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Electrochemical determination of the anticancer drug taxol at a ds-DNA modified pencil-graphite electrode and its application as a label-free electrochemical biosensor

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ABSTRACT

In this study a novel biosensor for determination of taxol is described. The interaction of taxol with salmon-sperm double-stranded DNA (ds-DNA) based on the decreasing of the oxidation signals of guanine and adenine bases was studied electrochemically with a pencil-graphite electrode (PGE) using a differential pulse voltammetry (DPV) method. The decreases in the intensity of the guanine and adenine oxidation signals after interaction with taxol were used as indicator signals for the sensitive determination of taxol. DPV exhibits a linear dynamic range of 2.0×10^{-7}–1.0×10^{-5} M for taxol with a detection limit of 8.0×10^{-8} M. Finally, this modified electrode was used for determination of taxol in some real samples.

1. Introduction

Electrochemical pre-treatment of pencil-graphite electrode (PGE) seems to be a simpler, less time-consuming and more applicable strategy in comparison with other modification procedures of the electrode surface. This strategy eliminates the use of some toxic compounds required in modification of electrode surface [1–4]. Using pencil graphite for disposable electrodes in electrochemical DNA sensing makes the method easier and more rapid compared with conventional DNA biosensors. The use of pencil-graphite electrodes has several advantages, such as avoidance of contamination among samples, ease of use due to lack of need for pre-treatment, constant sensitivity, selectivity and reproducibility. Testing of water, food, soil and plant samples for the presence of pathogenic microorganisms, carcinogens, drugs, mutagenic pollutants, etc., is one of the applications of DNA electrochemical biosensors [5–8]. Nucleic acids are powerful tools in the recognition and monitoring of many important compounds. Drugs that intercalate into ds-DNA have been extensively studied, and the field has been recently reviewed using a variety of techniques [9,10].

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 the nucleic recognition layer over an electrochemical transducer. Changes in the DNA structure during intercalation with DNA-binding molecules are detected by the nucleic acid recognition layer. Investigation based on DNA interaction is invaluable in order to understand the mechanisms of many drug compounds and to design new DNA biosensors. Electrochemical DNA biosensors are fabricated by immobilizing DNA on the electrode surface [8,11–13].

Taxol, first isolated in 1971 [14] from the stem bark of the Western yew tree, is one of the most effective natural anticancer agents, and is usually used in clinical practice for the medical treatment of ovarian, breast and non-small-cell lung cancers [15]. Because of its medical significance, there is an increasing demand for taxol from natural or artificial plants, chemical synthesis and biotechnology [16]. However, impurities similar in structure to taxol make it difficult to identify and isolate. Clinical trials have shown that taxol has a narrow therapeutic range, and its elimination half-life varies widely among patients. High-dose taxol treatment may also cause side effects [15].

There is an urgent need for a way of measuring taxol in a selective and rapid way to meet the requirements above. Several analytical methods are available for measuring taxol and its

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analogs, including high-performance liquid chromatography and tandem mass spectrometry. High-performance liquid chromatography has been widely used for evaluation of taxol and the other members of the taxane family. Tandem mass spectrometry coupled to high-performance liquid chromatography can achieve specificity and sensitivity. However, these methods are time consuming and expensive, and require sample pre-treatment. Due to the advantages of low cost, fast response, simple instrumentation, high sensitivity, facile miniaturization, and low power requirement, numerous voltammetric methods for taxol determination have been developed [17–19].

Therefore, in this work a novel, inexpensive, rapid and sensitive electrochemical DNA-biosensor for determination of taxol is constructed and applied for determination of taxol in paclitaxel injection, urine and human blood serum as real samples.

2. Experimental

2.1. Apparatus

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three-electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and PGE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements. UV–vis spectra were measured with a spectrophotometer, Carry 500, Varian, Australia.

A Noki pencil was used as a holder for Pentel graphite leads. Electrical contact with the lead was established by soldering a metallic wire to the metallic part. The pencil was held vertically with 12 mm of the lead extruded (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.

