Spectrochimica Acta Part A 81 (2011) 661–665

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Effect of inclusion complex on nitrous acid reaction with flavonoids

Lida Khalafi [a,*], Mohammad Rafiee [b], Sajjad Sedaghat [a]

[a] Department of Chemistry, Faculty of Science, Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran
[b] Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

ARTICLE INFO

Article history:
Received 27 March 2011
Received in revised form 18 June 2011
Accepted 26 June 2011

Keywords:
Catechin
Quercetin
Nitrous acid
β-Cyclodextrin

ABSTRACT

The kinetic of the nitrous acid reactions with quercetin and catechin has been studied using spectrophotometric method in aqueous solution. The results show that these antioxidants participate in oxidation reactions with nitrous acid which is derived from protonation of nitrite ion in mild acidic conditions. Corresponding *o*-quinones as relatively stable products were detected by spectrophotometric techniques. pH dependence of the reactions has been examined and the rate constants of reactions were obtained by non-linear fitting of kinetic profiles. The effect of β-cyclodextrin on the oxidation pathway was another object of this study. It is shown that β-cyclodextrin has an inhibitory effect on the oxidation reaction. The rate constants of oxidation reactions for complexed forms and their stability constants were obtained based on changes in the reaction rates as a function of β-cyclodextrin concentration.

1. Introduction

An antioxidant is defined as a molecule capable of slowing or preventing the oxidation of other molecules [1]. Among them flavonoids are the most widely used group which are isolated from a wide range of vascular plants [2]. They are found in substantial levels in commonly consumed fruits, vegetables and beverages. Flavonoids have aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. They have several biological activities including radical scavenging, anti-inflammatory, antimutagenic and anti-cancer activities [3]. It also has been shown that they enhance product stability, quality, and shelf life in food technology [4]. In seeds, they act in protection against predators and pathogens [5], increase seed coat (testa)-imposed dormancy [6,7] and protect against UV radiations [8]. Quercetin and catechin (Scheme 1) as natural flavonoids are selected for this study; quercetin is a frequent component of major dietary constituents, such as onions and apples. Catechin is extracted from plants and present in natural food and drinks, such as green tea.

They have been described extensively due to their broad biological properties, which are related to their antioxidant activity [9]. They are often referred as antioxidants on account of their ability of protecting against damages caused by reactive oxygen or nitrogen species, ROS/RNS [10].

On the other hand nitrous acid and nitrite ion are conjugate pair of species found at ambient levels in the atmosphere. They are significant components in environmental and biological chemistry [11–13]. Several studies have revealed that nitrous acid can react with flavonoids [14–16]. We have also studied the reaction of nitrous acid and nitrite ion with catechols and catecholamines in some kinetic details [13,17].

Finally cyclodextrins (CDs) are cyclic oligosaccharides composed of D(+)glucopyranose units with the shape of a torus with a hydrophilic exterior and a hydrophobic interior. They are well known in host guest chemistry due to their unique ability to form inclusion complexes with numerous compounds. The interaction of guest molecules with CD leads to apparent changes in their chemical properties, such as kinetic and thermodynamic parameters [18–20]. The actual or potential uses of CDs in pharmaceuticals, foods and cosmetics are well-known [21–24]. This paper focuses on the reactions of nitrous acid with quercetin and catechin in the presence of β-cyclodextrin (β-CD) and effect of inclusion complex on their reactions.

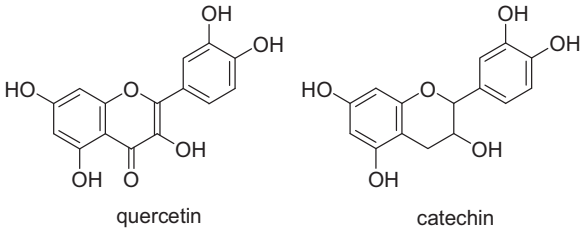
2. Materials and methods

2.1. Materials and solutions

All experiments were performed with analytical reagent grade chemicals purchased from E. Merck. These chemicals were used without further purification. The stock solutions of, catechin, sodium nitrite and β-CD were prepared fresh, daily by dissolving them in distilled water. The stock solution of quercetin was prepared daily by dissolving it in distilled water/ethanol (60/40) solution. The buffered solutions were prepared based on Kolthoff tables, and the concentration of the prepared buffers was 0.15 M [17]. Nitrogen gas with a purity of 99.999% was used to remove

* Corresponding author. Tel.: +98 2146842936; fax: +98 2146842936.
E-mail address: l_khalafi@yahoo.com (L. Khalafi).

