Nanochem Res 7(2): 79-84, Summer and Autumn 2022

RESEARCH PAPER

Electroreduction of Hydrogen Peroxide Using Direct Electrocatalysis of Cytochrome c on a Graphene-Modified Electrode

Fatemeh Norouz-Sarvestani[1], Seyyed Mehdi Khoshfetrat*[,2], Abdolkarim Abbaspour*[,1]

[1] Department of Chemistry, College of Sciences, Shiraz University, Shiraz, Iran

[2] Department of Chemistry, Faculty of Basic Science, Ayatollah Boroujerdi University, Boroujerd, Iran

ARTICLE INFO

Article History:
Received 9 March 2022
Accepted 14 March 2022
Published 1 May 2022

Keywords:
Graphene
Cytochrome c
Hydrogen peroxide
Electrochemical biosensor

ABSTRACT

In the mitochondrial intermembrane space, the redox protein heme-Cytochrome c (Cyt c) acts as an electron carrier. The translocation of Cyt c out of mitochondria triggers programmed cell death. In this study, direct electrochemistry of Cyt c adsorbed onto the surface of a graphene-modified electrode was investigated. Owing to the high electron mobility of one-atom thick graphene, it serves as a unique platform for facilitating direct electron transfer of proteins. The redox peak currents of the Cyt c-immobilized graphene increased linearly with increasing the scan rate, revealing a surface-controlled electrochemical process. The enzyme-mimetic activity of the Cyt c-immobilized graphene in the electroreduction of H_2O_2, from 2.0 µM to 4.0 mM with a detection limit of 0.4 µM, demonstrated that the graphene maintained the bioactivity of Cyt c. This intriguing enzyme-liked catalytic activity makes the Cyt c-modified graphene electrode a suitable candidate for fabricating H_2O_2 sensors. This direct electron-based electroreduction opens a new horizon for highly sensitive targeted bioanalysis with a functional nanomaterial design.

How to cite this article
Norouz-Sarvestani F., Khoshfetrat S.M., Abbaspour A. Electroreduction of Hydrogen peroxide using Direct Electrocatalysis of Cytochrome c on a Graphene-Modified Electrode . Nanochem Res, 2022; 7(2):79-84. DOI: 10.22036/ncr.2022.02.003

INTRODUCTION

The direct electron transfer (DET) of bio-molecules such as Cytochrome c (Cyt c) have been comprehensively studied for investigating their potential application in biofuels, enzyme mechanism and so on [1]. The water-soluble multi-functional hem-containing Cyt c at inner mitochondrial membrane [2] plays a significant role in the respiratory chain, acting as an interface for receiving electrons from the Cyt c reductase and transferring them to the Cyt c oxidase.

Cyt c with a spherical shape of 34°A in diameter (12 kDa) has a net positive charge of +5 to +6 at neutral pH (pI=10) [3]. Electrochemical studies have shown that proteins on a conventional bare electrode lead to substantial changes in their structure and function. Based on previous studies, DET between Cyt c and surface electrodes such as glassy carbon, Au, Hg, Ag and Pt is not possible. Most of the proteins are huge molecules and their redox center is embedded deeply in polypeptide chain structures, which hinder their direct electrochemical investigation. Furthermore, the proteins are being denatured on a bare electrode and poison the electrode surface [4]. Thus, modifying an electrode for preparing a biocompatible surface with a fast electron-transfer kinetic which prevents the denaturation of the protein is highly desirable [5, 6]. For this purpose, many innovative strategies have been proposed for modifying electrodes including self-assembled monolayers [7, 8], single-walled carbon nano tubes (SWNT) [9], multi-walled carbon nanotubes (MWNT) [10], nanomaterials [11, 12], and biomaterials modified electrodes [13]. These modifiers act as "tiny conducting wires" and increase the electron transfer rates between the

* Corresponding Author Email: *m.khoshfetrat@gmail.com*
abbaspour@chem.susc.ac.ir

This work is licensed under the Creative Commons Attribution 4.0 International License.
To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

protein redox center and the electrode.

Typically, graphene possesses a high surface area for immobilizing proteins [14-16], higher electron conductivity, and high mobility for transferring electrons [17], and strong irreversible adsorption [18].

Regarding the vital roles of hydrogen peroxide (H_2O_2) in pharmaceutics, food industry and other industries, and as a by-product of enzymatic reactions, its detection is a challenge [19].

