# PSA Nanobiosensor Using Graphene and Magnetic Nanoparticles

**H. Rashedi[1*], F.Yazdian[2]**
[1]*School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran*
[2]*Department of Life Science Engineering, Faculty of New Science and Technologies, University of Tehran, Tehran, Iran.*
*hrashedi@ut.ac.ir*

## Abstract
Prostate specific antigen (PSA) is the most common biomarker of prostate cancer, which can be used to detect the prostate cancer in early stages. A novel electrochemical biosensor based on aptamer and magnetic graphene oxide/gold nanocomposite as a label-free in the ferrocyanide and methylene blue-labeled in phosphate buffer was designed. To investigate the assembly process of the nanocomposite the cyclic voltammetry (CV), Squarewave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS) techniques were performed. In addition, the selectivity and specificity of the aptasensor to the PSA were examined. The result showed that the fabricated aptasensor possessed high selectivity toward the PSA in comparison to the other analytes such as glucose, fetal bovine serum (FBS), bovine serum albumin (BSA), carcinoembryonic antigen (CEA), and antibody. The limit of detection of the apatasensor was obtained 1.37 ng/mL with ferricyanide at R2 = 0.9777 and 1.57 pg/mL at R2 = 0.9676.

**Keywords:** Biomarker, Electrochemical Biosensor, Prostate Cancer, Prostate Specific Antigen

## *Introduction*
The prostate cancer is the third dangerous type of cancer in the worldwide. Early detection is the best and easiest strategy to prevent staging it, and prostate specific antigen (PSA) is the most common biomarker for this matter. Increasing the PSA more than 4 ng/mL shows the possibility of prostate cancer. There are several methods to diagnose the prostate cancer by detecting PSA such as, fluorescence, piezoelectricity, electroluminescence, surface enhancement Raman scattering, and electrochemistry [1–3]. The simplicity, cost effectiveness and high sensitivity of electrochemical methods [4], made them one of the best ways to detect the PSA. In electrochemical methods, a biosensor setup with three electrodes (working, reference, and counter electrodes) is used [2]. Most of the electrochemical methods for PSA detection are based on antibodies and aptamers as a receptor [5]. For more than three decades, antibodies were the most popular molecules for the molecular recognition. However, antibodies have been replaced by aptamers, and it is because of the advantages of aptamers such as, high stability, low immunogenicity properties, and high specificity and affinity to bind with a variety of targets [6–8]. Aptamers are peptide or oligonucleotide molecules such as single-stranded DNA (ssDNA) sequences or short RNA, when adopt a specific three-dimensional structure, is able to bind and attach to a specific target [6,9]. As a bio-material, aptamers have been applied for different purposes such as, developing new drugs, diagnostic and therapeutic tools, biosensors, etc. [10–12]. Usually, these sensors are based on

nanostructured electrodes, because of the advantages of nanomaterials as transducing elements. The analytical metrics of biosensors have been improved by nanomaterials due to their large electroactive surface area, which have reactive groups and simple to be functionalized to immobilize the aptamers [1]. Conductive nanomaterials promoted fast electron transfer at the surface of the electrode. Therefore, valid PSA aptasensors based on carbon nanotube [13], metal [2] and metal oxide [14] nanostructures, graphene derivative [15] and other nanomaterials [16] have been discussed.

Recently, graphene oxide have been noticed as a two-dimensional nanomaterials to provide new possibilities to develop next generation of biological sensing platforms. GO demonstrates great water dispersibility, high attachment properties for specific biomolecules, and biocompability, which are due to remarkable chemical, physical, and electrical properties [17,18]. In this research, an aptasensor based on magnetic graphene oxide/gold to detect the prostate cancer by measuring PSA concentration was designed. Cyclic voltammetry (CV), Squarewave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS) techniques were performed to investigate the changes in functional groups in every step of the nanocomposite synthetizing and incubation of the PSA. The electrochemical measurements were performed at different incubation time and different concentration of PSA. Moreover, methylene blue was used to label the electrode. The effect of different scan rates was studied via CV test.

## *Experimental*

Fabrication of Nanoprob and Aptasensor

98 µl of the GO/$Fe_3O_4$/Au solution (1.5 mg/mL) were incubated to 2 µl of aptamer for 24 h. Then, 2 µl of nafion were added to the 18 µl of the later mixture and left in a room temperature for 15 min.