1386-1425/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2011.06.070



Scheme 1. Structure of quercetin and catechin.

oxygen from solution. The kinetic experiment was initiated by the addition 0.5 ml of 5.0 mM stock solution of catechin or quercetin to 4.5 ml 3.3 mM sodium nitrite solution with desired pH and β-CD concentrations in a 5 ml flask. All of the solutions were thermostated at 25 °C before mixing. Measurements were also performed at this temperature. The reaction mixture was shaken and transferred to the spectrophotometer quartz cell immediately. The cell placed in cell holder and reaction monitored by full spectral scan with time.

2.2. Apparatus

Absorption spectra were obtained with a Scinco UV–vis Spectrophotometer S2100. In each experiment, the sample placed in a 1 mm path length quartz cells, and the measurements were performed at 25 °C. All of the calculations were performed in MATLAB 7.5 (Math Works, Cochituate Place, MA).

3. Results and discussion

3.1. Reaction study

Fig. 1 shows the absorption–time of 0.5 mM quercetin in the presence of 3.0 mM sodium nitrite at pH 3.40. At the initial time quercetin shows two absorption bands with λ_{max} of 251 and 365 nm respectively. Upon initiation of the reaction, the absorbance at 251 and 365 nm gradually decrease whereas the absorption band at 289 nm appears and its height increases with time. At the end of the reaction, the two peaks with λ_{max} 251 and 365 nm disappear, and the new band (λ_{max} 289 nm) reaches to its maximum value. The solution containing sodium nitrite in the same condition and absence of quercetin did not absorb in this region. These absorbance changes confirm the reactivity of quercetin toward nitrous acid at this pH. The absorbance with λ_{max} 289 nm is related to the oxidized form of quercetin [14]. Two possible oxidation products are semiquinone or quinine forms, but it has been reported that the semiquinone form is not stable in aqueous solutions and easily converts to the more stable quinonic form [25].

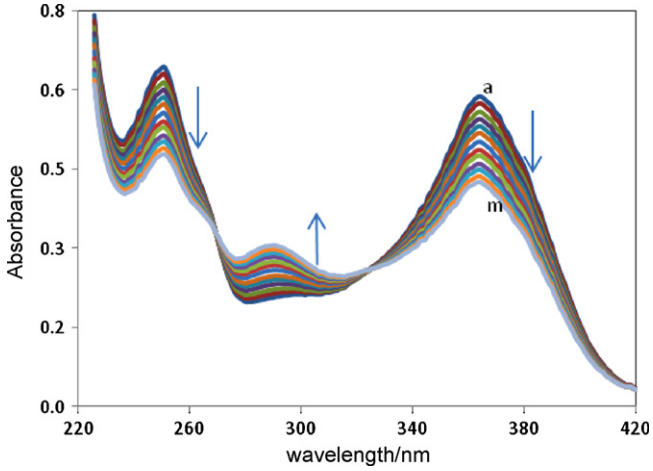


Fig. 1. Absorption spectra for 0.5 mM quercetin in the presence of 3.0 mM sodium nitrite with time, time intervals 30 s, pH 3.40, solvent 85/15 water/ethanol.

