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Modification of reduced graphene/Au-aptamer to develop an electrochemical based aptasensor for measurement of glycated albumin

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ABSTRACT

Herein, an electrochemical label-free biosensor designed for the detection of glycated albumin (GA) using reduced graphene oxide/Au nanoparticles (RGO/AuNPs) modified by anti-GA aptamer. For fast and simple modification of the electrode, the aptamer chain was thiolated. Transition electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) techniques were used to the characterization of synthesized materials. Structural analysis of nanomaterials shows that graphene sheets were synthesized very fine by average thickness of 2.5 nm and Au nanoparticles distributed on the surface of graphene sheets uniformly. Cyclic voltammetry (CV) square wave voltammetry (SWV) and impedance spectroscopy (EIS) were used to electrochemical study of the decorated electrode. Electrochemical studies described the potential of fabricated rGO/AuNPs-aptamer electrode to selectively determine GA properly in buffer solution at the range of 2–10 μg mL^{-1} by the detection limit of 0.07 μg. mL^{-1} for GA.

1. Introduction

Diabetes is one of the metabolic disease that attracted many attentions due to the number of patients in the world. The parameters that routinely helps to control the diabetes are the level of fasting blood sugar (FBS) and glycated hemoglobin (HbA1c). FBS indicates on how one's blood sugar is managed. and HbA1c is appropriate for monitoring the glycemic condition within 2–3 months [1,3–5]. Meanwhile, glycated albumin (GA) is very useful for monitoring the change in glycemic conditions when patients are under treatment, particularly when rapid treatment responses are required, because albumin have 12–21 days half-life in the blood [1,2,6,7]. In addition, in conditions such hemolytic anemia that influence the life span of erythrocytes or in cases of variants hemoglobin measurement of GA provide more accurate information about glycemic conditions [8–10]. Therefore, GA is thought to be more important and sensitive indicator than FBS and HbA1c for monitoring the glycemic condition in diabetic patients [1,2]. The serum concentration of GA is about 6–13% and 20–30% in healthy and diabetics, respectively as a result it is 2–5 times higher in diabetic patients than healthy individuals [1,11,12]. Up to now, there are some methods to measure concentration of GA including boronate affinity chromatography [13,14], immunoassay-related techniques [15,16] and enzymatic methods [7,17–19] that are relatively time consuming and expensive. Recently, aptamers as short single stranded DNA or RNA molecules that have presented high affinity, specificity and selectivity to their targets are frequently used in biosensors [20]. Several reasons support the preferential use of aptamers over antibodies in biosensors. The smaller aptamers are, the denser layer and therefore the higher affinity sensor can be generated, they can be manipulated and improved by rational design [21], thanks to their stability, aptasensor regeneration takes place more easily and finally, aptamers can be developed against wide range of targets including non-immunogenic or toxic components or even ions [22,23]. Among the sensors with optical, piezoelectric or thermal transducers, electrochemical sensors have the advantage of being portable, low cost and simple operation [24]. Typically, an electrochemical sensor consists of reference, counter and work electrodes. Working electrode is functionalized with capturing molecule and attachment of target will affect the current flow by conformational switching [25]. There are various materials that have been used for modification of electrode to enhance the sensitivity of the surface. Graphene with high thermal and electrical conductivities, large specific area and low cost has attracted intensive interests recently

