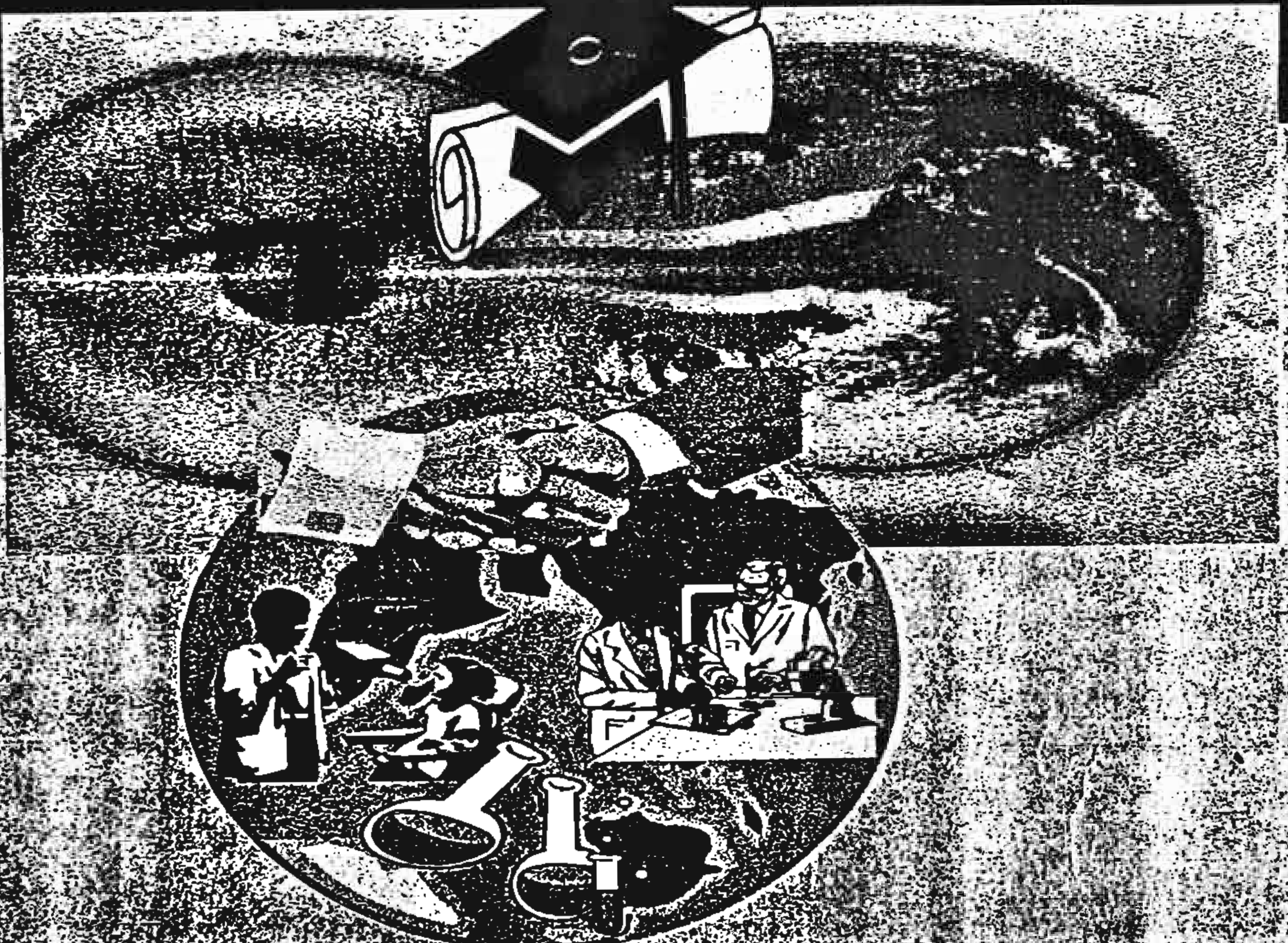

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Immobilized $K_3Fe(CN)_6$ and Glucose Oxidase in Polypyrrole Film on Au Microelectrode and Its Application as Glucose Sensors

