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An electrochemical study of the adsorptive behaviour of varenicline and its interaction with DNA

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Abstract: The electrochemical behaviour of a novel nicotinic $\alpha_4\beta_2$ subtype receptor partial agonist varenicline (VAR), which is used for smoking cessation, was investigated in Britton–Robinson buffers (pH 2.0–12.0) by cyclic, differential pulse and square wave voltammetry at a hanging mercury drop electrode (HMDE). The influence of pH, scan rate, concentration, accumulation potential and time on the peak current and potential suggested that the redox process was adsorption controlled in alkaline media. In addition, the experimental value of the surface coverage, $\Gamma = 1.03 \times 10^{-10} \text{ mol cm}^{-2}$, was used to determine the conditions when VAR was fully adsorbed at the electrode surface. Bearing in mind the potential high toxicity of VAR due to the presence of a quinoxaline structure, its interaction with double stranded-DNA (ds-DNA) was postulated and studied when both compounds were in the adsorbed state at a modified HMDE. Using the adsorptive transfer technique, changes in potential and decreases in the normalized peak currents were observed. The estimated value of the ratio of surface-binding constants indicated that the reduced form of VAR interacted with ds-DNA more strongly than the oxidized form. Subtle DNA damage under conditions of direct DNA–VAR interaction at room temperature was observed. The proposed type of interaction was intercalation. This study employed a simple electroanalytical methodology and showed the potential of a DNA/HMDE biosensor for investigation of genotoxic effects.

Keywords: adsorption; DNA; interaction; varenicline; electrochemistry.

INTRODUCTION

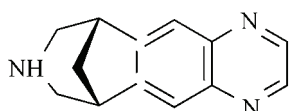
Varenicline (VAR, 7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino(2,3-*h*)(3) benzazepine), (Scheme 1) is a novel nicotinic $\alpha_4\beta_2$ subtype receptor partial ago-

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nist approved by the Food and Drug Administration (FDA) for smoking cessation.¹ VAR is similar in structure to nicotine but, as a partial agonist, it does not produce the full effect of nicotine. VAR competitively blocks the ability of nicotine to bind and stimulate the dopamine system. Only a small portion of VAR is metabolized, so most of the active compound is excreted renally.



Scheme 1. Chemical structure of varenicline.

Since VAR possesses a quinoxaline core, which is known as a cytotoxic structure,² it can be supposed that varenicline also shows some cytotoxic effects.

There are only few reports on determination of VAR or its degradation products and impurities by different chromatographic methods in tablets³⁻⁷ and plasma samples.⁸ Recently, the electrochemical behaviour of VAR at several electrodes was reported.⁹ VAR undergoes reduction of the two C=N bonds of the substituted quinoxaline ring. In two consecutive, one electron steps the C=N bonds in VAR are reduced to give dihydrovarenicline, which undergoes further hydrogen ion-catalyzed chemical transformation and after the addition of two protons and two electrons, it is reduced to the final product, tetrahydrovarenicline. The critical factor governing the reduction processes of VAR is the pH of the solution. The overall process was possible only in acid solution and is represented by two reduction peaks, separated by more than 0.5 V. In alkaline medium, dihydrovarenicline is formed as the final product, which can be reversibly oxidized back to VAR.

Since the VAR molecule has a planar structure, it can be assumed that it will show considerable adsorption at an electrode surface. Bearing in mind its high toxicity, interactions with DNA molecule may be postulated.

The best way to study these interactions is to measure the electrode signal when both compounds are in the adsorbed state at the electrode surface.

Electrochemical DNA-based biosensors¹⁰⁻¹² are often used for the determination of low-molecular weight compounds with affinity for nucleic acids and for the detection of the hybridisation reaction.¹³ There are many interesting applications of DNA biosensors for detection of different analytes in water, soil, plant, food samples and pharmaceuticals.¹⁴ It is well known that drugs bind to DNA both covalently and non-covalently. The covalent binding with DNA is irreversible and leads to complete inhibition of DNA processes and subsequent cell death. Non-covalent binding of small molecules to DNA can occur in three modes: intercalation into the base pairs, binding in the "major" or "minor" grooves, and by electrostatic interactions outside the helix.¹⁵ The intercalators are usually planar molecules which contain heterocyclic groups that stack between adjacent DNA base pairs and cause strong structural perturbations in the DNA molecule.

