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Redox mechanism of anticancer drug idarubicin and in-situ evaluation of interaction with DNA using an electrochemical biosensor

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ABSTRACT

Idarubicin (IDA), 4-demethoxydaunorubicin, is an anthracycline derivative and widely used treatment of leukemia. The electrochemical behavior of IDA was examined at a glassy carbon electrode (GCE) in different aqueous supporting electrolyte using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The oxidation process of IDA was found to be pH dependent and irreversible proceeding with a transfer of 1 proton and 1 electron under the diffusion controlled mechanism. The electroactive center is the hydroxyl group on the aromatic ring which produces a final quinonic product. The diffusion coefficient of IDA was calculated to be $D_{IDA} = 7.47 \times 10^{-6}$ cm^2 s^{-1} in pH = 4.3 0.1 M acetate buffer.
The interaction of IDA and double stranded deoxyribonucleic acid (ds-DNA) was investigated using electrochemical ds-DNA biosensor and incubation solution by means of DPV. The DNA damage was detected following the changes in the oxidation peaks of guanosine and adenosine residues. The results obtained showed that IDA interacts with DNA which causes the change in the DNA morphological structure. In addition to these polynucleotides, PolyG and PolyA, biosensors were also used to confirm the interaction between ds-DNA and IDA. However, no oxidation peaks of the purine base oxidation products, 8-oxoGua and 2,8-oxoAde, were observed.

1. Introduction

Idarubicin (IDA), 4-demethoxydaunorubicin, is an anthracycline derivative and widely used treatment of leukemia. It is a synthetic analogue of daunorubicin, Scheme 1. The absence of a methoxy group at position 4 of the anthracycline structure gives the high lipophilic character, therefore it is used orally. It binds in a non-covalent interaction to deoxyribonucleic acid (DNA), inhibits nucleic acid synthesis and interacts with the enzyme topoisomerase II [1,2].

Very few methods appear in the literature for the determination of IDA and its metabolites using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in bulk solutions, plasma, saliva, and urine with amperometric [3], UV [4], fluorescence [5–7], and mass spectrometry (MS) [8,9] detectors. In addition to these studies, only one voltammetric technique was used to investigate the electrochemical characterization of IDA using a multiwalled carbon nanotube modified glassy carbon electrode and pyrolytic graphite electrodes [10]. The modified electrodes were constructed for the determination of IDA in pharmaceutical dosage form using adsorptive stripping DPV.

DNA plays a major role in the life process because it carries heritage information and is responsible for the replication and transcription of genetic information in living cells. DNA is a cellular target for smaller molecules like drugs, metals, and carcinogens. Studies on binding mechanism are of great help to understand the mutation of genes and the origin of some diseases. Moreover the investigation of drug–DNA interaction is important for designing and monitoring new DNA-target drugs. There are two well characterized binding modes for small molecules with DNA: covalent and non-covalent. Non-covalent interaction includes groove binding and intercalation which are the most common modes to bind directly and selectively to DNA [11,12].

The interaction of DNA with drugs is among the important aspects of biological studies in drug discovery and pharmaceutical development. Various methods have been applied for the characterization of the interaction of drug with DNA such as UV–vis spectroscopy [13], IR and Raman spectroscopy [14], molecular modeling techniques [15], circular dichroism [16], fluorescence spectroscopy [17], capillary electrophoresis [18], and HPLC [19]. However, these methods need time, cost and more chemicals and also some of them have low sensitivity.

At the beginning of the sixties after discovery of electroactivty in nucleic acids, there has been a growing interest in the electrochemical investigation of interaction between anticancer drugs and DNA. The electrochemical techniques allow to evaluate and predict interactions and damage caused to DNA. These methods are suitable for investigation of drug–DNA interaction due to their advantages such as high sensitivity, fast response time, and low cost. In these techniques, DNA-electrochemical biosensors are one of the most important groups. Biosensors are small devices, which utilize biological reactions for detecting target analytes. Electrochemical DNA biosensors comprise a nucleic acid recognition layer, which is immobilized over an electrochemical

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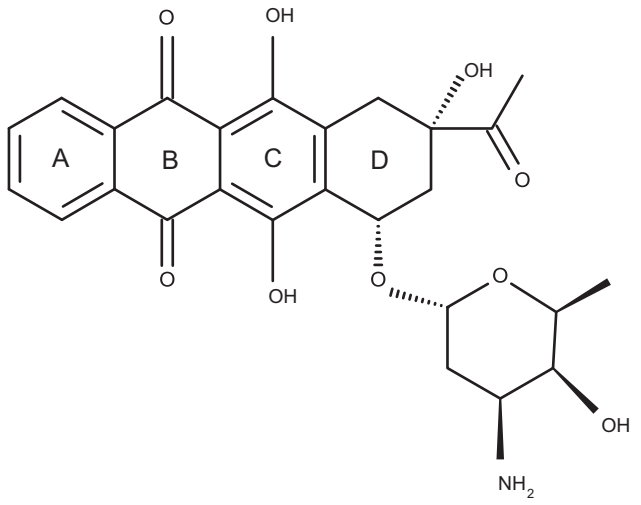


