

Cyclic Voltammetric Studies on Some Antioxidants with Carbon Paste-Based and Boron-Doped Diamond Electrodes

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Abstract: Some selected antioxidants and (poly)phenolic compounds occurring as basic skeletons in antioxidants were investigated with carbon paste and boron-doped diamond electrodes using cyclic voltammetry and hydrodynamic amperometry. The results show that the doped diamond electrode provides stable and reproducible responses which can be exploited for analytical purposes whereas with carbon paste strong adsorption of phenol and hydroquinone as well as of their oxidation products occurs.

Keywords: Antioxidants; Hydroquinone; Cyclic voltammetry; Hydrodynamic amperometry; Carbon paste electrode; Boron-doped diamond electrode.

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Introduction

Carbonaceous materials have gained enormous interest as electrode materials in the past few decades. Apart from graphite and glassy carbon (vitreous carbon), particularly heterogeneous composites containing graphite powder and a binder became extremely valuable due to their ease of preparation, handling and modification [1-7].

The milestone with highest impact was the invention of carbon paste with a liquid binder by Adams in 1958 [8]. In the 1980s screen printed electrodes, also called thick film electrodes, followed using a more robust solid polymeric matrix for the embedded graphite particles combined with screen printing technology, a concept which had been taken from electronic industry [5]. Recently the discovery of new allotropic modifications of carbon exerted a strong impetus on electrochemical sensor research. Whereas fullerenes seemed to present a more or less fashionable trend after the award of the Nobel prize for their practical discovery in 1996 (they had been theoretically predicted already by Osawa in the 1970s) it seems that carbon nanotubes seem to have a sustainable impact on sensor technology due to their catalytic properties in many electrochemical reactions.

Some 20 years ago boron-doped diamond (BDD) thin films were introduced by Fujishima [9] after doping of diamond had been suggested already by Japanese and Russian researchers [10, 11]. In the meanwhile the number of publications dealing with BDD has exceeded 3000 already, and quite a few reviews have appeared already ([12-20] and references therein). What makes this material so peculiar? BDD is usually generated as a thin film on a conductive substrate by chemical vapor deposition. Whereas diamond is a typical insulator the boron-doped variety shows good semiconductor properties (see Fig. 1, after [13]). Due to the insertion of boron atoms with three valence electrons into the three dimensional carbon structure typical p-doping results with empty levels of less than 0.4 eV above the valence band. Basically also n-doping with P and N atoms is possible but with less favorable energetic conditions with respect to the conduction band.

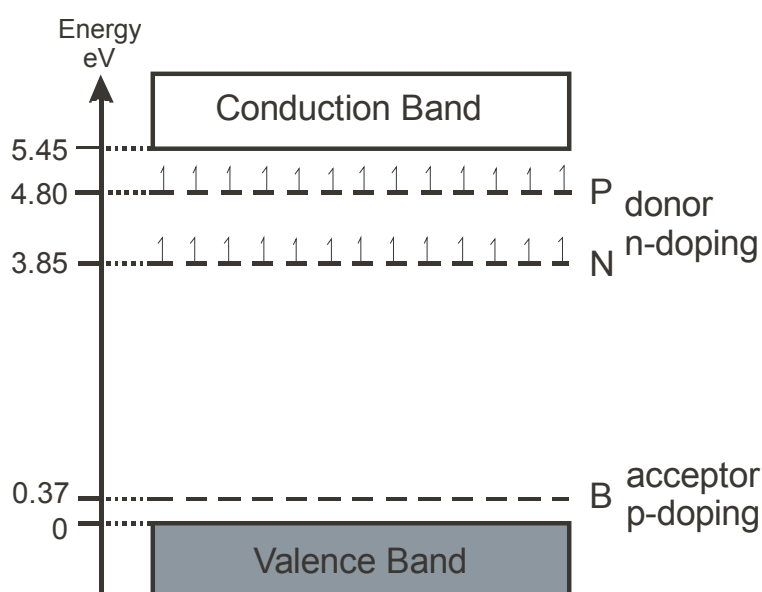


Fig. 1:
Approximate energy levels of p- and n-doped diamond.

BDD as electrode material is characterized by high chemical inertness and a wide applicable potential window particularly at the positive potential edge (up to 2 V vs. ref. and even more). Thus, it was soon discovered that the material can be used to design electrodes for electroanalysis [21].

Antioxidants are important secondary plant metabolites which are essential food constituents and play an important role in oxidative stress due to their ability to decrease the concentration of radicals and reactive oxygen species (ROS) in cells. In popular literature some of these substances (e.g., resveratrol from grapes and wine) have been associated even with “magic properties” for humans such as anti-aging and life-prolonging effects. Many of these propaganda issues are ambiguous and scientifically unproven, but without doubt antioxidants play an important role in detoxification of radicals and oxygen species in the human body. Though in common opinion antioxidants are related generally with positive health effects only, it is, nevertheless, worth to mention that on one hand some antioxidants (even ascorbic acid) seem to stimulate the production of radicals and ROS (pro-oxidants), and that, on the other, free radicals and oxidizing species (e.g., NO) are essential messengers in redox signaling which can be decoupled in their action by the presence of excess antioxidants. Usual methods for the determination of antioxidants rely on non-differentiating cumulative detection of certain groups or even all antioxidants (total antioxidant capacity TAOC) by their reducing effect in equivalents of a template antioxidant, e.g. the vitamin E derivative 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). Nevertheless, different chemical groups of antioxidants react differently, and this is the reason why often antioxidant capacities cannot be compared directly among each other.

An important class of substances with antioxidant properties is made up by phenols and polyphenols. Their determination can be done preferentially electrochemically because phenols show usually good electroactivity at different types of electrodes either by direct oxidation or by involvement of an oxygenase, such as phenol- or polyphenol-oxidase, tyrosinase or laccase.

The aim of this work is to study the ability of boron-doped diamond electrodes (BDDEs) for the direct oxidation of some selected phenols and polyphenols which can be found as fundamental structural element in more complex phenolic antioxidants like flavonoids, isoflavonoids and neoflavonoids. BDDEs will be compared with carbon paste electrodes (CPEs). Cyclic voltammetry (CV) and hydrodynamic amperometry (HA) are the methods of choice for electrochemical analysis.

Experimental

Chemicals, Materials, and Reagents

Deionized water was refined with a purification system (Milli-Q, Millipore) and had a specific resistivity of 18.2 M Ω cm; it was used throughout the experiments. All chemicals were of reagent grade and were purchased from Sigma-Aldrich (Vienna, Austria).

Graphite powder (RW-C) was obtained from Ringsdorff (Bad Godesberg, Germany); paraffin oil (Uvasol) was bought from Merck (Vienna, Austria). Carbon paste was prepared from graphite powder (1.00 g) and paraffin oil (350 μ L); the components were intimately mixed with a spatula for 15 minutes and were left to stand overnight at ambient laboratory conditions before use.

Boron-doped diamond lamellas (BDD thin films on Ti substrate with gold contact layer) were from Fraunhofer USA (Plymouth, MI). If not mentioned otherwise the supporting electrolyte was phosphate buffer solution (PBS, 0.1 M, pH 7.0) prepared by mixing aqueous NaH₂PO₄ and Na₂HPO₄ solutions (0.1 M) to achieve the desired pH.

Instrumentation

All electrochemical measurements were performed with a potentiostat (AutoLab PGStat101 equipped with dual-channel working electrode and a low current registration module, Metrohm, Switzerland) operated with a corresponding software (Nova, Metrohm). The electrochemical cell was equipped with an Ag/AgCl (3 M KCl)-reference electrode and a Pt-sheet as the counter electrode. The volume of solution in the cell was 20 mL. All potentials quoted below are referred to this reference electrode.

Methods and Procedures

Cyclic voltammetry was performed by polarizing the working electrode (BDDE or CPE) from -1.0 V to 1.4 V for BDDEs and from -0.5 to 1.2 V for CPEs. The scan rate was 50 mV s⁻¹. Hydrodynamic amperograms were recorded by application of the working potential (-1.0 V) and stirring the solution (PBS) until a stable base-line was obtained. Then corresponding amounts of the phenol dissolved in PBS were added. The heights were evaluated by the difference in height of the tangents fitted to the response curve before and after addition of the analyte.

Results and Discussion

A few representative antioxidants containing phenols with typical mono-, di- or trihydroxy-benzene skeletons were studied in detail in order to define the applicability of CPEs and BDDEs for the determination of the phenolic antioxidant capacity. A comparison is made with CPEs to uncover advantages and disadvantages of the different types of electrode.

Cyclic Voltammetry

Phenol can be oxidized at both types of electrodes under investigation; the cyclic voltammogram with a CPE is shown in Fig. 2; the curve recorded with a BDDE is almost identical and therefore not shown.