Since the structure of varenicline contains three planar heterocyclic rings, its potent interaction with DNA could be assumed.

Electrochemical analysis of DNA is based on the fact that the adenine and cytosine residues produce reducible signals, while guanine residues yield an anodic signal due to the oxidation of the guanine reduction product. For the detection of such signals, voltammetric techniques at a hanging mercury drop electrode (HMDE) are used most frequently.¹⁶ The interaction between drugs and DNA that cause DNA damage can be monitored by following the changes in the current intensity and the shift in the potential of the voltammetric peaks of DNA and investigated drug.

In this work, the preliminary results on the interaction of the nicotinic receptor agonist varenicline with calf thymus double-stranded DNA (ds-DNA) immobilized at a HMDE using adsorptive transfer stripping square wave voltammetry (AdTSSWV) are presented.

EXPERIMENTAL

Reagents and solutions

VAR was kindly donated by the Agency of Drugs and Medical Devices, Belgrade, Serbia. A stock solution (S_0) 1×10^{-3} mol dm⁻³ of VAR was prepared in redistilled water and stored in a freezer. More dilute solutions were prepared daily from the stock solution (S_0).

Double-stranded calf thymus DNA (MW = 10×10^6 – 15×10^6 g mol⁻¹) was purchased from Sigma-Aldrich (Deisenhofen, Germany). The stock solution of DNA was 1.3×10^{-4} mol dm⁻³ in 2×10^{-3} mol dm⁻³ phosphate buffer, pH 7.0, and its exact concentration was determined spectrophotometrically and related to the nucleotide content.

Britton–Robinson buffer solutions, used as the supporting electrolytes for VAR investigation, were prepared in the usual way.¹⁷ For the DNA studies, the adsorption was performed in phosphate buffer, and voltammetric measurements were realised in ammonium formate/sodium phosphate buffer. All the chemicals used for buffer preparation were of analytical grade.

Double distilled water was used throughout. All experiments were performed at room temperature.

Instrumentation

The measurements were performed with an μ AUTOLAB analyzer (EcoChemie, Utrecht, The Netherlands) connected to VA-Stand 663 (Metrohm, Herisau, Switzerland), and controlled by GPES 4.9 software. A standard three-electrode electrochemical cell was used. The working electrode was a hanging mercury drop electrode (HMDE) with drop area of 0.4 mm², the reference electrode was Ag/AgCl/3 mol dm⁻³ KCl, and a platinum wire was used as the auxiliary electrode.

Alternatively, in some voltammetric measurements, an Amel 433-A computerized polarographic analyzer was used, with a similar three-electrode system (working HMDE with a drop area of 1.9 mm², Ag/AgCl reference and Pt-auxiliary electrode).

A Radiometer PHM 220 pH meter with a Radiometer GK2401B combined pH electrode was used.

Cyclic (CV), differential pulse (DPV) and square wave (SWV) voltammetric techniques were used in this work. The CV mode was applied with the scan rates from 5 to 100 mV s⁻¹,

DPV was performed with pulse amplitude of 50 mV, pulse width 50 ms and scan rates of 20, 50 and 100 mV s⁻¹, while the parameters for the SWV mode were: amplitude 50 mV, scan increment 1–5 mV, frequency 125 Hz and sampling time 1 ms, and when required, the stirring speed of 300 rpm was applied. In the adsorptive stripping experiments, the adsorption accumulation potential and time were selected.

Procedure for the pH investigation

An appropriate volume of the supporting electrolyte of different pH values were placed in the electrochemical cell, de-aerated for 10 minutes with high purity nitrogen and then 0.15 ml of the VAR stock solution (S₀) was added to give a final VAR concentration of 1×10⁻⁵ mol dm⁻³ for cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The solution was purged for a further 3 min and the current–voltage curves were recorded.

