Full Paper

Redox Behavior of Anthocyanins Present in *Vitis vinifera L.*

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Abstract
Voltammetric techniques were employed to study the electrochemical behavior of several anthocyanins. The redox behavior of anthocyanins with the same basic structure, the influence of glycosylation on the redox behavior of anthocyanins derived from different anthocyanidins, and the influence of methoxylation were investigated. The anthocyanins used in this study were malvidin-3-*O*-glucoside chloride, malvidin-3,5-di-*O*-glucoside chloride, cyanidin-3-*O*-glucoside chloride, cyanidin-3,5-di-*O*-glucoside chloride, peonidin-3-*O*-glucoside chloride, delphinidin-3-*O*-glucoside chloride and the anthocyanidin petunidin chloride, all of them present in *Vitis vinifera L.* All hydroxyl groups of the anthocyanins can be electrochemically oxidized and the anthocyanins studied revealed a complex and pH dependent oxidation process, with the occurrence of adsorption and of oxidation products blocking the electrode surface.

Keywords: Anthocyanins, Flavonoids, Redox behavior, Oxidation, Reduction, Methoxylation, Glycosylation, Free radicals, *Vitis vinifera L.*

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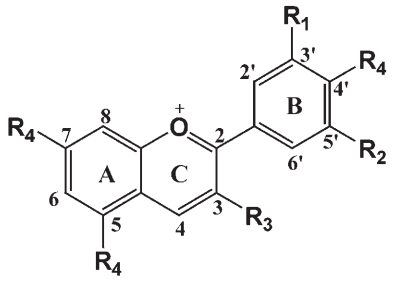
1. Introduction

Anthocyanins are plant polyphenols and one of the major groups of natural pigments widely distributed in nature. The differences between the more than 600 anthocyanins found in nature result mainly from the diverse hydroxyl and methoxyl substitutents on the B-ring, Scheme 1, and also from the type, the number and the position of the sugar moieties attached to the molecule [1, 2]. They are responsible for the attractive red, purple and blue colors of many flowers, fruits and vegetables. Anthocyanins belong to a group of plant compounds called flavonoids and possess a characteristic C6–C3–C6 carbon skeleton. They are glycosylated derivatives of the flavylium cation, and glycosylation, primarily at C3, confers higher stability and increased solubility, Scheme 1.

Anthocyanins display a remarkable number of biochemical and pharmacological activities which have been correlated with their antioxidant activity. Several studies report information concerning the antioxidant activity of anthocyanins [3–5]. Cyanidin and its glycosides represent one of the larger natural anthocyanins group with antioxidant properties [6].



Scheme 1. Flavylium cation structure. R1 and R2 are H, OH or OMe; R3 is a glycoside or H; and R4 is OH or a glycoside.

The electron deficiency of the flavylium cation makes the free aglycones (anthocyanidins) highly reactive, and they very rarely occur naturally [1, 2]. To date, seventeen anthocyanidins or aglycones have been identified which occur naturally, but only six of them are common in higher plants. The most commonly anthocyanidins found are pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp). Their chemical structure is given in Table 1.

Glycoside forms of anthocyanidins which are not methylated (Cy, Dp, Pg) are the anthocyanins most found in nature, which account for 80% in leaves, 60% on fruits and 50% on flowers of total anthocyanidins [1]. The substituent groups on the anthocyanin mainly influence reactivity and color by changing the electron distribution on the molecule [7]. Many published reports have described extraction methods of anthocyanins [8–10], and also their separation, determination and quantification using HPLC with ultraviolet diode-array [8, 11, 12], fluorescence [13] and MS detection [14].

In relation to stability, the anthocyanidins are less stable than the anthocyanins. Glycosylation of anthocyanidins increases stability and diglycosides are more stable than their corresponding monoglycosides [15, 16]. Additionally, anthocyanin sugar residues may be acylated with one or more moieties of aliphatic acids, phenolic benzoic acids or

Table 1. Chemical structure of the main anthocyanidins.

