ELSEVIER

Analytica Chimica Acta 385 (1999) 401–408

ANALYTICA CHIMICA ACTA

Electrochemical oxidation of mitoxantrone at a glassy carbon electrode

A.M. Oliveira Brett[a,*], T.R.A. Macedo[b], D. Raimundo[b], M.H. Marques[b], S.H.P. Serrano[c]

[a]*Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3000 Coimbra, Portugal*
[b]*Faculdade de Medicina, Universidade de Coimbra, 3000 Coimbra, Portugal*
[c]*Instituto de Química, Universidade de S. Paulo, CP 26077, 05599-970 S. Paulo, Brazil*

Received 28 July 1998; received in revised form 3 November 1998; accepted 8 November 1998

Abstract

Mitoxantrone is an anthracycline used as an antitumour antibiotic for leukaemia and breast cancer treatment, due to its interaction with DNA. However, the molecular mechanism of the antitumour action is not completely understood. Using a glassy carbon electrode the electrochemical oxidation of mitoxantrone was shown to be a complex, pH-dependent, irreversible electrode process involving several metabolites. Comparison of the electrochemical oxidation behaviour of mitoxantrone, ametantrone and aminantrone enabled a deeper understanding of the mechanism and showed the relevance of electrochemical data for the understanding of the cytotoxicity of mitoxantrone. Since mitoxantrone and its oxidation products adsorb strongly on the electrode surface, causing severe problems of electrode fouling, reproducible electroanalytical determinations could only be done at very low concentrations and in an aqueous buffer supporting electrolyte containing 30% ethanol. The detection limit obtained was 10^{-7} M.

Keywords: Bioelectrochemistry; Mitoxantrone oxidation; Voltammetry

1. Introduction

Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) (MTX) is an aminoanthraquinone, an anthracycline antibiotic, used as an antitumour antibiotic, due to its interaction with DNA [1]. It has a planar heterocyclic ring structure, the positively charged, nitrogen-containing side chains projecting out from the molecule, which stabilize the ring in between base pairs by intercalating with the negatively charged phosphate backbone of DNA [2,3]. Originally synthesized as stable dyes [3–5], anthracenediones are used as antitumour antibiotics for leukaemia and breast cancer treatment. MTX has been shown to induce condensation of nucleic acids but the most dominant molecular mechanism of antitumour action appears to be the induction of long-term DNA damage. Cytotoxic effects, although less than for other anticancer drugs, cause myelosuppression, cardiac toxicity and mucositis as well as other common effects. The mechanism of action of MTX is not completely understood but studies with an electrochemical DNA-biosensor [6], enabled the observation of DNA damage occurring with time which suggests that MTX intercalates with DNA and slowly interacts with it causing some breaking of the hydrogen bonds.

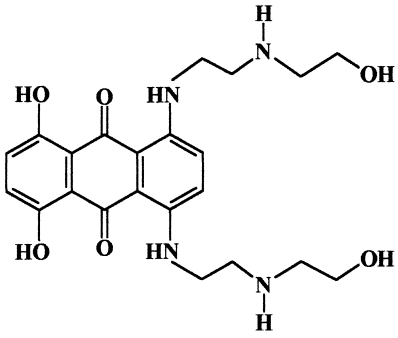
*Corresponding author. Tel.: +351-39-835295; fax: +351-39-835295; e-mail: brett@cygnus.ci.uc.pt

0003-2670/99/$ – see front matter
PII: S0003-2670(98)00807-1

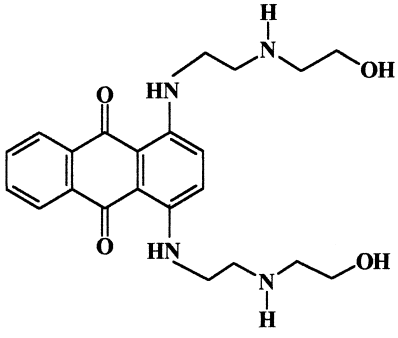
It was demonstrated that MTX is easily oxidized either by horseradish peroxidase producing free radicals that will bond to DNA [7,8] or by a myeloperoxidase [9,10] producing reactive intermediates. The substituents on MTX that are susceptible to oxidation are the hydroxyl and amino groups on the aromatic rings. The 5,8-hydroxyl substituents on the MTX anthracenedione chromophore are available to interact with the double helix [11]. Experiments with two anthraquinone derivatives structurally related to mitoxantrone, ametantrone and aminantrone [12–14], have been undertaken to assess the influence of the hydroxyl groups on the oxidation process. Both these compounds lack the hydroxy substituents at positions 5 and 8 and differ by the presence or absence of hydroxyethyl groups on the aminoalkyl side chains (see Scheme 1).

