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Cathodic reduction of coenzyme Q_{10} on glassy carbon electrode in acetic acid–acetonitrile solutions

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Abstract

The electrochemical reduction of coenzyme CoQ_{10} and CoQ_0 on glassy carbon (GC) has been investigated in mixed solvent containing 80 vol. % acetic acid and 20 vol.% acetonitrile. A combination of cyclic voltammetry (CV) and rotating disk electrode technique (RDE) was employed to elucidate the mechanism of electrode processes. The results obtained were interpreted in terms of an E_rE_q mechanism involving the inverted ordering of formal potentials, i.e. $E_2^{0'} > E_1^{0'}$. The cathodic processes of both compounds consist of two successive one-electron one-proton steps, whereas the second electron transfer is thermodynamically more facile than the first. The processes occur with the generation of unstable semiquinone radicals as primary products. The results presented can help in explanation of the biochemical properties of CoQ_{10} in the living cell.

Keywords: Coenzymes Q_{10} and Q_0; Electroreduction; Cyclic voltammetry; Potential inversion; Digital simulation

1. Introduction

Coenzyme Q (CoQ_n) is the most popular redox-active quinone derivative with a variable number of isoprene units (n), ranging from zero to twelve (Fig. 1).

In long lived mammals, the predominant homologue is CoQ_{10} (ubiquinone, ubidecarenone) which fulfils several biological functions in a living cell. It participates in electron and proton transport and ATP synthesis in the mitochondrial respiratory chain [1]. The overall reduction process in the proton-containing medium occurs as follows:

$$CoQ_{10} + H^+ + e \rightleftarrows CoQ_{10}H^\bullet \quad (1a)$$

$$CoQ_{10}H^\bullet + H^+ + e \rightleftarrows CoQ_{10}H_2 \quad (1b)$$

In this process, ubiquinone is reduced to ubiquinole ($CoQ_{10}H_2$) *via* semiubiquinone radical. Coenzyme Q_{10} is situated in the central hydrophobic part of cellular membranes, i.e. between the double layers of phospholipid fatty acids [2]. This allows the membranes to keep their structures in good condition and prevents several compounds and ions (eg. H_2O, K^+ or Mg^{2+}) from getting out of the cell [3]. $CoQ_{10}H_2$, the fully reduced form of coenzyme Q_{10}, exists in relatively high concentrations in mitochondria and cellular membranes [2,4]. It reveals an antioxidant activity as scavengers of reactive oxygen species or lipid radicals, enhances the antioxidative effect of α-tocopherol by regenerating it from its oxidized form — α-tocopheroxyl radical [2,5–11], and thus protects cells against peroxidative damage. However, the antioxidative activity of $CoQ_{10}H_2$ was found to be lower in comparison to that of vitamin E [5,6,11]. Semiubiquinone may also interact with oxygen and generate superoxide anion. This suggests that the $CoQ_{10}/CoQ_{10}H^\bullet$ redox couple may exert both antioxidant and prooxidant effects depending on the concentrations of the oxidized and semireduced forms of coenzyme Q_{10}, as well as molecular oxygen and superoxide [6].

Great importance of CoQ_{10} and its homologues in biochemistry causes that the electrochemical properties of these compounds have been widely investigated. Because of the strong hydrophobility of coenzyme Q_{10}, its electrochemical investigations were performed mainly from adsorbed layers in aqueous solutions (biphase electrochemistry) using pyrolytic graphite [12,13], glassy carbon [14], carbon-paste [15] and

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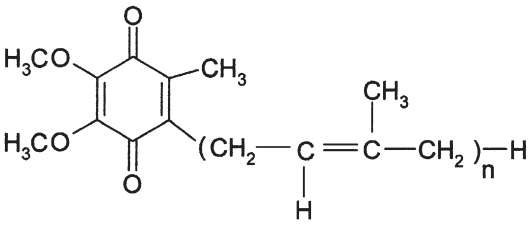

Fig. 1. Molecular structure of coenzymes Q (*n* ranging from 0 to 12).

