SPECTROELECTROCHEMICAL STUDY OF THE REDOX BEHAVIOUR OF QUESTIOMYCIN DRUGS IN APROTIC MEDIA

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The electrochemical reduction of questiomycin A (2-aminophenoxazin-3-one) has been studied by cyclic voltammetry, with stationary and rotating (Pt) electrode (RDE), coupled with UV-VIS absorption spectroscopy using *in situ* techniques. The cyclic voltammetry in dimethylsulfoxide evidences two successive monoelectronic transfers (ETs), assigned to the formation of an anion radical and, respectively, to a diamagnetic dianion. Extending the oxidation domain, on second and successive scans new redox couples appear in the range 0.3–0.8V, proving the oxidation of the products of a chemical step, occurring after the second ET. Analysis of the electrochemical data with the DigiSim 3.03 Software shows as most probable an ECEC mechanism for the reduction of 2-aminophenoxazone in aprotic and basic media. In order to evidence the intermediate species in the electrochemical reduction of 2-aminophenoxazin-3-one, the reverse process, that is the electrochemical oxidation of 2-aminophenol (questiomycin B), has also been studied. The results point out the formation of a reactive cation radical, followed by a rapid dimerization, leading finally to either 2-aminophenoxazone, or an electroactive polymer containing aminophenoxazine units. The semiempirical MO calculations allow a rationalisation of the experimental data, in terms of the electronic structure of the starting compounds and of different intermediates.

INTRODUCTION

Phenazines are biologically active aromatic natural products synthetized mainly by *Pseudomonas* and *Streptomyces* species.[1] For the culture of a Streptomyces species two tubercular antibiotics were obtained and named questiomycin A and B.[2, 3] The chemical structure was determined as 2-aminophenoxazin-3-one and, respectively, 2-aminophenol. Though phenoxazines are found in actinomycins, in various insect pigments and in some microorganism metabolites,[4] their biological effects remain obscure, except for actinomycin D, which shows strong anti-cancer effects by inhibiting DNA dependent RNA polymerase. Thus, it is conceivable that the phenoxazine compounds may act as anticancer drugs,[5] like actinomycin D. It has been also shown that 2-aminophenol may cause the reduction of methemoglobin, producing 2-aminophenoxazine-3-one, which has antitumor activity.[6]

For actinomycin D, several studies were dedicated to the interaction with DNA[7, 8] and to the electrochemical reduction in aprotic or protic media, in the absence or presence of oxygen.[9] This work evidenced the reducibility of 2-aminophenoxazin-3-one and the possibility of the intermediate reduction species to activate the molecular oxygen by electron transfer (ET). The biological implications of these processes were also discussed, in terms of semiempirical MO calculations.

In order to identify the intermediate species and for a better understanding of the role of the aminophenoxazone moiety in redox processes in various media, the behaviour of questiomycin A (Fig.1) in reduction processes in aprotic (dimethyl sulphoxide, DMSO) and basic media is investigated in the present work by coupling electrochemical and spectral techniques. As literature data[10-13] report the conversion of 2-aminophenol to polymers containing 2-aminophenoxazin-3-one units by different ways, the electrooxidation of 2-aminophenol in similar media is also investigated. The results are rationalised in terms of semiempirical MO calculations.
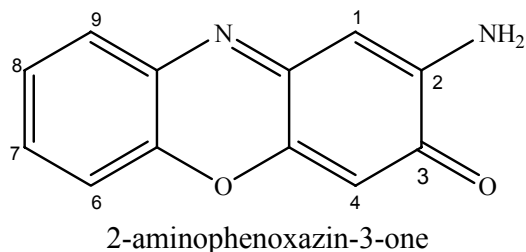
2-aminophenoxazin-3-one

Fig. 1 – The chemical structure of questiomycin A (2-aminophenoxazin-3-one).

RESULTS AND DISCUSSION

A. Cyclic voltammetry

The cyclic voltammograms of 2-aminophenoxazin-3-one in deaerated DMSO solution in the potential range $0.1 \div -1.6$ V (Fig. 2a) present two redox couples that were analysed separately. Both redox couples correspond to diffusion waves, as attested by the linear plot of the cathodic peak current (i_{pc}) *vs* the square scan rate ($v^{1/2}$) (r = 0.995, n = 6; and respectively r = 0.989, n = 6 for the first and second couples; r is the correlation coefficient and n is the number of experimental points). The first process is characterized by a well-shaped anodic counterpart and corresponds to a reversible (fast) electrochemical process. The relevant electrochemical data are presented in Table 1. The ratio of the peak currents i_{pa}/i_{pc} is smaller than unity but increases with the sweep rate, that presumes that the anion radical resulted from the first electron transfer is partly consumed in a rather slow chemical reaction. An EC-type process is indicated also by the slight decrease of the current function $i_{pc}/v^{1/2}$ with the sweep rate.
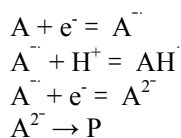
Table 1

