3-Mercapto propionic acid self-assembled on gold nano-particles applied for modification of screen-printed electrode as a new digoxin electrochemical aptasensor using graphene oxide-based signal-on strategy

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Abstract

In this study a new electrochemical aptasensor has been represented for digoxin determination using signal-on strategy to improve limit of detection. To construct the sensor, gold screen printed electrode (GSPE) was modified with electrodeposited gold nanoparticles (GNPs) followed by self-assembling a layer of 3-mercaptopropionic acid (MPA) on the GNPs. Amino-labeled digoxin specific aptamer was covalently attached to the surface via carbodiimide bond formation. Graphene oxide (GO) was accumulated on the electrode surface through interaction with aromatic nucleobases in aptamer structure and the monitored reduction signal of potassium ferricyanide as a redox probe was decreased. In the presence of digoxin, the immobilized GO left the surface and the current was increased and recorded as analytical signal. The proposed sensor was delivered a linear dynamic response over the range of 0.1 pM to 1.0 μ M with a detection limit of 0.050 pM. The ability of the aptasensor in real sample analysis was successfully evaluated by determination of digoxin in human blood plasma samples with no serious matrix interferences.

Keywords: digoxin; electrochemical aptasensor; graphene oxide; gold nanoparticles; 3-mercaptopropionic acid; self-assemble

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1. Introduction

The demand for a simple, sensitive, and rapid detection in bioanalysis is of great importance. A wide variety of biosensors have been designed with the aim of introducing reliable methods as alternatives for traditional analysis in recent years [1, 2]. In spite of unique properties of popular recognition elements such as enzymes and antibodies used in construction of biosensors, their instability is one of the limiting factors which restrict the use of these biomaterials [3]. As a good alternative, synthetic oligonucleotide-based ligands specified as aptamers, have been highly considered due to their ability in specific binding with different targets and excellent stability in comparison to enzymes and antibodies [4,5]. Aptamers which have been firstly introduced in 1990 are single-stranded RNA or DNA selected from a large pool of diverse randomized sequences via Systematic Evolution of Ligands by Exponential Enrichment (SELEX) and can be designed for various targets from small molecules to large proteins and even a whole cell [6,7]. Although aptamers have shown comparable binding affinity to that for antibodies, they are thermally stable, can be simply modified with different functional groups, fluorescent dyes, and electrochemical probes without any significant change in their affinity. Also the possibility of reproducible synthesis of aptamers via SELEX is another promising aspect of alternating the former bio-recognition elements with aptamers in construction of biosensors [8,9], consequently aptamer-based biosensors have been designed for a large diversity of analytes in recent years [10–17]. Different kinds of aptamer-based biosensors have been reported based on the utilized detection techniques [13,18–22]. Among them electrochemical aptasensors have been much more considered because of their simple instrumentation, low cost, ease of miniaturization, very low limits of detection, and the ability to perform rapid analysis [23–25].

Nanomaterial-based aptasensors have been widely developed because of the extraordinary physical and chemical properties of nanostructures. Amplification of the primary signal by the aid of using nanomaterials in aptasensors have been reported in the literature [8, 14, 26]. On the other hand, the sensitivity of electrochemical aptasensors has been significantly improved using nanomaterial-based labels. This improvement is due to the large surface to area of nanomaterials, their catalytic activity, and biocompatibility [27]. Graphene-based compounds have attracted much attention as a two-dimensional nanostructure because of the unique electronic properties originated from the planar framework [28]. The well-known π - π interaction between graphene and aromatic nucleobases in the aptamer structure is the base of innovative biosensing strategies [29,30].

In this work a sensitive electrochemical aptasensor have been designed for detection of very low levels of digoxin as a generally prescribed cardiac glycoside. According to the narrow therapeutic index of digoxin (0.8 ng/mL to 2 ng/mL) controlling its level in tehran and urine of patients is necessary to avoid toxicity. It is adsorbed from gastrointestinal tract with a half-life between 36-48 h. In all patients who survived the digoxin level in plasma ranged from 0.5 to 0.9 ng/ml after 30 days treatment and the mortality was reduced by 23%. By the way concentrations more than 2 ng/mL is toxic and controlling digoxin level is vital during the treatment [31]. Therefore, introducing a simple and sensitive method for monitoring digoxin in biological samples is of great importance. Herein, to fabricate an electrochemical digoxin aptasensor the surface of the GSPE was modified by electrodeposited GNPs followed by immobilizing of the amino-labeled digoxin specific aptamer. Graphene oxide was interacted strongly with aptamer via π - π interaction consequently the electrode surface was insulated. This caused a significant decrease in the reduction current of the potassium ferricyanide as a redox probe. In the presence

