รหัสโครงการ SUT1-102-56-12-31

รายงานการวิจัย

เอนไซม์ไบโอเซนเซอร์ที่ใช้ร่างแหตรึงของสารประกอบรีดอกซ์มอดิไฟด์ไคติน/ไคโตซานบนผิวคาร์บอนนาโนทิวบ์

Reagent-free enzyme biosensors with a redox-modified chitin/chitosan-carbon nanotube composite as immobilization matrix

ได้รับทุนอุดหนุนการวิจัยจาก
มหาวิทยาลัยเทคโนโลยีสุรนารี

ผลงานวิจัยเป็นความรับผิดชอบของหัวหน้าโครงการวิจัยแต่เพียงผู้เดียว

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คณะผู้วิจัย

หัวหน้าโครงการ

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28 สิงหาคม พ.ศ 2559

กิตติกรรมประกาศ

ข้าพเจ้าขอขอบคุณมหาวิทยาลัยเทคโนโลยีสุรนารีที่ให้ทุนสนับสนุนงานวิจัยในครั้งนี้ และขอขอบคุณศูนย์เครื่องมือ มหาวิทยาลัยเทคโนโลยีสุรนารี ที่ให้ใช้อุปกรณ์และเครื่องมือในห้องปฏิบัติการชีวเคมีเพื่อการวิจัย ทำให้งานวิจัยสำเร็จลุล่วงไปด้วยดี

รองศาสตราจารย์ ดร. อัลแบร์ต ชูลเทอ
อาจารย์ประจำสาขาวิชาเคมี สำนักวิชาวิทยาศาสตร์
มหาวิทยาลัยเทคโนโลยีสุรนารี
28 สิงหาคม พ.ศ. 2559

บทคัดย่อ

ในโครงการวิจัยนี้ได้ทำการพัฒนากลูโคสไบโอเซนเซอร์ที่มีอายุการใช้งานนานด้วยการเคลือบผิวหน้ากลมของแพลตทินัมอิเล็คโทรดด้วยคาร์บอนนาโนทิวบ์ตามด้วยไคโตซาน สารอนุพันธุ์เฟอโรซีน และเอนไซม์กลูโคสออกซิเดส ด้วยวิธีเคมีเชิงไฟฟ้าที่เรียกว่า ‘Electrophoretic Deposition Paint หรือ EDP’ การตรึงเอนไซม์ด้วยวิธีนี้ทำให้โมเลกุลของออกซิเจนและสารอนุพันธุ์เฟอโรซีนสามารถเคลื่อนที่เข้าไปทำปฏิกิริยากับเอนไซม์แล้วสร้างอิเล็คตรอนที่เคลื่อนที่จากโมเลกุลของเอนไซม์ไปยังผิวหน้าอิเล็คโทรดได้โดยตรง ทำให้เอนไซม์กลูโคสออกซิเดสสามารถทำปฏิกิริยากับกลูโคสใหม่ได้อย่างรวดเร็ว เป็นการช่วยฟื้นฟูบริเวณเร่งของเอนไซม์ให้เกิดปฏิกิริยาแบบต่อเนื่องได้ นอกจากนี้สารไคโตซานซึ่งมีคุณสมบัติเป็นชีวโพลีเมอร์ยังช่วยตรึงเอนไซม์กลูโคสออกซิเดสให้มีเสถียรภาพสูงและคงทนอยู่บนผิวหน้าอิเล็คโทรดได้เป็นระยะเวลานาน จากการวิเคราะห์เสถียรภาพการตอบสนองพบว่าสัญญาณที่ได้จากการวัดความเข้มข้นของสารละลายกลูโคสด้วยไบโอเซนเซอร์ CNT/Chit/Fc/GOx ในระบบโฟล สามารถวัดความเข้มข้นของสารละลายกลูโคสในความเข้มข้นต่าง ๆ ได้ ด้วยการวัดในระยะเวลาที่ยาวนานและต่อเนื่อง โดยสรุป การเคลือบด้วยชั้นบาง ๆ ของโพลิเมอร์ด้วยวิธีเคมีเชิงไฟฟ้า (EDP) บนผิวหน้าอิเล็คโทรด CNT/CHIT/Fc/GOx มีความเหมาะสมสำหรับการพัฒนาไบโอเซนเซอร์ประสิทธิภาพสูงที่สามารถวัดหาปริมาณกลูโคสในระยะเวลาที่ยาวนาน

ABSTRACT

In this project, long-live glucose biosensors have been developed from platinum disk working electrodes that are chemically modified with chitosan-soaked carbon nanotube deposits (CHIT-CNT), and subsequently loaded with a ferrocene species (Fc) and glucose oxidase (GOx) as functional sensing elements. The entire placement was easy to carry out by an electrochemical technique called 'Electrophoretic Deposition Paint or EDP', which at the end the CNT/CHIT/Fc/GOx thin films are top-coated to protect against loss of enzyme and/or synthetic redox mediator. This sophisticated but simple-to-make enzyme immobilization matrix allowed not only molecular O_2 and/or Fc to be active as native and/or synthetic redox-active enzyme partner but also made direct enzyme-to-electrode electron transfer (DET) possible. With the biosensor in operation all three options were capable possible for oxidized GOx recovery after enzymatic glucose conversion and restoration of the protein's catalytically active site for non-stop interaction with continuously arriving sugar targets. The integration of the marine biopolymer chitosan as part of the functional biosensor layer led to the predictable gain of a matrix biocompatibility that was well supportive of GOx long-term survival on the sensor surface and hence brought up analytical response stability. The stable and consistent manifestation of CNT/CHIT/Fc/GOx-based biosensor signals in a flow-based electrochemical cell for the duration of multiple day-long continuous exposures to adjusted glucose levels. From glucose calibration trials during uninterrupted flow cell operation showed that the immobilized GOx entities were entrapped gently enough in their polymeric chitosugar/CNT environment to maintain their bio-catalytic activity for long. In summary, EDP-covered CNT/CHIT/Fc/GOx thin films have the potential to be a suitable matrix for the development of long-time stable glucose biosensors with high performance.

สารบัญ

สารบัญภาพ

บทที่ 1
บทนำ (Introduction)

1.1. ความสำคัญและที่มาของปัญหาการวิจัย (Rationale/Motivation)

.(Amperometric) enzyme-based biosensors[1-5] are important tools in clinical diagnosis, health care, process control in food industry and biotechnology and also environmental monitoring. The variety and the complexity of potential samples and the request for cheap but reliable analysis are behind the motivation in the field to continuously improve the simplicity and reliability of enzyme biosensor assembly and permanently make sensor performance better in terms of sensitivity, selectivity, detection limits, minimal influence of interferences and long-term stability. This one-year project tried optimizing the chemical architecture of the porous polymeric immobilization layer that is placed on top of inert (carbon or noble metal) working electrodes for keeping in place (immobilizing) enzymes as the active key sensor component. Suggested as advanced immobilization layer was a novel composite made of (1) a dense network of electrically conductive carbon nanotubes (CNTs) that was (2) loaded with biopolymers chitin and/or chitosan and (3) the reversible redox compound ferrocene (or one of its derivatives). Expected was not only the gain of a very biocompatible immobilization layer for enzymes on electrochemical detector surfaces but also a considerable improvement of the effectiveness of the amperometric transduction scheme. Actually, ongoing substrate conversion by entrapped enzyme at any location in the functional coating would be electronically connected to the electrode via mediator interaction between the distributed ferrocene redox entities and the catalytic protein molecules and quantitative detection of the change in the mediator's redox state at the conductive CNT filaments. Potentially, the proposed sensor layout should be associated with an improved response behavior, in particular for low levels of targeted analyte (substrate), an independence from aerated measuring conditions due to the replacement of oxygen as enzyme redox partner, and an extended sensor life time because of enzyme embedment in a natural biocompatible matrix. Reach of this situation would be a significant achievement and a relevant input of the project to the highly active field of fundamental biosensor research and development and related scientific and industrial communities.

1.2. วัตถุประสงค์ของโครงการวิจัย (Objectives)

1. Addition of enzyme (here as a model: glucose oxidase, GOx) to a blend of CNTs with chitin or chitosan, and ferrocene-based redox entities and the placement of the composite as thin film modification on disk-shaped noble metal electrodes to form (glucose) biosensors with an improved layout of the immobilization layer.
2. Performance tests with the novel type of glucose biosensors and evaluation of their correct function and analytical figures of merit.

1.3. ขอบเขตของการวิจัย (Framework)

Enzyme-based electrochemical biosensors are widely used analytical tools that are subject of continuous research and development. Stable but mild (biocompatible) enzyme immobilization into polymer films on an electrode surface and efficient detection of enzyme/substrate interaction are in case of amperometric enzyme biosensors among the crucial issues determining satisfactory function. Work on the advancement of the building blocks of amperometric enzyme biosensors is thus an understandable requisite on the way to best possible analytical performance. This project aimed at contribution in terms of the improvements of the layout of immobilization layer of enzyme biosensors and a design adaptation that related to enhanced biosensor response towards analyte.

1.4. ประโยชน์ที่ได้รับจากการวิจัย (Proposed output) ประโยชน์ที่คาดว่าจะได้รับจากงานวิจัยนี้มี 4 ประการหลักคือ

1. Novel solutions for enzyme immobilization on noble metal disc electrodes.
2. Improved glucose/glucose oxidase biosensors with a CNT/Chitin or Chitosan/Ferrocene-based immobilization matrix.
3. Trained researchers with knowledge in modern electrochemical analysis/biosensing.
4. One publication in a peer-reviewed international journal with a good impact factor and dissemination of results on an international conference.

หน่วยงานที่จะนำผลงานวิจัยไปใช้ประโยชน์

The following institutions may benefit from the project deliveries:

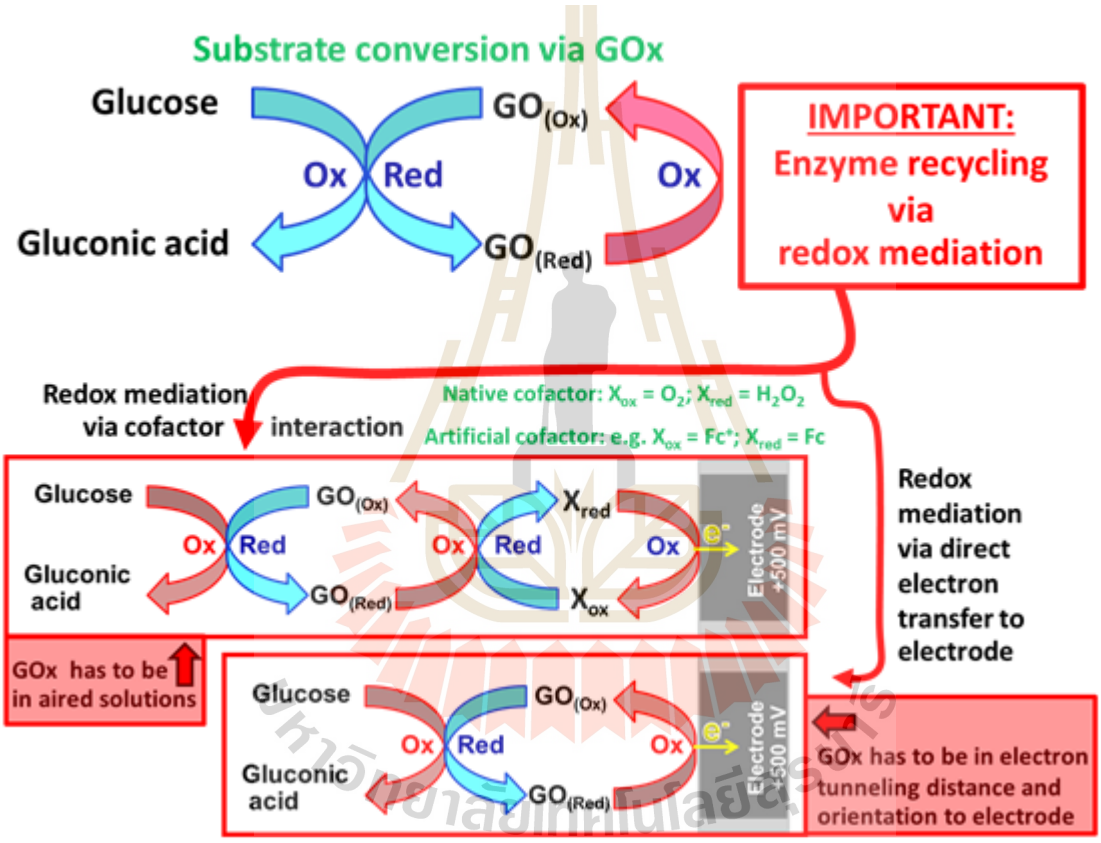
1. Public and private analytical laboratories
2. National and international academic research institutions

บทที่ 2

วิธีดำเนินการวิจัย (Methodology)

Scheme 1 is a graphical representation of the sequence of redox changeovers that are instantly induced when exposing immobilized glucose oxidase entities on a disk-shaped noble metal electrode to the substrate glucose while keeping the adjustable electrode potential at, for instance, an anodic value of +500 mV vs. reference electrode.