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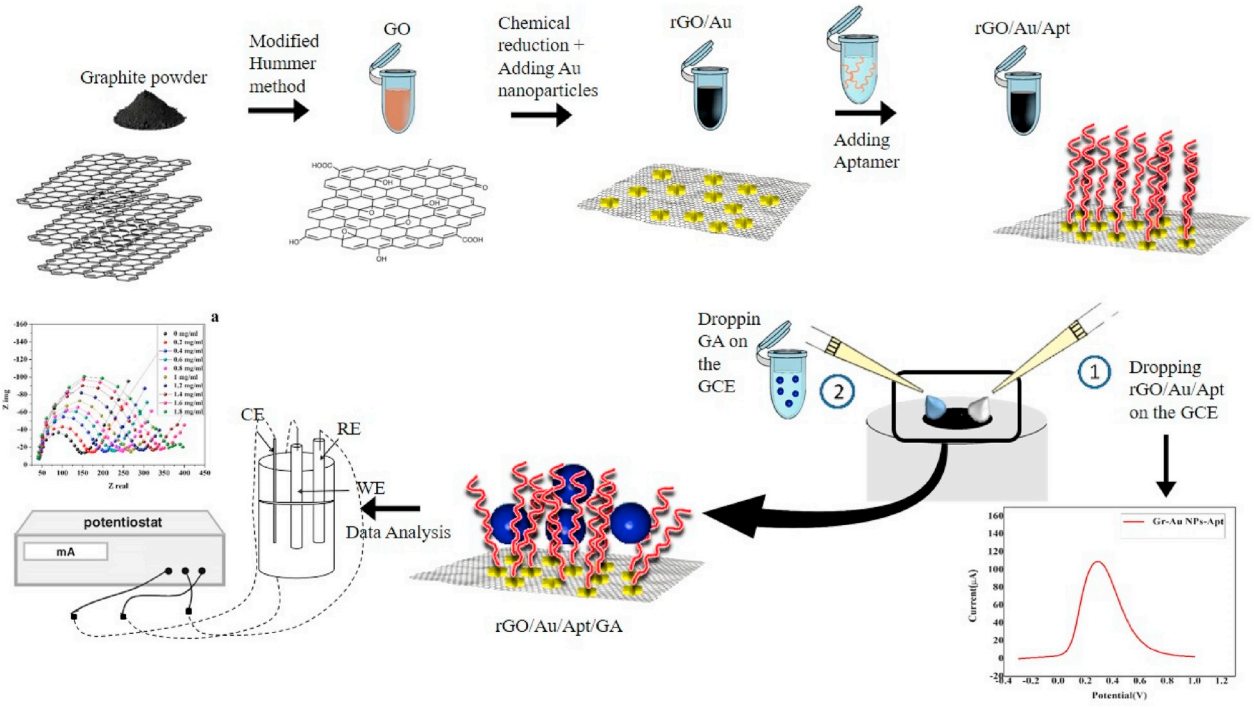

Scheme. 1. Electrochemical biosensor preparation process.

[26,27]. In addition, to enhance the sensitivity of electrode, graphene and gold nanoparticles were used simultaneously to benefit their synergistic effect [28]. In this paper, we report a graphene gold modified electrochemical based aptasensor against glycated albumin, a novel modification for electrode surface and a new technique for measurement of GA.

2. Materials and methods

2.1. Materials and equipment

Thiolated anti-GA aptamer designed by Tsukakoshi et al. [29] with some modification as 5′GGTGGCTGGAGGGGGCGCGAACGTTTTTTT TTT 3′– SH was synthesized and purified by Microsynth (Microsynth AG, Balgach, Switzerland). 4-(2- hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), $HAuCl_4$ was provided by Sigma-Aldrich (USA). All other chemicals were of analytical grade and were purchased from Merck co. Albumin was purchased from Sigma as lyophilized powder (10g). GA prepared through glycation of Albumin as presented in Fig S1.

2.2. Reduced graphene oxide/gold nanoparticle synthesis

Graphene oxide (GO) was synthesized through modified Hummers' method [30]. For synthesis of RGO/Au nano particles, at the first, 0.3 g GO was added to 50 mL H_2O and the suspension was sonicated using probe ultrasonic. The process was repeated twice with the first cycle lasting for 30 min and the second for 15 min and after that 15 mL HEPES as a reducing agent followed by the addition of 2 mL of 0.1 mol. L^{-1} $HAuCl_4$ and the mixture was kept at room temperature for 1.5 h. After that the product centrifuged at 10000 rpm twice and dried at 80 °C for 24 h. Finally RGO/Au NPs weighted and dispersed in double distiled water to concentration of 0.25 mg. mL^{-1}.

