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Square Wave Voltammetry Determination of Coenzyme Q_{10}

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ABSTRACT

An easy and extremely sensitive square wave voltammetric method for the determination of coenzyme Q_{10} has been evolved. The method is based on the direct measurement of the reduction peak current of the coenzyme Q_{10} at (-0.20 V)[(Ep) versus the Ag/AgCl reference electrode) at (pH = 7.3) and (25°C). The magnitude of the reduction peak current (at Ep) has been found to be related to the concentration of the coenzyme over the range of ($8.8 \times 10^{-8} - 5.4 \times 10^{-7}$ M) (r = 0.9893) and the relative standard deviation of the method at the (1.3×10^{-7} M) [113 ppb] level is (± 3.9 %) [n (number of samples) = 6]. The presence of albumin with CoQ_{10} causes a large depression in the reduction peak current of the coenzyme and its (Ep) moves to (-0.18 V).

تقدير مساعـد الانزيم Q_{10} بتقنية فولتامتري الموجة المربعة

الملخص

تم في هذا البحث استخدام طريقة لتقدير مساعد الانزيم Q_{10} اعتمادا على تقنية فولتامتري الموجـة المربعة. وتعتمد الطريقة على القياس المباشر لتيار القمة الاختزالي للمركب عند جهـد القمـة (V 0.2-) (مقابل القطب المرجعي: فضة/كلوريد الفضة) وعند الدالة الهيدروجينية (7.3) ودرجة الحرارة (25 °م). وقد لوحظ ان قيمة تيار القمة الاختزالي تتناسب مع تركيز مـساعد الانـزيم علـى مـدى (8.8×10^{-8}) مولاري الى (5.4×10^{-7}) مولاري (بمعامل ارتباط = 0.9893) وان درجة الانحراف القياسي للطريقـة عند التركيز (1.3×10^{-7}) مولاري (أي 113 جزء /بليون) هو (± 3.9%) (n=6). وقد لوحظ ان وجـود الالبومين مع مساعد الانزيم في المحاول يتسبب في انخفاض كبير لتيار القمة الاختزالي لمساعد الانـزيم مع انزياح جهده الاختزالي الى (0.18-) فولت.

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}) is a vitamin-like fat soluble antioxidant found in the highest concentration in vital organ such as the heart and pancreas. CoQ_{10} has been shown to be beneficial in disease conditions ranging from Parkinson's disease to cataracts, in addition to heart disease. Furthermore, it is now believed that CoQ_{10} is the key nutrient for generating 95 percent of the total energy required by the human body (Fonorow, 2003).

Moreover, treatment of patients having-(high-risk)-breast cancer with CoQ_{10} for 3 months has shown that the patient has become in excellent clinical condition with no residual tumor tissue (Sven and Folkers, 1994). It, therefore, seems very desirable to estimate CoQ_{10}.

The methods available for the determination of CoQ_{10} are only very few. A method for the determination of CoQ_{10} is accomplished using high pressure liquid chromatogaphy (HPLC) with electrochemical detection and internal standardization (Niklowitz et al., 2002). Another method is an invention of an electrochemical method using (HPLC) instrument (Fondacaro, 2003).

Square wave voltammetry (SWV) involves scanning the entire potential on a single drop of a dropping electrode and can be used to perform an experiment much faster than other related and non-related techniques. Square wave voltammetry employs scan rates up to (1 V/sec) for faster, allowing much faster determinations (Princeton, 1984; Wojcie chowski et al., 1985).

To the best of our available knowledge, no square wave voltammetry method has yet been established for the determination of CoQ_{10}. The present paper describes an easy square wave stripping voltammetric method for the determination of CoQ_{10} in at least pure aqueous solution. Albumin has been found to affect seriously the reduction peak current of the coenzyme.

EXPERIMENTAL

Reagents:

Chemicals used are of analytical grade.

Phosphate solution, (0.2 M). This solution is prepared by dissolving (7.8 g) of NaH_2PO_4 .$2H_2O$ (Fluka) in distilled water and diluting the volume to (250 ml) with distilled water, in a volumetric flask.

Phosphate buffer solutions, pH 6.5, 7.3 and 8.0. These phosphate buffer solutions are prepared by transferring (50 ml) of the above solution to a beaker, diluting the volume to about (90 ml) with distilled water and neutralizing the resulting solution to the required pH by dropwise addition of concentrated NaOH solution (Hopkin and Williams) then the volume is diluted to (100 ml) with distilled water in a volumetric flask.

