J Solid State Electrochem (2012) 16:2435–2440
DOI 10.1007/s10008-012-1707-6

ORIGINAL PAPER

Study of antioxidant properties of flavonoids by voltammetry

Elena I. Korotkova · Olesya A. Voronova · Elena V. Dorozhko

Received: 11 January 2012 / Revised: 18 February 2012 / Accepted: 26 February 2012 / Published online: 21 March 2012

Abstract Flavonoids are a large group of natural phenolic compounds contained in high concentrations in vegetables, fruits, etc. Antioxidant and redox properties of some flavonoids such as catechin, quercetin, dihydroquercetin, and rutin were investigated in this work. Optimal concentration and time of action of flavonoids were obtained. To determine the more effective range of antioxidant activity, mathematical models and the response surfaces of investigated flavonoids were determined using methods of experiment design. Oxidation potentials of the compounds were also obtained, E=0.3÷0.4 V. Moreover, the antioxidant activity of flavonoids depends on the redox properties and the structure of the flavonoids. The antioxidant activity of flavonoids, which is correlated to reversible potentials for this compound is good. Finally, the use of these substances as antioxidants with therapeutic effects has been recommended in human diet.

Keywords Antioxidant activity · Flavonoids · Oxygen electroreduction · Voltammetry

Introduction

Flavonoids (vitamins of P group) are a large group of natural phenolic compounds contained in vegetables, fruits, and plants. The basic features of all polyphenols structure are the presence of one or more hydroxylated benzene rings linked by an oxygencontaining heterocycle containing several hydroxyl groups (Fig. 1). According to their polyphenolic nature, flavonoids exhibit good antioxidant properties. The antioxidant effect of flavonoids can be due to both their radical scavenging activity and to their metal-chelating properties. Their antioxidant activities connects with ability to neutralize negative action of reactive oxygen species (ROS) such as superoxide $O_2^{\bullet-}$, hydroxyl $OH^{\bullet}$, peroxyl $ROO^{\bullet}$, etc. [1].

The ROS are highly reactive and toxic, and can cause oxidation of biomacromolecules as well as initiating radical chain oxidation in tissues. During normal biological processes, ROS are formed in small quantities, and natural antioxidant system organism effectively removes its. However, under certain conditions, such as intake of drugs, UV radiation, or metabolic dysfunction, these ROS can be generated in sufficient quantity exceeding the normal antioxidant defense capabilities of the organism. This can result in damage effects on tissues [2].

Therefore, flavonoids, which can react rapidly with ROS, are important natural antioxidants. They result in a decrease of ROS toxic action and stop the radical chain processes in organism. Flavonoids are able to cause many beneficial effects on human health, including anticancer activity, cardiovascular protection, cataract prevention, antiallergy activity, antiviral activity, etc. [3].

The search of novel analytical methods to evaluate the antioxidant activity of various foods has increased considerably during the last years [4]. There are many methods of antioxidant activity (AA) determination [5, 6]. They are generally based on the inhibition of model reactions by

E. I. Korotkova · O. A. Voronova (✉) · E. V. Dorozhko
Department of Phys Anal Chem, Tomsk Polytechnic University,
Lenin 30,
Tomsk, Russia
e-mail: oaa@tpu.ru

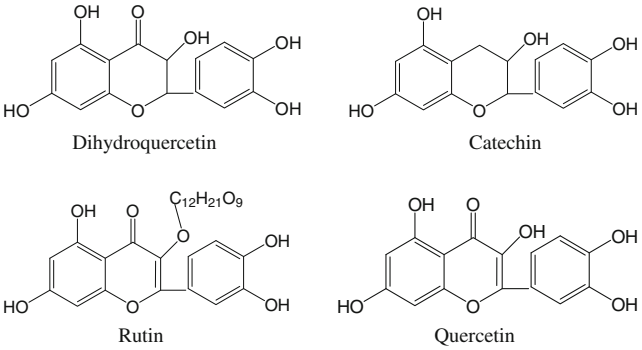


Fig. 1 Scheme of the flavonoids structures

antioxidants. The methods of radical generation as model reaction are most widely used [7]. Since, it was established that the antioxidant activity of flavonoids depends on variation and kind of functional groups of their chemical structure [8]. The traditional methods of AA determination of flavonoids include chemiluminescence [9], gas phase chromatography [10], electrochemical, fluorimetric [11], and other methods [12].