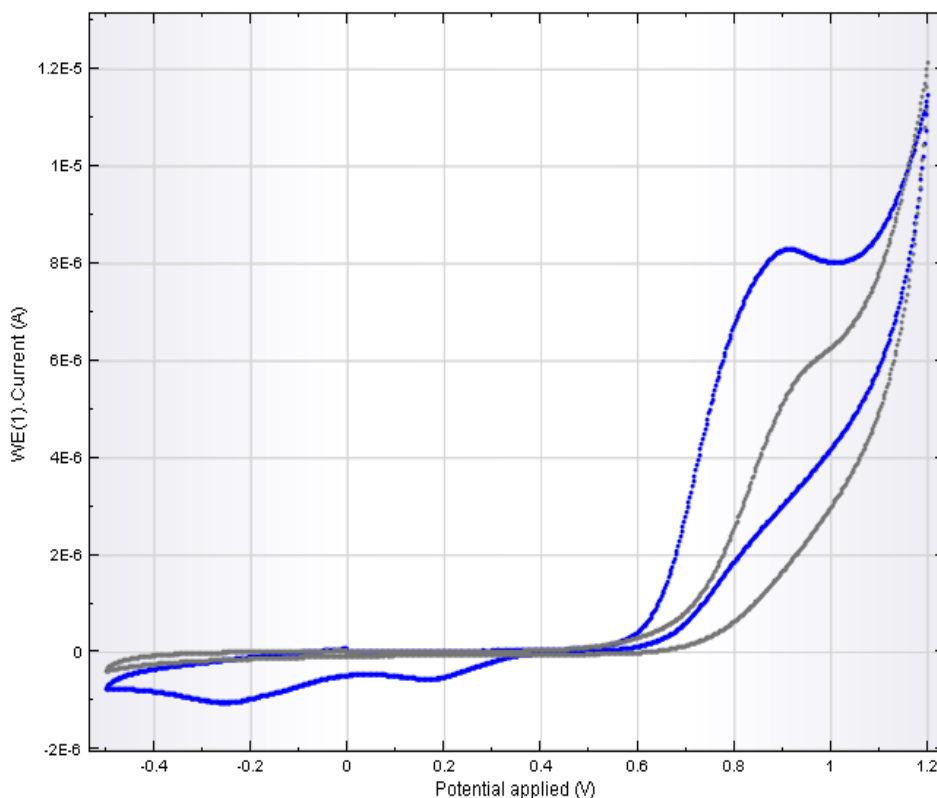
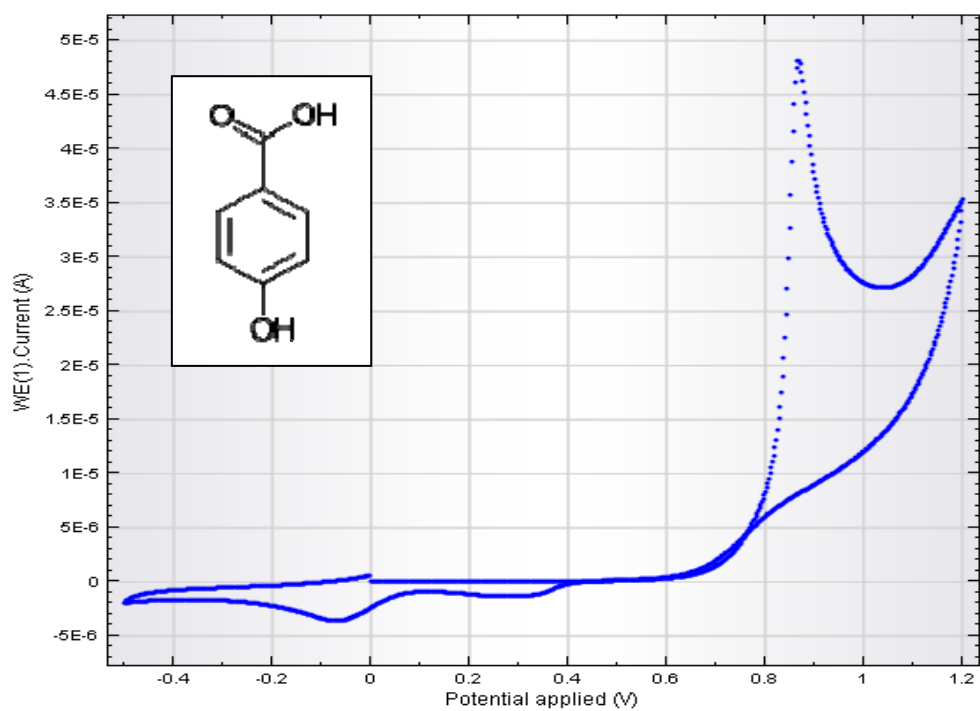


Fig. 2: Cyclic voltammogram of phenol with a CPE; PBS (0.1 M, pH 7); phenol, 2.5 mg/L.

Oxidation of phenol occurs at rather positive potentials (0.9 V) probably with hydroxylation in o- and/or p-position. The generated hydroquinone is further oxidized at positive potentials and then reduced at around -0.2 V. A reduction occurring at +0.2 V indicates that another product is formed, probably a dimer with 1,4-quinoid structure or a polymer.

4-Hydroxybenzoic acid is a monophenol with a carboxylic group in para position. The cyclic voltammograms can be seen in Fig. 3 overleaf:

(a)



(b)

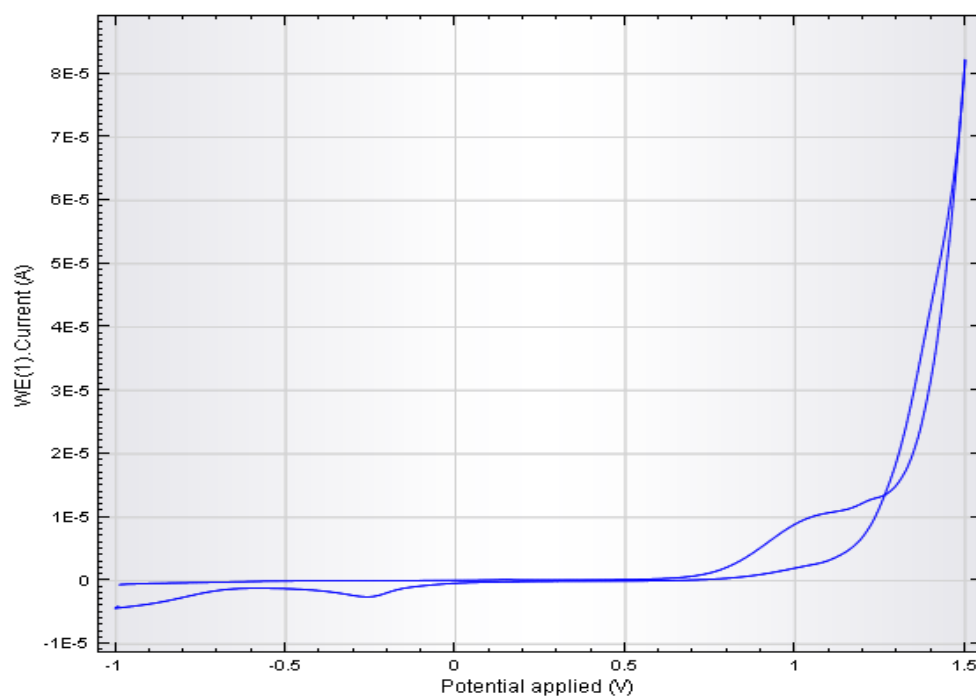


Fig. 3: Cyclic voltammograms of 4-hydroxybenzoic acid at a CPE (A) and a BDDE (B); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

Oxidation occurs again at rather positive potentials with formation of the 3,4-dihydroxy-derivative, which is after its oxidation to the quinone reduced to the hydroquinone. As position 1 is blocked there is no possibility for formation of *para*-quinoid structures, which could be the reason that the reduction signal at 0.2 V is absent compared to non-substituted phenol. The oxidation signal is much more pronounced with CPEs which indicates adsorption of the analyte at the electrode surface; this does not or only to a much smaller extent occur when employing the BDDE.

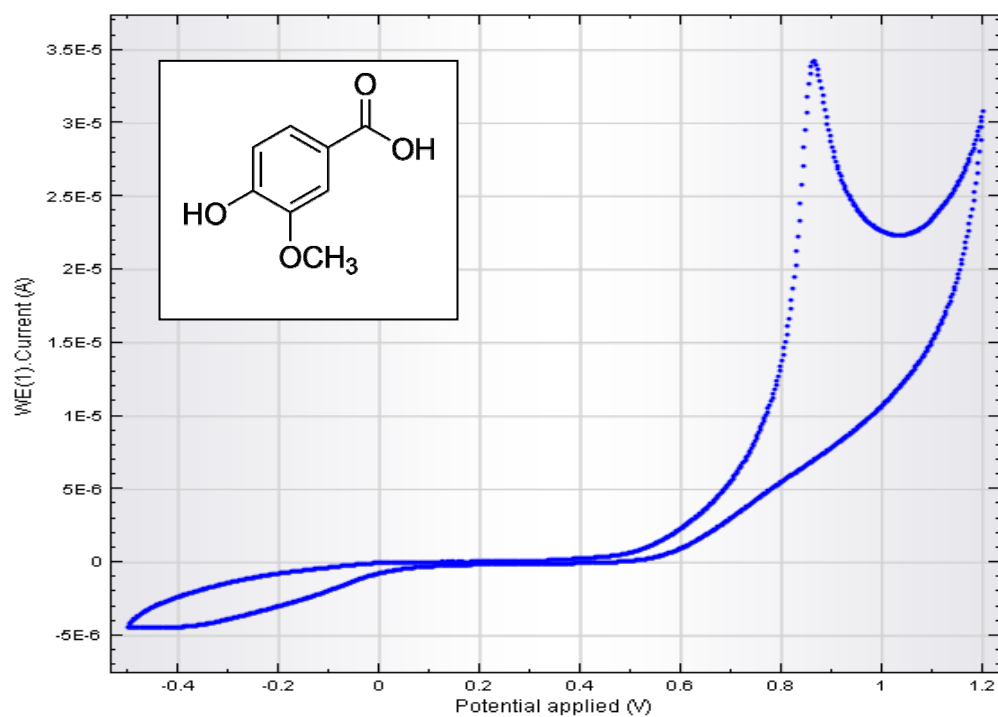
Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is used as a flavoring additive in food stuff. It contains a methoxy group in *m*-position to the carboxyl acid. Though it is a derivative of catechol (1,2-dihydroxybenzene) the cyclic voltammograms are similar to 4-hydroxybenzoic acid because the phenol group in position 3 is bound as an ether; see Fig. 4 overleaf. The signals also can be interpreted similarly as for the 4-OH-derivative. The reduction of the oxidized form shows some split of the signal at the BDDE the reason of which is unclear. A probable reason could be the formation of polymeric products.

Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) is a derivative of pyrogallol, but in fact has only one unblocked phenol group, similar to vanillic acid. The CVs (Fig. 5) differ from the latter probably because the ortho and para positions to the phenolic group are occupied. The main oxidation occurs at 0.9 V whereas on the BDDE significant reduction appears at -0.5 V with a shoulder at around -0.2 V not observable with CPEs. Oxidation(s) and reduction(s) seem rather irreversible probably due to some chemical rearrangements or to polymerization after oxidation. The mechanism is somehow unclear.

Coumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid) is a monophenol with a propenoic acid residue in *para*- position to the phenol group. The compound is irreversibly oxidized on both types of electrodes showing hardly any reduction current at negative potentials (Fig. 6). The first step is probably a hydroxylation in the neighboring position to the phenolic OH-group or just radical formation; nevertheless the double bond in the side chain seems to influence the electrochemical behavior. It is assumed that polymerization is the preferred follow-up reaction of the oxidized species.