The electrochemical reduction and oxidation of MTX have been studied but the mechanism was not fully established and detection limits at the nonmolar level were achieved using carbon paste electrodes only when a preconcentration time of 4 min was used and the electrode was polished between each measurement [12,15,16].

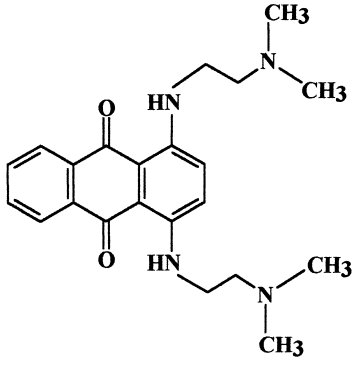
In this paper the electrochemical oxidation mechanism of MTX will be discussed and clarified by also studying the electrochemical oxidation of AMET and AMIN-1. It is hoped that this investigation may contribute to a better understanding of the cytotoxicity of MTX, which is also electroanalytically quantified in aqueous solution, without preconcentration, using a glassy carbon electrode.



Mitoxantrone (MTX)



Ametantrone (AMET)



Aminantrone-1 (AMIN-1)

Scheme 1.

2. Experimental

Mitoxantrone (MTX) (dihydroxyanthracenedione) was a synthetic sample solution of Novantrone® (mitoxantrone hydrochloride) that was supplied by Lederle Laboratories, Gosport, UK. The MTX sample stock solution of 2 mg ml^{-1} (4.5×10^{-3} M) was kept away from light to avoid photodecomposition and at a temperature of ~3°C. Solutions of 10^{-4} M MTX were always prepared just before the experiments from the sterilized stock solution. Solutions of 10^{-4} M ametantrone (AMET) and aminantrone (AMIN-1) were prepared from the stock solution just before the experiments.

The universal 0.3 M ionic strength buffer was prepared by mixing 0.8 M NaOH, 1.34 M KCl and an acid solution (0.16 M acetic acid, 0.16 M phosphoric acid and 0.16 M boric acid). All reagents were analytical grade and aqueous solutions were prepared using purified water from a Millipore Milli-Q system (con-

ductivity (0.1 μs cm^{-1}). The pH measurements were obtained with a Crison 2001 pH-meter with a combined glass electrode. All experiments were done at room temperature.

Voltammograms were recorded using a μAutolab potentiostat/galvanostat running with GPES version 3 software, from Eco-Chemie, Utrecht, Netherlands. A three-electrode cell with a glassy carbon working disc

