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Highly selective electrochemical biosensor for the determination of folic acid based on DNA modified-pencil graphite electrode using response surface methodology

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ABSTRACT

An electrochemical DNA biosensor was proposed as a screening device for the rapid analysis of folic acid using a pencil graphite electrode modified with salmon sperm ds-DNA. At first, immobilization of the ds-DNA on pencil graphite electrode was optimized using response surface methodology. Solution pH, DNA concentration, time of DNA deposition and potential of deposition was optimized each at three levels. The optimum combinations for the reaction were pH 4.8, DNA concentration of 24 μg mL^{-1}, deposition time of 304 s, and deposition potential of 0.60 V, by which the adenine signal was recorded as 3.04 μA. Secondly the binding of folic acid to DNA immobilized on a pencil graphite electrode was measured through the variation of the electrochemical signal of adenine. Folic acid could be measure in the range of 0.1–10.0 μmol L^{-1} with a detection limit of 1.06×10^{-8} μmol L^{-1}. The relative standard deviations for ten replicate differential pulse voltammetric measurements of 2.0 and 5.0 μmol L^{-1} folic acid were 4.6% and 4.3%, respectively. The biosensor was successfully used to measure folic acid in different real samples.

1. Introduction

One of the water-soluble vitamin B groups is folic acid that is a tasteless and odorless yellowish orange crystal. Folate has been identified as one of the most important vitamin for normal human metabolic function. The amount of folic acid perception is proper to neural tube defects (NTD) in newborns, cardiovascular diseases, colon cancer and certain anemia [1]. Also, folic acid is essential for cell growth and division and participates in lots of reactions in body and mainly in synthesis of nucleic acid and some important substances. It promotes the synthesis of protein from amino acid, in human body. Folate is not synthesized in humans, thus it should be obtained from dietary sources. In many countries mean folate intake was found to be lower than recommended or desire. To reduce the risk of woman of childbearing age to have a child with neural tube defect, some countries mandated folate fortification of staple food [1]. Recently, high-performance liquid chromatography (HPLC) [2,3], HPLC–MS [4], colorimetry [5], spectrophotometry [6], chemiluminescence [7], flouorimetric [8] and microbial methods [9] have been reported for the determination of folic acid. However, most of these methods are expensive and time consuming and/or suffer from many interfering compounds, and thus restrict their applications in food analysis.

It is well known that electrochemical methods are simple and inexpensive, in which analytical techniques require small amount of sample [2,10]. Electrochemical devices are easy to miniaturize, simple, and inexpensive compared with optical instrumentation [11]. Electrochemistry can overcome on the problem of coupling tiny chips with large readout optical systems. In addition, turbidity of sample is not a matter of concern and powerful sources of energy, for example lasers, are not required [12]. Indeed the use of electrochemical techniques instead of fluorescence allows for simpler and smaller detectors [13].

There are some studies about utilizing electrochemical methods for folic acid detection, but many of them are not selective, and sometimes are not sensitive sufficiently. Biosensors have higher selectivity than other sensors. One of the natural polymers is deoxyribonucleic acid (DNA) that has gained increasing attention in biosensor designing [14]. Since the discovery of electrochemical activity of nucleic acids by Palececk, huge promotion particularly by the development of electrochemical DNA biosensors based on the concept of chemically modified electrodes have been developed [12]. DNA is one of the most important biological molecules targeted by many small molecules [15]. Small molecule could bind with DNA by making covalent or non covalent binding. Intercalation between the stacked base pairs of native DNA, electrostatic interactions with the negative-charged sugar-phosphate at the out of DNA structure and binding interactions with both minor and major grooves of DNA double helix are three modes of non-covalent DNA interactions with small molecules [16,17]. The compound–DNA interaction can be irreversible, causing subsequent destabilization, breaking of the hydrogen bonds, and opening of the double helix [18]. This will lead to exposure of the DNA purine bases

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guanine and adenine, which can be oxidized at +0.80 V and +1.10 V respectively, on a carbon electrode; where quantification of the process enables DNA damage evaluation [19]. Characteristics such as low-cost, easy-to-use, portability, and miniaturizable, along with maintaining the level of accuracy and sensitivity of laboratory diagnostics, made DNA diagnostic systems highly desirable [20]. Given the fundamental importance of ds-DNA and specific interactions with selective compounds [21], we decided to investigate the interaction of folic acid and a ds-DNA by electrochemical methods.

