An electrochemical biosensor based on hemoglobin-oligonucleotides-modified electrode for detection of acrylamide in potato fries

Maryam Asnaashari, Reza Esmaeilzadeh Kenari, Reza Farahmandfar, Khalil Abnous, Seyed Mohammad Taghdisi

PII: S0308-8146(18)31298-6
DOI: https://doi.org/10.1016/j.foodchem.2018.07.150
Reference: FOCH 23265

To appear in: *Food Chemistry*

Received Date: 17 June 2017
Revised Date: 19 July 2018
Accepted Date: 23 July 2018

Please cite this article as: Asnaashari, M., Kenari, R.E., Farahmandfar, R., Abnous, K., Taghdisi, S.M., An electrochemical biosensor based on hemoglobin-oligonucleotides-modified electrode for detection of acrylamide in potato fries, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.07.150

An electrochemical biosensor based on hemoglobin-oligonucleotides-modified electrode for detection of acrylamide in potato fries

Maryam Asnaashari[1], Reza Esmaeilzadeh Kenari[1], Reza Farahmandfar[1], Khalil Abnous[2,3,*], Seyed Mohammad Taghdisi[4,5,*]

[1]Department of Food Science and Technology, Sari Agricultural Sciences & Natural Resources University (SANRU), Sari, Iran.

[2]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Department of Medicinal Chemistry, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

* Corresponding authors: Dr. Khalil Abnous (abnouskh@mums.ac.ir) and Dr. Seyed Mohammad Taghdisi (taghdisihm@mums.ac.ir), Tel.: +98 513 1801112**,** Fax.: +98 513 882 3255

Abstract

Acrylamide a neurotoxin and strong carcinogen, is found in various thermally processed foods. In this study, an electrochemical sensor for detection of acrylamide using double stranded DNA (dsDNA)/Hemoglobin (Hb)-modified screen printed gold electrode (SPGE) was designed. The immobilization of ssDNA1-SH on the surface of SPGE was confirmed by cyclic voltammetry, and the interaction between ssDNA2-NH_2 and Hb with the ratio 1:1 was characterized by agarose gel. The excellent response of the designed biosensor towards acrylamide due to acrylamide and Hb adducts and change of reduction/oxidation process of Hb-Fe(III)/Hb-Fe(II) was determined by square wave voltammetry (SWV). The biosensor showed the optimum response at pH 8.0. The linear working range for acrylamide was from 2.0×10^{-6} to 5.0×10^{-2} M with a detection limit of 1.58×10^{-7} M. The biosensor was suitable for direct determination of acrylamide in water extracted of potato fries and displayed good reproductivity and high stability.

Keywords: Acrylamide; Biosensor; Hemoglobin; Oligonucleotides; Potato fries; Screen printed gold electrode

1. Introduction

Acrylamide was found to be formed in thermally processed foods by Stockholm University (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002). The acrylamide formation in fried food is mainly due to Millard's reaction between reducing sugars and asparagine (Oracz, Nebesny, & Żyżelewicz, 2011). Acrylamide is rapidly absorbed by humans and distributed in brain, thymus, heart and liver and also causes various toxic disorders such as carcinogenicity, neurotoxicity and genotoxicity (Wenzl, Lachenmeier, & Gökmen, 2007).

Analysis of acrylamide focuses primarily on chromatographic methods such as GC–MS (Yamazaki, Isagawa, Kibune, & Urushiyama, 2012) and LC-MS/MS (Cheng, Kao, Shih, Chou, & Yeh, 2009) and HPLC-MS (Bortolomeazzi, Munari, Anese, & Verardo, 2012) for the advantages of sensitivity, high accuracy and selectivity. However, all of them are time consuming, expensive and complex. The difficulty of these methods and increasing demand for precise assessment of acrylamide has motivated researchers to develop alternative analytical methods. Currently new methods were proposed, including immune assay (Preston, Fodey, & Elliott, 2008), electrochemical biosensors (Batra, Lata, Sharma, & Pundir, 2013; Krajewska, Radecki, & Radecka, 2008; Stobiecka, Radecka, & Radecki, 2007) and quartz microbalance sensors (Kleefisch, Kreutz, Bargon, Silva, & Schalley, 2004). The biosensors offer great promise for acrylamide determination due to their rapidity, high sensitivity and simplicity.

In human body, acrylamide can bind DNA, hemoglobin (Hb), enzymes and serum albumin (Friedman, 2003; Hu, Xu, Li, Zhang, Wang, & Li, 2013). Investigations showed that acrylamide adducts with Hb as a result of the reaction between the α-NH_2 group of the N-terminal valine of Hb with the acrylamide molecules. So, as an analytical active element of biosensors, Hb has been used (Batra, Lata, & Pundir,

2013). Hb is an iron-containing protein consisting of four globular protein subunits, each of them with one not-protein heme group, which is the main component of red blood cells. The reversible conversion of Fe(III) to Fe(II) is responsible for its electroactivity. However, the rate of electron transfer is slow due to unfavorable orientation of Hb molecules on the electrode surface (Garabagiu & Mihailescu, 2011). It is possible to incorporate Hb molecules into pyrolytic graphite electrodes (L. Wang & Hu, 2001), poly-3-hydroxybutyrate membrane (Ma, Liu, Xiao, & Li, 2005), agarose hydrogel films (S.-F. Wang, Chen, Zhang, Shen, Lu, Pang, et al., 2005), onto carbon paste electrodes (Krajewska, Radecki, & Radecka, 2008), gold nanoparticles (H Bagheri, Talemi, & Afkhami, 2015; Zhang, Jiang, Wang, & Dong, 2005), carbon nanotubes modified electrodes (Hasan Bagheri, Afkhami, Khoshsafar, Hajian, & Shahriyari, 2017; W. Sun, Li, Wang, Zhao, & Jiao, 2009) or nanoparticles modified electrodes (Xie, Xu, & Cao, 2013). The principle of electrochemical biosensors is based on the reaction of acrylamide and Hb, which leads to Hb-acrylamide adduct formation, could alter its electroactivity properties (Stobiecka, Radecka, & Radecki, 2007). In the present study, a novel electrochemical sensor was designed for detection of acrylamide based on two strands of DNA and Hb on the screen printed gold electrode. Using two strands of DNA can facilitate the recovery of the electrode. Acrylamide was quantified by monitoring the current peak of cyclic votammogram and square wave voltammogram changes before and after the specific reaction.

2. Materials and methods

2.1. Materials

The single-stranded DNA, 5'-Thiol-CCCACCCATCCAACGCCTAC-3' (ssDNA 1) and its complementary strand, 5'-NH_2-CCACCGTAGGCGTTGGATGG-3' (ssDNA 2) were purchased from Bioneer (South Korea). Human hemoglobin (Hb), acrylamide (for electrophoresis, 99%), amoxicillin, potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$), potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6].3H_2O$), 6-mercaptohexanol (MCH), zinc sulphate heptahydrate, sodium chloride, sodium hydroxide and hexane, acrolein, asparagine, glycine, starch, glucose and Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were obtained from Sigma-Aldrich (St. Louis, MO). Screen printed gold electrodes (SPGEs) were purchased from DropSens (Spain, DRP-220BT). N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-n-ethyl-carbodiimide hydrochloride (EDC), Sodium dodecyl sulfate (SDS) and Dithiothreitol (DTT) were purchased from Fluka (Switzerland). Carrez I and Carrez II solutions were prepared by dissolving potassium hexacyanoferrate (II) trihydrate (28.8 g) and zinc sulphate heptahydrate (57.6 g) in deionized water (100 mL), respectively. All aqueous solutions were prepared using f deionized water 18 MΩ (Millipore Corp., USA). Phosphate Buffer Saline (PBS-0.1 M) was prepared by mixing monosodium phosphate with disodium phosphate at 1:4 stoichiometry, to obtain a pH value of 7.4. Sodium chloride 0.1 M was added to the final solution.

2.2. Apparatus

Square wave voltammetry (SWV) and cyclic voltammetry (CV) measurements were carried out using a μstat 400 portable Biopotentiostat/Galvanostat (DropSens, Spain). Data were assessed using DropView 8400 software.

2.3. Preparation of ssDNA2-Hb complex

Amine-modified ssDNA2 was conjugated to carboxyl groups of Hb using EDC/NHS technique. The solution of Hb and ssDNA2-NH_2 (40 µl, 25 µM) was incubated with EDC (300 µl, 400 mM) and NHS (100 µl, 100 mM) for 2 h. Then, Mixture was filtered through an Amicon filter (3 kDa MWCO) and washed three times with deionized water to remove the residual EDC and NHS.

2.4. Gel Electrophoresis

Agarose (2.5 %) gel electrophoresis was performed to verify Hb binding to the amine group of ssDNA2. The complexes in different ratios of Hb and ssDNA2-NH_2 (1:1, 5:1, 10:1 and 20:1 ratio of Hb:ssDNA2) were loaded on the gel (containing 0.1 µg/mL GelRed), and the gel was run in a tris-borate-EDTA (TBE) buffer at 80 V for 30 min. Then, the unbound ssDNAs were visualized using a standard transilluminator.

