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DNA-functionalized biosensor for riboflavin based electrochemical interaction on pretreated pencil graphite electrode

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ABSTRACT

The interaction of riboflavin with salmon sperm double-stranded DNA based on the decreasing of the oxidation signal of guanine and adenine bases was studied electrochemically with a pencil graphite electrode (PGE) using differential pulse voltammetry. The decrease in the intensity of the guanine and adenine oxidation signals after interaction with riboflavin was used as an indicator signals for the sensitive determination of riboflavin. Under the optimum conditions, a linear dependence of the guanine and adenine oxidation signals was observed for the riboflavin concentration in the range of 0.5–70 $\mu g\,mL^{-1}$ with a detection limit of 0.34 $\mu g\,mL^{-1}$ at ds-DNA modified PGE. The reproducibility and applicability of the analysis to pharmaceutical dosage forms and urine sample were also investigated. These results showed that this DNA biosensor could be used for the sensitive, rapid, simple and cost effective detection and determination of riboflavin-ds-DNA interaction. Pretreated pencil graphite electrode (PPGE) was also used for the determination of riboflavin by differential pulse adsorptive stripping voltammetry. With PPGE, a linear relationship was obtained for riboflavin over the concentration range of 0.003–0.88 $\mu g\,mL^{-1}$ with differential pulse adsorptive stripping voltammetric signal and with a detection limit of 0.076 $ng\,mL^{-1}$. Both determination methods were fully validated and applied for the analysis of riboflavin.

1. Introduction

Riboflavin (vitamin B_2) is a well-known vitamin, which plays an important role in biological oxidation and reduction (Ball, 2006). This vitamin is biochemically vital for proper utilization of carbohydrates, fats, and proteins as energy sources. It is a component of two coenzymes, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). In general, FAD and FMN, when tightly bound to specific enzymes, easily lose or gain one or two electrons or hydrogen atoms, and thus bring about oxidation–reduction reactions (Ball, 2006). The literature relating riboflavin with cancer is complex. Some studies indicate that riboflavin deficiency increases the risk of cancer at certain sites, whereas others point to a possible attenuating effect of riboflavin in the presence of some carcinogens and a protective effect of deficiency (Qiao, 1989; Rivlin, 1973). Riboflavin is a cellular photosensitizer that worsens the damage caused by solar radiation. The combination of riboflavin and ultraviolet-A radiation has recently been shown to increase gene mutations by seven times compared to UV-A radiation exposure alone (Besaratinia et al., 2007). The determination of trace amounts of riboflavin is important for the quality control of forages and vitaminized supplements (premixes) for animals and poultry, whose production and range increase from year-to-year. This, in turn, requires perfection of methods for determining vitamin B_2 and development of rapid, selective, and highly sensitive techniques to be used in complex multicomponent feed and vitaminized mixtures. The content of riboflavin varies in a wide range between 10 and 3500 mg/kg. Also, the concentration of riboflavin in urine is greatly influenced by many factors such as dietary intake, nutritional supplement use, health and physical condition, etc. Therefore, the determination of riboflavin in urine samples can considerably help to diagnose and treat some diseases. Various techniques have been used for determination of riboflavin. These include chemiluminescence (Zhang and Qi, 2002), high performance liquid chromatography (HPLC) coupled with fluorimetric (Analytical Methods Committee, 2000; Ndaw et al., 2000; Vinas et al., 2004) or ultraviolet (Albalá-Hurtado et al., 1997; Kozhanova et al., 2002), and mass spectrometry (Mandal et al., 2009), voltammetry (Anisomova et al., 2001; Bás et al., 2011; Gu et al., 2001; Kadara et al., 2006; Mielech, 2003; Shiu and Shi, 2000; Slepchenko et al., 2005), surface plasmon resonance (Caelen et al., 2004), fluorescence (Lorent-Martínez et al., 2006) and spectrophotometry (Perez-Ruiz et al., 1994). HPLC is widely used for determination of riboflavin in food samples because of its ability to effect rapid separation, and its high sensitivity of detection. The high cost of the required instrumentation and/or complicated and time consuming

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procedures are, however, a disadvantage. Electrochemical methods are a more attractive option because they are inexpensive, highly sensitive, and have long-term reliability and reproducibility.