Using pencil graphite as disposable electrodes (PGE) in electrochemical DNA sensing make the method easier and more rapid compared with the conventional DNA biosensors. The use of pencil graphite electrodes have several advantages, such as avoidance of contamination among samples, ease of use due to without any need to pretreatment, constant sensitivity, selectivity and reproducibility [22]. Testing of water, food, soil and plant samples for the presence of pathogenic microorganisms along with carcinogens, drugs [12], mutagenic pollutants [23], etc. is one of the applications of DNA electrochemical biosensors [12].

The objective of this study was to develop a disposable electrochemical DNA-sensor for folic acid detection based on new optimization method. Changes in the electrochemical signal of adenine in the salmon sperm ds-DNA used as an analytical signal for folic acid determination. The current study is broadly divided into two sections. The first part involves the design and optimization of the DNA sensor using response surface methodology (RSM) based on the changes of adenine signal on the modified PGE. The influence of pH, ds-DNA concentration, time of deposition, and potential of deposition on the oxidation peak current of the adenine was evaluated in the model system. In the second part, detection and determination of folic acid at the ds-DNA–PGE using differential pulse voltammetry was carried out.

2. Experimental

2.1. Chemicals

Reagent grade folic acid, Tris–HCl, EDTA, CH_3COOH, NaOH, and NaCl were purchased from Aldrich Chemicals (Milwaukee, USA).

Double-strand salmon sperm DNA (ds-DNA, was purchased from Sigma (St. Louis, USA). A salmon sperm ds-DNA stock solution (100 mg L^{-1}) was prepared in Tris–HCl (TE) buffer (pH 7.0) and kept frozen. More diluted solutions of the ds-DNA were prepared daily with acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl.

Stock solutions of folic acid (1.0 mmol L^{-1}) were prepared by dissolving accurately weighed amounts of folic acid in NaOH (0.10 mol L^{-1}). The solution was kept dark at 4 °C. Folic acid working solutions for voltammetric investigations were prepared by dilution of the stock solution with Tris buffer (pH 8.5). All solutions were prepared using reagent grade chemicals.

2.2. Apparatus

Pencil graphite electrode (2H, with two mm in diameter) composed of natural graphite and a polymeric binder and clay, was prepared from Rotring Co., Germany.

Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand, with GPES 4.9 software package (Eco Chemie, The Netherlands), was used for the electrochemical measurements. The raw data was treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction with a "peak width" of 0.01. The three-electrode system composed of a PGE or modified PGE as the working electrodes, an Ag/AgCl (3 mol L^{-1} KCl) as a reference electrode, and a platinum wire as an auxiliary electrode.

UV–Vis spectra were measured with a double beam spectrophotometer, Jasco Model V-750, using 1.0 cm quartz cells.

2.3. Preparation of PGE

The pencil lead's body was wrapped with Teflon bond, and the electrical contact was procured with joining a copper wire in one end. The cross section of another end that is contacted with solution was polished on a weighing paper before each use to a smoothed finish. The PGE was fixed vertically in the electrochemical cell [24].

2.4. Activation of PGE

The oxidation signal of guanine and adenine at untreated PGE could not be seen; that means that no ds-DNA was immobilized on the electrode surface and this could be resulted from impurity existed on the surface of PGE. So the polished PGE was immersed vertically in 10 mL acetate buffer (0.50 mol L^{-1}, containing 20 mmol L^{-1} NaCl) and a potential of 1.80 V was applied for 300 s without stirring the solution to activate PGE [24].

2.5. Immobilization of the ds-DNA

The ds-DNA was immobilized on the activated electrode by applying different potential at 5 levels in 10 mL of acetate buffer (0.50 mol L^{-1}, pH 4.8 containing 20 mmol L^{-1} NaCl) containing different concentrations of the ds-DNA by stirring. The electrode was then rinsed with acetate buffer (0.50 mol L^{-1}, pH 4.8 containing 20 mmol L^{-1} NaCl) [14]. After that, the oxidation signal of the adenine was obtained using differential pulse voltammetry (DPV), with a scan rate of 10 mV s^{-1} between +0.40 and +1.40 V. The data were baseline corrected with GPES software [25]. The optimum conditions were accumulation potential of 0.60 V, pH 4.8 (acetate buffer, 0.50 mol L^{-1}, pH 4.8 containing 20 mmol L^{-1} NaCl), the ds-DNA concentration of 24.0 μg mL^{-1} and deposition time of 304 s.

