Determination of vanillin in commercial food product by adsorptive stripping voltammetry using a boron-doped diamond electrode

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A R T I C L E   I N F O

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Commercial pudding powder

A B S T R A C T

A method for the determination of food additive vanillin was developed by adsorptive stripping voltammetry. Its determination was carried out at the anodically pre-treated boron-doped diamond electrode in aqueous solutions. Using square-wave stripping mode, the compound yielded a well-defined voltammetric response in phosphate buffer, pH 2.5 at +1.14 V (vs. Ag/AgCl) (a pre-concentration step being carried out at open-circuit condition for 60 s). A linear calibration graph was obtained in the concentration range of 0.5–15.0 μg mL⁻¹ (3.3 × 10⁻⁶–9.8 × 10⁻⁵ mol L⁻¹) with a detection limit of 0.024 μg mL⁻¹ (1.6 × 10⁻⁷ mol L⁻¹). As an example, the practical applicability of the proposed method was tested for the determination of this flavouring agent in commercial pudding powder of Kesikule (Turkish milk pudding with almond flour).

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1. Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a unique and highly prized food additive as flavor enhancer. This compound is widely used to contribute to the fragrance of commercial foods such as pudding, ice-cream, custard, cookies, chocolate, and beverages. Vanillin is the main aromatic compound in natural vanilla. The source of vanilla is the bean or pod of the tropical Vanilla plant, a member of the orchid family (Sinha, Sharma, & Sharma, 2008). However, the production of natural vanilla from vanilla pods covers only 0.2% of the market requirement, and its production cost is very high. Vanillin can also be synthesised from low-cost materials such as 2-methoxyphenol, eugenol, and lignin. Synthetic vanillin is used in both food and non-food applications, as a fragrance ingredient in perfumes and cosmetics, and as a flavoring in pharmaceutical preparations. Though synthetic one is cheaper and widely produced, it causes headaches, nausea and vomiting, and could affect liver and kidney functions when large amounts of this flavor enhancer is ingested. Consequently, due to food safety concerns controlling the quality of vanillin in foods is of great importance.

Several methods for determining vanillin from various food samples or vanilla extracts have been described and involve the use of high-performance liquid chromatography (de Jager, Perfetti, & Diachenko, 2007; Pyell, Pletsch-Viehmann, & Ramus, 2002), gas chromatography (Goodner, Jella, & Rouseff, 2000; Perez-Silva et al., 2006), thin layer chromatography (Gerasimov, Gornova, & Rudometova, 2003), micellar electrokinetic chromatography (Pyell et al., 2002), capillary electrophoresis (Ohashi, Omae, Hashida, Sowa, & Imai, 2007; Panossian, Mamikonyan, Torosyan, Gabrielyan, & Mkhitaryan, 2001), UV–vis spectrophotometry (Longares-Paltron & Canizares-Macias, 2006; Ni, Zhang, & Kokot, 2005) and chemiluminesmetry (Timothoeu-Potamia & Calokerinos, 2007). Recently, novel approach based on a carbon nanotube-modified screen-printed electrode as detector coupled in capillary electrophoresis has been tested as a new and promising material for electrochemical sensing of vanillin in both qualitative and quantitative determination in food samples (Crevillen, Avila, Pumera, Gonzalez, & Escarpa, 2007). Moreover, a supported liquid membrane-piezoelectric flow sensor with a molecularly imprinted polymer has also been reported for the determination of vanillin in food samples (Avila, Zougagh, Escarpa, & Rios, 2007). Most of the above mentioned methods offer very useful information in terms of identification and quantitation, excellent resolution and selectivity, however they require highly sophisticated instrumentation, and involve time-consuming sample pretreatment processes.

