

## Interaction between Cyanidin 3-glucoside and Cu(II) ions

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### Abstract

The interaction between cyanidin 3-glucoside (Cy 3-glc) and Cu(II) in a binary solvent (methanol–water, 1:1, (v/v)) and in a phosphate buffer was studied. It is demonstrated that Cy 3-glc and Cu(II) ions form a complex that is manifested by appearance of a new absorption band. In the complex, the reduction of Cu(II)–Cu(I) and oxidation of Cy 3-glc takes place. The association constant of the complex formation was calculated using the Rose–Drago method. The complex stoichiometry in the binary solvent was 1:1, and the association constant was  $K = (28,600 \pm 2600) \text{ M}^{-1}$ . Interaction of Cy 3-glc and Cu(II) ions has two stages “fast” and “slow”. The kinetics of these processes has been investigated. Thermodynamic data for the “fast” process: enthalpy ( $\Delta H^*$ ) and entropy ( $\Delta E^*$ ) of activation, were calculated.

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**Keywords:** UV–vis spectroscopy; Cyanidin 3-glucoside; Flavonoid; Cooper ions; Kinetics; Complexation

### 1. Introduction

Anthocyanins are natural pigments of the flavonoid family which are largely responsible for red, purple and blue colours displayed in plants. They are present in fruits and vegetables and are important components of the human diet (Brouillard, 1982). Anthocyanins, together with other phenolics, play an important biological role as antioxidant (Heim, Tagliaferro, & Bobilya, 2002; Noda, Kaneyuki, Mori, & Packer, 2002; Sarma, Sreelakshmi, & Sharma, 1977; Zheng & Wang, 2003). Recently, their anti-atherosclerosis and anticarcinogenic properties have been exploited in medicine (Kim & Park, 2006; Tsai, McIntosh, Pearce, Camden, & Jordan, 2002). The mechanism responsible for the antioxidant activity of anthocyanins appears to be dual. On one hand, they act as free radical scavengers and on the other, they are able to chelate metal ions, thereby reducing metal induced peroxidation (Ben-Allal el Amrani, Perello, Borrás, & Torres, 2000). Highly reduc-

ing flavonoids are air-sensitive compounds that may undergo autoxidation (Long, Clement, & Halliwell, 2000).

Recently, the interactions between flavonoids and iron and copper ions have been investigated (El Hajji, Nkhili, Tomao, & Dangles, 2006; Mira et al., 2002). Flavonoids together with iron and copper can create complexes and can reduce metal ions. The ability of flavonoids to chelate iron ions depends on the molecular structure and pH of the environment. Chelate formation is possible through “metal complexing sites” within a flavonoid molecule containing a hydroxyl group at 3, 5, and 3', 4' positions. Flavonoids should have more chelating sites at pH 7.4 than at pH 5 (El Hajji et al., 2006; Morel, Cillard, & Cillard, 1998).

Anthocyanins can bind various metal ions: Cu(II), Al(III), Fe(III) and this is manifested in large hyperchromic and bathochromic shifts in the molecular absorption spectra. UV–vis spectral and NMR analysis collected at the pH range 2–5 indicate that the complexes with metal ions are formed with both the coloured as well as the colourless forms of anthocyanins (Cherepy, Smestad, Grätzel, & Zhang, 1977; Dangles, Elhabiri, & Brouillard, 1994; George, Figueiredo, & Brouillard, 1999). George et al. (1999) identified complexation between the Fe(III) cation

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and the ( $C_Z$ ) - chalcone naturally occurring malvin in acidic aqueous solutions. Dangles et al. (1994) showed the kinetics of complexation between aluminium cation and a minor anionic quinoidal form of the anthocyanin at pH 2–5. The ability to form complexes is related to hydroxyl functional groups linked to the (B) ring, therefore cyanidin and delphinidin can form complexes while malvidin cannot. The methoxy group ortho in relation to position 4' on the ring B in malvidin lowers the ability to form complexes with metal ions (Satue-Gracia, Heinonen, & Frankel, 1997). Cyanidin with their 3',4'-dihydroxy group rapidly chelates metal ions with bathochromic shifts in the visible spectra (Sarma et al., 1977).

