



The Development of Glucose Biosensor Using Gold Core Platinum Shell Nanoparticles Implanted Chitosan-Carbon Nanotube System

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Abstract: The gold core-platinum shells nanoparticles ($\text{AuNP}_{\text{core}}\text{-Pt}_{\text{shell}}$) in the corporate system of carbon nanotube chitosan scaffolds linked glucose oxidase will be constructed for glucose determination. The nickel hexacyanoferrate (NiHCF) mediator will be implanted in the film as electron shuttle between electrode and electrolyte. The proposed biosensor exhibited good sensitivity for the detection of glucose.

Keywords: Glucose biosensor, Chitosan-CNT scaffolds, Nickel hexacyanoferrate, gold core-platinum shells nanoparticles

1. Introduction

The development of glucose biosensor in recent day becoming increasingly important in the clinical analysis since the lack of glucose can lead to serious complications, including higher risks of heart disease, kidney failure, and blindness in many patients. The glucose monitoring is very crucial for preventing a lack of glucose via the gluco-pump for taking the glucose into the circulate system of the diabetes patient. Diabetes mellitus is a metabolic disease marked by high levels of blood glucose, with the normal concentration range between 80 and 120 mg/dL ($4.4\text{--}6.6\text{ mmol L}^{-1}$) [1]. Formerly, the measuring levels of glucose in the blood of patients will take longer. If the patients with severe, treatment may be delayed and results in damage of patient organ.

The enzyme glucose oxidase (GOx) is used widely in the development of biosensors for detection of glucose due to an enzyme that is specific for glucose and sensitive to the reaction. For increase the efficiency of electron transfer, using gold nanoparticles combined with platinum shell ($\text{Au}_{\text{core}}\text{-Pt}_{\text{shell}}$) was activation [2]. Many research reported the electron transfer between the enzymes to the electrode surface enhanced by the nanotechnologies and it allows the enzyme to bind at the particles surface easily.

Chitosan (CHIT) is biomolecules that derivatives from chitin via deacetylation reaction by the chitosan molecular. The CHIT possess the functional group that can crosslink and occurring cavity or space. The nanomolecule such as carbon nanotube (CNT) led into

the gap of chitosan scaffold (CHIT-CNT) due to their specific structures and unique properties. Carbon nanotubes (CNT) could promote the electron-transfer reaction and improve the conductive property when they were used in the construction of biosensors. [3]. The nickel hexacyanoferrate (NiHCF) act as catalyst in the reduction reaction of the hydrogen peroxide.

This research was aimed to develop a sensitive and rapid glucose biosensor. The modification was done by immobilization of Ni, CHIT, $\text{AuNP}_{\text{core}}\text{-Pt}_{\text{shell}}$ and GOx to the surface of glassy carbon electrode ($\text{GOx}/\text{Au}_{\text{core}}\text{Pt}_{\text{shell}}\text{-CHIT-CNT}/\text{NiHCF}/\text{GCE}$). The mechanism of this biosensor show in Fig 1.

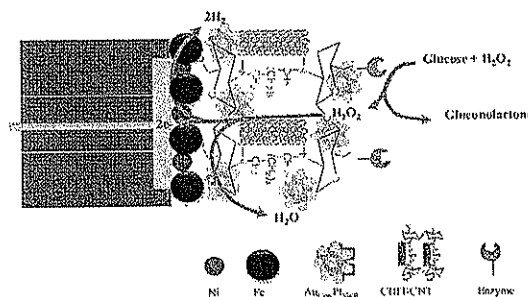


Figure 1. Schematic diagram of GCE/NiHCF/CHIT-CNT-AuNP_{core}-Pt_{shell}/GOx).

2. Experimental

2.1 Apparatus

The electrochemical experiments were conducted using a potentiostat (DigiIVY, model DY2000, USA). A three electrode; platinum wire, Ag/AgCl (3 M KCl) and glassy carbon (3 mm diameter) were used to be counter, reference and working electrode, respectively. The measurement were investigated in a 4 mL electrochemical cell.

2.2 Synthesis of gold core - platinum shell nano particle.

Heat 10 ml of 1% AuCl_4 at 120°C for 5 minutes, then add 1.24 mL of 10 mM sodium borohydride (NaBH_4) into the hot AuCl_4 and continue heat for an hour. Pipette 1.31 ml of 1.9 mM potassium



chloroplatinate into the heat solution and continuous heat for 30 minutes. The yellowed-brown color solution was obtained for Au_{core}-Pt_{shell}.

2.3 Synthesis of chitosan - carbon nanotubes scaffold

The CNT was purified with acid prior to use. Briefly, weighing 0.05 g of CNT and dispersed in Aqua regia acid. The dispersed solution was ultrasonic for 10 hour and keep rinsing by the ultrapure water until the pH is approximately 7, after that dried at 80 °C.