2.2. Chemicals

All solutions were freshly prepared with double-distilled water. Taxol, double-strand salmon-sperm DNA (ds-DNA), and reagent grade Tris–HCl, CH_3COOH, CH_3COONa, EDTA, NaCl and NaOH were purchased from Sigma Aldrich Chemicals (St. Louis, USA).

A salmon-sperm ds-DNA stock solution (100 mg L^{-1}) was prepared in Tris–HCl 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 M NaCl.

Stock solutions of taxol (1.0 mM) were prepared by dissolving accurately weighed amounts of taxol. Taxol working solutions for voltammetric investigations were prepared by dilution of the stock with acetate buffer (pH 4.8) containing 0.02 M NaCl.

2.3. Procedure

All the experiments were performed at room temperature. Each measurement was performed using a new graphite lead. Before immobilization of the ds-DNA, PGE was sonicated, washed with water and dried. Then the surface of the PGE was pre-treated by applying +1.40 V for 40 s followed by 200 s as an accumulation time at +0.50 V in an acetate buffer solution containing 12.0 mg L^{-1} ds-DNA with 200 rpm stirring. The electrode was then rinsed with acetate buffer solution for 5 s. After transferring the ds-DNA-modified PGE into an acetate buffer solution, the oxidation signals of guanine and adenine were obtained using DPV, with a scan rate of 10 mV s^{-1} between +0.5 and +1.3 V. The data were baseline corrected with GPES software. Five ds-DNA-modified PGE were prepared identically and the average signal of adenine and guanine was obtained prior to each experiment for taxol determination. A new ds-DNA-modified PGE was then prepared according to the recommended procedure to measure the amount of taxol in the solution. The ds-DNA-modified PGE was then immersed in a Tris–HCl buffer solution containing different concentrations of taxol with 200 rpm stirring for 100 s at an open circuit system. After the accumulation, the ds-DNA-modified PGE was rinsed and placed in taxol-free acetate buffer solution (pH 4.8), where DPVs were recorded. The analytical signal (I_s) represented the differences in the oxidation peak current of guanine and adenine before and after interaction with taxol at the ds-DNA-modified PGE. Repetitive measurements were carried out by renewing the surface and repeating the above assay formats.

2.4. Procedure of real samples preparation

One milliliter of a Paclitaxel ampoule was diluted to 10 mL with Tris–HCl buffer; then, different volumes of the diluted solution were transferred into each of a series of 25 mL volumetric flasks and diluted to the mark with Tris–HCl buffer. The taxol content was analyzed by the proposed method using standard addition.

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of the sample was centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 μm filter. Then, different volumes of the solution were transferred into a 25 mL volumetric flask and diluted to the mark with Tris–HCl buffer. The diluted urine sample was spiked with different amounts of taxol.

The serum sample was centrifuged and, after filtering, diluted with Tris–HCl buffer without any further treatment. The diluted serum sample was spiked with different amounts of taxol.

3. Results and discussions

3.1. UV–vis spectrophotometric analysis of the reaction of taxol and ds-DNA

UV–vis spectrophotometry was used to investigate the interaction between taxol and ds-DNA; the change in the UV–vis spectrophotometric signal was also observed as shown in Fig. 1. The peak intensity was weakened after adding ds-DNA into the drug solution (Fig. 1b). A notable decrease in the absorbance peak proves the existence of interaction between taxol and ds-DNA. UV–vis spectrophotometry combined with DPV is used to propose the most plausible mechanism for the interaction between taxol and ds-DNA. The decrease in the UV–vis absorption spectrum of taxol after the interaction with ds-DNA, along with the decrease of the DPV oxidation signal intensity of ds-DNA at PGE after the interaction with taxol, provide potent evidence for possible intercalation of taxol.