Nucleic acids offer a powerful tool in the recognition and monitoring of many important compounds such as drugs (Brabec et al., 1996; Hason et al., 2002a,b; Mascini et al., 2001; Palecek et al., 2002; Strasak et al., 2002; Wang et al., 1997). The interaction of ds-DNA with other molecules especially drug compounds is an important issue in life science. The investigation based on DNA interaction is invaluable in order to understand the mechanism of many drug compounds and designing of new DNA biosensors (Dogan-Topal et al., 2009). Electrochemical DNA biosensors are fabricated by immobilizing DNA on the electrode surface (Dogan-Topal et al., 2009; Mirmomtaz and Ensafi, 2009; Mirmomtaz et al., 2009; Yardıma et al., 2010).

As yet, electrochemical detection based on the interaction of riboflavin with a ds-DNA regarding the changes of the oxidation of guanine and adenine has not been carried out. Over the last two decades, mercury electrode and mercury-film electrode have provided valuable tools for adsorptive stripping voltammetry. Their main drawback, which affects our environment, is the extreme toxicity of mercury and the mercury salts employed for the preparation of mercury electrodes. As a result, electrode materials that can potentially replace mercury are continually being sought. One of these electrodes is pencil graphite electrode (PGE) with the advantages of cost effectiveness, commercial availability, and disposability. However, the electron transfer event on this dynamic electrode heavily depends on the prior history of pretreatment before it is actually used in the analysis. Various pretreatment (or activation) procedures, depending on the kind of analysis and nature of the redox system, have been adopted to achieve faster electron transfer rates and more reproducible results (Ozcan and Sahin, 2009, 2010). Such pretreatments are believed to result in an increase of surface quinone and other functional groups, which can then catalyze the oxidation/reduction of the analyte.

Current paper is broadly divided into two sections. In the first part, we report the construction of a biosensor for determination of riboflavin as DNA intercalation on salmon sperm ds-DNA-modified pencil graphite electrode (DNA-PGE). The second part involves the use of adsorptive stripping voltammetry on pretreated disposable PGE in aqueous solutions for the determination of riboflavin in real samples such as urine and tablets. The biosensor is highly selective, rapid, and sensitive for riboflavin determination.

2. Material and methods

2.1. Chemicals

All chemicals were of reagent grade quality and used as received without further purification. Riboflavin was purchased from Aldrich Chemicals. Double-stranded salmon sperm DNA (ds-DNA, catalog No. D8899) was purchased from Sigma (St. Louis, USA). Reagent grade Tris–HCl, CH_3COOH, CH_3COONa, EDTA, NaCl and NaOH were purchased from Aldrich Chemicals (Milwaukee, USA).

A salmon sperm ds-DNA stock solution (100 mg L^{-1}) was prepared in Tris–HCl buffer and kept frozen. More diluted solutions of the ds-DNA were prepared with acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl.

1.0 mmol L^{-1} riboflavin solution was prepared and stored at 4 °C. Riboflavin working solutions were prepared by diluting the stock with acetate buffer (pH 4.8) containing 0.02 mol L^{-1} NaCl.

Real samples pretreatments are described in Supplementary file.

2.2. Apparatus

Electrochemical measurements were performed using an Autolab PGSTAT 12, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand, with a GPES 4.9 software package (Eco Chemie, The Netherlands). An Ag/AgCl (3 mol L^{-1} KCl) was used as a reference electrode. A platinum wire was used as a counter electrode. A standard one-compartment three-electrode cell of 10 mL capacity was used in all the experiments. The renewable PGE described in the study of Erdem et al. (2003) was used in all the experiments. A Noki pencil was used as a holder for pentel graphite leads. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically, with 12 mm of the lead extruded outside (9 mm of which was immersed in the solution). All the electroanalytical measurements were performed at room temperature.

Association of Official Agricultural Chemists (AOAC) method of analysis for riboflavin was used based on fluorimetric determination (AOAC, 2005). The pretreatment of sample and the analysis procedures were performed accordingly. The fluorescence signal at 565 nm was measured using a Jasco FP750 spectrofluorometer with an excitation radiation at 440 nm.

