

1 **THEORETICAL DETERMINATION OF THE ELECTRODE POTENTIAL OF**
2 **CYANIDIN IN AQUEOUS SOLUTION**

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14
15 **Abstract**

16 The electrode potential of cyanidin was computed by means of *ab initio* methods, at HF/6-
17 311+G(d,p) level of theory. An isodesmic reaction scheme that uses the p-quinone/hydroquinone
18 couple as referenc molecules has been employed. Geometric parameters of the six more stable
19 conformers of cyanidin are computed, as well as properties like atomic charges and contribution
20 to the HOMO (Highest Occupied Molecular Orbital) energies of each hydroxyl group of the
21 cyanidin.

22 **Key words:** cyanidin, electrode potential, *ab initio* methods, atomic charges, antioxidant

23 **Running title:** ELECTRODE POTENTIAL OF CYANIDIN

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28 **Introduction**

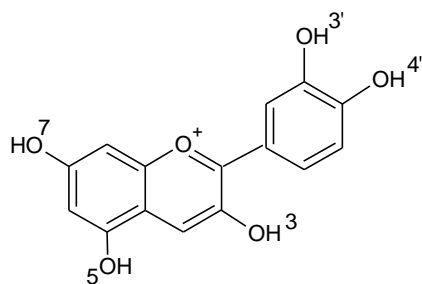
29 During the last years, antioxidants have gained an increased importance due to their large number
30 of applications in the pharmaceutical and medical field [1]. They were found to have a positive
31 role as adjuvants in treatment of diabetes [2] and cardiovascular diseases [3].

32 Among the various type of antioxidants, anthocyanidins are one of the polyphenolic derivatives
33 with highest antioxidant activity. There have been reported a number of studies that outlines the
34 enhanced antioxidant activity of the anthocyanidins both by experimental [4-7] and theoretical
35 [8,9] methods.

36 This way, the study of redox properties of a compound is a good possibility for predicting their
37 behavior in the more complex biological systems [10]. Also, calculation of electrode potential
38 with a high degree of accuracy may lead to valuable information regarding the nature of redox
39 reactions [10].

40 A literature survey regarding the theoretical determination of redox properties outlines a number
41 of studies within this field. Namazian et al. [11] have reported the computation of electrode
42 potential of a coumestan derivative, as well as a study regarding the electrode potential of
43 quinines with an accuracy of 0.03 V [12]. Tsutsui and Sakamoto [13] reported the correlation of
44 experimental electrode potentials for a series of silyl-substituted 1,4-benzoquinones with
45 theoretical calculations of LUMO energy levels. Namazian and Coote [14] have calculated the
46 absolute redox potential of rutin both by experimental and computational methods. Also, the
47 theoretical and experimental determination of oxidation potentials of dihydroxy-anthracene
48 thioxanthene derivatives [15] and the electrode potentials for substituted 1,2-dihydroxybenzenes
49 in aqueous solution [16] have been performed.

50 The present study deals with the evaluation, by theoretical methods, of the electrode potential of
51 cyanidin. Our research group has already reported a number of studies regarding the antioxidant
52 activity of various vegetal extracts [17], as well as a theoretical study regarding the evaluation of
53 the antioxidant activity of each OH group (by computation of BDE – Bond Dissociation Enthalpy
54 index) [18]. It is well known that that the antioxidant properties of cyanidin are given to the
55 presence of the five hydroxyl groups that are present both on the benzopyrilium and phenyl rings.



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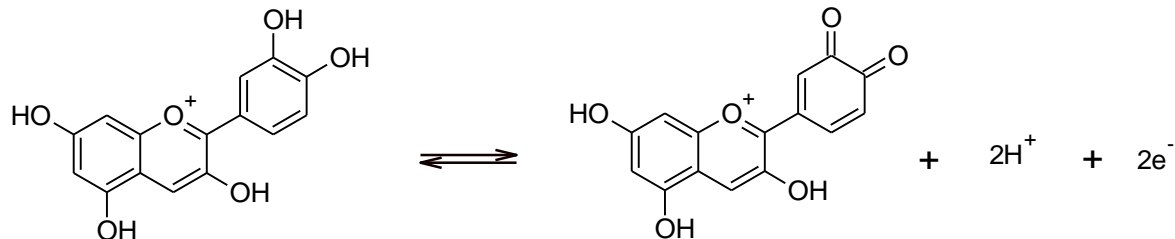
Figure 1. The structure of cyanidin

58 According to literature data, the highest antioxidant character is attributed to the OH groups in o-
 59 position on the phenyl ring (namely 3'-OH and 4'-OH), followed by the 3-OH group and, finally
 60 5-OH and 7-OH groups [19].