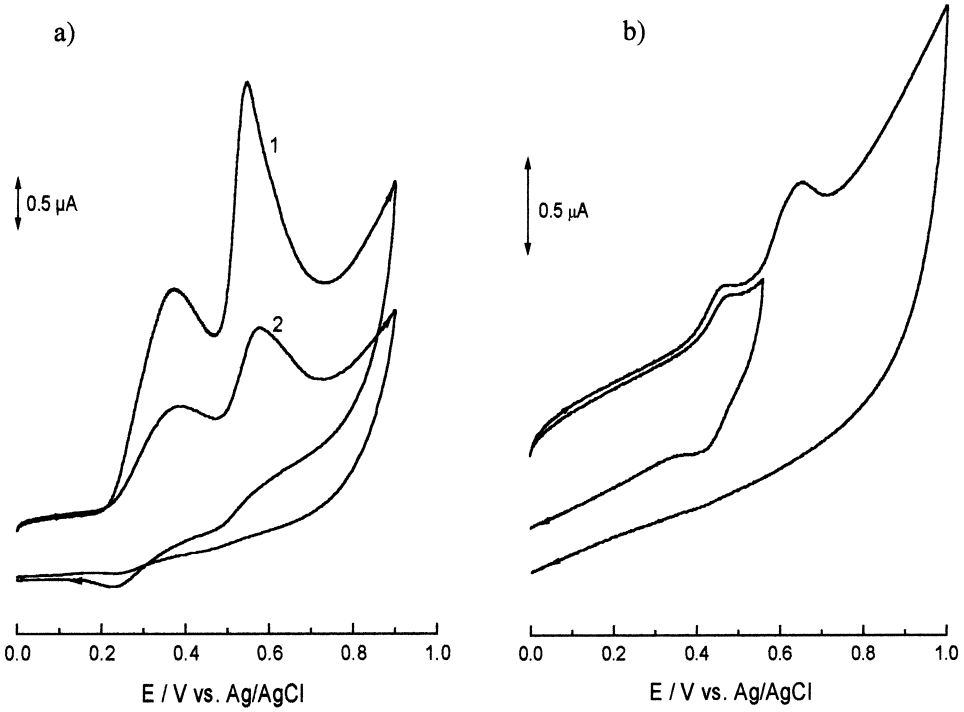


Fig. 1. Cyclic voltammograms for MTX in 0.3 M universal buffer in: (a) pH 8.09: 1×10^{-4} M and 2–2.25×10^{-5} M; (b) pH 7.25: 1×10^{-5} M. Scan rate 200 mV/s.

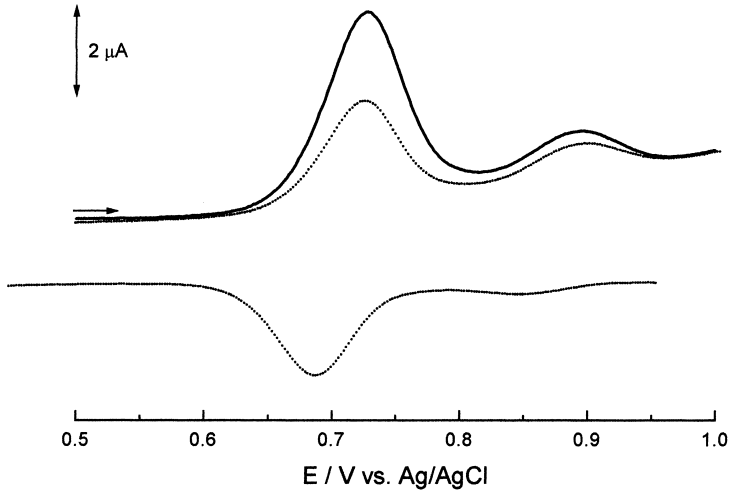


Fig. 2. Square wave voltammograms of MTX, 1×10^{-5} M in pH 1.13, 0.1 M perchloric acid: (———) net current and (. . .) I_f–I_b. Frequency 100 Hz.

electrode (1 mm diameter, Cypress Systems, Lawrence, Kansas, USA) together with an Ag/AgCl (3.0 M KCl) reference electrode, and a Pt-wire counter electrode were employed. The glassy carbon working electrode was polished using diamond spray (3 μm). After polishing, the electrode surface was thoroughly washed with purified water. All electrodes were contained in a one-compartment cell. Differential pulse voltammetry conditions were: pulse amplitude 100 mV, pulse width 70 ms and scan rate 5 mV s^{-1}. Square wave voltammetry conditions were: frequency 100 Hz, pulse amplitude 50 mV and scan increment 2 mV, corresponding to an effective scan rate of 200 mV s^{-1}.

3. Results and discussion

The electrochemical oxidation mechanism of MTX is a multistep process involving generation of free radicals and structural rearrangements as both the hydroxyl and the amino groups substituents on the aromatic rings of MTX are susceptible to oxidation (Figs. 1 and 2). MTX is a weak base with pK_{a1}=5.99 [17] and pK_{a2}=8.13 [18,19].

