THE INFLUENCE OF THE HOST–GUEST INTERACTION ON THE OXIDATION OF NATURAL FLAVONOID DYES

Šárka Ramešová[a1], Romana Sokolová[a2,*], Ilaria Degano[b1], Magdaléna Hromadová[a3], Miroslav Gál[a4], Viliam Kolivoška[a5] and Maria Perla Colombini[b2]

[a] *J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Dolejškova 3, 182 23 Prague, Czech Republic; e-mail:* [1] *sarka.tce@seznam.cz,* [2] *romana.sokolova@jh-inst.cas.cz,* [3] *magdalena.hromadova@jh-inst.cas.cz,* [4] *miroslav.gal@jh-inst.cas.cz,* [5] *viliam.kolivoska@jh-inst.cas.cz*

[b] *Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56100 Pisa, Italy; e-mail:* [1] *ilariad@dcci.unipi.it,* [2] *perla@dcci.unipi.it*

Received May 20, 2011
Accepted July 18, 2011
Published online January 4, 2012

Paper submitted for a special issue of CCCC devoted to "distinguished followers of the J. Heyrovský School of Polarography". This work is dedicated to Dr. L. Pospíšil on the occasion of his 70th birthday.

The influence of the molecular cavity protection on degradation processes of bioorganic compounds quercetin and luteolin used as the original dyes in old tapestries was studied. The degradation processes were studied by electrochemical methods in aqueous media. The products of the exhaustive electrolysis were separated and identified by GC-MS analysis. Cyclic voltammetry characteristics indicate that the inclusion complex is formed. The inclusion affects the redox potentials of both oxidation waves related to the different dissociation forms of the flavonoid molecule. It was shown that decomposition products formed by the oxidation of quercetin are stabilized in the cavity of β-cyclodextrin, including the main oxidation product 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one. The formation of the 1:1 inclusion complex of luteolin with β-cyclodextrin is supported by the enhancement of fluorescence intensity. In the case of quercetin, a decrease of fluorescence intensity occurs when 1:1 inclusion complex with β-cyclodextrin is formed.

Keywords: Oxidation; Inclusion complexes; Protection against oxidation; Flavonoids; Cyclic voltammetry; Natural products.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) and luteolin (3′,4′,5,7-tetrahydroxyflavone) are widely studied because of their pharmacological importance (Chart 1). These flavonoid compounds have antioxidant activity, antihepatotoxic and anti-inflammatory properties[1]. They belong to the his-

doi:10.1135/cccc2011106

torical natural dyes used already in 15th and 16th century. They were extracted from vegetal and animal sources; yellow dyes were achieved by extracting flavonoids and their glycosides from a number of widespread plants (some species of *Rhamnus*, quercitron bark, *Quercus velutina* L., Alnus jorullensis, Salvia sagittata)[2,3]. The original color of medieval textiles is transformed by ambient aerobic environment. The oxidative degradation processes involve the presence of subsequent chemical reactions such as hydroxylation or dimerization[4]. The oxidative degradation leads to the formation of low molecular weight compounds such as 3,4-dihydroxybenzoic acid and gallic acid[5,6].

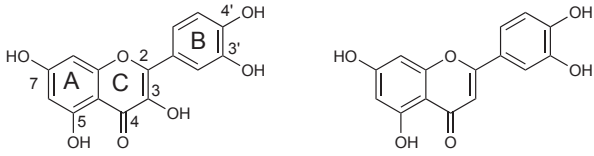


CHART 1

In order to establish the complexation properties of flavonoid molecules in the presence of host molecule it is necessary to have the knowledge of the dissociation constant of quercetin and luteolin. There are many studies dealing with estimation of dissociation constant in the literature. The controversy reported on the acid-base properties of both flavonoids was explained by strong influence of the oxygen on the stability of their dissociation forms in our recent paper[7,8]. We reported values of dissociation constant pK_1 = 5.87 ± 0.14 and pK_2 = 8.48 ± 0.09 of quercetin and pK_1 = 5.99 ± 0.32 and pK_2 = 8.40 ± 0.42 of luteolin, which were found under strict anaerobic conditions. The value of pK_1 of quercetin agrees with the lowest value reported in the literature by Escandar et al.[9].

The complexation properties of quercetin were mentioned in the case of its non-stable Cu(2+)-chelate[5]. The formation of a flavonoid-glutathione conjugate was shown by peroxidase-catalysed one electron oxidation of quercetin and luteolin in the presence of glutathione[10]. The stability constant of inclusion complex of undissociated quercetin with β-cyclodextrin (β-CD) determined by phase solubility study at pH 3 is 1028 M^{-1} according to Zheng et al.[11]. Values of stability constant found by fluorescence spectroscopy by Jullian et al.[12,13] at pH 7.4 correspond to mixture of dissociated and undissociated forms of quercetin or luteolin.

The present paper proves the protective properties of β-cyclodextrin against the oxidation process of the flavonoid dyes quercetin and luteolin. The protection properties of β-CD against the oxidation was found in case of catechin[14], chlorogenic acid[15], ferrocene derivatives[15,16] and chlorpromazine[17]. In addition, cyclodextrins have been successfully immobilized by physical or chemical bonds on the surface of fibres[18,19]. Since these flavonoid dyes undergo decomposition in the alkaline aqueous solution in the presence of oxygen[12], the study of the degradation processes of an anionic molecule of quercetin and luteolin encapsulated by β-CD under inert atmosphere of argon is proposed. Our preliminary results confirm that hydroxyl groups substituted on ring B of the flavonoids are protected against the oxidation by encapsulating by molecule of β-CD. The protection of these bioorganic molecules against the oxidation in the environment of cavity of a cyclodextrin has importance not only for restoration works and the best conservation of the tapestries and saving of the cultural heritage. The advantage of the complexation properties of these antioxidants turns profit in medical and cosmetic applications.