Dr. Orawon Chailapakul, Jitrudee Promnil, Dr. Mithran Somasundrum and Associate Prof. Dr. Morakot Tanticharoen

The purpose of this research is to prepare a glucose sensor using electrochemical polymerization of pyrrole onto a gold microelectrode in the presence of the enzyme glucose oxidase and ferricyanide at a potential of 0.70 V *vs.* Ag/AgCl. The enzyme glucose oxidase and ferricyanide are co-immobilized into the polypyrrole films during the electrochemical polymerization process. The two species compete for incorporation into the films so that on increasing the concentration of enzyme in the growth solution less ferricyanide is incorporated into the film. During the measurement of glucose, ferricyanide entrapped within the film will be used as a redox mediator for the oxidation of the enzyme at 0.45 V *vs.* Ag/AgCl, so that it avoids the oxidation of interferences at the higher potential . The effects of variation in charge passed during polymerization, pyrrole concentration and ferricyanide loading also are studied. Moreover, the stability of glucose sensor was investigated. The PPy/GOD/Ferri enzyme electrode was found more stable by storing in 100 nM ferricyanide and 0.1 M phosphate buffer pH 7 at 4 °C.

การตรึง $K_3Fe(CN)_6$ และ กลูโคสออกซิเดสในฟิล์มพอลิพิโรลบนอิเล็กโทรดทองขนาดไมโคร และการประยุกต์ใช้เป็นกลูโคสเซนเซอร์

ดร. อรวรรณ ชัยลภากุล จิตฤดี พรหมนิล ดร. มิทราน โซมาซันดรัม และ รศ. ดร. มรกต ตันติเจริญ

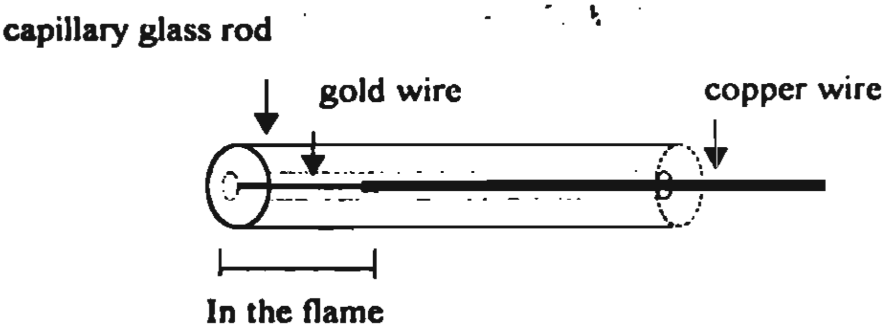
งานวิจัยนี้เป็นการเตรียมกลูโคสเซนเซอร์โดยการตรึงเอนไซม์กลูโคสออกซิเดส และ เฟอร์ริไซยาไนด์ลงในฟิล์มพอลิพิโรลบนอิเล็กโทรดทองขนาดไมโคร กลูโคสเซนเซอร์ดังกล่าวถูกเตรียมด้วยวิธีอิเล็กโตรเคมิคัลพอลิเมอร์ไรเซชันที่ศักย์ไฟฟ้า 0.70 โวลต์ เทียบกับขั้วอิเล็กโทรดอ้างอิง Ag/AgCl ปริมาณของเอนไซม์กลูโคสออกซิเดส หรือเฟอร์ริไซยาไนด์ที่จะถูกตรึงลงในฟิล์มพอลิพิโรล จะขึ้นอยู่กับความเข้มข้นของแต่ละสารที่มีอยู่ในสารละลายระหว่างที่ทำการพอลิเมอร์ไรซ์ ในงานวิจัยนี้ เฟอร์ริไซยาไนด์จะถูกใช้เป็นตัวกลางส่งผ่านอิเล็กตรอนขณะที่ทำการวัดปริมาณกลูโคสที่ 0.45 โวลต์เทียบกับขั้วอิเล็กโทรดอ้างอิง Ag/AgCl, เพื่อเลี่ยงการถูกรบกวนจากตัวแทรกสอดที่ศักย์ไฟฟ้า 0.7 โวลต์เทียบกับขั้วอิเล็กโทรดอ้างอิง Ag/AgCl นอกจากนี้ได้ศึกษาความเข้มข้นของพิโรล เฟอร์ริไซยาไนด์ และปริมาณไฟฟ้าค่าต่าง ๆ ที่ใช้ในการพอลิเมอร์ไรซ์ จากการตรวจสอบความเสถียรของกลูโคสเซนเซอร์ พบว่าเมื่อเก็บไว้ในสารละลาย 100 นาโนโมลาร์ของเฟอร์ริไซยาไนด์ใน 0.1โมลาร์ ฟอสเฟตบัฟเฟอร์ pH 7 ที่อุณหภูมิ 4 องศาเซลเซียส กลูโคสเซนเซอร์มีความเสถียรเพิ่มมากขึ้น