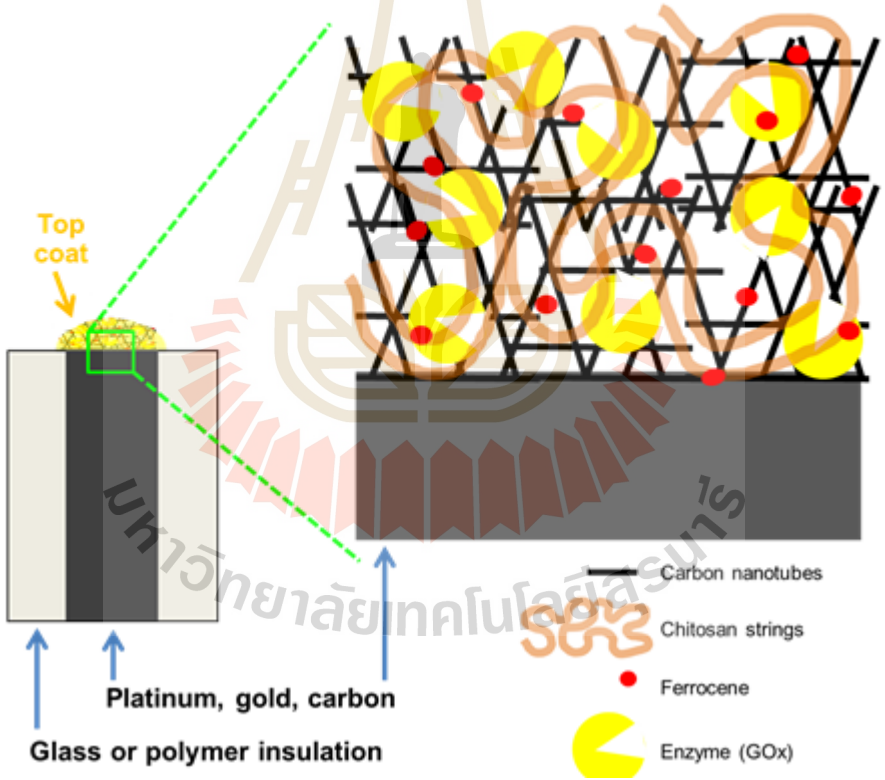
Scheme 1: Possible pathways for GOx redox recycling in the immobilization layer of an amperometric glucose biosensor after the entrapped catalytic protein converted glucose and changed from oxidized to reduced state.



In a few words, glucose is oxidized after conjugation with the catalytically active site of glucose oxidase while the protein itself acts as electron acceptor to become reduced. Depending on the particular biosensor design, up to three pathways may be available for turning the reduced GOx after substrate conversion back into original oxidized state and reset its catalytic site for the interaction with the next arriving target sugar molecule: (1) In aerated solutions the molecular interaction with dissolved oxygen as native redox active mediator can trigger the desired redox recycling; oxygen acts then as electron accepting species and facilitates via reduction into hydrogen peroxide the requisite $GOx_{red} \rightarrow GOx_{ox}$

redox state change, (2) In deaerated solution oxygen is obviously not available as redox partner of GOx; however, clever supplementation with a synthetic dissolved redox species in reduced form can provide a functional replacement that supports enzyme redox recycling; successfully used for this purpose have been, among others, potassium ferricyanide, ferrocene and ferrocene derivatives, and ruthenium-hexamine-chloride, (3) providing that enzyme molecules are immobilized in effective electron tunneling distance and orientation on the (noble metal or carbon) electrode surface direct electron transfer may work as support of the $GOx_{red} \rightarrow GOx_{ox}$ transition with the electron that is released by the process accepted by the electrode as oxidizing agent.

Scheme 2: The design of the developed composite immobilization layer in front of, for instance, a disk-shaped noble metal or carbon working electrode, which is the transducer of GOx action on glucose. Not shown in the zoom is the top coat of an electrodeposition paint is placed after solvent evaporation to hinder enzyme and/or ferrocene molecules to leave via diffusion out of the thin-film surface deposit.



Target here was a comparatively easy to create architecture that, in principle, offers utilization of all three options of glucose oxidase redox recycling, and thus forms biosensors that are well functional in aerated and de-aerated measuring conditions for efficient substrate quantification. Scheme 2 is a graphical representation of the design of the

composite immobilization layer in front of, for instance, a disk-shaped noble metal or carbon working electrode ("the transducer of GOx action on glucose").

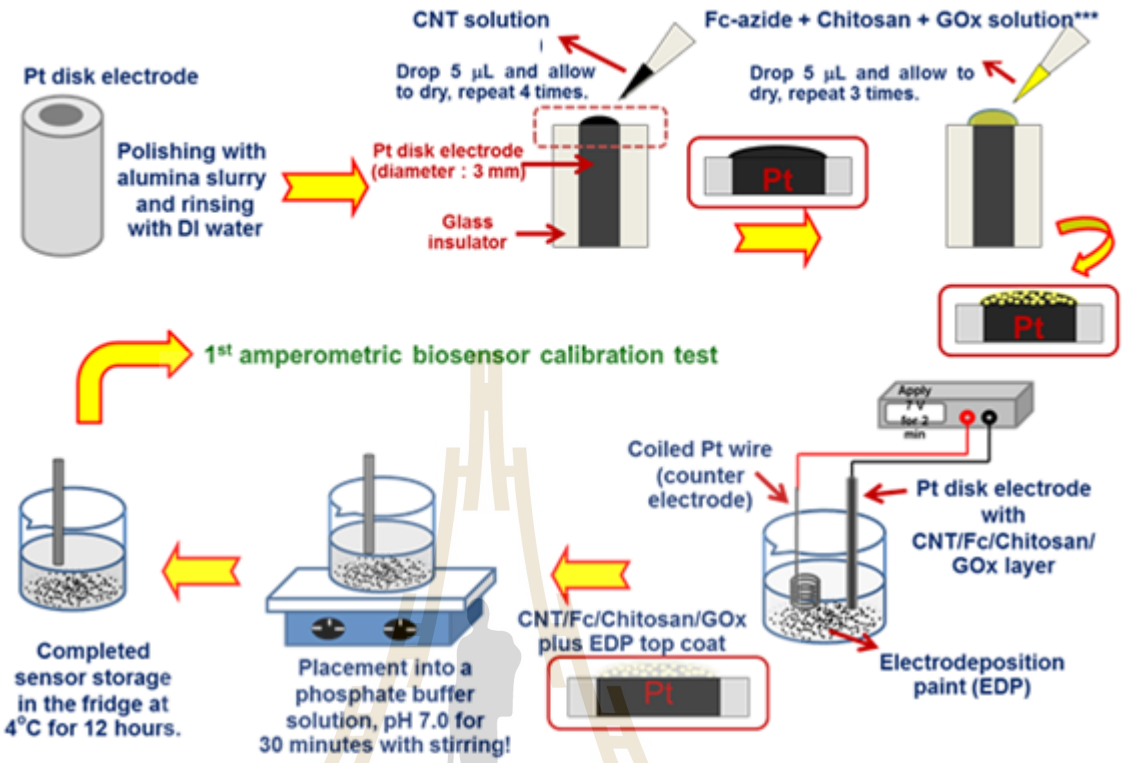


Figure 1: Schematic representation of the procedure that was used in this project for the fabrication of glucose biosensors with a CNT/Fc/Chitosan (or chitin)/GOx-based functional sensor layer on top of a polished 3-mm-diameter platinum disk working electrode (WE). First, a CNT suspension in water was dropped onto the WE disk and allowed to dry; 3 repetitions usually delivered a homogeneous and well-adhering CNT film in electrical contact to the electrode disk. Then, a second drop and dry procedure was carried out with an aqueous suspension holding the Fc compound (here, Fc-N3), GOx and chitosan, now three repetitions ensure proper load with enzyme and redox mediation in the CNT/chitosan matrix. As barrier against enzyme and mediator loss a thin film of electrodeposition paint was electrochemically put in place on top of the CNT/Fc-N3/GOx matrix. Note, that only simple drop coating procedures and, in the final step of top-coat placement, a simple electrodeposition of paint needed for sensor completion. The overall preparation has a potential to be customized for automation, if sensor mass fabrication desired.

Stock solution used for the various drop & dry coating steps usually had 2.5-5 mg/mL CNT, 10-15 mg/mL glucose oxidase and 1-3 mg/mL ferrocene azide suspended in solvent. The commercial chitosan solution was used as purchased.

Figure 1 is then a display of the procedure for the fabrication of glucose biosensors that follows the design suggested in Scheme 2. A nanoporous network of CNT filaments, simply drop-coated on the carrier disk electrode in a preceding step, got actually soaked with a few microliter of a suspension containing GOx, chitosan (or chitin) and ferrocene (or a ferrocene derivative). Solvent evaporation leaves the three functional components randomly distributed in the conductive CNT nano-grid, which itself is in good electrical contact to the

carrying electrode surface at e.g. + 500 mV detection potential vs. reference electrode. In this special configuration O_2 (in aerated solution), ferrocene (in aerated and de-aerated solution) and direct electron transfer redox mediation at CNT surfaces (in aerated and de-aerated solution) can be responsible for the processes of electron shuttling and, depending on the measuring conditions, all may jointly contribute to the current response of the biosensor that allows substrate quantification. The (marine) biopolymer chitosan (or chitin) was chosen to be included in the immobilization matrix for GOx to reach via gain of an inherent material biocompatibility a long-term stable sensor configuration that is suitable for continuous glucose monitoring over extended periods of many days or even weeks.

Sensors of the outlined designs were prepared and evaluated for their performance in common beaker-type three-electrode electrochemical cells via conventional amperometric biosensor calibration measurements and analytical redox voltammetry under aerated and de-aerated electrolyte conditions. Results of the measurements allowed judgments on the basic properties of the sensor response and the analytical figures of merit including linear range, sensitivity, detection limit, and type of redox mediation

Further assessments of the amperometric glucose response of the different established biosensors were carried out under aerated matrix conditions in a three electrode electrochemical flow cell flushed continuously with glucose-containing or -free running buffers with the intention to find out about the long-term stability tests and reproducibility of flow-based calibration curve construction.

บทที่ 3

ผลการทดลองและข้อวิจารณ์ (Results and Discussion)

The following summary of the outcome of this project first presents experiments that demonstrated the coexistence of native and artificial redox mediators and direct electron transfer redox mediation in glucose biosensors with GOx immobilized in a CNT/Fc/Chitosan electrode surface deposit. Data of trials with the sensors of the proposed type and of control versions of the immobilization layer architecture (lacking e.g. the ferrocene supplementation), either operated in aerated and de-aerated measuring buffers, will be presented and discussed. Then presented will be the performance for uninterrupted operation of the developed glucose biosensors in a flow-based system that allowed continuous exposure to substrate free or containing running buffer and scheduled injections of substrate at various time points after trial start.

