

Electrochemical behaviour of substances responsible for the vanilla aroma and a study of their structural analogues at the glassy carbon electrode

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In this article, the electrochemical behaviour of vanillin, ethylvanilin, and of their structural analogues has been studied at the glassy carbon electrode using cyclic voltammetry. Based on experimental observations and information from literature, the respective electrode reaction pathways were proposed according to the ECE mechanisms in all cases, when up to three oxidation products were identified from one reactant. It has been found that the electrode oxidation of vanillin and ethylvanillin occurs in one single step, followed by hydrolysis in ortho-position and/or cleavage of the corresponding alcohol, giving rise to two electrochemically reversible oxidation products.

Keywords: Vanillin; Ethylvanillin; *o*-Cresol; Guaiacol; 4-Hydroxybenzaldehyde

Introduction

Vanillin (VA) and ethylvanillin (EVA) are known as flavouring agents in the production of chocolate, biscuits, and other sweets. As indicated by the names themselves, both VA and EVA differ in the length of alkyl chain bonded by etheric oxygen atom, having also other structural analogues, namely: *o*-cresol (*o*-C), guaiacol (GUA), and 4-hydroxybenzaldehyde (4-HBA).

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All these substances are fairly electroactive and represent the attractive subjects for electrochemical studies at solid and solid-like electrodes [1,2], especially those, performed by cyclic voltammetry (CV [3]) in combination with the glassy carbon electrode (GCE [4]).

In this article, VA, EVA and the already mentioned structural analogues were selected for a comparative study, in which the individual pathways for their electrode oxidation were proposed — partially based on the data reported in literature [5–8] — and then experimentally examined by CV with the GCE. Regarding the selection of the VA and EVA analogues, 4-HBA can be considered as an EVA without ethoxy group ($\text{CH}_3\text{CH}_2\text{O}-\text{Ar}$), GUA as VA without an aldehyde functional group on the benzene ring ($\text{Ar}-\text{HC}=\text{O}$) and *o*-C as GUA bearing the methyl group ($\text{Ar}-\text{CH}_3$) instead of methoxy group ($\text{H}_3\text{CO}-\text{Ar}$). Based on the evaluation of the cyclic voltammograms (CVs) obtained, it was possible to elucidate possible pathways of the corresponding electrode reactions by considering the effect of the individual functional groups, and thus likely define the voltammetric behaviour of all the investigated substances.

Materials and methods

Chemicals and reagents

Vanillin ($\geq 97\%$), ethylvanillin ($\geq 98\%$), *o*-cresol ($\geq 99\%$), catechol ($\geq 99\%$), guaiacol ($\geq 99\%$), and 4-hydroxybenzaldehyde ($\geq 95.0\%$) were purchased from Merck (Darmstadt, Germany). Other chemicals, such as, sodium hydroxide and potassium chloride, hydrochloric acid (35%), ethanol (96%), disodium phosphate dihydrate, and disodium phosphate monohydrate (both solid; all originating from Lach-Ner (Neratovice, Czech Republic), were used to prepare the respective supporting electrolytes. Deionized water with a resistivity of $18.3 \text{ M}\Omega \text{ cm}$ was obtained from a Millipore Milli-Q[®] purification system (supplied by the above-mentioned Merck).

Instrumentation

All electrochemical measurements were carried out in a conventional three-electrode arrangement consisting of the glassy carbon electrode (GCE) with diameter of 3 mm (working), a silver chloride electrode (length 12.5 cm) with 3 mol L^{-1} KCl salt bridge (reference) and a platinum plate (auxiliary electrode). All these electrodes (from Metrohm, Czech division, Prague, Czech Republic) were connected to a potentiostat/galvanostat (model Autolab PGSTAT101) from the same company and operated via the NOVA 1.11 software.

Mechanical pretreatment of the glassy carbon electrode

Before each electrochemical experiment, the surface of GCE (with diameter of 3 mm; Metrohm) was mechanically renewed by polishing with alumina powder (with particle size of 0.3 μm) applied as a slurry (fine water dispersion) for 30 s. Furthermore, the surface was rinsed with a stream of deionized water and dried using a wood pulp paper.

Method

Cyclic voltammetry (CV) was chosen as the proper (diagnostic) technique to study electrochemical reactions of the substances responsible for the vanilla aroma. If not stated otherwise, the potential range (E_r) was set from -0.4 to $+1.6$ V vs. ref., step potential (E_{step}) as 2.5 mV, scan rate (ν) from 10 to 250 $\text{mV}\cdot\text{s}^{-1}$, when five repetitive cycles were used as typical conditions.

