COMPLEXATION OF ACTIVE INGREDIENT THYMOQUINONE OF *NIGELLA SATIVA* (BLACK SEED) WITH CHROMIUM(VI)

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Abstract

Thymoquinone is a principal and active ingredient of *Nigella sativa* (Black seed). In the present research its complexation with Cr(VI) has been studied using cyclic voltammetry. Mentioned electrochemical study was carried out at glassy carbon as working electrode, platinum as auxiliary and saturated calomel as reference electrode. Whole practical work was performed at 25 ± 1˚C in aqueous medium using NaCl as supporting electrolyte. Comparison of the voltammograms of metal, ligand and the complex suggest presence of pre - equilibrium charge transfer process in Cr(VI)-thymoquinone complex. In addition present study reflects effects of scan rates on the complex showing that the complex follows quasi-reversible electron transfer process. E˚ of complex is found to be 0.260 ± 0.01 V whereas diffusion coefficient was $3.37x10^{-5}$ cm^2s^{-1}. The values of transfer coefficients (α and β) were also determined. The value of α was found to be 0.593 ± 0.02 - 1.231 ± 0.01 whereas values of β were found in the range of 0.750 ± 0.01 – 1.171 ± 0.01. Since present research confirms complex formation between thymoquinone and Cr(VI), it is suggested that current research could be beneficial in eliminating Cr (VI) toxicity. This can be done either by complex formation between the thymoquinone and Cr(VI) or by reduction of Cr(VI) to Cr(III) by thymoquinone. In the former case Cr(VI) in complexed form will be no more harmful whereas in the later case it would be converted into useful form.

Introduction

Thymoquinone (2-methyl-5-isopropyl-1, 4-benzoquinone) (TQ) (Fig. 1) is one of the principal component of the *Nigella sativa* (NS) seed oil (Ali and Blunden, 2003). Several research studies have shown that most of the NS seed's beneficial effects are due to TQ (Mehta *et al*., 2009; Xin *et al*., 2008). Among the various bioactivities, examined for TQ, one of the most important is its antioxidant activity (Badary *et al*., 2003; Mansour *et al*., 2002). The compound was observed to decrease cellular oxidative stress (Mohamed *et al*., 2003). It has a potent chemo-preventive potential of inhibiting the process of carcinogenesis (Badary *et al*., 2007; Badary *et al*., 1999). In addition, several research studies have shown its other pharmacological activities such as anti-inflammatory (Syed, 2008; Gazzar *et al*., 2007), anti-tumor (Gali-Muhtasib *et al*., 2008; Shoieb *et al*., 2003), antidiabatic (Abdelmeguid *et al*., 2010; Fararh *et al*., 2005), antitussive (Hosseinzadeh *et al*., 2008), antimicrobial (Mouhajir *et al*., 1999) and apoptosis induction (El-Mahdy *et al*., 2005) activities. It also possesses neuro-protective effect (Al-Shabanah *et al*., 1998).

As most of the biological activities of TQ are due to its antioxidant properties, the electrochemical study of it is of potential importantance. Previously only polarographic behaviour of TQ has been investigated (Michelitsch and Rittmannsberger, 2003), but no sufficient work has been done on electrochemical properties of complexes of TQ hence there is a great need of research in this field. We have examined the electrochemical behavior of TQ and its complex with iron (III) in detail (Kishwar and Haq, 2013) and it was realized during the research that further study of TQ and its complexes with some other redox active metals could extremely beneficial. Keeping this point in view electrochemical study of complex of TQ with chromium (VI) has been performed.

Chromium is widely distributed in our entire environment including air, water, soil, plants and in animals. Cr(III) and Cr(VI) are the most common form of chromium (Lori and Mary, 2007; Eleanor and Sharon, 2002). Trivalent state is used as an essential mineral for human beings. It plays important role in metabolism of carbohydrate and lipid (Hellerstein, 1998; Mertz, 1998). Although its deficiency may cause elevated blood cholesterol and triglyceride levels but high levels could also create severe problems including renal failure (Lori and Mary, 2007). Specially Cr(VI) has been reported more toxic. It is a strong irritant; as a result it may cause different types of allergic reactions. Its inhalation can cause irritation and damage to nose, lungs, stomach and intestines and ingestion could result in stomach upsets and ulcers, convulsions, damage to kidneys and liver and even death. In case of chronic toxicity it might cause pulmonary fibrosis and lung cancer. However, reducing substances in the food reduces Cr(VI) to Cr(III) if it is ingested at low levels (Stoecker, 1999). Since seeds of NS are also used in food TQ can also serve the same purpose.

