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A novel sensitive DNA–biosensor for detection of a carcinogen, Sudan II, using electrochemically treated pencil graphite electrode by voltammetric methods

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ABSTRACT

A simple and inexpensive methodology was used to develop a novel electrochemical sensor for the determination of Sudan II. The interaction of Sudan II with salmon sperm ds-DNA on the surface of salmon sperm ds-DNA-modified pencil graphite electrode (PGE) and in solution phase was studied, using differential pulse voltammetry. The difference between adenine and guanine signals of the ds-DNA after and before interaction with Sudan II was directly proportional to Sudan II concentration, which used for quantitative inspections. Using PGE, a linear calibration curve (R^2 = 0.9958) was observed with 0.5–6.0 μg mL^{-1} Sudan II. Furthermore, the LOD of 0.4 μg mL^{-1} and linear range between 0.5 and 4.0 μg mL^{-1} were achieved in solution phase. In the second part, Sudan II was determined on a pretreated pencil graphite electrode by means of adsorptive stripping differential pulse voltammetry. The peak current was linearly dependent on Sudan II concentration over the range of 0.0015–0.30 μg mL^{-1}, with a detection limit of 0.00007 μg mL^{-1} Sudan II. Both ds-DNA-modified PGE and PPGE were applied to analyze Sudan II in real samples.

1. Introduction

Sudan dyes (Sudan I, II, III, and IV), which belong to a family of industrial dyes, are used as coloring agents in chemical industries such as oils, fats, plastics, waxes, petrol, shoes, printing inks, floor polishing and spirit varnishing [1]. The International Agency for Research on Cancer, a part of the World Health Organization, has assessed the Sudan dyes as group 3 geologic carcinogens. Due to this fact, any national and international food regulation does not permit the use of these colorings as food additives [2,3]. Different methods have been described in literature for Sudan II determination in food products, using different analytical methods such as HPLC/MS [4,5], HPLC/ultraviolet–visible [6,7], ionic liquid extraction/HPLC [8], HPLC/electrogenerated chemiluminescence [9], ultra-performance liquid chromatography [10], electrochemical methods [11,12], and resonance light scattering method [13]. However, most of these methods are expensive and time consuming, and thus restrict their applications in food safety examination. It is well known that electrochemical methods are simple and inexpensive as analytical techniques. But, available literature is very scanty dealing with measurement of Sudan II by electrochemical methods.

Nucleic acids offer an analytical chemist a powerful tool in the recognition and monitoring of many important compounds [14]. Molecules and ions interact with DNA in three significantly different ways: electrostatic, groove-binding and intercalation. These reactions cause changes in the structure of DNA and the base sequence, leading to perturbation of DNA replication. Electrostatic interactions are usually non-specific and consist in binding along the exterior of the ds-DNA helix. Groove-binding interactions involve direct interaction of the compound with the edges of the base pairs in the major or minor grooves of ds-DNA, extending to fit over many base pairs, and having very high sequence specificity. Intercalation encompasses inserting planar or nearly planar aromatic ring systems between the base pairs, causing unwinding and separation of base pairs [15]. Drugs that intercalate into ds-DNA have been extensively studied, and the field has been recently reviewed using variety techniques [16–25]. Electrochemical investigation of DNA–drug interaction can provide a rapid and inexpensive method for the determination of drugs. The construction of the electrochemical DNA–biosensors is based on immobilization of nucleic recognition layer over an electrochemical transducer. Changes in the DNA structure during intercalation with DNA binding molecules are detected by nucleic acid recognition layer.

Our experiment showed that a high degree of reproducibility could be obtained on a pencil graphite electrode (PGE). This high reproducibility together with other advantages such as ease of preparation, commercial availability, and low cost were the

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main reasons for choosing the disposable PGE. To the best of our knowledge, electrochemical determination of Sudan II based on its interactions with ds-DNA using PGE has not yet been reported. Current paper is broadly divided into two sections, in the first part, we report the construction of a biosensor for determination of Sudan II as a DNA intercalate on ds-DNA-modified PGE and in solution phase based on the differences of guanine and adenine oxidation signals before and after the intercalation. The differences between guanine and adenine oxidation signals were proportional to the Sudan II concentration and were used for quantitative investigations. The second part involves the use of adsorptive stripping voltammetry on pretreated pencil graphite electrode (PPGE) in aqueous solution for the determination of Sudan II. Furthermore, this study was used to develop new, inexpensive, rapid and sensitive voltammetric methods without any time-consuming extraction and separation steps for direct determination of Sudan II in real samples.

2. Experimental

2.1. Chemicals

All solutions were prepared using reagent grade chemicals and doubly distilled water was used through the work.

Sudan II was purchased from Aldrich Chemicals. Double-strand salmon sperm DNA (ds-DNA, catalog No. D-8899) 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 TE buffer (pH 7.0) and kept frozen. More diluted solutions of ds-DNA were prepared with acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl.

Stock solutions of Sudan II (1.0 mmol L^{-1}) were prepared by dissolving accurately weighed amounts of Sudan II. The solution was conserved at 4 °C, covered with aluminum foil and left to attain room temperature before use. Sudan II working solutions for voltammetric investigations were prepared by dilution of the stock with acetate buffer (pH 4.8) containing 0.02 mol L^{-1} NaCl.