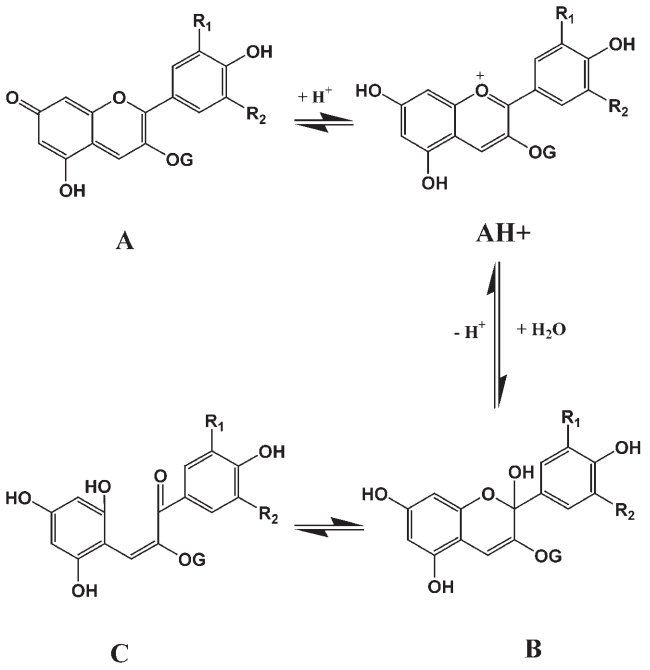
Name	Abbreviation	Substitution						Color
		3	5	7	3′	4′	5′	
Cyanidin	Cy	OH	OH	OH	OH	OH	H	orange/red
Delphinidin	Dp	OH	OH	OH	OH	OH	OH	blue/red
Malvidin	Mv	OH	OH	OH	OMe	OH	OMe	blue/red
Pelargonidin	Pg	OH	OH	OH	H	OH	H	orange
Peonidin	Pn	OH	OH	OH	OMe	OH	H	orange/red
Petunidin	Pt	OH	OH	OH	OMe	OH	OH	blue/red

phenolic cinnamic acids [15]. The stability of the heterocyclic π-electron structure of these compounds depends on the substituted positions as well as on the substituents themselves.

The most common glycosylic substitution positions in the flavylium cation are C3, C5, C7, C3′, C4′ and C5′. The sugar substituents are glucose, rhamnose, xylose, galactose, arabinose and fructose. Introduction of the sugar moieties in C3 or C5 position of aglycones causes a hypochromic shift and a decrease in the intensity of the absorption spectrum with increasing pH. Another explanation for the colour loss in 3-*O*-glucosides in relation to their aglycones may be attributed to an enhanced electrophilicity of the flavylium cation as a result of an electron-withdrawing effect [15]. At a given pH, anthocyanin 3-glucosides are more colored than 3,5- and 5-glucosides [17]. Different substituents have a marked effect upon the color and reactivity of anthocyanins. Generally, as the number of phenolic hydroxyls increases, the color changes from pink to blue. Methoxyl groups replacing hydroxyl groups reverses the trend: the hydroxyl group at C3 is particularly significant, as it shifts the color of the pigment from yellow-orange to red. Also differences in electroactive substituents on analogous structures can lead to characteristic differences in their voltammetric behavior [16]. The hydroxyl group as a substituent on position C4′, that is in the *para*-position of the ring B, and is associated with the increase in the delocalization of π-electrons in the chromophore [17].

In aqueous solution most anthocyanins behave as pH indicators, being red at low pH, colorless at intermediate pH and bluish at high pH. The appearance and disappearance of the different colored states of the same pigment is due to the high reactivity of the aglycone moiety [18]. In slightly acidic aqueous solution at ambient temperature, anthocyanins exist essentially with the four species in equilibrium, Scheme 2. At pH < 2 the anthocyanin exists primarily in the form of the red (R3 = O-sugar) or yellow (R3 = H) flavylium cation (AH+). As the pH is increased a rapid proton loss occurs to yield the red or blue quinoidal forms (A). On standing, hydration of the flavylium cation (AH+) occurs to give the colorless carbinol or pseudobase (B) in equilibrium at a slower rate to the open chalcone (C), which is also colorless [17, 19]. The pseudobase possesses more phenolic groups in its structure which can influence the electrochemical results. However, these groups will be oxidized at high potential values leading to lower currents than the flavylium cation hydroxyl groups. Further deprotonation of the quinoidal bases can take place between pH 6 and 7 with the formation of the bluish resonance-stabilized quinonoid anions [20]. At room temperature, and in slightly acidic media, the equilibrium between the carbinol and chalcone forms is very slow and takes hours to be reached. An increase of temperature displaces the equilibrium towards the chalcone forms [21]. The phenolic groups present on the B and the A rings of flavonoids, that means also in anthocyanins, can all be electrochemically oxidized [22].

Electrochemical studies reveal general trends in the electron-donating abilities of flavonoids. It was demonstrated that the catechol in the B-ring is more easily oxidizable than the resorcinol in the A-ring [23]. Comparison of the calculated deprotonation energies of the OH moieties in the flavylium cation of cyanidin and cyanidin-3-*O*-glucoside showed that 4′-OH is the group which preferentially deprotonates [15]. The pKa values were assigned to the B-ring according to reference [24].