of digoxin, because of its specific interaction with the aptamer, the adsorbed GO left the electrode surface and the electrochemical signal of the redox probe was increased. The percentage of this increase, which was proportional to digoxin concentration, recorded as an analytical signal. With the aid of electrode surface enhancement using GNPs, also the applied signal-on strategy the proposed aptasensor was utilized to detect digoxin concentrations as low as 0.1 pM. Also it was successfully used to determine the plasma digoxin level with no significant interference from the complicated matrix of the real sample.

2. Experimental

2.1. Reagents

The aptamer strand used in this study was received as a lyophilized powder which was purified by HPLC, from Biobasic Company (Canada) according to the following sequence [32]:

5'-(CH₂)₂-NH-AGCGAGGGCGGTGTCCAACAGCGGTTTTTTCACGAGGAGGT
TGGCGGTGG-3'

The stock aptamer solution was prepared by adding 500 µL salinized phosphate buffer solution (PBS) pH 7.4 to the purchased vial and vortexed well to obtain a 100 µM solution, then divided into 40 µL portions and stored at -20 °C.

Gold (III) chloride trihydrate (HAuCl₄·3H₂O) (>99.9%, trace metal bases), Silver nitrate (>99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (>98%), N-hydroxy-succinimide (NHS) (98%), and 3-mercaptopropionic acid (MPA) (>98%), were purchased from either Merck (Germany) or Sigma- Aldrich (USA) Companies. Digoxin was provided from Sigma-Aldrich Company. All other chemicals were in analytical grade and used without further purification.

2.2. Instrumentation

All electrochemical studies were performed on EmStat 2 from PalmSens Company (Netherland). Modification steps were monitored by cyclic voltammetry using potassium ferricyanide 5 μ M as a redox probe in 0.1 M KCl solution. Square wave voltammetry (SWV) was used for quantitative determination of digoxin. The SWV experimental parameters were as follows: initial potential, -0.5 V; final potential, 0.5 V; potential step, 0.005 V; amplitude, 0.001 V and frequency, 1 Hz. GSPEs of 220BT series with gold working and counter electrodes, and silver pseudo-reference electrode were provided from Dropsens Company (Spain). UV-Vis and FT-IR analysis were performed on Lambda 25 Perkin Elmer and Philips P900 (both US), respectively. UV-Vis spectrums were performed over the range of 200 to 800 nm, using quartz cuvette. The SEM images were carried out on a Mira scanning electron microscope (Czech).

2.3. Preparation of GO

GO was synthesized according to a well-known Hummer method by oxidizing graphite powder with the mixture of potassium permanganate, sodium nitrite, and concentrated sulfuric acid [33]. The prepared graphite oxide was washed several times with the aid of centrifuging (5000 rpm for 20 min) and re-dispersed in water, and sonicated in ultrasonic bath to achieve a thin-layer GO. Centrifuging separated the synthesized GO and finally dried overnight at 40 °C. SEM, FT-IR, and UV-Vis spectroscopy characterized the product.

2.4. The aptasensor development

GSPE used in this study was firstly cleaned by cyclic voltammetry applying five scans over a potential range of -0.35-1.5 V in H₂SO₄ 0.5 M. All modification steps have been illustrated in