INDEX KEY WORDS : polypyrrole, glucose sensors, ferricyanide, Amperometry

Immobilized $K_3Fe(CN)_6$ and Glucose Oxidase in Polypyrrole on Au Microelectrode and Its Application as Glucose Sensors

PROCEDURES

Preparation of Gold Microelectrodes



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Preparation of Glucose sensors: Electrochemical Polymerization of Pyrrole and Immobilization Glucose Oxidase and Ferricyanide

↓

Study the Effects of Charge Passed during Polymerization, Pyrrole Concentration and Ferricyanide Concentration

↓

Determination of Glucose, Study of Stability and Sensitivity

Immobilized $K_3Fe(CN)_6$ and Glucose Oxidase in Polypyrrole on Au Microelectrode and Its Application as Glucose Sensors

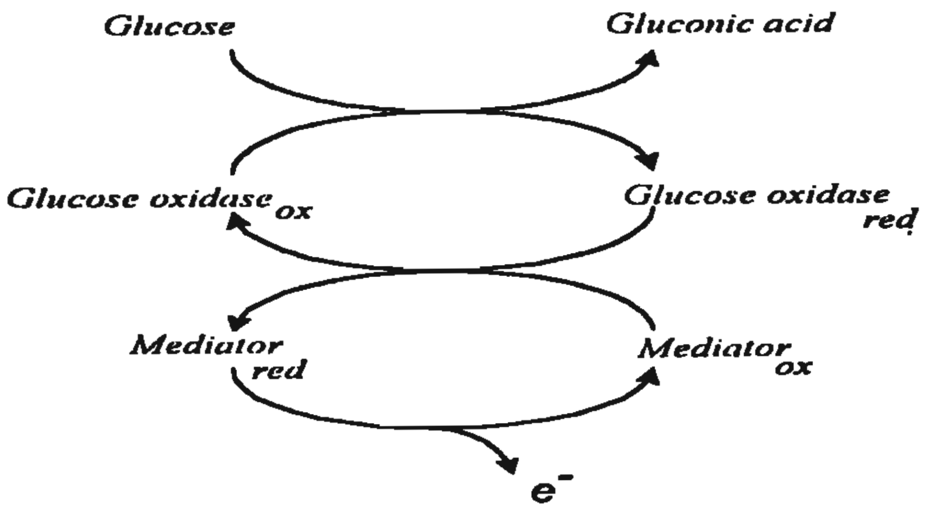

Fig. 1 Scheme reaction pathway utilizing a redox enzyme linked amperometric measurement via a redox mediator.