The simplicity, fast response and high sensitivity of electroanalytical methods make their adventure an available alternative of analysis for the electroactive compounds. However, there are few studies available on the voltammetric determination of vanillin although the molecule is electrooxidisable at several electrodes. When using conventional electrodes, such as glassy carbon or
metallic electrode, the reason is the problem of electrode surface fouling and regeneration, or high oxidation potential of vanillin where oxygen evolution current interferes. Some analytical procedures based on the amperometry, differential pulse voltammetry or square-wave voltammetry have been reported for its quantitative determination in vanilla extracts and/or in commercial products through the study of its oxidation. The electrochemical behaviour of vanillin in acetonitrile has been investigated using a platinum disc electrode by differential pulse voltammetry (Rifkin & Evans 1976). An adsorptive stripping voltammetry by using a carbon paste electrode containing diphenyl ether in Britton–Robinson buffer (pH 5) has been developed for the determination of vanillin in food samples (Cookes & Efstanthiou, 1992). The square-wave voltammetric method in ethyl acetate has been applied to the determination of vanillin in dehydrated pudding powder using a cylindrical carbon fibre microelectrode, 8 mm in length (Agui, Lopez–Guzman, Gonzalez-Cortes, Yanez-Sedeno, & Pingarron, 1999). A PVC-graphite composite electrode has been evaluated as an amperometric detector in a flow system for the determination of vanillin in food samples (Luque, Luque-Perez, Rios, & Valcarcel, 2000). The determination of vanillin in vanilla extract has been achieved by biphasic sonolectrovoltaammetry at glassy carbon disc and microelectrodes using square-wave mode and ethyl acetocetate as solvent (Hardcastle, Paterson, & Compton, 2001). Bettazzi, Palchetti, Sisalli, & Mascini (2006) reported on the electrooxidation of vanillin at a disposable screen-printed electrode in phosphate buffer (pH 7.4) by square-wave voltammetry. The method was applied to the determination of vanillin in natural concentrated vanilla extracts and in final products such as yoghurt and compote. In a very recent work, Au–Ag alloy nanoparticles modified glassy carbon electrode were used for the determination of vanillin in dehydrated pudding powder using a cylindrical carbon fibre microelectrode, 8 mm in length (Agui, Lopez-Guzman, Gonzalez-Cortes, Yanez-Sedeno, & Pingarron, 1999). A PVC-graphite composite electrode has been evaluated as an amperometric detector in a flow system for the determination of vanillin in food samples (Luque, Luque-Perez, Rios, & Valcarcel, 2000). The determination of vanillin in vanilla extract has been achieved by biphasic sonolectrovoltaammetry at glassy carbon disc and microelectrodes using square-wave mode and ethyl acetocetate as solvent (Hardcastle, Paterson, & Compton, 2001). Bettazzi, Palchetti, Sisalli, & Mascini (2006) reported on the electrooxidation of vanillin at a disposable screen-printed electrode in phosphate buffer (pH 7.4) by square-wave voltammetry. The method was applied to the determination of vanillin in natural concentrated vanilla extracts and in final products such as yoghurt and compote. In a very recent work, Au–Ag alloy nanoparticles modified glassy carbon electrode were used for the determination of vanillin in dehydrated pudding powder using a cylindrical carbon fibre microelectrode, 8 mm in length (Agui, Lopez-Guzman, Gonzalez-Cortes, Yanez-Sedeno, & Pingarron, 1999). A PVC-graphite composite electrode has been evaluated as an amperometric detector in a flow system for the determination of vanillin in food samples (Luque, Luque-Perez, Rios, & Valcarcel, 2000). The determination of vanillin in vanilla extract has been achieved by biphasic sonolectrovoltaammetry at glassy carbon disc and microelectrodes using square-wave mode and ethyl acetocetate as solvent (Hardcastle, Paterson, & Compton, 2001). Bettazzi, Palchetti, Sisalli, & Mascini (2006) reported on the electrooxidation of vanillin at a disposable screen-printed electrode in phosphate buffer (pH 7.4) by square-wave voltammetry. The method was applied to the determination of vanillin in natural concentrated vanilla extracts and in final products such as yoghurt and compote.

