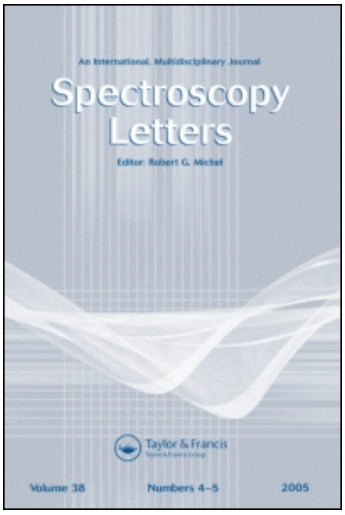


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Spectroscopic and Theoretical Study of Cyanidin-Aluminum (III) Complexes

Jasmina M. Dimitrić Marković[a]; Dragan S. Veselinović[a]; Jelisaveta M. Baranac[a]; Tanja P. Brdarić[a]
[a] Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia and Montenegro

To cite this Article Marković, Jasmina M. Dimitrić , Veselinović, Dragan S. , Baranac, Jelisaveta M. and Brdarić, Tanja P.(2008) 'Spectroscopic and Theoretical Study of Cyanidin-Aluminum (III) Complexes', Spectroscopy Letters, 41: 3, 104 — 115
To link to this Article: DOI: 10.1080/00387010701799704
URL: http://dx.doi.org/10.1080/00387010701799704

Spectroscopy Letters, 41:104–115, 2008

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010701799704

Taylor & Francis
Taylor & Francis Group

Spectroscopic and Theoretical Study of Cyanidin–Aluminum (III) Complexes

Jasmina M. Dimitrić Marković, Dragan S. Veselinović, Jelisaveta M. Baranac, and Tanja P. Brdarić
Faculty of Physical Chemistry, University of Belgrade, Belgrade, Serbia and Montenegro

ABSTRACT Complexation of aluminum (III) with cyanidin, a natural anthocyanidin molecule, has been investigated in methanol and buffered solutions of pH 3.0 and 4.0. Electronic absorption spectroscopy was performed to characterize the stoichiometry and stability of the complexes formed. In investigated solvents, aluminum bonded moderately to cyanidin requiring large mole ratios of the components (up to 200) for the access of complexation. Molar ratio plots showed the formation of only one complex with stoichiometry aluminum (III):cyanidin of 1:1 in both investigated media. Semiempirical calculations, performed in the Austin Model 1 parameterization, enabled the determination of the structural features of free compounds as well as complex structural modifications caused by chelation of Al(III).

KEYWORDS aluminum (III), complex formation, cyanidin, electronic spectra, semiempirical calculations, stability constants, stoichiometry

INTRODUCTION

Because of the remarkable array of biologic and pharmacologic activities, physiologic effects, possible industrial applications, and constantly increasing commercial interest, flavonoids, especially anthocyanins and flavones, have attracted the attention of many researchers during the past decade.[1–7]

Together with chlorophyll and carotenoids, flavonoids represent the main pigments in the plant world. These molecules are aromatic secondary plant metabolites, which belong to the class of plant polyphenolics. Structurally they are heterocyclic π-electron systems built upon a C_6H_5 (A)–C_3–C_6H_5 (B) flavone skeleton in which oxygen is the heteroatom. A group of flavonoids is differentiated in several classes according to the degrees of oxidation and unsaturation of the heterocyclic C ring.[8]

Anthocyanidins and their glycosylated forms, anthocyanins, represent one of the most important and the most widely spread classes of the flavonoids. These pigments as the most intensively colored are responsible for the existence of most red, blue, and purple colors in flowers and fruits.[9–12] The natural environments of anthocyanin pigments are cell vacuoles that are essentially aqueous, slightly acidic, or neutral.[8,13] Except in strongly acidic medium, which is not

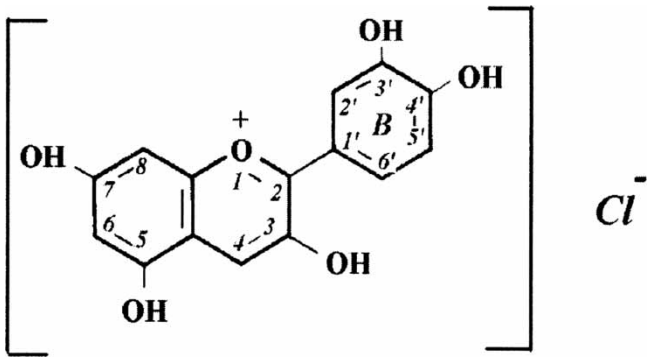
Received 5 March 2007; accepted 19 October 2007.

Address correspondence to Jasmina M. Dimitrić Marković, Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia and Montenegro. E-mail: markovich@ffh.bg.ac.yu

characteristic to the natural environment, the flavylium chromophore, responsible for the color of these molecules, is highly unstable and poorly colored.[13] The stability of the structure of these molecules *in vivo*, conditioning the stability of their color, is partially improved by glycosidation and acylation but also very dependent upon possible metal complexation, copigmentation, and self-association reactions.[8,9,13–21]

Metal complexation reactions play important and multiple roles in biologic systems. They are a very sensitive and powerful color stabilization mechanism developed in higher plants.[22–26] Accumulation of metals in peripheral tissues, enabled by these reactions, reduces the possibility of their migration to ecosystems[4,5] and also protects plants from pathogens and plant eaters. By chelating metal ions, anthocyanins, flavonoids generally, prevent the metal-mediated generation of free radicals and accordingly may protect the very important biologicly active molecules from oxidative stress.[27] Metal complexation reactions are also very often used for colorimetric purposes in the detection of metal traces in solutions.

Anthocyanidins and anthocyanins possess one or more structural features that can be involved in complexation reactions with metal ions. The current paper presents *in vitro* study of the cyanidin–aluminum (III) complex formation in acidic aqueous buffered solutions, pH 3.0 and pH 4.0, and in methanol. Cyanidin (Scheme 1) is, in terms of frequency and abundance, the most common anthocyanidin in nature,[28] which hydroxyl functions enable complex formation with trivalent aluminum. Cyanidin is used as a model compound because its aglycon form does not exist in nature. Using the aglycon form of cyanidin, apart from glyco and acyl moieties, the complexity of the complex structure could be reduced and consequently the semiempirical calculations much facilitated.



SCHEME 1 Structural Formula of Cyanidin.

The aim was to establish the possibility of compelexing in acidic medium to determine the stoichiometric composition and the stability of the complexes formed, as well as structural modifications caused by chelation of aluminum (III) ions.

MATERIALS AND METHODS

Materials

Cyanidin chloride (Pfaltz and Bauer, USA), $AlCl_3 \cdot 6H_2O$ (Fluka, Switzerland), acetic acid (Merck, USA), sodium chloride (p.a.; Merck, USA), sodium hydroxide (Merck, USA), methanol (Uvasol; Merck, USA), and hydrochloric acid (Merck, USA) were used. The aluminum chloride hexahydrate was used as received, and the purity of cyanidin was checked chromatographically according to the literature data.[29]

Solutions

Acetate buffered solutions pH 3.0 and pH 4.0 of constant ionic strength, adjusted by sodium chloride (5×10^{-1} moldm^{-3}), were used. The solutions were obtained by mixing acetic acid ($c = 5 \times 10^{-2}$ moldm^{-3}) and sodium hydroxide ($c = 1.5$ moldm^{-3}). Stock solution of cyanidin, $c = 1 \times 10^{-3}$ moldm^{-3}, prepared in methanol with the addition of one drop of 0.1% HCl, was left to equilibrate in the dark for 1 h. This solution was diluted to the original concentrations ($c = 5 \times 10^{-5}$ moldm^{-3}) by addition of the buffers and methanol. During all the measurements, the cyanidin concentration was kept constant. The stock solutions of the aluminum chloride hexahydrate, $c = 1 \times 10^{-2}$ moldm^{-3}, prepared in corresponding buffers and in methanol, were diluted to fit different metal:pigment mole ratios.