mercury electrodes [16–18]. The authors proved that the mechanism of the cathodic reduction of CoQ strongly depends on pH. The present process occurs in two stages according to Eqs. (1a) and (1b) in acidic, neutral and weak alkaline solutions, and, moreover, is irreversible. As opposed to that, only one-stage two-electron reversible reduction was observed in strong alkaline medium [12,13]. According to Gordillo and Schiffrin [16] who investigated the electrochemical properties of CoQ_{10} adsorbed on mercury in contact with aqueous electrolytes at pH<12, a two-electron two-proton process occurs with $CoQ_{10}H^{\bullet}$ as a primary product. This radical is unstable and undergo disproportionation reaction with $K_{disp}=10^{14}$. For pH>12, the products of the reduction of ubiquinone are charged species, i.e. $CoQ_{10}H^{-}$ and CoQ_{10}^{2-}. The authors estimated the formal potentials of the $CoQ_{10}/CoQ_{10}H^{\bullet}$ and $CoQ_{10}H^{\bullet}/CoQ_{10}H_2$ (at −0.157 and 0.710 V vs. SCE, respectively). The authors stated, that the electrode process is reversible at low sweep rates and high values of pH. In acidic solutions the electrode reaction is less reversible. Electroreduction of CoQ_{10} incorporated in a self-assembled monolayer of dioleoylphosphatidylcholine (DOPC) deposited on a mercury electrode was investigated by Moncelli et al. [17] by chronocoulometric technique in phosphate and borate buffers over the pH range from 7 to 9.5. This model is very important, because it can explain the electron-and proton-transfering properties of the CoQ_{10} in biological membranes. The reduction of this compound to $CoQ_{10}H_2$ in DOPC monolayers take place *via* the reversible uptake of one electron with formation of semiubiquinone radical anion $CoQ_{10}^{\bullet-}$ followed by the rate determining protonation of this anion with $CoQ_{10}H^{\bullet}$ formation. This neutral radical is more easily reduced than CoQ_{10} yielding the ubiquinol. The investigations of CoQ_{10} reduction to $CoQ_{10}H_2$ were carried out by Moncelli et al. [18] at the same electrode by cyclic voltammetry and confirmed the conclusions drawn from chronocoulometric measurements [17]. The overall electrode process was regarded as consisting of series of consecutive electron-transfer and chemical steps. The chemical steps are protonation of the radical anion $CoQ_{10}^{\bullet-}$ by hydrogen ions in CoQ_{10} reduction and deprotonation of the radical cation $CoQ_{10}H_2^{\bullet+}$ in $CoQ_{10}H_2$ oxidation by hydroxyl ions with formation of the $CoQ_{10}H^{\bullet}$ neutral radical which is instantaneously oxidized to CoQ_{10}. These chemical reactions are rate determining steps in the overall process.

In organic solvents, such as methanol [19], acetonitrile [20,21], dimethylphormamide (DMF) and dimethylsulphoxide (DMSO) [22], coenzymes Q undergo two successive one-electron reductions to form radical anions and dianions [20,21]. If proton donors are present in aprotic solvents, then the electron transfers are followed by the homogenous protonation reactions [20,21].

Until now, the electrochemical behaviour of CoQ_{10} was not widely investigated in organic solvents of acidic properties. Literature data concerning the electrode reactions of ubiquinones in the presence of acetic acid have rather qualititative character and relate to CoQ_6 but not to CoQ_{10} [21]. The aim of this work is to investigate the electrochemical properties of CoQ_{10} and CoQ_0 in acetic acid solutions using cyclic voltammetry at glassy carbon (GC) electrodes. CoQ_0 was applied for comparison as a water soluble homologue of CoQ_{10}. Acetic acid exhibits an ability to dissolve both hydrophobic organic compounds and their matrix (e.g. vegetable oils) as well as necessary supporting electrolyte. This solvent can denature peptides and therefore, one of many components of matrix can be removed from the analyzed sample. This procedure can facilitate quantitative analysis of coenzyme Q_{10} in real samples. This paper is a logical starting point for the development of a new simple voltammetric method for the determination of CoQ_{10} in pharmaceutical dosage forms. In our recent works acetic acid was employed as a medium to investigate the anodic oxidation of tocopherols [23], synthetic antioxidants such as BHQ, BHA and BHT [24] and to the voltammetric determination of α-tocopheryl acetate in pharmaceutical dosage forms [25]. On the other hand, acetic acid exhibits a low dielectric constant (ε=6.15 at 25 °C [26]), which results in a small degree of dissociation of electrolytes. This exerts a disadvantageous influence on the IR potential. However, the electric conductivity can be enhanced to some extent by the addition of acetonitrile (ε=37.5 [26]) and by the use of reasonably high concentration of supporting electrolyte.

