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One-electron redox reactions of troxerutin in aqueous solutions

A. SARKAR [1], N. BISWAS [1], S. KAPOOR [1,*], H. S. MAHAL [1], C. K. K. NAIR [2] and T. MUKHERJEE [1]

[1] *Radiation Chemistry and Chemical Dynamics Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*
[2] *Radiation Biology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India*

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Abstract—The oxidation of flavonoids is of great interest because of their action as antioxidants with the ability to scavenge radicals by means of electron-transfer processes. The redox reactions of the flavonoid derivative troxerutin, (2-[3,4-bis-(2-hydroxyethoxy) phenyl]-3[[6-deoxy-α-L-manno-pyranosyl)-β-(D-glucopyranosyl]-oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4H-1-benzo-pyran-4-one), were investigated over a wide range of conditions, using pulse radiolysis and cyclic voltammetry. The oxidation mechanism proceeds in sequential steps. One-electron redox potentials for troxerutin were found to be +1.196, +0.846 and −0.634 V *vs.* NHE.

Keywords: Troxerutin; pulse radiolysis; electron transfer.

INTRODUCTION

The major free radicals produced upon the irradiation of water are $H^{\cdot}$, $OH^{\cdot}$ and e^{-}_{aq}. These free radicals are very reactive [1] and react with many inorganic and organic compounds, including RNA, DNA, lipids and proteins [2]. Radiation therapy is one of the most common modalities of treatment for human cancers. In order to obtain better tumour control with higher doses of radiation, the normal tissues should be protected against radiation injury. Thus, radioprotecting compounds are of importance in clinical radiation therapy. A large number of these compounds have been synthesized. Unfortunately, most of them have been found to be toxic. Recently, promising results have been shown for WR 2721 and other protectors in its series [3–6]. One of the important features for an ideal radio protector is its

*To whom correspondence should be addressed. Tel.: (91-22) 2559-0298. Fax: (91-22) 2550-5151. E-mail: sudhirk@apsara.barc.ernet.in

water solublility, which facilitates administration. Naturally occurring antioxidant compounds, like flavonoids, polyphenols, vitamin E, etc., offer protection against deleterious effects of ionizing radiation because of their ability to scavenge free radicals [7–10]. Polyphenols, flavonoids and tannins are widely distributed in plants and are known to exhibit higher antioxidant activities. Humans consume substantial amounts of flavonoids in fruits, vegetables, herbs and beverages [11, 12]. It is known that flavonoids (i.e., rutin and quercetin) possess P vitamin activity. It has been shown that rutin can be used for treatment of Fanconi's anemia [13]. These effects of flavonoids are ascribed to their scavenging ability of superoxide anions, hydroxyl and peroxyl radicals, $NO^{\cdot}$ radicals and peroxynitrite [14–18]. Scavenging of reactive oxygen species results in the inhibition of lipid peroxidation [19, 20].

It is known that antioxidant activity of flavonoids is due to the aromatic OH groups [21]. The mechanism of their action as antioxidants seems to involve the ability of phenols to scavenge radicals by a pH-dependent electron-transfer process in which the phenol is converted into a phenoxyl radical. Since phenolic compounds have at least one parent pK_a ($ArOH + H_2O \Leftrightarrow ArO^- + H_3O^+$), at pH below this p$K_a$ the reduction potential increases with a slope of 0.059 V/pH [22]. In general, an electron donating substituent favours the oxidation of a compound. However, the extent of oxidation depends on the position of the substituent with respect to the C-atom having the $-$OH group. On the other hand, phenols with electron withdrawing groups have higher oxidation potential. This may resist the oxidation of phenols by milder oxidizing agents. Therefore, antioxidant ability of any compound can be correlated *a priori* with its pK_a. As mentioned above, one of the main thrust of synthesizing radioprotectors is to make them water-soluble. The flavonoid derivative troxerutin (2-[3,4-bis-(2-hydroxyethoxy) phenyl]-3[[6-deoxy-α-L-manno-pyranosyl)-β-(D-glucopyranosyl]-oxy]-5-hydroxy-7-(2-hydroxyethoxy)-4H-1-benzo-pyran-4-one) is water soluble and has been used therapeutically for treating various diseases, such as chronic venous insufficiency (CVI), varicosity, capillary fragility, etc. [23–30]. It has been shown to offer radioprotection to normal tissues in mice bearing tumours [31]. Troxerutin has undergone numerous clinical trials, animal studies and *in vitro* studies. Even with high doses, this compound had excellent safety and tolerability profiles [32, 33]. Troxerutin is a wide-spectrum drug. Therefore, it is important to know its reactivity towards various species produced during oxidative stress. This is important because there are reports in the literature that show that flavonoids can act as antioxidants or pro-oxidants. The pro-oxidant activities, due to their o-semiquinone and quinonoid products, are suggested to be responsible for their toxic physiological effects [34]. Studies of such species can be mimicked easily using radiolytic methods. In this study, we have used the pulse radiolysis technique to generate various radicals in aqueous medium and characterize the transient formed by their reaction with troxerutin by kinetic spectrophotometric technique. The reduction potentials were estimated using cyclic voltammetry at physiological pH.