To investigate the electrochemical changes of the nanoprob in every fabrication step, different electrochemical measurements were performed. A glassy carbon was used as a working electrode, and was polished with 5 µM alumina powder, then subsequently, sonicated in ethanol and water to eliminate absorbed particles. After that, 4 µL of GO, GO/$Fe_3O_4$, GO/$Fe_3O_4$/Au, GO/$Fe_3O_4$/Au/Naf, and GO/$Fe_3O_4$/Au/Naf/Apt were dropped on the surface of electrodes, separately, and left at room temperature for 10 min. The electrodes were investigated via CV, SWV, and EIS techniques. Then, PSA (2.5 ng/mL) was added to the later electrode and incubated for 30 min at room temperature. To remove non-bounded PSA particles, the surface of the electrode was incubated in 1 %w BSA for 30 min. The electrochemical measurements were repeated for this electrode. CV, SWV, and EIS were performed using ferricyanide ($Fe(CN)_6^{3-/4-}$) as electroactive indicator at a concentration of 2 mM. The potential range for the CV measurements were 0.7 to -0.5 V at a scan rate of 50 mV/s. SWV measurements were performed at the potential from 1 to -0.4 V and the scan rate was 200 mV/s. EIS measurements were obtained using a frequency range from 100 mH to 10 H, and the scan rate of 0.5 mV/s. The data were fitted by ZView software.

Different analytes were used to examine the selectivity of the aptasensor (the polished glassy carbon with 4 µL of GO/$Fe_3O_4$/Au/Naf/Apt on the surface) to PSA. The analytes consisting of glucose, fetal bovine serum (FBS), bovine serum albumin (BSA), carcinoembryonic antigen (CEA), and antibody. 10 µL of each analytes were dropped on the surface of the fabricated aptasensor, separately. After 30 min of incubation, the surface of the electrodes was washed with 1 %w BSA. SWV was performed on the electrodes.

In addition, to obtain the best incubation time of PSA with the aptasensor, the CV was performed in 15, 25, 35, 45, and 55 min of incubation. Moreover, the electrochemical

measurements were applied at the different concentration of PSA (2.5, 5, 7.5, 10, and 12.5 mM).

## *Results and discussion*

Electrochemical Characterization of Synthesized Particles

To investigate the changes in functional groups in every step of the GO/$Fe_3O_4$/Au/Nif/Apt synthetizing different electrochemical techniques were performed. Fig. 1a illustrated the CV curves of the different steps of the aptasensor fabrication and its response to the PSA analyte. The bare electrode showed the highest peak current. Adding GO on the electrode surface decreased the current and increased the electron transfer resistance, it due to the GO low conductivity [19,20]. In the next steps, magnetic GO particles and Au (GO/$Fe_3O_4$ and GO/$Fe_3O_4$/Au) enhanced the electron transfer and the current. However, the current was decreased at nifron and aptamer-modified electrodes (GO/$Fe_3O_4$/Au/Nif/Apt). The current of the electrode reached to the lowest value after PSA incubation. The similar conclusion can be drawn from SWV method (Fig. 1b).

EIS result provided a detailed information on the impedance changes of the electrode surface to investigate the assembly process steps of the aptasensor. Fig. 1c illustrated the Nyquist plots, which consist of a semicircle portion at higher frequencies, corresponds for electron transfer resistance ($R_{et}$), and the linear portion at the lower frequencies that represents the diffusion process. Increasing the diameter of the Nyquist loop demonstrates the increment of electron transfer resistance [21,22]. Tale 1 presented the $R_{et}$ value, which obtained by fitting the EIS data. $R_{et}$ of GO/$Fe_3O_4$/Au/Nif/Apt electrode increased significantly (265 Ω), compared to the bare electrode (130 Ω), indicating that the aptasensor reduced the electron conductivity pathway between the working electrode and electrolyte. After coating with GO/$Fe_3O_4$ and GO/$Fe_3O_4$/Au, $R_{ct}$ decreased in comparison with GO, indicating that $Fe_3O_4$ and Au were successfully assembled on the nanocomposite [23] and, created a barrier on the electrode surface, which blocked the electron transfer between electrode and the solution. However, the addition of nifron, aptamer, and PSA formed a barrier on the electrode surface, which blocked the electron transfer between the electrolyte and the electrode. There were no obvious changes in the linear part of Nyquist curves, which indicated the diffusion of ferricyanide toward the surface of electrode remained at the same condition [24].