Electronic Spectra

The complexation of cyanidin and aluminum was studied by spectroscopic measurements. Electronic spectra of free and complexed cyanidin in different media were recorded on a Cintra GB-10 UV-Vis spectrophotometer. Quartz cuvettes (Puy Unicam, England) of 10-mm optical pathlength were used. Each spectroscopic measurement was repeated three times.

pH Measurements

An Iskra MA 5730 (Kranj, Slovenia) pH meter with a Sentek (Essex, England) combined electrode was used for the pH measurements.

Theoretical

The stability constant values and the composition of the complexes formed were obtained by molar ratio method and by Nash method.[30] Jobb's method of continual variations could not be used because of the large molar ratios required for monitoring the reaction. Because the ligand molecule, cyanidin, absorbs in the same region as the complex formed, absorbance measurements in each applied method were performed away from the wavelength of absorption maximum (at $\lambda_{pH3} = 567$ nm, $\lambda_{pH4} = 563$ nm, and $\lambda_{MeOH} = 581$ nm), where the differences between absorbance values were the biggest.

Semiempirical Calculations

Taking into account the size of the molecules under investigation, the corresponding computer effort, and numerous literature data,[31–38] it is possible to conclude that among all semiempirical methods, the AM1 (Austin Model 1) method[39] is the most appropriate to determine the structure of the flavonoid compounds and to reproduce experimental data, particularly electronic or vibrational spectra. The geometry optimization has been performed at the restricted HF AM1 level[39] and implemented in the Hyperchem program (version 7.5).

RESULTS AND DISCUSSION

The obtained spectroscopic results confirm the complexation of cyanidin in methanol and also in acidic environment (pH 3.0 and pH 4.0), which generally is not the most favorable one.

Chosen pH values are close to an average, physiologic, pH value of the plant tissue[40,41] so the obtained results could give better insight into the processes of biological relevance *in vivo*. The pH interval in which the reaction was monitored was limited due to some reasons. The pH values below pH 3.0 were not investigated because they are not characteristic to natural media.[40,41] On the other hand, complexation reaction could not be monitored at higher pH values because of the strong hydrolytic properties of aluminum ion, which, at the concentrations used in the experiment, precipitated due to the formation of various types of aluminum hydroxo and acetate complexes.[42]

Aluminum (III) is an octahedrally hexacoordinated ion that can bind one, two, or three molecules of bidentate ligand with the formation of 1:1, 1:2, and 1:3 complexes, respectively. In the investigated systems, it bonded moderately to cyanidin requiring large mole ratios of the components, especially in the pH 3.0 solution, for the access of complexation. In the visible range of the spectra, free cyanidin exhibits an absorption band of cationic transformation form positioned at $\lambda_{max(pH3.0)} = 516$ nm (Fig. 1, curve 0) and $\lambda_{max(pH4.0)} = 518$ nm (Fig. 2, curve 0). The addition of aluminum to the buffered solutions results in the appearance of new, batochromically shifted bands that can be attributed to the metal complexes formed. Figures 1 and 2 present some of the spectra of the complexes formed at different aluminum–cyanidin mole ratios. The spectra cross clear isosbestic points ($\lambda^{is} = 519$ nm, Fig. 2; and $\lambda^{is} = 516$ nm, Fig. 3), which in both cases indicate fairly simple flavylium cation–aluminum equilibrium that involves only two species: free and complexed cyanidin molecule. By the positions of the new bands ($\lambda_{max(pH3.0)} = 551$ nm and $\lambda_{max(pH4.0)} = 553$ nm) and from the very well-known[13] complex structural equilibrium, which is generally established between colored (flavylium cation and anhydrobase) and colorless (pseudobase and chalcone) transformation forms, it is possible to conclude that these bands correspond with some of the anhydrobase transformation forms of cyanidin molecule. Because these pigment transformation forms are not characteristic to acidic environment, it is quite possible to presume the ability of the small, hard, charged aluminum (III) ion to deprotonate flavylium chromophore even in acidic buffered solutions and make the molecule adopt some of its anhydrobase forms that are normally characteristic to higher pH values. The earlier investigations[15] assumed that the anthocyanidin (anthocyanin) molecules preferentially enter complexation reactions not in acidic but in neutral or weekly alkaline aqueous solutions in which they adopt some of the anhydrobase structural forms.

To be able to compare the obtained results, the complexation was also investigated in methanol, which is well-known as a good complexing medium. In

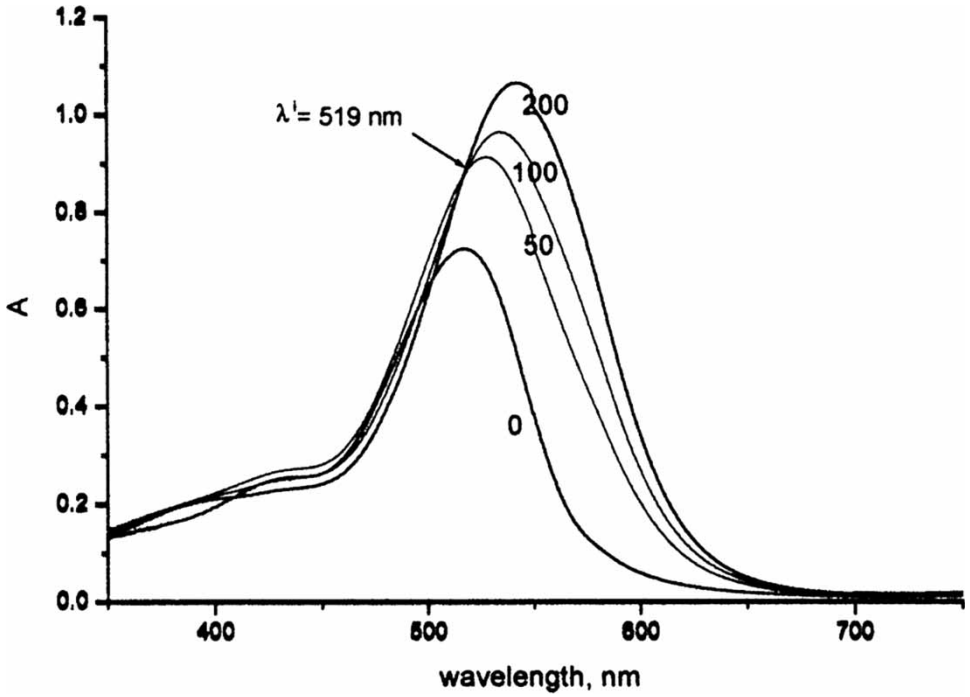


FIGURE 1 Electronic Spectra of Equilibrated Solutions of Cyanidin ($c = 4 \times 10^{-5}$ moldm^{-3}, $I = 5 \times 10^{-1}$ moldm^{-3}, $T = 20$°C) in pH 3.0 Buffer at Different Aluminum–Cyanidin Mole Ratios (Indicated on Spectra by numerical values).

methanol, complex formation also results in spectral modifications with the appearance of a new band batochromically shifted. Figure 3 presents electronic absorption spectra of equilibrated cyanidin–aluminum (III) solutions in methanol. The isosbestic point at $\lambda^{is} = 547$ nm also indicates simple equilibrium in the system.