61 Within the present study, the calculation of the electrode potential by means of an isodesmic
 62 reaction scheme has been performed. The hypothetical reaction is the following:



64 Due to the fact that the most reactive groups are 3'-OH and 4'-OH, the oxidation product of
 65 cyanidin is considered the following o-quinone derivative:



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Scheme 1. Oxidation mechanism of cyanidin

68 As reference molecules, the p-quinone/hydroquinone couple ($E^0 = 0.711V$) has been chosen.

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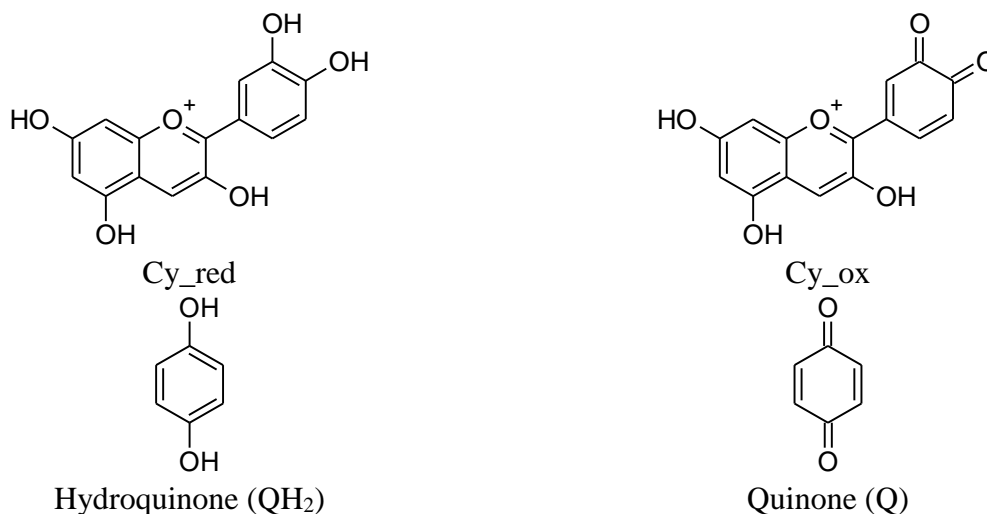
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78 All the investigated species are given in figure below:

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80 **Figure 2.** Reagents and products of the isodesmic reaction employed for the computation of
81 cyanidin electrode potential

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83 Methodology

84 The conformational analysis of cyanidin has been performed, and the most six stable conformers
85 have been chosen. The reduced and oxidized species have been optimized, no imaginary
86 frequencies being obtained. All the computations have been performed at HF/6-311+G(d,p) level
87 of theory, using gas-phase conditions, as well as in aqueous environment. For the solvent-phase
88 computations, the Polarizable Continuum Model has been chosen (namely IEF-PCM, integral
89 equation formalism polarizable continuum model, where the solute is characterized by the
90 electron density) [20].

91 The electrode potential of cyanidin (E^0) was computed was computed by means of equations (1)-
92 (4):

$$93 \quad \Delta G_T = -nF(E^0 - E_{Q/QH_2}^0) \quad (1)$$

$$94 \quad \Delta G_T = \Delta G_{gas} + \Delta \Delta G_{sol} \quad (2)$$

$$95 \quad \Delta G_{gas} = G_{gas}(products) - G_{gas}(reagents) \quad (3)$$

$$96 \quad \Delta \Delta G_{sol} = \Delta G_{sol}(products) - \Delta G_{sol}(reagents) \quad (4)$$

97

98 For the computations of Gibbs free energies, ZPE (Zero Point Energy) and thermal corrections
99 were taken into account. Gaussian 09W software [21] has been employed throughout the
100 calculations of the present study.