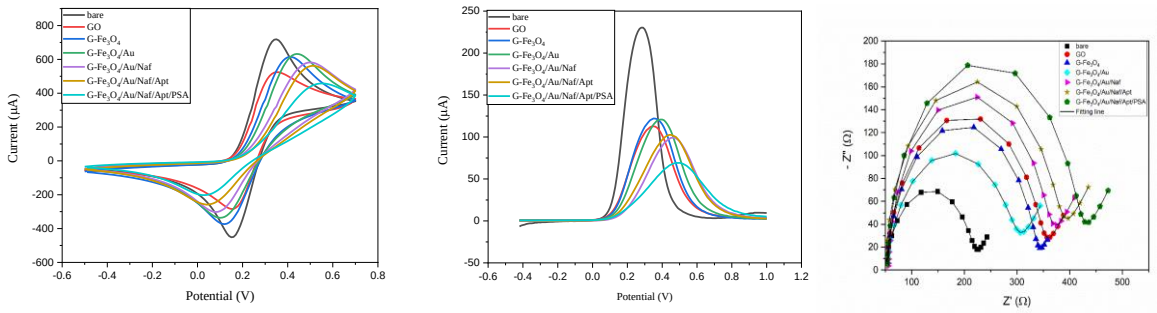


**Figure 1: a) CV curves, b) SWV curves, and c) EIS measurements curves of fabricated aptasensor with PSA incubation.**

Selectivity experiment was performed on the designed aptasensor with PSA, glucose, FBS, BSA, CEA and antibody. It was assumed the high specificity of the aptasensore to the PSA was because of the cavities shape in the imprinted nanoprobe matrix just fitting for the specific molecular structure of PSA, therefore, they cannot appropriately bind with other analytes.

To improve the PSA incubation on the surface of the aptasensor, incubation time is an important factor [25]. So, to obtain the best incubation time, CV test was performed at different times after PSA incubation to the electrode. The best incubation time was considered 35 min.

To detect the effect of different concentrations of PSA on the aptasensor performance CV, SWV, and EIS were applied to the electrodes incubated to the different concentrations of PSA (2.5 to 12.5 ng/mL). Under optimal conditions, the linear relation between current response (ΔI) and different concentrations of PSA were in the range 2.5 to 12.5 g/mL with the correlation coefficient (R2) of 0.97773 was obtained. The regression equation was Y = 2.5626 + 1.69112x.

$R_{et}$ was increased significantly with the increment of PSA indicating the increment of the electron transfer resistance (Table 1). The limit of detection (LOD) is estimated about 1.37 ng/mL as a signal to noise ratio of 3δ (δ= the standard deviation of the blank).

**Table 1. $R_{et}$ value extracted from EIS data, for different PSA concentration**

| Electrode | bare | 0 ng/mL | 2.5 ng/mL | 5 ng/mL | 7.5 ng/mL | 10 ng/mL | 12.5 ng/mL |
|---|---|---|---|---|---|---|---|
| $R_{et}$ (Ω) | 130 | 190 | 215 | 220 | 230 | 234 | 250 |

Methylene blue-labeled aptasensor

To examine the stability of the labeled electrode, 24 cycles of CV test were performed at scan rate of 50 mV/s and voltage of -0.4 to 0 V. There are no intense differences between cycles and 84 percent stability was observed after 24 CV tests. Therefore, repeating electrochemical measurements do not affect the sensitivity of the labeled aptasensor [26].

To describe the effect of the different scan rates on the peak current ($i_p$) in the labeled electrode Randles–Sevcik equation was used [27].

The D of the aptasensor can be determined by the defined relationship. Linear plots of $i_p$ versus $v^{1/2}$ was used to show the scan rate dependence of the peak currents and peak potentials.