3.1 Redox crosstalk (DET) of GOx and the CNT component of the CNT/Fc/Chitosan/EDP-based enzyme immobilization matrices

Direct electron transfer (DET) between GOx entities and the charged surface of electrodes avoiding an involvement of free-diffusing redox mediators is according to the Marcus theory not very feasible and only possible when the prosthetic group of the protein macromolecule is in suitable “electron tunneling distance to the solid and on top structurally oriented in a way that the inner catalytically active site is straight facing the interface. These two boundary conditions are not obtained easily and special care within the immobilization procedure of the biocatalyst and a priming of the electrode surface with, for instance, nanomaterial and/or conductive polymer coatings, have been reported as prerequisites for success with the achievement of an efficient DET contact between GOx and the superficial sensor disk. When, however, DET contact is accomplished, the enzyme and the electrode can reversibly exchange electrons and act in either direction as electron acceptor or donator. A convincing proof for a favorable GOX immobilization and electrochemistry with DET realization is thus the appearance of the redox wave for cathodic and anodic GOx reduction and oxidation in cyclic voltammograms that have been recorded with enzyme-modified sensors in redox mediator free de-aerated electrolyte solution that is free of dissolved reducible oxygen.

Glucose oxidase redox cyclic voltammograms have in the past been reported in several studies for electrodes with CNT thin films[6-8] and, as evidenced with the display of Figure 2, it was also accomplishable with the dropped & dried CNT deposit of the biosensors of this work. In good agreement with the earlier reports a pair of well-defined and nearly symmetrical cathodic and anodic current waves was detected when GOx a functional component of the CNT sensor coats and the peak currents scaled, as expected, linear with the square root of scan speed (not shown). In control experiments sensors with a bare CNT modification (no GOx added!) the two distinct redox waves disappeared clearly indicating that the waves in Figure 2 indeed are derived from a faradaic redox interaction between CNT and (adsorbed) enzyme molecules. Apparently, surfaces of the uniformly spread CNTs offered, to certain extent at least, an active graphitic platform for desired direct GOx redox mediation and it was expected to be able to benefit from this valuable effect also in the proposed biosensor configuration (CNT/Fc/Chitosan/GOx/EDP) as one contribution to analyte signaling, which would be particularly good in the absence of oxygen as native redox partner of the enzyme.

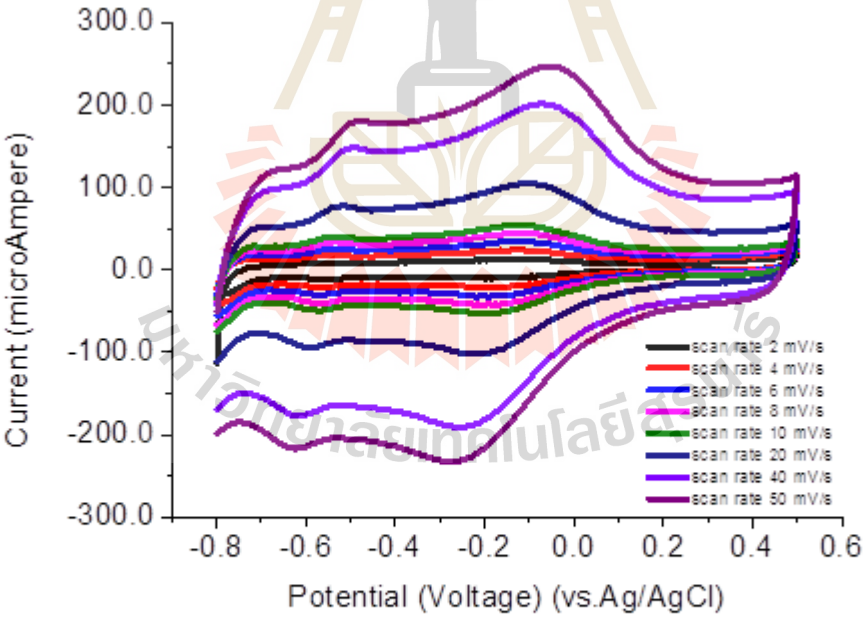


Figure 2: Cyclic voltammograms of the carbon nanotube/glucose oxidase-modified 3-mm-diameter platinum disk electrode in de-aerated 0.1 M KCl/PBS. (pH 7.0) at various scan rates from 2 to 50 mV/s.

After probability of an electron shuttle between the CNT nano-conduits and immobilized GOx was proven, the possibility of using extra artificial redox mediator, here a ferrocene derivative, for support of continuous enzyme recycling during substrate exposure had to be

tested. Tackled was this task with the amperometric glucose biosensor tests of the following section.

3.2 Ferrocene-assisted GOx redox mediation in CNT/Fc/Chitosan/GOx/EDP-sensor coatings

Three different biosensors have been prepared for comparative biosensor test trials concerning the achievement of **CNT & ferrocene**-based redox mediation in a thin-film CNT/Fc/Chitosan/EDP immobilization layer design as proposed in Scheme 2. They were:

- A *Chitosan/GOx/EDP biosensor* lacking both the CNT and the ferrocene component. Only chitosan was present as enzyme-entrapping matrix and EDP as top coat protection against diffusional enzyme loss (Figure 3A).

  For this sensor configuration enzyme redox mediation during substrate conversion is on the shoulders of oxygen alone. In de-aerated solution a full collapse of glucose-induced amperometric current response is expected.

- A *CNT/Chitosan/GOx/EDP biosensor* lacking both the ferrocene component but not the conducting CNT network. Again, chitosan was present as enzyme-entrapping matrix and EDP as top coat protection against diffusional enzyme loss (Figure 3B).

  Here, redox mediation during substrate conversion is not anymore on the shoulders of oxygen alone but, based on the findings in 3.1, may also be facilitated by DET between CNT and GOx entities. In de-aerated solution a distinct decrease of glucose-induced amperometric current response is expected but not a complete signal breakdown.

- The full *CNT/Fc/Chitosan/GOx/EDP glucose biosensor* design with (i) the conducting CNT network in contact with

- the carrier electrode for support of DET, with chitosan in place to add biocompatibility to the enzyme-entrapping matrix, with the ferrocene azide addition there as extra enhancer of redox mediation and, last but not least, with the EDP top coat applied as protection against diffusional enzyme loss (Figure 3C).

  For this sensor layout enzyme recycling during substrate conversion should be most efficient as in aerated measuring buffers it is a share of DET, oxygen as well

as ferrocene redox mediation and in de-aerated solutions still aided by the merger of DET and ferrocene.

A representative amperometric recording from calibration measurements with a type 3 sensor, with the CNT/Fc/Chitosan/GOx/EDP design, is shown in Figure 4. From such amperometric trials the corresponding calibration plots (biosensor current vs. glucose concentration) have been computed and looked at for interpretations of the effective redox mediation pathways in aerated and de-aerated situation.

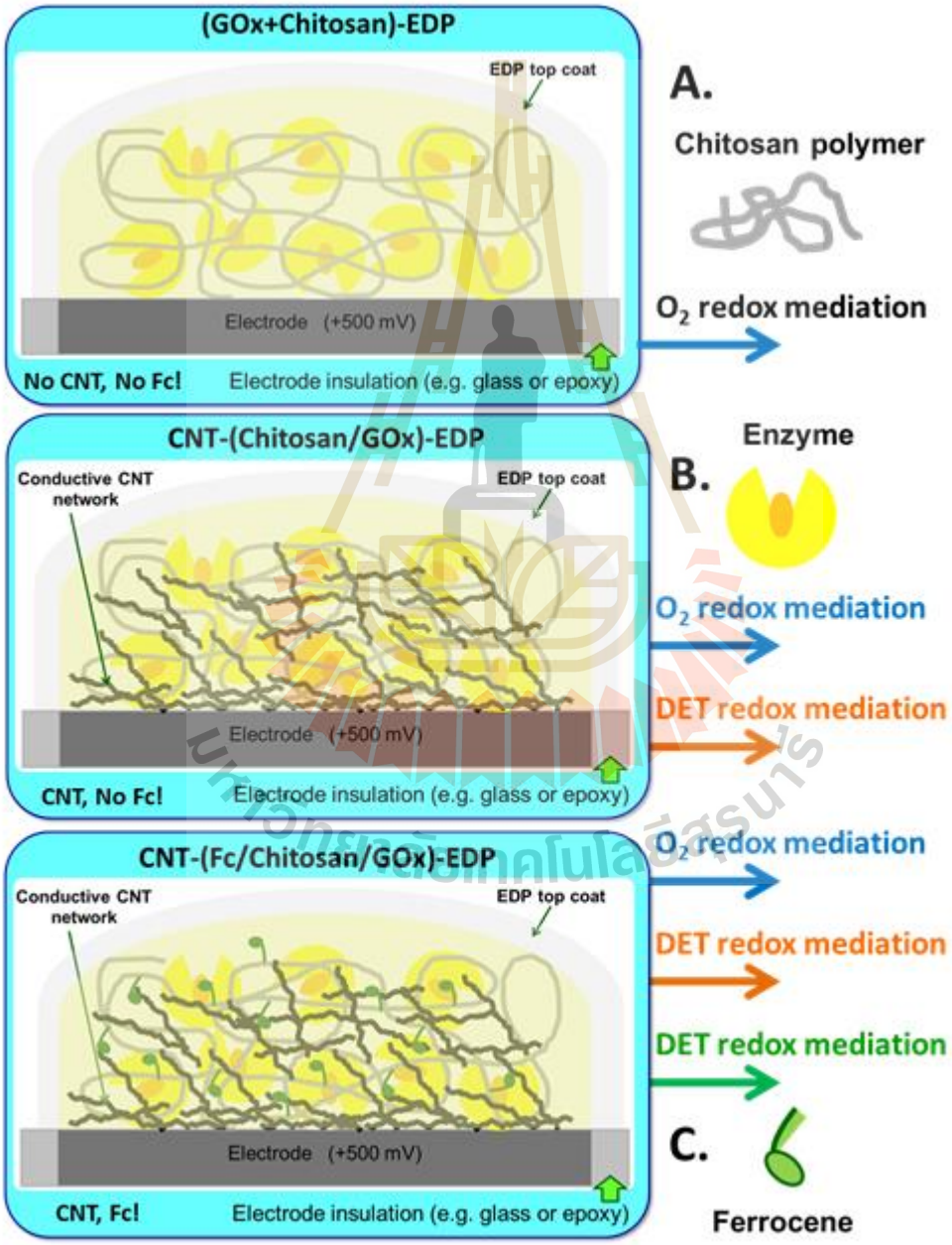


Figure 3: Graphical illustration of the three different types of biosensors that have been prepared for comparative biosensor test trials concerning the achievement of bare oxygen or additionally CNT & ferrocene-supported redox

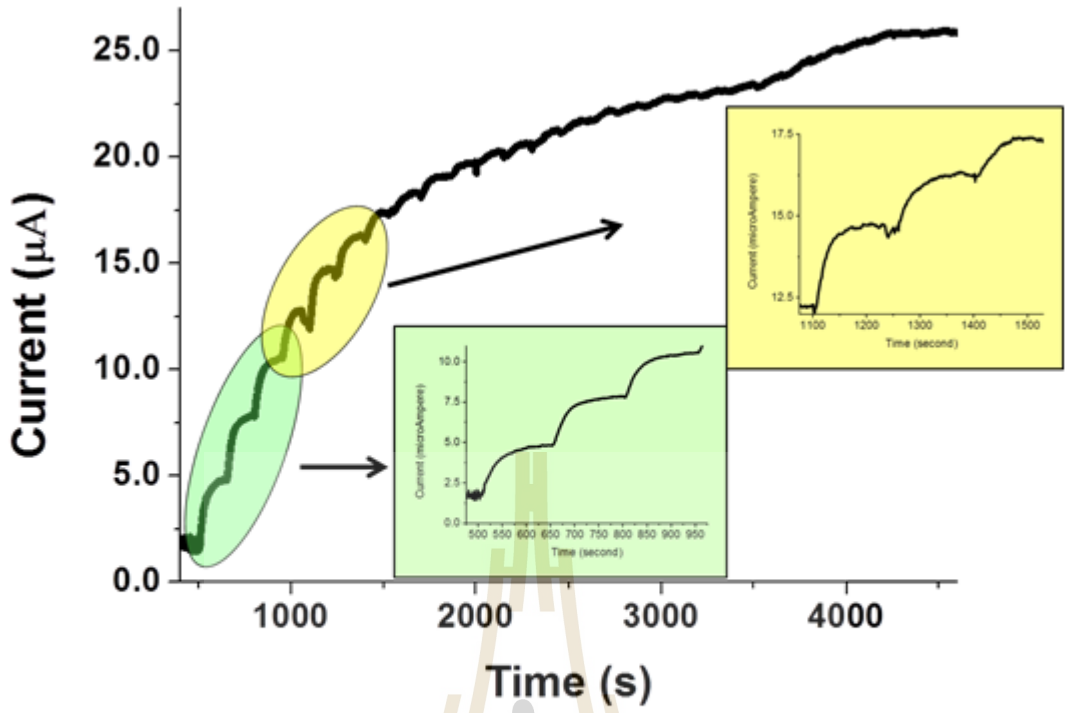


Figure 4: Typical steady-state current response of a CNT/CHIT/Fc/GOx/EDP biosensor on successive addition of small aliquots of glucose into stirred and aerated PBS /KCl (pH 7.2). Carrier electrode was a 3 mm Pt disk electrode. Anodic hydrogen peroxide detection was 500 mV vs. Ag/AgCl. A first set of 20 additions delivered 10 mM and a second set of nine additions 20 mM glucose each into the measuring buffer. Amperometric current traces as shown here were the basis for the calibration curve construction for particular sensor types.