Scheme 2. Acid-base and cyclization equilibria of anthocyanin-3-*O*-glycoside.

In this paper, the electrochemical oxidation mechanistic study of the anthocyanins described in Scheme 3, under identical conditions of pH, pigment concentration, temperature, ionic strength and solvent, was carried out.

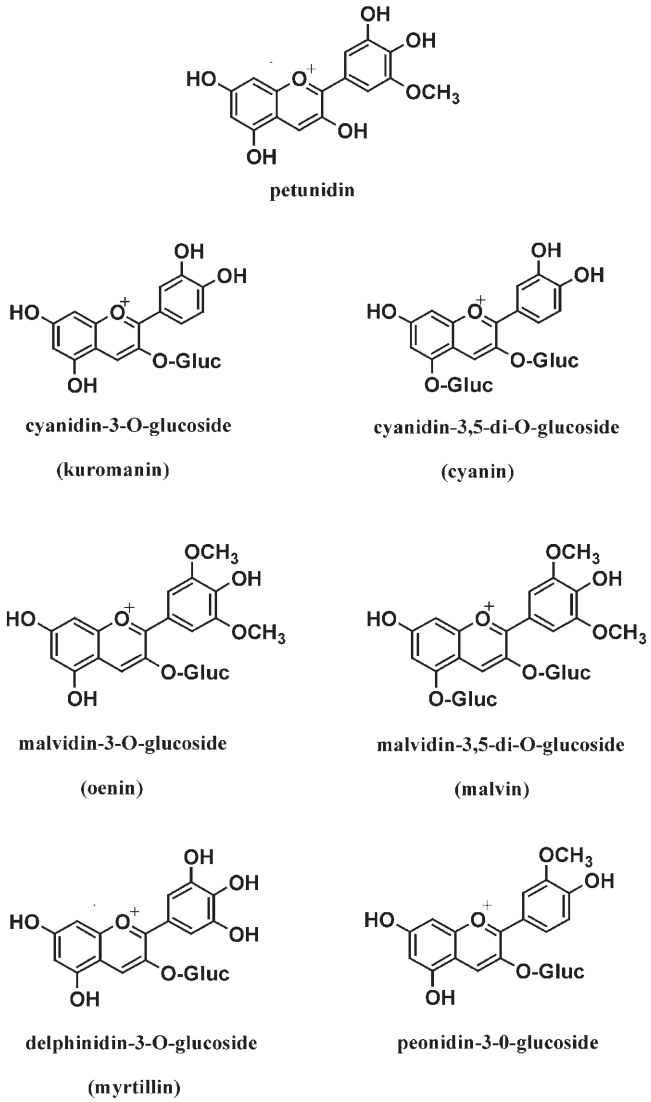
2. Experimental

The structures of the anthocyanins studied in their chloride salt forms are shown in Scheme 3. The anthocyanins peonidin-3-*O*-glucoside chloride, malvidin-3-*O*-glucoside chloride (oenin chloride), malvidin-3,5-*O*-diglucoside chloride (malvin chloride), cyanidin-3-*O*-glucoside chloride (kuromanine chloride), cyanidin-3,5-*O*-diglucoside chloride (cyanin chloride), delphinidin-3-*O*-glucoside chloride (myrtillin chloride) and the anthocyanidin petunidin chloride were from Extrasynthese, France. All the other reagents were Merck analytical grade. All solutions were made using deionised water obtained from a Millipore Milli-Q purification system (resistivity ≥ 18 MΩ cm).

Experiments were all carried out at room temperature ($25 \pm 1\,^{\circ}C$) and in the presence of dissolved oxygen. Solutions of buffer supporting electrolyte of ionic strength 0.2 were used in all experiments, Table 2. The pH measurements were carried out with a CRISON GLP 21 pH-meter.

The electrochemical experiences were done using an Autolab PGSTAT 10 running with GPES (General Purpose Electrochemical System) version 4.9 software (Eco-Chemie, Utrecht, The Netherlands). The voltammetric curves were recorded using a three electrode system in a small-volume electrochemical cell of capacity 2 mL (Cypress System, Inc., USA). The working electrode was a glassy carbon electrode of 1.5 mm diameter; Ag/AgCl (sat. KCl) was used as a reference electrode and a platinum wire as counter electrode.

In this work, all potentials were recorded versus Ag/AgCl (sat. KCl) electrode. The glassy carbon working electrode was polished with diamond spray (6 and 1 μm). Voltammetric scans were carried out in the potential range 0 to +1.4 V vs. Ag/AgCl. Differential pulse voltammetry (DPV) conditions used were pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mVs^{-1}. Square-wave voltammetry (SWV) conditions were frequency 13, 25 and 50 Hz, amplitude 50 mV and potential increment 2 mV.