Scheme 1. GNPs were electrodeposited on the cleaned electrode surface through cyclic scanning of the potential from -0.2 to -1.3 V for 20 cycles in 0.1% H₂AuCl₄ and 0.1 M KNO₃ solution [34]. Then, a layer of MPA was self-assembled on the GNPs-modified surface by dropping 8 μ L of 50 mM MPA aqueous solution. To avoid evaporation, the electrode put in a closed container and kept in refrigerator for 12 h. Putting 8 μ L of 80 mM EDC and 50 mM NHS in PBS pH 5 for 2 h at room temperature activated the carboxylic acid groups of the immobilized MPA. After rinsing the electrode an 8 μ L aliquot of the mixture of 1.2 μ M aptamer and 0.33 M magnesium chloride was dropped on the electrode surface for 1h. The 5'-amin terminated digoxin specific aptamer was covalently immobilized on the surface via carbodiimide bond formation. The 5'-amin terminated digoxin specific aptamer was covalently immobilized on the surface via carbodiimide bond formation. The as-prepared GO was dispersed in PBS pH 5 then 8 μ L of this solution was dropped on the aptamer modified surface and kept for 2 h. Graphene oxide was attached to the surface due to its strong interaction with aromatic rings of the nucleobases in the aptamer strand. The presence of the GO on the electrode caused the insulation of the surface consequently the electron transfer of potassium ferricyanide as the redox probe in the solution was decreased. In the presence of digoxin the immobilized GO left the surface because of the specific interaction of digoxin with the aptamer, therefore the reduction current of the probe was increased. This increase in the current monitored by square wave voltammetry was used as an analytical signal.

3. Result and discussion

DNA aptamer specific to digoxin was designed via SELEX in 2012 [32]. During this study three aptamers were characterized for digoxin (D₁, D₂, and D₃). According to the binding affinity studies dissociation constants, 8.2×10^{-9} , 44×10^{-9} , and 17.8×10^{-9} were reported for D₁, D₂, and D₃,

respectively. These constants are comparable to that for monoclonal antibodies raised against digoxin. Due to this research, aptamer strand D₁ could selectively bind to digoxin also successfully distinguished between digoxin and ouabain in binding studies. This is the significant advantage of the designed aptamer over anti-digoxin antibodies. Moreover the effect of D₁ on the isolated guinea-pig atrium was investigated and the aptamer successfully inhibited the digoxin effect. All these observations confirmed the ability of the DNA aptamer, D₁, to interact successfully with digoxin.

3.1. Characterization of the synthesized GO

The prepared GO was characterized by FT-IR and UV-Vis spectroscopy also scanning image microscopy (SEM) as illustrated in Fig. 1. The FT-IR spectrum of the GO represented different functional groups such as hydroxyl, carbonyl, carboxyl, and epoxy groups in the structure of GO. The resulted broad band in 3399 cm^{-1} , originated from the stretching vibration of O-H bonds while the appeared band in 1718 cm^{-1} is attributed to the stretching of C=O bonds in carbonyl and carboxyl groups. The peaks in 1617 cm^{-1} and 1049 cm^{-1} originated from the vibration of C=C and C-O, respectively. The UV-Vis spectrum of the aqueous dispersion of GO demonstrated a peak at 230 nm as the absorption characteristic of GO which is due to the π to π^* transition in C=C band [35]. Scanning image microscopy was used to morphology characterization of the synthesized GO. The thin layer folded sheet that is structural characteristic of GO was clearly observed in the image.

3.2. Electrochemical characterization of the modified electrode

Cyclic voltammetry, which is known as a simple tool for monitoring different electrode modification steps was performed after each successive step using potassium ferricyanide as a redox probe (Fig. 2A). At first, the surface of the cleaned GSPE was modified with electrodeposited GNPs subsequently characterized using SEM. Homogenously distributed GNPs were observed on the surface (Fig. 3). A significant increase in peak currents was observed as a result of the enhancement of surface area of the GNPs modified electrode. Due to the extended surface of the sensing platform, the loaded amount of the immobilized aptamer was enhanced; consequently the signal has been improved. The A layer of MPA was self-assembled on the GNPs-modified GSPE so negative charges developed over the electrode surface because of the carboxylic acid groups presented in the immobilized MPA structure. The electrostatic repulsion forces between negatively charged interface and the negative redox probe caused a decrease in the peak currents was observed. In the next stage, acidic groups were activated using EDC/NHS solution in PBS pH 5 and the 5'-amine terminated aptamer was covalently attached to the surface due to the imide bond formation between activated acidic groups and the amine in the aptamer strand. More negative charges were developed on the surface because of the phosphate backbone presented in the aptamer structure. So a decrease in peak currents was similarly observed. In the next step a suspension of the GO in PBS pH 4 was prepared and dropped on the aptamer-modified electrode. Graphene oxide was adsorbed on the surface according to the π - π interaction with the hydrophobic aromatic rings existed in the aptamer structure. As GO is an insulator, electron transfer of the redox probe was hindered again so the redox peak separation in cyclic voltammogram was increased also subsequent decrease in peak currents was observed. In the last step, digoxin solution in PBS pH 7.4 was dropped on the electrode surface. The strong specific interaction between digoxin and aptamer caused GO to leave the surface so electron transfer of

