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Voltammetric Probing of Molecular Assemblies of Ubiquinone-10 at the Air–Water Interfaces

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Abstract. The redox properties of ubiquinone 10 (UQ10) placed at the air–water interface were studied using the horizontal touching method with a thin mercury film (TMFE) working electrode and cyclic voltammetry. Changes of pH of the subphase affected the formal potential of the ubiquinone/ubiquinol system exhibiting the participation of protons in the overall reduction of UQ10. The protonation of the semiquinone transition product was found to be the rate determining step. This explains the dependence of the rate constant value on pH. The highest values of rate constants were found at pH over 13. Under these conditions the mechanism of the process is different. The concentration of protons is small, and the availability of the counter ions (i.e., K^+) becomes crucial for the kinetics of reduction. Their role is to neutralize the negative charge of the redox group following its reduction. The logarithm of rate constants was found to decrease linearly with the increase of surface concentration of ubiquinone. This reflects the influence of intermolecular interactions in the monolayer on the kinetics of the electrode process.

Key words: ubiquinone, monolayer, thin mercury film

1. Introduction

Ubiquinones (Figure 1) are organic compounds with an amphiphilic structure – possessing as the hydrophobic part a long isoprenoid side chain and a polar quinone center. The interest in ubiquinone chemistry is connected with its unique biological role [1]. Ubiquinone is located within mitochondrial membrane and acts as an electron and proton carrier in the mitochondrial respiratory chain. It is also known that ubiquinones have antioxidant and radical scavenging properties. It is assumed that location of ubiquinone within a phospholipid bilayer makes possible an oscillation of molecules toward both surfaces of the membrane [1]. The biological activity of ubiquinone is connected with its reversible redox process. The reduction of ubiquinone takes place through $1\bar{e}$ transfers: first with the formation of semiquinone radical anion, followed by a neutralization step and formation of a neutral radical, and second where the neutral radical is reduced to ubiquinol. The geometry of ubiquinone-1 and its radical anion was studied by Boesch and Wheeler [2]. The calculated data show good agreement with the X-ray diffraction studies.

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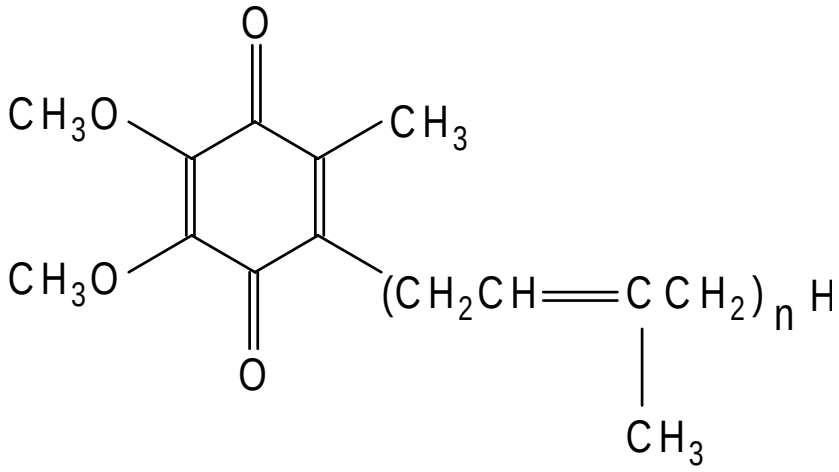

Figure 1. The structure of ubiquinone; n = 6 –10.

Despite the significant biological role of ubiquinone in membranes and its redox activity there are only few electrochemical studies of the behavior of this molecule at the interfaces. Because of the low solubility of ubiquinone in water, classical electrochemical measurements were carried out in non-aqueous solvents [3–5]. The redox behavior in aqueous solutions was studied using thin-layer voltammetry by Ksenzhek and coworkers [6]. The properties of ubiquinone adsorbed on a pyrolytic graphite electrode and glassy carbon electrode were studied by Schrebler and coworkers [7] and Takehara and Ide [8], respectively. The electrochemical behavior of ubiquinone-10 placed within a Langmuir–Blodgett monolayer containing octadecanol and octadecanethiol was investigated by Bilewicz [9] and within a phospholipid layer by Laval and Majda [10]. The reduction of ubiquinone incorporated into *n*-alkanethiol molecular assemblies on a gold electrode was presented by Takehara and co-workers [11]. The aim of the present work is to study the properties of ubiquinone at the air – water interface. This interface allows the ubiquinone molecules to assume the preferred orientation with the polar group towards the water surface and the hydrophobic tails orientation dependent on the surface excess. Voltammetry is shown to be a convenient tool for monitoring the redox properties and the increase of interaction between UQ10 molecules upon increasing surface excess.