Scheme 3. Chemical structures of anthocyanin.

3. Results and Discussion

Relevant aspects concerning the analytical electrochemistry of the anthocyanins and their standard aglycones chosen for this study were investigated using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square-wave voltammetry (SWV). The hydroxyl and methoxyl substituents on the B-ring and also the type, the number and the position of the various possible sugar moieties attached to the molecule, Scheme 1, account for most of the differences between more than 600 anthocyanins found in nature.

3.1. Cyclic Voltammetry

Figure 1 shows CVs of kuromanine (cyanidin-3-*O*-glucoside) and cyanin (cyanidin-3,5-*O*-diglucoside) chloride at pH 7.0. They exhibit two oxidation peaks, P1 and P2, associated with the two oxidizable centres in the molecules. Both anthocyanins posses a catechol group on the B-ring, and the oxidation peak corresponding to this moiety is

Table 2. Supporting electrolyte solutions.

Supporting electrolyte solutions			pH
0.2 M NaOAc	+	0.2 M HOAc	3.5
0.2 M NaOAc	+	0.2 M HOAc	4.5
0.2 M Na_2HPO_4	+	0.2 M NaH_2PO_4	7.0

designated P1. This peak occurs at the same potential value for both compounds, $E_{P1} = +0.30$ V. The oxidation products formed in P1 were reversibly reduced as confirmed in the second scan by reversing the potential scan just after P1.

The oxidized molecule could be further oxidized at higher potentials as shown in the second oxidation peak P2 related with oxidation of the 5,7-dihydroxyl moiety of the A-ring (resorcinol moiety). Due to the different sensitivities of the techniques, peak P2 by CV is lower than when using DPV, see Figure 2.

The reduction of kuromanine and cyanin peak P1 oxidation products occurred at $E_{P1}' = +0.23$ V. For the reversible P1 reaction, at 298 K, the values for cyanin, $E_{p,c} - E_{p/2,c} = 54$ mV, and kuromanine, $E_{p,c} - E_{p/2,c} = 55$ mV, were found. These indicate that one electron is involved in the first oxidation process of each compound. This approach is valid only for reversible systems; modifications for irreversible and quasireversible systems have been described in detail [25].

Cyanin and kuromanine have the catechol group on the B-ring (3′,4′-dihydroxyl) in common and differ only by the substituent on the A-ring, on C5, where cyanin has a glucoside group and kuromanine possesses a hydroxyl group. This difference is manifested in the second peak potential, P2, which is associated with the oxidation of the hydroxyl groups on the A-ring. The oxidation peak potential

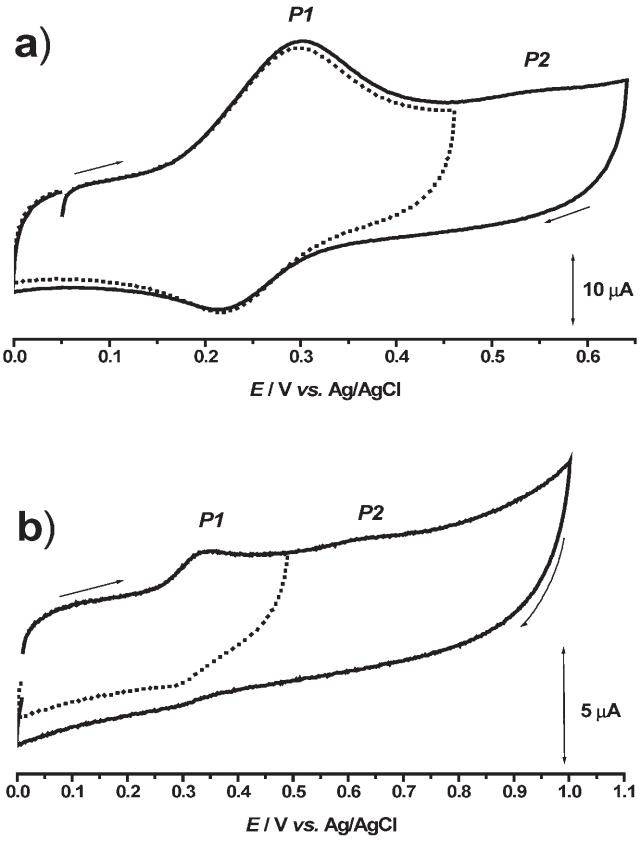


Fig. 1. Cyclic voltammograms in pH 7.0 0.2 M phosphate buffer: a) 0.1 mM kuromanine chloride and b) 0.1 mM cyanin chloride. Scan rate: 1 V s^{-1}.