Ferulic acid (3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid) is chemically similar to coumaric acid but is derived from catechol, where the second phenolic group is bound to methanol to form an ether.

(a)



(b)

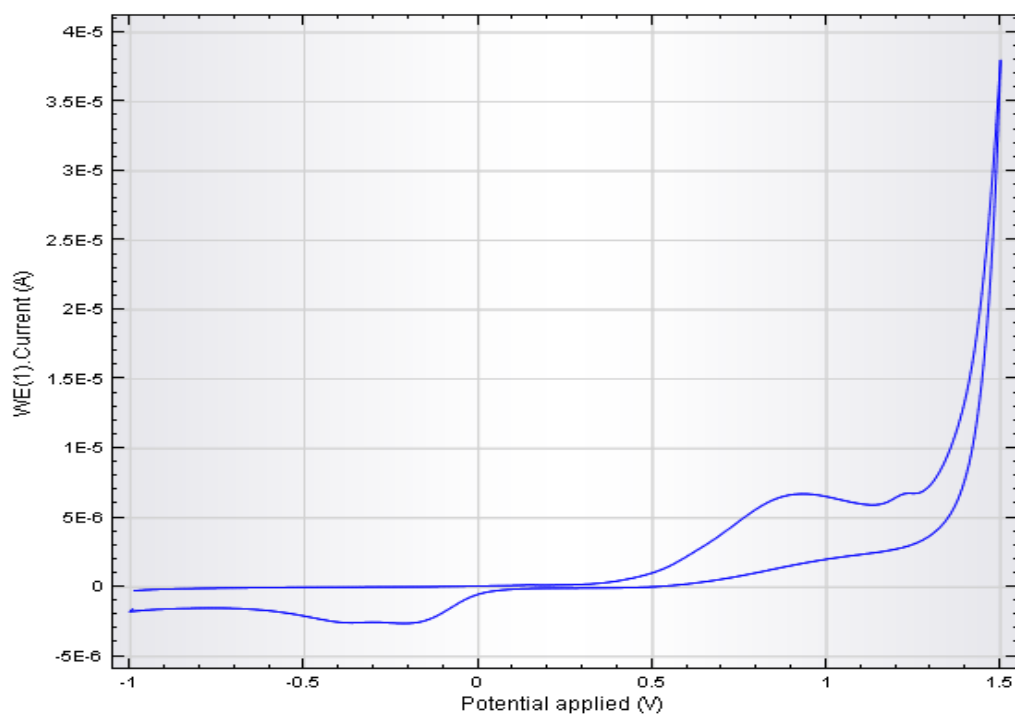


Fig. 4: Cyclic voltammograms of vanillic acid at a CPE (a) and a BDDE (b); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

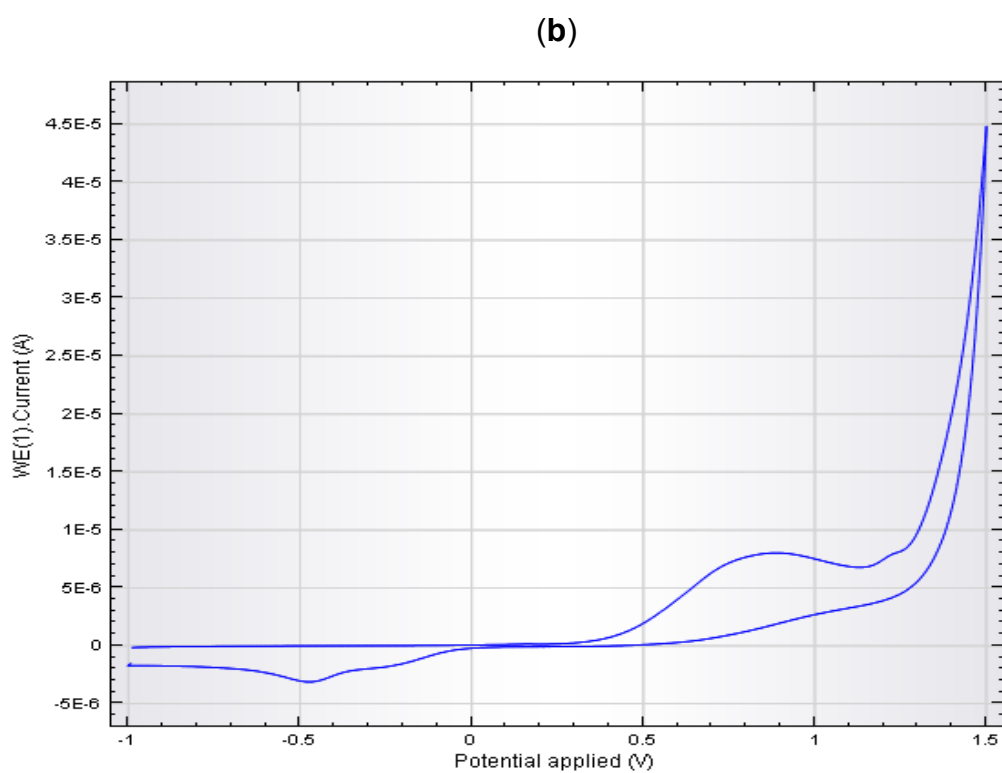
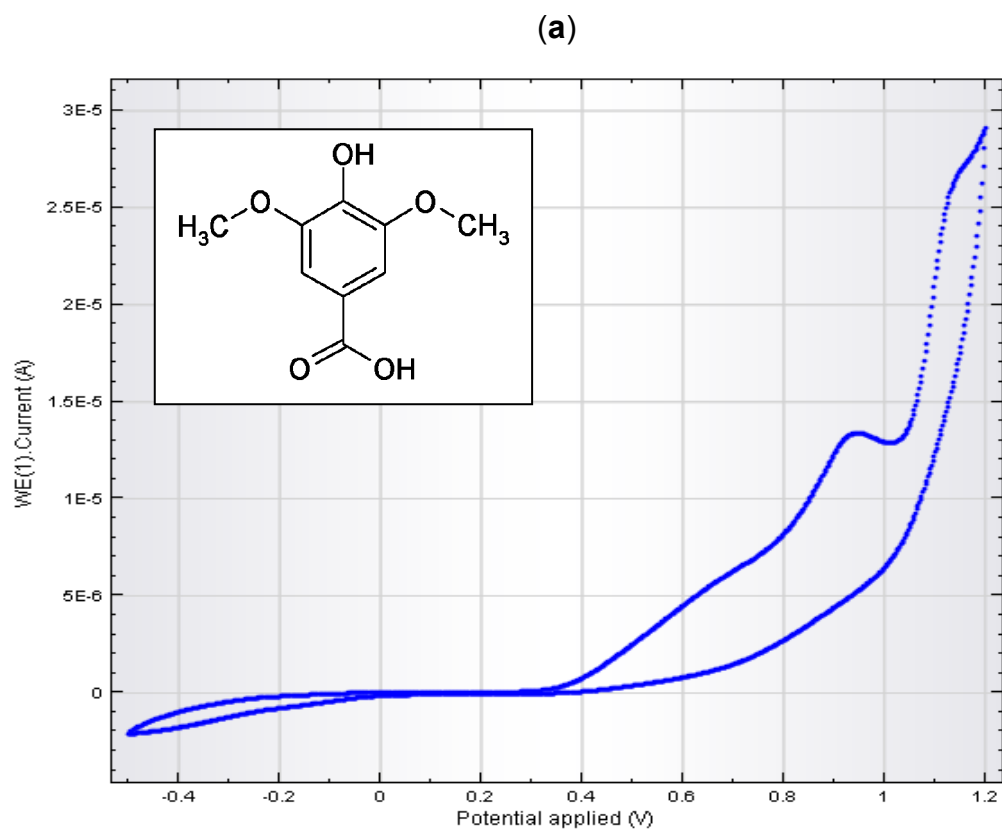


Fig. 5: Cyclic voltammograms of syringic acid at a CPE (a) and a BDDE (b); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

