Polish J. Chem., **78**, 1575–1582 (2004)

Anodic Oxidation of Trolox in Aqueous Solutions[*]

by J. Małyszko[**] and M. Mechanik

Institute of Chemistry, Akademia Świętokrzyska, PL-25020 Kielce, Poland

(Received February 12th, 2004; revised manuscript March 25th, 2004)

Electrochemical oxidation of trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was investigated on glassy carbon (GC) electrodes in buffered aqueous solutions in the pH range of 1.0–8.8. The results were interpreted in terms of the formation of phenoxyl radicals, and their subsequent dimerization (radical-radical coupling, *i.e.* DIM1). Dimerization rate constants were estimated from peak heights. pK_a value of the carboxyl group was determined by potentiometric acid/base titration.

Key words: anodic oxidation, carbon electrode, cyclic voltammetry, dimerization, trolox

Antioxidants are compounds of great interest due to their involvement in important biological and industrial processes. They reveal anti-cancer and anti-inflammatory activity, and are used against cardiovascular diseases. Phenols, flavonoids and vitamin E have recently gained significant interest among various antioxidants. Antioxidant Capacity (also called Antioxidant Activity) is widely used as a parameter to characterize anti-oxidative power of various compounds and materials. Within several methods that are currently used to assess antioxidant capacity, the Trolox Equivalent Antioxidant Capacity (TEAC) assay is simple to be performed and thus has achieved special popularity. This procedure, originally described by Miller *et al.* [1], and next improved by other researchers [2,3], is based on the reduction of the relatively stable blue-green 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anion ($ABTS^{\bullet -}$). TEAC value of an antioxidant can be estimated by comparing its scavenging capacity with that of trolox. Lien *et al.* [4] and Arnao [5] have presented a literature review concerning this domain of research.

Trolox (Fig. 1) is a water-soluble analogue of lipophilic α-tocopherol, the most biologically active component of vitamin E. In comparison to α-tocopherol, trolox molecule has no phytyl "tail". Instead, carbonyl group is attached to the chromanol "head" in the position 2. Consequently, trolox is more hydrophilic than α-tocopherol and can be used as antioxidant both in aqueous and non-aqueous solutions. The anti-oxidant action of trolox is associated with the free OH group.

---

[*] Dedicated to Prof. Dr. Z. Galus on the occasion of his 70th birthday.

[**] Corresponding author: jan.malyszko@pu.kielce.pl

There is a large number of papers focused on applications of trolox. By contrast, redox and electrochemical properties of this compound have been poorly described so far. Fundamental thermodynamic data for trolox were estimated indirectly. Its one-electron oxidation potential was derived by Steenken *et al.* [6–8] from pulse radiolytic measurements in aqueous solutions to be 0.192 V at pH 13.5 and 0.48 V at pH 7, presumably *vs.* SHE. The same authors calculated pK_a value of trolox as 11.91. Chevion *et al.* [9], and Chen *et al.* [10] were the only ones who published voltammetric curves of the anodic oxidation of trolox at GC electrode in phosphate buffer (pH 7.4) and citrate buffer (pH 4.6). However, they did not propose any mechanism of the electrode process.

The present paper aims at clarification of the mechanism of electrooxidation of trolox in aqueous solutions at different pH, and is a logical starting point for an investigation of this important compound in a series of solvents. Due to the poor solubility of trolox in water, water-organic solvent mixture was applied. Organic component of the mixture was methanol, since its acid-base properties are similar to those of water [11,12]. The equilibrium constant of the transfer of hydrogen ion from water to methanol in methanol-water solutions is close to 1 [13].

Since polymerization of free radicals is favoured at Pt electrodes, voltammetric measurements were performed at glassy carbon (GC) electrodes.