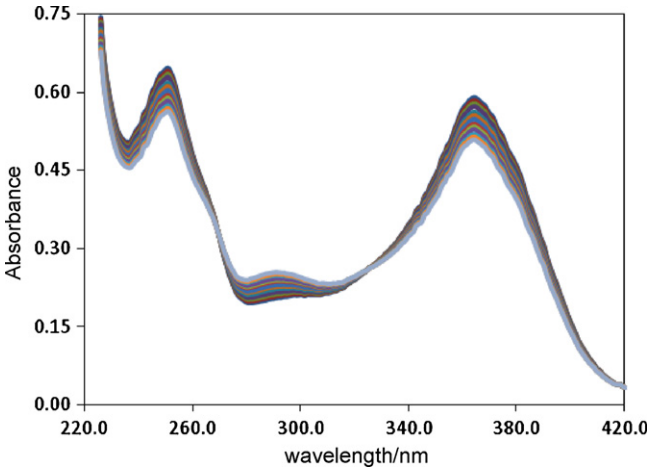


Fig. 2. Absorption spectra for 0.5 mM quercetin in the presence of 3.0 mM sodium nitrite with time, time intervals 30 s, pH 4.0.

The most probable reactions are oxidation with nitrous acid or catalytic oxidation by dissolved oxygen. The possibility of oxidation with dissolved oxygen has been examined and results did not show any considerable difference in the presence and absence of oxygen, it was removed by pumping nitrogen. Nitrous acid is a weak and monobasic acid with dissociation constant (K_a) which is generally quoted in the literature as 5.1×10^{-4} M, pK_a = 3.27 [26]. Considering this solution composition and pH value nitrous acid act as oxidant in the reaction. For more details we extended our studies at different pH values around the pK_a of nitrous acid. Fig. 2 shows the absorption time of quercetin in the presence of sodium nitrite at pH 4.10. Comparison of Figs. 1 and 2 shows that the reaction rate decreases with increasing pH. It is due to increase in nitrous acid concentration at lower pHs which has been discussed in our previous paper [13].

Spectrophotometric study of interaction of catechin has been performed in the presence of sodium nitrite at various pHs and shows the same results as quercetin. Fig. 3 shows the spectrum

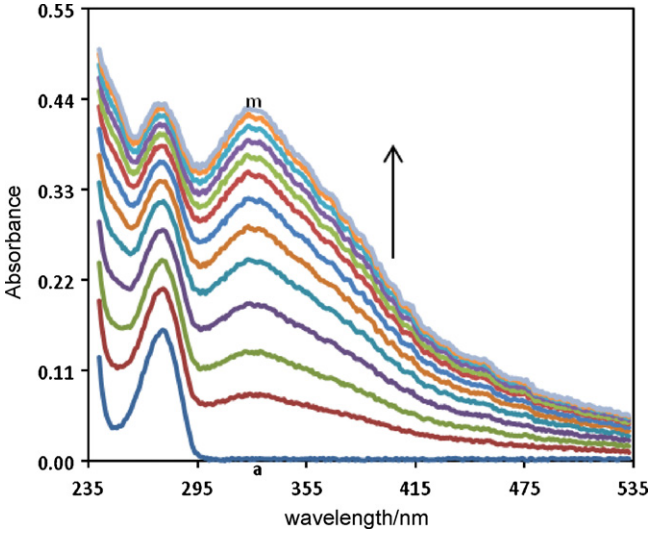


Fig. 3. Absorption spectra for 0.5 mM catechin in the presence of 3.0 mM sodium nitrite with time, time intervals 90 s, pH 3.40.

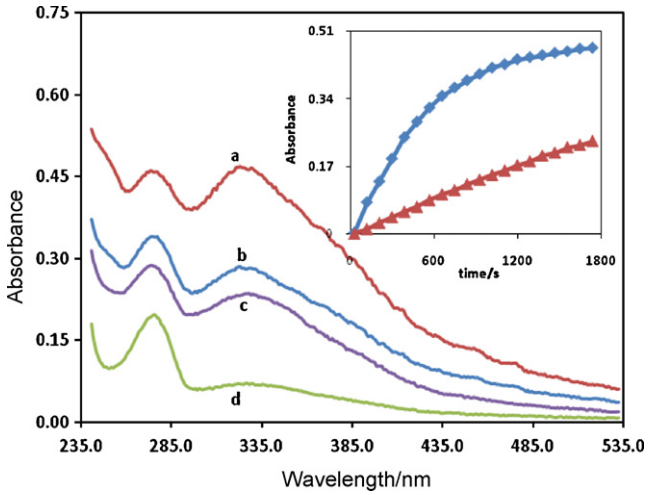


Fig. 4. Absorption spectra for 0.5 mM catechin in the presence of 3.0 mM sodium nitrite; (a and b) in the absence, (c and d) in the presence of 10.0 mM β-CD with time. (b and d) at 480 s (a and c) at 960 s. Inset: The absorbance–time plots of reactions at 330 nm.

of 0.5 mM catechin in the presence of 3.0 mM sodium nitrite at pH 3.40. An absorption band with λ_{max} 321 nm belong to quinonic form of catechin appears and its height increases with time.