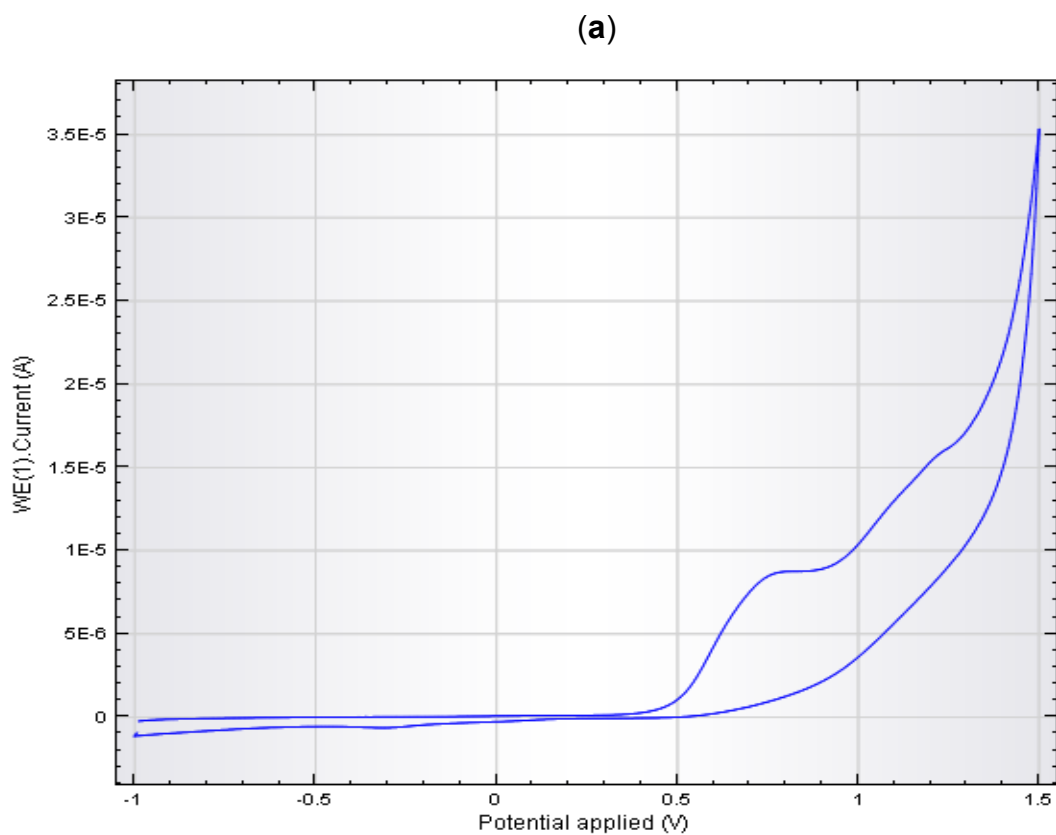
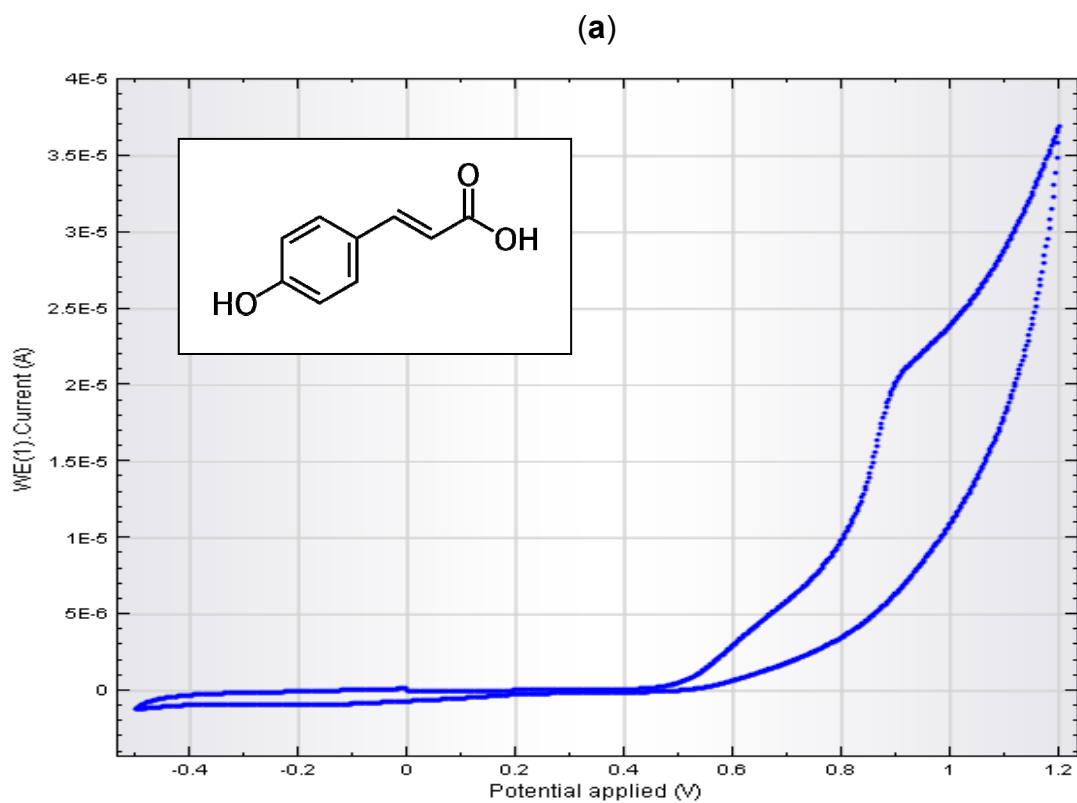


Fig. 6: Cyclic voltammograms of coumaric acid at a CPE (a) and a BDDE (b); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

Two distinct oxidations can be seen in the cyclic voltammograms (see Fig. 7, overleaf) where the one at around 0.5 V is much more pronounced with the BDDE whereas CPE yields a well-shaped peak at around +0.9 V.

It may be assumed that the oxidation to the o-dihydroxy derivative occurs at 0.9 V whereas the signal at 0.5 V could be connected to the double bond in the side chain. The reduction of the quinone to the hydroquinone occurs at -0.2 V. As the behavior is different from coumaric acid, exhibiting a reduction signal it seems probable that with coumaric acid some rearrangement of the molecule takes place involving the neighboring position of -OH which is blocked in ferulic acid.

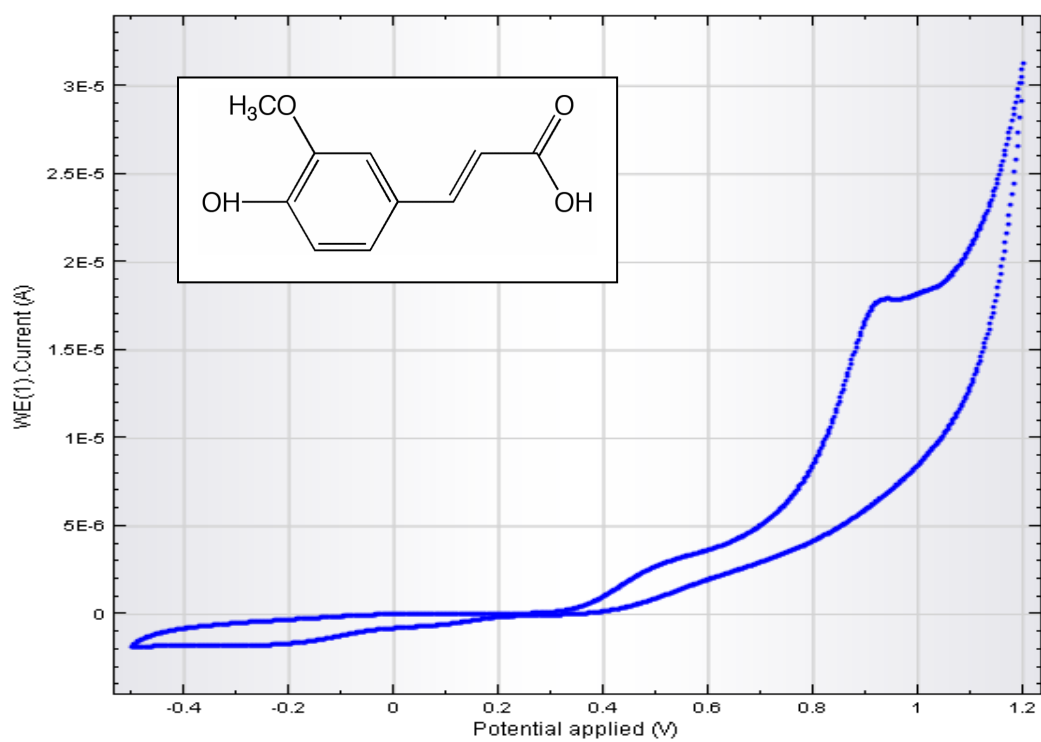
Hydroquinone (HQ) is the simplest polyphenol where the two hydroxyl-groups can be in o-, m- or p-position. p-Hydroquinone shows reversible or quasi-reversible electrochemical behavior with many solid electrodes. It also occurs naturally in glucosylated form as arbutin in a few plants and berries. Fig. 8 (shown below) shows the cyclic voltammograms as obtained with a BDDE (a) and a CPE (b).

HQ shows a well-formed current response of a redox couple with both electrodes. The oxidation occurs at around +0.4 V with formation of 1,4-benzoquinone, which is reduced again at around -0.2 V. The behavior is quasi-reversible due to the large potential separation of the redox processes. The shape shows that the electron transfer process is a bit sluggish; the ratio of the peak currents is about one supporting the classification of quasi-reversibility of the reaction, which is typical mainly for CPE [2,5].

Catechol (1,2-dihydroxybenzene) shows very similar behavior as p-hydroquinone; it displays a quasi-reversible redox signal at the carbon paste electrode (Fig.9); the voltammogram obtained with a BDDE is practical identical. This dihydroxyphenol is oxidized to the o-quinone which can be reduced again to the hydroquinone.

Resorcinol (1,3-dihydroxybenzene) can be oxidized at positive potentials and shows very small reduction currents in the negative potential range only. The cyclic voltammogram with a CPE is shown in Fig. 10; it is very similar to the voltammogram obtained with a BDDE. The oxidized product, probably some hydroxylated derivative (1,2,3- or 1,2,4-trihydroxybenzene) tends to undergo electrochemical polymerization; as a consequence, practically no reduction process can be observed.

(a)



(b)

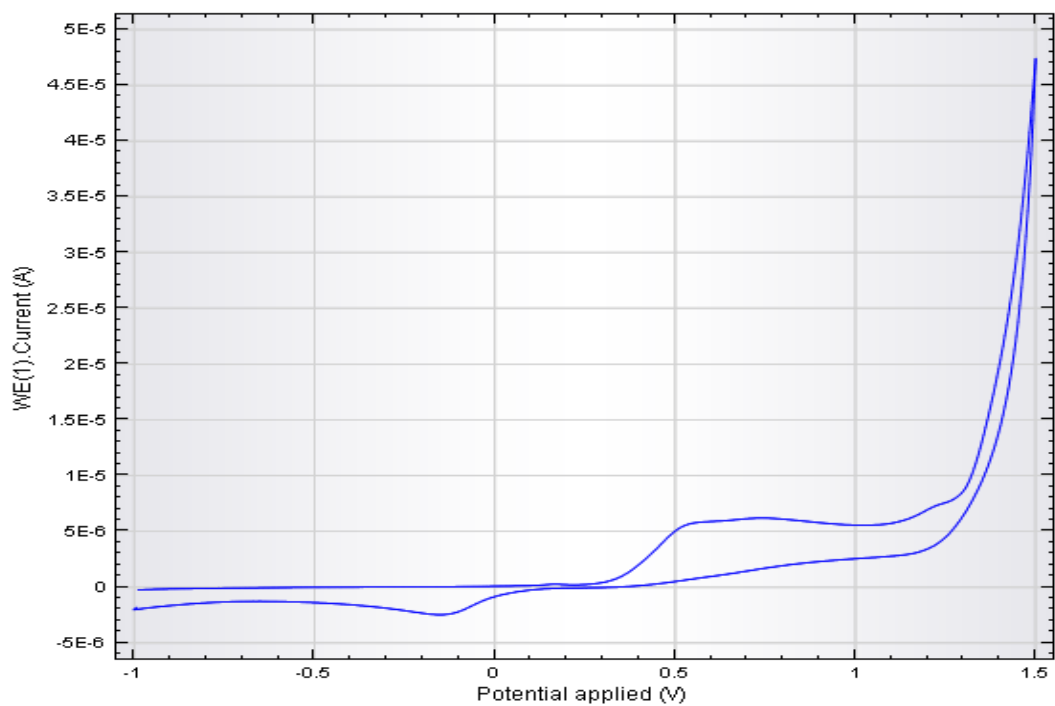


Fig. 7: Cyclic voltammograms of ferulic acid at a CPE (a) and a BDDE (b); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