2.3. Instrumental equipment

All electrochemical experiments were carried out by an Ivium stat potentiostat/galvanostat model vertex one (Eco-chime, Netherlands). A glassy carbon electrode, Platinum wire and an Ag/AgCl reference electrode as working electrode, counter and reference electrode, respectively, were used in the structure of the conventional three-electrode cell. All EIS analyses were carried out in the frequency range between 100 kHz and 15 mHz with a perturbation amplitude of 5 mV. Morphological investigations of the synthesized nano materials were carried out by using TEM (Philips XL 30). FT-IR spectra were obtained using a spectrometer (Thermo Incol). X-ray diffraction patterns were obtained from an X-ray diffractometer (PANalytical X'Pert-Pro) with a Cu-K^{α} monochromatized radiation source and a Ni filter.

2.4. Preparation of reduced graphene gold-aptamer electrode

The glassy carbon electrode (GCE) was polished on cotton soaked with alumina to make it clean with shiny appearance. 98 μL reduced Graphene-gold nanoparticle (0.25 mg. mL^{-1}) were mixed with 2 μL thiolated anti-GA aptamer (2 μ mol. L^{-1}) and incubated for 24 h in 4 °C. Five minutes before applying on electrode, graphene-gold-aptamer was mixed with 1% Nafion solution in proportion of 4:1. Then, 5 μL of the final solution were dropped on electrode and dried in air. The prepared electrode was used for tests.

2.5. Measurement of GA

40 μL of GA at various concentrations was applied on the surface of decorated nano probe electrode and kept at 100% moisture for 30 min. After that, electrode was gently rinsed with 100 mM phosphate buffer saline (PBS) (pH 7.4) and measurement was done in potassium hexacyanoferrate (II) media. For lable based measurments, Different concentrations of GA in the range of 0.2–2 mg. mL^{-1} were prepared from 50 mg. mL^{-1} stock by dilution in PBS. Then were tested by 0.2 mg. mL^{-1} rising steps.

