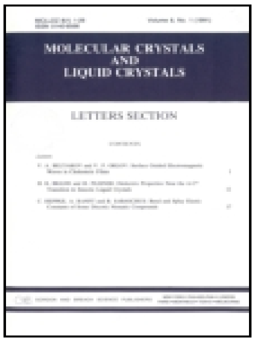

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Spectro-Electrochemical Properties of Pelargonidin-3-O-Glucoside

ION IOSUB,[1] FRANCOIS KAJZAR,[2] AURELIA MEGHEA,[2]
MARIA-LUIZA MIRCEA,[2] IRINA GEANA,[3]
AND ILEANA RAU[2,*]

[1]University of Piteşti, Pitesti, Romania
[2]University POLITEHNICA of Bucharest, Bucharest, Romania
[3]National Research and Development Institute for Cryogenic and Isotope, Technologies–ICIT Râmnicu Vâlcea, Romania

This paper presents the spectral and electrochemical properties correlated to antioxidant character attributed to anthocyans: Pelargonidin-3-O-Glucoside, Cyanidin-3-O-Glucoside, Delphinidin-3-O-Glucoside, Peonidin-3-O-Glucoside, Malvidin-3-O-Glucoside. The Anthocyans were obtained and purified from natural selective hydroalcoholic extracts using HPLC chromatography. A series of determinations through the chemical voltammetry method were performed in view of identifying the anthocyanins' oxidation and reduction potentials. These determinations were performed in electrochemical environments with different polarities and pH, in N, N-Dimethylformamide–Ethanol-Hydrochloric acid–Water systems. The results obtained offered data regarding the stability of anthocyans in the respective media. For Pelargonidin-3-O-Glucoside we performed detailed UV-VIS spectro-electrochemical measurements, using an optically transparent thin layer electrochemical cell. The results of these determinations offered information regarding the mechanism of redox processes.

Keywords Anthocyanins; cyclic voltammetry; spectro-electrochemisty; HPLC

Introduction

Anthocyanins are natural pigments soluble in water, present in flowers, fruit, leaves and other parts of the plants. The role of these compounds in the organism is not fully clear, but literature presents a series of health benefits due to the consumption of fruit containing a large amount of anthocyanins [1–7]. The presence of anthocyanins in plants is differentiated according to species, as well as the period of synthesis; for example, the fruit that reached maturity exhibit maximum of anthocyanin content as a result of successive steps of biosynthesis (*Aronia melanocarpa, Prunus cerasus, Vitis vinifera, Vaccinium myrtillus, Strawberry*). In other cases, the presence of anthocyanins is quasipermanent or permanent;

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[*] Address correspondence to Ileana Rau, University POLITEHNICA of Bucharest, 1 Polizu Street, Bucharest, Romania. E-mail: ileana_brandusa@yahoo.com

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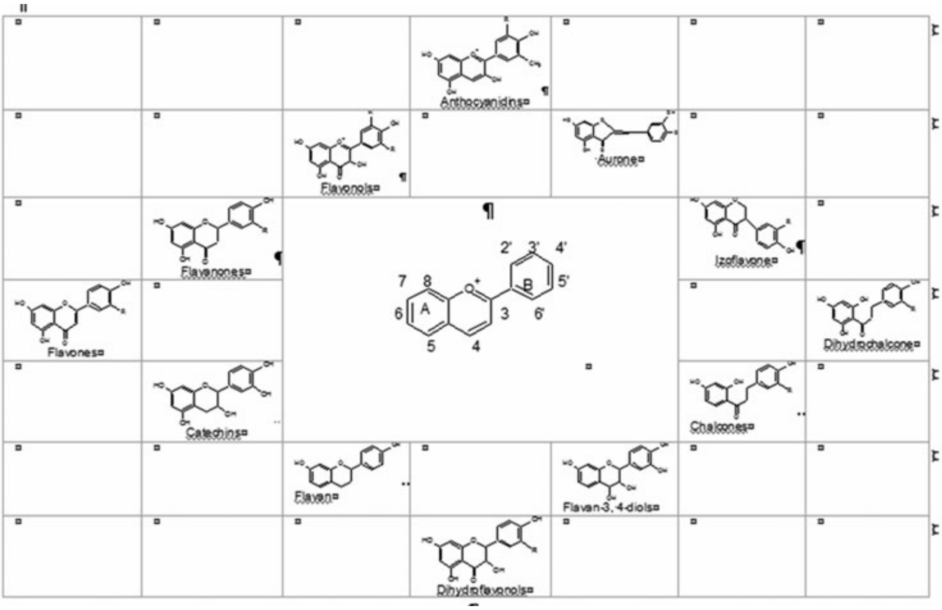