2. Experimental

2.1. Reagents

Chemicals used were coenzyme Q_{10} (CoQ_{10}) pract., >98%, coenzyme Q_0 (CoQ_0) pract., >99% (each Sigma–Aldrich), sodium acetate, CH_3COONa, anhydrous, fractopur (Merck). Acetic acid p.a. ACS, indifferent against chromic acid (Merck) and acetonitrile (AN) p.a. anhydride (Merck) were employed as solvents in all electrochemical experiments.

All freshly prepared solutions were kept in the dark and cool. Test solutions were deoxygenated before voltammetric measurements by ultrasonication and then by purging with a stream of solvent-saturated argon of high purity (>99.99%).

2.2. Apparatus

Voltammetric experiments were carried out with a three-electrode cell in which a glassy carbon (GC) electrode of 1 mm in diameter, $A=7.85\times10^{-3}$ cm^2 (Mineral, Warsaw) and a platinum wire were used as a working electrode and a counter electrode, respectively. Some experiments were performed with a home made rotating disk glassy carbon electrode, GC-RDE,

(A=0.287 cm^2). All potentials were measured and reported against the external silver chloride reference electrode with 1 M NaCl solution, which exhibits potential 22 mV more positive than the saturated calomel electrode with KCl solution. The reference electrode was isolated from the test solution by a frit of Vicor Glass. The surface of the working electrodes were polished on fine emery paper, and then with 0.3 μm alumina powder slurry on a polishing cloth. Finally, the electrodes were rinsed with water and dried before use.

All voltammetric experiments were performed using a Model EA9C electrochemical analyzer (Entech, Cracow) and controlled via Pentium computer using software EAGRAPH Version 4.0.

Electrochemical measurements were carried out at room temperature (25±1 °C).

Digital simulations were performed with DigiSim program (Bioanalytical Systems), version 3.03.

3. Results and discussion

Cathodic reduction of coenzyme Q_{10} was studied using the technique of cyclic potential-sweep voltammetry (CV). A 0.5 M sodium acetate was used as a supporting electrolyte. Such relatively high concentration of salt was needed to increase the electric conductivity of solutions and thus to diminish IR. The addition of acetonitrile (20 vol.%) to acetic acid was used for the same reason. The presence of AN in the solutions caused a considerable increase in specific conductivity compared with acetic acid (from 0.184 to 0.974 mS cm^{-1}). Preliminary tests showed that this solvent does not change the course of cyclic voltammetric curves for CoQ_{10} and CoQ_0. Fig. 2 presents typical CV curves recorded in the presence of the compounds and for supporting electrolyte alone.

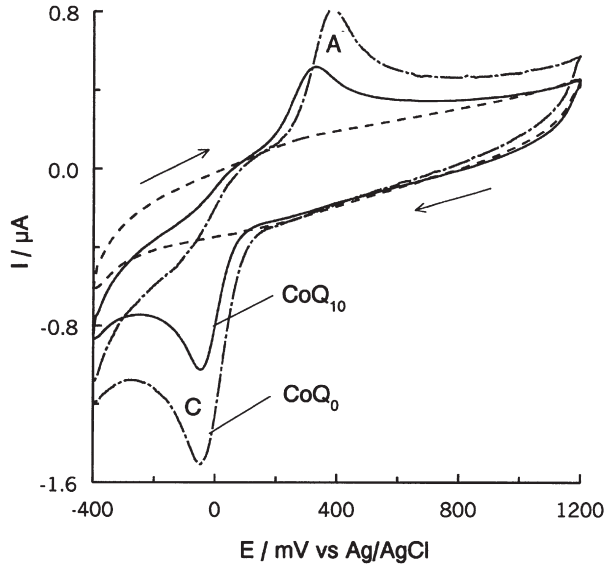

Fig. 2. Cyclic voltammetric curves obtained at GC electrode in acetic acid containing 0.5 mM CoQ_{10} or CoQ_0, 20% AN (v/v) and 0.5 M CH_3COONa. Dashed lines are residuals currents. Scan rate 50 mV s^{-1}. The potential scan started at 1200 mV in negative direction.