Scheme 1. Structural formula of idarubicin.

transducer. Different immobilization procedures are used for electrode surface modification such as formation of mono- or multi-layer DNA film, electrostatic adsorption, and evaporation. The investigations of interactions are based on the differences in the electrochemical behavior of DNA such as the decreases/increases of the peak currents and the shifts of the potentials related to guanine and adenine. These DNA probes using differential pulse voltammetry have great sensitivity for detecting small changes of DNA structure and have been successfully used to identify the interaction of anticancer drug with DNA [20].

In order to better understand the interaction, the other method used is the incubation procedure. For investigation of interaction in solution, drug and DNA are placed in the same solution and the changes in the electrochemical signals of drug or DNA are compared with the signals obtained with DNA or drug alone in the solution.

In previous studies, interaction of idarubicin and DNA was identified by electrochemical and spectroscopic techniques. These studies are based on the changes of spectra or only cyclic voltammograms before and after interaction. [21,22]. The electrochemical part of these studies is based on the following changes in the oxidation peak of IDA in the absence and presence of DNA.

In this study, the electrochemical oxidation of IDA and the mechanism of interaction of IDA with ds-DNA and polyhomonucleotides, poly[G] and poly[A], were carried out using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at a glassy carbon electrode (GCE). The results indicated that the intercalation played a predominant role in the interaction of IDA with DNA.

2. Experimental

2.1. Materials and reagents

Idarubicin (IDA) was kindly supplied by Mustafa Nevzat (Istanbul, Turkey). Double stranded (ds-DNA), polyadenylic acid (Poly[A]) and polyguanilic acid (Poly[G]) were obtained from Sigma and used without further purification. A stock solution of 1 mM IDA was prepared in deionized water and stored at 5 °C. Solutions of different concentrations of IDA were prepared by dilution of the appropriate quantity in supporting electrolyte. Stock solutions of 138 μg mL^{-1} ds-DNA, 83.2 μg mL^{-1} Poly[G], and 136.4 μg mL^{-1} Poly[A] were prepared in deionized water and diluted to the desired concentrations in 0.1 M pH = 4.5 acetate buffer. After dissolving the DNA fibers in deionized water, the purity of this solution was checked from the absorbance ratio A_{260}/A_{280}. The ratio of absorbance for the ds-DNA solution at 260–280 nm provides an estimate of the purity of DNA with respect to contaminant that absorbs in UV. DNA samples purified from biological sources such as tissue or cells have proteins as contaminants. Proteins absorb more strongly around 280 nm due to the absorbance of tryptophan with a lesser contribution from tyrosine, which decrease the ratio if they are present in a ds-DNA. A value of A_{260}/A_{280} ratio between 1.8 and 1.9 indicates that the protein concentration is negligible and no further deproteinization is required. The ratio of absorbance from ds-DNA solution was found to be 1.8, which indicated that the ds-DNA was free from any contamination.

Acetate (0.1 M, pH = 4.5 and 0.2 M, pH 3–5), phosphate (0.2 M, pH 6–8), borate (0.025 M, pH 8–9) buffers, HCl (0.2 M), and NaOH (0.2 M) were used as supporting electrolytes. All supporting electrolyte solutions were prepared using analytical grade reagents and deionized water.

2.2. Apparatus

Voltammetric experiments were carried out using a Gamry potentiostat (Model Reference 600, USA) with a three-electrode cell. A three electrode system was used, including a glassy carbon working electrode (GCE, d = 1.6 mm, BAS) (unmodified or modified), the Pt wire counter electrode and the Ag/AgCl (3 M KCl, BAS) reference electrode. The pH measurements were done by using a combined pH electrode with an Orion model 720 A pH meter. A Bondelin Sonorex RK 100H-type sonicator was used throughout this study. All experiments were done at room temperature.

The experimental conditions for DPV were: pulse amplitude of 50 mV, pulse width of 70 ms and scan rate of 5 mV s^{-1}.