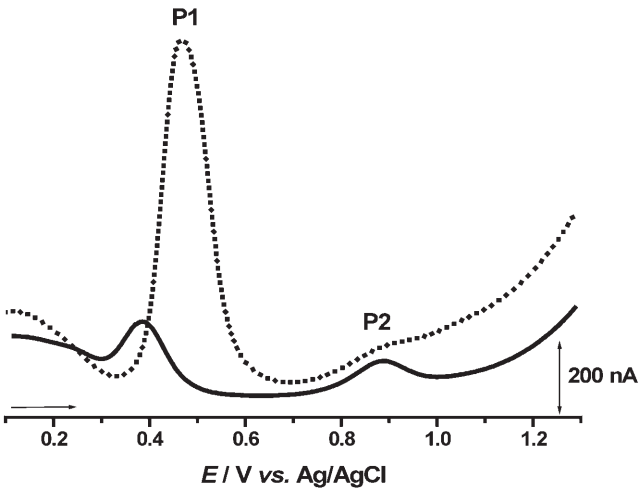


Fig. 2. Differential pulse voltammograms in pH 3.5 0.2 M acetate buffer: (–) 0.01 mM peonidin-3-*O*-glucoside chloride and (····) 0.1 mM kuromanine chloride. Scan rate: 5 mV s^{-1}.

P2 for kuromanin, with a *m*-dihydroxyl group on the A-ring, was $E_{P2} = +0.50$ V and, for cyanin, with just a hydroxyl group on C7, was $E_{P2} = +0.63$ V. In both cases, P2 corresponds to an irreversible oxidation process. The effect of substituent on P2 potential was also appreciable for oenin (malvidin-3-*O*-glucoside) and malvin (malvidin-3,5-diglucoside) chloride.

3.2. Differential Pulse Voltammetry

Table 3 summarises all the DPV results obtained for the oxidation of all seven anthocyanins including those not shown in the Figures. Comparing the oxidation potentials at different pHs provides very good information on the mechanism of anthocyanin oxidation and the dependence of the oxidation of these compounds on pH. An increase of pH was always associated with a linear decrease of the oxidation potential values. A strong adsorption of the oxidation products, which blocked the electrode surface, was also observed, since anthocyanin oxidation peaks decreased drastically in the second scan for all pH values. These results are supported by comparing Table 3 peak potentials with those obtained for the oxidation of the flavonoids rutin, catechin, chrysin and taxifolin [26–28].

DPVs show, Figure 2, the effect on the oxidation potential of peak P1 of replacing a methoxyl substituent by a hydroxyl group. Substituents on the B-ring, particularly those located in the *ortho*- or the *para*-positions to the –OH group, influence the acidity of the hydroxyl group due to resonance and/or inductive effects [22]. Comparing peonidin-3-*O*-glucoside chloride with kuromanine chloride at pH 3.5 a potential shift of 100 mV towards more positive values was observed when a methoxyl group substitutes a hydroxyl group. Kuromanine chloride showed an oxidation peak potential of $E_{P1} = +0.49$ V and peonidin-3-*O*-glucoside chloride of $E_{P1} = +0.39$ V. The number of electrons trans-

Table 3. Differential pulse voltammetry data for anthocyanin oxidation.

Compound	pH	P1 E (mV)	P2 E (mV)
Malvidin-3-*O*-glucoside chloride (*oenin chloride*)	3.5	540	880
	4.5	490	825
	7.0	380	500
Malvidin-3,5-*O*-diglucoside chloride (*malvin chloride*)	3.5	540	980
	4.5	490	910
	7.0	380	640
Cyanidin-3-*O*-glucoside chloride (*kuromanine chloride*)	3.5	490	885
	4.5	420	815
	7.0	310	500
Cyanidin-3,5-*O*-diglucoside chloride (*cyanin chloride*)	3.5	490	980
	4.5	420	910
	7.0	310	630
Delphinidin-3-*O*-glucoside chloride (*myrtillin chloride*)	3.5	390	880
	4.5	330	820
	7.0	195	490
Peonidin-3-*O*-glucoside chloride	3.5	390	885
	4.5	350	815
	7.0	210	600
Petunidin chloride	3.5	440	910
	4.5	395	845
	7.0	285	630

