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Antioxidative Properties of Silymarin, 7-aminoflavone, Neohesperidin Dihydrochalcone and Trihydroxyethylenorutin Studied by the Electrochemical Methods

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The electrooxidation of flavonoids has been investigated in non-aqueous solution at a platinum electrode. The process of oxidation and its kinetics has been investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The electrochemical oxidation of flavonoids is an irreversible reaction at a platinum electrode. For neohesperidin dihydrochalcone, silymarin, 7-aminoflavone and trihydroxyethylenorutin, the first step in the electrooxidation is the exchange of one electron during the oxidation of the hydroxyl group in ring B. The hydroxyl groups in ring A are likely oxidised in a subsequent step. Neohesperidin dihydrochalcone oxidation was the easiest, and trihydroxyethylenorutin was the hardest, which was confirmed by the half-wave potential ($E_{1/2}$) of the first electrode step in the electrooxidation of the flavonoids. Structural investigations of the flavonoids were conducted using FTIR spectroscopy. The voltammetric potentials were found to correlate well with computed highest occupied molecular orbital (HOMO) energies.

Keywords: flavonoids; electrooxidation; platinum electrode; FTIR spectroscopy

1. INTRODUCTION

Extensive studies of flavonoids are currently in progress [1-5]. Flavonoids, especially those naturally found in foods, are substances of increasing interest due to their biological properties, such as natural antioxidant, anti-thrombotic, anti-bacterial, anti-allergic and anti-inflammatory activities. These compounds are natural vegetable pigments synthesised from phenylalanine and impart colour to the blooming portions of plants [6-10]. In addition to their significant role in plants, flavonoids are

important to human health due to their activity as free radical acceptors. Flavonoids can protect against cancer by inhibiting the damage caused by oxidation processes and are distinguished by their capability to scavenge free radicals and active oxygen groups [11-13]. They have a C-15 skeleton, which is divided into three units, A, B and C. Unit C is an oxygen containing heterocyclic whose oxidation state and saturation levels define the major subclasses. Units A and B are aromatic rings in which four major types of substituents (i.e., hydroxyl, methoxyl, prenyl and glycosides) lead to over 8000 different flavonoids [4, 14-15] (Fig. 1).

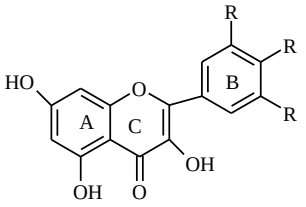


Figure 1. Flavonoid structure.

The presence of conjugate benzene rings and hydroxyl groups gives rise to antioxidant functions that are used in vitro or as elements of free systems through scavenging peroxide anions, oxygen singlets, peroxide radicals of lipids and free radical stabilisation in oxidation processes involving hydrogenation [16-17]. Flavonoids have been divided into the following classes: anthocyanins, flavones, flavonones, isoflavones, catechins and flavone-3-ols. The nature of flavonoids depends on their structural class, degree of hydroxylation, functional substitutions, conjugation and degree of polymerisation. Flavonoids contain a number of phenolic hydroxyl groups attached to ring structures that impart antioxidant capabilities to these compounds [1, 18-20]. Because of their antioxidant capacity, flavonoids can significantly slow the ageing process and prevent many diseases. This function finds practical use in the pharmacology and cosmetology industries [21-24]. The miscellaneous effects of flavonoids and their synthetic derivatives can be explored to search for new drugs. These compounds may also be used in the chemical industry as natural additives to stabilise polymeric materials [25].

Chalcone derivatives exhibit a wide variety of biological activities, including anti-inflammatory and anticancer activities [26-27]. They have two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system that give rise to the basic structure of chalcone (1,3-diphenyl-2-propenone), which serves as a unique template associated with biological activities [28-30]. The structure–activity relationships of chalcones have been reported for their anti-inflammatory and anticancer activities [28, 31]. Neohesperidin dihydrochalcone, which is a non-nutritive sweetening agent derived from citrus, has a potential therapeutic effect on reactive oxygen species (ROS)-related inflammatory diseases [32-33].

Many methods have been developed for the evaluation of the reducing/antioxidant capacity [34]. Most of the existing methods are based on scavenging capacity assays against specific ROS, including oxygen ions, free radicals and peroxides, which can be either inorganic or organic and can be generated in the human body by the normal metabolism of oxygen. Voltammetric methods, such as

cyclic and pulse voltammetry, are suitable methods for the determination of the antioxidative capacity of flavonoids. Voltammetric methods have many advantages in comparison to other methods, such as ORAC, TEAC and DPPH [35-36]. These methods do not require specific reagents and time-consuming sample preparation, and they are cost effective. In general, electrochemical methods are simple, rapid and based on physico-chemical properties of the studied substances. The application of voltammetric analysis results in the determination of the following parameters: potential and current of anodic peak as well as the half-wave potential. A lower oxidation potential can be attributed to a higher antioxidative power [37-39]. Due to these results, cyclic and differential pulse voltammetry can be applied to the evaluation of the antioxidative power of flavonoids [40-42]. In particular, it is important to determine the half-wave potentials of an antioxidant because a lower value indicates better free radical scavenging abilities [43-48].