EXPERIMENTAL

Materials

Troxerutin, NaN_3, KBr and sodium formate were obtained from Sigma and used as received. All other chemicals and reagents were of HPLC, AR or GR grade. IOLAR grade (purity > 99.9%) gases (N_2 or N_2O) used for purging the solutions were obtained from Indian Oxygen.

All solutions were prepared in a 10^{-3} mol/dm^3 phosphate buffer (equimolar mixtures of $Na_2HPO_4 \cdot H_2O$ and KH_2PO_4) unless stated otherwise. The alkaline pH was adjusted by NaOH to the required value. Water purified by Millipore was used for making solutions.

Radiolytic procedures

Pulse radiolytic studies were carried out by irradiating solutions in rectangular quartz cells of 1 cm optical path length. Pulses of 500 ns or 2 μs of 7 MeV electrons from a linear electron accelerator were employed. The details of the LINAC are given elsewhere [35]. An aerated 5×10^{-2} mol/dm^3 KSCN solution was used for dosimetry and the $(SCN)_2^{\cdot -}$ radical was monitored at 475 nm. The absorbed dose per pulse was calculated [36] assuming $G \times \varepsilon\ [(SCN)_2^{\cdot -}] = 2.6 \times 10^{-4}$ m^2/J at 475 nm. The dose employed was 16 Gy per pulse for 500 ns pulse. Experiments were carried out at room temperature (25°C).

The rates of reactions were determined by carrying out the experiments with at least three different concentrations of troxerutin, varying by at least a factor of 4. Bimolecular rate constants were derived from plots of the first-order rates *vs.* concentration. The rate constants reported are generally accurate to ±15%. Second-order rate constants were confirmed by changing the dose (from 16 to 80 Gy).

Generation of various primary and secondary radicals

Upon irradiating a dilute aqueous solution the following primary radicals are produced [1] from radiolysis of water:

$$H_2O \rightsquigarrow e_{aq}^-, H^{\cdot}, OH^{\cdot} \tag{1}$$

In order to selectively produce e_{aq}^-, 2-propanol was used to scavenge $H^{\cdot}$ and $OH^{\cdot}$ radicals. Reactions of $OH^{\cdot}$ radicals were studied in N_2O-saturated solution. This resulted in the scavenging of e_{aq}^-

$$e_{aq}^- + N_2O \xrightarrow{H_2O} N_2 + OH^- + OH^{\cdot} \tag{2}$$

Formate radicals were produced by irradating N_2O-saturated aqueous solution containing formate

$$HCO_2^- + OH^{\cdot}/H^{\cdot} \rightarrow CO_2^{\cdot -} + H_2O/H_2 \tag{3}$$

Secondary one-electron oxidants were produced by the reaction of $OH^{\cdot}$ radicals with inorganic anions [22] *via* the following reactions:

$$OH^{\cdot} + N_3^- \rightarrow N_3^{\cdot} + OH^- \quad (4)$$

$$OH^{\cdot} + Br^- \rightarrow Br^{\cdot} + OH^- \quad (5)$$

$$Br^{\cdot} + Br^- \rightleftarrows Br_2^{\cdot -} \quad (6)$$

Subsequent reactions of the secondary oxidants ($N_3^{\cdot}$ and $Br_2^{\cdot -}$) with troxerutin resulted in the generation of a one-electron-oxidised species of troxerutin.

