Hesperidin-dsDNA Interaction Based on Electrochemically Reduced Graphene Oxide and Poly-(2,6-Pyridinedicarboxylic Acid) Modified Glassy Carbon Electrode

Elektrokimyasal Olarak İndirgenmiş Grafen Oksit ve Poli(2,6-Piridindikarboksilik Asit) Modifiye Camsı Karbon Elektrot ile Hesperidin-Dsdna Tayini

Research Article

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ABSTRACT

In this work, electrochemically reduced graphene oxide (ERGO) was deposited on a glassy carbon electrode (GCE) and poly(2,6-pyridinedicarboxylic acid) (P(PDCA)) as a second layer was electrosynthesized on ERGO modified GCE and then, the prepared electrode was used to immobilize dsDNA. Electrochemical behaviour of GCE/ERGO/P(PDCA) was investigated by using cyclic voltammetry (CV) and compared with those of the bare GCE. Hesperidin (HDN), a promising anticancer agent, can be detected using GCE/ERGO/P(PDCA)/dsDNA biosensor using the differential pulse voltammetry (DPV). The decrease in the guanine oxidation peak current at +0.77 V was used as an indicator for the interaction in 0.5 M ABS (pH 4.8) containing 0.02 M NaCl. The experimental parameters such as dsDNA concentration, HDN concentration, dsDNA adsorption time and interaction time were optimized. The guanine oxidation peak currents were linearly proportional to the concentrations of the HDN in the range of in the range of 0.82-82 μM and detection limit was found to be 0.24 μM. The reproducibility, repeatability, and applicability of the analysis to human serum samples were also examined. In order to obtain more information about the interaction between dsDNA and HDN, UV-vis spectrophotometry measurements were also performed. The novel DNA biosensor could serve for sensitive, accurate and rapid determination of the HDN.

Key Words

Hesperidin, dsDNA, biosensor, electrochemistry.

ÖZ

Bu çalışmada, camsı karbon elektrot (GCE) yüzeyi elektrokimyasal olarak indirgenmiş grafen oksit (ERGO) ile kaplandı ve poli(2,6-piridindikarboksilik asit) (P(PDCA)) ikinci bir tabaka olarak ERGO modifiye GCE yüzeyinde biriktirildi. Hazırlanan elektrot dsDNA'yı immobilize etmek için kullanıldı. GCE/ERGO/P(PDCA) elektrodunun elektrokimyasal davranışı dönüşümlü voltametri (CV) yöntemi ile incelendi ve sonuçlar yalın GCE'den elde edilen değerler ile karşılaştırıldı. Ümit verici bir antikanser ajanı olarak kullanılan hesperidin (HDN), GCE/ERGO/P(PDCA)/dsDNA biyosensörü kullanılarak diferansiyel puls voltametrisi (DPV) tekniği ile tayin edildi. +0.77 V'daki guanin yükseltgenme pik akımındaki azalma 0.02 M NaCl içeren 0.5 M ABS (pH 4.8)'deki etkileşim için bir indikatör olarak kullanıldı. dsDNA konsantrayonu, HDN konsantrasyonu, dsDNA adsorpsiyon süresi ve etkileşim süresi gibi deneysel parametreler optimize edildi. Guanin yükseltgenme pik akımlarının 0.82-82 μM HDN konsantrasyon aralığında doğrusal olarak değiştiği bulundu ve gözlenebilme sınırı 0.24 μM olarak hesaplandı. Tekrar üretilebilirlik, tekrarlanabilirlik ve analizin insan serum örneklerine uygulanabilirliği incelendi. dsDNA ile HDN arasındaki etkileşim hakkında daha çok bilgi sağlamak amacıyla UV-görünür bölge spektrofotometri ölçümleri de alındı. Hazırlanan yeni dsDNA biyosensörünün hassas, doğru ve hızlı bir şekilde HDN tayini yapabildiği sonucuna varıldı.

Anahtar Kelimeler

Hesperidin, dsDNA, biyosensör, elektrokimya.

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INTRODUCTION

Hesperidin (HDN) (Figure 1), a polyphenolic bioflavonoid, is the predominant flavonoid in orange peel and other citrus fruits. HDN is an antioxidant that enhances the action of vitamin C to lower cholesterol levels [1]. It also possesses numerous beneficial health effects such as antioxidant, anticancer, anti-inflammatory, antihistaminic, and antiviral agent, and sedative activity [2,3]. Therefore, it is important to develop rapid and sensitive methods for the determination of HDN. Up to now, several chromatographic techniques, such as, high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) have been used for the determination of HDN. [4,5] Moreover, electrochemical techniques such as adsorptive stripping voltammetry (AdSV) [6] and differential pulse voltammetry (DPV) [7] were also used. However, to the best of our knowledge, the interaction of HDN with dsDNA using GCE/ERGO/P(PDCA) electrode has not been studied.

In recent years, polymer modified electrodes has received increasing attention in electrochemical biosensor applications [8,9]. The use of various conducting polymers (CPs) such as polyaniline, polypyrole and poly(3,4-ethylenedioxythiophene) have been proposed as a suitable matrix for immobilization of biomolecules especially enzymes, proteins or nucleic acids and they can be used to enhance the stability, sensitivity and selectivity [10,11]. Poly(2,6-pyridinedicarboxylic acid) P(PDCA) film has been used in biosensor applications with different immobilized biomolecules due to its rich electroactive center, excellent stability, biocompatibility, and good infusible and insoluble capabilities [12,13]. Graphene oxide (GO) which has a layered structure with a large specific area has been extensively used in the last years for sensing and biosensing applications [14]. Nowadays, graphene/conducting polymers composites have been considered as one of the most promising functional components due to their good electrical conductivity, chemical stability and high electrochemical capacity [15,16]. In this paper, we have prepared ERGO/P(PDCA) composite film modified glassy carbon electrodes (GCE/ERGO/(P(PDCA)) by a two-step electrochemical method. dsDNA was immobilized onto this modified electrode and GCE/ERGO/(P(PDCA)/dsDNA was prepared. The GCE/ERGO/(P(PDCA) electrode was characterized by cyclic voltammetry (CV) technique. The experimental conditions such as dsDNA concentration, adsorption time, HDN concentration and its interaction time with dsDNA were optimized. The obtained results exhibit that the GCE/ERGO/(P(PDCA)/dsDNA electrode showed high selectivity for the interaction of the HDN with dsDNA.