2.5. Preparation of the designed biosensor

Single-stranded DNA including thiol group (ssDNA1-SH) (1 µM final concentration) was pretreated with 10 mM TCEP in the immobilization buffer (10 mM Tris-HCl, 100 mM NaCl, one mM EDTA, pH 7.4) for 1 h at room temperature. After that, 9 µl of the solution was added onto the surface of SPGE and incubated at 4°C for 12 h under 100% humidity, followed by incubation with 1 mM MCH solution (10 µl) for 1 h to block the left sites of SPGE. Then, hybridization buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7.4) was used to rinse thoroughly the surface of the electrode. Finally, 10 µl of ssDNA2-Hb complex (10 µM) was added onto the surface of SPGE and incubated at refrigerator for two hours. Then, electrodes were stored on air at 4°C till use.

2.6. Characterization of the modified electrode

The interaction of Hb and the designed sensor was evaluated by electrochemical measurement. First, the immobilization of ssDNA1-SH on the surface of electrode was evaluated. Then, the addition of MCH and ssDNA2-Hb complex on the

surface of ssDNA1-modified electrode were determined. The modified electrode was immersed in 2 mM $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6].3H_2O$ and 0.1 M KCl. The electrochemical signals were measured using cyclic voltammetry by cycling the potential from -0.2 V to +0.7 V, with the scan rate 0.1 V/s, after incubation for 10 min at room temperature.

2.7. Optimization of pH

To determine optimum pH, the pH was varied between pH 5.0 to 9.0 when acrylamide was incubated with modified electrodes using 5 mL PBS solution containing 5 mM potassium ferrocyanide. The electrochemical signals were measured using CV.

2.8. Detection of acrylamide based on electrochemical measurement

The modified SPGEs were immersed in different concentrations of acrylamide ($0.1\text{-}10^{-7}$ M) in 5 mL PBS solution containing 5 mM potassium ferrocyanide (pH 8.0). After incubation at 4°C, the electrochemical signals were measured using SWV. SWV was performed in the same potential window with step potential of 2 mV, a square-wave frequency of 5 Hz, and amplitude of 2.5 mV. The dependence of the sensor response on the concentration of the acrylamide was expressed as the currents at the peak potential in SWV measured in a solution containing no analyte.

2.9. Selectivity of the developed electrochemical sensor

The selectivity was assessed in the presence of 0.01 M glucose, asparagine, starch, glycine, amoxicillin and acrolein as mentioned before Current intensity was shown as (I_0/I)-2.5 which I_0 and I were current intensity in the absence and presence of each substance in solution, respectively.

2.10. Preparation of potato fries water extracts

Crushed potato fries (4 g) were ground and mixed into deionized water (100 mL) and the sample was stored for 20 min for swelling. After the swelling time, the

sample was shaken for 1 h at 60°C (Ika, HS-B20 digital) and then centrifuged for about 20 min at 2268 g (MPW Med. instruments, MPW 350R). The supernatant solution was collected and defatted by extraction with hexane. The obtained aqueous solution was purified by adding Carrez I and Carrez II (2.5 mL). The supernatant was subsequently filtered by Whatman 1, and its acrylamide content was measured after diluted 100-fold with PBS to obtained final concentration 0.1 M (pH 8). before electrochemical measurements.

3. Results and discussion

In this work, we report an investigation concerning the electrochemistry of electrodes modified with Hb and oligonucleotides for acrylamide. The formation of ssDNA2-Hb complex was confirmed by gel assay (Figure 1). As shown in Figure. 1, in the presence of Hb the bands of ssDNA2-NH_2 was weak (for all the ratio) compared to the band of free ssDNA2-NH_2, confirming the formation of ssDNA2-Hb complex. Also, the 1:1 ratio of Hb and ssDNA2-NH_2 had the weakest band, indicating the ratio of 1:1 Hb and ssDNA2-NH_2 is the best ratio. The base of reaction is the conjugation of Hb to single-stranded DNA including NH_2 group.

3.1. Feasibility of the designed electrochemical sensor for acrylamide detection

Cyclic voltammetry measurements were used to determine the function of the designed biosensor. As shown in Figure. 2A, the bare electrode due to high capability of electron transfer, had the maximum redox peak (black curve). The electrochemical signal of the electrode was significantly reduced when ssDNA1-SH was added onto the surface of the electrode which could be attributed to the immobilization of DNA onto the surface of the electrode (purple curve) (Figure. 2A). Indeed, the phosphate groups of DNA inhibit the electron transfer of redox

probe through creating an electrostatic repulsive force to the negatively charged redox probe. In the presence of MCH, the electrochemical signal reduced more, because the accessibility of redox probe to the surface of electrode decreased (green curve) (Figure. 2A). After addition of ssDNA2-Hb complex, the electrochemical signal of electrode decreased more, which confirmed the immobilization of ssDNA2-Hb complex onto the surface of electrode (blue curve) via hybridization of ssDNA2 with ssDNA1(Figure. 2A). Finally, when the specific concentration of acrylamide was added, the electrochemical signal reduced more (red curve), which showed the adduct of Hb and acrylamide and formation of a physical barrier for the access of redox probe to the surface of electrode (Figure. 2A). The biosensor preparation was illustrated in the Figure. 2B. This process relies on the interaction between acrylamide and NH_2 group of the N-terminal valine of Hb (Figure. 2C). This adduct changed the Hb structure. So, it would be probable that accessibility of redox-active centers of Hb on the electrode surface causes a decrease on current of redox reaction values.

3.2. Effect of pH on the electrochemical signal of the designed SPGE

The oxidation peak current of acrylamide is closely related to the pH value of electrolyte solution. Therefore, the effect of pH was investigated using CV technique (Figure. 3A). The voltammetric response of modified electrode toward acrylamide was obtained in solutions with varying pH from 5.0 to 9.0 (Figure. 3A). It was found that the current of oxidation peaks increased gradually from pH 4.0 to 8.0, and then the current conversely decreased when the pH value reached 9.0 (Figure. 3B). So, pH 8.0 was chosen as the optimal experimental condition (Figure. 3B). The relationship between the oxidation peak potential and pH of electrolyte also indicates a potential shift towards negative potential and obeyed the following equation of Potential (V)=-0.0486pH+0.7772 with the correlation coefficient of

0.9714 which proved protons are directly involved in the oxidation of acrylamide (Figure. 3C). Indeed, the slope of this regression was determined as 0.0486 $V.pH^{-1}$ according to equation, which was close to the theoretical value of 0.0592 $V.pH^{-1}$ (25°C), indicating that the numbers of the electron and the proton were equal during the electrochemical oxidation process (Figure. 3C). Huang et al. (2016) designed an electrochemical biosensor based on ssDNA modified gold electrode for acrylamide detection. The pH optimization results indicated that the pH increased gradually from 7.0 to 8.0 and then, decreased from 8.0 to 9.0 and the optimum pH of the biosensor was 8.0 in 0.1 PB (NaH_2PO_4-Na_2HPO_4) solution.

3.3. Quantitative analysis of acrylamide based on electrochemical measurement

Cyclic voltammograms using the modified SPGE in 5 mM potassium ferrocyanide to 5 mL PBS solutions (as redox probe) were performed with various scan rates (Figure. 4A). The anodic peak potential was located at E_{an}=370 mV and Anodic $I_{p,a}$ [uA] peaks currents was linearly dependent on scan rate υ, ranging from 10 to 200 mV/s ($y = 418.41x + 44.039$; $R^2 = 0.9825$) (Figure. 4B). This indicates that redox reaction is not a diffusion-controlled process, but a surface-controlled one, as expected for an immobilized system.

For a reversible surface reaction, the surface concentration of electroactive species (Γ mol cm^{-2}) can be estimated to be about 3.5×10^{-9} mol cm^{-2} from the slope of linear plot of peak currents vs. scan rate according to the following equation:

$$I_P = \frac{n^2F^2A\Gamma\upsilon}{4RT} \qquad (1)$$

Where *A, n and v* is the geometrical surface area of the electrode, the number of electrons transferred and Faraday constant, respectively. (R = 8.314 J $mol^{-1}K^{-1}$, F = 96493 C/mol, T = 298 K) which indicates big surface and good efficiently in the electron transfer process.