2.6. Selectivity

Selectivity of the prepared nano probe was studied by

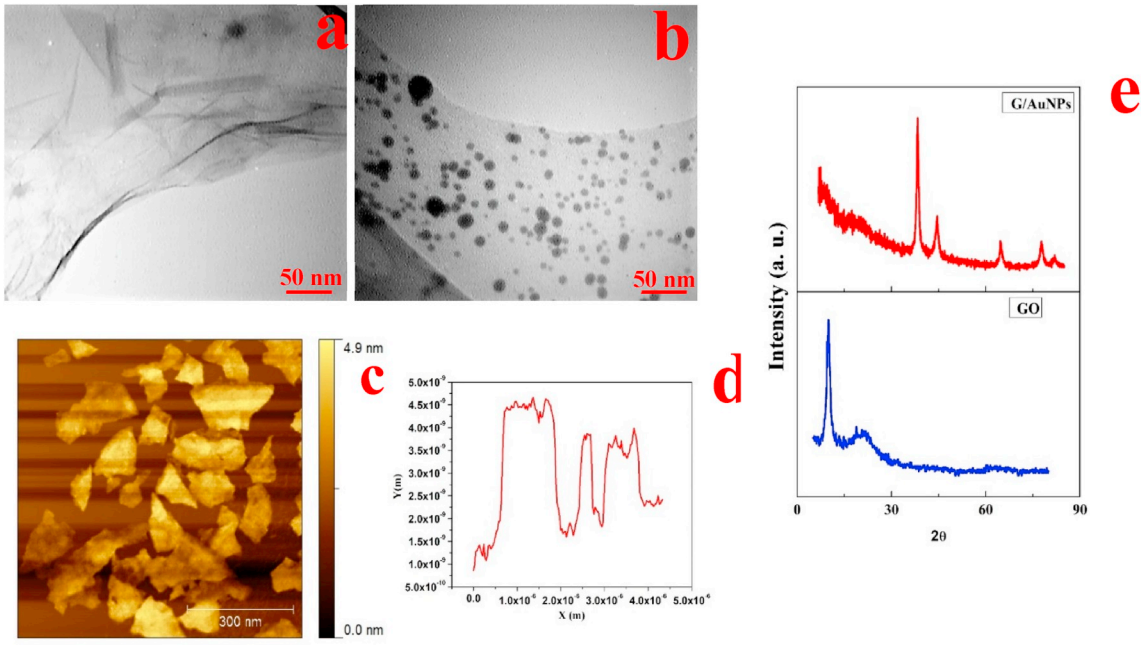

Fig. 1. a)TEM image of GO and b)RGO/Au NPs, c) AFM graph of RGO/Au NPs, d) height profile RGO/Au NPs, e) XRD pattern of GO and RGO/Au NPs.

measurements of non-glycated albumin (40 mg mL^{-1}), insulin (70 pmol. L^{-1}) and lysozyme (20 μg mL^{-1}) with SW volvametry and compare the results with the response of the nano probe to 0.2 mg. mL^{-1} concentration of GA.

3. Results and discussion

Scheme 1 presents the procedure of the nano probe. As illustrated, at the first Go particles were synthesized chemically and after that gold nano particles deposited on the surface of rGO sheets to result rGO/AuNP. Then aptamer chains decorated on rGO/AuNPs via S–Au interaction. By specific binding of GA to functionalized electrode surface, it blocked due to spatial avoidance GA molecules that results to decrease the current or increase the charge transfer resistance of electrode. TEM images of GO and rGO/AuNPs shows the uniform distribution of Au NPs on the surface of graphene sheets, demonstrating the suitable procedure for preparation of rGO/AuNPs with HEPES as a reduce agent. These uniform nano particles are good substrate for thiolated aptamer chains and due to high surface area of rGO, the magnitude of AuNPs on the surface of modified electrode are as enough as needed. The average diameter of AuNPsdecorated on the surface of rGO sheets was 20 nm (Fig. 1a and b). Investigation the morphology and structure of GO and rGO/AuNPs by AFM describes the fine structure of GO nano sheets with the thickness of 2.5 nm (Fig. 1c). Reduction of GO sheets to form rGO/AuNPs can be derived from the XRD patterns (Fig. 1e). As it can be seen, the sharp diffraction peak revealed at 2Θ = 10° characteristic to GO disappeared and replaced by strong peaks at 2Θ = 38.1°, 64.5∘and 77.5∘that correspond to (111), (220) and (311) plane of AuNPs. Furthermore, the diffraction peak appeared at 2Θ = 44° can be related to overlapping the reflection plane of Au and graphite.

Fig. 2a presents the cyclic voltammetry (CV) curves of modification process for GC electrode. As can be seen, each curves has a redox peak related to electrochemical reaction of Fe^{+2}/Fe^{+3} couple. After modification of GC electrode by rGO/AuNPs the peak current of CV curve increased due to enhancing the specific area of modified electrode and, therefore the number of active sites for Fe^{+2}/Fe^{+3} redox reaction. Increasing the resistance of electrode caused to changing the potential peaks for modified electrode; anodic peak potential shift to more positive and the cathodic peak potentials are slightly shifted toward cathodic direction. The addition of aptamer chains on the surface of rGO/AuNPs caused occupation of electrode active sites and therefore diminishing magnitude of the current peaks of Fe^{+2}/Fe^{+3} couple. In continue, GA binding to the coated aptamers results in a further decrease in the current and potential peaks in CV curves. Fig. 2b shows the Nyquist plots of the modification procedure of GC/rGO/AuNPs/Apt-GA electrode. As illustrated, each plot has a semicircle with various diameters that correspond to charge transfer resistance of Fe^{+2}/Fe^{+3} couple electrochemical reaction. By modification of GC electrode with rGO/AuNPs the conductivity of electrode increased and after modification of electrode with rGO/AuNPs/Apt and rGO/AuNPs/Apt-GA, the conductivity of electrode was decreased as mentioned above. For study the electrochemical performance of modified electrode at various concentration of GA, the electrode was dipped in various concentration of GA and after that placed in 0.1 M K_2 [Fe $(CN)_6$] media. CV is the most famous electrochemical techniques that give beneficial results about the performance of electrodes. Fig. 2c presents the SW curves of modified electrode at various concentration of GA. As presented, the maximum peak of SW curves were decreased by increase the concentration of GA due to hindering of surface electrode. Furthermore, the potential peak of SW curves were shifted to positive potentials by addition of GA molecules on the surface of modified electrode. Fig. 2d presents the calibration curves obtained from SW data. As it can be seen, calibration curves of GA molecules has two slopes; the first at the 0–0.6 mg ml^{-1} range and the latter at 0.8–1.6 mg ml^{-1} range. Appearing two slope in calibration plot can be related to changing the mechanism of aptamer folding at high GA concentration.

