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ELECTROCHEMICAL STUDIES OF THYMOQUINONE WITH COBALT(II)

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Abstract

In the present research complexation of thymoquinone with Co(II) has been investigated using cyclic voltammetry. In this electrochemical study glassy carbon, platinum and saturated calomel electrodes were used as working, auxiliary and reference electrodes respectively. During whole work temperature was 25±1˚C and NaCl was used as supporting electrolyte. Comparison of the voltammograms of Co(II), thymoquinone and their complex suggest existence of pre - equilibrium charge transfer process in the formation of Co(II)-thymoquinone complex. Effects of scan rates and successive cyclic scans on complex have also been examined. Results revealed quasi-reversible electron transfer process. E˚ of complex is found to be 0.275±0.01 V whereas diffusion coefficient was $2.412\text{x}10^{-5}$ cm^2s^{-1}. The values of transfer coefficients, α and β, were found to be in the range of 0.667±0.01- 0.873±0.01 and 0.738±0.01 – 1.091±0.01 respectively. Successive cyclic scans showed that no adsorption or deposition of complex occurred on the electrode surface. Present study confirms complex formation between thymoquinone, which is an active constituent of *Nigella sativa* (Black seed), and cobalt successfully. Former is used frequently in our food and confectionary items whereas later is used in the form of vitamin B_{12}. Hence this research reveals that simultaneous intake of cobalt mineral and *Nigella sativa* seeds may result in cobalt deficiency due to complexation of thymoquinone with cobalt. Therefore their intake should not be simultaneous.

Introduction

Medicinal plants are the source of many pharmacologically active compounds (Djipa *et al.*, 2000; Duley, 2000). *Nigella sativa* is one of such plants which contain hundreds of biologically active compounds and thymoquinone (Fig. 1) is one of them (Xin *et al.*, 2008). Its innumerable bioactivities are because of its antioxidant behaviour (El-Ghorab, 2003; Houghton et *al.*, 1995; Bourgou *et al.*, 2008; Al-Ghamdi, 2001).

Cobalt is an essential mineral for human (Eleanor and Sharon, 2002). It helps in red blood cells formation and nerve tissue maintenance. Vitamin B_{12} is the only source of cobalt as the body does not absorb it directly. Vitamin B_{12} is also needed for proper functioning of iron, calcium and vitamin B_6. Moreover, it helps in metabolism of carbohydrates, folic acid, protein and fats (Shane, 2000). Hence deficiency of cobalt can result in a number of complications including anemia, abnormalities in cell formation, nervous disorders, palpitation of heart, paralysis and even death (Baik and Russell, 1999). On the other hand cobalt toxicity may produce severe toxic effects including thyroid stimulation and polycythemia (Nourhashemi *et al.*, 2000). Co (II) is the most stable oxidation state of cobalt and it forms numerous complexes. Most of the complexes are octahedral or tetrahedral. In some cases it forms five-coordinate and square planer complexes also (Sharpe, 1996**;** Cotton and Wilkinson, 1988**).**

We have previously examined the complex forming ability of thymoquinone with some redox active metals, including Co(II), using potentiometry (Kishwar *et al.*, 2012). However electrochemical study of thymoquinone and its complex with Co(II) is important in order to understand the nature of the complex and mechanism of the reaction in complex formation hence present research work has been carried out.

Materials and Methods

Reagents and Glassware: Analytical grade reagents were used purchased from Merck and MP Biochemicals LLC. All glassware used was of standard quality. For present research cobalt acetate tetra hydrate, thymoquinone and sodium chloride were used.

Instrumentation

Electrical balance: For weighing Shimadzu AX 200 was used.

Cyclic voltammeter: CHI – 760 D Electrochemical work station, cyclic voltammeter was used. The electrodes used were a glassy carbon electrode as working electrode, a saturated calomel electrode as reference electrode and a platinum wire electrode as an auxiliary/ counter electrode. Re-polishing and resurfacing of working electrode was done time to time using Alumina polishing compound. Nitrogen purging was checked but its presence or absence made no change in cyclic voltammograms with in the examined potential range.