Results and discussion

Fig. 1 makes a comparison of repetitive cyclic voltammograms obtained in 0.1 mol L^{-1} phosphate buffer (PB; pH 6.0) containing always 5 % (v/v) ethanol (EtOH) and one of the substances investigated. At first glance, it is evident that substances having free *para* position on a benzene ring (o-C and GUA) have provided two redox couples in the subsequent cycles, where the oxidation peak height in the first cycle decreased with the number of replicates and the subsequent peaks increased, indicating an ECE electrode mechanism.

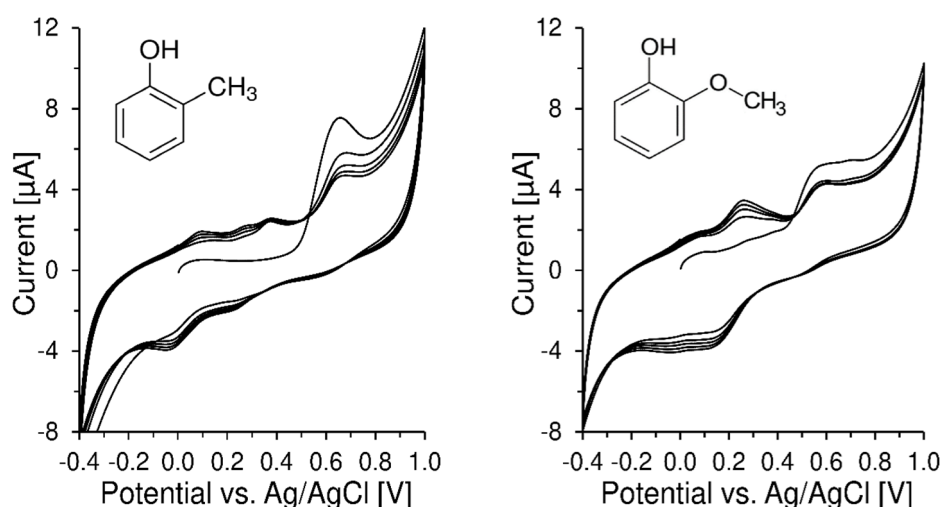


Fig. 1 A quartet of cyclic voltammograms of 0.5 mmol L^{-1} o-C, GUA, 4-HBA, and VA in 0.1 mol L^{-1} PB (pH 6) with 5% (v/v) EtOH recorded on GCE at a scan rate of 50 mV s^{-1}

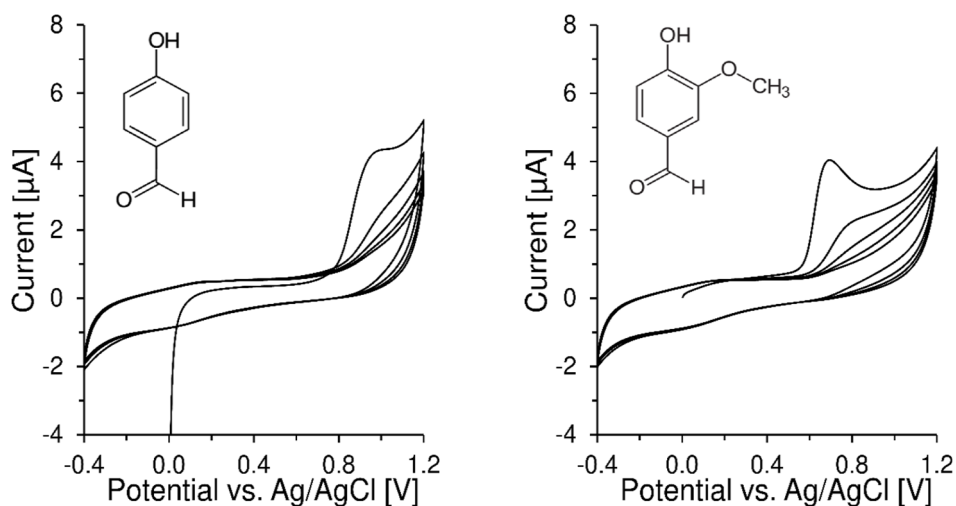


Fig. 1 (continued) A quartet of cyclic voltammograms of 0.5 mmol L^{-1} o-C, GUA, 4-HBA, and VA in 0.1 mol L^{-1} PB (pH 6) with 5% (v/v) EtOH recorded on GCE at a scan rate of 50 mV s^{-1}