The determination of acrylamide by SPGE modified with dsDNA/Hb has been examined using two techniques: CV and SWV in solution containing 5 mM potassium ferrocyanide to 5 mL PBS. SWV technique has been proved to be much more sensitive for quantitative measurements in comparison with CV (data not shown), because the SWV can suppress background currents. SPGEs modified with dsDNA/Hb showed various peak currents (from 15.43 to 34.42 µA) and peak potential (from 340 to 380 mV) in conditions of measurements (Figure. 5A). So, the parameter of relative electrochemical signal was used to evaluate their responses. The difference of SWV peak current in the presence of different concentrations of acrylamide to that in the absence of analyte (ΔI) was plotted versus the acrylamide concentration (Figure. 5B). The linearity of ΔI versus [log c] acrylamide was observed in the concentration range from 2.0×10^{-6} to 5.0×10^{-2} M (Figure. 5B). The detection limit was calculated as 1.58×10^{-7} M regarding the three times of the standard deviation of the blank/slope and was low enough to detect acrylamide in food products. The function is due to the binding between acrylamide and Hb immobilized onto the surface of SPGE and formation Hb/Acrylamide adduct, which decreases the electron transfer rate between the electrolyte and modified SPGE and altered the electroactivity of Hb. So, the potential peaks in square wave voltammograms decreased.

Reported detection limits of acrylamide in other studies were as following: 1.2×10^{-10} M for carbon paste electrode modified with single-walled carbon nanotubes/Hb in water extracts from potato crisps (Krajewska, Radecki, & Radecka, 2008),

1.2×10^{-10} M for carbon paste electrode (Stobiecka, Radecka, & Radecki, 2007), 4.48×10^{-5} M for ammonium ion selective electrode (Silva, Gil, Karmali, & Matos, 2009), 0.02×10^{-9} M for carboxylated multi-walled carbon nanotubes including Hb/iron oxide nanoparticles/chitosan composite film (Batra, Lata, & Pundir, 2013), 0.2×10^{-9} M for multiwalled carbon nanotube/copper nanoparticles/polyaniline hybrid film (Batra, Lata, Sharma, & Pundir, 2013), 4×10^{-5} M for gold electrode (W. Sun, Li, Wang, Zhao, & Jiao, 2009), 0.05×10^{-5} M for fluorescent sensor (Hu, Xu, Li, Zhang, Wang, Fu, et al., 2014), 4×10^{-8} M for gold nanoparticles modified electrode (Garabagiu & Mihailescu, 2011), 2×10^{-8} M for isocratic liquid chromatography (Casella, Pierri, & Contursi, 2006), 0.6 μg/kg for gas chromatography with electron capture detector (Zhu, Li, Duan, Chen, Zhang, & Li, 2008) and 0.85×10^{-6} M for chemiluminescence ELISA (Quan, Chen, Zhan, & Zhang, 2011). In comparison with most of these methods, the designed electrochemical sensor had acceptable LOD (Table 1).

3.4. Measurement of acrylamide in potato fries

The responses of the modified electrode with dsDNA/Hb towards acrylamide were measured in the presence of 100-fold diluted water extracts of potato fries (Figure. 5C). The sample solution also contained 5 mM potassium ferrocyanide to 5 mL PBS. The presence of the potato fries' matrix influenced slightly the electrode sensitivity towards acrylamide. The sensor response towards acrylamide such as linear range or LOD in aqueous and potato fries extract was not significantly different (Figure. 5D). However, the response measured in the synthetic solution was stronger. The weak influence of the natural matrix on the sensitivity of the developed sensor for acrylamide detection, claimed that this biosensor was resistant to interference coming from the food products' matrix. Moreover, the

sample preparation compared to other analytical methods for acrylamide measurement was so simple.

Moreover, the practicality of the sensor was also tested in the determination of acrylamide in potato fries using recovery assay (Table S1). As indicated in Table S1, known concentrations of acrylamide solution were spiked into potato fries water extracted, which diluted in PBS solution, followed by measuring the concentration of acrylamide using the presented sensing method. The values found for these samples were highly identical with the expected values, and the recoveries were from 91% to 120% with relative standard deviations between 0.08% and 6.66% for three determinations, addressing the good applicability of this modified SPGE for acrylamide detection in real food samples (Table S1).

3.5. Selectivity determination of the sensor for acrylamide detection

One of the most important parameters for a valuable biosensor is selectivity. The relative electrochemical signal of modified SPGE toward acrylamide was significantly higher than other compounds, including glucose, asparagine, starch, glycine, amoxicillin and acrolein (Figure. 5E). These results indicated high selectivity of the designed biosensor for acrylamide detection. The selectivity of the proposed electrochemical sensor is because of the formation of Hb-acrylamide adduct by the Michael-type nucleophilic addition reaction of a valine -NH_2 group to the acrylamide double bond.

3.6. Sensor reproductivity

The SPGEs modified with dsDNA/Hb were very stable. After preparation, they could be stored on air at 4°C during 2 months. The main disadvantage of this biosensor is that a strong irreversible covalent bond connect acrylamide to Hb which N-alkyl Edman degradation procedure could break it. Therefore, our

modified SPGE can only be used once and has to be modified again prior to a new measurement. However, the procedures for the deposition of the layers onto the electrodes are easy and inexpensive compared with regular detection methods for acrylamide (chromatographic techniques). For the biosensor reproductivity, the dsDNA/Hb complexes on the sensing interface could be effectively separated in 0.1% SDS and 50 mM DTT solution for 30 min without any destruction of the surface of the electrode. Therefore, it is possible to use this modification electrochemical setup to monitor low concentrations of acrylamide. Over 86.75% of the initial response can be observed after repeated use for four times (Figure. 5F).

4. Conclusion

In summary, a novel electrochemical biosensor for acrylamide was proposed based on dsDNA/Hb-modified SPGE. The interaction between ssDNA2-NH_2 and Hb with the ratio 1:1 was subsequently characterized by agarose gel, and the ssDNA1-SH was efficiently immobilized on the surface of SPGE. This dsDNA/Hb biosensor exhibited a simple response for acrylamide with long-term stability, high sensitivity and excellent reproducibility. Moreover, the designed biosensor was applied for acrylamide detection in potato fries with satisfactory results. The SPGE modified with dsDNA/Hb displays low detection limit (1.58×10^{-7} M) and wide dynamic range. Therefore, the proposed biosensor might be recommended for the direct determination of the acrylamide in the food samples.

Conflict of interest

There is no conflict of interest about this article.

Acknowledgment

Financial support of this study was provided by Mashhad University of Medical Sciences and Sari Agricultural Sciences & Natural Resources University (SANRU).

References

Bagheri, H., Afkhami, A., Khoshsafar, H., Hajian, A., & Shahriyari, A. (2017). Protein capped Cu nanoclusters-SWCNT nanocomposite as a novel candidate of high performance platform for organophosphates enzymeless biosensor. *Biosensors and Bioelectronics, 89*(Part 2), 829-836.

Bagheri, H., Talemi, R., & Afkhami, A. (2015). Gold nanoparticles deposited on fluorine-doped tin oxide surface as an effective platform for fabricating a highly sensitive and specific digoxin aptasensor. *RSC Advances, 5*(72), 58491-58498.

Batra, B., Lata, S., & Pundir, C. (2013). Construction of an improved amperometric acrylamide biosensor based on hemoglobin immobilized onto carboxylated multi-walled carbon nanotubes/iron oxide nanoparticles/chitosan composite film. *Bioprocess and biosystems engineering, 36*(11), 1591-1599.

Batra, B., Lata, S., Sharma, M., & Pundir, C. (2013). An acrylamide biosensor based on immobilization of hemoglobin onto multiwalled carbon nanotube/copper nanoparticles/polyaniline hybrid film. *Analytical biochemistry, 433*(2), 210-217.

Bortolomeazzi, R., Munari, M., Anese, M., & Verardo, G. (2012). Rapid mixed mode solid phase extraction method for the determination of acrylamide in roasted coffee by HPLC–MS/MS. *Food chemistry, 135*(4), 2687-2693.

Casella, I. G., Pierri, M., & Contursi, M. (2006). Determination of acrylamide and acrylic acid by isocratic liquid chromatography with pulsed electrochemical detection. *Journal of Chromatography A, 1107*(1–2), 198-203.

Cheng, W.-C., Kao, Y.-M., Shih, D. Y.-C., Chou, S.-S., & Yeh, A.-I. (2009). Validation of an improved LC/MS/MS method for acrylamide analysis in foods. *Journal of Food and Drug Analysis, 17*(3).

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide. A review. *Journal of agricultural and food chemistry, 51*(16), 4504-4526.

Garabagiu, S., & Mihailescu, G. (2011). Simple hemoglobin–gold nanoparticles modified electrode for the amperometric detection of acrylamide. *Journal of electroanalytical chemistry, 659*(2), 196-200.

Hu, Q., Xu, X., Li, Z., Zhang, Y., Wang, J., Fu, Y., & Li, Y. (2014). Detection of acrylamide in potato chips using a fluorescent sensing method based on acrylamide polymerization-induced distance increase between quantum dots. *Biosensors and Bioelectronics, 54*, 64-71.

Hu, Q., Xu, X., Li, Z., Zhang, Y., Wang, J., & Li, Y. (2013). Rapid detection of acrylamide in food using a fluorescent sensing method based on functional CdSe/ZnS quantum dots. In *SENSORS, 2013 IEEE*, (pp. 1-4): IEEE.