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). 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 reference electrode was Ag/AgCl (3 mol L^{-1} KCl) and the counter electrode was a platinum wire. A standard one-compartment three-electrode cell of 10 mL capacity was used in all experiments. The renewable PGE that was described in the study of Ozsoz and co-workers [26] were used in all 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 hold vertically with 12 mm of the lead extruded outside (9 mm of which was immersed in the solution). The pencil leads were used as received. All the electroanalytical measurements were performed at room temperature.

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

Metrohm pH meter (Model 827) with a glass electrode (Corning) was used to adjust the pH of solution.

2.3. Interaction of Sudan II with ds-DNA

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

DNA and RNA could be easily immobilized on the mercury [25,26] and carbon [25] electrodes. In this study, prior to immobilization of ds-DNA on the surface of PGE, the surface of the 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 pretreated electrode was immersed in a stirred (200 rpm) 10 μg mL^{-1} ds-DNA containing (0.5 mol L^{-1} acetate buffer solution and 0.02 mol L^{-1} NaCl, pH 4.8) for a short time (optimum time was 200 s) at +0.50 V. After this step, the electrode was washed with acetate buffer (0.5 mol L^{-1} at pH 4.8) for 5 s to remove the unbounded ds-DNA on the electrode surface. The DNA-modified electrode was immersed in the background electrolyte 0.5 mol L^{-1} acetate buffer (pH 4.8) containing 0.02 mol L^{-1} NaCl, free of ds-DNA, and a positive-going differential pulse potential scan (from 0.40 to 1.40) was performed. The oxidation signals of guanine and adenine were recorded. This technique was called adsorptive transfer stripping voltammetry. The voltammetric stripping step was performed with potential amplitude of 50 mV, a modulation time of 0.05 s, and a step potential of 8 mV. For inspection of interaction between Sudan II with ds-DNA, the procedure was repeated with a new PGE. The ds-DNA modified-PGE was immersed into a stirred (200 rpm) Tris buffer solution (pH 7.0) containing different concentrations of Sudan II for 300 s in an open circuit system. After the accumulation step, the ds-DNA-modified PGE was rinsed and placed in Sudan II free acetate buffer solution (pH 4.8) containing 0.02 mol L^{-1} NaCl, and differential pulse voltammograms were recorded. The obtained curves before and after interactions between Sudan II and ds-DNA were compared with each other.

2.3.2. Interaction between the ds-DNA and Sudan II in solution phase

The inspection of interaction between the ds-DNA and Sudan II in solution phase was carried out according to the following procedure. After the activation step of PGE (as described in Section 2.3.1), different Sudan II concentrations were added to 0.5 mol L^{-1} acetate buffer solution containing 0.02 mol L^{-1} NaCl (pH 4.8) and 10.0 μg mL^{-1} ds-DNA (stirred at 200 rpm for 300 s) in an open circuit condition. Then, the electrode was washed with the acetate buffer and transferred into a voltammetric cell containing acetate buffer (pH 4.8). The oxidation signals of guanine and adenine were recorded using DPV. Operating conditions are described in Section 2.3.1. The differences between the guanine and adenine signals before and after interaction with Sudan II were recorded and used for quantitative investigations.

2.4. Adsorptive stripping voltammetric assay

2.4.1. Preparation of the activated electrode

Two methods were used to prepare the activated electrode. In the first method, PGE was treated at different constant potentials for a short period of time (from 0.5 to 3.0 min). In the second method, the potential was cycled between two preselected potentials at a scan rate of 100 mV s^{-1} for five cycles. The results showed that applying the cyclic voltammetric (CV) method for pretreatment had higher responses than the constant potential method. Among the potential ranges applied for pretreatment of the electrode with CV method, the highest response was achieved at the potential range between −0.20 and +2.00 V. Therefore, this potential range was selected for electrochemical treatment throughout the work. PGEs obtained after electrochemical activation were called PPGE in this study.

2.4.2. Adsorptive stripping differential pulse voltammetric (AdSDPV) measurements

Adsorption of Sudan II on the surface of PPGE was done by dipping the electrode into phosphate buffer solution (PBS, 0.1 mol L^{-1}, pH 4.0) containing the desired Sudan II concentration for a given time with stirring (400 rpm). After adsorption of Sudan II on PPGE surface, the potential was scanned between −0.50 and 1.00 V in PBS (0.1 mol L^{-1}, pH 4.0), and differential pulse voltammograms was recorded. The operating conditions are described in Section 2.3.1. Each measurement was performed with a fresh PPGE and repeated three times.

2.5. Preparation of real samples

For preparation of real samples, we worked according to the procedure of Shouguo and coworkers [27]. Amount of 2.00 g of sample (chili and ketchup sauce) was accurately weighed and added into 50 mL ethanol followed, by ultrasonicating for 1 h. After filtration with a filter membrane, the filtrate was collected in 100 mL volumetric flask and diluted to the mark with ethanol. For determination of Sudan II with fabricated DNA sensor and PPGE, 20 μL sample solution was transferred into a 10 mL of Tris buffer solution (pH 7.0) for the DNA interaction study, and in PBS (pH 4.0) on PPGE.

3. Results and discussion

3.1. Interaction of Sudan II with the ds-DNA at PGE

The electrochemical interaction between Sudan II and the ds-DNA was investigated using two different interaction processes: first, interaction on the PGE surface and second in the solution phase. It is known that planar condensed aromatic ring systems in Sudan II play a major role in their interaction with DNA, primarily involving stacking interactions.