3.2. Interaction of taxol with ds-DNA at modified PGE using DPV

The interaction of taxol with the ds-DNA-modified PGE was studied by DPV analysis as shown in Fig. 2. Different concentrations of taxol were used to interact with guanine and adenine at the surface of the ds-DNA-modified PGE. The changes in the oxidation signals of the guanine and adenine were obtained before and after interaction with taxol. It was found that the oxidation peak currents decreased with a concomitant increase in taxol concentration. The proposed method involves monitoring of guanine and adenine oxidation currents before and after interaction with taxol, which decrease in the presence of taxol. Therefore, preliminarily ds-DNA

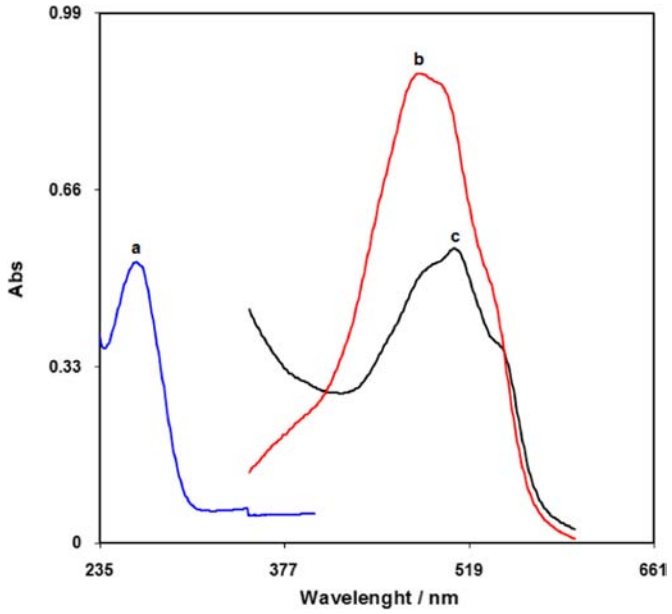

Fig. 1. UV–vis spectra of 10.0 μg mL^{-1} ds-DNA: (a) 3.0 μg mL^{-1} taxol before (b) and after (c) the reaction with 10.0 μg mL^{-1} ds-DNA.

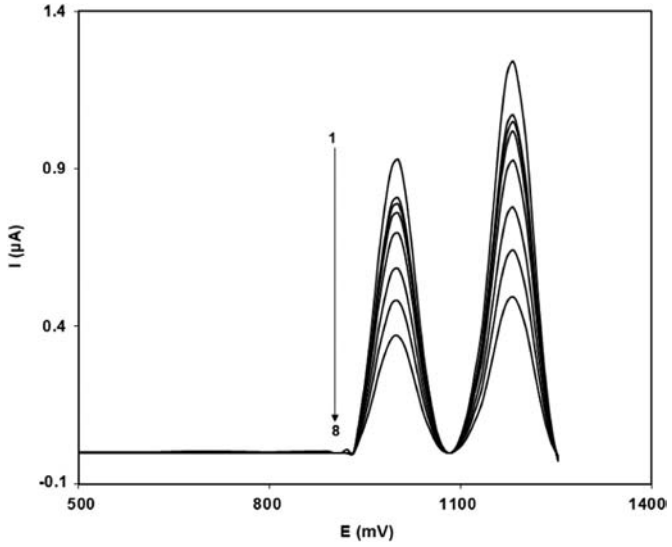

Fig. 2. DPVs for the interaction of taxol at the ds-DNA-modified PGE. The oxidation signals of guanine and adenine after interacting with 0.0, 0.2, 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 μM of taxol (1, 2, 3, 4, 5, 6, 7 and 8, respectively). Conditions: ds-DNA immobilization (12.0 μg/mL) on PGE (at=0.50 V) during 100 s in acetate buffer (pH 4.8); taxol incubation in open-circuit system during 200 s in Tris–HCl buffer (pH 7.0). Measurement: scanning between +0.5 and 1.3 V in acetate buffer (pH 4.8).

was immobilized at the surface of PGE in acetate buffer solution by adsorptive stripping voltammetry. To optimize the experimental parameters, the parameters affecting the magnitude of guanine and adenine peak currents at the ds-DNA-modified PGE were investigated. Subsequently, the developed ds-DNA-modified PGE was immersed in the Tris–HCl buffer solutions containing different concentrations of taxol. The incubation time for interaction of taxol and ds-DNA immobilized at the surface of PGE was optimized. Finally, the decreases in the guanine and adenine oxidation signals were obtained in acetate buffer solution after its interaction with taxol. These decreases were considered as analytical signals and were proportional with the concentration of taxol.