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

2.3. Procedure

2.3.1. Interaction between ds-DNA and riboflavin at the ds-DNA-modified electrode

For adsorptive transfer of the ds-DNA on a PGE, the surface of PGE was pretreated at +1.40 V for 60 s in a quiescent solution (10 mL) of 0.5 mol L^{-1} acetate buffer containing 0.02 mol L^{-1} NaCl (pH 4.8). Then, the ds-DNA was immobilized on a pretreated PGE by applying a potential at +0.50 V for different preselected times (optimum time of 200 s) from a stirred (200 rpm) solution containing 10.0 μg mL^{-1} ds-DNA in the acetate buffer containing 0.02 mol L^{-1} NaCl. The electrode was then gently rinsed with the acetate buffer for 5 s to remove unbounded ds-DNA. The voltammetric stripping step was performed by employing a positive-going differential pulse potential scan (from 0.40 to 1.40), using a pulse amplitude of 50 mV, a modulation time of 0.05 s, and a step potential of 8 mV in the blank solution (the acetate buffer plus 0.02 mol L^{-1} NaCl). The oxidation signals of guanine and adenine were recorded, and labeled as I_b. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the GPES software moving average baseline correction, using a "peak width" of 0.01. The procedure was repeated using a new PGE. The ds-DNA-modified PGE was then immersed into a Tris buffer solution (pH 7.0) containing different concentrations of riboflavin when the solution was stirred at 200 rpm for 300 s at an open circuit system. After accumulation of riboflavin, the ds-DNA-modified PGE was rinsed and placed in riboflavin free acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl. Then, differential pulse voltammograms were recorded and labeled as I_s. Calibration curve was constructed as the net signal ($I_b - I_s$) vs. riboflavin concentration.

2.3.2. Adsorptive stripping voltammetric assay

Cyclic voltammetry was used for the electrochemical treatment of PGE in the subsequent experiments. The potential was cycled between ((−1.00 and +2.00 V), (−0.20 and +2.00 V), (+0.30 and +2.00 V), (+0.80 and +2.00 V) and (+1.30 and +2.00 V)) at a scan rate of 100 mV s^{-1} for five cycles. The electrode surface became colored after the activation. Pencil graphite electrodes obtained after electrochemical activation were designated as PPGE in this study. PPGE was first immersed into a stirred sample solution (400 rpm) containing the desired riboflavin concentration in phosphate buffer solution (PBS 0.1 mol L^{-1}, pH 7.0) for a given time and accumulation potential. Then, it was rinsed with PBS. Finally, voltammetric stripping step was performed by applying a positive-going differential pulse potential scan (from −1.00 to 1.00 V) in a blank solution, PBS

(0.1 mol L^{-1}, pH 7.0). The operating conditions were as described in section 2.3.1. Each measurement was performed with a fresh PPGE and repeated three times.

3. Results and discussion

3.1. Interaction of riboflavin with ds-DNA at the modified PGE using differential pulse voltammetry

Pretreatment of PGE was carried out before immobilization of the ds-DNA, to activate PGE for the immobilization. The redox behavior of original ds-DNA immobilized PGE exhibited two oxidation processes of guanine (ca. +1.02 V) and adenine (ca. +1.28 V) residues (Dryhurst and Elving, 1968; Dryhurst, 1971). The results showed no oxidation signals of adenine and guanine without pretreatment of PGE. These observations proved that the ds-DNA was not immobilized on the surface of PGE without pretreatment and activation of the surface. The reason for the low activity may result from the lack of functional groups on the surface (Engstrom, 1982). However, there are other possible explanations such as presence of impurities on the surface of PGE that hinder the immobilization of the ds-DNA.