Cyclic voltammetry parameters for the first reduction wave of 2-amino-phenoxazin-3-one (2×10^{-3}M) in DMSO, under anaerobic conditions

v (V/s)	$-E_{pc}$ (V)	$-E_{pa}$ (V)	$-\Delta E_p$ (V)	i_{pa}/i_{pc}	$i_{pc}/v^{1/2} \times 10^4$ (mA·s$^{1/2}$/V$^{1/2}$)
0.1	0.759	0.654	0.105	0.733	0.142
0.2	0.759	0.635	0.124	0.763	0.124
0.3	0.756	0.626	0.130	0.817	0.126
0.4	0.753	0.626	0.133	0.845	0.127
0.5	0.753	0.613	0.140	0.857	0.120
0.6	0.756	0.607	0.149	0.867	0.118

The second electron transfer is obviously a slower process. From the linear dependence of the peak potential on the sweep rate: $E_{pc} = f (\log v)$ (r = 0.991, n = 6), a value of the transfer coefficient $\alpha = 0.47$ was obtained. This process was assigned to the reduction of the anion radical to the dianion.

The results obtained using the rotating disc electrode, presented in Fig. 2(a) as inset for the first wave, confirm that both reduction steps are monoelectronic, as determined from the plots $i = f (\omega^{1/2})$ (r = 0.991; n = 10), where ω is the rotating angular rate, and $E = f(\ln(i_l-i)/i)$, (r = 0.985; n = 9), where i_l is the limiting current.

The simulation of the voltammogram with the DigiSim Program using the default parameters values ($\alpha = 0.5$ and the diffusion coefficient $D = 10^{-5}$cm^{2}s^{-1}) is presented in Fig. 2b. The use of the default options is justified by the preliminary analysis of the experimental data according to the data in Table 1 ($\alpha = 0.47$, $D = 2.52 \times 10^{-5}$cm^{2}s^{-1}). A good agreement with the experimental results in the range $0 \div -1.6$ V is obtained for an ECEC mechanism.

$$A + e^- = A^{\cdot -} \qquad\qquad E^0_1 = -0.705V \qquad\qquad k_s = 0.5 cm/s$$
$$A^{\cdot -} + H^+ = AH^{\cdot} \qquad\qquad\qquad\qquad\qquad k_f = 0.1 M^{-1}s^{-1}; K_{eq} = 10 \ M^{-1}$$
$$A^{\cdot -} + e^- = A^{2-} \qquad\qquad E^0_2 = -1.2V \qquad\qquad k_s = 5 \times 10^{-3} cm/s$$
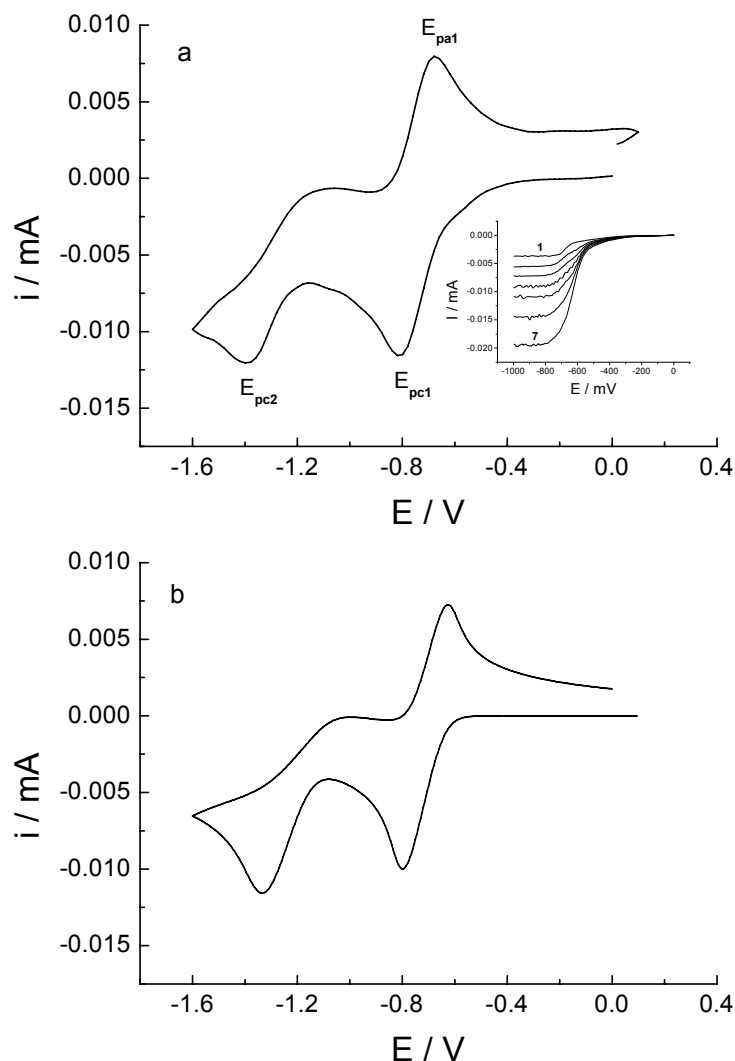$$A^{2-} \rightarrow P \qquad\qquad\qquad\qquad\qquad\qquad k_f = 100 \ s^{-1}; K_{eq} = 15$$

