UNIVERSITI PUTRA MALAYSIA

FABRICATION AND CHARACTERIZATION OF DNA SENSORS FROM METAL OXIDES NANOPARTICLES AND CHITOSAN-MODIFIED GLASSY CARBON ELECTRODES

NOR AMIRAH BINTI MOHD MAAMOR

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2011

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By

NOR AMIRAH BINTI MOHD MAAMOR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

MAY 2011

Dedicated to my beloved parents,

Haji Mohd Maamor Bin Radiman Hajah Ngaliah Binti Barindi

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

FABRICATION AND CHARACTERIZATION OF DNA SENSORS FROM METAL OXIDES NANOPARTICLES AND CHITOSAN-MODIFIED GLASSY CARBON ELECTRODES

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MAY 2011

Chairman: Tan Wee Tee, PhD

Faculty: Science

Voltammetric studies of a sensitive electrochemical deoxyribonucleic acid (DNA) sensor based on nano particles and multi-walled carbon nanotube (MWCNTs) for DNA immobilization is described. In this study, two nanoparticles were used, zirconium oxide (ZrO_2) and titanium oxide (TiO_2). Layer deposition technique was used to prepare nanoparticles/MWCNTs/chitosan (CHIT)-modified glassy carbon electrode (GCE) and DNA were immobilized to the GCE. The immobilization of DNA on the electrode was monitored by cyclic voltammetry (CV) analysis by measuring the change of peak currents using electroactive methylene blue (MB) as an indicator.

Compared with previous DNA sensor with DNA directly incorporated on carbon electrodes, this carbon nanotube-based assay with its large surface area and good charge-transport characteristics increased DNA attachment quantity. Parameters used on this study including electrochemical characterization, scan rate study and pH optimization as well as chronoamperometry (CA) and chronocoulometry (CC). The

electrochemical reduction and oxidation of the redox couples of MB (as a DNA indicator) can be recognized easily by the solid-phase voltammetry of nanoparticles. The cyclic voltammograms for the three differently modified electrodes, nanoparticles/CHIT, MWCNTs/CHIT and nanoparticles/MWCNTs/CHIT, showed that there are 2 major peaks due to the redox couple of MB. Electrochemical characterization of the two modified electrodes, nanoparticles/MWCNTs/CHIT, shows that both of this modified electrode give highest peak current, evident that the composite mixtures cause the increase in the redox peak current of MB.

For hybridization study, the current enhancement observed in three differently fabricated DNA sensor based on DNA/TiO_2/CHIT/GCE, DNA/MWCNTs/CHIT/GCE and DNA/TiO_2/MWCNTs/CHIT/GCE, responding to redox couple of MB are compared. The sensor DNA/TiO_2/MWCNTs/CHIT/GCE was found to the most be sensitive towards hybridized DNA. Parameters, used in this study include hybridization time, hybridization temperature, accumulation time, effect of varying concentration of probe, calibration curve, selectivity of DNA sensor, reproducibility and repeatability. From the studies, the optimized condition of hybridization for target ssDNA reaching the ssDNA probe is 30 min at 30 ^{o}C. The accumulation time of MB, time taken for MB to bind with dsDNA is 150 seconds, while, 100 μM of ssDNA probe was found to be the appropriate dosage for the fabrication of the sensor

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

FABRIKASI DAN PENCIRIAN DNA SENSOR DARI NANOPARTIKEL OKSIDA LOGAM DAN KITOSAN-TERUBAHSUAI ELEKTROD-ELEKTROD KARBON BERKACA

Oleh

NOR AMIRAH BINTI MOHD MAAMOR

MEI 2011

Pengerusi: Tan Wee Tee, PhD

Fakulti: Sains

Kajian voltametri sensor DNA yang peka menggunakan nanopartikel dan multi-lapisan nanotiub karbon (MWCNTs) untuk pemegunan DNA dijelaskan. Dalam kajian ini, dua nanopartikel digunakan iaitu zirkonium oksida (ZrO_2) dan titanium oksida (TiO_2). Teknik lapisan pemendapan digunakan untuk menyediakan nanopartikel/MWCNTs/kitosan(CHIT)-modifikasi elektrod karbon berkaca (GCE) dan DNA dipegunkan di atas GCE tersebut. Proses pemegunan DNA pada elektrod dipantau oleh voltametri siklik (CV) dengan mengukur perubahan arus puncak menggunakan metilen biru (MB) yang elektroaktif sebagai penunjuk.

Dibandingkan dengan sensor DNA sebelumnya dengan DNA yang dipegunkan secara langsung pada elektrod karbon, nanotiub karbon diuji berasaskan ciri-ciri luas permukaan yang besar dan cas-pengangkutan yang baik yang meningkatkan pengikatan DNA. Parameter yang digunakan pada kajian ini termasuk pencirian elektrokimia, kadar imbasan yang berbeza dan pengoptimuman pH serta kronoamperometri (CA) dan

kronokoloumetri (CC). Penurunan dan pengoksidaan elektrokimia dari pasangan redoks MB (sebagai penunjuk DNA) boleh dikenali dengan mudah oleh voltammetri fasa pepejal oleh nanopartikel. Siklik voltamogram untuk tiga modifikasi elektrod yang berbeza, nanopartikel/CHIT, MWCNTs/CHIT dan nanopartikel/ MWCNTs/CHIT, menunjukkan dua puncak utama redoks MB. Pencirian elektrokimia bagi dua modifikasi elektrod, nanopartikel/MWCNTs/CHIT, menunjukkan kedua-dua modifikasi elektrod ini memberikan arus puncak yang tertinggi membuktikan bahawa campuran komposit ini menyebabkan peningkatan arus puncak bagi MB.

Untuk kajian pengacukan, perbandingan peningkatan arus diamati dalam tiga DNA sensor yang berbeza, DNA/TiO_2/CHIT/GCE, DNA/MWCNTs/CHIT/GCE dan DNA/TiO_2/MWCNTs/CHIT/GCE, berdasarkan pasangan redoks dari MB tersebut. DNA/TiO_2/MWCNTs/CHIT/GCE sensor didapati paling peka terhadap pengacukan DNA. Parameter yang digunakan dalam kajian ini termasuk tempoh pengacukan, suhu pengacukan, masa pengumpulan, kesan perbezaan kepekatan prob, lengkung kalibrasi, selektiviti DNA sensor, kebolehulangan dan kebolehhasilan. Dari kajian, keadaan optimum bagi pengacukan untuk ssDNA sasaran mencapai ssDNA prob adalah 30 min pada 30 ^{o}C. Masa pengumpulan MB, iaitu masa yang diperlukan untuk MB terikat pada dsDNA adalah 150 saat dan didapati 100 μM ssDNA prob adalah dos yang sesuai untuk fabrikasi sensor.

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I certify that a Thesis Examination Committee has met on **3 May 2011** to conduct the final examination of Nor Amirah Binti Mohd Maamor on her thesis entitled "**Fabrication and Characterization of DNA Sensors from Metal Oxides Nanoparticles and Chitosan Modified Glassy Carbon Electrode**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Examination Committee were follows:

Aspollah Hj. Md Sukari, PhD
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisor Committee were as follows:

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HASANAH MOHD. GHAZALI, PhD
Professor and Dean
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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NOR AMIRAH BINTI MOHD MAAMOR

Date: 3 May 2011

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