2. Materials and methods

2.1. Chemicals

A standard stock solution of 6 mg mL\(^{-1}\) vanillin (Acros, USA) was prepared by dissolving vanillin in ethanol. On the day of the experiment working solutions were prepared by appropriate dilution of the stock with a selected supporting electrolyte. Four different supporting electrolytes, namely Britton–Robinson (0.1 M, pH 2–9), acetate (0.1 M, pH 4.8) and phosphate (0.1 M, pH 2.5 and 7.4) buffers or perchloric acid solution (0.1 M) were used. All the optimisation experiments were performed using about 1% (v/v) of ethanol in the supporting electrolyte solution. All stock solutions were preserved at +4 °C when not in use and protected from daylight during use in the laboratory. All other chemicals were prepared from analytical reagent grade chemicals. Water from a Milli-Q purification system (Millipore) was used in all cases.

2.2. Apparatus

The cyclic (CV), linear sweep (LSV) and square-wave (SWV) voltammetric experiments at a boron-doped diamond (BDD) electrode were performed using a Autolab type III electrochemical analyzer controlled with the GPES 4.9 software (EcoChemie, The Netherlands). All SW voltammograms were smoothed using a Savicky and Golay algorithm and baseline-corrected by the moving average method (peak width of 0.01 V), using the GPES software. A three-electrode cell system was used: a BDD working electrode (Windsor Scientific Ltd.; Ø: 3 mm, diameter), a Pt-wire auxiliary electrode, and an Ag/AgCl (3 mol L\(^{-1}\) NaCl) (Model RE-1, BAS, USA) reference electrode to which all electrode potentials hereinafter are referred. The pH values of solutions were measured using a WTW inoLab pH 720 meter with a combined electrode (glass-reference electrodes).

A procedure similar to that proposed in our previous work (Yardim & Erez, 2010) was followed for the pre-treatment of BDD electrode. This electrode was firstly polarised in a 0.5 mol L\(^{-1}\) H\(_2\)SO\(_4\) by applying +3.0 V during 180 s; thus, the BDD surface was made predominantly oxygen-terminated. Afterwards, the electrode was pre-treated for 30 s under the same experimental conditions. In this study, the first anodic surface pre-treatment was daily performed before starting the experimental work. The other step in the procedure was applied before each voltammetric experiment. The pre-treatment procedure was carried out in an independent electrochemical cell.

2.3. Adsorptive stripping voltammetric procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: the required aliquot of the vanillin working solutions was placed in a cell containing a selected supporting electrolyte at a desired pH. The previously treated electrode was placed in the cell, and the solution was stirred at 400 rpm at a chosen accumulation potential throughout the selected accumulation period. Following the pre-concentration period, the stirring was stopped, and after a 10 s had elapsed, anodic scans were carried out over the range +0.5 to +1.6 V using the SWV technique. All measurements were made in a standard 10-mL voltammetric cell, at a room temperature.
The best instrumental parameters for SWV which was used for investigating the determination of vanillin were as follows: frequency, 100 Hz; pulse amplitude, 40 mV; scan increment, 12 mV. Successive measurements were carried out by repeating the above assay protocol on the working electrode.

2.4. Preparation of the commercial food samples

The analysed sample was commercial pudding powder of Kesrkule (Turkish milk pudding with almond flour) purchased at a local supermarket; containing sugar, corn starch, ground almonds, almond aroma, vanillin, colouring agents (Beta carotenes). The pudding powder samples were submitted to a simple preparation procedure prior to electrochemical analysis. Powder samples taken from five package of the same brand were previously mixed in a mortar. An accurately weighed portion of powder (about 1 g of sample) was transferred into a centrifuge tube with a screw tap, and dissolved in 5 mL ethanol. The mixture was sonicated for 90 min and undissolved material was allowed to settle. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with ethanol/phosphate buffer, pH 2.5 in order to obtain a final solution of 1:99 ethanol/respective buffer mixture. Each solution was transferred to a voltammetric cell and analysed in the day of preparation according to the procedure developed for the pure electrolyte.