Figure 1. Basic structure and the main classes of flavonoids.

for example, in the leaves of certain species of plants [8, 9]. (leaf of *Prunus cerasifera ‘Atropurpurea’*)

Anthocyanins are part of a large group of the natural compounds of Flavonoids. This group also includes many natural pigments that have been divided into 12 classes of basic compounds [10]: anthocyanidins, flavonols, flavones, flavanones, catechins, flavan, dihydroflavonols, flavan-3, 4-diols, chalcones, dihydrochalcones, isoflavones, aurones (see Fig. 1).

In natural pigments, anthocyanidins are found only in more stable forms, such as methylated, acilated or glycosylated, represented by anthocyanins. The biosynthesis of anthocyanidins and anthocyanins takes place in plants through the transformation of the different flavonoids [11–16], following the process presented in Fig. 2.

The best known natural anthocyanins are those with basic and methylated structures. Their chemical structures are presented in Fig. 3. These compounds have the absorption spectra similar to those of the corresponding anthocyanidines, due to the presence of the same chromophore group in the structure, but with chemical shifts determined by the nature and the number of the substitution functional groups, the pH or the solvent nature.

Experimental Part

Firstly, we isolated natural anthocyans based on ethanolic extracts obtained from vegetal raw materials. The vegetal materials used were selected depending on the high specific content for each anthocyan. We used petals of *Geranium* flower (for Pelargonidin-3-O-Glucoside), black berries fruits *Rubus fructicosus* (for Cyanidin-3-O-Glucoside), black grape peel *Vitis vinifera* (Delphinidin-3-O-Glucoside, Malvidin-3-O-Glucoside), fresh cranberries peel *Vaccinium myrtillus* (Peonidin-3-O-Glucoside), black chokeberries fruit