GUA provided two characteristic redox signals, described by values of the peak potentials $E_p^a = +0.588 \text{ V}$ and $E_p^c = -0.037 \text{ V}$. Nearly identical values were also obtained for *o*-quinone/*o*-catechol redox pair (with $E_p^a = +0.589 \text{ V}$ and $E_p^c = -0.040 \text{ V}$), even though another GUA reduction peak at $+0.151 \text{ V}$ was observed. This finding corresponds to the fact that methanol is cleaved to form *o*-quinone. Moreover, the second redox couple corresponding to the formation of yet another product also participates in a reversible electrode reaction (with $E_p^a = +0.098 \text{ V}$ and $E_p^c = -0.017 \text{ V}$ for o-C; and with $E_p^a = +0.056 \text{ V}$ and $E_p^c = -0.056 \text{ V}$ for GUA).

In comparison with similar electrochemical behaviour of o-C and GUA, just a trace of a redox couple was observed for 4-HBA ($E_p^a = +0.168 \text{ V}$ and $E_p^c = +0.044 \text{ V}$) and VA ($E_p^a = +0.151 \text{ V}$ and $E_p^c = +0.037 \text{ V}$) having *para* position on the benzene ring occupied by aldehyde functional group. Hence, it can be assumed here that a hydroxylation of the benzene ring of o-C and GUA (as the nucleophilic addition of water with the subsequent detachment of H^+ ion) probably occurs in the *para* position to form *p*-quinone with methyl and methoxy groups in *ortho* position, respectively.

The proposed o-C electrochemical oxidation pathway is shown in Fig. 2. The electrochemical behaviour of o-C could be considered as a general scheme for *ortho*-substituted phenols. In special literature [1,2], it has already been emphasised that the electrochemical oxidation of phenol is pH-dependent (with typical shift of the peak potential) and of irreversible character (seen as the decreasing oxidation peak with the pronounced cycle).

