Investigation of the Electrochemical Reduction of 3R-Claussequinone, in "Dry" DMF and Mixed Water-DMF Solvent: A system Involving Self-Protonation.

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A redução da 3R-claussequinona, CQ, em DMF "seco" e solvente misto água/DMF, foi investigada por técnicas eletroquímicas e espectroscópicas. Os resultados experimentais revelam um equilíbrio ácido-base ($pK_a^* \sim 10,7$) envolvendo as espécies protonada e desprotonada da CQ. Em solvente misto, uma única transferência reversível de dois elétrons foi observada. Em DMF "seco" foram observadas duas transferências de um elétron cada, sendo apenas a primeira reversível. Para todas as condições experimentais investigadas, detectou-se a participação de prótons no mecanismo de redução. Comparando-se o comportamento eletroquímico da CQ ao de seu derivado acetilado, é estabelecida, claramente, a ocorrência de um passo de auto-protonação no mecanismo eletródico. São propostos mecanismos detalhados para ambos os solventes.

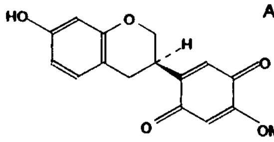
The reduction of 3R-claussequinone, CQ, in "dry" DMF and mixed water-DMF solvent was investigated by electrochemical and spectroscopic techniques. Experimental results support an acid-base equilibrium ($pK_a^* = 10.7$) involving protonated and unprotonated forms of CQ. In mixed solvent a single reversible two-electron transfer is observed. In "dry" DMF two one-electron transfers are observed, the first being reversible. For all experimental conditions investigated, protons participate in the reduction mechanism. The comparison of the behavior of CQ with its acetylated derivative clearly established the occurrence of a self-protonation step in the electrode mechanism. Detailed electrode mechanisms are proposed for both media.

Key words: *claussequinone; reduction mechanism; self-protonation.*

Introduction

Quinonoid compounds actively participate in electron-transfer processes in biological systems. Extensive reviews of the different electrochemical aspects involved have appeared in the literature[1-3]. In particular, the quinone-hydroquinone couple has been exhaustively investigated because it is an excellent model system or because it is directly involved in biological processes[4]. Several quinones exhibit an intense pharmacological activity, some of them being strongly bound to the DNA molecule[5]. This property, together with the electrochemical surface behavior of these drugs, permits one to investigate their interactions with DNA by following the decrease of the quinone reduction wave in the presence of DNA[6,7]. The anti-tumor activity, observed with some quinones, has been related to the alkylation of the quinonemethide function, obtained on reduction of the quinonoid radical[8]. Electrogenerated quinone anion radicals and dianions can be alkylated and arylated, these reactions being useful synthetic routes[9]. Therefore, knowledge of the basic electrochemical behavior is of fundamental importance to the understanding of these processes in more complex systems.

Claussequinone, CQ, a natural isoflavanquinone isolated from *Cyclolobium clausseni* Benth[10], has strong anti-malarial activity. In this paper we present the results of our electrochemical investigation of CQ, **1**, and its acetylated derivative CQ-OAc, **2**. The studies were carried out in mixed water-dimethylformamide (DMF) as well as in "dry" DMF.



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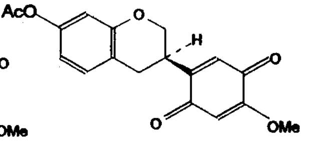

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Experimental

Equipment. D.c.$_{\text{tast}}$ and a.c.$_{\text{tast}}$ polarograms were recorded on a Polarecord E 506 polarograph equipped with a model E 505 DME stand and Ag/AgCl, Cl^-(sat) reference and Pt wire auxiliary electrodes (all Metrohm, Swiss). General working conditions were as follows: drop time = 1.4 s; height of mercury column = 44 cm; mercury flux = 0.740 mg/s; a.c. frequency = 75 Hz and a.c. amplitude = 10 mV p/p.

Cyclic voltammetric investigations were done with a PAR model 173 potentiostat/galvanostat equipped with a model 175 Universal Programmer (PAR, USA); the output signal was recorded on a HP model 7045A XY recorder. A HMDE model E-410 from Metrohm ($A = 2.22 \pm 0.07\ mm^2$) was used as working electrode. The reference electrode, either Ag/AgCl, Cl^-(sat) or Ag/AgI, I^-(sat), was separated from the solution with a Luggin capillary closed at its end by a Vycor^R tip.

This experimental setup was also used for the electrolysis experiments. For these experiments the auxiliary electrode (graphite) was separated from the solution by a glass tube closed at its end by a sintered, medium-porosity, frit.

Coulometric experiments were carried out with the model 173 potentiostat equipped with a digital coulometer model 179, a model 377 synchronous stirring motor and a model 377A coulometric cell, all from PAR.

Data were extracted from the experimental polarograms with the aid of a digitalizing device (Da Vinci/Digigraph, Brazil). The communication software was home-written[11] Interpretation of the experimental data by means of the Gauss-Newton algorithm[12] was done on an IBM PC-AT compatible microcomputer.