The study of relationship between electrochemical parameters and antioxidant activity of flavonoids could be of interest for investigation. The AA of flavonoids such as catechin, quercetin, dihydroquercetin, and rutin (Fig. 1) were evaluated in this work. The redox potentials of these compounds were also measured to compare with data of antioxidant activity. For these aims, we have applied an effective and convenient voltammetric approach for the determination of the antioxidant activity of the flavonoids [13]. To determine the more effective range of antioxidant activity of investigated flavonoids, mathematical models and the surfaces of response were obtained using methods of experiment design.

Experimental

Chemicals and reagents

Catechin, quercetin, dihydroquercetin, and rutin were supplied by Sigma (Germany). Investigation solutions of 3.6×10^{-3} mol l^{-1} catechin, 3.3×10^{-3} mol l^{-1} quercetin, 3.3×10^{-2} mol l^{-1} dihydroquercetin, and 8.2×10^{-2} mol l^{-1} rutin were prepared in ethanol. These solutions were diluted to convenient concentration after mixing buffer supporting electrolyte. As a supporting electrolyte, 0.025 mol l^{-1} (equimolar mixtures of Na_2HPO_4 and KH_2PO_4, pH 6.86) phosphate buffer was used. Nanopure water and ethanol were used for making solutions.

Table 1 The basic characteristics of the experiment

Name of characteristic	X_1, (C)	X_2, (t)
Zero level	0.3	12
Interval of a variation	0.2	8
Top level	0.5	20
Bottom level	0.1	4

Where, C 10^{-2} (gram per liter) is flavonoid concentration and t (minute) is time of the interaction between oxygen, its radicals and antioxidant

Instrumentations

A voltammetric analyzer TA–2 ("Tomanalyt", Tomsk, Russia) in connection with PC was used in this work. Voltammetric curves were recorded at room temperature in a three-electrode electrochemical cell connecting to the analyzer. A working glassy carbon electrode, a silver–silver chloride electrodes with saturated ($Ag|AgCl|KCl_{sat}$) KCl, as reference and counter electrodes were used. An open-type cell was used in this investigation.

A working glassy carbon electrode was used with length of 0.5 cm and diameter of 0.13 cm. It was constant for all measurements. In order to remove residual-adsorbed impurities, the indicator electrode was subjected to 20 voltammetric cycles between 0.0 and −2.0 V at 0.1 Vs^{-1}.

The counter, reference, and indicator electrodes were held in the electrochemical cell. The thermostatic cell was maintained at 25.0±0.5 °C. The pH was measured using digital pH meter model M64 (Belorussia). The oxygen concentration in the solutions was monitored with an oxygen analyzer model P5972 (Poland).

Voltametric measurement

A volume of 10 ml phosphate buffer was placed in the electrochemical cell. The measurement involved the recording of voltammograms of the cathodic reduction of oxygen by differential pulse voltammetry without and with the investigated substances under the following conditions: potential rate scan, 0.05 Vs^{-1}; potential range, E=0.0÷−0.8 V;

Table 2 Mathematical models of the flavonoids

Substance name	Mathematical model
Dihydroquercetin	$y=0.0140+0.0045x_1+0.0099x_2$
Quercetin	$y=0.0479+0.0376x_1-0.0099x_2-0.0645x_1x_2$
Rutin	$y=0.0083+0.0023x_1+0.0038\ x_2$
Catechin	$y=0.033+0.0021x_1+0.0036\ x_2$

Table 3 Mathematical models of the second order of the flavonoids

Substance name	Mathematical model
Rutin	$y=0.0699+0.0212x_1-0.0112x_2+0.0078x_1^2$
Quercetin	$y=0.1504-0.0373x_2-0.0112x_1x_2-0.0731x_1^2+0.0250x_2^2$
Dihydroquercetin	$y=0.0859-0.029x_1-0.0134x_2-0.0116x_1x_2+0.125x_1^2$
Catechin	$y=0.0275+0.0083x_1+0.0090x_2+0.0030x_1x_2-0.0104x_1^2-0.0144x_2^2$