the redox probe was accelerated. The binding affinity of aptamers to the target is much greater than that for other molecules. In most of the previous reports dissociation constants of different aptamers and their targets are 2 to 6 folds smaller than for compounds even with closely related structures. So in the presence of digoxin, GO was replaced due to the much stronger interaction between digoxin and aptamer [36]. Impedance spectroscopy was also utilized to characterize the surface, in each modification step. The diameter of the semicircle in Nyquist plot (R_{ct}) represents the charge transfer resistance. As it is clearly observed in Figure 2B, prior to modification of the electrode surface with MPA, the R_{ct} of the GNPs modified electrode is too small which represented the low resistance of surface to the electron transfer of the probe. This is due to the presence of the electrodeposited GNPs. After self-assembling a monolayer of MPA on GSPE-GNPs surface, R_{ct} which indicates the interfacial electron transfer resistance, was increased because of the repulsion forces between the negative MPA modified surface and $[\text{Fe}(\text{CN})_6]^{-3/4}$ probe used in impedance spectroscopy. In the next step digoxin aptamer was immobilized on the electrode surface via carbodiimide chemistry. A remarkable increase in the charge transfer resistance was observed which is due to the presence of negative phosphate backbone of the aptamer strand. The presence of the aptamer also insulated the surface consequently the electron transfer of the probe was decreased; consequently R_{ct} was significantly increased. After aptamer immobilization, GO solution was dropped on the modified electrode and the surface was more insulated because of the presence of GO. As a consequence R_{ct} was more increased in this step. In the final step in the presence of **digoxin**, it was specifically interacted with aptamer strand, therefore GO left the surface. The amount of GO which left the electrode surface is relevant to digoxin concentration. Because digoxin is a small molecule, when GO was replaced by digoxin

the surface was less insulated so the electron transfer resistance was decreased. Results have been demonstrated in Fig. 2.B.

For quantification, the oxidation current of potassium ferricyanide was monitored using square wave voltammetry. It was carried out at step potential and amplitude of 10 mV and 25 mV, respectively. The applied frequency was 15 Hz. The percentage of the increase in the current, which demonstrated as analytical signal, was proportional to the digoxin concentration and used to construct a calibration curve. Calibration curve was obtained in the optimum conditions.

3.3. Optimization of experimental parameters

Different experimental conditions were optimized to achieve the maximum analytical signal. To immobilize the aptamer on the electrode surface 7 μL of 2.4 μM aptamer were mixed with different concentrations of magnesium chloride then an 8 μL aliquot of the mixture was dropped on the MPA modified surface. The aptamer concentration was an important parameter, which should be optimized. Increasing the aptamer concentration from 0.4 μM to 1.2 μM significantly increased analytical signal. At higher amounts the response of the aptasensor was dramatically decreased which is probably due to the intermolecular interactions between the immobilized aptamer strands or maybe because of the spatial hindrances (Fig. 4.a). The presence of magnesium chloride is necessary in order to immobilize the aptamer on the electrode surface. In the presence of Mg^{+2} ions the repulsion forces between negative backbones of the aptamer strands were reduced so the optimization of magnesium chloride is of great importance. Increasing MgCl_2 concentration up to 0.33 M caused to decrease these repulsions; as a consequence much more aptamer amount was immobilized and the analytical signal was improved. At higher MgCl_2 concentrations even more aptamer was loaded on the electrode

surface. Due to the intermolecular interactions between aptamer strands, which were mentioned before the analytical signal, was decreased at the higher MgCl_2 concentrations (Fig. 4.b). The incubation time of the GO on aptamer modified surface also the time of the interaction between aptamer and digoxin were optimized to achieve maximum response (Fig. 4.c, d).