Anodic peaks due to the oxidation of adenine and guanine residues in DNA [28,29] are observed at around +1.0 and +1.3 V, respectively. The oxidation peak of Sudan II is observed at +0.10 V.

3.1.1. Interaction of Sudan II with the ds-DNA at the modified-PGE using DPV

DPV oxidation signals of guanine and adenine at ds-DNA-modified PGE, before and after interaction with Sudan II on the electrode surface, are shown in Fig. 1A. Fig. 1A(a) shows the background voltammogram of acetate buffer (pH 4.8) at on a bare PGE. The oxidation signals of guanine and adenine obtained with the ds-DNA-modified electrode before interaction of Sudan II was higher than the one obtained after interaction with Sudan II (Fig. 1A(b)). The electrochemical response of Sudan II on a bare PGE in a Tris buffer (pH 7.0) containing 4.0 μg mL^{-1} Sudan II for 300 s in an open circuit condition was investigated (not shown here). It was observed that Sudan II had no electrochemical response in the potential range (+0.40 to 1.40 V) scanned in this study. Thus, any changes of the oxidation signals of the guanine and adenine were attributed to the interaction of Sudan II with the guanine and adenine (from the ds-DNA that immobilized on the surface of ds-DNA-modified PGE) as shown in Fig. 1A(b). This decreasing trend could be explained as a possible damage or shielding of the oxidizable groups of guanine and adenine bases while Sudan II interacts with the ds-DNA either on PGE surface or in solution phase. Our obtained results showed that PGE might be used for the direct detection of Sudan II interaction with ds-DNA.

Absorption spectrophotometry was used to prove the interaction between Sudan II and the ds-DNA. The absorption spectra of Sudan II and ds-DNA before and after interaction with each other are shown in Fig. 1B. The maximum absorbance of Sudan II is located at 520 and 560 nm (Fig. 1B(a)) and the maximum absorbance of the ds-DNA is located at 290 nm (Fig. 1B(c)). The absorption spectra of the mixture of Sudan II and the ds-DNA are shown in Fig. 1B(b). A notable decrease of absorbance of Sudan II at 520 and 560 nm proved the existence of interaction between Sudan II and the ds-DNA. The obtained results from absorption spectrophotometry and decreasing of adenine and guanine DPV signals are good pieces of evidence for the interaction between Sudan II and ds-DNA.

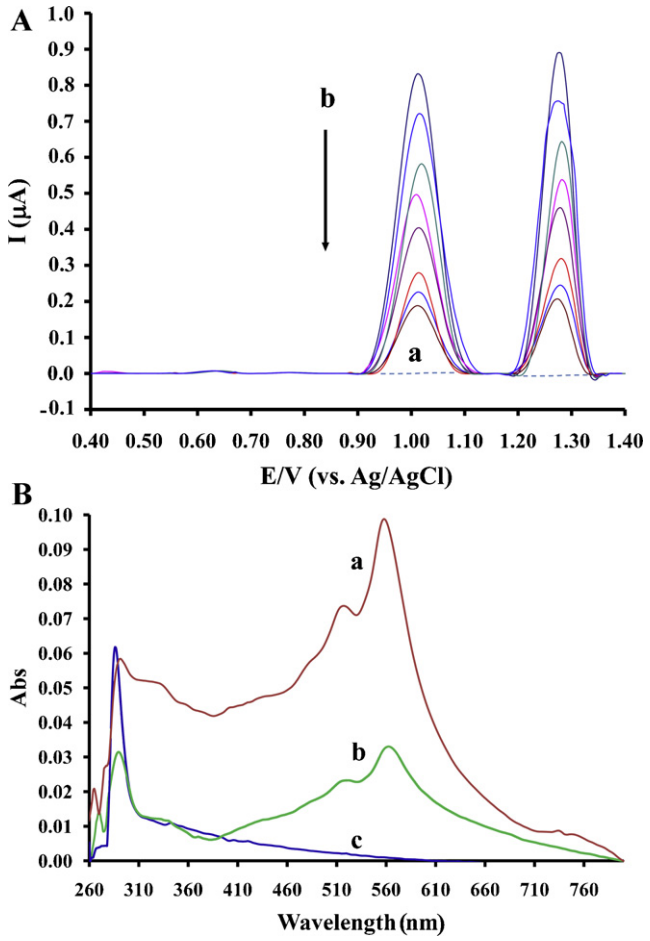

Fig. 1. (A): (a) Differential pulse voltammogram of acetate buffer (pH 4.8) at a bare PGE (the background signal). (b) Differential pulse voltammograms for the interaction of Sudan II at the ds-DNA-modified PGE; The oxidation signal of guanine and adenine after interaction with 0.0, 0.5, 2.0, 3.0, 4.0, 5.0, 5.5 and 6.0 μg mL^{-1} Sudan II (from up to down). Conditions: The ds-DNA immobilization (10.0 μg mL^{-1}) on PGE (at +0.50 V) during 200 s in acetate buffer (pH 4.8); Sudan II incubation: at open circuit system during 300 s in TE buffer (pH 7.0); Measurement: scanning between +0.40 and +1.40 V in acetate buffer (pH 4.8). (B) UV–vis spectra of 4.0 μg mL^{-1} Sudan II before (a) and after (b) the reaction with 10.0 μg mL^{-1} ds-DNA, and (c) just ds-DNA (10.0 μg mL^{-1}) in the absence of Sudan II.