Before each measurement the GCE was polished mechanically using the Al_2O_3 slurry. After polishing, it was rinsed thoroughly with deionized water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.3. Incubation procedure and DNA-biosensor preparation

IDA and ds-DNA interaction was identified in two ways; ds-DNA biosensor and incubation procedure. Before modification, a working electrode was mechanically polished with slurry of alumina powder. The thin layer ds-DNA-modified electrode was prepared depositing three drops of 5 μL each containing 50 μg mL^{-1} ds-DNA on the GCE surface. After each dropping step, the biosensor was allowed to dry. The prepared ds-DNA-electrochemical biosensor was incubated in 100 μM IDA solution during different times. Then, an electrode was rinsed carefully with deionized water to remove unbound IDA molecules from the electrode surface and transferred to a fresh supporting electrolyte. After DP voltammograms were recorded, the ds-DNA film was removed from the electrode surface. A new biosensor was prepared for each experiment. Poly[G] and Poly[A]-electrochemical biosensors were prepared from a 25 μg mL^{-1} solution, using the same procedure. pH = 4.5 0.1 M acetate buffer was used as a supporting electrolyte for all procedures.

In the incubation procedure, 100 μg mL^{-1} ds-DNA was mixed with 2 μM IDA in pH = 4.5 0.1 M acetate buffer, and 100 μg mL^{-1} Poly[G] or Poly[A] was mixed with 5 μM IDA in pH = 4.5 0.1 M acetate buffer, and then incubated at room temperature during different time periods. Control solutions of IDA, ds-DNA, Poly[G] and Poly[A] were also prepared in pH = 4.5 0.1 M acetate buffer and stored during the same time periods and in similar conditions as the IDA–ds-DNA, IDA–Poly[G] and IDA–Poly[A] incubated solutions. Systematic studies to clarify the interaction mechanism of IDA with ds-DNA, Poly[A] and Poly[G] were carried out at the modified GCE using DPV that enables the rapid detection of minor changes in the ds-DNA morphological structure and of DNA oxidative damage, because of its high sensitivity and selectivity.

3. Results and discussion

The electrochemical behavior of IDA was investigated using cyclic voltammetry and differential pulse voltammetry at the GCE surface using various buffer solutions. Systematic studies to explain the mechanism of interaction of IDA with ds-DNA, Poly[A] and Poly[G] were carried out, with multilayer modified electrodes or in incubated solutions, using the GCE and DPV. DPV was used due to its high sensitivity which enabled the detection of minor changes on the DNA double helical structure and DNA oxidative damage.

3.1. Electrooxidation behavior of idarubicin

3.1.1. Cyclic voltammetry

CV of 200 μM IDA at a GCE in pH = 4.3 0.1 M acetate buffer showed one distinct and well defined anodic peak (E_{pa}) 1_a at $E_{pa} = +0.616$ V, Fig. 1. Reversing the potential after oxidation, a new reduction peak (E_{pc}) 1_c appeared at $E_{pc} = +0.512$ V. The unequal anodic and cathodic peak currents pointed to the irreversible nature of the overall electrochemical process. With increasing number of scans, the decrease in the anodic peak 1_a current of IDA, under the same conditions, indicated adsorption of IDA and/or its oxidation product on the GCE surface.

The variation in voltammetric response indicated the strong dependency of IDA redox mechanism on the pH of supporting electrolyte. Therefore, the effect of pH on the electrochemical oxidation behavior of IDA was investigated in aqueous buffered solutions within pH range between 2.0 and 12.0. Cyclic voltammograms showed that in strong acidic buffer solutions, pH ≤ 2, no IDA oxidation peak was observed. For pH > 2 the voltammograms showed two peaks 1_a and 1_c. The oxidation process was pH-dependent and the voltammetric peak potentials of IDA shifted to negative potentials with increasing pH indicating that proton is directly involved in the reaction.

The effect of the scan rate between 5 and 500 mV s^{-1} on the peak current and peak potential of 200 μM IDA solution, in pH = 4.3 0.1 M acetate buffer, was also examined to clarify process character (Fig. 2). Increasing the scan rate, the peak 1_a potential was slightly shifted to more positive potentials and current values increased, showing a typical behavior of irreversible electrochemical reactions. It is found that the anodic peak current (I_p) of IDA is linear to the square root of the scan rate ($\nu^{1/2}$) according to the following Eq. (1):

$$I_p(A) = 6.79 \times 10^{-7}\nu^{1/2} - 1.51 \times 10^{-6}\ r = 0.994. \quad (1)$$

The linearity of I_p vs $\nu^{1/2}$ shows the character of a diffusional system. This evidence is confirmed by plotting the logarithm of peak current

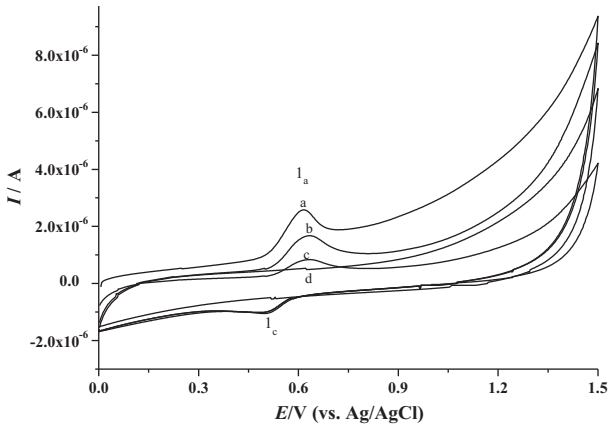


Fig. 1. Cyclic voltammograms of 200 μM IDA in pH = 4.3 0.1 M acetate buffer solution, N_2 saturated; (a) first, (b) second, and (c) third scans and (d) acetate buffer, $\nu = 100$ mV s^{-1}.