3.2. The effect of β-cyclodextrin

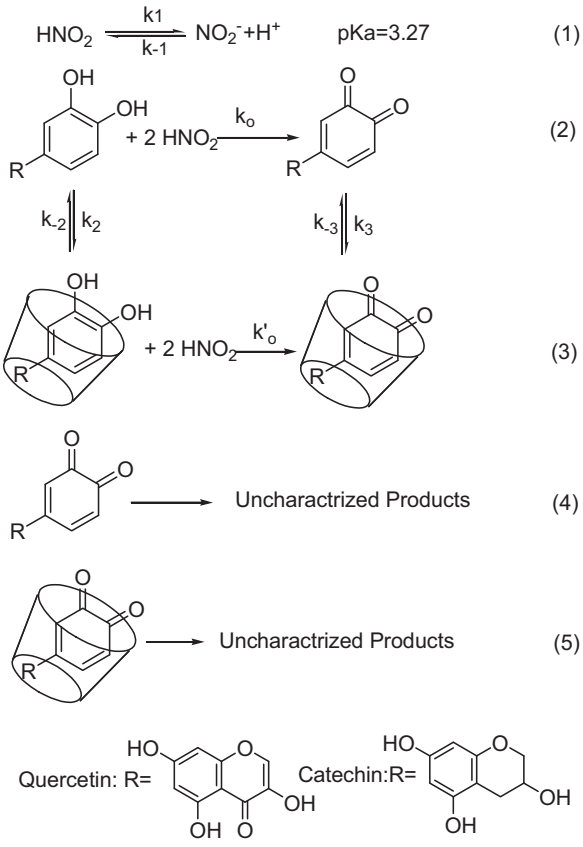
Fig. 4 shows the absorption spectra for the reaction of catechin with sodium nitrite; in the absence and presence of β-CD at pH 3.40. In order to compare the reaction rates the absorbance changes at 320 nm were monitored and the kinetic profiles are shown in the inset of this figure.

It shows that in the presence of β-CD the rate of oxidation reaction decreases drastically. Addition of β-CD to the quercetin causes to formation of a 1:1 inclusion complex between them. The decrease in the rate of the reaction by addition of β-CD indicates that the rate constant for the oxidation of the complexed form of quercetin is smaller than for its free form [19]. The results for the spectrophotometric study of quercetin in the presence of various concentrations of β-CD are the same as the results that obtained for catechin. Based on these results the following pathway is presented for the reaction of these flavonoids in the presence of β-CD.

The acid base reaction of nitrous acid and nitrite ion is an equilibrium. The formation of inclusion complex between flavonoids and β-CD is also reversible and equilibrium reaction. Presence of equilibrium between the reactants of these parallel reactions follows a complicated reaction pathway. The observed reaction rates are roughly proportional to the equilibrium concentration of free and complex forms of H_2Q and nitrous acid. Based on the proposed reaction mechanism consumption of each reactants shifts the equilibriums to counteract the imposed changes. For example consumption of H_2Q due to its higher rate constants shifts the complexation reaction to produce the free form. Also in the right side of the reaction the ratio of Q to QCD is the function of their production, formation constant of QCD and β-CD concentration. Produced quinones and their complex forms undergo some side reactions such as nitration, cyclization and degradation. But the results show that they are relatively stable and the rates of their conversion are negligible in the time scale of our spectrophotometric study.