3.3. Electrochemical impedance spectroscopic studies of different electrodes

The electrochemical characterization of bare-pencil-graphite electrode (curve a), ds-DNA/PGE (curve b) and taxol-dsDNA/PGE (curve c) was made by means of electrochemical impedance spectroscopy in the presence of 1.0 mmol/L $Fe(CN)_6^{3-/4-}$ containing 0.1 mol/L KNO_3 (acetate buffer) was used as a probe. The Nyquist plots show a significant difference in the response for all the three electrodes, shown in Fig. 3. A semicircle with larger diameter is observed for taxol-dsDNA/PGE in the frequency range of 10^2–10^6 Hz. However, the diameter of the semicircle diminished with the bare-pencil electrode. This implies that charge transfer resistance of the electrode surface increases and the charge transfer rate decreases on deploying ds-DNA/PGE or taxol-dsDNA/PGE.

3.4. Effect of ds-DNA concentration on immobilization at PGE surface

The electrochemical DNA biosensor was prepared by immobilizing ds-DNA on the PGE surface. Fig. 4 displays intensities of guanine and adenine oxidation signals as the function of ds-DNA concentration after 200 s accumulation at 0.50 V at the surface of PGE in acetate buffer solution. Different concentrations of ds-DNA were immobilized, and then the guanine and adenine oxidation

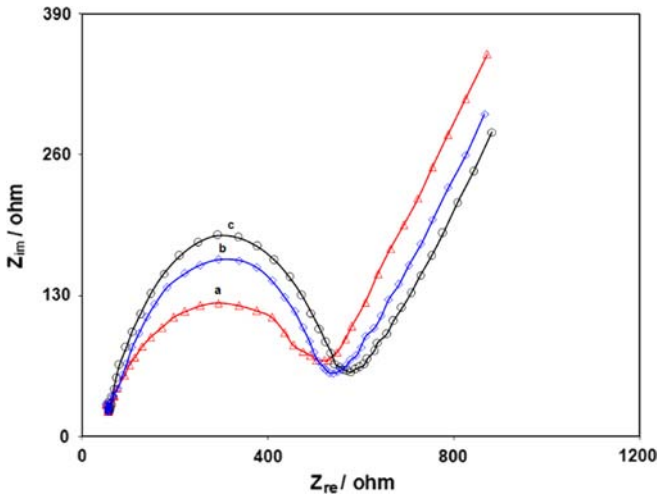

Fig. 3. Nyquist diagrams of bare-pencil-graphite electrode (curve a), ds-DNA/PGE (curve b) and taxol-dsDNA/PGE (curve c) in 1.0 mmol/L $Fe(CN)_6^{-3/-4}$ containing 0.1 mol/L KNO_3 (acetate buffer).

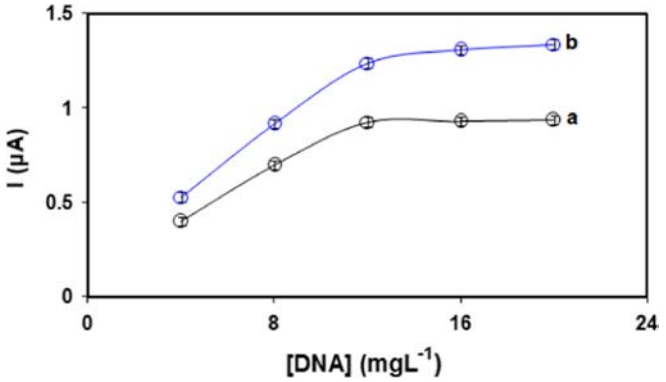

Fig. 4. Dependence of DPV oxidation signals of guanine (a) and adenine (b) upon increasing ds-DNA concentration. Conditions: ds-DNA immobilization on PGE at +0.50 V during 200 s.