As can be seen, the single-stage reduction of CoQ_{10} and CoQ_0 on GC takes place, which results in a well-shaped peak C at the potential of about −50 mV. When the direction of polarization was reversed, one anodic peak A is observed. This well-shaped peak is situated at the potential of about 400 mV. A similar course of CV curves for both compounds confirms the earlier observations that the length of the side chain exerts no influence on the mechanism of the electrode process [12]. The considerably higher current of the reduction of CoQ_0 compared with that of CoQ_{10} results from a higher diffusion coefficient, which is consequently caused by the absence of a long side chain in the molecule (Fig. 1). It should be stressed that very good reproducibility of successively recorded curves was observed. A similar shape of curves was observed by Cauquis and Marbach [21] while investigating the reduction of coenzyme Q_6 in acetonitrile containing small quantities of water or acetic acid. They attributed the cathodic peak to the two-electron reduction of the coenzyme accompanied by the reaction of radical anion protonation (ECE mechanism). During the anodic polarization only one wide peak of the oxidation of anion QH^-, which is the final product of the reduction, was observed. Nevertheless, the authors did not give explicit evidence to confirm the mechanism proposed.

The heights of all peaks considered increase with an increase in the potential scan rate, v (Fig. 3A). Moreover, the cathodic peak potential, E_{pc}, shifts negative with the increase of v, whereas the anodic peak A shifts in oppositive direction.

The dependence of the cathodic peak current, I_{pc}, on $v^{1/2}$ is linear only for low v, below 100 mV s^{-1} (Fig. 3B). A deviation from the linearity is observed above this value. The nature of the dependence $I_p=f(v^{1/2})$ shows that the cathodic process approaches the reversible one at small v. Similar results were obtained during the reduction of CoQ_0.

Judging from Fig. 3, the current of anodic peak, A is considerably smaller than those of cathodic one, thus indicating that reduction product of CoQ_{10} can participate in the successive homogenous reaction. It is to note that I_{pa}/I_{pc} ratio increases with the increasing potential scan rate and thus the chemical reaction occurs shorter.

CV curves recorded with different turn back potentials E_λ (Fig. 4) can provide useful information on the mechanism of CoQ_{10} reduction. As seen in this figure, the height of the peak A increases with the increase of reduction time. Irrespective of the E_λ value, the anodic peak is considerably lower than its cathodic counterpart. This fact indicates that the primary product of the electron-transfer is chemically unstable and undergoes a follow-up heterogenous reaction, likely a disproportionation one.

In order to determine the number of electrons involved in the reduction of ubiquinone, n_{app}, the method based on the analysis of changes in the limiting current, I_L/A, during the electrolysis at the constant potential on the rotating disc electrode of glassy carbon (GC-RDE) was used. To shorten the time of electrolysis, the electrode of large surface (0.287 cm^2) was used. The current of the reduction of CoQ_{10} and CoQ_0 was recorded at the constant potential −300 mV that corresponds to the limiting currents of the reduction of these

