Springer

Dear Author,

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For fax submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/ corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
  Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL: http://dx.doi.org/[DOI].
If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information go to: http://www.springerlink.com.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

Please note: Images will appear in color online but will be printed in black and white.

ArticleTitle	Study of ellagic acid electro-oxidation mechanism	
Article Sub-Title		
Article CopyRight	Springer-Verlag (This will be the copyright line in the final PDF)	
Journal Name	Monatshefte für Chemie - Chemical Monthly	
Corresponding Author	Family Name	**Verbić**
	Particle	
	Given Name	**Tatjana Ž.**
	Suffix	
	Division	Faculty of Chemistry
	Organization	University of Belgrade
	Address	Studentski Trg 12-16, 11158, Belgrade, Serbia
	Email	tatjanad@chem.bg.ac.rs
Author	Family Name	**Simić**
	Particle	
	Given Name	**Aleksandra Z.**
	Suffix	
	Division	Faculty of Chemistry
	Organization	University of Belgrade
	Address	Studentski Trg 12-16, 11158, Belgrade, Serbia
	Email	
Author	Family Name	**Sentić**
	Particle	
	Given Name	**Milica N.**
	Suffix	
	Division	Faculty of Chemistry
	Organization	University of Belgrade
	Address	Studentski Trg 12-16, 11158, Belgrade, Serbia
	Email	
Author	Family Name	**Vojić**
	Particle	
	Given Name	**Mirjana P.**
	Suffix	
	Division	Faculty of Chemistry
	Organization	University of Belgrade
	Address	Studentski Trg 12-16, 11158, Belgrade, Serbia
	Email	
Author	Family Name	**Juranić**
	Particle	
	Given Name	**Ivan O.**
	Suffix	

	Division	
	Organization	Institute of Chemistry, Technology, and Metallurgy
	Address	Njegoševa 12, 11000, Belgrade, Serbia
	Email	
Author	Family Name	**Manojlović**
	Particle	
	Given Name	**Dragan D.**
	Suffix	
	Division	Faculty of Chemistry
	Organization	University of Belgrade
	Address	Studentski Trg 12-16, 11158, Belgrade, Serbia
	Email	

Schedule	Received	15 June 2012
	Revised	
	Accepted	28 August 2012

Abstract

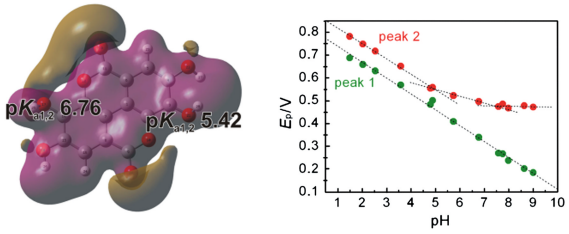
Abstract:

Ellagic acid is a biologically active polyphenol found in numerous fruits and vegetables. However, not many papers dealing with the electrochemical properties and protolytic equilibria of ellagic acid have been published so far. The electro-oxidation mechanism of ellagic acid was studied in methanol aqueous media (1:1, v/v) within the pH range of 1.5–9.0, $t = 25 \pm 1$ °C, using cyclic voltammetry on a glassy carbon electrode, and by semiempirical calculations. Results show that oxidation of ellagic acid is a pH-dependent, two-step quasireversible process. The slope of peak 1 indicates the exchange of the same number of electrons and protons within the whole studied pH range; the slope of peak 2 changes with the increase of pH, and three different regions are visible. As protolytic equilibria studies revealed that ellagic acid acts as a diprotic acid in the studied conditions (acidity constants were potentiometrically determined as $pK_{A1} = 5.42 \pm 0.01$ and $pK_{A2} = 6.76 \pm 0.01$), it is obvious that the electro-oxidation occurs at the hydroxyl group subjected to dissociation. The three different regions are therefore recognized as regions with different dominating species: unionized molecule (H_4A), monoanion (H_3A^-), and dianion (H_2A^{2-}). UV/Vis spectral changes confirmed the proposed equilibria. Heat of formation and electron densities calculated at semiempirical level were used to propose the hydrogen and electron abstraction sites. According to the obtained results, a new mechanism of ellagic acid electro-oxidation is proposed.

Keywords (separated by '-')	Acidity constants - Cyclic voltammetry - Potentiometry - Semiempirical calculations - UV/Vis spectroscopy
Footnote Information	

Metadata of the article that will be visualized in OnlineAlone

Graphical abstract:



Monatsh Chem
DOI 10.1007/s00706-012-0856-8

ORIGINAL PAPER

Study of ellagic acid electro-oxidation mechanism

Aleksandra Z. Simić · Tatjana Ž. Verbić · Milica N. Sentić · Mirjana P. Vojić · Ivan O. Juranić · Dragan D. Manojlović

Received: 15 June 2012 / Accepted: 28 August 2012

Abstract Ellagic acid is a biologically active polyphenol found in numerous fruits and vegetables. However, not many papers dealing with the electrochemical properties and protolytic equilibria of ellagic acid have been published so far. The electro-oxidation mechanism of ellagic acid was studied in methanol aqueous media (1:1, v/v) within the pH range of 1.5–9.0, $t = 25 \pm 1$ °C, using cyclic voltammetry on a glassy carbon electrode, and by semiempirical calculations. Results show that oxidation of ellagic acid is a pH-dependent, two-step quasireversible process. The slope of peak 1 indicates the exchange of the same number of electrons and protons within the whole studied pH range; the slope of peak 2 changes with the increase of pH, and three different regions are visible. As protolytic equilibria studies revealed that ellagic acid acts as a diprotic acid in the studied conditions (acidity constants were potentiometrically determined as $pK_{A1} = 5.42 \pm 0.01$ and $pK_{A2} = 6.76 \pm 0.01$), it is obvious that the electro-oxidation occurs at the hydroxyl group subjected to dissociation. The three different regions are therefore recognized as regions with different dominating species: unionized molecule (H_4A), monoanion (H_3A^-), and dianion (H_2A^{2-}). UV/Vis spectral changes confirmed the proposed equilibria. Heat of formation and electron densities calculated at semiempirical level were used to propose the hydrogen and electron abstraction sites. According to the obtained results, a new mechanism of ellagic acid electro-oxidation is proposed.