101 The equations (5)-(7) have been employed for the computations of the electronic properties of the
102 six conformers of cyanidin, namely chemical potential (μ), hardness (η) and electrophilicity index
103 (ω) [22]:

$$104 \quad \mu = (E_{\text{HOMO}} + E_{\text{LUMO}})/2 \quad (5)$$

$$105 \quad \eta = (E_{\text{LUMO}} - E_{\text{HOMO}})/2 \quad (6)$$

$$106 \quad \omega = \mu^2/2\eta \quad (7)$$

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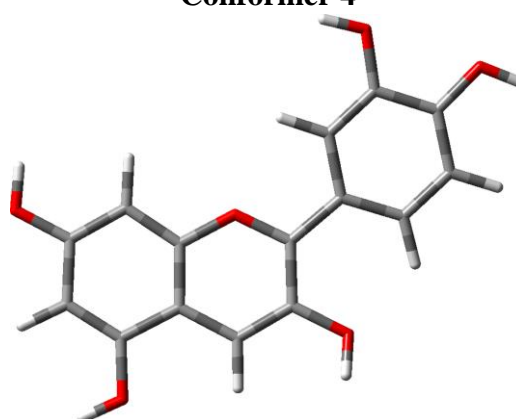
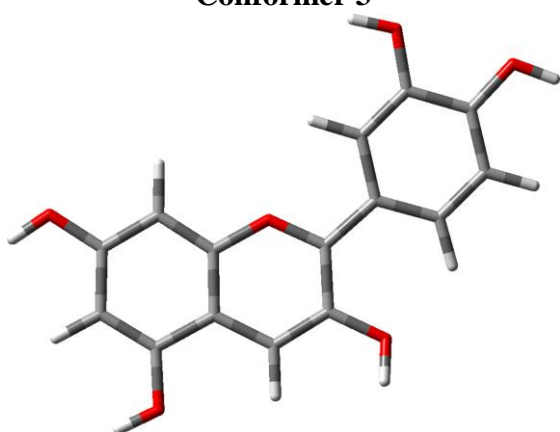
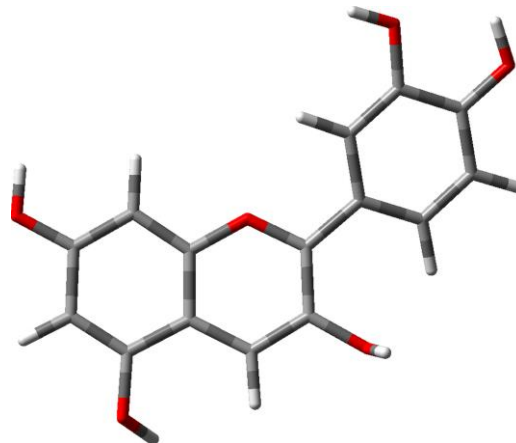
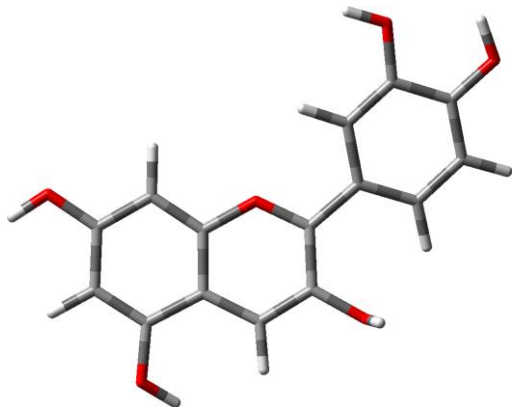
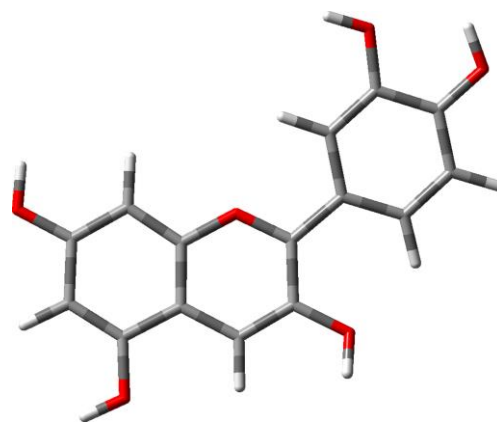
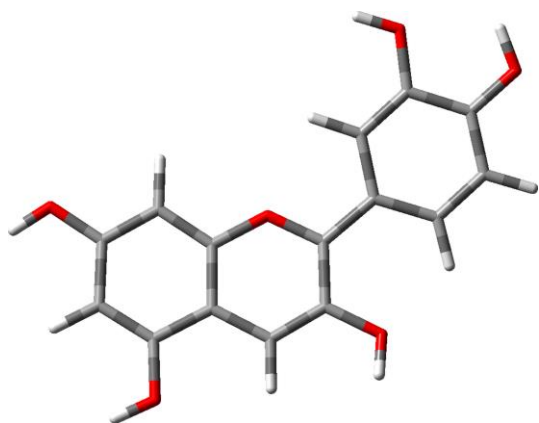
108 **Results and discussion**