2. Experimental

2.1. METHOD AND MATERIALS

Electrochemical experiments were carried out in a three electrode arrangement with platinum foil as the counter electrode, a saturated calomel electrode as the reference electrode and a thin mercury film electrode (TMFE) on gold as the

working electrode [12, 13]. Voltammograms were recorded using a PAR 273A potentiostat. Supporting electrolyte solutions were prepared using acetic acid (PCh Odczynniki), sodium acetate, boric acid, borax, potassium hydroxide (PChO) and water distilled and purified by passing through the Milli-Q system. Ubiquinone was purchased from Sigma. The spreading solvent was pentane (Merck).

2.2. PREPARATION OF THE UBIQUINONE LAYER

The experiments were done using the horizontal touching method [14–17]. The layer of ubiquinone at the air–water interface was prepared by spreading ubiquinone solution in pentane (1 mg/ml) on the surface of the deaerated supporting electrolyte solution. A constant flow of argon was kept over the solution surface during measurements. Voltammograms were recorded when the thin mercury film electrode was touching the electroactive ubiquinone monolayer placed at the air–water interface (Figure 2).

3. Results and Discussion

Due to the amphiphilic structure of the molecule, ubiquinone forms stable monolayers at the air–water interface. The voltammogram recorded under conditions when TMFE was touching the ubiquinone molecular layer at the air–water interface is shown in Figure 3. The shape of the voltammetric curve indicates that reduced and oxidized forms of ubiquinone are in contact with the electrode surface. The presence of adsorptive interactions between the electrode surface and molecules of UQ10 was shown by the following experiment. TMFE, after being in contact with the ubiquinone layer, was transferred to another electrochemical cell with deaerated supporting electrolyte solution. A couple of peaks appears on the voltammogram recorded in this solution which may be attributed to reduction and oxidation of the ubiquinone remaining adsorbed on the TMFE surface following the transfer.

At the air–water interface in spite of adsorption, the dependence of the peak current on scan rate deviates from linearity. At higher scan rates the peak current is lower than theoretically predicted which suggests that the additional electron transfer requires time and occurs laterally between neighboring quinone redox centers. This effect was observed by Daifuku and co-workers for bipyridine osmium complexes in monolayer assemblies on SnO_2 electrode [18]. Because of the participation of protons in the overall ubiquinone reduction, the formal potential of the ubiquinone/ubiquinol system is pH dependent (Figure 4). Three regions can be distinguished, where the dependence on pH is different:

$$\mathrm{UQ} + 2\bar{e} + 2\mathrm{H}^+ \rightarrow \mathrm{UQH_2} \quad \mathrm{pH} < 13 \tag{1}$$

$$\mathrm{UQ} + 2\bar{e} + \mathrm{H}^+ \rightarrow \mathrm{UQH}^- \quad 13 < \mathrm{pH} < 13.5 \tag{2}$$

$$\mathrm{UQ} + 2\bar{e} \rightarrow \mathrm{UQ}^{2-} \quad \mathrm{pH} > 13.5 \tag{3}$$