EXPERIMENTAL

The following compounds were used: trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Aldrich), sodium perchlorate monohydrate p.a. (Merck), perchloric acid 60% p.a. (Merck), glacial acetic acid p.a., (Merck), sodium acetate p.a (Merck), sodium dihydrogencitrate (Aldrich), disodium succinate hexahydrate (Sigma). All reagents were used as received. In the most of voltammetric studies a 2.5×10^{-4} M trolox solution was used. pH of the solution was maintained between 3 and 9 using the following buffers: citrate, acetate, succinate, phosphate and borate. The most acidic solutions (pH between 1 and 2) were prepared using perchloric acid. Ionic strength of the solutions was kept constant (0.425) by the appropriate addition of $NaClO_4$. Final concentration of methanol (initially present in trolox stock solution) was 4% (v/v). Stock standard solutions were prepared daily before use. They were protected from light and air. Final solutions were prepared by the appropriate dilution of standard solutions with triply distilled water. They were used within a few hours. Solutions were degassed with argon of high purity (> 99.99%).

2 mm-in-diameter glassy carbon disc electrode (Mineral, Warsaw) was used as the working electrode. Its surface was polished on fine emery paper, and then with 0.3 μm alumina slurry on a polishing cloth. Finally, the electrode was rinsed with water and dried before use. Electrode surface was regenerated at moderately negative potentials in the hydrogen evolution region. Potentials were measured against Ag/AgCl/3 M NaCl electrode. Auxiliary electrode was a GC rod of 2 mm in diameter.

Voltammograms were recorded using an M161 electrochemical analyser (Entech, Cracow), controlled via Eagraph Version 3.0 software. Electrochemical measurements were performed in a three-electrode cell, at the room temperature (22 ± 1 °C). Electrochemical cell was enclosed in a grounded Faraday cage in order to minimise the influence of external electric fields.

RESULTS AND DISCUSSION

According to the molecular structure of trolox (Fig. 1), it exists in aqueous solutions as either neutral molecule (COOH/OH form), dissociated mono-anionic (COO^-/OH) or di-anionic species (COO^-/O^-), depending on pH. Since pK_{a1}, related to the dissociation of single proton from the carboxylic group, is not known in the literature, we have made an attempt to determine its value by potentiometric acid-base titration. For this purpose, 2×10^{-3} M trolox solution in water-methanol mixture (70/30, v/v) was titrated with standard solution of NaOH in the same solvent mixture. Bearing limitations of the Henderson-Hasselbach equation in mind [14], we have calculated an approximate value of pK_{a1} to be 3.6.

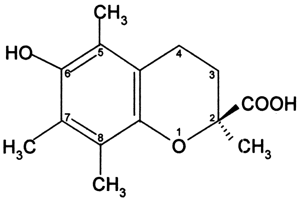

Figure 1. Molecular structure of trolox.

The dependence of average number of dissociable protons bound to trolox molecule on the pH (Bjerrum plot) is presented in Fig. 2. pK_{a2} value of the hydroxyl group was taken from the literature [7]. According to this diagram, trolox is fully protonated at pH < 2, whereas the mono-anionic COO^-/OH form predominates in the pH range 5÷10.5.

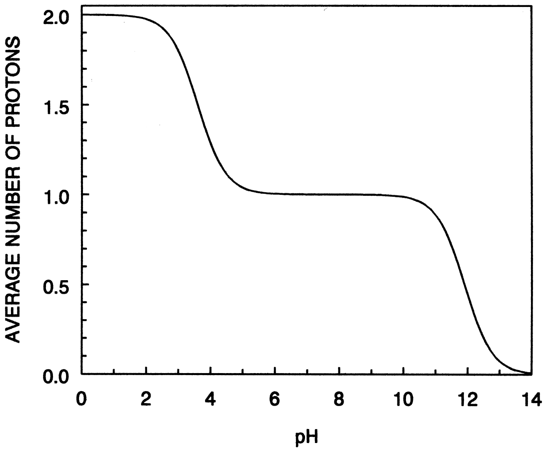

Figure 2. Average proton number in trolox molecule as a function of pH (Bjerrum plot).