Experimental

Reagents and materials

Fish sperm dsDNA was purchased from Serva Electrophoresis GmbH Co. (Germany). HDN, PDCA, sodium acetate, sodium chloride, and acetic acid were provided by Sigma Chemical Co., Ltd. Graphite powder (Fluka, Germany), H_2SO_4 and H_2O_2 (Sigma-Aldrich, Germany), $KMnO_4$ (Merck, Germany), $NaNO_3$ and HCl (J.T Baker, USA). 1.0 mg/mL of dsDNA stock solution was prepared in ultrapure water and stored at –20 °C. Solutions of different concentrations of dsDNA and HDN were prepared by dilution in 0.5 M ABS (pH 4.8). Solutions of 1 mg/mL HDN were prepared in N,N-dimethylformamide (DMF) and kept away from light to avoid photochemical decomposition. Other chemicals were analytical grade and used without further purification.

Apparatus

Electrochemical measurements were performed with an Autolab PGSTAT 302N potentiostat/galvanostat measurement system using Nova 11.1 software (Eco Chemie, The Netherlands). The experiments were conducted in a voltammetric cell (Bioanalytical Systems, Inc., USA) using a conventional three-electrode system. Silver/silver chloride (BAS MF2052), platinum wire (BAS MF1032), and bare or GCE/ERGO/P(PDCA) electrodes were used as the reference, counter and working electrodes, respectively. The pH measurements were obtained using an ORION Model 720A pH meter (Thermo Scientific, USA). The ultrapure water (18.2 MΩcm) from Purelab Elga system (Veolia Water Systems Ltd., UK) was employed for preparing all solutions. All measurements were carried out at room temperature.

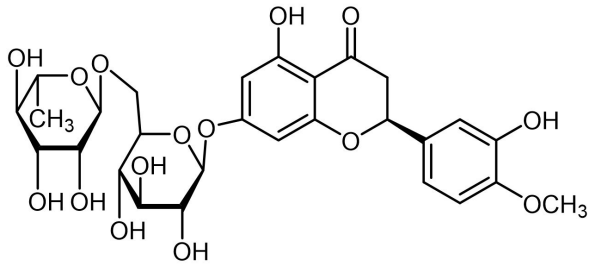

Figure 1. The chemical structure of hesperidin.

Electrochemical characterizations of GCE/ERGO/P(PDCA) and GCE/ ERGO/P(PDCA)/dsDNA were achieved in a 0.1 M KCl solution including 5.0 mM $Fe(CN)_6^{3-/4-}$ by cyclic voltammetry (CV) technique. The cyclic voltammograms (CVs) were obtained by scanning in the potential window from –0.3 V to 0.6 V at a scan rate of 50 mVs–1. The differential pulse voltammograms (DPVs) were obtained in 0.5 M pH 4.8 acetate buffer solution (ABS) by scanning in the potential range from +0.4 to +1.3 V. The experimental conditions for the differential pulse voltammetry were step potential: 0.003 V; modulation amplitude: 0.05 V; modulation time: 0.02 s; interval time: 0.2 s and scan rate: 0.015 Vs^{-1}. The anodic current was +0.8 V, which corresponds to the guanine oxidation, and it was employed as an analytical signal.

UV-vis absorption spectra were obtained with a Shimadzu 1700 (Pharma Spec) double beam spectrophotometer (Shimadzu Corporation, Tokyo, Japan) using quartz cuvettes of 1 cm path length at room temperature in the wavelength range of 200–500 nm. The spectra were recorded for free dsDNA and dsDNA-HDN for the calculation of the binding constant of the reaction occurring between the HDN and the dsDNA.

Synthesis of graphene oxide

Graphene oxide was synthesized from natural graphite powder by modified Hummers method according to ref [17]. In a typical synthesis, 2 g of graphite powder was added into cooled 350 mL of 98% H_2SO_4 (0°C). After that, 1 g of $NaNO_3$ was added into the mixture and stirred for 5 min. Then, 8 g of $KMnO_4$ was slowly added to the mixture. It was warmed to a 30°C and stirred for 30 min. 250 mL of ultra-pure water was slowly added and the temperature was increased to 98°C. The reaction mixture was maintained at that temperature for 30 min. Later, 500 mL of ultra-pure water and 40 mL of 30% H_2O_2 were added into the reaction mixture. The colour of the mixture altered to brilliant yellow. This change indicates that the pristine graphite is oxidized to graphite oxide. Afterwards, the mixture was filtered and treated with diluted HCl to remove metal ions. Finally, the product was washed with ultra-pure water until the pH was 7. The graphite oxide was obtained after drying. The graphite oxide was re-dispersed in ultra-pure water, and the exfoliation of graphite oxide to produce GO sheets was performed by ultrasonication for 1 h. The resultant aqueous dispersion of brown GO sheet was stable [17,18].