Anthocyanins are soluble in water, and they undergo structural transformations. In a slightly acid aqueous solution, four main species of anthocyanins exist in equilibrium. These are the blue quinoidal base (A), the red flavylium ion ( $AH^+$ ), the colourless hemiacetal base (B), and the colourless chalcone form (C). Depending on the pH level, quinoidal base (A) can exist in different structures in equilibrium with flavylium ion ( $AH^+$ ). Hemiacetal base (B), which is formed by a nucleophilic attack of water at carbon 2, also exists in equilibrium with the flavylium ion (Brouillard, 1982).

Anthocyanins, for example, cyanidin 3-glucoside, are present in plants in the form of glycosides and in that form they are absorbed by the human body. The best assimilation is in the small intestine (pH 7.4), however, in this environment anthocyanins are easily degraded by bacteria (Cao, Muccitelli, Sanchez-Moreno, & Prior, 2001; Yi, Akoh, Fischer, & Krewer, 2006). Assimilation of anthocyanins may be diminished by the ionic presence in the human body as a result of environmental pollution. It is worth noting that oxidation of anthocyanins by metal ions may occur in the different steps of production and preservation of food. Multivalent metal ions such as iron, copper and manganese in food have been shown to be catalysts in the oxidation process (El Hajji et al., 2006; Makris & Rossiter, 2002).

In this context, investigations of the interaction of cyanidin glycosides with metal ions are important from the technological and medical points of view.

The aim of these studies was to investigate the interactions between Cu(II) ions and cyanidin 3-glucoside (Cy 3-glc) in a water medium as well as determination of the stoichiometry and association constant of the Cu(II)–Cy 3-glc complex formation. Cyanidin 3-glucoside was selected for this study as one of the anthocyanins most widely occurring in nature which is capable of chelating metal ions. The studies were carried out in a binary solvent (methanol–water, 1:1, v/v), which is most often used as an eluent and as a solvent of anthocyanin pigments.

## 2. Experimental

### 2.1. Reagents

Cyanidin 3-glucoside (Cy 3-glc) as a chloride salt was purchased from The Department of Fruit and Vegetable

Technology, Wrocław Agricultural University (Wrocław, Poland). Its purity was checked by HPLC, NMR and MS methods (Kucharska & Oszmiański, 2002). Methanol (spectral grade) was from Fluka (Buchs, Switzerland) and all other analytical grade chemicals were from Merck (Darmstadt, Germany) and POCH (Poland).

### 2.2. Sample preparation

Cyanidin 3-glucoside (Cy 3-glc) was dissolved in a binary solvent (methanol–water, 1:1, v/v). Stock solutions ( $2.0\text{--}2.5 \times 10^{-4} \text{ mol dm}^{-3}$ ) were left to equilibrate in the dark at  $2\text{--}5^\circ\text{C}$  for about 24 h, and were subsequently diluted to a final concentration of  $1.5 \times 10^{-5} \text{ mol dm}^{-3}$ . The concentration of stock solution of copper chloride ( $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ) was  $5 \times 10^{-2} \text{ mol dm}^{-3}$ . Samples of cyanidin 3-glucoside with Cu(II) ions were prepared at pH 6.8 both in a binary solvent and in 0.1 M phosphate buffer. The molar ratio of the metal to ligand ( $C_M:C_L$ ) ranged from 1:10 to 40:1.

Prior to measurements, the samples of cyanidin 3-glucoside were left in a water bath to equilibrate for 2 h at  $24^\circ\text{C}$ , and after this period, the absorption spectra of the samples were stable for at least 24 h.

### 2.3. Measurement of Cu(I)

The ability of anthocyanins to reduce Cu(II) was evaluated by monitoring the formation of the complex between Cu(I) and bathocuproine disulphonic acid disodium salt (BCDS) (Blair & Diehl, 1961). The absorbance was read at 482 nm ( $\lambda_{\text{max}}$  of the complex  $(\text{BCDS})_2\text{-Cu(I)}$ ,  $\epsilon = 13.900 \text{ M}^{-1} \text{ cm}^{-1}$ ). The mole ratio of BCDS–Cu(II) was equal 10 and was kept constant in all samples. The mixtures were incubated at  $24^\circ\text{C}$  for 20 min.

### 2.4. Methods

Steady state absorption spectra and kinetic measurements were performed on a Cary 300 spectrophotometer equipped with thermostated accessory and a magnetic stirrer placed in a 1 cm cuvette. All measurements were done at  $(24.0 \pm 0.1)^\circ\text{C}$ . The temperature measurements were carried out in the range of  $11\text{--}40^\circ\text{C}$ .