EXPERIMENTAL

Reagents

Quercetin and luteolin were purchased from Fluka. The reagents used as supporting electrolyte as tetrabutylammoniumhexafluorophosphate and acetonitrile were of reagent grade.

All the solvents were Carlo Erba (Milan, Italy) HPLC grade except from ethyl acetate (AcOEt), Anal R, BDH. Hexadecane and 2,4-dihydroxybenzophenone, used as internal standards, and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were purchased from Sigma (Milan, Italy). Standard solutions of analytes for GC-MS analysis were prepared in methanol (Carlo Erba, Milan, Italy).

All reagents and chemicals were used without any further purification.

Methods

Electrochemical measurements were done using an electrochemical system for cyclic voltammetry. It consisted of a fast rise-time potentiostat interfaced to a personal computer via an IEEE-interface card (AdvanTech, model PCL–848) and a data acquisition card (PCL-818) using 12bit precision. Cyclic voltammetry was also conducted using a PGSTAT 12 AUTOLAB potentiostat. A three-electrode electrochemical cell was used with an Ag|AgCl| 1 M LiCl reference electrode separated from the test solution by a salt bridge. The working electrodes were platinum electrode (0.8 mm) and glassy carbon electrode (0.7 mm). The auxiliary electrode was cylindrical platinum net. Oxygen was removed from the solution by passing a stream of argon. The oxidation products of quercetin were prepared by exhaustive electrolysis of its 0.7×10^{-3}–1×10^{-3} M solutions on carbon paste electrode.

Gas chromatography. The products were identified using a Trace GC gas chromatograph (Thermo Electron Corporation, USA) equipped with a PTV injection port and a mass spectrometric detector based on an ion trap analyzer (Polaris Q, Thermo Electron Corporation, USA). The PTV injector was in the CT 'splitless with surge' mode at 280 °C with a surge pressure of 100 kPa, and the mass spectrometer parameters were: electronic impact ionization (70 eV), ion source temperature 230 °C, scan range *m/z* 50–700 and interface temperature 280 °C. Chromatographic separation was performed on a DB-5MS chemically bonded fused silica capillary column (J & W Scientific, Agilent Technologies) with stationary phase 5% phenyl–95% methylpolysiloxane, and of dimensions 0.25 mm i.d., 0.1 μm film thickness, 25 and 30 m length. The gas chromatographic conditions were as follows: initial temperature 57 °C, 2 min isothermal, then ramped at 10 °C/min up to 200 °C, 3 min isothermal, then ramped at 20 °C/min up to 300 °C and then isothermal for 20 min. The carrier gas was He (purity 99.9995%), at a constant flow rate of 1.2 ml/min. The peak assignment was based on comparison with analytical reference compounds and materials with library mass spectra (NIST 1.7) and with mass spectra reported in the literature.

In order to perform GC-MS analysis, electrolysis products were derivatised with a silylating agent *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Derivatisation conditions are: 10 μl of 2,4-dihydroxybenophenone (solution in isopropanol; internal standard IS1) is added to the sample; the solution is dried and 30 μl of the derivatisation agent BSTFA in 50 μl of AcOEt is added; the reaction takes place in closed glass vials at 60 °C for 30 min. Just before injection, 10 μl of hexadecane (solution in isooctane; internal standard IS2) and 150 μl of AcOEt is added; 2 μl of the final solution is injected in the GC system.

Fluorescence spectroscopy. Fluorescence spectra were measured on LS50B luminescence spectrometer (Perkin–Elmer) in 1 cm^2 quartz cuvettes with both excitation and emission slit set at 10 nm. In the case of both flavonoids, quercetin and luteolin, the emission spectra were the recorded at the excitation wave length λ = 365 nm, where the highest sensitivity of emission was obtained. Since the flavonoid compounds decompose in the presence of air oxygen, all the solutions were prepared under the deaerated conditions. Data were statistically analyzed according to Eckschlager[20].

RESULTS AND DISCUSSION

The Electrochemical Properties

The electrochemical properties of quercetin and luteolin in aqueous media are related to the presence of their dissociation forms in solution. Similar behaviour is known in the case of the oxidation of other flavonoid compounds in aqueous solution[21]. In acidic and neutral solution cyclic voltammetry of quercetin on glassy carbon electrode yields three oxidation waves up to the potential 1.2 V (curve *1*, Fig. 1). The first oxidation wave belongs to two electron oxidation of 3',4'-dihydroxymoiety on ring B [4,22,23]. The oxidation wave of luteolin (curve *2*, Fig. 1) is more reversible in comparison with the oxidation wave of quercetin. The quinoid oxidation product formed by oxidation of quercetin and luteolin undergoes further chemical

