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# Study of ellagic acid electro-oxidation mechanism

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**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

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Institute of Chemistry, Technology, and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia 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

## 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].



## 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  $(9.7 \,\mu\text{g/cm}^3)$ , while in methanol the solubility increases (671  $\mu$ g/cm<sup>3</sup>) [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 processs 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;  $c_{\text{EA}} = 2 \times 10^{-4}$  M; scan rate 100 mV/s

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 ( $\nu$ ) 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 ( $\Delta pK_a \ge 4$ ) 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<sub>2</sub>O (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:

$$E(\text{mV}) = 380.8 \pm 0.2 - 57.3 \pm 0.02 \text{p}c_{\text{H}}, \quad R = -1.000.$$
(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



**Fig. 5** UV/Vis spectra of ellagic acid ( $c_{\rm EA} = 1 \times 10^{-5}$  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  $\pm$  0.01), as is expected to uncover visible spectral differences in a solution with pH  $\approx pK_{a1} - 1$ . 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  $\approx pK_{a2} - 1$  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  $\approx pK_{a2} + 1$ ), 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 ringopening 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  $\pi$ -electron delocalization in the EA monoanion. Extended  $\pi$ -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  $\pi$ -electron delocalization is possible for another aromatic ring.

To verify this assumption, the heat of formation  $(\Delta H_{\rm f}^{\theta})$  and net atomic charges were calculated at semiempirical level (MOPAC 2007, MNDO-RM1 Hamiltonian [33, 34]). The results for  $\Delta H_{\rm f}^{\theta}$  suggest that the first dissociation happens at the C<sub>3</sub> –OH group ( $\Delta H_{\rm f}^{\theta} = -1,383.407$  kJ/mol), not at C<sub>2</sub> – OH ( $\Delta H_{\rm f}^{\theta} = -1,377.813$  kJ/mol). As for further dissociation and formation of dianion (H<sub>2</sub>A<sup>2–</sup>), four combinations are possible:

C<sub>3</sub> and C<sub>8</sub> –OH groups ( $\Delta H_{\rm f}^{\theta}$  = 1,325.697 kJ/mol), C<sub>3</sub> and C<sub>7</sub> –OH groups ( $\Delta H_{\rm f}^{\theta}$  = 1,320.086 kJ/mol), C<sub>2</sub> and C<sub>8</sub> –OH groups ( $\Delta H_{\rm f}^{\theta}$  = 1,320.045 kJ/mol), C<sub>2</sub> and C<sub>7</sub> –OH groups ( $\Delta H_{\rm f}^{\theta}$  = 1,290.821 kJ/mol),

with the results for  $\Delta H_{\rm f}^{\theta}$  values suggesting that protons dissociate from C<sub>3</sub> and C<sub>8</sub> –OH groups. Calculated net atomic charges for oxygen atoms in monoanion C<sub>3</sub>–O (-0.4370) and C<sub>2</sub>–O (-0.2873), and in dianion C<sub>3</sub>–O



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

(-0.5014), C<sub>2</sub>–O (-0.3050), C<sub>8</sub>–O (-0.5014), and C<sub>7</sub>–O (-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  $E_p = f(pH)$  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 < 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 electrooxidation 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  $\pi$ -electron delocalization shown in Scheme 1, the phenoxyl radical formed by electro-oxidation of the –OH group at the C<sub>2</sub> atom (Scheme 1a) is more stable than the one formed by the oxidation of the –OH group at the C<sub>3</sub> atom (Scheme 1b). Even in the case of an anion, this will hold. Our results show that the first dissociation occurs on the C<sub>3</sub> atom, but  $\pi$ -electron delocalization will favor formation of the radical at the C<sub>2</sub> atom. This is confirmed by the calculated heat of formation



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

 $(\Delta H_{\rm f}^{\theta})$  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<sub>2</sub> atom if the anion is at C<sub>3</sub> or at C<sub>7</sub> atom, emphasizing that an *ortho* radical-anion configuration is thermodynamically favorable:

