Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 10 (2014) 173 – 178

Procedia Chemistry

XV International Scientific Conference "Chemistry and Chemical Engineering in XXI century" dedicated to Professor L.P. Kulyov

# Investigation of Coenzyme $Q_{10}$ by Voltammetry

E.V. Petrova[a*], E. I. Korotkova[a], B. Kratochvil[b] , O.A. Voronova[a], E.V. Dorozhko[a], E. V. Bulycheva[a]

[a]*National Research Tomsk Polytechnic University, 30 Lenin ave., 634050, Russia*

[b] *Institute of Chemical Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic*

**Abstract**

A simple, accurate and rapid voltammetric method has been developed for the quantitative determination of coenzyme $Q_{10}$. Studies with direct current voltammetry were carried out using a glassy carbon electrode (GCE) in a phosphate buffer solution (pH 6.86). A well-defined oxidation peak of $CoQ_{10}$ was obtained at -0.600 V vs Ag/AgCl. The magnitude of the oxidation peak current has been found to be related to the concentration of the coenzyme over the range of ($2.0 \cdot 10^{-5}$ to $2.0 \cdot 10^{-4}$ M) (r = 0,991). Antioxidant activity of $CoQ_{10}$ was investigated.



Peer-review under responsibility of Tomsk Polytechnic University

*Keywords:* voltammetry, coenzyme $Q_{10}$, glassy carbon electrode, antioxidant activity, determination

## 1. Introduction

Coenzyme $Q_{10}$ (2,3-dimetoxy-5-methyl-6-decaprenyl-1,4-benzoquinone, $CoQ_{10}$) is a fat soluble, vitamin-like quinone commonly known as ubiquinone or ubidecarenone. $CoQ_{10}$ (fig. 1) is widely distributed in the living system of plants, animals and humans[1]. It is essential for cell respiration and electron transfer and it acts as an electron carrier and translocator of hydrogen ions in mitochondria[2]. Coenzyme $Q_{10}$ exhibits antioxidative and prooxidative properties[3].

* Corresponding author. Tel.: +7-923-401-14-78
*E-mail address*: evp_89@mail.ru



Peer-review under responsibility of Tomsk Polytechnic University
doi:10.1016/j.proche.2014.10.030

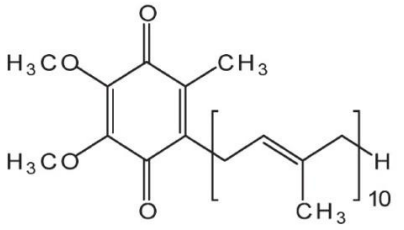


Fig. 1. Structural formula of coenzyme $Q_{10}$

The earliest observations of an antioxidant function of $CoQ_{10}H_2$ (fig. 2) date back to the sixties, and show that this substance could, like α-tocopherol, protect against the light-catalysed prooxidation of mitochondrial lipids. These observations have been extensively confirmed during the last two decades. Coenzyme $QH_2$ is the only lipid-soluble antioxidant synthesised by animal tissues *in vivo*. Coenzyme $Q_{10}H_2$ can act as an antioxidant directly, by preventing initiation and propagation of mitochondrial lipid peroxidation, or indirectly, by recycling α-tocopherol[4]. Coenzyme $Q_{10}H_2$ can also protect against oxidative damage to other important cellular macromolecules such as proteins and DNA.

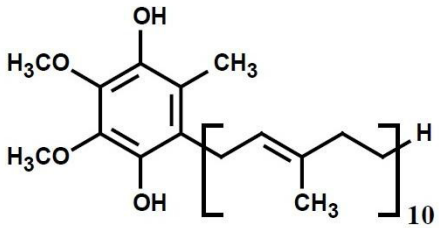


Fig. 2. Structural formula of ubiquinol $CoQ_{10}H_2$

The reactivity of $CoQ_{10}H_2$ with peroxyl radicals is much slower than that of α-tocopherol, and because $CoQ_{10}H_2$ is a highly hydrophobic molecule located in the middle of the phospholipid bilayer, it has considerably less intramembrane mobility than α-tocopherol, which would tend to decrease its radical scavenging potential. Therefore, this suggests that α-tocopherol, rather than $CoQ_{10}H_2$, acts as a direct scavenger of peroxyl radicals within the mitochondrial inner membrane.

Coenzyme $Q_{10}$ is an effective scavenger of free radicals and takes part in the elimination process of products resulting from metabolic reactions. Both $CoQ_{10}$ ($CoQ_{10}$ and $CoQ_{10}H_2$) and vitamin E efficiently detoxify cells from toxins. The coenzyme also prevents cells and membranes not only against generating free radicals but also from oxidative modifications of proteins, lipids and DNA[5].