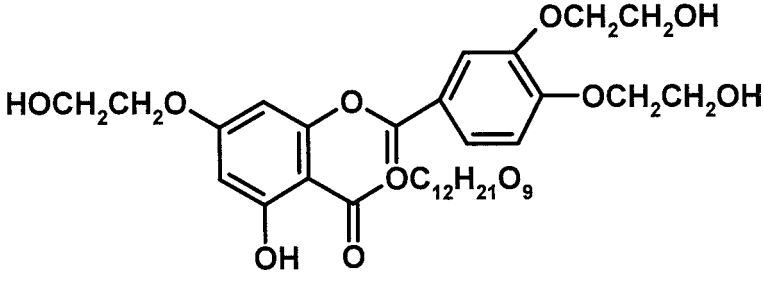
Cyclic voltammetry experiments were carried out employing a potentiostat, Autolab 100 electrochemical system (Eco Chemie, Netherlands) driven by GPES software. The electrochemical system was coupled to a cell comprising a glassy carbon disc (GC) as a working electrode (3 mm diameter), a saturated calomel reference electrode (SCE) and a platinum rod as the counter electrode. The scan rate was 50 mV/s. For differential pulse voltammetry step potential and pulse amplitude were 1 mV and 25 mV, respectively. The experiments were performed in a N_2-bubbled aqueous solution at 25°C. The potential values obtained against SCE were converted to those against the normal hydrogen electrode (NHE) by adding 0.24 V.

RESULTS AND DISCUSSION

The structure of troxerutin is shown in Scheme 1. Figure 1 shows the variation in absorbance at 347 nm against the pH of the solution. It can be noted that absorbance at 347 nm falls sharply at pH more than 8.5. Concomitantly the absorption due to the formation of quinone increases at 280 nm. The pK_a value of troxerutin was found to be 9.5, which is closer to the reported pK_a for phenol. The pK_a value of troxerutin is much higher than that reported for rutin 3.2 [37]. This could be due to the absence of polyhydroxy groups in troxerutin.

Reactions of inorganic radicals with troxerutin

The kinetics of formation of the phenoxyl radical with $N_3^{\cdot}$ and $Br_2^{\cdot -}$ as the oxidizing radicals were measured at pH 11. Figure 2 shows the spectra of the transient



Scheme 1. Troxerutin.

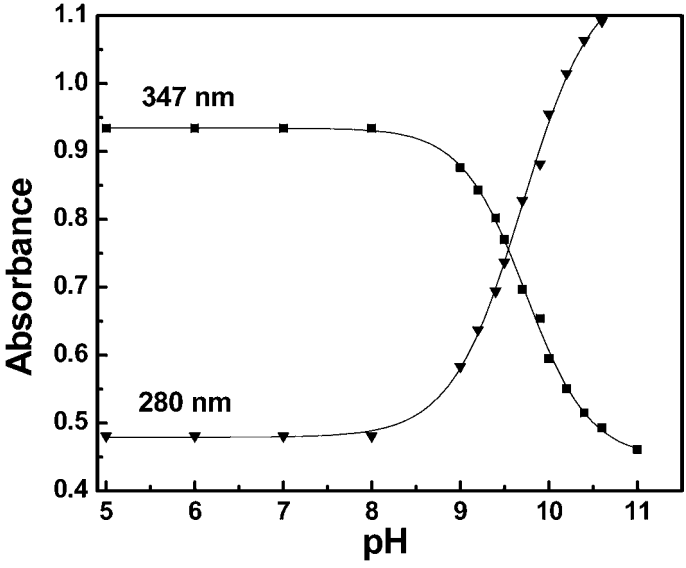


Figure 1. Variation of absorbance with pH of the solution (▼, 280 nm; ■, 347 nm).

obtained in a N_2O-saturated solution containing 2×10^{-4} mol/dm^3 troxertuin and 10^{-2} mol/dm^3 NaN_3. It can be seen that the transient absorption spectrum shows a strong bleaching at around 390 nm. The observed spectrum is similar to that previously assigned to the rutin phenoxyl radical [21]. The bimolecular rate constant for the formation of the phenoxyl radical was not determined due to the strong bleaching signal. Similar result was obtained when $Br_2^{-\cdot}$ was used as one-electron oxidant. It should be pointed out here that one-electron oxidation of troxerutin was not observed at pH 6.8.