A Metrohm model E500 pHmeter, equipped with a combination glass electrode, was used for all pH measurements. The equipment was standardized, over the pH range investigated, with N.B.S. buffers. Values, in the mixed water-DMF solvent, are not corrected for the solvent effect, so pH values reported are apparent values.

Reagents. All products, obtained from commercial sources, were of A.R. purity with the exception of DMF which was Uvasol (Merck). All products were used as received.

Stock solutions, 0.01M substrate (CQ and CQ-OAc) in pure DMF were used to prepare all working solutions. Working solutions in mixed solvent, of appropriate pH and CQ concentration, were prepared by transferring appropriate volumes of stock solutions of the buffer components, the substrate stock solution and DMF to a volumetric flask. Mc Ilvaine buffers[13] were used to control the solution pH. Total ionic strength was kept constant at 1.0 M through addition of the appropriate volume of 6 M $NaClO_4$ solution.

In the mixed solvent investigation a 7:3 (v:v) mixture of H_2O and DMF was used. In this solvent both substrates show a reasonable solubility. The residual water content of the commercial Uvasol DMF was lowered further through the addition of alumina 90 (Merck), activated at 150°C. After this treatment the residual water content of this "dry" DMF, as determined by the Karl-Fischer method, was found to be around 6 mM. In the latter solvent, 0.1 M tetrabutylammonium perchlorate (TBAP), was used as supporting electrolyte. TBAP was prepared according to the House[14] procedure. The isolated product was recrystallized from ethyl acetate and vacuum-dried for two days.

The acetylated derivative of CQ was prepared according to the literature[16]. The purified substrate showed a melting point of 168-171°C. As far as we know, the complete proton attribution of the ^1H NMR spectrum, for both CQ and CQ-OAc, has never been reported in the literature. Proton attributions for both compounds are as follows:

3R-Claussequinone (CQ) : ^1H NMR [$(CD_3)_2CO$, δ , 250 MHz]: 8.15 (s, O<u>H</u>, disappears with D_2O); 6.89 (d, J = 8Hz, H-5); 6.50 (d, J = 1.3 Hz, H-6'); 6.38 (dd, J = 8; 2.5 Hz, H-6); 6.27 (d, J = 2.5 Hz, H-8); 6.07 (s, H-3'); 4.24 (ddd, J = 10.2, 3.0, 1.5, H-2); 3.99 (dd, J = 10.2, 6.2, 1.2 Hz, H-2); 3.84 (s, O<u>Me</u>); 3.38-3.33 (m, 1H, H-3); 2.92 (ddl, J = 16.2, 6.2, H-4); 2.80 (dd, J = 16.2, 7.5 Hz; H-4).

3R-Claussequinone-OAc (CQ-OAc): ^1H NMR ($CDCl_3$, δ, 250 MHz), 7.04 (d, J = 8.0 Hz, H-5); 6.63 (dd, J = 8.00, 2.5 Hz, H-6); 6.58 (d, J = 2.5 Hz, H-8); 6.48 (d, J = 1.3 Hz, H-6'); 5.98 (s, H-3'); 4,28 (ddd, J = 10.2, 3, 1.5 Hz, H-2); 4.06 (ddd, 10.2, 6.2, 1.2 Hz, H-2); 3.82 (s, OMe); 3.42-3.53 (m, H-3); 3.07 (dd, J = 16.2, 6.2 Hz, H-4); 2.79 (dd, J = 16.2, 7.5 Hz, H-4); 2.27 (s, OCOC<u>H3</u>).

Results

D.c. and a.c. Polarographic investigation in mixed H_2O-DMF solvent. The most acid limit experimentally accessible, approximately pH 4.5, is dictated by the instability of the CQ

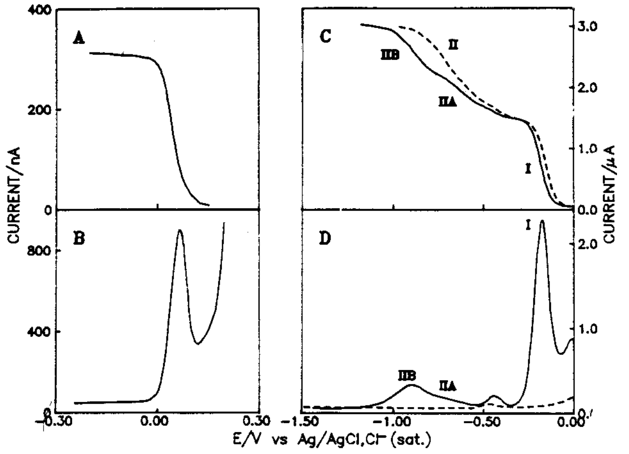

Figure 1. *Polarographic behavior of claussequinone. (A) d.c. polarogram: solvent H_2O:DMF (7:3, v:v); pH* 6.14; [CQ] = 1.00×10^{-4}M; 25°C; (B) a.c. polarogram: same conditions as (A); (C) d.c. polarograms: solvent "dry" DMF (full) and 150 mM H_2O added to DMF (dashed); [CQ] = 1.00×10^{-3} M; f = 75 Hz; 10 mV p/p; 25°C (dashed = supporting electrolyte).*

solution. Representative d.c. and a.c. polarograms are shown in Figure 1 (A, B).