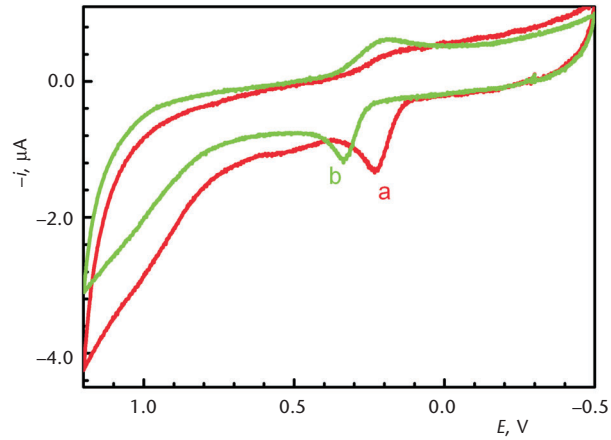


FIG. 1
Cyclic voltammogram of 2×10^{-4} M quercetin (a) and luteolin (b) in the solution of Britton–Robinson buffer and ethanol 3:2 (v/v) at pH 6.9 on glassy carbon microelectrode. The scan rate is 0.25 V s^{-1}

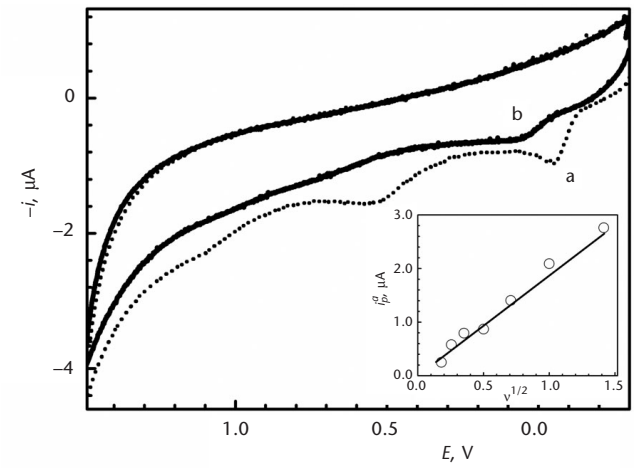


FIG. 2
Cyclic voltammogram of 6.5×10^{-4} M quercetin in 0.1 M KCl and 3.6×10^{-3} M KOH on glassy carbon microelectrode at different concentration of β-cyclodextrin: 0 (a), 1×10^{-3} M (b). The scan rate is 0.065 V s^{-1}. The dependence of the anodic peak current on scan rate is shown in inset

reaction in the case of quercetin and the oxidation product 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one is formed[24]. Its oxidation occurs at the second oxidation wave[28]. The complexation study is performed in alkaline aqueous solution because of poor solubility of both compounds and to avoid using of ethanol. The molecule of quercetin and luteolin is under alkaline conditions dissociated (pK_{que} = 5.87 and pK_{lut} = 5.99)[8,9]. Cyclic voltammetry of quercetin in alkaline solution on glassy carbon electrode shows three oxidation waves up to the potential 1.5 V (curve *1*, Fig. 2). The first oxidation wave at 0.05 V corresponds to the one electron oxidation of dissociated form of quercetin[25,26]. The consumption of one electron was confirmed coulometrically. From the linear dependence of peak current on square root of scan rate was found that this one electron process is diffusion controlled. At the potential of second oxidation wave a two electron oxidation of quercetin takes place. The third oxidation wave belongs to the oxidation of the oxidation product 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one.

When the β-cyclodextrin is present in the solution, the potential of the first oxidation wave is shifted to the more positive values (curve *2*, Fig. 2). The height of the oxidation wave is significantly lower in the presence of β-CD compare to the height of a free quercetin. This indicates that quercetin forms a complex with β-CD. The oxidation of complexed quercetin is more difficult and requires the dissociation of an inclusion complex, i.e. CE mechanism is involved in the oxidation mechanism (Eq. (*1*))[27]

$$[\mathrm{AH{\cdot}CD}] \underset{K_S}{\overset{K_d}{\rightleftharpoons}} \mathrm{AH} + \mathrm{CD}$$

$$\Big\updownarrow \text{electron transfer}$$

$$\text{products} \qquad (1)$$

where K_S is the constant of stability, K_d is the dissociation constant of a complex.

This one electron process is diffusion controlled, what was proved by linear dependence of peak current on square root of scan rate (see inset in Fig. 2). The dependence of the peak potential on the scan rate $\delta E/\delta \log v$ = 19 mV indicates that a disproportionation reaction takes place. The higher value of potential in the presence of β-cyclodextrin proves that a cavity of β-CD serves as a stabilizer of the molecule of quercetin.

A stability constant of the inclusion complex can be evaluated from the change of the standard redox potential in the absence and presence of complexing agent (Eq. (*2*))[28,29]

$$(E^0)_{\text{complex}} - (E^0)_{\text{free}} = \frac{RT}{nF}\ln\sqrt{\frac{D}{D^*}} - \frac{RT}{nF}\ln K_S - \frac{RT}{nF}\ln[\text{CD}] \qquad (2)$$

where D and D^* represent the diffusion coefficient of the free and complexed molecule, respectively, K_S is a stability constant of the complex. The values of standard redox potential were determined from cyclic voltammograms of quercetin in the absence and presence of β-CD on platinum microelectrode (Fig. 3). The inset of Fig. 3 shows the dependence of the peak current on square root of scan rate and confirmes that the oxidation process is diffusion controlled. The difference of standard redox potentials extrapolated from values of the anodic and cathodic potential (Fig. 4) is included in Table I. The estimation of standard redox potential for luteolin in the absence and presence of β-CD is in Fig. 5. The constants stability determined from the Eq. (*2*) are summarized in Table I for both flavonoid compounds, quercetin and luteolin. The cyclic voltammetry in the presence and in the absence of β-cyclodextrin (Fig. 2) shows that both dissociation forms are affected by the presence of β-CD. The shift of the peak potentials