The interaction of riboflavin with the ds-DNA-modified PGE was studied by differential pulse voltammetric analysis as shown in Fig. 1A. Different concentrations of riboflavin were used to interact with the ds-DNA on the surface of the ds-DNA-modified PGE. The changes in the current of the oxidation signals of guanine and adenine were obtained before and after interaction with riboflavin. It was found that the oxidation peak current decreased with a concomitant increase in riboflavin concentration. To investigate the electroactivity of riboflavin, a bare PGE was immersed into the Tris buffer containing 5.0 μg mL^{-1} riboflavin for 300 s in an open circuit. No remarkable oxidation signal of riboflavin was obtained at the bare PGE in the potential range between +0.40 and +1.40 V. Thus, any changes of the oxidation signals of the guanine and adenine (immobilized on the surface of ds-DNA-modified PGE electrode) were attributed to their interaction with riboflavin. The decrease of the oxidation signal of guanine and adenine bases was attributed to the binding of riboflavin to these electroactive DNA bases. This decrease could be explained as a possible damage or shielding of the oxidizable groups of guanine and adenine bases while riboflavin interacts with the ds-DNA on PGE surface. These types of experiments are very important to determine DNA sites and rational design of new DNA-targeted molecules for the applications in cancer therapy. Our obtained results showed that ds-DNA-modified PGE might be used for the detection of riboflavin interaction with the ds-DNA.

UV–vis absorption is one of the spectroscopic techniques generally employed to investigate drug–DNA interactions. The absorption spectrum of riboflavin exhibited bands at 273, 281, 355 and 392 nm (Fig. 1B(a)), and the peak position of the ds-DNA appeared at 265 and 290 nm (Fig. 1B(c)). As is evident from Fig. 1B(b), the absorbance of riboflavin decreased at 355 and 392 nm (with red shift) and vanished at 273 and 281 nm with the addition of the ds-DNA. These results revealed that there is a strong interaction between riboflavin and the ds-DNA. The intercalative binding of molecules to ds-DNA helix has been characterized by an appreciable shift in wavelength (red shift ≥15 nm) due to the interaction of a ds-DNA π stack with the ligand, while outside binders (groove binders) display a smaller red shift ($\Delta\lambda \leq 8$ nm) (Guo et al., 2007). The red shift for absorption peaks at 355 and 392 nm is about 5 nm. In addition, the decrease in the differential pulse voltammetric oxidation signal intensity of the ds-DNA at PGE after its interaction with riboflavin could further demonstrate that riboflavin interacted with the ds-DNA. Therefore, we propose groove binding mode between riboflavin and the ds-DNA.