Keywords Acidity constants · Cyclic voltammetry · Potentiometry · Semiempirical calculations · UV/Vis spectroscopy

A. Z. Simić · T. Ž. Verbić (✉) · M. N. Sentić · M. P. Vojić · D. D. Manojlović
Faculty of Chemistry, University of Belgrade, Studentski Trg 12-16, 11158 Belgrade, Serbia
e-mail: tatjanad@chem.bg.ac.rs

I. O. Juranić
Institute of Chemistry, Technology, and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia

Introduction

Ellagic acid (EA, Fig. 1) is a natural polyphenol dimeric derivative of gallic acid contained in natural products in the form of ellagitannins and only in small quantities in free form [1, 2].

It is found in many fruits such as grapes [3], strawberries [2, 4, 5], cloudberries, and red raspberries [5]. EA constantly attracts much attention for its potential to protect against a variety of human diseases. It shows antimutagenic [6], antioxidant [7], and antiinflammatory activity in vitro and in vivo [8]. It was also reported that EA possesses antihyperglycemic effect in streptozotocin-induced diabetes in rats [9]. Recent studies showed that EA is effective against malaria parasites in vitro and in vivo, and that it potentiates the effect of antimalarial drugs (chloroquine, artesunate, mefloquine, and atovaquone) in vitro [10]. When administrated orally (50 mg/kg of body weight), EA protects rat liver from oxidative damage caused by the immunosuppressor cyclosporine A [11]. Oral pretreatment with EA is also very effective in cardioprotection from isoproterenol-induced myocardial infarction in rats [12]. Anticancer properties of EA are well documented: it was found that EA is effective in fighting various forms of cancer such as pancreatic cancer [13], lung tumours [14], and colon cancer [15].

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | ☐ LE | ☐ TYPESET
MS Code : MOCHEM-D-12-00266 | ☑ CP | ☑ DISK

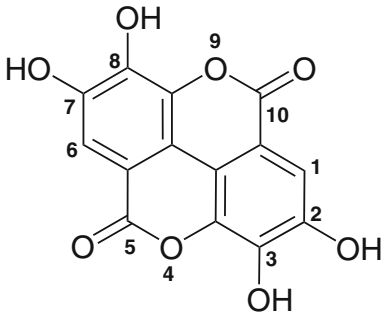


Fig. 1 Ellagic acid

Many health benefits of EA are attributed to its antioxidant properties [11, 12, 14, 16]. Studies show that polyphenols react with free radicals through three possible mechanisms: hydrogen atom transfer (HAT), single-electron transfer-proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) [17–20]. From a thermodynamic point of view, it was found that hydrogen atom transfer (HAT) is the most important free-radical scavenging mechanism for EA [20].

Recently, several research groups have investigated electrochemical techniques as tools for EA determination in natural samples [21–26]. One of the problems in studying EA is its poor solubility in water (9.7 μg/cm^3), while in methanol the solubility increases (671 μg/cm^3) [27]. In order to raise the solubility, but still keep the aqueous surroundings, we studied the electrochemical behavior of EA on a glassy carbon (GC) electrode in methanol aqueous media (1:1, v/v) within the pH range of 1.5–9.0. This range was selected because, in strongly alkaline solutions, spontaneous lactone ring-opening occurs [27, 28]. As EA has four hydroxyl groups in its structure, it is clear that pH and pK_A values may significantly influence its electrochemical behavior. However, data about acidity constants of EA are generally sparse in the literature; For example, spectral data were used to draw conclusions about the pH range where the first two dissociation processes occur [28], but no precise pK_A values were reported. It was also stated in literature that the symmetry of the molecule would explain the existence of only two chemically distinguishable pK_A values, corresponding to simultaneous ionization of both *p*-OH and both *m*-OH groups [29]. Another group of authors used potentiometric titration in aqueous media to determine pK_A values, but just one value (pK_A = 6.54) was reported [30]. To the best of our knowledge, to date, no consecutive EA pK_A values have been published for aqueous or mixed solvent media with subsequent correlation to aqueous media. We have used potentiometry and spectrophotometry to study the protolytic equilibria of EA in methanol aqueous media (MeOH:H_2O = 1:1, v/v) and in pure aqueous media. Semiempirical molecular orbital (MO) calculations were done to corroborate the validity of the proposed dissociation and electro-oxidation mechanisms.

Because of the apparent parallelism between electrochemical oxidations and antioxidant reactions of EA, it is likely that study of its electrochemical oxidation could contribute, at least in part, to understanding of the complex mechanism of EA oxidation reactions in vitro, and perhaps, in vivo, too.

Results and discussion

Typical cyclic voltammograms of EA oxidation in methanol aqueous buffer solutions at pH 1.49 and 9.0 are given in Fig. 2.