In this study, a negative surface of the nafion/reduced graphene oxide (rGO)-modified glassy carbon electrode (GCE) acts as a suitable substrate for the immobilization of a positively charged protein. Cyt c shows a fast electron transfer kinetic on the graphene-modified electrode, stemming from electron transfer the mediating/accelerating effect of graphene. The immobilized Cyt c retains its electrocatalytic activity while immobilized on the electrode surface. Thus, an electrochemical hydrogen peroxide biosensor is constructed in which Cyt c catalyzes its reduction to water.

EXPERIMENTAL

Reagents

Extra pure graphite powder (particle size ≤50 μm) and equine heart Cytochrome c were purchased from Merck. The nafion solution in alcohol (0.5 wt.%) was purchased from Fluka. The experiments were conducted in the phosphate buffer solution (PBS, 0.1 M, pH 7.0).

Apparatus

The electrochemical signals were recorded using a μAutolabe type III potentiostat/galvanostat, controlled via aGPES4.9 software. A conventional electrochemical system with a working glassy carbon electrode, reference Ag|AgCl| 3 M KCl and counter electrode of Pt were used for recording the signals. In order to remove the O_2 from the solution, deaeration was applied using ultra-pureN_2 gas for 10 min.

PROCEDURE

Synthesis of rGO

Modified Hummer's method graphene oxide (GO) was prepared according to previous studies [20, 21]. To prepare rGO, 40 μL N_2H_4 was injected to stirred 1 mL GO (4 mg mL^1) and, subsequently, reacted at 80 °C for 24h. The color changed from brown to black, demonstrating the reduction of the oxygen group in GO.

Preparation of modified GC electrode

The bare glassy carbon electrode was cleaned with alumina slurries and water, then sonicated in ethanol, and finally exposed to 10μl of the dispersed rGO solution (1.0 mg/ml) with 100 μl of the nafion solution (0.08%) (Fig. 1). After that, the Cyt c-nafion-rGO/GC electrode was prepared by drop-casting the 1.0 mg/mL Cyt c solution (prepared PBS) for 24h at 4°C. In the same manner, a Cyt c-nafion/GC electrode was also prepared without using the rGO.

RESULTS AND DISCUSSION

DET of Cyt c on nafion/rGO GCE

To confirm the immobilization of Cyt c, the modification of the electrode was assessed using cyclic voltammograms (CVs). Fig. 2 shows CVs of the electrodes in PBS at a scan rate of 10 mV/s. Fig. 2a demonstrates the CV of a bare GC electrode, and Fig. 2b displays the CV of a nafion-rGO/GC electrode. As illustrated, no signal from both the Cyt c/GCE and nafion-rGO/GCE appeared. Notably, the nafion-rGO/GC electrode shows a high capacitive current resulting from the deposition of the graphene sheets. As shown in Fig. 2c, no characteristic redox peaks of Cyt c on a nafion/GC electrode was found, indicating that nafion alone either cannot adsorb Cyt c or fails to facilitate electron transfer to Cyt c due to its insulating properties (Fig. 2b). Interestingly, the defined redox peak currents on the nafion-rGO/Cyt c/GCE are attributed to wiring of graphene to the electroactive center of the immobilized Cyt c (Fig. 2d). In addition, it is revealed that graphene has a significant role in DET between Cyt c and the GCE. The Cyt c molecules are positively charged at pH 7.0 PBS. Therefore, hydrophobic and electrostatic interactions can contribute in immobilizing Cyt c on a graphene-modified electrode [22, 23].

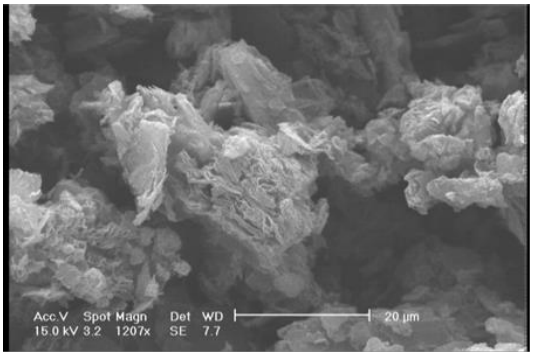


Fig. 1. SEM image of rGO.