Methylene blue was used to perform the selectivity experiment. The PSA concentration effect was showed by recording CV and SWV at each concentration, and plotting the ΔI versus concentration. After labeling electrode by methylene blue, to examine the operation of the labeled electrode and estimating LOD of the labeled electrode, CV and SWV tests were taken from 2.5, 5, 7.5, 10 and 12.5 pg/mL of PSA in PBS (Fig. 2). According to CV spectra (Fig. 2a), when concentration of PSA increased, the current reduced. As observed in Fig. 2b, the results of SWV are similar to the CV result. Calibration curve of the responses is linear with R 2=0.96764 and the LOD was estimated about 1.57 pg/ml.

## *Conclusions*

A novel aptasensor with GO/$Fe_3O_4$/Au/Nif/Apt was synthesized to recognize the PSA. Electrochemical tests performed on the methylen blue-labeled electrode at different concentrations of PSA in phosphate buffer medium showed that as the concentration of analyte increased, the peak obtained in CV tests changed. After calibration of the results, the R2 = 0.9676 and the limit of detection was obtained 1.571 pg/mL. The results demonstrated that using the magnetic graphene/gold nanocomposite showed an acceptable increase in the limit of detection of the PSA.

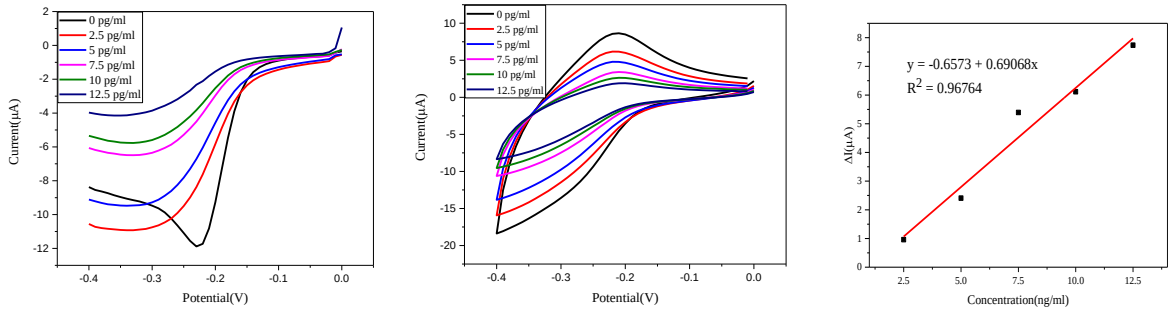


**Figure 2: (a) CV tests, (b) SWV of PSA detection of labeled electrode by methylene blue in PBS, and (c) calibration curve of SWV**

## *References*

[1] Argoubi W, Sánchez A, Parrado C, Raouafi N and Villalonga R 2018 Label-free electrochemical aptasensing platform based on mesoporous silica thin film for the detection of prostate specific antigen *Sensors Actuators, B Chem.*

[2] Rahi A, Sattarahmady N and Heli H 2016 Label-free electrochemical aptasensing of the human prostate-specific antigen using gold nanospears *Talanta*

[3] Ruddon R W 2007 *Cancer biology* (Oxford University Press)

[4] Grieshaber D, MacKenzie R, Vörös J and Reimhult E 2008 Electrochemical biosensors - Sensor principles and architectures *Sensors*

[5] Chang Y, Wang M, Wang L and Xia N 2018 Recent progress in electrochemical biosensors for detection of prostate-specific antigen *Int. J. Electrochem. Sci* **13** 4071–84

[6] Hidding J 2017 A therapeutic battle: Antibodies vs. Aptamers *Nanosci. master Progr.*

[7] Zhou J and Rossi J 2017 Aptamers as targeted therapeutics: Current potential and challenges *Nat. Rev. Drug Discov.*

[8] Lakhin A V, Tarantul V Z and Gening L 2013 Aptamers: problems, solutions and prospects *Acta Naturae (англоязычная версия)* **5**

[9] Zhang Y, Lai B S and Juhas M 2019 Recent advances in aptamer discovery and applications *Molecules*

[10] Figueroa-Miranda G, Feng L, Shiu S C C, Dirkzwager R M, Cheung Y W, Tanner J A, Schöning M J, Offenhäusser A and Mayer D 2018 Aptamer-based electrochemical biosensor for highly sensitive and selective malaria detection with adjustable dynamic response range and reusability *Sensors Actuators, B Chem.*