The aim of the investigations described in this paper was to determine the electrochemical behaviour of specific flavonoids (i.e., neohesperidin dihydrochalkone, silymarin, 7-aminoflavone and trihydroxyethylrutin) during electrooxidation at a platinum electrode. The experiments were performed in non-aqueous medium. Structural investigations of flavonoids were conducted using FTIR spectroscopy.

2. EXPERIMENTAL

2.1. Reagents and solutions

The following polyphenol derivatives with varying numbers of -OH groups and substituents were used:

a) 7-aminoflavone $C_{15}H_{11}NO_2$),
b) neohesperidin dihydrochalkone ($C_{28}H_{36}O_{15}$),
c) trihydroxyethylrutin ($C_{33}H_{42}O_{19}$),
d) silymarin ($C_{25}H_{22}O_{10}$).

These pure compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Acetonitrile (CH_3CN, pure p.a.) was used to prepare the amino acid solutions and was from Sigma-Aldrich. Tetrabutylammonium perchlorate ($(C_4H_9)_4NClO_4$) was from Fluka (Germany) and was used as a supporting electrolyte. The substrates solutions were prepared by dissolving in 0.1 mol L^{-1} ($(C_4H_9)_4NClO_4$ in acetonitrile. Concentrations of flavonoids it was 2.0×10^{-3} mol L^{-1}. All reagents used were of analytical grade.

2.2. Measurement methods

Methods of cyclic (CV) and differential pulse (DPV) voltammetry were used in electrochemical measurements with an Autolab PG-STAT30 Electrochemical Analyser (Eco-Chemie B.V., Utrecht, The Netherlands) equipped with GPES. A three-electrode cell system including a ferricinium/ferrocene electrode (Fc^+/Fc) as a reference electrode, a platinum wire as an auxiliary

electrode, and the platinum (geometric surface area of 0.5 cm^2) as the working electrode, was applied in the electrochemical studies. Before measurements, the solutions were purged with argon in order to remove dissolved oxygen. During measurements, argon layer was kept over the solutions. CV and DPV recorded in the potential range from 0 to 2 V vs. Fc^+/Fc. The effect of the scan rate on the electrooxidation of flavonoids in non-aqueous medium was assessed. All experiments were carried out at room temperature.

FTIR analysis. IR spectra were recorded within the wavelength range of 3000-700 cm^{-1} using an FTIR Nicolet 6700 FT–IR (Thermoscientific). The measurement parameters were as follows: 128 scans, resolution 8 cm^{-1}, DTGS/KBr detector. The FT–Raman spectrum of the compound was also recorded over the range of 3000–1000 cm^{-1}.

The quantum chemical calculations were performed using the AM1 method with HyperChem program packages. The molecular structures of amino acids in gas phase were fully optimized by using ab-initio quantum chemical calculations at the restricted Hartree–Fock (RHF) level of theory.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behaviour of flavonoids at a Pt electrode

Flavonoids are a large group characterised by good antioxidant activity. The activity of flavonoids depends on their ring structure, which is characterised by the conjugation of double bonds and the presence of hydroxyl groups on ring B. To estimate the specific properties of flavonoids, electroanalytical research has been conducted on different flavonoids structures with different numbers of hydroxyl groups.

The electrochemical reactions that proceed at the electrode are characterised by the dependence of the current on the electrode potential. The electrode reactions that characterise the electrochemical oxidation of flavonoids at a platinum electrode were studied using CV and DPV voltammetry. DPV voltammetry achieves a higher resolution and enables improved peak separation to characterise subsequent steps in the electrooxidation. The half-wave potential of a peak in a cyclic voltammogram corresponds to the potential of a peak occurring in a differential pulse curve and is characteristic for each of the subsequent steps in the investigated electrode reaction. Selected cyclic and differential pulse voltammograms are recorded in the flavonoid solutions and the supporting electrolyte are presented in Figs. 2–4. Within the potential range in which the flavonoids oxidation peaks appear, the supporting electrolyte (tetrabutylammonium perchlorate in acetonitrile, 0.1 mol L^{-1}) exhibits no characteristic peaks apart from charging the electrical double layer (Figs. 2–4, curve 3). However, a small wave appears in the supporting electrolyte in the potential range from 0.5 to 1.0 V in the voltammograms. This wave can be attributed to the presence of $(C_4H_9)_4NClO_4$ and the oxidation of perchlorate ions. However, this wave's current is relatively low in comparison to those of the peaks attributed to polyphenol oxidation. Zieja, Gadowska-Trzos, and Stojek [49] reported that this wave can also be caused by the oxidation of impurities, such as water and other organic substances.