CoQ_{10} solution, (5.5×10^{-4} M). This solution is prepared by dissolving (0.0048 g) of the compound (Fluka) in ethanol (Fluka) (warming is necessary) and the volume is diluted, with the same solvent, to (10 ml) in a volumetric flask. This solution is stored in a dark bottle where it is stable for at least two weeks at room temperature. Less concentrated solutions are prepared by dilution with ethanol.

Instruments:

Square wave measurements are performed with an EG and G Princeton Applied Research Model 384B Polarographic Analyzer. An EG and G PARC Model 303A static mercury drop electrode (SMDE) in the hanging mode is used as the working electrode. An Ag/AgCl is used as the reference electrode and a platinum wire as the auxiliary electrode. The SMDE is equipped with an EG and G PAR Model 305 stirrer. The data are plotted with the Hiplot DMP-40 series digital plotter.

The pH adjustments are made using a Sargent-Weleh Model NX pH meter.

Temperature variations are carried out using Radiometer type VIS 31 water thermostat.

Recommended Procedure:

To the EG and G PARC polarographic cell, (5 ml) of (0.1 M) phoshpate buffer (pH 7.3) solution is transferred and after 240 sec purging with N_2 gas, a square wave voltamogram is run under the default conditions (fixed in the polarographic analyser) (Table 1).

Table 1: Polarographic analyzer default conditions.

Technique: SWV
Initial E: 0.000 V
Final E: -1.2 V
Purging time: 240 sec (for blank) and 30 sec (for standard)
Scan increment: 2mV
Conditioning time: 0 sec
Conditioning potential: 0V
Equilibrium time: 5 sec
Deposition time: 0 sec
Frequency: 100 Hz
Pulse height: 0.02V

This run corresponds to the blank which is automatically stored in the memory of the 384B analyzer. Then standard solution of the compound (CoQ_{10}) of (1.1×10^{-5} M solution) was injected into the supporting electrolyte (buffer). The solution was purged for 30 sec, and a sample run was performed and value of the blank is subtracted electronically from the sample. Typical voltammogram of $5x10^{-7}$ M CoQ_{10} are shown in Fig. (1A).

RESULTS AND DISCUSSION

The effect of various parameters related to and affecting the square wave voltammetric behaviour of CoQ_{10} has been studied and optimum conditions have been selected.

Deposition time and drope size:

The deposition time is an important experimental parameter in stripping voltammety (Petrson and Wong, 1981). A deposition time of (0-400) sec has been tried with small, medium and large drop size. The experimental data reveal that the peak height (Ip) increases as the deposition time increases up to about 200 sec and as the drop size becomes larger. In the subsequent experiments, a deposition time of 200 sec and a large drop size are selected.

Conditional time and conditional potential:

A conditional time of 60 sec and a conditional potential of (-0.500V) are found suitable in the method. The equilibrium time of (5-10) sec is also suitable.

Pulse height, frequency and scan increment:

A pulse height of (0.02-0.04 V) are tried for maximum sensitivity and resolution. A 0.03V pulse height gives maximum reduction peak current.

A frequency of (60-120 Hz) has been examined for optimum peak height. The experimental data show that the peak current increases as the frequency increases and the

maximum frequency of 120 Hz has been recommended for the procedure.

A scan increment of (1-4) mV is tested for maximum reduction peak current. The results show that (1-2 mV) scan increments give best results (peak height and shape). A (2 mV) scan increment is selected fore the subsequent experiments.

pH and temperature:

The effect of pH and temperature has been investigated from the physiological point of view, since CoQ_{10} occurs in the respiratory electron transport chain (Stryer, 1988). A (0.1 M) phosphate buffer (and as a supporting electrolyte) solution of pH 6.5 (acidosis), 7.3 (normal) and 8.0 (alkalosis) and a temperature of (25°C, 37°C and 45°C have been investigated for their effect on the square wave voltammetric behaviour of CoQ_{10}. The experimental data indicates that the reduction peak potential (Ep) shifts to more negative value, at all temperatures, when the pH change from 6.5 to 8.0. At the higher temperature, the (Ip) decreases as the pH moves upwards. At (25°C), maximum peak current is observed at (pH 7.3). For the subsequent work, (0.1 M) phosphate buffer solution of (pH 7.3) and (25°C) are continually used.

Quantitative determination of CoQ_{10} :

Using the optimum conditions obtained above, pH 7.3 of phosphate buffer and the experimental parameter show in Table (2), the square wave voltammetery of CoQ_{10} were recorded separately at different concentrations.

Table 2 : Optimum experimental parameters.