3. Results and discussion

3.1. Electrochemical behaviour on the boron-doped diamond electrode

To the best of our knowledge, no work using BDD electrode to determine vanillin, either as pure electrolyte or in complex samples, has been reported. Thus, preliminary experiments using CV, LSV and SW-AdSV were performed to characterise the voltammetric behaviour of vanillin at the anodically pre-treated BDD.

Prior to the experimental design stage, a preliminary conclusion indicated that BDD electrode without pre-treatment was not free from passivating problems, and no suitable electroanalytical responses could be obtained when vanillin solutions were analysed, and thus a way to restore the initial activity of the BDD electrode surface was necessary. Three different cleaning procedures were considered. First, the electrode was treated by mechanical cleaning (polishing manually with 0.01-lum alumina slurries). A second procedure consisted in a cathodic cleaning (+3.0 V for 30 s). Finally, the third procedure consisted in an anodic one (+3.0 V for 180 s). In order to decrease the background current, the acidic media of buffers, or perchloric acid solution. All of these solutions had a concentration of 0.1 M. Cyclic voltammograms recordings were obtained within the range +0.5 to +1.6 V at a scan rate of 100 mV s⁻¹ for 100 µg mL⁻¹ vanillin. The electrochemical behaviour of the compound at this electrode over the pH range investigated yielded one broad irreversible oxidation wave. A representative cyclic voltammogram of vanillin in phosphate buffer at pH 2.5 (optimised response at around +1.15 V) is shown in Fig. 1A. All the published works have demonstrated that the voltammetric responses present only single peak. This process took place at more positive potential than those previously reported for the oxidation of vanillin at modified glassy carbon electrode at about the same pH (Zheng et al., 2010). It indicates that the oxidation process of the compound requires higher overpotential at the diamond surface because of a slower electron transfer kinetic, compared to other carbon electrodes. However, the wider potential window available for BDD with respect to the glassy carbon electrode allows vanillin to be analysed. Multi-scan CV recordings revealed that the waves decreased upon the second and subsequent scans at the same BDD electrode, pointing to certain adsorption activity at the electrode surface.

The effects of scan rate on the oxidation of vanillin at the BDD electrode were checked by LSV in phosphate buffer, pH 2.5. As shown in Fig. 1B, the oxidation peak shifted slightly toward more positive potentials as the scan rate increased; a behaviour typical of irreversible electrochemical reactions. The oxidation peak current (Iₒ) of vanillin increases linearly with the scan rate (v) particularly at low scan rate values (10–200 mV s⁻¹) (Iₒ (µA) = 0.049 r (mV s⁻¹) + 4.984, r = 0.994), which suggests that the electrode reaction is controlled by the adsorption process. However, the peak current started to decrease slightly with faster scan rates. According to the Laviron theory (Laviron, 1979) for an irreversible electrode process, the peak potential (Eₒ) and v have the following relationship:

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 1. The repetitive cyclic voltammograms (A), and the linear sweep voltammograms at different scan rates of 10, 25, 50, 75, 100, 150 and 200 mV s⁻¹ (B) of 100 µg mL⁻¹ vanillin solutions in phosphate buffer, pH 2.5. CV condition: Scan rate, 100 mV s⁻¹. Dashed lines represent background current. Inset of (B) depicts plots of peak current (Iₒ) vs. scan rate (r).
where $\alpha$ is charge transfer coefficient and $n$ the number of electrons involved in the redox reaction, the other terms having their usual meaning. The slope obtained from the $E_p$ vs. log $\nu$ was 0.051; thus, by means of the Eq. (1), the value equals to 1.157 was determined for $n$. Generally, the value of $n$ is assumed as equal to 0.5 in totally irreversible electrode process. Therefore, the value of $n = 2.31 \approx 2$ was obtained. This result indicates that the irreversible oxidation of vanillin involves two electrons per molecule at BDD electrode. The conclusion confirms the previous result for the electrooxidation of vanillin at Au–Ag alloy nanoparticles modified glassy carbon electrode (Zheng et al., 2010).