Huang, S., Lu, S., Huang, C., Sheng, J., Zhang, L., Su, W., & Xiao, Q. (2016). An electrochemical biosensor based on single-stranded DNA modified gold electrode for acrylamide determination. *Sensors and Actuators B: Chemical, 224*, 22-30.

Kleefisch, G., Kreutz, C., Bargon, J., Silva, G., & Schalley, C. A. (2004). Quartz microbalance sensor for the detection of acrylamide. *Sensors, 4*(9), 136-146.

Krajewska, A., Radecki, J., & Radecka, H. (2008). A voltammetric biosensor based on glassy carbon electrodes modified with single-walled carbon nanotubes/hemoglobin for detection of acrylamide in water extracts from potato crisps. *Sensors, 8*(9), 5832-5844.

Ma, X., Liu, X., Xiao, H., & Li, G. (2005). Direct electrochemistry and electrocatalysis of hemoglobin in poly-3-hydroxybutyrate membrane. *Biosensors and Bioelectronics, 20*(9), 1836-1842.

Oracz, J., Nebesny, E., & Żyżelewicz, D. (2011). New trends in quantification of acrylamide in food products. *Talanta, 86*, 23-34.

Preston, A., Fodey, T., & Elliott, C. (2008). Development of a high-throughput enzyme-linked immunosorbent assay for the routine detection of the carcinogen acrylamide in food, via rapid derivatisation pre-analysis. *Analytica chimica acta, 608*(2), 178-185.

Quan, Y., Chen, M., Zhan, Y., & Zhang, G. (2011). Development of an enhanced chemiluminescence ELISA for the rapid detection of acrylamide in food products. *Journal of agricultural and food chemistry, 59*(13), 6895-6899.

Silva, N., Gil, D., Karmali, A., & Matos, M. (2009). Biosensor for acrylamide based on an ion-selective electrode using whole cells of Pseudomonas aeruginosa containing amidase activity. *Biocatalysis and Biotransformation, 27*(2), 143-151.

Stobiecka, A., Radecka, H., & Radecki, J. (2007). Novel voltammetric biosensor for determining acrylamide in food samples. *Biosensors and bioelectronics, 22*(9), 2165-2170.

Sun, W., Li, X., Wang, Y., Zhao, R., & Jiao, K. (2009). Electrochemistry and electrocatalysis of hemoglobin on multi-walled carbon nanotubes modified carbon ionic liquid electrode with hydrophilic EMIMBF 4 as modifier. *Electrochimica Acta, 54*(17), 4141-4148.

Sun, X., Ji, J., Jiang, D., Li, X., Zhang, Y., Li, Z., & Wu, Y. (2013). Development of a novel electrochemical sensor using pheochromocytoma cells and its assessment of acrylamide cytotoxicity. *Biosensors and Bioelectronics, 44*, 122-126.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of agricultural and food chemistry, 50*(17), 4998-5006.

Wang, L., & Hu, N. (2001). Direct electrochemistry of hemoglobin in layer-by-layer films with poly (vinyl sulfonate) grown on pyrolytic graphite electrodes. *Bioelectrochemistry, 53*(2), 205-212.

Wang, S.-F., Chen, T., Zhang, Z.-L., Shen, X.-C., Lu, Z.-X., Pang, D.-W., & Wong, K.-Y. (2005). Direct electrochemistry and electrocatalysis of heme proteins entrapped in agarose hydrogel films in room-temperature ionic liquids. *Langmuir, 21*(20), 9260-9266.

Wenzl, T., Lachenmeier, D. W., & Gökmen, V. (2007). Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. *Analytical and bioanalytical chemistry, 389*(1), 119-137.

Xie, L., Xu, Y., & Cao, X. (2013). Hydrogen peroxide biosensor based on hemoglobin immobilized at graphene, flower-like zinc oxide, and gold nanoparticles nanocomposite modified glassy carbon electrode. *Colloids and Surfaces B: Biointerfaces, 107*, 245-250.

Yamazaki, K., Isagawa, S., Kibune, N., & Urushiyama, T. (2012). A method for the determination of acrylamide in a broad variety of processed foods by GC–MS using xanthydrol derivatization. *Food Additives & Contaminants: Part A, 29*(5), 705-715.

Zhang, L., Jiang, X., Wang, E., & Dong, S. (2005). Attachment of gold nanoparticles to glassy carbon electrode and its application for the direct electrochemistry and electrocatalytic behavior of hemoglobin. *Biosensors and Bioelectronics, 21*(2), 337-345.

Zhu, Y., Li, G., Duan, Y., Chen, S., Zhang, C., & Li, Y. (2008). Application of the standard addition method for the determination of acrylamide in heat-processed starchy foods by gas chromatography with electron capture detector. *Food Chemistry, 109*(4), 899-908.

Figure captions

Figure 1. Agarose gel assay analysis of Hb and ssDNA-NH_2 bioconjugate. From left to light: only ssDNA-NH_2, Hb: ssDNA-NH_2; 1:1, Hb: ssDNA-NH_2; 5:1, Hb: ssDNA-NH_2; 10:1 and Hb: ssDNA-NH_2; 20:1. The results showed that sequences captured on the Hb can not intercalate with gel red.

Figure 2. (A) Cyclic voltammetry responses of bare SPGE (black curve), ssDNA1- modified SPGE (purple curve), ssDNA1- modified SPGE + MCH (green curve), ssDNA1- modified SPGE + MCH + ssDNA2-Hb complex (blue curve), Modified electrode in 10^{-5} M of acrylamide solution (red curve). $[Fe(CN)_6]^{3-/4-}$ was used as redox probe. (B) Schematic description of the biosensor preparation (C) Hb–acrylamide adduct formation.

Figure 3. (A) Influence of pH on cyclic voltammograms of acrylamide at surface of the modified SPGE (B) Current-pH curve C) Potential-pH curve for electrooxidation of 10^{-6} M of acrylamide at modified SPGE with a scan rate 100 mV/s.

Figure 4. (A) The CV curves for the modified SPGE measured in solution containing 5 mM potassium ferrocyanide to 5 mL Phosphate Buffer Saline (PBS) solution vs. scan rates: 10, 20, 50, 100 and 200 mV/s. (B) Linear relationship between anodic peak current vs. scan rate.

Figure 5. (A)The SWV curves for modified sensor measured in the presence of various concentrations of acrylamide. The electrolyte composition: 5 mM potassium ferrocyanide to 5 mL Phosphate Buffer Saline (PBS) solution. The concentration of analyte was, as follows: (a) 0, (b) 2.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 5.0×10^{-5} M, (e) 1.0×10^{-4} M, (f) 5.0×10^{-3} M, (g) 1.0×10^{-3} M, (h) 1.0×10^{-2} M, (i) 5.0×10^{-2} M. (B) Linear dependence of acrylamide concentration on the peak current. (C) The SWV curves for modified sensor measured in the presence of various concentrations of acrylamide in water extract of potato fries. The electrolyte composition: 5 mM potassium ferrocyanide to 5 mL Phosphate Buffer Saline (PBS) solution. The concentration of analyte was, as follows: (a) 0, (b) 2.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 5.0×10^{-5} M, (e) 1.0×10^{-4} M, (f) 5.0×10^{-3} M, (g) 1.0×10^{-3} M, (h) 1.0×10^{-2} M, (i) 5.0×10^{-2} M. (D) Linear dependence of acrylamide concentration on the peak current in water extract of potato fries. (E) Relative SWV electrochemical signals of modified SPGE in the presence of various interferences (Current intensity ((I_0/I)-2.5) in the presence (I) and absence (I_0) of each substance in solution).

(F) Reproductivity of electrochemical signals of modified SPGE using SWV: blue curve (new GSPE), red (1 time), green (2 time), purple (3 time) and black curves (4 time) (GSPE after reproduction).

