Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 60 (2003) 11–19
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Electrochemical monitoring of the interaction of doxorubicin with nicotinamide and Fe(III) ions under aerobic and anaerobic conditions

S. Çakír*, E. Biçer, E. Coşkun, O. Çakír

Department of Chemistry, Faculty of Arts and Sciences, Nineteen May University, 55139 Kurupelit, Samsun, Turkey

Received 18 October 2002; received in revised form 21 February 2003; accepted 25 February 2003

Dedicated to Professor Dr. Osman Çakír on the occasion of his 50th birthday

Abstract

The interaction of doxorubicin with Fe(III) ions and nicotinamide (NA) has been followed by square-wave voltammetry, cyclic voltammetry and UV–VIS. spectroscopy techniques at aerobic and anaerobic conditions. Fe(III)–doxorubicin complex gives a 1-electron reversible step at −0.494 V and a shoulder at 580 nm. Further, the Fe(III)–doxorubicin complex was found to be more stable at aerobic conditions. In the presence of NA, an intermediate (NA–Fe(III)–DQ) forms at −0.462 V under aerobic conditions. Because of the formation of this intermediate, nicotinamide may reduce the cardiotoxic effect of doxorubicin and cause to its detoxification.

Keywords: Doxorubicin; Nicotinamide; Fe(III)/Fe(II); Electrochemistry; UV–VIS spectroscopy

1. Introduction

Doxorubicin (also called adriamycin [1], Scheme 1) is an anthracycline antibiotic. It is a potent antineoplastic agent that is active against a wide range of human cancers [2]. However, its cardiotoxicity limits the clinical use.

Metal ions play an important role in altering the biochemical properties of the anthracyclines, and indicate a new direction in the pursuit of chemotherapeutic efficacy and lowering toxicity of these drugs. The binding of metal ions may cause a significant influence on the redox property of these drugs [3–6]. Iron is an important metal in that it participates in the action of drug functioning as a redox center, which can free radicals in the presence of dioxygen under reducing conditions and damage cell components [7]. Doxorubicin can be reduced to semiquinone form by biological reducing agents such as NADH and NADPH. Superoxide and hydrogen peroxide can be produced via dioxygen receiving electron from the semiquinone. This event occurs in the presence of transition metal [8]. It is important that the concentration of the metal (iron and copper) ions be kept at a minimum. Transition metals may simultaneously bound to biomolecule and dioxygen and may often act as a bridge between molecule and dioxygen [8]. Therefore, it is very important to investigate the structure and characteristic of the metal–doxorubicin complexes. Several papers have described the doxorubicin–Fe(III) or Fe(II) complexes [9,10]. Furthermore, the synthesis and physicochemical properties of Fe(II) [11] and Fe(III) [12] complexes with nicotinamide have been investigated. It has been reported [11,12] that nicotinamide is coordinated to Fe(II) and Fe(III) through the nitrogen atom of its heterocyclic ring. However, we were not able to trace any references on spectroscopic and electrochemical behavior of the interaction of Fe(III)/Fe(II) with doxorubicin in the presence of nicotinamide (Scheme 1) in the relevant literature.

In this work, the interaction of Fe(III)/Fe(II) with doxorubicin in the presence of nicotinamide under anaerobic and aerobic conditions was monitored by using spectroscopic and electrochemical techniques.

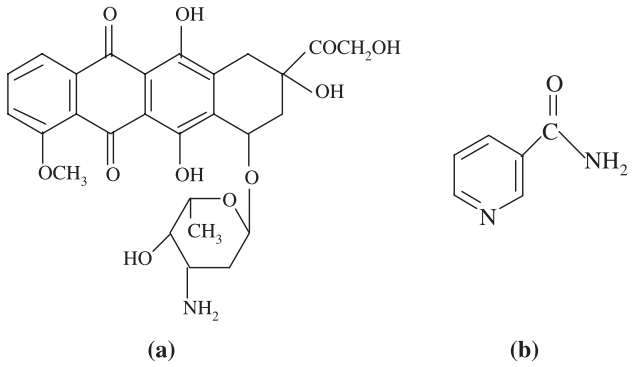
2. Experimental

2.1. Instrumentation

Voltammograms were recorded using an EG&G PARC Model 303A polarographic analyzer (Princeton, NJ, USA)

* Corresponding author. Tel.: +90-362-4576020x5097; fax: +90-362-4576081.
E-mail address: scakir@omu.edu.tr (S. Çakír).

1567-5394/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1567-5394(03)00040-9



Scheme 1. Chemical structures of doxorubicin (a) and nicotinamide (b).

coupled with a Houston Instrument DMP-40 plotter (Austin, TX, USA). Electrochemical experiments were performed in a three-electrode cell, at ambient temperature (approximately 20 °C). Potentials are referred to as Ag|AgCl|$KCl_{sat.}$ reference electrode. The working electrode is a static mercury drop electrode (SMDE). The auxiliary electrode was a Pt wire.

Electronic spectra were recorded on Unicam V2-100 UV–VIS spectrophotometer in the 800–200 nm range at 1-cm cell length.

2.2. Reagents and solutions

Doxorubicine hydrochloride were purchased from Sigma. Nicotinamide was obtained from Merck. Standard Fe(II) and Fe(III) solutions were prepared with analytical-grade $FeCl_2{\cdot}4H_2O$ and $FeCl_3{\cdot}6H_2O$, respectively. Stock standard solutions were prepared fresh daily in ultrapure triply distilled water and protected from light and air. Solutions with lower concentrations were prepared by dilution with deionized triply distilled water and were used within a few hours. 0.1 M $Na_4P_2O_7$ solution (pH 9) was used as the supporting electrolyte. The pH value of supporting electrolyte was adjusted with 0.1 M H_3PO_4 solution.