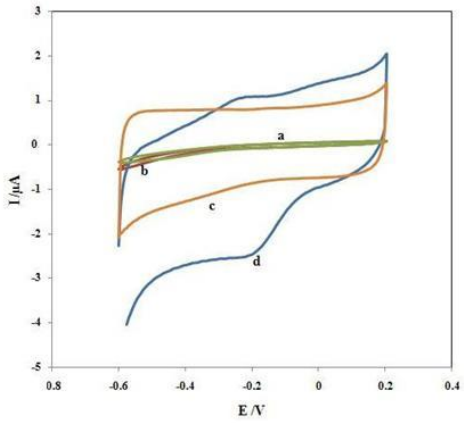


Fig. 2. Cyclic voltammograms of a bare GC electrode (a), nafion-rGO/GC electrode (b) Cyt c on nafion/GC electrode (c), and Cyt c on nafion-rGO/GC electrode (d). All the voltammograms are recorded in a PBS buffer pH 7 at a scan rate of 10 mV/s.

The high stability of the nafion-rGO/Cyt c/GCE, with no significant redox peaks of Cyt c (30 cycles), demonstrated strong interactions between Cyt c and the graphene sheets. The immobilized protein shows an $E_{1/2}$ = -256 mV and peaks separation (ΔE_p) of 86 mV at a scan rate of 20 mV s^{-1} in PBS (pH 7.0), indicating a rapid and quasi-reversible electron-transfer process. This phenomenon is attributed to the heterogeneous dispersion of Cyt c on the electrode due to various kinetic/thermodynamic balances on uneven nafion-rGO/GCE [24]. Further, the scan rate effect was studied on the redox currents of Cyt c. The Linear increase in redox peak currents (cathodic and anodic) (Fig. 3) with the increment scan rate with correlation coefficients of 0.996 and 0.994, respectively, reveals an adsorption-controlled electrochemical process. Based on the the Laviron's equation [25]:

$$I_p = n^2F^2\nu A\Gamma/4RT$$

Where, n is the number of electrons transferred; I_p is the cathodic peak current; F is the Faraday constant; A is the electrode surface area; ν is the scan rate; Γ is the surface coverage, and T is the temperature (K); R is the gas constant. The surface coverage of Cyt c on nafion-rGO/GC electrode was estimated as 1.8 pmol/cm^{-2}, close to values obtained for a monolayer coverage of Cyt c on an electrode surface [26]. Using the Laviron's equation [25], the obtained heterogeneous electron transfer of K_s=1.3±0.09 s^{-1} suggests a high kinetic electron transfer.

Electroreduction of H_2O_2

Based on previous studies, heme-proteins such as hemin, horseradish peroxidase, myoglobin, hemoglobin, could be used as a biocatalyst for the electroreduction of H_2O_2 [27]. For this reason, the electroactivity of Cyt c, immobilized on to the graphene-modified electrode, towards the reduction of H_2O_2 was studied.

The redox peak of nafion-rGO/Cyt c/GCE in the absence of H_2O_2 is observed (Fig. 4a). Upon the addition of 1.0 mM H_2O_2 to the PBS (Fig. 4b), the voltammetric signal of the relative electrode changed significantly. In the presence of H_2O_2, the reduction peak of Cyt c increased considerably while its oxidation peak decreased.

The change in redox reaction of H_2O_2 is attributed to the electroactivity of Cyt c towards H_2O_2 (Scheme