2.6. Interaction of folic acid with the ds-DNA and electrochemical analysis

A new ds-DNA–modified PGE was used by dipping it into 10 mL Tris–HCl buffer (pH 8.5) containing different concentrations of folic acid with stirring for different times at an open circuit system. After the accumulation, the ds-DNA–modified PGE was rinsed with acetate buffer (0.50 mol L^{-1}, pH 4.8 containing 20 mmol L^{-1} NaCl) for 10 s and it was placed in 10 mL of the acetate buffer solution, where differential pulse voltammograms were recorded. The analytical signal (I) represented the differences in the oxidation peak current of adenine before and after interaction with folic acid at the ds-DNA–modified PGE [25].

2.7. Real sample preparation

One tablet (5.0 mg) was grinded and dissolved in 0.10 mol L^{-1} NaOH in a 100 mL standard flask, and after 15 min stirring, the mixture was filtered. After that, a suitable aliquot (100 μL) of the clear filtrate was diluted with Tris–HCl buffer (pH 8.5) to prepare appropriate sample solution [26]. Fortified wheat flour (fortified with 1.5 ppm folic acid) was prepared by the method of Xiao et al. with some modification [27]. The wheat flour was dispersed in appropriate amount of 0.10 mol L^{-1} NaOH solution and stirred. Then, the solution was centrifuged for 10 min at 12,000 rpm. The mixture was filtered with 0.45 μm micropore membrane. The pH of the resulting solution was adjusted at 8.5 (using the Tris buffer) for the voltammetric determination.

For measuring folic acid in spinach, 1000 g of spinach was cleaned, washed with water and dried at room temperature. Then, 100 g of chopped spinach was weighed and transferred into a 1000 mL

standard flask containing 100 mL of 0.10 mol L^{-1} NaOH and homogenized for 10 min, after that it was centrifuged for 10 min in 12,000 rpm. The supernatant was filtered with 0.45 μm micropore membrane. The pH of the resulting solution was adjusted at 8.5 for the voltammetric determination.

2.8. Experimental design

Minitab software, version 14.0, was employed for experimental design, data analysis and model building. The central composite design with 4 variables was used to determine the response pattern and then to establish a model. In the study four variables used each at three levels as presented in Table 1. The experimental and predicted values from these models are presented in Table 2. The actual set of experiments performed (experimental runs 1–31) is shown in Table 2. Seven replicates at the center of the design were used to allow for estimation of a pure error of the sum of the squares. A full quadratic equation or the diminished form of this equation was used as:

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j X_j + \sum_{j=1}^{k}\beta_{jj}X_j^2 + \sum\sum_{i<j}^{k}\beta_{ij}X_iX_j \quad (1)$$

Where Y is the estimated response and β_0, β_j, β_{jj}, and β_{ij} are the regression coefficients for the intercept, linearity, square, and interaction terms, respectively. X_i and X_j are the coded independent variables.

3. Results and discussion

3.1. DNA sensor behavior, optimization the ds-DNA–modified PGE

Folic acid has specific interaction with the salmon sperm ds-DNA. Changes in the oxidation signal of adenine in the salmon sperm ds-DNA used as an analytical signal for folic acid determination. The sensor material and the degree of surface coverage influence the biosensor response. Those are also a critical issue in the development of a DNA electrochemical biosensor for rapid detection of DNA interaction. The redox behavior of original ds-DNA immobilized PGE exhibited two oxidation processes of adenine and guanine residues. The interaction of folic acid with the ds-DNA leads to variation in the initial signal height of the adenine, whereas the signal of guanine did not change significantly. For this reason, only adenine signal was considered as a prob. To optimize the conditions for the preparation of the ds-DNA-modified PGE, different parameters including the pH of ds-DNA solution, accumulation potential, the ds-DNA concentration, and deposition time were optimized using RSM.