For all pH values investigated a single, well defined, d.c. polarographic wave (a.c. peak) was observed. D.c. half-wave potentials coincide with a.c. peak potentials. When the pH of the medium is increased, the wave (peak) is displaced to more negative potentials. In the $9.5 < pH < 11.5$ interval, a splitting of the d.c. wave is observed. Similar behavior is observed in the a.c. polarogram where a second peak, localized at more negative potentials, is observed in the above mentioned pH region.

For the $pH < 11$ region, and for those polarograms which showed a single d.c. wave, the logarithmic analysis of the wave resulted in a single straight line having a 30 mV slope. The value of the slope increases to 60 mV for higher pH values. Simultaneously, the limiting current of the d.c. wave, in the strongly basic pH region, decreases to approximately half of the limiting current value displayed by the wave, in the more acid pH region investigated. For the a.c. polarographic peak a width at half-height, $E_{p/2}$, of 46 mV was observed, in the $pH < 11$ region. At higher pH values a $E_{p/2} = 60$ mV was obtained.

The displacement of the half-wave potential, $E_{1/2}$, as a function of pH is shown in Figure 2. Similar behavior was obtained for the a.c. polarographic peak potential.

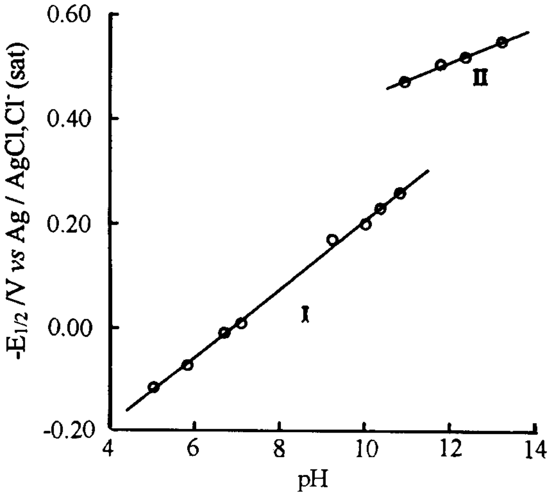

Figure 2. *$E_{1/2}$ as a function of pH* in mixed H_2O:DMF (7:3, v:v) solvent; [CQ] = 1.00×10^{-3} M; 25°C.*

Two linear regions, designated I and II, can be observed in the $E_{1/2}$ *vs* pH graph. Line I, corresponding to the lower and intermediate pH region, has a slope of 60 mV/pH. For the strongly basic pH region, line II, the slope is 30 mV/pH.

The experimental d.c. limiting current, i_l, is proportional to the a.c. peak current, i_p, over the complete pH region investigated. The behavior of i_p as a function of pH is shown in Figure 3. This figure shows that around pH 12 the initial peak, observed at the lower acidities, vanished completely and was replaced by the peak at the more cathodic potential. From the limiting current of the polarograms, obtained at $4.7 < pH < 7.0$, a diffusion coefficient of 3.19×10^{-6} cm^2/s (2.19×10^{-6} cm^2/s) was calculated for CQ from the Ilkovic (Koutecky) equation.

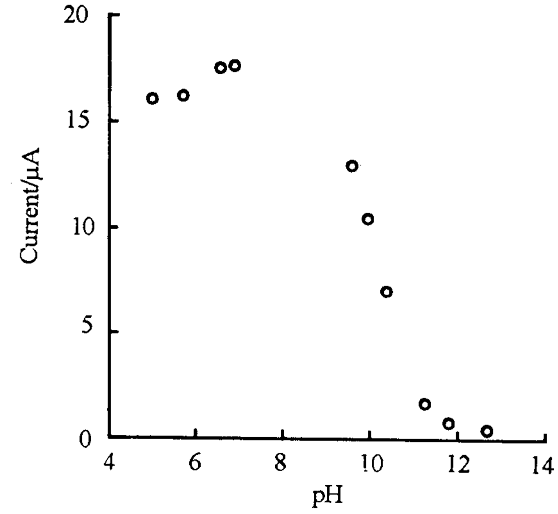

Figure 3: *Peak current (a.c. polarography) as a function of pH*. Solvent: H_2O:DMF (7:3, v:v); [CQ] = 1.30×10^{-3} M; 25°C.*

The influence on the polarographic behavior of the height of the mercury column, h_{Hg}, and the drop time, t_d, was investigated at pH 6.6. A linear dependence of i_l with $h_{Hg}^{1/2}$ and $t_d^{1/6}$, was obtained. Other values for the exponents of h_{Hg} and t_d were tested and found not to result in straight lines. A study of the influence on the limiting current of the CQ concentration, investigated in the 0.1-3.0 mM interval, resulted in a linear dependence, showing the current to be diffusion controlled. The half-wave potential was independent of CQ concentration. When the influence of temperature, over the 25-57°C range on the limiting current was investigated, a linear dependence with a temperature coefficient of 2.5%C^{-1} was observed.