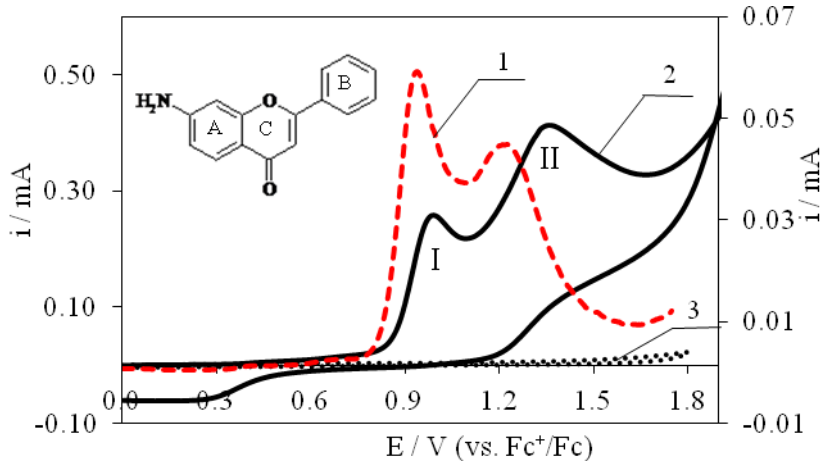


Figure 2. Voltammograms of 7-aminoflavone oxidation at a Pt electrode; c = 2.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: differential pulse curve, 2: cyclic voltammogram, 3: cyclic voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

7-Aminoflavone is a flavonoid that contains one amino group in ring A. Cyclic and differential pulse voltammograms recorded in the flavonoid solutions (Fig. 2) indicate two peaks characterising at least two electrode steps in the 7-aminoflavone electrooxidation in the potential range below which the electrolyte decomposes (1.8 V). The electrooxidation of 7-aminoflavone is irreversible. As determined by cyclic voltammetry, the half-wave potential ($E_{1/2}$) of the first step in the 7-aminoflavone oxidation is 0.92 V, which corresponds to the peak potential from the differential pulse voltammetry measurements. The half-wave potential ($E_{1/2}$) of the second step is 1.27 V.

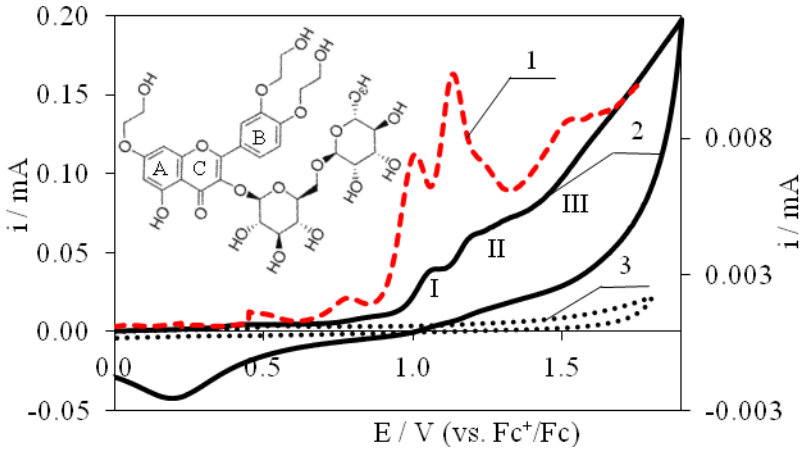


Figure 3. Voltammograms of trihydroxyethylrutin oxidation at a Pt electrode; c = 2.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: differential pulse curve, 2: cyclic voltammogram, 3: cyclic voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

Trihydroxyethylrutin has a large number of hydroxyl groups. Trihydroxyethylrutin is irreversibly oxidised in a three-step electrode process. As determined by differential pulse voltammetry (Fig. 3, curve 1) and cyclic voltammetry (Fig. 3, curve 2), the peak potential ($E_{1/2}$) of the first step of trihydroxyethylrutin oxidation is 1.05 V, the second is 1.12 V, and the third is 1.52 V.

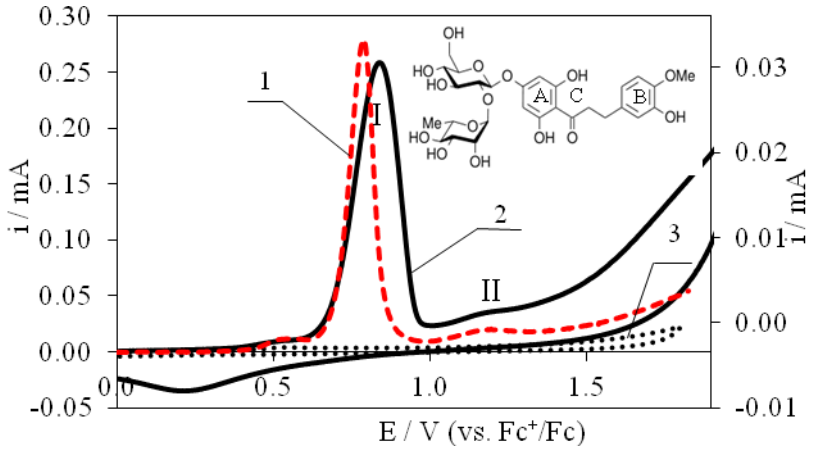


Figure 4. Voltammograms of neohesperidin dihydrochalcone oxidation at a Pt electrode; c = 2.0 × 10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: differential pulse curve, 2: cyclic voltammogram, 3: cyclic voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

Neohesperidin dihydrochalcone is irreversibly oxidised in two electrochemical steps (Fig. 4, curves 1 and 2). The potential of the first oxidation peak for this compounds is 0.85 V ($E_{1/2}$ is 0.804 V), and the second is 1.15 V. The potential peak, with an increasing speed of the test electrode polarisation, shifted towards positive values.