Radical at C<sub>2</sub>, anion at C<sub>3</sub> atom ( $\Delta H_{\rm f}^{\theta} = -1,328.318$  kJ/mol), Radical at C<sub>2</sub>, anion at C<sub>7</sub> atom ( $\Delta H_{\rm f}^{\theta} = -1,314.187$  kJ/mol), Radical at C<sub>3</sub>, anion at C<sub>8</sub> atom ( $\Delta H_{\rm f}^{\theta} = -1,312.621$  kJ/mol), Radical at C<sub>2</sub>, anion at C<sub>8</sub> atom ( $\Delta H_{\rm f}^{\theta} = -1,303.042$  kJ/mol).

As further electron transfer leads to quinone formation,  $\Delta H_{\rm f}^{\theta}$  was also calculated for four possible combinations. The results show that the most stable form is the quinone with carbonyl oxygen at C<sub>2</sub> and C<sub>3</sub> atoms:

Carbonyl oxygen at C<sub>2</sub> and C<sub>3</sub> atoms  $(\Delta H_{\rm f}^{\theta} = 1,011.494 \text{ kJ/mol}),$ Carbonyl oxygen at C<sub>2</sub> and C<sub>7</sub> atoms  $(\Delta H_{\rm f}^{\theta} = 929.572 \text{ kJ/mol}),$ Carbonyl oxygen at C<sub>3</sub> and C<sub>8</sub> atoms  $(\Delta H_{\rm f}^{\theta} = 808.114 \text{ kJ/mol}),$ Carbonyl oxygen at C<sub>2</sub> and C<sub>8</sub> atoms  $(\Delta H_{\rm f}^{\theta} = 800.063 \text{ kJ/mol}).$ 

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<sub>4</sub>A), 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  $E_p = f(pH)$  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.

#### Conclusions

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

Scheme 1

Scheme 2



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 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 (p $K_{a1} = 5.68 \pm 0.01$  and p $K_{a2} =$  $7.02 \pm 0.01$ ), 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,  $\pi$ -electron delocalization favors radical formation at the C<sub>2</sub> 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  $(c_{\text{buff}}^{\text{tot}} = 0.1 \text{ M})$ . 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 counterelectrode. Before each experiment, the GC electrode was polished with alumina powder (0.5  $\mu$ m), 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<sub>2</sub>O (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<sub>2</sub>O (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 ( $c_{\text{EA}} = 5 \times 10^{-4}$  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<sup>3</sup> of the standard 0.1 M HCl solution was added to 15.00 cm<sup>3</sup> of the working EA solution ( $c = 5 \times 10^{-4}$  M). All probes were titrated with 5 mm<sup>3</sup> increments of the standard 0.1 M NaOH solution. Measured pH values were converted to  $pc_{\rm H}$  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<sub>w</sub> value (pK<sub>w</sub> = 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  $pc_{\rm H}$  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  $(c = 1 \times 10^{-3} \text{ M})$  was prepared in methanol. Working solutions  $(c = 1 \times 10^{-5} \text{ M})$  were prepared in deionized water (methanol concentration 1 %, vol.) in the  $pc_{\text{H}}$  range of 1.1–12.5 [I = 0.1 M (NaCl)]. HCl solutions were used for the  $pc_{\text{H}}$  range of 1.1–4.0, acetate buffers for  $pc_{\text{H}}$  4.0–6.0, phosphate buffers for  $pc_{\text{H}}$  6.0–8.5 and  $pc_{\text{H}}$  11.0–12.0, carbonate buffers for  $pc_{\text{H}}$  8.5–11.0 ( $c_{\text{buff}}^{\text{tot}} = 0.01 \text{ M}$ ), and NaOH solutions for  $pc_{\text{H}}$  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  $(\Delta H_{\rm f}^{\theta})$  and net atomic charges were calculated at semiempirical level (MOPAC 2007, MNDO-RM1 Hamiltonian [33, 34], including the simulation solvent as dielectric continuum, COSMO).

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