Reaction of the hydroxyl radical with troxerutin

Figure 3 shows the absorption spectrum of the transient obtained upon the pulse radiolysis of an aqueous N_2O-saturated solution of troxerutin (2×10^{-4} mol/dm^3) at pH 6.8 (dose = 16 Gy). The transient shows an absorption maximum at 450 nm along with strong bleaching signal at wavelengths lower than 375 nm. In troxerutin there are several sites available for an attack of the $OH^{\cdot}$ radical. Because the $OH^{\cdot}$ radical can abstract hydrogen from the phenolic group, it can add to the phenolic ring and can also form an adduct or abstract hydrogen from the glucose moiety. All of these processes can take place in parallel. On comparing the spectrum of the transient with that obtained in Fig. 2, the absorption at above 390 nm could be assigned due to a cyclohexadienyl-type radical. The kinetics of the reaction of $OH^{\cdot}$ radical with troxerutin was monitored at 450 nm and the bimolecular rate constant was found to be 7.5×10^9 dm^3 mol^{-1} s^{-1}. The transient decayed by second-order kinetics and $2k/\varepsilon l = 1.3 \times 10^5$ s^{-1}.

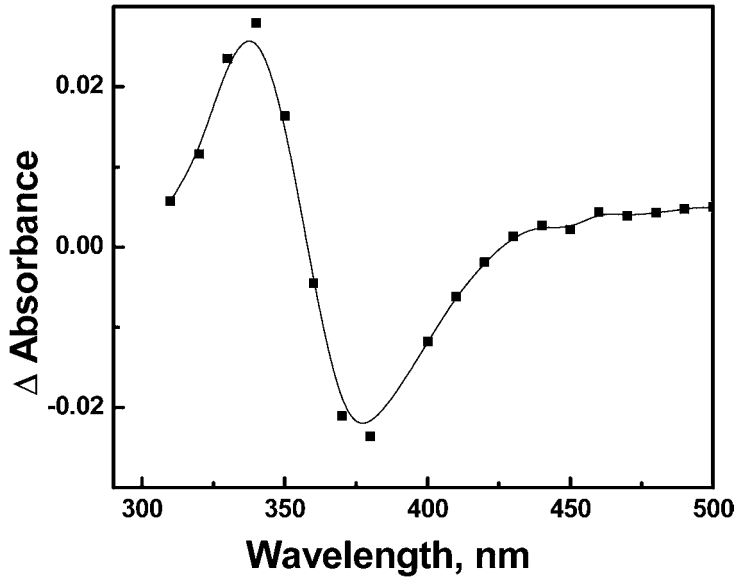


Figure 2. Transient absorption spectrum obtained at 20 μs after pulse radiolysis of a N_2O-saturated aqueous solution containing 1×10^{-4} mol/dm^3 troxerutin and 1×10^{-2} mol/dm^3 NaN_3 at pH 11. Dose = 16 Gy.

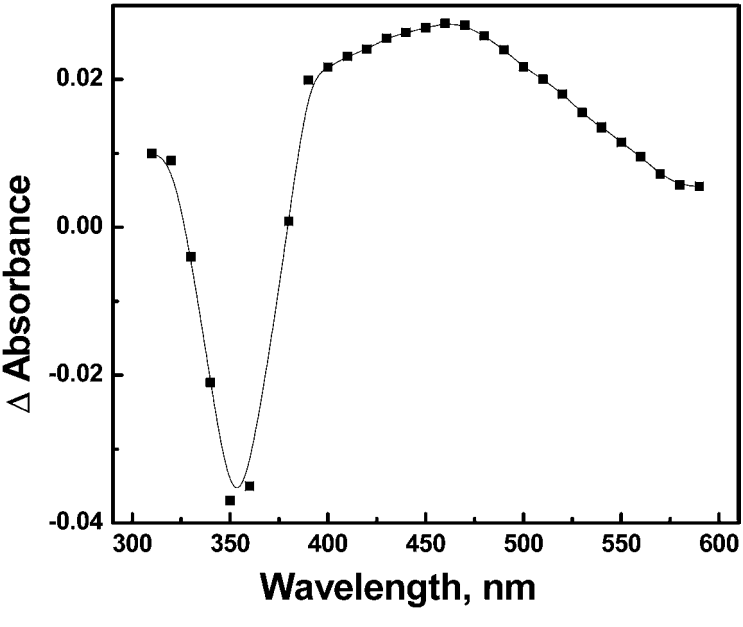


Figure 3. Time-resolved transient absorption spectra obtained at 10 μs after pulse radiolysis of a N_2O-saturated aqueous solution containing 1×10^{-4} mol/dm^3 troxerutin at pH 6.8.