Another flavonoid, which has very good antioxidant properties, is silymarin. Silymarin (SMR) is a mixed extract of polyphenolic flavonoids isolated from the dried seeds of the milk thistle plant [50]. SMR is a complex mixture of four flavonolignan isomers (silybin, isosilybin, silydianin and silychristin) and has the empirical formula $C_{25}H_{22}O_{10}$. Among the isomers, silybin is the major and most biologically active component, representing approximately 60–70% followed by silychristin (20%), silydianin (10%), and isosilybin (5%) [50-51]. This flavonoid is one of the most powerful natural substances and has the ability to protect and rebuild liver cells damaged by alcohol and other toxic substances [51-52].

The exemplary cyclic and differential pulse (with higher resolution) voltammograms of silymarin electrooxidation are shown in Fig. 5. The voltammograms presented in Fig. 5 (curve 1, 2 and 3) show that silymarin is likely oxidised irreversibly in a potential range below that at which the electrolyte decomposes (1.8 V). Two peaks (I at 0.84 V and II at 1.22 V) visible in the differential pulse and cyclic voltammograms correspond to silymarin electrooxidation.

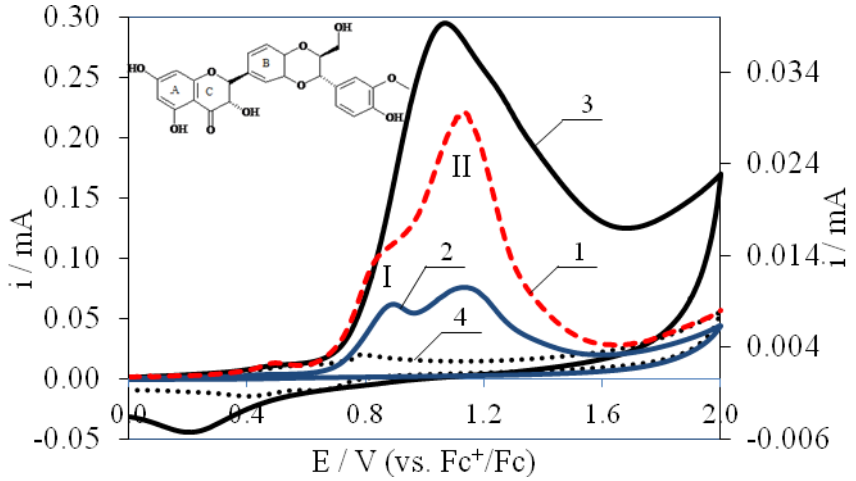


Figure 5. Voltammograms of silymarin oxidation at a Pt electrode; c = 2.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}, 1: differential pulse curve, 2: cyclic voltammogram, v = 0.01 V s^{-1}, 3: cyclic voltammogram, v = 0.1 V s^{-1}, 4: cyclic voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

The compounds that contain hydroxyl groups in ring (B or A and C) are more easily oxidised (i.e., have a lower positive first peak potential in the cyclic voltammograms) than those containing only one hydroxyl group or amino group in this ring. We propose that the first peak observed in the voltammograms recorded in the solutions of substrates with hydroxyl groups can be attributed to the electrooxidation of the hydroxyl groups. The electrooxidation of each hydroxyl group is connected to an exchange of one electron and the formation of a proper quinone.

3.2. Influence of the scan rate

The effect of the polarisation rate on the electrooxidation of flavonoids was investigated using cyclic voltammetry with scan rates of 0.01 to 1.0 V s^{-1}. These voltammograms were used in determination of peak potential (E_p) and current (i_p). The peak potential and current were determined for the first step in the oxidation of flavonoids at the electrode, but only the peak potential was measured for the second step. Two widely used approaches for studying the reversibility of electrochemical reactions and determining whether a reaction rate is controlled by adsorption or diffusion involve the analyses of the i_p as a function of $v^{1/2}$ and the ln i_p as a function of ln v curves. Figure 6 shows these plots for the first oxidation peak of the flavonoids in acetonitrile. For reversible or irreversible systems without kinetic complications, i_p varies linearly with $v^{1/2}$ and intersects the origin of the coordinates. The plot of i_p on $v^{1/2}$ presented in Fig. 6 (A) is linear and it does not cross the origin of the axes.

