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Voltammetric determination of coenzyme Q_{10} in pharmaceutical dosage forms

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ABSTRACT

A simple and rapid voltammetric method has been developed for the quantitative determination of coenzyme Q_{10} (CoQ_{10}) in pharmaceutical preparations. Studies with differential pulse voltammetry (DPV) were carried out using a glassy carbon electrode (GCE) in a mixed solvent containing 80 vol.% acetic acid and 20 vol.% acetonitrile. A well-defined reduction peak of CoQ_{10} was obtained at −20 mV vs. Ag/AgCl. The voltammetric technique applied provides a precise determination of CoQ_{10} using the multiple standard addition method. The statistical parameters and the recovery study data clearly indicate good reproducibility and accuracy of the method. The accuracy of the results assessed by recovery trials was observed to be within the range of 101.1% to 102.5%. The detection and quantification limits were found to be 0.014 mM (12 mg L^{-1}) and 0.046 mM (40 mg L^{-1}), respectively. An analysis of real samples containing CoQ_{10} showed no interferences with common additives and excipients, such as unsaturated fatty acids and soya lecitine. The method proposed does not require any pretreatment of the pharmaceutical dosage forms. A spectrophotometric determination of CoQ_{10} in real samples diluted in mixtures containing ethanol and *n*-hexane was also performed for comparison.

1. Introduction

Coenzyme Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, CoQ_{10}) is a fat soluble, vitamin-like quinone commonly known as ubiquinone or ubidecarenone. This naturally occurring compound fulfils several biological functions in a living cell. It participates in electron and proton transport and ATP synthesis in the mitochondrial respiratory chain [1]. In this process, ubiquinone is reduced to ubiquinole ($CoQ_{10}H_2$) *via* semiubiquinone radical ($CoQ_{10}H^{\bullet}$). Ubiquinone is located in the cellular membranes and thus prevents several compounds and ions from getting out of the cell [2,3]. $CoQ_{10}H_2$, the fully reduced form of coenzyme Q_{10}, exerts important effects against the oxidative damage of polyunsaturated fatty acids. The antioxidative activity of $CoQ_{10}H_2$ was found to be lower in comparison with that of vitamin E [4–8]. In addition, the antioxidant functions of coenzyme Q_{10} reveals in the synergistic interaction with α-tocopherol by regenerating it from its oxidized form — α-tocopheroxyl radical [2,4,8–12], and thus protects cells against peroxidative damage. It is to be noted that CoQ_{10} is known to be the only endogenously synthesized lipid soluble antioxidant [13]. However, the biosynthesis of ubiquinone decreases with age and the deficiency of this species cause cardiac disorders, blood circulation and neurodegenerative diseases [13,14]. Therefore, their treatment involves pharmaceutical supplementation or an increased consumption of coenzyme Q_{10} with meals.

The great biochemical importance of coenzyme Q_{10} causes that the electrochemical properties of this compound have been widely investigated. Owing to the strong hydrophobicity of CoQ_{10}, its electrochemical investigations were performed mainly from adsorbed layers in aqueous solutions (biphase electrochemistry) using pyrolytic graphite [15,16], glassy carbon [17], carbon-paste [18] and mercury electrodes [19–21] or in such organic solvents as methanol [22], acetonitrile [23,24], dimethylphormamide (DMF) and dimethylsulphoxide (DMSO) [25]. A cathodic reduction of CoQ_{10} to $CoQ_{10}H_2$ in acidic, neutral and weak alkaline aqueous solutions occurs in two one-electron one-proton stages with $CoQ_{10}H^{\bullet}$ as a primary product. Moreover, it is irreversible. This radical is unstable and undergoes disproportionation. In contrast to that, only one-stage two-electron reversible reduction was observed in a strong alkaline medium [15,16]. The products of the reduction of ubiquinone in this medium are charged species, i.e. $CoQ_{10}H^{-}$ and CoQ_{10}^{2-}. The electroreduction of CoQ_{10} to $CoQ_{10}H_2$ incorporated in self-assembled phospholipids [20] or phosphatidylcholine [21] monolayers were investigated by Moncelli et al. in phosphate and borate buffers over the pH range from 7 to 9.5. The reduction of this compound takes place *via* the reversible uptake of one electron with the formation of semiubiquinone radical anion $CoQ_{10}^{\bullet-}$ followed by the rate determining the protonation of this anion with formation of $CoQ_{10}H^{\bullet}$. This neutral radical is more easily reduced than CoQ_{10} yielding ubiquinol. In organic solvents, ubiquinones undergo two successive one-electron reductions to form radical anions and dianions. If proton donors are present in aprotic solvents, then the electron transfers are followed by the homogenous protonation reactions [23–25].