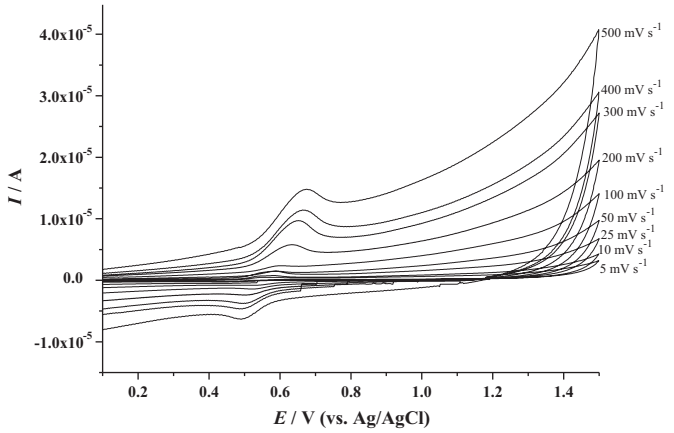


Fig. 2. Cyclic voltammograms of 200 μM IDA in pH = 4.3 0.1 M acetate buffer solution, at different scan rates.

($\log I_p$) versus the logarithm of the scan rate ($\log \nu$). Eq. (2) is shown below:

$$\log I_p(A) = 0.99 \log\nu - 5.99\ r = 0.999. \quad (2)$$

The slope of the equation is close to the theoretical value of 1.0, which is indicated for an ideal reaction the adsorption effected diffusion controlled. Due to the partial adsorption character of IDA on GC electrode surface, stripping techniques were also examined. However, no appropriate results have been achieved. Therefore, the oxidation of IDA on GC electrode was worked as diffusional controlled.

From voltammograms, the value of difference in potential (E_{pa}) between the potential at half height of peak ($E_{p/2}$), ($|E_{pa} - E_{p/2a}|$) was found to be ~65 mV which indicated a one electron transfer process. Since for a diffusion-controlled irreversible system $|E_{pa} - E_{p/2a}| = 47.7/(\alpha_a n')$ where α_a is the charge transfer coefficient and n′ the number of electrons in the rate-determining step [23], the value of $\alpha_a n' = 1.36$ was calculated. The peak current for a diffusion-controlled irreversible system is given by I_{pa} (A) $= -2.99 \times 10^5\ n\ (\alpha_a n')^{1/2}\ A\ [O]_\infty\ D_O^{1/2}\ \nu^{1/2}$ where n is the number of the electrons transferred during the oxidation of IDA (n = 1 as shown below, Section 3.1.2), A is the electrode area in cm^2, D_O is the diffusion coefficient in $cm^2\ s^{-1}$, $[O]_\infty$ is the concentration in mol cm^{-3} and ν is in V s^{-1}. The GCE electroactive area of $A = 0.0068\ cm^2$ was determined as described elsewhere [24]. The diffusion coefficient of IDA was calculated to be $D_{IDA} = 7.47 \times 10^{-6}\ cm^2\ s^{-1}$ in pH = 4.3 0.1 M acetate buffer.

3.1.2. Differential pulse voltammetry

DPV was used to investigate the effect of pH on the electrochemical oxidation of 200 μM IDA, peak 1_a, in aqueous supporting electrolytes over a pH range from 2.0 to 12.0 (Fig. 3A). As already found by CV, in strong acidic buffer solution, no IDA oxidation peak was observed. In addition, recorded successive DP voltammograms in different buffer solutions showed that with increasing scan number, IDA wave decreased. The reproducibility of peak current and potential was also examined and the values of standard error were found less than 0.1.