Cyclic voltammetry of 2.5×10^{-4} M trolox solutions of different pH was performed on a glassy carbon electrode at scan rates (ν) $0.005 \div 1$ V s^{-1}. pH ranged from 1 to 8.8. All the solutions contained 4% of methanol (v/v) as a co-solvent. Typical *I-E* responses recorded in citrate buffer (pH 3.44) are presented in Fig. 3. In this experiment, the direction of potential scanning was reversed to the cathodic one at successively more positive potentials, and thereby at increasing of the oxidation time of trolox. As it can be seen in this Figure, the voltammogram is characterized by only one anodic peak (A_1) at 0.32 V. Anodic peak current, I_{pa}, was proportional to $\nu^{1/2}$ within the entire range of scan rates applied. This relationship is described by the following empirical equation:

$$I_{pa} = 0.04(\pm0.05)\,\mu A + 8.21(\pm0.05)\,\mu A/(V\,s^{-1})^{1/2},\ r = 0.997.$$

The numbers in parentheses denote standard deviations of the intercept and the slope, respectively, and r is the correlation coefficient (at 95% significance level). Thus, the peak current was governed by the Randles-Ševčik equation. Oxidation peak was shifted to more positive potentials with the increasing ν.

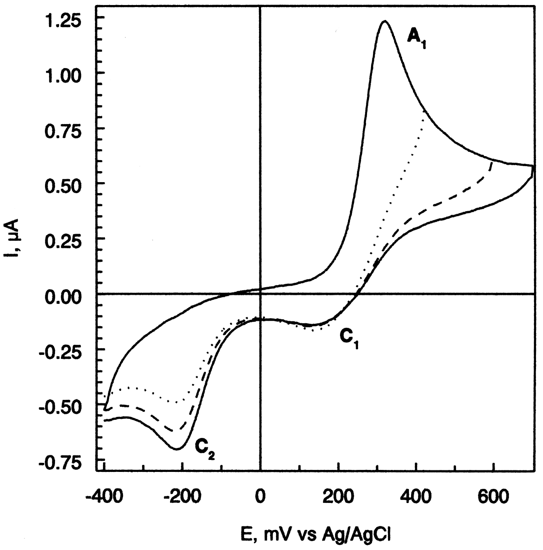

Figure 3. Cyclic voltammograms of trolox (2.5×10^{-4} M) in citrate buffer (pH 3.44). Conditions: scan rate: 10 mV s^{-1}, ionic strength: 0.425 mol dm^{-3} (adjusted with $NaClO_4$), initial potential: –400 mV, reverse potential: 420 (dotted line), 600 (dashed line) and 700 mV (solid line). Potentials were measured *vs.* Ag/AgCl/3 M NaCl.

The number of electrons, *n*, involved in the anodic process was evaluated using the method originally proposed by Malachesky [15], and then improved by Möller and Knaack [16], and Amatore *et al.* [17]. The method is based on the comparison of currents measured in two voltammetric techniques: anodic peak current in cyclic voltammetry (CV), and the limiting current in chronoamperometry (CA). Combination of Randles-Ševčik and Cottrell equations results in the formula:

$$n^{1/2} = 0.202\ (I_{\mathrm{pa}}\, v^{-1/2}/I_{\mathrm{L}}\, t^{1/2}) \qquad (1)$$

where v is the scan rate in CV, I_{L} and t denote anodic limiting current and time of electrolysis in CA, respectively. This approach is easy, since the knowledge of the diffusion coefficient is not necessary. The only requirement is reversibility of the electrode reaction, and linear plots of I_{pa} *vs.* $v^{1/2}$ and I_{L} *vs.* $t^{-1/2}$. The occurrence of chemical follow-up reaction results in a slight decrease of the slope of I_{pa} *vs.* $v^{-1/2}$ plot [18]. In our opinion, however, this effect is unimportant and negligible in the calculations of n from equation (1).