Materials and Methods

Reagents and Glassware: The reagents used were of analytical grade, purchased from Merck, and MP Biochemicals LLC. The glassware used was of standard quality. They were properly cleaned and rinsed with distilled- deionized water. For electrochemical studies potassium dichromate ($K_2Cr_2O_7$), thymoquinone (TQ) and sodium chloride (NaCl) were used.

Instrumentation

Electric balance: Shimadzu AX 200 was used for weighing.

Cyclic voltammeter: CHI – 760 D Electrochemical work station, cyclic voltammeter was used for cyclic voltammetric studies. The instrument consists of a computer under Windows environment, a potentiostat and a cell assembly. The cell assembly includes a cell stand and a cyclic voltammetric glass cell. There were three electrodes, a glassy carbon electrode as working electrode, a saturated calomel electrode as reference electrode and a platinum wire electrode as an auxiliary or counter electrode. Re-polishing and re-surfacing of working electrode was performed using Alumina polishing compound. Finally, nitrogen purging was checked for the system, but its presence or absence was found to produce no change in voltammograms.

Sample preparation

Supporting Electrolyte Solution: 2.92g of NaCl was transferred in a 500mL volumetric flask and then dissolved in distilled deinoized water to prepare 0.1M solution.

Analyte Solutions: 0.005M solution of TQ and equimolar solution of $K_2Cr_2O_7$ were prepared as analyte by using NaCl (0.1 M) as electrolyte solution. 10% methanol was also used in the preparation of analyte's solutions.

Cyclic Voltammetric Studies: Every time fresh solutions were prepared. At first the base-line of the supporting electrolyte was taken and then 15.0mL of analyte was run to get overlay of NaCl (0.1 M), Cr(VI) (5×10^{-4}M), TQ ($5x\ 10^{-4}$M) and Cr(VI) – TQ (5×10^{-4}M). The scan rate was 0.1 V and current sensitivity was $1x10^{-4}$ A/V. The potential range was set from -0.40 V to +0.80 V and then reversed back to -0.40 V. In order to observe effect of scan rate, the complexation was studied at different scan rates having a range from 0.05V/s to 0.50V/s whereas all other parameters were kept constant. The metal-ligand ratio of the complex solution was 1:3.

Results and Discussion

Current study gave following important information:

Confirmation of complex formation: Complex formation was checked by comparing voltammograms of supporting electrolyte NaCl (0.1M), Cr(VI) ($5x10^{-4}$M), TQ ($5x10^{-4}$M) and Cr(VI)-TQ complex (1:1) ($5x10^{-4}$M) (Fig. 2). The linearity of the baseline in overlay of the voltammograms reveals absence of impurities in supporting electrolyte and cleanliness of working electrode. Linear response of the metal showed its redox inactivity within applied potential scan range. The overlay shows significant differences in the cyclic voltammograms of Cr(VI), TQ and Cr(VI)–TQ complex which shows complexation between Cr(VI) and TQ. In case of complex an oxidative wave in the potential range of forward scan could be seen going from -0.4V to +0.8V. Then during the reverse scan the oxidized species is reduced back from +0.8V to -0.4V. The anodic peak for the complex was noted at +0.179V, whereas cathodic peak was seen at -0.210V. The comparison shows that there is a great difference between values of E_{pa} and E_{pc} of TQ and complex, pointing out that complex formation has taken place between Cr(VI) and TQ (Table-1).