As far as we know, there is no study that reported an electrochemical mechanism for vanillin oxidation. Though the elucidating the mechanism of vanillin electrochemical oxidation is beyond the aim of this study, however, from these results and considering the proposed oxidation mechanism for some other phenolic compounds (Medeiros, Rocha-Filho, & Fatibello-Filho, 2010), the electrochemical oxidation of vanillin is believed to occur by a two-electron mechanism.

The observed responses suggested the possibility of setting up a method to determine vanillin by AdSV. Among the stripping waveforms, the SW modulation combines good sensitivity with high speed, and reduces problems with poisoning of the electrode surface. As a consequence, further work was dedicated towards studying the influence of nature and acidity of the supporting electrolyte upon the SW response, after performing an accumulation step at BDD electrode. In Fig. 2A, this parameter was established in the pH range 2.0–9.0 of Britton–Robinson buffer by carrying out adsorptive measurements on 10 μg mL$^{-1}$ vanillin solution, with an open-circuit mode at 120 s. As can be seen from the figure, decreasing the solution acidity resulted in a decrease of the peak heights. For a solution with pH between 2.0 and 5.0, vanillin oxidation peak potential did not depend practically on pH. On increasing pH > 5.0, peak potential was slightly displaced to less positive values till pH 6.0 then became again almost pH independent (from +1.08 V at pH 2.0 to +1.21 V at pH 9.0). The $pK_a$ value of vanillin is reported to be 7.38 which are attributed to the deprotonation of the phenol group (Robinson & Kiang, 1955). In the case of vanillin oxidation when pH is below ~6.0 it is predominantly present in its protonated form. At pH well above ~9.0, the unprotonated form of vanillin (becoming negatively charged) predominates in the supporting electrolytes. Bearing in mind that aqueous alkaline solutions change the morphology of the BDD surface resulting in surface degradation (Griesbach, Pütter, & Comninellis, 2005), the strongly alkaline media at pH > 9 were avoided. Fig. 2B depicts the SW voltammograms in various supporting electrolytes. Using 0.1 M perchloric acid, phosphate buffer pH 2.5, acetate buffer pH 4.8 and phosphate buffer 7.4, anodic peak potentials of +1.07, 1.11, 1.08 and 1.20 V were obtained, respectively, together with the decrease of the anodic peak currents with different degrees (1.85, 1.90, 1.20 and 0.58 μA), which are in agreement with the results in Britton–Robinson buffer. According to the obtained results, the 0.1 M phosphate buffer solution at pH 2.5 are the most suitable media for analytical purposes, yielding the high peak current, better peak shape, and also the best background signal, which was chosen for further experiments and development of the methodology.

Pre-concentration of the analysed compound on the surface of BDD electrode is one of the essential conditions for highly sensitive determinations. Next, the attention was turned to the effect of pre-concentration/stripping conditions, such as accumulation potential and time (data not shown). The accumulation potential on the stripping peak was evaluated at open-circuit condition or at a potential range from +0.1 V to +0.3 V for a pre-concentration period of 120 s in stirred 10 μg mL$^{-1}$ vanillin solution. Similar values of peak current were obtained in all cases. Since the baseline were distorted in the range +0.1 V and +0.3 V, so the accumulation in the rest of experiments was carried out under open-circuit. The influence of the accumulation time upon the analytical signal was examined in the range 15–240 s. The current increased linearly with accumulation time till 60 s beyond which it remained constant, indicating that electrode surface becomes saturated with the analyte molecules. The accumulation time of 60 s is very short and doubtlessly advantageous for practical use of this electrode. Therefore, this accumulation time was selected for all the AdSV experiments.