Fig. 2 – (a) Cyclic voltammogram of 2-aminophenoxazin-3-one (2×10^{-3}M) in the potential range $0.1\div-1.6$V in dearerated DMSO, at a 0.4V/s sweep rate; inset − family of curves for the first reduction wave of 2-aminophenoxazin-3-one (2×10^{-3}M) with rotating (Pt) electrode (RDE): 1-7, $\omega = 100$-1000 rpm; (b) Simulation of the cyclic voltammogram with the Digisim Program.

The data referred to for the above reactions are: the standard electrode potential (E^0), the standard electrochemical rate constant (k_s), rate constant of the forward reaction (k_f), and the equilibrium constant (K_{eq}).

The first process is a rapid electron transfer, followed by a slow chemical reaction, probably the protonation by the protic impurities in the solvent, estimated at a concentration 10^{-4}M for the commercial DMSO used without further purification. The second ET corresponds to a quasireversible process also followed by a chemical reaction.

Extending the domain to oxidation potentials (Fig. 3), on second and successive scans, a new redox couple appears at potential values of $0 \div 0.5$ V with the reduction peak of 2-aminophenoxazin-3-one shifted towards less negative potentials (from –0.774 to –0.649 V) and decrease in intensity. Also, after the first scan, the second reduction wave of 2-aminophenoxazin-3-one disappears. This is an indication that the substrate is consumed in the chemical step following the second ET. A possible reaction which can occur under the influence of the electro generated bases (EGB) is a cleavage of the phenoxazone ring, leading to products which can be oxidized on the anodic scan.

However, because of the limitations of the DigiSim Program, these processes could not be included in the mechanism and therefore the simulation is limited to the potential range of $0 \div -1.6$ V.
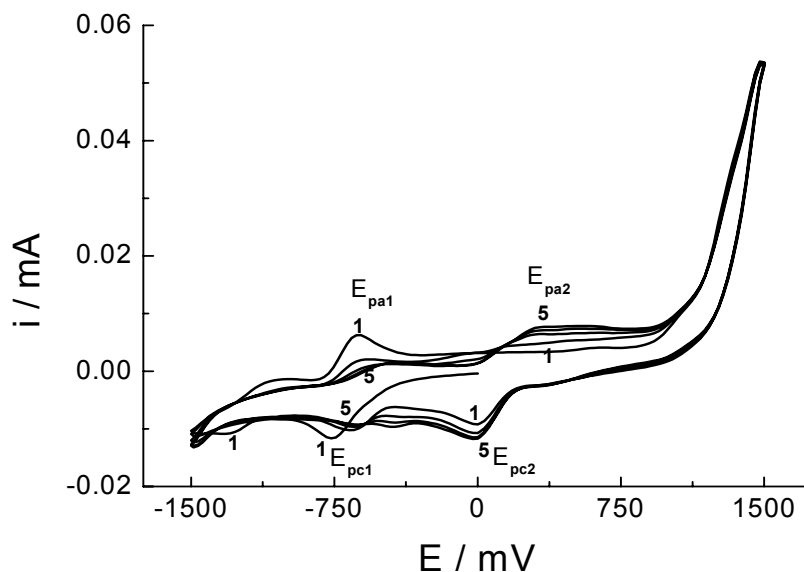

Fig. 3 – Cyclic voltammograms of 2-aminophenoxazin-3-one (c=3.33×10⁻³M) in DMSO in the potential range −1,5÷+1,5V; the first five successive scans.