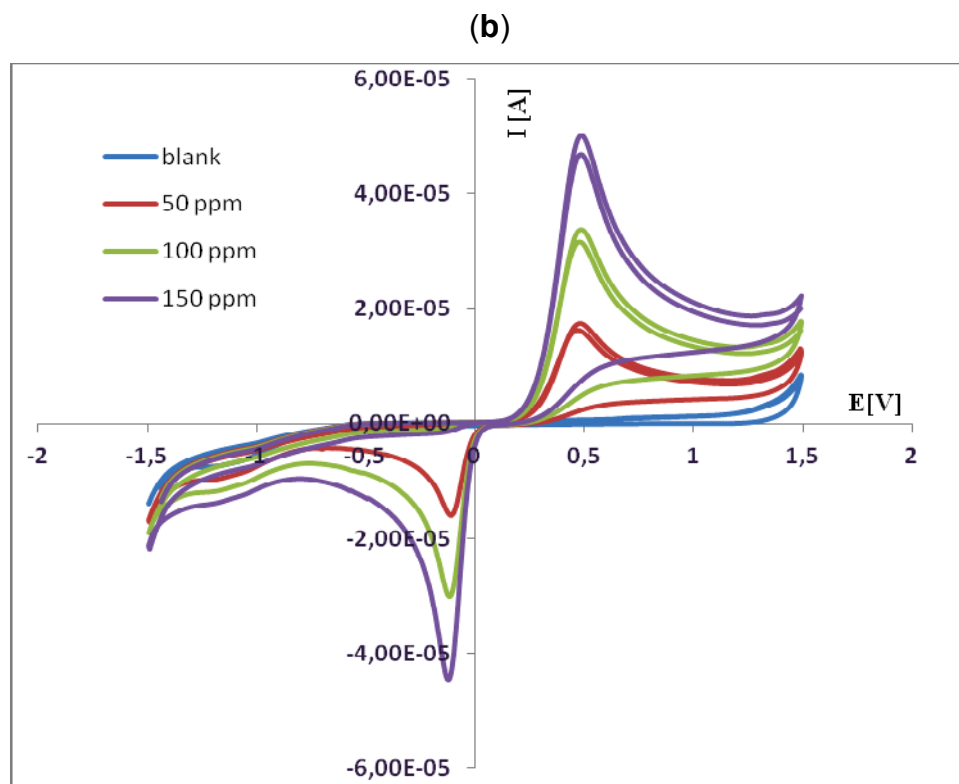
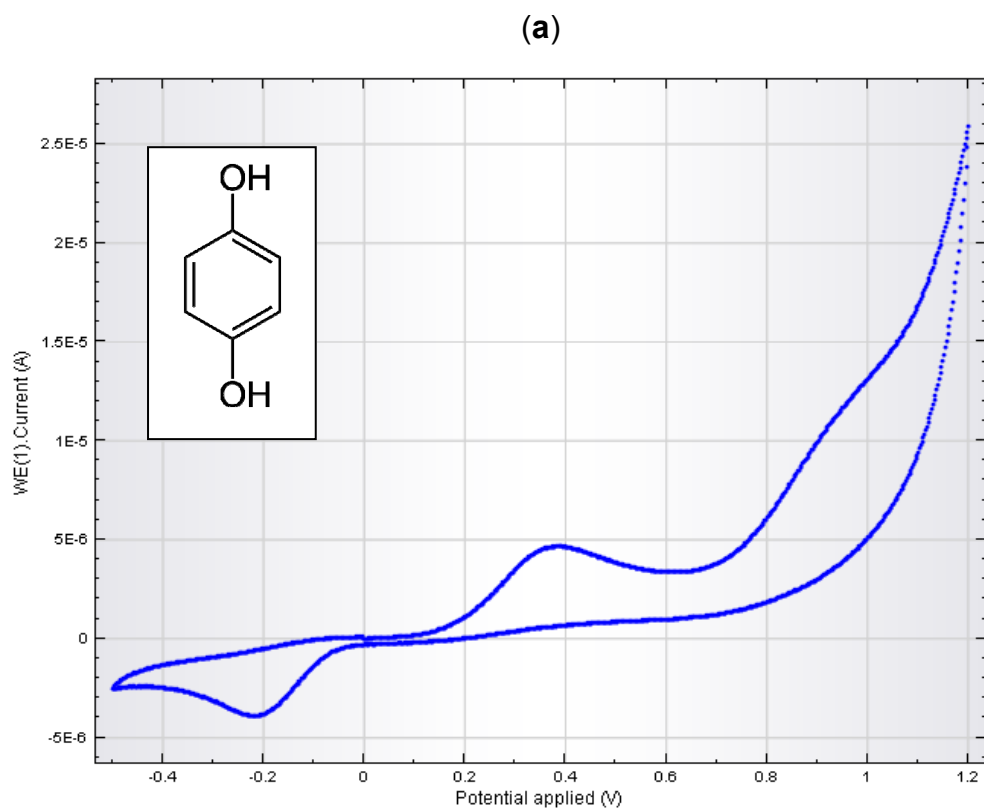


Fig. 8: Cyclic voltammograms of hydroquinone (HQ) at a CPE (a) and a BDDE (b); $c(\text{HQ}) = 2.5 \text{ mg/L}$ in PBS (0.1 M, pH 7).

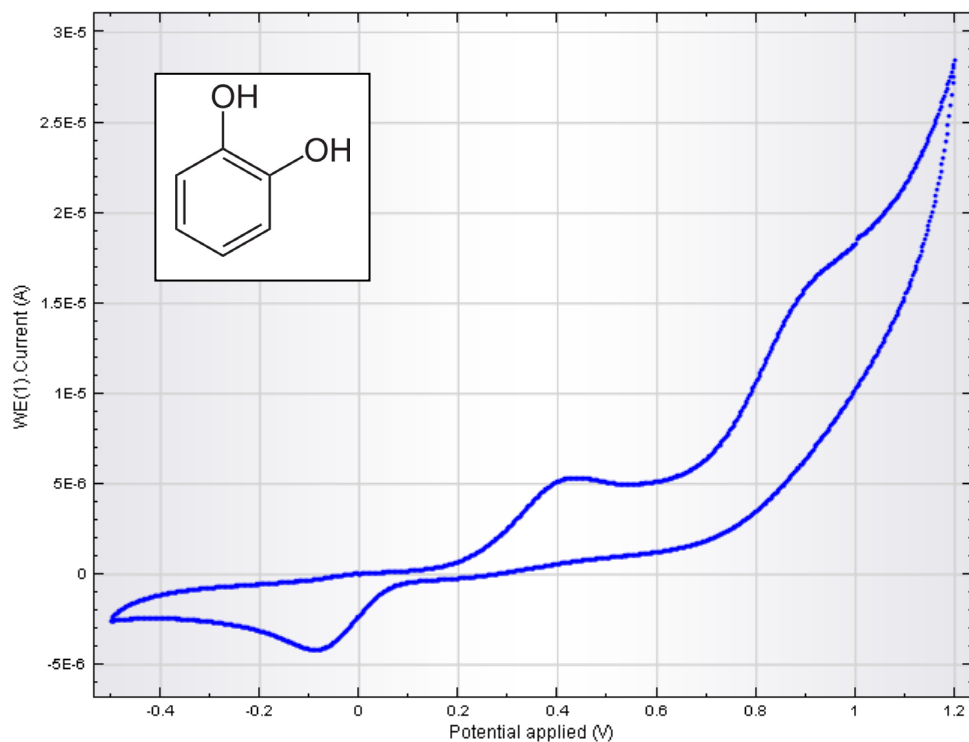


Fig. 9: CV of catechol at a CPE; analyte concentration, 2.5 mg/L in PBS (0.1 M, pH 7).

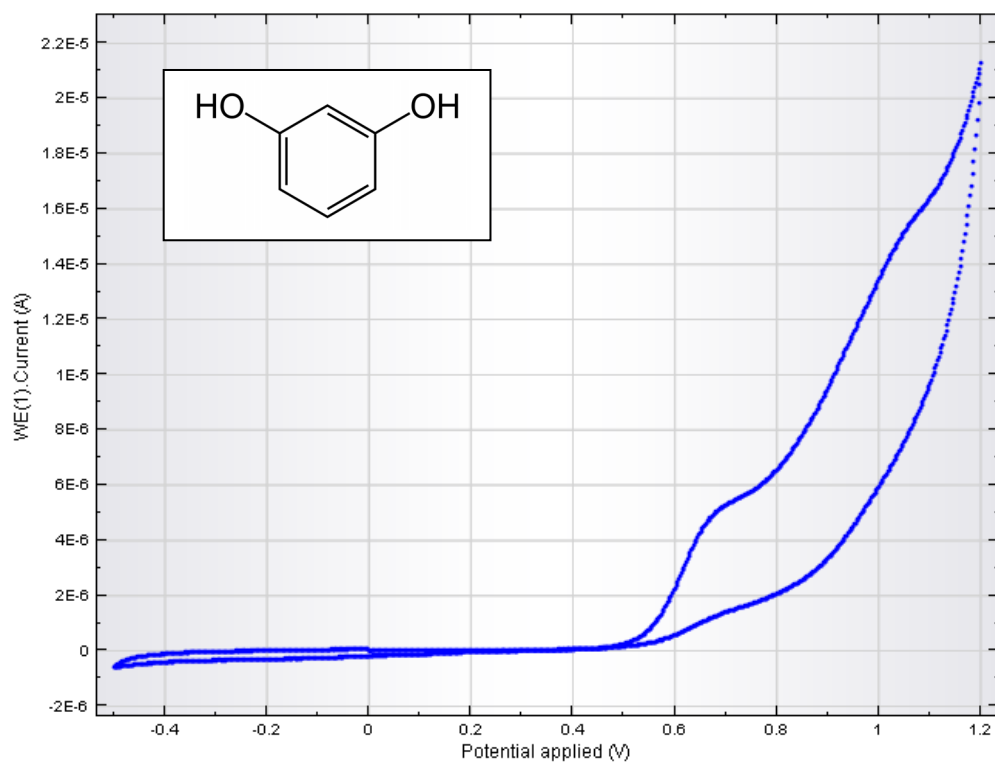


Fig. 10: Cyclic voltammograms of resorcinol at a CPE; analyte concentration, 2.5 mg/L in PBS (0.1 M, pH 7).

