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Electrochemical Study of Ellipticine Interaction with Single and Double Stranded Oligonucleotides

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Abstract: Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole) is an alkaloid that has been isolated from plants of an *Apocynaceae* family. It is one of the simplest naturally occurring alkaloids with a planar structure. Over the past decades, ellipticine became a very promising antitumor agent. Interaction with DNA is one of the most studied ellipticine effects on cell division. This phenomenon is not clearly explained so far. In our experiments we studied interaction of ellipticine with single-stranded and double-stranded oligonucleotides by electrochemical methods on mercury electrode. Differential pulse voltammetry was applied for ellipticine (Elli) and CA peak detection. Square wave voltammetry was applied for G peak detection. The effect of the interaction time and ellipticine concentrations on interactions of ellipticine with single- and double-stranded oligonucleotides was tested too.

Keywords: Adsorptive transfer stripping technique, DNA, differential pulse voltammetry, ellipticine, hetero-nucleotides, intercalation, square wave voltammetry, cancer, therapy.

1. INTRODUCTION

Ellipticine and its 9-methoxyderivate are natural substances isolated from some plant of an *Apocynaceae* family such as *Ochrosia borbonica* and *Excavatia coccinea*. These substances have been shown in the treatment of several tumours such as myeloblastic leukaemia and bone metastases of breast-cancer with this drug only by the limitation in haematological toxicity and fairly limited toxic side effects but strong antitumor effects from clinical point of view [1-6]. Ellipticine within the low concentration range (10^{-10} to 10^{-6} M) induces growth arrest and cells death in breast adenocarcinoma [7-9], leukaemia [10], colon cancer [11], neuroblastoma [12], glioblastoma [13, 14], lung carcinoma [15] and hepatocellular carcinoma [16] cells. Due to the high efficiency (antitumor activity) of ellipticine and its derivatives against various types of cancer, the interest on this drug has been increasing. New directions for development and preparation of targeted drugs based on ellipticine are still explored. Furthermore, ellipticine also exhibited anti-HIV activity [17].

Cytotoxic and mutagenic effects of ellipticine are attributed mainly to two mechanisms. The first is inhibition of topoisomerase II [18] and the intercalation into the structure of DNA double helix [19]. The mechanisms of the ellipticine cytotoxic effect mediated by DNA damage are that ellipticine induces DNA strand breaks connected with topoisomerase II inhibition [20, 21] and it also forms covalent DNA adducts after its activation with peroxidase and cytochrome P450 enzymes [3-6, 22]. The induction of DNA strand breaks was described for the first time in DNA from L1210 cells [23]. The unsuccessful reparation of DNA faults leads to apoptosis of cancer cells. The major study about ellipticine-topoisomerase activity identified topoisomerase II as the primary cellular target for this drug [24]. Cytochrome P450 enzymes metabolize ellipticine to five metabolites (13-hydroxyellipticine, 12-hydroxyellipticine and N2-oxide of ellipticine, 7-hydroxyellipticine and 9-hydroxyellipticine) [5, 6, 25]. 12-Hydroxyellipticine, 13-hydroxyellipticine and the ellipticine N2-oxide (generating spontaneously 12-hydroxyellipticine) are responsible for formation of ellipticine-12/13-ylium that react with nucleophilic centres of the deoxyguanine residues in DNA, generating two DNA adducts [3, 5, 6, 22, 25, 26]. In the case of peroxidases, ellipticine is oxidized to radicals providing ellipticine dimer (major product) and, in a minority, the N2-oxide of ellipticine. Using the ^{32}P-post-labeling method and [^{3}H]-labelled ellipticine, Stiborova *et al.* showed that ellipticine binds covalently to DNA after its activation both by peroxidases [22, 27] and cytochromes P450 [3-6, 25].

The other mechanism is the intercalation effect. Ellipticine itself is not too strong intercalator, but more effective from this point of view are its hydroxyderivates. The 9-hydroxyellipticine shows the best affinity to the guanine - cytosine (GC) [1-6]. Several studies showed that the planar (hetero)-aromatic structure intercalated between pairs of bases of double-stranded DNA usually leads to its stabilization, strengthening of conformations, elongation and partially untangle [1-6]. These changes usually lead to functional inhibition of the numerous enzyme processes acting on DNA, such as transcription and replication [3, 6].

Nowadays electrochemistry is regarded as one of the most sensitive method for DNA study [3, 24] and this method can be used for detection of DNA adducts through determination of DNA peaks (mostly cytosine-adenine (CA) peak and guanine peak (G) [28]) and peak connected with drugs. CA peak can be determined by differential pulse voltammetry (DPV) and G peak by square wave voltammetry (SWV). In this study results from the characterization of ellipticine and its interaction with single (ssODN) and double (dsODN) strand oligonucleotides electrochemically by adsorptive transfer stripping technique (AdTS) at hanging mercury drop electrode (HMDE) are presented.

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2. EXPERIMENTAL PART

2.1. Chemicals

Two sequences of oligonucleotides (GL6 P2 - 5´-ATGGAACATT; GL6 P2K - 5´-AATGTTCCAT) were purchased from Sigma-Aldrich (USA). Sodium acetate, acetic acid, ellipticine and water were purchased from Sigma Aldrich (USA) in ACS purity unless noted otherwise. Standard solutions of the oligonucleotides (10 μg/ml) were prepared with American Chemical Society (ACS) analytical water and stored in dark at -20°C. The concentration of standard ellipticine (powder) was 0.05 M dissolved in dimethyl sulfoxide (DMSO). For electrochemical measurements, two electrolytes were used as acetate buffer (pH 5.0), which consist of 0.2 M CH_3COOH + 0.2 M CH_3COONa, and sodium phosphate buffer (50 mM, pH 6.9) with 0.3 M ammonium formate.

2.2. Differential Pulse and Square Wave Voltammetric Measurements

Electrochemical measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland) and 797 VA computrace (Metrohm, Switzerland) with three electrodes. Details are published in [2].

2.2.1. Ellipticine Determination

Ellipticine was electrochemically detected by differential pulse voltammetry (DPV) in the presence of 0.2 M acetate buffer (pH 5.0). Volume of samples was 5 μl, volume of electrolyte was 695 μl. Experimental parameters were as follows: purge time 2 min, potential step 5 mV, initial potential 0 V, end potential -1.6 V, scan rate 49.5 mV/s, pulse amplitude 0.02502 V, pulse width 0.00495 V. The analysed samples were deoxygenated by argon (99.999%), purging time was 120 s.