D.c. and a.c. Polarographic investigation in "dry" DMF. The change from a mixed water-DMF solvent to pure DMF solvent introduces some remarkable changes in the polarographic behavior of CQ. Representative polarograms are

Table I - *Electrochemical parameters of the CQ d.c. polarographic wave in "dry" DMF and "dry" DMF + 150 mM H_2O; 0.1 M TBAP; [CQ] = 1.00 mM; t = 25°C.*

wave	"dry" DMF			"dry" DMF + 150 mM H_2O		
(see Fig 1C)	$E^a_{1/2}$ V	slope mV	i_l μA	$E^a_{1/2}$ V	slope mV	i_l μA
I^b	-0.184	60	1.46	-0.158	63	1.44
IIA^c	-0.621	89	0.455	-	-	-
IIB^c	-0.863	123	0.645		-	-
II^b	-	-	-	-0.730	154	1.00

[a] $E_{1/2}$ /V vs Ag/AgCl,Cl$^-$(sat.).

[b] Parameters obtained by classical logarithmic treatment.

[c] Parameters obtained by the Gauss-Newton algorithm[12]

shown in Figures 1C (d.c.) and 1D (a.c.). The result of the deliberate introduction of 150 mM H_2O into "dry" DMF, so as to study the influence of a proton donor, is also included in Figure 1C. In "dry" DMF, when compared to the mixed solvent, the reduction of CQ occurs in two consecutive steps, the first being a single wave, I, the second being split into two waves, IIA and IIB, of approximately the same height; the sum of i_{IIA} and i_{IIB} is almost identical to i_I. The intensity of the a.c. signal of peak I is much more pronounced than the signal of peaks IIA and IIB. The introduction of 150 mM of water into the "dry" DMF has three significant effects on the polarographic behavior: (1) the polarogram is shifted to less negative potentials, the displacement being important for wave IIB while less significant for wave I; (2) waves IIA and IIB merge into a single wave II; (3) water has no influence on the limiting currents. The electrochemical parameters resulting from the analysis of the waves (peaks) are shown in the Table I.

From the limiting current of the first wave and from the fact that this wave is a one-electron process (see coulometric results), a diffusion coefficient of 7.69×10^{-6} $cm^2 s^{-1}$ (6.33×10^{-6} $cm^2 s^{-1}$) was calculated for CQ from the Ilkovic (Koutecky) equation.

Comparing the a.c. currents in the presence and absence of the electroactive material, one observes that outside the peak regions both currents coincide. This behavior suggests that none of the species involved in the electrode mechanism is strongly adsorbed onto mercury in the DMF solvent.

The width at half-height of the first a.c. polarographic peak has a value of 91 mV, very close to the theoretical value of 92 mV for a reversible one-electron transfer[17]. The $E_{p/2}$ value for peak IIB is close to 210 mV, suggesting the electron transfer to be reversible with an irreversible chemical step coupled to it. Since a.c. polarography, for the experimental conditions employed in this study, does not give rise to a signal when the electron transfer is irreversible[18] and since a small peak current (~0.3 μA) was observed, irreversibility of the electron transfer represented by wave IIB can be excluded.

Cyclic voltammetric behavior of CQ in "dry" DMF. The influence of sweep rate on the cyclic voltammetric behavior of CQ (1.0 mM) in DMF containing 0.1 M TBAP is shown in Figure 4.

Three cathodic peaks (I, IIA and IIB) were observed over the complete sweep-rate, v, interval investigated ($5 < v < 500$ mV s^{-1}). Of these only peak I shows an anodic counterpart. The analysis of the influence of v on the electrochemical parameters related to the pair of peaks I suggests the electron transfer representing these peaks to be reversible. In fact it was found that $i_p^a/i_p^c = 1$, the peak potential separation is 64 $mV^{(*)}$, i_p^c has a linear dependence with $v^{1/2}$, and E_p^c is independent of v. All of this criteria[19] point towards a reversible electron transfer for peak I.

The influence of v on peaks IIA and IIB is much more complex. With increasing sweep rates a displacement towards more negative values, at a rate of ~70 mV $decade^{-1}$, is observed for E_p^{IIB} (E_p^{IIA} cannot be evaluated accurately because of its overlapping with peak IIB). Besides the observed cathodic displacement, the peak-current ratio i_p^{IIA}/i_p^{IIB} is also drastically affected on increasing v. For very low sweep rates ($v < 10$ mV s^{-1}) the current for peak IIA is langer than that of peak IIB. For $v > 10$ mV s^{-1} this pattern is inverted (i_p^{IIA} i_p^{IIB}). For $v \sim 500$ mV s^{-1}, peak IIA almost vanishes completely from the cyclic voltammogram, showing up only as an almost imperceptible shoulder at the foot of peak IIB. For these conditions the difference between E_p^{IIB} and the potential at half-height, E_p $E_{p/2}$, is close to 80 mV. The theoretical value for a single reversible electron transfer is $E_p - E_{p/2} = 56/n$ mV^{19}. The addition of CH_3I did not affect the cyclic voltammetric behavior.