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Recently, we investigated the cathodic reduction of coenzyme Q_{10} and CoQ_0 on a glassy carbon electrode (GCE) in acetic acid containing 20 vol.% acetonitrile (AN) and 0.5 M CH_3COONa as a supporting electrolyte using cyclic potential-sweep voltammetry (CV) [26]. The addition of acetonitrile and the use of reasonably high concentration of salt enhanced the electric conductivity of the solutions tested and diminished the *IR* potential. Preliminary tests showed that AN does not change the course of cyclic voltammetric curves. A single-stage cathodic reduction of CoQ_{10} and CoQ_0 was shown to occur, which results in a well-shaped peak at potentials about −50 mV vs. Ag/AgCl. When the direction of polarization was reversed, one anodic peak at about 400 mV was observed. Considering the results obtained, we claimed that the cathodic peak corresponds to the quasireversible, diffusion controlled by two consecutive one-electron one-proton steps giving unstable semiquinone radicals ($CoQ_{10}H^{\bullet}$) as primary products. 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, semiubiquinone radicals can undergo electrochemical disproportionation. This reaction results in a decrease of the anodic peak in comparison to the cathodic one.

Since ubiquinone is of great importance, it is necessary to investigate CoQ_{10} in real samples. This species has been determined mainly by high-performance liquid chromatography (HPLC) with different methods of detection: diode-array UV–Vis (DAD) [27–29], electrochemical (ECD) [29–33] or mass spectrometry (MS) [34]. The HPLC methods showed clear advantages over other techniques due to their separation ability and specify. Thus, they are regarded as suitable for the quantification of CoQ_{10} at low concentration in complicated matrix, e.g. human serum or plasma. The main problems involved in using such methods are the need for derivatisation or time-consuming multisolvent extraction procedures, slightly expensive instrumentation and high running costs. Being faster, simpler and less expensive than chromatography, spectrophotometry is also applied for the determination of coenzyme Q_{10}. In the case of real samples, the direct reading of absorbance from the spectra cannot give satisfactory results due to the large overlap of the spectra and requires an initial separation of analyte or an application of derivative spectrophotometry. The derivatisation of spectra eliminates the influence of the matrix and thus can be successfully applied to determine CoQ_{10} in pharmaceuticals and plasma [35,36]. European Pharmacopoeia [37] recommends the use of liquid chromatography with spectrophotometric detection at 275 nm or spectrophotometry for the identification and determination of CoQ_{10} in pharmaceutical dosage forms. Electroanalytical methods can be an alternative for HPLC and spectrophotometric techniques in the determination of CoQ_{10}. These techniques are not, however, often applied in laboratory practice. The main problems involved in using these methods are the strong hydrophobicity of the analyte and the flexibility to adsorption at the electrode. The solvent applied should dissolve both hydrophobic organic compounds and their matrix (e.g. vegetable oils), as well as a necessary supporting electrolyte. Long et al. [38] used simultaneous EPR/electrochemistry to determine CoQ_{10} in real samples diluted in ethanol–water mixtures. This method is based on the measurements of the radical intermediate (ubisemiquinone) formed during the reduction of CoQ_{10} at the silver electrode by *in situ* EPR spectroelectrochemical techniques. The ability of CoQ_{10} for the adsorption on mercury electrodes was applied to determine this analyte using differential pulse voltammetry [39,40]. In recent years Litescu et al. [41] proposed a simple, sensitive and accurate voltammetric method with the use of a glassy carbon electrode (GCE)) to determine CoQ_{10} in commercially available capsules. Experiments were carried out in a hexane/methanol (1:2 v/v) mixture containing 0.12 M H_2SO_4 as a supporting electrolyte. However, the determination of ubiquinone in real samples performed by the standard addition method required four consecutive steps of compound extraction with hexane, followed by the appropriate dilution.

To our knowledge, acetic acid has not been used as a medium to determine ubiquinone. In comparison to other organic solvents (eg. acetonitrile alone), this solvent exhibits an ability to dissolve both hydrophobic organic compounds and a large amount of their matrix (e.g. vegetable oils) as well as a necessary supporting electrolyte. In addition, acetic acid can denature peptides and, therefore, one of many components of matrix can be removed from the analyzed sample. This procedure can facilitate the quantitative analysis of real samples. In addition, this solvent guarantees a good stability of the analyte and their matrix. In our recent works acetic acid was successfully employed as a medium to investigate the anodic oxidation of tocopherols [42], synthetic antioxidants such as BHQ, BHA and BHT [43] and in the voltammetric determination of α-tocopheryl acetate in pharmaceutical dosage forms [44].