2.2.2. Oligonucleotide Determination

CA peak was measured by DPV method coupled with the adsorptive transfer stripping technique (AdTS DPV) in the presence of 0.2 M acetate buffer pH 5.0. Experimental parameters of the method were as follows: purge time 2 min, potential step 5 mV, initial potential 0 V, end potential -1.6 V, scan rate 49.5 mV/s, pulse amplitude 0.02502 V, pulse width 0.00495 V. The analysed samples were deoxygenated by argon (99.999%) prior to measurement, purging time was 120 s. Drop volume was 5 μl, volume of electrolyte (acetate buffer pH 5.0) was 695 μl.

2.2.3. Ellipticine Interaction with ssODN and dsODN - Elli and CA Peak

Interaction of ellipticine with oligonucleotides was measured by AdTS DPV method in the presence of 0.2 M acetate buffer pH 5.0. Drop volume of oligonucleotide was 5 μl and accumulation time of ODN was 2 min. In the first step, oligonucleotide was accumulated on the mercury surface, next was immersed into the ellipticine drop of 5 μl volume. Accumulation time was 2 min or 8 min. Samples accumulated by this way were measured in the presence of 1500 μl electrolyte (acetate buffer pH 5.0). Experimental parameters of electrochemical methods were as follows: potential step 5 mV, initial potential 0 V, end potential -1.6 V. The analysed samples were deoxygenated by argon (99.999%) prior to measurement, purging time was 2 min.

2.2.4. Ellipticine Interaction with ssODN and dsODN - G Peak

G peak was measured by square wave voltammetry (SWV) in 50 mM sodium phosphate buffer with 0.3 M ammonium formate, pH 6.9. Measurements were carried out in the electrochemical cell; total volume of buffer with sample was 1100 μl. The volume of samples (ODN, ODN with ellipticine) was added request concentration of sample. Experimental parameters of measurements are as follows: potential step 5 mV, frequency 280 Hz, time of accumulation 2 min, initial potential -1.85 V, end potential 0 V, conditioning potential -1.85 V and duration time of conditioning potential 5 s. All experiments were carried out at room temperature (20°C).

2.3. dsODN Preparation

Double-stranded ODNs were prepared by mixing of GL6 P2 and GL6 P2K single-stranded ODN for 3 min. The mixture was heated at 99 °C for 5 min. After heating the solution was slowly cooling down. Standard solution of the double strand oligonucleotide (concentration 10 μg/ml) was prepared and stored in dark at -20°C. These steps were done according to the protocol Anneal complementary pairs of oligonucleotides from Thermo Scientific (TECH TIP # 45, alternative annealing methods, option 1) [29].

2.4. Preparation of Deionised Water and pH Measurement

The deionised water was prepared using reverse osmosis equipment Aqual 25 (Czech Republic) and the pH was measured using pH meter WTW inoLab (Weilheim, Germany) (details in [30]).

2.5. Mathematical Treatment of Data and Estimation of Detection Limits

Mathematical analysis of the data and their graphical interpretation was realized by software Matlab (version 7.11., Czech Republic). Results are expressed as mean ± standard deviation (S.D.) from at least five parallel determinations unless noted otherwise that was calculated by EXCEL® (Microsoft, Czech Republic). and the detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner [31]. The expression "relative peak height" means relative value related to the maximum value from the fixed interval.

3. RESULTS AND DISCUSSION

As it was mentioned above, intercalation of the drug into DNA is one of the current accepted mechanisms of the effect of this drug on cells. Forty years ago, Palecek discovered that nucleic acids gave two signals: i) redox signal of adenine and cytosine (CA peak), and ii) oxidative signal of guanine [32, 33]. Moreover it has been described that cytosine, adenine, thymine and guanine gave signals on carbon electrodes [34-36]. Based on these facts, we decided to explore interaction of ODNs with ellipticine using electrochemical methods as a suitable tool for detection of nucleic acids [26, 37-52]. For quantification of the rate of the interaction, three various types of measured peaks were used (Fig. **1**). For the first, Elli peak characterizing ellipticine was determined by DPV method (Fig. **1A**) together with CA peak (Fig. **1B**), which characterized adenine and cytosine bases of ODNs (Fig. **1C**). The last one is called G peak, which characterizes guanine bases.

3.1. Influence of Ellipticine Concentration on the Height of Elli Peak

To quantify the interactions between ellipticine and ODNs, determination of the presence of the individual parts is very advantageous. Differential pulse voltammetry was applied to detect Elli peak ([2]) and CA peak ([42], [53]). The influence of the accumulation time on Elli peak height is shown in Fig. **2A**. In this case the accumulation time means the time period during which the compound is accumulated (absorbed) on the mercury drop. In Fig. **2A** there are two break points on the presented curve. The first one is at 120 s and the second one at 480 s. These values were subsequently used for the next interaction studies as characteristic time points. The dependence of the accumulation time on Elli peak potential was investigated too (Fig. **2B**). This dependence has increasing linear character according to the equation: E(V) = 0.00005 t(s) - 0.497, R^2 = 0.982, n = 3. For determination of an ellipticine calibration curve, the accumulation time of 120 s was chosen as the first break point value of an accumulation curve. Ellipticine concentrations were changed from 1.25 to 1000 μM and the dependence has linear character according to the equation: I(nA) = 0.138 c(μM) + 5.897, R^2 = 0.988, n = 3 (Fig. **2C**). The analytical parameters of the applied method and conditions