One of the most important parameters, which considered in optimization, was the acidity of the GO solution. GO interacted with aromatic rings in the structure of the aptamer strand through π - π stacking. To improve the interaction between aptamer and GO, the electrostatic repulsion forces between negatively charged aptamer and GO should be minimized. To investigate optimum conditions, GO dispersed in different phosphate buffer solutions over the range of 4 to 9 and dropped on the aptamer-modified surface for 2 h. After washing the electrode, cyclic voltammetry was performed in 5 μM of potassium ferricyanide and 0.1 M KCl solution. In the presence of the adsorbed GO the electrode surface was insulated so peak separation of the redox probe was enhanced. Also a decrease in the currents was observed. As it is demonstrated (Fig.4e), the maximum adsorption of GO on the surface was achieved at pH 4.

GO possesses different functional groups containing oxygen in its structure with the acidity close to benzoic acid ($\text{pK}_a = 4.2$) and acetic acid ($\text{pK}_a = 4.7$). So it is expected in the pH values around 4 the surface of GO neutralized consequently the repulsion forces between GO and the aptamer minimized [37]. Under these circumstances the interaction between GO and hydrophobic nucleobases is in its greatest extent.

3.4. Investigating the performance of the aptasensor

To evaluate the linear response range and minimum detectable concentration by the designed sensor, square wave voltammetry was performed in the presence of potassium ferricyanide as a

redox probe. As it is mentioned before in the presence of digoxin, the adsorbed GO on the aptamer modified electrode left the surface so a consequent increase in the reduction current of the probe was observed. The percentage of the increased current was recorded against digoxin concentration to construct a calibration curve. The calibration plot demonstrated a good linear relationship between the monitored signal and the logarithm of digoxin concentration over the range of 0.1 pM to 1 μ M with a detection limit of 0.05 pM. Table 1 shows a comparison between characteristics of the different digoxin aptasensors [18,38,39]. As seen, the proposed aptasensor delivered much better limit of detection, also a wide linear dynamic range. Modifying the electrode surface with GNPs caused to enhance the specific surface area of the electrode consequently the loaded aptamer on the surface was increased. Also a designed signal-on amplification strategy provided the improved limit of detection.

Using GNPs for modifying the surface also utilizing GO to design a signal-on aptasensor provided amplification of the analytical signal. As a consequence the designed sensor was able to detect digoxin concentrations as low as 0.1 pM.

To evaluate the stability of the sensing surface the aptamer modified electrode was kept in PBS pH 7.4 in the refrigerator. After 5 days the electrode was used for the determination of digoxin and a decrease of 5% was observed in the analytical signal in comparison to that for the same digoxin concentration. This results suggested good stability for the produced aptasensor.

3.5. Real sample analysis

The plasma sample of healthy volunteer was obtained from the Tehran Blood Transfusion Organization. In this section, all experiments were performed in compliance with the relevant laws and institutional guidelines. To estimate whether the constructed aptasensor was able to

determine digoxin in real samples, it was used to detect digoxin level in human plasma samples. To do this the known amounts of digoxin were added to the portions of 20 mL untreated plasma samples without any dilution, to achieve solutions with final digoxin concentrations in the range of 1.0×10^{-10} M to 5.0×10^{-8} M. Subsequently the prepared samples were analyzed with the aid of the proposed aptasensor and recoveries over a range of 90% to 110% were obtained. As a result the fabricated aptasensor was successfully evaluated to use in real sample analysis with no severe matrix interferences.

To ensure that the proposed sensor can successfully determine digoxin in the presence of interferences, three samples were prepared: a control sample which was 1 nM to digoxin, a solution containing the same concentration (1 nM) of digoxin, ascorbic acid, glucose, Mg^{+2} , Ca^{+2} , K^+ , Na^+ , and Zn^{+2} and another solution in which digoxin concentration was kept constant and concentration of all other interferences were increased five times. Then digoxin was measured in these three samples. Results showed that in the first sample not serious interference from matrix were detected consequently not significant changes were seen in comparison to the control sample. In the second sample analytical signal showed a 7% increase compared to the control sample, which is due to the presence of interferences. These experiments, also the ability of the proposed sensor to determine digoxin in plasma sample without any treatment confirm the ability of the sensor to analysis digoxin in complicated matrix of the real sample.

4. Conclusion

Herein a highly sensitive electrochemical aptasensor for determination of digoxin has been reported. In the construction of this aptasensor two signal amplification strategies were used to obtain lower limit of detection. Surface of the GSPE was modified with electrodeposited GNPs

to achieve higher surface area consequently more aptamer on the surface was immobilized. Also a designed signal-on strategy based on the interaction of GO with aptamer strand is another approach to obtain a sensitive aptasensor which can detect concentrations as low as 0.1 pM of digoxin. Also the proposed sensor was successfully utilized to determine digoxin human plasma level.