Sample preparation

Supporting Electrolyte Solution: 0.1M solution of NaCl was used as supporting electrolyte.

Analyte Solutions: $5x10^{-3}$M solution of thymoquinone and equimolar solution of cobalt acetate were prepared as analyte solutions.

Cyclic Voltammetric Studies: Fresh solutions were used every time. The cell assembly was rinsed with the analyte solution before starting the work. First of all, the supporting electrolyte was run in order to take base-line and then 15.0 mL of Co(II) solution (5 x 10^{-4}M), thymoquinone (5 x 10^{-4}M) and Co(II) – thymoquinone solution (5 x 10^{-4}M) were run separately to get an overlay. The scan rate was 0.1 V/s while the current sensitivity was $1x10^{-4}$ A/V and the potential range was set from -0.40 V to +1.00 V and then reversed back to -0.40 V.

In addition to complex formation effects of variation of scan rate and successive cyclic scans on complexation of Co(II) and thymoquinone was also investigated. To observe effect of scan rate Co (II)-thymoquinone complex was analyzed at scan rates ranging from 0.05- 0.5V/s whereas all other parameters were kept constant. Repeated scan of the complex was recorded up to 16 sweep segments.

Results and Discussion

Valuable information were obtained from cyclic voltammetric studies of Co(II)-thymoquinone complex which are as follows:

Complex formation: To investigate this, solutions of NaCl (0.1M), Co(II) ($5x10^{-4}$M), thymoquinone ($5x10^{-4}$M) and a solution having 1:1 Co(II) to thymoquinone ratio ($5x10^{-4}$M) were run at 0.1 V/s. The base line was horizontally straight which indicates absence of impurities and cleanliness of working electrode. The comparison of the cyclic voltammograms showed that the metal gave a linear response which shows its redox inactivity within applied potential scan range. Thymoquinone showed very prominent anodic and cathodic peaks, whereas the complex gave cathodic peak only and no anodic peak was observed (Fig. 2). These drastic differences in the cyclic voltammograms as well as significant drop in the cathodic peak current of the complex solution confirms complex formation between Co(II) and thymoquinone (Table- 1).

The shape of the cyclic voltammogram of Co(II)-thymoquinone complex indicates coupling of oxidation with reduction (Fig. 2). Significant drop in the peak current and positive shift in the peak potential of the complex as compared to the ligand reveal presence of pre equilibrium charge transfer process in Co(II)-thymoquinone complex which could be represented as follows:

$$Co^{n+} \rightleftharpoons Co^{(n+)+1} + 1e^-$$

$$Co^{(n+)+1} + L \rightleftharpoons [Co^{(n+)+1}, L]$$

Effect of scan rate on Co(II)-thymoquinone complex: Effect of scan rate from 0.05 V/s - 0.5 V/s was examined on the complex solution ($5x10^{-4}$M) having 1:3 metal-ligand ratio and different electrochemical parameters i.e., E_{pa}, E_{pc}, I_{pa}, I_{pc} etc., were obtained (Table- 2). Cyclic voltammograms seems to obey the criteria of quasi-reversible behaviour (Bard and Faulkner, 2001; Greef *et al.*, 1985; Nicholson, 1965) (Fig.3). This fact is further confirmed by the plot of I_p versus $\nu^{1/2}$ (Fig. 4) which shows enhanced I_p with the increase of $\nu^{1/2}$ but it is not proportional to $\nu^{1/2}$. Secondly, I_{pa}/I_{pc} was not found equal to 1(Table-2). Thirdly, E_{pa}-E_{pc} was observed to be greater than 59/n mV and an increase in the ΔEp was observed with the increase in potential scan rate (ν).Moreover a negative shift in E_{pc} with the increase of scan rate also favours quasi-reversible mechanism.