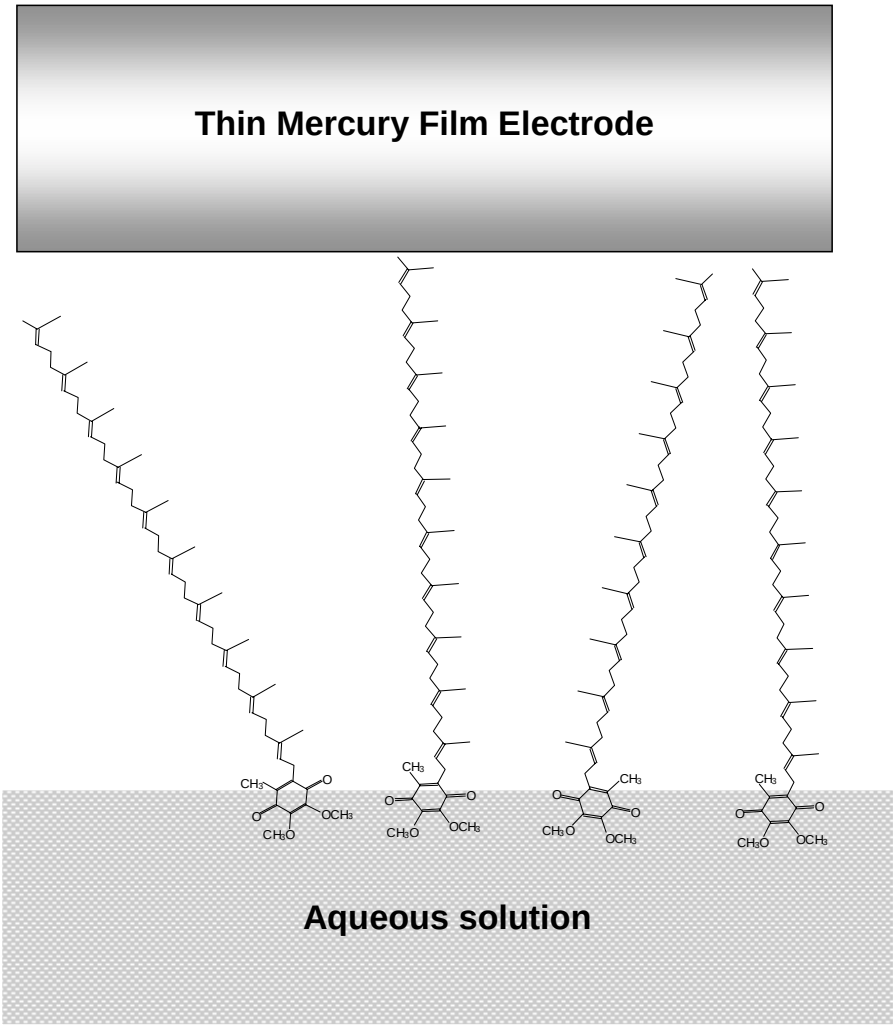

Figure 2. Experimental system for the horizontal touching cyclic voltammetry of ubiquinone placed at the air–water interface.

At pH higher than 13 the negative charge of the ubiquinone moiety is neutralized by cations from the supporting electrolyte solution (i.e., K^+). Assuming that changes in the slope of the formal potential-pH plots are connected with changes in the stoichiometry of the reaction, we can determine the pK_a values [19]:

$$UQH_2 \leftrightarrow UQH^- + H^+; \quad pK_{a1} = 13 \tag{4}$$

$$UQH^- \leftrightarrow UQ^{2-} + H^+; \quad pK_{a2} = 13.5 \tag{5}$$

The standard rate constant of ubiquinone reduction is dependent on pH. Using Laviron theory [20] the transfer coefficients were obtained from the slopes of the

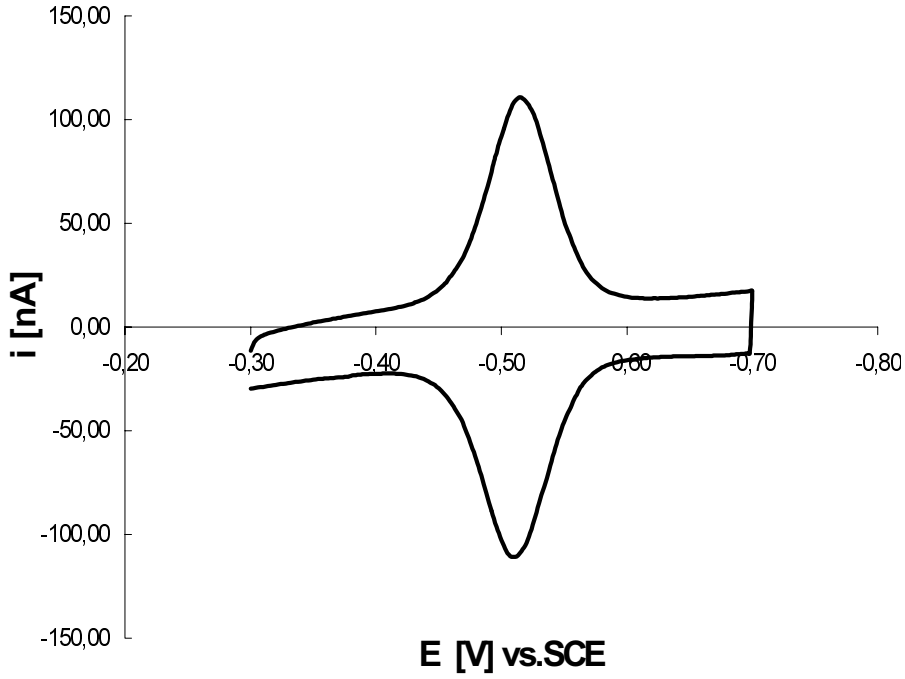

Figure 3. Cyclic voltammogram of the thin mercury film electrode in contact with a layer of ubiquinone placed at the air–water interface. Supporting electrolyte: 0.1 M KOH; pH = 13; scan rate v = 0.05 V/s.