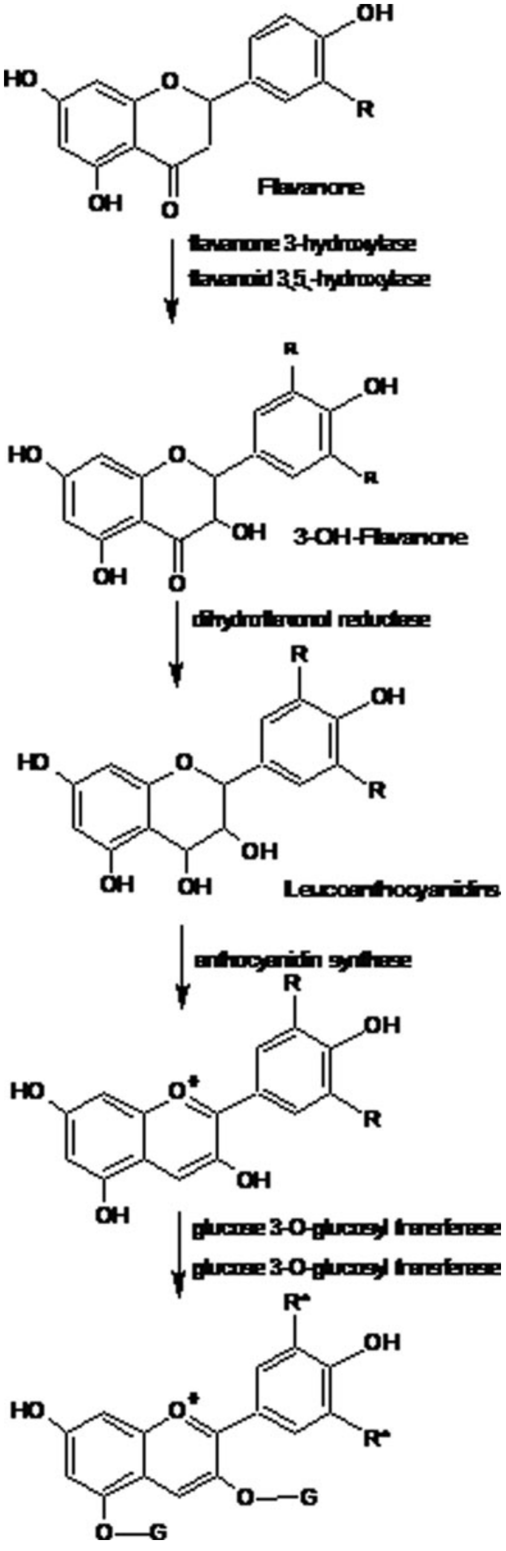

Figure 2. Biosynthesis of anthocyanins natural pigments starting from flavanone R* = —H, —OH, —O—CH_3, G = —O—G.

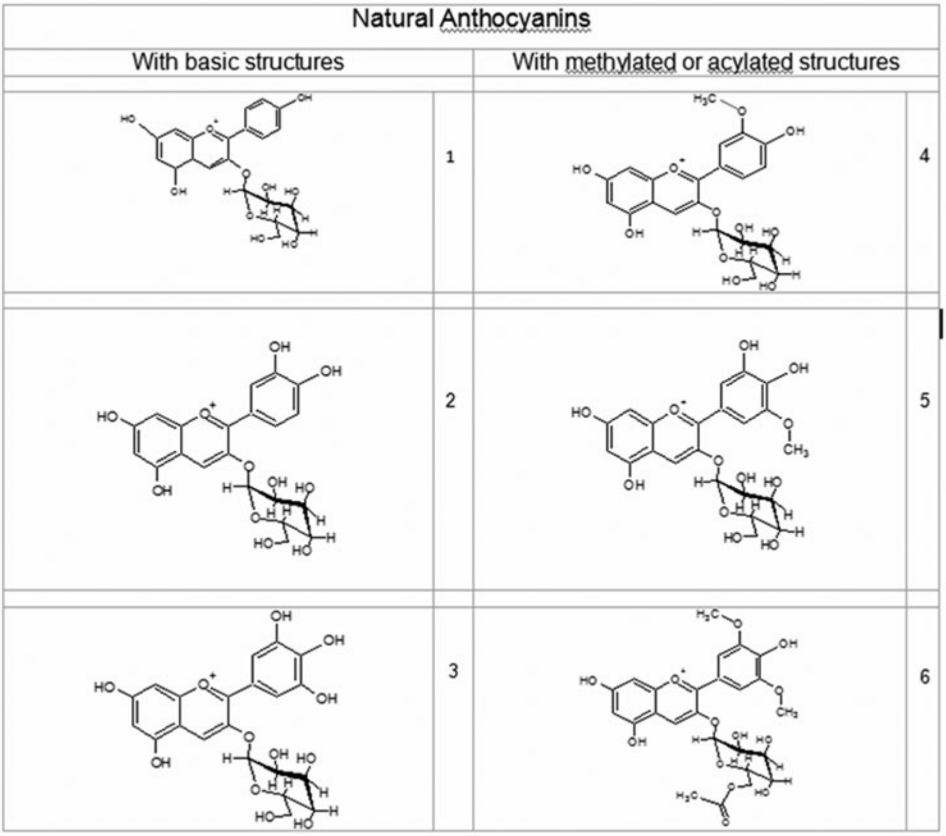

Figure 3. The main natural anthocyanins 1. Pelargonidin-3-O-Glucoside, 2. Cyanidin-3-O-Glucoside, 3. Delphinidin-3-O-Glucoside, 4. Peonidin-3-O-Glucoside, 5. Petunidin-3-O-Glucoside, 6. Malvidin Acetyl-3-O-Glucoside.

peel (*Aronia melanocarpa*). The methods of concentration and purification were based on HPLC liquid chromatography. The Solid Phase Extraction (SPE) is performed by reverse-phase chromatography on C18 column, using as mobile phase: ethanol/hydrochloric acid 1M: (90/10) described in literature [14, 17, 18]. The properties of the anthocyans obtained were compared with those of the corresponding standards (Merck).