The optimum concentration of the ds-DNA and accumulation time were studied to obtain reproducible signals of the guanine and adenine as given in our previous paper [16]. It was observed that the oxidation peak currents of guanine and adenine increased with the ds-DNA concentration up to 10 μg mL^{-1}, and then levelled off. Therefore, 10.0 μg mL^{-1} of the ds-DNA concentration was selected and used in all other subsequent experiments.

To study the influence of accumulation time at PGE surface on guanine and adenine oxidation signals, nine different times between 30 and 300 s were applied. These studies were performed at 0.50 V using 10.0 μg mL^{-1} of ds-DNA [16]. The results showed that DPV signals of guanine and adenine increased till 200 s and then levelled off. Therefore, 200 s was selected as an optimum adsorption time for adsorption of ds-DNA at PGE.

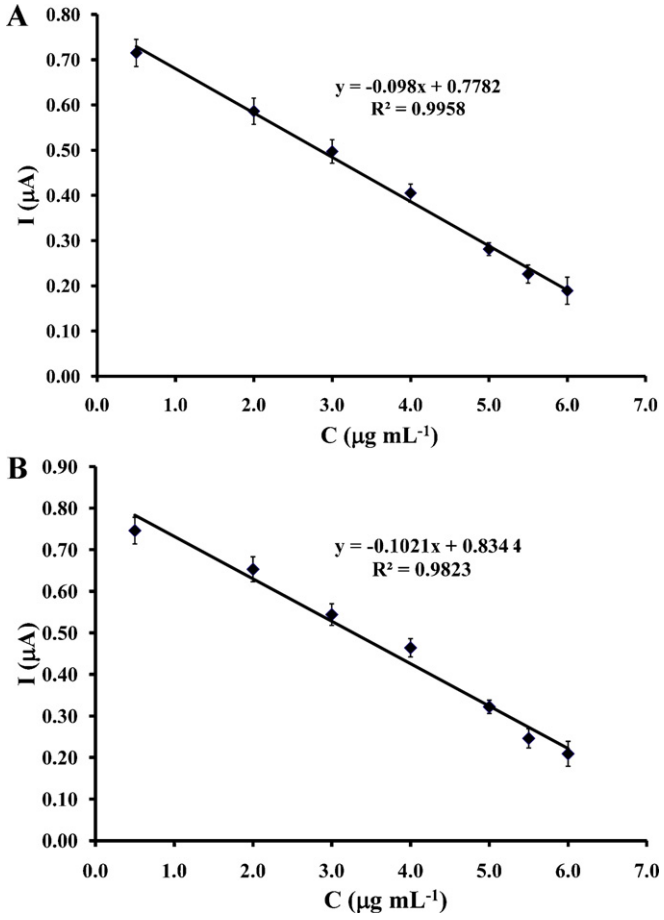

Fig. 2. Calibration curve for the determination of Sudan II based on the changes of the oxidation signals of guanine (A) and adenine (B) after interaction of Sudan II on ds-DNA-modified PGE. (Error bars show the relative standard deviation, $n = 3$.)

The binding of the Sudan II to the ds-DNA depends on the interaction (incubation) time. The incubation time for the interaction of Sudan II with ds-DNA-modified PGE surface was optimized. For this purpose, different times between 50 and 400 s were selected and the results showed that during the increasing of the interaction time till 300 s, there was a dramatic decrease in the oxidation signal of guanine and then it almost levelled off for longer incubation time. Similarly, a significant decrease was obtained in the oxidation signal of adenine till 300 s, and then levelled off. Accordingly, 300 s was selected as the optimum time for the interaction of Sudan II with the ds-DNA-modified PGE.

Increasing the concentration of Sudan II caused a corresponding decrease in the guanine and adenine oxidation signals. As shown in Fig. 2A, the oxidation signals of guanine were linear with Sudan II concentration over the range of 0.5–6.0 μg mL^{-1} with a correlation equation of $I_s(\mu A) = (0.7782 \pm 0.0040) - (0.0981 \pm 0.0020)C$ with $R^2 = 0.9958$ ($n = 7$). In addition, using the same procedure and following the change in the oxidation signal intensity of adenine after the interaction with Sudan II in the concentration range of 0.5–6.0 μg mL^{-1}, the regression equation was $I_s(\mu A) = (0.8344 \pm 0.0050) - (0.1021 \pm 0.0010)C$ with $R^2 = 0.9823$ ($n = 7$), where C is Sudan II concentration in μg mL^{-1} (Fig. 2B). The limit of detection (3 s/m, where s is the standard deviation of the blank signal for five replicates measurements and m is the slope of the calibration curve) based on guanine and adenine were estimated as 0.43 and 0.48 μg mL^{-1} Sudan II, respectively.

The RSD values for the change of guanine signals after interaction with 3.0 and 5.0 μg mL^{-1} Sudan II were 4.2% and 3.9% ($n = 10$), respectively. This inspection was done for adenine signal and RSD values of 4.9% and 4.0% were obtained.