Preparation of GCE/ERGO/P(PDCA)

GCE/ERGO/P(PDCA) was prepared by a two-step electrochemical method. Prior to modification, the GCE was polished with alumina suspension and rinsed thoroughly with ultra-pure water. The polished GCE was sonicated in ethanol and ultra-pure water for 5 min, respectively, and dried in air. 5 μL of the graphene oxide suspension was dropped onto the GCE surface and dried at 40°C to get graphene oxide modified GCE (GCE/GO). The GCE/GO was then transferred to 0.1 M PBS (pH 7.0). Five successive potential sweeps were applied between +0.60 V and –1.50 V at a scan rate of 25 mVs^{-1} to form the electrochemically reduced graphene oxide modified GCE (GCE/ERGO). After washing with ultrapure water, the GCE/ERGO was treated in 0.1 M KCl containing 1.0 mM of PDCA by carrying out voltammetric cycling between 0.0 and +2.0 V at 60 mVs^{-1} with 18 cycles [19]. After this procedure, the GCE/ERGO was covered with a thin film of P(PDCA), and this electrode was washed with ultrapure water. A pre-treatment procedure for the polymer film

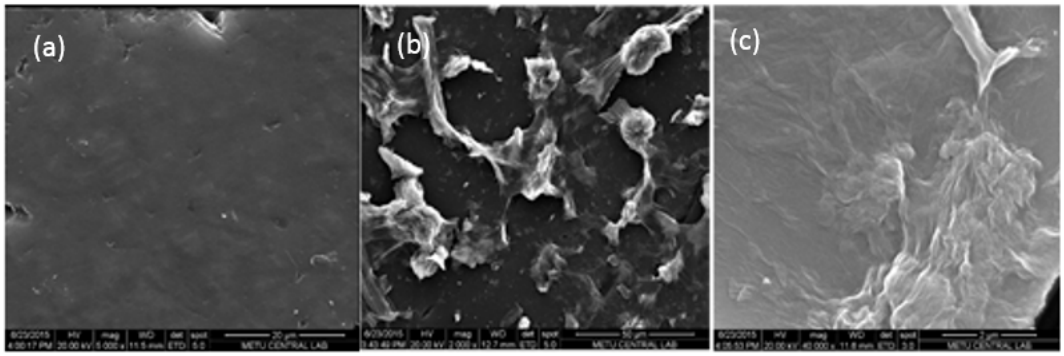

Figure 2. SEM images of bare GCE (a) and GCE/ERGO/P(PDCA) (b,c)

is required to accomplish immobilization of the negatively charged dsDNA. For this purpose, the polymer was activated by applying ten successive DPVs in the potential range from +0.4 to +1.3 V (vs. Ag/AgCl) in 0.5 M ABS (pH 4.8). Unless otherwise stated, the pre-treated polymer film was used in the experimental studies. Finally, pre-treated P(PDCA) modified GCE/ERGO electrode (GCE/ERGO/P(PDCA)) was obtained and thoroughly washed with ultrapure water to remove unadsorbed substances and dried in the air.

Immobilization of dsDNA onto GCE/ERGO/P(PDCA)

For the adsorption of the dsDNA, the modified electrodes were immersed into vials containing 40 μg/mL dsDNA in 0.5 M ABS (0.02 M NaCl) for 15 min. After adsorption of the DNA, the electrode was washed with acetate buffer (0.5 M at pH 4.8) for 5 s.

Interaction of dsDNA with HDN

To investigate the interaction between the HDN and the dsDNA, the the guanine oxidation signal was used as an indicator. After the adsorption of dsDNA onto GCE/ERGO/P(PDCA), the modified electrode was immersed into in 0.5 M ABS (pH 4.8) containing different concentration of HDN and stirred at 200 rpm for selected times. After the interaction, the electrode was rinsed and placed in HDN-free acetate buffer solution, where differential pulse voltammogram was recorded.

Preparation of real samples

Human serum samples were supplied from healthy individuals and stored at –20°C until the assay procedure. Afterward, a 10 μL volume of serum was transferred into vial containing 90 μL ABS, a certain volume of the stock solution of HDN was added into the vial. This mixture was transferred to an electrochemical cell, and allowed to interact with the GCE/ERGO/P(PDCA)/dsDNA electrode for the selected time. After the interaction, the GCE/ERGO/P(PDCA)/dsDNA electrode was rinsed and placed into blank ABS and the DPVs were recorded.

RESULTS and DISCUSSION

Surface characterization of various electrodes

The surface morphology of the GCE/ERGO/P(PDCA) electrode was characterized by SEM. Figure 2(a) displays the SEM image of the bare GCE and this electrode had a uniform and smooth structure. As shown in Figure 2(b,c), the ERGO/P(PDCA) film deposited on the GCE exhibits a rough surface with many wrinkles, showing a three-dimensional (3D) morphological microstructure due to the presence of GO sheets [16]. This 3D structure of ERGO/P(PDCA) can provide a large surface area for dsDNA adsorption.

Electrochemical characterization of the modified electrodes

The electrochemical performance of the developed electrodes was investigated using cyclic voltammetry in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Figure 3 shows CVs of bare GCE, GCE/ERGO and GCE/ERGO/P(PDCA) at a scan rate of 50 mV s^{-1}. Well-defined oxidation and reduction peaks are observed, and the highest peak current is noticed on the GCE/ERGO/P(PDCA).

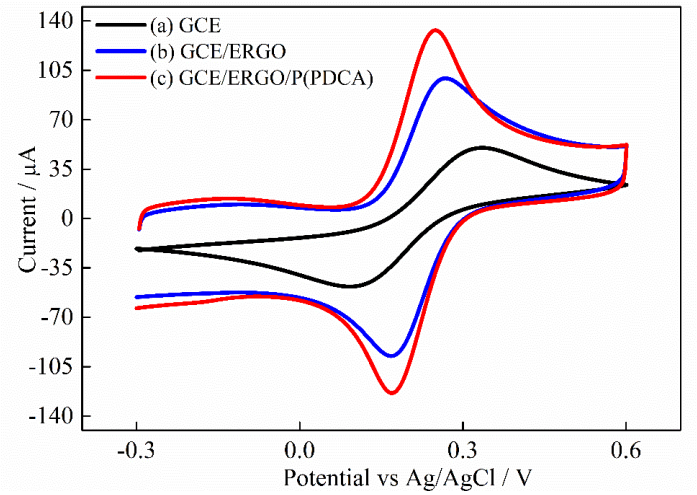

Figure 3. Cyclic voltammogram of (a) GCE, (b) GCE/ERGO, (c) GCE/ERGO/P(PDCA) in 0.1 M KCl solution containing 5.0 mM $Fe(CN)_6^{3-/4-}$.