Cyclic voltammograms of MTX, Fig. 1, showed that the complex electrochemical oxidation at a glassy carbon electrode is an irreversible electrode process. The first oxidation peak corresponds to the oxidation of the hydroxyl substituents at positions 5 and 8 and the second peak to the oxidation on the aminoalkyl side chains after a tautomeric structural rearrangement [15]. This was further investigated using differential pulse voltammetry and square wave voltammetry. In fact the first oxidation step is reversible, which is easily confirmed if the scan is stopped at a E=+0.57 V just before the beginning of the second oxidation peak (Fig. 1(b)) and has also previously been observed [15]. Square wave voltammograms (Fig. 2) show the net current and the difference between the forward and backward currents; the reversibility of the first oxidation step is very clearly demonstrated in pH 1.13, 0.1 M perchloric acid, supporting electrolyte.

The electrochemical oxidation of MTX was also studied using square wave voltammetry for a wide range of pH (1.1–12.8), and it followed a two electron two proton transfer pathway over all the pH range studied (Fig. 3(a)). The peak currents diminished with

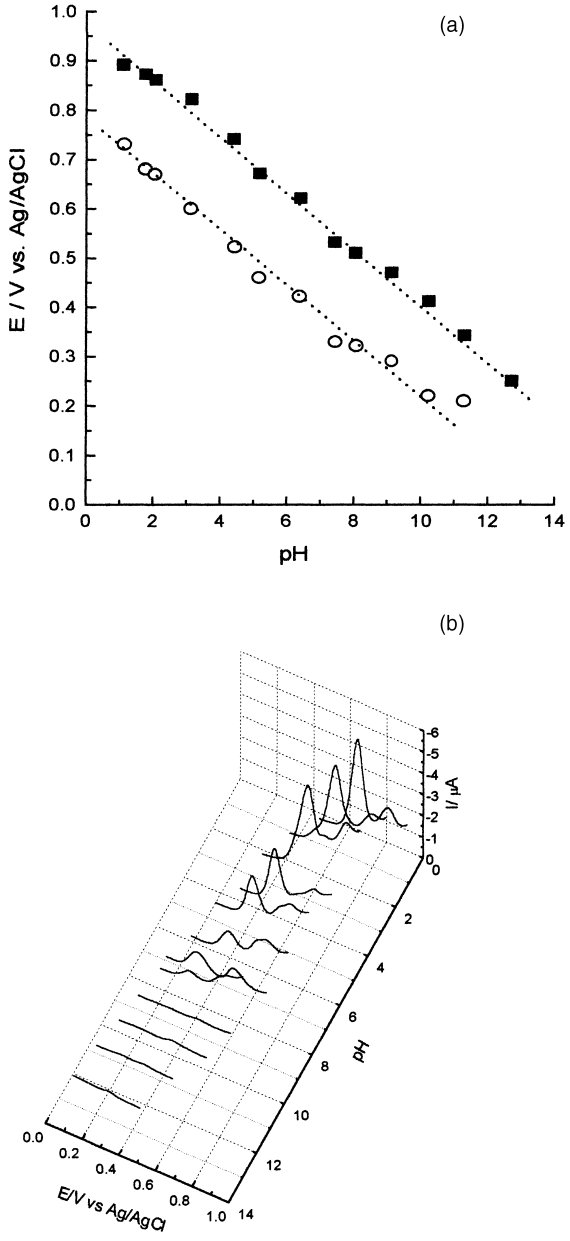


Fig. 3. (a) Plot of E_p vs pH for 10^{-5} M solutions of MTX in 0.3 M universal buffer electrolyte. Dotted line corresponds to a slope of 59 mV per unit of pH. Frequency 100 Hz. (b) 3D plot of square wave voltammograms as a function of pH.

increasing pH and the peaks almost disappeared for pH values higher than 9 (Fig. 3(b)).

In order to clarify the electrochemical mechanism and to assess the influence of the hydroxyl groups on