Spectrophotometric Measurements

The spectral properties of Pelargonidin-3-O-Glucoside were determied in a series of solutions, using solvents with different polarities: N,N-Dimethylformamide (non polar), ternary systems (N,N-Dimethylformamide/Ethanol/Water), water-polar. The spectra were recorded in solutions where the concentration of Pelargonidin-3-O-Glucoside was kept constant, using the Spekord 600 spectrophotometer. The spectra recorded are presented in Fig. 4.

The analysis of the spectral data presented in Table 1 reveals a linear increase in absorbance with solvent polarity increasing.

The bathochromic shift recorded while reducing the proton concentration and increasing the nonpolar character of the solvent proves the transformation of the flavilium ion

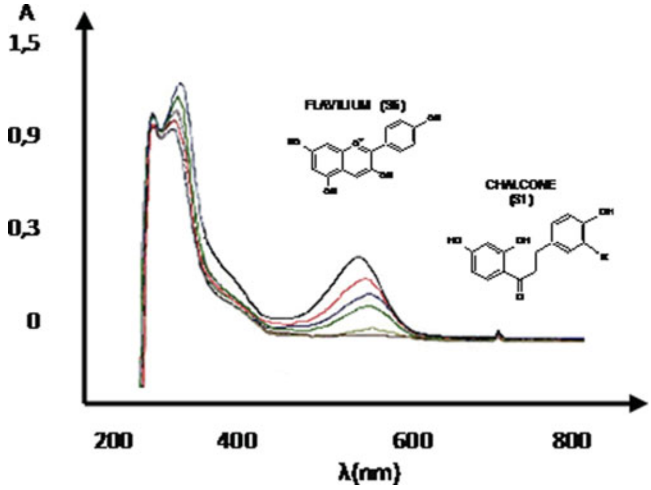

Figure 4. The spectra of Pelargonidin-3-O-Glucoside (0.012 mg/mL) in solvents with different polarities S1–S6.

of the Pelargonidin-3-O-Glucoside anthocyan in the form of chalcones (see Fig. 5). With the decrease of the solvent polarity, a change in the pH was recorded. The stability of the anthocyan structure in the form of the flavilium ion decreases and the colorless form appears (chalcone).

Electrochemical Measurements

Cyclic voltammetry method was applied in order to establish the oxidation and the reduction potentials in view of their correlation with the antioxidant activity of anthocyans [9–11]. The cyclic voltammetry method was used to outline certain antioxidant properties of anthocyans and the stability of the electroactive forms in different electrolytic media. These are based on a NaCl 0.5 M support electrolyte, the electroactive species represented by a hydro-ethanolic anthocyan solution with a concentration of magnitude order 10^{-4} ML^{-1}. The cyclic voltammetry method was applied by using a PGSTAT Voltalab, Master 4. The

Table 1. Spectral data of solutions of Pelargonidin 3-O-Glucoside in solvents with different polarities

	Ternary system				
Nr.	Ethanol ($HCL_{(aq)}$ 1%) (% vol.)	DMF (% vol.)	Pelargonidin-3-O-Glucoside (mg/mL)	A	λ (nm)
S1	0	100	0.01	0.01	509
S2	20	80	0.01	0.05	508
S3	40	60	0.01	0.18	506
S4	60	40	0.01	0.26	505
S5	80	20	0.01	0.34	504
S6	100	0	0.01	0.48	502