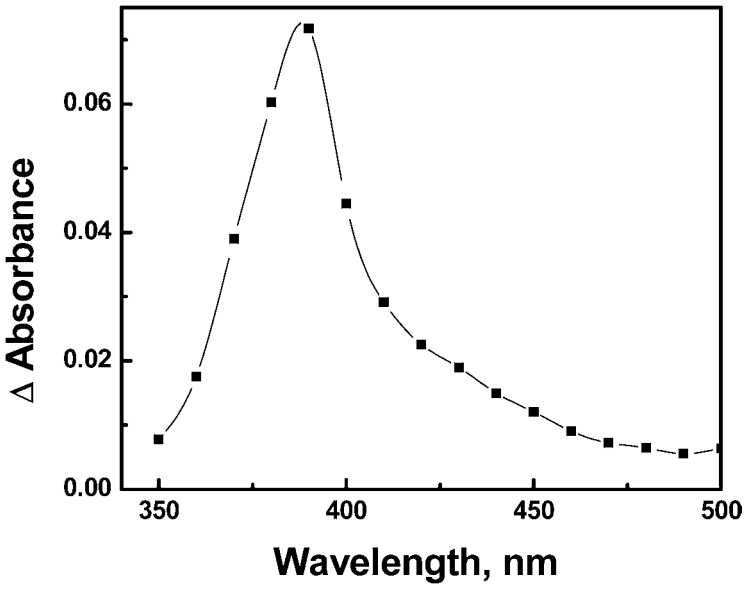


Figure 4. Absorption spectra of transient species formed at 5 μs after pulse radiolysis of a N_2-bubbled aqueous solution containing troxerutin (2.0×10^{-4} mol/dm^3) and 0.1 mol/dm^3 2-propanol at pH 6.8.

Reactions with e^-_{aq}

Figure 4 shows the transient absorption spectrum obtained on bombardment of an N_2-bubbled aqueous solution containing 2×10^{-4} mol/dm^3 troxerutin and 0.1 mol/dm^3 2-propanol at pH 6.8. The observed transient showed an absorption maximum at 390 nm. The bimolecular rate constant for the reaction of e^-_{aq} with troxerutin was measured by monitoring the decay of e^-_{aq} at 700 nm and found to be 1.5×10^{10} dm^3 mol^{-1} s^{-1}. The observed transient shows a strong absorption in the wavelength region where the ground state absorption spectrum of troxerutin appears. As can be seen from Fig. 4, no bleaching is observed in the transient absorption spectrum. This shows that the transient formed on reaction with e^-_{aq} has a high molar absorptivity. The possible site of attack of e^-_{aq} in troxerutin is the carbonyl group and the radical formed gets delocalilzed in the fused ring. This could be the possible reason for high molar absorptivity. The radical thus formed decayed by second-order kinetics with $2k/\varepsilon l = 1.1 \times 10^5$ s^{-1}.

Reaction with $CO^{\cdot}_2{}^-$

Figure 5 shows the transient absorption spectrum obtained on pulse irradiation of N_2O-saturated aqueous solution containing 2×10^{-4} mol/dm^3 troxerutin and 10^{-2} mol/dm^3 formate at pH 6.8. The transient absorption spectrum showed a maximum at around 390 nm. It can be seen that the observed spectrum in Fig. 5 is significantly different from that observed in Fig. 4. The insets of Fig. 5 show the formation of the transient at 390 nm at different time windows. A closer look