## 3. Results

The absorption spectra and changes in the absorbance at selected wavelengths were measured for cyanin without Cu(II) and after its addition in a binary solvent.

The addition of Cu(II) ions resulted in the appearance of a new band with a maximum at 584 nm (Fig. 1, curve 2).

Having reached the maximum, the band disappeared. Concomitantly, new bands were observed at shorter wavelengths. Fig. 1 (curves 2, 3 and 4) indicates that directly after the Cu(II) ions addition and after several hours of storage, the absorption spectrum of Cy 3-glc was signifi-

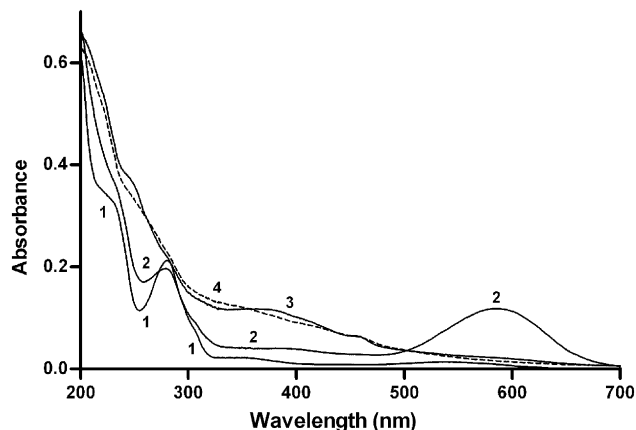


Fig. 1. Time dependence of absorption spectrum of Cy 3-glc under the influence of Cu(II) ions in a binary solvent. Molar ratio of Cu(II) ions to Cy 3-glc,  $C_M:C_L = 5:1$ . Curve 1 – absorption spectrum of Cy 3-glc, curves 2–4 – spectra of Cy 3-glc with Cu(II) ions after about 13 min, 170 min and 470 min, respectively.

cantly different as compared to the Cy 3-glc spectrum (Fig. 1, curve 1). No changes were noted in the absorbance of Cy 3-glc without Cu(II) ions over the measurement period. The absorbance was additionally monitored as a function of time for three wavelengths: 590 nm, 458 nm and 370 nm, at molar ratio  $C_M:C_L = 5:1$  (Fig. 2) and at a constant concentration of  $C_L$  and at different concentrations of  $C_M$  (Fig. 3).

Fig. 2 indicates that time coursed experiment can be divided into two time regions: an initial, shorter one (“fast” process) involving an increase in the absorbance at 590 nm up to reaching the maximum (see also Fig. 3) and second one – a much longer (“slow” process) when decrease of the absorbance was observed.

The increase of absorbance at 590 nm lasted from a few minutes to one hour (Figs. 3 and 2, curve 1 – “fast”), and the decrease lasted several hours (Fig. 2, curve 1 – “slow”).

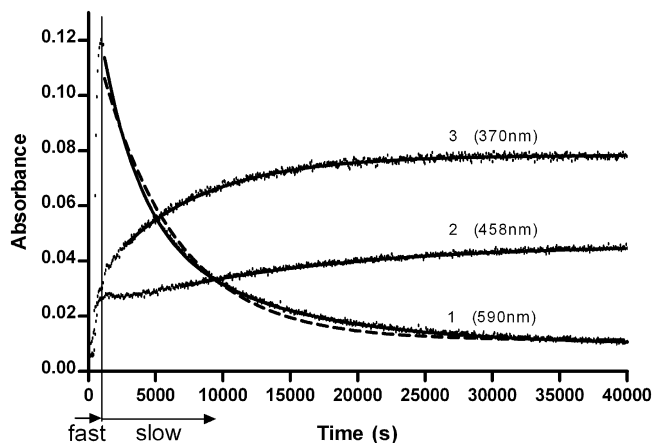


Fig. 2. Time dependence of Cy 3-glc absorbance after addition of Cu(II) ions in binary solvent registered at 590 nm – curve 1, 458 nm – curve 2 and 370 nm – curve 3. Solid and dashed lines – fitted exponential functions. “fast” – fast processes, “slow” – slow processes.

