Bioelectrochemistry 87 (2012) 3–8

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Nucleoside analogue electrochemical behaviour and in situ evaluation of DNA–clofarabine interaction

H. Eda Satana [a,b], A. Dora R. Pontinha [a], Victor C. Diculescu [a], Ana Maria Oliveira-Brett [a,*]

[a] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535, Coimbra, Portugal
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330, Etiler, Ankara, Turkey

ARTICLE INFO

Article history:
Received 14 April 2011
Received in revised form 18 July 2011
Accepted 18 July 2011
Available online 27 July 2011

Keywords:
Clofarabine
DNA
Oxidation
Voltammetry

ABSTRACT

Nucleoside analogues have had a substantial impact on the treatment of cancer, especially haematological malignancies. The electrochemical behaviour of the nucleoside analogue clofarabine (CLF) was investigated at a glassy carbon electrode using cyclic, differential pulse and square wave voltammetry in different pH supporting electrolytes. The oxidation process of CLF is irreversible and pH-dependent with transfer of two protons and two electrons, following a diffusion controlled mechanism. The oxidation mechanism of CLF involves deprotonation and leads to the formation of a hydroxylated species that undergoes reversible redox reactions. The interaction of DNA and the antileukemia drug CLF was investigated using a dsDNA–electrochemical biosensor in incubated solutions by differential pulse voltammetry. The CLF–DNA interaction leads to changes in the DNA morphological structure, confirmed using the purinic homo-polynucleotide single stranded sequences of guanosine and adenosine, poly[G] and poly[A]-electrochemical biosensors.

1. Introduction

Cancer is a disease in which cells grow beyond the normal limits and destroy the tissues. Cancer is treated with surgery, chemotherapy, radiation or sometimes a combination of these treatments. Chemotherapy utilises chemicals that interfere with the cell division process, damaging proteins or DNA, so that cancer cells undergo apoptosis.

There are many types of medicines used to treat cancer. Nucleoside analogues comprise a major class of chemotherapeutic agents for the treatment of cancer, especially leukaemia, and viral diseases [1]. Because of their structural similarity, these analogues are taken up by cells, turned to their triphosphate species and incorporated into DNA or RNA [2].

Clofarabine (CLF), (2-chloro-9-(2-deoxy-2-fluoro-beta-D-arabinofuranosyl) adenine), Scheme 1, is a new generation of adenosine analogues with higher stability in the plasma and acidic gastric media. Like other purine analogues, CLF includes the 2-halogenated aglycone, which provides resistance to deamination by adenosine deaminase. In addition, CLF also contains fluorine at the second position of the sugar, which protects from bacterial purine nucleoside phosphorylase and from acid hydrolysis. The stability of CLF in acidic media makes its oral usage possible in cancer treatment. Moreover, it exhibits the highest lipophilicity when compared to related analogues [3]. The mechanism of its anti-cancer activity is a combination of inhibition of DNA synthesis, RNA reductase and stimulation of apoptosis.

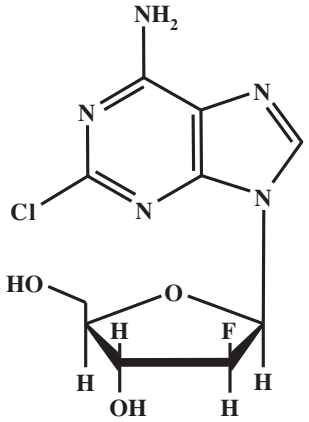
After entry into cells, CLF is phosphorylated to its monophosphate and finally to triphosphate derivatives by deoxycytidine kinase and deoxyguanosine kinase [4,5], which incorporate into DNA, leading to inhibition of nucleic acid synthesis and repair mechanism, finally causing cell death. Although there are no evidence of direct interaction between CLF and DNA, there is biological relevance to investigate its interaction with DNA because this drug has easy access to the interior of the cells.

Due to the importance of CLF in biological systems the development of analytical methods for its determination in biological fluids as well as interaction with important biomolecules is of great interest. Being a relatively new drug, very few methods have appeared in the literature for the determination of CLF. Most of them are based on high performance liquid chromatography. For the determination of CLF in mouse plasma, the chemical extraction of the compound was achieved using either ultra performance liquid chromatography or micro column HPLC coupled to either quadrupole ion trap mass spectrometry or triple quadrupole mass spectrometry [6]. The same method was also used for the determination of CLF in serum samples of patients undergoing hemodialysis [7]. Another study is based on the identification and separation of impurities and metabolites using 1H NMR [8].