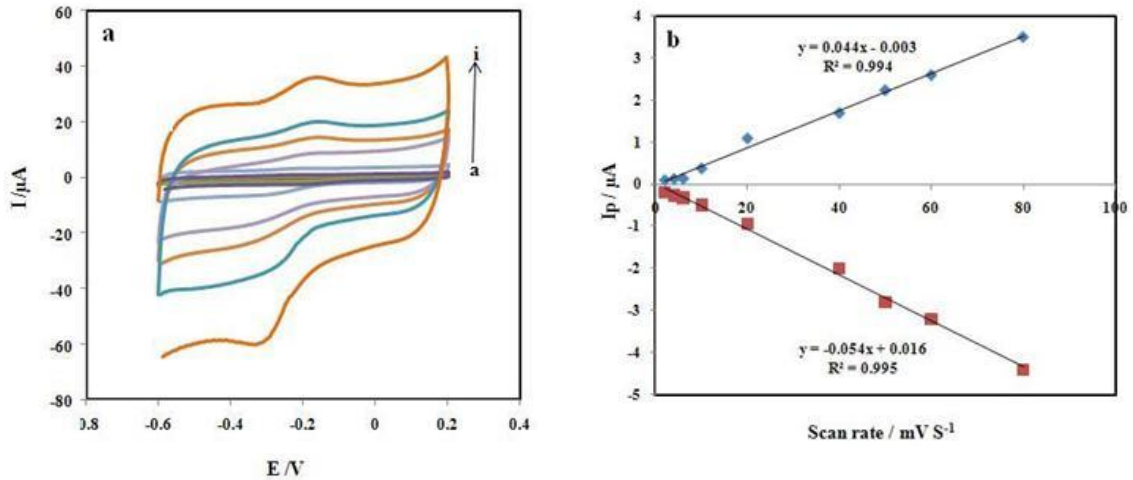


Fig. 3. (a) Cyclic voltammograms of Cyt c at differentscan rates: 2, 4, 6, 10, 20, 40, 50, 60 and 80 mV/s from a to i. (b) Relationship between the Cyt c redox peak currents ($I_{p,a}$ and $I_{p,c}$) vs. the scan rates.

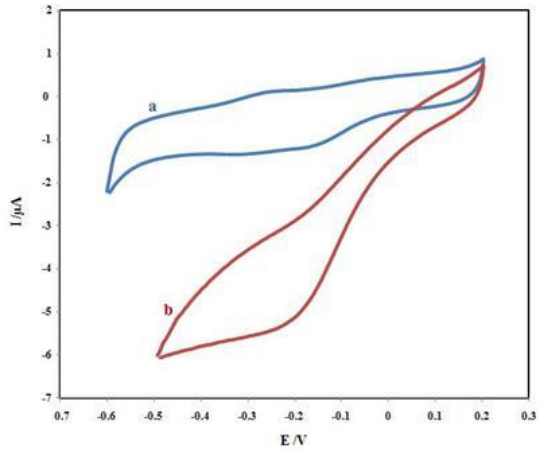
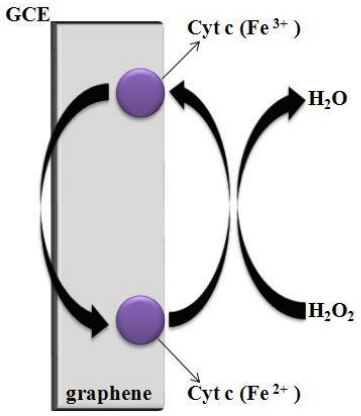


Fig. 4. CVs of Cyt c on a nafion-rGO/GC electrode in the absence (a) and in the presence of a 1.0 mM H_2O_2 (b).



Scheme 1. Representation of electron transfer between immobilized Cytc and Cyt c-nafion-GR/GC electrode during electrocatalytic activity for the reduction of H_2O_2.

1). Many research groups have investigated the mechanism of such an electrocatalytic reaction [28, 29], and the electrocatalytic process can be expressed as follow:

(1)

$$Fe^{3+}\text{-Cyt c} + e^- \longrightarrow Fe^{2+}\text{- Cyt c}$$

(2)

$$Fe^{2+}\text{-Cyt c} + 1/2H_2O_2 + H^+ \longrightarrow Fe^{3+}\text{-Cyt c} + H_2O$$

(3)

$$1/2H_2O_2 + H^+ + e^- \xrightarrow{Fe^{2+}/Fe^{3+}\text{-Cyt c}} H_2O$$

Based on this mechanism, Fe^{3+}-Cyt c is electrochemically reduced to Fe^{2+}-Cyt c on the electrode surface (Eq. 1). H_2O_2 diffuses to the electrode surface where it can take an electron from electrochemically reduced Cyt c (Fe^{2+}-Cyt c) (Eq. 2). In this process, Fe^{2+}-Cyt c is oxidized back to its original oxidation state of Fe^{3+}-Cyt c. The overall redox reaction taken place on the electrode surface is indicated in Eq. 3. The bimolecular electron transfer between H_2O_2 and Fe^{2+}-Cyt c is rather a rapid process. Thus, in the presence of H_2O_2 the surface concentration of Fe^{3+}-Cyt c increases while that of Fe^{2+}-Cyt c decreases. This phenomenon results in increasing the reduction peak current of Fe^{3+}-Cyt c with increasing H_2O_2 concentration (Fig. 5).