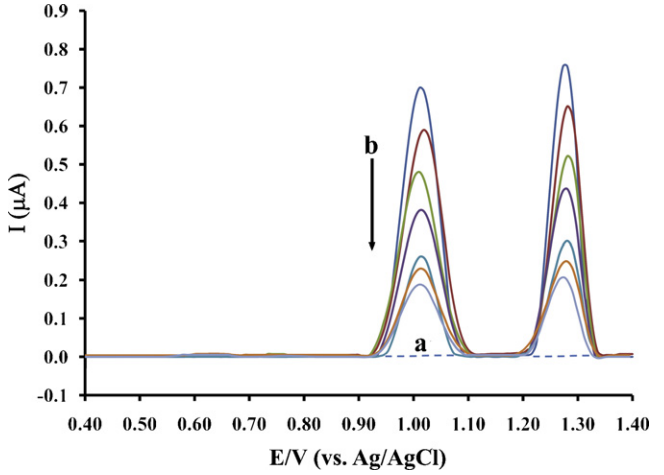

Fig. 3. (a) Differential pulse voltammogram of acetate buffer (pH 4.8) at pretreated PGE; (b) differential pulse voltammograms for the interaction of Sudan II with the ds-DNA in solution phase at pretreated PGE; oxidation signal of guanine and adenine after their interactions with Sudan II concentration of 0.5, 1.2, 2.0, 2.5, 3.2, 3.5, and 4.0 μg mL^{-1} (from top to down).

3.1.2. *Interaction of ds-DNA and Sudan II in solution phase*

DPV oxidation signals of guanine and adenine, before and after interaction with Sudan II in solution phase are shown in Fig. 3. Fig. 3a shows differential pulse voltammogram of acetate buffer (pH 4.8) at pretreated PGE, whereas Fig. 3b shows differential pulse voltammograms for the interaction of Sudan II with the ds-DNA in solution phase at pretreated PGE The oxidation signals of guanine and adenine obtained before interaction of Sudan II was higher than the one obtained after interaction with Sudan II. To find the optimum concentration of the ds-DNA in solution, ten different concentrations of the ds-DNA (between 2.0 and 50 μg mL^{-1}) were studied. These studies were performed in an open circuit condition using 300 s as an accumulation time. A deviation of the linearity was observed for the ds-DNA concentrations higher than 10.0 μg mL^{-1}. Therefore, 10.0 μg mL^{-1} of the ds-DNA was selected and used in all the further experiments. Furthermore, for finding the influence of accumulation time on adsorption of the ds-DNA on PGE surface, nine different times between 30 and 300 s were selected. These studies were performed in an open circuit condition using 10.0 μg mL^{-1} of the ds-DNA. The results showed that DPV signals of guanine and adenine increased till 120 s and then levelled off. Therefore, 120 s was considered as an optimum adsorption time for the interaction of ds-DNA with Sudan II in solution phase.

DPV signals of guanine and adenine were recorded after addition of different amounts of Sudan II into a solution containing 10.0 μg mL^{-1} ds-DNA. The changes in the electrochemical signals were obtained from Sudan II–ds-DNA complex and compared with the signals of the pure ds-DNA in solution. The influence of Sudan II concentration (in the range of 0.5–6.0 μg mL^{-1}) on the guanine signal was studied. The results showed that guanine oxidation signal decreased with increasing concentration of Sudan II till 4.0 μg mL^{-1} and then almost levelled off till 6.0 μg mL^{-1}. By means of DPV method and following the change of guanine oxidation signal after the interaction with Sudan II (in the range of 0.5–4.0 μg mL^{-1}), the regression equation was $I_s(\mu A) = (0.7650 \pm 0.0051) - (0.1504 \pm 0.0020)C$ with $R^2 = 0.9918$ ($n = 7$), where C is Sudan II concentration in μg mL^{-1} (Fig. 4A). In addition, using the same procedure and following the change in the oxidation signal intensity of adenine after the interaction with Sudan II in the concentration range of 0.5–4.0 μg mL^{-1}, the regression equation was

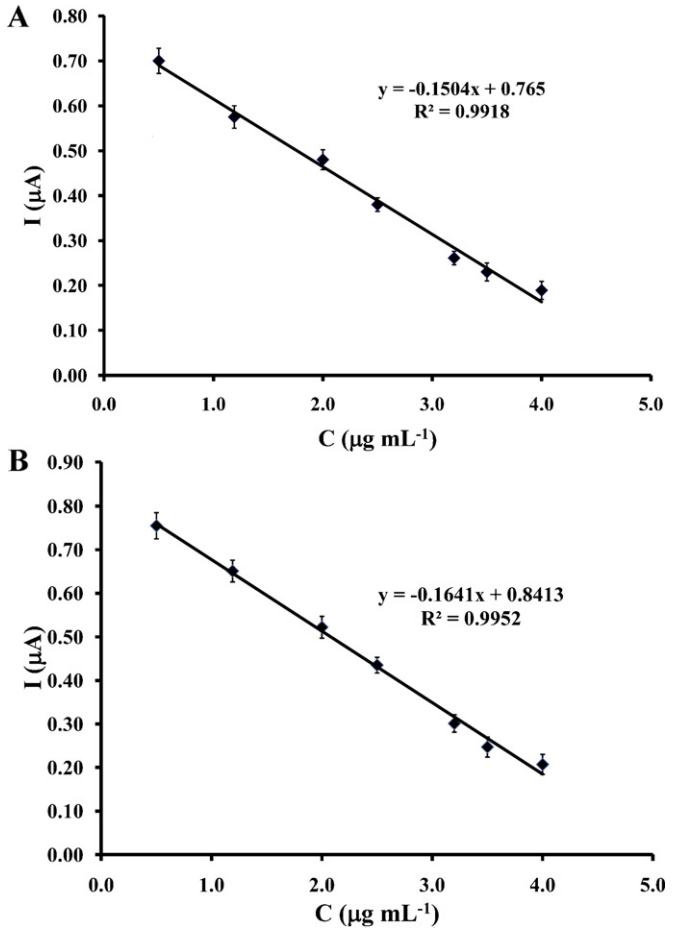

Fig. 4. Calibration curve for the determination of Sudan II based on the changes of the oxidation signals of guanine (A) and adenine (B) after their interaction with Sudan II in solution phase. (Error bars show the relative standard deviation, $n = 3$.)