Due to their high sensitivity, voltammetric methods have been successfully used for the detection and determination of many biological compounds. Moreover, investigations of the redox behaviour of biologically occurring compounds by means of electrochemical

* Corresponding author. Tel./fax: +351 239 835295.
E-mail address: brett@ci.uc.pt (A.M. Oliveira-Brett).

1567-5394/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2011.07.004



Scheme 1. Chemical structure of CLF.

techniques have the potential for providing valuable insights into the redox reaction mechanisms of these molecules.

Interaction of a damaging agent with DNA causes changing of the electrochemical properties of the DNA recognition layer and this effect is turned to measurable electrical signals. DNA damage and interaction caused by hazardous compounds, such as metals or pharmaceutical drugs, can be detected [9–12].

The electrochemical oxidation of CLF was investigated for the first time using cyclic, square wave and differential pulse voltammetry at a glassy carbon electrode. The DNA–electrochemical biosensor, an electrode with DNA immobilised on the surface, was used to investigate the CLF–DNA interaction. The results presented will contribute to the clarification of the mechanism by which CLF can interact with DNA.

2. Experimental

2.1. Materials and reagents

Clofarabine (CLF), double stranded (dsDNA), polyadenylic acid (Poly[A]) and polyguanilic acid (Poly[G]) were obtained from Sigma and used without further purification. A stock solution of 1.6 mM CLF was prepared in deionised water and stored at 5 °C.

Solutions of different concentrations of CLF were prepared by dilution of the appropriate quantity in supporting electrolyte. Stock solutions of 186 μg mL^{-1} dsDNA, 477 μg mL^{-1} Poly[G], and 587 μg mL^{-1} Poly[A] were prepared in deionized water and diluted to the desired concentrations in pH 4.5 0.1 M acetate buffer.

All supporting electrolyte solutions (Table 1) were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1}).

Table 1
Supporting electrolyte buffer solutions.

pH	Composition
2.0	HCl + KCl
3.4	HAcO + NaAcO
4.3	HAcO + NaAcO
4.5	HAcO + NaAcO
5.4	HAcO + NaAcO
6.1	$NaH_2PO_4 + Na_2HPO_4$
7.0	$NaH_2PO_4 + Na_2HPO_4$
8.0	$NaH_2PO_4 + Na_2HPO_4$
9.2	$NH_3 + NH_4Cl$
10.5	$NH_3 + NH_4Cl$
12.0	NaOH + KCl

2.2. Apparatus

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a three-electrode system in a 0.5 mL one-compartment electrochemical cell (Cypress System Inc., USA). Glassy carbon electrode (GCE, d = 1.5 mm) was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 M KCl) reference electrode.

The pH measurements were carried out with a Crison micropH 2001 pH-metre with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. For square wave (SW) voltammetry a frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s^{-1} were used.

The GCE was polished using diamond spray, particle size 3 μm, (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q 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. Acquisition and presentation of voltammetric data

All the voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artefact, although the peak intensity is, in some cases, reduced (<10%) relative to that of the untreated curve. This mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. Nevertheless, the values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

2.4. Incubation procedure and DNA–biosensor preparation

In the incubation procedure, 100 μg mL^{-1} dsDNA were mixed with 2 μM CLF in pH 4.5 0.1 M acetate buffer, and 100 μg mL^{-1} Poly[G] or Poly[A] were mixed with 5 μM CLF in pH 4.5 0.1 M acetate buffer, and then incubated at room temperature during different time periods. Control solutions of CLF, dsDNA, 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 CLF–dsDNA, CLF-Poly[G] and CLF-Poly[A] incubated solutions.

The thin layer dsDNA-modified electrode was prepared depositing three drops of 5 μL each containing 50 μg mL^{-1} dsDNA on the GCE surface. After placing each drop on the electrode surface, the biosensor was allowed to dry under the constant flux of N_2.

The dsDNA–electrochemical biosensor was incubated in 100 μM CLF solution during different times. Then, in order to remove unbound molecules from the surface, the electrode was carefully rinsed with deionized water and transferred to supporting electrolyte. Afterwards DP voltammograms were recorded and the dsDNA 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. Systematic studies to clarify the interaction mechanism of CLF with dsDNA, Poly[A] and Poly[G] were carried out at the modified GCE using DP voltammetry that, because of its high sensitivity and

selectivity, enables the rapid detection of minor changes in the dsDNA morphological structure and of DNA oxidative damage.