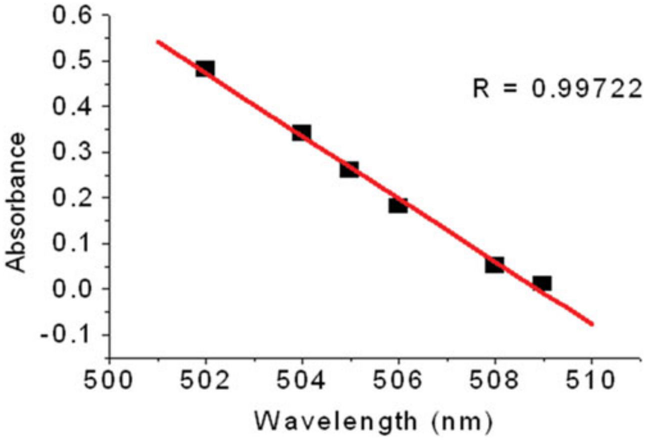

Figure 5. Bathochromic shift observed while decreasing the solvent polarity Pelargonidin-3-O-Glucoside (0.01 mg/mL) in solvents.

work electrode used was a carbon electrode, the reference electrode was Ag/AgCl and the auxiliary electrode was Pt. The potential domain ranging between −400 mV and 800 mV, at a scan rate of 10 mV/s. We obtained voltammograms for: Pelargonidin-3-O-Glucoside, Cyanidin-3-O-Glucoside, Definidin-3-O-Glucoside, Peonidin-3-O-Glucoside, Malvidin-3-O-Glucoside, with the same concentrations $c = 2 \times 10^{-4}$ ML^{-1}, in NaCl 0.5 ML^{-1} as support electrolyte (Fig. 6).

The electrochemical data obtained by applying the cyclic voltammetry method are presented in Table 2. The cyclic voltammetry measurements generally outlined two waves: the first wave quasireversible around the value of 312 mV and the second is an irreversible oxidation wave at 588 mV. These were used for establishing the oxidation potentials in the spectro-electrochemical measurements.

Table 2. Electrochemical data from cyclic voltammograms of redox processes, for Pelargonidin-3-O-Glucoside, Malvidin-3-O-Glucoside, Cyanidin-3-O-Glucoside, Delphinidin-3-O-Glucoside, Peonidin-3-O-Glucoside, Petunidin-3-O-Glucoside

Redox process potential	1 Epc1 (mV)	2 Epa1 (mV)	ΔE1 (mV)	Epc2 (mV)	Epa2 (mV)	ΔE2 (mV)
Pelargonidin-3-O-Glucoside	268	312	54	537	588	51
Malvidin-3-O-Glucoside	112	172	60	500	559	59
Cyanidin-3-O-Glucoside	276	302	36	529	589	60
Delphinidin-3-O-Glucoside	192	236	44	571	603	32
Peonidin-3-O-Glucoside	250	318	68	530	561	31
Petunidin-3-O-Glucoside	180	208	28	554	561	7