2.3. Procedure

2.3.1. Voltammetry

For anaerobic conditions, the solution was purged with oxygen-free nitrogen for 10 min and during the measurements, nitrogen was passed above the solution in the cell. The addition of doxorubicin and/or nicotinamide to the cell containing metal ions were carried out and the voltammograms were recorded under the aerobic and anaerobic conditions. Potential scans were recorded using the square-wave and cyclic voltammetry modulations and the following optimum parameters (if not stated otherwise): pulse height, 20 mV; frequency, 100 Hz; drop size medium; and equilibrium time 5, s. Each measurement was carried out on a fresh mercury drop.

2.3.2. Spectroscopy

The electronic spectra of mixtures with ambient mole ratio of both metal ions and doxorubicin and/or nicotinamide aqueous solutions were recorded, following the changes in absorbance at the wavelength of maximum absorption.

3. Results and discussion

3.1. Doxorubicin in the presence and absence of Fe(III) ions

Because doxorubicin contains both quinone and hydroquinone, it can be electrochemically reduced or oxidized (Scheme 1). Under anaerobic conditions, the cyclic voltammogram of 3.44×10^{-6} M doxorubicin in 0.1 M $Na_4P_2O_7$ solution (pH 9) is shown in Fig. 1. As can be observed in Fig. 1, the voltammogram of doxorubicin is characterized by only one peak couple at −0.700 V. The peak potentials for the oxidation and reduction reactions are almost similar (Fig. 1). The peak couple observed at −0.700 V can be attributed to redox process, involving the transformation of quinone to hydroquinone [13]. Moreover, the electrode reaction is controlled by the adsorption phenomena since the peak current for the reduction reaction is different from that of the oxidation reaction, in which the drug is adsorbed. The reversible electrochemical process has been found for doxorubicin-monolayer (Fig. 1), because the immobilized doxorubicin molecules are fixed at the electrode surface. Also, Oliveira-Brett et al. [14] have reported that adriamycin adsorbs some electrode (i.e., glassy carbon and highly oriented pyrolytic graphite) surfaces.

Under the same experimental conditions, square-wave voltammogram of fresh solutions of Fe(III) exhibits current maxima at −0.396 (E_{pc}) and −1.114 V (E_{pc}), respectively (Fig. 2). The former maximum was assigned to Fe(III)/Fe(II) reduction and the latter to Fe(II)/Fe(0) reduction. In the cyclic voltammograms (Fig. 2, inset), Fe(II)/Fe(III) oxidation (the anodic peak) was clearly seen. In cyclic voltammograms, the difference between the anodic and cathodic peak potentials of Fe(III)/Fe(II) redox process is about 85 mV, which may arise from the slow electron transfer. In relation to other peaks, the intensity of Fe(III)/Fe(II) reduction current is small. For this observation, it may be noted that Fe(III) aggregates, or turns mostly into Fe(II) species.

The reproducibilities of the electrochemical signals with time were studied by cyclic voltammetry under anaerobic conditions (Fig. 3). As can be observed in Fig. 3, the currents of Fe(III)/Fe(II) reduction, Fe(II)/

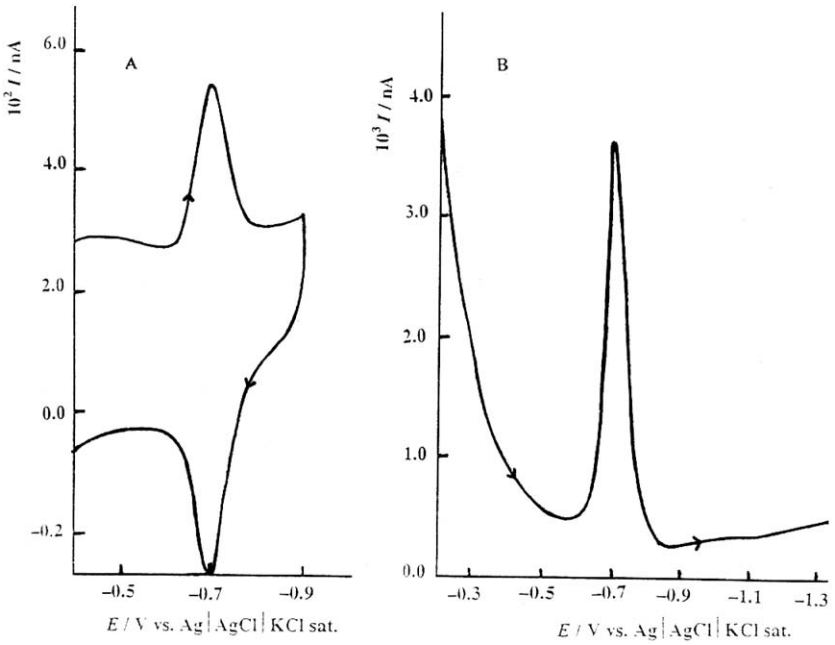


Fig. 1. Cyclic (A) and square-wave (B) voltammogram of 3.44×10^{-6} M doxorubicin at 0.1 M $Na_4P_2O_7$ solution (pH 9). Experimental conditions: pulse height, 20 mV; frequency, 100 Hz; drop size, medium; scan rate, 200 mV s^{-1} (500 mV s^{-1} for CV) and equilibrium time, 5 s.

Fe(III) oxidation and Fe(II)/Fe(0) reduction do not considerably change with time (at least up to 167 min). At aerobic conditions, cyclic voltammograms show that both the current maxima of Fe(II)/Fe(III) oxidation and Fe(II)/Fe(0) reduction increase with respect to the anaerobic conditions (Fig. 2, inset).