Of note, all sensors carried identical amounts of the individual functional components (CNT, CHIT, Fc, GOx and EDP) in their surface modifications to facilitate sensor response comparison.

Chitosan/GOx/EDP biosensor

Figure 5 is the calibration curve for the biosensor that had the active enzyme entrapped in a biocompatible chitosan matrix for gentle immobilization but not CNT as DET facilitator. A look to calibration curve reveals that as expected this GOx biosensor did not respond in de-aerated measuring buffer not respond with a concentration-dependent H_2O_2 current since there is no GOx redox recycling possible (red trace, Figure 5; all redox partners - O_2, CNT and Fc - are absent). Aeration and supply of oxygen turned the biosensor into an active tool and the typical shape of plots of the measured anodic H_2O_2 current vs. glucose concentration was revealed (brown trace, Figure 5; O_2 available as enzyme redox partner but not CNT and Fc). The current at any concentration is entirely a current produced by

continuous redox interaction of freely diffusing molecular oxygen with enzyme that just completed a substrate conversion cycle. As usual for enzyme sensors signal saturation (reach of I_{lim}) was observed at higher substrate concentration (here: ~ 70-80 mM), indicating reach of maximum turnover for all biocatalyst entities within the immobilization layer.

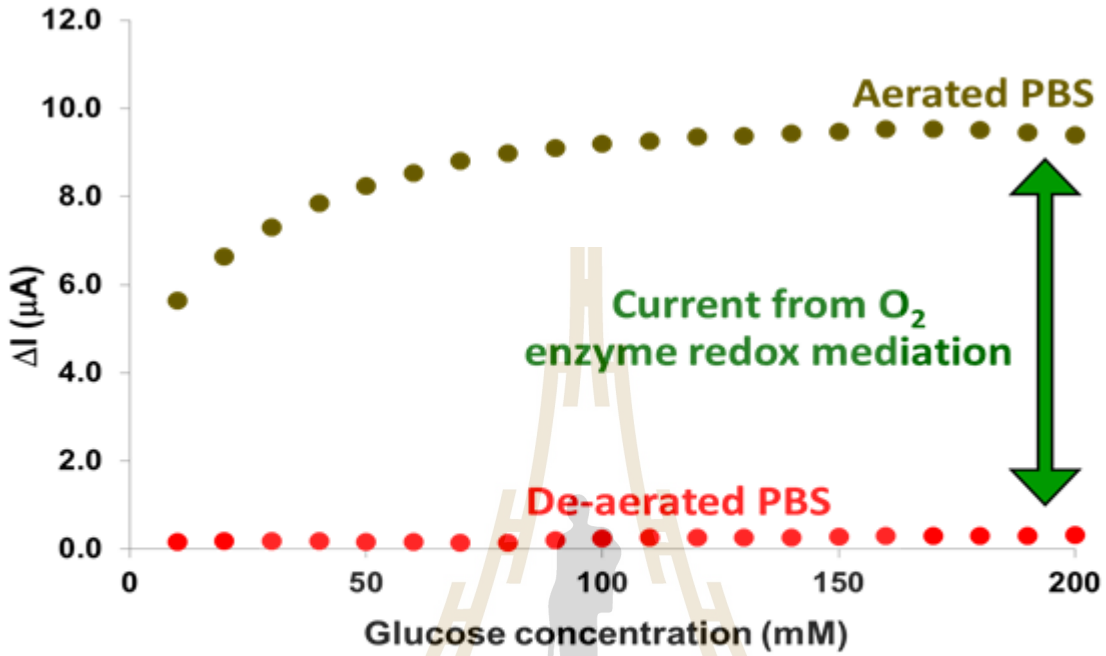


Figure 5: Typical glucose calibration curve of a CHIT/GOx/EDP biosensor. The bottom red trace is valid for an operation in de-aerated PBS/KCl measuring buffer of pH 7.2 while the upper brown trace is the calibration response in aerated solution. Hydrogen peroxide was detected at +500 mV vs. reference electrode. Removal of oxygen from the measuring buffer leads to disappearance of enzyme-based glucose signaling because of elimination of the only option of glucose oxidase redox regeneration after substrate conversion.

CNT/Chitosan/GOx/EDP biosensor

The addition of CNT to the otherwise barely chitosan-based immobilization matrix of the biosensors of this study had a well-positive effect on their performance for the anodic detection of the substrate glucose. In contrast to the above first (CNT free!) sensor case the CNT-holding version produced under de-aerated conditions a hydrogen peroxide oxidation current response upon addition of glucose to measuring buffer, and the signal magnitude developed with the increase of substrate concentration in the characteristic manner reaching a limiting (saturation) value at high enough glucose levels (here: 50-60 mM) (refer to Figure 6 for the calibration curve). The capacity of a CNT/Chitosan/GOx/EDP-biosensor to produce a “normal” amperometric glucose under full exclusion of oxygen as enzyme reaction partner meant manifestation of reasonable DET between immobilized enzyme and CNT network and confirmed the observation from the cyclic voltammetry experiment in

section 3.1 (Figure 2). Not surprisingly, the current at enzyme saturation, could for this sensor design got amplified in course of aeration and related provision of oxygen as extra redox partner for reduced enzyme re-oxidation (refer to the inset of Figure 6). So far confirmed were actually the active involvements of two of the three pathways that have been highlighted in Scheme 1 as possibilities for enzyme biosensor signal establishment with the proposed system.

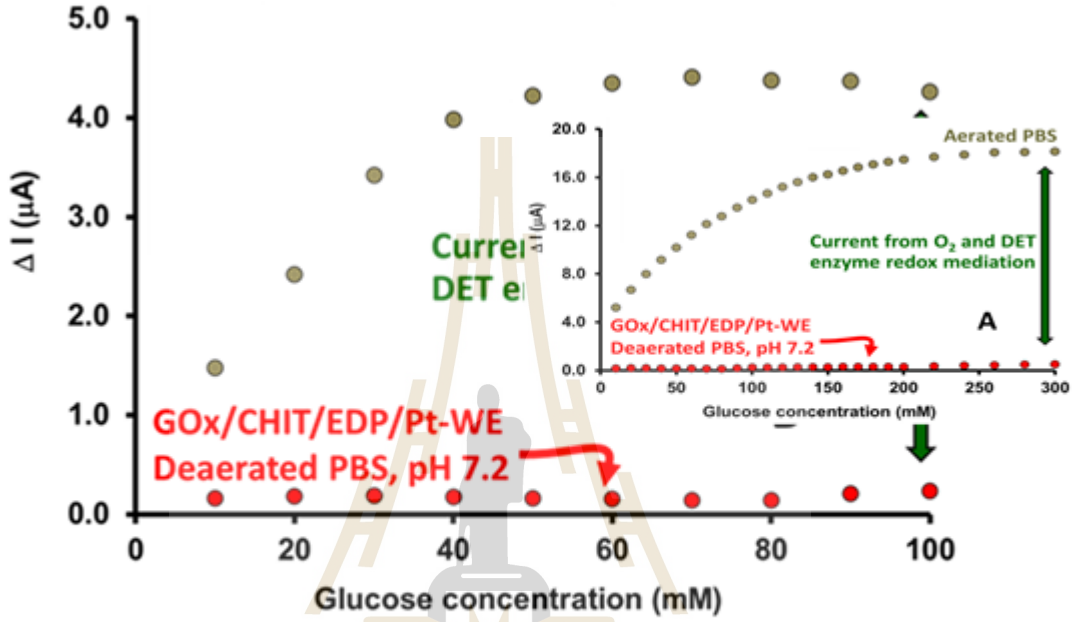


Figure 6: Typical glucose calibration curve of a CNT/CHIT/GOx/EDP biosensor. Hydrogen peroxide was detected at +500 mV vs. reference electrode. The current response of the biosensor is due to effective redox communication between CNT nanowires and GOx molecules and thus possible cyclic redox state regeneration for GOx molecules after substrate conversion. The inset displays the response of the same biosensor in aerated PBS/KCl solution.

CNT/Fc/Chitosan/GOx/EDP biosensor

Amperometric calibration trials with sensors that had a supplemental incorporation of ferrocene azide were executed to get evidence that the redox active iron compound indeed could replace dissolved oxygen as electron-accepting partner for GOx in anaerobic solution. Of note, the ferrocene species was not kept in place in the immobilization matrix of the CNT/CHIT/Fc/GOx/EDP versions via special chemical cross-linking to the network of enzyme-entrapping CNT/CHIT immobilization matrix but rather as randomly dispersed unbound molecule that found position in course of a simple drop & dry placement of the functional electrode coating. An electrodeposition paint top coat was practical leak protection for freely movable ferrocene (and GOx) molecules. Evidence for successful reach of the desired leak protection for Fc (but also active GOx) will be provided in the following Section 3.3 in

which the flow-based long-term stability tests are described. Figure 7 presents for a CNT/CHIT/Fc/GOx/EDP biosensor the calibration curves that were derived from measurements in PBS/KCl measuring buffers with and without oxygen presence.