As shown in Fig. 5A and B, the CVs of Cyt c on a nafion-rGO/GCE in different concentrations of H_2O_2 in PBS (0.1M, pH 7) increased linearly in the range of 2.0 μM to 1.0 mM. As indicated above, the reduction peak current of Cyt c increases with the increase in the H_2O_2 concentration, thus providing a means for constructing an H_2O_2 sensor.

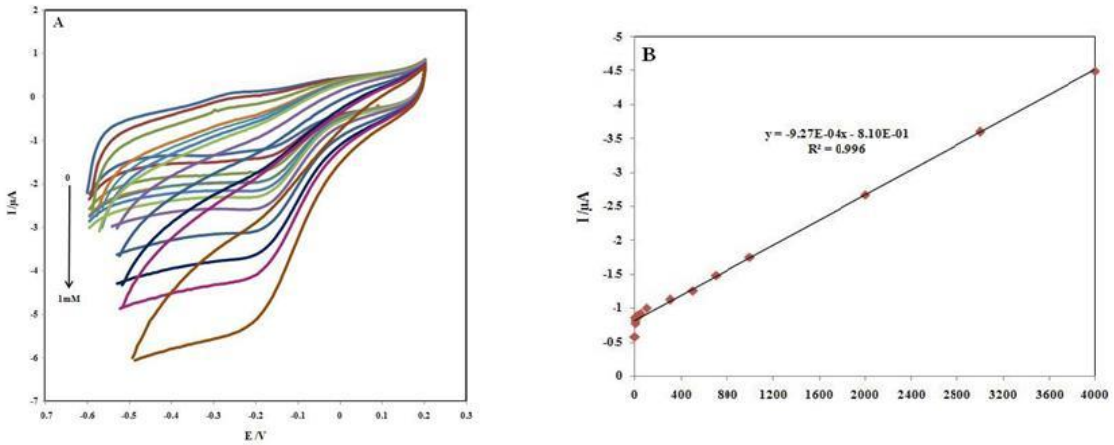


Fig. 5. (A) CVs of a Cyt c on a nafion-rGO/GC electrode in a set of H_2O_2 solutions with different concentrations (0, 2, 4, 8, 10, 20,50, 100, 300,500, 700, 1000μM).(B) Linear calibration curve for H_2O_2 sensor based on the data extracted from CVs in part A. All the voltammograms are recorded in a PBS pH 7ata scan rate of 10mV/s.

The detection limit of the proposed sensor towards H_2O_2 was 0.4 µM at a signal-to-noise ratio of 3.

To investigate the selectivity of the proposed electrode, not only the 10-fold concentration of Cl^-, CO_3^{2-}, K^+, Ca^{2+}, Na^+, and SO_4^{2-} does not interfere in the determination of H_2O_2, but also, no significant effect was observed in the presence of dopamine, glucose, and ascorbic acid.

CONCLUSION

In this study, successfully oriented immobilization of Cyt c as well as preserving its electroactivity on a nafion/rGO-modified electrode was reported. The electron-transfer rate constant of Cyt c was calculated, confirming a high kinetic electron transfer of Cyt c on the nafion/rGO-modified electrode. The high kinetic electron transfer of Cyt c on the modified electrode results from the excellent electron-transfer mediating effects of graphene. Cyt c showed an outstanding electrocatalytic activity towards the reduction of hydrogen peroxide. Thus, a hydrogen peroxide biosensor with an extended dynamic range and acceptable detection limit was constructed.

ACKNOWLEDGMENT

The authors express their gratitude to Shiraz University Research Council for the support of this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Yan X, Ma S, Tang J, Tanner D, Ulstrup J, Xiao X, et al. Direct electron transfer of fructose dehydrogenase immobilized on thiol-gold electrodes. Electrochimica Acta. 2021;392:138946. https://doi.org/10.1016/j.electacta.2021.138946

[2] Qi G, Li H, Zhang Y, Li C, Xu S, Wang M, et al. Smart Plasmonic Nanorobot for Real-Time Monitoring Cytochrome c Release and Cell Acidification in Apoptosis during Electrostimulation. Analytical Chemistry. 2019;91(2):1408-15. https://doi.org/10.1021/acs.analchem.8b04027

