Immobilization of Glucose Oxidase in Anthracene-Based Semi-Conducting Polymer: Application on Glucose Biosensing

Dhekra A*, Khaled H, Mina S, Mustapha M and Ali O

Laboratory of Advanced Materials and Interfaces (LIMA), University of Monastir, Monastir, Tunisia

Abstract

A glucose biosensor based on the immobilization of the enzyme glucose oxidase (GOx) onto an anthracene-based polymer analogue of poly (phenylene sulfide) (PAnS)/Gold electrode was elaborated. The building of the bridge system was evaluated by electrochemical impedance spectroscopy measurement (EIS) and cyclic voltammetry (CV). The PAnS semi-conducting polymer exhibits a good film forming, stability and adhesion on gold Electrode. The choice of the immobilization of GOx in thin film polymer is essentially based on the facility of the synthesis of the polymer also on the surface properties. The immobilized GOx was proved by cyclic voltammetry, contact angle and UV-visible spectroscopy. The GOx/PAnS/Au bioelectrode showed a linear response to glucose in the concentration ranging from $10^{-8}$ M to $10^{-3}$ M with a correlation coefficient of 0.98 and a sensitivity of 2.66 (kΩ M$^{-1}$ cm$^{-2}$). Indeed, The GOx/PAnS/Au structure could make it a promising bioelectrode for detection of glucose in the biological samples.

Keywords: Semi-conducting polymer; Anthracene; Thin film; Glucose oxidase immobilization

Introduction

A biosensor defined as a compact analytical device incorporating a biological or biologically derived sensitive element integrated or associated with a physio-chemical transducer [1]. Clark et al. was the first to develop glucose oxidase (GOx) immobilized electrode to quantify the glucose concentration [2]. Since then, the detection of biomolecules has become important in a variety of areas including bioreactors, medical diagnostics, veterinary medicine and viral diagnostic and bacterial diagnostic [3,4]. Electrochemical glucose biosensors based on biomolecules immobilized on an electrode surface play a leading role in this direction specially that the diabetes mellitus is one of the great modern health issues. Several studies have been carried out in order to improve performance of this type of sensor. Sensors continue to have an important impact in everyday life. There has been a strong demand to produce highly, sensitive, selective, responsive, and cost effective sensors [5-7].

Great efforts have been devoted to immobilization of glucose oxidase (GOx) on an electrode surface, which is one of the main factors that affect the performance of glucose biosensors. In order to have a stable, selective and sensitive response, the surface of the electrode has been modified [8]. This can be realized by conductive polymers such as polypyrrole [9], polythiophene [10], and polyaniline [11], because of a number of favorable characteristics, they afforded stable surface for the immobilization of the biocomponent and play the role of a transducers to convert a chemical signal into an electrical one [12,13]. Also, conducting polymers are known for their ability to be compatible with biomolecule in neutral aqueous solutions [14]. Biosensors, prepared using conducting polymers as a support material, have a high storage, a fast response time with an operational stability [15]. In this context, we have used a new semi-conducting polymer based on anthracene analogue of poly(phenylene sulfide) PPS as support of enzyme. This polymer was reported in our previously work [16].

Anthracene presented a good photophysical properties and it is the first aromatic material used in organic light emitting diode (OLEDs). The first experiments were carried out by Pope in the early 1960s [17]. Soon after, several reports on single-crystal anthracene-based OLEDs were published and good quantum yields were obtained (up to 5%). Nevertheless, such devices are thick therefore they require very high operating voltage (over 100 V) and also semi-conductor layer in OFET [18]. However, the anthracene tends to recrystallize with diode operating time, which led to a degradation of device performance. But, actually, the anthracene-based-polymers show the most greatly reported in organic electronics [19-23].

While, only few works were done on anthracene use in chemical or biochemical sensor [24,25]. However, Poly (p-phenylenesulfide) (PPS) is a high-performance thermoplastic with many desirable characteristics, such as thermal behaviour, oxidative and chemical resistance [26]. Also, it exhibits a greater electrical conductivity (>10 S.cm$^{-1}$), when doped with AsF$_5$, [16]. In fact, the anthracene-based polymer analogue of poly (phenylene sulfide) (PAnS) is soluble, exhibits a good thin film quality and good thermal stability. The electrochemical gap determined by cyclic voltammetry measurements was about 2.34 eV. In this contribution, we reported the glucose oxidase enzyme immobilization based on soluble semi-conducting polymer based on anthracene (PAnS). This elaborated bioelectrode was evaluated by electrochemical impedance spectroscopy measurement (EIS) and cyclic voltammetry (CV).