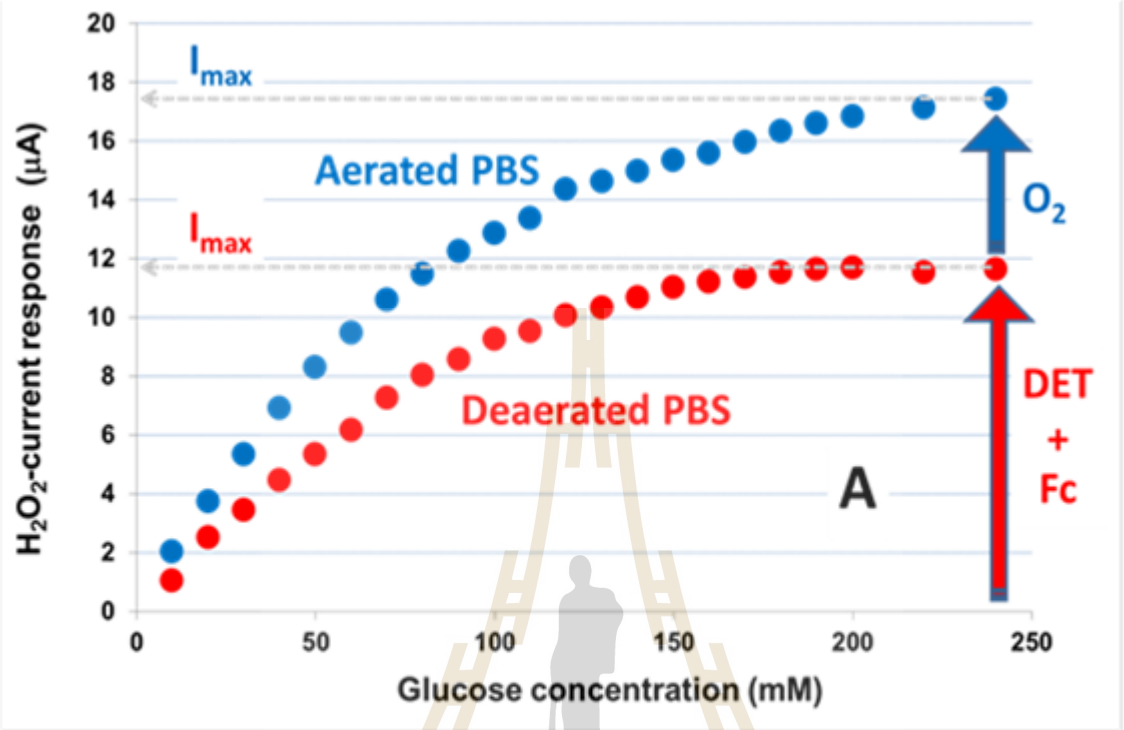


Figure 7: Typical glucose calibration curves of a CNT/CHIT/Fc/GOx/EDP biosensor in presence and absence of oxygen as native enzyme redox mediator. Hydrogen peroxide was detected at +500 mV vs. reference electrode. The current response of the biosensor in the presence of dissolved oxygen in the measuring buffer (blue trace) is due to a sum of the redox communications between CNT nanowires, O_2 and Fc with GOx molecules that need re-oxidation after substrate conversion. After removal of oxygen from the measuring buffer only Fc and CNT via DET can support enzyme recycling and maintenance of the biosensor signal (red trace).

A comparison of the current vs. [GOx] plots available in Figure 7 with the ones in Figure 6 is illustrating that the addition of Fc as synthetic redox active species to the active biosensor matrix indeed is bringing up a positive effect in terms of the efficacy of the recycling of the enzyme's redox state after reaction with substrate. For the trial under anaerobic condition the biosensor current for the Fc-containing sensor variant was at saturation level about doubled over the Fc-lacking equivalent with an otherwise identical supplementation apparently helps increasing the number of GOx entities in the immobilization matrix that are offered the opportunity of a regenerating redox contact. Upon aeration of the measuring buffer oxygen can move in as third partaker of cyclic GOx redox state regeneration and accordingly a further amplification of the current response at sensor saturation is observed.

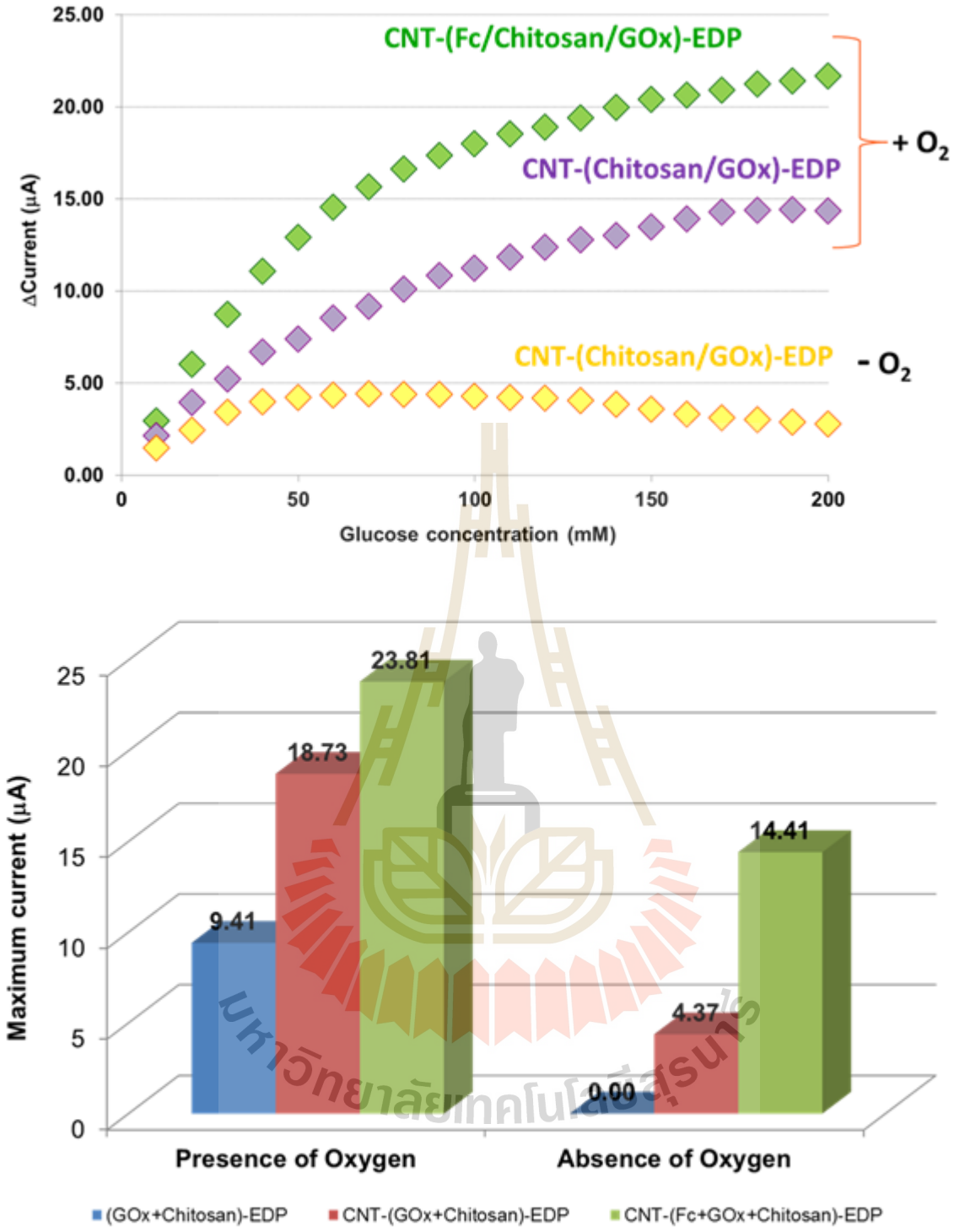


Figure 8: Comparison of typical amperometric glucose calibration curves of CNT/CHIT/GOx/EDP and CNT/CHIT/Fc/GOx/EDP biosensors in presence and absence of oxygen as native enzyme redox mediator (top). Column chart comparison of amperometric biosensor currents at saturation level for CHIT/GOx/EDP, CNT/CHIT/GOx/EDP and CNT/CHIT/GOx/EDP tool versions (bottom). For both displays all experimental conditions for original data recordings were identical except for the composition of the immobilization matrix.

Above situation is graphically concluded in Figure 8, which offers a combination of the calibration curves of CNT/CHIT/Fc/GOx/EDP and CNT/Chitosan/GOx/EDP biosensors in

oxygen-free and oxygen-containing solution (top display) and a column chart comparison of the amperometric sensor currents at saturation level for Chitosan/GOx/EDP, CNT/Chitosan/GOx/EDP and CNT/Chitosan/GOx/EDP, all valid experimental conditions that were identical except for the composition of the immobilization matrix.

3.3 Amperometric biosensor stability and calibration tests in electrochemical flow cells

The biosensors for the trials in above section used a 3-mm-diameter Pt disk electrode as carrier of the enzyme-loaded CNT/CHIT matrix a 3-mm-diameter but for flow-based sensor stability tests Au disks were the transducers as they were available with a design that allowed use as working electrode in the electrochemical flow cell of the device shown in Figure 9.

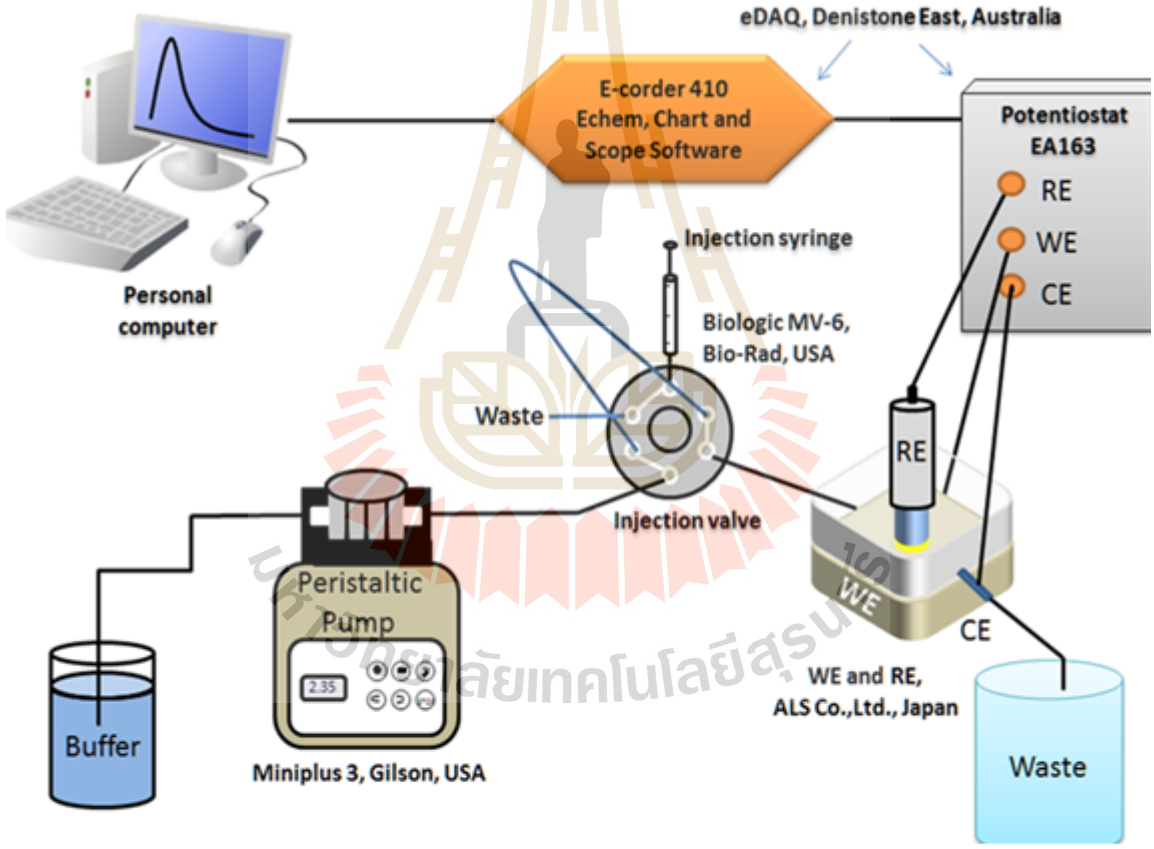


Figure 9: Schematic of the workstation that was used for flow-based amperometric stability and calibrations tests with the CNT/CHIT/Fc/GOx/EDP of this study.