Recently the cathodic reduction of coenzyme Q_{10} and CoQ_0 on a glassy carbon electrode (GCE) in acetic acid containing 20 vol.% acetonitrile and 0.5 M CH_3COONa as a supporting electrolyte was reported [26]. Considering obtained results, a simple, rapid, reproducible and accurate voltammetric method was developed to determine of CoQ_{10} in pharmaceutical preparations. The results were compared with those obtained using the spectrophotometric method according to the European Pharmacopoeia [37] procedure.

2. Experimental

2.1. Reagents

Chemicals used were coenzyme Q_{10} (CoQ_{10}) pract., >98%, (Sigma-Aldrich), sodium acetate, CH_3COONa, anhydrous, fractopur (Merck) and oleic acid p.a. (Fluka). 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. Solutions for spectrophotometric measurements were prepared using ethanol, dried, >99.8% (Merck) and *n*-hexane ultra resi-analyzed (Baker).

Soft gel capsules from Vita Care (Jeno-Pharm A/S Stege, Danmark), Bioqunone (Pharma Nord Aps, Danmark), Walmark (Chech Republik), Naturell AB (Swedish) and Naturcaps (Hasco-Lek S.A., Poland) were obtained from commercial sources. Each capsule contained 10, 15 or 30 mg of coenzyme Q_{10} mixed with different excipients such as soya lecithin, soya oil, glycerol, gelatine, glucose syrup and magnesium stearate. Two preparations (Bioquinone and Naturell AB) additionally contained α-tocopherol as an antioxidant.

2.2. Apparatus

Voltammetric experiments were carried out with a three-electrode cell in which the glassy carbon electrode (GCE) of 1 mm in diameter, $A = 7.85 \times 10^{-3}$ cm^2 (Mineral, Warsaw) and platinum wire were used as a working electrode and a counter electrode, respectively. All potentials were measured and reported against the external silver chloride reference electrode with 1 M NaCl solution. The reference electrode was isolated from the test solution by a frit of Vicor Glass. The surface of the working electrode was polished on fine emery paper, and then with 0.3 μm alumina powder slurry on a polishing cloth. Finally, the electrode was rinsed with water and dried before use.

All voltammetric experiments were performed using a Model EA9C electrochemical analyzer (Entech, Cracow) and controlled *via* PC computer using software EAGRAPH Version 4.0. The software used was equipped with a program for semiautomatic determination of the quantification limit based on the error propagation method [45]. Another program provided an analytical determination by the standard addition technique.

The absorption spectra were recorded with a double-beam Specord M500 UV–Vis spectrophotometer (Carl Zeiss, Germany) equipped with quartz cells of 1 cm light path and connected to the computer for data acquisition. The decomposition of spectra of real samples into

appropriate constituents and the isolation of spectra corresponding to CoQ_{10} was carried out using program Origin.

All measurements were carried out at room temperature (22±1 °C).

2.3. Preparation of test solutions

Standard solutions for electrochemical experiments were prepared by dissolving a suitable amount of coenzyme Q_{10} in a mixed solvent containing 80 vol.% glacial acetic acid and 20 vol.% acetonitrile. The stock solution was stored in the dark and cool. An aliquot of this solution was appropriately diluted with the same mixed solvent and CH_3COONa was added as a background electrolyte. The final sample solutions contained CoQ_{10} in the concentration range of 0.36–1.0 mM and 0.5 M CH_3COONa.

Solutions for testing the proposed voltammetric determination of coenzyme Q_{10} using multiple standard addition method were prepared in 100 mL volumetric flask by dissolving about 30 mg of the analyte in a mixed solvent containing 0.5 M CH_3COONa as well as about 500 mg of oleic acid taken as a hydrophobic matrix for CoQ_{10}. The amount of oleic acid corresponds with the mean concentration of excipients accompanying CoQ_{10} in real samples.

The solutions of real samples were prepared from commercially available capsules containing CoQ_{10} and other components. Since the CoQ_{10} concentration in capsules is usually high, only one capsule was opened with a scalpel, and its content was quantitatively transferred into a 100 mL volumetric flask and dissolved in a mixed solvent containing 0.5 M CH_3COONa. The content of the flask was sonicated for about 15 min and then diluted to the mark with the same mixed solvent. In order to remove insoluble particles and denaturated peptides from the solutions, they were next filtrated through an ordinary filtration paper. The solutions were directly analyzed according to the proposed procedure without the need for another pretreatment or extraction steps. All stock solutions were stored 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%).