The complex formation between cyanidin and aluminum (III) proceeds most probably by proton detachment from the cyanidin molecule and can be

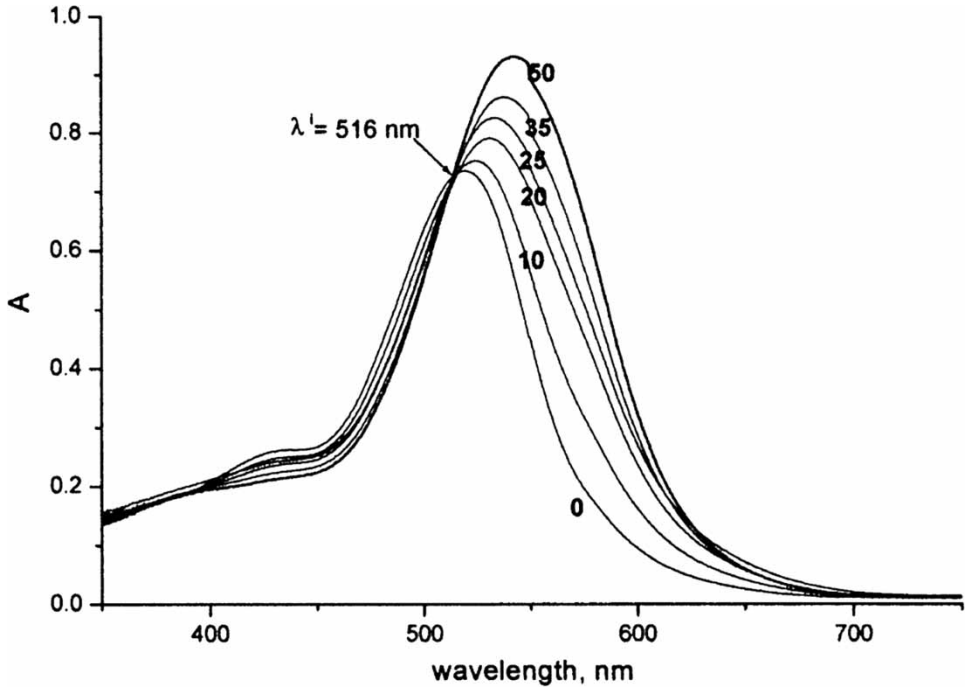


FIGURE 2 Electronic Spectra of Equilibrated Solutions of Cyanidin ($c = 5 \times 10^{-5}$ mdm^{-3}, $I = 5 \times 10^{-1}$ moldm^{-3}, $T = 20$°C) in pH 4.0 Buffer at Different Aluminum–Cyanidin Mole Ratios (Indicated on Spectra by numerical values).

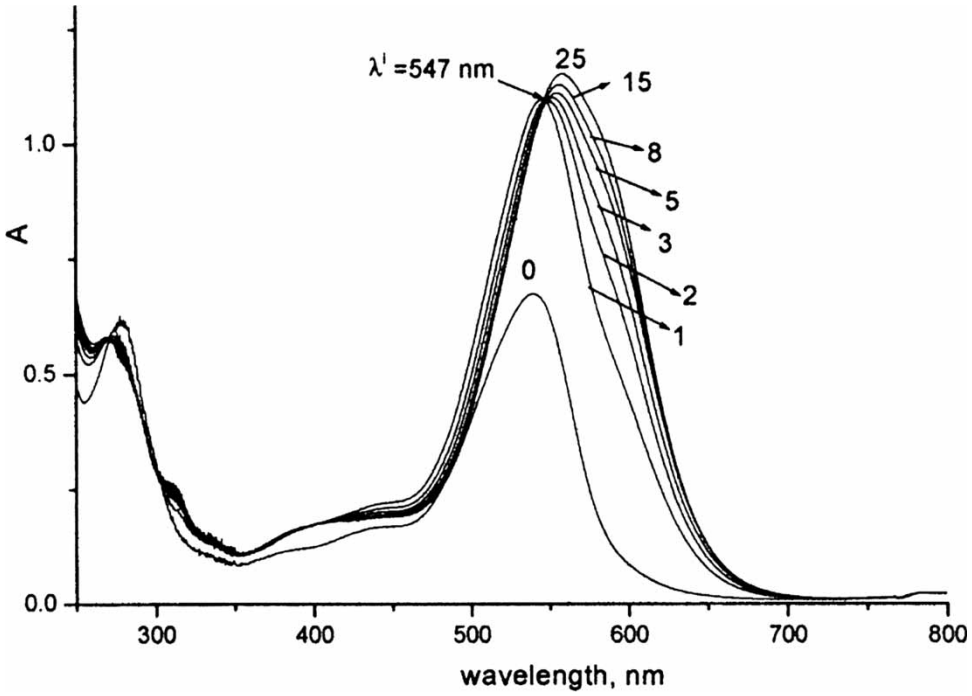


FIGURE 3 Electronic Spectra of Equilibrated Solutions of Cyanidin ($c = 5 \times 10^{-5}$ mdm^{-3}, $I = 5 \times 10^{-1}$ moldm^{-3}, $T = 20$°C) in Methanol at Different Aluminum–Cyanidin Mole Ratios (Indicated on Spectra by numerical values).

accounted from the Eq. (1):[30]

$$m\mathrm{Al}^{3+} + \mathrm{nH_5C} \Longleftrightarrow [\mathrm{Al}_m(\mathrm{H}_{5-y}\mathrm{C})_n]^{3m-yn} + ny\mathrm{H}^+ \quad (1)$$

where m and n are the numbers of aluminum ions and cyanidin molecules bound in the complex structure, and y is the number of H^+ ions detached from one cyanidin (C) molecule upon complex formation.

The corresponding stability constant value is:

$$\gamma = \frac{[\mathrm{Al}_m(\mathrm{H}_{5-y}\mathrm{C})_n]^{3m-yn}[\mathrm{H}^+]^{ny}}{[\mathrm{Al}^{3+}]^m[\mathrm{H_5C}]^n} \quad (2)$$

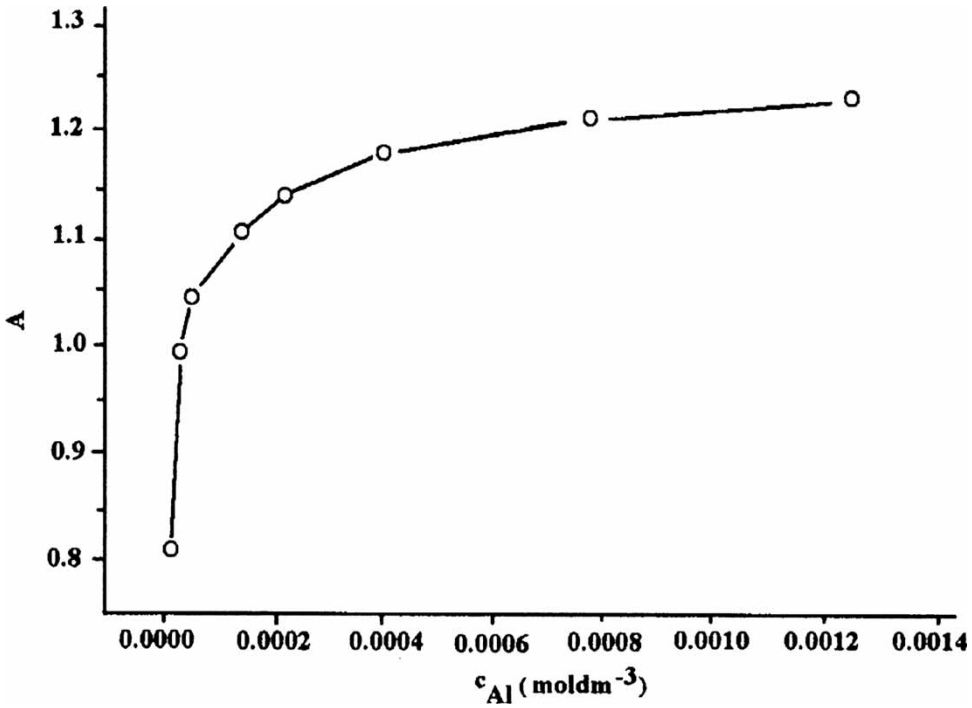


FIGURE 4 The Changes of the Absorbance Values of the Complex (Measured at λ = 581 nm) versus Aluminum (III) Concentration in Methanol.