Experiments

Material and methods

The glucose oxidase (from Aspergillus niger, E.C. 1.1.3.4) were purchased from Sigma Aldrich France. Chemical Glucose was obtained from Beijing Chemical Reagent and the stock solution of glucose was allowed to mutorotare at room temperature overnight before every use.

*Corresponding author: Dhekra A, Faculty of Medicine, Laboratory of Advanced Materials and Interfaces (LIMA), University of Monastir, 5019 Monastir, Tunisia, Tel: 216 73 500 280; Fax: +216 73 500 278; E-mail: ayeddhekra@rocketmail.com

Received: October 25, 2017; Accepted: November 04, 2017; Published: November 10, 2017

Citation: Dhekra A, Khaled H, Mina S, Mustapha M, Ali O (2017) Immobilization of Glucose Oxidase in Anthracene-Based Semi-Conducting Polymer: Application on Glucose Biosensing. J Bioengineer & Biomedical Sci 7: 239. doi: 10.4172/2155-9538.1000239

Copyright: © 2017 Dhekra A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
(K,Fe(CN)₃H₂O) Potassium ferrocyanidetrihydrate, (K,Fe(CN)₆)₃ potassium ferricyanide disodium hydrogenorthophosphate, Chloroform was purchased from Aldrich and potassium dihydrogen orthophosphate were obtained from Sigma Aldrich France. Hydrogen peroxide (30%) was received from Prolabo France. All reagents were of analytical grade. Phosphate buffer solutions (PBS) (0.16 mol L⁻¹, pH 7.2) prepared with NaH₂PO₄ and Na₂HPO₄ were used as supporting electrolyte. Pure water was employed throughout, which was obtained using a Millipore Q water purification apparatus (resistivity over18 MΩcm). Anthracene-based semi-conducting polymer analogue of poly(phenylene sulfide)(PPS) was used to elaborate a biosensor. The synthesis and chemical properties have been reported previously [16]. The chemical structures are shown in Figure 1.

Electrochemical measurements were carried out using a Volta Lab 40 (PGZ 301, Villeurbanne, France) with standard three-electrode configuration. A platinum counter electrode was used as the counter electrode, a saturated calomel electrode Hg/Hg₂Cl₂/KCl(+0.248 V vs. SHE) as the reference electrode, and the gold electrode as the working electrode, placed in a Faraday cage at room temperature. Two electrochemical techniques were applied:

- Cyclic voltammetry (CV) performed in 5 mM [Fe(CN)₆³⁻] redox probe in PBS solution (0.16 M, pH 7.2) at a scan-rate of 50 mV s⁻¹.
- Electrochemical impedance spectroscopy (EIS) based upon inducing a disturbance in the electrochemical reaction from its steady-state by applying an alternating potential with amplitude of -450 mV at the frequency range from 0.1 Hz to 100 kHz.

The impedance data were represented in the complex impedance plot (Nyquist plot), taking into account the electrode surface (0.07 cm²), and the fitting program of Z view was employed to analyze the impedance spectra using an appropriate Randles equivalent electrical circuit.

- Contact angle measurements were carried out by a GBX-Digidrop Scientific Instrument (Romans, France) to examine the hydrophilic/hydrophobic nature of the various modification steps of the surface by the static sessile drop method.

Electrode modification and immobilization of the enzyme glucose oxidase

As a first step of experiment, the working gold electrode was ultrasonically cleaned by acetone, ethanol, and distilled water for 20 min, respectively. Then, this electrode was put in Piranha solution (3:7 v/v of H₂O₂ and H₂SO₄) during 3 min. Finally, the gold surface were washed extensively with water, absolute ethanol and dried under a stream of nitrogen. The (2.10⁻² M) PAnS solution was prepared in chloroform; the film was spin-coated onto a gold substrate and using a Millipore Q water purification apparatus (resistivity over18 MΩcm). Anthracene-based semi-conducting polymer analogue of poly(phenylene sulfide)(PPS) was used to elaborate a biosensor. The synthesis and chemical properties have been reported previously [16]. The chemical structures are shown in Figure 1.

Electrochemical measurements were carried out using a Volta Lab 40 (PGZ 301, Villeurbanne, France) with standard three-electrode configuration. A platinum counter electrode was used as the counter electrode, a saturated calomel electrode Hg/Hg₂Cl₂/KCl(+0.248 V vs. SHE) as the reference electrode, and the gold electrode as the working electrode, placed in a Faraday cage at room temperature. Two electrochemical techniques were applied:

- Cyclic voltammetry (CV) performed in 5 mM [Fe(CN)₆³⁻] redox probe in PBS solution (0.16 M, pH 7.2) at a scan-rate of 50 mV s⁻¹.
- Electrochemical impedance spectroscopy (EIS) based upon inducing a disturbance in the electrochemical reaction from its steady-state by applying an alternating potential with amplitude of -450 mV at the frequency range from 0.1 Hz to 100 kHz.