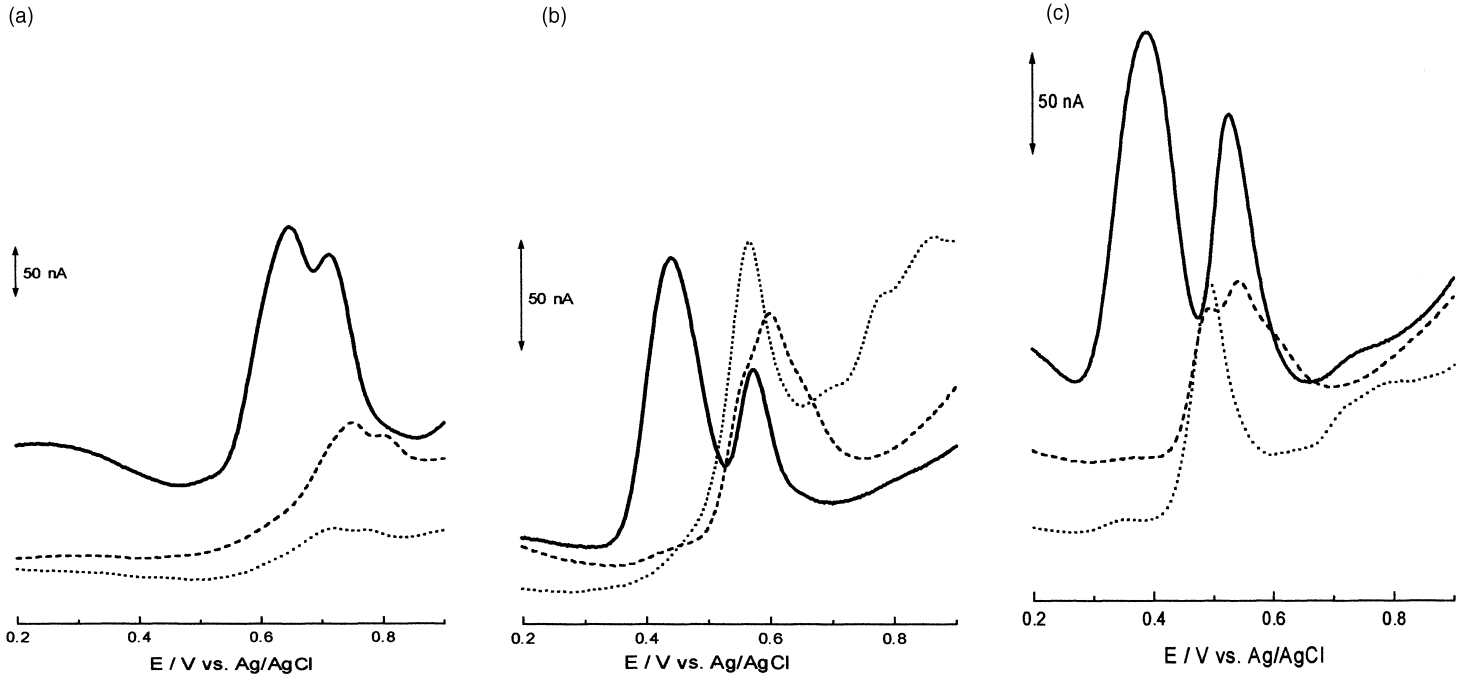
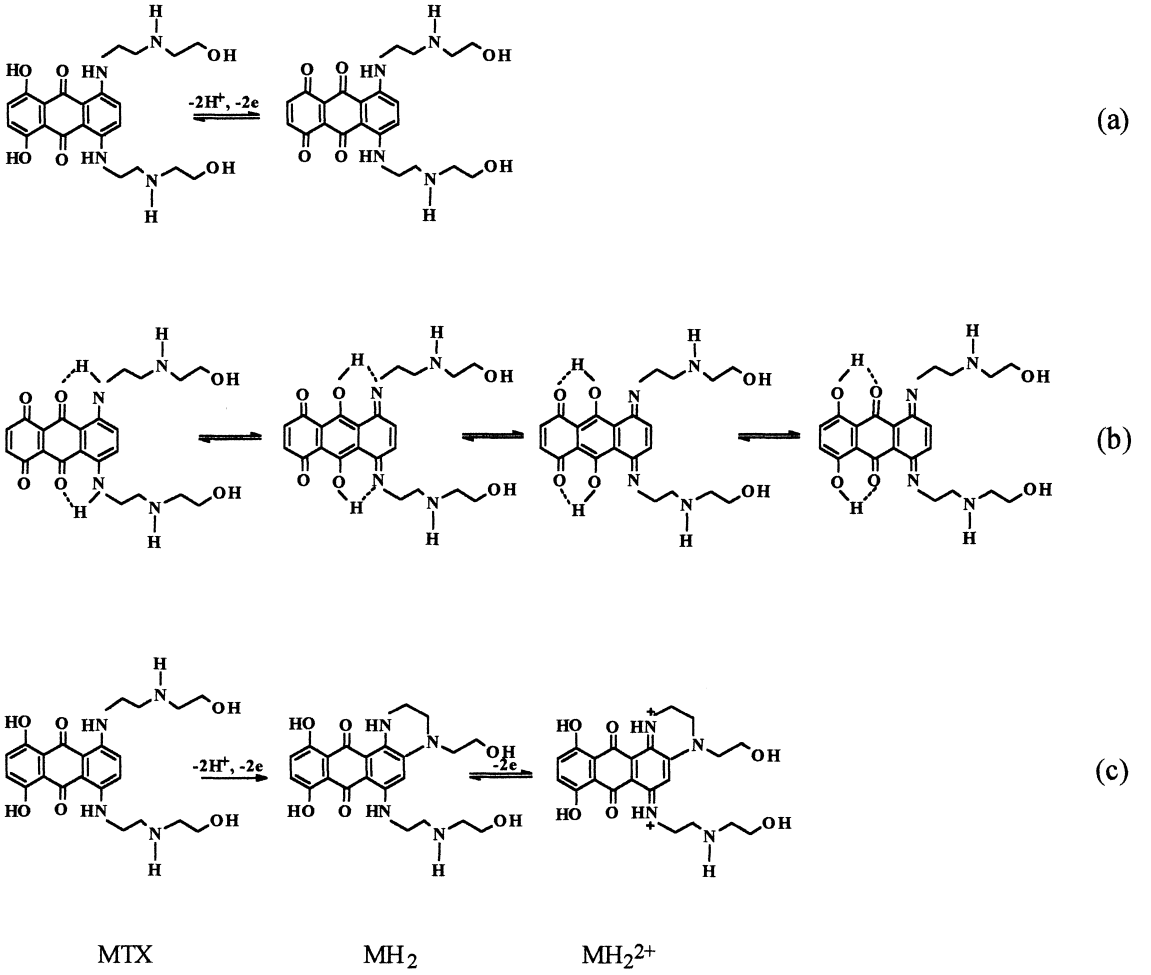