The chitosan scaffold was synthesized using the glutaraldehyde as cross linking agent. Briefly, weighing 0.05 g of chitosan flake, put into 60 ml of water, then add concentrated hydrochloric acid until receive clear solution. The solution was adjusted to pH 5 by 1.0 M sodium hydroxide. The solution was adjusted to 100 ml with the ultrapure water to receive 1% chitosan and store at 4°C when not in use.

Glutaraldehyde was dissolved in chitosan solution at the ratio of 1:200 mol. The slurry was sonicated in ultrasonic bath overnight. Then, extracted the unreact by ethyl ether for 5 time. The purified CNT was mixed into scaffold slurry and sonicate for 4 hours.

2.4 Modification of GOx/Au_{core}Pt_{shell}-CHIT-CNT/NiHCF/GCE

The glassy electrode was cleaned by polish on the emery paper containing Al₂O₃ powder prior to use. The film of NiHCF was fabricate at the first step. Briefly, bring 1.5 ml of 1.2 mM nickel chloride and 1.5 ml of 0.5 mM potassium hexacyanoferrate (III) and 1.5 mL of 0.05 M potassium chloride into the electrochemical cell. The cyclic voltammetric technique was used to electrodeposit the NiHCF at the glassy carbon electrode by cycling potential in the range of -0.1 to 1.0 V at scan rate 50 mV.s⁻¹ for 25 cycles. Then, a drops of CHIT-CNT-AuNP_{core}-Pt_{shell} was applied onto the electrode surface and left dried in air. An aliquot of the enzyme glucose oxidase was spread on the electrode surface and left it dried to obtain GOx/Au_{core}Pt_{shell}-CHIT-CNT/NiHCF/GCE.

3. Results and Discussion

3.1 The cyclic voltammogram of modified electrode

The cyclic voltammogram of many electrodes show in figure 2 in 0 mM (curve 1), 1 mM (curve 2) and 5 mM (curve 3) glucose. The cyclic voltammogram of bare GCE (figure 2A) show no any signal from the substance since there are no glucose oxidase. The GOx/GCE (figure 2B) show a curve at 0.68 V that the current is increase along with the higher concentration of glucose. For the GOx/NiHCF/GCE (figure 2C), the peak of Fe^{2/3} in NiHCF dominated at 0.48 V, The NiHCF can catalyze the hydrogent peroxide through the Fe^{2/3}. While adding the CHIT-CNT into the system of GOx/NiHCF/GCE

(figure 2D), we found that the background current is higher than without CHIT-CNT. This phenomena mean that the CHIT-CNT is barrier to the electrode transfer. While we put Au_{core}-Pt_{shell} into the previous electrode as GOx/Au_{core}Pt_{shell}-CHIT-CNT/NiHCF/GCE (figure 2E), the effect of Au_{core}-Pt_{shell} can reduce the background current and resulting in reduce the detection limit of biosensor.

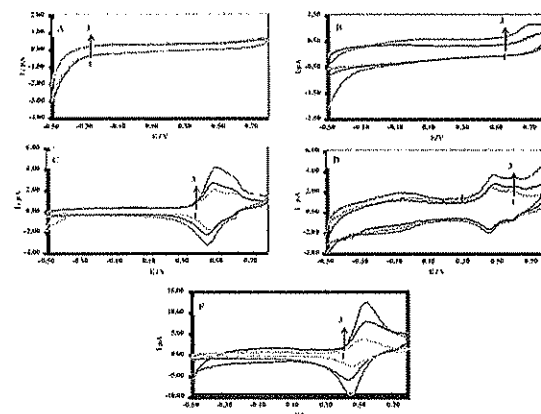


Figure 2. Cyclic voltammogram of (A) bare GCE, (B) GOx/GCE, (C) GOx/NiHCF/GCE, (D) GOx/CHIT-CNT/NiHCF/GCE and (E) GOx/CHIT-CNT-Au_{core}Pt_{shell}/NiHCF/GCE in solution (1) 0 mM, (2) 1 mM and (3) 5 mM glucose. Condition; scan rate, 50 mVs⁻¹; supporting electrolyte, 0.1 M PBS.

3.3 The figure of merits

The GOx/CHIT-CNT-Au_{core}Pt_{shell}/NiHCF/GCE was used for parameter optimization by spiking the glucose to 5 mM into 0.1 M phosphate buffer. The applied potential was varied from 0.2 – 0.7 V. A plot of applied potential against obtained current was showed in figure 3. We found that at the applied potential between 0.2 – 0.4 V, the current were low and they were immediately increasing at 0.5 – 0.6 V. However, we found that the maximum current was obtained at 0.55 V. The applied potential of 0.55 V was selected for next experiment.

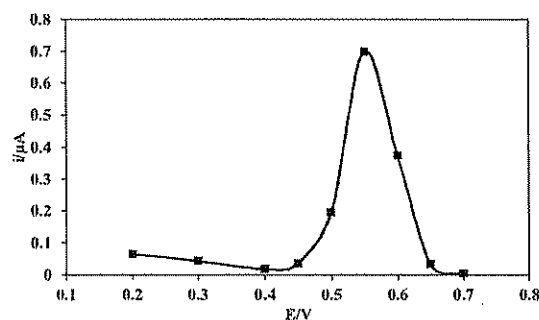


Figure 3. The i-E curve for GOx/Au_{core}Pt_{shell}-CHIT-



CNT/NiHCF/GCE. Conditions; spiked glucose, 5 mM; supporting electrolyte, 0.1 M PBS.