Cyclic voltammetric behavior of CQ-OAc in "dry" DMF. Representative cyclic voltammograms for the acetylated derivative of CQ (1.25 mM) in "dry" DMF, containing 0.1M TBAP, are shown in Figure 5. Compared to the parent substrate, CQ, remarkable changes are observed in the CQ-OAc voltammetric behavior.

For identical sweep rates, peak IIA, observed at low v values in the CQ voltammogram, is entirely absent in the CQ-OAc cyclic voltammogram. Now, only two cathodic peaks of approximately the same intensity are observed, together with an anodic peak pertaining to the first cathodic peak. This pair of peaks I was characterized as being reversible[19] on the bases of the following electrochemical parame-

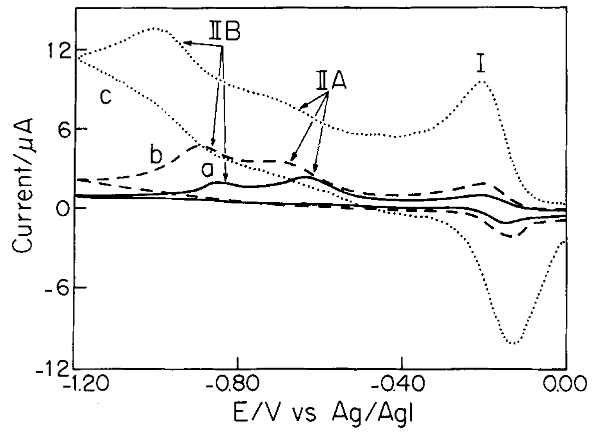

Figure 4 *Cyclic voltammetry of C.Q. in "dry" DMF-0.1M TBAP; [CQ] = 1.00 × 10^{-3} M; 25°C; working electrode = HMDE. v: 5 mV/s (A), 20 mV/s (B), 500 mV/s(C).*

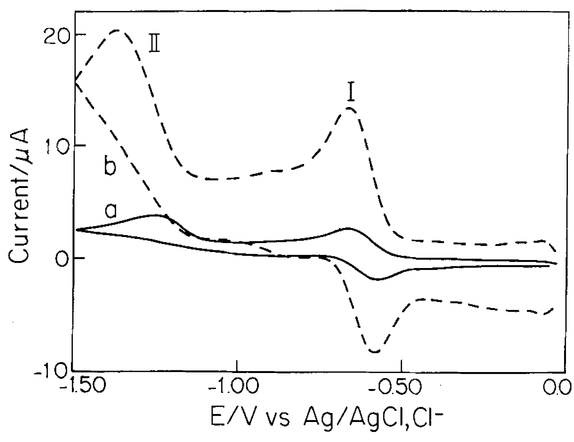

Figure 5. *Cyclic voltammetry of CQ-OAc in "dry" DMF/0.1M TBAP; [CQ-OAc] = 1:00 × 10^{-3} M; 25°C; working electrode = HMDE. v: 10 mV/s (a), 500 mV/s (b).*

ters: (1) $i_p^a/i_p^c = 1$; (2) E_p^I is independent of sweep rate; (3) i_p^I has a linear dependence on $v^{1/2}$; (4) anodic and cathodic peak potentials are separated by ~67 $\text{mV}^{(*)}$.

Increasing the sweep rate results in a displacement of E_p^{II} of 78 mV per decade of v. The $E_p - E_{p/2}$ parameter has a value of 70 mV at v=500 mV s^{-1}. The presence of CH_3I did not affect the CQ-OAc voltammetric behavior.

Controlled-potential coulometric behavior of CQ and CQ-OAc. A number of controlled-potential coulometric (CPC) experiments of CQ and CQ-OAc were conducted in DMF with 0.1 M TBAP as supporting electrolyte. Working potentials after the first and second peak potentials were explored. The influence of CH_3I, at a concentration of at least 10 times the substrate concentration, was also investigated. The results of the CPC experiments, together with some experimental details, are shown in Table II.

Controlled-potential electrolysis of CQ-OAc. After deaeration of 40 ml of DMF-0.1 M TBAP, 0.040 g (aprox. 0.13 mmol) of CQ-OAc were added together with 0.083 g (ca. 0.59 mmol) of CH_3I. The mixture was electrolyzed at -1.00 V vs. Ag/AgBr at a mercury pool working electrode (A = 22 cm^2). The experiment was stopped after 2 F mol^{-1} of charge were passed. It was observed that the initial yellow color of the mixture turned brown in the early stage of the experiment. During the progression of the experiment the solution became more faintly colored, acquiring a clear yellow color at the end. At this point the current flowing through the cell was very close to the residual current. The complete isolation procedure was executed under a strong N_2 flux in order to avoid decomposition. Initially, 25 ml of H_2O was added to the catholyte and the organic material extracted with successive 20 ml portions of diethyl ether. After drying of the extract with anhydrous $MgSO_4$ and evaporation of the solvent, 40 mg of a pasty mixture (CQ-OAc-R) was obtained. This material suffers decomposition, as evidenced by its darkening on prolonged standing; therefore, its analysis by ^1H NMR was done immediately after the isolation procedure.