Procedure for adsorptive stripping voltammetric investigation

An aliquot of 15.00 ml of supporting electrolyte (BR buffer) solution was introduced into the electrochemical cell and de-aerated with pure nitrogen for 10 min. A selected accumulation potential was applied to the mercury drop for a selected accumulation period, while the solution was stirred at 300 rpm. The stirring was then stopped and after the rest period, adsorptive stripping square wave voltammetry was applied for reduction process over the range –0.2 to –1.2 V vs. Ag/AgCl. After the background voltammogram had been recorded, an adequate aliquot of the stock VAR solution was added to the cell and under the same conditions, the AdSSW voltammogram was recorded at a new drop.

Procedure for adsorptive transfer stripping technique (AdTS, ex situ)

DNA was adsorbed from a 5 µl sample drop onto the HMDE surface for 120 s. Then the electrode with an adsorbed DNA layer was washed with water and transferred into the background electrolyte, where the voltammetry was performed. The same procedure was employed with VAR under the same experimental conditions. The measurements were performed in the absence of oxygen, by passing nitrogen through the solution for 5 minutes before starting the measurements.

Alternatively, VAR and DNA were mixed, and after the selected incubation period (2–30 min) this mixture was adsorbed from a 5 µl drop onto the HMDE. The electrode modified with a VAR–DNA layer was washed and transferred into the background electrolyte and the SW voltammogram was recorded.

RESULTS AND DISCUSSION

Effect of pH

The study of the redox processes of VAR was performed in the pH range 2.0 to 12.0 in BR buffers. VAR exhibited electrochemical activity at all the investigated pH values. The voltammetric behaviour of VAR was examined using CV and DPV. Some representative voltammograms are presented in Fig. 1. Two reduction peaks (I and II) were present. Peak I was present over the whole pH range, and its reversibility increased with increasing pH. On the other hand, peak II was irreversible and not well developed. This peak appeared at negative potentials (–0.7 V < E_p < –1.0 V) and disappeared in neutral and alkaline media while the peak I attained full reversibility.

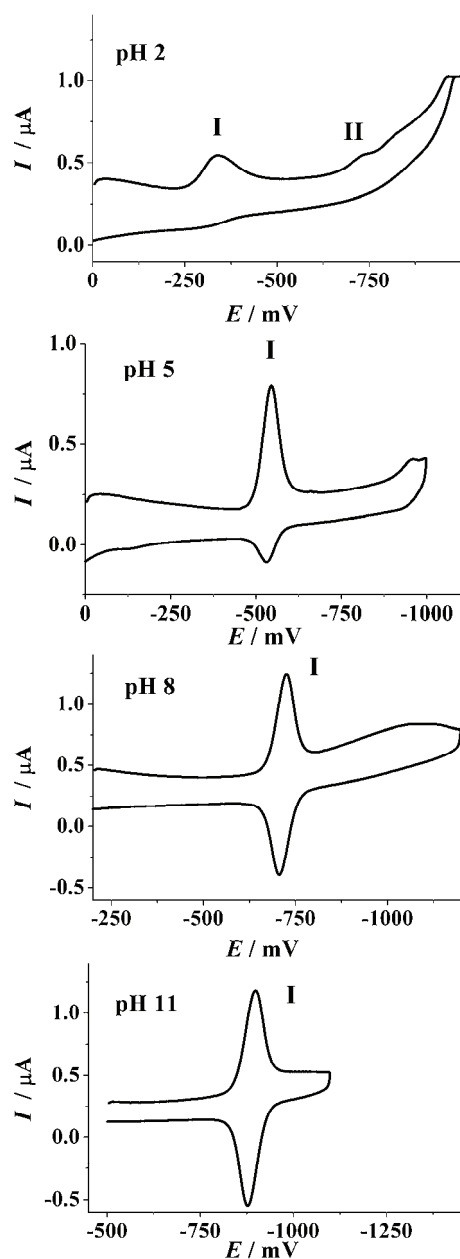


Fig. 1. Representative CV voltammograms of 1×10^{-5} mol dm^{-3} VAR in BR buffer: pH 2.0, 5.0, 8.0 and 11.0. Scan rate: 100 mV s^{-1} .

Attention was focussed on peak I, which is the consequence of the two-electron reduction of the C=N bond in VAR to give dihydrovarenicline.⁹ The peak current increased with the increasing pH, (Fig. 2A), indicating a higher rate of drug adsorption at the electrode surface in neutral and alkaline media. At the

same time, the peak potential was shifted towards more negative potentials, and linearly varied from -0.3 V at pH 2.0 up to -0.96 V at pH 12.0 (Fig. 2B), proving that protons participate directly in the reduction process.