Table 1. Previously reported sensors for AA detection

Type of sensor	Detection range	LOD	Applicability	Reference
CdSe/ZnS Quantum dots fluorescence sensor	5×10^{-7} -1× 10^{-2} M	5×10^{-7} M	Not tested in real sample	(Hu, Xu, Li, Zhang, Wang, & Li, 2013)
Carbon paste electrode modified with single-walled carbon nanotubes/Hb sensor	1.3×10^{-11}-5.6×10^{-3} M	1.0×10^{-9} M	potato crisps	(Krajewska, Radecki, & Radecka, 2008)
Single-stranded DNA modified gold electrode	0.4×10^{-6}-2×10^{-4} M	8.1×10^{-9}M	Tap water and potato crisps	(Huang, Lu, Huang, Sheng, Zhang, Su, et al., 2016)
Carbon paste electrode	1.3×10^{-11}- 5.6×10^{-3} M	1.2×10^{-10} M	Potato crisps	(Stobiecka, Radecka, & Radecki, 2007)
Carboxylated multi-walled carbon nanotubes including Hb/iron oxide nanoparticles/chitosan composite film	3×10^{-9}-90×10^{-9} M	0.02×10^{-9} M	Potato crisps	(Batra, Lata, & Pundir, 2013)
Ammonium ion selective electrode	0.1-4.0×10^{-3} M	4.48×10^{-5} M	Industrial effluents	(Silva, Gil, Karmali, & Matos, 2009)
Multiwalled carbon nanotube/copper nanoparticles/polyaniline hybrid film	5×10^{-9}- 7.5×10^{-2} M	0.2×10^{-9} M	Potato crisps	(Batra, Lata, Sharma, & Pundir, 2013)
Fluorescent sensor based on polymerization-induced distance between quantum dots	0.05×10^{-5} - 0.05 M	0.05×10^{-5} M	Potato crisps	(Hu, et al., 2014)
Gold nanoparticles modified electrode	10^{-8}-10^{-5} M	4×10^{-8} M	Not tested in real sample	(Garabagiu & Mihailescu, 2011)
Electrochemical sensor using pheochromocytoma cells and acrylamide cytotoxicity	10^{-4}–5×10^{-3} M	4×10^{-5} M	Not tested in real sample	(X. Sun, Ji, Jiang, Li, Zhang, Li, et al., 2013)

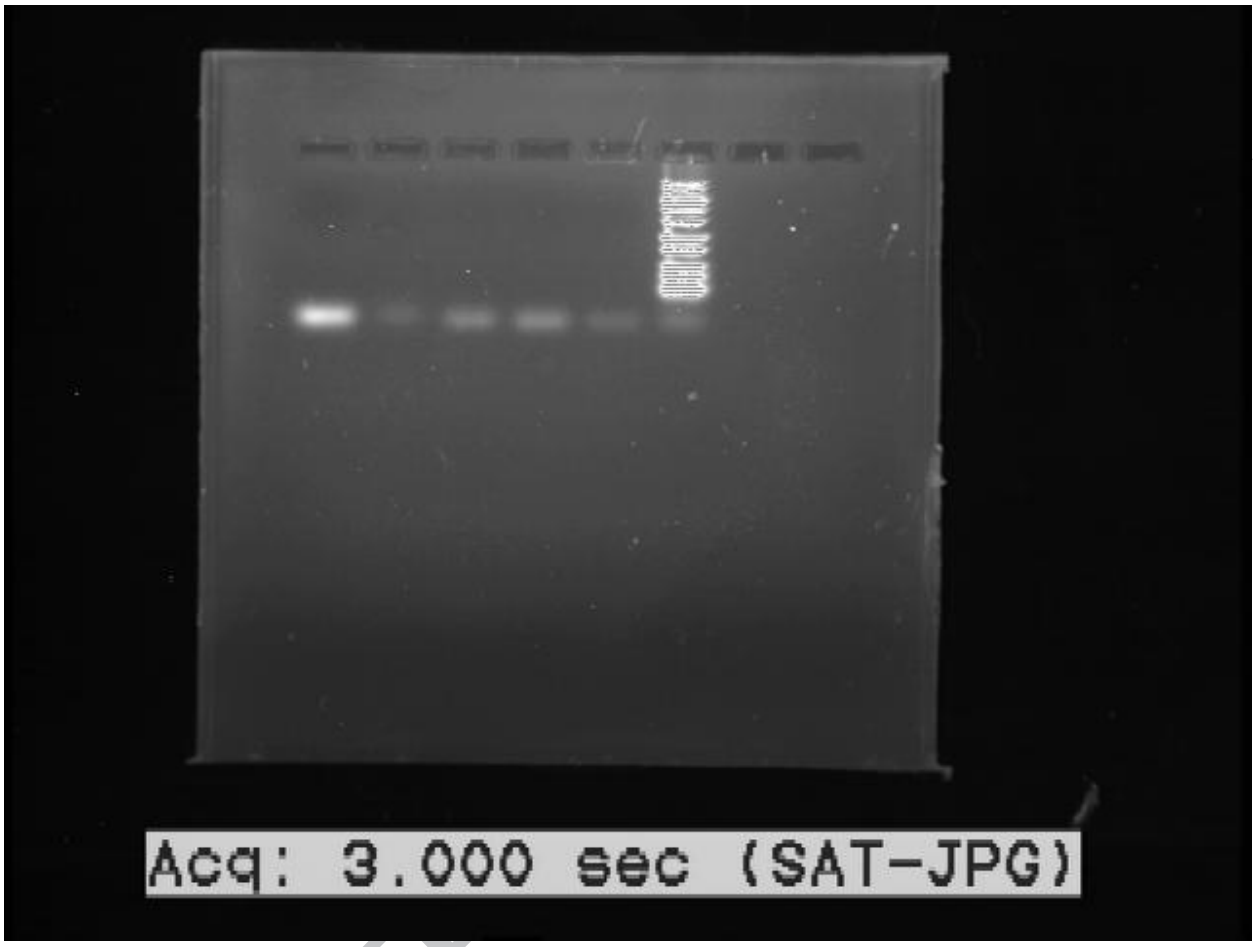
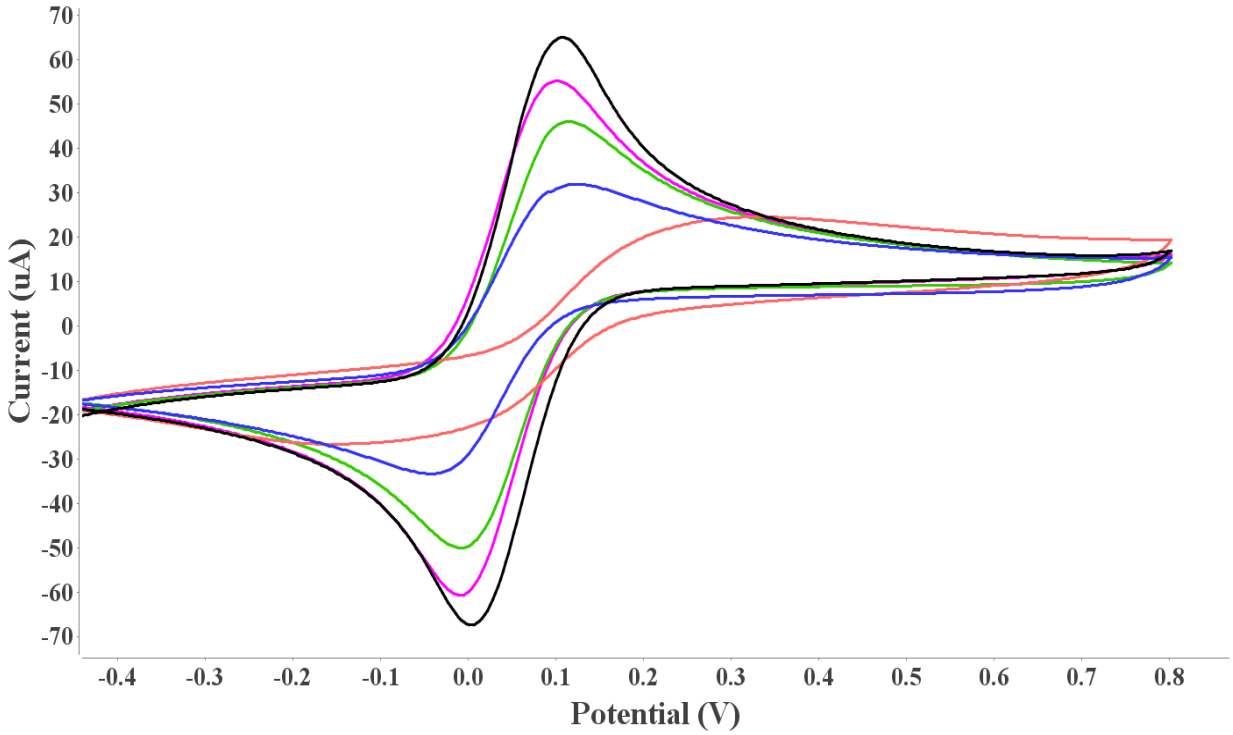
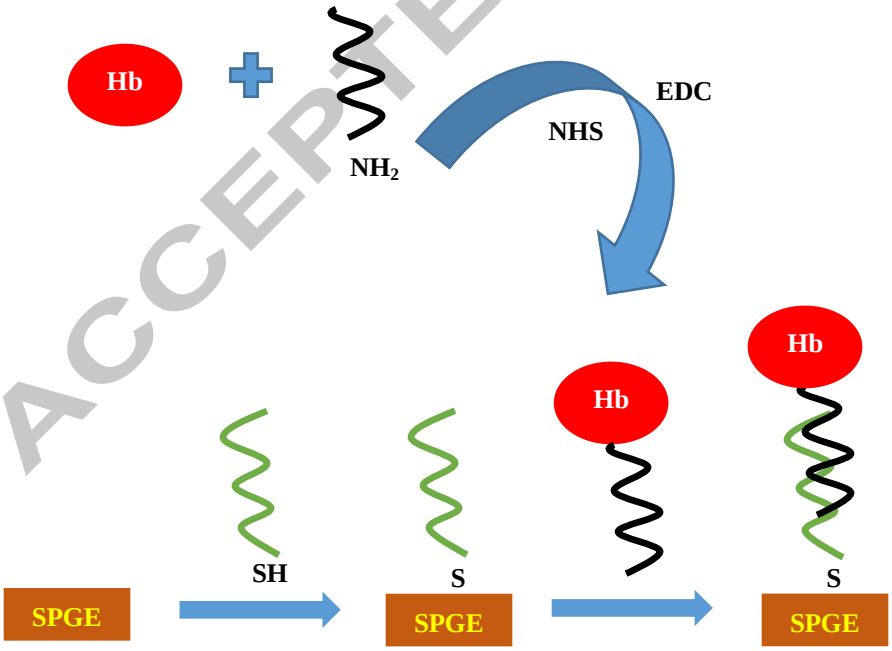