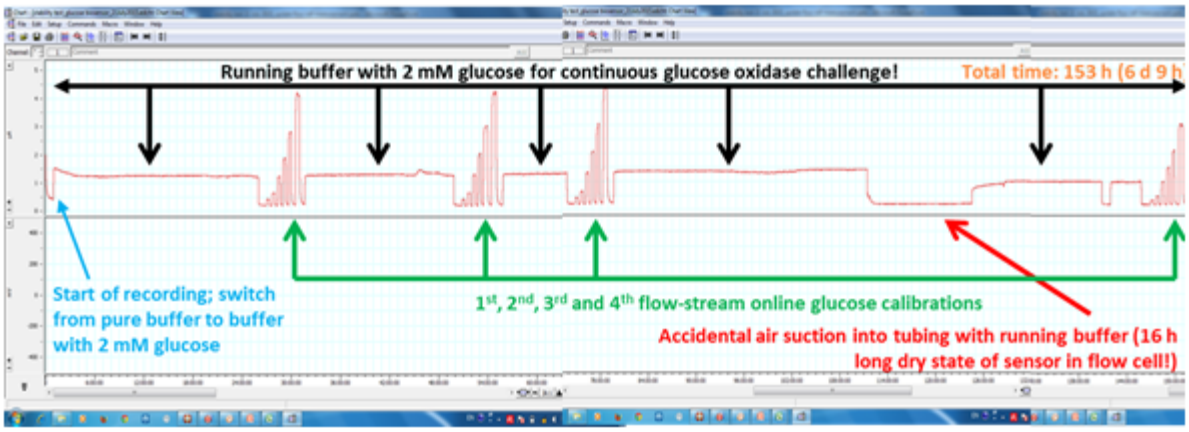


Figure 10: Screen-shot of the I/t trace acquired in course of a long-term glucose oxidase biosensor stability test with timed calibration assessments in the flow-based workstation shown in Figure 9. Most of the time running buffer (PBS/KCl, pH 7.0) spiked with 2 mM glucose was moving through the electrochemical flow cell, challenging the enzyme in the biosensors immobilization layer continuously to perform bio-catalysis on its substrate. At certain time points the running buffer is changed to glucose free condition to allow calibration trials to be carried out. Please note: There was a 16 h long interruption of buffer flow in this experiment after third calibration, due to accidental removal of running buffer tube from the running buffer reservoir. The experiment had been continued to find out how GOx survived the extended period of dryness of its immobilization matrix.

Prior to the inspection in flow operation freshly prepared biosensors with a CNT/CHIT/Fc/GOx/EDP functional matrix have been exposed to amperometric glucose calibrations trials in aerated and de-aerated measuring buffers and obtained data was used to judge on proper responsiveness of the analytical tool. The schedule of the representative trial addressing on-line biosensor stability testing with intermittent calibration trials during un-interrupted flow is shown in Figure 10.

Figure 11-15 are displays of the original amperometric current traces for the five calibrations that have been gained in the flow-system at the 26-32 (1st), 50-56 (2nd), 74-80 (3rd), 146-152 (4th) and 170-176 (5th) hour intervals after trial start together with the corresponding calibration plots. Worth mentioning that valuations # 4 and 5 happened after an accidental 16 hour long break of buffer stream with flow cell and electrodes running dry.

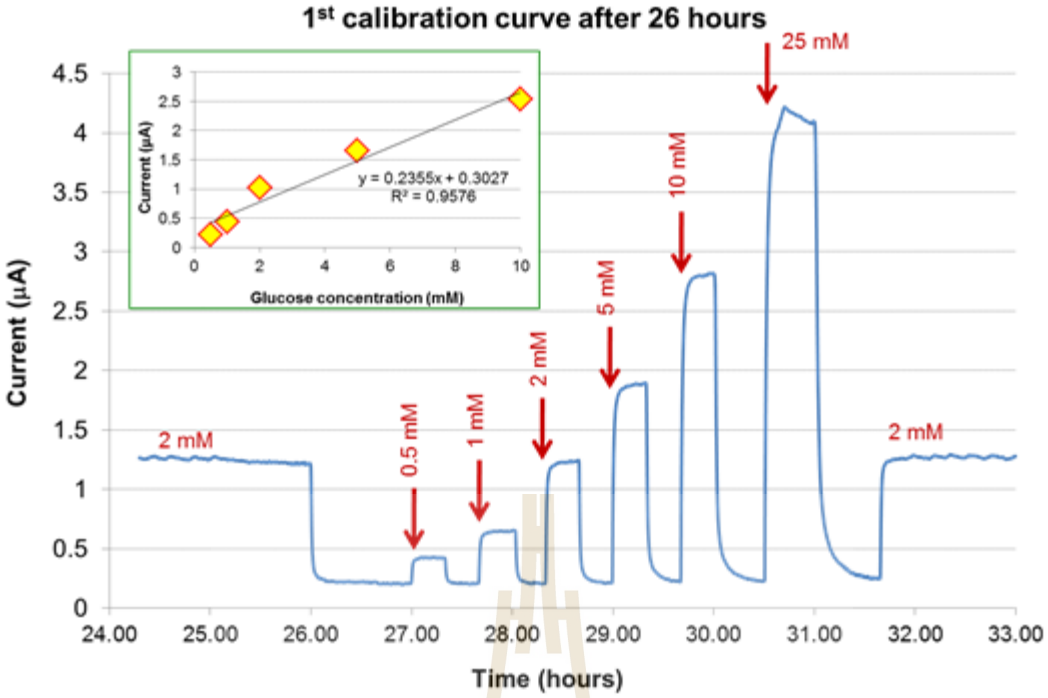


Figure 11: 1st of five glucose biosensor calibrations that acquired via amperometric hydrogen peroxide detection in a workstation for flow-based analysis (see Figure 9) in a 153 hour long measuring cycle. Glucose test levels ranged from 500 µM to 25 mM. Before and after the calibration run the glucose biosensor was challenged nonstop with 2 mM glucose exposure and related nonstop catalytic substrate conversion with enzyme given no rest.

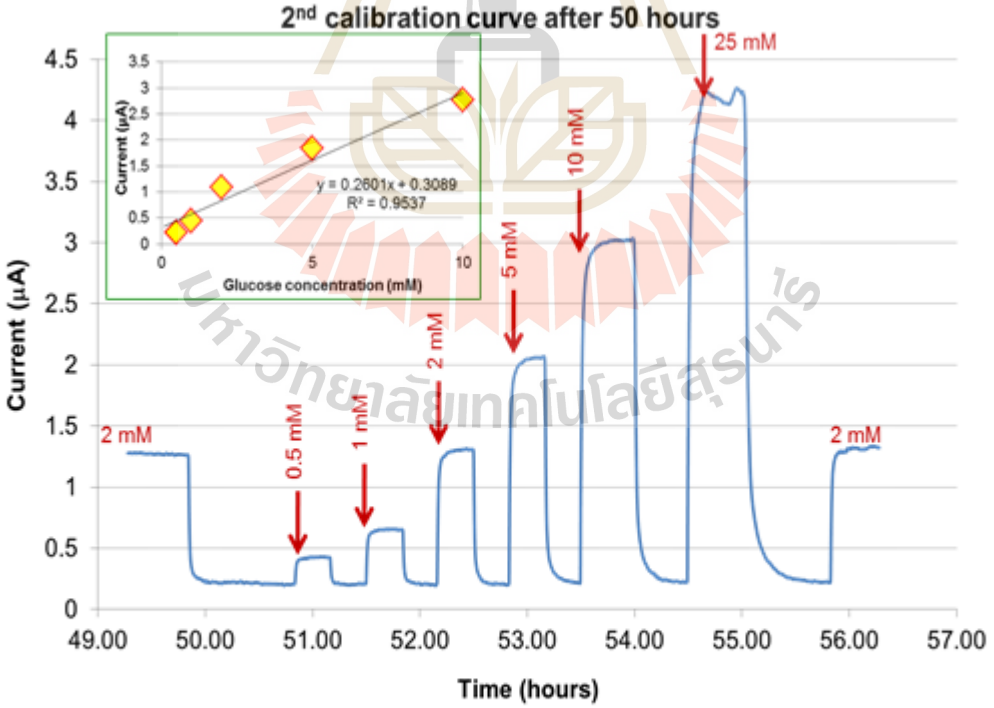


Figure 12: 2nd of five glucose biosensor calibrations that acquired via amperometric hydrogen peroxide detection in a workstation for flow-based analysis (see Figure 9) in a 153 hour long measuring cycle. Glucose test levels ranged from 500 µM to 25 mM. Before and after the calibration run the glucose biosensor was challenged nonstop with 2 mM glucose exposure and related nonstop catalytic substrate conversion with enzyme given no rest.

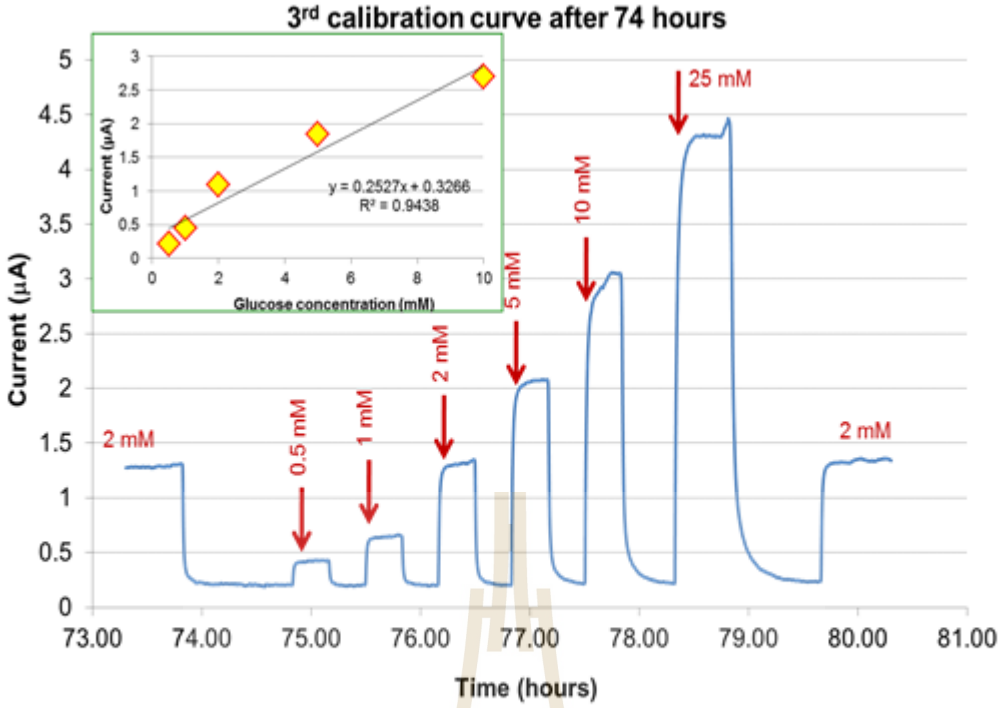


Figure 13: 3rd of five glucose biosensor calibrations that acquired via amperometric hydrogen peroxide detection in a workstation for flow-based analysis (see Figure 9) in a 153 hour long measuring cycle. Glucose test levels ranged from 500 µM to 25 mM. Before and after the calibration run the glucose biosensor was challenged nonstop with 2 mM glucose exposure and related nonstop catalytic substrate conversion with enzyme given no rest.

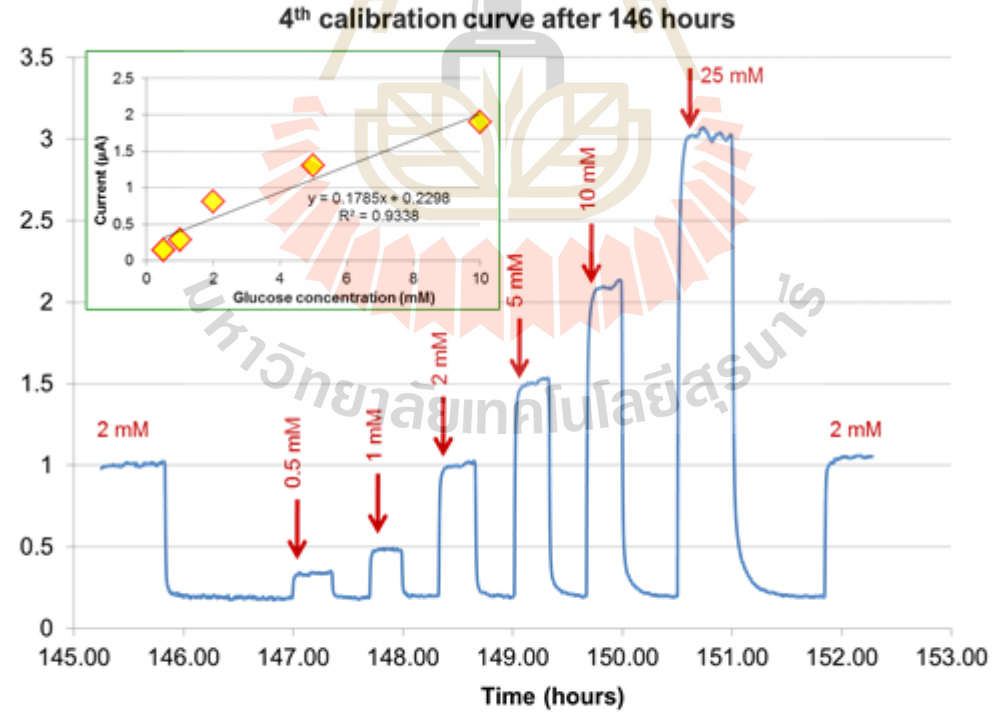


Figure 14: 4th of five glucose biosensor calibrations that acquired via amperometric hydrogen peroxide detection in a workstation for flow-based analysis (see Figure 9) in a 153 hour long measuring cycle. Glucose test levels ranged from 500 µM to 25 mM. Before and after the calibration run the glucose biosensor was challenged nonstop with 2 mM glucose exposure and related nonstop catalytic substrate conversion with enzyme given no rest.