3. Results and discussion

The electrochemical behaviour of CLF was investigated by CV, DP, and SW voltammetry using a GCE. The electrochemical measurements were performed in different pH electrolyte buffer solutions.

The interaction between CLF and DNA was investigated in incubated solutions and with the multilayer dsDNA, and polyhomonucleotides, poly[G] and poly[A]-electrochemical biosensors using DP voltammetry.

3.1. Electrooxidation behaviour of CLB

3.1.1. Cyclic voltammetry

CVs were performed in 300 μM CLF in all buffer electrolytes, scan rate 100 mV s^{-1}. In the first anodic scan in pH 4.3 0.1 M acetate buffer, peak 1_a, at $E^1_{pa} = +1.47$ V, and in the reverse scan peak 2_c, at $E^2_{pc} = +0.30$ V, were observed, Fig. 1. Changing the scan direction, on the second voltammogram, the anodic peak 2_a, at $E^2_{pa} = +0.33$ V, occurred. Peaks $2_c - 2_a$ correspond to the redox processes of the CLF oxidation product formed on GCE surface. The CLF oxidation peak 1_a current decreased in successive voltammograms, Fig. 1A, due to the adsorption of CLF oxidation product on the electrode surface.

For 2.0<pH<8.0, a similar behaviour was observed, the peak potentials being pH-dependent. The current of peak 2_c increased with decreasing pH. For pH<2.0 no oxidation peak of CLF was observed but two anodic peaks for pH>8.0 occurred.

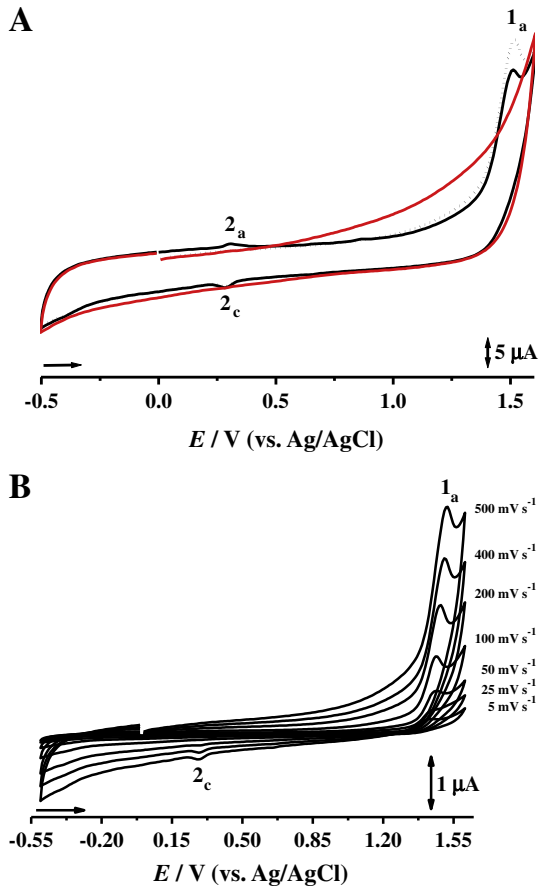


Fig. 1. CVs in 300 μM CLF in pH 4.3 0.1 M acetate buffer solution, N_2 saturated: (A) (•••) first, (—) second scans and (—) first scan of acetate buffer at $\nu = 500$ mV s^{-1}, (B) different scan rates.

The effect of scan rate was also evaluated and CVs were recorded in 300 μM CLF solution in pH 4.3 0.1 M acetate buffer at scan rates between 5 and 500 mV s^{-1}, Fig. 1B. Between measurements, the electrode surface was always polished in order to ensure a clean surface to avoid possible problems due to adsorption. The peak 1_a always appeared on the first scan and the pair of peaks $2_a - 2_c$ on subsequent scans. Also, peak 1_a current increased with increasing scan rate, following a linear dependence with square root of scan rate, in agreement with a diffusion-controlled process [13].

3.1.2. Differential pulse voltammetry

The electrochemical behaviour of CLF was also examined in 2.0<pH<12.0 buffer supporting electrolytes with 300 μM CLF using DP voltammetry, Fig. 2A.

For 2.0<pH<8.0, the oxidation of CLF is pH-dependent and the variation of peak 1_a potential is linear, $E_{pa}(V) = 1.640 - 0.059$ pH, Fig. 2B. The slope of the line, 59 mV per pH unit indicates that the same number of proton and electron are involved in the oxidation of CLF. The width at half-height of peak 1_a was $W_{1/2} \sim 46$ mV for the lowest concentrations used (between 1.6 and 25.3 μM, see below Section 3.1.5) close to the theoretical value for the transfer of two electrons [13]. Thus, the oxidation of CLF occurs with a transfer of two electrons and two protons, and the peak current increases up until pH = 4.0 and afterwards decreases with increasing pH, Fig. 2A.