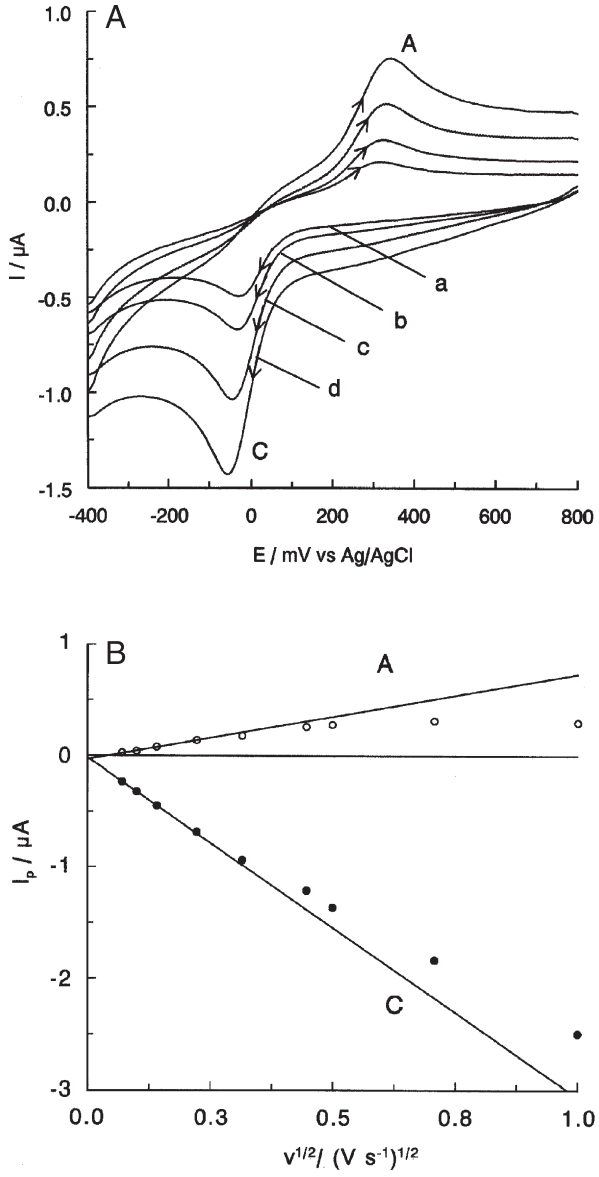

Fig. 3. (A) Cyclic voltammetric curves obtained at GC electrode in solutions containing 0.5 mM CoQ_{10} at different scan rates: a) 10, b) 20, c) 50 and d) 100 mV s^{-1}. Other components of the test solutions as in Fig. 2. (B) Relationship between cathodic (C) and anodic (A) peak currents (I_p) and square root of scan rate, $v^{1/2}$.

compounds. The results obtained were analysed using the following dependence [27]:

$$\log(I_L/A) = \log(I_{L,0}/A) - I_{L,0}t[2.303\ n_{app}FVc_0]^{-1} \quad (2)$$

where $I_{L,0}$/A is the initial limiting current at bulk concentration c_0/M, F is Faraday constant, V/l is the volume of the solution and t/s denote the time of electrolysis. Curves of the dependence of $\log(I_L/A)$ on time for the reduction of CoQ_{10} and CoQ_0 are shown in Fig. 5. According to the Eq. (2), slopes of the curves obtained are inversely proportional to the number of electrons involved, n_{app} and are 2.2 and 2.1, respectively for the reduction of CoQ_{10} and CoQ_0. It is to note that n_{app} does not significantly

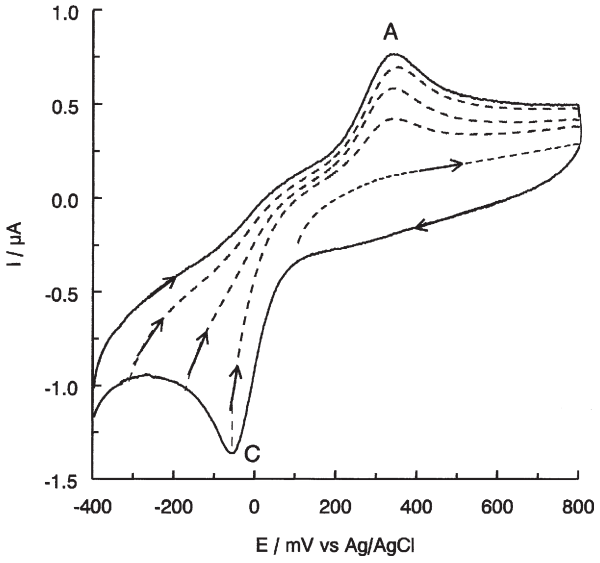

Fig. 4. Cyclic voltammetric curves obtained on GC electrode in solutions containing 0.5 mM CoQ_{10}. Other components of the solutions as in Fig. 2. Directions of electrode polarization were reversed from cathodic to anodic at potentials E_λ: −400, −300, −200, −50 and 50 mV. Scan rate 100 mV s^{-1}.

deviate from the ideal value of 2. This indicates that the cathodic peak C may be attributed to the two-electron reduction of the coenzymes.