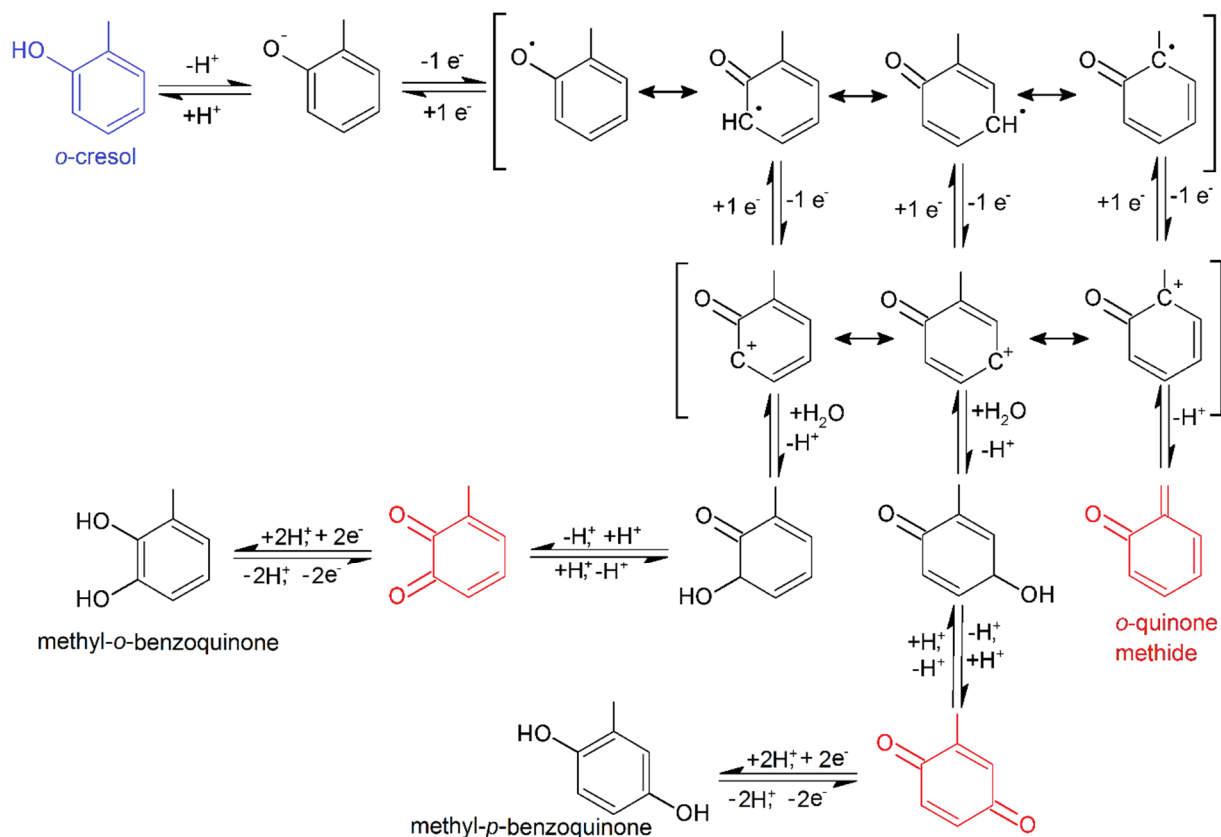


Fig. 2 Proposed pathway of electrochemical oxidation of *o*-cresol

Basically, the oxidation occurs in one step, followed by hydrolysis in *ortho*- and *para*-positions, leading to two oxidation products, hydroquinone and catechol [5]. Analogically, phenols having occupied *para* surface by a substituent and free *ortho* position — or vice versa — undergo a hydroxylation at the free position during their electrochemical oxidation.

According to information given in the previous paragraph, GUA represents an *ortho*-substituted phenol, and therefore, it should undergo the same electrochemical oxidation pathway as that for *o*-C. However, as one can learn from the literature [6–8], methanol is cleaved to form *o*-quinone similarly as other *o*-methoxyphenols, especially capsaicinoids [9–11]. Based on these findings, three products of the electrochemical oxidation of GUA can be expected, namely 2-methoxy-1,4-benzoquinone, 3-methoxy-1,2-benzoquinone, and 1,2-benzoquinone (see red-coloured chemical structures in Fig. 3). While the blue-coloured chemical structures indicate the original substances.