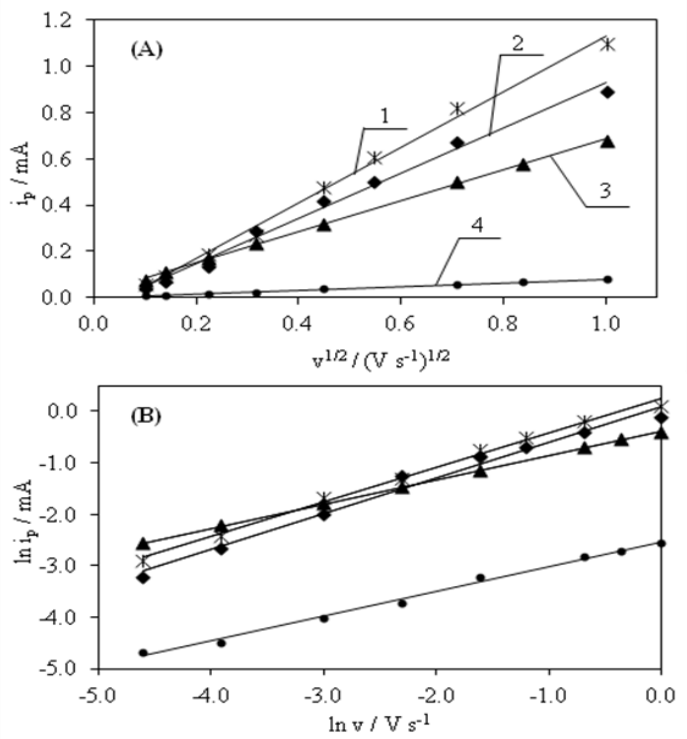


Figure 6. (A) The dependence of the anodic peak current (i_p) on the square root of the potential scan rate (v); (B) The dependence of the anodic peak current on the potential scan rate in double logarithm coordinates for the oxidation of flavonoids in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile at the Pt electrode; curve 1 – silymarin, 2 – neohesperidin dihydrochalcone, 3 – 7-aminoflavone and 4 – trihydroxyethylenorutin.

This proves that the process can be diffusion controlled. A dependence of i_p on $v^{1/2}$ for anodic peak can be described by the following equations:

$i_p = \{1.208[v(V\ s^{-1})]^{1/2}\}mA - 0.075mA$ $R^2 = 0.995$ for silymarin,

$i_p = \{0.978[v(V\ s^{-1})]^{1/2}\}mA - 0.049mA$ $R^2 = 0.991$ for neohesperidin dihydrochalcone,

$i_p = \{0.673[v(V\ s^{-1})]^{1/2}\}mA + 0.016mA$ $R^2 = 0.999$ for 7-aminoflavone,

$i_p = \{0.079[v(V\ s^{-1})]^{1/2}\}mA + 0.001mA$ $R^2 = 0.995$ for trihydroxyethylenorutin.

This linear fit does not precisely intersect the origin of the coordinates (Fig. 6A). Diffusion character of flavonoids electrooxidation was confirmed by a dependence of ln ip on ln v which is linear (Fig. 6B). This dependence is described by the equation:

$\ln i_p = \{0.668 \ln v\ (V\ s^{-1})\}mA + 0.243mA$ $R^2 = 0.994$ for silymarin,

$\ln i_p = \{0.694 \ln v\ (V\ s^{-1})\}mA + 0.099mA$ $R^2 = 0.983$ for neohesperidin dihydrochalcone,

$\ln i_p = \{0.473 \ln v\ (V\ s^{-1})\}mA - 0.377mA$ $R^2 = 0.999$ for 7-aminoflavone,

$\ln i_p = \{0.484 \ln v\ (V\ s^{-1})\}mA - 2.530mA$ $R^2 = 0.993$ for trihydroxyethylenorutin.

The slope is 0.67 for silymarin, 0.69 for neohesperidin dihydrochalcone, 0.47 for 7-aminoflavone and 0.48 for trihydroxyethylenorutin indicating diffusion control of the electrode processes. A slope close to 0.5 is expected for diffusion-controlled electrode processes, and a slope close to 1.0 is expected for adsorption-controlled processes [53-54]. Under the conditions defined in this experiment, the peak currents can be analysed as though they result solely from diffusion. In the

flavonoids, the first step of their electrooxidation was controlled by substrate diffusion toward the electrode surface. Therefore, the kinetics of the processes required investigation.