In order to elucidate an exact mechanism of the electrode process, digital simulations were performed with DigiSim program. The voltammetric and amperometric data obtained for the CoQ_{10} reduction can be rationalized under assumption that the electrode process consist of two one-electron transfer reactions and occurs with the inversion of formal potentials [28–31]. The potential inversion takes place when the introduction of the first electron occurs at potentials more negative

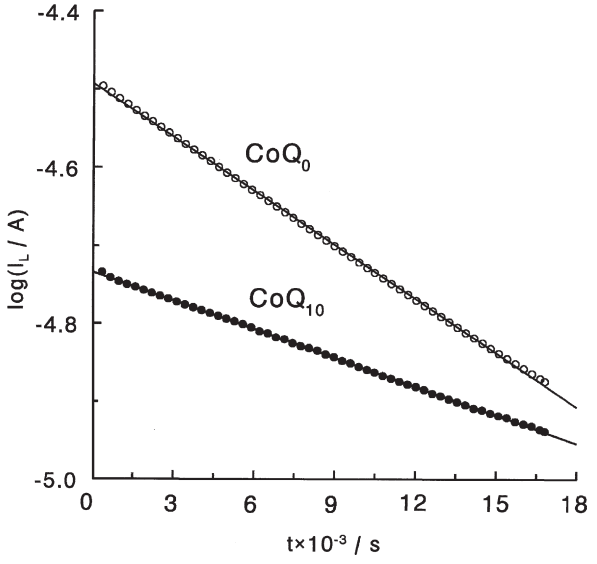

Fig. 5. Semi-logarithmic plots of the limiting currents against time during the cathodic reduction of 0.2 mM CoQ_{10} and CoQ_0 at a GC-RDE in CH_3COOH and AN (20% v/v) mixture containing 0.5 M CH_3COONa. Limiting currents were measured at −300 mV vs Ag/AgCl. Rotation frequency 8 Hz; solution volume 15 ml.

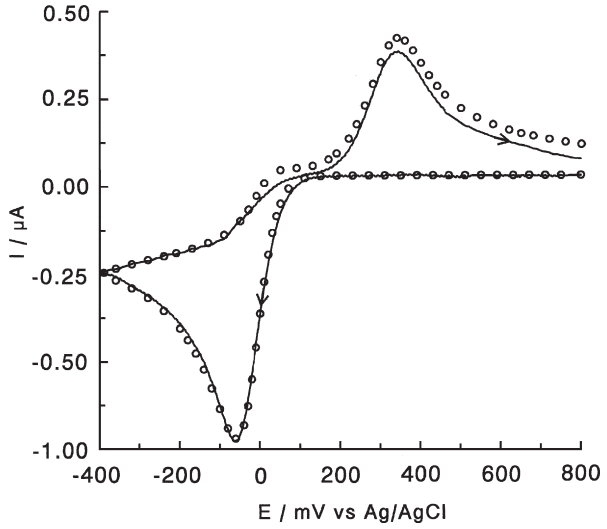

Fig. 6. Experimental (line) and simulated (circles) cyclic voltammograms of 0.5 mM CoQ_{10} in acetic acid+acetonitrile (20%, v/v) mixed solvent containing 0.5 M CH_3COONa. Potential scan rate 100 mV s^{-1}. The experimental curve was obtained upon substracting the residual current from the corresponding current in the presence of CoQ_{10}.

of that of the second electron transfer reaction, i.e. $E_1^{0'} < E_2^{0'}$. Under these circumstances, only a single cathodic peak, C, situated on the potential axis between the two formal potentials is observed during the negative-going potential scan [31]. Its height and shape are consistent with an overall two-electron reaction. On the other hand, the kinetics of the electron transfer reactions can exert a significant effect on the current response. Voltammograms presented in Fig. 3A correspond to the case when addition of the second electron occurs relatively sluggish [31].

A voltammogram (line) of 0.5 mM CoQ_{10} at 100 mV s^{-1} is shown in Fig. 6 along with simulation (circles) according to the E_rE_q mechanism. The experimental curve was obtained upon substracting the residual current from the corresponding current in the presence of CoQ_{10}.