The increment in the peak current of Fe(II)/Fe(III) oxidation may be due to the acceleration of production of

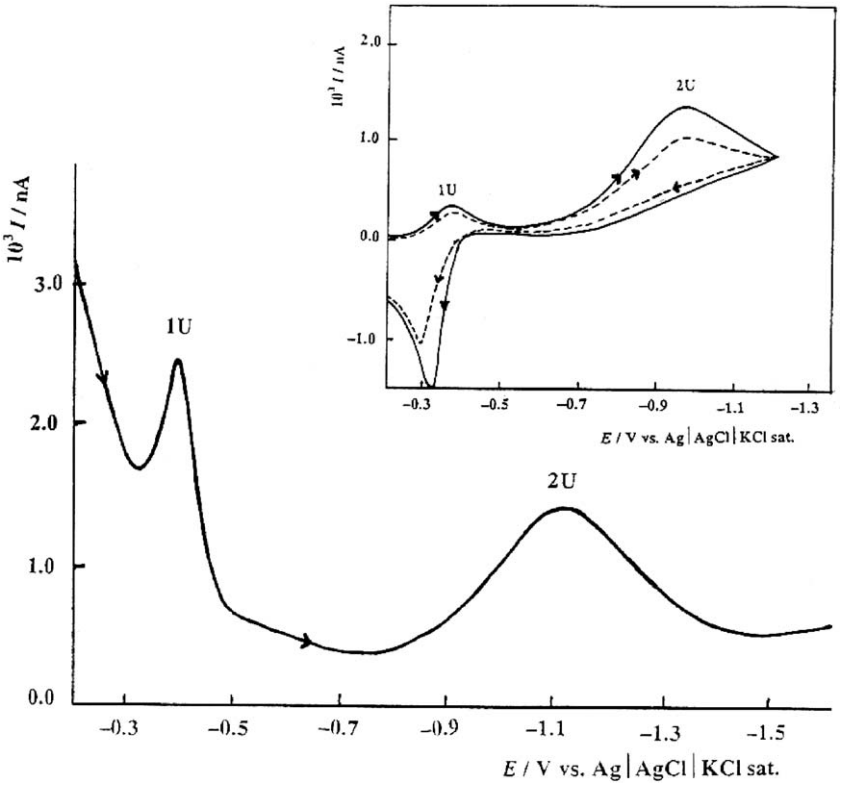


Fig. 2. Square-wave voltammogram of 5×10^{-4} M Fe(III) solution at 0.1 M $Na_4P_2O_7$ solution (pH 9). Inset: Cyclic voltammogram of 5×10^{-4} M Fe(III) solution at aerobic (——) and anaerobic conditions (- - - -). Other conditions are as in Fig. 1.

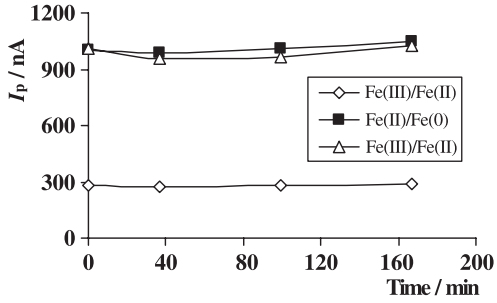


Fig. 3. The peak currents of Fe(III)/Fe(II), Fe(II)/Fe(0) and Fe(II)/Fe(III) processes as a function of time for 5×10^{-4} M Fe(III) solution at anaerobic conditions.

Fe(II) from Fe(III). The reactions involved can be represented by reactions (1) and (2).

$$O_2 + e^- \rightarrow O_2^{\cdot -} \quad (1)$$

$$Fe^{3+} + O_2^{\cdot -} \rightarrow Fe^{2+} + O_2 \quad (2)$$

When gradually increasing amounts of doxorubicin were added to 5×10^{-4} M Fe(III) solution, the peak current of

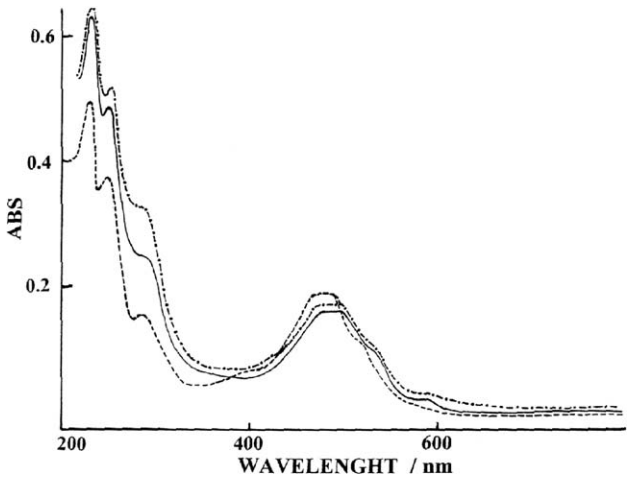


Fig. 5. Electronic spectra of 1×10^{-5} M doxorubicin in the presence (——) and absence (- - - -) of 1×10^{-5} M Fe(III) at 0.1 M $Na_4P_2O_7$ solution (pH 9). Electronic spectra of doxorubicin in the presence (–●–●–●–●–) of 1×10^{-5} M Fe(II) at 0.1 M $Na_4P_2O_7$ solution (pH 9).