Solutions of real samples for spectrophotometric determinations were prepared according to the European Pharmacopoeia [37]. The content of one capsule was quantitatively transferred into a 50 mL volumetric flask, 1.0 mL of hexane was added and diluted to the mark with ethanol. The content of the flask was sonicated for about 15 min. The solutions were next filtrated through an ordinary filtration paper. In order to measure the absorbance not exceeding about 0.6, the solutions were diluted to suitable concentration with ethanol.

3. Results and discussion

In our recent publication [26] we reported that CoQ_{10} is reduced at GCE in acetic acid containing 20 vol.% AN and 0.5 M CH_3COONa as a supporting electrolyte. Several concentrations of AN were investigated, and, it was found that 20 vol.% of this solvent in connection with the salt of reasonably high concentration provided good electric conductivity of the solutions and sufficient solubility of the hydrophobic components. A larger amount of AN limited the solubility of the analyte, especially in the presence of a large amount of their matrix. Preliminary investigations showed that sodium acetate is a good supporting electrolyte for the reduction of coenzyme Q_{10}. In comparison to $NaClO_4$, this salt provides very good reproducibility of the results and stability of the solutions of CoQ_{10}, especially of the real samples containing the hydrophobic matrix. Voltammetric curves recorded in the presence of sodium perchlorate were not reproducible. Changes in the peak potentials and peak currents were observed. This may result from the chemical oxidation of the components of the solutions by perchlorate ions. This process arises from the high oxidative power of these ions. Because the lack of the reproducibility may be due to changes in the composition of solutions, CH_3COONa, instead of $NaClO_4$, was used as a supporting electrolyte. A well-defined and highly reproducible peak at about −50 mV vs. Ag/AgCl is observed on cyclic voltammetric curves (Fig. 1) in the presence of sodium acetate. This peak is attributed to two consecutive one-electron one-proton electrode reactions.

Following this process, a new voltammetric method was developed to determine CoQ_{10} in pharmaceutical preparations. In order to enhance rapidity, sensibility and selectivity of the proposed method, differential pulse voltammetry (DPV) was applied. This technique is considered a convenient method because of the wide range of linearity, excellent reproducibility, low experimental costs and the attainment of low quantification limit [46]. In DPV, the peak current increases with increasing the pulse amplitude (ΔE) and this improves the voltammogram shape, but the peak width also increases and diminishes the signal resolution. The pulse amplitude of 20 mV was chosen in order to obtain good sensibility and selectivity of the method. A scan rate of 20 mV s^{-1} and the pulse width 80 ms was chosen as a compromise between fast recording and good resolution. As can be seen in Fig. 1, using this technique, a well-defined one cathodic peak attributed to the reduction of CoQ_{10} is observed. This peak is situated at potential −20 mV vs. Ag/AgCl and corresponds with the peak observed using the cyclic voltammetry (CV).

The applicability of the proposed voltammetric method to determine CoQ_{10} was examined by measuring the peak current as a function of concentration of the analyte. The linearity was checked by preparing standard solutions for 13 different concentrations of CoQ_{10}. As can be seen from Fig. 2, the height of this peak increases with increasing the concentration of the analyte. It is to be noted that the peak potential is concentration independent and the peak currents observed were highly reproducible. The RSD did not exceed 3.5% for $n=10$. As can be seen from Fig. 2, the peak corresponding to the reduction of CoQ_{10} is preceded by a broad peak at about 300 mV. This peak is independent of the concentration of CoQ_{10} and exists in all recorded curves, in a residual current as well (dashed line in Figs. 2 and 3). Its origin is not clear, but it can probably be connected with the presence of acetate anions in the solutions. It should be noted that this peak occurs at the curves recorded at a platinum working electrode in the same potential range. In our previous investigations with the use of electrochemical quartz crystal microbalance (EQCM) [47] we claimed that these ions strongly adsorb on the surface electrode and desorbs in the potential range where the peak mentioned exists. It seems probable that acetate anions adsorb at GCE at the initial potential of recorded curves. The cathodic polarization of this electrode causes desorption and formation of the observed broad peak at about 300 mV.