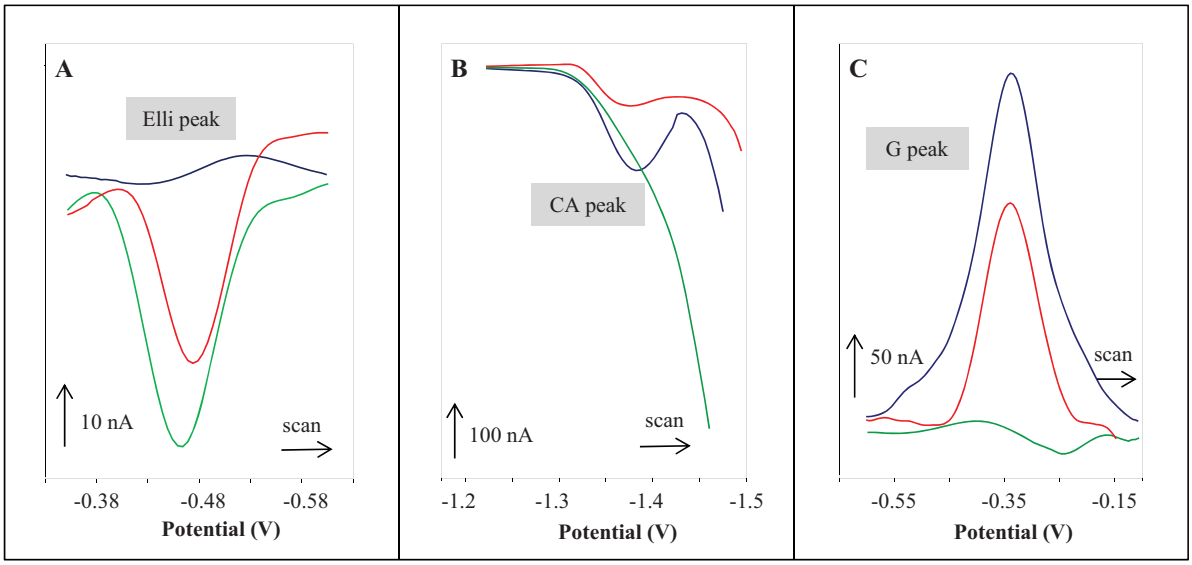

Fig. (1). Voltammograms (dependence of current on applied potential) of 2.5 µg/ml dsODN (blue line), ellipticine (green line) and interaction of ellipticine with dsODN (red line). (**A**) Detected Elli peak at potential about -0.480 V measured by AdTS technique coupled with DPV method, accumulation time of ellipticine 2 min, volume of drop was 5 µl. (**B**) CA peak at potential about -1.380 V measured by AdTS technique coupled with DPV method, accumulation time of ellipticine 2 min, volume of drop was 5 µl. (**C**) G peak at potential about -0.330 V measured by SWV method in the electrochemical cell, accumulation time of ellipticine 2 min.

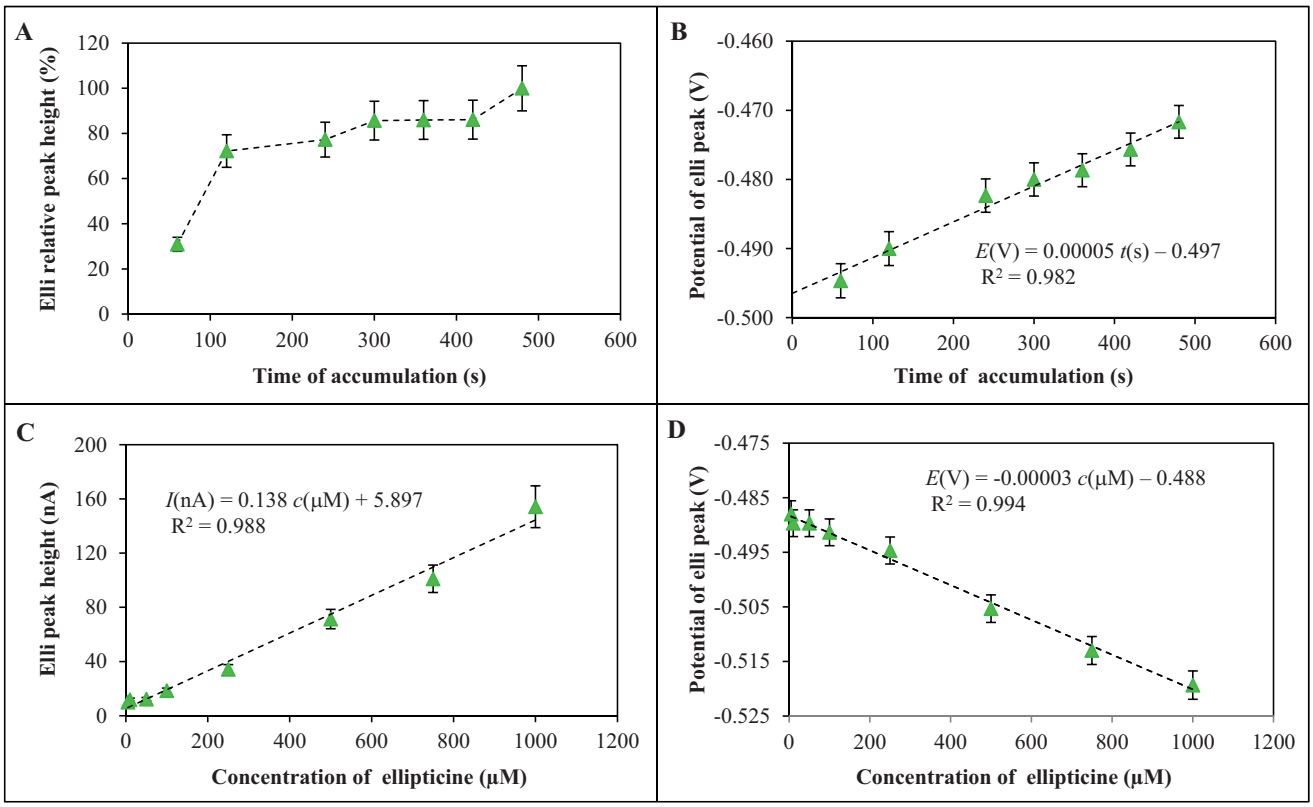

Fig. (2). Optimization of ellipticine electrochemical detection. (**A**) Dependence of relative peak height of ellipticine on accumulation time. (**B**) Effect of accumulation time on ellipticine peak potential. Applied concentration of ellipticine in both dependences were 500 µM. (**C**) Ellipticine calibration curve in a concentration range of 0-1000 µM. Time of accumulation was 2 min. (**D**) Effect of ellipticine concentration on ellipticine peak potential. All dependences were measured by DPV method.

are shown in Table **1**. The influence of the ellipticine concentration on Elli peak potential is shown in Fig. **2D**. This dependence has decreasing linear character according to the equation: E(V) = -0.00003 c(µM) - 0.488, R^2 = 0.994, n = 3. The measured peak potential moved within the range from -0.487 V to -0.519 V.

Table 1. Analytical parameters of electrochemical determination of ellipticine.