3.2. Model fitting and analysis

The mathematical models representing the adenine signal height (μA) as a function of the independent variables within the region of investigation are expressed by the following equations:

$$Y = 1.56 + 0.06X_1 + 0.15X_2 + 0.08X_3 + 0.16X_4 - 0.03X_1^2 - 0.02X_2^2 - 0.02X_3^2 + 0.03X_4^2 \quad (2)$$

Table 1
Coded settings for different parameters according to a central composite design.

Independent variable	Symbols	Range and levels				
		−2	−1	0	1	2
pH	X_1	4.2	4.5	4.8	5.1	5.4
DNA concentration (μg mL^{-1})	X_2	4	12	20	28	36
Deposition time (s)	X_3	50	175	300	425	550
Deposition potential (V)	X_4	0.2	0.3	0.4	0.5	0.6

Table 2
Experimental design used in the RSM studies and real responses and predicted value with RSM.

Runs	Variables Codes				Adenine peak height (μA)	Predicted value
	X_1	X_2	X_3	X_4		
1	2	0	0	0	1.40	1.56
2	1	1	−1	−1	1.32	1.49
3	1	−1	−1	−1	0.77	1.19
4	−2	0	0	0	0.81	1.32
5	−1	−1	1	1	1.34	1.55
6	1	−1	−1	1	1.34	1.51
7	0	0	0	0	1.56	1.56
8	0	0	0	0	1.62	1.56
9	0	0	−2	0	0.63	1.32
10	0	0	0	0	1.54	1.56
11	1	1	−1	1	1.89	1.81
12	0	−2	0	0	0.57	1.18
13	1	−1	1	1	1.44	1.67
14	1	1	1	1	2.11	1.97
15	−1	1	1	−1	1.20	1.53
16	0	0	0	0	1.57	1.56
17	−1	1	−1	−1	1.12	1.37
18	0	2	0	0	2.04	1.78
19	−1	−1	−1	−1	0.65	1.07
20	1	1	1	−1	1.81	1.65
21	0	0	0	0	1.56	1.56
22	0	0	0	0	1.54	1.56
23	0	0	0	0	1.59	1.56
24	−1	1	1	1	1.83	1.85
25	0	0	0	2	3.19	2.5
26	−1	−1	1	−1	0.70	1.23
27	0	0	0	−2	1.35	1.36
28	0	0	2	0	1.87	1.64
29	1	−1	1	−1	1.15	1.35
30	−1	1	−1	1	1.85	1.69
31	−1	−1	−1	1	1.28	1.39

As shown in Table 2 the predicted value of response and experimental data are slightly different. In Table 3, the analysis of variance and error are presented. The p-value of model is <0.001 that means the model is significant.

3.3. Effect of different variables on the adenine signal height

As shown in Fig. 1, a, b and c, the optimum pH that produced maximum adenine signal height was about 4.8 while lower or higher pH cause decreasing the signal height. Deoxy ribonucleic acid contains adenine and guanine base that oxidized in acidic pH on pencil graphite electrode [28]. Fig. 1, a, d and e show the influence of the ds-DNA concentration (on the preparing the modified PGE) on the adenine signal height. The results showed that increasing in the concentration of ds-DNA leads to increase in the signal height. This could be resulted from availability of more ds-DNA for the immobilization at the surface of PGE. Fig. 1b, e, and f showed that by increasing in the deposition time, the ds-DNA had longer time to reach to electrode

Table 3
Analysis of the variance (ANOVA) for the fit of the experimental data to response surface model.

Source	Degree of freedom	Sum of squares	Mean square	F-value	p-Value
Regression	14	7.97	0.56	15.03	0.00[a]
Linear	4	5.97	1.49	39.41	0.00[a]
Square	4	1.84	0.46	12.18	0.00[a]
Interaction	6	0.15	0.02	0.68	0.67[ns]
Residual error	16	0.60	0.03		
Total	30	8.58			

[ns] Not significant at $p > 0.5$.
[a] Significant at $p < 0.001$.