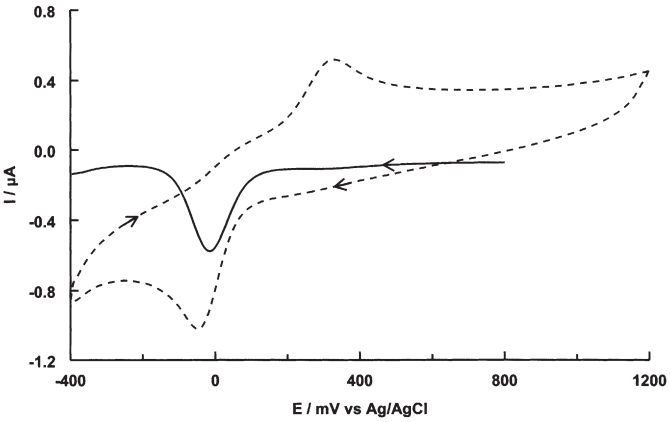

Fig. 1. Voltammograms of 1 mM CoQ_{10} recorded at GCE (1 mm diameter) in acetic acid containing 20 vol.% acetonitrile and 0.5 M CH_3COONa (a) by CV, $v=50$ mV s^{-1} and (b) by DPV, $\Delta E=20$ mV, $v=20$ mV s^{-1}.

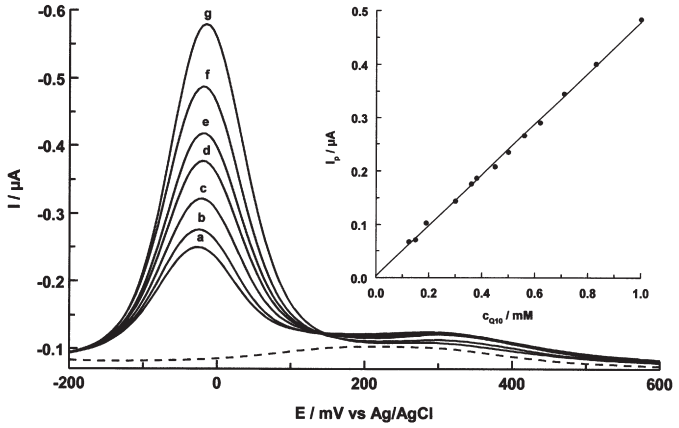

Fig. 2. Cathodic reduction curves of CoQ_{10} recorded at GCE (1 mm diameter) in acetic acid containing 20 vol.% acetonitrile and 0.5 M CH_3COONa by DPV (ΔE=20 mV, v=20 mV s^{-1}) at different concentrations of analyte: (a) 0.36, (b) 0.42, (c) 0.50, (d) 0.62, (e) 0.71, (f) 0.83 and (g) 1.0 mM. Dashed line is residual current. Inset: calibration plot for CoQ_{10} obtained by DPV technique.

The calibration graph of the peak current versus concentration was constructed using data from these measurements and the least-squares were evaluated using the linear regression method. The results of the measurements are summarized in Table 1.

The linearity range was found to be up to 1 mM with the coefficient r=0.9989 (inset in Fig. 2). The calculated limit of the detection value, LOD=0.014 mM (12 mg L^{-1}) with RSD=6.3% was obtained as a S/N ratio to be 3 [46]. The limit of the quantification (LOQ) value was determined using a program for the semiautomatic determination of the quantification limit based on the error propagation method [45]. The LOQ value calculated in this way was 0.046 mM (40 mg L^{-1}) with RSD=3.8%.

To develop a reliable voltammetric method, a test solution containing CoQ_{10} and oleic acid taken as a matrix was prepared as described above. 5 mL of this solution containing a known amount of the analyte was added to the electrolytic cell. Then, the electrodes were immersed and differential pulse voltammograms were recorded. The multiple standard addition method was applied by spiking (four spikes for each sample) small volumes of the standard solution of CoQ_{10} (0.376 mg $spike^{-1}$). Before the addition of the standard solution and after each spike, DPV voltammograms were recorded. Three measurements were performed in the absence and in the presence of

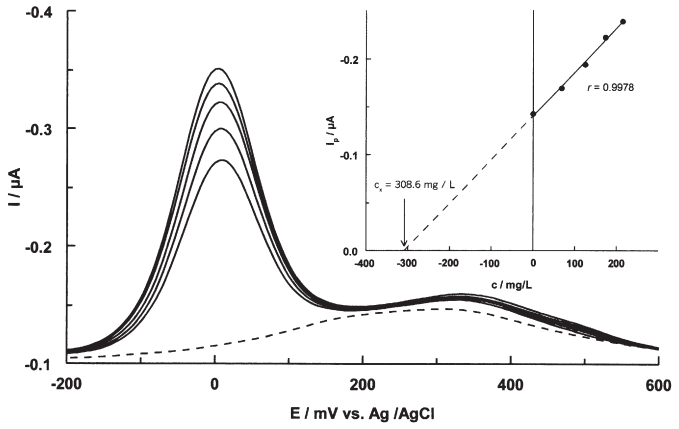

Fig. 3. DPV voltammograms of 309.0 mg/L (the lowest current peak) and after spiking the sample 0.376 mg of CoQ_{10} each time. Test solution contained 0.5 M CH_3COONa and 5 g L^{-1} oleic acid (added as a matrix). Dashed line is residual current. Inset: calibration plot for the quantification of CoQ_{10} in test solution. Arrow indicates amount of analyte determined in this medium.