As can be seen in Fig. 2a, in acidic media, two anodic and two small cathodic peaks were observed within the investigated potential range (E = 0.0–1.2 V). The first oxidation peak appears as a shoulder of the second one, but in neutral and alkaline media (Fig. 2b) the shoulder develops into the separate peak 2, and in alkaline media a broad peak 3 arises around E_p = 0.80 V. In the reverse scan at pH 1.49 two small peaks appear, which indicates that electro-oxidation of EA is a quasireversible process followed by a chemical reaction. The absence of cathodic peaks in the reverse scan in alkaline media (Fig. 2b) indicates that an oxidation process was followed by a

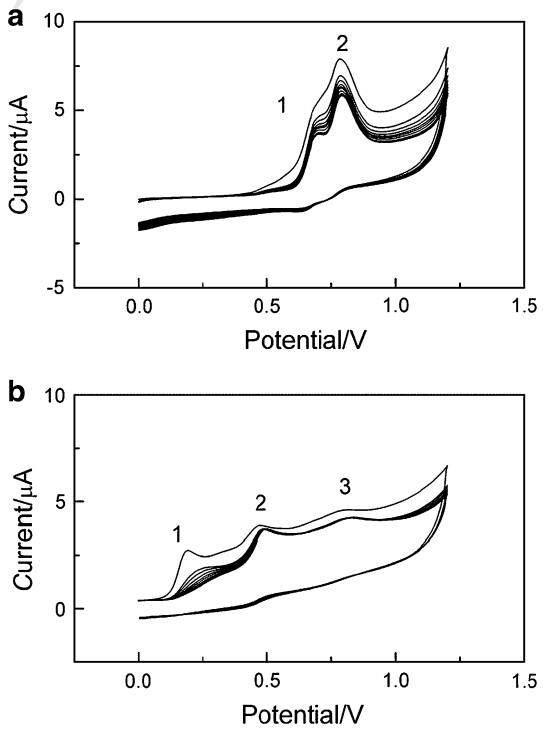


Fig. 2 Cyclic voltammograms of EA oxidation in methanol aqueous buffer solutions: **a** pH 1.49 and **b** pH 9.0; $c_{EA} = 2 \times 10^{-4}$ M; scan rate 100 mV/s

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | LE | TYPESET
MS Code : MOCHEM-D-12-00266 | CP | DISK

chemical reaction which rapidly removed the generated product. Repeated cycling led to a decrease in oxidation current (I), presumably due to generation of electro-inactive species which adsorb on and block the GC electrode surface.

Influence of the scan rate

The influence of the scan rate (v) was studied in the 25–500 mV/s range. If the scan rate is reduced to 25 mV/s, the voltammogram of EA consists of two well-defined peaks even in acidic media (Fig. 3).

At higher scan rate, in acidic media, two peaks overlap, making the determination of the true peak I_p values impossible. In neutral medium (Fig. 4a), the slope for peak 1 is 0.65 ($R = 0.983$) and for peak 2 is 0.59 ($R = 0.978$). This value is higher than the theoretical value of 0.5 for diffusion-controlled processes, but is less than 1, which is the theoretical value for an adsorption-controlled electrode process.

Therefore, we can conclude that the peak current does not arise only from oxidation of EA molecules that reach the electrode surface by diffusion, but also from those molecules that were adsorbed on the electrode surface before the oxidation. The gathered results are in fairly good agreement with previous studies [24]. However, with increase of pH, as dissociation proceeds, the effect of adsorption decreases. In alkaline media (Fig. 4b) the slope values are around 0.5 (0.50, $R = 0.999$ for peak 1; 0.51, $R = 0.999$ for peak 2) and thus correspond to the theoretical value for diffusion-controlled electrode processes.

Study of protolytic equilibria

To examine the effects of pH on the electrochemical behavior of EA, the dissociation reactions were studied and pK_A values determined. As the molecule is symmetrical, it

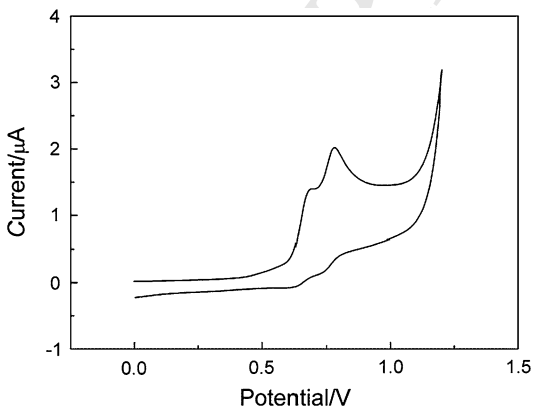


Fig. 3 Cyclic voltammogram of EA oxidation in methanol aqueous buffer solution pH 1.49; $c_{EA} = 2 \times 10^{-4}$ M; scan rate 25 mV/s

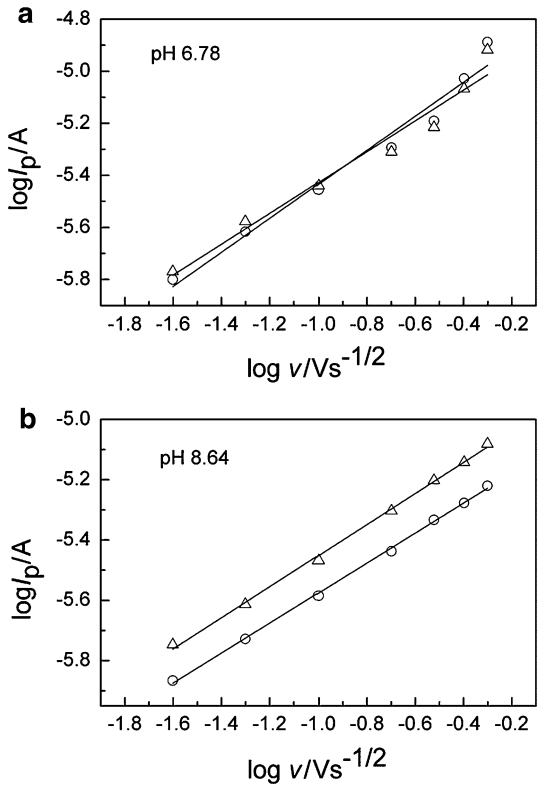


Fig. 4 Plots of first (*circles*) and second (*triangles*) anodic peak current at pH 6.78 (**a**) and 8.64 (**b**) as a function of scan rate