The reduction of 2-aminophenoxazin-3-one in basic media was also investigated (Fig. 4). Cyclic voltammetry in the presence of added tetra butyl ammonium hydroxide (TBAOH) ($C_{TBOH}/C_{substrate}$ =2:1 molar ratio) shows a similar pattern to that observed in Fig. 3 on the first reduction scan, but on the oxidation scan, new irreversible waves are observed, consistent with the cleavage of the phenoxazone ring, already signaled in the literature for strong basic media.[7] In order to identify the final cleavage product, presumably 2-aminophenol, *i.e.*, the reverse process, the electrochemical oxidation of 2-aminophenol was also investigated in similar experimental conditions.
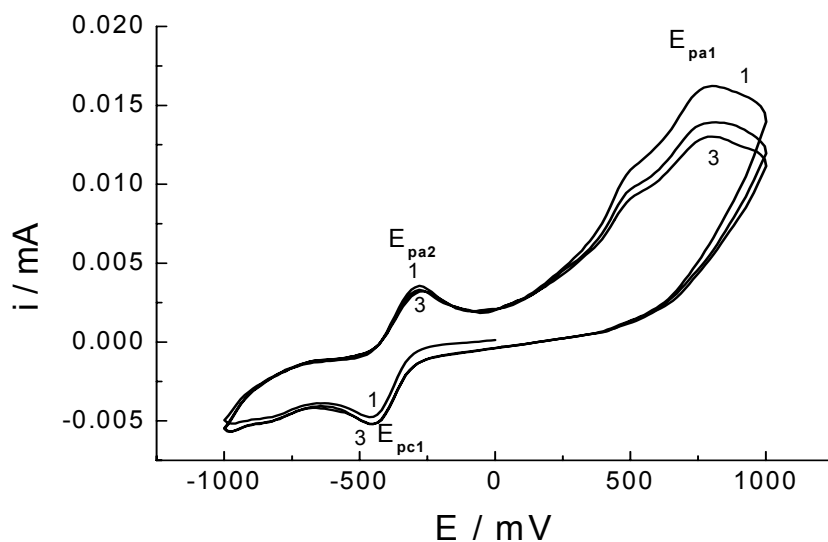

Fig. 4 – Cycling of the voltammogram of 2-aminophenoxazin-3-one (2×10⁻³M) in the potential range -1÷+1V in dearerated DMSO with TBAOH added (1:2 molar ratio); 1-3, three successive scans.

The electrochemical oxidation of 2-aminophenol in deaerated DMSO is illustrated in Fig. 5a. The i/E curves recorded towards negative potentials show no reduction waves on the first scan, and an irreversible oxidation wave at E = 0.843 V. On second and successive scans two new redox couples appear in the same potential range of the reduction couple of 2-aminophenoxazin-3-one, thus the cyclic voltammogram becoming practically identical to that in Fig. 3, excepting the oxidation peak at 0.843V.
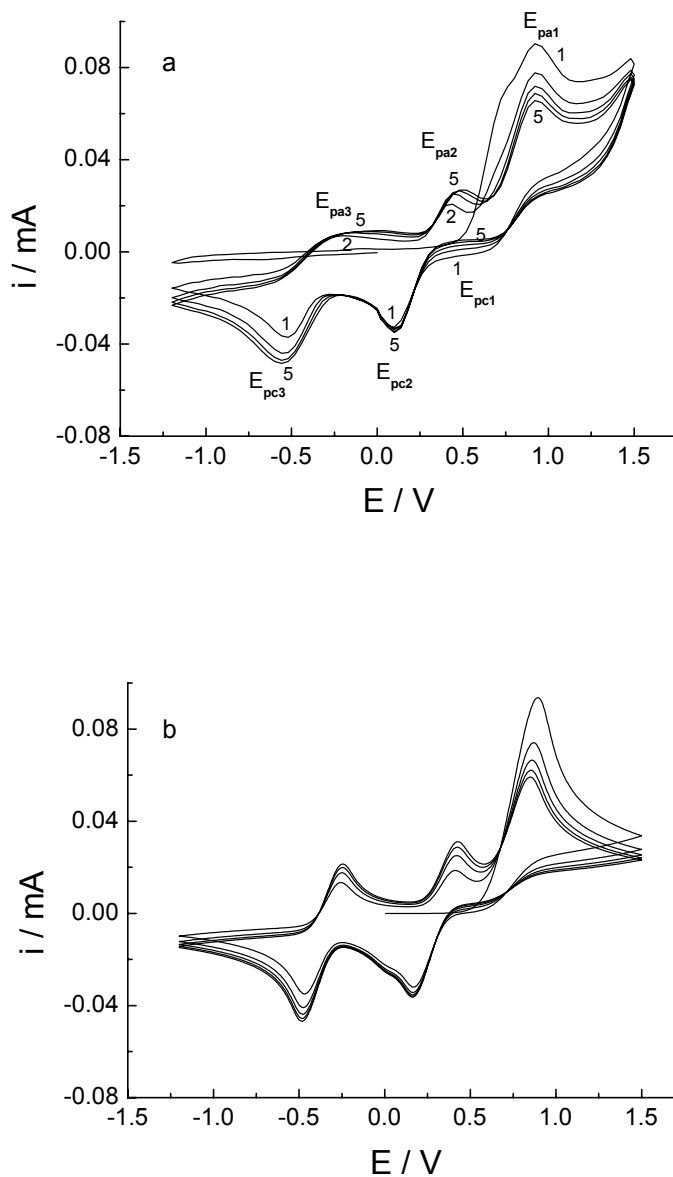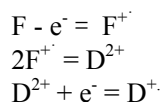