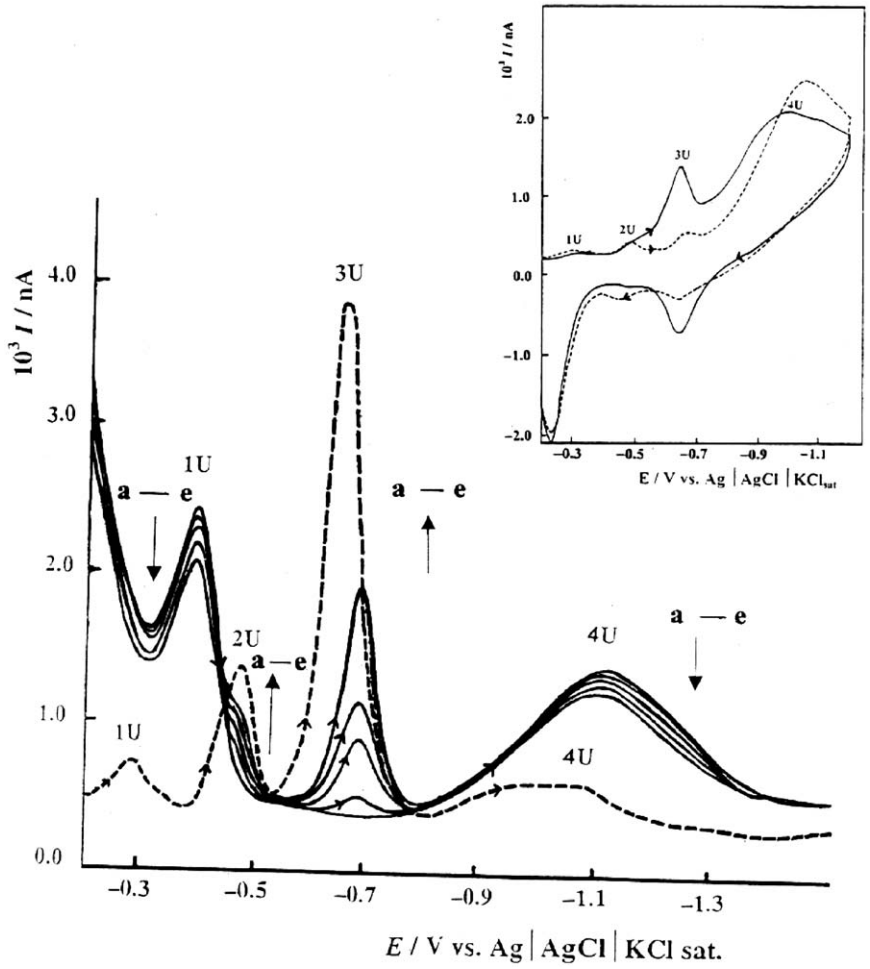


Fig. 4. Square-wave voltammograms 5×10^{-4} M Fe(III) solution containing 0 M (a); 4.3×10^{-7} M (b); 1.29×10^{-6} M (c); 2.15×10^{-6} M (d); 3.44×10^{-6} M (e) doxorubicin at anaerobic conditions (——); Square-wave voltammogram of 1.12×10^{-5} M doxorubicin and 5×10^{-4} M Fe(III) solution, was waited under aerobic conditions for a day (- - - -). Inset: Cyclic voltammogram of 5×10^{-4} M Fe(III) solution containing 1.12×10^{-5} M doxorubicin at anaerobic conditions (——) and at aerobic conditions (- - - -). 1U, Fe(III)/Fe(II); 2U, Fe(III)–DQ complex; 3U, DQ; 4U, Fe(II)/Fe(0). Other conditions are as in Fig. 1.

Table 1
The electronic spectra data under aerobic conditions

Solutions	λ_{max} (exp.) [nm]
NA	260, 300(sh)
DQ	232, 255(sh), 280(sh), 391, 478(br)
DQ–NA	252(sh), 280(sh), 372, 495
Fe(II)–NA	223, 245, 280(sh), 513
Fe(III)–NA	260, 280(sh), 340(sh)
Fe(III)–DQ	232, 252, 280(sh) 482, 580(sh)
Fe(II)–DQ	232, 252, 280(sh) 494, 580(sh)
Fe(III)–DQ–NA	254, 280(sh), 482

NA: nicotinamide; DQ: doxorubicin; sh: shoulder, br: broad.

both Fe(III) and Fe(II) decreased under anaerobic conditions and then a little shoulder occurred at − 0.470 V (Fig. 4). The shoulder at − 0.470 V was seen as a peak (− 0.494 V) at the aerobic solution, was sustained for a day. Although this peak is not well defined at anaerobic conditions, it was well determined after the cell, including doxorubicin and Fe(III), persisted under aerobic conditions for a day. This reversible peak (− 0.494 V), which belongs to the complexation of doxorubicin with Fe(III) ions, is observed (Fig. 4).

According to the presented voltammetric data, it can be said that Fe(III)–DQ complex, under aerobic conditions, is more stable than under anaerobic conditions; furthermore, its stability is a function of time.

The electronic spectra of doxorubicin in the presence and absence of Fe(III) ions were recorded in 0.1 M $Na_4P_2O_7$ (pH 9.0) under aerobic conditions (Fig. 5). The electronic spectra data are given in Table 1. As can be seen in Fig. 5, doxorubicin exhibits four absorption bands at 232, 255(sh), 280(sh), 391, 478(br) nm, respectively. In the presence of Fe(III) ions, the overlapping bands near 478 nm of doxorubicin shift to higher wavelength and lose intensity. The loss of the 478-nm absorption was associated with the formation of a new shoulder at 580 nm, which may be attributed to the complexation. In addition, the absorption spectra of Fe(III) and doxorubicin appears identical to that of aerobic solution of Fe(II) and doxorubicin (Fig. 5). These spectroscopic results are in good agreement with those reported for Fe(II)–adriamycin [9] and Fe(III)–adriamycin [15] systems.

Doxorubicin is complexed and oxidized by aqueous iron (III) [16]. Gianni et al. [15] demonstrated that the