3.3. Kinetic evaluation

By assuming the formation of a 1:1 inclusion complex between the antioxidants and their oxidized forms with β-CD and considering Eqs. (1)–(3) in Scheme 2 as the main route of reaction we can write the following equations:



Scheme 2. Proposed mechanism for the reactions of quercetin and catechin with nitrous acid.

$$\frac{d[HNO_2]}{dt} = -k_1[HNO_2] + k_{-1}[NO_2^-][H^+] - 2k_o[H_2Q][HNO_2] - 2k'_o[H_2QCD][HNO_2] \quad (6)$$

$$\frac{d[NO_2^-]}{dt} = k_1[HNO_2] - k_{-1}[NO_2^-][H^+] \quad (7)$$

$$\frac{d[H_2Q]}{dt} = -2k_o[H_2Q][HNO_2] - k_2[H_2Q][CD] + k_{-2}[H_2QCD] \quad (8)$$

$$\frac{d[Q]}{dt} = 2k_o[H_2Q][HNO_2] - k_3[Q][CD] + k_{-3}[QCD] \quad (9)$$

$$\frac{d[H_2QCD]}{dt} = -2k'_o[H_2QCD][HNO_2] + k_2[H_2Q][CD] - k_{-2}[H_2QCD] \quad (10)$$

$$\frac{d[QCD]}{dt} = 2k'_o[H_2QCD][HNO_2] + k_3[Q][CD] - k_{-3}[QCD] \quad (11)$$

where H_2Q and Q stand for uncomplexed forms of flavonoids and their oxidized products respectively and H_2QCD and QCD stand for their complexed forms. Considering this fact that equilibrium acid base and complex formation reactions are very fast in comparison with desired reaction we can write:

$$\frac{[NO_2^-]}{[HNO_2]} = \frac{k_1}{k_{-1}[H^+]} = \frac{K_a}{[H^+]} \quad (12)$$

$$\frac{[H_2QCD]}{[H_2Q]} = \frac{k_2[CD]}{k_{-2}} = K_{fH_2Q}[CD] \quad (13)$$

Table 1
Kinetic and thermodynamic data for the reaction of nitrous acid in the presence of β-CD.

Compound	K_{fH_2Q} (M^{-1})	K_{fQ}(M^{-1})	k_o*(M^{-1} s^{-1})	k'_o (M^{-1} s^{-1})*	Lack of fit %**
Quercetin	2700	1900	1.9	0.4	2.3
Catechin	2060	1450	2.3	0.5	1.8

* Reported rate constants are the average of three measurements and fitting at pHs 3.0, 3.2 and 3.4.

** Lack of fit (%) $= 100 \times \sqrt{\sum_{ij} e_{ij}^2 / \sum_{ij} d_{ij}^2}$ where e_{ij} are residuals and d_{ij} are the elements of the experimental data matrix at pH 3.4.

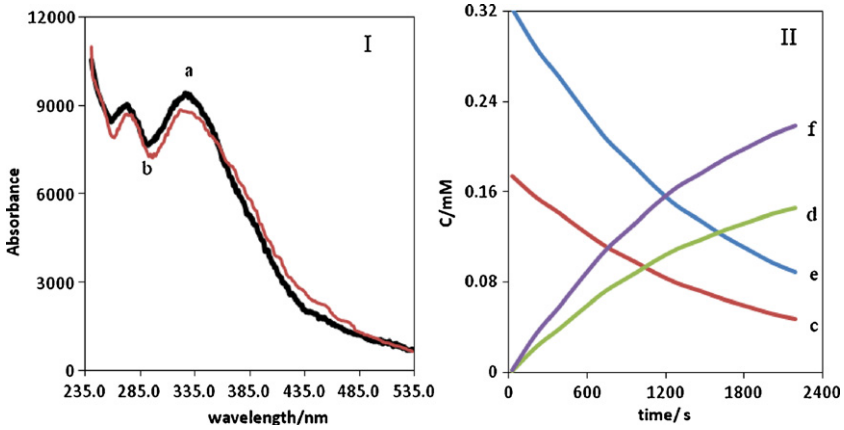


Fig. 5. (I) Resolved spectral profiles for (a) free and (b) complex form of reaction product for catechin and (II) concentration profiles for (c) H_2Q, (d) Q, (e) H_2QCD and (f) QCD for the reaction of 0.5 mM catechin with 3.0 mM sodium nitrite in the presence of 10.0 mM of β-CD.

