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Abrasive Stripping Square Wave Voltammetry of Some Natural Antioxidants

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Square wave voltammetric net peak potentials of investigated microparticles increase in the order delphinidin < epigallocatechin gallate < epigallocatechin < cyanidin < myricetin < pelargonidin < epicatechin gallate. This order is explained by the structural differences.

Keywords: Anthocyanidins, catechins, myricetin, microparticles, voltammetry.

1. INTRODUCTION

Among the methods for investigation of electrochemical properties of solids, the voltammetry of microcrystals, also known as abrasive stripping voltammetry, is particularly useful [1 - 3]. Its procedure consists of mechanical immobilization of microparticles of water-insoluble matter on the surface of graphite electrode, which is then used as the working electrode in voltammetric experiment [4 - 6]. This method can be applied for qualitative analysis of alloys [1, 4], inorganic [2, 3] and organic compounds [5 - 9] and medicaments [10, 11]. We have used it for the determination of oxidation potentials of powders of several anthocyanidins [12], catechins [13] and myricetin [14]. These compounds are natural antioxidants and may exhibit antiviral, anti-inflammatory and antitumor activities [15 - 26]. In the reaction of antioxidant with free radical either electron or proton can be transferred first, or the transfers of both can occur simultaneously [27 - 29]. This reaction order depends on the ionization potential and the enthalpies of proton and O-H bond dissociations. Nucleophilic radicals are scavenged by the one-step transfer of hydrogen atom, while electrophilic free

radicals are deactivated through single electron transfer [27, 30]. For the latter reaction the activity of compound can be estimated from its electrooxidation potential [31 - 35].

In this short communication the results of above mentioned abrasive stripping square wave voltammetric measurements of several antioxidants are summarized and compared with literature data.

2. EXPERIMENTAL

Cyanidin chloride ($C_{15}H_{11}O_6Cl$), delphinidin chloride ($C_{15}H_{11}O_7Cl$), pelargonidin chloride ($C_{15}H_{11}O_5Cl$) and myricetin (HPLC grade, ≥ 95 %) were purchased from Extrasynthese (France). The catechins (-)-epigallocatechin gallate (≥ 95 %), (-)-epigallocatechin (≥ 95 %) and (-)-epicatechin gallate (> 95 %) (all Sigma-Aldrich, St Louis, USA) were used as received. For the supporting electrolyte analytical grade KNO_3 and HNO_3 (Kemika, Zagreb) were used. All buffer solutions (pH 3-11) were obtained from Kemika, Zagreb, analytical grade. Purified water from a Millipore Milli-Q system (resistivity 18.2 MΩ cm) was used throughout the study. The liquid electrolyte was 0.1 M KNO_3 buffered to the particular pH.

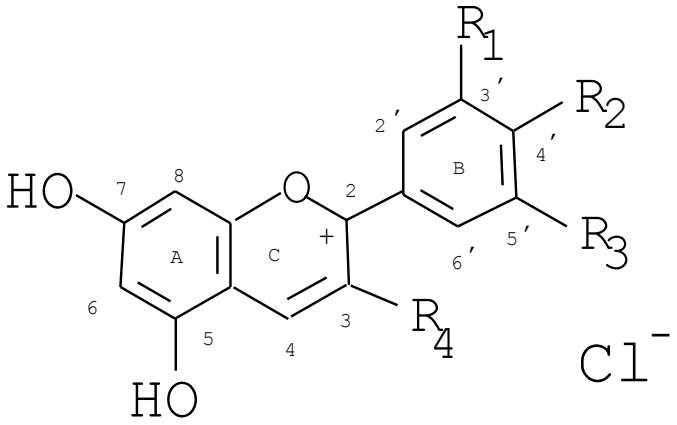
Voltammetric measurements were carried out using the computer-controlled electrochemical system Autolab PGSTAT 30 (Eco-Chemie, Utrecht, Netherlands). A three-electrode system (Methrom, Switzerland) with a spectral-grade paraffin-impregnated graphite rod (diameter 5 mm, length 50 mm) as the working electrode, an Ag/AgCl (3 M KCl) electrode as a reference electrode (E = 0.210 V *vs* SHE at 20 ºC) and a platinum wire counter electrode was used. Working electrode was mechanically cleaned before each run. Its circular surface was rinsed with distilled water, polished on a wet polishing cloth, rinsed again, dried with a fine-grade paper tissue and carefully polished on a dry, white paper sheet. Then it was contaminated with microparticles of anthocyanidins, catechins or myricetin by pressing it into a small pile of substance powder on a highly glazed ceramic tile and moving it with a circular motion. The working electrode was immersed in the electrolyte only during the voltammetric measurements. Less than 1 mm of the graphite rod was immersed in the electrolyte.

The solutions were degassed with high-purity nitrogen prior to the electrochemical measurements. A nitrogen blanket was maintained thereafter. All experiments were performed at room temperature.