Table 4 Effective concentration and time of the interaction between oxygen, its radicals and antioxidant

Substance name	C_{eff}, μmol l^{-1}	t_{eff}, min
Rutin	0.82±0.04	40
Catechin	1.40±0.06	20
Quercetin	1.55±0.04	45
Dihydroquercetin	3.20±0.03	30

and amplitude, 10 mV. After substance addition, the solution was stirred for about 20 s. After the stirring is stopped, the potential was scanned negatively, causing oxygen reduction, giving a current first wave electroreduction of oxygen (ER O_2). Its value was proportional to the amount of oxygen in the bulk of the solution. Oxygen concentration was monitored by oxygen analyzer. Based on the ammetric measurements, the concentration of oxygen in phosphate buffer at 25.0±0.5 °C was $2.56\pm0.05\cdot10^{-4}$ mol l^{-1}.

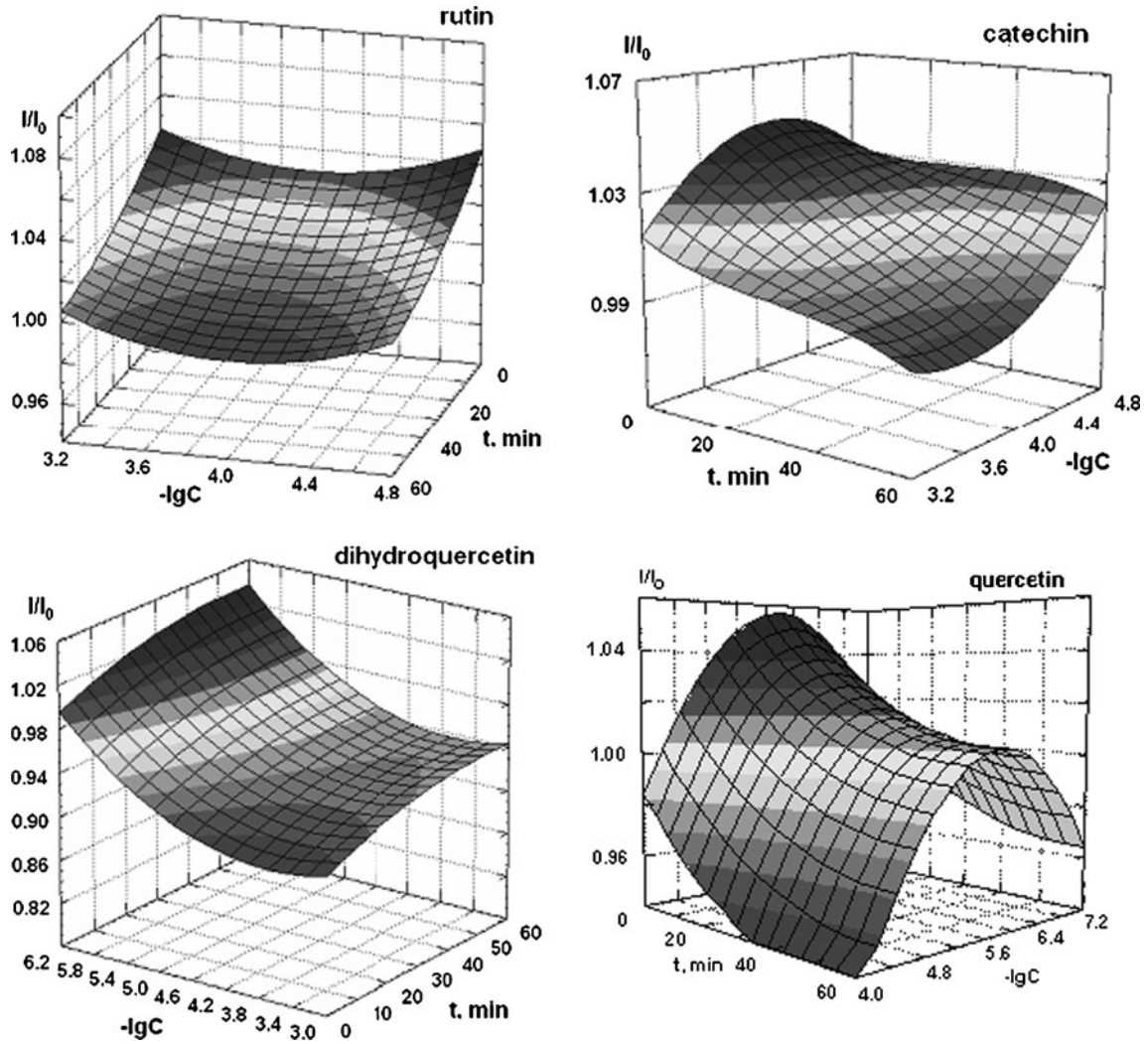


Fig. 2 The surfaces of the response of function $Y=I/I_0$ against flavonoids concentration and time of the interaction between ROS and antioxidant in minutes

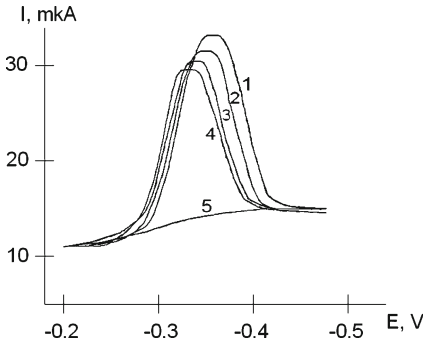


Fig. 3 Voltammograms of the ER O_2 current in phosphate buffer (0.025 M, pH 6.86) without (*1*) and with 0.27 mmol l^{-1} of dihydroquercetin at *t*=10 min (*2*), *t*=20 min (*3*), *t*=30 min (*4*). *Scan rate* 0.05 Vs^{-1}. For other conditions, see "Voltammetric measurement"