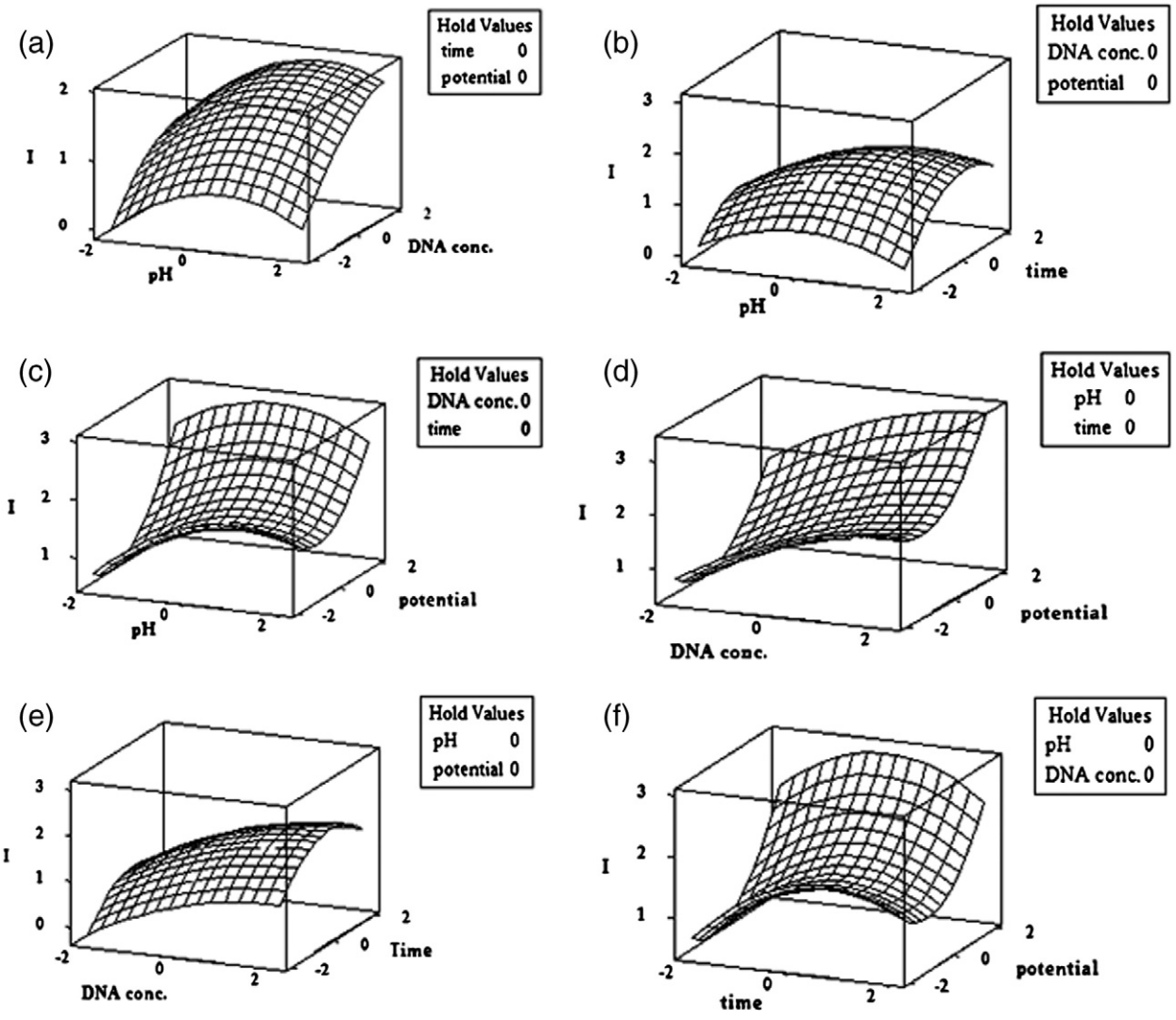

Fig. 1. 3D plot of the effect of different parameters on the adenine oxidation peak current (variable values are shown as coded value).

surface, thus the signal was increased. This increasing was continued up to 400 s, whereas after 400 s the signal was decreased. This is due to the fact that the electrode destruction occurred after performing high potential in longer time. The optimum potential for the deposition was 0.70 V (Fig. 1(c, d, f)) that is higher than those of reported in the other studies [17,18,22]. The deposition potential that applied during the immobilization causing the enhancement in the stability of the probe through the electrostatic attraction between the positively charge surface of the electrode and the negatively charged sugar-phosphate backbone of DNA [29]. It is probable that further positive charges were engendered in 0.70 V than 0.50 V, thus more ds-DNA was immobilized.