Substance	Regression Equation	Linear Dynamic Range (µM)	Linear Dynamic Range (µg/ml)	$R^{2,*}$	LOD[1] (µM)	LOD (µg/ml)	LOQ[2] (µM)	LOQ (µg/ml)	RSD[3] (%)
Ellipticine	I(nA) = 0.138 c(µM) + 5.897	1.25 – 1000	0.31 – 246	0.988	1.6	0.4	5.3	1.3	2.38

*…coefficient of determination
1…limit of detection (3 S/N)
2…limits of quantification (10 S/N)
3…relative standard deviation of ellipticine in DMSO

3.2. Electrochemical Determination of ODN Bases

In our experiments, a single-stranded oligonucleotide of the size of ten bases containing all four various types of bases was used. The double stranded variant was tested, too. As it was mentioned above, electrochemical methods allowed determining various bases signals. In our experiments, we aimed at CA and G peaks.

3.2.1. Influence of ODN Concentration on the Height of CA Peak

CA peak as the second peak that is detectable in one scan together with Elli peak is placed at potential of about -1.380 V. Opposite to this, Elli peak is located at potential of about -0.480 V, which is far away from CA peak and therefore CA peak is not influenced by this electrochemical process. The influence of the accumulation time on the CA peak height was tested within the time interval from 0 to 360 s (Fig. **3A**) for both ssODN and dsODN. As it is obvious from presented dependences, the accumulation time of 120 s is sufficient for determination of CA peak. A further increase in the accumulation time caused only very small changes in a detected signal. Concentration dependences of CA peaks from ssODN and dsODN are shown in Fig. **3B**. This dependence was measured within the concentration range from 0.04 µM to 10 µg/ml ODN. The maximum concentration of ssODN and dsODN that changed height of CA peaks is of 5 µg/ml. Further increasing concentrations of ODNs had no influence on height of CA peak. Basic analytical parameters for ssODN and dsODN of the applied method and conditions for CA peak detection are indicted

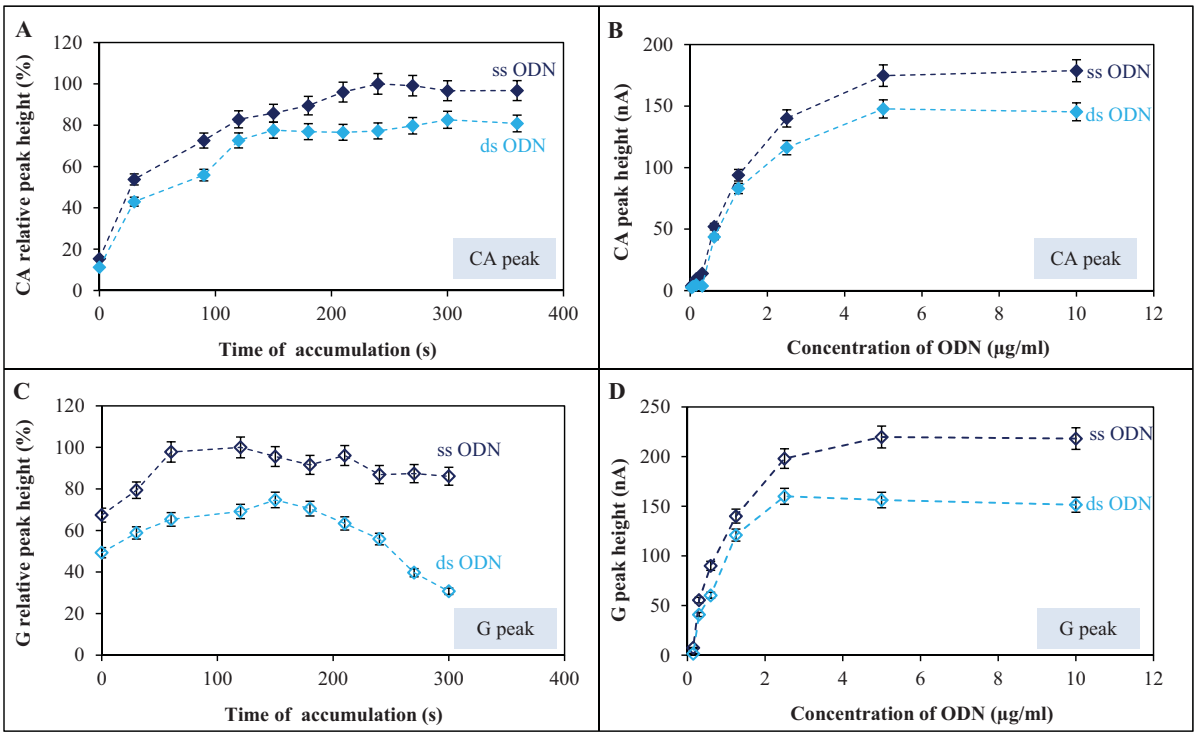

Fig. (3). Electrochemical signals of single/double strand oligonucleotide. (**A**) CA relative peak height as a function of accumulation time, ssODN (dark blue) and dsODN (light blue). (**B**) CA peak height as a function of ODN concentration, accumulation time 2 min. There were CA peaks measured by AdTS DPV technique. (**C**) G relative peak height as a function of accumulation time, ssODN (dark blue) and dsODN (light blue). (**D**) G peak height as a function of ODN concentration, accumulation time 2 min. Dependences of G peaks were measured by SWV in electrochemical cell.

Table 2. Parameters of electrochemical detection of CA peak.

Substance	Peak	Regression Equation	Dynamic Range (µg/ml)	$R^{2,*}$
ssODN	CA	I(nA) = -9.514 c^2(µM) + 82.688 c(µM)- 1.719	0.04 – 5	0.993
dsODN	CA	I(nA) = -8.337 c^2(µM) + 71.828 c(µM)- 4.108	0.04 – 5	0.983

*…coefficient of determination

in Table **2**. dsODN has lower intensity of CA peaks than ssODN in the whole concentration range.

3.2.2. Influence of ODN Concentrations on the Height of G Peak

Except CA peak, G peak is the other parameter in the characterization of used ODNs sequences. This peak was determined by square wave voltammetry (SWV). Dependence of the height of G peak on the accumulation time for both ssODN and dsODN is shown in Fig. **3C**. The maximum of the height of G peak for ssODN was measured at 60 s, for dsODN at 150 s, but a sufficient value (90 %) was measured at 60 s, too. The height of G peak decreased after 150 s of the accumulation time for dsODN. The effect of ODN concentrations (ranging from 0.04 µM to 10 µg/ml ODN) on the height of G peak is shown in Fig. **3D**. The maximum concentration of ssODN and dsODN, which changed the height of G peaks, was 2.5 µg/ml. The height of G peak was lower for dsODN compared to ssODN in the whole concentration range. Increasing concentrations of ODNs had no influence on the height of G peak. The basic parameters of applied method and conditions for G peak detection for ssODN and dsODN are shown in Table **3**.