When the pH value is constant, Eq. (2) is transformed into Eq. (3) giving rise to stability constant β:

$$\beta = \frac{[\gamma]}{[H^+]^{ny}} = \frac{[Al_m(H_{5-y}C)_n]^{3m-yn}}{[Al^{3+}]^m[H_5C]^n} \qquad (3)$$

Equation (3) shows that the stability constant value β is the relative one, and in the case the complexation reaction proceeds without proton detachment, it equals $\beta = \gamma$. By the molar ratio method,[30] Eq. (3) may transform into Eq. (4) which correlates all the parameters in Eq. (3) with the spectroscopically obtained

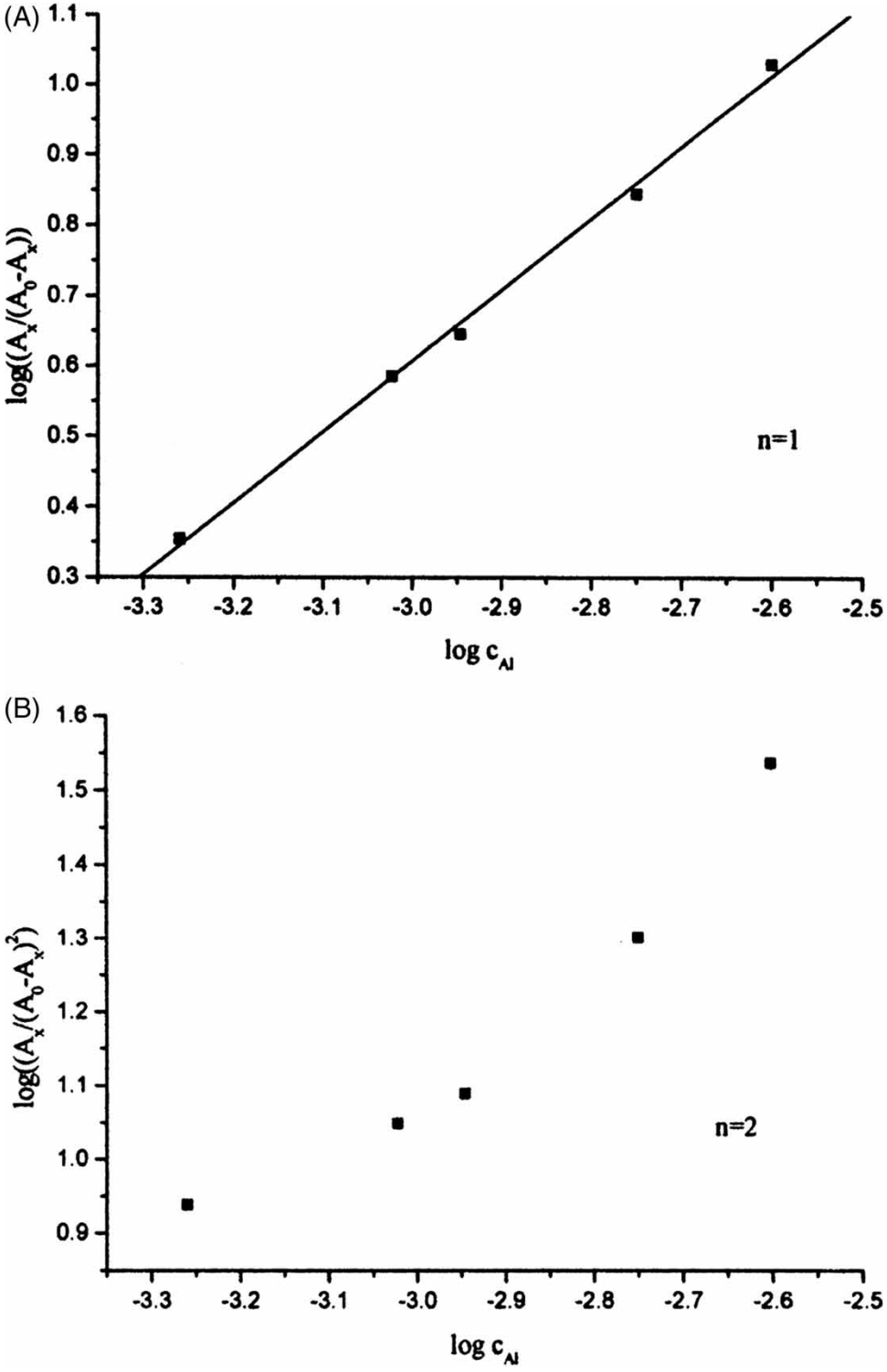


FIGURE 5 Relative Absorbance Values, log $(A_x/(A_0 - A_x)^n)$, verses Aluminum Concentration in the pH 4.0 Buffer: (A) $n = 1$; (B) $n = 2$.

TABLE 1 Stability Constant Values and Stoichiometric Ratios of the Components for the Cyanidin–Aluminum (III) Complex Formation Calculated by Nash and Molar Ratio Methods

	Nash method		Molar ratio method	
Complexing medium	log β	n	log β	n
pH 3.0	2.74	1	2.91	1
pH 4.0	3.64	1	3.80	1
Methanol	4.32	1	4.44	1

results:

$$\log\frac{A_x}{(A_0 - A_x)^n} = m\log c_{Al^{3+}} + \log\beta \qquad (4)$$

where (A_x) and $(A_0 - A_x)$ represent absorbance values that correspond with equilibrium concentrations of the complex and ligand, respectively. A_0 is the absorbance value of the horizontal part of the curve that presents the dependence of the complex absorbance values verses aluminum (III) concentration. Figure 4 presents such dependence in methanol. The horizontal part of the curve, reached in each medium at certain aluminum concentrations, corresponds with the completely complexed cyanidin molecule. For certain number of ligand molecules and metal ions bound in the complex structure (n and m values, respectively), Eq. (4) gives linear dependence of $\log A_x/(A_0 - A_x)^n$ verses $\log c_{Al^{3+}}$ with an intercept that equals $\log \beta$. Figure 5A, 5B presents molar ratio plots for the complexation in the pH 4.0 buffer solution taking $n = 1$ and $n = 2$, respectively, and $m = 1$ in both cases. From Fig. 5A it is evident that only the $n = 1$ value gives the linear dependence indicating 1:1 stoichiometry for the complex formed. The same stoichiometry is also obtained for the complexation in pH 3.0 buffered solution and in methanol. Quite similar results are also obtained by the Nash method, which gives the same stoichiometry for the complexes formed in buffered solutions and methanol. The total dominance of 1:1 species in both solvents could be expected as the experimental data was possible to collect only at a considerable excess of aluminum (III). Relative stability constant values, obtained by both methods, are listed in Table 1. From Eq. (3), which can also be presented as $\log\beta = \log\gamma + \text{pH}$, it is evident that the more pH increases, the more the complex is stable, and consequently at pH 4.0 the stability constant value is greater than at pH 3.0 (Table 1). These findings go along with what is generally observed in the large majority of the cases. The calculated constant values are the relative ones, and our results, as well as the literature data, have not been corrected for the ligand dissociation. The fact that the difference between two stability constant values in buffered solutions ($\approx$0.9) almost corresponds with the difference in pH values (1.0) confirms the assumption that the complex formation mechanism proceeds with the detachment of H^+ ion. The question, which possibly can arise from the obtained result, is whether or not the proton detachment is a consequence of the cyanidin deprotonation and the bond formation with aluminum (III) on the same oxygen atom. The answer to this question is probably yes. The known fact is that a large majority of compounds with phenolic (OH) groups enter complexation reactions upon hydroxyl group (or groups) deprotonation and metal binding on the same oxygen atom. The main driving force for binding metal ions in such complexes is larger binding energy in the complex compared with binding energy of H^+ ion. We suppose that the same reason stands also for complex formation between cyanidin and aluminum (III).