3.4. Optimization of adenine signal

From the computed predictions, the optimal conditions to obtain the highest adenine signal height were determined as 3.0 μA. Getting this value seems to be suitable in the lowest DNA concentration and time of deposition. However, there is not any limitation in the solution pH and the deposition voltage selection. Therefore, in levels of pH and deposition voltage that produced highest level of current (pH 4.8 and deposition potential of 0.70), the lowest level of DNA concentration and time of deposition (DNA concentration of 24 μg mL^{-1} and deposition time of 304 s) was selected. By this level of variables the current was higher than 3.0 μA. These values are not significantly different ($p > 0.05$) from the predicted values of 3.0058.

3.5. Interaction of folic acid with the ds-DNA at the modified-PGE

After electrochemical deposition of the ds-DNA, anodic peaks due to the oxidation of adenine and guanine residues in the ds-DNA could be observed at around +1.00 and +1.30 V, respectively [30]. On the other hand, any damage in these signals is due to the interaction of the ds-DNA with folic acid occurred at adenine and/or guanine. As shown in Fig. 2, an increase in the folic acid concentration leads to a higher decrease in the adenine signal height. Folic acid affects the adenine signal greatly, whereas the guanine signal does not considerably change. This may be due to the topology and structure of folic acid. It seems that folic acid could interact with adenine in the ds-DNA chain, whereas guanine could not interact with folic acid. Therefore, change in the signal height of adenine (decreasing) after this interaction was considered as a suitable probe for folic acid determination.

To study the interaction of folic acid with the ds-DNA, different time and pH values were examined. The effect of solution pH was studied from 7.0 to 11.0. The results showed that pH 8.5 was the best and that folic acid interacts with the ds-DNA even in low concentration, but the ds-DNA was not damaged. In addition different times from 180 s to 600 s as an interaction time, between folic acid and the

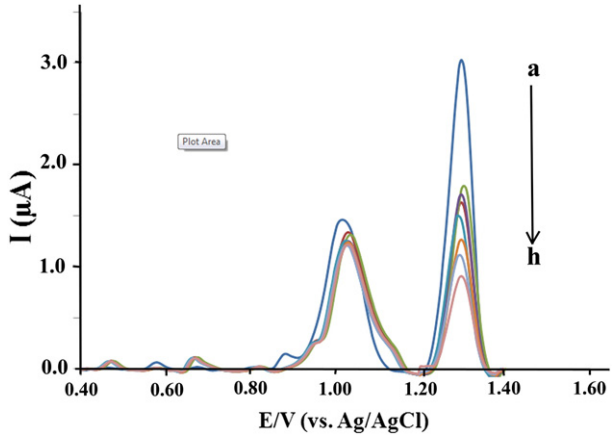

Fig. 2. Effect of folic acid concentration on the adenine and guanine peak current at DNA modified-PGE: a) in acetate buffer (pH 4.8), b): 0.10 µmol L^{-1} folic acid in the acetate buffer; c): 0.50 µmol L^{-1} folic acid in the acetate buffer; d) 1.0 µmol L^{-1} folic acid in the acetate buffer; e) 2.0 µmol L^{-1} folic acid in the acetate buffer; f) 5.0 µmol L^{-1} folic acid in the acetate buffer; g) 7.0 µmol L^{-1} folic acid in the acetate buffer; and h) 9.0 µmol L^{-1} folic acid in the acetate buffer.

ds-DNA, was studied on the signal sensitivity. The results confirmed that with 420 s as an interaction time, the system reaches to the maximum interaction. Higher incubation time ($t > 420$ s) could not create significant change in the responses of the electrode, because the immobilized DNA was saturated with folic acid after 420 s. Therefore, 420 s was selected as the optimum incubation time.

4. Figures of merit

The oxidation peak current of adenine at pH 4.8 and at the optimized electrochemical conditions were used to construct the calibration graphs. As shown in Fig. 3 the decrease in the oxidation signal of adenine was linear in the range of 0.1–10.0 µmol L^{-1} with a correlation equation of $I\ (\mu A) = -0.0941C + 1.7491$ ($R^2 = 0.9863$, $n = 7$) where, C is folic acid concentration in µmol L^{-1}.