3.3. Interaction of Ellipticine with ssODN and dsODN

The electrochemical study of interactions between ellipticine and ODNs (single stranded and double stranded) was the basic aim of this study. Therefore, we focused on electrochemical experiments, which can give us some information about the intercalation of the drug into DNA. Electrochemical tools (detection of CA and G peak) can bring additional information about these interactions.

3.3.1. Influence of the Interaction Time on the Heights of CA, G and Elli Peaks

The time of the interaction is the first important condition. The time of interaction was changed within the range from 0 to 480 s. This range was selected according to the characteristic points of accumulation dependences of Elli, CA and G peaks. The applied concentration of ss/ds ODN was 2.5 µg/ml and the concentration of ellipticine was 400 µM. Interaction curves, namely the dependences of individual peak heights, on the time of interaction for both ssODN and dsODN are shown in Figs. **4A**, **4B** and **4C**. The dependence of CA peak for ssODN and dsODN on the interaction time is indicated in Fig. **4A**. For ssODN, CA peaks increased linearly within the range from 30 s to 120 s and then it had linear

Table 3. Parameters of electrochemical detection of G peak.

Substance	Peak	Regression Equation	Dynamic Range (µg/ml)	$R^{2,*}$
ssODN	G	I(nA) = -32.355 c^2(µM) + 160.4 c(µM) - 2.153	0.15 – 2.5	0.979
dsODN	G	I(nA) = -35.482 c^2(µM) + 151.5 c(µM) - 13.097	0.15 – 2.5	0.984

*…coefficient of determination

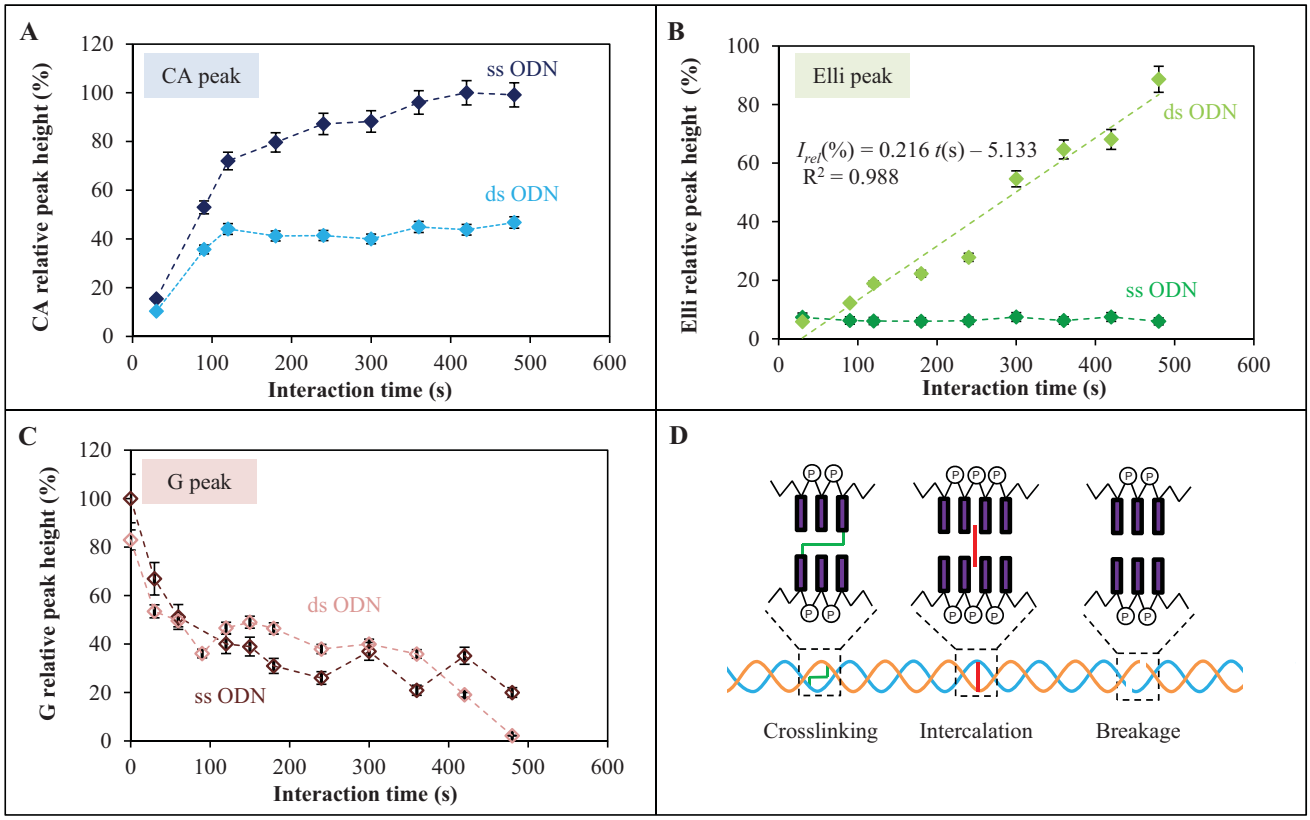

Fig. (4). Influence of ellipticine interaction time on intensity of CA, Elli and G peaks by interaction of ellipticine with ODN. Concentration of ss/ds ODN was 2.5 µg/ml and concentration of ellipticine 400 µM. CA and Elli peaks were detected by AdTS DPV technique, where ODN drop was accumulated 2 min. **(A)** CA relative peak height as a function of interaction time, ssODN (♦) and dsODN (♦). **(B)** Elli relative peak height as a function of interaction time, ssODN (♦) and dsODN (♦). G peaks were detected by SWV method measuring in electrochemical cell where mixture of ellipticin and ODN was placed. **(C)** G relative peak height as a function of interaction time, ssODN (◊) and dsODN (◊). **(D)** Schematic view of possible ways of ellipticine interaction with dsODN.