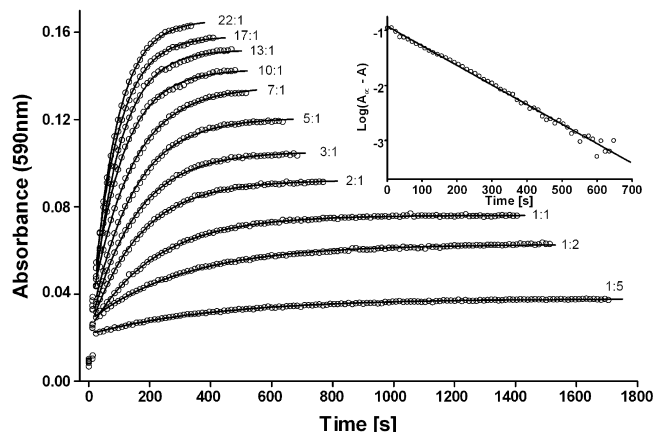


Fig. 3. Time dependence of Cy 3-glc absorbance after addition of Cu(II) ions in binary solvent registered at 590 nm. Molar ratio  $C_M:C_L$  from 1:10 to 22:1. Solid curves – fitted one exponential growth functions. Inset – logarithmic graph for  $C_M:C_L = 5:1$ .  $A_\infty$  – extrapolate absorbance, proportional to the product concentration,  $A$  – real absorbance at the moment of measurement.

Absorbance changes at 370 nm showed a continuous increase up to the maximum (Fig. 2, curve 3). An initially irregular, and then continuous, increase in the absorbance up to the maximum reached can be also observed for the 458 nm (Fig. 2, curve 2).

The analysis of results obtained for the “slow” kinetics at 590 nm demonstrated two molecular processes manifested by two exponential decays of the absorbance. Simultaneously an increase in the absorbance observed at 458 nm and 370 nm for the “slow” process was one exponential for these two wavelengths. The calculated reaction rate constants are presented in (Table 1).

A comparison of these data indicates that the rate constants of the slow processes ( $k_s$ ), related to the disappearance of complex band, were different from those connected with appearance of new bands in shorter wavelength calculated at 370 nm and 485 nm in the “slow” processes. Moreover, the values of the rate constant in this process differed from each other for both of the wavelengths.

In the present study, attention was paid mainly to changes in the absorbance of Cy 3-glc in a binary solvent

Table 1  
Kinetic rate constants of “fast” and “slow” processes in 24 °C for  $C_M:C_L = 5:1$

Solvent	$\lambda$ (nm)	Fast processes $k_f \times 10^5$ (s <sup>-1</sup> )	Slow processes $k_s \times 10^5$ (s <sup>-1</sup> )
Binary solvent	590	252 ± 5	9.6 ± 1.0 <sup>a</sup>
	Methanol–water	–	36.6 ± 0.4 <sup>a</sup>
	1:1 (v/v)	–	6.8 ± 0.2
Phosphate buffer	370	–	14.5 ± 0.2
	590	144 ± 2	9.0 ± 0.2
	330	–	9.1 ± 0.5

<sup>a</sup> Kinetic rate constant calculated for two exponential decay.

in the long wavelength band range that is typical of the cyanin and Cu(II) ions complex. Fig. 3 depicts kinetic curves of the complex formation at different molar ratios  $C_M:C_L$  at 590 nm in the binary solvent. It was found that in all cases, the observed increase could be described with a strictly single exponential growth function. The reaction was found to be of the first order because, in all cases, a very good linear dependence  $\ln(A_\infty - A)$  on time was obtained (Fig. 3 inset). The reaction rate constants of fast processes ( $k_f$ ) were calculated by analyzing the rate of product formation.

The thermodynamic data of the complex formation was also determined. Changes in the absorbance of the Cy 3-glc solution after Cu(II) ions addition ( $C_M:C_L = 5:1$ ) were measured at 590 nm at several temperatures ranging from 11 °C to 40 °C. Throughout the entire temperature range used, the reaction was of the first order. In order to determine enthalpy ( $\Delta H^*$ ) and entropy ( $\Delta S^*$ ) of activation, the Eyring equation was applied. The results obtained are presented in Fig. 4.

The parameters for the 1:1 and 1:2 stoichiometry of the complex were calculated with the use of the Rose–Drago method (Rose & Drago, 1959). The moment of reaching the absorbance maximum at 590 nm was chosen for each sample (Fig. 3). The absorption spectra measured at this moment are shown in Fig. 5.