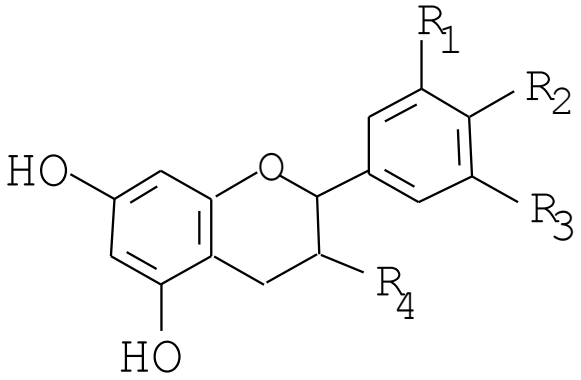
3. RESULTS AND DISCUSSION

The following compounds were investigated: delphinidin, pelargonidin, cyanidin [12] (Scheme 1), epigallocatechin gallate, epigallocatechin, epicatechin gallate [13] (Scheme 2) and myricetin [14] (Scheme 3). Electrochemical properties of their microcrystals immobilized on the surface of graphite electrode were measured by square wave voltammetry. Two examples are shown in Figure 1. The details of voltammetric responses were published previously [12 - 14]. All electrode reactions appeared reversible. The net peak potentials of voltammograms of investigated substances are reported in the Table 1. It can be noted that the potentials increase from delphinidin to epicatechin gallate. This

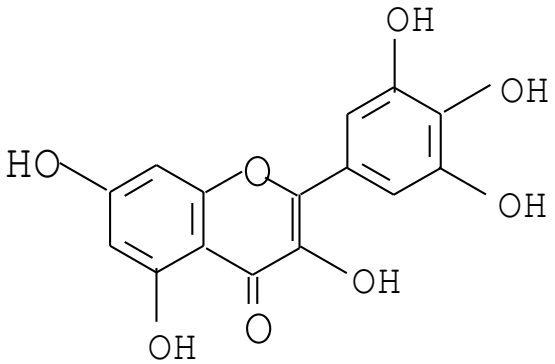
is in agreement with literature data [15, 19, 28, 30, 32, 35 - 46]. It was demonstrated previously that oxidation peak potentials of anthocyanidins increase in the order delphinidin < cyanidin < pelargonidin [32, 38, 39].



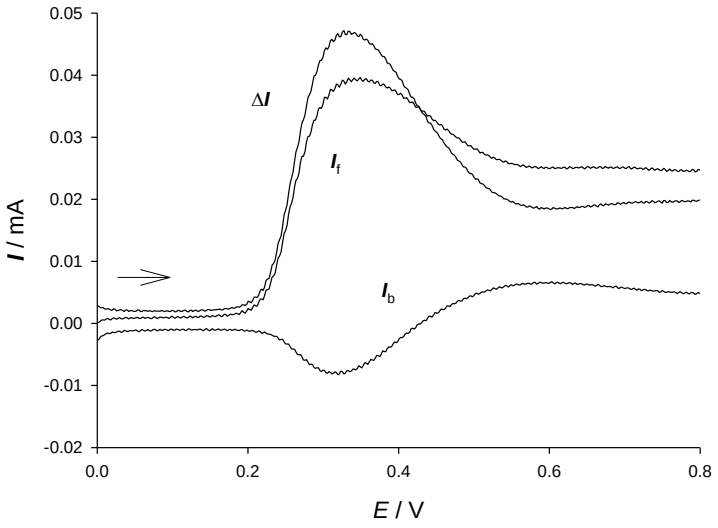
Scheme 1. Structural formulae of cyanidin (R_1: OH, R_2: OH, R_3: H, R_4: OH), delphinidin (R_1: OH, R_2: OH, R_3: OH, R_4: OH) and pelargonidin (R_1: H, R_2: OH, R_3: H, R_4: OH).



Scheme 2. Structural formulae of epigallocatechin gallate (R_1: OH, R_2: OH, R_3: OH, R_4: galloyl group), epigallocatechin (R_1: OH, R_2: OH, R_3: OH, R_4: OH) and epicatechin gallate (R_1: OH, R_2: OH, R_3: H, R_4: galloyl group).