The peak potential of anodic peak of IDA is shifted linearly towards more negative values and peak current also increased up to pH = 4.3 and afterwards decreased with increasing pH values (Fig. 3A). The relationship between pH and E_{pa} was linear following the equation E_{pa} (mV) = 850.41 − 61.37 pH, Fig. 3B. The slope of the line is close to theoretical value of 59 mV per pH unit and shows that the mechanism of oxidation involves the same number of electrons and protons. The number of electrons transferred n, was determined by the peak width at half height, $W_{1/2}$ ~ 87 mV, and is close to the theoretical value of

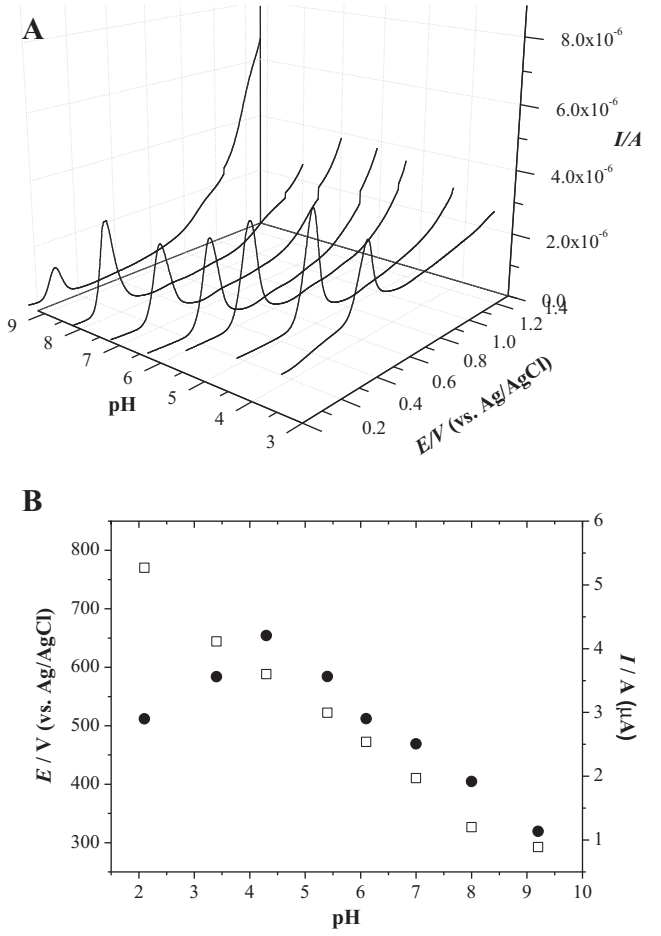


Fig. 3. (A) 3D plots of base-line corrected DP voltammograms obtained in 200 μM IDA; (B) plot E_{pa} vs. pH (□) and I_{pa} vs. pH (●) of peak 1_a.

90 mV, corresponding to an electrochemical reaction involving the transfer of one electron.

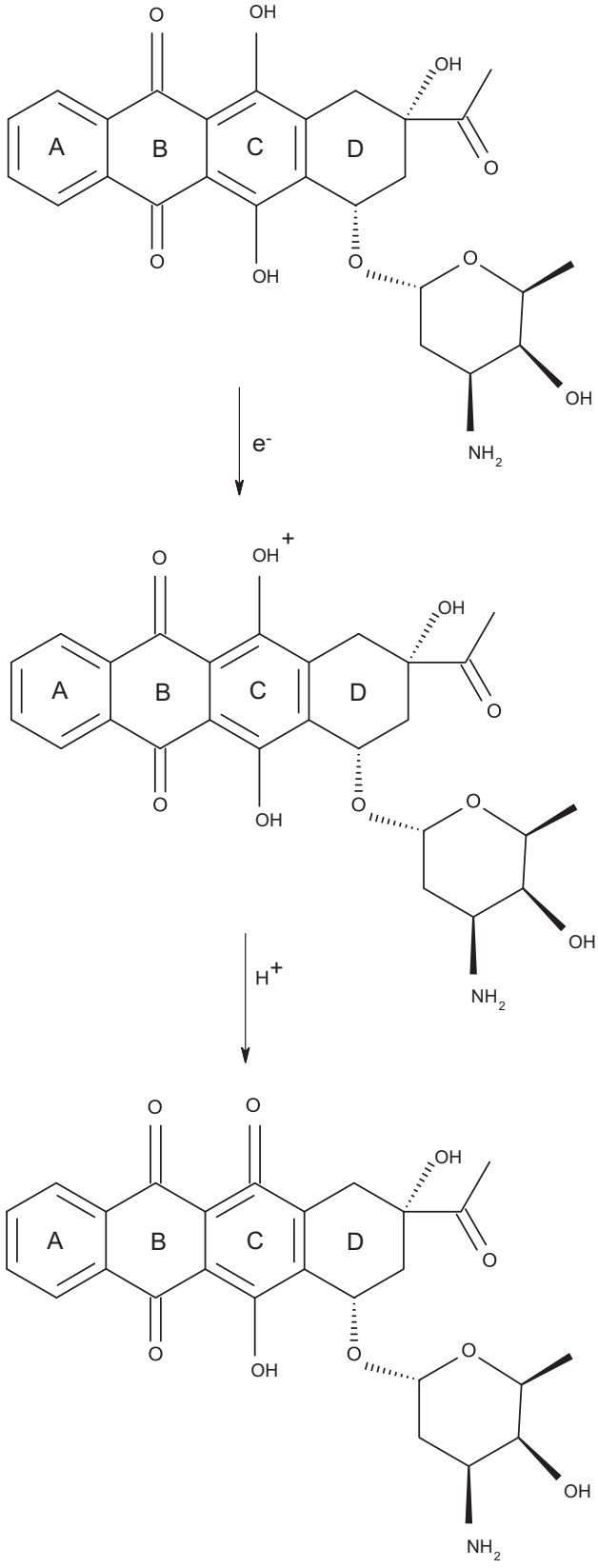
The IDA has a quinonic structure and the electrochemical behaviors of quinone–hydroquinone redox complexes are well-known. It is presumed that it begins to form a semiquinone free radical via a one-electron reaction [25]. Based on this information, the oxidation mechanism of IDA is an irreversible process and the electroactive center is the hydroxyl group on the aromatic ring. It can be assumed that this paradioxybenzene part of IDA is first oxidized and then reduced at the GCE surface. According to these results, the oxidation of IDA occurs with a transfer of one electron and one proton to produce a final quinonic product, Scheme 2.