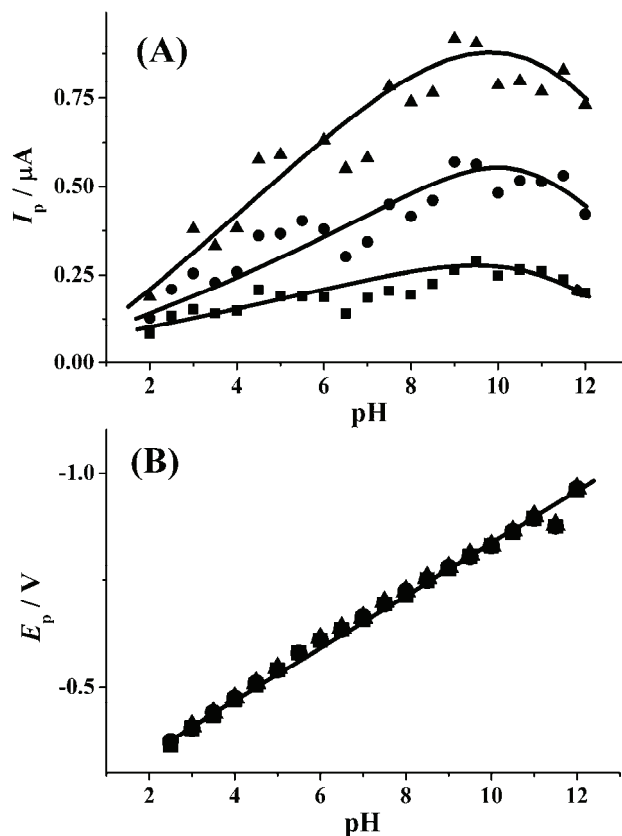


Fig. 2. The influence of pH on CV peak current (A) and peak potential (B) of 1×10^{-5} mol dm^{-3} VAR in BR buffer at different scan rates: 20 (■), 50 (●) and 100 mV s^{-1} (▲).

Using a VAR concentration of 1×10^{-5} mol dm^{-3} at different pH values and varying the scan rate over the range 5–100 mV s^{-1} , the voltammetric peak current was measured. At $\text{pH} < 4.0$, the I_p vs. ν dependence is nonlinear, but at $\text{pH} > 4.0$, a linear dependence was obtained, suggesting that the reduction process is controlled by adsorption. According to the values of the obtained slopes (Table I), it seems that the most pronounced adsorption of VAR at the mercury surface was observed at pH around 8. This means that the adsorbed form of VAR undergoes an electrode reduction process under these conditions.

The slope of the linear dependences of $\log I_p$ vs. $\log \nu$ at $\text{pH} > 4.0$ increases and becomes equal to the theoretical value of 1.0 at pH 8.0, which is characteristic for an entirely adsorption controlled processes. In addition, the small de-

crease of the slopes in the linear dependences $\log I_p$ vs. $\log v$ at $\text{pH} > 8.0$ expresses a weakened adsorption in highly alkaline solutions.

TABLE I. Regression equations of the $I_p = f(v)$ and $\log I_p = f(\log v)$ linear dependences, in acid and alkaline medium

pH	$I_p / \mu\text{A} = f(v / \text{V}^{-1} \text{ s})$	r	$\log I_p = f(\log v)$	r
2.0	Nonlinear	–	$\log I_p = 0.4831 \log v - 0.269$	0.997
4.0	Nonlinear	–	$\log I_p = 0.664 \log v + 0.267$	0.997
6.0	$I_p = 5.740v + 0.0466$	0.996	$\log I_p = 0.861 \log v + 0.668$	0.995
8.0	$I_p = 7.218v + 0.0143$	0.997	$\log I_p = 1.001 \log v + 0.882$	0.998
10.0	$I_p = 7.217v + 0.0851$	0.995	$\log I_p = 0.741 \log v + 0.642$	0.999
12.0	$I_p = 6.965v + 0.0553$	0.995	$\log I_p = 0.628 \log v + 1.292$	0.997

Adsorptive character of the drug

To improve the sensitivity for monitoring the accumulated drug, adsorptive stripping square-wave voltammetry was applied. The signal intensity of the AdSSW voltammetry was found to be 30 and 600 times higher than those of differential pulse (DP) and linear sweep (LS) adsorptive technique, respectively. The advantage of the application of an SW waveform was documented by trace analysis of several drugs that exhibited adsorption at the electrode surface.^{18–21} The SW response markedly depended on the parameters of the excitement signal. In order to obtain the maximum peak current, the optimum instrumental conditions (frequency of 125 Hz, scan increment of 5 mV and pulse amplitude of 50 mV) were applied in the further work.