Electrochemical impedance spectroscopy (EIS) is a comprehensive electrochemical system to study the performance of electrochemical biosensors. Fig. 3a, presents the Nyquist plots of modified electrode at various concentration of GA. As mentioned, each plot has a semi-circle related to electrochemical charge transfer reaction of Fe^{+2}/Fe^{+3} couple at the surface of modified electrode. GA binding on the surface of modified electrode caused to increase the charge transfer resistance of Fe^{+2}/Fe^{+3} electrochemical reaction. As shown, the conductivity of modified electrode and the diameter of semi circles were decreased and increased by increasing the concentration of GA, respectively. Calibration curve of modified electrode obtained from EIS spectra was shown in Fig. 3b. The limit of detection (LOD) was calculated using

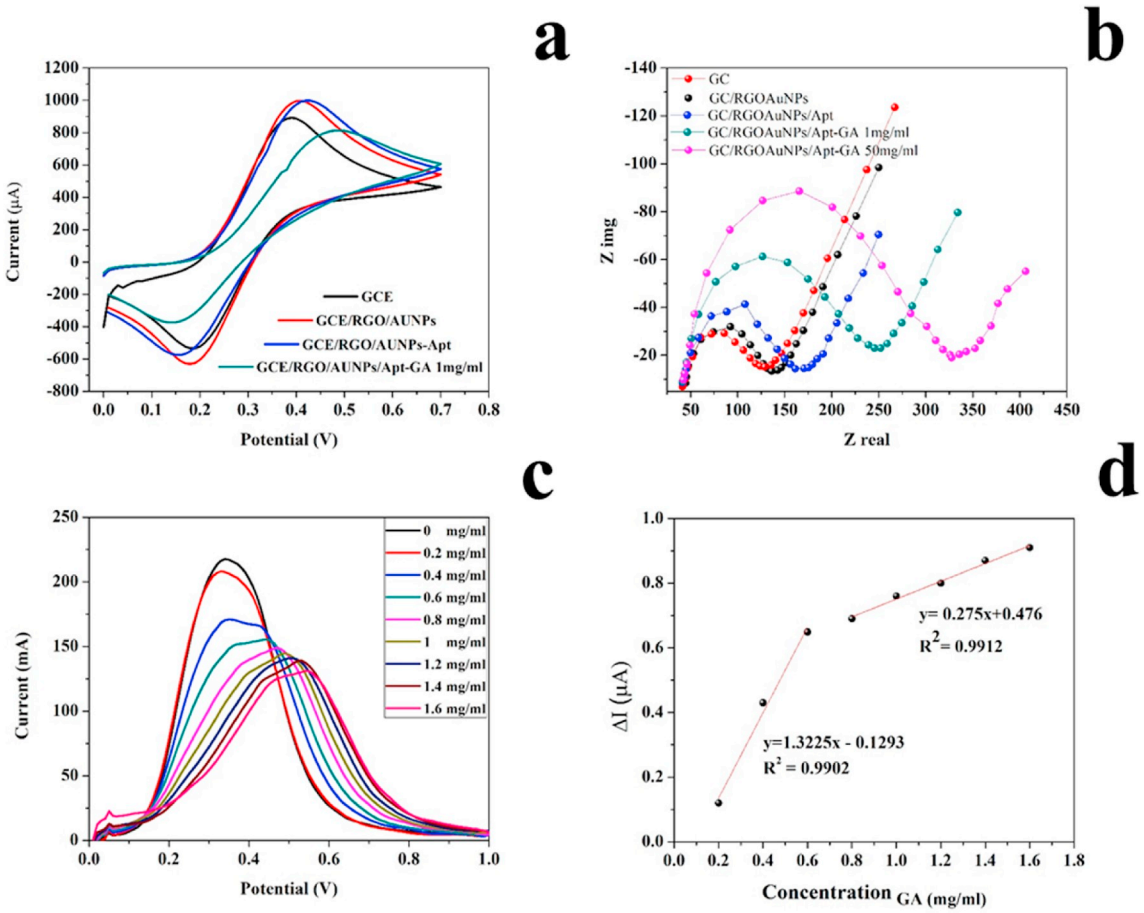

Fig. 2. CV curves (a), and EIS spectra (b) of bare GC and modified electrode with RGO/Au NPs, RGO/Au NPs-Apt and RGO/Au NPs-Apt-GA in K_4 [$Fe(CN)_6$] media. Evaluation of various concentration of GA by SW (c) and its calibration curve (d).

Equation (1):

$$\mathrm{LOD} = \frac{3s_b}{m} \tag{1}$$