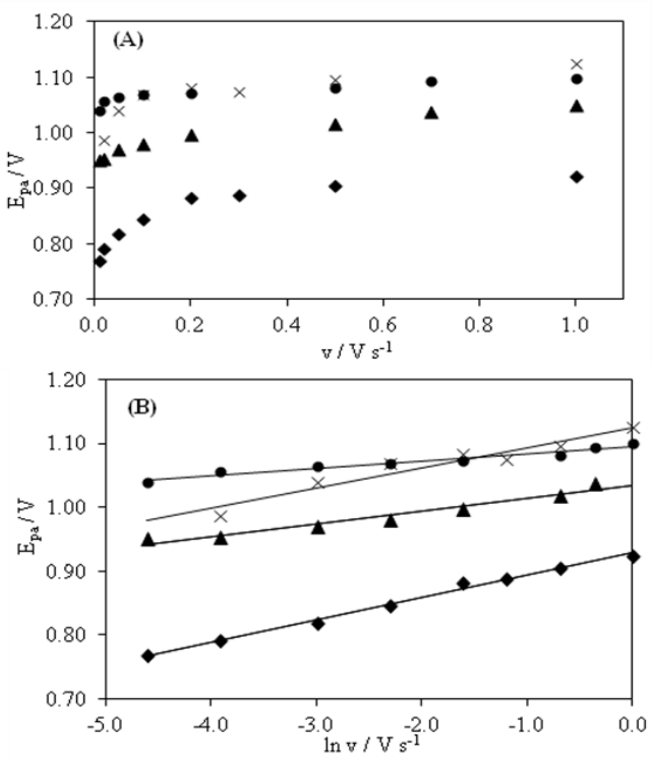


Figure 7. (A) Dependence of the peak potential (E_p) on the potential scan rate (v) for the oxidation of polyphenols in 0.1 mol L^{-1} $NaClO_4$ at the Pt electrode. (B) Dependence of the peak potential on ln v for the oxidation of polyphenols in 0.1 mol L^{-1} $NaClO_4$ at the Pt electrode; x – silymarin, ▲– 7-aminoflavone, ♦ – neohesperidin dihydrochalcone, ● – trihydroxyethylenorutin.

Fig. 7A shows the dependence of E_p on the scan rate determined from cyclic voltammograms recorded for the polyphenols electrooxidation. If the electrochemical reaction is reversible, then E_p is independent of v. Therefore, it can be concluded that heterogeneous electron transfer in polyphenol electrooxidation is irreversible because E_p increases as the scan rate increases.

In addition, the value of the overall electron transfer coefficient for the reaction can be obtained from the following equation [54]:

$$E_p = (\frac{RT}{2\beta n_\beta F})\ln v + const \qquad (1)$$

where E_p is the peak potential (V), R is the universal gas constant (8.314 J K^{-1} mol^{-1}), F is the Faraday constant (96,487 C mol^{-1}), T is the temperature in Kelvin (298 K), βn_β is the anodic transfer coefficient and v is the scan rate (V s^{-1}).

This equation is valid for a totally irreversible diffusion-controlled process. The dependence of the anodic peak potential on the logarithm of the potential scan rate is linear and is described by the following equations (Fig. 7B):

E_{pa} = {0.030[ln v(V s^{-1})]}V + 1.034 V R^2 = 0.973 for silymarin

E_{pa} = {0.035[ln v(V s^{-1})]} V + 0.928 V R^2 = 0.992 for neohesperidin dihydrochalcone
E_{pa} = {0.020[ln v(V s^{-1})]} V + 1.033 V R^2 = 0.955 for 7-aminoflavone
E_{pa} = {0.011[ln v(V s^{-1})]} V + 1.095 V R^2 = 0.945 for trihydroxyethylenorutin.

Using the dependence of the anodic peak potential on the logarithm of the potential scan rate (Fig. 7B), the value of the overall electron transfer coefficient (βn_β) was determined to be 0.41 for silymarin, 0.37 for neohesperidin dihydrochalcone, 0.64 for 7-aminoflavone and 1.15 for trihydroxyethylenorutin.

3.3. Kinetic parameters of flavonoids electrooxidation

The recorded voltammograms, under linear diffusion in the first electrooxidation step, were used to determine the peak potential (E_{pa}), half-peak potential ($E_{pa/2}$) and half-wave potential ($E_{1/2}$). In addition, the voltammograms were used to calculate an anodic transition coefficient (βn_β) and a heterogeneous rate constant (k_{bh}) for the electrode process at the half-wave potential (see Table 1) [55]. The heterogeneous rate constant (k_{bh}) determined for a specified potential E characterises the transfer rate of an electron through the electrode-solution interface. The electron transition coefficient characterises the symmetry of the activated barrier of an electrode reaction.

Table 1. Values of the peak potential (E_{pa}), half-peak potential ($E_{pa/2}$), half-wave potential ($E_{1/2}$), anodic transition coefficient (βn_β) and heterogeneous rate constant (k_{bh}) determined for the half-wave potential of the first electrode step in the electrooxidation of polyphenols at a platinum electrode) and E_{HOMO}; c = 2 × 10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile, v = 10 mV s^{-1}.

Compound	E_{pa} (V)	$E_{pa/2}$ (V)	$E_{1/2}$ (V)	βn_β	D_{red} cm^2 s^{-1}	$k_{bh\ E1/2}$ cm s^{-1}	E_{HOMO} (eV)
Neohesperidin dihydrochalcone	0.86	0.73	0.81	0.38	3.43×10^{-6}	6.49×10^{-4}	-8.978
Silymarin	0.90	0.78	0.84	0.41	4.17×10^{-6}	6.29×10^{-4}	-9.158
7-aminoflavone	0.95	0.88	0.92	0.64	6.01×10^{-6}	3.89×10^{-4}	-9.278
Trihydroxyethylenorutin	1.06	1.01	1.03	1.11	3.18×10^{-6}	2.28×10^{-4}	-8.911

The diffusion coefficient (D_{red}) was estimated according to Hayduk and Laudie's equation [56]:

$$D = \frac{13.26 \times 10^{-5}}{\mu^{1.4} V_o^{0.589}} \qquad (2)$$

where μ is the viscosity of a solvent (centipoises) and v_o is the molar volume (cm^3 g^{-1} $mole^{-1}$).