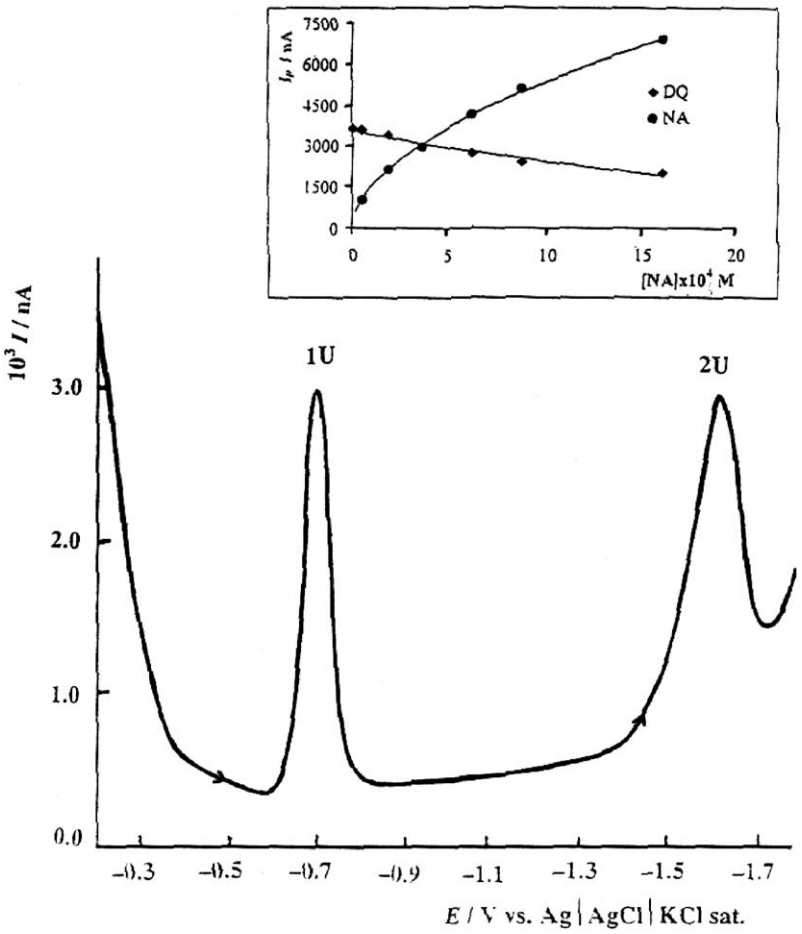


Fig. 6. Square-wave voltammogram of 3.44×10^{-6} M doxorubicin solution containing 4×10^{-4} M nicotinamide at anaerobic conditions. Inset: The dependence of the cathodic peak current of doxorubicin with the nicotinamide concentration at anaerobic conditions. 1U, DQ; 2U, NA. Other conditions are as in Fig. 1.

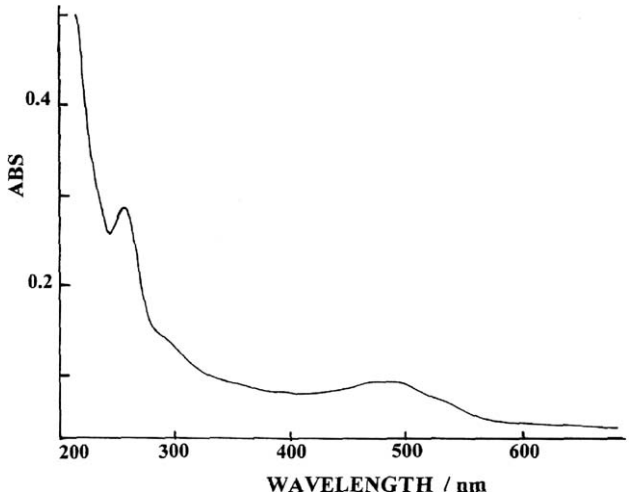


Fig. 7. Electronic spectra of 1×10^{-5} M nicotinamide in the presence of 1×10^{-5} M doxorubicin at 0.1 M $Na_4P_2O_7$ solution (pH 9).

Fe^{3+}–doxorubicin complexes cycle to reduce molecular oxygen. As Fe^{3+} is reduced to Fe^{2+}, doxorubicin free radical is formed, which may mediate the toxic effects of the drug [10,15]. In addition, Fe^{3+}–doxorubicin chelate leads to the formation of reactive oxygen species [17]. It has been reported that, in alkaline solutions, the hydroquinone moiety of doxorubicin undergoes autoxidation, forming free radicals, leading to oxygen consumption [17]. Doxorubicin free radical is relatively unstable in aerobic conditions and readily reduces oxygen to superoxide [10,18].

According to the data presented above, the reactions can be represented as follows:

$$Fe^{3+} + DQ \rightarrow Fe^{3+} - DQ \quad (3)$$

$$Fe^{3+} - DQ + e^- \rightleftarrows Fe^{2+} - DQ \quad (4)$$

In addition, the mechanism at aerobic conditions can given to be the iron-catalyzed Haber–Weiss reaction [19]:

$$Fe^{3+} - DQ + O_2^{\cdot -} \rightleftarrows Fe^{2+} - DQ + O_2 \quad (5)$$

$$Fe^{2+} - DQ + H_2O_2 \rightarrow Fe^{3+} - DQ + OH^{\cdot} + OH^- \quad (6)$$

Although the CV curve (Figs. 2 and 4, insets) at aerobic conditions does not show any signal due to the reduction of O_2 in the range -0.5 to -1.2 V, this signal can be probably hidden by the reduction peak of Fe(II)/Fe(0). According to