Where S_b is the standard deviation for blank (peak current at 0 M concentration of GA) and m is the slope of calibration curve. The magnitude of LOD using Equation (1) was obtained at about 0.09 mg mL^{-1} that derived from Nyquist plot due to higher sensitivity and wide dynamic range of calibration plot.

Although, Potassium Ferrocyanide produce suitable electrochemical signal, application of this material restricted due to its hazardous nature. For this reasons use of some non-dangerous materials that produce electrochemical signal is necessary. Furthermore MB can be used for label based aptasensor in buffer media. MB is one of the redox compounds that can be non-specifically attached to aptamer chains and generate an electrochemical signal. Fig. 4a, presents the CVs of GC/RGO-AuNPs/Apt-MB electrode in PBS media. As presented, a redox peak cleared in curves that related to electrochemical reaction of MB molecules that attached on the surface of anti-GA aptamers. By increasing the sweep rate, the peak current of CV curves enhanced and

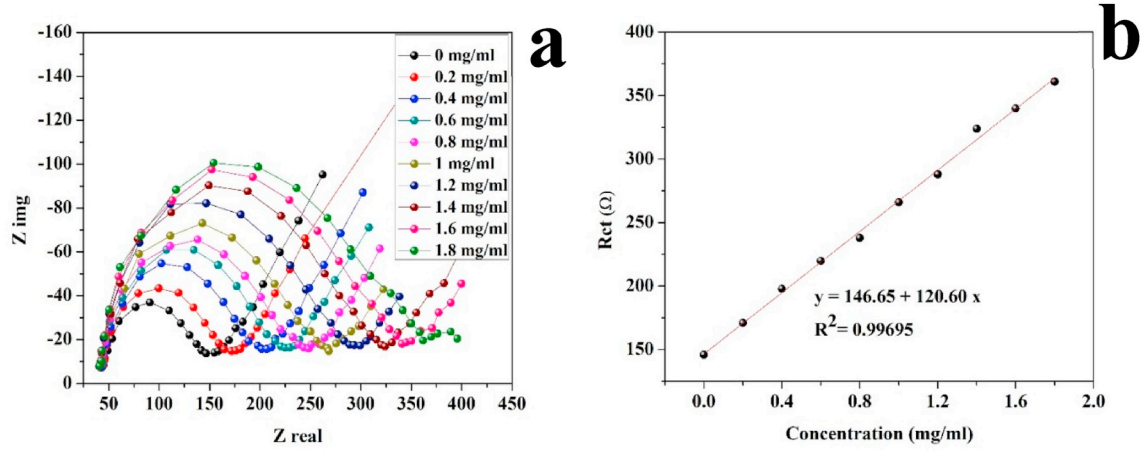

Fig. 3. Evaluation of various GA concentration by EIS spectra (a) and the calibration curve (b).