Effect of accumulation time (t_{acc}) and potential (E_{acc})

The effect of accumulation time on the peak current for $1 \times 10^{-7} \text{ mol dm}^{-3}$ VAR was studied in the range from 1 to 30 s. The peak current increased with increasing accumulation time up to 10 s when adsorptive saturation on the mercury electrode surface was finally achieved. Under such conditions, interactions among the drug molecules in the adsorbed state became noticeable and the peak current commenced to decrease slightly. Hence, accumulation times longer than 10 s are required for work with an electrode surface covered with a VAR layer (Fig. 3A).

The potential range of -0.1 to -0.8 V was examined for $1 \times 10^{-7} \text{ mol dm}^{-3}$ VAR after a pre-concentration time of 10 s, to define the optimal accumulation potential. The highest current peak was obtained with a deposition potential of -0.4 V at pH 5.0 and -0.6 V at pH 8.0 (Fig. 3B). The observed gradual decrease in peak current intensity may be the consequence of desorption of the drug at much higher or lower potentials compared to the potential of zero charge, when the maximum adsorption of uncharged organic molecules is expected.

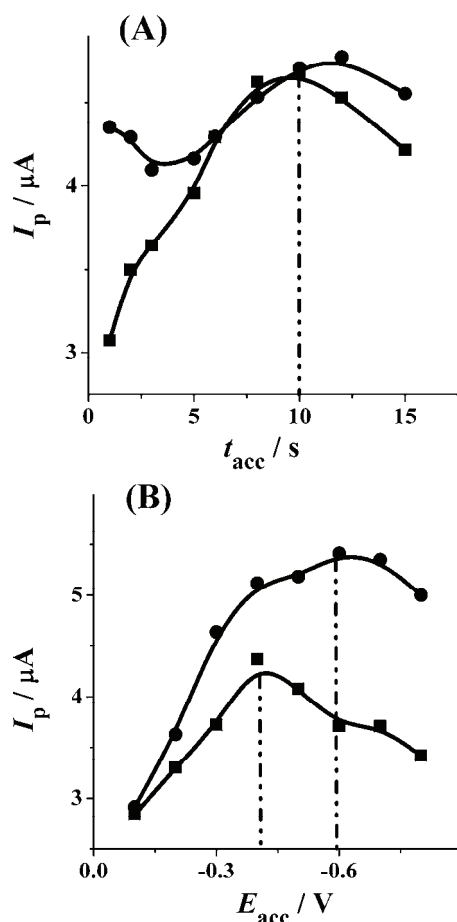


Fig. 3. The influence of (A) accumulation time (t_{acc}) and (B) accumulation potential (E_{acc}) on the AdSSWV current peak for 1×10^{-7} mol dm $^{-3}$ VAR in BR pH 5.0 (■) and 8.0 (●) (B: $t_{\text{acc}} = 10$ s).

Effect of the drug concentration

Applying AdSSW voltammetry under the chosen optimal conditions for VAR concentrations above 10^{-7} mol dm $^{-3}$, the peak height increased linearly up to 4×10^{-7} mol dm $^{-3}$, and then slightly decreased. Simultaneously, the peak potential was almost constant (Fig. 4).