Significant drop in the peak current of the complex as compared to the ligand was observed, as well as positive shift in the peak potential was also noted suggesting presence of pre equilibrium charge transfer process in Cr(VI)-TQ complex (Table-1), which could be expressed as follows:

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

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

Effect of scan rate on voltammograms of Cr(VI)-TQ complex: For this purpose cyclic voltammograms of the complex solution ($5x10^{-4}$M) were observed at different scan rates i.e. 0.05 V/s - 0.5 V/s. The overlay of the voltammograms clearly reveals the presence of a quasi-reversible electron transfer (Fig.3). Continuous increase in I_{pa} and I_{pc} was observed with the increase of scan rates, furthermore the ratio of these peak currents (I_{pa}/I_{pc}) was not found equal to 1. Another quasi-reversible behavior was exhibited by ΔE_p, which was observed greater than 59/n mV and it increased as the scan rate was enhanced (Table 2). I_p was also observed to increase with the square root of scan rate (Fig. 4). Quasi-reversible behavior was further favoured by negative shift of E_{pc} with

increase of ν (Table 2 and 3). The plot of I_p versus $\nu^{1/2}$ gave a straight line which reveals that the reaction may be diffusion controlled (Fig.4).

At higher scan rates anodic peak of the complex was observed to be distorted, but an increase in current was examined which shows presence of some chemical reaction. This distortion of the peak may be due to superimposition of two closely located waves resulting in a broadened anodic wave or due to adsorption of any specie on the electrode (Fig. 3).

The plot of peak potentials against log of scan rate gave straight lines with good R^2 values (Fig. 5). The values of transfer coefficient (α and β) was also calculated for complex solution according to the relation $0.048/(E_p-E_{p/2})$ at different scan rates which were found to be 0.593 ± 0.02 - 1.231 ± 0.01 and 0.750 ± 0.01 - 1.171 ±0.01 respectively (Table 2).

Analysis of diffusion coefficient for Cr(VI)-TQ complex: Diffusion coefficient of the complex was found to be entirely different than that of TQ which confirms complexation between Cr(VI) and TQ (Table- 4(a)). It was determined using Randles- Sevcik (Greef *et al.*, 1985) equation. Its values were calculated at different scan rates also and no reasonable effect was observed (Table- 4(b)). Area of electrode (A) was 0.0706 cm^2.

Analysis of E˚, a characteristic property: For Cr(VI)-TQ complex values of E˚ were determined at different scan rates and were found to be approximately constant (Table 5).

Conclusion

Cyclic voltammetric study of Cr(VI)-TQ complex was performed at Glassy carbon electrode against Standard calomel electrode. Horizontal base line (NaCl) indicates the purity of system. The qualitative and quantitative analyses were performed for this complex. Qualitative analysis clearly showed the formation of complex on mixing of Cr(VI) and TQ. Quantitative analyses include determination of E˚, D, α and β. Effects of varying scan rates on complexation was also observed. In case of Cr(VI)-TQ complex significant 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. The data at different scan rates showed that the complex exhibited quasi-reversible behaviour. The complex seems to be stable at lower values of scan rates. 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 constant. Diffusion coefficient was calculated using Randles- Sevick equation. The values of transfer coefficients, α and β were also determined at different scan rates. Furthermore present research reveals that in case of metal toxicity by chromium, especially by Cr(VI), TQ could be helpful in removing it from the body by forming complex with Cr(VI). This metal after formation of complex will be unable to absorb in the body and then excreted from the body in the form of complex. Hence intake of *Nigella sativa* seeds in case of Cr(VI) toxicity could be beneficial without any side effects.

Table 1. Electrochemical parameters of cyclic voltammograms of TQ, Cr(VI), and Cr(VI)- TQ Complex.