The impedance data were represented in the complex impedance plot (Nyquist plot), taking into account the electrode surface (0.07 cm²), and the fitting program of Z view was employed to analyze the impedance spectra using an appropriate Randles equivalent electrical circuit.

- Contact angle measurements were carried out by a GBX-Digidrop Scientific Instrument (Romans, France) to examine the hydrophilic/hydrophobic nature of the various modification steps of the surface by the static sessile drop method.

Results and Discussion

Enzyme immobilization in a PAnS film

Wetting properties: The wettability of the bare gold and modified electrodes (PAnS/Au and GOx/PAnS/Au) was evaluated using contact angle measurements. Figure 3 shows the increase in the contact angle for PAnS/Au (73°) in comparison with the bare gold electrode (38°). This result indicates the hydrophobe property of thin film polymer. On the other hand, we noted the decrease in contact angle (10°) following the modification of the surface by the enzyme glucose oxidase. This result proves that the surface became more hydrophilic by immobilization of GOx in the modified surface. In fact, we can suggest that semi-conducting polymer film provides an attractive matrix for effective immobilization of glucose oxidase.

UV-Vis absorption: The optical characteristic of PAnS and PAnS/GOx was investigated by spectroscopy UV-vis absorption. This analysis was determined in dilute solution (5.10⁻⁵ mol.L⁻¹) and in thin film layer. The Table 1 summarizes the obtained optical data. The optical band gaps (Eg) of PAnS film was estimated from the absorption edges, using the Tauc relation [27].

$$\alpha h\nu = A (h\nu - E_g)^{\alpha}$$

Where hν is the absorbed photon energy and α is the corresponding absorption coefficient. The Eg value was obtained also from extrapolation to α = 0 of the straight line portion in the (αhν)² versus hν plot (Figure 4); it was found 2.14 eV. In solid state, the PAnS and PAnS/GOx spectra showed the same feature with one shoulder and
Effect of glucose on PANs/GOx/Au biosensor

The electrochemical measurement of materials was carried out by Electrochemical Impedance Spectroscopy (EIS) at potential (-450 mV). The surface characterization was investigated in aqueous solution of 0.16 M PBS (pH 7.2). Figure 7a shows in the impedance spectra Nyquist plots the relationship between the real and imaginary components of complex impedance of the GOx-PAnS modified electrode after adding the different glucose concentrations. The spectra are dominated by the presence of capacitive lines. They comprise a high frequency intercept on the real Z axis and the beginning of a semicircular arc across the high to low frequency range. The high frequency intercept is representative of a combination of electrolyte ionic resistance and the resistance of the contacts to the potentiostat. This decrease in -Z is attributed to the transmission of the glucose to gluconic acid and H₂O₂ in the presence of GOx enzyme. To more explain that, glucose oxidase (GOx) is a type of oxydo-reductase enzyme that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-d-lactone, [28,29] as described by the reaction given in the following equations: GOx enzyme with Fe(CN)₆³⁻/⁴⁻ as the redox probe with scan rate of 50 mV/s.

Effect of glucose on PANs/GOx/Au biosensor

The electrochemical measurement of materials was carried out by Electrochemical Impedance Spectroscopy (EIS) at potential (-450 mV). The surface characterization was investigated in aqueous solution of 0.16 M PBS (pH 7.2). Figure 7a shows in the impedance spectra Nyquist plots the relationship between the real and imaginary components of complex impedance of the GOx-PAnS modified electrode after adding the different glucose concentrations. The spectra are dominated by the presence of capacitive lines. They comprise a high frequency intercept on the real Z axis and the beginning of a semicircular arc across the high to low frequency range. The high frequency intercept is representative of a combination of electrolyte ionic resistance and the resistance of the contacts to the potentiostat. This decrease in -Z is attributed to the transmission of the glucose to gluconic acid and H₂O₂ in the presence of GOx enzyme. To more explain that, glucose oxidase (GOx) is a type of oxydo-reductase enzyme that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-d-lactone, [28,29] as described by the reaction given in the following equations: GOx enzyme with Fe(CN)₆³⁻/⁴⁻ as the redox probe with scan rate of 50 mV/s.
The impedimetric responses are presented in a linear relationship between the relative variation of $R_{CT}$ and the logarithmic value of glucose concentrations in the range of $10^{-3}$ M to $10^{-8}$ M. The slope of the averaged dataset was determined to be 2.66 (kΩ·M$^{-1}$·cm$^{-2}$) of glucose. This relationship has an associated R-squared value of 0.98. In order to ensure that the decrease of the $R_{CT}$ value after adding different glucose concentrations, we repeated the same experiment in the absence of the enzyme (PAnS/Au electrode), enzymatic reaction of glucose and glucose oxidase, we repeated the inhibition study: The activity of GOx enzyme can be inhibited by the binding of specific small molecules and ions such as (Ag$^+$, Cd$^2+$) [32]. In the first step, following stabilization of the baseline, a defined concentration ($10^{-5}$M) of glucose was added. The choice of the glucose concentration was determined by the calibration curve between the electron transfer resistance and different concentrations of glucose before adding the inhibitor. Inhibitor solution of Ag$^+$ (1 μM) is then injected (second step) to evaluate the decrease of enzymatic activity. The surface characterisation was investigated by electrochemical measurements (CV) and (EIS) in aqueous solution of 0.1 M PBS (pH 7.2). As shown in Figure 8 the decrease of oxidation peak of current proves the decrease of quantity of detected substrate by the enzyme after injection of the inhibitor. This results confirms the activity of the enzyme glucose oxidase immobilized on the surface of the polymer PAnS.