The CLF peak 1_a current decreased for different pHs in successive DP voltammograms. The oxidation peak 1_a disappeared in the second scan in strong acidic media, pH<2.0. For pH>8.0, peak 1_a was split into two peaks, Fig. 2A, due to the degradation of CLF in alkaline media.

On the second and following scans a new oxidation pH-dependent peak 2_a related with the oxidation of CLF oxidation product occurred.

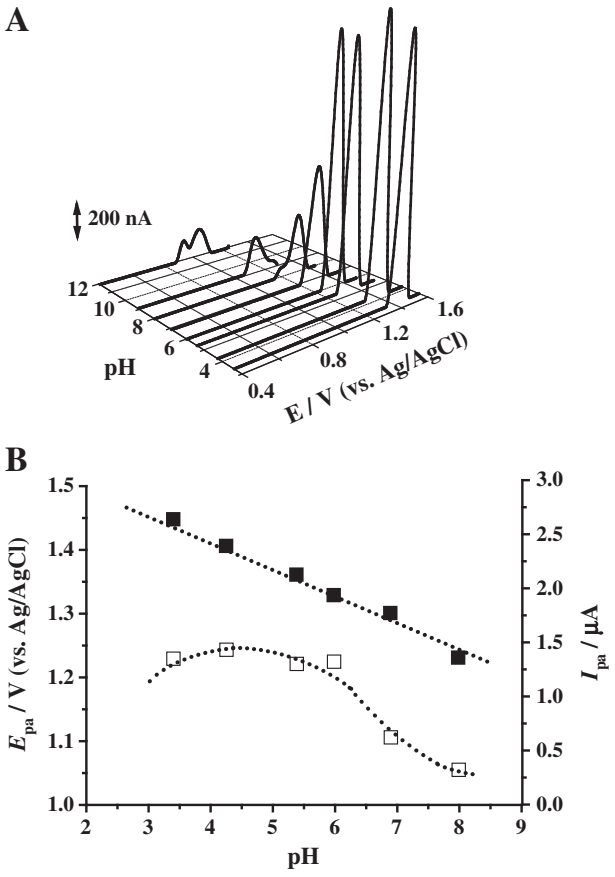


Fig. 2. (A) 3D plots of DP voltammograms base-line corrected in 300 μM CLF; (B) Plot of peak 1_a (■) E_{pa} and (□) I_{pa} versus pH. Dotted line corresponds to 59 mV per pH unit.

Increasing the pH, peak 2_a potential decreased. The slope of the E^2_{pa} versus pH was −59 mV per pH unit, as the width at half-height of the peak 2_a was $W_{1/2}$ ~ 81 mV, close to the theoretical value for the transfer of one electron [13], the mechanism of peak 2_a oxidation involves the transfer of one electron and one proton.

3.1.3. Square wave voltammetry

SW voltammetry has some advantages such as greater speed of analysis, lower consumption of the electroactive species, and less blocking of electrode surface by the compounds. Since in SW voltammetry the current is sampled in both positive and negative--going pulses, oxidation and reduction peaks of the electroactive compound at the electrode surface can be obtained simultaneously and the reversibility of the electron transfer reaction monitored by plotting the forward and backward components of the total current.

SW voltammetry was carried out for CLF over the same pH range. The first scan in 300 μM CLF in pH 4.3 0.1 M acetate buffer solution confirmed the irreversibility of CLF oxidation peak 1_a, at $E^1_{pa} = +1.45$ V, Fig. 3A. On the second SW voltammogram in the same solution without cleaning the GCE surface a second oxidation peak 2_a, at $E^2_{pa} = +0.33$ V, related with the CLF oxidation product occurred. The reversibility of peak 2_a was confirmed by plotting the forward and backward components where the reduction and oxidation currents are equal, Fig. 3B.

3.1.4. Oxidation mechanism of CLF

The CLF oxidation is an irreversible process and the electroactive centre is the adenine moiety. The oxidation involves the transfer of two electrons and two protons to produce a cation radical, which undergoes hydrolysis and yields a final hydroxylated product, Scheme 2. The hydroxylated product is also oxidised, peak 2_a, in a reversible process involving the transfer of one electron and one proton.