Fig. 5 – Cyclic voltammograms of 2-aminophenol (8×10^{-3}M) in the potential range $-1.5 \div 1.5$V, the first five successive scans: (a) experimental; (b) simulation with Digisim Program.

The simulation of the first five cycles with the DigiSim Programme (Fig. 5b) leads to a good agreement with the experimental data with an ECEEC mechanism:

$$F - e^- = F^{+\cdot} \qquad E^0_1 = 0.40 \text{ V} \qquad k_s = 5\times10^{-5}\text{cm/s}$$
$$2F^{+\cdot} = D^{2+} \qquad\qquad\qquad k_f = 1000\text{s}^{-1}\text{M}^{-1}; K_{eq}=10^4\text{M}^{-1}$$
$$D^{2+} + e^- = D^{+\cdot} \qquad E^0_2 = 0.30 \text{ V} \qquad k_s = 3\times10^{-3}\text{cm/s}$$
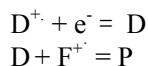
$$D^{+\cdot} + e^- = D \qquad\qquad E^0_3 = -0.35 \text{ V} \qquad\qquad k_s = 4\times10^{-3}\text{cm/s}$$
$$D + F^{+\cdot} = P \qquad\qquad\qquad\qquad\qquad\qquad k_f = 10^6 \text{ s}^{-1}\text{M}^{-1}; K_{eq}=10 \text{ M}^{-1}$$