The anodic oxidation of coenzyme $Q_{10}$ on a glassy carbon electrode (GSE) in a phosphate buffer solution (pH=6.86) was investigated. Considering obtained results, a simple, reproducible, rapid and accurate method was developed to determine $CoQ_{10}$.

## 2. Materials and Methods

Chemicals used were coenzyme $Q_{10}$ ($CoQ_{10}$), concentration > 98%, (Sigma-Aldrich).

Standard solution for electrochemical experiments was prepared by dissolving a suitable amount of coenzyme $Q_{10}$ in an ethyl alcohol followed by heating to a temperature not exceeding $25^0$C. The stock solution was stored in the dark and cool. Background solution was prepared by dissolving standard powder of $Na_2HPO_4$ in 1 L volumetric flask.

All voltammetric experiments were performed using an electrochemical analyzer "TA-2" ("Tomanalyt", Tomsk) with a three-electrode cell connected to it in which the glassy carbon electrode of 1 mm in diameter and silver chloride electrode were used as a process electrode and reference electrode, respectively. All potentials were measured and reported on the external silver chloride electrode with 1M NaCl solution. A method of determining electrochemical properties was made by cyclic voltammograms without addition and with following addition of the

test substances in a supporting electrolyte solution. The range of potential was from -1.5 to 1.5 V. Oxygen was removed by bubbling nitrogen through the electrochemical cell.

Antioxidant Activity Estimation

The antioxidant activity was estimated using an electrochemical method. The experimental method consisted in the recording of a voltammogram of the cathode electroreduction of oxygen by means of AOA Analyzer (RU.C.31.113.A N28715) connected to a PC. The antioxidant activity of the tested samples was calculated according to kinetic criterion K (in micromoles per liter·minute) indicating the quantity of the reactive oxygen species in time and determined using the formula [6]:

$$K = \frac{C_o}{t}(1 - \frac{I}{I_o})$$

where

$C_{O2}$ - oxygen concentration in the initial solution without a substance, in micromoles per liter;

$I_i$ - electric current of oxygen electroreduction, in microampere;

$I_o$ - electric current of oxygen electroreduction without a substance in the solution, in microampere;

$t$ - process running time, in minute.

## 3. Results and Discussion

Initially the effect of the supporting electrolyte pH on the analytical signal of coenzyme Q10 at different concentrations in solution was considered. As a result the cyclic voltammogram with redox peaks of coenzyme Q10 on the GCE at a scan rate 30 mV/s was obtained (Figure 3).

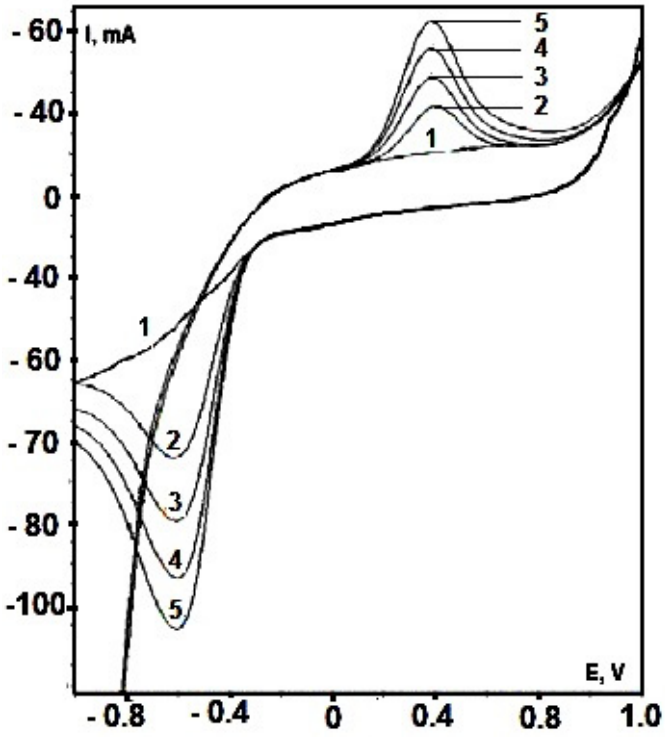


Fig. 3. Cyclic voltammogram of $CoQ_{10}$ on GCE ($v$ = 30 mV/s) at different concentration $CoQ_{10}$ in the solution: (1) background electrolyte without $CoQ_{10}$ ; (2) $0.5 \cdot 10^{-4}$ M $CoQ_{10}$; (3) $1.0 \cdot 10^{-4}$ M $CoQ_{10}$; (4) $1.5 \cdot 10^{-4}$ M $CoQ_{10}$; (5) $2.0 \cdot 10^{-4}$ M $CoQ_{10}$ in the phosphate buffer solution (pH=6.86).