was expected that the difference between pK_{A1} and pK_{A2} values would not be sufficient ($\Delta pK_A \geq 4$) for classical spectrophotometric determination [31]. Thus, classical potentiometric acid–base titration and HyperQuad 2008 software [32] were used to determine pK_{A1} (5.68 ± 0.01) and pK_{A2} (7.02 ± 0.01) values and to evaluate the dissociation scheme in the studied pc_H range (2.7–10.6). It was shown that the E–pH dependence in the used experimental regime [MeOH:H_2O (1:1, v/v), $I = 0.1$ M (NaCl), $t = 25 \pm 1$ °C] is linear within the pc_H range of 2.0–11.7 with a slope that is very close to the Nernstian slope for monovalent ions:

$$E(\text{mV}) = 380.8 \pm 0.2 - 57.3 \pm 0.02 pc_H, \quad R = -1.000. \qquad (1)$$

Therefore, the experimentally determined pK_W value and correction factor (A) were used to convert pK_{A1} and pK_{A2} values determined in methanol aqueous media to pK_{A1} (5.42 ± 0.01) and pK_{A2} (6.76 ± 0.02) values in aqueous media.

Although spectrophotometry was not the most appropriate method for pK_A determination, in this case, as spectral changes obtained in aqueous buffer solutions in the pH range 1.1–12.5 (Fig. 5) are in very good agreement with obtained pK_A values for aqueous media, we may

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | LE | TYPESET
MS Code : MOCHEM-D-12-00266 | CP | DISK

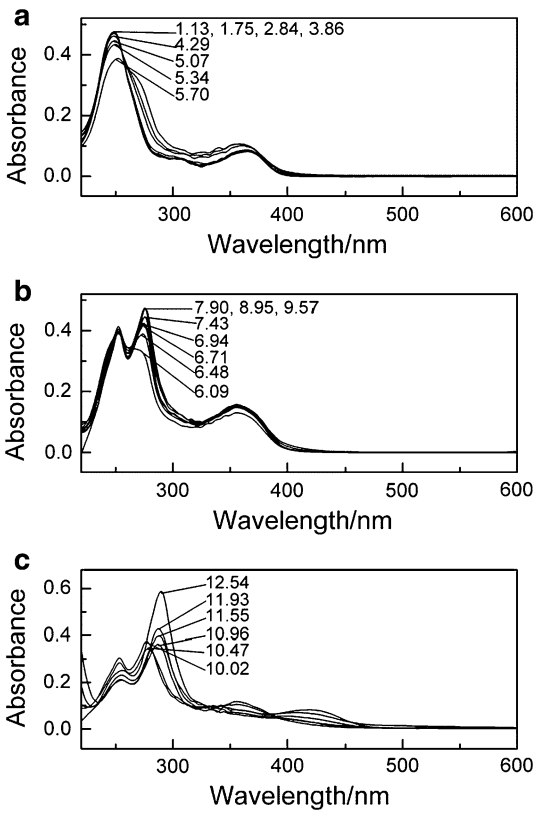


Fig. 5 UV/Vis spectra of ellagic acid ($c_{EA} = 1 \times 10^{-5}$ M) in aqueous solutions of different pH values (given on figure): **a** pH range 1.13–5.70, **b** pH range 6.09–9.57, and **c** pH range 10.02–12.54; $t = 25 \pm 1$ °C; scan rate 500 nm/min

consider this as a confirmation of the potentiometrically determined pK_A values.

As observed in Fig. 5a, spectra are overlapping in the pH range 1.13–3.86. Small but obvious differences are visible in the spectrum recorded in solution with pH 4.29; these data indicate initiation of the dissociation process. This is in very good agreement with the obtained pK_{A1} value (5.42 ± 0.01), as is expected to uncover visible spectral differences in a solution with pH ≈ pK_{A1} − 1. In a solution with pH 5.70, the shape of the spectrum starts to change, indicating the beginning of the second dissociation process, which is, again as expected, in good agreement with the pH ≈ pK_{A2} − 1 value. Spectral changes are visible within the pH range 6.09–7.43 (Fig. 5b) as the second –OH group dissociation proceeds. In the pH region 7.90−9.57 (pH ≈ pK_{A2} + 1), spectra are overlapping again, confirming that the second −OH group is completely deprotonated. As the pH value is raised above pH 10, the main absorption maximum is moved toward higher wavelength (bathochromic shift). The lack of isosbestic points in this region indicates that this is no more just protolytic equilibria; the reason lies in a reversible lactone ring-opening reaction. The lactone ring-opening reaction rate was monitored in several solutions with pH >10 in aqueous as well as methanol aqueous media. The same conclusion was drawn: in both studied media the reaction rate is considered to be significant (absorbance at the absorption maximum is lowered by at least 10 % in 10 min) in solutions with pH >10.6.

Based on the experimentally determined pK_{A1} and pK_{A2} values, a representative distribution diagram of EA was calculated (Fig. 6).

Data about specific dissociation sites of EA are diverse in the literature [28, 29]. The dissociation of –OH groups at C_3 and C_8 atoms (Fig. 1) seems more probable than the dissociation of -OH groups at C_2 and C_7 atoms. This can be explained by possible π-electron delocalization in the EA monoanion. Extended π-electron delocalization represented by a quinoid structure is possible just for C_3 and C_8 -OH group(s) dissociation. The EA dianion (H_2A^{2-}) is even more stabilized, as the same π-electron delocalization is possible for another aromatic ring.