The plot of I_{pc} versus $\nu^{1/2}$ showed good linearity but the plot of I_{pa} showed linear relationship only up to 0.25 V/s. It shows that the reaction is diffusion controlled between scan rates 0.05 V- 0.25 V but further increase in scan rate results probably in any chemical complication. This may be in the form of adsorption of any specie or any other chemical reaction (Fig. 4). The plot E_{pa} and E_{pc} against log of ν at different scan rates showed that later increases with the increase of scan rates, whereas former remains approximately constant (Fig. 5).

The values of αn_a and βn_b were also calculated at different scan rates. The values of α were found 0.667 ± 0.01 to 0.873 ± 0.01, whereas values of β were obtained in the range of 0.738 ± 0.01 to 1.091 ± 0.01.

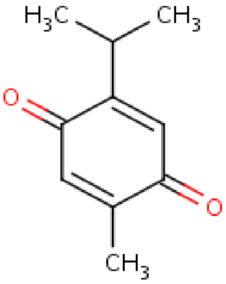

Fig. 1. Structure of Thymoquinone.

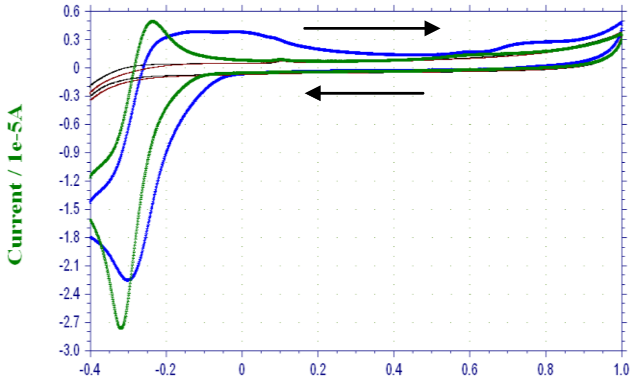

Fig. 2. Cyclic voltammograms of Thymoquinone, Co(II) and Co(II)-thymoquinone Complex at 0.1 V/s (Baseline = NaCl (0.1 M), Metal = Co(II) (5x10^{-4} M), Ligand = Thymoquinone (5x10^{-4} M), Complex = Co(II)-Thymoquinone (5x10^{-4}) M).

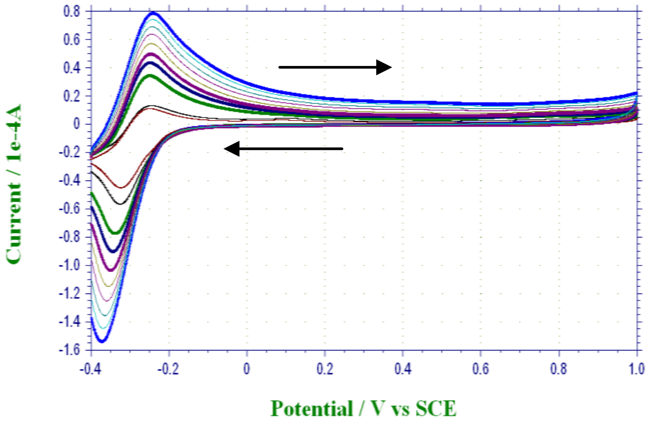

Fig. 3. Cyclic voltammograms of Co(II)-thymoquinone complex at different scan rates.

(0.05 V/s, 0.1 V/s, 0.15V/s, 0.2V/s, 0.25 V/s, 0.3 V/s, 0.35 V/s, 0.4 V/s, 0.45 V/s, 0.5 V/s)