The first step, that is the oxidation of 2-aminophenol to the corresponding cation radical, is slow and is followed by a fast dimerisation of the radical species leading to an intermediate dimeric cation (D^{2+}) that can be reduced in the next successive ET steps to the cyclic dimer (Scheme 1a). This dimer can either be oxidised to 2-aminophenoxazone as reported for metabolic processes,[15] or polymerise to an electroactive polymer containing aminophenoxazine units (P), according to the last reaction, as literature data have recently pointed out.[12]
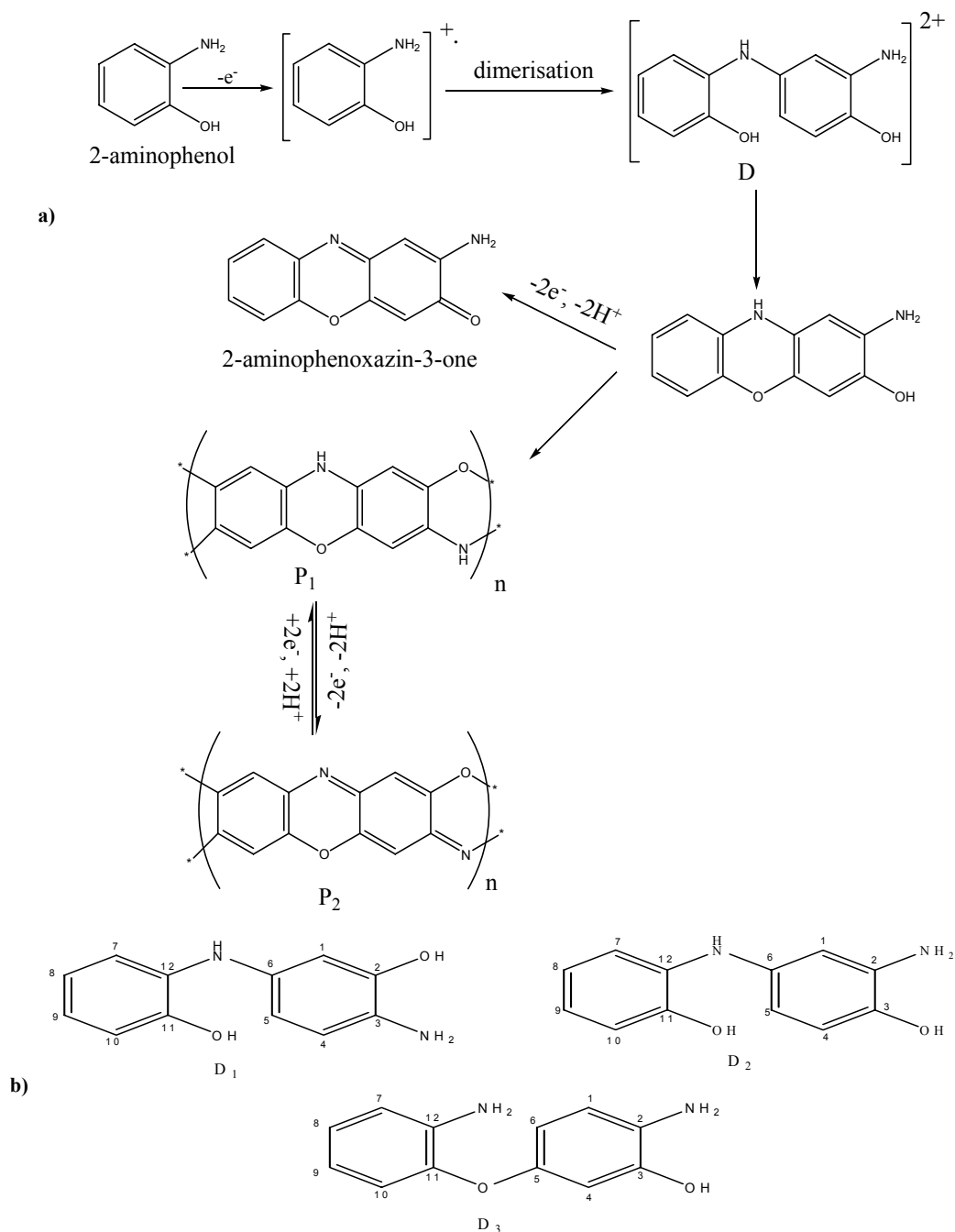
Scheme 1 – (a) Proposed mechanism for the electrochemical oxidation of 2-aminophenol;
(b) structures and numbering used in the MO calculations for the open dimmers.

The support for this mechanism is given by the similitude of the cyclic voltammogram obtained at the reduction of 2-aminophenoxazone (in Fig. 3) after cycling, with that in Fig. 5 obtained at the oxidation of 2-aminophenol. Further support is given by the optical spectra recorded during the electrochemical oxidation of 2-aminophenol, discussed below.

In alkaline media (Fig. 6), (C_{TBOH} / $C_{substrate}$= 2:1 molar ratio), a new oxidation wave at E = 0.38 V appears, which decreases in intensity on cycling, and which was assigned to the oxidation of the phenolate anions (existing in solution in basic media) to phenoxy radicals, followed by oxidation and electropolymerization reactions. In this last process, as shown by literature data,[13] the polymerisation involves the –OH group by the formation of C-O-C bond. The obtained insulating polymers have potential application in the protection against corrosion.
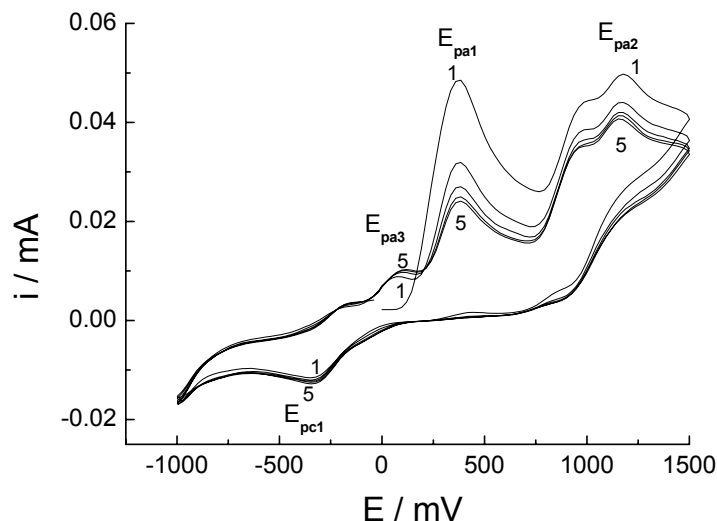