109 **a. Geometry details**

110 The structure of cyanidin consists in a planar construction of a benzopyrylium ring that has a
111 phenyl moiety in position 2. All the compounds within the anthocyanidins class have a variable
112 number of hydroxyl and methoxy groups; in the specific case of cyanidin, there are five hydroxyl
113 groups distributed in positions 3, 5, 7, 3' and 4'. This leads to the possibility of a large number of
114 conformers, as a function of the position of the O-H bonds towards the corresponding rings. As a
115 result, after performing a conformational analysis of the cyanidin, six more stable conformers
116 were chosen for the present study. Along with the results for the most stable conformer, the same
117 computations were performed for the other 5 structures, in order to evaluate the influence of the
118 O-H bond orientation on the properties of each conformer.

119 The optimized geometries of the 6 investigated structures are depicted in figure below:

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Figure 1. Structures investigated within the present study

125 **Table 1.** Total energy, dipole moment and HOMO energy of the investigated structures

Compound	Energy / a.u.	Dipole moment / D	E_{HOMO} / a.u.
Conformer 1	-1023.645985	6.846	-0.412464
Conformer 2	-1023.646411	4.640	-0.413827
Conformer 3	-1023.637457	4.160	-0.421845
Conformer 4	-1023.636135	2.217	-0.422812
Conformer 5	-1023.638734	7.170	-0.411516
Conformer 6	-1023.639350	4.497	-0.412631

126
 127 According to these results, the most stable structure is the **conformer 2**. The geometric
 128 parameters of the six conformers are given in Table 2, and show that the two structures (namely 3
 129 and 4) where the ³OH groups are non-coplanar with the phenyl) chromenylium ring are the least
 130 stable ones.

131 **Table 2.** Geometric parameters of the O-H groups

Compound	<i>Dihedral angle / °</i>				
	C3'-C4'-O4'-H	C4'-C3'-O3'-H	C4-C3-O3-H	C6-C5-O5-H	C6-C7-O7-H
Conformer 1	-0.054	-178.360	-15.470	0.211	-0.170
Conformer 2	0.190	-175.388	-20.576	0.240	-179.730
Conformer 3	-0.216	178.673	-95.912	158.185	0.502
Conformer 4	0.059	-176.179	-95.110	-23.566	-178.909
Conformer 5	179.968	-179.891	-17.256	0.193	-0.135
Conformer 6	179.984	-179.680	-18.625	0.187	-179.898

132
 133 Due to the fact that the antioxidant character is strongly correlated with the ability of donating
 134 electrons, higher HOMO energies outline an increased antioxidant character. Total HOMO
 135 energies are depicted in Table 1 and show only insignificant differences among the six
 136 compounds; instead, computations of the contribution to the HOMO energy of each group
 137 suggest that ⁴OH, ³OH and ³OH have a more pronounced antioxidant character. There are
 138 noticed the lower values obtained for the contributions of the ³OH and ⁵OH groups of the non-
 139 planar conformers 3 and 4.

140 **Table 3.** Contributions to E_{HOMO} / a.u.

Compound	³OH	⁴OH	³OH	⁵OH	⁷OH
Conformer 1	0.267	0.348	0.325	0.168	0.243
Conformer 2	0.237	0.339	0.295	0.149	0.210
Conformer 3	0.273	0.345	0.191	0.114	0.221
Conformer 4	0.275	0.335	0.184	0.077	0.215
Conformer 5	0.298	0.350	0.296	0.159	0.230
Conformer 6	0.292	0.347	0.285	0.137	0.224

141 An experimental study reported by de Lima [19] showed that the OH groups of the phenyl ring
 142 are the first that undergo oxidation, followed by the ³OH group. The least reactive are the OH
 143 groups ⁵OH and ⁷OH. This appears to be in good agreement with the results presented in Table
 144 3. The same study reports a value of 564 mV for the first oxidation peak (corresponding to the
 145 oxidation mechanism that we have presented in Scheme 1).