3.2. DNA–idarubicin interaction

IDA is a synthetic analogue of daunorubicin and used in the treatment of leukemia. It binds in a non-covalent interaction to DNA. Therefore the investigation of the interaction mechanism between IDA and DNA is very important.

The mechanism of interaction of IDA and DNA was investigated in incubated solutions or with a multilayer GC modified electrode. This investigation is mainly based on the differences in the electrochemical behaviors of ds-DNA in the absence or presence of IDA. The electrochemical signals of guanine and adenine residues were measured before and after interaction.

The electrochemical behavior of ds-DNA and IDA was shortly reconsidered to identify the peaks depending on ds-DNA–IDA interaction.



Scheme 2. Proposed oxidation mechanism of idarubicin.

DP voltammograms showed one peak at $E_{pa} = +0.59$ V for IDA and two well defined peaks related to oxidation of desoxyguanosine (dGuo) at $E_{pa} = +1.11$ V and desoxyadenosine (dAdo) at $E_{pa} = +1.30$ V for ds-DNA.

3.2.1. In situ DNA–idarubicin interaction

Multilayer ds-DNA-, Poly[G] and Poly[A]-electrochemical biosensors were prepared and used to investigate the in situ DNA–idarubicin interaction. The biosensor surface was covered by the multilayer to make sure that the undesired adsorption of IDA on GCE could not occur. These type of biosensors have some advantages such as low cost, fast response time, simple design, and high detection limit.

The ds-DNA-electrochemical biosensor was prepared as explained in Section 2.3. The prepared biosensor was incubated in 100 μM IDA solution for different times at 5, 10 and 15 min, and transferred in a fresh supporting electrolyte. In order to remove unbounded IDA, the ds-DNA-electrochemical biosensor was carefully washed with deionized water before being transferred to pH = 4.5 0.1 M acetate buffer where the DP voltammograms were recorded, Fig. 4. As expected, dGuo and dAdo oxidation peaks were obtained. In these voltammograms, the observed peaks can only be caused by the interaction between ds-DNA and IDA. After each measurement, the ds-DNA film was removed from the electrode surface and the experiments were always performed with a newly prepared biosensor. The results showed that with increasing incubation time in the IDA solution, dGuo and dAdo oxidation peaks decrease after the interaction of IDA and DNA. This is caused by the changes of the ds-DNA morphological structure, due to the interaction with IDA and this is detected by the event of decreasing peaks with increasing incubation time. However, in this experimental conditions, no oxidation peaks of the purine base oxidation products, 8-oxoGua and 2,8-oxoAde, were observed [26,27]. This result proved that during the incubation time IDA intercalated to double helix, but there was no DNA oxidative damage.

Polynucleotide biosensors, Poly[G] and Poly[A]-electrochemical biosensors, were also prepared, Section 2.3, and incubated in 100 μM IDA in pH = 4.5 0.1 M acetate buffer for different times, in order to obtain more information on the interaction between ds-DNA and IDA, Fig. 5.

A DP voltammogram obtained in pH = 4.5 0.1 M acetate buffer shows only one peak at $E_{pa} = +1.30$ V, corresponding to dAdo oxidation. After preparing a new biosensor, it was incubated for varying time in drug solution and two peaks corresponding to the oxidation of Poly[A] and IDA were observed. Obtained voltammograms showed that oxidation peak of Poly[A] decreased with increasing incubation time, and no oxidation peak for 2,8-oxoA was observed, Fig. 5A.

Alike Poly[A] experiments, the Poly[G]-electrochemical biosensor was prepared, incubated in IDA solution and DP voltammograms were recorded. These voltammograms showed both IDA and dGuo at $E_{pa} = +0.59$ V and $E_{pa} = +1.11$ V, respectively. Similar results were obtained, confirming that IDA interacts with Poly[A] and ds-DNA. Furthermore, no oxidation peak for 8-oxoGua was observed, Fig. 5B.