Table 2. *Controlled-potential coulometric results and experimental details.*

Substrate	E_{ap}/V *vs* SSCE	n_{ap}	[c]/mM
CQ (pH 6.26)[a]	-0.16	2.20	0.042
CQ (pH 13.2)[a]	-0.57	1.05	0.056
CQ	-0.50	0.45	0.535
CQ[b]	-0.50	0.50	0.944
CQ[b]	-1.18	1.50	0.944
CQ + CH_3I	-0.50	0.96	1.985
CQ	-1.18	1.54	0.944
CQ	-1.30	1.49	0.944
CQ-OAc	-0.70	0.93	0.559
CQ-OAc	-1.50	2.09	0.826

[a] Experiments in mixed H_2O-DMF solvent. All other experiments in "dry" DMF.

[b] Same experimental conditions. In second experiment, potential was jumped to -1.18 V after exhaustive electrolysis at -0.50 V. Mercury pool cathode (Area = 12.5 cm^2).

On the basis of the integration of the 5.98 ppm CQ-OAc residual NMR signal, a 95% conversion was obtained.

Results of the analysis of the CQ-OAc-R are shown below. The numbers in parenthesis were calculated using the number of protons of the acetoxyl group as a reference. From this it was found that methylation occurred with a 56% yield. One of the isolated products showed a signal inferior to unity which can be related to a trimethoxylated derivative.

CQ-OAc-R : ^1H NMR ($CDCl_3$, 250 MHz, δ) : 7.2-7.09 (m,1);
6.7-6.51 (m,4); 6.41 (s, 0.4); 4.36-4.25 (m, 1.2);
4.09-3.92 (m, 1.2); 3.9 (2s, 1.81); 3.9 (s, 1.90);
3.81 (2s, 2.54); 3.67-3.42 m,1); 3.08-2.82 (m, 2.1);
2.28 (s, 3, $OCOC\underline{H_3}$).

Discussion and Conclusions

The results of the polarographic investigations in mixed solvent and the different criteria investigated clearly show that the electron transfers are reversible processes with proton uptake coupled to them. Further, depending on the acidity of the medium, CQ exists as the protonated form or its conjugated base (the unprotonated form), both forms being reduced at different potentials. So, in mixed solvents, CQ is reduced according to the classical reduction mechanism of quinones, Scheme I, being followed for moderately acid conditions and Scheme II for strongly basic conditions:

Scheme I (pH < 7.0)

$$CQ + H^+ \rightleftharpoons CQH^+ \qquad (1)$$

$$CQH^+ + e^- \rightleftharpoons CQH^\bullet \qquad (2)$$

$$CQH^\bullet + e^- \rightleftharpoons CQH^- \qquad (3)$$

$$CQH^- + H^+ \rightleftharpoons CQH_2 \qquad (4)$$

Scheme II (pH 12)

$$CQ + e- \rightleftharpoons CQ^{-\bullet} \qquad (5)$$

$$CQ^\bullet + H+ \rightleftharpoons CQH^\bullet \qquad (6)$$

In the intermediate pH range (7-11.5) CQH^+ and CQ exist in equilibrium, both species giving rise to a d.c. wave (a.c. peak), with the protonated form, CQH^+, being reduced at a less negative potential. For the pH < 7 region the experimental finding ($E_p = E_{1/2}$; the 30-mV slope obtained from the logarithmic treatment of the wave; the 60 mV/pH slope of the $E^I_{1/2}$ vs pH graph; and the coulometric result of n = 2), besides being strong evidence for the reversibility of the electron transfer[17,19], also supports the participation of two protons in the reduction mechanism[15]. The fact that a single two-electron polarographic wave is observed means that the second one-electron transfer (Scheme I, step 3) occurs at less negative potentials than the first electron uptake (scheme I, step 2). The occurrence of step 1 in Scheme I is strongly supported by the i_l *vs* pH behavior (Fig. 3), and permits us to evaluate the polarographic dissociation constant of the protonation reaction to be $pK_a^* = 10.7$.

For the strongly basic pH region the experimental results (the polarographic limiting current has approximately half the value of il under moderately acid conditions; the 60-mV slope of the logarithmic treatment of the wave coincides with the theoretical value for a reversible one-electron transfer; the 30 mV/pH slope of the $E^{II}_{1/2}$ vs pH graph; the $n_{ap} = 1$ coulometric result at pH = 13.2) all support a one-electron, one-proton mechanism[15] to form the stable radical, which does not undergo further reduction, under these experimental conditions.