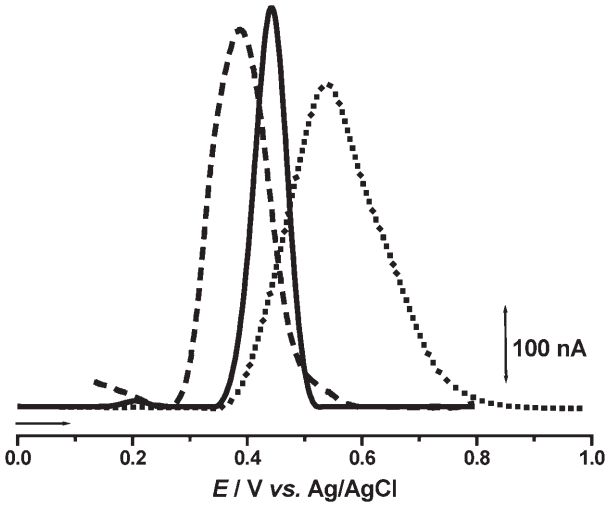


Fig. 3. Background-subtracted differential pulse voltammograms in pH 3.5 0.2 M acetate buffer: (–) 0.1 mM myrtillin chloride, (- - -) 0.1 mM petunidin chloride and (····) 0.1 mM oenin chloride. Scan rate: 5 mV s^{-1}.

ferred, n, was determined for P1. Since the value of $W_{1/2}$ obtained for peonidin-3-*O*-glucoside chloride was 85 mV and for kuromanine chloride was 103 mV, was concluded that the first oxidation process for both compounds corresponded to the oxidation of the hydroxyl group on C4′ and involved one electron.

The oxidation products formed in the first oxidation were reversibly reduced but the molecule could still be oxidized at higher potentials, oxidation peak P2 related with the oxidation in the 5,7-dihydroxyl moiety of the A-ring (resorcinol moiety). Both 3-*O*-glucosylated anthocyanins, showed the same oxidation potential value for peak P2, $E_{P2} = +0.885$ V. The value of $W_{1/2}$ obtained for this peak was 100 mV for kuromanine chloride and 93 mV for peonidin-3-*O*-glucoside chloride, thus indicating again that one electron is involved in each oxidation processes.

Myrtillin presented an oxidation peak P1 related with the hydroxyl group on C4′ at $E_{P1} = +0.390$ V whereas oenin chloride showed an oxidation potential of $E_{P1} = +0.540$ V, Figure 3. However, the oxidation peak potential P1 from petunidin chloride, $E_{P1} = +0.440$ V, has an intermediate value between oenin chloride and myrtillin chloride. In all three cases oxidation corresponded to the transfer of one electron associated with the oxidation of the hydroxyl group on C4′. These results can be explained because this hydroxyl group on C4′ is more acidic when is placed in the *ortho*-position to the two methoxyl groups, in oenin, than when is placed in the *ortho*-position to just one, in petunidin, or no methoxyl group, in myrtillin [29].

The fact that peak P1 corresponds to the oxidation of the catechol group by a single electron transfer when B ring has two oxidizable hydroxyl groups was confirmed with measurements done in the presence of oxygen [30].

3.3. Square-Wave Voltammetry

The square-wave voltammetry conditions chosen, frequency 25 Hz, amplitude 50 mV, corresponding to an effective scan rate of 50 mV s^{-1}, led to well-defined voltammograms, Figure 4. A great advantage of the square-wave method is the possibility to see during one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both the positive and the negative-going pulses, peaks corresponding to the oxidation or reduction of the electroactive species at the electrode surface are obtained in the same experiment.

An important feature was found for both malvin chloride and oenin chloride (not shown), that each have two methoxyl groups in the *ortho*-position to hydroxyl group on C4′ on their B-ring, Scheme 3. In these compounds an irreversible oxidation reaction occurred shown in oxidation peak P1, Figure 4a. This behavior was found over all the range of pH studied. The number of electrons involved in the first oxidation process was determined by the peak width at half height ($W_{1/2}$), using the differential pulse voltammograms. The value of $W_{1/2}$ obtained was 100 mV for oenin chloride and 85 mV for malvin chloride, thus indicating that one electron is involved in each oxidation processes.