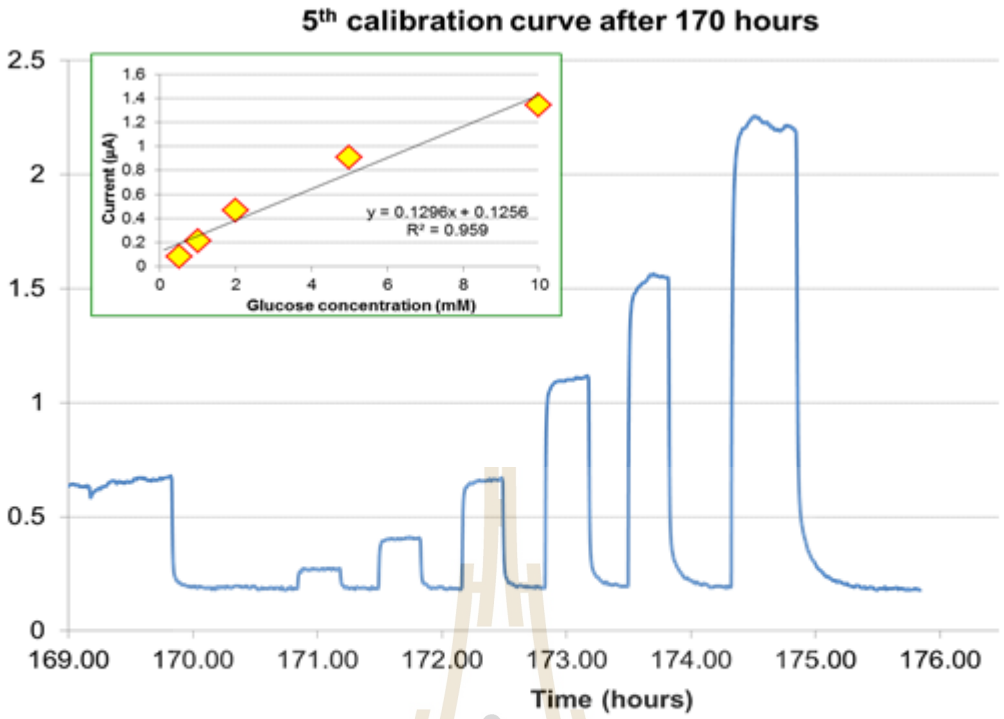


Figure 15: 5th of five glucose biosensor calibrations that acquired via amperometric hydrogen peroxide detection in a workstation for flow-based analysis (see Figure 9) in a 153 hour long measuring cycle. Glucose test levels ranged from 500 µM to 25 mM. Before and after the calibration run the glucose biosensor was challenged nonstop with 2 mM glucose exposure and related nonstop catalytic substrate conversion with enzyme given no rest.

Important observations of the outcome of flow-based sensor stability and calibration testsas presented in Figures 10-15 were:

- CNT/CHIT/Fc/GOx/EDP-based biosensors were capable to respond to the constant 2 mM glucose level that was in the running buffer before and after the first three individual calibration trials for more than 100 h of continuous operation with a very stable hydrogen peroxide oxidation current.
- Sub-mM glucose levels, here for instance 500 µM, produced current signals that rose well above background noise/baseline; The sensitivity of CNT/CHIT/Fc/GOx/EDP-based biosensor was competitive; predicted detection limit in the flow system is in the order of about 100 µM.
- The current elevations for the 0.5, 1, 2, 5, 10 and 25 mM glucose exposure of the first three calibrations (before the happening of sensor/electrochemical cell dry out) were virtually identical for corresponding sugar concentrations. As data acquisition for the first, second and third calibration experiments took more than 80 hours the reproduction of equal magnitudes of sensor current for the six tried glucose concentrations is another sign of well-expressed sensor stability.

- 26 hour of air-flow through the electrochemical cell and related system drying did lead to some enzyme death; however, the remaining quantity of active GOx was enough to ensure sensor functioning. Evidences were a stable amperometric biosensor current for unbroken flow cell flushing with 2 mM glucose and the ability to transfer 0.5, 1, 2, 5, 10 and 25 mM glucose exposures still into reasonable amperometric calibration plots

The operation of CNT/CHIT/Fc/GOx/EDP-based biosensors in the flow system exposed a satisfactorily performance. The tool actually could work nonstop for multiple days with a conservation of sensitivity and linearity for the amperometric monitoring of hydrogen peroxide from GOx/glucose interaction. The gold working electrode showed in the electrochemical flow cell a constant anodic activity for H_2O_2 sensing and entrapped GOx molecules in course of the day-long acquisition trials did not experience a major gradual degradation. Quite the opposite, the structural and functional enzyme integrity was well-maintained for the immobilized biocatalysts in the CNT/CHIT matrix. Apparently, the

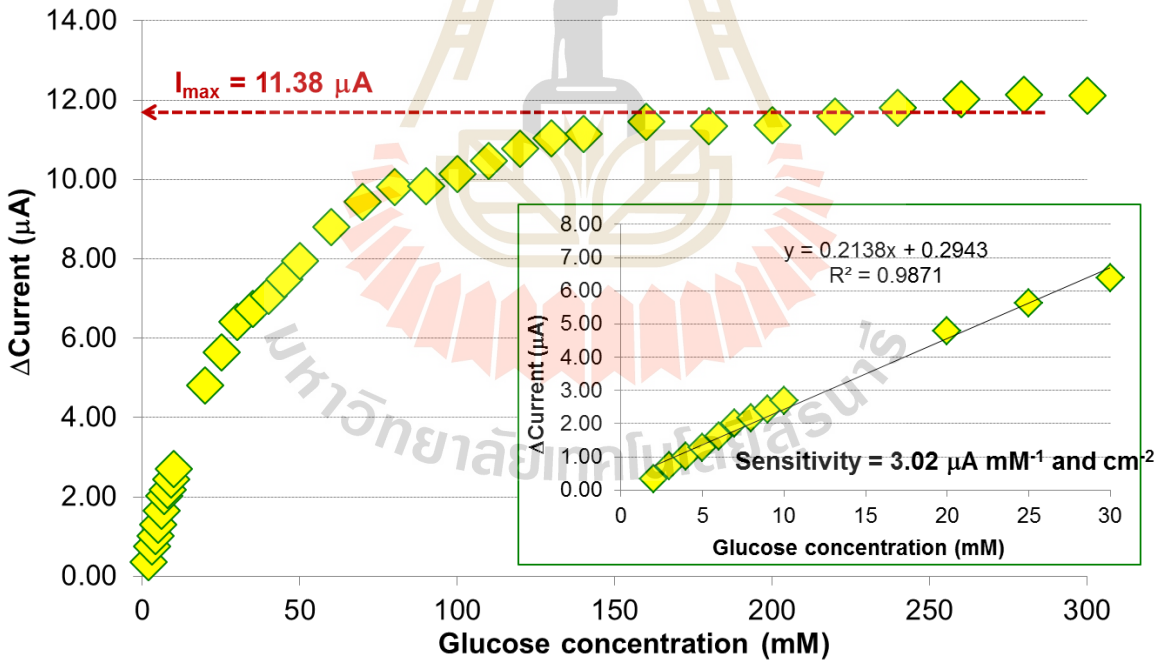


Figure 16: *Calibration curve of a CNT/CHIT/Fc/GOx/EDP biosensor that was obtained after the tool has been used more than 150 hours in the flow system of Figure 9 for continuous glucose monitoring.*

บทที่ 4

บทสรุป (Conclusion/Summary)

A merger of carbon nanotubes with the biopolymer chitosan and a redox active ferrocene derivative has been explored for use as immobilization matrix of amperometric glucose oxidase biosensors with anodic hydrogen peroxide readout. The placement of the target sensor modification as thin films on disk shaped noble metal precursor electrodes was successful with a sequence of very simple drop & dry coating procedures. A top coat of electrodeposition paint, also easily to apply, provided finally an effective loss of active sensor components from CNT/CHIT/FC/GOx-modified sensor disks via diffusional leakage. Proven was for completed biosensors the coexistence of three redox pathways for the regeneration of GOx after substrate conversion. Namely, molecular redox contact with native oxygen and ferrocene as well as direct electron transfer at CNT surfaces could help resetting the enzyme from reduced to oxidized state, effectively facilitating its cyclic biocatalytic activity. Sensors of the established design offered an analytical performance that was well competitive to published similar options. Particular positive feature were (1) an excellently wide linear range that was paired with reasonable analyte sensitivity and signal independence from the presence of dissolved O_2 and (2) an outstanding sensor stability that allowed, for instance, almost a week of non-stop operation for glucose measurements in a flow-based electrochemical workstation. The biosensors of this study were rather easy to make without involvement of critical chemical cross-linking reagents or supplementation with other nanomaterials than bare CNTs and the procedure adaptation for manufacture automation is feasible. In summary, the convenient EDP-covered CNT/CHIT/Fc/GOx thin films showed good potential to be a competent matrix for the development of long-time stable glucose biosensors with an acceptable quality of analytical performance. Prototypes here used glucose oxidase as model; however, other the transfer of the methodology to sensors with other redox enzymes is practical and recommended.

บรรณานุกรม

(References)

1. Jomma, E.Y., Ding, S.-N. *Current Analytical Chemistry*, 12 (**2016**) 5-21.
2. Wang, J. *Electroanalysis*, 23 (**20166**), 1289-1300.
3. Wang, J. *Chemical Reviews*, 108 (**2008**), 814-825.
4. Wang, J. *RSC Analyst*, 130 (**2005**), 421-426.
5. Wang, J. *Electroanalysis*, 17 (**2005**), 7-426/
6. Liu, Y., Wang, M., Zhao, F., Xu, Z., Dong, S. *Biosensors and Bioelectronics*, 21 (**2005**) 984-988.
7. Mani, V., Devadas, B., Chen, S.-M. Biosensors and Bioelectronics, 41 (**2013**) 309-315.
8. Wooten, M., Karra, S., Zhang, M., Gorski, W. *Analytical Chemistry*, 86 (**2014**) 752-757.

ภาคผนวก ก
(Appendix 1)
ผลงานตีพิมพ์และการเผยแพร่

1. ผลงานตีพิพม์ในวารสารนานาชาติ 1 ผลงาน

   Publication

   A publication is in preparation for a peer-reviewed international journal of good reputation. Intended title of the report will be “Practical carbon nanotube- chitosan-glucose oxidase-ferrocene electrode coatings for long-term glucose biosensing”.

   Oral and poster presentations on international meetings:

   The work was presented in an invited oral session contribution on “The 64rd Annual Meeting of Japanese Society of Applied Glycoscience/Symposium for Applied Glycoscience”, September 16-18, 2015 in Nara, Japan.