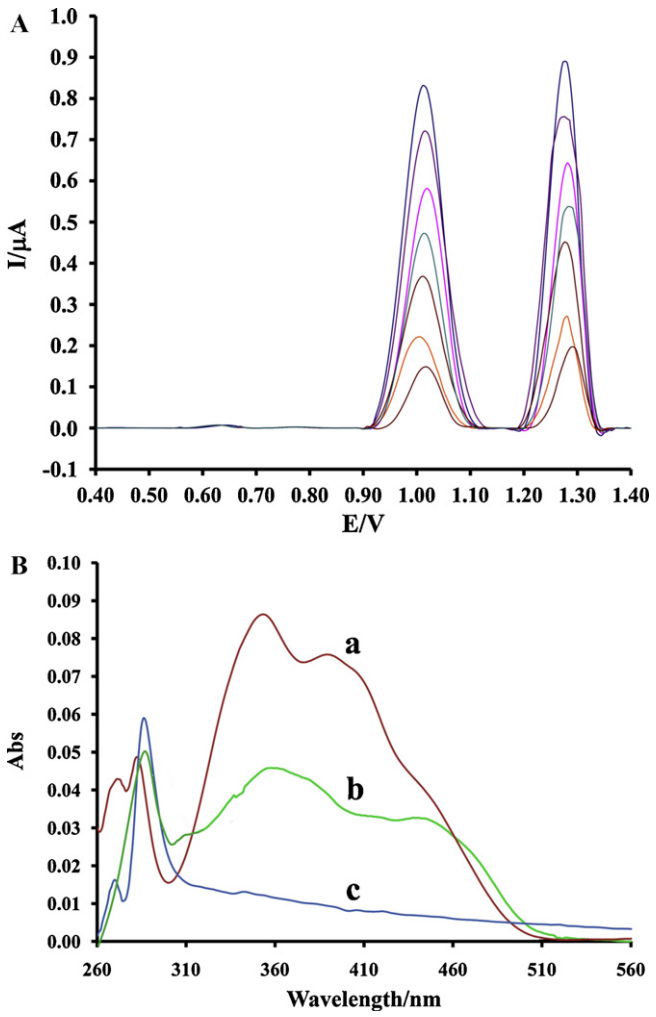

Fig. 1. (A) Differential pulse voltammograms for the interaction of riboflavin with ds-DNA-modified PGE; oxidation signal of guanine and adenine after interaction with (from up to down) 0.0, 0.5, 15.0, 30.0, 40.0, 60.0 and 70.0 μg mL^{-1} riboflavin at surface of ds-DNA-modified PGE. Conditions: ds-DNA immobilization (10.0 μg mL^{-1}) on PGE at +0.50 V during 200 s in acetate buffer (pH 4.8); Riboflavin incubation: at open circuit system during 300 s in Tris buffer (pH 7.0); Conditions: scanning potential between +0.40 and +1.40 V in acetate buffer (pH 4.8). (B) UV–vis spectra of 4.0 μg mL^{-1} riboflavin before (a) and after (b) the reaction with 10.0 μg mL^{-1} ds-DNA, and (c) ds-DNA (10.0 μg mL^{-1}).

The optimum conditions for the ds-DNA concentration and accumulation time were studied to obtain the maximum guanine and adenine signals. To find the optimum concentration of the ds-DNA, ten different concentrations of the ds-DNA (between 2.0 and 50 μg mL^{-1}) were selected at 0.50 V using 300 s as an accumulation time. The results showed that the guanine and adenine signals increased by increasing the DNA concentration up to 10.0 μg mL^{-1}, and then leveled off (Fig. S-1). Therefore, 10.0 μg mL^{-1} of salmon sperm ds-DNA was selected. To find the optimum accumulation time for the ds-DNA on PGE surface, nine different times between 30 and 300 s were selected and studied at 0.50 V using 10.0 μg mL^{-1} ds-DNA. Differential pulse voltammetric signals of guanine and adenine increased till 200 s as an accumulation time and then leveled off (Fig. S-2). Therefore, 200 s was considered as an optimum adsorption time.

The binding of riboflavin with the ds-DNA depends on the interaction (incubation) time. The incubation time for the interaction of riboflavin with the ds-DNA-modified PGE surface was optimized (using 50–400 s as an incubation time of riboflavin). The results showed that during the increasing of the interaction time up to

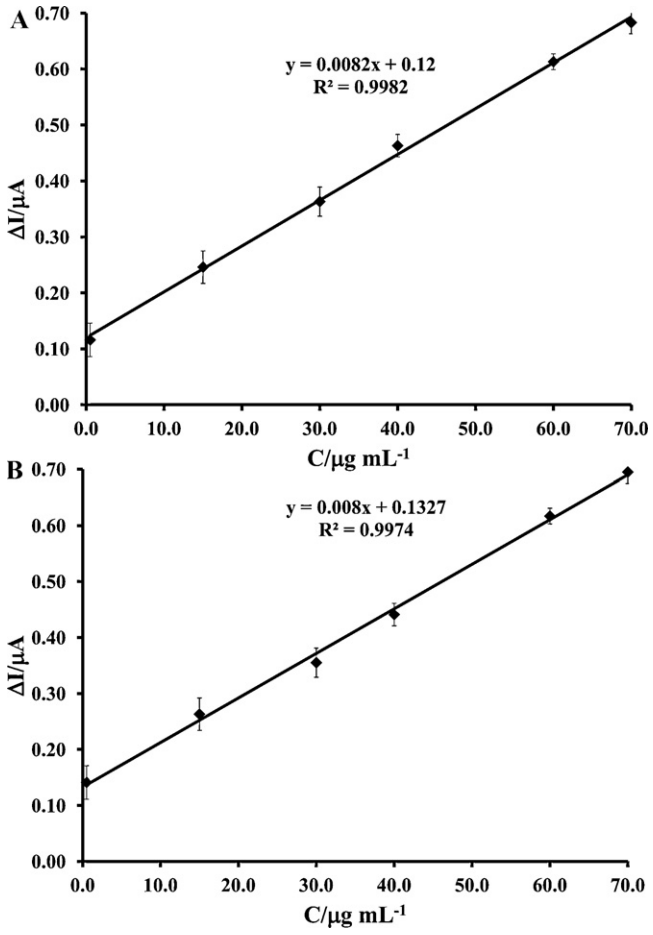

Fig. 2. Calibration curve for determination of riboflavin on the changes of oxidation signal of guanine (A) and adenine (B) after interaction with riboflavin on ds-DNA-modified PGE.