Comparing these results with peonidin, Figure 4b, the influence of only one methoxyl group in the *ortho*-position to hydroxyl group of C4′ can be observed. In this case a reversible oxidation peak P1 was obtained, since the forward and backward currents are similar and the oxida-

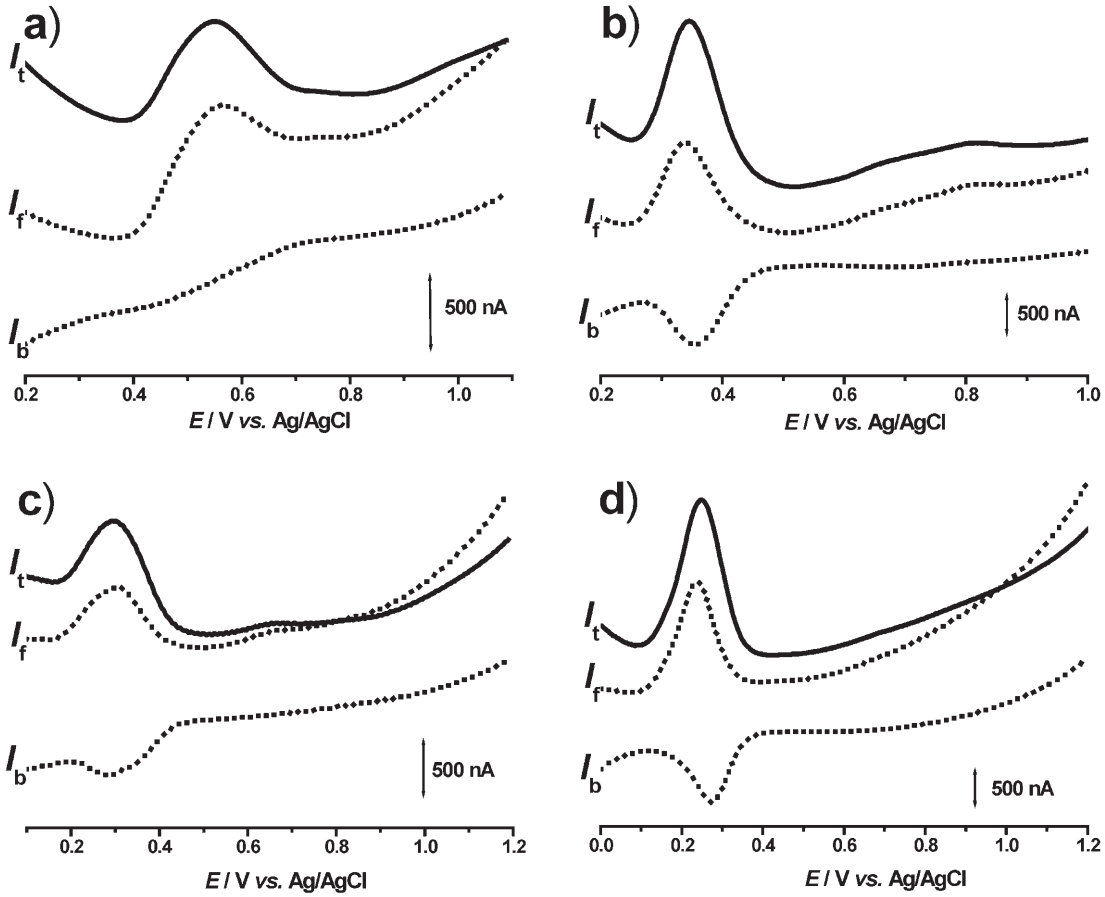


Fig. 4. Square-wave voltammograms, frequency 25 Hz, amplitude 50 mV: a) 0.1 mM malvin chloride pH 3.5 0.2 M acetate buffer; b) 0.1 mM peonidin-3-*O*-glucoside chloride pH 4.3 0.2 M acetate buffer; c) 0.1 mM petunidin chloride pH 7.0 0.2 M phosphate buffer; and d) 0.1 mM kuromanine chloride pH 7.0 0.2 M phosphate buffer. Effective scan rate: 50 mV s^{-1}. I_t: total current, I_f: forward current, I_b: backward current.

tion and reduction peaks occur at the same potential, denoting some adsorption of the analyte. The anthocyanidin petunidin, with one methoxyl group on the B-ring, as in peonidin-3-*O*-glucoside, but with a catechol group (i.e. two hydroxyl groups), also showed a reversible oxidation, Figure 4c. A square-wave voltammogram of kuromanine chloride, with a catechol group on the B-ring, and absence of methoxyl groups, Figure 4d, shows a reversible behavior.

The oxidation peak potentials for all compounds studied by square-wave voltammetry, at different pH, agree with the potentials obtained by differential pulse voltammetry shown in Table 3 The effect of having a catechol group on the B-ring or only a hydroxyl group in the *ortho*-position to a methoxyl group, comparing petunidin with peonidin-3-*O*-glucoside, demonstrates that it is easier to oxidize a hydroxyl group than a catechol group in the *ortho*-position to a hydroxyl group. The second oxidation reaction in all anthocyanins studied always showed irreversibility. These results obtained by SWV compare very well with those from cyclic and differential pulse voltammetry.