Cyclic voltammetry was performed in the electrochemical cell containing phosphate buffer and the investigated flavonoids in concentrations of 1–10 μmol l^{-1}. The potential range is *E*=0.0÷1.0 V. The potential scan rate was 0.05 Vs^{-1}. The working electrode was cleaned before each measurement by dipping in ethanol for 10 min, then polishing with a cleaning paste. The solution being analyzed was deoxygenated by passing nitrogen for 10 min prior measurements.

Results and discussion

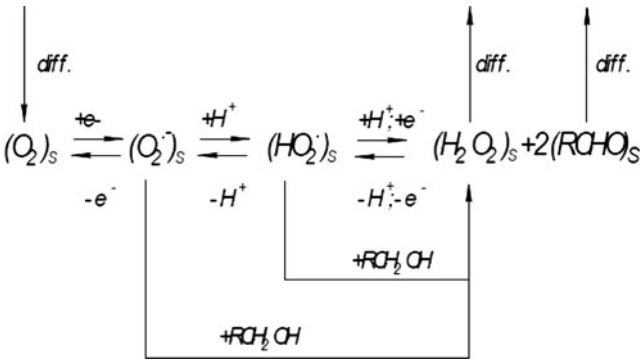
The electroreduction of oxygen at the working electrode has been treated as a "model" reaction because of similar processes of ER O_2 and the oxygen reduction in tissues. It proceeds at the cathode in several stages with formation of the ROS, such as $O_2^{\bullet-}$ and $HO_2^{\bullet}$ according to stages (1–3):

$$O_2 + e^- \xrightarrow{k_O} O_2^{\bullet-} \quad (1)$$

$$O_2^{\bullet-} + H^+ \rightleftarrows HO_2^{\bullet} \quad (2)$$

$$HO_2^{\bullet} + H^+ + e^- \rightleftarrows H_2O_2 \quad (3)$$

The concentration of oxygen and its radicals is decreased at the electrode, and the current of ER O_2 also decreases. We have suggested the following mechanism of these interactions:



where, RCH_2OH is the reduced form of the antioxidant and RCHO is the oxidized form of one.