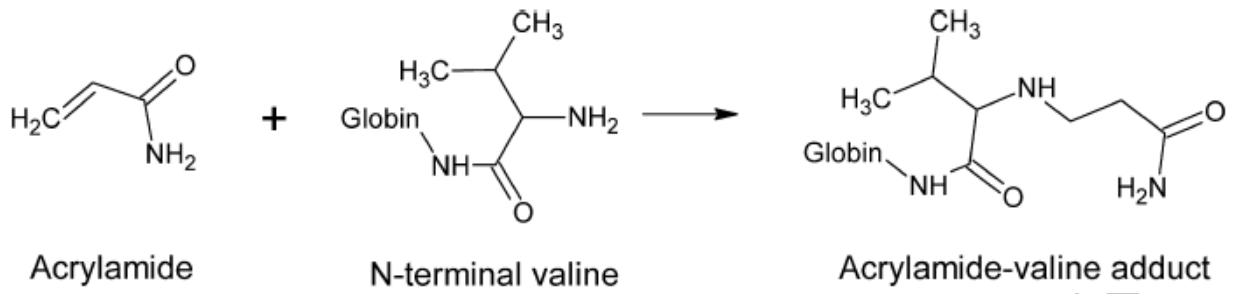
Figure 1. Agarose gel assay analysis of Hb and ssDNA-NH_2 bioconjugate. From left to light: only ssDNA-NH_2, Hb: ssDNA-NH_2; 1:1, Hb: ssDNA-NH_2; 5:1, Hb: ssDNA-NH_2; 10:1 and Hb: ssDNA-NH_2; 20:1. The results showed that sequences captured on the Hb can not intercalate with gel red.



(A)

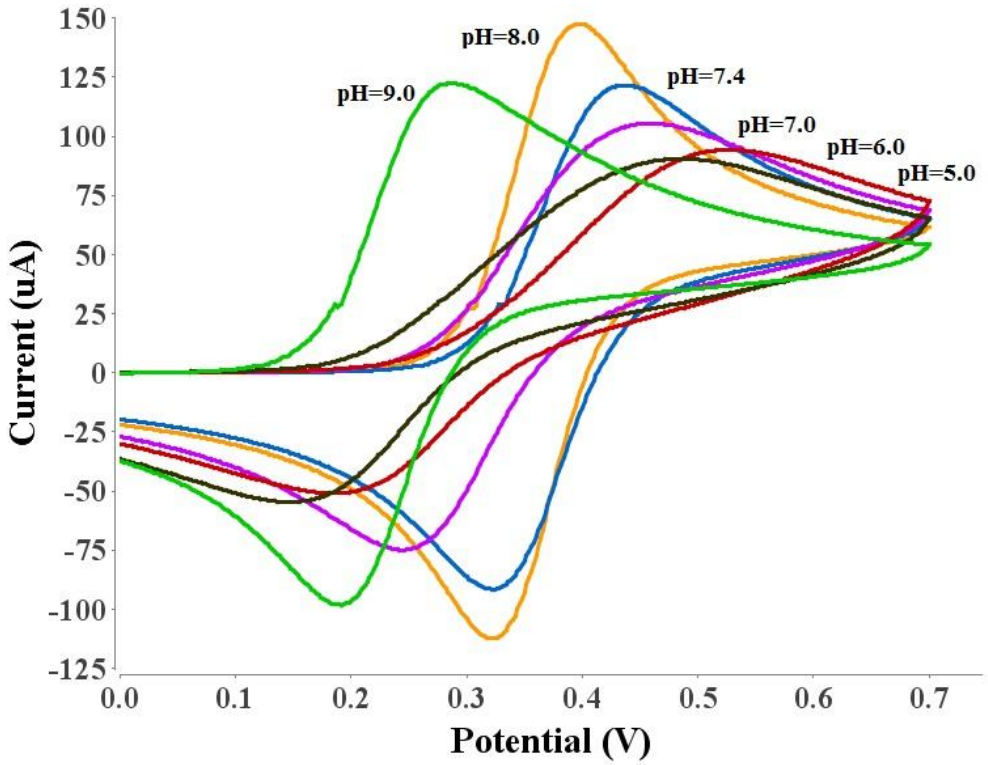


(B)

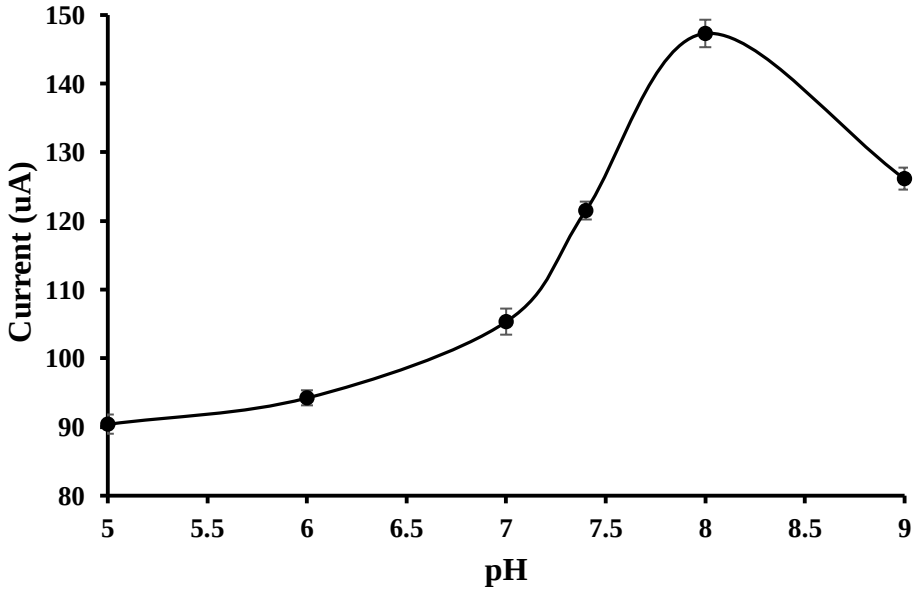


(C)

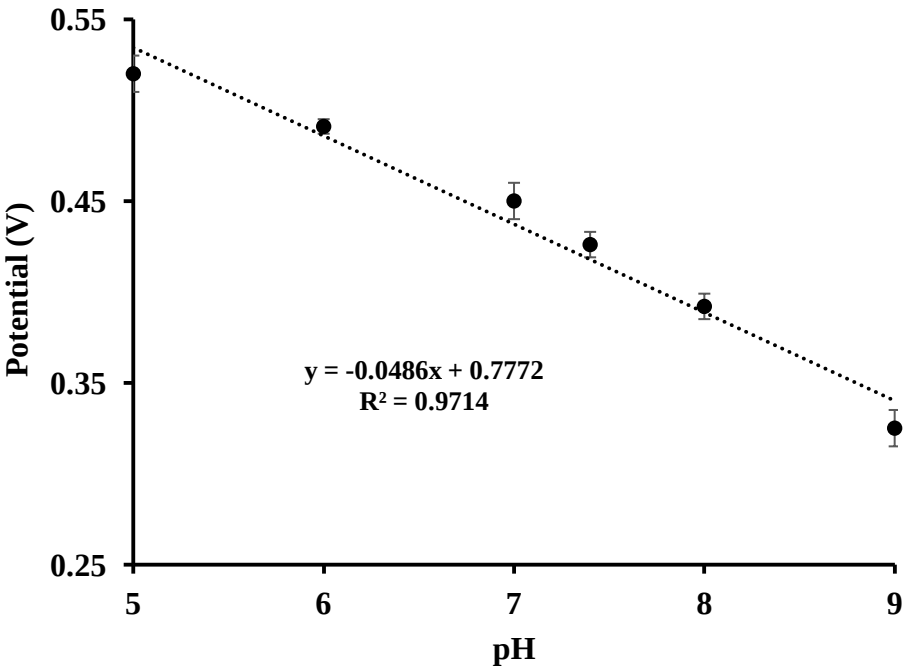
Figure 2. (A) Cyclic voltammetry responses of bare SPGE (black curve), ssDNA1- modified SPGE (purple curve), ssDNA1- modified SPGE + MCH (green curve), ssDNA1- modified SPGE + MCH + ssDNA2-Hb complex (blue curve), Modified electrode in 10^{-5} M of acrylamide solution (red curve). $[Fe(CN)_6]^{3-/4-}$ was used as redox probe. (B) Schematic description of the biosensor preparation (C) Hb–acrylamide adduct formation.