Table 1
Characteristic of CoQ_{10} calibration plot in acetic acid containing 20 vol.% acetonitrile and 0.5 M CH_3COONa at glassy carbon electrode using DPV technique

Parameter	
Regression equation	I_p (µA)=0.475c (mM)+0.003
Standard error of slope	0.006
Standard error of intercept	0.004
Correlation coefficient, r	0.9989
Linearity range, mM	0.12–1.0 (100–860 mg/L)
Number of data points	13
LOD, mM	0.014 (12 mg/L)
LOQ, mM	0.046 (40 mg/L)

the added standard. Only the mean of measured currents was considered. The reduction peak currents were measured from residual current baseline. This current was estimated from the voltammogram of oleic acid sample without CoQ_{10}. Fig. 3 presents responses obtained and the resulting calibration plot (inset). Changes in the solution volume were taken into account. As can be seen, the calibration plot yields good linearity, with a regression coefficient of 0.9982. It is to be noted that very good reproducibility of the peak potential and the peak current were observed. The peak potential was stable and the RSD of peak currents did not exceed 4% for n=10 and for different concentrations of ubiquinone.

The slope of the standard addition calibration plot was (0.391 ± 0.014) (µA mM^{-1}), which is lower than that of the standard calibration plot (see Table 1) thus revealing the matrix effect. Next, the concentration of coenzyme Q_{10} was determined by linear regression using a program used in the analytical determination by the standard addition technique. The determinations were performed for five independent measurements. The statistic analysis of CoQ_{10} determination in a synthetic mixture was given in Table 2. The results are given with a confidence limit calculated for Student's t-test coefficient equal to 95%. The accuracy of the analysis, named a recovery trial (%R) was determined by calculating the relative error between the measured mean and the concentrations involved. The results obtained are in good agreement with a real amount of the analyte in solutions tested. The results obtained indicate a good accuracy of the methodology proposed.

The utility of the developed method was further tested by determining CoQ_{10} in five commercially available soft gel capsules containing different amounts of the analyte. The preparation of the solutions tested was previously described. The analytical procedure was the same as for the synthetic mixture. Voltammograms recorded in real solutions are presented in Fig. 4. As can be seen, the peak potentials changes in comparison with the standard. All these peak potentials are shifted into the negative direction of the electrode potential. This indicates that the cathodic reduction of CoQ_{10} in real samples is hindered by excipients accompanying the analyte. Differences in peak potentials result from the different composition of the matrix. When pharmaceutical preparations contained the same components, peak potentials were identical (curves c and d in Fig. 4). Apart from this,

Table 2
Statistic analysis of assay of CoQ_{10} in synthetic mixtures determined using differential pulse voltammetry (DPV)

CoQ_{10} added, mg	CoQ_{10} found, mg
30.90	30.86
30.90	31.02
30.90	30.98
30.90	31.15
30.90	31.09
Mean, mg	31.02
Standard deviation, mg	0.11
RSD,%	0.36
95% Confidence limit, mg	0.14
%R[a]	100.4

[a] %R=(mean/added)×100%.

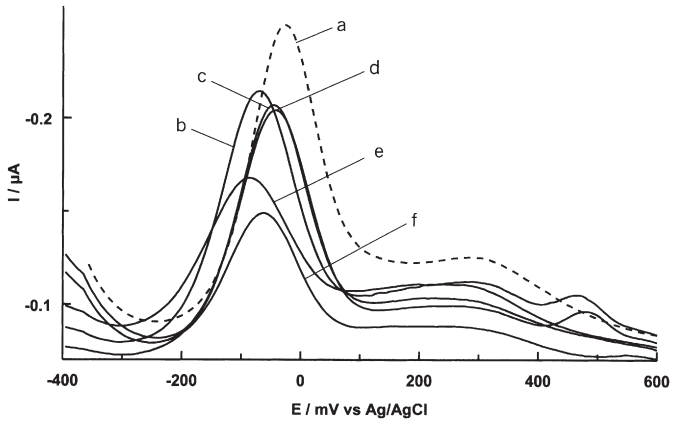

Fig. 4. DPV voltammograms for CoQ_{10} recorded in solutions of (a) standard 310 mg L^{-1} and of real samples containing this coenzyme: (b) Bioquinone (300 mg L^{-1}), (c) Naturcaps (300 mg L^{-1}), (d) Naturell (300 mg L^{-1}), (e) Walmark (150 mg L^{-1}) and (f) Vita Care (100 mg L^{-1}) Other parameters as in Fig. 2.