The n value determined in citrate buffer was 1.00 ± 0.03, which confirmed that oxidation of trolox at GC is one-electron process leading to the formation of the appropriate phenoxyl-type radical. Hence, A_1 peak was diffusive-controlled and represented oxidation of trolox to its phenoxyl radical cation, which in turn underwent an immediate deprotonation to chromanoxyl radical. The Tomeš criterion of electrochemical reversibility for one-electron transfer ($E_{3p/4} - E_{p/4} \approx 44$ mV) [19] was almost fulfilled at low scan rates. For the scan rates larger than 0.1 V s^{-1} the difference ($E_{3p/4} - E_{p/4}$) slightly increased (up to 55 mV at 1 V s^{-1}) and anodic process became quasi-reversible.

In the backward scan, two cathodic peaks appeared. Apparently C_1 peak at 0.15 V corresponded to the anodic peak A_1. As the oxidation time was increased, C_1 peak height decreased with respect to the baseline of the cathodic current. Moreover, the $|I_{\mathrm{pc}}|/I_{\mathrm{pa}}$ current ratio significantly increased with the increase of the scan rate. This effect originated from the primary product being present at the electrode surface, and its reduction back to trolox. Simultaneously, another cathodic peak (C_2) was formed at –0.15 V. Its height increased with an increase of the oxidation time. All of the above results are in accordance with the reaction sequence involving an irreversible chemical reaction following the electron transfer (EC mechanism) [20]. Consequently, peak C_2 can be attributed to the cathodic reduction of a secondary product formed during the following chemical step.

Similar CV curves were obtained for the solutions of different pH from the range $1 \div 5.3$. As expected, voltammograms were shifted to more negative potentials as pH of the solution was increased. Only one anodic peak appeared in each voltammogram. Noteworthy, anodic responses obtained in the solutions of pH 1.02 and 1.91 were fairly reversible (according to the Tomeš criterion) in the entire range of scan rates applied. In less acidic solutions (pH up to 5.3) anodic process was reversible only at slow scan rates. For pH above 5.3 oxidation became irreversible. Peak heights, I_{pa}, were correlated with the square of the scan rate, according to the Randles-Ševčik equation. The data obtained for the solutions of pH ≤ 4.6 show linear dependencies of I_{pa} *vs.* $v^{1/2}$, with slopes previously determined at pH (with $r > 0.99$). At pH ≥ 5.3, the correlation was increasingly non-linear, due to the irreversibility of the anodic process. We attributed this behaviour to the gradual conversion of charge-neutral COOH/OH form into the mono-anion COO^-/OH one, which stops approximately at the pH of 5.5 (see Fig. 2).

In Fig. 4 the anodic half-peak potentials, $E_{pa/2}$, measured at the scan rate of 10 mV s^{-1} are plotted *vs.* pH. At pH less than 5.3, the plot is linear of the slope of –50 mV per one pH unit. This value indicates equal numbers of protons and electrons being involved in the electrode process. At higher pH values, one can observe a distinct deviation from the linearity, which is caused by the irreversibility of the system. Extrapolation of the linear part of the plot to pH 7 provides a value of 83 mV against Ag/AgCl/3 M NaCl, *i.e.* 290 mV *vs.* SHE. This value disagrees significantly (by about 0.2 V) with that of one-electron redox potential obtained indirectly from pulse radiolytic measurements reported by Steenken and Neta [7].

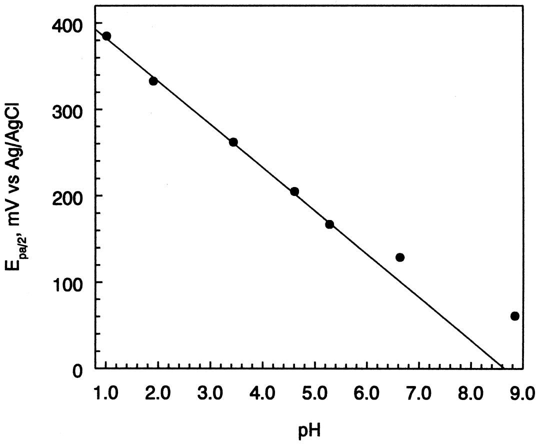

Figure 4. pH dependence of the half-peak oxidation potential of trolox (2.5×10^{-4} M); $v = 0.01$ V s^{-1}.