The glucose biosensor was operated for the linearity and detection limit. Figure 4 shows linearity of this biosensor that it was in the range of 0.5 mM – 20 mM ($y=0.0038x - 0.0017$, $R^2= 0.9947$). The calculated detection limit was obtained to be 0.21 mM.

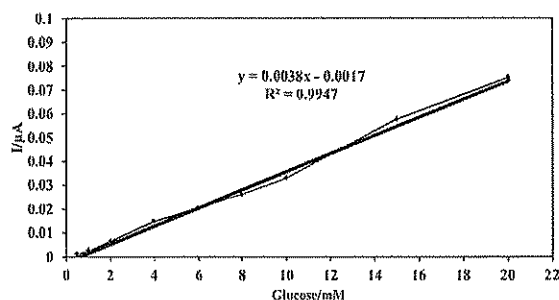


Figure 4 Linearity of the GOx/Au_{core}Pt_{shell}-CHIT-CNT/NiHCF/GCE. Conditions; applied potential, 0.55 V ; supporting electrolyte, 0.1 M PBS.

4. Conclusion

The glucose biosensor based on the GOx assisted Au_{core}Pt_{shell} and NiHCF in the incorporated system of CHIT-CNT scaffolds was successfully developed. The biosensor are easy to use, quickly, accurately, sensitivity, and reliable for measuring glucose and also can be applied for glucose determination in real sample in the near future.

Acknowledgements

This project was supported by the Thailand Research Fund (TRF) and partial financial support by the Faculty of Science, Maejo University.

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ที่ ศธ.5808 /สธบ./ 244

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เรื่อง ขอเรียนเชิญเข้าร่วมงานประชุมวิชาการระดับนานาชาติ (ภูมิภาคอาเซียน) ครั้งที่ 2
ASEAN Plus 2013: The 2nd Regional Symposium on Biosensors, Biodiagnostics and Biochips

เรียน ท่านผู้ทรงคุณวุฒิ

ด้วยมหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรีร่วมกับมหาวิทยาลัยมหิดล มหาวิทยาลัยแม่ฟ้าหลวง และศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ ดำเนินการจัดโครงการประชุมวิชาการระดับนานาชาติทางวิทยาศาสตร์ (ภูมิภาคอาเซียน) ในหัวข้อ ASEAN Plus 2013: The regional symposium on Biosensors, Biodiagnostics and Biochips โดยกำหนดจัดขึ้นระหว่าง 11-13 ธันวาคม 2556 ณ มหาวิทยาลัยแม่ฟ้าหลวง จังหวัดเชียงราย โดยมีวัตถุประสงค์เพื่อส่งเสริมให้เกิดการเผยแพร่ผลงานวิจัยสู่การนำไปใช้ประโยชน์ต่อสาธารณะ รวมทั้งเป็นเวทีในการแลกเปลี่ยนความคิดเห็นของนักวิชาการ อาจารย์ นักศึกษาจากสถาบันต่างๆ อันจะนำไปสู่การสร้างสรรคผลงานทางวิชาการที่มีคุณค่าต่อประเทศชาติ รวมถึงความสัมพันธ์อันดีต่อประเทศเพื่อนบ้าน

คณะผู้จัดการประชุมวิชาการ ขอเรียนเชิญบุคลากรที่มีความสนใจ หรือผู้มีผลงานวิจัย เข้าร่วมประชุมและนำเสนอผลงานทางวิชาการดังกล่าว พร้อมทั้งขอความอนุเคราะห์ประชาสัมพันธ์งานสัมมนาไปยังผู้สนใจ ทั้งนี้ผู้เข้าร่วมฝึกอบรมที่ได้รับอนุมัติการเข้าร่วมจากผู้บังคับบัญชาจะไม่ถือเป็นวันลา และสามารถเบิกค่าใช้จ่ายจาก ต้นสังกัดได้ตามหนังสือของกระทรวงการคลังที่ กค 0409.6/ว.122 ลงวันที่ 27 ธันวาคม 2545 สำหรับพนักงานรัฐวิสาหกิจและองค์การบริหารส่วนท้องถิ่นสามารถเบิกค่าใช้จ่ายจากต้นสังกัดได้ตามระเบียบของแต่ละหน่วยงาน

จึงเรียนมาเพื่อโปรดพิจารณาเข้าร่วมโครงการ ซึ่งจะยังประโยชน์ให้เกิดการพัฒนาเทคโนโลยี รวมถึงเพิ่มศักยภาพในการปฏิบัติงานในลำดับต่อไป

ขอแสดงความนับถือ

(นางบุษยา นูนาค)

ผู้อำนวยการ

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