A correction related to the added volume of the salt solution of Cu(II) both for Cu(II) ions and Cy 3-glc was made. Calculations using the Rose–Drago method were carried out for 11 wavelengths within the range 550–650 nm at 10 nm intervals, for 5 solutions with a molar ratio of  $C_M:C_L$  ranging from 5:1 to 17:1. Fig. 6 presents a plot of the  $(1/K)$  against  $(\epsilon)$  at 590 nm for the stoichiometry 1:1. The calculated value of the association constant of the complex was  $K = (28600 \pm 2600) \text{ M}^{-1}$ . For the stoichiometry, a 1:2 intersection point has not arisen.

The long wavelength absorption band calculated using the extinction coefficient from Rose–Drago method, as presented in Fig. 5 (a dotted curve), can be considered as the complex band.

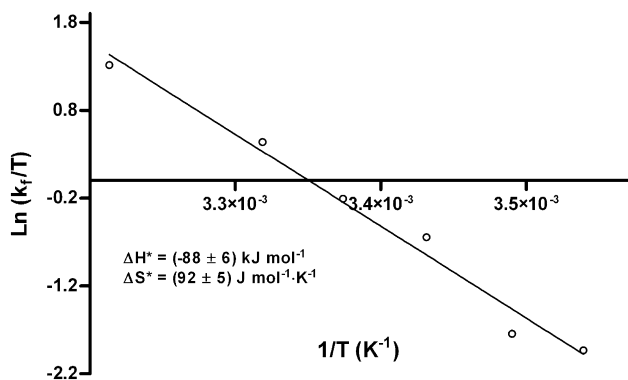


Fig. 4. Logarithmic dependence of  $k_f/T$  versus  $1/T$  for the data from temperature study.  $H^*$  – enthalpy of activation,  $\Delta S^*$  – entropy of activation.

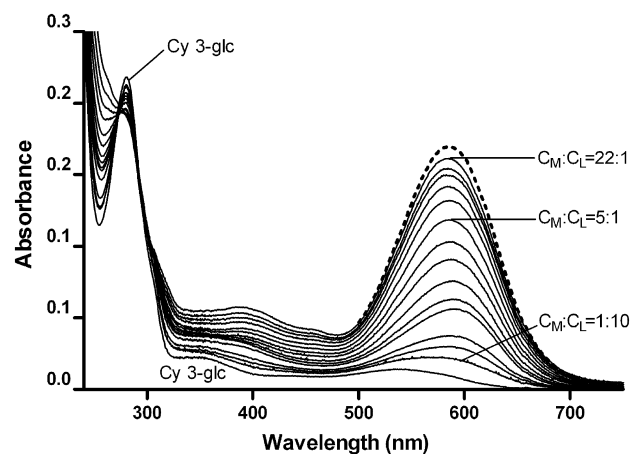


Fig. 5. Absorption spectra of Cy 3-glc after addition of Cu(II) ions in binary solvent measured during reaching the absorbance maximum at 590 nm (see Fig. 3). Molar ratio  $C_M:C_L$  was changed from 1:10 to 22:1. Dotted line – spectrum of the complex calculated using iterative method.

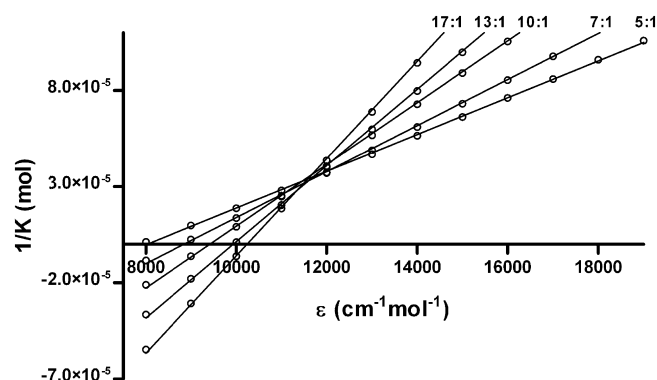


Fig. 6. Rose–Drago plot of the Cy 3-glc – Cu(II) complex for the stoichiometry 1:1 at 590 nm.

In order to stabilize pH, kinetic studies were carried out in a phosphate buffer at pH 6.8. The changes observed in Cy 3-glc absorption spectrum after Cu(II) ions addition, for the system of  $C_M:C_L = 5:1$  and wavelengths of 330 nm and 590 nm, are presented in Figs. 7 and 8.