$$\frac{[H_2QCD]}{[H_2Q]} = \frac{k_3[CD]}{k_{-3}} = K_{fQ}[CD] \quad (14)$$

K_a is the acidic constant of nitrous acid. K_{fH_2Q} and K_{fQ} are the inclusion stability constants of H_2Q and Q with β-CD respectively. During the consecutive reactions the total concentrations of NO_2^-, HNO_2, H_2QCD and H_2Q are varying with time but the actual ratio of NO_2^-/HNO_2 depends on the pH of solution and K_a value. The H_2QCD/H_2Q is also constant and depends on β-CD concentration and K_f value.

A two-way data matrix **Y** can be formed by measuring absorbance under different wavelengths at a series of chosen times (kinetic spectra). This matrix can be decomposed into the product of matrix **C**, column wise, containing the concentration profiles of the absorbing species and matrix **A**, row wise, containing their molar absorptivities. Matrix **E** is the residual.

$$\mathbf{Y} = \mathbf{CA} + \mathbf{E} \quad (15)$$

Propose of the fitting consists of finding set of parameters for which the sum over all the squares, *ssq*, over all the elements of the error matrix, *E*, is minimal.

$$ssq = \sum E_{ij}^2 \quad (16)$$

The kinetic profiles were obtained by numerical integration using MATLAB's solver, ode45, which is the standard Runge–Kutta algorithm [27]. Also the values of rate constants and equilibrium constants as nonlinear parameters which define the matrix **C** were calculated by fitting through the data of the concentration profiles using Newton–Gauss algorithm. The molar absorptivities of the H_2Q and H_2QCD are known and they are used during the fitting process. The molar absorptivities of the Q and QCD are calculated as linear parameters based on beer equation ST = C + D, where C+ is the pseudo-inverse of the matrix C [28].

The spectral time data are obtained at pHs lower than 4.0 in the absence and presence of various concentrations of β-CD. The values of the rate constant for the reaction of H_2Q (k_o) are obtained in the absence of β-CD considering pK_a equal to 3.27 for HNO_2. The k'_o, K_{fH_2Q} and K_{fQ} are obtained by using the same method and substituting the obtained value for k_o in the absence of β-CD. The rate and formation constants of catechin and quercetin are listed in Table 1. A very good fit of the data is observed (small values for the lack of fit), which indicates the validity of the underlying model.

Fig. 5 shows the absorption spectra (5I) for Q and QCD and concentration profiles (5II) related to the analysis of the data for catechin in presence of 3.0 mM $NaNO_2$ and 10.0 mM β-CD. Interestingly, the calculated spectra and concentration profiles are well consistent with the spectral changes in the data collected.

Estimation of K_f for the investigated flavonoids and their oxidized forms indicates that they can form relatively stable complexes with β-CD. The rate constants of complex forms are considerably lower than the free forms and inclusion cause to decrease in the rate of oxidation reaction. The formation constants of the oxidized forms are lower than related parent molecules. The higher complex formation constants of reduced forms and inhibitory effect of β-CD for flavonoid oxidation may be due to the hydrogen bonding of their hydroxyl groups with hydroxyl groups of β-CD [19].

4. Conclusion

The above work describes the oxidation of catechol containing flavonoids exposed to HNO_2 in aqueous solution. Reaction mechanisms were proposed and related *o*-quinones were detected as relatively stable products. The rate and equilibrium constants of reactions were obtained by chemometrics techniques. Reaction is pH dependent and grater reaction rates at lower pH values are due to higher percentage of HNO_2 as oxidant. One of the more significant findings to emerge from this study is the effect of inclusion complex on the antioxidant properties of selected flavonoids. The results show that the formation of inclusion complex decreases the rate of oxidation reaction. On one hand this complexation lead to more stability of these antioxidants toward the nitrous acid as reactive nitrogen species. On the other hand more stability of antioxidants maybe cause to weaker protecting effect in damage

of other molecules and further experimental investigations are needed to explore these effects.