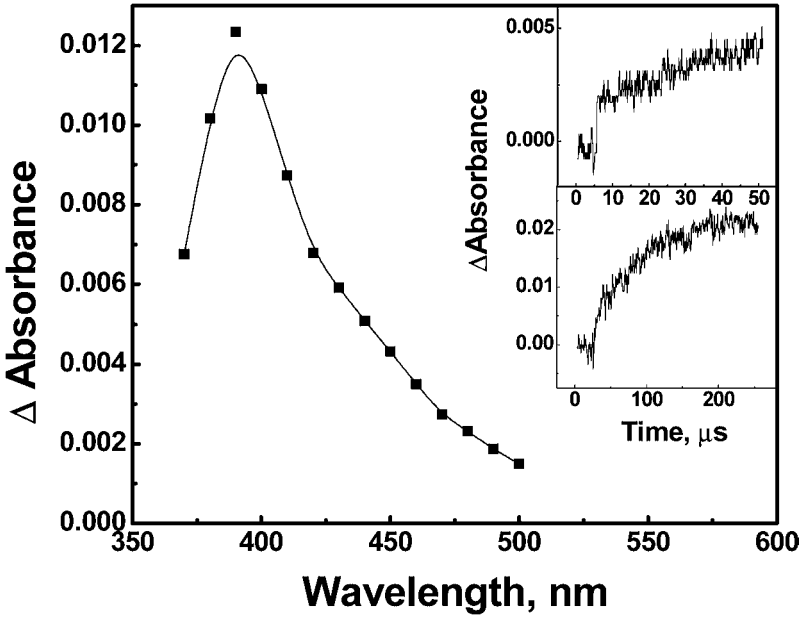


Figure 5. Absorption spectra of transient species formed at 400 μs after pulse radiolysis of a N_2O-saturated aqueous solution containing troxerutin (2.0×10^{-4} mol/dm^3) and 1×10^{-2} mol/dm^3 formate at pH 6.8. Insets: formation of the transient at 390 nm.

of these insets clearly shows a two-step formation. It appears that formate radical reacts with troxerutin by two paths: (i) reduction and (ii) addition. The contribution of the latter seems to be higher as the optical absorbance yield of the transient formed by one electron reduction is significantly low.

One-electron reduction potential

Determining the one-electron reduction potential of an anti-oxidant/radioprotector is very important to obtain information about the ease with which it can undergo an electron-transfer reaction with an oxidizing radical. Electrochemical studies reveal general trends in the electron-donating abilities of the molecule. The reduction potential of troxerutin was determined by cyclic voltammetry (CV) of a de-aerated aqueous solution containing 0.1 mol/dm^3 KCl, 1.0×10^{-3} mol/dm^3 phosphate buffer and 1.0×10^{-3} mol/dm^3 troxerutin. The results are shown in Fig. 6. Only one oxidation peak with a shoulder was observed in the differential pulse voltammetric scan (Fig. 6A). This shows that the electron transfer occurs at the ring having OH group and at the glucose moiety. The reduction process has been observed clearly in the cyclic voltammetric scan at -0.634 V *vs*. NHE (Fig. 6B). This peak comprises the one-electron reduction of the carbonyl group present in the moiety. One-electron oxidation and reduction potentials for troxerutin were found to be $+1.196$ and -0.634 V *vs*. NHE.

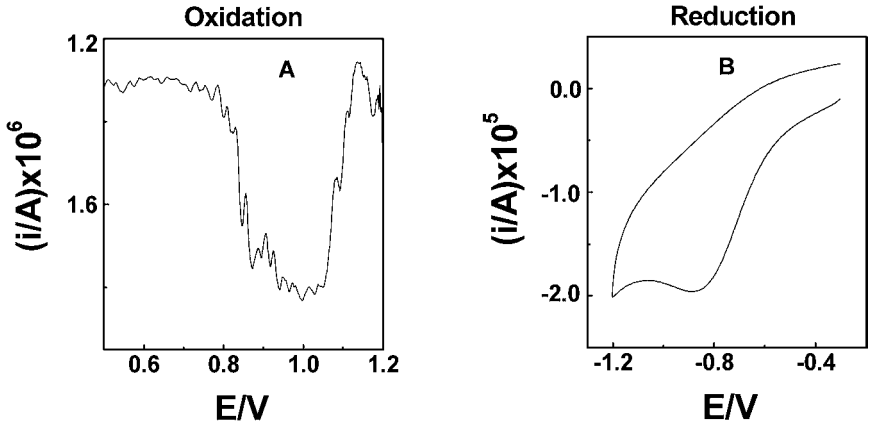


Figure 6. (A) Differential pulse voltammetry of 1×10^{-3} mol/dm^3 troxerutin in a N_2-bubbled aqueous solution containing 0.1 mol/dm^3 KCl and 1×10^{-3} mol/dm^3 phosphate buffer. (B) Cyclic voltammetry of the same solution.

CONCLUSIONS

The present study indicates that troxerutin, a cardiovascular drug, exhibits high reactivity towards primary radicals of radiolysis. Under hypoxic conditions the fact remains to be seen whether troxerutin could act as a radiosensitizer, as we have observed high reactivity with e^-_{aq}. Unlike known radiosensitizers the present drug does not possess a nitro group. However, N-ethyl maleimide, that has no nitro group, has high reactivity towards e^-_{aq} and acts as a hypoxic radiosenstizer [38]. The higher one-electron oxidation potential value could be due to the phenolic OH and the lower one due to the glucose moiety. Similar results were obtained for the water soluble vitamin E derivative, tocopherol monoglucoside [39]. The sequential oxidation of troxerutin may be one of the reasons for DNA radioprotection by troxerutin [31].

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