**Conclusion**

The elaboration of glucose biosensor based on the immobilization of the enzyme glucose oxidase (GOx) onto an anthracene-based polymer analogue of poly (phenylene sulfide) (PAnS)/Gold electrode was evaluated. The hydrophobicity of the bare gold and modified electrodes (PAnS/Au and GOx/PAnS/Au) was investigated using contact angle measurements. The optical characteristic of the modified surfaces was examined by spectroscopy UV-vis absorption. Results clearly suggest that semi-conducting polymer film (PAnS) provides an attractive matrix for effective immobilization of glucose oxidase GOx. The GOx/PAnS/Au bioelectrode showed a linear response to glucose in the concentration ranging from $10^{-6}$ M to $10^{-3}$ M with a correlation coefficient of 0.98 and a sensitivity of 2.66 (kΩ·M$^{-1}$·cm$^{-2}$). The fabrication of the glucose sensor based on (PAnS) is inexpensive, fast and simple. The biosensor seems promising for being applied in environmental analysis.

**Acknowledgments**

This study was supported by the Ministry of Higher Education and Scientific Research of Tunisia.

### References

1. Tunner APF, Karube I, Wilson GS (1987) Biosensors -- fundamentals and applications. Oxford University Press, New York. pp: 719-800.

2. Clark L, Lyons C (1962) Electrode systems for continuous monitoring in cardiovascular surgery. Ann NY Acad Sci 102: 29-45.

3. Sadana A (2006) Binding and dissociation kinetics for different biosensor applications using fractals. Elsevier, US. pp: 219-242.

4. Liu G, Lin Y (2005) Electrochemical sensor for organophosphate pesticides and nerve agents using zirconia nanoparticles as selective sorbents. Anal Chem 77: 5894-5901.

5. Ma W, Jiang Q, Yu P, Yang L, Mao L (2013) Zedicid imidazolate framework-based electrochemical biosensor for in vivo electrochemical measurements. Anal Chem 85: 7550-7557.

6. Song Y, Liu H, Wang Y, Wang L (2013) A novel bi-protein bio-interphase of cytochrome c and glucose oxidase: Electron transfer and electrocatalysis. Electrochim Acta 93: 17-24.

7. Yang Z, Tang Y, Li J, Zhang Y, Hu X (2014) Facile synthesis of tetragonal columnar-shaped TiO2 nanorods for the construction of sensitive...
electrochemical glucose biosensor. Biosens Bioelectron 54: 528-533.

8. Norouzi P, Mirzaei GT, Rashedi H, Zamani HA, Ganjali MR (2010) Ultrasensitive flow-injection electrochemical method using fast fourier transform square-wave voltammetry for detection of vitamin B1. Int J Electrochem Sci 5: 639-652.

9. Cho JH, Shin MC, Kim HS (1996) Electrochemical adsorption of glucose oxidase onto films prepared by electrochemical copolymerization of 3-methylthiophene and thiophene-3-acetic acid for amperometric sensing of glucose: Effects of polymerization conditions on sensing properties. Eur Polym J 43: 3264-3276.

10. Liu C, Kuwahara T, Yamazaki R, Shimomura M (2007) Covalent immobilization of glucose oxidase on films prepared by electrochemical copolymerization of 3-methylthiophene and thiophene-3-acetic acid for amperometric sensing of glucose: Effects of polymerization conditions on sensing properties. Eur Polym J 43: 3264-3276.