References

[1] B. Halliwell, J.M. Gutteridge, Free Radic. Biol. Med. 18 (1995) 125–126.
[2] J. Kwanmoon, S. Takayuki, J. Agric. Food Chem. 57 (2009) 1655–1666.
[3] J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481–504.
[4] A. Agnieszka, B. Grzegorz, C. Ana, D. Gunars, H. Lubica, L. Wojciech, M. Magdalena, O. Andreani, R. Lucia, S. Elzbieta, S. Milan, S. Miriam, T. Gunars, V.P. Rimantas, V. Jana, V. Panagiota, Z. Neven, Free Radic. Res. 44 (2010) 1216–1262.
[5] R.A. Dixon, L. Achnine, P. Kota, C.J. Liu, M. Reddy, L. Lang, J. Mol. Plant Pathol. 3 (2002) 371–390.
[6] I. Debeaujon, K.M.L. Kloosterziel, M. Koomneef, Plant Physiol. 122 (2000) 403–414.
[7] L. Pourcel, J.M. Routaboul, L. Kerhoas, M. Caboche, L. Lepiniec, Plant Cell 17 (2005) 2966–2980.
[8] B. Winkel-Shirley, Curr. Opin. Plant Biol. 5 (2002) 218–223.
[9] I. Erlund, Nutr. Res. 24 (2004) 851–874.
[10] I. Crespo, M.V. Garcia-Mediavilla, M. Almar, P. Gonzalez, M.J. Tunon, S. Sanchez-Campos, J. Gonzalez-Gallego, Food Chem. Toxicol. 46 (2008) 1555–1569.
[11] B.D. Beake, R.B. Moodie, J.P.B. Sandall, J. Chem. Soc. Perkin Trans. 2 (5) (1994) 957–960.
[12] G. Lammel, J.N. Cape, Chem. Soc. Rev. 25 (1996) 361–369.
[13] M. Rafiee, L. Khalafi, Electrochim. Acta 55 (2010) 1809–1813.
[14] U. Takahama, T. Oniki, S. Hirota, J. Agric. Food Chem. 50 (2002) 4317–4322.
[15] S.Y.H. Lee, B. Munerol, S. Pollard, K.A. Youdim, A.S. Pannala, G.C. Kuhnle, E.S. Debnam, C. Rice-Evans, J.P.E. Spencer, Free Radic. Biol. Med. 40 (2006) 323–334.
[16] C. Oldreive, K. Zhao, G. Paganga, B. Halliwell, C. Rice-Evans, Chem. Res. Toxicol. 11 (1998) 1574–1579.
[17] L. Khalafi, M. Rafiee, J. Hazard. Mater. 174 (2010) 801–806.
[18] A. Afkhami, L. Khalafi, Anal. Chim. Acta 569 (2006) 267–274.
[19] A. Afkhami, L. Khalafi, Bull. Chem. Soc. Jpn. 80 (2007) 1542–1548.
[20] A. Afkhami, L. Khalafi, Supramol. Chem. 19 (2008) 579–586.
[21] J. Szejtli, Chem. Rev. 98 (1998) 1743–1754.
[22] V.T. Karathanos, I. Mourtzinos, K. Yannakopoulou, N.K. Andrikopoulos, Food Chem. 101 (2007) 652–658.
[23] F. Van-de-manakker, T. Vermonden, C.F. Van Nostrum, W.E. Hennink, Biomacromolecules 10 (2009) 3157–3175.
[24] H.J. Buschmann, E. Schollmeyer, J. Cosmet. Sci. 53 (2002) 185–191.
[25] M. Rafiee, D. Nematollahi, Electroanalysis 19 (2007) 1382–1386.
[26] E. Riordan, N. Minogue, D. Healy, P. Odriscoll, J.R. Sodeau, J. Phys. Chem. A 109 (2005) 779–786.
[27] M. Maeder, Y.M. Neuhold, Practical Data Analysis in Chemistry, Elsevier, Amsterdam, Netherlands, 2007.
[28] M. Hasani, M. Shariati-Rad, H. Abdollahi, Anal. Chim. Acta 636 (2009) 175–182.