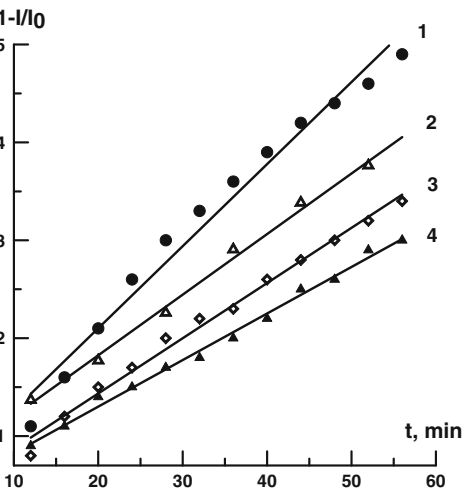


Fig. 4 Dependences on the relative change of the ER O_2 current against time of the interaction between ROS and antioxidant at the working electrode in phosphate buffer (0.025 M, pH=6.86) for 0.27 mmol l^{-1} quercetin (*1*), catechin (*2*), dihydroquercetin (*3*), and rutin (*4*)

Table 5 Antioxidant and electrochemical properties of the flavonoids

Substance name	*K*, mol l^{-1} min^{-1}	E_{ox}, *V*	E_{red}, *V*
Rutin	0.12±0.09	0.48±0.02	0.31±0.03
Catechin	0.23±0.05	0.41±0.04	0.20±0.03
Quercetin	0.27±0.03	0.37±0.03	0.14±0.02
Dihydroquercetin	0.14±0.06	0.44±0.04	0.24±0.02

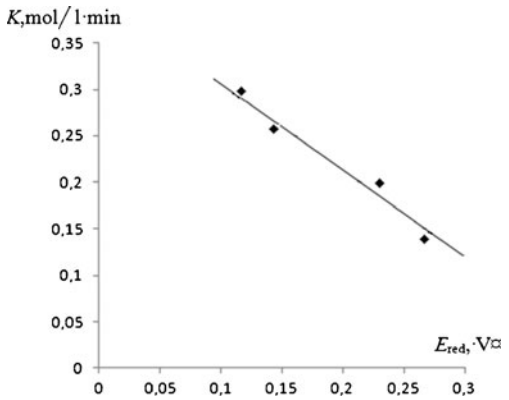


Fig. 5 Dependences of antioxidant activity of flavonoids on reversible potentials for these compounds

In this work, AA coefficient of substances were used in micromole per liter per minute [14]

$$K = \left(1 - \frac{I}{I_o}\right)\frac{C^0}{t} \quad (4)$$

where, C^0 (micromole per liter) is the oxygen concentration at the electrode in absence antioxidant, I (Ampere) is the ER O_2 current with the investigated substance addition in the solution, I_o (Ampere) is the limiting ER O_2 current without substance in the solution, and t (minute) is the time of interaction between oxygen, its radicals and antioxidant at the working electrode.

It should be noted that K depends on the time of the interaction between oxygen, its radicals and antioxidant (t), as well as on the antioxidant concentration (C^0_{ROH}) in solution. In order to estimate the significance of these factors and to determine the more effective range of antioxidant activity of investigated flavonoids, mathematical models and the surfaces of response were obtained using methods of experiment design [15].

The basic characteristics of the full-factor experiment, such as zero level, interval of a variation, top level, and bottom level are established in Table 1. The function of response (Y) was chosen as the response of relative change of the ER O_2 current (equivalent the antioxidant activity): $Y=I/I_0$. The matrix of experiment for two factors, such as C and t has been constructed. The number of experiments is $N=2^2=4$.

For all investigated flavonoids, mathematical models were obtained. Fisher's criterion was used to check hypothesis of mathematical model adequacy. The estimation of the significant coefficient of the mathematical models (Table 2) has been carried out using Student's criterion. All linear coefficients of mathematical models of the flavonoids were significant. For quercetin, the interaction coefficient between X_1 and X_2 factors was significant too. For all investigated flavonoids, mathematical models were adequate in local range of factor space. The second factor (time of the interaction between oxygen, its radicals and antioxidant) had greater influence on the antioxidant activity for all flavonoids.

To estimate the mathematical models of second order in the optimal range of factor space, the method "The central orthogonal composite planning" has been used. Fisher's criterion was used for check of a hypothesis of adequacy of mathematical model. The estimation of the significance of coefficients of the mathematical models (Table 3) has been carried out using Student's criterion. All mathematical models were adequately in local range of factor space.