To verify this assumption, the heat of formation (ΔH_f^θ) and net atomic charges were calculated at semiempirical level (MOPAC 2007, MNDO-RM1 Hamiltonian [33, 34]). The results for ΔH_f^θ suggest that the first dissociation happens at the C_3 −OH group ($\Delta H_f^\theta = -1{,}383.407$ kJ/mol), not at C_2 −OH ($\Delta H_f^\theta = -1{,}377.813$ kJ/mol). As for further dissociation and formation of dianion (H_2A^{2-}), four combinations are possible:

C_3 and C_8 −OH groups ($\Delta H_f^{\theta=1{,}325.697}$kJ/mol),
C_3 and C_7 −OH groups ($\Delta H_f^{\theta=1{,}320.086}$kJ/mol),
C_2 and C_8 –OH groups ($\Delta H_f^{\theta=1{,}320.045}$kJ/mol),
C_2 and C_7 –OH groups ($\Delta H_f^{\theta=1{,}290.821}$ kJ/mol),

with the results for ΔH_f^θ values suggesting that protons dissociate from C_3 and C_8 –OH groups. Calculated net atomic charges for oxygen atoms in monoanion C_3–O (−0.4370) and C_2–O (−0.2873), and in dianion C_3–O (−0.5014), C_2–O (−0.3050), C_8–O (−0.5014), and C_7–O

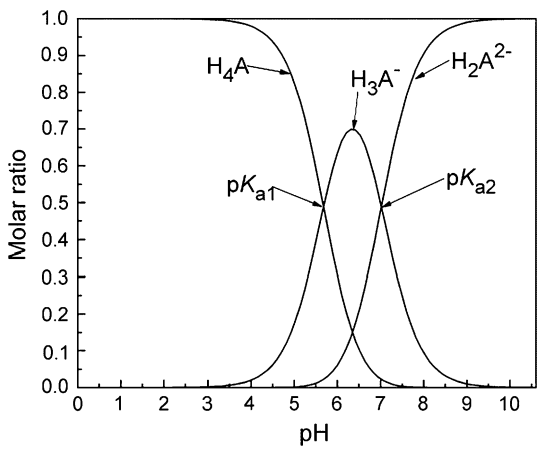


Fig. 6 Distribution diagram of ellagic acid in methanol aqueous media (1:1, v/v)

Journal : Large 706 Dispatch : 16-9-2012 Pages : 8
Article No. : 856 LE TYPESET
MS Code : MOCHEM-D-12-00266 CP DISK

(−0.3049) are in good agreement with the suggested dissociation scheme.

Influence of pH on electrochemical behavior

The effect of pH value on electro-oxidation potentials of peaks 1 and 2 is shown in Fig. 7.

It is observed that, within the studied pH range, the increase of pH shifts the peak 1 E_p toward less positive values. The obtained slope (−69 ± 1 mV/pH; $R = 0.997$) is close to Nernstian, indicating that the electrode reaction involves equal number of protons and electrons. For peak 2, the $E_p = f(\text{pH})$ dependence is divided into three regions. For pH <4.8, a linear decrease in peak potential by −67 ± 2 mV/pH ($R = 0.998$) was observed. The obtained slope indicates that in this region the oxidation mechanism involves equal number of electrons and protons. However, in the $4.8 < \text{pH} < 7.6$ region, the slope changes to −30 ± 2 mV/pH ($R = 0.992$), whereas in solutions with pH >7.6 the peak 2 potential remains constant, suggesting that protons no longer participate in the electrode reaction.

It is usually accepted that the first step in the electro-oxidation of phenols involves the formation of a phenoxyl radical (Scheme 1). Since the EA molecule is symmetrical, there are actually two possible sites for phenoxyl radical formation: –OH group at C_2 (i.e., C_7) atom and –OH group at C_3 (i.e., C_8) atom.

As can be seen from the π-electron delocalization shown in Scheme 1, the phenoxyl radical formed by electro-oxidation of the –OH group at the C_2 atom (Scheme 1a) is more stable than the one formed by the oxidation of the –OH group at the C_3 atom (Scheme 1b). Even in the case of an anion, this will hold. Our results show that the first dissociation occurs on the C_3 atom, but π-electron delocalization will favor formation of the radical at the C_2 atom. This is confirmed by the calculated heat of formation (ΔH_f^θ) for various combinations of hydrogen and electron abstraction sites. A difference of 14.2 kJ/mol is observed when comparing the radical formed at the C_2 atom if the anion is at C_3 or at C_7 atom, emphasizing that an *ortho* radical-anion configuration is thermodynamically favorable:

Radical at C_2, anion at C_3 atom ($\Delta H_f^\theta = -1{,}328.318$ kJ/mol),
Radical at C_2, anion at C_7 atom ($\Delta H_f^\theta = -1{,}314.187$ kJ/mol),
Radical at C_3, anion at C_8 atom ($\Delta H_f^\theta = -1{,}312.621$ kJ/mol),
Radical at C_2, anion at C_8 atom ($\Delta H_f^\theta = -1{,}303.042$ kJ/mol).

As further electron transfer leads to quinone formation, ΔH_f^θ was also calculated for four possible combinations. The results show that the most stable form is the quinone with carbonyl oxygen at C_2 and C_3 atoms:

Carbonyl oxygen at C_2 and C_3 atoms ($\Delta H_f^{\theta=1{,}011.494}$ kJ/mol),
Carbonyl oxygen at C_2 and C_7 atoms ($\Delta H_f^{\theta=929.572}$ kJ/mol),
Carbonyl oxygen at C_3 and C_8 atoms ($\Delta H_f^{\theta=808.114}$ kJ/mol),
Carbonyl oxygen at C_2 and C_8 atoms ($\Delta H_f^{\theta=800.063}$ kJ/mol).

These observations allow us to propose the pathway for EA electro-oxidation (Scheme 2).