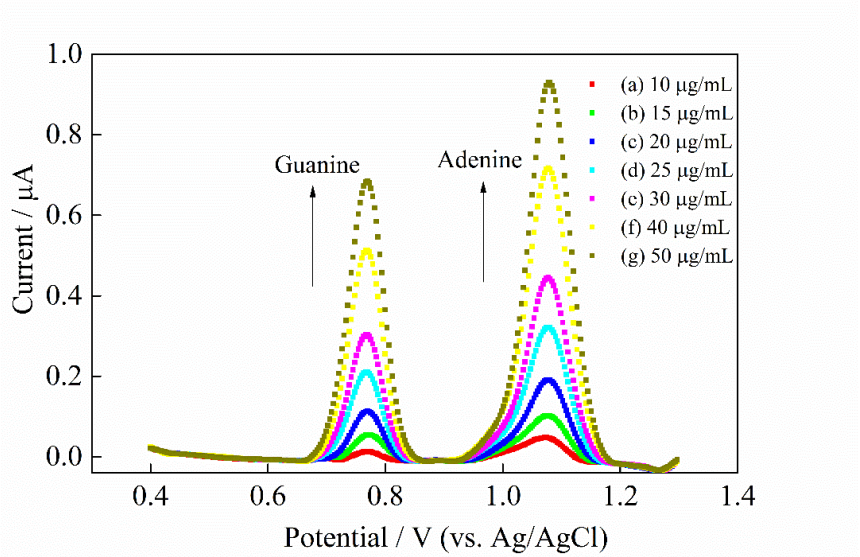

Figure 4. DPVs of the oxidation peaks of the guanine and adenine at the dsDNA concentrations varying from 10 to 50 µg/mL in ABS (pH 4.8) at the GCE/ERGO/P(PDCA).

dsDNA immobilization onto the GCE/ERGO/P(PDCA) electrode

In order to determine the optimization of the experimental parameters related to dsDNA, the guanine oxidation peak current was used as an indicator [20]. Figure 4 exhibits the DPVs of the different concentrations of the dsDNA at the GCE/ERGO/P(PDCA) electrode. The oxidation peak of guanine and adenine were shown at approximately +0.77 V and +1.08 V, respectively. The oxidation current of the guanine increased with increasing concentration of dsDNA adsorbed onto the GCE/ERGO/P(PDCA). This can be ascribed to the increased number of guanine molecules suitable for oxidation [21]. For finding the optimum concentration of dsDNA adsorbed onto the GCE/ERGO/P(PDCA) electrode was studied different concentrations from 10 to 50 µg/mL. Figure 5(A) exhibits that the guanine oxidation current increases with the dsDNA concentration from 10 to 40 µg/mL, and reaches to the maximum value at 40 µg/mL. Thus, 40 µg/mL of the dsDNA was selected as optimum value in order to obtain a high sensitivity in the subsequent experiments. The effect of the dsDNA adsorption time on the oxidation of guanine at GCE/ERGO/P(PDCA) was evaluated between 5 and 30 min. As shown in Figure 5(B), the oxidation peak current of guanine