	I_{pa} (A)	I_{pc} (A)	E_{pa} (V)	E_{pc} (V)
TQ	$1.017x10^{-5}$±0.01	$2.717x10^{-5}$±0.01	-0.242±0.01	-0.326±0.01
Cr(VI)	-	-	-	-
Cr(VI)-TQ Complex	$2.463x10^{-6}$± 0.01	$1.142x10^{-5}$± 0.01	0.179 ± 0.01	-0.210 ± 0.01

Table 2. The values of E_p, $E_{p/2}$, E_p-$E_{p/2}$, E_{pa}-E_{pc} and I_p from cyclic voltammograms of Cr(VI)- TQ Complex with 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}	$\beta n_b = 0.048/E_{pa}$-$E_{pa/2}$
0.05	-0.238±0.01	-0.279±0.01	0.041±0.01	1.180±0.01	0.459±0.01	1.171± 0.01
0.10	-0.236±0.01	-0.282±0.01	0.046±0.01	1.431±0.01	0.434±0.01	1.043± 0.01
0.15	-0.233±0.02	-0.288±0.01	0.055±0.01	1.709±0.02	0.474±0.02	0.873± 0.01
0.20	-0.229±0.01	-0.290±0.01	0.061±0.01	1.957±0.01	0.503±0.01	0.787± 0.01
0.25	-0.228±0.02	-0.292±0.01	0.064±0.01	1.811±0.02	0.405±0.02	0.750± 0.01
0.35	a	-	-	-	-	-
0.40	a	-	-	-	-	-
0.45	a	-	-	-	-	-
0.50	a	-	-	-	-	-

a = Peak distorted

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	-0.336± 0.01	-0.291±0.01	-0.045± 0.01	0.098±0.01	2.568± 0.01	1.067± 0.01
0.10	-0.344± 0.01	-0.303±0.01	-0.041± 0.01	0.108±0.01	3.300± 0.01	1.171± 0.01
0.15	-0.354± 0.01	-0.315±0.01	-0.039±0.01	0.121±0.01	3.604± 0.01	1.231± 0.01
0.20	-0.370± 0.01	-0.315±0.01	-0.055±0.01	0.141±0.01	3.888± 0.01	0.873± 0.01
0.25	-0.379± 0.01	-0.315±0.01	-0.064±0.01	0.151±0.01	4.471± 0.01	0.750± 0.01
0.35	-0.403± 0.02	-0.335±0.02	-0.068±0.02	-	4.915± 0.02	0.706± 0.02
0.40	-0.410± 0.02	-0.339±0.02	-0.071±0.02	-	5.061± 0.02	0.676± 0.02
0.45	-0.415± 0.02	-0.339±0.02	-0.076±0.02	-	5.215± 0.02	0.632± 0.02
0.50	-0.419± 0.02	-0.338±0.02	-0.081±0.02	-	5.417± 0.02	0.593± 0.02

Table 3. Comparison of diagnostic criteria for quasi-reversible systems at 25 ± 1˚C and results obtained from Cr(VI)- TQ Complex.

S.No	Criteria for Quasi-reversible System*	Results obtained for Cr(VI) –TQ Complex
1	lI_pl is not proportional to $\nu^{1/2}$, but increases with increase in $\nu^{1/2}$	lI_pl was not found proportional to $\nu^{1/2}$, but it increased with the increase in $\nu^{1/2}$
2	$lI_{pa}/I_{pc}l = 1$, provided $\alpha_c = \alpha_a = 0.5$	$lI_{pa}/I_{pc}l \neq 1$
3	E_{pa}-E_{pc} > 59/n mV and increases with increase in ν	E_{pa}-E_{pc} > 59/n mV and it was found to increase with the increase in ν
4	E_{pc} shifts negatively as ν increases	As ν increases E_{pc} shifts negatively

References*= (Bard and Faulkner, 2001; Greef *et al*., 1985; Nicholson, 1965).

Table 4(a). Comparison of the Diffusion Coefficients of TQ, Cr(VI), and Cr(VI)-TQ Complex $D^{1/2} = I_p/ (2.69x10^5) (n)^{3/2} AC (\nu)^{1/2}$.