Two processes proceeding up to reaching the maximum absorption can be deduced from Fig. 7. The first is a very rapid and lasts seconds, whereas the second one spans several minutes. These processes correspond to the “fast” process in the binary solvent (Figs. 3 and 2, curve 1).

In the long wavelength band, after reaching of absorption maximum, the slow process lasting several hours was commenced, as in the case of the binary solvent (Fig. 8, inset – curve 1). The absorption spectra during the slow process created isosbestic points (Fig. 8), indicating an equilibrium between two forms in the solution.

Bathocuproine disulfonic acid disodium (BCDS) is a well-known molecule which is sensitive to Cu(I) ion presence. BCDS was added to the solution of Cu(II)–Cy 3-glc of three molar ratios ( $C_M:C_L$ ): 1:1, 5:1 and 10:1, while the absorbance reached the maximum at 590 nm. In each case,

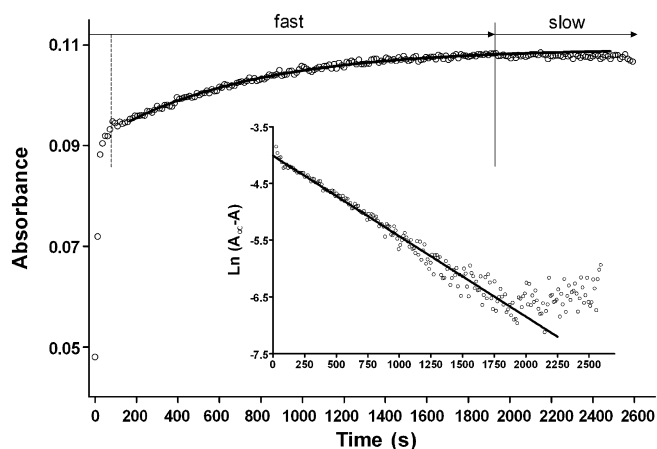


Fig. 7. Time dependence of Cy 3-glc absorbance after addition of Cu(II) ions in 0.45 M phosphate buffer – “fast” processes. Molar ratio  $C_M:C_L = 5:1$ . Inset – the same graph in logarithmic scale.

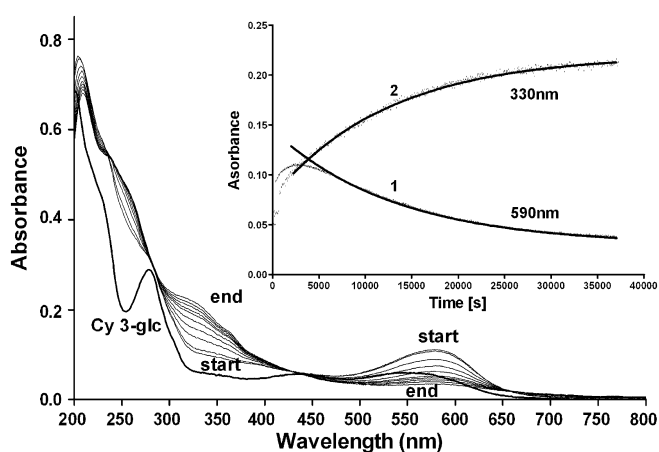


Fig. 8. Time dependence of Cy 3-glc–Cu(II) complex in 0.45 M phosphate buffer – “fast” processes. Molar ratio  $C_M:C_L = 5:1$ . Inset – changes of absorbance registered at 590 nm – curve 1 and 330 nm – curve 2. Solid lines – fitted one exponential functions.

a strong absorption increase was observed for 482 nm. At this wavelength, it is the maximum of the complex of  $(BCDS)_2$ –Cu(I). Simultaneously, absorbance of the complex at 590 nm was decreased.

#### 4. Discussion

The results of the investigation presented indicate that interaction between cyanidin 3-glucoside and Cu(II) ions leads to a complex formation which undergoes further transformation. Literature data (Brouillard, 1982; Figueiredo, et al., 1994; Março, Levi, Scarminio, Poppi, & Trevisan, 2005) and the absorption spectra presented here indicate that both in the binary solvents and in the buffer solution (pH 6.8), different forms of Cy 3-glc can exist. The  $AH^+$  and A forms show the absorption spectra in the long wavelength range (Figs. 1 and 2). In this region, the band of Cy 3-glc is the sum of two bands with a maximum at 520 nm ( $AH^+$ ) and 580 nm (A).