Fig. 4. Differential pulse voltammograms in 0.3 M universal buffer electrolyte of 1×10^{-5} M of MTX (———), AMET (. . .) and AMIN-1 (- - -): (a) pH 1.8; (b) pH 4.7 and (c) pH 7.3. Frequency 100 Hz.

the oxidation process, the electrochemical oxidation of MTX was compared with the electrochemical oxidation, at the same glassy carbon electrode, of the two anthraquinone derivatives structurally related to MTX: AMET and AMIN-1 and for pH values of 1.8, 4.7 and 7.3 (Fig. 4(a), (b) and (c)). These both compounds lack the 5,8-hydroxyl substituents on the MTX and AMIN-1 also has no hydroxyl substituents on the side chain. The oxidation of AMET and AMIN-1 can occur only at the aminoalkyl substituent. This is at a more positive potential than the first oxidation peak of MTX, which corresponds to the oxidation of the 5,8-hydroxyl substituents, and at a similar potential to the second oxidation peak of MTX, corresponding to the oxidation of the aminoalkyl substituents.

As a resulting of these comparative studies, the detailed mechanism proposed for the electrochemical oxidation of MTX can be described by the reactions given in Scheme 2.

The oxidation of 5,8-hydroxyl substituents on MTX (reaction (a)) corresponding to the first reversible oxidation peak of the voltammograms occurs at less positive potentials. This peak does not occur for AMET and AMIN-1 which is in agreement with their molecular structure not having 5,8-hydroxyl substituents. A tautomeric equilibrium (reaction (b)) was proposed [18] to explain the formation of a tautomer, where the intramolecular addition of the side chain nitrogen was favoured, and which corresponds to the second oxidation peak. After the tautomeric structural



Scheme 2.

rearrangements, oxidation of the aminoalkyl substituent in MTX (reaction (c)) yields the stable metabolite cyclic hexahydronaphthol-[2,3-f]-quinoxaline-7,12-dione (MH_2) and the reaction proceeds via formation of free radical species from $MH_2^{\bullet}$ leading to further conversion to an unstable, fully oxidized diimino compound (MH_2^{2+}), which shows electrophilic character [18,19] and has been structurally identified [20,21]. So far 10 metabolites of the MTX oxidation have been identified but the structures of many of them are still unknown. Fig. 4 shows that the oxidation of the aminoalkyl substituents in AMET and AMIN-1 is a multistep process with the kinetics of the electron transfer mechanism being pH-dependent and with the possible formation of intermediate radicals and dimerization products.