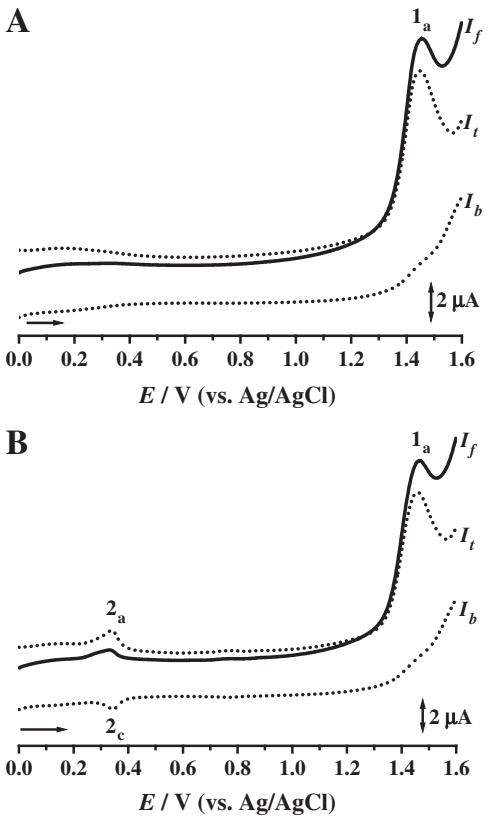


Fig. 3. SW voltammograms in 300 μM CLF in pH 4.3 0.1 M acetate buffer solution: (A) first and (B) second scan: I_t: total, I_f: forward and I_b: backward.

3.1.5. Analytical determination of CLF

DP voltammetry has advantages such as low detection limit and a good discrimination against the background current and was used for the development of a methodology for the electroanalytical determination of CLF.

Experiments were carried out by the standard addition method in electrolytes with different pHs. Between measurements the GCE surface was always cleaned to ensure a fresh electrode surface and to avoid the adsorption of CLF oxidation product. The best condition for analytical determination was in acetate buffer at pH = 4.3. A good correlation was obtained between 1.6 and 25.3 μM. For higher concentrations, the loss of linearity was probably due to the adsorption of CLF on the electrode surface. The line parameters are given in Table 2. The detection limit, LOD = 0.08 μM, and the quantification limit, LOQ = 0.26 μM, were calculated according to the 3 s/m and 10 s/m, where *s* is the standard deviation of the peak current and *m* is slope of calibration curve.

3.2. CLF–dsDNA interaction

3.2.1. Incubated solutions

The electrochemical behaviour of dsDNA was recorded as control for clarifying the CLF–dsDNA interaction. The DP voltammogram of dsDNA showed two well defined peaks corresponding to the oxidation of desoxyguanosine (dGuo), at $E_{pa} = +0.98$ V, and desoxyadenosine (dAdo), at $E_{pa} = +1.24$ V, Fig. 4.

The electrochemical study of the CLF–dsDNA interaction was carried out incubating 2 μM CLF with 100 μg mL^{-1} dsDNA in pH = 4.5 0.1 M acetate buffer and, after different incubation periods, DP voltammograms were recorded. Control solutions in buffer containing 100 μg mL^{-1} dsDNA or 2 μM CLF were also prepared and analysed after the same periods of time. After each measurement, the GCE electrode surface was cleaned to avoid blocking of the surface by adsorption of electroactive species or their oxidation products.

The DP voltammogram recorded immediately after addition of CLF to the dsDNA solution, showed three peaks due to the oxidation of the purinic dsDNA bases dGuo, at $E_{pa} = +0.98$ V, dAdo, at $E_{pa} = +1.24$ V and the oxidation of CLF, at $E_{pa} = +1.36$ V, Fig. 4. Increasing incubation time, the peak currents corresponding to dGuo and dAdo oxidation peaks decreased. The interaction CLF–dsDNA caused morphological structural changes of condensation and/or aggregation of dsDNA, Fig. 4. No additional peaks for 8-oxoGua or 2,8-oxoAde, meaning that CLF does not cause oxidative damage to DNA, were observed.