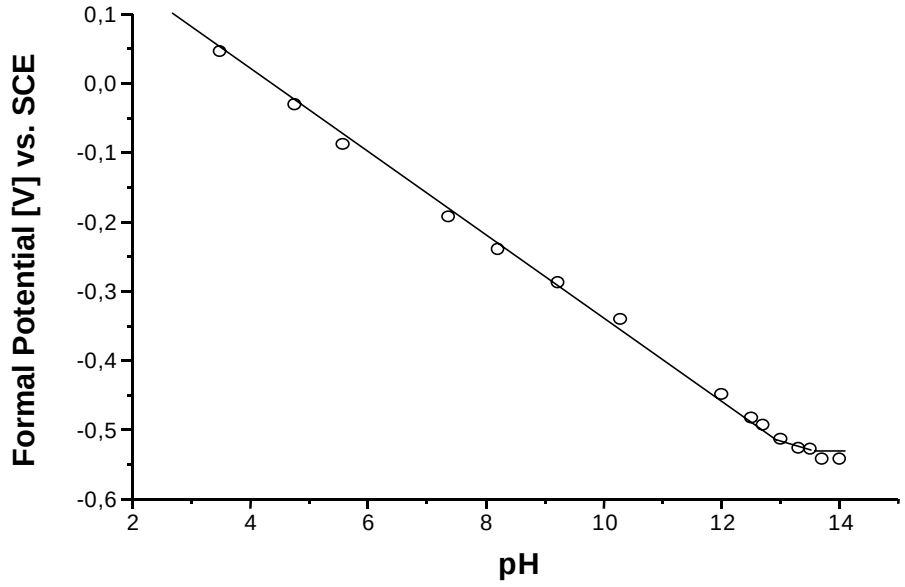

Figure 4. Dependence of the formal potential of the ubiquinone/ubiquinol system at the air–water interface on pH. Three regions can be distinguished with slopes equal to 0.06 V/decade; 0.03 V/decade and 0.00 V/decade. (Other conditions as in Figure 3).

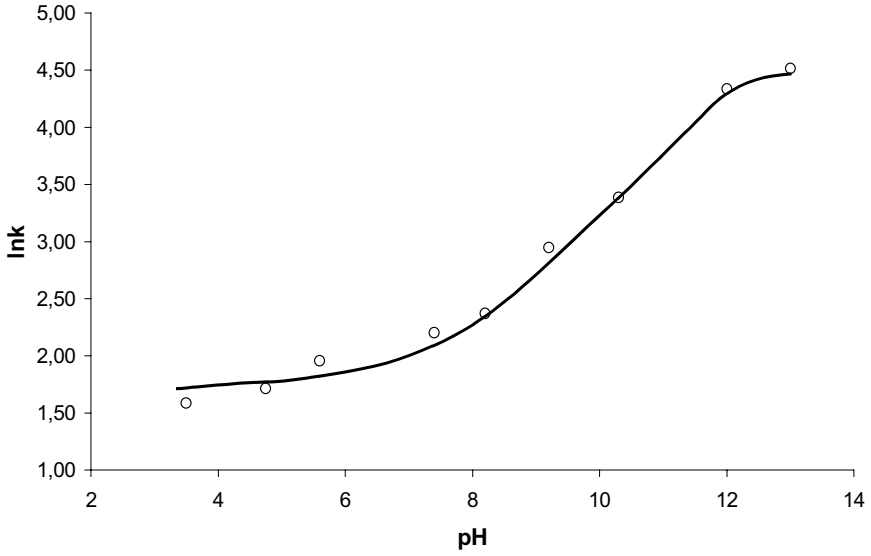

Figure 5. Dependence of the logarithm of standard rate constant on pH. (Other conditions as in Figure 3).

plot of peak potential vs. $\ln v$. The standard rate constants for various values of pH were calculated using the equation:

$$\ln k = \alpha \ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha)\frac{nF\,\Delta E_p}{RT}, \qquad \text{(I)}$$

where k is the standard rate constant; α and $(1-\alpha)$ are cathodic and anodic transfer coefficients respectively; v, scan rate; ΔEp, difference between peak potential of the anodic and cathodic peaks; R, T, n, F have their usual significance.