According to our results, peak 1 originates from the formation of EA phenoxyl radical by release of one electron and one proton. As is obvious from the distribution diagram (Fig. 6), significant dissociation does not occur below pH 4.8. Consequently, in this region the phenoxyl radical formed in the first oxidation step undergoes further one-electron, one-proton charge-transfer reaction leading to peak 2 (Scheme 2). In pH region 4.8–7.6, all three EA species are present: unionized molecule (H_4A), monoanion (H_3A^-), and dianion (H_2A^{2-}), and the oxidation of all present species is occurring, resulting in the change of slope for peak 2 in the $E_p = f(\text{pH})$ dependence (Fig. 7). However, at pH higher than 7.6, as the EA dianion becomes the dominating species, protons no longer participate in the second oxidation step. Resulting semiquinone and quinone forms may further undergo dimerization or polycondensation reactions, producing compounds that can also be oxidized. This may explain the existence of the third peak.

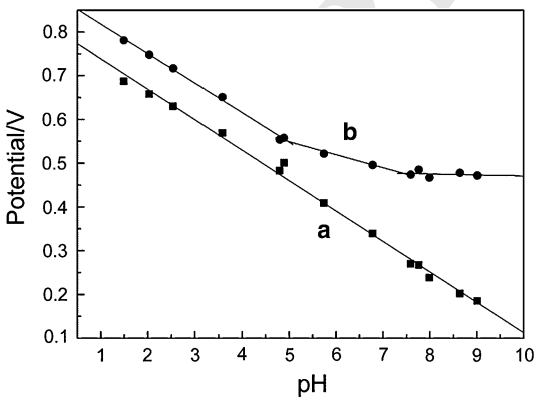


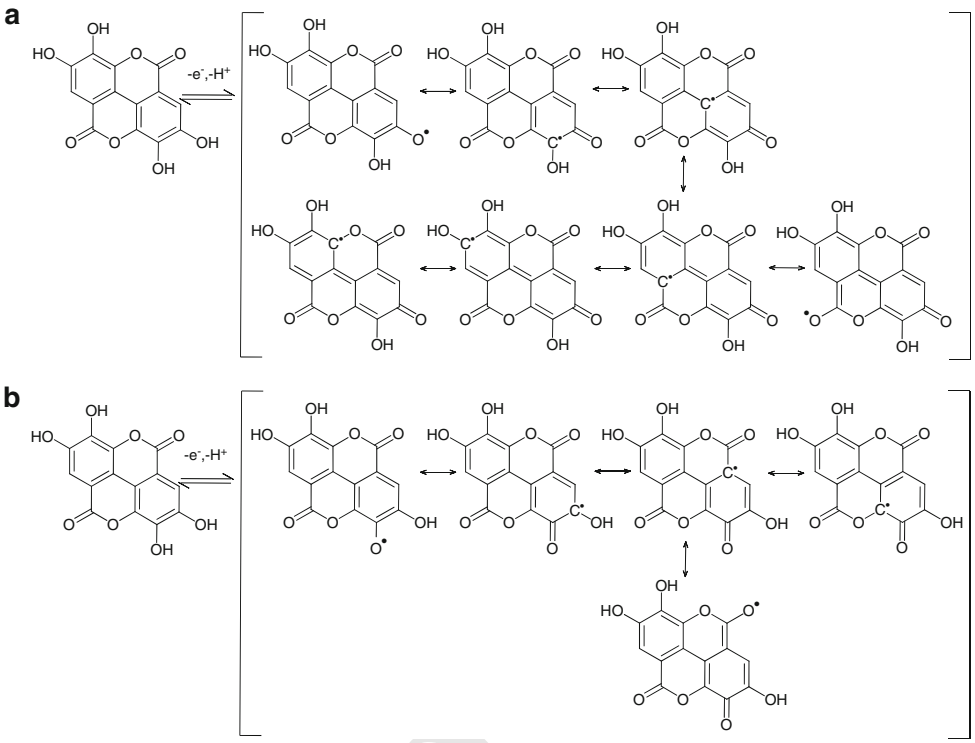
Fig. 7 E_p versus pH of ellagic acid ($c_{EA} = 2 \times 10^{-4}$ M): peak 1 (*a*) and peak 2 (*b*) obtained from cyclic voltammograms at scan rate of 25 mV/s

Conclusions

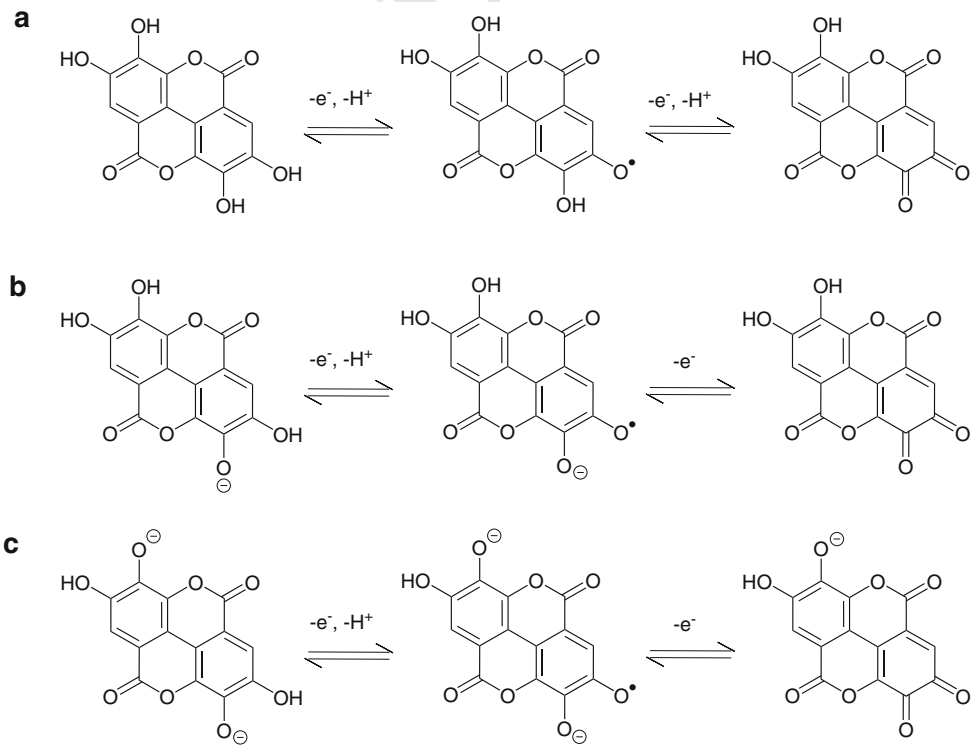
Cyclic voltammetry was used to investigate the electrochemical behavior of EA. Oxidation at GC electrode in