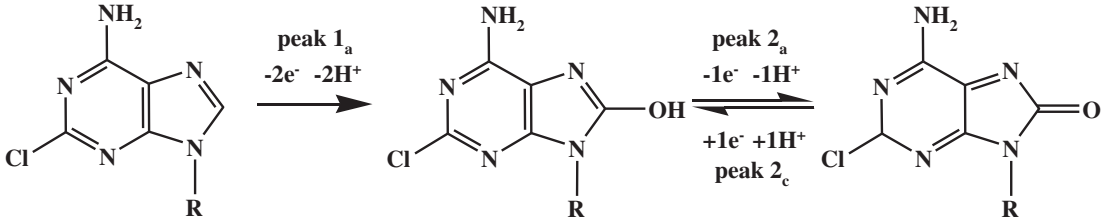
Experiments were also carried out incubating in pH 4.5 0.1 M acetate buffer 5 μM CLF with 100 μg mL^{-1} poly[A] and poly[G] solutions. DP voltammograms recorded after different incubation periods showed similar results as with dsDNA confirming the decrease of dGuo, Fig. 5A and dAdo, Fig. 5B peaks and no oxidative damage was observed.

3.2.2. In situ study using the dsDNA–electrochemical biosensor

During the experiments carried out in incubated solutions, a mixed multilayer of adsorbed DNA, CLF and CLF–dsDNA is formed at the GCE surface. For better understanding of the CLF–dsDNA interaction, a multilayer DNA–electrochemical biosensor was prepared.

The dsDNA biosensor consists of an electrode with DNA immobilised on the surface and the DNA film completely covers the GCE surface. The interaction of the compound with surface immobilised dsDNA causes changes of the DNA structure which can be seen by the alterations in the DNA oxidation peaks.

The freshly prepared dsDNA–electrochemical biosensor was incubated for 5 min in 100 μM CLF, was washed carefully with deionized water to remove unbound CLF molecules and transferred to acetate buffer where a DP voltammogram was recorded, Fig. 6. The DP voltammograms showed the peaks due to the dsDNA base oxidation,



Scheme 2. Proposed CLF oxidation mechanism.

smaller relative to the control, followed by a peak at a higher potential due to CLF oxidation.

This experiment was repeated, always using a newly prepared biosensor, testing incubation times of 10 and 15 minutes, and a decrease of dsDNA base oxidation peaks with increasing incubation time was observed. The CLF oxidation peak increased with time due to incorporation and pre-concentration into the DNA film.

The decrease in dGuo and dAdo oxidation peaks with increasing incubation time in CLF corresponds to the condensation of the dsDNA morphological structure. Nevertheless, no oxidative DNA damage occurred during CLF–dsDNA interaction, since no peaks corresponding to 8-oxoGua or 2,8-oxoA were observed.

In order to obtain more detailed information about the nature of the interaction between CLF–dsDNA interaction, the GCE surface was modified, as described in Section 2.4, and Poly[G] and Poly[A]-electrochemical biosensors were prepared.

The control Poly[G]-electrochemical biosensors showed only the dGuo peak, at $E_{pa} = +0.98$ V, Fig. 7A. In a new experiment, newly prepared Poly[G]-electrochemical biosensors were incubated in 100 μM CLF during different times and the peaks corresponding to the oxidation of dGuo and CLF decreased with increasing incubation time, Fig. 7A.

The control Poly[A]-electrochemical biosensors showed only the dAdo peak, at $E_{pa} = +1.24$ V, Fig. 7B. In another experiment, newly prepared Poly[A]-electrochemical biosensors were also incubated in 100 μM CLF during different times and the peaks corresponding to the oxidation of dAdo and CLF decreased with increasing incubation time, Fig. 7B.

It was confirmed that the interaction CLF–dsDNA is not specific to a given type of dsDNA base and does not cause oxidative damage.

4. Conclusions

The electrochemical behaviour of CLF, a drug used to treat various lymphocytic cancers, was studied using cyclic, differential pulse and square wave voltammetry and a glassy carbon electrode. The oxidation of CLF is irreversible, pH-dependent, and leads to a hydroxylated product. The oxidation product of clofarabine undergoes reversible oxidation.

The interaction between dsDNA and CLF in incubated solutions and using dsDNA, Poly[G], and Poly[A]-electrochemical biosensors was investigated showing that CLF interacts with dsDNA, confirmed by decreasing of the dGuo and dAdo oxidation peaks with increasing incubation time in solutions containing CLF.

The CLF–dsDNA interaction causes condensation of the dsDNA morphological structure, is not specific to a given type of base, and does not cause oxidative damage.

Table 2
Regression and validation data of CLF.