(A)

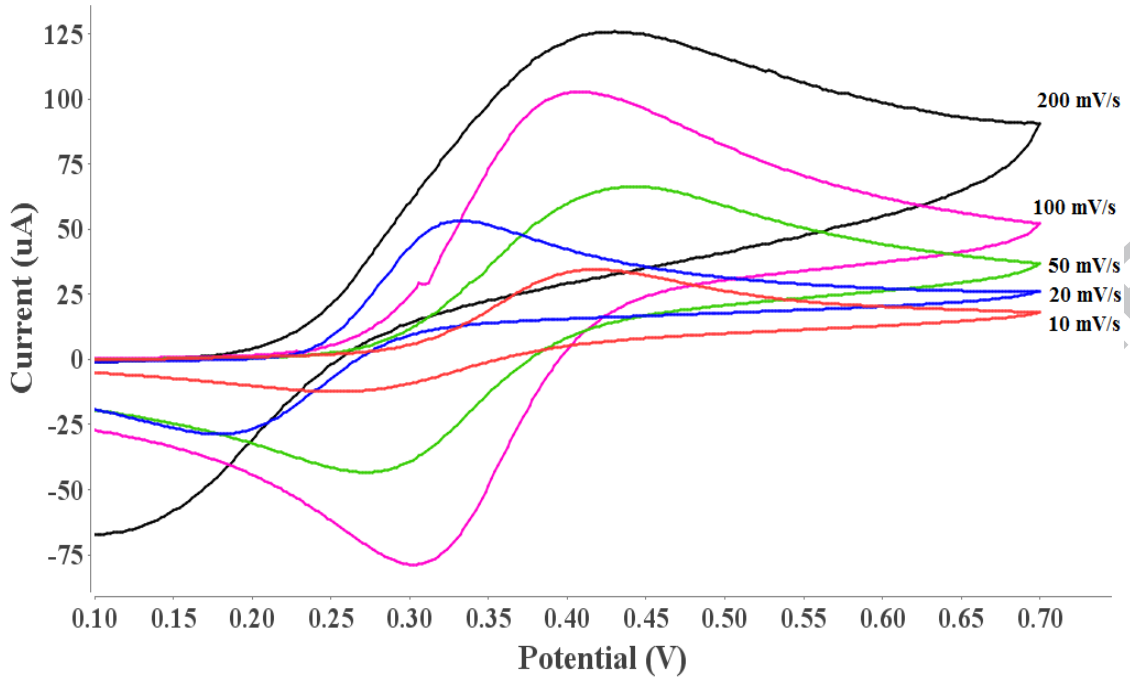


(B)

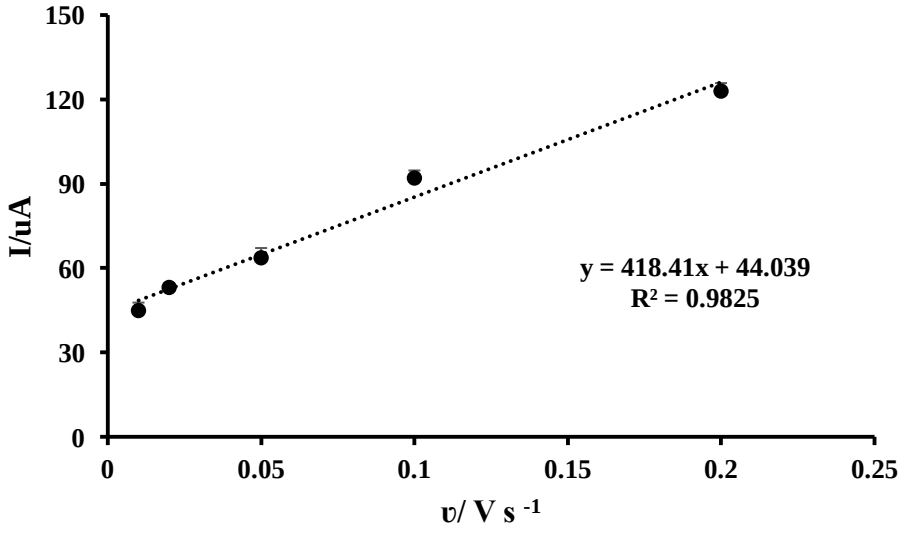


(C)

Figure 3. (A) Influence of pH on cyclic voltammograms of acrylamide at surface of the modified SPGE (B) Current-pH curve C) Potential-pH curve for electrooxidation of 10^{-6} M of acrylamide at modified SPGE with a scan rate 100 mV/s.

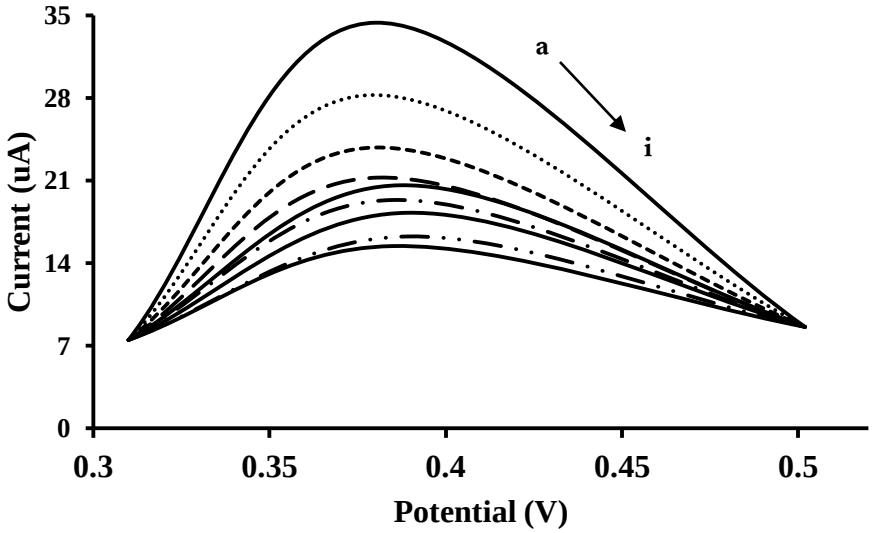


(A)

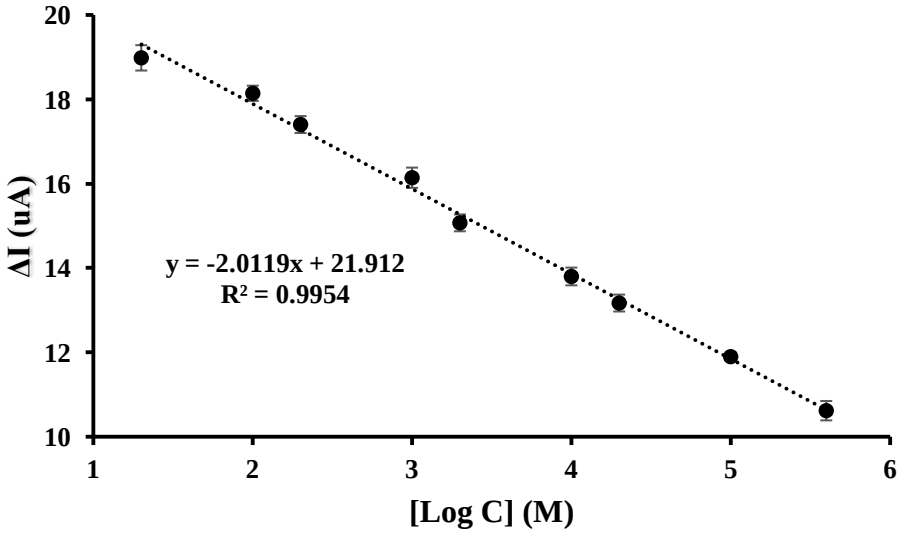


(B)