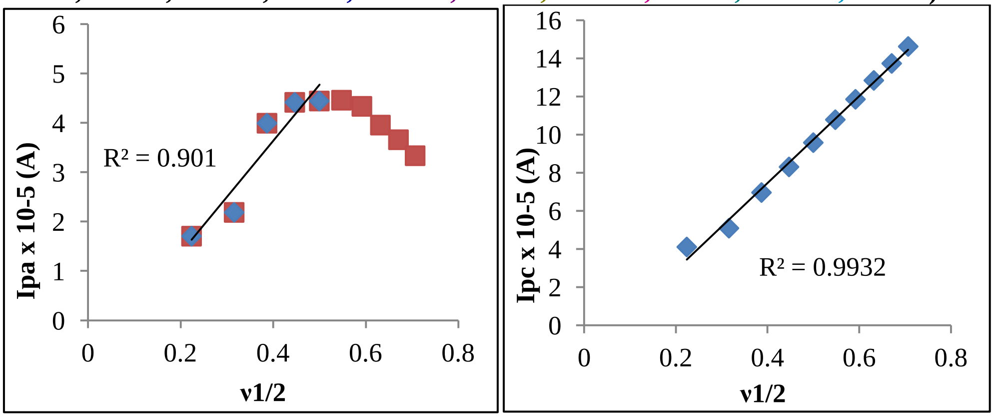

Fig. 4. Variations of anodic and cathodic peak current with square root of sweep rate from the cyclic voltammograms of Co(II)-thymoquinone complex.

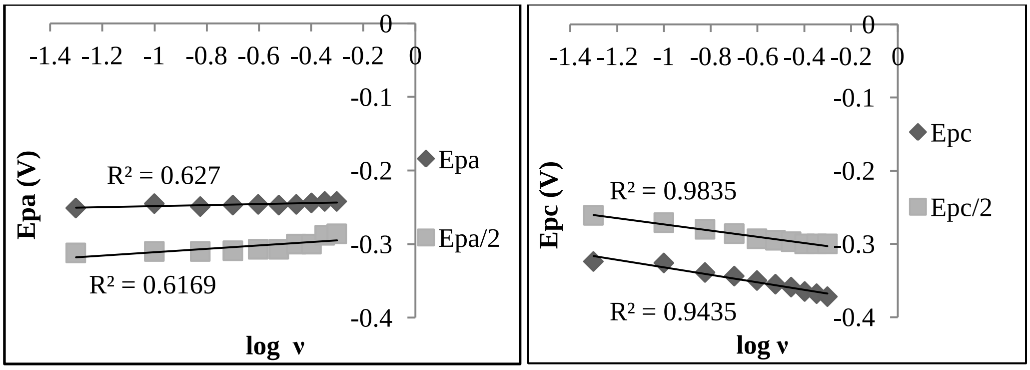

Fig. 5. Variation of anodic and cathodic peak potentials with sweep rate from cyclic voltammograms of of Co(II)-thymoquinone complex.

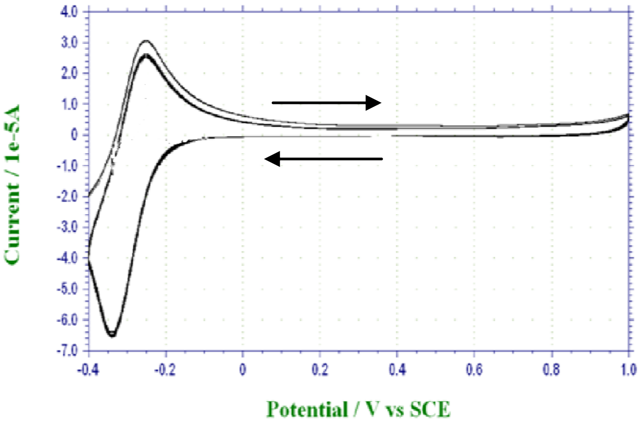

Fig. 6. Reapeted scan cyclic voltammograms of Co(II)-thymoquinone complex at 0.1 V/s.

Effect of repeated scanning: Effect of successive cyclic scans on Co(II)-thymoquinone complex was examined, for which $5x10^{-4}$ M complex solution was analyzed up to 16 sweep segments at 0.1V/s and $1x10^{-4}$ Amp/Volt (Fig. 6).