Addition of Cu(II) ions to the solution of Cy 3-glc was manifested in a new absorption band with a maximum at 584 nm. At the same time, absorbance around 330 nm was increased (Figs. 1, 5 and 8). This is a typical region of the oxidized forms of flavonoids. The rate of this process was dependent on the amount of Cu(II) ions added to the solution (Fig. 3). To sum up, the BCDS resulted in complex formation with Cu(I), which was manifested in a new absorption band appearing with a maximum at 482 nm. Such observation indicates the reduction of Cu(II)–Cu(I) after complexation and oxidation of the Cy 3-glc molecule. The same situation was observed by El Hajji et al. (2006) for quercetin in an aqueous environment. Cyanin is regarded as one of the flavonoids which easily undergoes oxidation and acts as an electron donor. This property was used, for example, to prepare photoelectrochemical cell (Cherepy et al., 1977) with the complex of cyanin and  $TiO_2$ .

The  $pK_a$  of the four OH groups in Cy 3-glc may vary from  $pK_a$  4–5 through 6–8 up to 9–11, similar to the case of morin sulfone derivative (Bujonek, 1993). Dangles et al. (1994) reported that for the cyanidin derivative (3',4',7-trihydroxy-3-methoxyflavylium ion), dissociation constants of the OH groups in the aqueous solution were  $pK_{a1} = 4.40$  and  $pK_{a2} = 7.62$ . Thus, pH stabilisation with the use of a buffer does not decrease the number of ionic and structural forms of cyanin present in the solution. The changes in the absorption spectrum in the buffer demonstrated an increase in the absorbance at 590 nm (fast process) after Cu(II) ion addition (Figs. 7 and 8), similar to the binary solvent (Figs. 3 and 5). These changes may, thus, be ascribed to the Cu(II) – Cy 3-glc complex formation. The “fast” process in the buffer may be divided into two time ranges: shorter and longer (Fig. 7). Due to the measuring technique applied in this study, the “fast” process was studied in the longer time range. Both in the binary solvent as well as in the buffer, the fast processes proceeded as the first order kinetics, however, in a buffer at a lower rate constant ( $k_f$ ) (Table 1). The similarity of the processes in both solvents was also confirmed by the fact that the absorption spectra of Cu(II)–Cy 3-glc, normalised at the maximum of the long wavelength complex band, demonstrated no quality differences in that range. It can be stated that the proceeding processes were alike, differing only in reaction rate.

The quantitative differences in the absorption spectra of Cy 3-glc in the presence of Cu(II) and different values of the reaction rate constants ( $k_f$ ) in the binary solvent and in the buffer can be explained by the influence of methanol. As demonstrated in studies (Yilmaz, 2002) into the dielectric properties of methanol–water mixtures, the value of a dielectric constant ( $\epsilon$ ) of the mixture decreases with an increase in the molar fraction of methanol content. It was found that at a molar fraction equal to 0.5, the value of the dielectric constant ( $\epsilon$ ) decreased by ca. 40% as compared to the ( $\epsilon$ ) of water, and the excess dielectric constant ( $\epsilon^E$ ) reached the minimum. Such changes in the dielectric parameters of the solvent can affect the interactions

between Cu(II) ions and Cy 3-glc. Cy 3-glc molecules are subject to a hydrophilic attack of water molecules so the presence of methanol diminished such an attack and facilitated a complex formation.

The fast process seems to be linked with the oxidation of the Cy 3-glc molecule and Cu(II)–Cu(I) reduction. Positive change of entropy and enthalpy of activation calculated for the binary solvent additionally supports this process. This indicates that the transition state of the reaction is highly disordered compared to the ground state. The proceeding structural changes result in an increase in degrees of freedom of the system. The two similar processes of a rapid signal increase – the first one very rapid and the second one slower – were observed in the studies of quercetin and its derivative interactions with metal ions: Zn(II), Cu(II), Mg(II) and Fe(II) (El Hajji et al., 2006; Spickett, Wojtoń, Kopacz, & Oleksyn, 2002).