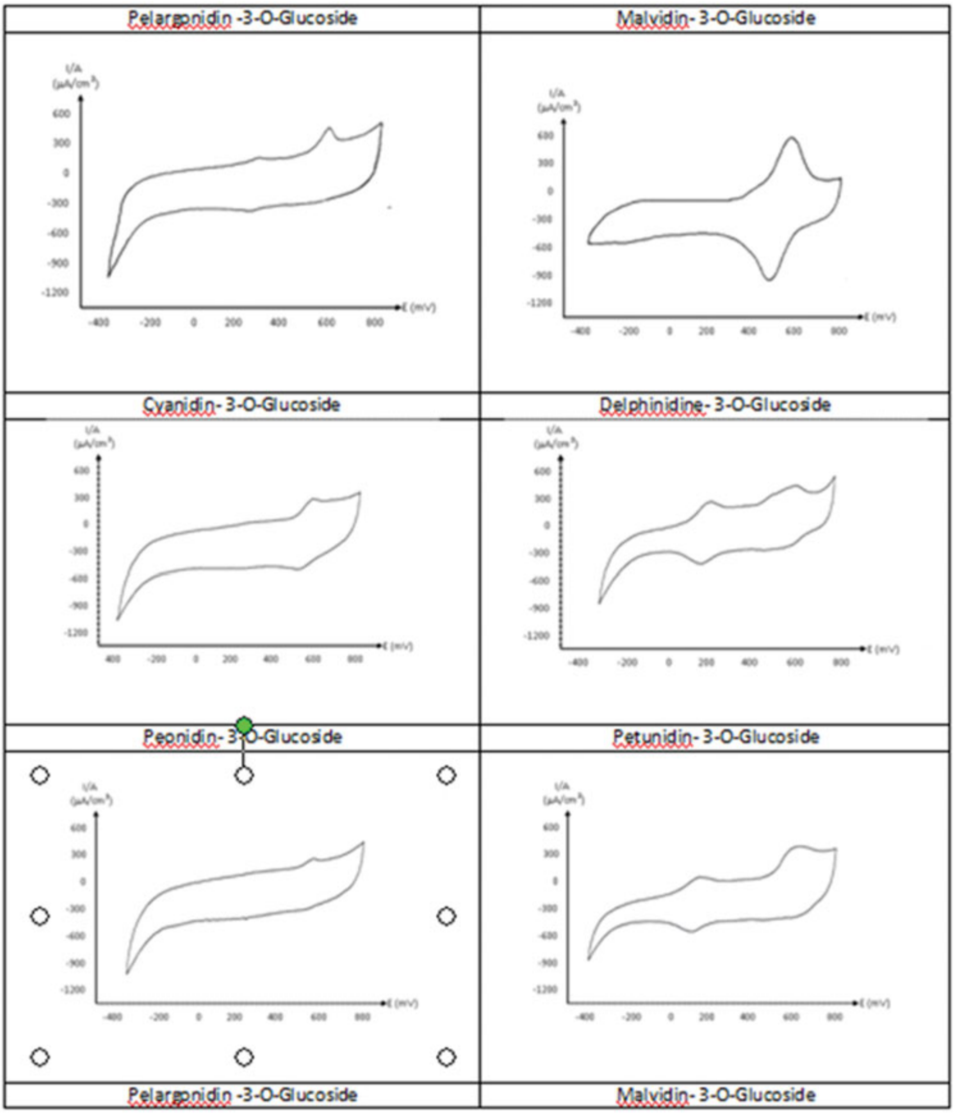

Figure 6. Cyclic voltammograms for the main natural anthocyans chromographically separated.

Spectro-Electrochemical Measurements

These measurements aimed at assessing the ECE steps in the redox mechanism by identifying the shifts in the molecular absorption spectra. For this purpose, the absorption spectra were recorded successively, at the same time by imposing a potential identical with that recorded in the cycle voltammetry determination. Optically Transparent Thin Layer Electrochemical (OTTLE) cell was used. The successive spectra for Pelargonidin-3-O-Glucoside, were recorded in two versions: in unbuffered solution and in buffered solution

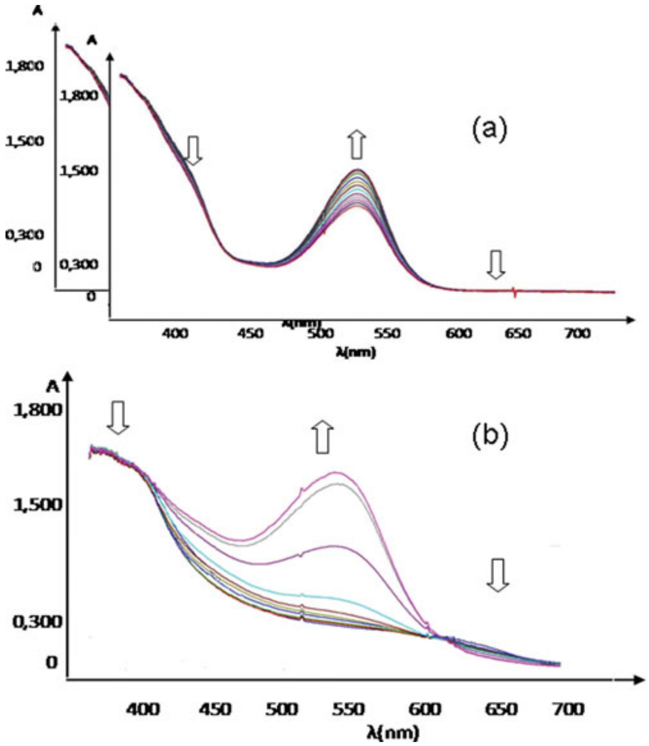

Figure 7. The spectro-electrochemistry of Pelargonidin-3-O-Glucoside 2×10^{-4} ML^{-1}, in NaCl 0.5 ML^{-1} solution, at the second oxidation wave potential (588 mV): (a) unbuffered solution; (b) in buffer solution pH = 3.