In "dry" DMF, a solvent known to promote the stabilization of anion-radicals, the reduction of CQ (CQ-OAc) proceeds in two, well-separated, one-electron transfers. The experimental evidence, emerging from the different electrochemical techniques applied, indicates that the first one-electron transfer is reversible. For the second electron transfer, all experimental evidence supports an EC type mechanism in which the chemical step involved is irreversible and the electron transfer reversible. Also, as seen by the splitting of the second wave and by the anodic displacement of the waves on addition of a proton donor, proton uptake is involved in the reduction mechanism. An electrode mechanism which adequately describes all of the experimental results for the CQ reduction in "dry" DMF is presented below (Scheme III).

Scheme III ("dry" DMF)

$$\text{HO-CQ}^{(1)} + e^- \rightleftharpoons \text{HO-CQ}^{-\bullet} \qquad \text{(peak I)} \quad (5)$$

$$\text{HO-CQ}^{-\bullet} + H^+ \rightleftharpoons \text{HOCQH}^{\bullet} \qquad (6)$$

$$\text{HO-CQ}^{-\bullet} + \text{HO-CQ} \rightleftharpoons \text{HO-CQH}^{\bullet} + {}^-\text{OCQ}^{(2)} \qquad \text{(self-protonation reaction)(6a)}$$

$$\text{HO-CQ}^{-\bullet} + e^- \rightleftharpoons \text{HO-CQ}^{=} \qquad \text{(peak IIB)} \quad (7a)$$

$$\text{HO-CQH}^{\bullet} + e^- \rightleftharpoons \text{HO-CQH}^- \qquad \text{(peak IIA)} \quad (7b)$$

$$\text{HO-CQ}^{=} + 2H^+ \rightleftharpoons \text{HO-CQH}_2 \qquad (8a)$$

$$\text{HO-CQH}^- + H^+ \rightleftharpoons \text{HO-CQH}_2 \qquad (8b)$$

(1) CQ is here represented by HO-CQ to emphasize the presence of a -oH group in the molecule.
(2) ⁻OCQ is supposed to be electroinactive (see details).

The protons for the acid-base equilibrium (Scheme III, steps 6 and 6a) involving the anion-radical, formed after the first one-electron transfer, can have two possible sources. Since "dry" DMF still contains small amounts of H_2O (~ 6 mM), this can act as a proton donor, explaining the splitting of the second d.c. polarographic wave (a.c. peak) into wave (peak) IIA (reduction of the HO-CQH$^{\bullet}$ species) and wave (peak) IIB (reduction of the more-difficult-to-reduce HO-CQ$^{\bullet}$ species). Depending on the proton concentration the protonated and unprotonated forms can exist simultaneously, giving rise to two d.c. waves (a.c. peaks). The deliberate introduction of more H_2O (150 mM) increases the proton concentration of the solution, resulting in a complete displacement of equilibrium 6 (Scheme III) to the right, explaining the merging of the two waves (peaks) into a single d.c. wave (a.c. peak) and the anodic displacement observed. An alternative as a proton source is HO-CQ itself. In fact the -OH group of CQ (see molecular structure), which in water behaves as a very weak acid, when in a non-aqueous solvent with proton-acceptor properties (such as DMF), behaves as a much stronger proton donor. So it is reasonable to consider the self-protonation reaction (Scheme III, step 6a) as an alternative way to protonate the anion-radical HO-CQ$^{-\bullet}$. Self-protonation was extensively investigated by Vianello *et al.*[20,21]. Obviously, the simultaneous occurrence of steps 6 and 6a cannot be excluded.

Results of the cyclic voltammetric investigation support Scheme III. The influence of the sweep rate (and thus the time window of the experiment) on the i_pIIA/i_pIIB ratio permits further conclusions on the protonation rates of steps 6 and 6a. At low sweep rates i_pIIA > i_pIIB (Figure 4a). So, at this low sweep rate, the time of window is such as to favor significantly the protonation of the HO-CQ$^{-\bullet}$ species ([HO-CQH$^{-\bullet}$] [HO-CQ$^{-\bullet}$]). A slight increase, however, of the sweep rate is enough to invert this situation, whereas a modest sweep rate of 500 mV/s eliminates peak IIA almost completely from the HO-CQ cyclic voltammogram. This behavior suggests that the protonation reactions are not fast reactions. The cyclic voltammetric behavior of the acetylated form of CQ furnishes important evidence about the relative importance of steps 6 and 6a as a source for HO-CQ$^{-\bullet}$ protonation. In fact, the acetylation of the -OH group of the CQ molecule eliminates its proton as a source of protonation so that the self-protonation alternative can no longer be considered. Since the CQ-OAc cyclic voltammogram does not show any splitting of the second peak, it must be concluded that the splitting of the second peak, observed with the non-acetylated form of CQ, has as its principal cause the self-protonation reaction and not the proton-donation capacity of the residual water. This latter contribution, for the experimental conditions used, must be considered negligible.