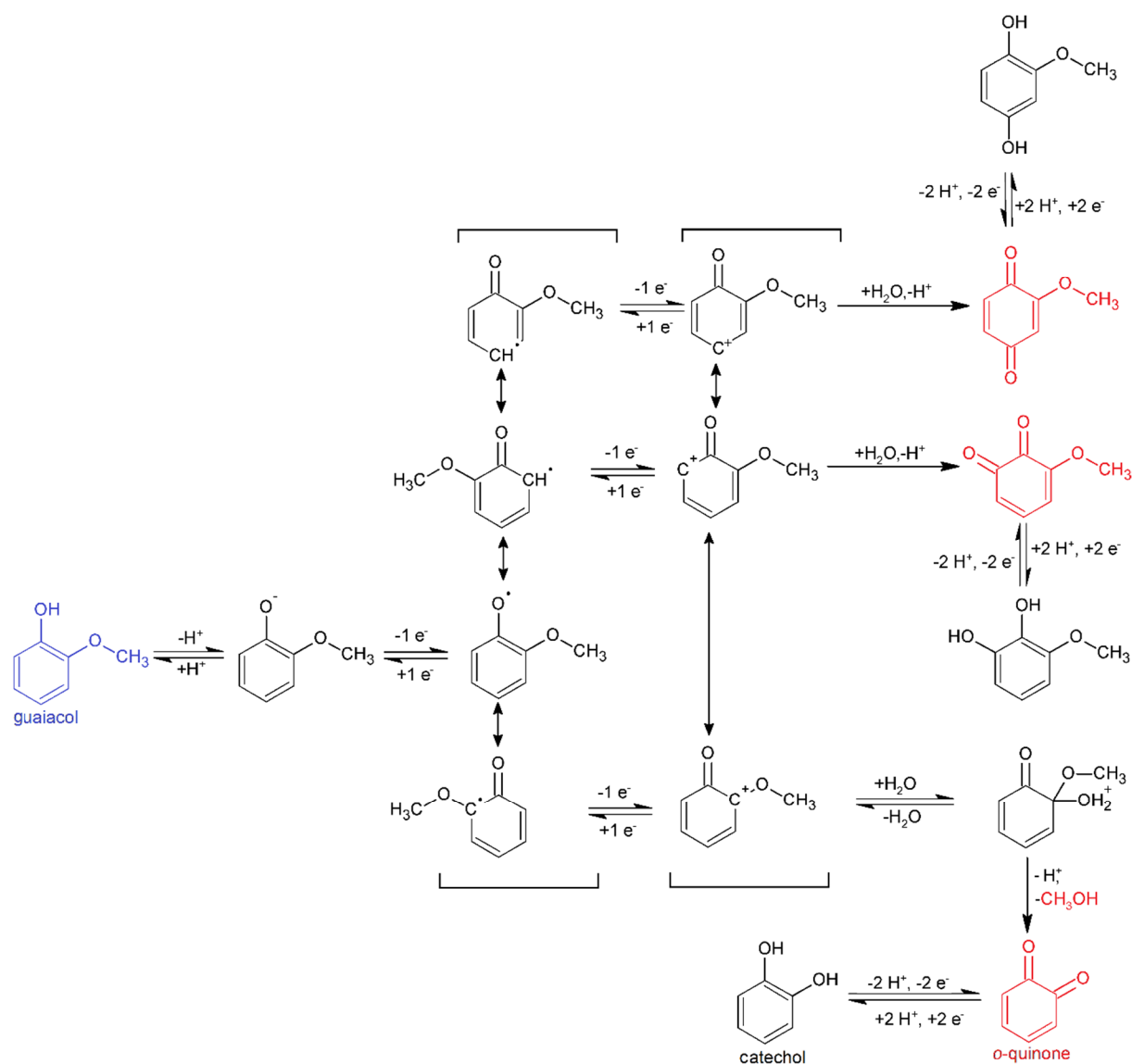


Fig. 3 Proposed pathway of the electrochemical oxidation of guaiacol

The 4-HBA does not have the methoxy (VA) or ethoxy (EVA) group in its molecular structure. Due to the occupation of the *para* position by aldehyde group, it is clear that solely the free *ortho* position may be a site to be hydroxylated, as demonstrated in Fig. 4. In the acidic working medium, 4-HBA provided a distinguishable redox couple ($E_p^a = +0.339$ V and $E_p^c = +0.178$ V) at the subsequent repetitive cycles. Very similar redox pairs have been observed for VA ($E_p^a = +0.320$ V and $E_p^c = +0.180$ V) and EVA ($E_p^a = +0.320$ V and $E_p^c = +0.176$ V) bearing aldehyde group in *para* position. Hence, it seems that VA and EVA exhibit the electrochemical oxidation pathway like that for 4-HBA

VA, EVA, and 4-HBA provided only one oxidation peak in neutral aqueous solution (in the first cycle), which could be attributed to the oxidation of hydroxyl group with the participation of two electrons and one proton.