Although the experimental results and calculations do not precisely indicate the structure of the complexes formed, the existence of one catechol unit in the B ring of cyanidin (Scheme 1), the strong affinity of aluminum to this structural feature, and the obtained 1:1 stoichiometry implicate the formation of chelate structure complex, $[\text{Cy Al}]^+$, with the participation of catechol C-4′–C-3′ unit as a chelating site. This assumption is consistent with the fact that the hydroxyl group at C-4′, due to conjugation effect, undergoes deprotonation first followed by the deprotonation of the hydroxyl group at C-3′.[13] Consequently, it is possible to predict the formation of the chelate complex structure. The results are also consistent with the literature data concerning complexation of some synthetic and natural anthocyanidin molecules.[43] Because the complexation constant β is the relative one, it is possible to presume that there is more than one H^+ ion that detaches.

The constant values calculated by both methods agree rather well with the data[44] referring to the deprotonation constant values (varying from pK = 4.3 to 5.6, $I = 0.5$ moldm^{-3}, and $T = 25^\circ$C) of more than 20

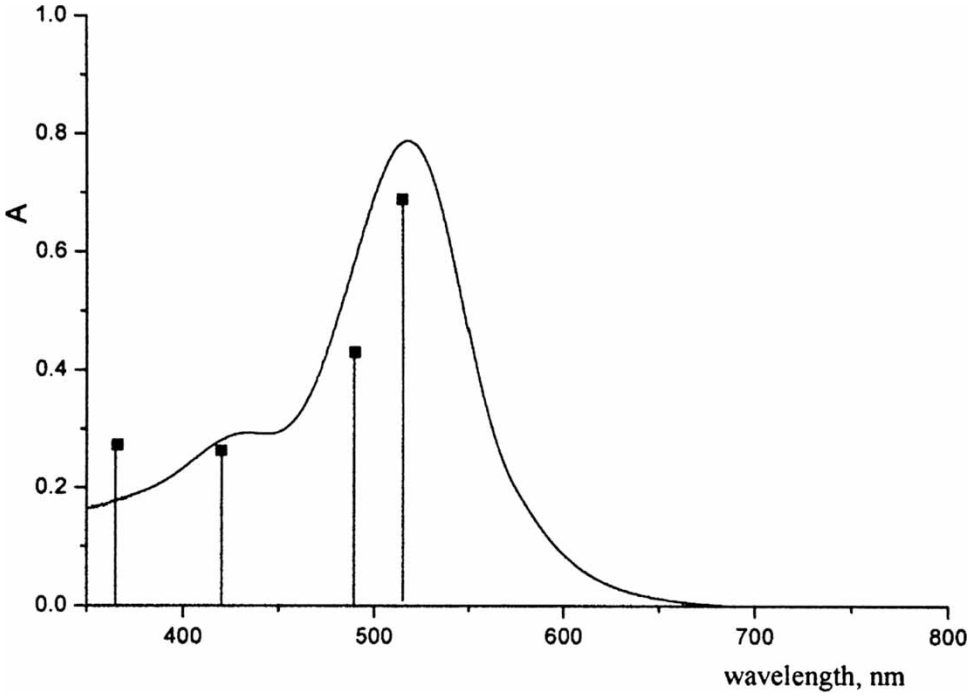


FIGURE 6 **Comparison of Calculated and Experimental Electronic Spectra of Free Cyanidin at pH 3.0. The Vertical Lines Represent Some of the Theoretical Band Positions Calculated by AM1 Method for Optimal Cyanidin Conformation.**

differently substituted cyanidin derivates and the deprotonation constant values of the flavylium transformation forms of six anthocyanin molecules in water ($pK' = 3.50$ to 4.85).[45] Somewhat bigger stability constant value in methanol (Table 1) goes along with the change of the relative permittivity value of the solvent. The solvatation process in methanol is much less pronounced compared with water, so the complexation is probably driven by stronger electrostatic forces. The stability constant values in methanol (Table 1) are also rather consistent with the data[36–39] referring to the complexation of aluminum (III) with differently substituted flavones, molecules structurally very similar to anthocyanidins.

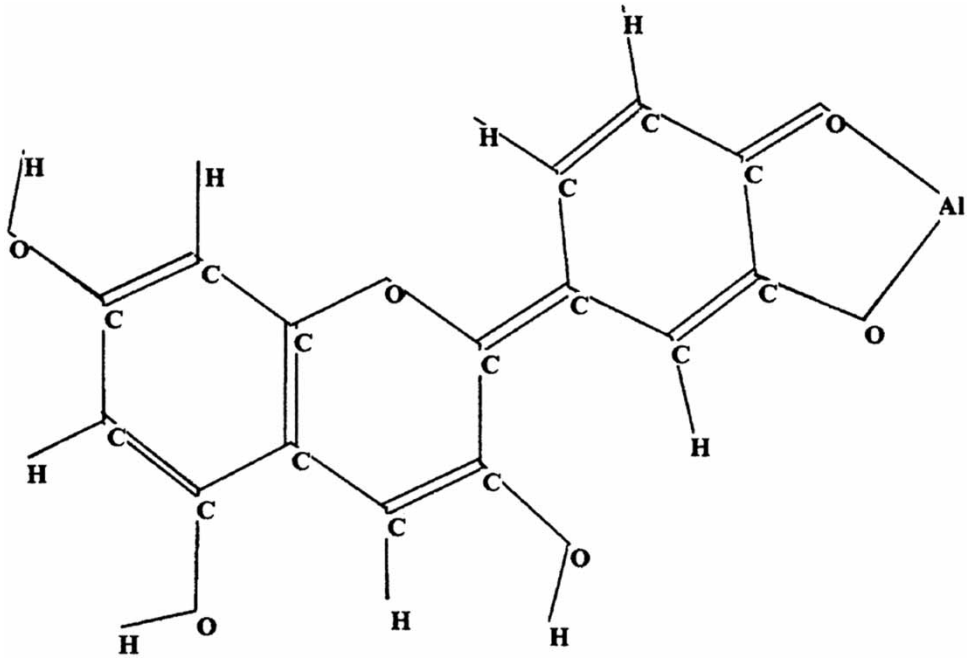


FIGURE 7 **Geometry Optimized Structure of Complex I (pH 4.0) (Water Molecules have been Omitted).**