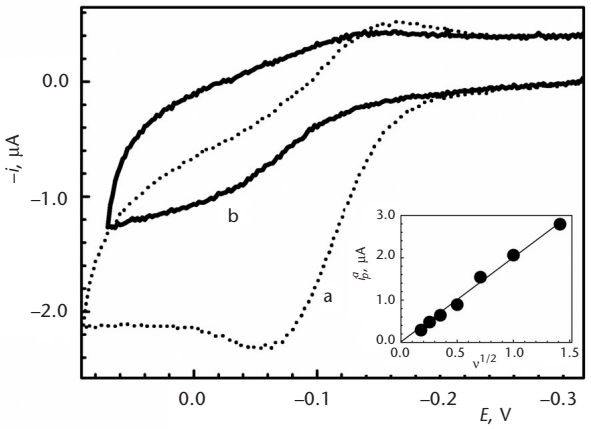


FIG. 3

Cyclic voltammogram of 6.5×10^{-4} M quercetin in 0.1 M KCl and 3.6×10^{-3} M KOH on Pt microelectrode at different concentration of β-cyclodextrin: 0 (a), 1×10^{-3} M (b). The scan rate is 0.032 V s^{-1}. The dependence of the anodic peak current on scan rate is shown in the inset

to the higher values confirms that both oxidation places, catechol ring B and hydroxyl group OH-7 interact with the cavity of β-cyclodextrin as was calculated by Yan et al.[30]. Bergonzi et al.[31] found that the interaction of B-ring of quercetin with β-cyclodextrin is the most favored. Jullian et al.[13] recently found that in case of the inclusion complex luteolin:β-cyclodextrin the B-ring of luteolin is oriented toward the primary ring of β-CD, while the A-ring of luteolin remains exposed to the external surface by the secondary ring.

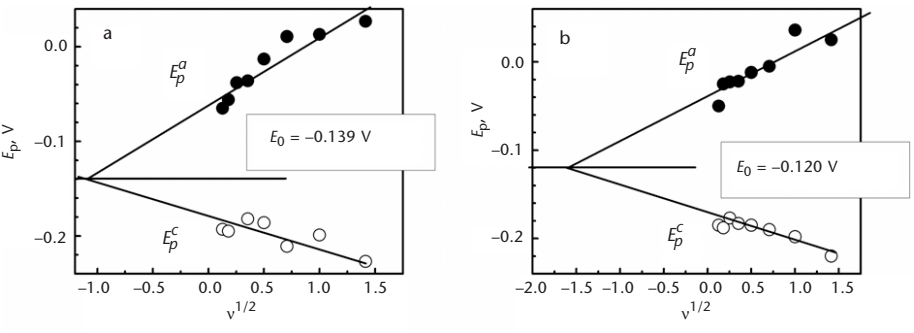


FIG. 4
The dependence of the peak potential on the square root of scan rate of 6.5×10^{-4} M quercetin in 0.1 M KCl and 3.6×10^{-3} M KOH on Pt microelectrode in the absence (a) and in the presence of 1×10^{-3} M β-cyclodextrin (b)

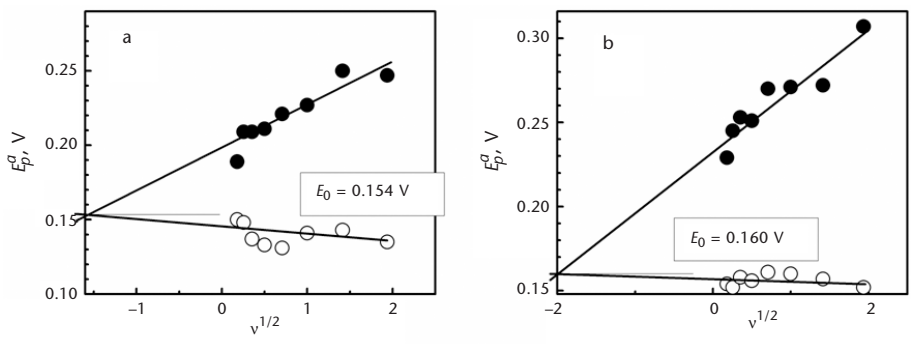


FIG. 5
The dependence of the peak potential on the square root of scan rate of 1×10^{-3} M luteolin in 0.1 M KCl and 3.6×10^{-3} M KOH on Pt microelectrode in the absence (a) and in the presence of 2×10^{-3} M β-cyclodextrin (b)

TABLE I
The determination of the stability constant of the inclusion complex of dissociated flavonoid:β-cyclodextrin

	Cyclic voltammetry			Fluorescence
	$(E^0)_{free}$, V	$(E^0)_{complex}$, V	K, M^{-1}	K, M^{-1}
Luteolin	0.1541 ± 0.0049	0.1601 ± 0.0058[a]	520 ± 220	720 ± 170
Quercetin	–0.1392 ± 0.0038	–0.1202 ± 0.0041[b]	600 ± 200	515 ± 50

[a] The ratio of the concentrations luteolin:β-cyclodextrin is 1:2. [b] The ratio of the concentrations quercetin:β-cyclodextrin is 1:2.