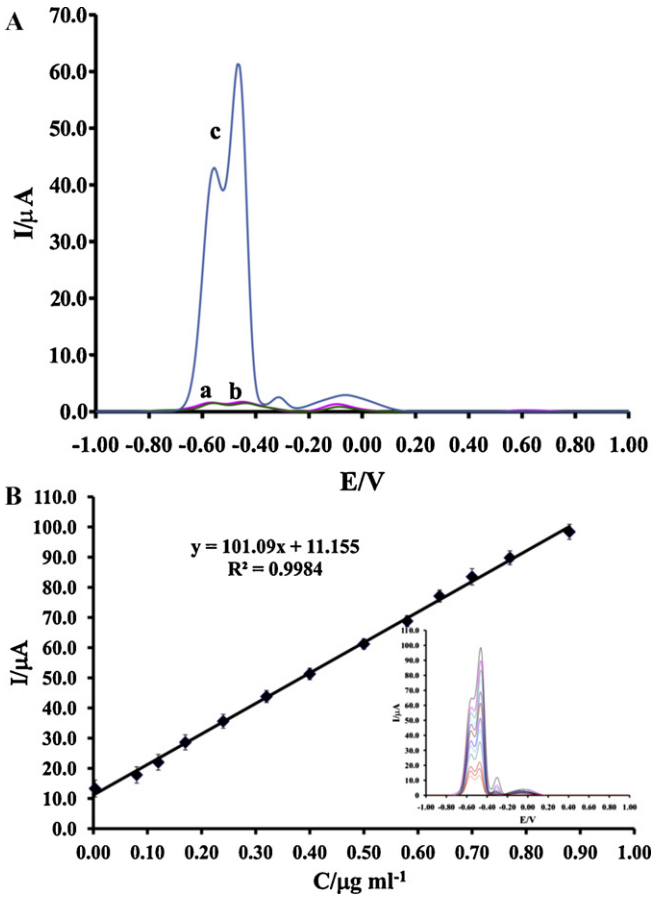

Fig. 3. (A) Differential pulse voltammograms for 0.1 μg mL^{-1} riboflavin in pH 7.0 PBS at a bare PGE (a) and a pre-anodized PGE (b and c). In (a and c) the PGE was preconcentrated for 300 s. (B) Calibration curve for the determination of riboflavin on the changes of stripping oxidation signal on PPGE under the optimum conditions.

300 s, there was a dramatic decrease in the oxidation signal of guanine till 300 s and then it almost leveled off for the longer incubation time. Similarly, a significant decrease was obtained in the oxidation signal of adenine till 300 s while it leveled off from 300 to 400 s (Fig. S-3). Accordingly, 300 s was selected as the optimum time for the interaction of riboflavin with the ds-DNA-modified PGE.

The dependence of riboflavin concentration on the adenine and guanine signals was studied, too. The results showed that guanine and adenine oxidation currents decreased with increasing concentration of riboflavin up to 70.0 μg mL^{-1} and then almost leveled off. This is due to the fact the modified electrode surface was saturated by riboflavin in higher concentration of the analyte. Using differential pulse voltammetry and following the change in the oxidation signal intensity of guanine after the interaction with riboflavin concentration in the range of 0.5–70.0 μg mL^{-1}, the regression equations were as follows: $I_s(\mu A) = (0.1200 \pm 0.0030) + (0.0082 \pm 0.0003)C_{Riboflavin}$ with $R^2 = 0.998$ ($n = 6$), where C is riboflavin concentration in μg mL^{-1} (Fig. 2A). In addition, using the same procedure and following the change in the oxidation signal intensity of adenine after the interaction with riboflavin in the concentration range of 0.5–70.0 μg mL^{-1}, the regression equations were as follows: $I_s(\mu A) = (0.1322 \pm 0.0020) + (0.0080 \pm 0.0003)C_{Riboflavin}$ with $R^2 = 0.997$ ($n = 6$), where C is riboflavin concentration in μg mL^{-1} (Fig. 2B).The detection limit of the proposed method corresponded to 0.34 μg mL^{-1}of riboflavin using the proposed procedure.