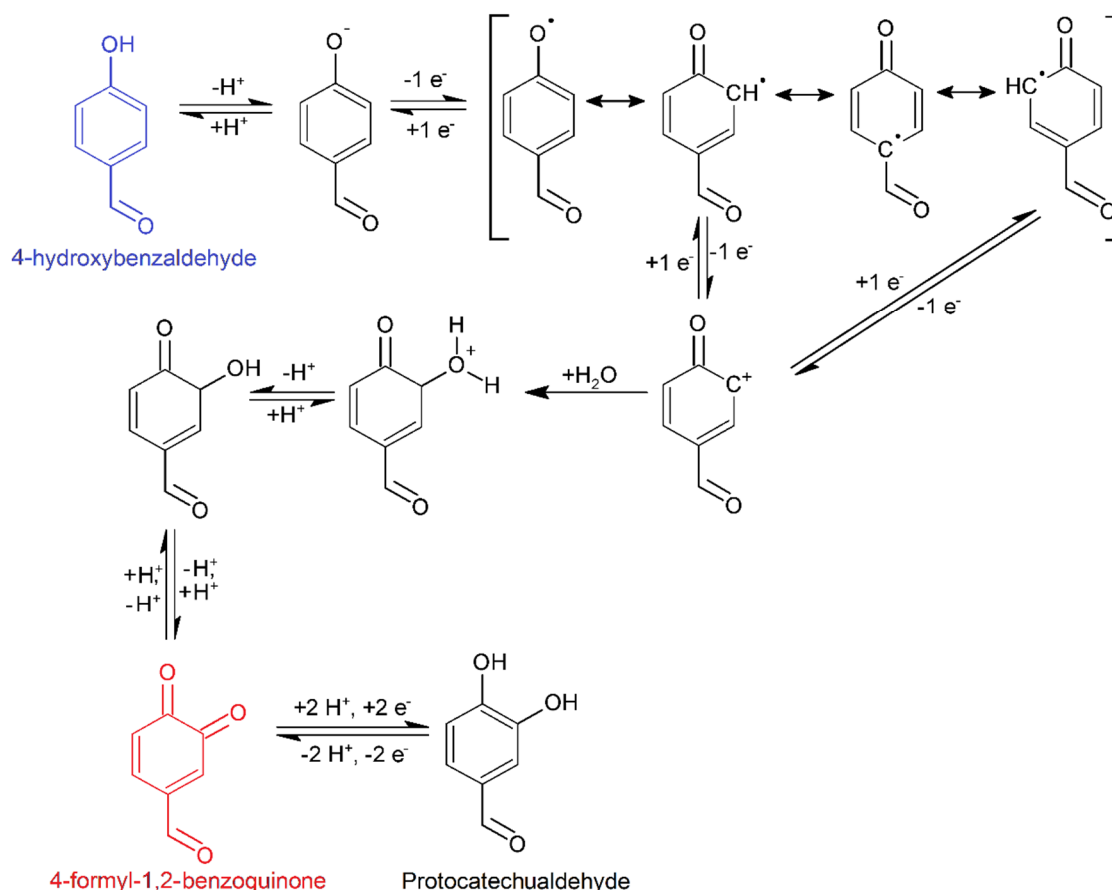


Fig. 4 Proposed pathway of the electrochemical oxidation of 4-hydroxybenzaldehyde

However, the situation was markedly different when voltammetric measurements were performed in acidic or basic media (compare the voltammograms for 4-HBA and EVA in Fig. 5). Since EVA differs from 4-HBA only in the presence of an ethoxy group, it could be deemed from that this functional group has contributed significantly to the electrode and/or subsequent chemical reaction. The 4-HBA provided always one oxidation peak regardless of pH of the working medium or the scan rate (ν) used. On the contrary, two partially overlapping oxidation peaks were observed for EVA whose position (the peak potentials, respectively) had depended upon the pH used. In addition, it is important to notice the similarities of the reduction signals (number, position and shape), especially those for o-C, GUA, and EVA that have given rise to the reduction peaks at +0.479, +0.459, and +0.457 V in 0.01 mol L⁻¹ HCl with 0.1 mol L⁻¹ KCl (pH 2.05), respectively.