The quantitative analysis of MTX is complicated because of the instability of this drug in plasma at room temperature, nonspecific binding of mitoxantrone to proteins, and (in aqueous solutions) adsorption onto many materials, including filters and glass surfaces. Its oxidation and reduction products also adsorb strongly on the electrode surface causing severe problems of electrode fouling [12,15,16].

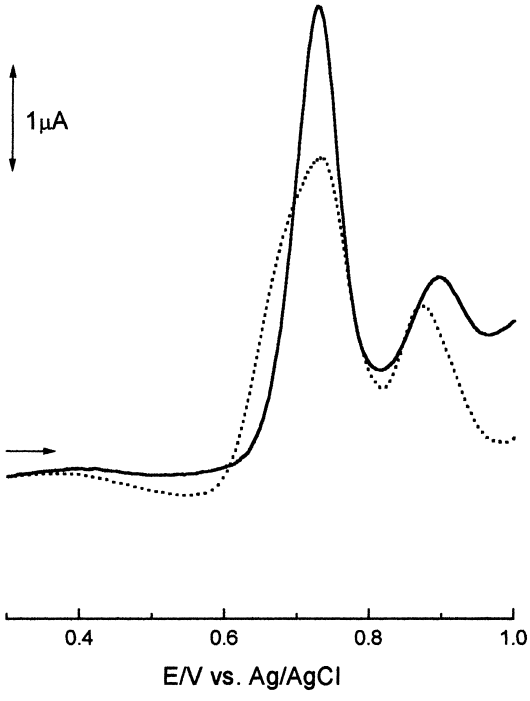


Fig. 5. Square wave voltammograms of MTX, 10^{-5} M in pH 1.13, 0.1 M perchloric acid, (———) with 30% ethanol and (. . .) without ethanol. Frequency 100 Hz.

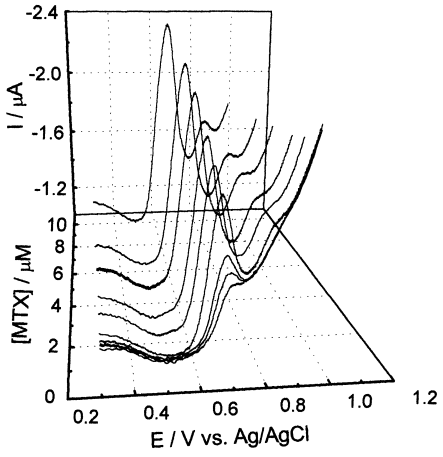


Fig. 6. Sucessive square wave voltammograms in 0.3 M universal buffer electrolyte pH 2.03 of MTX with 30% ethanol: (1) 0.19; (2) 0.39; (3) 0.57; (4) 0.99; (5) 1.88; (6) 2.76; (7) 4.45; (8) 6.12; (9) 10 μM. Frequency 100 Hz.

In fact, MTX adsorbs very strongly on the electrode surface as soon as it is immersed in the MTX/supporting electrolyte solution, and in order to minimize this effect, 30% ethanol was added to the supporting electrolyte which causes a much slower adsorption of the drug on the electrode surface but does not change the electrochemical reaction. This effect of increasing peak height and decreasing peak width in square wave voltammograms, is shown in Fig. 5.

Electroanalytical quantification of MTX was done in this mixed supporting electrolyte using square wave voltammetry in the range of 10^{-7}–10^{-5} M (Fig. 6). It was not necessary to clean the electrode between experiments. From the square wave voltammograms of the standards, a straight line calibration plot was obtained between 10^{-7}M and 10^{-6} M, described by the equation $I(\mu A)=0.546$ [MTX]/μM-0.00479 ($r=0.996$, $n=6$), for MTX and the detection limit was 10^{-7} M, based on three times the noise level.