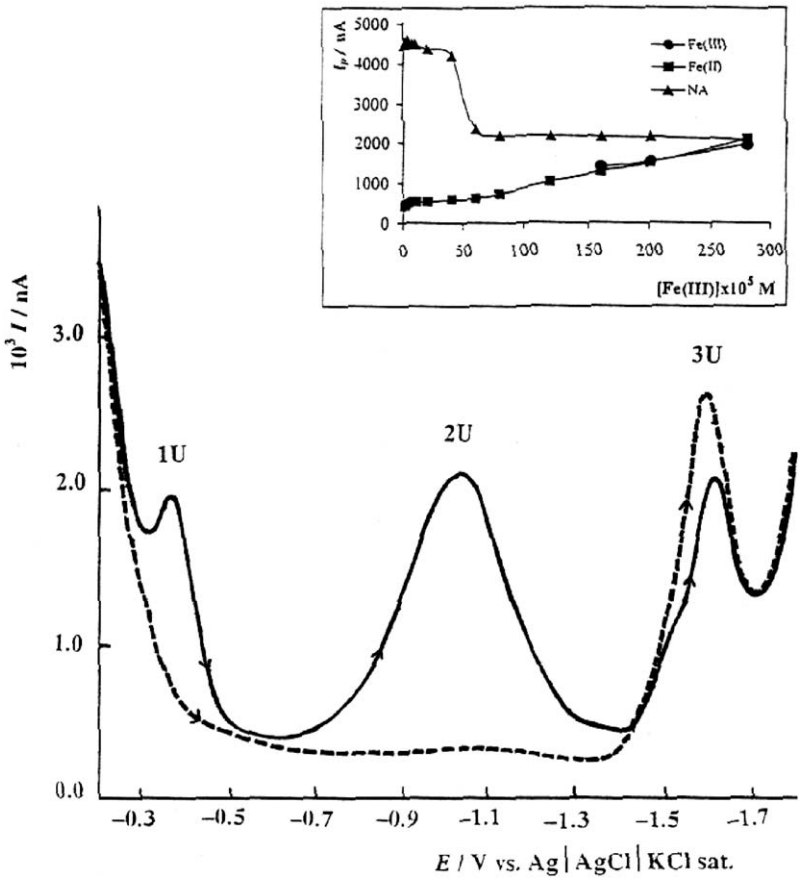


Fig. 8. Square-wave voltammograms of 4×10^{-4} M nicotinamide (- - - -); 4×10^{-4} M nicotinamide solution containing 2.8×10^{-3} M Fe(III) (——) at anaerobic conditions. Inset: The dependence of the cathodic peak currents of nicotinamide, Fe(III) and Fe(II) with the Fe(III) concentration. 1U, Fe(III)/Fe(II); 2U, Fe(II)/Fe(0); 3U, NA. Other conditions are as in Fig. 1.

the obtained results, the observation of Fe(III)–DQ complex in the presence of O_2 may indicate that iron complexes can catalyze a reaction between $O_2^{\cdot -}$ and H_2O_2, thereby forming $OH^{\cdot}$. Indeed, $OH^{\cdot}$ is extraordinarily reactive and can attack all organic compounds [20]. And assuming the prevention of reaction (6) by nicotinamide, the experiments described below have been carried out.

3.2. Nicotinamide in the presence and absence of doxorubicin

Usually, for nicotinamide in aqueous solution at pH 9.0 and at a mercury electrode, one irreversible peak is observed at −1.594 V (Fig. 6). The peak at −1.594 V (Fig. 6) may be attributed to an irreversible 4-electron reduction of an amide group to an alcohol [21]. However, the electrocatalytic rates of the reduction is generally slow to produce observable catalytic hydrogen current.

When nicotinamide (4.78×10^{-5}–1.61×10^{-3} M) was added to the bulk solution containing doxorubicin (3.44×10^{-6} M), an obvious change of SWV was observed (Fig. 6). The peak current of doxorubicin decreases gradually while the peak current of nicotinamide increases (Fig. 6), and its peak potential shifts to slightly positive values (from −1.628 to −1.618 V). The observed behaviour results from the adsorption effect or intermolecular interaction on the electrode surface. For final clarification of this behaviour, the electronic spectra data were used.

The electronic spectra of the mixing solution of doxorubicin with nicotinamide clearly show the changes at the overlapping bands of doxorubicin (Fig. 7). In the presence of nicotinamide, the overlapping bands with maximum absorptions at 391 and 478 nm are expanded to those with maximum absorptions at 372 and 495 nm. Doxorubicin in the conjugates of doxorubicin was given a maximum absorption band at 495 nm [22].

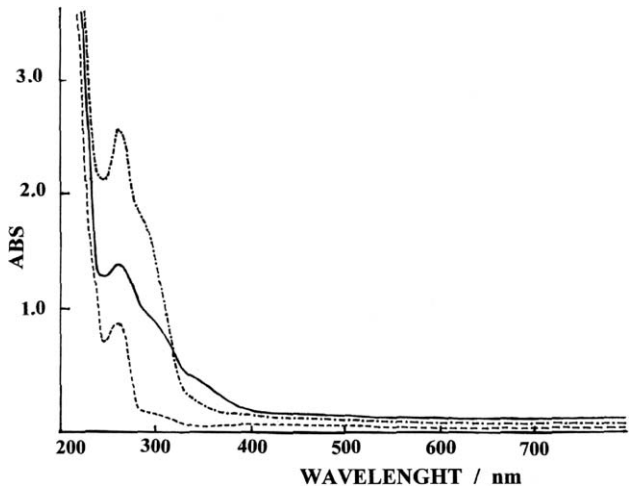


Fig. 9. Electronic spectra of 1×10^{-5} M nicotinamide in the presence (——) and absence (- - - -) of 1×10^{-5} M Fe(III) at 0.1 M $Na_4P_2O_7$ solution (pH 9). Electronic spectra of doxorubicin in the presence (– • – • – • – • –) of 1×10^{-5} M Fe(II) at 0.1 M $Na_4P_2O_7$ solution (pH 9).

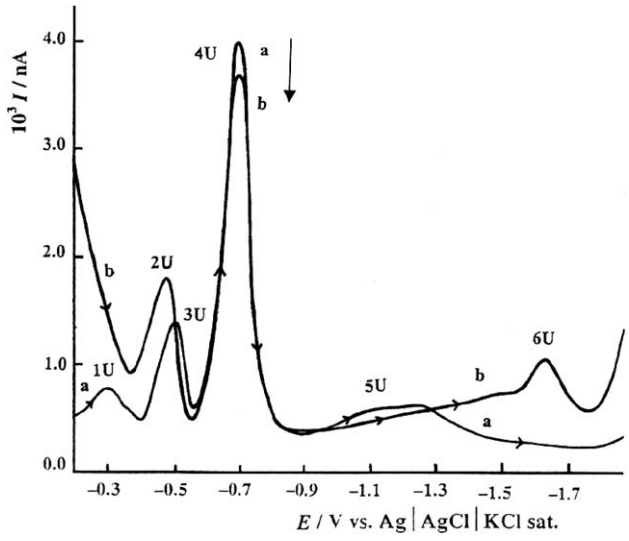


Fig. 10. Square-wave voltammograms of 5×10^{-4} M Fe(III) and 1.12×10^{-5} M doxorubicin solution, was waited under aerobic conditions for a day in the presence of 0 M (a); 5×10^{-5} M (b) nicotinamide at aerobic conditions. 1U, Fe(III)/Fe(II); 2U, NA–Fe(III)–DQ pre-association intermediate; 3U, Fe(III)–DQ complex; 4U, DQ; 5U, Fe(II)/Fe(0); 6U, NA. Other conditions are as in Fig. 1.