At first sight, the controlled-potential coulometry experiments (see Table II) reveal some surprising results such as n_{ap} = 0.5 (E_{ap} at first peak) and n_{ap}=1.5 (E_{ap} at second peak). Since CPC is an exhaustive technique and since, at a working potential localized immediately after the first peak, the only electron-transfer process possible is step 5 in Scheme III. The experimental value of 0.5 F/mol is explained as the result of step 5 and 6a, not only confirming the self-protonation process but also the significant contribution of H_2O as a proton donor when "dry" DMF is used as solvent. It seems very probable that the phenolate interfere in the electrodic process, decreasing the effective number of transferred electrons, due to addition on the carbonyl of the quinonoid group, altering its electrochemistry drastically[9].

The explanation of n_{ap} = 1.5 is straightforward. This fact also explains why $i_L^{IIA}+i_L^{IIB}$ is approximately 30% less than i_L^{I}. When experimental conditions are slightly changed, so that one introduces a substrate such as CH_3I capable of competing with protonation steps 6 and 6a of the HO-CQ$^{-\bullet}$ anion-radical, the expected 1 F/mol is obtained for an CPC experiment executed with Eap at the first peak. For these experimental conditions, $[CH_3I] > [CQ]$. As the result the methylation reaction

$$\text{HO-CQ}^{\bullet} + \text{CH}_3\text{I} \rightleftharpoons \text{HO-CQCH}_3 + \text{I}^-$$

is favored over the self-protonation step so that rate of methylation >> rate of self-protonation. Electrolysis experiments in the presence of CH_3I executed under similar conditions showed the main products to be the di- and trimethylated forms of CQ.

CPC experiments of CQ-OAc (the CQ form with the -OH group protected by acetylation so that the self-protonation reaction of the anion-radical is avoided) with Eap at a potential localized after the first and second voltammetric peaks resulted in the expected theoretical values of 1 F mol^{-1} and 2, respectively, demonstrating the importance of the self-protonation reaction in the electrode mechanism describing the reduction of HO-CQ.

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References

1. J. Q. Chambers, *The Chemistry of the Quinonoid Compounds*, Ed. S. Patai (John Wiley & Sons, New York, 1974), pp.719-791.
2. J. Q. Chambers, *The Chemistry of the Quinonoid Compounds*, Ed. S. Patai (John Wiley & Sons, New York, 1988), pp.719-757
3. G. E. W. Wolstenhome, C. M. O'Connor (Eds.), *Quinones in Electron Transport* (Churchill, London, 1961).
4. J. H. Jongejan, J. A. Duine (Eds.), *PQQ and Quinoproteins* (Kluwer Academic Publishers, Dordrecht, 1989).
5. W. D. Wilson, R. L. Jones, *Adv. Pharm. Chemoter.* **18**, 177(1981).
6. G. Molinier-Jumel, B. Malfoy, J. A. Reynaud, G. Aubel-Sadron, *Biochem. Biophis. Res. Commun.* **84**, 441 (1978).
7. J. A. Plambeck, J. W. Lown, *J. Electrochem. Soc.*, **131**, 2556(1984).
8. E. B. Skibo, *J. Org. Chem.*, **51**(4), 522(1986).
9. J. H. P. Utley, C. Z. Smith, M. O. F. Goulart, Y. L. Chiu, G. Yaping, *Electro-organic Reactions. Part 39. Novel Electrosynthetic Transformations of Quinones, Quinonemethides and Quinonemethanes in Modern Methodology in Organic Synthesis*, proceedings of ISOR-1991 (Kodanska-VCH,1992).
10. A. B. Oliveira, O. R. Gottlieb, J. M. M. Gonçalves, G. G. de Oliveira, A. S. Pereira, *Phytochem.*, 14, 2495(1975).
11. J. F. C. Boodts, O. L. Bottecchia, *Quimica Nova* **12**(1), 97(1989).
12. L. Degreve, O. L. Bottecchia, J. F. C. Boodts, *J. Electroanal. Chem.* **206**, 81(1986).
13. D. D. Perrin, B. Dempsey, *Buffers for pH and Metal Ion Control* (John Wiley & Sons, New York, 1974).
14. H. O. House, E. Ferg, N. P. Peet, *J. Org. Chem.* **36**, 2371(1971).
15. P. Zuman, C. Perrin, *Organic Polarography* (John Wiley & Sons, New York, 1969), p.232.
16. L. Velluz, G. Nominé, G. Amiard, V. Torelli, J. Cérède, *Compt. Rend.* **257**, 3086(1963).
17. B. Breyer, H. H. Bauer, *Alternating Current Polarography and Tensammetry* (Insterscience Publishers, New York, 1963), chap. 2.
18. S. Hacobian, *Australian J. Chem.* **6**, 211(1953).
19. R. S. Nicholson, I. Shain, *Anal. Chem.* **37**, 1351(1965).
20. F. Maran, S. Roffia, M. G. Severin, E. Vianello, *Electrochimica Acta* **35**, 81(1990).
21. M. C. Arevalo, G. Farnia, M. G. Severin, E. Vianello, *J. Electroanal. Chem.* **220**, 201(1987).