Journal : Large 706 Dispatch : 16-9-2012 Pages : 8
Article No. : 856 LE TYPESET
MS Code : MOCHEM-D-12-00266 CP DISK

Scheme 1



Scheme 2



acidic media produces two anodic and two small cathodic peaks, corresponding to a quasireversible oxidation process. In neutral and alkaline media, two anodic peaks are separated and a third one is formed at potential around 0.80 V. Inspection of the oxidation peak current variation with the scan rate of EA suggests mixed adsorption–diffusion control in acidic media and diffusion-controlled electrode process in alkaline media. The obtained E_p–pH dependence emphasizes the influence of protolytic equilibria on the EA electro-oxidation process. It was shown

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | LE | TYPESET
MS Code : MOCHEM-D-12-00266 | CP | DISK

that, although EA has four −OH groups, it acts as a diprotic acid under these experimental conditions, as in alkaline media (pH >10) lactone ring-opening reaction occurs. pK_A values were potentiometrically determined in methanol aqueous media ($pK_{A1} = 5.68 \pm 0.01$ and $pK_{A2} = 7.02 \pm 0.01$), and converted to values in pure water: $pK_{A1} = 5.42 \pm 0.01$ and $pK_{A2} = 6.76 \pm 0.01$. Although the calculated heats of formation suggest that proton dissociation occurs at C_3 and C_8 –OH groups, π-electron delocalization favors radical formation at the C_2 atom in the EA unionized form, as well as in anionic form. According to our results, it seems that, when pH is lower than pH 4.8, EA is oxidized to quinone through the mechanism which involves two consecutive one-electron, one-proton steps. In solution with pH between 4.8 and 7.6, all three species are present in solution, so the second oxidation step may occur with or without the participation of a proton. However, if the pH is raised above 7.6, the dianion becomes the dominating species and the proton no longer participates in the second step of the electrochemical reaction. Within this pH region semiquinone and quinone may undergo further dimerization or polycondensation reactions and form products that can generate the third peak upon electrochemical oxidation.

Experimental

All chemicals were of analytical reagent grade and were purchased from Fluka or Merck.

Working solutions of EA ($c = 2 \times 10^{-4}$ M) for cyclic voltammetry were prepared daily in methanol and diluted with the same volume of aqueous buffer solutions (c^{tot}_{buff}= 0.1 M). HCl solutions were used for pH 1.5–4.3, acetate buffers for pH 4.8–6.8, and phosphate buffers for pH 7.6–9.0. pH values were measured in aqueous buffers, as well as in working solutions. All experiments were performed on a Metrohm VA 797 Computrace instrument (Herisau, Switzerland). The triple-electrode system consisted of a working GC electrode, a reference Ag/AgCl, KCl (3 M) electrode, and a platinum wire as a counter-electrode. Before each experiment, the GC electrode was polished with alumina powder (0.5 μm), rinsed thoroughly with distilled water, and sonicated for 60 s in water. To prevent possible air oxidation of the tested substance, nitrogen was bubbled through solutions for 3 min. All measurements were done in 0.0–1.2 V potential range at room temperature.

Acidity constants of EA were potentiometrically determined in MeOH:H_2O (1:1, v/v) at $t = 25 \pm 1$ °C and at constant ionic strength [$I = 0.1$ M (NaCl)]. Solutions of NaOH (0.1 M) and HCl (0.1 M) were prepared in MeOH:H_2O (1:1, v/v) and potentiometrically standardized. EA dihydrate was dissolved in methanol and diluted with the equivalent volume of aqueous 0.2 M NaCl ($c_{EA} = 5 \times 10^{-4}$ M). Titrations were performed using a TTT-60 titrator equipped with an ABU-12 autoburette (Radiometer Copenhagen, Denmark), and pH was measured with a PHM240 pH-Meter (Radiometer) with a combined GK2401B electrode (Radiometer).

Prior to titration, 250 mm^3 of the standard 0.1 M HCl solution was added to 15.00 cm^3 of the working EA solution ($c = 5 \times 10^{-4}$ M). All probes were titrated with 5 mm^3 increments of the standard 0.1 M NaOH solution. Measured pH values were converted to pc_H according to the relation [35]: $pc_H = -\log[H_3O^+] = pH - A$, where A is the correction factor ($A = 0.26$) as determined by the potentiometric titration of the standard HCl solution with the standard NaOH solution under experimental conditions. The pK_W value ($pK_W = 13.84 \pm 0.01$) was calculated from the same set of titrations. HyperQuad 2008 software [32] was used to evaluate the dissociation scheme in the studied pc_H range (2.7–10.5) and to calculate the values of acidity constants from four repeated titrations. The determined pK_W value and correction factor were used to convert pK_{A1} and pK_{A2} values determined in methanol aqueous media to values in pure aqueous media.