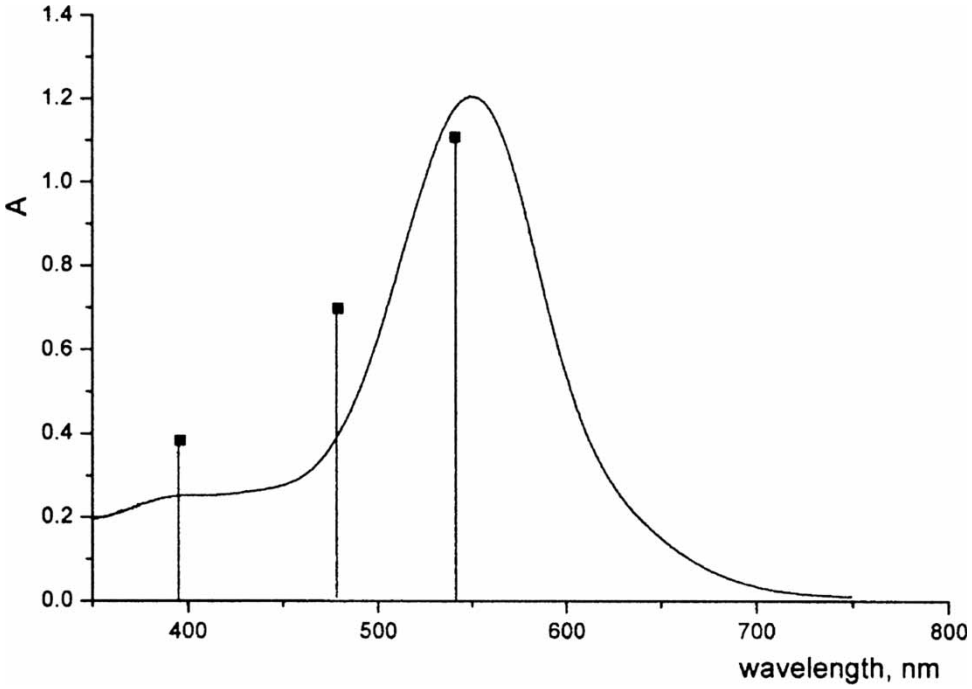


FIGURE 8 **Comparison of Calculated and Experimental Electronic Spectra of Complex I (pH 4.0). The Vertical Lines Represent Some of the Theoretical Band Positions Calculated by AM1 Method for Optimal Complex Conformation.**

Considering the fact that the dissociation constant values of hydrogen atoms in phenolic groups are not known, it is possible to suppose that chelate complex is bearing one positive charge, $[Cy\ Al]^{+}$. Positive charge of the cyanidin cationic form presented in Scheme 1 does not point to ionic character of cyanidin. It denotes only the center of the positive charge of the chromophore[8–10,13] and cannot be taken into account in calculating complex charge. This also goes in favor of the assumption that upon deprotonation of cyanidin molecule, aluminum binds on the same oxygen atom. Binding on the oxygen

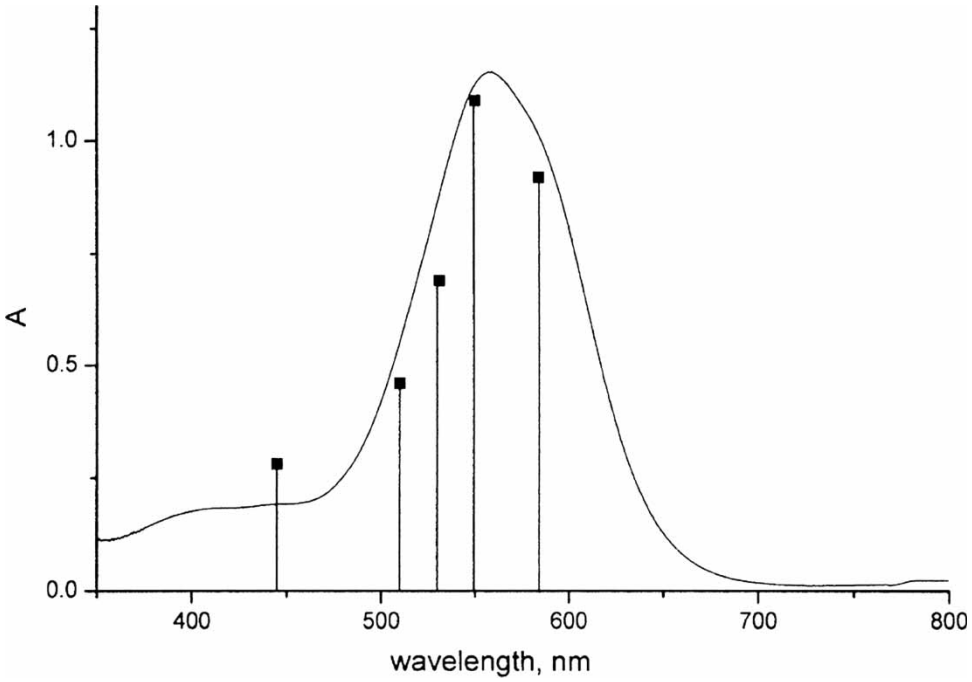


FIGURE 9 **Comparison of Calculated and Experimental Electronic Spectra of Complex II (in methanol). The Vertical Lines Represent Some of the Theoretical Band Positions Calculated by AM1 Method for Optimal Complex Conformation.**

atom that is bearing the positive charge (Scheme 1) would not be possible as the aluminum is also positively charged.

Semiempirical calculations enabled the determination of the structural features of free cyanidin and structural modifications caused by aluminum (III) binding. The geometry optimization performed at the restricted HF AM1 level[32] provides the opportunity to describe the electronic spectra of both free and complexed cyanidin molecule.

Conformation search is performed to determine stable conformers of cyanidin. Optimal structure is used for further investigations. The geometries of the free and complexed molecules are fully optimized without restriction. The results of the energy minimization show that free cyanidin and complexes formed can adopt several conformations. The almost planar conformation of free cyanidin is realized with minimum energy and consequently is the most stable one as the B ring is not much distorted in response to orientation of hydroxyl group in C-3 position. The electronic spectrum of free cyanidin at pH 3.0 (in its cationic form) and the spectral positions of the calculated transitions for optimal conformation are represented in Fig. 6. Calculated electronic transitions (vertical lines) are relative to the values of oscillator strengths. It is evident that the theoretically calculated transitions, especially those with minimal energy, maximal wavelength, are in rather good agreement with the experimentally obtained spectrum. This can validate the chosen semiempirical method, which reproduces the experimental results rather well.

The complex model obtained by the AM1 calculations indicates the formation of chelate complex in which cyanidin bonds to aluminum with the loss of two protons and the breaking of the hydrogen bonds. Although the experimental results do not directly indicate the loss of two protons, the theoretical model does so by calculating chelate complex structure as optimal configuration, the one that is obtained with minimal energy.

For energy minimization purposes of each complex structure solvent, molecules are coordinated to aluminum in order to obtain proper metal coordination. Geometry optimized structure of the 1:1 complex formed in the pH 4.0 buffered solution (complex I) is presented in Fig. 7. For clarity, water molecules are omitted from Fig. 7. The experimental electronic spectrum of the complex I is presented in Fig. 8, and the spectrum of the 1:1 complex formed in methanol (complex II) is presented in Fig. 9. Obtained electronic transitions (vertical lines), calculated by taking into account the pigment as well as metal structure, are relative to the values of oscillator strengths and are rather consistent with the absorption coefficients of the experimental spectra.