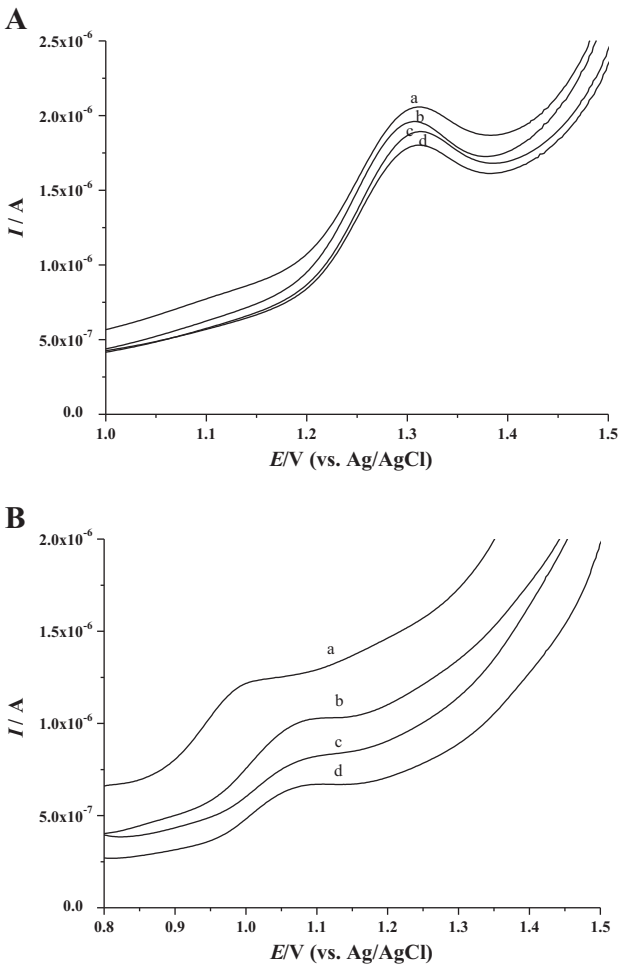


Fig. 5. DP voltammograms obtained in pH = 4.5 0.1 M acetate buffer with (A) Poly[A] and (B) Poly[G] biosensors (a) before and after incubation during (b) 5, (c) 10 and (d) 15 min in a solution of 100 μM IDA.

These experiments using polyhomopurinenucleotide single stranded sequences, Poly[G] and Poly[A]-electrochemical biosensors, confirm the results obtained using the ds-DNA-electrochemical biosensor.

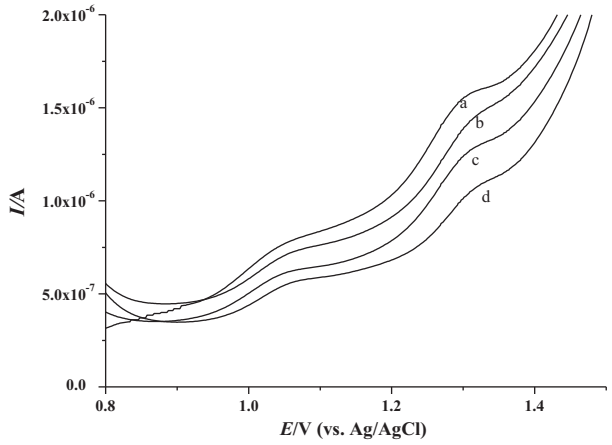


Fig. 4. DP voltammograms obtained in pH = 4.5 0.1 M acetate buffer with ds-DNA biosensors (a) before and after incubation during (b) 5, (c) 10 and (d) 15 min in a solution of 100 μM IDA.

3.2.2. DNA–idarubicin interaction in incubated solutions

In order to investigate the interaction between IDA and ds-DNA, incubated solutions including 2 μM IDA and 100 μg mL^{-1} ds-DNA, in pH = 4.5 0.1 M acetate buffer, were prepared and DP voltammograms were recorded for different incubation times. Control solutions of ds-DNA and IDA were also prepared in the same concentrations, and DP voltammograms were recorded for the same incubation times. After each measurement the GCE surface was cleaned to avoid the blocking of the electrode surface by adsorption of the IDA and ds-DNA.

Before the incubation studies in order to identify the peaks of IDA and ds-DNA, DP voltammograms were recorded in pH = 4.5 0.1 M acetate buffer.