On repeated scanning the shape of the cyclic voltammogram was observed to be unchanged in terms of peak potentials (E_{pa} and E_{pc}) and no appearance of any pre or post-peak was observed. It shows that neither adsorption nor deposition of complex took place on electrode surface.

Analysis of diffusion coefficient: Diffusion coefficient (D) for the Co(II)-thymoquinone complex was determined using Randles-Sevcik equation. Area of electrode (A) was 0.0706 cm^2 and number of electron transferred (n) was equal to 1. Drastic difference in diffusion coefficient of thymoquinone and complex was observed which confirms complexation (Table-3(a)). In order to examine effect of scan rate it was calculated at different scan rates. It was noted that the values of diffusion coefficient remained approximately unaffected (Table-3(b)) however diffusion coefficient for reverse scan was observed to be in the range of 10^{-4}. It points out towards some chemical complication including some sort of coupled reaction or adsorption of any specie on the electrode.

Analysis of E°: E°, being a characteristic property, is unaffected by variation of different parameters. For Co(II)-thymoquinone complex effect of scan rate was observed on it and no reasonable change in its value was noted (Table- 4).

Conclusion

Electrochemical studies of Co(II) and thymoquinone was carried out using cyclic voltammetry. Comparison of cyclic voltammograms of Co(II), thymoquinone and Co(II),-thymoquinone solution revealed complex formation between metal and the ligand. Clear drop in the peak current of the complex as compared to the ligand and a positive shift in the peak potential suggested the presence of pre equilibrium charge transfer process in the complex formation. Data from effect of varying scan rate on complex exhibited quasi-reversible behavior. The reaction seems to be diffusion controlled between scan rates 0.05 V- 0.25 V but further increase in scan rate results probably in any chemical complication which rendered the reaction free from diffusion control. Values of E˚, D, α and β were also calculated. E˚ being a characteristic property is constant for a particular system. In present study effect of scan rate was observed on E˚ and it was found to be approximately same. Diffusion coefficient was calculated using Randles- Sevick equation. The values of transfer coefficients, α and β were also determined at different scan rates.

In addition present research points out that simultaneous intake of cobalt mineral and *Nigella sativa* seeds, which are often used in our food, may result in cobalt deficiency due to complexation of thymoquinone with cobalt. Hence it is better not to take seeds and the element cobalt simultaneously.

Table 1. Electrochemical parameters of cyclic voltammograms of thymoquinone, Co(II), and Co(II)- thymoquinone complex.

	I_{pa} (A)	**I_{pc} (A)**	**E_{pa} (V)**	**E_{pc} (V)**
Thymoquinone	1.017x10^{-5}±0.01	2.717x10^{-5}±0.01	-0.242±0.01	-0.326±0.01
Co(II)	-	-	-	-
Co(II)-thymoquinone complex	-	1.743x10^{-5}± 0.01	-	-0.302 ± 0.01

Table 2. The values of E_p, $E_{p/2}$, E_p-$E_{p/2}$, E_{pa}-E_{pc}, I_p, α and β from cyclic voltammograms of Co(II)- thymoquinone complex at different scan rates.