A series of ten repetitive differential pulse voltammetric measurements of the change in the oxidation peak current of guanine and adenine after those interactions with riboflavin concentration of 3.0 and 6.0 μg mL^{-1} resulted in the relative standard deviations of 4.3% plus 3.9%, and 4.8% plus 4.4%, respectively.

3.2. *Differential pulse adsorptive stripping voltammetry of riboflavin at PPGE*

It is generally believed that the pre-anodization of carbon electrode surfaces results in porous graphite oxide films with various oxygen-containing functional groups such as quinone, carbonyl, hydroxyl, carboxyl, phenolic groups, etc (Mc Creery, 1991). These functional groups increase organics adsorption capacities. Fig. 3A shows differential pulse voltammogram of 0.5 μg mL^{-1} riboflavin at pH 7.0 PBS with different electrode conditions. This figure shows that no oxidation peak is observed for riboflavin at PGE without preconcentration, whereas a very small anodic stripping signal ($E_{pa} = -0.445$ V and $i = 1.48$ μA) is observed after the preconcentration (Fig. 3A(a)). On the surface of PPGE (PGE after pre-anodization) (Fig. 3A(b)), a signal of riboflavin at $E_{pa} = -0.466$ V with a current of 1.72 μA was detected. A very pronounced stripping signal with E_{pa} and current corresponding to −0.468 V and 61.20 μA, respectively, was obtained for PGGE (c). The current obtained value in (Fig. 3A(c)) is about forty one times higher than the current value of (a and b).

The stripping anodic signal of riboflavin at PPGE was observed at about −0.47 V accompanied by two shoulders at −0.57 V and −0.32 V. These waves should be related to the incorporation of riboflavin species at PPGE. It should be pointed out that no

significant incorporation of riboflavin was observed at pencil graphite electrode without electrochemical activation.

Preparation of real samples, optimizations of variables including influence of sample solution pH, accumulation potential and time on the sensitivity, figures of merit for PPGE method plus the influence of potential interfering substances on riboflavin determination are described in Supplementary file.

Fig. 3B shows the calibration curve and differential pulse voltammetric peak current for adsorbed riboflavin as a function of concentration in the exposure solution. The preconcentration process was conducted under controlled potential at −1.00 V for 180 s with stirring (400 rpm). The peak current increased linearly with riboflavin concentration over the range from 0.003 to 0.88 μg mL^{-1} in 0.1 mol L^{-1} PBS (pH 7.0). Saturated adsorption was attained when the concentration was higher than 0.88 μg mL^{-1}. A very large slope of 101.09 μA mL μg^{-1} with a correlation coefficient of 0.999 was obtained. The detection limit ($3S_b/m$, where S_b is the standard deviation and m is the slop of the calibration curve) was estimated to be 0.076 ng mL^{-1}, proving that the proposed method is more sensitive than the other reported electrochemical methods.

3.3. Analytical performance

Before evaluating the sensors for analysis of riboflavin in real samples, the interference studies were carried out with several species. The results showed that (Supplementary file) the biosensor and PPGE are highly selective for riboflavin analysis. Here, both ds-DNA-modified PGE and PPGE were applied to the analysis of riboflavin in real samples. Commercial pharmaceutical formulation samples of multivitamin tablet plus urine were evaluated for riboflavin analysis under the optimum conditions. The results obtained from the proposed biosensor were compared with those determined by standard AOAC official method (AOAC Official Method 970.65). Table 1 compares the results of the analysis. It should be stressed that the results obtained using the differential pulse adsorptive stripping voltammetric methods at the ds-DNA-modified electrode and PPGE are in good agreement with those obtained by the AOAC method and with the data declared by the manufacturer. The statistical comparisons of the values obtained by these methods for the determination of riboflavin were made by Student's t-test and the variance ratio F-test. As can be seen from Table 1, the experimental t-values at $P = 0.05$ for both techniques did not exceed the theoretical ones. The differences in the amounts of riboflavin on labeled and measured means (bias%) were usually higher for the reference method. Those results indicate that the accuracy of the proposed methods is slightly better than that of the spectrofluorimetric method (AOAC official method 970.65). In addition, the reference method is considerably more laborious (with respect to the preparation of a sample) than the developed methods. However, the proposed methods could be successfully applied for riboflavin assay in tablet dosage form without any interference.