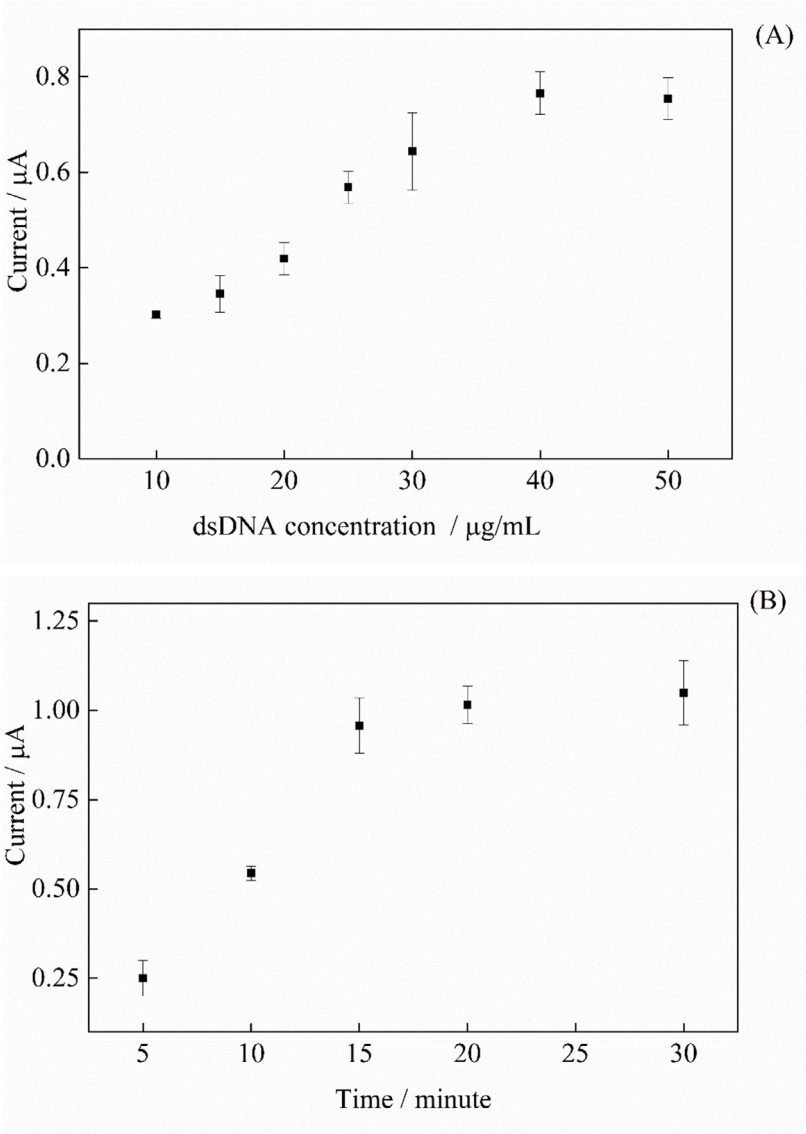

Figure 5. DPVs of the oxidation peaks of the guanine and adenine at the dsDNA concentrations varying from 10 to 50 μg/mL in ABS (pH 4.8) at the GCE/ERGO/P(PDCA).

increased up to 20 min of the adsorption time and then levelled off. Thus, the optimum adsorption time was determined as 20 min.

HDN-dsDNA interaction at the GCE/ERGO/P(PDCA) electrode

The binding of the HDN to dsDNA is dependent on the interaction time. As shown in Figure 6, the oxidation peak current of guanine decreased as the time increased up to 120 s, after which the decrease in the guanine peak current levelled off and remained constant up to 180 s. Thus, in all subsequent experiments, a 120 s as interaction time was used. The decrease of the guanine oxidation peak currents corresponds to the binding of HDN to the guanine electroactive base. This decrease could be explained a possible damage in the oxidizable groups of electroactive bases while HDN interacts with dsDNA at the surface of GCE/ERGO/P(PDCA) [22,23].

The effect of the concentration of the HDN on the DPV signals in the presence of 40 μg/mL dsDNA was evaluated in the concentration range of 0.5 μg/mL and 40 μg/mL. Figure 7(A) displays the HDN, guanine and adenine oxidation signals at the GCE/ERGO/P(PDCA) electrode for the different concentrations of HDN. Increasing the concentration of the HDN caused a decrease in the oxidation peak current of the guanine and

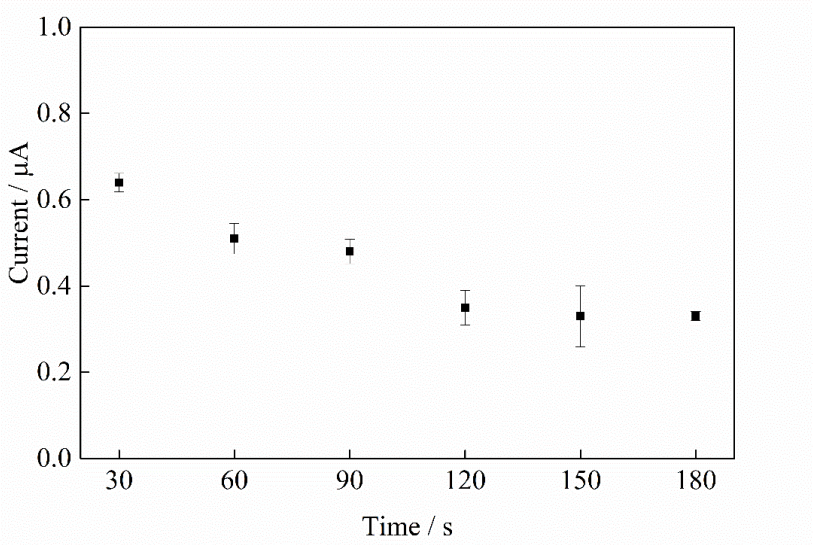

Figure 6. DPVs of the oxidation peaks of the guanine and adenine at the dsDNA concentrations varying from 10 to 50 μg/mL in ABS (pH 4.8) at the GCE/ERGO/P(PDCA).

adenine which corresponds to the binding of the HDN to those electroactive bases. If there is a negative or positive shift in the oxidation peak potential, this can be considered to be an electrostatic or intercalative interaction, respectively [24]. As can be seen from Figure 7(A), a positive shift was observed in the peak potential of HDN. The oxidation peak currents of guanine were linear with the HDN concentration over the range of 0.82 – 82 μM with a linear equation of $I(\mu A) = 0.0153C+1.198$ with $R^2 = 0.995$ (n = 3) where C is the HDN concentration (Figure 7(B)). The limit of detection (LOD) and limit of quantification (LOQ) from the calibration curve were found to be 0.24 and 0.81 μM, respectively. The LOD and LOQ values confirmed the sensitivity of the modified electrode which was calculated using the following equation (Equation 1) [25].

LOD= 3*s/m*, LOQ=10*s/m*

where s is the standard deviation of the current (three runs) for the lowest concentration of the linearity range, m is the slope of the related calibration curve. The oxidation peak currents of adenine (Figure 7(C)) and (Figure 7(D)) were linear with the HDN concentration over the range of 4.1 – 65.6 μM with a linear equation of $I(\mu A) = 0.0117C + 1.598$ ($R^2 = 0.998$) and 1.64 – 32.8 μM with a linear equation of $I(\mu A) = 0.0478C + 0.019$ ($R^2 = 0.984$), respectively.