Scan rate (V/s)	**E_{pa} (V)**	**$E_{pa/2}$ (V)**	**E_{pa}-$E_{pa/2}$ (V)**	**I_{pa} x10^{-5}(A)**	**I_{pa}/I_{pc}**	**βn_b =0.048/E_{pa}-$E_{pa/2}$**
0.05	-0.251 ± 0.01	-0.312 ±0.01	0.061 ±0.01	1.706 ± 0.01	0.415 ±0.01	0.787 ± 0.01
0.10	-0.245 ±0.01	-0.310 ±0.01	0.065 ±0.01	2.188 ± 0.01	0.430 ±0.01	0.738 ± 0.01
0.15	-0.249 ±0.02	-0.310±0.01	0.061 ±0.02	3.994 ± 0.02	0.574±0.02	0.787 ± 0.02
0.20	-0.247 ±0.01	-0.309 ±0.01	0.062 ±0.01	4.417 ± 0.01	0.532 ±0.01	0.774± 0.01
0.25	-0.246 ±0.02	-0.307 ±0.02	0.061 ±0.02	4.444 ± 0.02	0.464 ±0.02	0.787 ± 0.02
0.30	-0.247 ±0.01	-0.307 ±0.01	0.060 ±0.01	4.460 ± 0.01	0.414 ±0.01	0.800 ± 0.01
0.35	-0.246 ±0.01	-0.300 ±0.01	0.054 ±0.01	4.336 ± 0.01	0.366 ±0.01	0.889 ± 0.01
0.40	-0.244 ±0.01	-0.300 ±0.01	0.056 ±0.01	3.956 ± 0.01	0.308 ±0.01	0.857 ± 0.01
0.45	-0.242 ±0.01	-0.288 ±0.01	0.046 ±0.01	3.660 ± 0.01	0.267 ±0.01	1.043 ± 0.01
0.50	-0.242 ±0.01	-0.286 ±0.01	0.044 ±0.01	3.336 ± 0.01	0.228 ±0.01	1.091 ± 0.01
Scan rate (V/s)	**E_{pc} (V)**	**$E_{pc/2}$ (V)**	**E_{pc}-$E_{pc/2}$ (V)**	**E_{pa}-E_{pc} (V)**	**I_{pc} x10^{-5}(A)**	**αn_a =0.048/E_{pc}-$E_{pc/2}$**
0.05	-0.324 ± 0.01	-0.261 ± 0.01	-0.063± 0.01	-0.073±0.01	4.106 ± 0.01	0.761 ± 0.01
0.10	-0.326 ± 0.01	-0.271 ± 0.01	-0.055± 0.01	-0.081±0.01	5.090 ± 0.01	0.873 ± 0.01
0.15	-0.339 ± 0.01	-0.280 ± 0.01	-0.059± 0.01	-0.090±0.01	6.961 ± 0.01	0.814 ± 0.01
0.20	-0.344 ± 0.01	-0.286 ± 0.01	-0.058± 0.01	-0.097±0.01	8.304 ± 0.01	0.828 ± 0.01
0.25	-0.350 ± 0.01	-0.293 ± 0.01	-0.057± 0.01	-0.104 ±0.01	9.577 ± 0.01	0.842 ± 0.01
0.30	-0.355 ± 0.01	-0.295 ± 0.01	-0.060± 0.01	-0.108 ±0.01	10.78± 0.01	0.800 ± 0.01
0.35	-0.359 ± 0.01	-0.297 ± 0.01	-0.062± 0.01	-0.113 ±0.01	11.85± 0.01	0.774 ± 0.01
0.40	-0.365 ± 0.02	-0.300± 0.02	-0.065± 0.02	-0.121 ±0.01	12.84± 0.01	0.738 ± 0.01
0.45	-0.368 ± 0.01	-0.300 ± 0.01	-0.068± 0.01	-0.126 ±0.01	13.73± 0.01	0.706 ± 0.01
0.50	-0.372 ± 0.02	-0.300 ± 0.01	-0.072± 0.02	-0.130 ±0.01	14.62± 0.01	0.667 ± 0.01

Table 3(a). Comparison of the Diffusion Coefficients of Thymoquinone, Co(II), and Co(II)-thymoquinone complex $D^{1/2} = I_p/ (2.69x10^5) (n)^{3/2} AC (\nu)^{1/2}$

	I_{pa} (A)	$D^{1/2}$	D ($cm^2\ s^{-1}$)
Thymoquinone	$1.017x10^{-5}\pm 0.01$	3.39×10^{-3}	1.15×10^{-5}
Co(II)	-	-	-
Co(II)-thymoquinone	-	-	-
	I_{pc} (A)	**$D^{1/2}$**	**D ($cm^2\ s^{-1}$)**
Thymoquinone	$2.717x10^{-5}\pm0.01$	9.06×10^{-3}	8.20×10^{-5}
Co(II)	-	-	-
Co(II)-thymoquinone	$1.743\ x10^{-5}\pm0.01$	5.81×10^{-3}	3.38×10^{-5}