Fluorescence Spectroscopy

In order to support the formation of an inclusion complex of studied flavonoids with β-cyclodextrin the fluorescence measurements were provided. The fluorescence spectra of luteolin recorded at different concentrations of β-cyclodextrin are shown in Fig. 6. The increasing concentration of β-cyclodextrin causes the enhancement of fluorescence intensity of luteolin. Since β-cyclodextrin does not yield emission under the same experi-

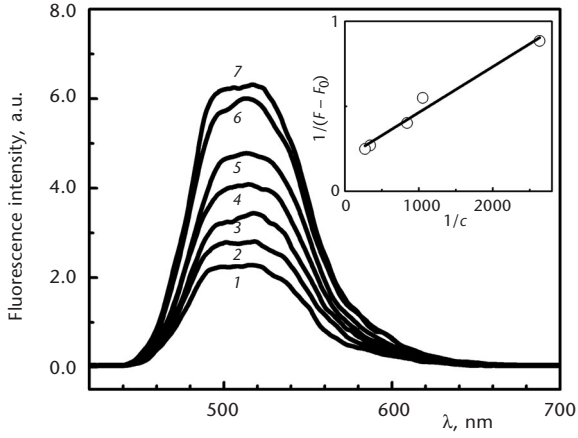


FIG. 6
Fluorescence emission spectra of 3×10^{-4} M luteolin in 3.6×10^{-3} M KOH at different concentration of β-cyclodextrin: 0 (*1*), 2.3×10^4 (*2*), 3.8×10^4 (*3*), 9.5×10^4 (*4*), 1.2×10^3 (*5*), 2.9×10^3 (*6*), 3.7×10^3 M (*7*) at λ_{exc} = 365 nm. The Benesi–Hildebrand plot for 1:1 luteolin:β-cyclodextrin inclusion complex is shown in inset

mental conditions, this behaviour indicates the formation of an inclusion complex.

The stability constant (K_S) of the inclusion complex can be calculated by the use of the modified Benesi–Hildebrand equation[32,33]

$$\frac{1}{F - F_0} = \frac{1}{F_\infty - F_0} + \frac{1}{(F_\infty - F_0)K_S[\mathrm{CD}]_0} \qquad (3)$$

where $[\mathrm{CD}]_0$ is the initial concentration of cyclodextrin, F_0 denotes the fluorescence intensity of flavonoid in the absence of cyclodextrin, F_∞ is the fluorescence intensity when all of the flavonoids molecules are essentially complexed with cyclodextrin, F is the observed fluorescence at each tested concentration of cyclodextrin, and K_S is the stability constant of the complex. The inset of the Fig. 6 shows the dependence of $1/(F - F_0)$ on $1/[\mathrm{CD}]$ and a straight line is obtained (coefficient of determination $D = 100R^2 = 96.83$). This indicates that the ratio luteolin:β-CD is 1:1. The same ratio was confirmed also in the case of quercetin and agrees with findings in literature[13,34]. The continuous variation method confirmed also the ratio 1:1 (Fig. 7). The emission band at 530 nm is characteristic for pyrilium tautomers[35]. The intensity of fluorescence of luteolin is very low due to the faible formation of a pyrilium form upon intramolecular proton transfer from

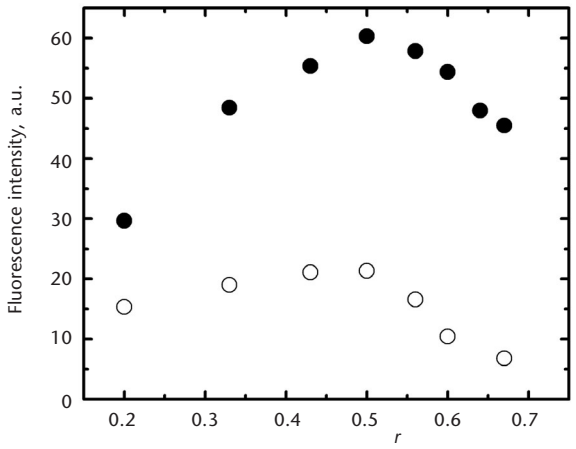


FIG. 7
Continuous variation plot for quercetin:β-cyclodextrin (●) and luteolin:β-cyclodextrin (○) inclusion complex (Jobb plot)

OH-5 to O-4. The binding into the β-cyclodextrin cavity causes the protonation of O-4 and the enhancement of fluorescence is obtained. Dangles et al.[41] underlined the fluorescence behaviour of pyrilium cations formed during intramolecular protonation of some flavonoids. The fast intramolecular proton transfer from OH-7 group to the 4-oxo group was obtained in the case of 3-hydroxyflavone and 7-hydroxyflavone[36,37].

The change of the fluorescence spectra of quercetin shows a reversed trend (Fig. 8). The intensity of emission spectra of quercetin decreases with increasing concentration of β-cyclodextrin. The stability constant of an inclusion complex of dissociated quercetin with β-CD was determined from the linear dependence shown in the inset of Fig. 8 (D = 99.77). The stability constants are summarized in Table I. The ratio of the guest:β-cyclodextrin was confirmed also by the continuous variation method (see Fig. 7). The values found by fluorescence measurement are in agreement with values obtained from cyclic voltammetry. The strong fluorescence of quercetin is consistent with a pyrilium-like structure formed by the intramolecular protonation of O-4 by OH-3 group. The binding into the cavity of β-cyclodextrin probably avoid the intramolecular protonation of O-4 by OH-3 group. Nevertheless, the fluorescence intensity in the presence of β-CD remains still higher than in the case of luteolin. The protonation of O-4 must occur in the cavity of β-CD.