signals were obtained using DPV. It was found that the guanine and adenine oxidation peak currents increased with increases in ds-DNA concentration. A deviation of the linearity was exhibited for ds-DNA concentrations higher than 12.0 mg L^{-1}. Therefore, 12.0 mg L^{-1} of ds-DNA was selected and used in all the further experiments.

3.5. Effect of adsorption time on immobilization of ds-DNA at PGE surface

One important parameter for the immobilization of ds-DNA is the adsorption (accumulation) time. Fig. 5 shows the voltammetric response for 12.0 mg L^{-1} ds-DNA accumulated at +0.50 V on PGE following different accumulation periods. Well-defined signals of guanine and adenine were obtained at the selected concentration of ds-DNA, even after short accumulation times. The DPV signals of guanine and adenine increased up to 200 s accumulation, and then levelled off. Therefore, 200 s can be considered as an optimum adsorption time for ds-DNA for preparation of ds-DNA-modified PGE.

3.6. Effect of incubation time of taxol on analytical signals

The binding of the taxol to ds-DNA depends on the interaction (incubation) time. The incubation time for the interaction of taxol with the ds-DNA-modified PGE surface was optimized. The electrochemical detections were assessed and obtained in the range of 0–200 s incubation time of taxol. The results shown in Fig. 6 show that during the increasing of the incubation time up to 200 s, there was a dramatic decrease in the oxidation signal of guanine up to 100 s, after which point it was almost levelled off. Similarly, a significant decrease was obtained in the oxidation signal of adenine up 100 s, after which the decrease became gradual from 100 to 200 s. Accordingly, 100 s was selected as the optimum time for the interaction of taxol with the ds-DNA-modified PGE.

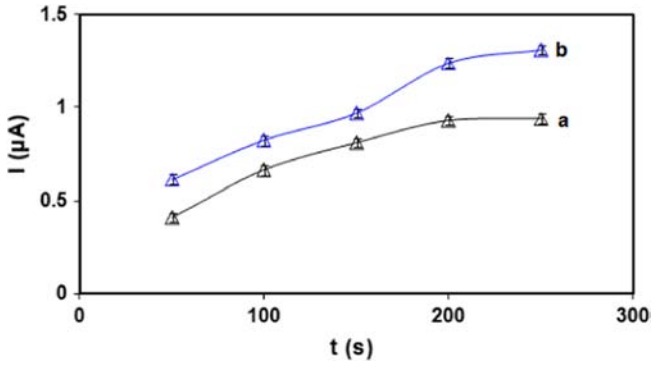

Fig. 5. Effect of accumulation time of ds-DNA at PGE surface on guanine (a) and adenine (b) oxidation signals in solution containing 12.0 mg L^{-1} of ds-DNA.

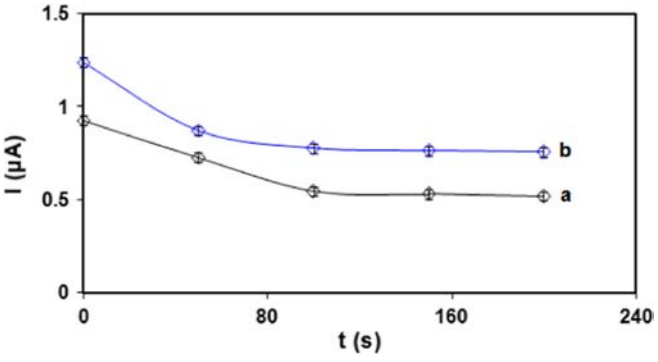

Fig. 6. Effect of incubation time of 25.0 μM taxol with ds-DNA-modified PGE on guanine (a) and adenine (b) oxidation signals.