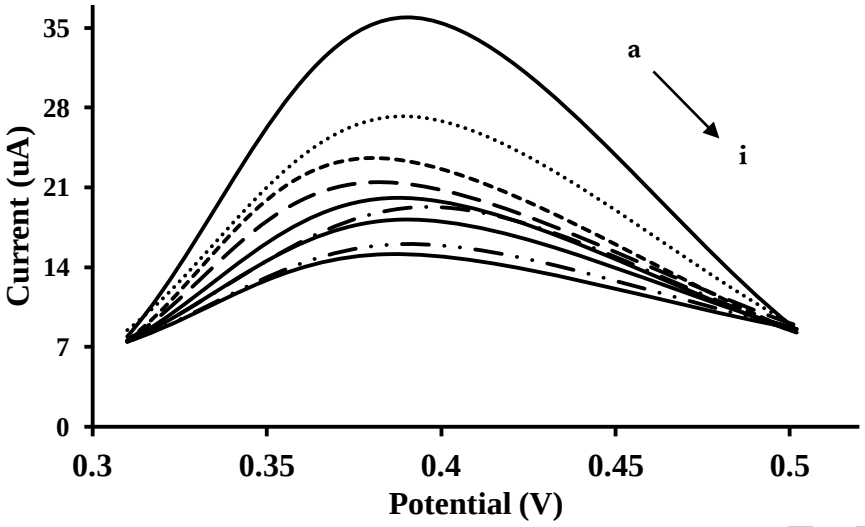
Figure 4. (A) The CV curves for the modified SPGE measured in solution containing 5 mM potassium ferrocyanide to 5 mL Phosphate Buffer Saline (PBS) solution vs scan rates: 10, 20, 50, 100 and 200 mV/s. (B) Linear relationship between anodic peak current vs. scan rate



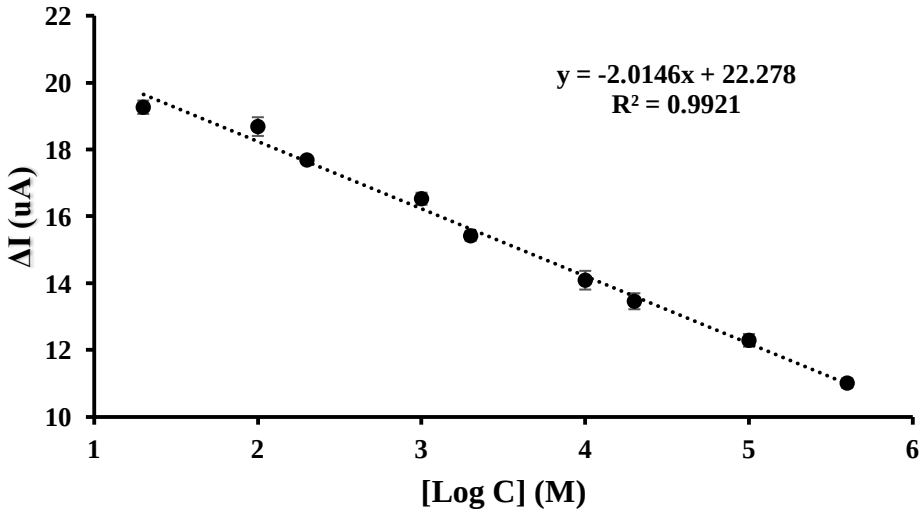
(A)



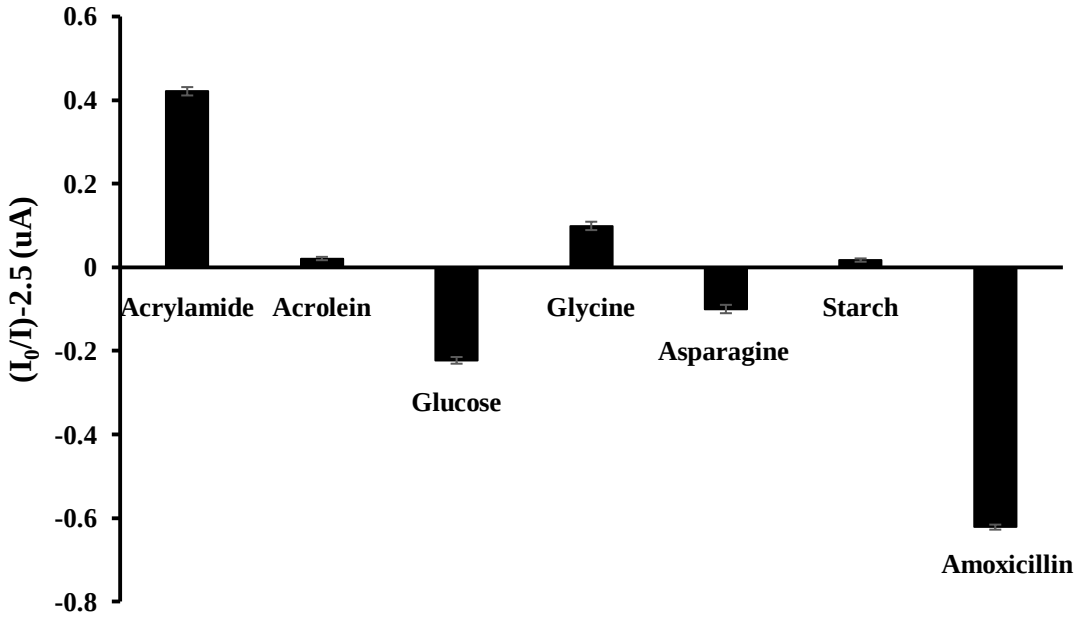
(B)



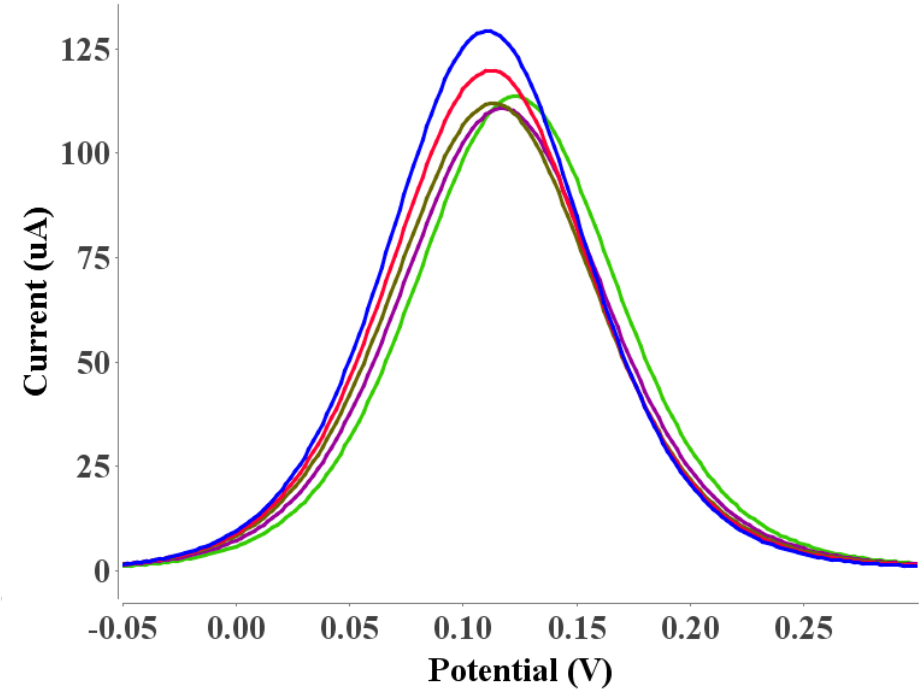
(C)



(D)



(E)



(F)

Figure 5. (A)The SWV curves for modified sensor measured in the presence of various concentrations of acrylamide. The electrolyte composition: 5 mM potassium ferrocyanide to 5

mL Phosphate Buffer Saline (PBS) solution. The concentration of analyte was, as follows: (a) 0, (b) 2.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 5.0×10^{-5} M, (e) 1.0×10^{-4} M, (f) 5.0×10^{-3} M, (g) 1.0×10^{-3} M, (h) 1.0×10^{-2} M, (i) 5.0×10^{-2} M. (B) Linear dependence of acrylamide concentration on the peak current. (C) The SWV curves for modified sensor measured in the presence of various concentrations of acrylamide in water extract of potato fries. The electrolyte composition: 5 mM potassium ferrocyanide to 5 mL Phosphate Buffer Saline (PBS) solution. The concentration of analyte was, as follows: (a) 0, (b) 2.0×10^{-6} M, (c) 1.0×10^{-5} M, (d) 5.0×10^{-5} M, (e) 1.0×10^{-4} M, (f) 5.0×10^{-3} M, (g) 1.0×10^{-3} M, (h) 1.0×10^{-2} M, (i) 5.0×10^{-2} M. (D) Linear dependence of acrylamide concentration on the peak current in water extract of potato fries. (E) Relative SWV electrochemical signals of modified SPGE in the presence of various interferences (Current intensity ((I_0/I)-2.5) in the presence (I) and absence (I_0) of each substance in solution). (F) Reproductivity of electrochemical signals of modified SPGE using SWV: blue curve (new GSPE), red (1 time), green (2 time), purple (3 time) and black curves (4 time) (GSPE after reproduction).

Highlights

*A novel hemoglobin-oligonucleotides-modified electrochemical biosensor was fabricated for acrylamide detection.

*The sensor allows to measure acrylamide in a wide range of concentrations 2.0×10^{-6} to 5.0×10^{-2} M with a detection limit of 1.58×10^{-7} M.

*The electrochemical sensor was successfully used for acrylamide detection in potato fries.