UV-vis spectrophotometric studies for interaction of HDN with dsDNA

To investigate the HDN-dsDNA interaction, UV-vis absorption spectroscopy was also used. Generally, a bathochromic/hypsochromic shift for the absorption peak can indicates a drug-DNA interaction [26]. Figure 8 exhibits the UV-vis spectra of the HDN alone (a) and the dsDNA-HDN mixtures (b-e). As can be seen from Figure 8, the HDN exhibited one absorption peak at about 273 nm. The hyperchromic effect in the absorption peaks of HDN was observed with the addition of dsDNA (5-40 μg/mL) to the fixed concentration of the HDN (65.6 μM). It is reported that, compounds binding with dsDNA via intercalation are generally characterized by hypochromism/hyperchromism and significant bathochromism (red shift) [27]. Therefore, the observed hyperchromic effect and slight red shift indicate the partially intercalative binding of HDN with dsDNA base pairs [28]. The binding constant (*K*) can be calculated from the equation (Equation 2) based on the variations in the absorbance of the of HDN-dsDNA complexes:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \times \frac{1}{K[\mathrm{DNA}]}$$

where *K* is the binding constant; A_0 and A are the absorbance values of the HDN and its complex with dsDNA, respectively; and, ε_G and ε_{H-G} are the absorption coefficient of the drug and the HDN–dsDNA complex, respectively. The binding

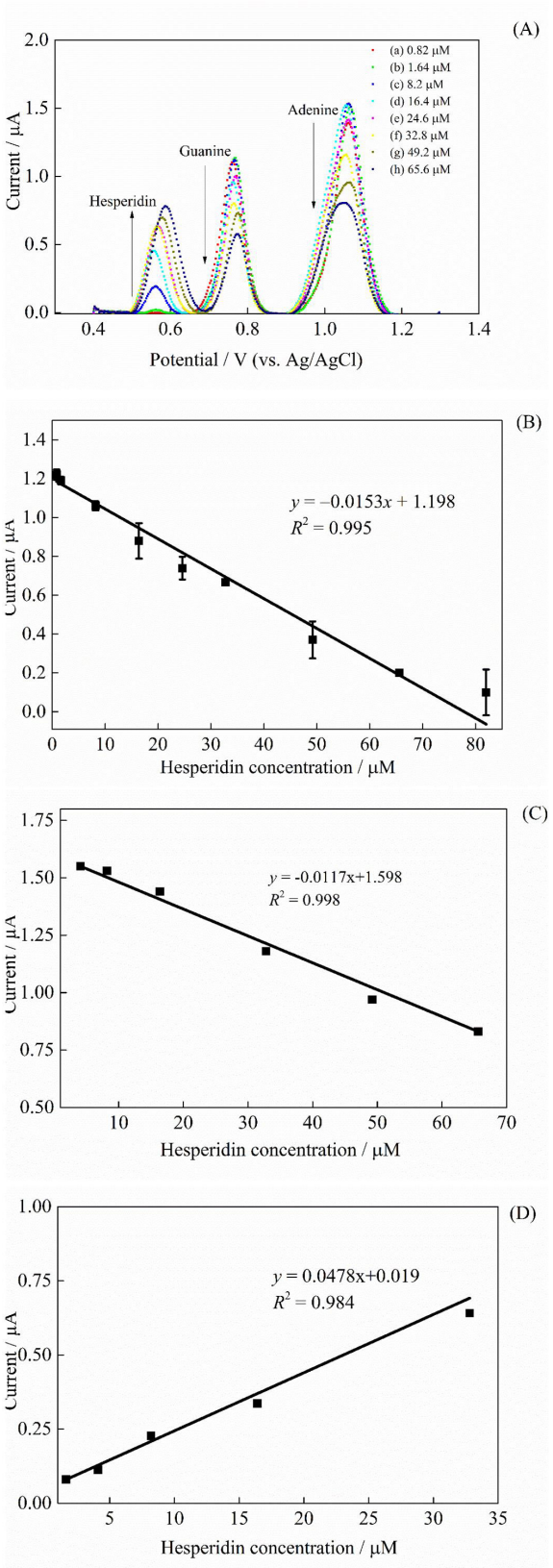

Figure 7. The effect of the HDN concentration of the HDN with dsDNA on DPV signals (A). A linear dependence of HDN concentration on the peak current of guanine (B), adenine (C) and hesperidin (D).

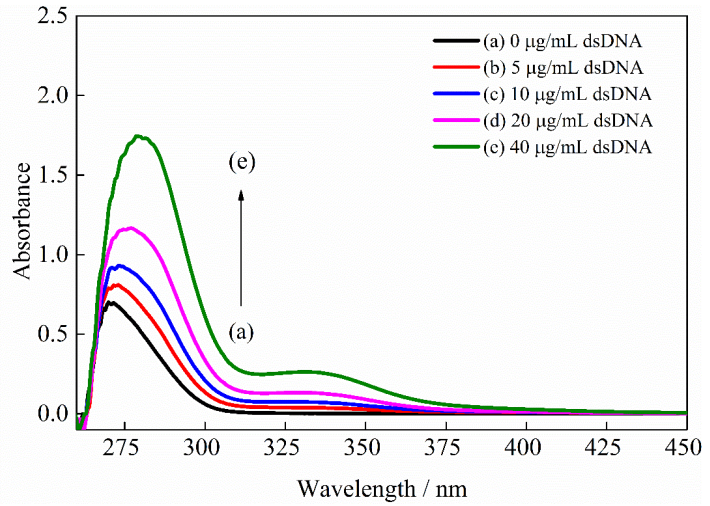

Figure 8. UV-vis spectra of HDN (65.6 μM) in the absence and presence of dsDNA in the range of 5.0–40.0 μg/mL.