DP voltammograms, obtained immediately after the addition of IDA to ds-DNA solution, showed three peaks corresponding to the oxidation of dGuo at $E_{pa} = +1.11$ V, dAdo at $E_{pa} = +1.30$ V, and IDA at $E_{pa} = +0.59$ V. Increasing the incubation time, the peak current of dGuo and dAdo decreased. This result indicated that ds-DNA structure was modified due to the interaction, which makes more difficult the access of the ds-DNA bases to the electrode surface hindering their oxidation. However, in the DP voltammograms, no peaks related

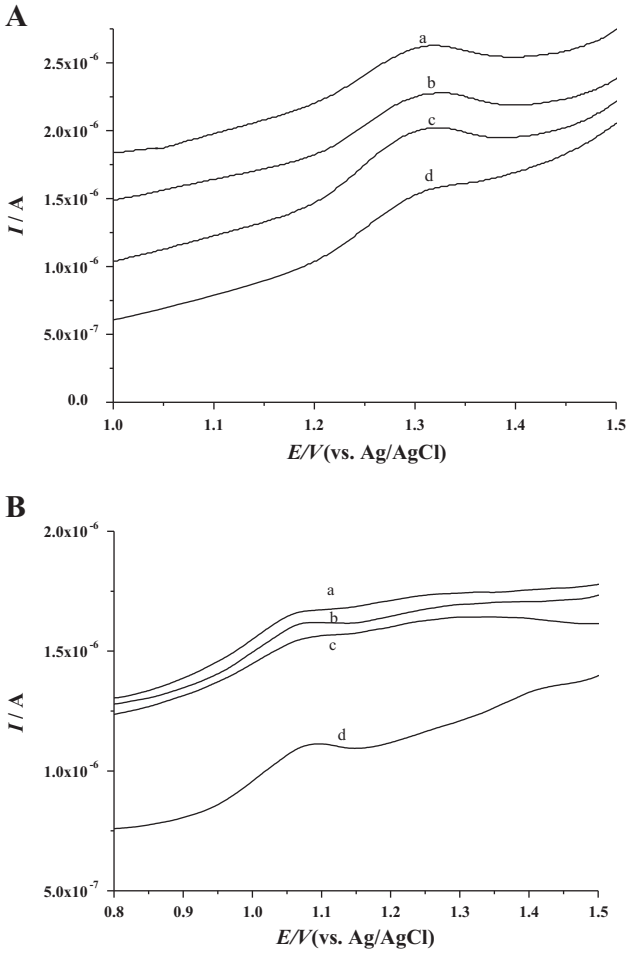


Fig. 6. DP voltammograms in a solution of 100 μg mL^{-1} of (A) Poly[A] and (B) Poly[G] in pH = 4.5 0.1 M acetate buffer (a) before and after incubation with 5 μM IDA during (b) 0, (c) 2 and (d) 4 h.

to the presence of oxidative damage biomarkers, 8-oxoGua or 2,8-oxoAde, were observed.

To better understand the interaction of IDA and ds-DNA, experiments were also carried out in incubated solutions of 5 μM IDA and 100 μg mL^{-1} purinic base solutions, Poly[A] or Poly[G], in pH = 4.5 0.1 M acetate buffer, Fig. 6. DP voltammograms showed similar results as with ds-DNA, confirming the decrease of the oxidation peaks of dGuo and dAdo and no oxidative damage.

IDA is a planar organic molecule containing aromatic condensed rings and these types of molecules often bind to DNA in an intercalative mode [28]. In addition, the obtained results from in-situ and incubation solution experiments support this conclusion by decreasing of the dGuo and dAdo oxidation peaks.

4. Conclusion

The electrochemical behavior of idarubicin was investigated on the glassy carbon electrode in different aqueous buffer solutions using cyclic voltammetry and differential pulse voltammetry. The oxidation mechanism of idarubicin is irreversible, pH dependent and occurs with one electrode and one proton transfer. The diffusion coefficient was found to be $D_{IDA} = 7.47 \times 10^{-6}$ cm^2 s^{-1} in pH = 4.3 0.1 M acetate buffer.

The mechanism of interaction of IDA with ds-DNA, Poly[A] and Poly[G] was identified in incubated solutions or with multilayer GC modified electrodes. The obtained results explained that IDA interacts with ds-DNA in an intercalative mode which causes modifications in the ds-DNA morphological structure and strand breaks confirmed by decreasing of the dGuo and dAdo oxidation peaks with increasing incubation time. However, no oxidation peaks of the purine base oxidation products, 8-oxoGua and 2,8-oxoAde, were observed.

The results presented lead to the proposal of a mechanism through which idarubicin causes a direct in vivo damage to DNA. The proposed voltammetric methods could be applied directly without any separation, evaporation, or difficult sample preparation as there was no interference present from the endogenous substances. These methods are suitable for studies of biological systems, since they are low cost, fast, and have high sensitivity and simple design. It can be undoubtedly said that the studied techniques are high-quality analytical alternatives for evaluating drug–DNA interaction.

Acknowledgments

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