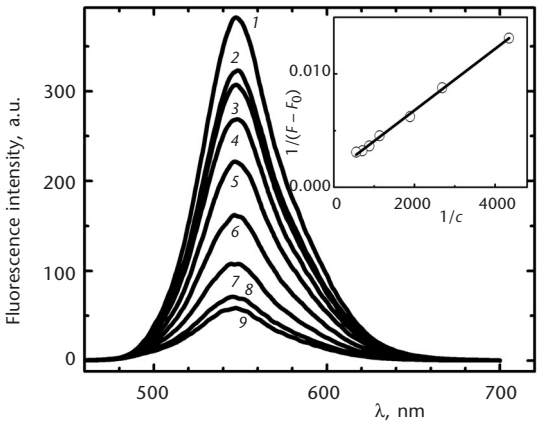


FIG. 8
Fluorescence emission spectra of 3×10^{-4} M quercetin in 3.6×10^{-3} M KOH at different concentration of β-cyclodextrin: 0 (*1*), 6.2×10^{5} (*2*), 2.3×10^{4} (*3*), 3.7×10^{4} (*4*), 5.3×10^{4} (*5*), 8.8×10^{4} (*6*), 1.1×10^{3} (*7*), 1.4×10^{3} (*8*), 1.8×10^{3} M (*9*) at λ_{exc} = 365 nm. The Benesi–Hildebrand plot for 1:1 quercetin:β-cyclodextrin inclusion complex is shown in the inset

The higher value of the stability constant of luteolin inclusion complex compare to quercetin reflects the hydrophobicity of a guest molecule. Our measurements are performed in alkaline solution where both flavonoid molecules are dissociated. In the case of quercetin the values of the stability constant of anion:β-cyclodextrin 602 M^{-1} (found electrochemically) or 515 M^{-1} (fluorescence spectroscopy) are in agreement with finding in literature by Zheng et al.[12]. Zheng et al. demonstrated that the stability constant of the inclusion complex of undissociated quercetin with hydroxypropyl-β-cyclodextrin (HPβ-CD) is significantly higher than the stability constants of its dissociated form (1009 M^{-1}). The latter value of stability constant of dissociated form with HPβ-CD is higher than our value of stability constant of the complex with β-CD (Table I). This suggests that the anion is bound more strongly to HPβ-CD than to β-CD. The similar order was found in the case of undissociated quercetin[12] and in the case of a mixture of anion and undissociated quercetin complexed with β-CD [14]. As expected, the value of stability constant of dissociated quercetin found in this work[12] is lower than the constant for undissociated molecule 1028 M^{-1}.

The strong influence of air oxygen on emission spectra was found in case of both flavonoids. This was the reason why all the fluorescence measurements were performed under deaerated conditions as in case of electrochemical studies. In the case of luteolin exposed to the air (Fig. 9a) the fluorescence intensity at 495 nm significantly increases and is accompanied by blue shift up to 488 nm. The increase of emission band at 488 nm most

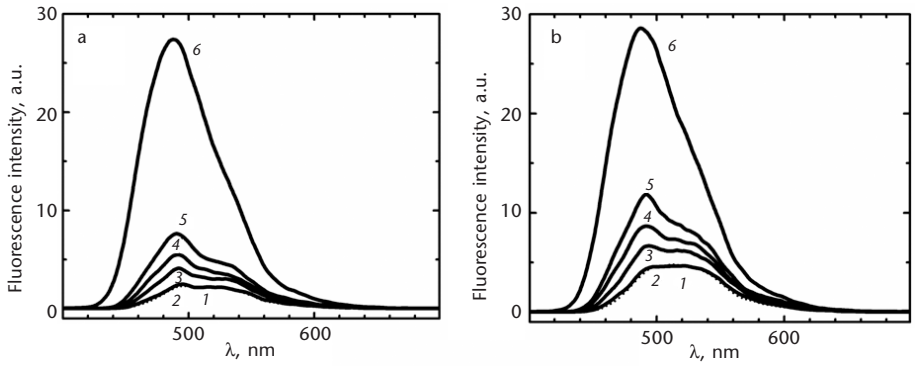


FIG. 9
Fluorescence emission spectra of 3×10^{-4} M luteolin in 3.6×10^{-3} M KOH in the absence (a) and in the presence of 1.2×10^{3} M β-cyclodextrin (b) recorded at the different time (in min) of exposure to the air oxygen: 0 (*1*), 54 (*2*), 76 (*3*), 119 (*4*), 163 (*5*), 938 (*6*). The excitation wavelength is λ_{exc} = 365 nm

likely belongs to an oxidation product of luteolin. When β-CD is present in the solution of luteolin, the presence of oxygen causes the same effect as in the absence of β-CD (Fig. 9b). The difference of fluorescence intensities at 488 nm of the curves exposed 15 h and 38 min to the presence of air in the absence and presence of β-CD is negligible (compare curves *6* in Fig. 9a and 9b). So, the part of molecule of luteolin, which undergoes the oxidation, is not hidden in the cavity of β-cyclodextrin. This implies that the oxidation of luteolin takes different reaction pathway than the oxidation process of quercetin. The cyclic voltammetry of luteolin (curve *2*, Fig. 1) shows higher reversibility of the oxidation process than in the case of quercetin (curve *1*, Fig. 1). This indicates that a presence of a chemical reaction following the electron transfer plays an important role in the case of quercetin. This is in agreement with the fast formation of the oxidation product 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one, which was found as the oxidation product of quercetin in acetonitrile[28].