The dependence of $\ln k$ on pH is presented in Figure 5. The most significant changes of $\ln k$ are observed over the range of pH from 6 to 12. It indicates that in this pH range the protonation of the semiquinone radical anion is the rate determining step. The mechanism can be described by the following equations:

$$\mathrm{UQ} + \bar{e} \leftrightarrow \mathrm{UQ}^{\bullet -} \qquad (7)$$

$$\mathrm{UQ}^{\bullet -} + \mathrm{H}^{+} \rightarrow \mathrm{UQH}^{\bullet} \quad \text{rds} \qquad (8)$$

$$\mathrm{UQH}^{\bullet} + \mathrm{H}^{+} + \bar{e} \leftrightarrow \mathrm{UQH}_2. \qquad (9)$$

A similar behavior for ubiquinone was postulated by Moncelli and coworkers [21] in the lipid monolayer environment.

At pH above 12, changes of $\ln k$ are small, because of the low concentration of protons and a change in the mechanism of the process. A small increase of $\ln k$ can be caused by increasing the concentration of cations (K^+) in base electrolyte solution. At pH < 6 the protonation step described by Equation (8) does not determine

the rate of the overall reduction any more since the concentration of protons is in sufficient excess. The highest values of rate constants for ubiquinone reduction were found at pH above 13. Subsequent experiments were carried out at pH = 13 where the influence of protons on the redox process can be neglected.

The shape of the voltammograms allows us to estimate the parameters of interactions between the adsorbed molecules. Upon increasing the surface concentration of UQ10 the width of the peak at half height is increased. This effect is due to the presence of repulsive interactions between the redox centers of the molecular assembly contacting the electrode surface [22, 23]. These interactions diminish in mixed monolayers where the redox centers are well separated [24, 25]. In our case mixed molecular layers containing octadecanol and ubiquinone (1 mol%) were prepared, however, the value of peak half-widths remained always larger than predicted theoretically for a two electron process (45.2 mV) [22]. The value obtained in this study was 58 mV which indicates, that larger widths of reduction peaks may result not only from repulsive interactions, which should be decreased by diluting the layer with octadecanol. The additional reason is the sequential two electron transfer processes with similar but not identical formal potentials. This observation leads us to postulate that the radical anion is stable also at these conditions. The semiquinone radical anion is of course most stable in non-aqueous solutions [26].

An increase of surface concentration of UQ10 over the range from 8.7×10^{-12} to 1.92×10^{-10} mol/cm^2 leads to a decrease of the standard rate constant of electron transfer. The dependence of $\ln k$ vs. surface concentration is linear and can be described by the following equation:

$$\ln k = 5.38 - 2 \times 10^{10}\Gamma, \tag{II}$$

where Γ is the surface concentration of ubiquinone obtained from the cathodic peak area (anodic peak area was almost identical).

In the rate constant calculated on the basis of Laviron equation (Equation (I)) the presence of intermolecular interactions is neglected. The error due to this simplification becomes significant at higher electrode surface coverages. Therefore, the coverage dependent formal rate constant $k^{0\prime}$ would be described by the following equation [18]:

$$k^{0\prime} = (1/2)k \exp\{-\alpha(\lambda + \mu)\theta_T - (1 - \alpha)(\gamma + \beta)\theta_T\}, \tag{III}$$

where k is the rate constant described by Equation (I); μ, λ, γ, β are interaction coefficients independent of potential and θ_T is the total coverage of the electrode by oxidized and reduced forms.

4. Conclusions

The properties of ubiquinone placed at the air-water interface can be conveniently probed by voltammetry using the electrode horizontal touching method.

The dependence of the rate constant of electron transfer on pH suggests that at physiological pH the protonation step is rate determining. The highest value of rate constant was found at pH > 13.

Even in molecular assemblies where ubiquinone molecules are diluted with octadecanol, hence when the separation of redox centers is large, the value of the peak half-width is higher than the theoretically expected value of 45.2 mV. This observation is explained assuming two electron transfer processes taking place at similar but not identical potentials.

The linear dependence of the rate constant of electron transfer on the surface concentration of UQ10 indicates the presence of intermolecular interactions in the monolayer.

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