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References:

- [1] M.S. Kumar, S. Ghosh, S. Nayak, A.P. Das, Recent advances in biosensor based diagnosis of urinary tract infection, *Biosens. Bioelectron.* 80 (2016) 497–510.
- [2] L. Du, Y. Zhang, Y. Du, D. Yang, F. Gao, D. Tang, A novel label-free aptasensor based on target-induced structure switching of aptamer-functionalized mesoporous silica nanoparticles, *RSC Adv.* 5 (2015) 100960–100967.
- [3] G. Gundlach, E. Luttermann-Semmer, The Effect of pH and Temperature on the Stability and Enzymatic Activity of Prostatic Acid Phosphatase Studies on the Optimization of a Continuous Monitored Determination of Acid Phosphatase, II., *Clin. Chem. Lab. Med.* 25 (1987) 441–446.
- [4] T. Hermann, D.J. Patel, Adaptive recognition by nucleic acid aptamers., *Science.* 287 (2000) 820–825.
- [5] G.S. Baird, Where are all the aptamers?, *Am. J. Clin. Pathol.* 134 (2010) 529–531.
- [6] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase., *Science.* 249 (1990) 505–510.
- [7] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands., *Nature.* 346 (1990) 818–822.
- [8] I. Palchetti, M. Mascini, Electrochemical nanomaterial-based nucleic acid aptasensors, *Anal. Bioanal. Chem.* 402 (2012) 3103–3114. doi:10.1007/s00216-012-5769-1.
- [9] A. Sassolas, L.J. Blum, D.L.B. Béatrice, Electrochemical aptasensors, *Electroanalysis.* 21 (2009) 1237–1250.
- [10] B. Jean, S. Prezioso, S.A. Haughey, K. Campbell, C.T. Elliott, S.L. Degrasse, Sensors and Actuators B : Chemical An improved immunoassay for detection of saxitoxin by surface

- plasmon resonance biosensors, *Sensors Actuators B. Chem.* 156 (2011) 805–811.
- [11] N. Duan, X. Ding, L. He, S. Wu, Y. Wei, Z. Wang, Selection, identification and application of a DNA aptamer against *Listeria monocytogenes*, *Food Control*. 33 (2013) 239–243.
- [12] J.T. Wen, K. Bohorquez, H. Tsutsui, Polydiacetylene-coated polyvinylidene fluoride strip aptasensor for colorimetric detection of zinc(II), *Sensors Actuators B Chem.* 232 (2016) 313–317.
- [13] N. Formisano, P. Jolly, N. Bhalla, M. Cromhout, S.P. Flanagan, R. Fogel, J.L. Limson, P. Estrela, Optimisation of an electrochemical impedance spectroscopy aptasensor by exploiting quartz crystal microbalance with dissipation signals, *Sensors Actuators B Chem.* 220 (2015) 369–375.
- [14] A. Mokhtarzadeh, J. Ezzati Nazhad Dolatabadi, K. Abnous, M. de la Guardia, M. Ramezani, J.E.N. Dolatabadi, K. Abnous, M. de la Guardia, M. Ramezani, Nanomaterial-based cocaine aptasensors, *Biosens. Bioelectron.* 68 (2015) 95–106.
- [15] X. Liu, L. Su, L. Zhu, X. Gao, Y. Wang, F. Bai, Y. Tang, J. Li, Hybrid material for enrofloxacin sensing based on aptamer-functionalized magnetic nanoparticle conjugated with upconversion nanoprobe, *Sensors Actuators B Chem.* 233 (2016) 394–401.
- [16] Z. Zhang, S. Zhang, S. Liu, M. Wang, G. Fu, L. He, Y. Yang, S. Fang, Electrochemical aptasensor based on one-step synthesis of Cu₂O@aptamer nanospheres for sensitive thrombin detection, *Sensors Actuators B Chem.* 220 (2015) 184–191.
- [17] S. Amin, M.T. Soomro, N. Memon, A.R. Solangi, T. Qureshi, A.R. Behzad, Disposable screen printed graphite electrode for the direct electrochemical determination of ibuprofen in surface water, *Environ. Nanotechnology, Monit. Manag.* 1–2 (2014) 8–13.