This is underlined by the electrochemical behavior of the before-mentioned trihydroxybenzenes pyrogallol and phloroglucinol which can be oxidized, but do not give a reduction current in the negative potential range (see Fig.11, overleaf).

Trihydroxybenzene derivatives in general seem to have a tendency for polymerization which could be the reason for the disappearance of a reduction signal; this is demonstrated with 2,3- dihydroxy- and 2,3,4-trihydroxybenzoic acid (Fig 12). The former shows a distinct reduction peak, whereas the triol does not.

Caffeic acid (3-(3,4-dihydroxyphenyl)-2-propenoic acid) shows a well developed quasi-reversible redox signal at the BDDE electrode (Fig. 13). At the CPE some significant part of the quinone is further oxidized (probably due to adsorption) to a polymer, which is also reflected in a diminished reduction signal in the negative potential range.

It may be concluded from the cyclic voltammetric studies that all phenolic compounds under investigation are oxidized at a potential of 1 V; most of them at even significantly lower values. Trihydroxy derivatives tend to undergo irreversible electropolymerization where the polymeric form cannot be reduced anymore.

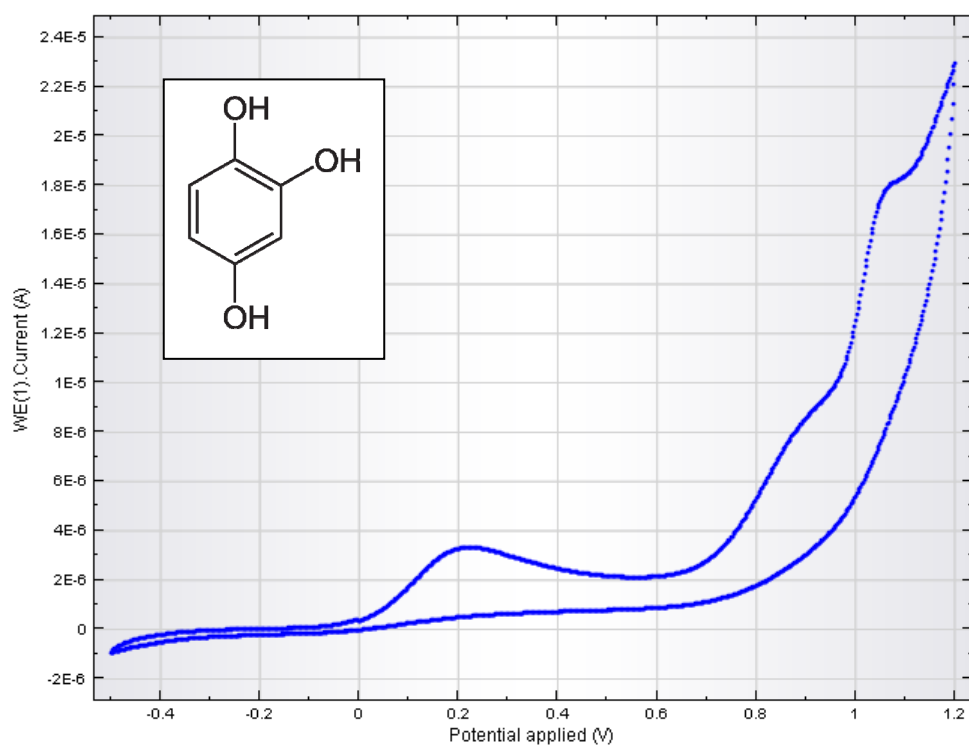
Hydrodynamic Amperometry

Based on the results from cyclic voltammetry it can be concluded that for hydrodynamic amperometric investigations a potential of 1 V is sufficient to detect antioxidants present in samples. Typical hydrodynamic amperograms for hydroquinone on both electrodes, CPE and BDDE, are shown in Fig. 14 (see overleaf).

Both electrodes exhibit a completely different behavior. With CPEs (curve a) a peak-shaped signal is obtained after addition of the analyte, which quickly decreases; subsequent additions yield only a fractional part of the signal of the previous one. This behavior can be explained by strong adsorption of the oxidation product of hydroquinone, i.e., quinone, which blocks the electrode surface subsequently.

BDDE (curve b) displays different characteristics: the signal steps after each constant concentration increment are practically constant, and the oxidation current does not decrease but remains also unchanged. Due to the fact that under otherwise identical conditions (electrode surface area, etc.) the signal is much higher with a carbon paste electrode it may be concluded that also the analyte, hydroquinone, is strongly adsorbed to the this type of electrode material, which is not the case with the BDD.

(a)



(b)

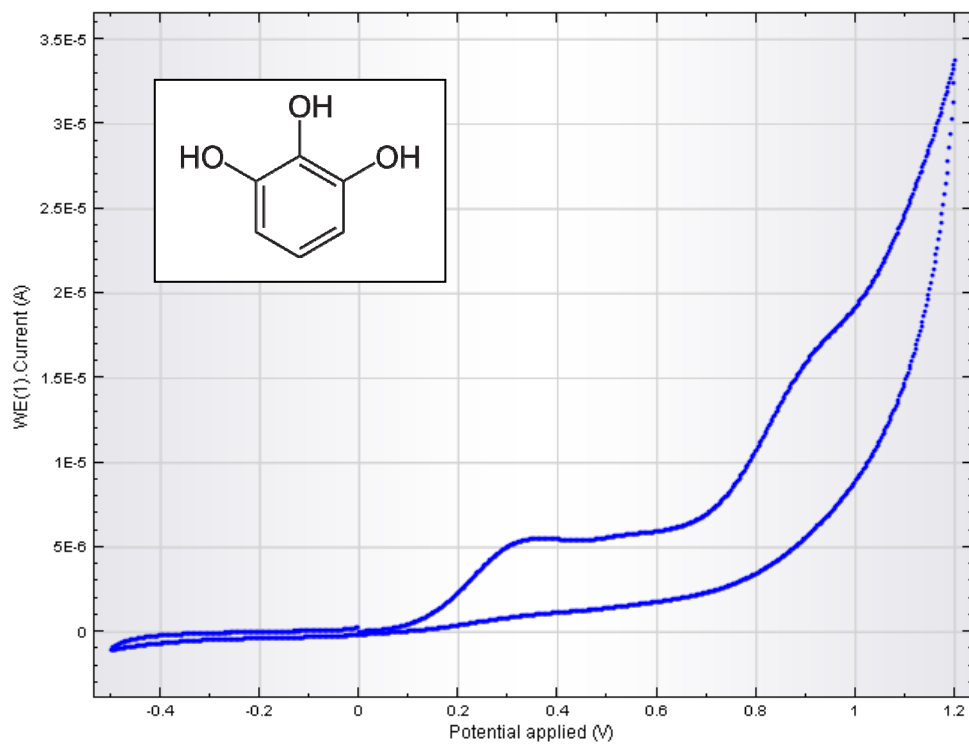
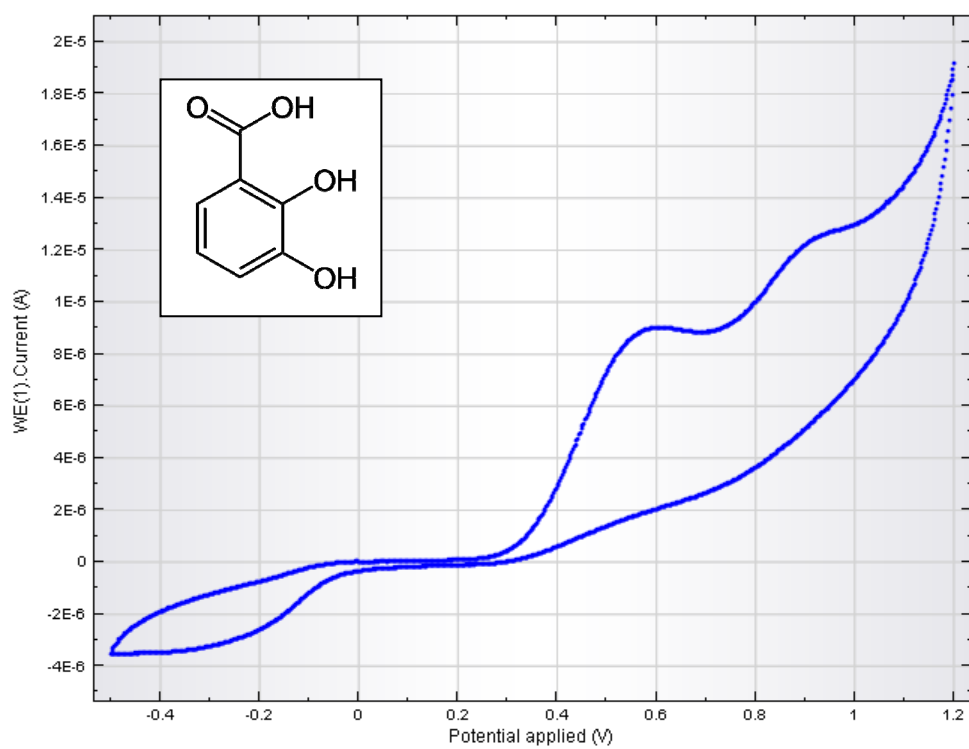


Fig. 11: Cyclic voltammograms of 1,2,4-trihydroxybenzene (a) and pyrogallol (b) at a CPE; analyte concentration, 2.5 mg/L in PBS (0.1 M, pH 7).