For UV/Vis spectrophotometry a stock solution of EA ($c = 1 \times 10^{-3}$ M) was prepared in methanol. Working solutions ($c = 1 \times 10^{-5}$ M) were prepared in deionized water (methanol concentration 1 %, vol.) in the pc_H range of 1.1–12.5 [$I = 0.1$ M (NaCl)]. HCl solutions were used for the pc_H range of 1.1–4.0, acetate buffers for pc_H 4.0–6.0, phosphate buffers for pc_H 6.0–8.5 and pc_H 11.0–12.0, carbonate buffers for pc_H 8.5–11.0 (c^{tot}_{buff} = 0.01 M), and NaOH solutions for pc_H 12.0−12.5. UV/Vis spectra were recorded on a GBC Cintra 6 spectrophotometer (GBC Dandenong, Australia) with a 1-cm quartz cuvette against the corresponding blank in the 220–600 nm wavelength range, with 500 nm/min scan rate.

The heat of formation (ΔH^{θ}_f) and net atomic charges were calculated at semiempirical level (MOPAC 2007, MNDO-RM1 Hamiltonian [33, 34], including the simulation solvent as dielectric continuum, COSMO).

Acknowledgments The Ministry of Education and Science of Serbia supports this work (grants 172030 and 172035). The reported computational work makes use of results produced by the High-Performance Computing Infrastructure for South East Europe's Research Communities (HP-SEE), a project cofunded by the European Commission (under contract number 261499) through the Seventh Framework Programme HP-SEE (http://www.hp-see.eu/).

References

1. Zafrilla P, Ferreres F, Tomás-Barberán A (2001) J Agric Food Chem 49:3651

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | LE | TYPESET
MS Code : MOCHEM-D-12-00266 | CP | DISK

2. Da Silva Pinto M, Lajolo FM, Genovese MI (2008) Food Chem 107:1629
3. Lee JH, Talcott ST (2004) J Agric Food Chem 52:361
4. Williner MR, Pirovani ME, Güemes DR (2003) J Sci Food Agric 83:842
5. Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J, Törrönen R (1999) Food Res Int 32:345
6. Loarca-Pina G, Kuzmicky PA, de Mejía EG, Kado NY, Hsieh DPH (1996) Mutat Res. Environ Mutagen Relat Subj 360:15
7. Priyadarsini KI, Khopde SM, Kumar SS, Mohan H (2002) J Agric Food Chem 50:2200
8. Rogerio AP, Fontanari C, Borducchi É, Keller AC, Russo M, Soares EG, Albuquerque DA, Faccioli LH (2008) Eur J Pharmacol 580:262
9. Malini P, Kanchana G, Rajadurai M (2011) Asian J Pharm Clin Res 4:124
10. Soh PN, Witkowski B, Olagnier D, Nicolau ML, Garcia-Alvarez MC, Berry A, Benoit-Vical F (2009) Antimicrob Agents Chemother 53:1100
11. Pari L, Sivasankari R (2008) Fundam Clin Pharmacol 22:395
12. Kannan MM, Quine SD (2011) Eur J Pharmacol 659:45
13. Edderkaoui M, Odinokova I, Ohno I, Gukovsky I, Go VLW, Pandol SJ, Gukovskaya AS (2008) World J Gastroenterol 14:3672
14. Khanduja KL, Gandhi RK, Pathania V, Syal N (1999) Food Chem Toxicol 37:313
15. Umesalma S, Sudhandiran G (2011) Eur J Pharmacol 660:249
16. Yüce A, Ateşşahin A, Çeribaşi AO (2008) Basic Clin Pharmacol Toxicol 103:186
17. Wright JS, Johnson ER, DiLabio GA (2001) J Am Chem Soc 123:1173
18. Vafiadis AP, Bakalbassis EG (2005) Chem Phys 316:195
19. Musialik M, Litwinienko G (2005) Org Lett 7:4951
20. Zhang J, Xiong Y, Peng B, Gao H, Zhou Z (2011) Comp Theor Chem 963:148
21. Ghoreishi SM, Behpour M, Khayatkashani M, Motaghedifard MH (2011) Dig J Nanomater Bios 6:625
22. Ghoreishi SM, Behpour M, Khayatkashani M, Motaghedifard MH (2011) Anal Methods 3:636
23. Thakur K, Pitre KS (2008) J Chin Chem Soc 55:143
24. Cuartero M, Ortuño JA, Truchado P, García MS, Tomás-Barberán FA, Albero MI (2011) Food Chem 128:549
25. Komorsky-Lovrić Š, Novak I (2011) J Food Sci 76:C916
26. Komorsky-Lovrić Š, Novak I (2011) Int J Electrochem Sci 6:4638
27. Bala I, Bhardwaj V, Hariharan S, Ravi Kumar MNV (2006) J Pharm Biomed Anal 40:206
28. Hasegawa M, Terauchi M, Kikuchi Y, Nakao A, Okubo J, Yoshinaga T, Hiratsuka H, Kobayashi M, Hoshi T (2003) Monatsh Chem 134:811
29. Muñoz-Muñoz JL, Garcia-Molina F, Garcia-Molina M, Tudela J, García-Cánovas F, Rodriguez-Lopez JN (2009) IUBMB Life 61:171
30. Queimada AJ, Mota FL, Pinho SP, Macedo EA (2009) J Phys Chem B 113:3469
31. Albert A, Serjeant EP (1971) The determination of ionization constants, 2nd edn. Chapman and Hall, London
32. Gans P, Sabatini A, Vacca A (1996) Talanta 43:1739
33. Stewart JJP (1990) J Comput Aid Mol Des 4:1
34. Stewart JJP (2007) MOPAC 2007, Stewart computational chemistry, Colorado Springs, CO. http://OpenMOPAC.net Accessed 28 March 2012
35. Irving HM, Miles MG, Pettit LD (1967) Anal Chim Acta 38:475

Journal : Large 706 | Dispatch : 16-9-2012 | Pages : 8
Article No. : 856 | ☐ LE | ☐ TYPESET
MS Code : MOCHEM-D-12-00266 | ☑ CP | ☑ DISK