- [18] A.S. Emrani, S.M. Taghdisi, N.M. Danesh, S.H. Jalalian, M. Ramezani, K. Abnous, A novel fluorescent aptasensor for selective and sensitive detection of digoxin based on silica nanoparticles, *Anal. Methods*. 7 (2015) 3814–3818.
- [19] G. Evtugyn, U. Cherkina, A. Porfireva, J. Danzberger, A. Ebner, T. Hianik, Electrochemical aptasensor based on ZnO modified gold electrode, *Electroanalysis*. 25 (2013) 1855–1863.
- [20] J. Jia, J. Feng, H.G. Chen, H.Q. Luo, N.B. Li, A simple electrochemical method for the detection of ATP using target-induced conformational change of dual-hairpin DNA structure, *Sensors Actuators B Chem*. 222 (2016) 1090–1095.
- [21] T.T. Moghadam, B. Ranjbar, Heat induced aggregation of gold nanorods for rapid visual detection of lysozyme, *Talanta*. 144 (2015) 778–787.
- [22] S. Song, L. Wang, J. Li, C. Fan, J. Zhao, Aptamer-based biosensors, *TrAC Trends Anal. Chem*. 27 (2008) 108–117.
- [23] S. Beiranvand, A.R. Abbasi, M. Roushani, Z. Derikvand, A. Azadbakht, A simple and label-free aptasensor based on amino group-functionalized gold nanocomposites-Prussian blue/carbon nanotubes as labels for signal amplification, *J. Electroanal. Chem*. 776 (2016) 170–179.
- [24] F. Shahdost-Fard, M. Roushani, An impedimetric aptasensor based on water soluble cadmium telluride (CdTe) quantum dots (QDs) for detection of ibuprofen, *J. Electroanal. Chem*. 763 (2016) 18–24.
- [25] L. Wu, Y. Yao, Z. Li, X. Zhang, J. Chen, A new amplified impedimetric aptasensor based on the electron transfer ability of Au nanoparticles and their affinity with aptamer, *J. Electroanal. Chem*. 757 (2015) 243–249.

- [26] F.J. Hernandez, V.C. Ozalp, Graphene and other nanomaterial-based electrochemical aptasensors, *Biosensors*. 2 (2012) 1–14.
- [27] F. Gao, L. Du, Y. Zhang, F. Zhou, D. Tang, A sensitive sandwich-type electrochemical aptasensor for thrombin detection based on platinum nanoparticles decorated carbon nanocages as signal labels, *Biosens. Bioelectron.* 86 (2016) 185–193.
- [28] J. Ping, Y. Zhou, Y. Wu, V. Papper, S. Boujday, R.S. Marks, T.W.J. Steele, Recent advances in aptasensors based on graphene and graphene-like nanomaterials, *Biosens. Bioelectron.* 64 (2015) 373–385.
- [29] J. Björk, F. Hanke, C. A. Palma, P. Samori, M. Cecchini, M. Persson, Adsorption of Aromatic and Anti-Aromatic Systems on Graphene through π – π Stacking, *J. Phys. Chem. Lett.* 1 (2010) 3407–3412.
- [30] S. Mehdi Khoshfetrat, M. a. Mehrgardi, Dual amplification of single nucleotide polymorphism detection using graphene oxide and nanoporous gold electrode platform, *Analyst*. 139 (2014) 5192–5199.
- [31] C. Arrhythmias, D. Concentration, I.N. Plasma, U. In, P. Treated, W. Digoxin, Cardiac arrhythmias, electrolytes, and digoxin concentration in plasma and urine in patients treated with digoxin, *Acta Med. Scand.* 197 (1975) 391–401.
- [32] Z. Kiani, M. Shafiei, P. Rahimi-Moghaddam, A.A. Karkhane, S.A. Ebrahimi, In vitro selection and characterization of deoxyribonucleic acid aptamers for digoxin, *Anal. Chim. Acta.* 748 (2012) 67–72.
- [33] J. William S. Hummers, R.E. Offeman, Preparation of Graphitic Oxide, *J. Am. Chem. Soc.* 80 (1958) 1339.
- [34] A. J. Saleh Ahammad, Y.H. Choi, K. Koh, J.H. Kim, J.J. Lee, M. Lee, Electrochemical