(a)



(b)

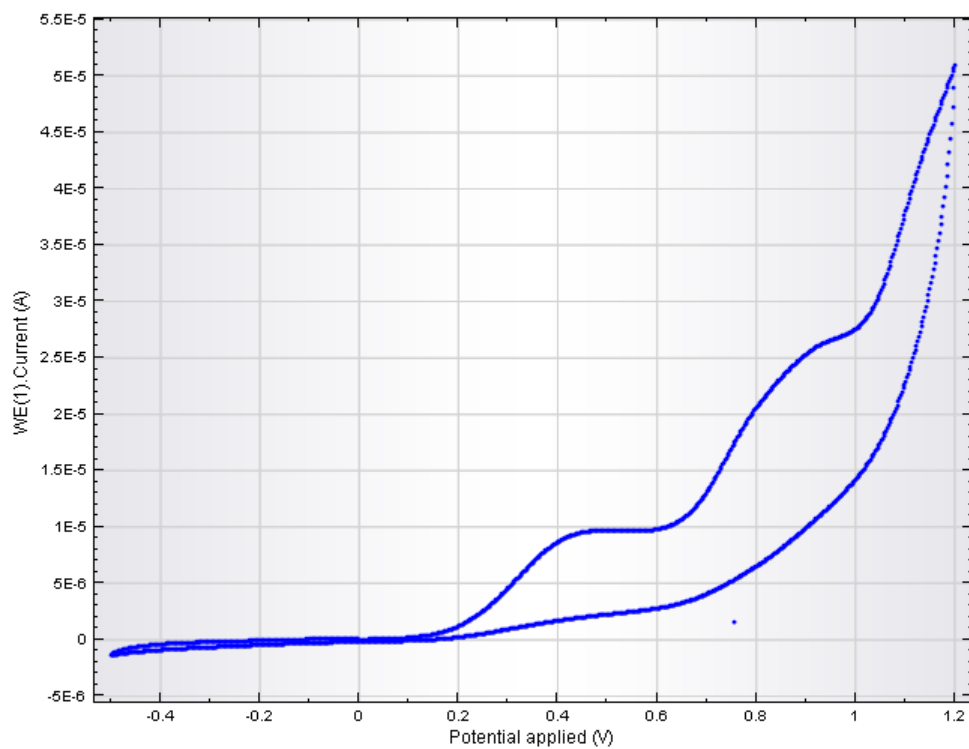


Fig. 12: CVs of 2,3-dihydroxybenzoic acid (a) and 2,3,4-trihydroxybenzoic acid (b) at a CPE; analyte concentration, 30 mg/L in PBS (0.1 M, pH 7).

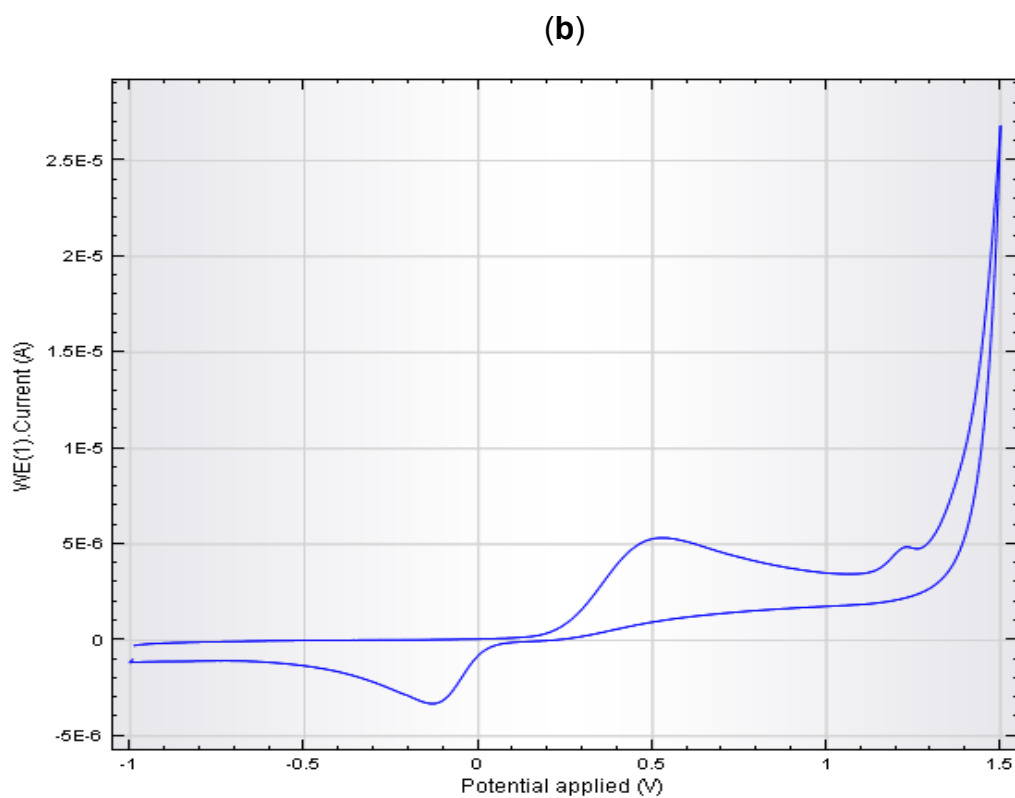
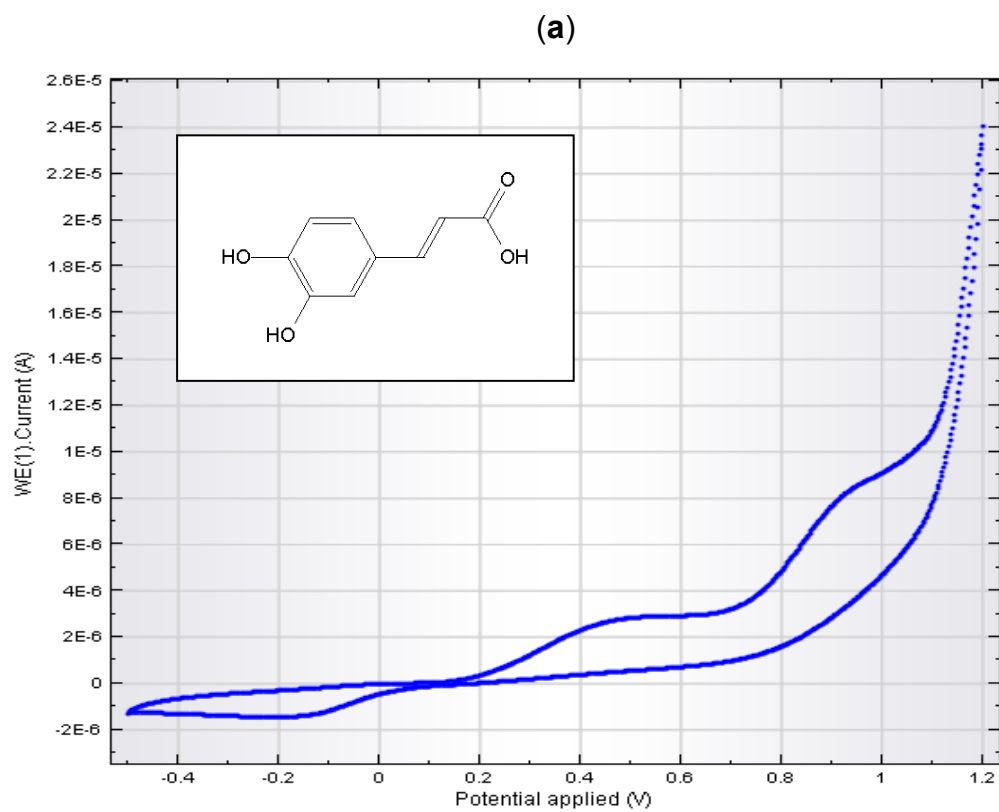


Fig. 13: Cyclic voltammograms of caffeic acid at a CPE (a) and a BDDE (b); PBS (0.1 M, pH 7); analyte concentration, 30 mg/L.