The influence of pH supporting electrolyte on redox signal coenzyme $Q_{10}$ on GCE was investigated (fig. 4).

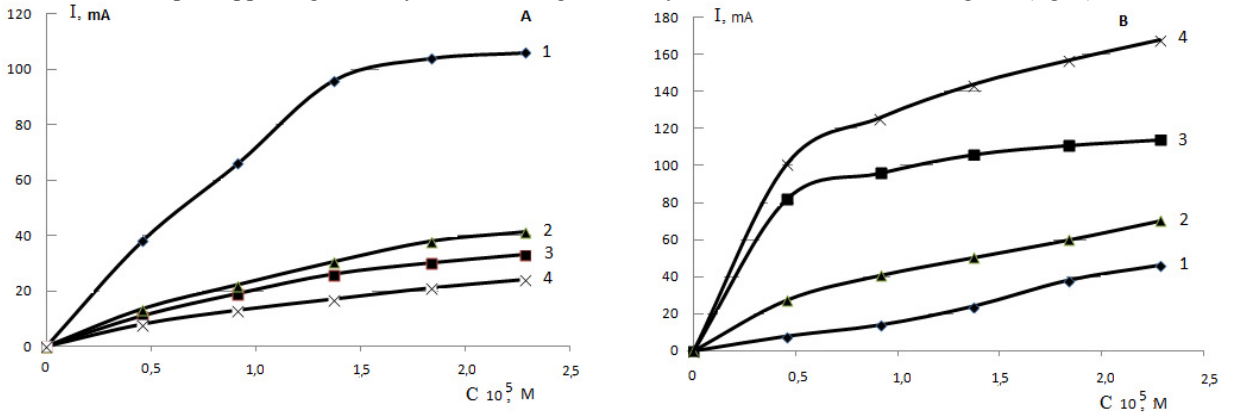


Fig. 4. Dependence of the oxidation current (A) and reduction current (B) of coenzyme Q10 on the GCE from their concentration in the solution at different pH value: 1.65 (1); 4.01 (2); 6.86 (3); 9.18 (4).

With addition of the analyte in the acidic and alkaline buffer lower values of the analytical signal in the anode and cathode area, respectively, are observed. It is assumed that protonation of coenzyme $Q_{10}$ molecule occurring in acidic medium facilitates its oxidation. In alkaline medium analytical signal due to complete dissociation of hydroxyl groups (-OH) of hydroquinone group of coenzyme $Q_{10}$ molecule increases and as a result facilitates the recovery process.

For the future investigation as a supporting electrolyte the phosphate buffer solution with pH 6.86 was chosen. This caused the stability of the hydroquinone group of coenzyme $Q_{10}$ in a neutral supporting solution.

On the basis of obtained data and literature [7] a redox mechanism of coenzyme $Q_{10}$ on GCE can be suggested. In aqueous medium at neutral pH, the reduction is either by two protons two electrons (fig. 5).

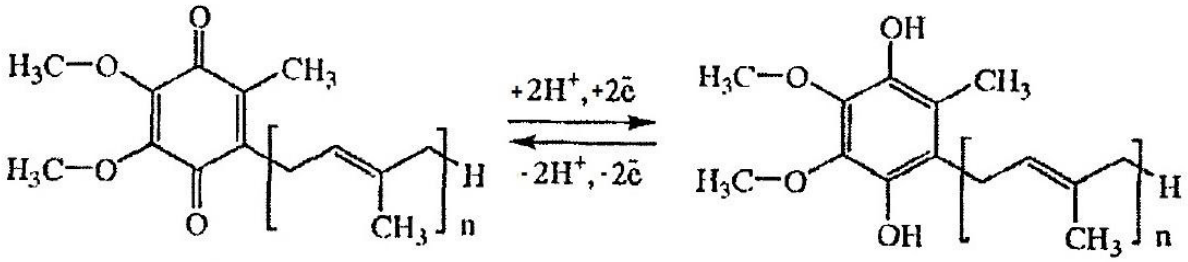


Fig. 5. Redox scheme of $CoQ_{10}$ on GCE in aqueous medium at neutral pH.

On a glassy carbon electrode, at pH 6.86 coenzyme $Q_{10}$ shows two sets of waves: one at a positive (+ 0.4 V) potential and another at a negative (- 0.6 V) one. The oxidation peak at + 0.4 V is reversible and involves the oxidation of the hydroquinone group. The reduction peak at negative potential of -0.6 V was similar to the previously reported reduction of ubiquinon to ubiquinol.

The calibration curve (fig. 6) of the peak current versus concentration is constructed using the linear regression method. The calibration curves are linear within the range of concentration from $2.0\times10^{-5}$ to $2.0\times10^{-4}$ M. This range of concentration is available for determination coenzyme $Q_{10}$ in pharmaceutical and cosmetics objects.