	I_{pa} (A)	$D^{1/2}$	D ($cm^2 s^{-1}$)
TQ	1.017x10^{-5}± 0.01	3.39 x 10^{-3}	1.15 x 10^{-5}
Cr(VI)	-	-	-
Cr(VI)-TQ Complex	0.246x10^{-5} ± 0.01	5.68 x 10^{-4}	3.23 x 10^{-7}
	I_{pc} (A)	$D^{1/2}$	D ($cm^2 s^{-1}$)
TQ	2.717x10^{-5}±0.01	9.06 x 10^{-3}	8.20 x 10^{-5}
Cr(VI)	-	-	-
Cr(VI)-TQ Complex	1.142 x10^{-5}±0.01	2.64 x 10^{-3}	6.97 x 10^{-6}

Table 4(b). Diffusion coefficients of Cr(VI)-TQ Complex at different scan rates.

v (V/s)	$v^{1/2}$	I_{pa} $x10^{-5}$(A)	$D^{1/2}$	D (cm^2 s^{-1})
0.05	0.224	1.180 ± 0.01	3.85×10^{-3}	1.48×10^{-5}
0.10	0.316	1.431± 0.01	3.31×10^{-3}	1.09×10^{-5}
0.15	0.387	1.709 ± 0.02	3.23×10^{-3}	1.04×10^{-5}
0.20	0.447	1.957 ± 0.01	3.20×10^{-3}	1.02×10^{-5}
v (V/s)	**$v^{1/2}$**	**I_{pc} $x10^{-5}$ (A)**	**$D^{1/2}$**	**D (cm^2 s^{-1})**
0.05	0.224	2.568± 0.01	8.37×10^{-3}	7.01×10^{-5}
0.10	0.316	3.300± 0.01	7.63×10^{-3}	5.82×10^{-5}
0.15	0.387	3.604± 0.01	6.80×10^{-3}	4.63×10^{-5}
0.20	0.447	3.888± 0.01	6.35×10^{-3}	4.04×10^{-5}
0.25	0.500	4.471± 0.01	6.53×10^{-3}	4.27×10^{-5}
0.35	0.592	4.915± 0.02	6.06×10^{-3}	3.68×10^{-5}
0.40	0.632	5.061± 0.02	5.85×10^{-3}	3.42×10^{-5}
0.45	0.671	5.215± 0.02	5.68×10^{-3}	3.22×10^{-5}
0.50	0.707	5.417± 0.02	5.60×10^{-3}	3.13×10^{-5}

Table 5. Half wave potential ($E^\circ = E_{1/2}$) for Cr(VI)-TQ Complex at different scan rates.

Scan rates (v) V/s	$(E^\circ)_a$ (V)
0.05	0.259 ± 0.01
0.10	0.259 ± 0.01
0.15	0.261 ± 0.01
0.20	0.259 ± 0.01
0.25	0.260 ± 0.01

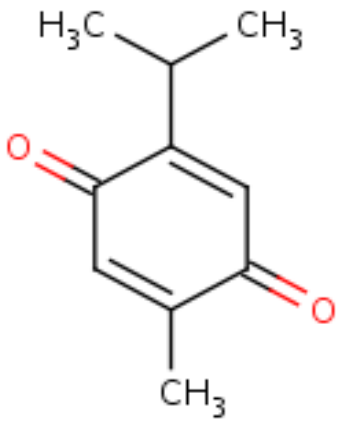

Fig. 1. Structure of TQ

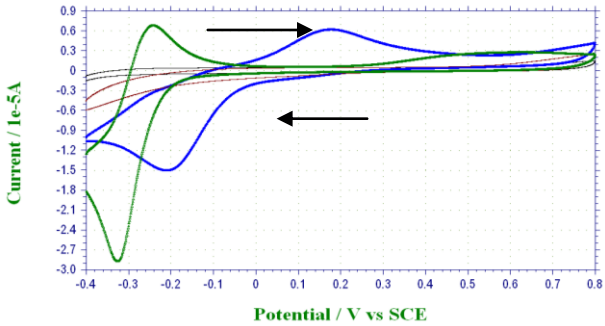

Fig. 2. Cyclic- Voltammograms of TQ, Cr(VI) and Cr(VI)-TQ Complex in NaCl at 0.1V/sec (Baseline= NaCl (0.1 M), Metal= Cr(VI) ($5x10^{-4}$ M), Ligand= TQ ($5x10^{-4}$ M), Complex= Cr(VI)-TQ ($5x10^{-4}$M)).