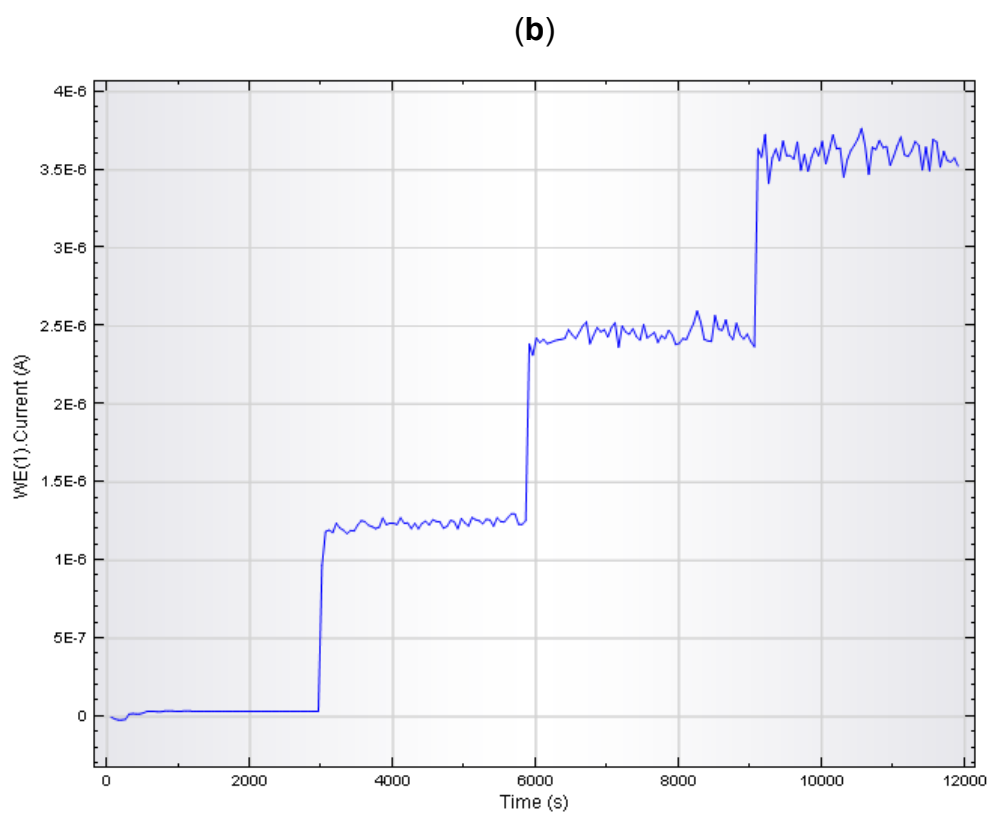
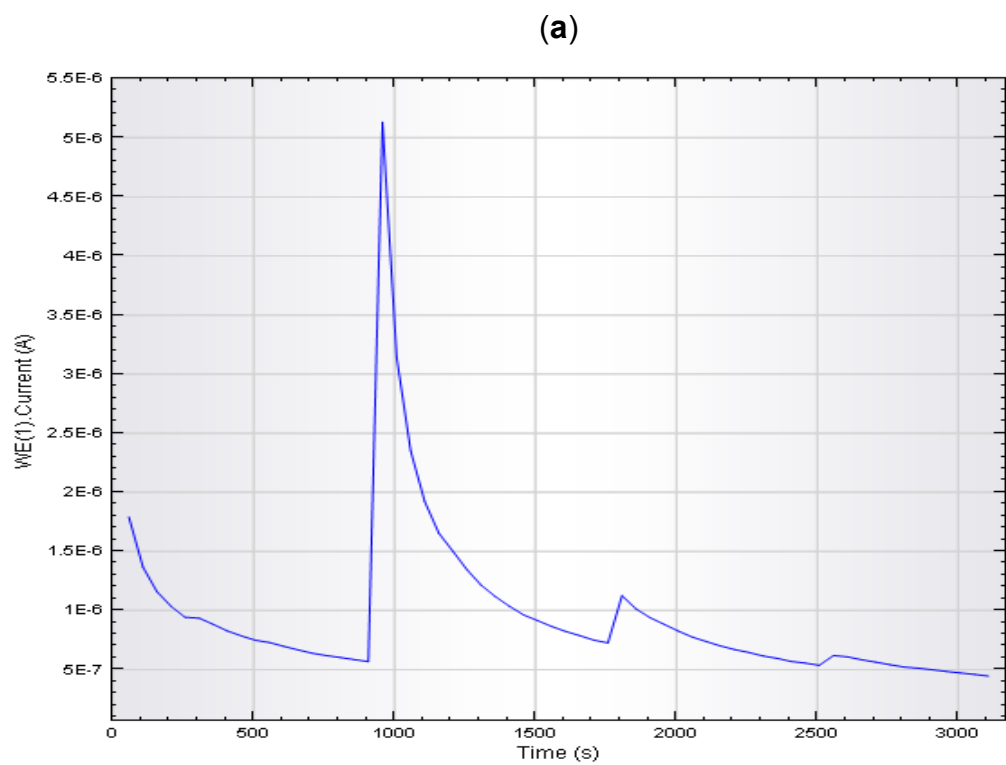


Fig. 14: Hydrodynamic amperograms of hydroquinone recorded with a CPE **(a)** and a BDDE **(b)**. Experimental conditions: PBS (0.1 M, pH 7), stirring speed: 400 rpm; concentration of HQ per addition, 2.5 mg/L.

As a consequence, BDDE is the preferential electrode for the direct determination of hydroquinone in the case that the electrode process is accompanied by adsorption as an undesired effect (under non-stripping conditions).

From all other antioxidants investigated in this study (see above), phenol showed strong adsorption on CPEs as well. Di- and trihydroxybenzenes showed low adsorption tendency due to their increased hydrophilicity. 1,2,4-trihydroxybenzene also shows a peak-like decrease not vanishing almost completely (as in case of HQ) but only to a constant oxidation current which increases with each addition. This is probably due to the formation of an electrically conductive polymer. Pyrogallol shows signal steps where each one is linearly decreasing due to polymer formation; as the polymer seems not or only a bit electrically conductive, a subsequent step is smaller than the preceding one.

The different behavior of HQ towards carbon paste and boron-doped diamond is also reflected with the calibration curves (see Fig.15, overleaf). BDDE gives a linear relation between signal and concentration up to 1000 mg/L HQ whereas the graph for CPE shows a typical non-linear behavior due to strong adsorption. At high concentrations, a quasi-linear behavior can be approximated as the model; but, in fact, the curve approaches asymptotically a maximum value.

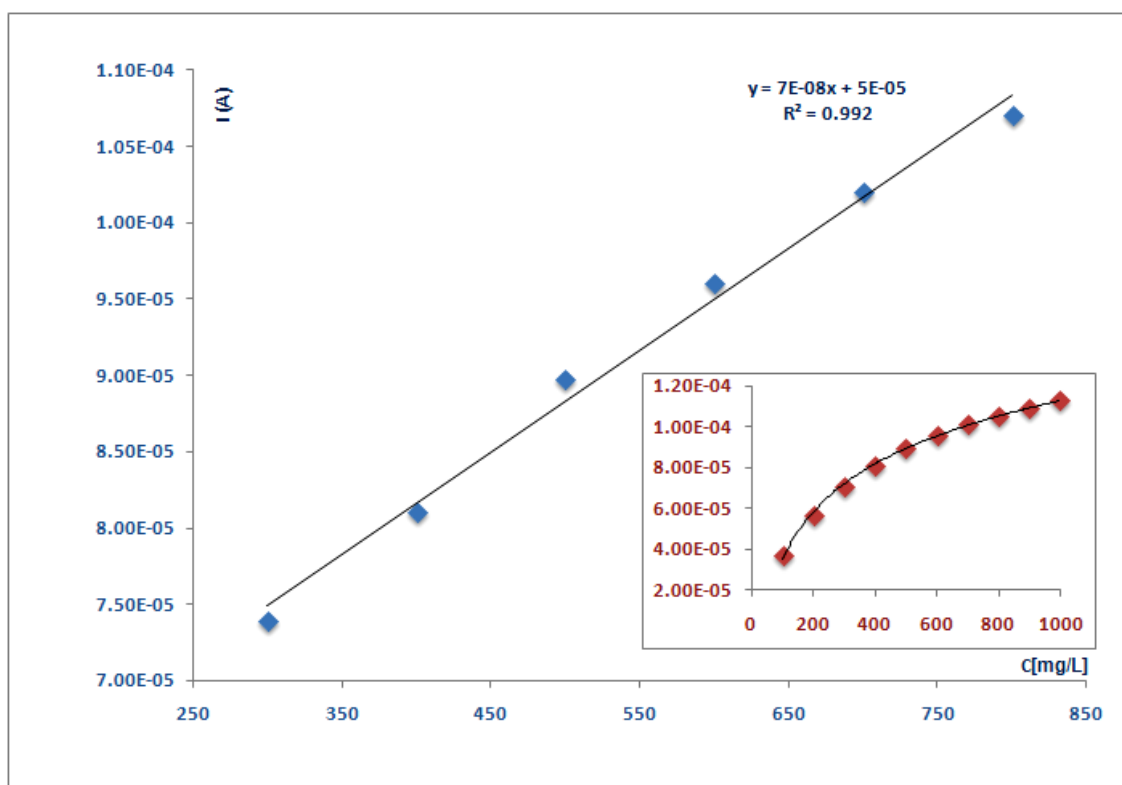
Based on these fundamental investigations an amperometric method had been worked out using a BDDE for the amperometric determination of phenolic antioxidants in ethanolic extracts of food processing residues (berries) [22]. According to the results presented there, HQ can be used as a template antioxidant similar to *Trolox*[®] providing hydroquinone equivalents which are directly proportional to *Trolox* equivalents. Studies with berry extracts showed excellent correlation between *Trolox* and hydroquinone equivalents in the samples obtained by both methods, photometry and amperometry.

Conclusions

Cyclic voltammetric and hydrodynamic amperometric studies with some model antioxidants and phenols representing basic structural elements in polyphenolic antioxidants showed that boron-doped diamond can be used as a well suited electrode material for the determination of antioxidant capacity.

Compared to carbon paste the latter seems less suitable because many phenolic substances adsorb strongly on its surface which imposes difficulties on its handling, because the electrode history (contact time with analyte solution, multiple measurements) influences the signal for direct measurements significantly.

(a)



(b)

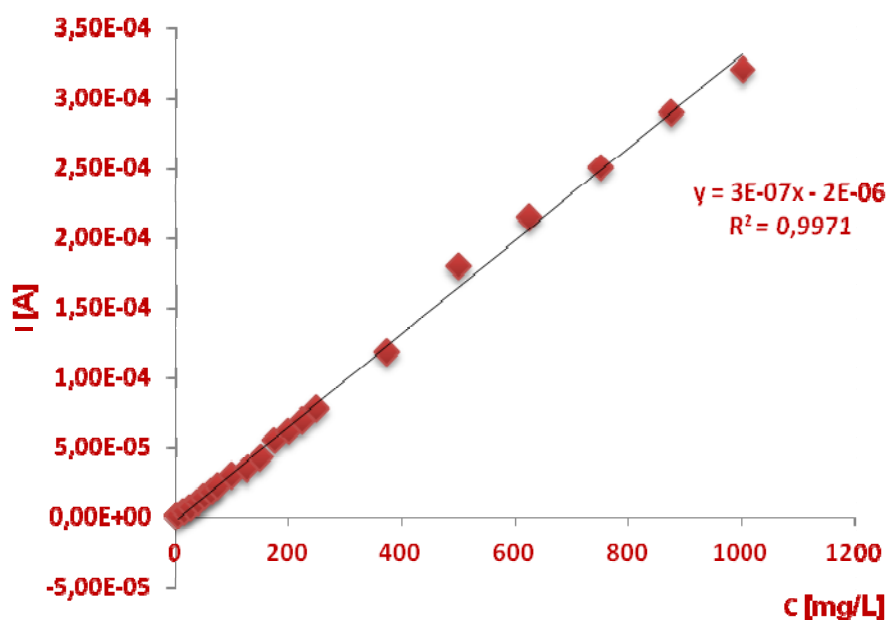


Fig. 15: Calibration curve of hydroquinone with a CPE (a) and a BDDE (b) as obtained with the aid of hydrodynamic amperometry; PBS (0.1 M, pH 7)

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