146 The results of the global electronic parameters of the investigated structures are listed in Table 4
 147 and show no significant differences among the six conformers:

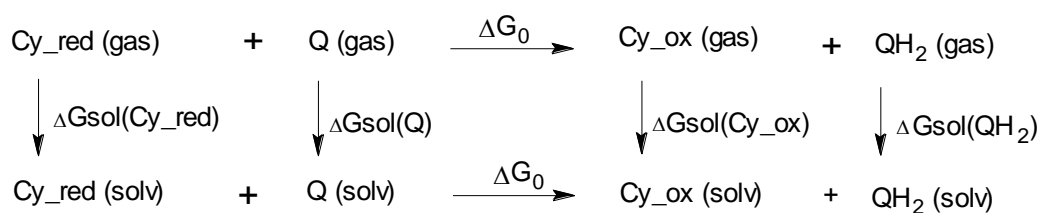
148 **Table 4.** Chemical potential (μ), hardness (η) and electrophilicity (ω) of cyanidin

Compound	μ / eV	η / eV	ω / eV
Conformer 1	-7.31	3.91	6.83
Conformer 2	-7.33	3.92	6.86
Conformer 3	-7.50	3.97	7.09
Conformer 4	-7.52	3.98	7.11
Conformer 5	-7.29	3.90	6.82
Conformer 6	-7.32	3.91	6.85

149

150 b. Electrode potential computations

151 In order to estimate the theoretical potential electrode of cyanidin, the following thermodynamic
 152 cycle has been employed:



153

154 **Scheme 1.** Thermodynamic cycle employed for the computation of electrode potential of
 155 cyanidin

156 The Gibbs free energies for the oxidized and reduced forms of cyanidin, as well as for
 157 quinone/hydroquinone couple are given in Table 5. The electrode potential was computed by
 158 using the equations (1)-(4); according to Scheme 1, there is a change of two electrons ($n = 2$).
 159 The value of Faraday constant that was employed throughout the study is $F = 96,485$ kJ/mol ($F =$
 160 $23,061$ kcal/mol), and the electrode potential for the quinone/hydroquinone couple is $0,711$ V.

161

162 **Table 5.** Values of Gibbs free energies (including thermal corrections and ZPEs (Zero Point
 163 Energies)

Compound	Gas-phase energies / a.u.	Aqueous energies / a.u.
Cy_red 1	-1023.185602	-1023.262942
Cy_red 2	-1023.185484	-1023.263143
Cy_red 3	-1023.176890	-1023.256810
Cy_red 4	-1023.176658	-1023.256874
Cy_red 5	-1023.178122	-1023.259674
Cy_red 6	-1023.178254	-1023.260038
Cy_ox 1	-1022.003135	-1022.099094
Cy_ox 2	-1022.003437	-1022.100028
Cy_ox 3	-1021.993969	-1022.092122
Cy_ox 4	-1022.003437	-1022.100028
Cy_ox 5	-1022.003135	-1022.099091
Cy_ox 6	-1022.003437	-1022.100028
Quinone (Q)	-379.177939	-379.189273
Hydroquinone (QH ₂)	-380.318435	-380.330543

164
 165 The results obtained for the electrode potential of all the six conformers are listed in Table 6:

166 **Table 6.** Electrode potential of the conformers of cyanidin (HF/6-311+G(d,p))

Compound	Electrode potential / V
Conformer 1	0.420
Conformer 2	0.414
Conformer 3	0.393
Conformer 4	0.490
Conformer 5	0.450
Conformer 6	0.460

167
 168 A value of 0.414 V was determined for the electrode potential of the most stable cyanidin
 169 conformer. The results obtained for the other structures are within the range 0,393 V – 0,490 V;
 170 the differences are believed to be due to the values of solvation energies of the oxidized and
 171 reduced form of cyanidin. Smaller differences between the absolute values of changes in free
 172 energies of reaction (1) (both in gas-phase and aqueous conditions) led to higher values of the
 173 electrode potential (as the case of conformers **4-6**).

174 Undervaluation of the solvation energies of cyanidin when PCM computations are employed (due
 175 to the neglecting of the hydrogen bonds establish by the hydroxyl groups with the aqueous
 176 environment) is a possible explanation for the lower value of electrode potential (when
 177 compared to the experimental results).

178 **Conclusions**

179 The electrode potential of six conformers of cyanidin in aqueous solution has been calculated by
180 means of an isodesmic reaction scheme, at HF/6-311+G(d,p) level of theory. The data range is
181 0,393 V – 0,490 V, an electrode potential of 0,414 V being obtained for the most stable
182 conformer. Comparison with experimental results leads to a calculation error of 0,1 V, which is
183 attributed to the undervaluing of the free energies computed within the PCM model.

184 As regards the electronic properties of the six conformers, global parameters like electrophilicity,
185 chemical potential and hardness are sparingly influenced by the geometry of the molecules.
186 Instead, local parameters like contribution to EHOMO or the atomic charges are dependent by the
187 planarity of the OH groups with the phenyl-benzopyrylium skeleton.

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