Linearity (μM)	Slope (A M^{-1})	Intercept (A)	R^2	LOD (μM)	LOQ (μM)	RSD%
1.6–25.3	1.87×10^{-8}	-2.87×10^{-9}	0.9997	0.08	0.26	2.14

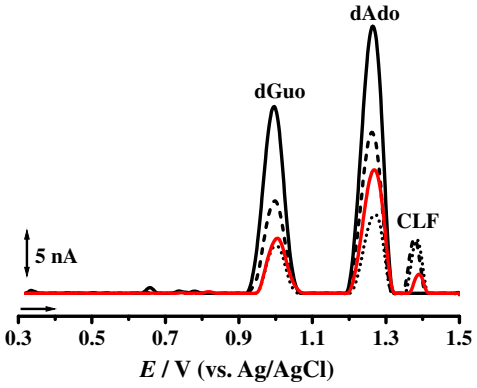


Fig. 4. DP voltammograms base-line corrected in 100 μg mL^{-1} dsDNA in pH 4.5 0.1 M acetate buffer (—) control and after incubation with 2 μM CLF during (- - -) 0 h, (•••) 2 h and (—) 4 h.

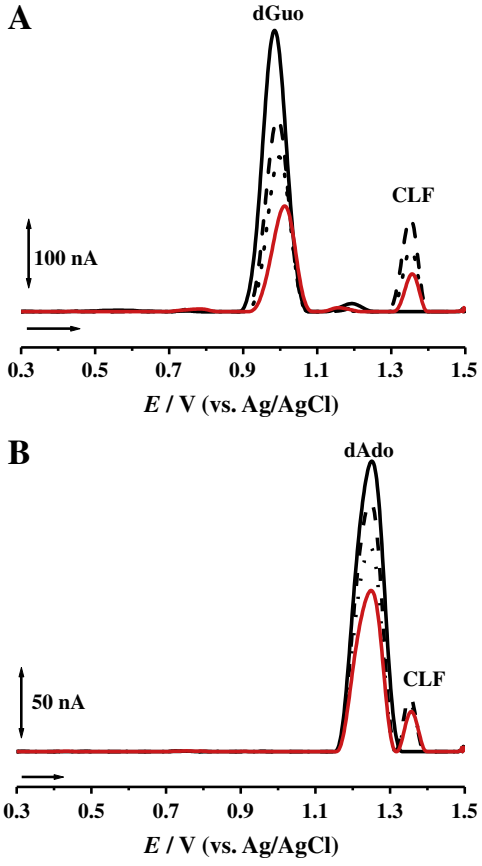


Fig. 5. DP voltammograms base-line corrected in 100 μg mL^{-1} of (A) Poly[G] and (B) Poly[A] in pH 4.5 0.1 M acetate buffer: (—) control and after incubation with 5 μM CLF during (- - -) 0 h, (•••) 2 h and (—) 4 h.

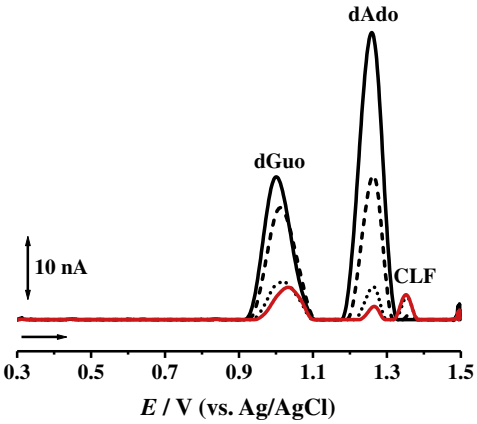


Fig. 6. DP voltammograms base-line corrected in pH 4.5 0.1 M acetate buffer with dsDNA-electrochemical biosensors (—) control and after incubation with 100 μM CLF during (- - -) 5 min, (•••) 10 min and (—) 15 min.

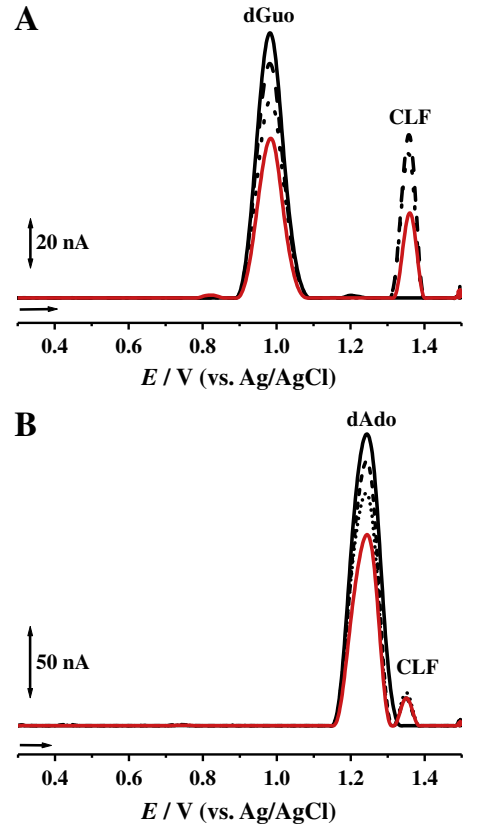


Fig. 7. DP voltammograms base-line corrected in pH 4.5 0.1 M acetate buffer with (A) Poly[G] and (B) Poly[A]-electrochemical biosensors: (—) control and after incubation with 100 μM CLF during (- - -) 5 min, (•••) 10 min and (—) 15 min.