4. Conclusions

The electrochemical oxidation of mitoxantrone is a complex irreversible electrode process that is pH-dependent and involves several metabolites. Compar-

ison of the electrochemical oxidation behaviour of mitoxantrone, ametantrone and aminantrone enables a deeper understanding of the mechanism and shows the relevance of the electrochemical data for the understanding of the cytotoxicity of MTX.

Both mitoxantrone and its oxidation products adsorb strongly on the electrode surface causing severe problems of electrode fouling. Electroanalytical determinations could be done only for very low concentrations and in an aqueous buffer supporting electrolyte containing 30% ethanol. The detection limit was 10^{-7} M.

Acknowledgements

Ametantrone (AMET) and aminantrone (AMIN-1) were kindly supplied by Dr. P.L. Gutierrez, University of Maryland Cancer Center, Division of Developmental Therapeutics and Department of Biological Chemistry, Baltimore, USA, and prepared by Dr. L.H. Patterson, Department of Pharmacy, School of Applied Sciences, DeMontfort University, Leicester, UK.

References

[1] C.E. Riggs, in: M.C. Perry (Ed.), The Chemotherapy Source Book, Williams and Wilkins, 1992, Maryland, USA, p. 318.
[2] K.Y. Millery, D.D. Newton, Biopolymers 21 (1982) 633.
[3] K.C. Murdock, R.G. Child, P.F. Fabio, R.B. Angier, R.E. Wallace, F.E. Durr, R.V. Citarella, J. Med. Chem. 22 (1979) 1024.
[4] R.K.-Y. Zee-Cheng, C.C. Cheng, J. Med. Chem. 21 (1978) 291.
[5] I.E. Smith, Cancer Treat. Rev. 10 (1983) 103.
[6] A.M. Oliveira Brett, T.R.A. Macedo, D. Raimundo, M.H. Marquese, S.H.P. Serrano, Biosensors and Bioelectronics, 13 (1998) 861.
[7] K. Reszka, J.A. Hartley, P. Kolodziejczyk, J.W. Lown, Biochem. Pharmacol. 38 (1989) 4253.
[8] G.R. Fisher, L.H. Patterson, J. Pharm. Pharmacol. 43 (1991) 65.
[9] C. Panousis, A.J. Kettle, D.R. Phillips, Biochem. Pharmacol. 48 (1994) 2223.
[10] C. Panousis, A.J. Kettle, D.R. Phillips, Anti-Cancer Drug Des. 10 (1995) 593.
[11] C. Bailly, S. Routier, J.L. Bernier, M.J. Waring, Febs Lett. 379 (1996) 269.
[12] B. Nguyen, P.L. Gutierrez, Chem. Biol. Interactions 74 (1990) 139.
[13] R.K. Johnson, R.K.-Y. Zee-Cheng, W.W. Lee, E.M. Acton, D.W. Henry, C.C. Cheng, Cancer Treat. Rep. 63 (1979) 425.
[14] J.W. Lown, A.R. Morgan, S.-F. Yen, Y.-H. Wang, W.D. Wilson, Biochemistry 24 (1985) 4028.
[15] J.C.C. Villar, A.C. Garcia, P.T. Blanco, J. Pharm. Biomed. Anal. 10 (1992) 263.
[16] J. Zimák, M. Zimáková, J. Volke, Pharmazie 46 (1991) H8.
[17] V. Vukovic, I.F. Tannock, Br. J. Cancer 75 (1997) 1167.
[18] K. Mewes, J. Blanz, G. Ehninger, R. Gebhardt, K.-P. Zeller, Cancer Res. 53 (1993) 5135.
[19] A.M.J.A. Duchateau, Pharm. Weekbl. 122 (1987) 286.
[20] P. Kolodziejczyk, K. Reszka, J.W. Lown, Free Rad. Biol. Med. 5 (1988) 13.
[21] J. Blanz, K. Mewes, G. Ehninger, B. Proksch, D. Waidelich, B. Greger, K.-P. Zeller, Drug Metabol. and Dispos. 19 (1991) 871.