Molecular features calculated for optimal conformations of free and complexed cyanidin indicate that the structural modifications caused by the chelation of aluminum (III) are mainly localized on the C ring and on the chelating sites of the B ring. Bond lengths and valence angles of free cyanidin and complexes I and II are reported respectively in Tables 2 and 3. The main changes occur on O-1–C-2, C-2–C-3, and also on C-3–C-4, C-4–C-10, and C-9–C-10 bonds in the C ring (Table 2). The changes are also evident on possible chelating sites, C-3′–C-4′ as well as on the C-2–C-1′, C-2′–C-3′, and the other bonds in the B ring that decrease upon complexation indicating

TABLE 2 Bond Lengths Calculated by AM1 Method for Optimal Conformations of Free Cyanidin and Complexes I and II

Bond length (Å)	Free cyanidin	Complex I	Complex II
O(1)–C(2)	1.241	1.367	1.364
O(1)–C(9)	1.373	1.357	1.361
C(2)–C(3)	1.494	1.353	1.361
C(3)–C(4)	1.391	1.348	1.348
C(4)–C(10)	1.405	1.344	1.344
C(5)–C(10)	1.433	1.343	1.347
C(5)–C(6)	1.387	1.344	1.344
C(6)–C(7)	1.417	1.342	1.342
C(7)–C(8)	1.405	1.342	1.342
C(8)–C(9)	1.396	1.342	1.342
C(9)–C(10)	1.417	1.343	1.343
C(2)–C(1′)	1.510	1.357	1.357
C(1′)–C(2′)	1.364	1.348	1.339
C(2′)–C(3′)	1.394	1.340	1.341
C(3′)–C(4′)	1.416	1.351	1.353
C(4′)–C(5′)	1.408	1.352	1.365
C(5′)–C(6′)	1.362	1.342	1.347
C(6′)–C(1′)	1.376	1.351	1.353
C(4)–H(4)	1.106	1.103	1.103
C(3)–O(3)	1.375	1.359	1.217
C(5)–O(5)	1.362	1.361	1.360
C(7)–O(7)	1.357	1.360	1.360
C(3′)–O(3′)	1.378	1.355	1.360
C(4′)–O(4′)	1.364	1.212	1.217
C(5′)–O(5′)			
O(3′)–Al		1.820	1.805
O(4′)–Al		1.830	1.799

TABLE 3 Bond Angles Calculated by AM1 Method for Optimal Conformations of Free Cyanidin and Complexes I and II

Bond angles (°)	Free cyanidin	Complex I	Complex II
O(1)–C(2)–C(3)	118.5	117.3	117.4
C(2)–C(3)–C(4)	118.6	118.6	118.2
C(3)–C(4)–H(4)	121.3	116.5	116.5
C(3)–C(4)–C(10)	119.3	124.7	124.7
C(4)–C(10)–C(5)	124.0	122.4	122.4
C(10)–C(5)–C(6)	121.1	118.1	118.1
C(5)–C(6)–C(7)	119.3	122.6	122.6
C(6)–C(7)–C(8)	121.8	117.4	117.4
C(7)–C(8)–C(9)	117.5	121.9	121.9
C(8)–C(9)–C(10)	123.1	119.1	119.1
C(8)–C(9)–O(1)	118.1	120.9	120.2
C(9)–O(1)–C(2)	126.0	122.5	121.5
O(1)–C(2)–C(1′)	119.3	117.2	118.0
C(2)–C(1′)–C(2′)	117.6	126.7	120.1
C(1′)–C(2′)–C(3′)	118.8	123.4	124.2
C(2′)–C(3′)–C(4′)	119.9	120.8	119.7
C(3′)–C(4′)–C(5′)	119.3	117.3	117.0
C(4′)–C(5′)–C(6′)	118.7	120.5	121.0
C(5′)–C(6′)–C(1′)	121.6	123.3	123.8
C(4′)–C(3′)–O(3′)	125.0	112.7	115.6
C(5′)–C(4′)–O(4′)	115.4	126.4	123.8
C(3′)–O(3′)–H	109.5		
C(4′)–O(4′)–H	110.4		
C(3′)–O(3′)–Al		112.1	105.5
C(4′)–O(4′)–Al		115.9	109.4
O(3′)–Al–O(4′)		82.9	90.3

the delocalization of the π bond between two plains. The magnitudes of the bond orders correspond with the bond lengths. Bond angle values (Table 3) also change upon complexation. The majority of these values change in the range of 1 to 5°. The biggest changes occur on the C-4′–C-3′–O-3′ bond angle values, which are reduced approximately 12° and 10° upon formation of complexes I and II, respectively. These changes can be caused by the deprotonation of the hydroxyl groups, which happens upon chelation of aluminum.

TABLE 4 Some of the Torsion Angles Calculated by AM1 Method for Optimal Conformations of Free Cyanidin and Complexes I and II

Torsion angles (°)	Free cyanidin	Complex I	Complex II
O(1)–C(2)–C(1′)–C(2′)	177.66	−179.94	−179.95
C(2′)–C(3′)–O(3′)–Al	/	−179.25	179.65
C(5′)–C(4′)–O(4′)–Al	/	−179.51	−179.76
C(3)–C(2)–C(1′)–C(6′)	178.78	−179.97	−179.99
C(2′)–C(3′)–O(3′)–H	−180	/	/
C(3′)–C(4′)–O(4′)–H	0	/	/

The presence of aluminum also causes steric hindrance and consequently induces some changes in torsion angle values. The new torsion angles, C-2′–C-3′–O-3′–Al and C-5′–C-4′–O-4′–Al, formed upon aluminum chelation, indicate that aluminum is coplanar with the B ring in the complex. Table 4 lists some of the main torsional angles of free cyanidin and complexes I and II.

CONCLUSIONS

The study of complexation of aluminum (III) with cyanidin in aqueous buffered and methanol solutions was performed using electronic absorption spectroscopy and quantum chemical calculations.

The results indicate the total dominance of the 1:1 species in both solvents. The complex formed in methanol is somewhat more stable compared with the complexes formed in acidic buffered solutions. The existence of one catechol unit in the B ring of cyanidin, the strong affinity of aluminum to this structural feature, and the obtained 1:1 stoichiometry implicate the formation of chelate structure complex.

The theoretical treatment, performed in the AM1 parameterization, fairly reproduces the experimental results. Considering the cyanidin molecular structure and the results of the semiempirical calculations, it is possible to implicate catechol C-3′–C-4′ group as the one with the predominant chelating power.

Knowing that glycosidation and acylation of cyanidin molecule *in vivo* almost always proceeds via the substituents in the A and C rings, it is quite possible to presume that the results obtained *in vitro*, pointing to complexation via C-4′–C-3′ group, fairly represent the possible complex formation of these molecules *in vivo*.

REFERENCES

1. Middelton, E.; Kandaswami, C. Effects of flavonoids on immune and inflammatory functions. *Biochem. Pharmacol.* **1992,** *43*, 1167–1173.
2. Kinsell, J. E.; Frankel, E.; German, B.; Kanner, B. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *J. Food Tecnol.* **1993,** April 85–92.
3. Saija, A.; Trombetta, D.; Tomaino, A.; Lo Cascio, R.; Princi, P.; Uccella, N.; Bonina, F.; Castelli, F. "In vitro" evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. *Int. J. Pharm.* **1998,** *166*, 123–130.