appropriate peaks increased linearly with the addition of the standard solution. Two additional sharp peaks at the potential about 500 mV can be seen in Fig. 4 (curves b and d). The existence of these peaks can be attributed to the reduction of the oxidized form of α-tocopherol. This compound is present in two preparations investigated and fulfils the antioxidative function preventing the oxidation of hydrophobic components of the drug. In our recent work [42] we claimed that this antioxidant undergoes reversible oxidation in acetic acid in this potential range.

The precision and accuracy of the proposed method were investigated by intra-day and inter-day determinations of CoQ_{10} in samples tested. Intra-day accuracy and precision were evaluated by the analysis of five samples with five determinations per sample in the same day. The inter-day studies were performed for five days over two weeks (after 1, 5, 8, 11 and 14 days of storage in a refrigerator at 4 °C). The precision of the analysis was expressed as a relative standard deviation (%RSD) and the accuracy, named a recovery trial (%R), was determined by calculating the relative error between the measured mean and the concentrations labeled. The results obtained for intra-day and inter-day precision and accuracy are presented in Table 3. As can be seen, the RSD values of the measurements were not greater than 1.17% and 1.23% for intra-day and inter-day determinations, respectively, and the accuracy of determination was not different from labeled values by more than 2.7%. These results confirm the good precision and accuracy of the proposed method and indicate that there is no interference from the common excipients used in the pharmaceutical dosage forms. Both the intra-day and inter-day reproducibilities of the voltammetric method were fairly good. This indicates that solutions containing pharmaceutical preparations remained stable for at least two weeks of storage at 4 °C. In this period no changes were observed in the peak potentials and peak currents. The peak potentials were stable and RSD of reduction currents of CoQ_{10} in all samples did not exceed 4% for $n=10$. Additional results obtained by a repeated recording of voltammetric curves in mixtures stored for over two weeks showed a gradual loss of reproducibility. This may be due to changes in the composition of solutions resulting in the oxidation of their components. Therefore, for practical reasons the solutions can be kept in a refrigerator up to 2 weeks before analysis.

For comparison, the quantitative determination of CoQ_{10} in pharmaceutical dosage forms was also performed by the spectrophotometric method according to the procedure recommended by the European Pharmacopoeia [37]. The preparation of test solutions was described above. The spectra of real samples were recorded in ethanol containing small amounts of hexane. The direct reading of absorbance corresponded to CoQ_{10} is impossible due to the large overlap of the spectra of the components. In order to eliminate the influence of the matrix, the spectra were mathematically transformed using a program Origin. The spectra corresponded to CoQ_{10} were analyzed at 275 nm. The content of the coenzyme was calculated taking the specific absorbance to be 169 [37]. This value corresponds with the concentration of CoQ_{10} equal to 10 g L^{-1}. Five independent determinations were performed for all samples. The results were statistically examined in the same way as for voltammetric determinations and are presented in Table 4.

It should be stressed that the results obtained using the voltammetric method are in good accordance with those obtained by the spectrophotometric method and with the data declared by the manufacturer. The statistical comparison of the values obtained by these methods for the determination of CoQ_{10} was done by Student's *t*-test and the variance ratio *F*-test. As can be seen from Table 4, the experimental *t*-values at $P=0.05$ for samples A, C and D did not exceed the theoretical ones. This indicates that there are no significant differences between the results obtained by the proposed voltammetric and reference spectrophotometric methods. Only in the two preparations investigated (B and E) statistical differences between the means were observed. These differences may arise from an especially large overlap of the spectra of the components in these preparations. This causes the difficulties in the isolation of spectra corresponding to CoQ_{10}, and, thus the results of these spectrophotometric determinations can be less accurate. A comparison of the calculated *F*-values with tabulated ones indicates that there are no significant differences in precision between the methods (samples B, C and D) or that the voltammetric

Table 3
Summary of the accuracy and precision of CoQ_{10} determination in commercial capsules by differential pulse voltammetry (DPV)