(0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH titrated to pH = 3 with 0.2 M NaOH- Britton Robinson buffer) (Fig. 7(a), 7(b)).

If the spectro-electrochemical measurements are performed without the pH control, as the chemical steps are influenced by the hydronium ion, the presence of the anion radical will be relatively difficult to be observed (Fig. 7a). In the presence of a buffer with pH 3, the formation of the anion radical is obvious and it is more stable. While applying the oxidation potential, significance changes appear in the successive spectra in the sense of increase in the absorbance on the interval 425 nm and 625 nm, between the two isosbestic points. These spectral changes are linked with the transformation of the flavilium ion into hemiketal or chalcones species [19]. The absorption band located at 518 nm is attributed to formation of an $A^{-\bullet}$ anion radical following the process presented by Fig. 8.

In this scheme AH2 represents the nondissociated molecule of Pelargonidin-3-Glucoside. In the chemical process of dissociation (mechanism C) the hydrogen of hydroxyl groups from positions 4′ and 7 is successively given up to form conjugated bases represented by AH^- and A^{2-}. In the electrochemical process (mechanism E) $A^{-\bullet}$ anion radical is formed, outlined by the successive spectra recorded during electrolysis at constant potential. The final compound A represents the oxidated form resulted following the oxidation of the anion radical (mechanism E).

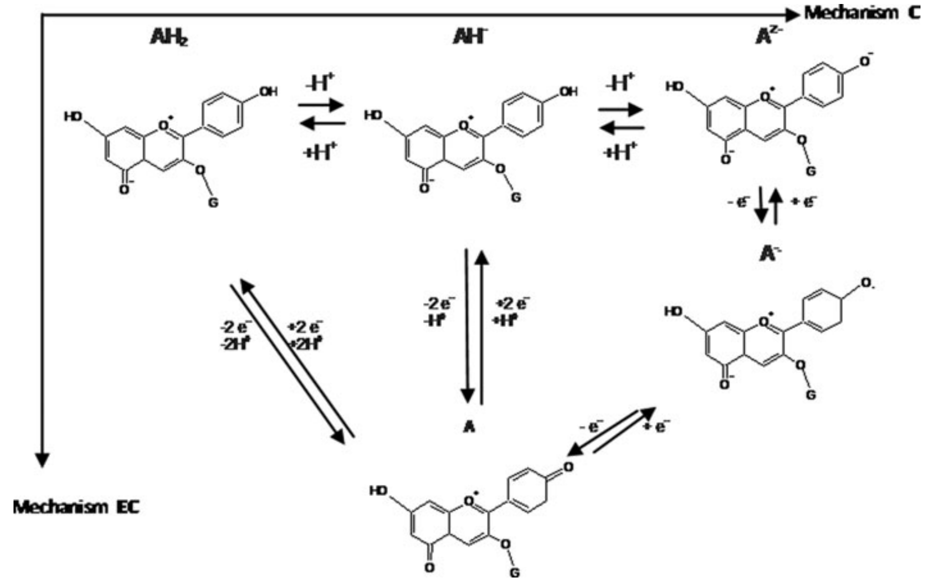

Figure 8. Representation of the chemical and electrochemical steps for the redox mechanism of Pelargonidin-3-Glucoside in the field of potential −400 mV −800 mV.

Conclusions

This paper presents interesting results outlining the spectral and electrochemical properties of anthocyans obtained from natural extracts. These properties refer to the influence of the solvent's polarity and of the pH on the stability of anthocyans solutions. From the cyclic voltammograms resulted the pH influence over the oxidation potential. The presence of various equilibrium and redox forms in solutions at different pH of Pelargonidin-3-O-Glucoside is demonstrated.

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