4. Pilon-Smits, E.; Pilon, M. Phytoremediation of metals using transgenic plants. *Crit. Rev. Plant Sci.* **2002,** *21* (5), 439–456.
5. Hale, K. L.; Tufan, H. A.; Pickering, I. J.; George, G. N.; Terry, N.; Pilon, M.; Pilon-Smits, E. Anthocyanins facilitate tungsten accumulation in Brassica. *Physiologia Plantarum.* **2002,** *116* (3), 351–358.
6. Harborne, J. B.; Williams, C. A. Anthocyanins and other flavonoids. *Natural Product Reports* **1995,** *12*, 639–657.
7. Harborne, J.; Williams, C. Anthocyanins and other flavonoids. *Natural Product Reports* **1998,** *15*, 631–652.
8. Harborne, J. B. *Chemistry and Biochemistry of Plant Pigments*; Ed. Goodwin, T. W., Academic Press: New York, 1976; p. 736.
9. Harborne, J. B.; Grayer, R. *The Flavonoids–Advances In Research since 1980*; Chapman and Hall: London, 1988; p. 1.
10. Brouillard, R. *The Flavonoids–Advances In Research since 1980*; Chapman and Hall: London, 1988; pp. 525–616.
11. Brouillard, R.; Dangles, O. *The Flavonoids. Advances In Research since 1986*; Chapman and Hall: London, 1994; pp. 565–588.
12. Rommel, A.; Wrolstad, R.; Heatherbell, D. Blackberry juice and wine: Processing and storage effects on anthocyanin composition, color and appearance. *J. Food. Sci.* **1992,** *57* (2), 385–391.
13. Timberlake, C. F.; Bridle, P. Flavylium salts, anthocyanidines and anthocyanins. *Sci. Food. Agric.* **1967,** *18*, 473–478.
14. Harborne, J. B.; Baxter, H. *Handbook of Natural Flavonoids*; Wiley: Chichester, 1999.
15. Bayer, E.; Egeter, H.; Fink, A.; Nether, K.; Wegmann, K. The complex formation and flower colors. *Angew. Chem. Int. Ed. Engl.* **1966,** *5*, 791–798.
16. Elhabiri, M.; Figueiredo, P.; Toki, K.; Saito, N.; Brouillard, R. Anthocyanin-aluminum and gallium complexes in aqueous solution. *J. Chem. Soc. Perkin Trans. 2* **1997, 355–362.**
17. Dangles, O.; Saito, N.; Brouillard, R. Kinetic and thermodynamic control of flavylium hydration in the pelargonidin-cinnamic acid complexation. Origin of the extraordinary flower color diversity in *Pharbitis nil*. *J. Am. Chem. Soc.* **1993,** *115*, 3125–3232.
18. Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry* **1993,** *34*, 119–124.
19. Figueiredo, P.; Elhabiri, M.; Saito, N.; Brouillard, R. Anthocyanin intramolecular interactions. A new mathematical approach to account for the remarkable colorant properties of the pigments extracted from Matthiola incana. *J. Am. Chem. Soc.* **1996,** *118*, 4788–4793.
20. Goto, T.; Kondo, T. Structure and molecular stacking of anthocyanins-flower color variation. *Angew. Chem. Int. Ed. Engl.* **1991,** *30*, 17–33.
21. Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry* **1993,** *34*, 119–124.
22. Hoshino, T.; Matsumoto, U.; Goto, T. The stabilizing effect of the acyl group on the copigmentation of acylated anthocyanins with C-glucosylflavones. *Phytochemistry* **1980,** *19*, 663–667.
23. Hoshino, T.; Matsumoto, U. Evidences of the self-association of the anthocyanins I. Circular dichroism of cyanin anhydrobase. *Tetrahedron Lett.* **1980,** *21*, 1751–1754.
24. Hoshino, T.; Matsumoto, U.; Goto, T. Self-association of some anthocyanins in neutral aqueous solution. *Phytochemistry* **1981,** *20* (8), 1971–1976.
25. Hoshino, T.; Goto, T. Effects of pH and concentration on the self-association of malvin quinoidal base, electronic and circular dichroic studies. *Tetrahedron Lett.* **1990,** *31* (11), 1593–1596.
26. Sakata, K.; Saito, N.; Honda, T. Ab initio study of molecular structures and excited states in anthocyanidins. *Tetrahedron* **2006,** *62*, 3721–3731.
27. Pietta, P. G. Flavonoids as antioxidants. *J. Nat. Prod.* **2000,** *63*, 1035–1042.
28. Ishikura, N.; Sugahara, K. A survey of anthocyanins in fruits of some angiosperms. *Bot. Mag. Tokyo* **1979,** *92*, 157–162.
29. Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: London, 1982.
30. Inczedy, J. *Analytical Applications of Complex Equilibria*; Ellis Horwood: New York, 1976.
31. Vrielynck, L.; Cornard, J. P.; Merlin, J. C.; Bopp, P. Conformational analysis of flavone: vibrational and quantum mechanical studies. *J. Mol. Struct.* **1993,** *287*, 227–234.
32. Vrielynck, L.; Cornard, J. P.; Merlin, J. C. Semi-empirical and vibrational studies of flavone and some deuterated analogues. *Spectrochim. Acta* **1994,** *50A*, 2177–2188.
33. Boudet, A. C.; Cornard, J. P.; Merlin, J. C. Conformational and spectroscopic investigation of 3-hydroxyflavone-aluminum chelates. *Spectrochimica Acta* **2000,** *56*, 829–839.
34. Cornard, J. P.; Boudet, A. C.; Merlin, J. C. Complexes of Al(III) with 3′4′-dihydroxy-flavone: characterization, theoretical and spectroscopic study. *Spectrochimica Acta* **2001,** *57*, 591–602.
35. Cornard, J. P.; Merlin, J. C. Structural and spectroscopic investigation of 5-hydroxyflavone and its complex with aluminum. *J. Mol. Struct.* **2001,** *569*, 129–138.
36. Cornard, J. P.; Merlin, J. C. Spectroscopic and structural study of complexes of quercetin with Al (III). *J. Inorg. Biochem.* **2002,** *92*, 19–27.
37. Cornard, J. P.; Merlin, J. C. Complexes of aluminum (III) withisoquercitrin: spectroscopic characterizationand quantum chemical calculations. *Polyhedron* **2002,** *21*, 2801–2810.
38. Cornard, J. P.; Merlin, J. C. Comparison of the chelating power of hydroxyflvones. *J. Mol. Struct.* **2003,** *651*, 381–387.
39. Dewar, M. J. S.; Zoebisch, E. G.; Healey, E. F.; Stewart, J. J. AM1: a new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* **1985,** *107*, 3902–3909.
40. Stewart, R. N.; Noris, K. H. Microspectrophotometric measurement of pH and pH effect on color of petal epidermal cells. *Phytochemistry* **1975,** *14*, 937–942.
41. Asen, S.; Stewart, R. N.; Norris, K. H. Anthocyanin, flavonol, and pH responsible for larkspur flower color. *Phytochemistry* **1975,** *14*, 2677–2683.
42. Stevenson, F. J.; Vance, G. F. *The Environmental Chemistry of Aluminum*; Sposito, G., Ed.; CRC: Boca Raton, 1982; Ch. 4: Aqueous Polynuclear Aluminium Species, p. 145.
43. Pereira, G. K.; Galembeck, S. E. Comutational study of the electronic excitations of some anthocyanidins. *Spectrochimica Acta A* **1998,** *54*, 339–348.
44. Redus, M.; Baker, D. C.; Dougall, D. Rate and equilibrium constants for the dehydration and deprotonation reactions of some monoacylated and glycosylated cyanidin derivates. *J. Agric. Food Chem.* **1999,** *47*, 3449–3454.
45. Brouillard, R. *Anthocyanins as Food Colors*; Eds. Markakis, P. Academic Press: 1982; 1–38.