Based on the results provided in Table 1, neohesperidin dihydrochalcone, with $E_{1/2}$=0.81 V, was most easily oxidised while trihydroxyethylenorutin, with $E_{1/2}$=1.03 V, was least easily oxidised. The calculated anodic transition coefficient (βn_β) for neohesperidin dihydrochalcone, which was oxidised at the highest rate (i.e., 6.49 × 10^{-4} cm s^{-1}), was 0.38. Silymarin, 7-aminoflavone and trihydroxyethylenorutin were oxidised more slowly with heterogeneous rate constants (k_{bh}) equal to

6.29×10^{-4}, 3.89×10^{-4} and 2.28×10^{-4} cm s^{-1}, respectively. Their anodic transition coefficients ranged from 0.41 to 1.11.

3.4. FTIR spectra of flavonoids

FTIR spectroscopy (Fig. 8) was used for the evaluation this type of compounds [57-59].

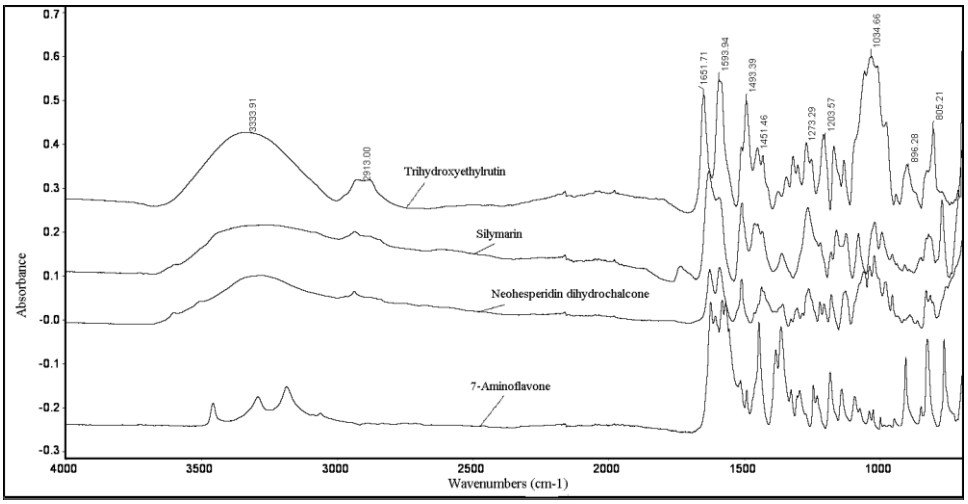


Figure 8. The FTIR spectra of the flavonoids.

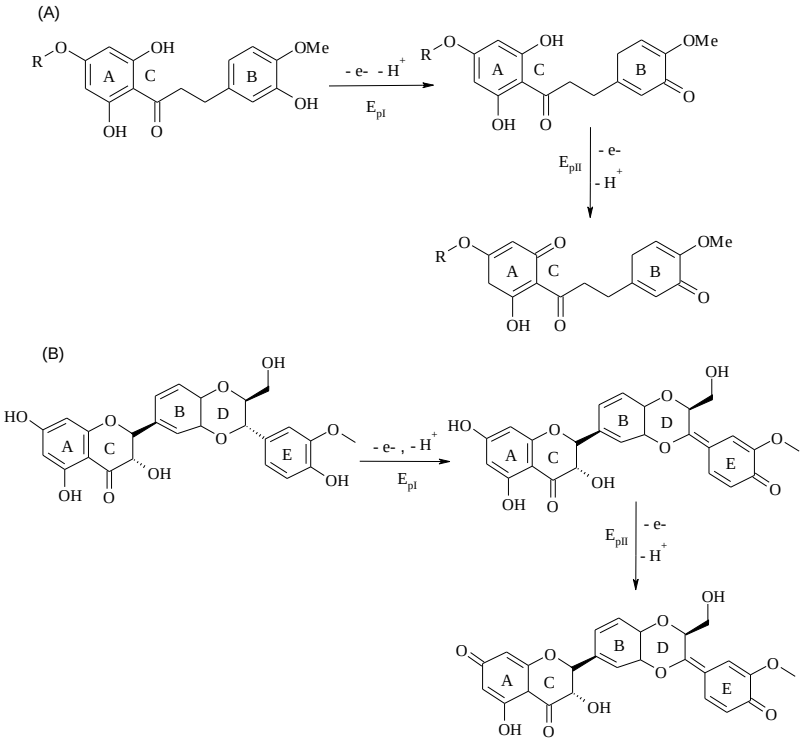
The FTIR spectrum of the tested flavonoids exhibited a broad band at (3600-3200) cm^{-1} corresponding to the hydroxyl group (bonded). The presence of this band indicates the presence of the phenol (-OH) stretch. The bands below 900 cm^{-1}, which are attributed to C-H bending, indicate the presence of aromatic protons. The band at 3195.74 cm^{-1} indicates the possible presence of an aromatic ring (=CH) stretch in the region of 3100-3010 cm^{-1}. The presence of a band at 1652 cm^{-1} indicates the possible presence of an aromatic compound (C=C stretch) in the region of ~1600 cm^{-1} (silymarin, trihydroxyethylorutin, neohesperidin dihydrochalcone, 7-aminoflavone). The band at approximately 1710-1720 cm^{-1} is due to the highly polar C=O bond (silymarin). The most characteristic band of the amine groups is due to the N-H bond stretch, which appears as a weak to medium and somewhat broad band. This band is positioned at the left end of the spectrum in the range of approximately 3200-3600 cm^{-1} (7-aminoflavone). The band at 1665 cm^{-1} can be attributed to the carbonyl group, and the bands below 829 cm^{-1} indicate the existence of an alfa-D-glycoside linkage (Fig. 8).