[11] Ma X, Jiang Y, Jia F, Yu Y, Chen J and Wang Z 2014 An aptamer-based electrochemical biosensor for the detection of Salmonella *J. Microbiol. Methods*

[12] Dehghani S, Nosrati R, Yousefi M, Nezami A, Soltani F, Taghdisi S M, Abnous K, Alibolandi M and Ramezani M 2018 Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): A review *Biosens. Bioelectron.*

[13] Tahmasebi F and Noorbakhsh A 2016 Sensitive electrochemical prostate specific antigen aptasensor: effect of carboxylic acid functionalized carbon nanotube and glutaraldehyde linker *Electroanalysis* **28** 1134–45

[14] Deng Y L, Xu D D, Pang D W and Tang H W 2017 Target-triggered signal turn-on detection of prostate specific antigen based on metal-enhanced fluorescence of Ag@SiO2@SiO2-RuBpy composite nanoparticles *Nanotechnology*

[15] Ueno Y, Furukawa K, Matsuo K, Inoue S, Hayashi K and Hibino H 2015 On-chip graphene oxide aptasensor for multiple protein detection *Anal. Chim. Acta*

[16] Shi J, Lyu J, Tian F and Yang M 2017 A fluorescence turn-on biosensor based on graphene quantum dots (GQDs) and molybdenum disulfide (MoS2) nanosheets for epithelial cell adhesion molecule (EpCAM) detection *Biosens. Bioelectron.*

[17] Cruz S M A, Girão A F, Gonçalves G and Marques P A A P 2016 Graphene: The missing piece for cancer diagnosis? *Sensors (Switzerland)*

[18] Nag A, Mitra A and Mukhopadhyay S C 2018 Graphene and its sensor-based applications: A review *Sensors Actuators, A Phys.*

[19] Feng L, Chen Y, Ren J and Qu X 2011 A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells *Biomaterials*

[20] Yan M, Sun G, Liu F, Lu J, Yu J and Song X 2013 An aptasensor for sensitive detection of human breast cancer cells by using porous GO/Au composites and porous PtFe alloy as effective sensing platform and signal amplification labels *Anal. Chim. Acta* **798** 33–9

[21] Sharma P, Bhalla V, Dravid V, Shekhawat G, Jinsong-Wu, Prasad E S and Suri C R 2012 Enhancing electrochemical detection on graphene oxide-CNT nanostructured electrodes using magneto-nanobioprobes *Sci. Rep.* **2** 877

[22] Lee C Y, Fan H T and Hsieh Y Z 2018 Disposable aptasensor combining functional magnetic nanoparticles with rolling circle amplification for the detection of prostate-specific antigen *Sensors Actuators, B Chem.*

[23] Wang Y-S, Wang Y, Xia H, Wang G, Zhang Z-Y, Han D-D, Lv C, Feng J and Sun H-B 2016 Preparation of a Fe 3 O 4–Au–GO nanocomposite for simultaneous treatment of oil/water separation and dye decomposition *Nanoscale* **8** 17451–7

[24] Li X, Wang X, Li L, Duan H and Luo C 2015 Electrochemical sensor based on magnetic graphene oxide@gold nanoparticles-molecular imprinted polymers for determination of dibutyl phthalate *Talanta*

[25] Keighley S D, Li P, Estrela P and Migliorato P 2008 Optimization of DNA

immobilization on gold electrodes for label-free detection by electrochemical impedance spectroscopy *Biosens. Bioelectron.*

[26] Taghdisi S M, Danesh N M, Nameghi M A, Ramezani M, Alibolandi M, Hassanzadeh-Khayat M, Emrani A S and Abnous K 2019 A novel electrochemical aptasensor based on nontarget-induced high accumulation of methylene blue on the surface of electrode for sensing of α-synuclein oligomer *Biosens. Bioelectron.*

[27] Kumar V, Shorie M, Ganguli A K and Sabherwal P 2015 Graphene-CNT nanohybrid aptasensor for label free detection of cardiac biomarker myoglobin *Biosens. Bioelectron.*