Sample[a]	Amount labeled (mg)	Intra-day ($n=5$)			Inter-day[c] ($n=5$)		
		Amount found[b] (mg)	*R* (%)	RSD (%)	Amount found (mg)	*R* (%)	RSD (%)
A	30	30.33±0.30	101.1	0.80	30.25±0.31	100.8	0.95
B	30	30.61±0.44	102.0	1.17	30.51±0.47	101.7	1.23
C	30	30.76±0.17	102.5	0.45	30.82±0.23	102.7	0.58
D	15	15.11±0.08	100.7	0.43	15.19±0.10	101.3	0.61
E	10	10.18±0.10	101.8	0.76	10.23±0.15	102.3	0.83

[a] A — Naturell, B — Naturcaps, C — Bioquinone, D — Walmark, E — Vita Care.
[b] $x=\bar{x}\pm t_{0.95}S_{\bar{x}}$ for $n=5$ and $t_{0.95}=2.776$ (tabulated). $S_{\bar{x}}$ denote standard deviation of mean.
[c] Inter-day precision was determined from five different runs over a 2-week period.

Table 4
Results of the CoQ_{10} determination in commercial capsules by differential pulse voltammetry (DPV) compared with a reference spectrophotometric method

Real sample[a]	Amount labeled (mg)	Amount found (mg)					
		DPV method[b]	RSD (%)	Reference method	RSD (%)	*t*-test[c]	*F*-test
A	30	30.33±0.30 (101.1%)	0.80	30.37±0.89 (101.2%)	2.37	0.1180 (<2.306)	8.779 (>6.388)
B	30	30.61±0.44 (102.0%)	1.17	29.78±0.58 (99.3%)	1.63	3.077 (>2.306)	1.837 (<6.388)
C	30	30.76±0.17 (102.5%)	0.45	30.67±0.33 (102.2%)	0.88	0.6630 (<2.306)	3.803 (<6.388)
D	15	15.11±0.08 (100.7%)	0.43	15.25±0.22 (101.7%)	1.11	1.728 (<2.306)	6.760 (>6.388)
E	10	10.18±0.10 (101.8%)	0.76	10.33±0.11 (103.3%)	0.97	2.650 (>2.306)	1.677 (<6.388)

The quantities of the drugs expressed as percentage of the label claim (%*R*) are given in parentheses.
[a] A — Naturell, B — Naturcaps, C — Bioquinone, D — Walmark, E — Vita Care.
[b] $x=\bar{x}\pm t_{0.95}S_{\bar{x}}$ for $n=5$ and $t_{0.95}=2.776$ (tabulated). $S_{\bar{x}}$ denote standard deviation of mean.
[c] Values in parenthesis are tabulated *t* and *F* at $P=0.05$.

method is more precise (A and E). However, the relative standard deviations (RSD) of the spectrophotometric results were considerably higher compared with those obtained by the DPV technique. Differences in the amounts labeled and measured means (%R) were usually higher for the reference method. These results indicate that the accuracy and the precision of the DPV method are slightly better than those of the spectrophotometric one. In addition, the reference method is considerably more laborious (with respect to the preparation of a sample and the need of the separation of the spectra corresponded to CoQ_{10}) than the developed method.

4. Conclusions

The electrochemical method using differential pulse voltammetry at a glassy carbon electrode based on the cathodic reduction of coenzyme Q_{10} was applied in the determination of this species in pharmaceutical dosage forms. The principal advantages of the proposed method over the already published chromatographic and spectrophotometric procedures are the use of nontoxic mixture of solvents containing 80 vol.% acetic acid and 20 vol.% acetonitrile and the rapidity of analysis that involves no sample preparation other than dissolving samples and filtration in order to remove insoluble particles. The analysis of the real samples containing CoQ_{10} showed no interferences with common additives and excipients, such as unsaturated fatty acids, soya lecithin, glycerol and glucose syrup. In addition, acetic acid can denature peptides and therefore one of many components of the matrix can be removed from analyzed samples. This procedure facilitates the quantitative analysis of real samples. The results obtained using voltammetric method are in good accordance with those obtained by the spectrophotometric method and with the data declared by manufacturers. The proposed DPV method is slightly more precise. In addition, the spectrophotometric method is considerably more laborious because of its need of separation of the spectra corresponded to CoQ_{10} or the initial separation of the analyte from the matrix in time-consuming extraction steps. Consequently, the above-presented method is sensitive, rapid, reliable, simple to perform and suitable to determine coenzyme Q_{10} in pharmaceutical preparations.

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