This behaviour suggests that with increasing concentration, the electrode surface reaches its full coverage, and it can be assumed that at a VAR bulk concentration above 5×10^{-7} mol dm $^{-3}$, a monolayer is completely formed and only the adsorbed VAR molecules undergo electron transfer processes. The CV response involving only the adsorbed species is characterized by symmetric cathodic and anodic peak shapes, which are noticeable in Fig. 1. The relative position of cathodic and anodic peaks depends on the relative adsorption energies of the oxidized and reduced species, and the corresponding peak current is given by:

$$I_p = \frac{n^2 F^2}{4RT} \nu A \Gamma \quad (1)$$

where n represents the number of electrons transferred in a reversible electrode reaction, A is the electrode surface area and Γ is the surface coverage in moles of adsorbed molecules per surface area. Applying this equation to the results obtained for the CV curves of $1 \times 10^{-5} \text{ mol dm}^{-3}$ VAR two electron reduction at pH 8.0, the amount of electroactive specie on the surface was evaluated, and the experimental surface coverage was found to be $\Gamma = 1.03 \times 10^{-10} \text{ mol cm}^{-2}$. To check whether this corresponds to a monolayer, the theoretical surface coverage was estimated by considering the varenicline molecule as a rectangle with an area of 1.5 nm^2 ($0.75 \text{ nm} \times 2 \text{ nm}$ corresponding to the bond lengths²²). A dense arrangement of these molecules should lead to a coverage of $\Gamma = 1.107 \times 10^{-10} \text{ mol cm}^{-2}$. This confirms that in the limit of the approximations made, the electrode was covered with a monolayer of varenicline.

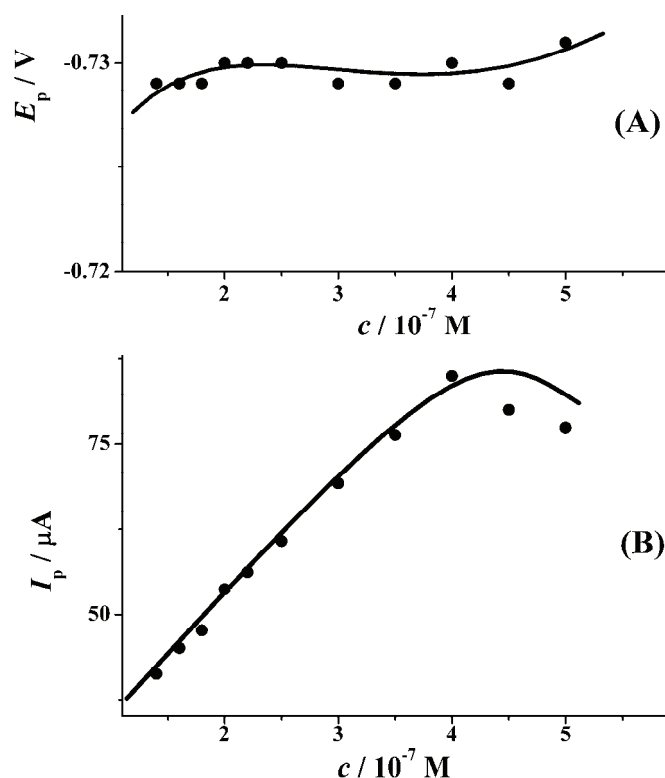


Fig. 4. Dependence of the AdSSWV peak potential (A) and current (B) on the VAR concentration in BR buffer at pH 8.0.

VAR–DNA Interaction

For the study of VAR–DNA interaction, DNA was pre-incubated with VAR in equal concentrations for different incubation periods. After this incubation period, the HMDE was modified with this mixture and the interaction was studied by comparing the obtained voltammogram with the signals obtained for HMDE/VAR and HMDE/DNA modified electrodes under the same conditions. The conditions pH (pH 7.0), ionic strength (5×10^{-2} mol dm⁻³ NaCl), concentrations (20 – 100×10^{-6} mol dm⁻³), and incubation time (2–30 min) were selected in order to ensure that both components are fully adsorbed at the electrode surface. After the incubation period, SWV measurements were performed using the adsorptive transfer stripping technique (AdTS).

The usual reduction and subsequent oxidation and electron transfer reactions characteristic for both components are shown in Fig. 5. The reversible reduction peak at -0.654 V corresponds to the two-electron reduction of VAR (curve 1, peak V), while DNA produces a cathodic peak at -1.426 V due to the reduction of adenine and cytosine (curve 2a, peak A/C) and an anodic peak of re-oxidized guanine at -0.253 V (curve 2b, peak G) after its reduction at extremely negative potentials.