[3] Zhang M, Zheng J, Nussinov R, Ma B. Release of Cytochrome C from Bax Pores at the Mitochondrial Membrane. Scientific Reports. 2017;7(1):2635. https://doi.org/10.1038/s41598-017-02825-7

[4] Harper-Leatherman AS, Wallace JM, Long JW, Rhodes CP, Graffam ME, Abunar BH, et al. Redox Cycling within Nanoparticle-Nucleated Protein Superstructures: Electron Transfer between Nanoparticulate Gold, Molecular Reductant, and Cytochrome c. The Journal of Physical Chemistry B. 2021;125(7):1735-45. https://doi.org/10.1021/acs.jpcb.0c09688

[5] Lebègue E, Smida H, Flinois T, Vié V, Lagrost C, Barrière F. An optimal surface concentration of pure cardiolipin deposited onto glassy carbon electrode promoting the direct electron transfer of cytochrome-c. Journal of Electroanalytical Chemistry. 2018;808:286-92. https://doi.org/10.1016/j.jelechem.2017.12.024

[6] Abdoli M, Nami N, Hossaini z. A facile and efficient synthesis of indole and acridine derivatives using (MWCNTs)-COOH/La2O3 nanostructure. Nanochemistry Research. 2021;6(2):178-87.

[7] Lee I, Loew N, Tsugawa W, Lin C-E, Probst D, La Belle JT, et al. The electrochemical behavior of a FAD dependent glucose dehydrogenase with direct electron transfer subunit by immobilization on self-assembled monolayers. Bioelectrochemistry. 2018;121:1-6. https://doi.org/10.1016/j.bioelechem.2017.12.008

[8] Mehdi khoshfetrat S, Mehrgardi MA. Electrochemical Genotyping of Single-Nucleotide Polymorphisms by using Monobase-Conjugated Modified Nanoparticles. ChemElectroChem. 2014;1(4):779-86. https://doi.org/10.1002/celc.201300221

[9] Bollella P, Hibino Y, Kano K, Gorton L, Antiochia R. Enhanced Direct Electron Transfer of Fructose Dehydrogenase Rationally Immobilized on a 2-Aminoanthracene Diazonium Cation Grafted Single-Walled Carbon Nanotube Based Electrode. ACS Catalysis. 2018;8(11):10279-89. https://doi.org/10.1021/acscatal.8b02729

[10] Aghamiri ZS, Mohsennia M, Rafiee-Pour H-A. Immobilization of cytochrome c on polyaniline/polypyrrole/carboxylated multi-walled carbon nanotube/glassy carbon electrode: biosensor fabrication. Journal of Solid State Electrochemistry. 2019;23(7):2233-42. https://doi.org/10.1007/s10008-019-04300-x

[11] Zhang M, Zheng J, Wang J, Xu J, Hayat T, Alharbi NS. Direct electrochemistry of cytochrome c immobilized on one dimensional Au nanoparticles functionalized magnetic N-doped carbon nanotubes and its application for the detection of H2O2. Sensors and Actuators B: Chemical. 2019;282:85-95. https://doi.org/10.1016/j.snb.2018.11.005

[12] Guo C, Wang J, Chen X, Li Y, Wu L, Zhang J, et al. Construction of a Biosensor Based on a Combination of Cytochrome c, Graphene, and Gold Nanoparticles. Sensors. 2019;19(1). https://doi.org/10.3390/s19010040

[13] Omidfar K, Ahmadi A, Syedmoradi L, Khoshfetrat SM, Larijani B. Point-of-care biosensors in medicine: a brief overview of our achievements in this field based on the conducted research in EMRI (endocrinology and metabolism research Institute of Tehran University of medical sciences) over the past fourteen years. Journal of Diabetes & Metabolic Disorders. 2020. https://doi.org/10.1007/s40200-020-00668-0

[14] Geim AK, Novoselov KS. The rise of graphene. Nanoscience and Technology: Co-Published with Macmillan Publishers Ltd, UK; 2009. p. 11-9. https://doi.org/10.1142/9789814287005_0002

[15] Khoshfetrat SM, Mehrgardi MA. Amplified electrochemical genotyping of single-nucleotide polymorphisms using a graphene-gold nanoparticles modified glassy carbon platform. RSC Advances. 2015;5(37):29285-93. https://doi.org/10.1039/C5RA03794H