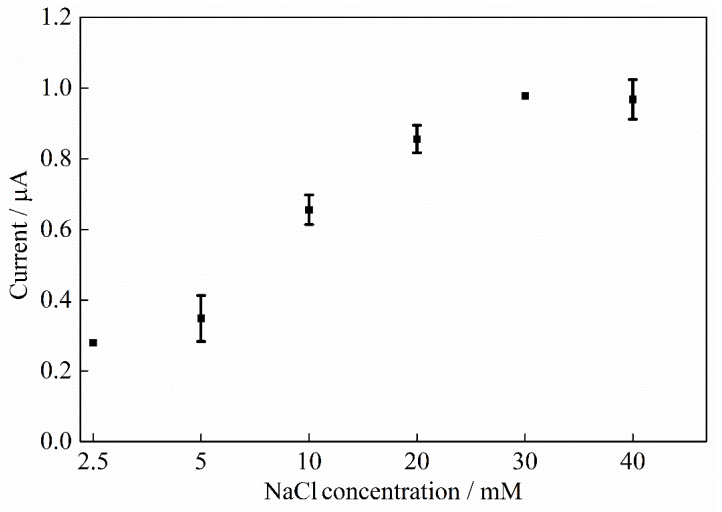

Figure 9. UEffect of the Na^+ concentrations varying from 2.5 mM to 40 mM on the interaction of the HDN with dsDNA (HDN = 65.6 μM, dsDNA = 40 μg/mL).

constant, *K*, can be obtained from the intercept-to-slope ratio of the $A_0/(A-A_0)$ vs. 1/[dsDNA] plot. The value of K for the HDN–dsDNA interaction was calculated to be 2.51×10^5 $L.mol^{-1}$. The calculated value of K is very close to the value of the classic intercalation binding such as the EtBr-DNA complex ($K=6.58\times10^4$ $L.mol^{-1}$) [29].

Effect of NaCl concentration on the interaction of HDN with dsDNA

To confirm the interaction mode of the HDN with the dsDNA immobilized at the GCE/ERGO/P(PDCA) surface, the effect of the salt concentration on the guanine oxidation signal was investigated. DPVs were recorded in 0.5 M ABS (pH 4.8) containing di–erent concentrations of NaCl (5.0-40 mM). As shown in Figure 9, the peak current of the guanine fairly changed with the increasing salt concentration of the acetate buffer. The electrostatic interaction between the small molecule and the dsDNA is one of non-covalent binding, which served as an auxiliary mode to assist the intercalation and groove binding. If the electrostatic interaction occurs between the small molecule and dsDNA, the strength of the interaction decreases with an increase in the salt concentration in the solution [30,31]. The results indicated that there was no significant electrostatic binding between the HDN and dsDNA.

Table 1. Determination of HDN in serum samples.

Sample no	Amount of added (μg/mL)	Amount of found (μg/mL)	Recovery (%)	RSD* (%)	Bias (%)
1	5.00	5.21	101.00	1.43	-1.00
2	10.00	10.15	101.50	1.70	-1.50
3	20.00	19.43	97.15	8.31	2.85

*each value is the mean of three measurements

Real sample analysis

In the biological sample analysis, standard addition method was used to determine the concentration of HDN and the different amounts of HDN that were spiked into the test sample (Table 1). These results demonstrate the applicability of the proposed electrochemical biosensor based on GCE/ERGO/P(PDCA)/dsDNA for the determination of HDN in human serum samples.

CONCLUSIONS

In conclusion, a study of the binding of hesperidin with dsDNA was investigated using GCE/ERGO/P(PDCA) electrode. The modified GCE improved the rate of electron transfer at the solution/electrode interface according to the results obtained with CV method in $Fe(CN)_6^{3-/4-}$ as redox probe. In order to determine the interaction of the HDN with dsDNA, the DPV and UV-vis spectroscopy techniques were used. The results confirmed that the binding mode of HDN to dsDNA is mainly based on intercalative binding under our conditions. The results offer an opportunity to use dsDNA as an electrochemical probe for determination of HDN drug concentration. The present study is of potential importance in understanding the mechanism of interaction of HDN with dsDNA and has a potential in emerging drug development.

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References

1. C.S. Seo, J.-A. Lee, D. Jung, H.-Y. Lee, J.K. Lee, H. Ha, M.-Y. Lee, H.K. Shin, Simultaneous determination of liquiritin, hesperidin, and glycyrrhizin by HPLC-photodiode array detection and the anti-inflammatory effect of Pyungwi-san, Arch. Pharm. Res., 34 (2011) 203-210.
2. L.M. Loscalzo, C. Wasowski, A.C. Paladini, M. Marder, Opioid receptors are involved in the sedative and antinociceptive effects of hesperidin as well as in its potentiation with benzodiazepines, Eur. J. Pharmacol., 580 (2008) 306-313.
3. A. Balakrishnan, V.P. Menon, Effect of hesperidin on matrix metalloproteinases and antioxidant status during nicotine-induced toxicity, Toxicology, 238 (2007) 90-98.
4. I. Saeidi, M.R. Hadjmohammadi, M. Peyrovi, M. Iranshahi, B. Barfi, A.B. Babaei, A.M. Dust, HPLC determination of hesperidin, diosmin and eriocitrin in Iranian lime juice using polyamide as an adsorbent for solid phase extraction, J. Pharmaceut. Biomed., 56 (2011) 419-422.
5. T. Wu, Y. Guan, J. Ye, Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection, Food Chem., 100 (2007) 1573-1579.
6. J. Hu, Q. Li, X. Tan, Study on the Adsorptive Behaviour of Hesperidin and Its Adsorptive Stripping Voltammetry, Anal. Lett., 29 (1996) 1779-1789.
7. D. Sun, F. Wang, K. Wu, J. Chen, Y. Zhou, Electrochemical determination of hesperidin using mesoporous SiO2 modified electrode, Microchim. Acta, 167 (2009) 35-39.
8. G. Aydoğdu, G. Günendi, D.K. Zeybek, B. Zeybek, Ş. Pekyardımcı, A novel electrochemical DNA biosensor based on poly-(5-amino-2-mercapto-1,3,4-thiadiazole) modified glassy carbon electrode for the determination of nitrofurantoin, Sensor. Actuat. B-Chem. 197 (2014) 211-219.
9. F. Berti, S. Todros, D. Lakshmi, M.J. Whitcombe, I. Chianella, M. Ferroni, S.A. Piletsky, A.P.F. Turner, G. Marrazza, Quasi-monodimensional polyaniline nanostructures for enhanced molecularly imprinted polymer-based sensing, Biosens. Bioelectron., 26 (2010) 497-503.