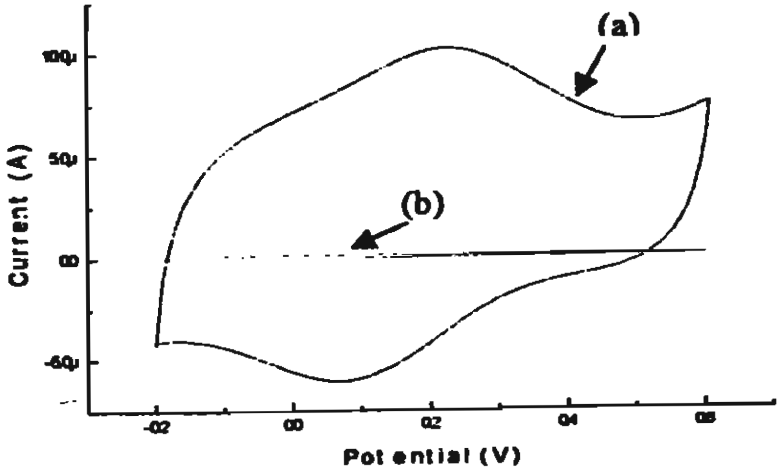

Fig. 2 A typical cyclic voltammograms for oxidation-reduction of ferricyanide in films (a) compared with that of the background (b)

Immobilized $K_3Fe(CN)_6$ and Glucose Oxidase in Polypyrrole on Au Microelectrode and Its Application as Glucose Sensors

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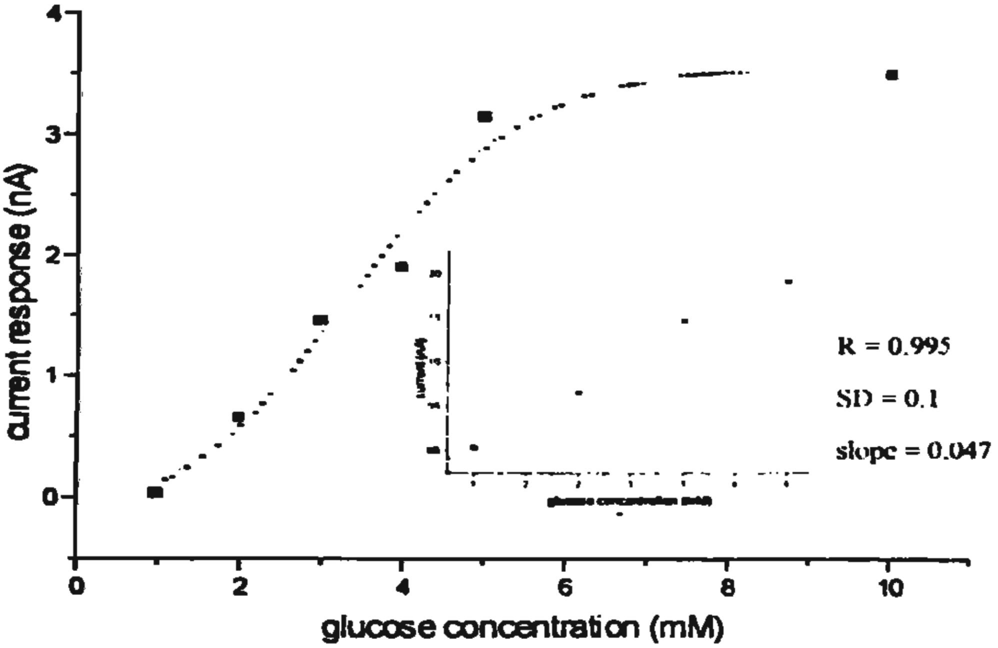

Fig. 3 Calibration curve of glucose sensor at 0.45 V using ferricyanide as mediator

References

1. Holdcroft, S.; Funt, B.L. (1988) *J. Electroanal. Chem.* , **240**, 89 - 95

2. Bartlett, P.N.; Whitaker, R.G. (1987) *J. Electroanal . Chem* ., **224**, 27-35