[16] Khoshfetrat SM, Seyed Dorraji P, Shayan M, Khatami F, Omidfar K. Smartphone-Based Electrochemiluminescence for Visual Simultaneous Detection of RASSF1A and SLC5A8

Tumor Suppressor Gene Methylation in Thyroid Cancer Patient Plasma. Analytical Chemistry. 2022;94(22):8005-13. https://doi.org/10.1021/acs.analchem.2c01132

[17] Ahmadi A, Khoshfetrat SM, Kabiri S, Fotouhi L, Dorraji PS, Omidfar K. Impedimetric Paper-Based Enzymatic Biosensor Using Electrospun Cellulose Acetate Nanofiber and Reduced Graphene Oxide for Detection of Glucose From Whole Blood. IEEE Sensors Journal. 2021;21(7):9210-7. https://doi.org/10.1109/JSEN.2021.3053033

[18] Chen RJ, Choi HC, Bangsaruntip S, Yenilmez E, Tang X, Wang Q, et al. An Investigation of the Mechanisms of Electronic Sensing of Protein Adsorption on Carbon Nanotube Devices. Journal of the American Chemical Society. 2004;126(5):1563-8. https://doi.org/10.1021/ja038702m

[19] Jung E, Shin H, Hooch Antink W, Sung Y-E, Hyeon T. Recent Advances in Electrochemical Oxygen Reduction to H2O2: Catalyst and Cell Design. ACS Energy Letters. 2020;5(6):1881-92. https://doi.org/10.1021/acsenergylett.0c00812

[20] Mehdi Khoshfetrat S, Mehrgardi MA. Dual amplification of single nucleotide polymorphism detection using graphene oxide and nanoporous gold electrode platform. Analyst. 2014;139(20):5192-9. https://doi.org/10.1039/C4AN01171F

[21] Chen Y, Zhang X, Yu P, Ma Y. Stable dispersions of graphene and highly conducting graphene films: a new approach to creating colloids of graphene monolayers. Chemical Communications. 2009(30):4527-9. https://doi.org/10.1039/b907723e

[22] Kumar V, Sachdev A, Matai I. Self-assembled reduced graphene oxide-cerium oxide nanocomposite@ cytochrome c hydrogel as a solid electrochemical reactive oxygen species detection platform. New Journal of Chemistry. 2020;44(26):11248-55. https://doi.org/10.1039/D0NJ02038A

[23] Zuo X, He S, Li D, Peng C, Huang Q, Song S, et al. Graphene Oxide-Facilitated Electron Transfer of Metalloproteins at Electrode Surfaces. Langmuir. 2010;26(3):1936-9. https://doi.org/10.1021/la902496u

[24] Mousavi MF, Amiri M, Noori A, Khoshfetrat SM. A Prostate Specific Antigen Immunosensor Based on Biotinylated-Antibody/Cyclodextrin Inclusion Complex: Fabrication and Electrochemical Studies. Electroanalysis. 2017;29(12):2818-31. https://doi.org/10.1002/elan.201700363

[25] Laviron E. The use of linear potential sweep voltammetry and of a.c. voltammetry for the study of the surface electrochemical reaction of strongly adsorbed systems and of redox modified electrodes. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry. 1979;100(1):263-70. https://doi.org/10.1016/S0022-0728(79)80167-9

[26] Fuku X, Iftikar F, Hess E, Iwuoha E, Baker P. Cytochrome c biosensor for determination of trace levels of cyanide and arsenic compounds. Analytica Chimica Acta. 2012;730:49-59. https://doi.org/10.1016/j.aca.2012.02.025

[27] Gu H-Y, Yu A-M, Chen H-Y. Direct electron transfer and characterization of hemoglobin immobilized on a Au colloid-cysteamine-modified gold electrode. Journal of Electroanalytical Chemistry. 2001;516(1):119-26. https://doi.org/10.1016/S0022-0728(01)00669-6

[28] Liu H, Tian Y, Deng Z. Morphology-Dependent Electrochemistry and Electrocatalytical Activity of Cytochrome c. Langmuir. 2007;23(18):9487-94. https://doi.org/10.1021/la700817y

[29] Moghaddam AB, Ganjali MR, Dinarvand R, Ahadi S, Saboury AA. Myoglobin immobilization on electrodeposited nanometer-scale nickel oxide particles and direct voltammetry. Biophysical Chemistry. 2008;134(1):25-33. https://doi.org/10.1016/j.bpc.2008.01.001