10. R.J. Geise, J.M. Adams, N.J. Barone, A.M. Yacynych, Electropolymerized films to prevent interferences and electrode fouling in biosensors, Biosens. Bioelectron., 6 (1991) 151-160.
11. B.D. Malhotra, A. Chaubey, S.P. Singh, Prospects of conducting polymers in biosensors, Anal. Chim. Acta, 578 (2006) 59-74.
12. T. Yang, W. Zhang, M. Du, K. Jiao, A PDDA/poly(2,6-pyridinedicarboxylic acid)-CNTs composite film DNA electrochemical sensor and its application for the detection of specific sequences related to PAT gene and NOS gene, Talanta, 75 (2008) 987-994.
13. J. Yang, T. Yang, Y. Feng, K. Jiao, A DNA electrochemical sensor based on nanogold-modified poly-2,6-pyridinedicarboxylic acid film and detection of PAT gene fragment, Anal. Biochem., 365 (2007) 24-30.
14. Y. Fang, E. Wang, Electrochemical biosensors on platforms of graphene, Chem. Commun., 49(83) (2013) 9526-9539.
15. Y. Fan, J.-H. Liu, C.-P. Yang, M. Yu, P. Liu, Graphene–polyaniline composite film modified electrode for voltammetric determination of 4-aminophenol, Sensor. Actuat. B-Chem., 157 (2011) 669-674.
16. N. Hui, S. Wang, H. Xie, S. Xu, S. Niu, X. Luo, Nickel nanoparticles modified conducting polymer composite of reduced graphene oxide doped poly(3,4-ethylenedioxythiophene) for enhanced nonenzymatic glucose sensing, Sensor. Actuat. B-Chem., 221 (2015) 606-613.
17. T.A. Pham, J.S. Kim, J.S. Kim, Y.T. Jeong, One-step reduction of graphene oxide with l-glutathione, Colloids and Surfaces A., 384 (2011) 543-548.
18. D.R. Dreyer, S. Park, C.W. Bielawski, R.S. Ruoff, The chemistry of graphene oxide, Chem. Soc. Rev., 39 (2010) 228-240.
19. G.A. Tığ, B. Zeybek, Ş. Pekyardımcı, Electrochemical DNA biosensor based on poly(2,6-pyridinedicarboxylic acid) modified glassy carbon electrode for the determination of anticancer drug gemcitabine, Talanta, 154 (2016) 312-321.
20. E. Pale–ek, R. Kizek, L. Havran, S. Billova, M. Fojta, Electrochemical enzyme-linked immunoassay in a DNA hybridization sensor, Anal. Chim. Acta, 469 (2002) 73-83.
21. A. Troisi, G. Orlandi, The hole transfer in DNA: calculation of electron coupling between close bases, Chem. Phys. Lett., 344 (2001) 509-518.
22. A. Erdem, B. Kosmider, R. Osiecka, E. Zyner, J. Ochocki, M. Ozsoz, Electrochemical genosensing of the interaction between the potential chemotherapeutic agent, cis-bis(3-aminoflavone)dichloroplatinum(II) and DNA in comparison with cis-DDP, J. Pharmaceut. Biomed., 38 (2005) 645-652.
23. B. Dogan-Topal, B. Uslu, S.A. Ozkan, Voltammetric studies on the HIV-1 inhibitory drug Efavirenz: The interaction between dsDNA and drug using electrochemical DNA biosensor and adsorptive stripping voltammetric determination on disposable pencil graphite electrode, Biosens. Bioelectron., 24 (2009) 2358-2364.
24. M.T. Carter, M. Rodriguez, A.J. Bard, Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt(III) and iron(II) with 1,10-phenanthroline and 2,2′-bipyridine, J. Am. Chem. Soc., 111 (1989) 8901-8911.
25. A.A. Ensafi, B. Rezaei, M. Amini, E. Heydari-Bafrooei, A novel sensitive DNA–biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods, Talanta, 88 (2012) 244-251.
26. M. Rahban, A. Divsalar, A.A. Saboury, A. Golestani, Nanotoxicity and Spectroscopy Studies of Silver Nanoparticle: Calf Thymus DNA and K562 as Targets, J. Phy. Chem-US., C 114 (2010) 5798-5803.
27. M. Sirajuddin, S. Ali, A. Badshah, Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry, J. Photoch. Photobio. B., 124 (2013) 1-19.
28. F. Cui, R. Huo, G. Hui, X. Lv, J. Jin, G. Zhang, W. Xing, Study on the interaction between aglycon of daunorubicin and calf thymus DNA by spectroscopy, J. Mol. Struct., 1001 (2011) 104-110.
29. S. Nafisi, A.A. Saboury, N. Keramat, J.-F. Neault, H.-A. Tajmir-Riahi, Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue, J. Mol. Struct., 827 (2007) 35-43.
30. J.H. Shi, J. Chen, J. Wang, Y.-Y. Zhu, Binding interaction between sorafenib and calf thymus DNA: Spectroscopic methodology, viscosity measurement and molecular docking, Spectrochim. Acta A., 136. (2015) 443-450.
31. L. Strekowski, B. Wilson, Noncovalent interactions with DNA: An overview, Mutat. Res-Fund. Mol., 623 (2007) 3-13.