11. Pechhayasatien S, Decho O, Seipairoj S, Aramcharoensuk S, Wongcharoen D (2006) Electrochemical study of polyaniline modified glassy carbon electrode for ascorbic acid determination. Int J Electrochem Sci 10: 1208-1220.

12. Popovic N, Jugovic B, Knezevic-Jugovic Z, Stevanovic J, et al. (2015) Electrochemical template-free synthesis of nanofibrous polyaniline modified electrode for ascorbic acid determination. Int J Electrochem Sci 10: 1208-1220.

13. Bhadra D, Das M, Datta M, Malhotra BD (2011) Recent advances in polyaniline based biosensors. Biosens. Bioelectron 26: 2811-2821.

14. Popovic N, Jugovic B, Knezevic-Jugovic Z, Stevanovic J, et al. (2015) Electrochemical template-free synthesis of nanofibrous polyaniline modified electrode for ascorbic acid determination. Int J Electrochem Sci 10: 1208-1220.

15. Gerard M, Chauvey A, Malhotra BD (2002) Application of conducting polymers to biosensors. Biosens Bioelectron 17: 345-359.

16. Shaolin M, Chi Y, Cao K, Xue, Lu R (2013) Synthesis of monodisperse oligocarbazoles-functionalized anthracene with intense blue-emitting. Tetrahedron Lett 54: 594-599.

17. Ben Chaabane R, Jaballah N, Benzarti-Ghédira M, Chaieb A, Majdoub M, et al. (2009) Synthesis and thin films characterization of new anthracenecore molecules for opto-electronic. Physica B Condensed Matter 404: 1912-1916.

18. Gondek E, Kityk IV, Daniel A (2008) Some anthracene derivatives with N, N-dimethylamine moieties as materials for photovoltaic devices. Mater Chem Phys 112: 301-304.

19. Meng H, Sun F, Goldfinger MB, Gao F, Londono DJ, et al. (2005) High-performance, stable organic thin film field effect transistors based on bis-(5'-alkylthiophen-2'-yl)-2,8 anthracene semiconductors. J Am Chem Soc 127: 2406-2407.

20. Li Y, Kim TH, Zhao Q, Kim EK, Han SH, et al. (2008) Synthesis and characterization of a novel polymer based on anthracene moiety for organic thin film transistor. J. Polym Sci Part A: PolymChem. 46: 5115-5122.

21. Chung DS, Park JW, Yun WM, Cha H, Kim YH, et al. (2010) Solution-processed organic photovoltaic cells with anthracene derivatives. Chem Sus Chem 3: 742-748.

22. Raghunath P, Ananth Reddy M, Gouri C, Bhanuprakash K, Jayathirtha V, et al. (2006) Electronic properties of anthracene derivatives for blue light emitting electroluminescent layers in organic light emitting diodes: a density functional theory study. J Phys Chem A 110: 1152-1159.

23. Serevicius T, Komsris K, Adomenas P, Adomeniene O, Jankauskas V, et al. (2014) Non symmetric 9,10 diphenenylanthracene based deep-blue-emitters with enhanced charge transport properties. Phys ChemChemPhys 16: 7089-7101.

24. Lopez. LC, Wilkes GL (1989) Poly(p-phenylene sulfide) - an overview of an important engineering thermoplastic. J Macromol Sci 29: 83-151.

25. Peng KY, Chen SA, Fann WS, Chen SH, Su AC (2005) Well-packed chains and aggregates in the emission mechanism of conjugated polymers. J Phys Chem B 109: 9368-9373.

26. Gibson DH, Swoboda B, Massey V (1964) Kinetics and mechanism of action of glucose oxidase. Biol Chem 239: 3927-3934.

27. Duke FR, Weibel M, Phage DS, Bulgrin VS, Luthy JJ (1969) The glucose oxidase mechanism. Enzyme activation by substrate. Chem Soc 9: 3904.

28. Amberger SA, Shirley JA (1980) Glucose oxidase, Glucose dehydrogenase, Glucose isomerase, β-Galactosidase and Invertase. In: Rose AH (ed.), Microbial Enzymes and Biocconversion. Academic Press, New York. pp: 173-226.

29. Yamaguchi M, Mitsuomori M, Kano Y (1998) Noninvasively measuring blood glucose using saliva. IEEE Eng Med Biol Mag 17: 59-63.

30. Guascito MR, Maitestes C, Mazzotta E, Turco A (2008) Inhibitive determination of metal ions by an amperometric glucose oxidase biosensor: Study of the effect of hydrogen peroxide decomposition. Sens Actuator B-Chem 131: 394-402.