ภาคผนวก ข

(Appendix 2)

ประวัตินักวิจัย (Personal Resume/CV)

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Marital status Married, one child

Current Position Associate Professor in Analytical Chemistry

Research Interest

1. Electrochemical biosensing
   Enzyme biosensors, Immunosensors
2. Automated electroanalysis in microtiter plates
   Food analysis, Drug analysis, Environmental analysis
3. Electroanalysis in ultrasmall volumes
4. Electrochemical scanning probe microscopy
   Scanning electrochemical microscopy, Electrochemical scanning tunneling microscopy

List of publications (2007-2016)

1. JAIKAEW, W., PATANAKUL, R., **SCHULTE, A.*** Electrical Cable-based Copper Disk Electrodes as Oxidase Biosensor Platforms with Cathodic H2O2 Readout. Electroanalysis, Article in Press. (JIF2015 = 2.471).

2. CHUMJAN, W., WINTERHALTER, M., **SCHULTE, A.**, BENZ, R., SUGINTA, W. Chitoporin from the marine bacterium vibrio harveyi: Probing the essential roles of Trp136 at the surface of the constriction zone. J. Biol. Chem. 290 (2015) 19184-19196. JIF2015 = 4.258).
3. THEANPONGKRANG S., SUGINTA W., WEINGART H., WINTERHALTER M., **SCHULTE A***. Robotic voltammetry with carbon-nanotube-based sensors: a superb blend for convenient high-quality antimicrobial trace analysis. Int. J. Nanomedicine 10 (2015) 859-868 (JIF2013 = 4.195).
4. CHAOCHAROEN W, SUGINTA W, LIMBUT W, RANOK A., NUMNUAM A., KHUNKAEWLA P, KANATHARANA P, THAVARUNGKUL P*, **SCHULTE A*.** Electrochemical detection of the disease marker human chitinase-3-like protein 1 by matching antibody-modified gold electrodes as label-free immunosensors. Bioelectrochem. 101 (2015) 106-113 (JIF2013 = 3.870).
5. CHOTINANTAKUL K., SUGINTA W., **SCHULTE A***. Advanced amperometric respiration assay for antimicrobial susceptibility testing. Anal. Chem. 86 (2014) 10315. (JIF2013 = 5.825).
6. AUNKHAM A., **SCHULTE A.**, WINTERHALTER M., SUGINTA W.* Porin involvement in cephalosporin and carbapenem resistance of Burkholderia pseudomallei. PLoS ONE. 2014. 9, e95918. (JIF2013 = 3.534).
7. LALGEE L.J., GRIERSON L., FAIRMAN R.A., JAGGERNAUTH G.E., **SCHULTE A.**, BENZ R., WINTERHALTER M.* Synthetic ion transporters: Pore formation in bilayers via coupled activity of non-spanning cobalt-cage amphiphiles. Biochim. Biophys. Acta - Biomembr. 2014. 1838, 1247-1254. (JIF2013 = 3.431).
8. SUGINTA W., KHUNKAEWLA P., **SCHULTE A*.** Biosensor Applications of the Polysaccharides Chitin and Chitosan. Chemical Reviews. 113 (2013) 5458–5479. (JIF2013 = 45.661)
9. SUGINTA W., CHUMJAN W., MAHENDRAN K. R., JANNING P., **SCHULTE A.**, WINTERHALTER M. Molecular Uptake of Chitooligosaccharides through chitoporin from the marine bacterium Vibrio harveyi. PLoS ONE 8/1 (2013): e55126. (JIF2013 = 3.534)
10. SUGINTA W., CHUMJAN W., MAHENDRAN K. R., **SCHULTE A.**, WINTERHALTER M. Chitoporin from Vibrio harveyi, a channel with exceptional sugar specificity. J. Biol. Chem. 288 (2013). 11038–1104. (JIF2013 = 4.600)

11. NEBEL M., GRUETZKE S., DIAB N., **SCHULTE A.**, SCHUHMANN W. Visualization of oxygen consumption of single living cells by scanning electrochemical microscopy: The influence of the faradaic tip reaction. Angew. Chem. Int. Ed., 52 (2013) 6335 -6338. (JIF2013 = 13.336)
12. NEBEL M., GRUETZKE S., DIAB N., **SCHULTE A.**, SCHUHMANN W. Microelectrochemical visualization of oxygen consumption of single living cells. Faraday Transactions. 2013, in print, DOI: 10.1039/C3FD00011G (JIF2013 = 4.194)
13. SRIPIROM J., KUHN S., JUNG U. MAGNUSSEN O., **SCHULTE A***. Pointed carbon fiber ultramicroelectrodes: A new probe option for electrochemical scanning tunneling microscopy. Anal. Chem. 85 (2013) 837-842. (IF2013 = 5.825)
14. INTARAKAMHANG S., SCHUHMANN W., **SCHULTE A.*** Robotic heavy metal anodic stripping voltammetry: ease and efficacy for trace lead and cadmium electroanalysis. J. Solid State Electrochem. 17 (2013) 1535-1542. (JIF2013 = 2.234)
15. Intarakamhang S., **Schulte A*.** Automated electrochemical free radical scavenger screening in dietary samples. Anal. Chem. 84 (2012) 6767–6774. (JIF2013 = 5.825)
16. **SCHULTE A***, NEBEL M., SCHUHMANN W. Single live cell topography and activity imaging with the shearforce-based constant-distance scanning electrochemical microscope. Methods Enzymol. 504 (2012) 1535-1542 (JIF2013 = 2.194)
17. BORGMANN S., **SCHULTE A.**, NEUGEBAUER S., SCHUHMANN W. Amperometric biosensors. Book chapter in: Advances in Electrochemical Sciences and Engineering, Vol. 13 “Frontiers in Bioelectrochemistry” (Eds. Alkire, Kolb, Lipkowski, Ross); Wiley-VCH Verlag GmbH, Weinheim, Germany. 2012, p.1. doi: 10.1002/9783527644117.ch1 (Book series, no IF)
18. SHKIL H., **SCHULTE A.**, GUSCHIN D.A., SCHUHMANN W. Electron transfer between genetically modified Hansenula polymorpha yeast cells and electrode surfaces via Os-complex modified redox polymers. Chem. Phys. Chem. 12 (2011) 806–813. (JIF2013 = 3.360)
19. SUGINTA W.*, MAHENDRAN K.R., CHUMJAN W., HAJJAR E., **SCHULTE A.**, WINTERHALTER M., WEINGART H.*. Molecular analysis of antimicrobial agent translocation through the membrane porin BpsOmp38 from an ultraresistant Burkholderia pseudomallei strain. BBA-Biomembr. 1808 (2011) 1552-1559. (JIF2013 = 3.431)

20. SRIPIROM J., NOOR S., KÖHLER U., **SCHULTE A*.** Easily made and handled carbon nanocones for scanning tunneling microscopy and electroanalysis. Carbon 49 (2011), 2402-2412. (JIF2013 = 6.160)
21. INTARAKAMHANG S., LESON C., SCHUHMANN W., **SCHULTE A.*** A novel automated electrochemical ascorbic acid assay in the 24-well microtiter plate format. Anal. Chim. Acta 687 (2011) 1-6. (JIF2013 = 4.517)
22. NEBEL M., ECKHARD K., **SCHULTE A.**, ERICHSEN T., SCHUHMANN W.* 4D Shearforce-based constant distance mode scanning electrochemical microscopy (SECM). Anal. Chem. (2010) 7842–7848. (JIF2013 = 5.825)
23. HUSSEIN E. M., SCHUHMANN W., **SCHULTE A*.** The shearforce constant-distance scanning electrochemical microscope as fabrication tool for needle-like carbon-fiber nanoelectrodes. Anal. Chem. 82 (2010) 5900. (JIF2013 = 5.825)
24. **SCHULTE A.***, NEBEL M., SCHUHMANN W.* Scanning electrochemical microscopy in neuroscience. Ann. Rev. Anal. Chem. 3 (2011) 299. (JIF 2013 = 7.814)
25. **SCHULTE A.**, RUAMCHAN S., KHUNKAEWLA P., SUGINTA W.* The outer membrane protein VhOmp of Vibrio harveyi: pore-forming properties in black lipid membranes. J. Membr. Biol. 230 (2009) 101. (JIF2013 = 2.174)
26. RUHLIG D., GUGEL H., **SCHULTE A.**, THEISEN W., SCHUHMANN W.* Visualization of local electrochemical activity and local nickel ion release on laser-welded NiTi/steel joints using combined alternating-current mode and stripping-mode SECM. Analyst 133 (2008) 1700-1706. (JIF2013 = 3.906)
27. ECKHARD K., ETIENNE M., **SCHULTE A.**, SCHUHMANN W.* Constant distance AC_SECM for the visualization of corrosion pits. Electrochem. Comm. 9 (2007) 1793-1797. (JIF2013 = 4.287)
28. **SCHULTE A.***, SCHUHMANN W.* Single cell microelectrochemistry. Angew. Chem. Int. Ed. 46 (2007) 8760-8777. (JIF2013 = 13.336)

Selected Conference Contributions 2010-2016

1. KULLAWONG P., **SCHULTE A*.** Untainted carbon nanotube networks as competitive immobilization matrix for amperometric enzyme biosensors. Regional Electrochemistry Meeting of South-East Asia 2010 (REMSEA 2010); 16th – 19th November 2010, Bangkok, Thailand. (Oral Presentation)
2. **SCHULTE A.***. Electrochemistry and Nanoscience: The world of ultrasmall electrodes, nanoscale sensor modifiers, and electrochemical microscopy schemes. (Invited Oral Presentation). German-Thai Symposium on Nanoscience and Nanotechnology (GTSNN2011); 13th – 16th September, 2011, Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima, Thailand. (Oral Presentation)
3. SRIPIROM J., KUHN S., JUNG U., MANGUSSEN O., **SCHULTE A.***. Pointed carbon microelectrodes with nanometer tip radii: Potential tools for EC-STM and voltammetry. 12th International Fischer Symposium on "Frontiers in Nanoelectrochemistry"; 3rd – 7th June 2012, Luebeck Germany. (Oral Presentation)
4. **SCHULTE A.***, SCHUHMANN W., INTARAKAMHANG S. Robotic stripping voltammetry of trace lead and cadmium in water and soil samples. The 63rd Annual Meeting of the International Society of Electrochemistry: Electrochemistry for Advanced Materials, Technologies and Instrumentation, 19-24 August, 2012, Prague, Czech Republic. (Oral Presentation)
5. **Schulte A.***, THEANPONKRANG, S., WEINGART H., WINTERHALTER M. Robotic drug electroanalysis in microtiter plates: Convenience paired with potential. Bioelectrochemistry 2013, 12th Topical Meeting of the International Society of Electrochemistry & XXII international Symposium on Bioelectrochemistry and Bioelectrochemical Society, 17th – 21st March 2013, Bochum, Germany. (Oral Presentation)
6. Sripirom J., **Schulte A.***. Easy and economical pharmaceutical drug electroanalysis in microliter volumes of blood. ASEAN Plus 2013: The 2nd Regional Symposium on Biosensors. 11- 13 December, 2013, Mae FahLuang University, Chiangrai, Thailand. (Oral Presentation)
7. **Schulte A.***. Electrochemical probe microscopy at solid/liquid interfaces: Topographys and/or reactivity imaging with high-resolution zoom. 39th Congress on Science and

Technology of Thailand “Innovative Sciences for a Better Life”. October 21 - 23, 2013, BITEC, Bangkok, Thailand. (Oral Presentation)

8. Teanphonkrang S., Sripirom J., **Schulte A.***. A simple three-electrode cell assembly for convenient and effective analytical voltammetry in 50 microliter of sample solution. Electrochemistry 2014: Basic science and key technology for future applications. 22 – 24 September, 2014, Mainz, Germany. (Poster Presentation)
9. Rernglit, W., Suginta, W., **Schulte, A.*** Chitosan-enzyme-ferrocene-carbon nanotube electrode coatings for advanced glucose biosensing. The 64rd Annual Meeting of Japanese Society of Applied Glycoscience/Symposium for Applied Glycoscience. September 16-18, 2015 in Nara, Japan. (Invited oral presentation)