In order to estimate the more effective range of factor space, the decision to investigate a surface of the response by a method of steepest ascent was accepted. For all investigated flavonoids, the two-factorial surface of the response is represented in Fig. 2. According Fig. 2, more effective range of flavonoids concentration and time of the interaction between oxygen, its radicals and antioxidant in minutes were determined in Table 4.

In order to investigate the antioxidant activity of flavonoids, voltammograms of ER O_2 current were recorded as a function potential at the working electrode in supporting electrolyte containing the substances under investigation (Fig. 3).

As a result, the curves of the relative change of the ER O_2 current ($1 - \frac{I}{I_0}$) against time of the interaction between oxygen, its radicals, and antioxidant at the working electrode in supporting electrolyte at effective concentration of antioxidant were plotted (Fig. 4). All the curves represent straightforward lines in the range of effective antioxidant concentrations. Coefficients of antioxidant activity were evaluated by Eq. (4). Data on K are shown in Table 5 for investigation of flavonoids. The antioxidant activity of investigating flavonoids correlate well with those obtained from kinetic conductivity method, which was developed for the measurements of reaction rate constants of

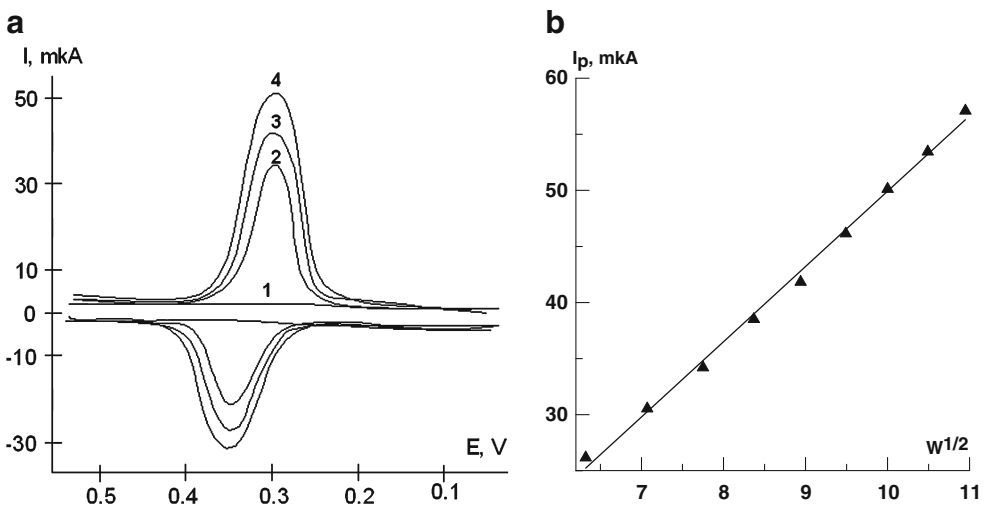
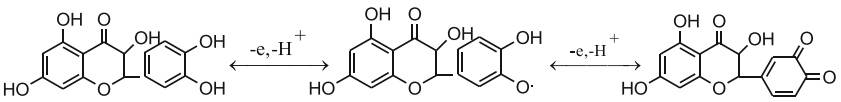


Fig. 6 **a** CV of dihydroquercetin in phosphate buffer (0.025 M, pH 6.86) on the glassy carbon electrode (GCE) at a concentration of dihydroquercetin (in millimole per liter); 0 (*1*), 0.1 (*2*), 0.2 (*3*), and 0.3 (*4*). Scan rate 0.05 Vs^{-1}. **b** The dependence of dihydroquercetin anodic current I_p on potential scan rates in phosphate buffer (0.025 M, pH 6.86) of the GCE

the superoxide radical reactions with flavonoids in aqueous solution [16] and obtained from "ferric reducing antioxidant power" assay in aqueous solution [17].

The investigated flavonoids have hydroxyl groups in its structure (Fig.1), which could be reduced and oxidized at the electrode. Investigation of their reduction and oxidation potentials is very important to obtain information about its electron transfer reactions. Moreover, the antioxidant activity of flavonoids depends on the redox properties and the structure of the flavonoids (Table 5). The antioxidant activity of flavonoids which is correlated to reversible potentials for this compound is good (Fig. 5).