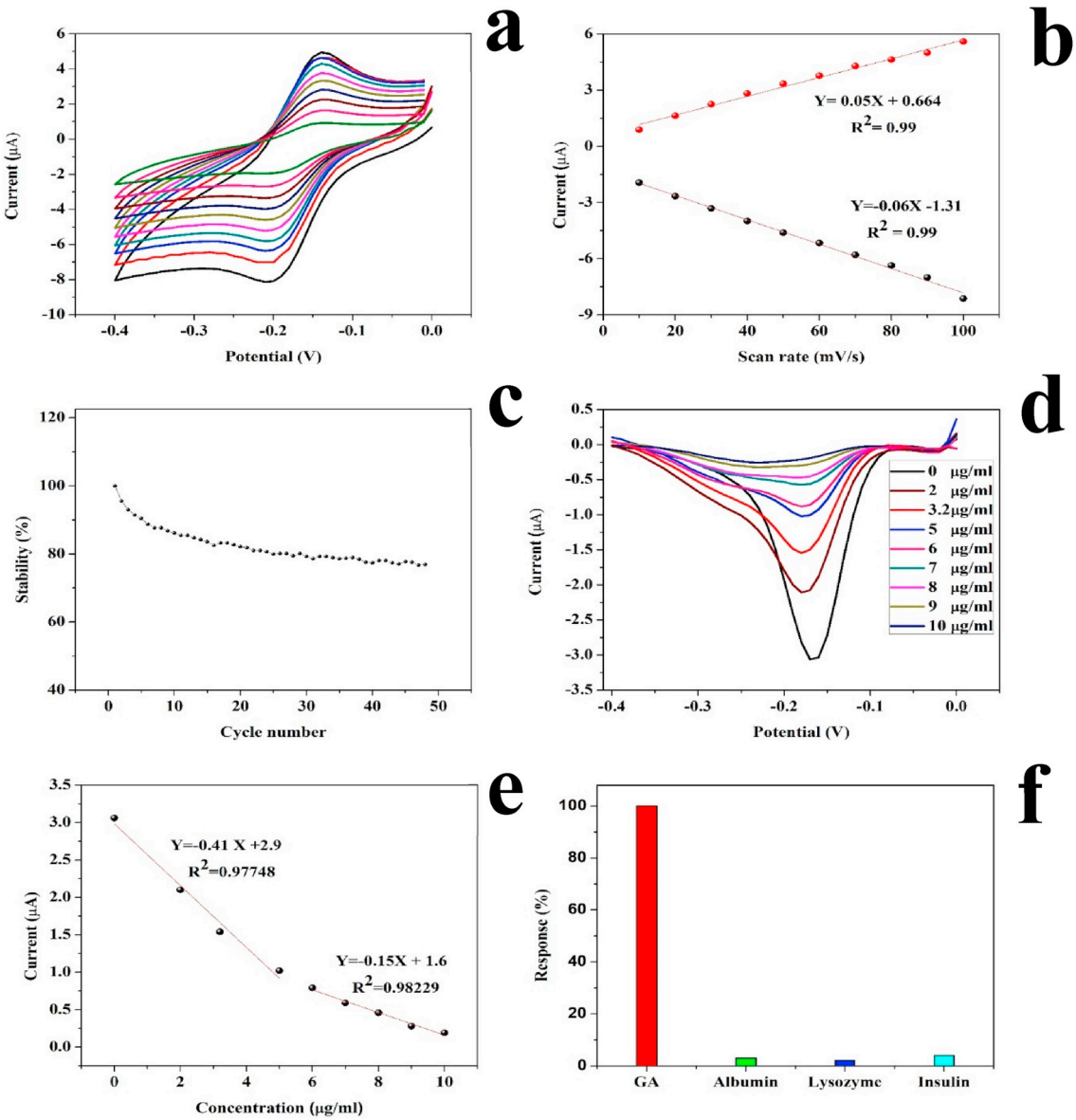

Fig. 4. a) CV curves of GC/RGO-AuNPs/Apt-MB electrode at various scan rates in PBS solution, b) Current peak to scan rate dependency of GC/RGO-AuNPs/Apt-MB electrode, c) stability of GC/RGO-AuNPs/Apt-MB electrode thorough continues CVs, d) SW curves of GC/RGO-AuNPs/Apt-MB at different concentrations of GA, e) related calibration curve of GC/RGO-AuNPs/Apt-MB electrode, f) selectivity of GC/RGO-AuNPs/Apt-MB electrode against some other biomaterials.

the potential of peak currents shifted to positive and negative potentials for oxidation and reduction half cycles, respectively. Dependency of peak currents to scan rate shows that the electrochemical reaction of MB is under diffusion control (Fig. 4b). Capability of SW voltammetry caused to full application of this electrochemical techniques in biosensors.

Stability of modified electrode is one of critical parameters that could not be neglected in constructing electrochemical biosensors. Fig. 4c shows the stability of modified electrode against 50 cycles. Thorough continuous cycles, some of MB molecules that attached to aptamer chains weakly separated from the electrode and therefore the current peaks at oxidation and reduction half cycles were decreased. Fig. 4d presents the SW curves of GC/rGO-AuNPs/Apt-MB electrode in the presence of various concentrations of GA. As it can be seen, the peak current of SW curves were decreased by increasing the concentration of GA, probably due to the leakage of MB molecules that attached to aptamers to the solution. Upon of GA binding on anti-GA aptamer, MB molecules de-attached and lose from the surface of electrode. Calibration curve of GC/rGO-AuNPs/Apt-MB electrode obtained from Fig. 4d shows that by increasing the GA concentration, the mechanism of GA interaction is changed (Fig. 4e). The LOD of prepared electrode was calculated at about 0.07 μg ml^{-1}, using Equation (1). Moreover, selectivity of the sensor was verified using non-glycated