In each case, two cathodic peaks resulting from the reduction of phenoxyl radical and dimer are evident in the voltammogram. According to the aforementioned explanations, such a pattern is indicative of the follow-up dimerization at the limited rate. Cathodic peak at more negative potentials can be attributed to the reduction of dimer likely formed from two phenoxyl radicals. It is known from the literature [21] that meta-stable dimers frequently undergo reduction at much more negative potentials than their parent radicals do.

The dependence of the anodic peak potential on the scan rate provides more details on the type of the follow-up chemical step. In Figure 5 anodic peak potential, E_{pa}, is plotted *vs.* the logarithm of the scan rate at different pHs. In the solution of pH 1.0, E_{pa} is shifted by 20 mV in the positive direction for each tenfold increase of v. Similarly in other solutions the slope remains about 20 mV per scan rate decade at low scan rates (mostly up to 0.1 V s^{-1}). This is consistent with theoretical value expected for fast electron transfer followed by the coupling of two phenoxyl radicals to form a dimer (DIM1 mechanism) [22]. However, at higher scan rates E_{pa} *vs.* log v plots exhibit larger slope, which may account for transition from a DIM1 to a DIM2 mechanism. On the other hand, it is more likely that changes in peak potential are caused by the slow electron transfer kinetics.

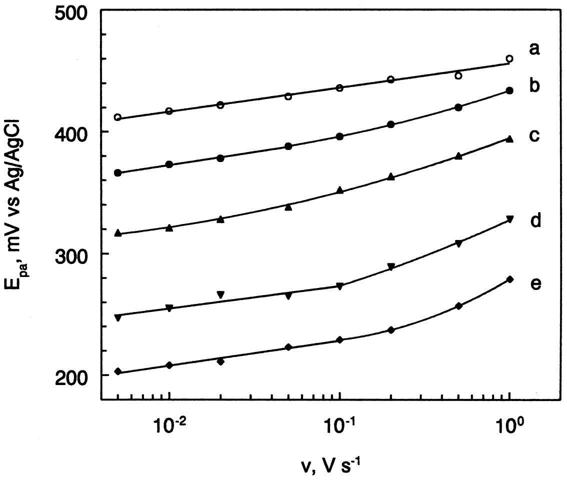

Figure 5. The relationship between the anodic peak potential of trolox (2.5×10^{-4} M) and the scan rate. pH: (a) 1.02, (b) 1.91, (c) 3.44, (d) 4.61, (e) 5.28.

For the cathodic-to-anodic peak ratio $|I_{pc}/I_{pa}| < 1$, kinetic data can be obtained. The kinetics of DIM1 mechanism under CV conditions was theoretically treated by Olmstead *et al.* [23]. Accordingly, one can determine the rate constant by comparing experimental I_{pc}/I_{pa} ratios to those plotted as a function of $\log k\tau$ in the working curve. τ is the time required for the potential to be scanned from the reversible half-wave value $E_{1/2}$ to that at which the polarization direction is reversed, E_λ. The rate constant can be calculated using the following equation:

$$\log k_{\mathrm{dim}} = \log \omega/(c\tau) - 0.034(a\tau) \qquad (2)$$

where ω is a theoretically derived function of I_{pc}/I_{pa} and c denotes bulk concentration of the primary compound. $E_{1/2}$ was determined as the potential corresponding to the 85% of the anodic peak height. For one-electron perfectly reversible anodic process, the quantity $a\tau$ is defined as [23]:

$$a\tau = (\mathrm{F/RT})\,(E_\lambda - E_{1/2}) \qquad (3)$$

Using equation (3) k_{dim} values were estimated for pH 3.44, 4.61 and 5.28: $(1.5\pm0.2)\times10^3$, $(1.0\pm0.2)\times10^3$ and $(3\pm1)\times10^2$ dm^3 mol^{-1} s^{-1}, respectively. These data were obtained at slow scan rates, ranging from 0.005 to 0.02 V s^{-1}, in order to provide reversible anodic responses. The error accompanying determination of k_{dim} was relatively large (up to 40% [19]). Nevertheless, it follows from the above results that DIM1 rate constant decreased with the increase in pH. Within the applied pH range, proton dissociation in the carboxylic group intensifies significantly, leading to the formation of mono-anions (COO^-/OH). It seems that appropriate anions are formed during oxidation of trolox. Thus, electrostatic repulsion of radicals occurring in the form $COO^-/O^\bullet$ can reasonably account for the decrease of k_{dim}.

Acknowledgment

Helpful discussion with Professor M.K. Kalinowski from Warsaw University is gratefully acknowledged.

REFERENCES

1. Miller N.J., Rice-Evans C.A., Davies M.J., Gopinathan V. and Milner A., *Clinical.Sci.*, **84**, 407 (1993).
2. van den Berg R., Haenen G.R.M.M., van den Berg H. and Bast A., *Food Chem.*, **66**, 511 (1999).
3. Arts M.J.T.J., Dallinga J.S., Voss H.-P., Haenen G.R.M.M. and Bast A., *Food Chem.*, **80**, 409 (2003).
4. Lien E.J., Ren S., Bui H.-H. and Wang R., *Free Radic. Biol. Med.*, **26**, 285 (1999).
5. Arnao M.B., *Trends Food Sci. Technol.*, **11**, 419 (2000).
6. Steenken S. and Neta P., *J. Phys. Chem.*, **83**, 1134 (1979).
7. Steenken S. and Neta P., *J. Phys. Chem.*, **86**, 3661 (1982).
8. Jovanovic S.V., Steenken S., Tosic M., Marjanovic B. and Simic M.G., *J. Am. Chem. Soc.*, **116**, 4846 (1994).
9. Chevion S., Berry E.M., Kitrossky N. and Kohen R., *Free Radic. Biol. Med.*, **22**, 411 (1997).
10. Chen J., Gorton L. and Akesson B., *Anal. Chim. Acta*, **474**, 137 (2002).
11. Hulanicki A., Reactions of Acids and Bases in Analytical Chemistry, Ellis Horwood, Chichester 1987, Chapter 2.16.
12. Marcus Y., The Properties of the Solvents, John Wiley, Chichester 1998.
13. Abrash H.I., *J. Chem. Educ.*, **78**, 1496 (2001).
14. Po H.N. and Senozan N.M., *J. Chem. Educ.*, **78**, 1499 (2001).
15. Malachesky P.A., *Anal. Chem.*, **41**, 1493 (1969).
16. Möller D. and Knaack J.D., *J. Electroanal. Chem.*, **63**, 303 (1975).
17. Amatore C., Azzabi M., Calas P., Jutand A., Lefrou C. and Rollin Y., *J. Electroanal. Chem.*, **288**, 45 (1990).
18. Greef R., Peat R., Peter L.M., Pletcher D. and Robinson J., Instrumental Methods in Electrochemistry, Ellis Horwood, Chichester 1985, p. 195.
19. Electroanalytical Methods for Determining Physicochemical Constants (in Polish), Ed. Z. Galus, PWN, Warszawa 1979, pp. 204, 309.
20. Nicholson R.S. and Shain I., *Anal. Chem.*, **36**, 706 (1964).
21. Webster R.D., *J. Chem. Soc., Perkin Trans. 2*, 263 (1999), and literature cited therein.
22. Andrieux C.P., Nadjo L. and Saveant J.M., *J. Electroanal. Chem.*, **42**, 223 (1973).
23. Olmstead M.L., Hamilton R.G. and Nicholson R.S., *Anal. Chem.*, **41**, 260 (1969).