3.3. Nicotinamide in the presence of Fe(III)

The interaction of nicotinamide with Fe(III) ions was also electrochemically monitored. With increasing Fe(III) concentration, the peak current of nicotinamide at −1.594 V decreases while the reduction peak current of Fe(II) ions

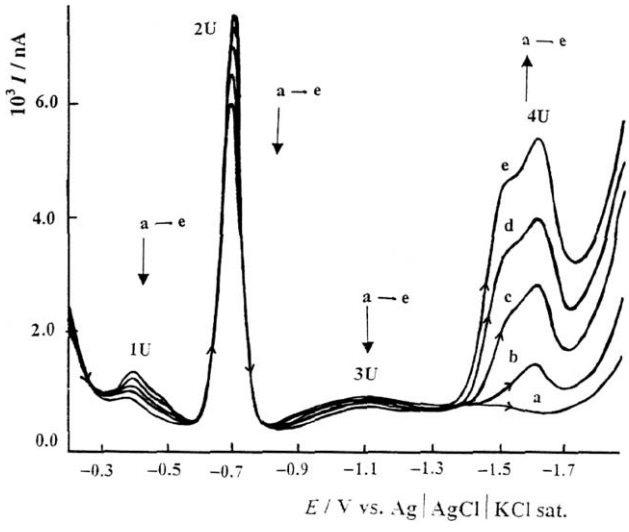


Fig. 11. Square-wave voltammograms of 1.4×10^{-5} M doxorubicin and 5×10^{-4} M Fe(III) solution in the presence of 0 (a); 2×10^{-4} M (b); 7×10^{-4} M (c); 1.2×10^{-3} M (d); 2×10^{-3} M (e) nicotinamide at anaerobic conditions. 1U, Fe(III)/Fe(II); 2U, DQ; 3U, Fe(II)/Fe(0); 4U, NA. Other conditions are as in Fig. 1.

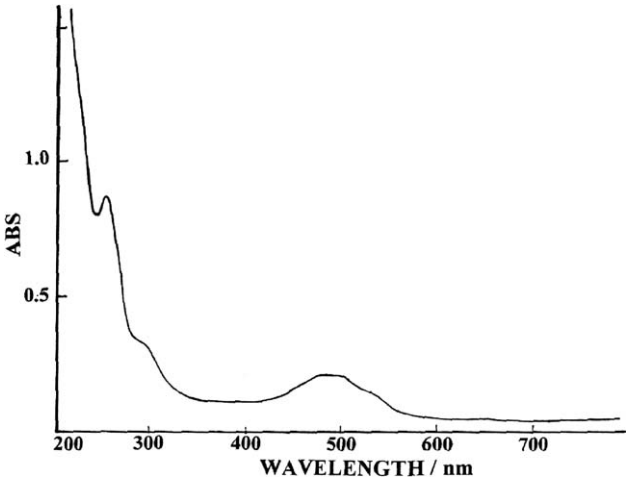


Fig. 12. Electronic spectra of 1×10^{-5} M nicotinamide and 1×10^{-5} M doxorubicin in the presence of 1×10^{-5} M Fe(III) at 0.1 M $Na_4P_2O_7$ solution (pH 9).

at -1.026 V increases (Fig. 8). Moreover, the reduction peak of Fe(III) at -0.368 V also appears at high ferric ion concentrations ($\geq 1.6 \times 10^{-3}$ M) (Fig. 8). The presented results show that an equilibrium forms between the reduced and oxidized species (reaction (7)).

$$Fe^{3+} + NA \rightleftarrows Fe^{2+} + NA^{+} \quad (7)$$

Although a weakly bonded Fe(III)–nicotinamide complex can form with monodendate binding through a single nitrogen atom of the pyridine ring, the peak of Fe(III)–nicotinamide complex is not seen on the voltammograms. For this situation, two comments can be given: first, the complex is electro-inactive and second, the peak of complex fits into that of Fe(III)/Fe(II) reduction.

On the other hand, the observation of Fe(III) under aerobic conditions can be attributed to the reaction between formed Fe(II) species and H_2O_2. As regards the presented data, however, nicotinamide partially prevents the oxidation reaction of Fe(II).

The UV–VIS spectrum of Fe(III)–NA solutions exhibits a new shoulder at 340 nm, which is not present in the Fe(II)–NA spectrum (Fig. 9). The shoulder (340 nm) may be assigned to the charge transfer band [23,24].

3.4. Nicotinamide in the presence of Fe(III) and doxorubicin

When increasing amounts of nicotinamide are added to the cell including Fe(III)–DQ complex at aerobic conditions, the peak current of Fe(III)–doxorubicin complex increases and its peak potential (-0.494 V) shifts to more positive potential (-0.462 V) while the peak current of free doxorubicin decreases (Fig. 10). This potential value (-0.462 V) is different from the reduction potential (-0.494 V) of Fe(III)–doxorubicin complex. The peak at -0.462 V can be probably attributed to the formation of a NA–Fe(III)–DQ pre-association intermediate. Under anaerobic conditions, the disappearance of NA–Fe(III)–DQ intermediate on the voltammogram (Fig. 11) can explain that the intermediate is unstable under anaerobic conditions.

The UV–VIS spectrum of Fe(III)–DQ–NA mixing solution exhibits the absorption bands at 254, 280(sh) and 482 nm (Fig. 12). As can be observed in Fig. 12, the absorption bands at 232 and 580 nm in the Fe(III)–DQ spectrum disappear in the presence of nicotinamide. Also, the electronic spectra of this ternary system do not exhibit the absorption band at 340 nm, attributed to the charge transfer between NA and Fe(III). This case is probably due to the formation of NA–Fe(III)–DQ intermediate.