Fig. 6 – Cycling of the voltammogram of 2-aminophenol (8×10^{-3}M) in the potential range $-1.5 \div 1.5$V, in dearated DMSO with TBAOH added (1:2 molar ratio); 1-5, five successive scans

B. Optical spectra

In order to identify the intermediate species in the reduction mechanism of 2-aminophenoxazin-3-one, the absorption spectra were performed during the electrochemical reduction (Fig. 7a). The absorption band of the compound located at 416 – 454 nm decreases in time and two new absorption regions at 625nm and, respectively, ~330 nm are apparent. Two isobestic points at 385 nm and at 534 nm are also observed. As the ratio of intensities of the two bands varies in time, the bands do not belong to the same species and were assigned to the anion-radical (625 nm) and, respectively, to an intermediate (presumably the dianion; ~330 nm), or a reduction product.

The family of curves obtained during the electrochemical oxidation of 2-aminophenol (Fig. 7b) evidences the occurrence of the absorption band of 2-aminophenoxazin-3-one (416 – 454 nm). A brown precipitate was observed during the electrolysis process, and this fact supports the idea that the oxidation of 2-aminophenol is irreversible and leads to a polymeric structure containing 2-aminophenoxazone units.

C. MO calculations

The MO calculations were intended to account for the following experimental results:
– the pathways of the electrochemical reduction of 2-aminophenoxazone in aprotic and basic media, leading finally to 2-aminophenol;
– the electrochemical oxidation of 2-aminophenol, producing either 2-aminophenoxazone or an electroactive polymer containing aminophenoxazine units, as shown by spectral and cyclic voltammetry data.
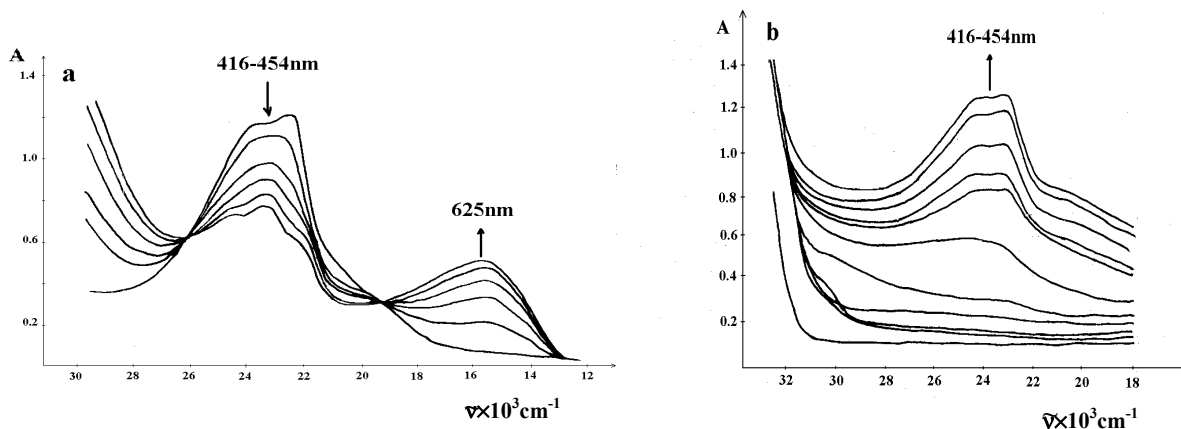
Fig. 7 – Family of absorption spectra recorded during: (a) the electrochemical reduction of 2-aminophenoxazin-3-one;
(b) the electrochemical oxidation of 2-aminophenol.

The electronic parameters relevant in the discussion of the redox properties of 2-aminophenoxazin-3-one and 2-aminophenol were calculated and are presented in Table 2. It may be observed that 2-aminophenoxazin 3-one is characterised by high absolute and adiabatic electronegativities and high adiabatic electron affinity, in agreement with its reducibility. The first reduction step leads to a fairly stable anion radical, evidenced by cyclic voltammetry, as well as by the optical spectra (absorption at 625 nm). The charge distribution in this anion radical, together with the spin distribution, illustrate the main contribution of a –C· –O⁻ structure ($q_O^- = -0.444$ *vs.* $q_O = -0.294$ in the neutral molecule, and high spin density on the carbon of the carbonyl group), meaning that the quinone-imine moiety of the molecule is involved in the reduction process. These results confirm the previous EPR results for actinomycin D, where the phenoxazone moiety is responsible for the reduction behaviour.[9] The evolution of the N-C bond orders, in the central ring, in the reduction process, which decrease in the order: 1.720 (neutral) > 1.335 (anion radical) > 1.055 (dianion), and the ring C-O bond order in the range 1.045-0.999 makes predictable the breaking of these bonds in the reduction process and is in agreement with the experimental data in alkaline media, where 2-aminophenol is the final reduction product of questiomycin A.