Technique: SWV
Initial E: 0.000 V
Final E: -0.500 V
Purging time: 240 sec (for blank) and 30 sec (for standard)
Calculated drop time: 270 sec
Scan increment: 2mV
Conditioning time: 60 sec
Conditioning potential: -0.500V
Equilibrium time: 10 sec
Deposition time: 200 sec
Cycles : 1
Frequency: 120 Hz
Pulse height: 0.03V
Replications: 1
Blank subtraction: Yes
Tangent fit: No
Peak location: Yes
Derivatives: No

The results indicate that the peak current Ip was proportional to the concentration and a good calibration graphs were obtained. Some typical results for a series of standard solution of the CoQ_{10} are show in Table (3). The solutions were prepared by adding appropriates aliquot of the stock solution (40-260 µl of 1.1 x 10^{-5} M CoQ_{10}) to 5 ml of phosphate buffer at pH 7.3 as supporting electrolyte.

Table 3 : Standard curve of CoQ_{10}.

µl of 1.1x10^{-5} M CoQ_{10} solution	**Final concentration (M)**	**Corrected Ip, nA**
40	8.765 x 10^{-8}	961
60	13.043 x 10^{-8}	1489
80	17.323 x 10^{-8}	2031
100	2.157 x 10^{-7}	2489
140	2.996 x 10^{-7}	3145
160	3.411 x 10^{-7}	3375
180	3.822 x 10^{-7}	4260
200	4.231 x 10^{-7}	4400
220	4.636 x 10^{-7}	4670
240	5.038 x 10^{-7}	4860
260	5.437 x 10^{-7}	5000

The relationship between reduction peak current (Ip) and molar concentration (C) of CoQ_{10} is given by the formula:
$Ip = 9x10^{9}$ conc. + 391.72
and the correlation coefficient (r) of the formula is (0.9899).

The precision of the method (relative standard deviation percent) is tested and found to be (± 3.9 % (n=6) at the 1.3 x 10^{-7} M (113 ppb CoQ_{10}) level.

The presence of albumin:

The existence of albumin with CoQ_{10} leads to a large decrease in the reduction peak current of CoQ_{10} (Fig. 1B). This is not unexpected since albumin is a surfactant and hence will act as a maximum suppressor. Therefore, albumin should be removed when CoQ_{10} is assayed in serum. Similar results have been observed for the interaction of NAD^{+} with albumin (Sulaiman, 2003).

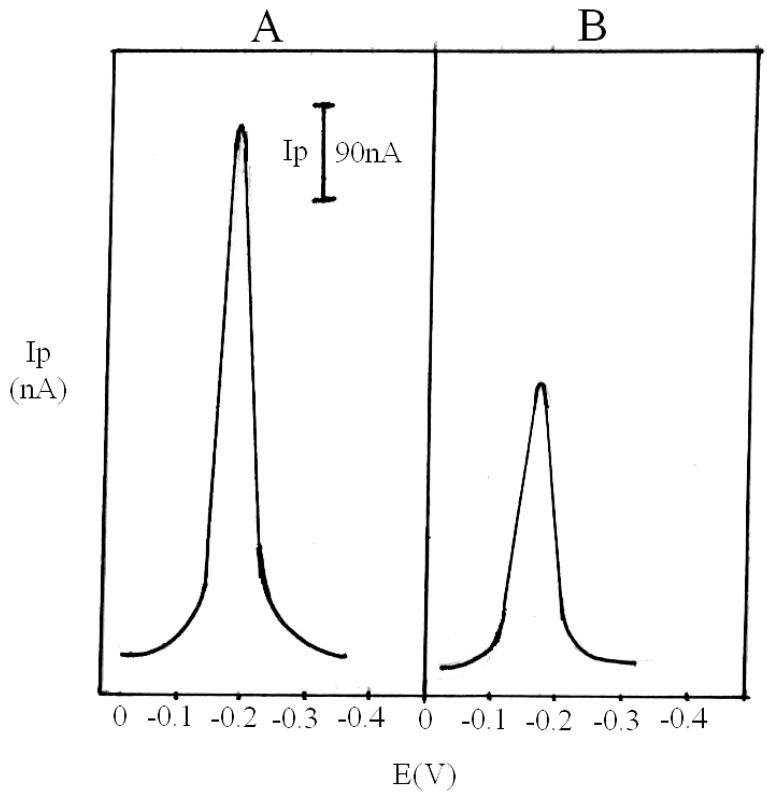

Fig. 1: Square Wave Voltammogram of $5x10^{-7}$ M CoQ_{10} in Phosphate buffer at pH 7.3. (A) in the absence of albumin, (B) in the presence of albumin.

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