After the incubation of VAR and DNA, the obtained voltammogram showed changes in the peak heights and potentials (curves 3a and 3b). All peak currents decreased, and the VAR peak potential was shifted to a more positive potential. This indicates that VAR and DNA interact and that the ds-DNA structure can be distorted as a result of subtle damage by this drug.

The changes in the current responses caused by VAR–DNA interaction were expressed as normalized peak currents: V (%), A/C (%), and G (%) using Eqs. (2)–(4):

$$V (\%) = 100I_{p,V}/I_{p0,V} \quad (2)$$

$$A/C (\%) = 100I_{p,A/C}/I_{p0,A/C} \quad (3)$$

$$G (\%) = 100I_{p,G}/I_{p0,G} \quad (4)$$

where $I_{p,V}$, $I_{p,A/C}$ and $I_{p,G}$ and $I_{p0,V}$, $I_{p0,A/C}$ and $I_{p0,G}$ are the peak currents of varenicline, adenine/cytosine and guanine after and before interaction, respectively. The obtained results are summarized in Table II. The normalized peak current of both compounds decreased after incubation.

The phenomenon of a change in the nature of the interaction from electrostatic to intercalative with increasing ionic strength was reported.²³ According to this study, the formal potential shift in the negative direction at low ionic strengths shows that the behaviour of the ds-DNA on the electrode surface was dominated by electrostatic interactions, while the formal potential shift in the positive direction at higher ionic strengths indicates intercalative interactions.

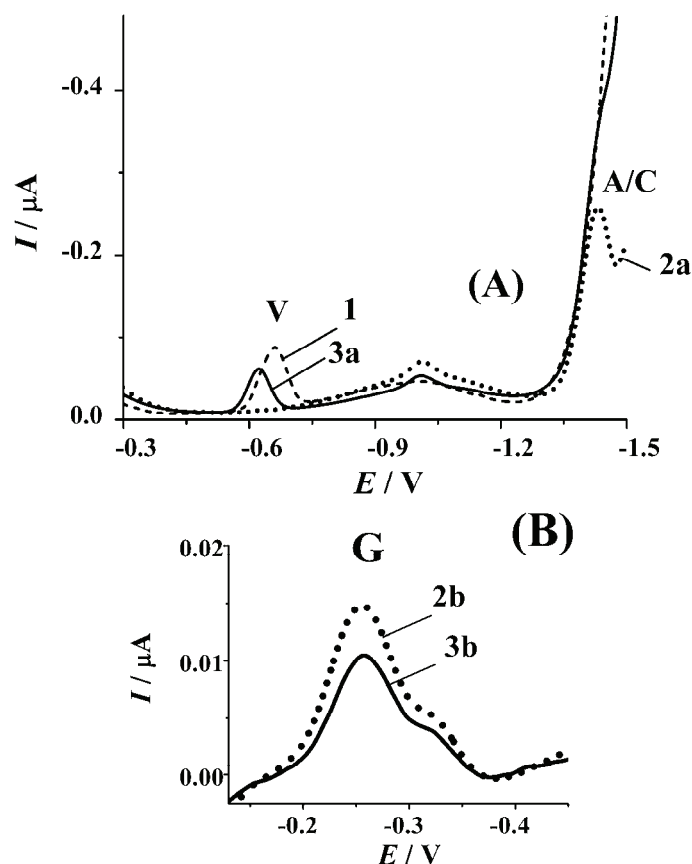


Fig. 5. AdTSSW voltammograms of: 1×10^{-5} mol dm^{-3} VAR (1 - - -), ds-DNA (2a, 2b \cdots) and VAR-DNA mixture (3a, 3b \rightarrow). The compounds were adsorbed from 0.05 mol dm^{-3} NaCl with 0.002 mol dm^{-3} phosphate buffer, pH 7.0, for $t_{\text{acc}} = 120$ s, $E_{\text{acc}} = \text{OCP}$. The HMDE with adsorbed layers of (1), (2) or (3) were transferred to the blank background electrolyte 0.3 mol dm^{-3} ammonium formate/sodium phosphate buffer, pH 6.86 and the SW voltammograms were recorded (frequency 25 Hz, amplitude 200 mV, potential range from 0 to -1.85 V). A) cathodic; B) anodic peaks.