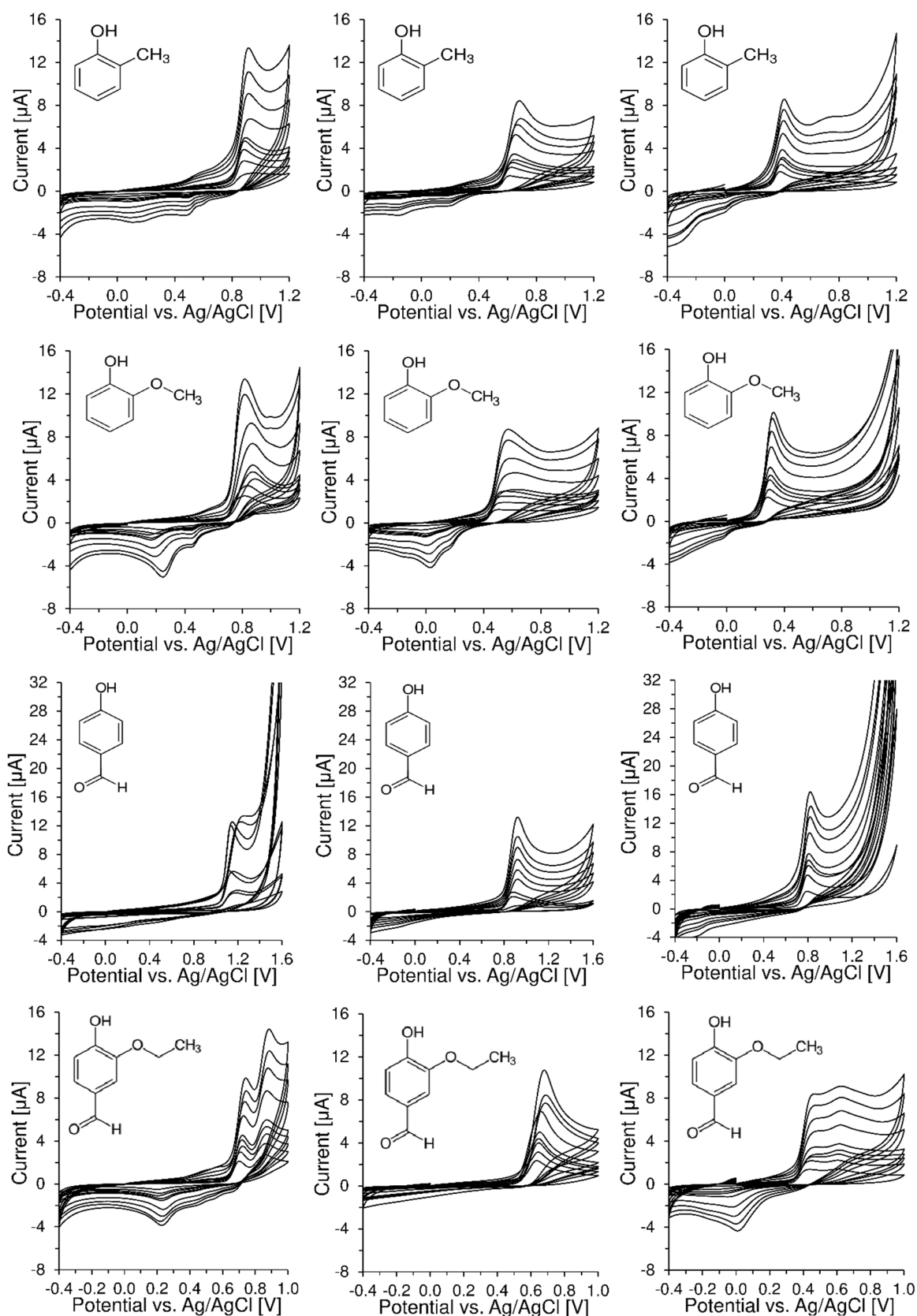


Fig. 5 Cyclic voltammograms of 0.5 mmol L^{-1} o-C, GUA, 4-HBA, and EVA in 0.01 mol L^{-1} HCl with 0.1 mol L^{-1} KCl, 0.1 mol L^{-1} PB (pH 6) with 5% (v/v) EtOH, and 0.01 mol L^{-1} NaOH with 0.1 mol L^{-1} KCl (order from left to right) recorded on GCE at scan rate of 10, 20, 30, 40, 50, 100, 150, 200, and 1250 mV s^{-1}

Further, it was found that similar reduction peaks at +0.256 V for GUA and at +0.237 V for EVA were obtained (compare with reduction peak at +0.138 V for o-C). In highly alkaline medium of 0.01 mol L⁻¹ NaOH containing 0.1 mol L⁻¹ KCl (pH 11.8), EVA exhibited at +0.020 V markedly higher reduction peak than other organic compounds investigated. As shown in Fig. 6, it can be assumed that EVA is electrochemically oxidised to form 4-formyl-1,2-benzoquinone and 3-ethoxy-5-formyl-1,2-benzoquinone.