Analyses of the “slow” processes kinetics, i.e. after reaching the absorption maximum in the buffer, demonstrated one strictly exponential decay curve at the 590 nm (Fig. 7, inset), contrary to the binary solvent in which was two exponential (Fig. 2). The rate constant  $k_s$  of the slow reaction in the buffer is comparable to the lower value of the reaction rate constant obtained for the two exponential decay in the binary solvent (Table 1). In addition, the rate constants  $k_s$  for decrease (at 590 nm) and increase (at 330 nm) in the buffer were equal (Table 1). The very good isosbestic points formed in the absorption spectra (Fig. 8) indicate the existence of equilibrium between the oxidized form and the complex during the process of decomposition. Such isosbestic points were not so well-defined in the binary solvent. In previous research (Pliszka, Smyk, Mieszko, Oszmiański, & Drabent, 2005), while studying the effect of Cu(II) on anthocyanins in a binary solvent at pH 5, a similar situation was observed.

This long-term process, after reaching the absorption maximum at 590 nm, is probably connected with further oxidation of the Cy 3-glc molecule following after Cu(II) binding. A similar process ascribed to the oxidation was observed in the case of a sulfone derivative of quercetin (El Hajji et al., 2006; Kopacz, Kopacz, & Skuba, 2000) with and without metal ions.

The most probable site of Cu(II) ions binding to a Cy 3-glc molecule are the two OH groups of the (B) ring. It is common knowledge that hydroxylation of anthocyanins at 3' and 4' position of the (B) ring enhances the chelating properties of anthocyanins to metal ions (Cherepy et al., 1977; Sarma et al., 1977; Satue-Gracia et al., 1997). The possibility of Cu(II) ions binding to two OH groups of the A ring is quite unlikely due to the steric effect, i.e. both the groups are too far from one another. The kinetic data of this process obtained in this work suggests that only one of the possible forms of Cy 3-glc is preferable, since the absorbance in the range of long wavelength band of the complex increased in a one exponential mode (Figs. 3 and 2 – inset, Fig. 7). The binding of the Cu(II) ions to two Cy 3-glc molecules through four OH groups from the (B) ring (stoichiometry 1:2) is unli-

kely because of a lack of the intersection point of straight lines in the method of Rose–Drago. The stoichiometry of 1:1 and association constant  $K = (28,600 \pm 2600) \text{ M}^{-1}$  was obtained for Cy 3-glc complex for big excess of metal ions to Cy 3-glc due to small value of association constant and overlapping of absorption bands of bound and no bound form of Cy 3-glc. The Rose–Drago equation with such a large excess of one of the reactants practically becomes a Benesi–Hildebrand equation (Rose & Drago, 1959). Stoichiometry of 1:1 was also achieved by other authors for complexes of Zn(II) and Cu(II) ions with catechin (Salinas, Esparza, Gomez, Santamaria, & Fernandez, 2005) and quercetin with Cu(I), Cu(II), Fe(II) and Fe(III) ions (El Hajji et al., 2006). On the contrary, for complexes of Fe(III) ions with Cy 3-glc, the stoichiometry reached 1:2, similar to the case of complexes of Al(III) ions with Cy 3,5-glc at pH 4.5 (Salinas et al., 2005).

The results presented indicate that Cu(II) ions create a complex with Cy 3-glc and then proceed to a reduction to Cu(I) and cause oxidation of Cy 3-glc molecule in the “fast” process. In next step (“slow” process) the complex dissociates and Cy 3-glc undergoes further oxidation.

## 5. Conclusions

Studies into the interactions of Cu(II) ions with Cy 3-glc showed that a complex was formed in both the binary solvent and phosphate buffer. The association constant of the complex determined from the Rose–Drago method is equal:  $K = (28,600 \pm 2600) \text{ M}^{-1}$  and the stoichiometry of the complex reached 1:1. The thermodynamic data:  $\Delta H^* = (88 \pm 6) \text{ kJ mol}^{-1}$  and  $\Delta S^* = (92 \pm 5) \text{ J mol}^{-1} \text{ K}^{-1}$  enabled deducing that the binding of Cu(II) ions to Cy 3-glc caused an increase in the degrees of freedom of the system in the transition state. In addition, during the entire process of Cu(II) ions binding to cyanin, two stages were distinguished according to two different time scales: “fast” and “slow”. The “fast” processes can be divide into two processes: binding of the Cu(II) ions and a redox reaction of Cu(II) and Cy 3-glc resulted in the reduction of Cu(II)–Cu(I) and the oxidizing of the Cy 3-glc molecule. The “slow” processes are related to degradation of the complex and the further oxidation of Cy 3-glc molecule.

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