4. Conclusions

This study demonstrates that doxorubicin generates a cycle of iron with oxidation–reduction reactions. However, doxorubicin binds to ferric ion, yielding a ferric–doxorubicin complex. This complex is more stable in the presence of oxygen. Furthermore, in the presence of nicotinamide, an intermediate product (NA–Fe(III)–DQ) can form. The formation of the NA–Fe(III)–DQ intermediate reveals that nicotinamide may reduce the cardiotoxic effect of the drug and induce its detoxification. It is evident that free radicals play a role in many human diseases. The results of this study should provide a way following the clinical progression of a disease and for devising strategies.

References

[1] C. Yi, M. Gratzl, Continuous in situ electrochemical monitoring of doxorubicin efflux from sensitive and drug-resistant cancer cells, Biophys. J. 75 (1998) 2255–2261.
[2] B.C.L. Cheung, T.H.T. Sun, J.M. Leenhauts, P.L. Cullis, Loading of doxorubicin into liposomas by forming Mn^{2+}–drug complex, Biochim. Biophys. Acta 1414 (1998) 205–216.
[3] R.B. Martin, in: H. Siegel (Ed.), Metal Ions in Biological Systems, vol. 19, Dekker, New York, 1985.
[4] T. Allman, R.E. Lenkinski, Adriamycin complexes of Pd(II) and Pt(II), J. Inorg. Biochem. 30 (1991) 35–43.
[5] J.R. Muindi, B.K. Sinha, L. Gianni, C.E. Myers, Hydroxyl radical production and DNA damage induced by anthracycline-iron complex, FEBS Lett. 172 (1984) 226–230.
[6] F.T. Greenaway, J.C. Dabrowiak, The binding of copper ions to daunomycin and adriamicin, J. Inorg. Biochem. 16 (1982) 91–107.
[7] H. Beralso, A. Garnier-Suillerot, L. Tosi, F. Lavelle, Iron(III)–adriamycin and iron(III)–daunorubicin complexes: physicochemical characteristics, interaction with DNA, and antitumor activity, Biochemistry 24 (1985) 284–289.
[8] N. Sibmooh, R. Udomsangpetch, A. Kijjao, U. Chantharaksri, S. Mankhetkorn, Redox reaction of artemisinin with ferrous and ferric ions in aqueous buffer, Chem. Pharm. Bull. 49 (2001) 1541–1546.
[9] M. Feng, Y. Yang, P. He, Y. Fang, Spectroscopic studies of copper(II) and iron(II) complexes of adriamycin, Spectrochim. Acta A56 (2000) 581–587.
[10] K. Jung, R. Reszka, Mitochondria as subcellular targets for clinically useful anthracyclines, Adv. Drug Deliv. Rev. 49 (2001) 87–105.

[11] S. Çakır, E. Biçer, K. Aoki, E. Coşkun, Structural features of new $[Fe(nicotinamide)_2(H_2O)_4]\cdot[Fe(H_2O)_6]\cdot(SO_4)_2\cdot 2H_2O$ complex, in preparation.
[12] S.C. Mojumdar, I. Ondrejkovicova, L. Nevidanska, M. Melník, Thermoanalytical and IR-spectral investigation of Fe(III) complexes with nicotinamide, J. Anal. Appl. Pyrolysis 64 (2002) 59–69.
[13] G.M. Rao, J.W. Lown, J.A. Plambeck, Electrochemical studies of antitumor antibiotics: III. Daunorubicine and adriamycine, J. Electrochem. Soc. 125 (1978) 534–539.
[14] A.M. Oliveira-Brett, J. Antonio, P. Piedade, A.-M. Chiorcea, Anodic voltammetry and AFM imaging of picomoles of adriamycin adsorbed onto carbon surfaces, J. Electroanal. Chem. 538–539 (2002) 267–276.
[15] L. Gianni, J.L. Zweier, A. Levy, C.E. Myers, Characterization of the cycle of iron-mediated electron transfer from adriamycin to molecular oxygen, J. Biol. Chem. 260 (11) (1985) 6820–6826.
[16] S.S. Massoud, R.B. Jordan, Kinetic and equilibrium studies of the complexation of aqueous iron(III) by daunomycin, quinizarin-2-sulfonate, Inorg. Chem. 30 (1991) 4851–4856.
[17] K.J. Rezska, M.L. McCormick, B.E. Britigan, Peroxidase- and nitrite-dependent metabolism of the anthracycline anticancer agents daunorubicin and doxorubicin, Biochemistry 40 (2001) 15349–15361.
[18] H. Nohl, W. Jordan, $OH^{\cdot}$-generation by adriamycin semiquinone and H_2O_2; an explanation for the cardiotoxicity of anthracycline antibiotics, Biochem. Biophys. Res. Commun. 114 (1983) 197–205.
[19] G.R. Buettner, Activation of oxygen by metal complexes and its relevance to autoxidative processes in living systems, Bioelectrochem. Bioenerg. 18 (1987) 29–36.
[20] E.L. Smith, R.L. Hill, I.R. Lehman, R.J. Lefkowitz, P. Handler, A. White (Eds.), Principles of Biochemistry: General Aspects, 7th Edition, McGraw-Hill, Tokyo, 1983, p. 381.
[21] S. Çakır, İ. Bulut, E. Biçer, E. Coşkun, O. Çakır, Electrochemical study of the interaction of nicotinamide with tryptophan in the presence and absence of nickel(II), J. Electroanal. Chem. 511 (2001) 94–100.
[22] S.-C. Song, C.O. Lee, Y.S. Sohn, Synthesis and antitumor activity of poly(organophosphazene)/doxorubicin conjugates, Bull. Korean Chem. Soc. 20 (1999) 250–252.
[23] A. Vadas, H.G. Monbouquette, E. Johnson, I. Schröder, Identification and characterization of a novel ferric reductase from the hyperthermophilic Archaeon *Archaeoglobus fulgidus*, J. Biol. Chem. 274 (1999) 36715–36721.
[24] E.S. Henle, Y. Luo, W. Gassmann, S. Lin, Oxidative damage to DNA constituents by iron-mediated Fenton reactions—the deoxyguanosine family, J. Biol. Chem. 271 (1996) 21177–21186.