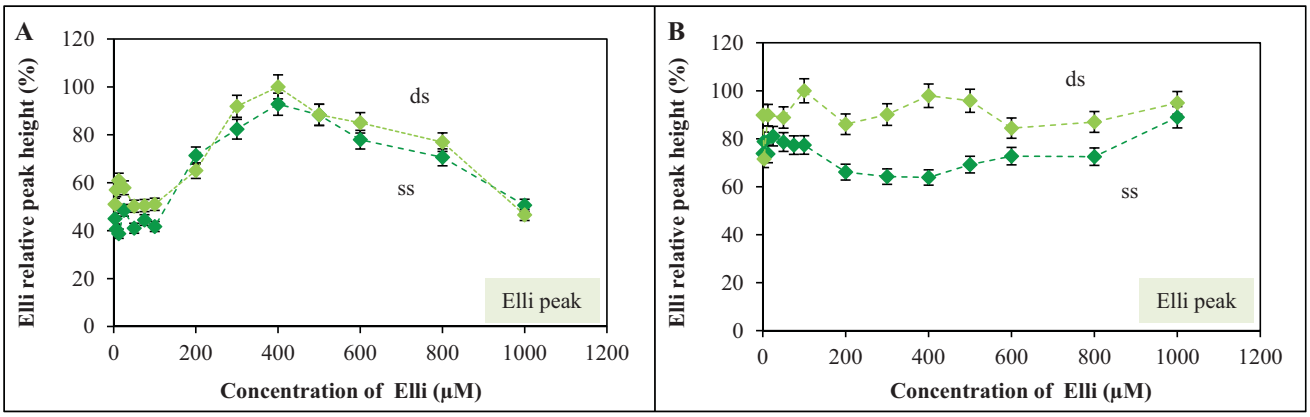

Fig. (5). Influence of ellipticine concentration on electrochemical signals of Elli peaks for 2 and 8 minute interaction. Concentration of ODN was 2.5 µg/ml. AdTS technique with DPV method was applied for detection of Elli (ssODN (♦) and ds ODN (♦)) peaks. (**A**) Elli relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 2 min. (**B**) Elli relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 8 min.

dependence from 120 s to 480 s, too. dsODN had two linear parts of the dependence. The limits of linear parts are the same as ssODN. dsODN demonstrated the lower values of the height of CA peak than ssODN, these values ranged between 5 % to 53 %.

A change in Elli peak during the interaction period was dramatically dependent on the type of ODN (Fig. **4B**). While ssODN showed no changes in Elli peak within the whole time interval, dsODN caused a linear increase in the height of Elli peak according to the following equation: $I_{rel.}(\%) = 0.216\ t(s) - 5.133$, $R^2 = 0.988$, $I_{rel.}$ is relative peak height. The increase in the height of Elli peak at interactions with dsODN is probably caused by the formation of ellipticine-ODN complex. This complex is probably formed by the intercalation process because interaction with ssODN has no changes in the height of Elli peak. Ellipticine molecules are probably intercalated and accumulated in dsODN.

The height of G peak decreased within the whole time interval from 0 to 480 s (Fig. **4C**). This phenomenon is caused by the affinity of ellipticine to the guanine bases. This fact is confirmed by results shown in Fig. **4A**, where a signal of adenine and cytosine is growing. The greatest decrease in its height is well evident during the first 120 s, where the decrease in signal intensity was for about 60 %. The signal is stable from 120 s to 150 s and after it, further decrease to the value of about 20 % of original value is determined. Differences between the effect of ssODN and dsODN are negligible and both curves have similar shape.

3.3.2. Influence of Ellipticine Concentrations on the Heights of CA, G and Elli Peaks

Influence of Ellipticine Concentration on the Height of Elli Peak

In general, ellipticine could have various effects on ODN *via* their mutual interactions. For the first, as other intercalators, ellipticine could intercalate into the ODN structure, it could, under some conditions, create cross links or caused breakage of double helix (Fig. **4D**). All these possibilities are probable and can probably depend on the concentration of ellipticine. The effect of concentrations of ellipticine on the interaction between ellipticine and ODN was tested within the time intervals from 120 to 480 s. Concentrations of ellipticine were changed in an interval from 0 to 1000 µM. The changes of Elli peak in these times are shown in Figs. **5A** and **5B**. After the 120 s long interaction, both curves (for ssODN and dsODN) have maximum at 400 µM of ellipticine (Fig **5A**). After the 480 s long interaction, curves had similar shape and no significant extremes were evident.

Influence of Ellipticine Concentrations on the Height of CA Peak

These interactions were well evident also from the CA peak. Changes in the height of CA peak according to ellipticine concentrations are shown in Figs. **6A** and **6B**. These two dependences are related to the two times of interaction - 120 s and 480 s. The concentration of ellipticine was changed within the range from 0 to 1000 µM. The whole ellipticine concentration interval was divided into three basic parts which correspond to ssODN and dsODN (see schematic view shown in Figs. **6A** and **6B** as parts I-III).

The interaction of ellipticine with ssODN in the time of interaction of 120 s is shown in Fig. **6A** - the dark columns. The simple schemes of individual interaction parts are shown in inset in Fig. **6A**. The first part started without ellipticine (0 µM ellipticine), which is a control value and continued up to 6.25 µM with the increasing height of CA peak. In this part, we observed no interaction between ODN and ellipticine. From 12.5 µM ellipticine up to its concentration of 600 µM, the height of CA peak increased very slowly. In this part, the interaction process of ellipticine with ODN is assumed. This process could be determined by weak binding forces and affinity of ellipticine molecules to the bases in ODN. The concentration of ellipticine higher than 600 µM ellipticine led to the decrease in CA signal. This is probably caused by strengthening of binding forces of ellipticine molecules to the ODNs bases and influencing the electron distribution in the oligonucleotide strand.

The interaction of ellipticine with dsODN after 120 s of interaction is shown in Fig. **6A** - the light columns. The simple schemes of individual interaction parts are presented in the bottom inset in Fig. **6A**. The first part of ellipticine-dsODN interactions ended at the concentration of ellipticine of 200 µM. In this concentration range, CA peak increased very slowly. In this case we assumed mainly intercalation processes of ellipticine. An increase in a CA signal is probably caused by the change in electron distribution in individual bases due to spatial changes in ellipticine interaction with dsODN. There was a rapid increase in a CA signal within the concentration range from 300 to 600 µM ellipticine. This phenomenon could be explained by the separation of individual dsODN strands due to accumulation of elllipticine molecules in the double helix structure. After that the CA signal decreased again as in the case of interaction of ssODN with ellipticine. The reason for this change is probably the same as for ssODN. The separation of individual ODNs strand allowed to form stronger adduct of ellipticine and ODN.