albumin, insulin and lysozyme (Fig. 4f). Accordingly, the modified electrochemical biosensor has an excellent selectivity to GA. Table 1 presents the performance of some reported results about the GA detection to compare with prepared nano-probe. Results show that prepared nano-probe has performance properly for detection of GA macromolecules.

4. Conclusion

In this work, collectively, an electrochemical aptasensor was designed by modification of rGO with AuNPs for the determination of GA. For simple and straightforward decoration of biosensor electrode, a thiolated aptamer that incubated with methylene blue was used. Results show that the modified electrode has a suitable sensitivity to GA in the range of 2–10 μg ml^{-1} by the LOD about 0.07 μg ml^{-1}. Furthermore, the selectivity of the decorated electrode among various

Table 1
Comparison the performance of prepared nano probe with other reports.

Detector molecule	Type	Detection limit	Reference
Enzyme	Electrochemical	6.6 μg mL^{-1}	[31]
Aptamer	Optical (graphene oxide based sensor)	50 μg mL^{-1}	[32]
Enzyme	electrochemical	30 μg mL^{-1}	[33]
Aptamer	Electrochemical	2.6 ng mL^{-1}	[34]
Enzyme	Optical (colorimetric)	0.5 mg mL^{-1}	[35]
Aptamers	Optical	0.05 mg mL^{-1}	[36]
Aptamer	Optical (gold quantum dot)	67 ng mL^{-1}	[37]
Aptamer	Electrochemical	0.07 μg mL^{-1}	This work

biological elements was tested that proved the capability of using this electrode as a commercial GA biosensor Moreover, the fabricated GA biosensor is proved to be selective against GA which has been tested by measurements of non-glycated albumin, insulin and lysozyme. In conclusion, an electrochemical GC/rGO-AuNPs/Apt biosensor has been fabricated which is successfully detects and quantifies GA as a preferred candidate to evaluate a prolonged hyperglycemic condition which is thought to be important to diagnose type 2 diabetes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.120722.

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