$I_s(\mu A) = (0.8413 \pm 0.0040) - (0.1641 \pm 0.0010)C$ with $R^2 = 0.9952$ ($n = 7$) where C is Sudan II concentration in $\mu g\,mL^{-1}$ (Fig. 4B).

The limit of detection of the proposed method based on guanine and adenine were estimated 0.40 and 0.42 $\mu g\,mL^{-1}$, respectively. The limit of detection was estimated 0.40 $\mu g\,mL^{-1}$ of Sudan II using the proposed procedure.

A series of 10 repetitive DPV measurements of the change in the oxidation peak current of guanine after its interaction with Sudan II concentration of 2.0 $\mu g\,mL^{-1}$ was carried out and RSD of 4.8% was obtained.

3.2. AdSDPV of Sudan II at PPGE

Fig. 5 shows the differential pulse voltammograms of 0.1 $\mu g\,mL^{-1}$ Sudan II at pH 4.0 in different conditions. As can be seen, no signal is observed on a PGE without preconcentration (Fig. 5a), whereas a very small anodic stripping signal ($E_{pa} = -0.055$ V and $i = 1.87$ μA) is observed after preconcentration (Fig. 5b). On the surface of PPGE (without preconcentration), a signal at $E_{pa} = 0.056$ V with a current of 1.72 μA was detected (Fig. 5c). A very pronounced stripping signal with E_{pa} and i corresponding to 0.095 V and 21.87 μA, respectively, was obtained on PPGE after 300 s accumulation (Fig. 5d). The obtained i value in Fig. 5d is 12-time higher than the i value of Fig. 5b and c. The stripping anodic signal of Sudan II on PPGE was observed at 0.095 V. It should be pointed out that no significant incorporation of Sudan II was observed on PGEs without electrochemical activation.

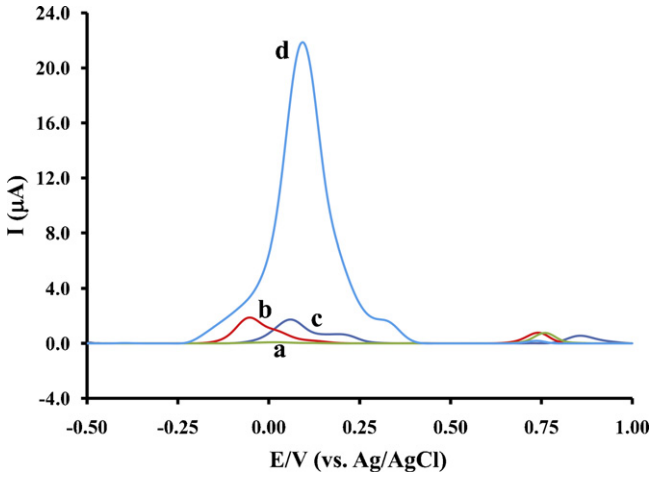

Fig. 5. Differential pulse voltammograms for 0.10 $\mu g\,mL^{-1}$ Sudan II at pH 4.0 PBS at: (a) a bare PGE without preconcentration; (b) a bare PGE with preconcentration; (c) a pre-anodized PGE without preconcentration; and (d) a pre-anodized PGE with preconcentration. Condition: preconcentration time, 300 s.

Mc-Creery and coworkers [30,31] categorized redox systems according to their kinetic sensitivity to carbon surface modification. Several intermediates including $Ru(NH_3)_6^{2+/3+}$, are insensitive to surface modifications and are considered outer sphere. $Fe^{3+/2+}$, $V^{2+/3+}$, and $Eu^{2+/3+}$ are catalyzed by surface carbonyl groups and are very sensitive to the removal of surface oxides or derivatization of –C=O groups. A similar explanation can also be provided in the present case for the increased current in Fig. 5d. Ascorbic acid and $Fe(CN)_6^{3-/4-}$ constitute a third group which are not catalyzed by oxides, but which do require a specific surface interaction. It is therefore expected that the obvious increase in the detecting signal of Sudan II upon pre-anodization of PGE is due to the participation of certain surface functional groups.

The effect of sample solution pH on the adsorption of Sudan II on PPGE was also investigated in 0.1 mol L^{-1} PBS containing 0.1 $\mu g\,mL^{-1}$ Sudan II with stirring at different pH levels in the range of 2.0–9.0. The variations of the peaks potential and the currents of the main peak with pH were investigated using AdSDPV. The maximum peak current value was obtained at pH of 4.0 for the stripping oxidation of Sudan II. Therefore, the optimum pH of the measurement solution was found to be 4.0.