The detection limit was found as 1.06×10^{-8} mol L^{-1} folic acid based on adenine signal height.

The repeatability and stability of the modified electrode were investigated by differential pulse voltammetric measurements of 2.0 and 5.0 µmol L^{-1} folic acid at the optimum conditions. The results of our studied showed the relative standard deviations of 4.6% and 4.3%, respectively for ten successive assays. When using four different sensors (prepared independently in 4 days) with the same procedure and used on the same samples (1.0 µmol L^{-1} folic acid) the RSD% for four measurements was 4.9%. In addition, the response of the biosensor to 10.0 µmol folic acid during experiment after 15, 30 and 45 min remains stable during the experimental time (a decrease of only 3.1% observed in the current after 45 min). This is due to the fact that no diffusion of DNA from the electrode to the solution occurred and no inhibition effect observed from the oxidation products of folic acid or probable contaminant for the modified electrode surface. In addition, reproducibility is of the modified electrode was checked. Therefore, the DNA modified pencil graphite electrode has an excellent and stable operation for the measurement of folic acid. Here the proposed electrode (biosensor) is a single use electrode. Single-use sensors have several advantages, such as avoidance of contamination among samples, constant sensitivity and reproducibility and ease of use because no pretreatment is needed. Avoidance of contamination due to its disposability caused to increase in repeatability.

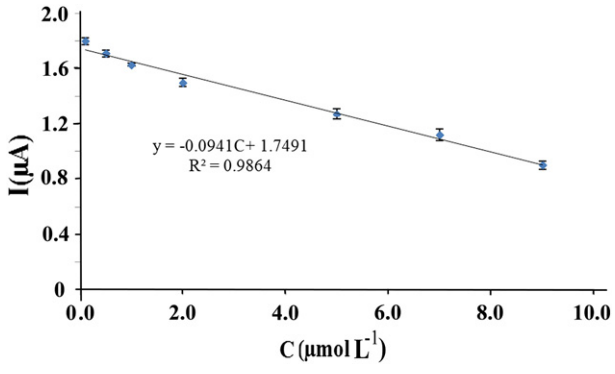

Fig. 3. Calibration curve for folic acid at the surface of the ds-DNA modified-PGE (error bars show the standard deviation for five replicate measurements).

UV–Vis absorption is one of the spectroscopic techniques generally employed to investigate drug–DNA interactions. The interaction between folic acid and the ds-DNA was studied with absorption spectrophotometry. The absorption spectra of folic acid before and after its interaction with the ds-DNA are shown in Fig. 4. This figure showed that the absorbance of folic acid decreased with the addition of the ds-DNA. These results revealed that there is a strong interaction between folic acid and the ds-DNA. In addition, because the red shift for the absorption peaks is less than 10 nm we propose groove binding [31] mode between folic acid and the ds-DNA.

Table 4 shows the figures of merit of the proposed sensor vs. reported electrochemical methods for analysis of folic acid [10,26,32–37]. These results confirm that the proposed method is more selective for folic acid determination than other pervious reported electrochemical methods.

5. Analytical performance

Before real sample analysis, the effect of potential interferences compounds on the performance of the biosensor in folic acid determination was studied with several potential interfering substances. The potential interfering substances were chosen from the group of substances commonly found with folic acid in cereal. This study was done at the optimum conditions and 420 s was used as an incubation time. The voltammetric responses resulting from the presence of interfering molecules were compared with those obtained for pure folic acid. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ± 3% for determination of folic acid. The results showed that 1000-fold of glucose, sucrose, ascorbic acid, Ca^{2+} and Fe^{2+}, 500-fold of vitamin B_1, vitamin B_2, vitamin B_6, nicotinamide and tannic acid did not affect the selectivity. These results confirm that the proposed method is selective for folic acid determination.

Folic acid tablet, Fortified wheat flour and spinach were chosen as real sample for folic acid analysis with standard addition method. The results are given in Table 5, which shows that the new biosensor is capable to measure folic acid in different real samples.