The increase in the time of interaction to 480 s changed observed concentration dependences (Fig. **6B**). There were still three basic parts of interaction for both ssODN and dsODN. For interaction of ssODN with ellipticine, the first part ended under the concentration of 12.5 µM ellipticine. The height of the CA signal

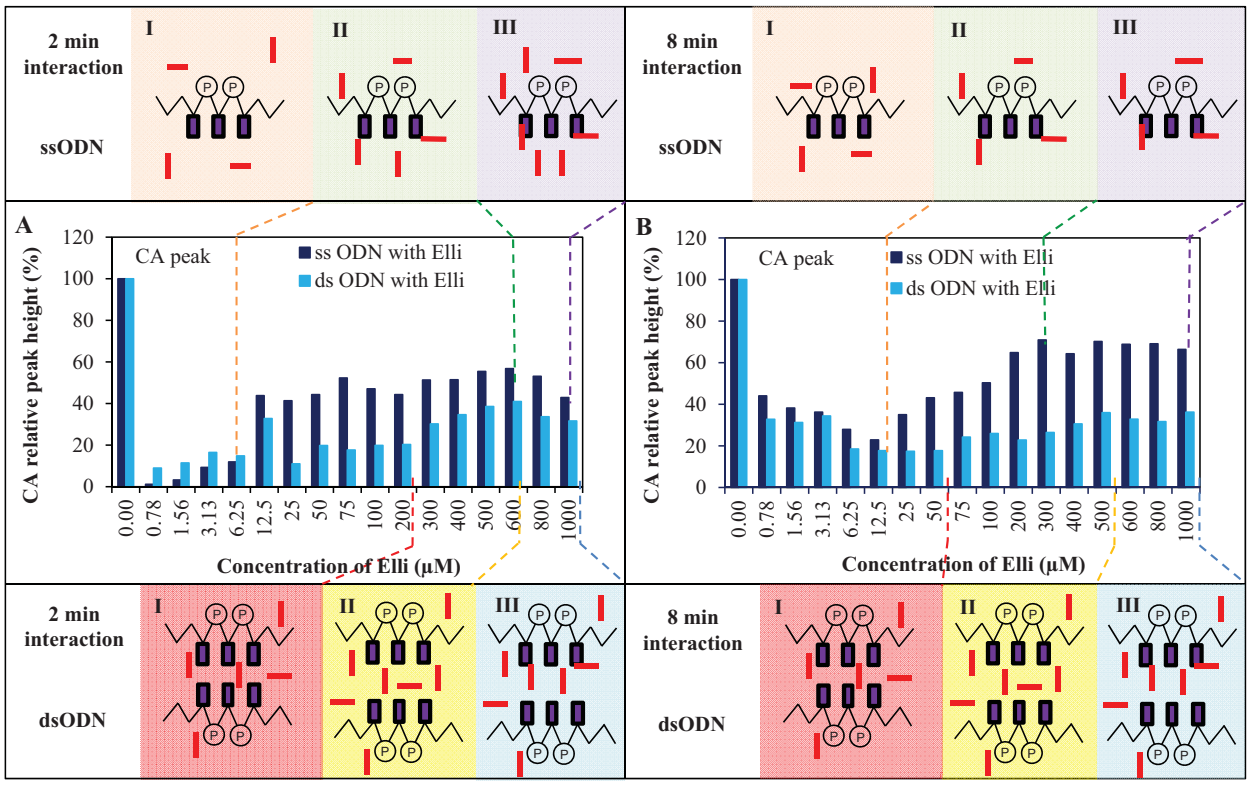

Fig. (6). Influence of ellipticine concentration on electrochemical signals of CA and Elli peaks for 2 and 8 minute interaction. Concentration of ODN was 2.5 µg/ml. AdTS technique with DPV method was applied for detection of CA (ssODN (■) and dsODN (■)) after interaction with ellipticine. (**A**) CA relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 2 min. (**B**) CA relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 8 min. (**I-III**) Three phases of the interaction process related to the changing in ellipticine concentration in interval 0 - 1000 µM). Red lines show ellipticine.

decreased in this phase. The change in the height of CA peak in this interaction time is more controlled by the change in concentration opposite kinetic aspect in 120 s. The second part lied within the concentration range from 25 µM to 300 µM ellipticine. There was steeper increase in the height of CA peak within this concentration range compared to 120 s long interaction. This fact could be caused by the longer time of interaction of ellipticine and ODN, which is closer to the equilibrium point. Due to this fact the concentration effect is stronger. Concentrations of ellipticine higher than 300 µM (to 1000 µM) had no obvious effects on the height of CA signal. It can be assumed that the complex of ellipticine and ssODN is in a stable form and no changes due to changing ellipticine concentrations were detected. This stable complex is formed by the same aspects as at 120 s of the time of interaction. We assumed the strengthening of forces binding the ellipticine molecules to the ODNs bases.

The dsODN interaction after 480 s ended the first part at the concentration of 50 µM ellipticine. The height of a CA signal decreased very slowly in this concentration range. This trend is inversed to the 120 s of interaction and in accordance with 480 s of ssODN. The influence of concentration change is here stronger than kinetic aspect, too. In the second part the height of CA signal increased (up to concentration 500 µM ellipticine) and after that it did not changed. It seems that concentrations of ellipticine higher than 500 µM ellipticine cause no further changes in the formation of complex between ellipticine and ODN.

Influence of Ellipticine Concentrations on the Height of G Peak

The last part of investigated interactions was determination of G peak. The obtained results were divided in the same order as for Elli and CA peak: interaction for ssODN and dsODN, interaction times 120 s and 480 s (Figs. **7A** and **7B**). The G peak characterized the ODN part of the interaction complex as the CA peak but the G peak is directly connected with guanine bases.

The change in the G peak at interaction of ssODN with ellipticine after 120 s is shown in Fig. **7A**. Up to 50 µM ellipticine has no effect on the height of G peak with changing ellipticine concentrations and it is the similar value as for ssODN only. It seems that the lower concentrations of ellipticine did not affect the behavior of guanine bases in ssODN. Within the concentration range from 50 µM to 600 µM ellipticine, the decrease (to 100 µM) and no change (to 600 µM) in the height of G peak were observed. The concentrations of ellipticine higher than 600 µM led to the rapid decrease in the height of G peak. This fact can be connected with formation of stronger binding of ellipticine with ODN and therefore elimination of guanine redox availability.

The interaction of ellipticine with dsODN in the view of G peak had ended the first part of the process at the concentration of 200 µM ellipticine. In this phase the height of G peak slowly increased up to a maximum value at the concentration of 200 µM ellipticine. This process suggests the gradual accumulation of ellipticine in the double helix structure due to weak binding forces. The next part where the height of G peak demonstrated no changes was detected within the concentration range from 200 µM to 600 µM ellipticine. In this part, the gradual separation of individual dsODN strands could take place and the next accumulation of ellipticine molecules in the double helix structure was possible. The concentrations of ellipticine higher than 600 µM led to the rapid decrease in the height of G peak as in the case of ssODN.