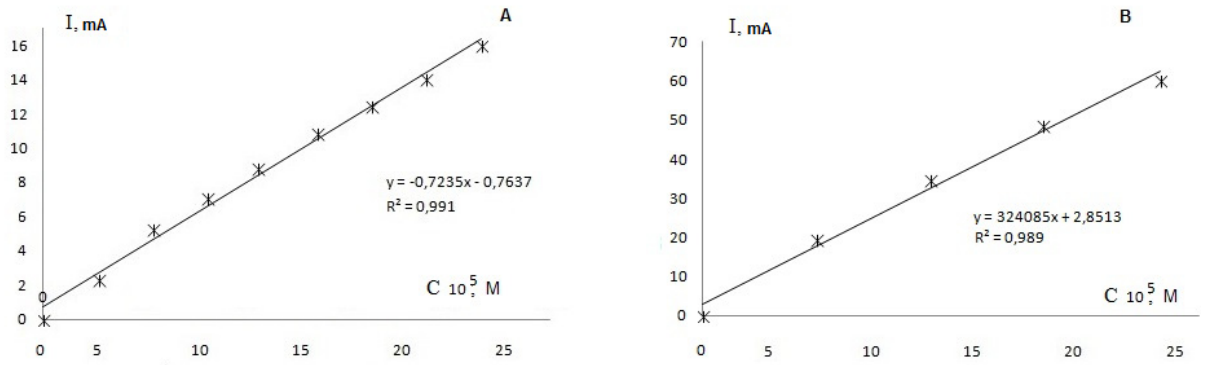


Fig. 6. Calibration curve of the redox peak current versus concentration coenzyme $Q_{10}$ (oxidation (A) and reduction (B))

Also the antioxidant activity of coenzyme $Q_{10}$ has been investigated. Results are shown in Table 1.

Table 1. Values of the antioxidant activity at various concentrations of coenzyme $Q_{10}$ in the solution

| $C \times 10^5$, M | K, mkmol/(L•min) |
|---|---|
| 0.1 | 0.32±0.09 |
| 1.0 | 0.25±0.05 |
| 10.0 | 0.18±0.06 |

As it is seen from the table 1 the activity of coenzyme $Q_{10}$ decreases with increasing concentration of the object in a solution. However, the change of coenzyme $Q_{10}$ antioxidant activity does not strongly depend on its concentration.

## 4. Conclusion

As results electrochemical properties of coenzyme $Q_{10}$ by cyclic voltammetry were investigated. The redox mechanism of coenzyme Q10 on GCE in aqueous solution has been assumed. The range of linear dependence of the analytical signal height on its concentration in the solution has been found. This method is suitable for determination of $CoQ_{10}$ in pharmaceutics. The main advantages of this method are significantly shortening of analysis time, low cost of analysis and widespread access to apparatus.

## Acknowledgments

This work was supported by the Grant of the President of the Russian Federation for state support of young Russian scientists MK – 7366.2013.8 and Ministry of Education and Science of Russian Federation (project "Science" № 4.1619.2014/к dated of 11.06.2014).

## References

1. Crane F. L., Biochemical functions of coenzyme $Q_{10}$, *Journal of the American College of Nutrition*, 2001; **20**: 6, p 591–598.
2. Lenaz G., Fato R., Formiggini G., Genova M. L., The role of coenzyme Q in mitochondrial electron transport, Mitochondrion 2007; **7**: 8-33.
3. Ruiz-Jimenez J., Priego-Capote F., Mata-Granados J. M., Quesada J. M., Luque de Castro M. D., Determination of the ubiquinolo-10 and ubiquinone-10 (coenzyme $Q_{10}$) in human serum by liquid chromatography tandem mass spectrometry to evaluate theoxidative stress. *J. Chromatogr. A*. 2007; **1175**: 242-248.
4. Indyk H, Littlejohn VC, Lawrence RJ. Liquid chromatographic determination of vitamin K1 in infant formulas and milk. *Journal of AOAC International*. 1995; **78**: 719-723.
5. Siemeniuk E., Skrzydlewska E., Coenzyme $Q_{10}$: its biosynthesis and biological significance in animal organisms and in humans. *Prog. Hyg. Exp. Med.* 2005, **59:** 150-159.

6. Dmitrova S., Pavlova K., Lukanov L., Korotkova E., Petrova E., Zagorchev P., Kuncheva M., Production of Metabolites with antioxidant and emulsifying properties by Antarctic strain sporobolomyces salmonicolor $AL_1$, *Appl Biochem Biotechnol*. 2013; **169**: 301-311.
7. Guin P. C., Das S., Mandal P. C. Electrochemical reduction of Quinones in different media: a review, *International Journal of Electrochemistry*, 2011.