3.5. Oxidation processes of flavonoids

The as-determined parameters were confirmed using quantum chemical calculations. The distribution of the electron charges in the investigated molecules was non-uniform and determined the reactivity of the particular positions [60]. The energy of the highest filled orbital (E_{HOMO}, or ionisation

potential) determines the ease with which electrons are given up and indicates the site most susceptible to oxidation. The E_{HOMO} molecular orbital energies were calculated using the AM1 method, as implemented using HyperChem software. The observed half-wave potential ($E_{1/2}$) should be linearly dependent on the energy of the HOMO (E_{HOMO}) [61]. The E_{HOMO} values for all of the studied flavonoids were determined via calculation and correlation to the $E_{1/2}$ of the first electrooxidation step (Table 1). Based on the designated $E_{1/2}$ and calculated E_{HOMO} for the flavonoids, neohesperidin dihydrochalcone was oxidised most easily, followed by silymarin and 7-aminoflavone. Trihydroxyethylenorutin was the most difficult to oxidise.

The highest electron density in the neohesperidin dihydrochalcone molecule was observed in rings A and B, which suggested an ease of oxidation for the hydroxyl groups in these rings. The oxidation mechanism for neohesperidin dihydrochalcone in the subsequent electrode steps and its antioxidant activity are connected to the number of hydroxyl groups and their position in the two aromatic rings of this compound (B and A). Neohesperidin dihydrochalcone exhibit conjugation between rings A and B and different pharmacophores, including the moiety in ring B and hydroxyl group in rings A. At the highest positive potential (the first electrode step), the hydroxyl group of ring B was oxidised, and one electron and one proton were exchanged. The current of peak I was high compared to that of peak II, which is in agreement with the higher radical scavenging activity associated with the oxidation of the ring B moiety. Silymarin is irreversibly oxidised in two electrochemical steps. Peak I reflects the oxidation of the OH group in the ring E. This result is in good agreement with the pulse radiolysis results [62]. Peak II is most likely associated with the oxidation involving the OH group in ring A [48, 63].



Scheme 1. Proposed mechanism for neohesperidin dihydrochalcone (A) and silymarin (B) electrooxidation.

Based on the electroanalytical investigations and the literature data [33, 63], mechanisms, which is shown in Scheme 1, have been proposed for the oxidation of neohesperidin dihydrochalcone and silymarin.

4. CONCLUSIONS

The flavonoids were irreversibly oxidised at a platinum electrode. The first step in flavonoid electrooxidation was controlled by substrate diffusion towards the electrode surface. Neohesperidin dihydrochalcone was oxidised most easily, followed by silymarin and 7-aminoflavone. Trihydroxyethylenorutin was the most difficult to oxidise. The low oxidation potential of the flavonoids indicates that they are excellent scavengers of free radicals. If the substrate contained hydroxyl groups in the aromatic rings (A, B or C), the first peak of its oxidation can be attributed to the oxidation of the hydroxyl groups on ring B. This result was associated with an exchange of one or two electrons, depending on the number of hydroxyl groups in ring B. The hydroxyl groups in ring A were oxidised at higher potentials in the subsequent electrode steps. Therefore, subsequent oxidation peaks appeared in the voltammograms.

Flavonoids, which are antioxidants derived from natural sources, including vegetables and fruits, exhibited high reduction activity in the oxidation process, which confirmed the known antioxidant properties that make them successful anti-ageing substances. Of the flavonoids studied, neohesperidin dihydrochalcone exhibited the best antioxidant properties (lowest half-wave potential $E_{1/2}$). Electrochemical research and quantum chemistry calculations (the energy of the highest occupied molecular orbital) allowed for the proposal of a mechanism for the electrochemical oxidation of neohesperidin dihydrochalcone and silymarin.

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