3.7. Calibration plot and limit of detection

Increasing the concentration of taxol caused a corresponding decrease in the guanine and adenine oxidation signals. As shown in Fig. 3, the oxidation signals of guanine were linear with taxol concentration over the range 0.2–10.0 μM with a correlation equation of I_s (μA)=(0.120)+(0.043)C with R^2=0.999 (n=5) (Fig. 7A). In addition, using the same procedure and following the change in the oxidation signal intensity of adenine after the interaction with taxol in the concentration range of 0.2–10.0 μM, the regression equation was I_s(μA)=(0.162)+(0.058)C with R^2=0.999 (n=5), where C is taxol concentration in μM (Fig. 7B). The limit of detection (3 s_b/m, where s is the standard deviation of the blank signal for five replicated measurements and m is the slope of the calibration curve) based on guanine and adenine were estimated as 0.08 and 0.09 μM taxol, respectively. These values are comparable with values reported by other research groups for determination of taxol at the surface of electrodes (see Table 1).

The precision of the method was evaluated by repeating the experiments on the same days (within day reproducibility) with different standard solutions (freshly prepared at the same concentration). It was not possible to use the electrodes for more than one measurement, and therefore they had to be replaced each time.

The RSD% values (n=5) for the change of guanine signals after interaction with 1.0 and 10.0 μM taxol were 4.2% and 3.9% (n=10), respectively. For adenine signal the RSD values of 4.4% and 4.3% were obtained. These results indicate that the whole protocol to determine taxol, using ds-DNA/PGE, is reproducible.

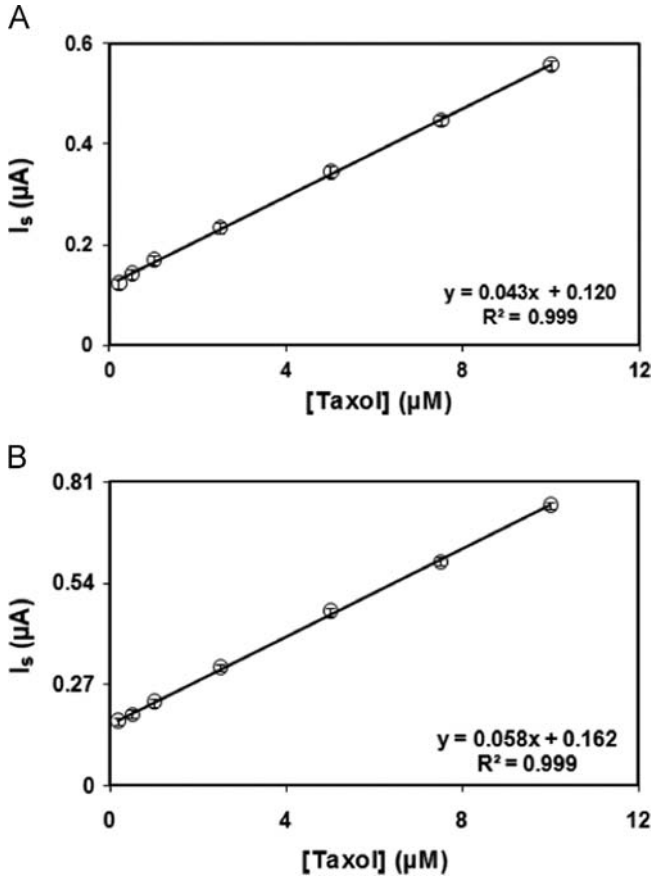

Fig. 7. Calibration curve for determination of taxol on the changes of oxidation signal of guanine (A) and adenine (B) after interaction with taxol on ds-DNA-modified PGE (error bars show the relation deviation, n=5).

Table 1
Comparison of the efficiency of some modified electrodes used in the determination of taxol.