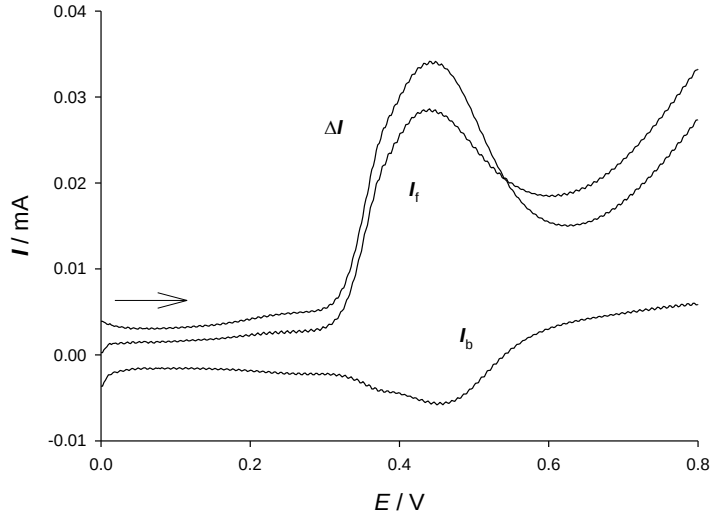


Scheme 3. Structural formula of myricetin.



A

Figure 1A



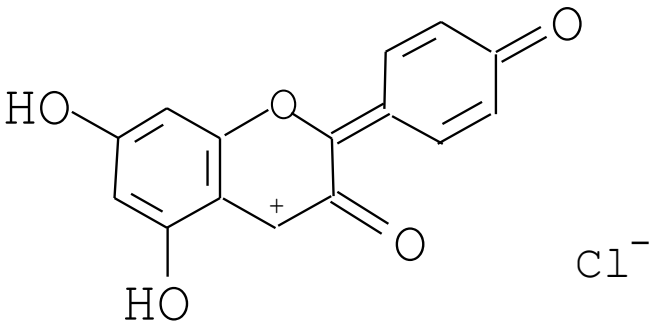
B

Figure 1. Abrasive stripping square-wave voltammetry of delphinidin (A) and pelargonidin (B) on the paraffin-impregnated graphite electrode in 0.1 mol/L KNO_3 at pH 2. A net response ($\Delta\boldsymbol{I}$) and its forward ($\boldsymbol{I}_f$) and backward ($\boldsymbol{I}_b$) components are shown. The frequency is 8 Hz, the pulse amplitude is 50 mV, the potential increment is 2 mV, the starting potential is 0 V *vs.* Ag/AgCl and the scan direction is positive.

Table 1. Net peak potentials of voltammograms of immobilized microparticles at pH 2

Compound	$E_{p,1}$ / V *vs* Ag/AgCl	$E_{p,2}$ / V *vs* Ag/AgCl
delphinidin	0.327	
epigallocatechin gallate	0.365	0.486
epigallocatechin	0.373	
cyanidin	0.403	
myricetin	0.415	
pelargonidin	0.440	
epicatechin gallate		0.480

Also, it is known that cyclic voltammogram of epigallocatechin gallate exhibits two peaks that appear at potentials which are close to potentials of oxidation maxima of epigallocatechin and epicatechin gallate, respectively, and that the difference between these two peak potentials is about 0.1 V [40, 41]. Oxidation potentials reported for quercetin are similar to our results obtained for myricetin [42 – 44, 47 - 49] and the latter was shown to be weaker scavenger for electrophilic radicals than epigallocatechin [30]. Furthermore, the products of oxidation of morin and pelargonidin are similar (Scheme 4) and the oxidation peak potential of morin is 0.48 V *vs* Ag/AgCl at pH 2 [41, 45]. Finally, epicatechin gallate and taxifolin share the same electroactive moiety and have similar oxidation potentials, which is 0.500 V at pH 2 for the latter [46, 50].



Scheme 4. Structural formula of the oxidized pelargonidin.

The difference in oxidation peak potentials shown in Table 1 is caused by the difference in structure of these compounds [15, 19]. Previously it was discovered that the superoxide radical scavenging activity of myricetin and epigallocatechin was much higher compared to quercetin and epicatechin, which showed the superiority of pyrogallol over catechol as the antioxidant [32, 41, 51, 52]. We agree that the most important is the number of conjugated hydroxyl groups in the molecule [36]. In delphinidin and myricetin this number is 4 and in epigallocatechin gallate and epigallocatechin it is 3. The second factor is the electron-donor double bond between the positions 3 and 4 in the C ring of delphinidin [27, 32, 37, 53, 54] comparing to the electron-withdrawing ketone group on the position 4 in the C ring of myricetin [32, 35, 55]. This is the reason for the difference in oxidation potentials between these two compounds.

4. CONCLUSIONS

Electrooxidation potentials of seven antioxidants were measured by the abrasive stripping voltammetry. All investigated electrode reactions were reversible. Square-wave voltammetric peak potentials of immobilized microparticles are consistent with available knowledge, which confirms that this technique may be used for the estimation of antioxidant activity of solids. Its advantage is that there is no influence of adsorption of oxidation products on the electrode surface.

The results obtained in this work show that the pyrogallol group is more easily oxidized than the catechol group. The oxidation potential is particularly low if the hydroxyl group on the position 3 in the C ring of molecule is conjugated to pyrogallol group in the B ring, but the ketone group on the position 4 in the C ring inhibits the oxidation.

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