For urine sample analysis, different amounts of riboflavin were spiked into the test sample and standard addition method was applied using the proposed methods (Table 2).

Table 1
Recovery of riboflavin in multivitamin tablets.

	ds-DNA-modified PGE	PPGE	AOAC official method
Labeled claim (mg)	1.65	1.65	1.65
Amount found[a] (mg)	1.61 ± 0.10	1.63 ± 0.08	1.59
R.S.D%	1.88	0.73	0.90
Bias%	2.42	1.21	3.64
$t_{calculated}$	1.112	0.956	$t_{theoretical}$ = 2.306
$F_{calculated}$	2.245	1.879	$F_{theoretical}$ = 6.388
Added (mg)	10.0	10.0	–
Found[a] (mg)	10.18 ± 0.50	10.08 ± 0.30	–
R.S.D.%	2.87	0.98	–
Bias%	−1.8	−0.8	–

[a] Average of five replicate measurements.

Table 2
Recovery of riboflavin in urine samples.

Method	Sample No.	Added (μg mL^{-1})	Expected (μg mL^{-1})	Found[a] (μg mL^{-1})	Recovery%
ds-DNA modified PGE	1	–	–	<Limit of detection	–
	2	2.00	2.00	1.94 ± 0.18	97.0
	3	4.00	4.00	3.81 ± 0.26	96.0
PPGE	1	–	–	<Limit of detection	–
	2	0.100	0.100	0.103 ± 0.017	103.0
	3	0.600	0.600	0.607 ± 0.080	101.2

[a] Average of four replicate measurements

Table 3
Comparison of the efficiency of reported electrchemical methods in the determination of riboflavin.

Electrode	Method	Limit of detection (μg mL^{-1})	Linear dynamic range (μg mL^{-1})	pH	Reference
C-18 modified gold electrode	SWV[a]	2.3	–	2.0	Blanco et al. (2009)
Screen-printed carbon electrode	DPV[b]	0.9	1–23	6.0	Kadara et al. (2006)
Glassy carbon electrode	DPV	0.008	–	0.1 mol L^{-1} HCl	Mikheeva et al. (2009)
Silver liquid amalgam film-modified silver Solid amalgam annular band electrode	ASDPV[c]	0.009	0.05–3	7.5	Bás et al. (2011)
DNA carbon nano tube paste electrode	SWASV[d]	0.0000002	1×10^{-6}–1×10^{-5} 1×10^{-5}–1.7×10^{-4}	0.1 mol L^{-1} H_3PO_4	Ly et al. (2011)
ds-DNA-modified PGE	DPV	0.36	0.5–70	7.0	This work
PPGE	ASDPV	0.000076	0.003–0.88	7.0	This work

[a] Square wave voltammetry.
[b] Differential pulse voltammetry.
[c] Differential pulse adsorptive stripping voltammetry.
[d] Square wave adsorptive stripping voltammetry.

4. Conclusion

In this article we showed that differential pulse adsorptive transfer stripping voltammetry could monitor the interaction of riboflavin with the ds-DNA. In the case of the interaction of riboflavin with the ds-DNA immobilized on the PGE, riboflavin binds preferentially to the sites of the duplex oriented towards the solution. The ds-DNA is electrostatically adsorbed on the PGE surface through the negatively charged sugar–phosphate backbone by forming a flat layer on the electrode surface. The side of the duplex that is in intimate contact with the electrode surface is not easily accessible. The coupling of an adsorptive stripping voltammetry step and effective correction of the background current allowed the sophisticated quantification of low levels of riboflavin. In addition, electrochemically activated pencil graphite electrode showed excellent sensitivity and selectivity for the preconcentration and determination of riboflavin in aqueous solutions. PPGEs are also very promising in the electroanalytical applications because of their easy preparation procedure and disposable character. Both of the proposed methods reported in this paper have similar advantages. No pretreatment of riboflavin sample was necessary prior to electroanalysis. High organic absorption capacity of PPGE decreased the limit of detection of riboflavin and enhanced the linear dynamic range. Although the ds-DNA-PGE has better selectivity, it is better to be used for clinical analysis. However, PPGE has a better limit of detection and longer linear dynamic range compared with the ds-DNA-PGE (Table 3).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.10.050.

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