Acknowledgements

Financial support from The Scientific and Technological Research Council of Turkey (TUBITAK) — International Postdoctoral Research Fellowship Programme (BIDEB) (H.E. Satana) and Fundação para a Ciência e Tecnologia (FCT), PhD grant SFRH/BD/46026/2008 (A.D.R. Pontinha), Post-Doctoral Grant SFRH/BPD/36110/2007 (V.C. Diculescu), project PTDC/QUI/65255/2006, PTDC/QUI/098562/2008 and PTDC/SAU-BEB/104643/2008, POCI (co-financed by the European Community Fund FEDER), CEMUC-R (Research Unit 285) are gratefully acknowledged.

References

[1] T. Robak, A. Korycka, M. Kasznicki, A. Wrzesien-Kus, P. Smolewski, Purine nucleoside analogues for the treatment of hematological malignancies: pharmacology and clinical applications, Curr. Cancer Drug Targets 5 (2005) 421–444.
[2] A. Zhenchuk, K. Lotfi, G. Juliusson, F. Albertioni, Mechanism of anti-cancer action and pharmacology of clofarabine, Biochem. Pharmacol. 78 (2009) 1351–1359.
[3] V. Reichelova, J. Liliemark, F. Albertioni, Liquid chromatographic study of acid stability of 2-chloro-2'-arabino-fluoro-2′-deoxyadenosine, 2-chloro-2′-deoxyadenosine and related analogues, J. Pharmaceut. Biomed. 13 (1995) 711–714.
[4] A.H. Sjoberg, L. Wang, S. Eriksson, Substrate specificity of human recombinant mitochondrial deoxyguanosine kinase with cytostatic and antiviral purine and pyrimidine analogs, Mol. Pharmacol. 53 (1998) 270–273.
[5] X. Wang, F. Albertioni, Effect of clofarabine on apoptosis and DNA synthesis in human epithelial colon cancer cells, Nucleosides Nucleotides Nucleic Acids 29 (2010) 414–418.
[6] Y. Hsieh, C.J.G. Duncan, S. Lee, M. Liu, Comparison of fast liquid chromatography/tandem mass spectrometric methods for simultaneous determination of cladribine and clofarabine in mouse plasma, J. Pharmaceut. Biomed. 44 (2007) 492–497.
[7] P.G. Steinherz, N. Shukla, R. Kobos, S. Latcha, Effect of hemodialysis on the plasma levels of clofarabine, Pediatr. Blood Cancer 55 (2010) 196–198.
[8] B.G. Anderson, W.E. Bauta, W.R. Cantrell, T. Engles, D.P. Lovett, Isolation, synthesis, and characterization of impurities and degradants from the clofarabine process, Org. Proces. Res. Dev. 12 (2008) 1229–1237.
[9] F. Jelen, M. Fojta, E. Palacek, Voltammetry of native double-stranded, denaturated and degraded DNA, J. Electroanal. Chem. 427 (1997) 49–56.
[10] A.M. Oliveira-Brett, J.A.P. Piedade, L.A. Silva, V.C. Diculescu, Voltammetric determination of all DNA nucleotides, Anal. Biochem. 332 (2004) 321–329.
[11] X. Cai, G. Rivas, H. Shirashi, P. Farias, J. Wang, M. Tomschikb, F. Jelenb, E. Palecek, Electrochemical analysis of formation of polynucleotide complexes in solution and at electrode surfaces, Anal. Chim. Acta 344 (1997) 65–76.
[12] A.M. Chiorcea-Paquim, O. Corduneanu, S.C.B. Oliveira, V.C. Diculescu, A.M. Oliveira-Brett, Electrochemical and AFM evaluation of hazard compounds — DNA interaction, Electrochim. Acta 54 (2009) 1978–1985.
[13] C.M.A. Brett, A.M. Oliveira Brett, Electrochemistry. Principles, Methods and Applications, Oxford University Press, UK, 1993.