Identification of Products

The products obtained by an exhaustive electrolysis at 1.02 V were separated and GC-MS analysis confirmed the formation of 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one (**P5**) as the main product. The main fragments of the derivatized **P5**-5TMS correspond to *m/z* values:

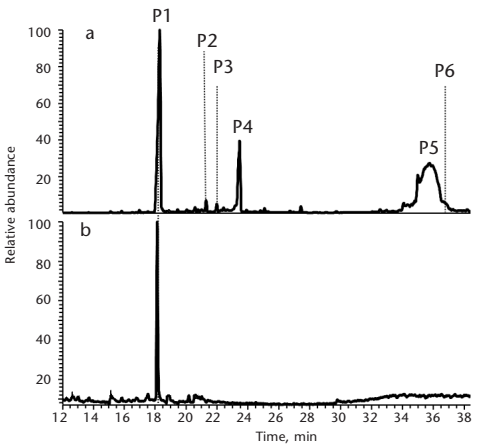


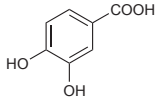
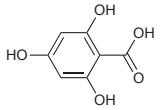
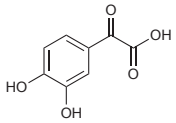
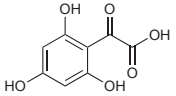
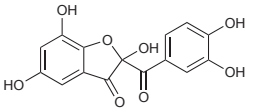
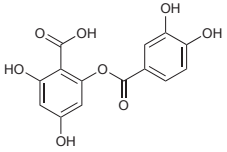
FIG. 10
Gas chromatogram of the solution of quercetin after the exhaustive electrolysis at 1.1 V after the removing of the precipitate (see the text) in the presence of 1×10^{-2} M β-cyclodextrin (a) and in the absence of β-cyclodextrin (b)

663 ([M – CH_3]$^{\bullet+}$), 397 ([M – ring B]$^+$), 369 ([M – ring B – CO]$^+$), 281 ([M – ring B – CO – OTMS]). This oxidation product was found also in the literature as the one final oxidation product in nonaqueous media[28,38] and agrees with reports[5,39].

The difference in the distribution of oxidation products of quercetin in the presence and in the absence of β-CD is shown in Fig. 10. Oxidation

TABLE II

The products of oxidation of 1 mM quercetin in 0.1 M KCl/3.6 mM KOH at the potential of the second oxidation wave in the presence of 10 mM β-cyclodextrin

Peak	Structure	T_r, min	Main *m/z*	Molecular weight	
				Not derivatized	Derivatized
P1		18.1	370, 355, 193	154	370
P2		21.2	443, 353	170	458
P3		22.0	383, 281	182	398
P4		23.4	471, 369	198	468
P5		35.6	678, 369, 281	318	678
P6		37.3	651, 355	306	666

products labeled as **P1–P6** are summarized in Table II. Gas chromatogram of solution after the electrolysis without presence of β-CD (Fig. 10b) yields 3,4-dihydroxybenzoic acid. The gas chromatogram of solution after electrolysis shows the presence of the main oxidation product **P5** and other compounds. Since the strong influence of the presence of oxygen on the decomposition of **P5** was found[8], it is evident that the cavity of β-CD protects the oxidation of **P5** and stabilizes its oxidation products such as oxoacetic acid **P4**.

CONCLUSION

A poor stability of quercetin while kept in the presence of air oxygen was confirmed by fluorescence spectroscopy. The study proved a protective formation of inclusion complex of quercetin with β-cyclodextrin. It was shown that decomposition products formed by the oxidation of quercetin are stabilized in the cavity of β-cyclodextrin, mainly 2(3′,4′-dihydroxybenzoyl)-2,4,6-trihydroxybenzofuran-3(2*H*)-one. This oxidation product decomposes in the presence of oxygen to 3,4-hydroxybenzoic acid and other hydroxy compounds, when β-cyclodextrin is not present in solution. The formation of inclusion complex plays a role in the stabilization of oxoacetic acid found as one of oxidation products in the presence of oxygen.

In the contrary, molecule of luteolin encapsulated in the cavity of β-CD is oxidised at part, which is not hidden in the cavity. In long time scale, the molecule of β-cyclodextrin does not protect luteolin against the oxidation by air oxygen. Nevertheless, the oxidation of luteolin complexed with β-cyclodextrin is more difficult than the oxidation of its free molecule.

Electrochemical behaviour of quercetin and luteolin confirmed that both dissociation forms are affected by the interaction with the cavity of β-cyclodextrin. Nevertheless, values of constants stability 515 ± 50 and 720 ± 170 M^{-1} for the complex of β-cyclodextrin and dissociated molecule of quercetin and luteolin, respectively, determined by fluorescence spectroscopy are more precise than values found electrochemically. All the measurements were done under inert atmosphere to avoid the influence of oxygen.

This work is supported by the Czech Science Foundation (grants 203/09/1607 and 203/08/1157).