The redox potentials, the standard electron transfer rate constants and the diffusion coefficients were optimized as follows:

$$E_1^{0'} = -15 \text{ mV}, k_{s1} = 1 \text{ cm s}^{-1}, \alpha = 0.5, D = 2 \times 10^{-6} \text{cm}^2 \text{ s}^{-1};$$
$$E_2^{0'} = 150 \text{ mV}, k_{s2} = 7 \times 10^{-5} \text{ cm s}^{-1}, \alpha = 0.5.$$

Due to similarity in size of the electrode reagents, their diffusion coefficients, D were considered to be equal. As seen in this figure, a good fit between the simulated and experimental data (background subtracted) was obtained. Considering the results presented, two electrons can be assumed to take part in the cathodic reduction as follows:

$$CoQ + e^- + HAc \rightleftarrows CoQH^{\bullet} + Ac^-(E_1^{0\prime}, k_{s1}) \quad (3)$$

$$CoQH^{\bullet} + e^- + HAc \rightleftarrows CoQH_2 + Ac^-(E_2^{0\prime}, k_{s2}) \quad (4)$$

CoQ is an oxidized form of coenzyme Q_{10} or Q_0, and HAc denotes acetic acid. The semiubiquinone radical produced in the reaction (3) undergoes electrochemical disproportionation:

$$2CoQH^{\bullet} \rightarrow CoQ + CoQH_2 \quad (5)$$

The electrochemical disproportionation constant [28]:

$$K_{disp} = [CoQ][CoQH_2]/[CoQH^{\bullet}]^2 \quad (6)$$

was calculated using the formula:

$$\ln K_{disp} = (F/RT)(E_2^{0'} - E_1^{0'}) \quad (7)$$

to give a value of $K_{disp} = 9.1\ (\pm 0.5) \times 10^2$. The probability of the participation of the disproportionation of the primary product of two-electron reduction of coenzyme Q in the presence of proton donors is confirmed by the literature data [6]. It also accompanies the cathodic reduction of 1,4-benzoquinone in acidified solutions of acetonitrile [32] and CoQ_{10} at pH < 12 in aqueous solutions [16].

4. Conclusions

The results presented show that the cathodic processes of coenzymes Q_{10} and Q_0 on a glassy carbon electrode in acetic acid are two consecutive one-electron one-proton reductions giving unstable semiquinone radicals ($CoQH^{\bullet}$) as primary products (E_rE_q mechanism coupled with protonation reaction). The existence of these radicals as a primary products of reduction of CoQ_{10} was confirmed by Gordillo and Schiffrin [16] in aqueous electrolytes at pH < 12. Similar courses of CV curves for CoQ_{10} and CoQ_0 indicate that the length of the side chain exerting no influence on the mechanism of the electrode process. Within the range of small scan rates of the potential, this process is reversible. Electrode reactions occur with the potential inversion i.e. $E_1^{0'} < E_2^{0'}$, which means that the second electron transfer is thermodynamically more facile than the first. Nevertheless, the kinetics of the second electron transfer is relatively sluggish. Consequently, semiquinone radicals can undergo the electrochemical disproportionation. This reaction results in a decrease of the anodic peak in comparison to the cathodic one. Possibility of appearance of this process at pH < 12 in aqueous solutions confirm the results obtained by Gordillo and Schiffrin [16]. The proposed mechanism of electrode reaction with CoQ_{10} and CoQ_0 is in good agreement with that obtained from adsorbed layers in aqueous solutions [16–18]. Similar CV behaviour of CoQ_6 were observed by Cauquis and Marbach [21] in acetonitrile containing small quantities of water or acetic acid. They attributed the cathodic peak to the two-electron reduction of the coenzyme accompanied by protonation of the radical anion (ECE mechanism). During the anodic scan one wide peak of the oxidation of anion QH^-, the final product of the reduction, was observed. However, the authors did not give any explicit evidence of the mechanism proposed.

The results presented show, that mixture of acetic acid and acetonitrile can be a good medium for voltammetric determination of CoQ_{10} in pharmaceutical dosage forms.

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