The formal potential shift can be expressed as:

$$\Delta E^{0'} = E_{\text{surf}}^{0'} - E_{\text{sol}}^{0'} = \frac{RT}{nF} \ln \frac{K_{\text{Red}}}{K_{\text{Ox}}} \quad (5)$$

where $E_{\text{surf}}^{0'}$ and $E_{\text{sol}}^{0'}$ represent the formal potentials of the redox species immobilized on the electrode surface and in the solution, and K_{Red} and K_{Ox} are the surface-binding constants for the reduced and oxidized forms of the redox species, respectively. In the case of VAR, which undergoes a reversible, two-electron transfer at relatively high ionic strength (0.05 mol dm^{-3}), the ratio of $K_{\text{V,Red}}/$

$/K_{V,Ox}$ was estimated and is presented in Table II. These results indicate that the reduced form of VAR interacts with the ds-DNA on the modified electrode surface more strongly (3–14 times, depending on the incubation period) than the oxidized form. This behaviour is characteristic of intercalative interactions.²³ The shift in VAR peak potential, E_p , towards more positive potentials after VAR–DNA incubation supports this hypothesis.

TABLE II. The VAR–DNA interaction expressed by normalized values of the SW voltammetric responses, change in the peak potential, and surface binding constants ratio

Peak	E_p / V	$100I_p/I_{p0}$		$\Delta E_p / V$		$K_{V,Red}/K_{V,Ox}$	
		10	30	10	30	10	30
V	-0.654	74.9	31.1	34.0	15.0	14.0	3.0
A/C	-1.426	31.0	6.95	–	–	–	–
G	-0.253	72.5	68.7	–	–	–	–

CONCLUSIONS

The novelty of these investigations is based on the adsorption effects of VAR at a mercury surface, which enables the application of AdSSW voltammetry for studying the redox processes of VAR in the adsorbed state. The obtained values of surface coverage and accumulation potential and time were used to determine the conditions under which VAR is fully adsorbed at the electrode surface.

Association interaction of calf thymus ds-DNA with VAR at the modified HMDE was investigated. At room temperature and selected ionic strength, subtle DNA damage in the direct DNA–VAR interaction was observed, suggesting intercalation as the type of the interaction. The results lead to the conclusion that the toxicity of the investigated drug could be caused by this interaction. This study used simple electroanalytical methodology and showed the potential use of a DNA/HMDE biosensor for investigation of genotoxic effects.

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ИЗВОД

ЕЛЕКТРОХЕМИЈСКО ИСПИТИВАЊЕ АДСОРПЦИЈЕ И ИНТЕРАКЦИЈЕ ВАРЕНИКЛИНА И ДНК

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Електрохемијско понашање варениклина, новог парцијалног агонисте $\alpha_4\beta_2$ никотинског рецептора, који се користи за одвикавање од пушења, испитано је цикличном, диференцијално пулсном и волтаметријом правоугаоних таласа у Бритон–Робинсоновом пуферу (рН 2,0–12,0). На основу утицаја рН, брзине промене потенцијала, концен-

трације, потенцијала и времена акумулације на висину и положај пика, закључено је да је процес редукције у алкалној средини контролисан адсорпцијом варениклина. Коришћењем експериментално добијене вредности површинске запоседнутости, $\Gamma = 1,03 \times 10^{-10} \text{ mol cm}^{-2}$, одређени су услови под којима је варениклин потпуно адсорбован на површини електроде. Имајући у виду могућу високу токсичност варениклина, с обзиром на присуство хиноксалинског прстена у структури, претпостављено је да варениклин интерагује са ДНК када су оба молекула адсорбована на модификованој живиној електроди. Ова интеракција је испитана коришћењем „адсорптивне трансфер технике“ и примећене су промене потенцијала и смањење нормализованих струја волтаметријских пикова. На основу израчунате вредности односа константи везивања закључено је да се редуктовани облик варениклина јаче везује за ДНК од оксидованог. Претпостављено је да директна варениклин-ДНК интеракција на собној температури доводи до извесног оштећења ДНК и да је тип интеракције – интеркалација. Ова једноставна електроаналитичка методологија могла би наћи примену у виду потенцијалног биосензора за испитивање генотоксичних ефеката.

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