- detection of cardiac biomarker troponin I at gold nanoparticle-modified ITO electrode by using open circuit potential, *Int. J. Electrochem. Sci.* 6 (2011) 1906–1916.
- [35] D. Li, M.B. Müller, S. Gilje, R.B. Kaner, G.G. Wallace, Processable aqueous dispersions of graphene nanosheets., *Nat. Nanotechnol.* 3 (2008) 101–105.
- [36] M.H. Mashhadizadeh, N. Naseri, M.A. Mehrgardi, A digoxin electrochemical aptasensor using Ag nanoparticle decorated graphene oxide, *Anal. Methods.* 8 (2016) 7247–7253.
- [37] H. Park, S.-J. Hwang, K. Kim, An electrochemical detection of Hg^{2+} ion using graphene oxide as an electrochemically active indicator, *Electrochem. Commun.* 24 (2012) 100–103.
- [38] A. Sarreshtehdar Emrani, N.M. Danesh, P. Lavaee, S.H. Jalalian, M. Ramezani, K. Abnous, S.M. Taghdisi, Sensitive and selective detection of digoxin based on fluorescence quenching and colorimetric aptasensors, *Anal. Methods.* 7 (2015) 3419–3424.
- [39] H. Bagheri, R.P. Talemi, a. Afkhami, Gold nanoparticles deposited on fluorine-doped tin oxide surface as an effective platform for fabricating a highly sensitive and specific digoxin aptasensor, *RSC Adv.* 5 (2015) 58491–58498.

Figures Legends:

Figure 1. Characterization of the synthesized GO. a) FT-IR spectrum, b) UV-Vis spectrum of the dispersed GO in deionized water, c) SEM image of GO.

Figure 2. (A) Cyclic voltammograms of different modification steps of the GSPE in the presence of 5 μM $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.1 M KCl. a) GNPs modified GSPE, b) bare GSPE, c) MPA-modified GSPE, d) aptamer-modified GSPE, e) adsorption of GO on the aptamer-modified GSPE. In all experiments scan rate and potential step were 0.05 $\text{V} \cdot \text{sec}^{-1}$ and 0.005 V respectively; (B) Impedance spectrums of different modification steps. Experiments were performed in the presence of 5 mM of $\text{Fe}(\text{CN})_6^{-3/4}$ containing 0.1 M KCl. The impedance spectrums were achieved at DC potential equal to the open circuit over the frequency range of 100 KHz to 0.1 HZ and in the oscillation potential of 5 mV. a) GNPs modified GSPE, b) MPA modified GSPE, c) aptamer-modified GSPE d) adsorption of GO on the aptamer-modified GSPE e) interaction of digoxin with aptamer

Figure 3. SEM image of the GNPs-modified GSPE surface.

Figure 4. Optimization of experimental conditions: charts demonstrate the analytical signal versus; a) aptamer concentration, b) magnesium chloride concentration, c) GO incubation time, d) interaction time between aptamer and digoxin, and e) Effect of pH; a) 8.5, b) 7.5, c) 5.5, and d) 4.5. Cyclic voltammograms were performed in the presence of 5 μM $\text{K}_3\text{Fe}(\text{CN})_6$ and 0.1 M KCl in phosphate buffer.

Figure 5. SWVs of GO modified-GSPE in solutions with different digoxin concentration range of 0.1 pM to 1.0 μ M. Inset is a calibration curve for the fabricated aptasensor. The experiment was performed over the potential range of 0 to 0.5 V at the step potential of 0.01 V. The amplitude and frequency were 0.025 V and 15 Hz respectively. digoxin in the

Scheme 1. Schematic preview of different modification steps in the construction of the aptasensor.

(a)

(b)

(c)

Figure 1

(A)

(B)

Figure 2

Figure 3

(a)

(b)

(c)

(d)

(e)

Figure 4

Figure 5

Scheme 1

Table 1. A comparison between different digoxin aptasensors.

Method of detection	LOD	LDR	Ref
Fluorescence	566 pM	0-60 nM	[18]
Fluorescence	392 pM	0-30 nM	[38]
Colorimetry	571 pM	0-30 nM	[38]
Electrochemistry (DPV)	12.8 pM	25 pM-2.5 nM	[39]
Electrochemistry (DPV)	0.05 pM	1 μ M-0.1 pM	This work