The mechanism of flavonoids oxidation is shown on an example of dihydroquercetin oxidation [18]:



An experiment was carried out using cyclic voltammetry (CV) in a deoxygenated (by N_2) aqueous solution containing 0.025 mol l^{-1} phosphate buffer in the −0.2 to +1.5 V potential range. All investigated flavonoids have received reversible peaks in the 0.3–0.4 V field of potentials. The cyclic voltammograms of the dihydroquercetin at a glassy carbon electrode at different concentrations and potential scan rates are shown in Fig. 6a and b, correspondingly.

The one oxidation peak of dihydroquercetin was obtained at E=0.44±0.04 V at the glassy carbon electrode in phosphate buffer, which is related to the deprotonation of the catechol moiety, 3′-OH electron-donating group. The height of the oxidation peak proportionally increased with concentration of flavonoid in solution. A reduction peak of 0.24±0.02 V corresponds to reduction of the oxidation product. This cyclic voltammogram clearly shows the reversible character of dihydroquercetin electron transfer oxidation reaction. The mass transfer-limited nature of the oxidation of dihydroquercetin is confirmed by the linearity of observed plot of I_p vs. $W^{1/2}$ (Fig. 6b). Similar results were obtained for catechin, quercetin, and rutin. The CV data for the oxidation of flavonoids are given in Table 5. The electrochemical data were in good agreement with the results of AA obtained by the voltammetric approach. The easy electron transfer is obtained in the order rutin–dihydroquercetin–catechin–quercetin, and the antioxidant activity is increased too.

Conclusion

The antioxidant activity of some flavonoids was measured by differential pulse voltammetry and their redox potentials were determined by cyclic voltammetry. More effective range of flavonoids concentration, time of their interaction with oxygen, and its radicals were determined. A good correlation between the antioxidant activities and electrochemical parameters of the flavonoids was observed. The antioxidant activity of flavonoids depends on the redox properties and the structure of the flavonoids. The antioxidant activity of flavonoids which is correlated to reversible potentials for this compound is good. Finally, the use of these substances as antioxidants with therapeutic effects has been recommended in human diet.

Acknowledgments This work was financially supported by the Ministry of Education and Science of the Russian Federation (FCP, contract № 14.740.11.1369).

References

1. Frankel EN (1998) Lipid oxidation. The Oily Press, Dundee
2. Forest SE, Stimson MJ, Simon JD (1999) J Phys Chem B 103 (19):3963–3964
3. Abramov JI, Oksengendler GI (1985) Human and antioxidative substances. Science Press, Leningrad
4. Prior RL, Wu X, Schaich K (2005) J Agric Food Chem 53:4290–4302
5. Antolovich M, Prenzler PD, Patsalides E, Donal SMc, Robards K (2002) Analyst 127:183–198
6. Roginsky V, Lissi EA (2005) Food Chem 92:235–254
7. Arnao MB, Cano A, Acosta M (1999) Free Rad Res 31:89–96
8. Arora A, Nair MG, Strasburg GM (1998) Free Radic Biol Med 24 (9):1355–1363
9. Arzamasceva AP, Shkarina EI, Maksimova TV (1999) Khimiko-Farmatsevticheskii Zhurnal 11:17–20
10. Denisov ET (1996) Uspekhi Khimii 65(6):547–563
11. Campanella L, Bonanni A, Bellantoni D, Favero G, Tomassetti M (2004) J Pharm Biomed Anal 36(1):91–99
12. Blasco AJ, Rogerio MC, Gonz'alez MC, Escarpa A (2005) Anal Chim Acta 539:237–244
13. Korotkova EI, Karbainov YA, Shevchuk AV (2002) J Electroanal Chem 518:56–60
14. Korotkova EI, Avramchik OA, Angelov TM, Karbainov YA (2005) Electrochim Acta 51:324–332
15. Derffel K (1994) Statistics in analytical chemistry. Mir, Moscow
16. Jovanovic SV, Steeden S, Tosic M, Marjanovic B, Simicg MG (1994) J Am Chem Soc 116:4846–4851
17. Firuzia O, Lacannaa A, Petruccib R, Marrosub G, Saso L (2005) Biochim Biophys Acta 1721:174–184
18. Oliveira Brett AM, Mariana-Emilia Ghica (2003) Electroanalysis 15(22):1745–1750