Table 2

AM1 calculated redox properties of 2-aminophenoxazin-3-one and 2-aminophenol

	ΔH^f (kcal/mol)	ε_{HOMO}	ε_{LUMO}	X_v [1] (eV)	IP_{ad} [2] (kcal/mol)	EA_{ad} [3] (kcal/mol)	X_{ad} [4] (eV)
2-aminophenoxazin-3-one	24.92	-8.455	-1.503	4.979	181.94	50.201	5.035
2-aminophenol	-18.89	-8.204	0.633	3.785	178.303	6.056	3.892

[1] the absolute electronegativity, $X_v = -1/2(\varepsilon_{HOMO} + \varepsilon_{LUMO})$
[2] the adiabatic ionisation potential, IP_{ad} defined as ΔH for the reaction: $A \rightarrow A^+ + e^-$
[3] the adiabatic electron affinity, EA_{ad} defined as $-\Delta H$ for the reaction: $A + e^- \rightarrow A^-$
[4] the adiabatic electronegativity, $X_{ad} = 1/2 (IP_{ad} + EA_{ad})$

For 2-aminophenol the higher ε_{HOMO} energy, the lower absolute and adiabatic electronegativities and the low adiabatic electron affinity confirm that it is a good electron donor, leading to cation radicals, the reactive species being implied in the electro polymerisation of 2-aminophenol. Also, for 2-aminophenol, the charge distribution shows the greatest negative charge in the *p*-position *versus* the amine group, indicating this position as preferred for the attack of the cation radical leading to the open dimer (Scheme 1b). As there are three possibilities for the head-to-tail dimers: D_1 and D_2 (N-linked) or D_3 (O-linked), all these structures were calculated. The results indicated that D_3 is more difficult to be oxidised than D_1 or D_2 ($\varepsilon_{HOMO} = -8.466$eV *vs.* -7.913eV for D_1 and -8.045eV for D_2). The charge distribution shows greater negative charge

on C_9 (-0.171) than C_6 (-0.141), favourable to further attack of the cation radical to site 9 as against the closing of the phenoxazine ring. This is in agreement with the open structure of the polymer[13] obtained in basic media through C-O-C bonds. The charge distribution calculated for D_1 and D_2 shows large negative charges (–0.178 and –0.189) for the *p*–position *vs*. the –OH or –NH_2 groups, favouring the closing of the ring leading to phenoxazine. Further attack of the cation radical to D_1 or D_2 produces either to a polymer with phenoxazine units (D_1), consistent with the results in the literature,[11] or 2-aminophenoxazone (D_2). The phenoxazine units may be further oxidised to a quinone-imine structure (P_2 in Scheme 1a). The MO calculations account satisfactorily for the redox equilibrium between the two polymeric structures: higher ε_{HOMO} values (–7.905eV for P_1 *vs*. -8.644eV for P_2) indicate the possibility to oxidise P_1 to P_2, while the higher ε_{LUMO} (-0.078eV for P_1 *vs*. –1.502eV for P_2) is in agreement with the reduction of P_2 to P_1.

In conclusion, the performed MO calculations account for the main experimental results obtained at the electrochemical reduction of questiomycin A (2-aminophenoxazone) in aprotic and basic media, as well as for the electrochemical oxidation of questiomycin B (2-aminophenol) in similar conditions.

EXPERIMENTAL

Cyclic voltametry experiments with both stationary and rotating disc electrode (RDE) were performed in DMSO with 0,1M tetra *n*-buthyl ammonium perchlorate (TBAP) as supporting electrolyte. A VOLTALAB-32 electrochemical device and a cell with platinum (2 mm diameter) working and counter electrodes as well as Ag-quasi reference electrode were used.

The optical spectra were registered during the electrochemical and chemical reduction using *in situ* techniques developed in our laboratory using C. Zeiss Jena and UNICAM-UV 4 spectrophotometers. The semiempirical MO calculations were performed using the AM1 Hamiltonian in the MOPAC program package and RHF (ROHF) formalism for closed and, respectively, open-shell structures. All structures were fully optimised using the EF optimisation algorithm. The cyclic voltammetry simulations were carried out using DigiSim 3.03 software.[16]

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