The interaction of ellipticine with ODN after 480s changed the height of G peak reversely as the 120s long interaction. This

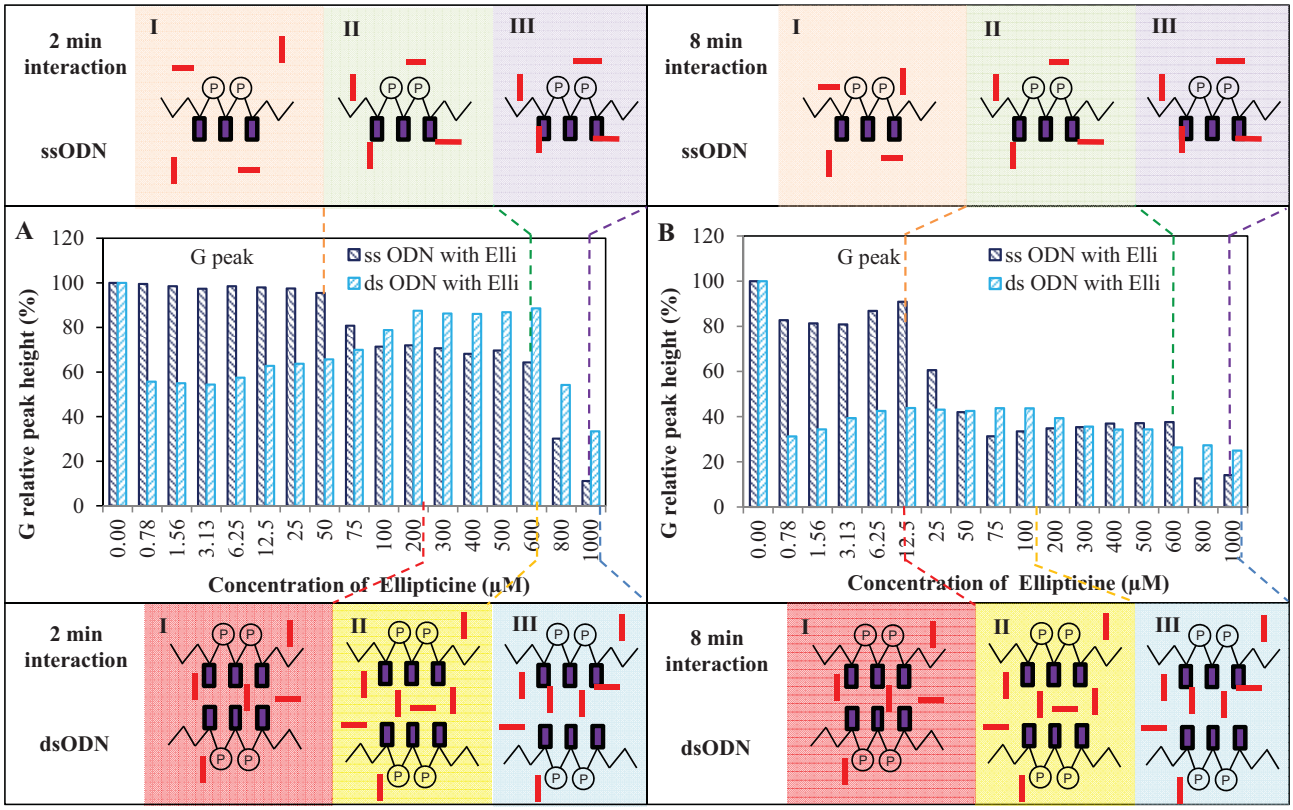

Fig. (7). Influence of ellipticine concentration on electrochemical signals of G peak for 2 and 8 minute interaction. Concentration of ODN was 2.5 µg/ml. G peaks (ssODN (■) and dsODN (■)) were detected by SWV method measuring in electrochemical cell where mixture of ellipticine and ODN was placed. (**A**) G relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 2 min. (**B**) G relative peak height as a function of ellipticine concentration, accumulation time of ODN 2 min, ellipticine accumulation 8 min. (**I-III**) Three phases of the interaction process related to the changing in ellipticine concentration. Red lines show ellipticine.

decrease is obvious for both ssODN and dsODN (Fig. **7B**). The end of the first part of ssODN interaction moved to the concentration of 12.5 µM opposite 50 µM ellipticine in the 120 s interaction. There was found approximately the same signal intensity in this phase. It means that the change in ellipticine concentrations in this range had no significant effect on the guanine bases in ssODN. The next part started with a dramatic decrease in the height of G peak (up to 75 µM ellipticine) and after it, a stabilization of a signal (up to 600 µM ellipticine) was observed. This effect indicated a possible interaction of ellipticine (weak bounded) and the next saturation of the ssODN strand. The last part ran from the concentration of ellipticine higher than 600 µM, where a decrease in the height of G peak was recorded.

The dsODN interaction according to the ellipticine concentration ended the first part at 12.5 µM ellipticine. To this concentration, the dependence had an increasing character. The move of this characteristic point from 200 µM in 120 s to 12.5 µM in 480 s is obvious. The longer equilibrium time had the significant effect on the achieved effect of ellipticine on the height of G peak. After that, the signal height was stable at the same value just up to 100 µM of ellipticine. The next part (from 100 µM ellipticine) is characteristic by a gradual decrease in the height of G peak. This decrease is probably caused by the stronger affecting of guanine bases by ellipticine molecule. Formation of some stronger elli-ODN complex is expected.

4. CONCLUSIONS

In preclinical experiments and clinical trials, ellipticine and several of its more soluble derivatives exhibited significant antitumor and anti-HIV activities [1-6,17]. Intercalation of ellipticine into DNA is one of its possible effects on the division. This phenomenon is not clearly explained so far. For decades electrochemistry is one of the best methods for the detection of nucleic acid. Application of various electrochemical methods for studying interactions of ellipticine with ssODN and dsODN was carried out. Changes in the CA and G signals of DNA (dsODN) found in this study can be caused by intercalation of ellipticine into the DNA structure. The exact mechanism of interaction of ellipticine with DNA is not fully understood but the next steps in explanation of ellipticine interaction with DNA could be essential for application of these drugs for treatment of malignant tumours.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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