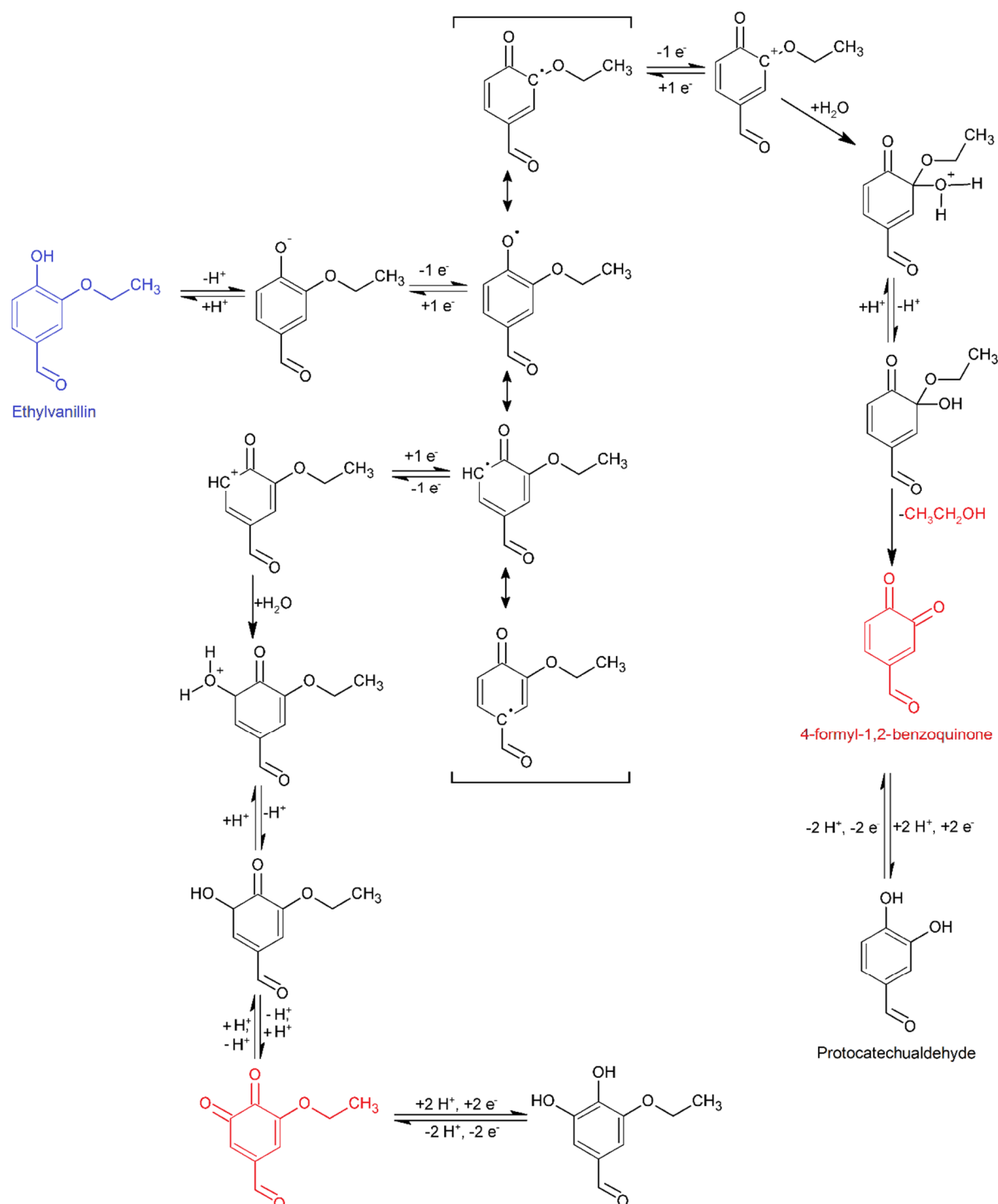


Fig. 6 Proposed pathway of the electrochemical oxidation of ethylvanillin (EVA)

At the first anodic scan, all investigated phenolic compounds provided only one sensitive oxidation peak differing in the value of the peak potential. Under buffered neutral pH conditions, oxidation peak potentials of +0.706 V, +0.664 V, +0.591 V, +0.991 V, and +0.669 V for VA, EVA, GUA, 4-HBA, and o-C, were found. All these substances gave rise to the anodic peak(s) corresponding to the oxidation of the hydroxy group (HO-Ar) and hence, it is clear that the present substituents (“electron acceptors or donors”) significantly affect the value of the potential required to complete this electrode reaction.

For example, an aldehyde group (OHC-Ar) in the *para* position with a moderate negative mesomeric effect draws electron density away from the aromatic ring, and therefore, the oxidation of 4-HBA is more difficult than those having in *ortho* position an ether functional group (Ar-O-R) with positive mesomeric effect. For that reason, GUA is the most easily oxidisable phenolic substance. The methoxy group represents an electron donor being stronger than the methyl group (o-C). In the case of VA and EVA, the aldehyde group in the *para* position is affected by the presence of either methoxy (Ar-O-CH₃) or ethoxy group (Ar-O-CH₂CH₃), respectively.

Conclusions

Herein, it can be concluded that the electrochemical oxidation of vanillin and ethylvanillin is pH-dependent and irreversible as in the case of their structural analogues (*o*-cresol, guaiacol, and 4-hydroxy-benzaldehyde). In general, the electrochemical oxidation of vanillin and ethylvanillin occurs in one step, followed by hydrolysis in *ortho* position and/or cleavage of the corresponding alcohol, leading to the two electrochemically reversible oxidation products. Based on these observation and results, the ECE mechanism should be accepted.

Acknowledgements

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