3.4. Influence of the A-Ring Glucosylation on the Redox Behavior of Anthocyanins with the same Basic Structure

Anthocyanins are usually found glycosylated in nature. The most common substitution positions in the flavylium cation are C3, C5, C7, C3′, C4′ and C5′ where the sugar substituents are glucose, rhamnose, xylose, galactose, arabinose and fructose. Comparing the cyclic voltammograms of kuromanine (cyanidin-3-*O*-glucoside) and cyanin (cyanidin-3,5-*O*-diglucoside) chloride, Figure 1, it is demonstrated that with diglucosylation on C3 and C5 the oxidation peak potential of the hydroxyl group on the A-ring occurs at higher values than when the glucosylation is only at C3. These two anthocyanins both possess a catechol group on the B-ring, and the oxidation peak P1, corresponding to this catechol moiety, occurs at the same potential value for both compounds. This means than the hydroxyl groups in the B-ring are not affected on their oxidation peak potential by the effect of the glucosylation on the A-ring.

3.5. Influence of Methoxylation on the Redox Behavior of Different Anthocyanins and Anthocyanidins

The different substituents have a marked effect upon the color and reactivity of anthocyanins. Also differences in electroactive substituents on analogous structures can lead to characteristic differences in their voltammetric behaviour. The influence of methoxylation on the redox behavior of different anthocyanins and anthocyanidins is shown, Scheme 3, Table 3. The only difference between them is on the B-ring structure and that the anthocyanidin, petunidin, has a hydroxyl group on C3. All these effects are rationalized on the basis of the electron-donating properties of the substituents. Comparing peonidin-3-*O*-glucoside chloride with kuromanine chloride a potential shift towards positive potential values is observed when a methoxyl group substitutes a hydroxyl group, Figure 2. In this case, the hydroxyl group substitution by a methoxyl group makes the hydroxyl group more oxidizable because the phenol acidity decreased. The hydroxyl substitution increases the positive charge in the adjacent carbon and hydrogen atoms, whereas the negative charge is increased on the hydroxyl oxygen atom due to the inductive effect that causes delocalisation of the ionic charge [31].

Three different compounds which all possess the hydroxyl or the methoxyl groups on their carbons C3′, C4′ and C5′ on the B-ring were compared. The introduction of $-OH$, $-OCH_3$ or $-CH_3$ groups into the aromatic ring, all of them electron-donating groups, could make the compound more easily oxidizable. Comparing myrtillin chloride, which possesses a pyrogallol group on the B-ring, with oenin chloride, where the hydroxyl group on C4′ is in the *ortho*-position to two methoxyl groups, the inverse effect was observed, i.e., it was easier to oxidize the hydroxyl group from pyrogallol on the B-ring than the hydroxyl group in the *ortho*-position to two methoxyl groups from oenin chloride, Figure 3. These results are in agreement with the literature because two methoxyl groups in the *ortho*-position to a hydroxyl group renders the hydroxyl group more acidic than when is just one methoxyl group [29].

Petunidin chloride possesses a catechol group in the *ortho*-position to a methoxyl group on the B-ring. Accordingly just one methoxyl group in the *ortho*-position to a hydroxyl group renders the hydroxyl group oxidation peak potential less positive, than observed in oenin. These results demonstrate that a pyrogallol group is easier to oxidize than a catechol group in the *ortho*-position to a methoxyl group and the oxidation potential value is approximately the same as when the B-ring possesses just one hydroxyl group in the *ortho*-position to a methoxyl group (peonidin-3-*O*-glucoside).

4. Conclusions

The mechanism of electron transfer of several anthocyanins, all of them present in *Vitis vinifera L.*, was clarified using electrochemical methods, cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry. It was demonstrated than it was easier to oxidize the hydroxyl group on C4′ from pyrogallol on the B-ring, in myrtillin chloride, than the hydroxyl group in the *ortho*-position to two methoxyl groups, in oenin chloride, which was also easier to oxidize than the catechol group in the *ortho*-position to a methoxyl group, in petunidin chloride. A reversible oxidation reaction occurs only with a methoxyl group in the *ortho*-position to the hydroxyl group on C4′. The experiments showed an irreversible reaction corresponding to the oxidation of the hydroxyl group on C4′ of the B-ring for the compounds where this hydroxyl group is located in the *ortho*-position to two methoxyl groups, such as in malvin and oenin chloride. Hydroxyl groups from the B-ring do not present variation on their oxidation peak potential values by the effect of glucosylation on the A-ring. At higher positive potentials an irreversible oxidation reaction was observed corresponding to the oxidation of the hydroxyl groups of the A-ring.

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