In order to test the influence of accumulation potential and time on the amount of Sudan II adsorption on PPGE, the preconcentration step was performed at controlled potential and time, using accumulation time of 300 s in a stirred solution containing 0.1 $\mu g\,mL^{-1}$ Sudan II. The experimental results showed that the electrode potential applied during preconcentration had not any effect on the voltammetric signal. Fig. 6 shows the effects of accumulation time on the anodic peak current of the adsorbed Sudan II. When the preconcentration was performed at open-circuit potential, it showed that the peak current increased gradually with the accumulation time and reached its maximum at 300 s. Thus, for further analytical studies, the accumulation stage was carried out in an open circuit condition for a preconcentration time of 300 s.

Fig. 7 shows the DPV peak current for the adsorbed Sudan II as a function of its concentration in the exposure solution. The preconcentration process was done under open circuit condition for 300 s with stirring (400 rpm). The peak current increased linearly with Sudan II concentration over the range of 0.0015–0.30 $\mu g\,mL^{-1}$ in the PBS (pH 4.0). Saturated adsorption was attained when the concentration was higher than 0.3 $\mu g\,mL^{-1}$. A very large slope of 115.88 $\mu A\,mL\,\mu g^{-1}$ and a correlation of 0.9962 were also obtained. The LOD (3 s) was estimated 0.07 ng mL^{-1}. The characteristics of the

Table 1
Regression data of the calibration lines for the determination of Sudan II by the proposed methods.

	ds-DNA-modified PGE		Solution phase		PPGE
	Guanine	Adenine	Guanine	Adenine	
Linearity range ($\mu g\,mL^{-1}$)	0.5–6.0	0.5–6.0	0.5–4.0	0.5–4.0	0.0015–0.3
Slope ($\mu A\,mL\,\mu g^{-1}$)	−0.098	−0.1021	−0.1504	−0.1641	115.88
Intercept (μA)	0.7782	0.8344	0.765	0.8413	10.811
Correlation coefficient	0.9958	0.9823	0.9918	0.9952	0.9962
Limit of detection ($\mu g\,mL^{-1}$)	0.43	0.48	0.40	0.42	0.00007

Table 2
Comparison of the efficiency of previously electrochemical studies in the determination of Sudan II.

Electrode	Technique	Dynamic range ($\mu g\,mL^{-1}$)	Limit of detection ($\mu g\,mL^{-1}$)	Reference
MWNTs-IL-Gel/GC	Amperometry	0.005–20	0.001	[11]
Mercury	DPP[a]	0.082–27.63	0.082	[12]
ds-DNA modified PGE	DPV[b]	0.5–6	0.43	This work
Interaction in solution phase	DPV	0.5–4	0.40	This work
PPGE	AdSDPV	0.0015–0.3	0.00007	This work

[a] Differential pulse polarography.
[b] Differential pulse voltammetry.

Table 3
Interference study for the determination of Sudan II.

Species	Tolerant limit (w/w) At the ds-DNA Modified PGE	Tolerant limit (w/w) Interaction with ds-DNA in solution phase	Tolerant limit (w/w) on PPGE
Capsanthin, Capsaicin, β-Carotene, Cryptoxanthin	100	100	100
Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Cu^{2+}, SO_4^{2-}, CO_3^{2-}, NO_3^-	500	500	500

calibration plots are summarized in Table 1. This value of detection limit and the linear dynamic range for Sudan II observed for the ds-DNA-modified PGE and PPGE are comparable and better than those obtained for several other previous studies (Table 2).

3.3. Interference study

Interference studies were carried out with several species, prior to the application of the proposed methods for the assay of Sudan II in real samples such as chili and ketchup sauce. The potential interfering substances were chosen from the group of substances commonly found with Sudan II in food products. Tolerance limit was defined as the maximum concentration of the potential interfering substance that caused an error less than 3% for determination of Sudan II concentration. The results for the effect of the several potential interference compounds on the determination of Sudan II are given in Table 3. The obtained data showed that the proposed method is selective for Sudan II determination. Different Sudan II concentrations used for this investigation with ds-DNA-modified electrode, in solution phase and on PPGE were 5.0, 3.0, and 0.1 $\mu g\,mL^{-1}$, respectively. The selectivity of the ds-DNA-modified PGE for measuring Sudan II is due to weak interaction of the other substances with the ds-DNA in the selected conditions.

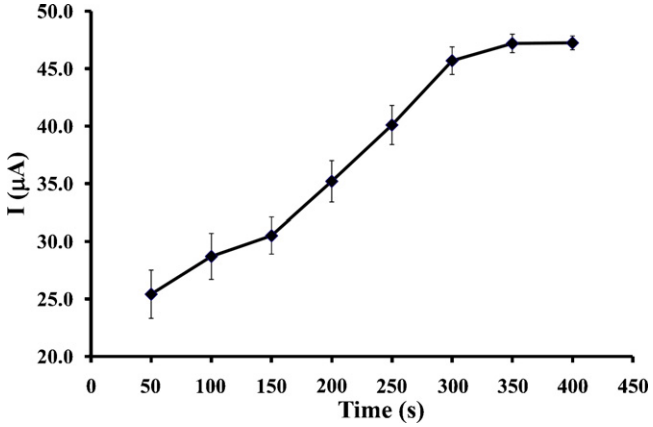

Fig. 6. Effect of accumulation time of Sudan II on the stripping oxidation current in 0.1 mol L^{-1} PBS containing 0.1 $\mu g\,mL^{-1}$ Sudan II at an open-circuit condition and with stirring. (Error bars show the relative standard deviation, $n = 3$.)