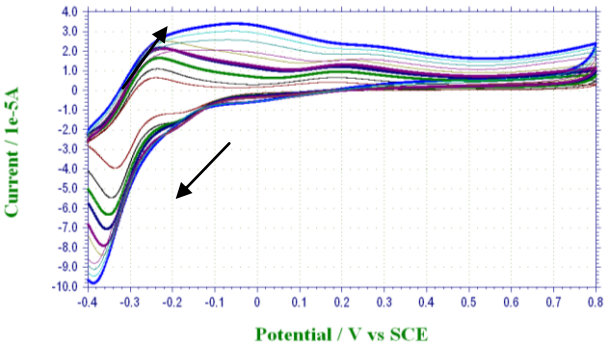

Fig. 3. Cyclic voltammograms of Cr(VI)-TQ Complex at Different Scan rates (0.05 V, 0.1 V, 0.15V, 0.2V, 0.25 V, 0.3 V, 0.35 V, 0.4 V, 0.45 V, 0.5 V).

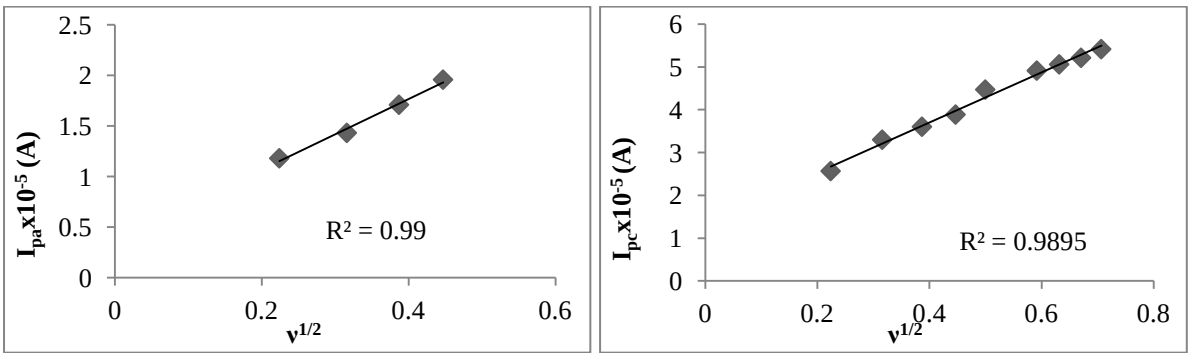

Fig. 4. Variations of anodic and cathodic peak current with square root of sweep rate from the cyclic voltammograms of Cr(VI)-TQ Complex.

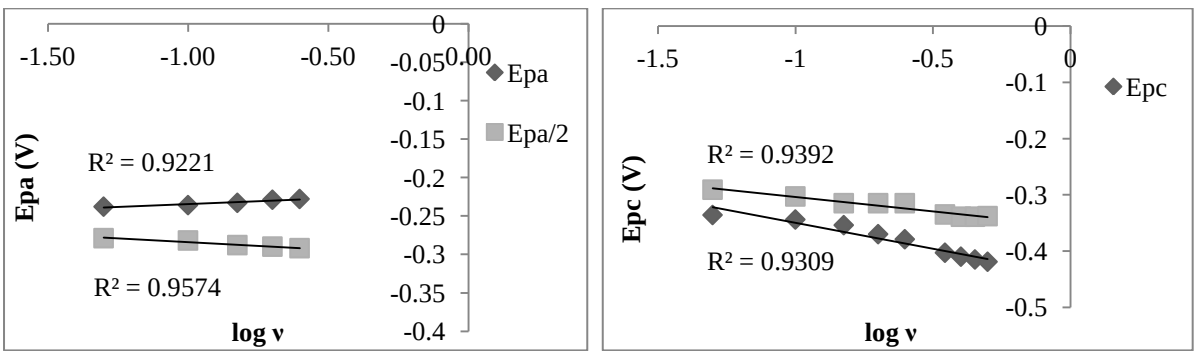

Fig. 5. Variation of anodic and cathodic peak potentials with sweep rate from cyclic voltammograms of of Cr(VI)-TQ Complex.

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