REFERENCES

1. Di Carlo G., Mascolo N., Izzo A. A., Capasso F.: *Life Sci.* **1999**, *65*, 337.

2. Hofenk de Graaf J.: *The Colourful Past, Origins, Chemistry and Identification of Natural Dyestuffs*. Abegg-Stiftung and Archetype Publications Ltd., London 2004.
3. Wouters J., Rosario-Chirinos N.: *J. Am. Inst. Conserv.* **1992**, *31*, 237.
4. Hendrickson H. P., Kaufman A. D., Lunte C. E.: *J. Pharm. Biomed. Anal.* **1994**, *12*, 325.
5. Makris D. P., Rossiter J. T.: *J. Food Compos. Anal.* **2002**, *15*, 103.
6. Zhou A., Kikandi S., Sadik O. A.: *Electrochem. Commun.* **2007**, *9*, 2246.
7. Ramešová Š., Sokolová R., Degano I., Žabka J., Bulíčková J., Gál M.: *Anal. Bioanal. Chem.* **2012**, *402*, 975.
8. Ramešová Š.: *Diploma Thesis*. Charles University in Prague, Department of Analytical Chemistry, Prague 2011.
9. Escandar G. M., Sala L. F.: *Can. J. Chem.* **1991**, *69*, 1994.
10. Galati G., Moridani M. Y., Chan T. S., O'Brien P. J.: *Free Radical Biol. Med.* **2001**, *30*, 370.
11. Zheng Y., Haworth I. S., Zuo Z., Chow M. S. S., Chow A. H. L.: *J. Pharm. Sci.* **2005**, *94*, 1079.
12. Jullian C., Cifuentes C., Alfaro M., Miranda S., Barriga G., Olea-Azar C.: *Bioorg. Med. Chem.* **2010**, *18*, 5025.
13. Jullian C., Moyano L., Yañez C., Olea-Azar C.: *Spectrochim. Acta, Part A* **2007**, *67*, 230.
14. Fayad N., Marchal L., Billaud C., Nicolas J.: *J. Agric. Food Chem.* **1997**, *45*, 2442.
15. Matsue T., Evans D. H., Osa T., Kobayashi N.: *J. Am. Chem. Soc.* **1985**, *107*, 3411.
16. Strelets V. V., Mamedjarova I. A., Nefedova M. N., Pysnograeva N. I., Sokolov V. I., Pospíšil L., Hanzlík J.: *J. Electroanal. Chem.* **1991**, *310*, 179.
17. Takamura K., Inoue S., Kusu F., Otagiri M., Uekama K.: *Chem. Pharm. Bull.* **1984**, *32*, 839.
18. Kistamah N., Carr C. M., Rosunee S.: *J. Mater. Sci.* **2006**, *41*, 2195.
19. Zyschka R., Brückmann R., Kammerer B., Schreiber H.: *Textilveredlung* **2004**, *39*, 10.
20. Eckschlager K.: *Chemometrie*. Univerzita Karlova v Praze, Karolinum, Praha 1991.
21. Sokolová R., Degano I., Hromadová M., Bulíčková J., Gál M., Valášek M.: *Collect. Czech. Chem. Commun.* **2010**, *75*, 1097.
22. Zare H. R., Namazian M., Nasirizadeh N.: *J. Electroanal. Chem.* **2005**, *584*, 77.
23. Kang J., Lu X., Zeng H., Liu H., Lu B.: *Anal. Lett.* **2002**, *35*, 677.
24. Sokolová R., Degano I., Ramešová Š., Bulíčková J., Hromadová M., Gál M., Fiedler J., Valášek M.: *Electrochim. Acta* **2011**, *56*, 7421
25. Richards J. A., Whitson P. E., Evans D. H.: *J. Electroanal. Chem.* **1975**, *63*, 311.
26. Morrow G. W.: Anodic Oxidation of Oxygen-Containing Compounds in "Organic Electrochemistry" (H. Lund and O. Hammerich, Eds), 4th ed., p. 589. Marcel Dekker Inc., New York 2001.
27. Pospíšil L., Trsková R., Colombini M. P., Fuoco R.: *J. Inclusion Phenom.* **1998**, *31*, 57.
28. Koryta J.: *Electrochim. Acta* **1959**, *1*, 26.
29. Heyrovský J., Kůta J.: *Základy polarografie*, pp. 111–118. Nakladatelství Československé akademie věd, Praha 1962.
30. Yan C., Xiu Z., Li X., Teng H., Hao C.: *J. Inclusion Phenom. Macrocycl. Chem.* **2007**, *58*, 337.
31. Bergonzi M. C., Bilia A. R., Di Bari L., Mazzi G., Vincieri F. F.: *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5744.
32. Muñoz de la Peña A., Salinas F., Gómez M. J., Acedo M. I., Sánchez Peña M.: *J. Inclusion Phenom.* **1993**, *15*, 131.
33. Shamsipur M., Yari A., Sharghi H.: *Spectrochim. Acta, Part A* **2005**, *62*, 372.

34. Kim H., Choi J., Jung S.: *J. Inclusion Phenom.* **2009**, *64*, 43.
35. Dangles O., Dufour C., Bret S.: *J. Chem. Soc., Perkin Trans. 2* **1999**, 737.
36. Schipfer R., Wolfbeis O. S., Knierzinger A.: *J. Chem. Soc., Perkin Trans. 2* **1981**, 1443.
37. Itoh M., Adachi T.: *J. Am. Chem. Soc.* **1984**, *106*, 4320.
38. Jorgensen L. V. Cornett C., Justesen U., Skibsted L. H., Dragsted L. O.: *Free Radical Res.* **1998**, *29*, 339.
39. Jungbluth G., Ruhling I., Ternes W.: *J. Chem. Soc., Perkin Trans. 2* **2000**, 1946.