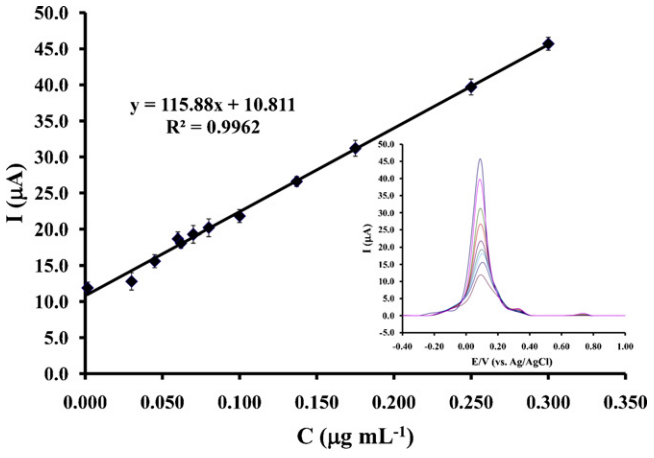

Fig. 7. Calibration curve for the determination of Sudan II on PPGE under the optimum conditions. Differential pulse voltammograms of Sudan II at different concentrations of 0.30, 0.25, 0.175, 0.14, 0.10, 0.07, 0.06, 0.045, and 0.0015 $\mu g\,mL^{-1}$ (from up to down). (Error bars show the relative standard deviation, $n = 3$.)

Table 4
Recovery of Sudan II in chili and ketchup sauce using PPGE.

Sample	Sample No.	Sudan II added ($\mu g\,mL^{-1}$)	Sudan II expected ($\mu g\,mL^{-1}$)	Sudan II found[a] ($\mu g\,mL^{-1}$)	Recovery%	RSD%
Chili sauce	1	–	–	–	–	–
	2	0.080	0.080	0.082	102.5	3.1
	3	0.200	0.200	0.194	97.0	2.8
Ketchup sauce	1	–	–	–	–	–
	2	0.080	0.080	0.078	97.5	2.7
	3	0.200	0.200	0.205	102.5	3.5

[a] Average of four replicate measurements.

Table 5
Recoveries of Sudan II in chili and ketchup sauce.

Sample	Method	Sample No.	Added ($\mu g\,mL^{-1}$)	Expected ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Recovery%	RSD%	$t_{calculated}$[b]	$F_{calculated}$[b]
Chili sauce	ds-DNA modified PGE	1	–	–	<Limit of detection	–	–	–	–
		2	2.00	2.00	1.93	96.5	4.1	–	–
		3	4.00	4.00	3.81	95.2	3.9	–	–
	Solution phase	1	–	–	–	–	–	–	–
		2	2.00	2.00	1.90	95.0	4.8	–	–
		3	3.00	3.00	3.18	106.0	4.3	–	–
Ketchup sauce	ds-DNA modified PGE	1	–	–	–	–	–	–	–
		2	2.00	2.00	1.94	97.0	3.8	1.143	4.478
		3	4.00	4.00	3.85	96.2	3.6	–	–
	Solution phase	1	–	–	–	–	–	–	–
		2	2.00	2.00	2.12	106.0	3.5	1.073	3.311
		3	3.00	3.00	2.86	95.3	4.3	–	–

[a] Average of four replicate measurements.
[b] Theoritical *t*-student test value = 3.182 for $n = 4$ ($p = 0.05$), and theoritical *F*-value = 9.277 for $n = 4$ ($p = 0.05$).

3.4. Analytical performance

On the basis of the above results, both ds-DNA-modified PGE and PPGE were applied to analyze Sudan II in real samples. Food products such as chili and ketchup sauce were evaluated for Sudan II analysis (according to the described procedure). For real sample analysis, different amounts of Sudan II were spiked into the test sample and standard addition method was applied. Because the linear calibration ranges and detections limit of PGE and in solution phase method have near the same therefore, we compared the "solution phase" data vs. PGE data (as a reference). The results are given in Tables 4 and 5.

4. Conclusions

Highly sensitive techniques for determination of carcinogenic additives in food products are particularly interesting in relation to the newly developed DNA sensors. At the present work, a sensitive DNA–biosensor was developed for the determination of Sudan II in real sample such as food products. The interaction of Sudan II with ds-DNA was characterized by voltammetric methods. As a result of the interaction between Sudan II in different concentrations with ds-DNA, a decreasing trend of the response based on the signals of guanine and adenine was observed. The results demonstrated that the DNA/Sudan II could be used as a biosensor not only to explore the interaction of the ds-DNA with Sudan II, but also to detect DNA damage caused by Sudan II. This was a fast, simple, sensitive, selective and cost effective method for recognition and evaluation of Sudan II-mediated DNA damage. In the second part of this work, AdSDPV method using PPGE was applied to determine the trace amount of Sudan II. Pretreatment of PGE before accumulation step resulted in increasing the signal and the sensitivity. Furthermore, this technique has capability for determination of Sudan II without any pretreatment such as separation steps. Although the ds-DNA-modified PGE has not better sensitivity than the reported electrochemical methods based on MWNTs-IL-Gel/GC [11] and Hg-electrode [12], but the proposed biosensor is highly selective for Sudan II determination. In addition, the proposed method based on PPGE has more sensitivity and better detection limits plus selectivity than those reported electrochemical methods [11,12]. The advantages of the proposed method include extremely low limit of detection and suitability for the online and in situ measurements.

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