Electrode	Modifier	pH	LOD (M)	LD (M)	Ref.
Graphite pencil	–	7.0	2.46×10^{-9}	7.0×10^{-7}–3.0×10^{-6}	18
Glassy carbon	–	7.0	1.23×10^{-8}	1.0×10^{-6}–1.0×10^{-5}	20
Gold	Hemoglobin immobilized on the DDAB/SWNTs	7.0	2.8×10^{-6}	1.4×10^{-5}–1.0×10^{-4}	17
Gold	DNA immobilization onto the azide-terminated monolayers	7.4	1.2×10^{-8}	1.2×10^{-7}–1.5×10^{-6}	21
Pencil graphite	Ds-DNA	7.0	8.0×10^{-8}	2.0×10^{-7}–1.0×10^{-6}	This work

Table 2
The application of modified electrode for determination of taxol in real samples. All concentrations are in μM ($n=5$).

Sample	Spiked (μM)	Found (μM)	Recovery (%)	R.S.D. (%)
Paclitaxel injection	0	0.55	–	3.3
	1.0	1.54	99.3	2.9
	3.0	3.63	102.2	1.9
	5.0	5.63	101.4	3.1
Urine	0	ND[a]	–	–
	2.50	2.45	98.0	1.8
	5.00	4.95	99.0	3.2
	7.50	7.71	102.8	2.8
Human blood serum	0	ND[a]	–	–
	3.00	3.10	103.3	3.4
	6.00	5.95	99.2	1.7
	9.00	9.12	101.3	2.6

[a] ND: not detected.

3.8. Interferences study

Interference studies were carried out with several species prior to the application of the proposed methods for the assay of taxol in real samples. Tolerance to the maximum concentration of the potential interfering substance led to an error of less than 3% for determination of 10.0 μM taxol. The results revealed that a 1000-fold increase of glucose, sucrose, lactose, fructose, citric acid, ascorbic acid, methanol, ethanol, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Al^{3+}, NH_4^+, F^-, alanine, phenylalanine, methionine, glycine, glutamic acid, tryptophan, aspirin, and thiourea, a 500-fold increase in cysteine and cystine, and a 250-fold increase of urea and uric acid did not affect the selectivity. The obtained data show that the proposed method is selective in the determination of taxol. The selectivity of the ds-DNA-modified PGE for taxol is due to the insufficient interaction of other substances with the ds-DNA in the selected conditions.

3.9. Real sample analysis

3.9.1. Determination of taxol in injection sample

In order to evaluate the analytical applicability of the proposed method, it was also applied to the determination of taxol in Paclitaxel injection, based on the repeated DPV responses ($n=5$) of the diluted analytes and the samples that were spiked with specified concentration of taxol for determination of taxol concentration in pharmaceutical preparation. The results are listed in Table 2.

The reliability of the proposed modified electrode was also evaluated by comparing the obtained results with those declared in the label of the pharmaceutical preparations (300 mg/50 mL). The results in Table 2 show the relative standard deviations (RSD%), and the recovery rates of the spiked samples are acceptable. Thus, the modified electrode can be efficiently used for determination of taxol in pharmaceutical preparation.

3.9.2. Determination of taxol in human blood serum and urine samples

In order to evaluate the analytical applicability of the proposed method, it was also applied to the determination of taxol in human blood serum and urine samples. The results for determination of taxol in real samples are given in Table 2. Satisfactory recovery of the experimental results was found for taxol. The reproducibility of the method is demonstrated by the mean relative standard deviation (RSD).

4. Conclusion

A novel taxol DNA-biosensor was constructed and studied using spectrophotometric and voltammetric approaches. The decrease in UV–vis absorption spectrum of taxol after the interaction with ds-DNA, and the decrease of the DPV oxidation signal intensity of ds-DNA at PGE after the interaction with taxol provide potent evidence for possible intercalation of taxol. A linear dynamic range between taxol concentration and ds-DNA signals was obtained of 2.0×10^{-7}–1.0×10^{-5} M with a detection limit of 8.0×10^{-8} M using DPV. Finally, this modified electrode was used for determination of taxol in some real samples.

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