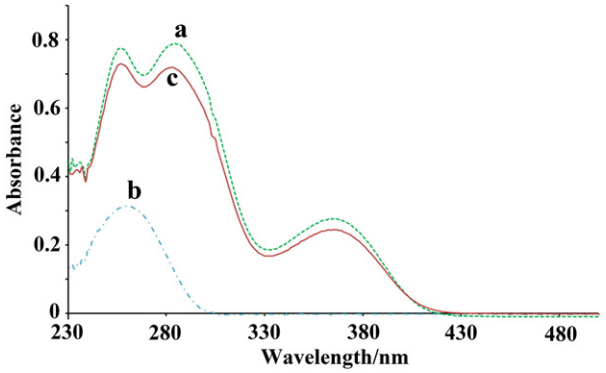

Fig. 4. UV–Vis spectra of a) 1.0×10^{-5} mol L^{-1} folic acid; b) 20.0 µg mL^{-1} ds-DNA; and c) 1.0×10^{-5} mol L^{-1} folic acid plus 20.0 µg mL^{-1} ds-DNA. Conditions: Tris–HCl buffer (pH 8.5).

Table 4
Analytical parameters for several modified electrodes for determination of folic acid using electrochemical methods.

Folic acid sensor	Detection limit (μmol L^{-1})	Linear range (μmol L^{-1})	Interference (Tolerance limit)					Reference
			B_1[a]	B_2	B_3	B_6	C	
MWCNT/GC	0.134	0.3–80	NS[b]	NS	NS	NS	NS	[10]
SWCNT/GC	0.001	0.01–100	NS	NS	NS	NS	100	[32]
AgSAE–Hg	0.0005	Not reported	NS	NS	NS	NS	NS	[26]
Calixarenes/CP	0.0012	8.79×10^{-3}–1.93	NS	10	10	10	1	[33]
Ni/POA/CP	90	100–5000	NS	NS	NS	NS	NS	[34]
CuO-MWCNT/GC	0.0015	0.01–0.9 2–24	NS	NS	NS	NS	NS	[35]
MBT/SAM/Au	0.01	0.008–1	NS	NS	NS	NS	NS	[36]
MWCNT/Au	0.01	0.02–1	50	1	NS	NS	50	[37]
DNA/PGE	0.0106	0.1–10.0	500	500	500	500	1000	This work

[a] B_1: thiamine; B_2: riboflavin; B3: niacin; B6: pyridoxine hydrochloride; C: ascorbic acid; GCE: glassy carbon electrode; MWCNT: multiwall carbon nanotubes; SWCNT: single wall carbon nanotube; AgSAE: silver solid amalgam electrode; Hg: mercury electrode; CP: carbon paste electrode; POA: poly(orto-anisidine); MBT: mercapto benzothiazole; SAM: self assembly monolayer; Au: gold electrode.
[b] Not studied.

Table 5
Recoveries of folic acid in wheat flour.

Sample	Number	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery%
Tablet (5.0 mg)	1	–	1.08	–
	2	3.00	4.06	99.0
	3	4.00	5.02	98.4
Fortified wheat flour	1	–	0.40	–
	2	3.00	3.43	100.8
	3	4.00	4.37	99.3
Spinach	1	–	0.25	–
	2	3.00	3.22	99.1
	3	4.00	4.28	100.0

6. Conclusion

At the present work, a selective DNA-biosensor was introduced for the determination of folic acid in real sample such as wheat flour and spinach. As a result of the interaction between folic acid in different concentrations with the ds-DNA, a decreasing trend of the response based on the signal of adenine was observed. The differences between the oxidation signal intensity of adenine before and after its interaction with folic acid was used for the determination of folic acid. RSM modeling was used to optimize the DNA modified-PGE preparation. A sensitive and highly selective DNA biosensor could in principle reduces and even eliminates the need for sample pre-separation. In addition this sensor is fast responding, and simple to use without the need for target labeling or the reagent. Pencil graphite electrode is ready to use and there is no need to any time for making (like carbon paste electrode) or pretreatment (gold electrode) before utilizing. As shown on Table 4, although some of the reported electrochemical methods are more sensitive than the proposed biosensor, but they suffer from many interfering compounds, whereas the proposed biosensor is highly selective for folic acid detection.

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