Table 3(b). Diffusion coefficients of Co(II)-thymoquinone complex at different scan rates $D^{1/2} = I_p/ (2.69x10^5) (n)^{3/2} AC (\nu)^{1/2}$

ν (V/s)	$\nu^{1/2}$	I_{pa} $x10^{-5}$(A)	$D^{1/2}$	D ($cm^2\ s^{-1}$)
0.05	0.224	1.706 ± 0.01	8.02×10^{-3}	6.43×10^{-5}
0.10	0.316	2.188 ± 0.01	7.29×10^{-3}	5.32×10^{-5}
0.25	0.500	4.444 ± 0.02	$9.36\ x10^{-3}$	$8.76\ x10^{-5}$
0.30	0.548	4.460 ± 0.01	$8.57\ x10^{-3}$	$7.35\ x10^{-5}$
0.35	0.592	4.336 ± 0.01	$7.71\ x10^{-3}$	$5.95\ x10^{-5}$
0.40	0.632	3.956 ± 0.01	$6.59\ x10^{-3}$	$4.35\ x10^{-5}$
0.45	0.671	3.660 ± 0.01	$5.74\ x10^{-3}$	$3.30\ x10^{-5}$
0.50	0.707	3.336 ± 0.01	$4.97\ x10^{-3}$	$2.47\ x10^{-5}$
ν (V/s)	**$\nu^{1/2}$**	**I_{pc} $x10^{-5}$ (A)**	**$D^{1/2}$**	**D ($cm^2\ s^{-1}$)**
0.05	0.224	-4.106 ± 0.01	1.93×10^{-2}	3.73×10^{-4}
0.10	0.316	-5.090 ± 0.01	1.70×10^{-2}	2.88×10^{-4}
0.15	0.387	-6.961 ± 0.01	1.90×10^{-2}	3.59×10^{-4}
0.20	0.447	-8.304 ± 0.01	1.96×10^{-2}	3.83×10^{-4}
0.25	0.500	-9.577 ± 0.01	2.02×10^{-2}	4.07×10^{-4}
0.30	0.548	-10.78± 0.01	2.08×10^{-2}	4.30×10^{-4}
0.35	0.592	-11.85± 0.01	2.11×10^{-2}	4.45×10^{-4}
0.40	0.632	-12.84± 0.01	2.14×10^{-2}	4.58×10^{-4}
0.45	0.671	-13.73± 0.01	2.16×10^{-2}	4.65×10^{-4}
0.50	0.707	-14.62± 0.01	2.18×10^{-2}	4.75×10^{-4}

Table 4. Half wave potential ($E^{\circ}= E_{1/2}$) for Co(II)-thymoquinone complex at different scan rates.

Scan rates (ν) V/s	$(E^{\circ})_a$ (V)	Scan rates (ν) V/s	$(E^{\circ})_c$ (V)
0.05	0.282 ± 0.01	0.05	0.291 ± 0.01
0.10	0.278 ± 0.02	0.10	0.299 ± 0.01
0.15	0.280 ± 0.01	0.15	0.310 ± 0.01
0.20	0.278 ± 0.01	0.20	0.315 ± 0.01
0.25	0.277 ± 0.01	0.25	0.322 ± 0.01
0.30	0.277 ± 0.01	0.30	0.325 ± 0.01
0.35	0.273 ± 0.01	0.35	0.328 ± 0.03
0.40	0.272 ± 0.01	0.40	0.333 ± 0.02
0.45	0.265 ± 0.01	0.45	0.334 ± 0.02
0.50	0.264 ± 0.02	0.50	0.336 ± 0.02

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