Finally, in order to optimise the SWV parameters that affect the response, voltammograms of 2.5 μg mL$^{-1}$ vanillin in phosphate buffer pH 2.5 following pre-concentration for 60 s under open-circuit were recorded using various instrumental conditions (not shown): (square-wave frequency, 10 Hz ≤ f ≤ 125 Hz; pulse amplitude, 20 mV ≤ a ≤ 60 mV; and scan increment, 4 mV ≤ ΔE ≤ 16 mV). The variation in the $f$ values shown that its increase promoted an increase in the peak current due to the increase in the effective scan rate. However the background current and noise were also increased at $f$ values higher than 100 Hz. The voltammetric responses for vanillin determination as a function of variation in
a demonstrated that peak current values increased upon increase of this parameter. However, the peak shape became wider at values of \( a \) higher than 40 mV, which represent a considerable loss in analytical selectivity. In addition, at higher values of 12 mV, an increase in \( \Delta E \) resulted in a decrease in peak current. To account for the results, in subsequent experiments, values of \( f = 100 \) Hz, \( a = 40 \) mV, and \( \Delta E = 12 \) mV were adopted.

3.2. Application of methodology

Once the most suitable chemical conditions and instrumental parameters for the adsorptive determination were established, SW stripping voltammograms at different concentrations of vanillin were recorded to estimate the analytical characteristics of the developed method. For this, aliquots from the vanillin stock solution were consecutively added to the electrochemical cell and the voltammetric responses were evaluated for each addition. The SWV responses at a potential of +11.4 V showed that the dependence of peak currents on the vanillin concentration was linear, in the range of concentration from 0.5 to 15 \( \mu \)g mL\(^{-1}\) (3.3 \( \times 10^{-6} \)–9.8 \( \times 10^{-3} \) mol L\(^{-1}\)). The analytical curves together with the obtained linear relationship between peak currents and concentrations (as the inset) are presented in Fig. 3. Table 1 shows the results of the statistical analysis of the experimental data. From the data obtained by the analytical curves, the detection (LOD) and quantification (LOQ) limits were calculated using the formulas \( 3 \sigma / C \) and \( 10 \sigma / C \), respectively.

Table 2 shows a comparison of the efficiency of the electrodes in the determination of vanillin. Intra-day reproducibility of peak current (RSD, %) and inter-day reproducibility of peak current (RSD, %) for the results, in subsequent experiments, values of \( n = 3 \), \( n = 8 \), and \( n = 1 \) were adopted.

![Fig. 3. The stripping voltammograms in phosphate buffer, pH 2.5 containing different concentration of vanillin, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 12.5, 15 \( \mu \)g mL\(^{-1}\). Inset depicts a corresponding calibration plot for the quantitation of vanillin. Pre-concentration period, 60 s at open-circuit condition; SWV parameters: frequency, 100 Hz; scan increment, 12 mV; pulse amplitude, 40 mV.](image)

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration range (( \mu )g mL(^{-1}))</td>
<td>0.5–15</td>
</tr>
<tr>
<td>Slope (( \mu )A mL ( \mu )g (^{-1}))</td>
<td>0.5984</td>
</tr>
<tr>
<td>Standard deviation of slope (( \mu )A mL ( \mu )g (^{-1}))</td>
<td>0.0028</td>
</tr>
<tr>
<td>Intercept (( \mu )A)</td>
<td>(-0.3513)</td>
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<tr>
<td>Standard deviation of intercept (( \mu )A)</td>
<td>(0.0071)</td>
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<tr>
<td>Correlation coefficient (r)</td>
<td>0.999</td>
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<td>LOD (( \mu )g mL(^{-1}))</td>
<td>0.024</td>
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<tr>
<td>LOQ (( \mu )g mL(^{-1}))</td>
<td>0.072</td>
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<tr>
<td>Intra-day reproducibility of peak current (RSD, %)</td>
<td>2.33</td>
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<tr>
<td>Inter-day reproducibility of peak current (RSD, %)</td>
<td>2.95</td>
</tr>
<tr>
<td>Intra-day reproducibility of peak potential (RSD, %)</td>
<td>0.81</td>
</tr>
<tr>
<td>Inter-day reproducibility of peak potential (RSD, %)</td>
<td>1.51</td>
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</table>

\( a = 3 \), \( n = 8 \), and \( n = 1 \) for the results, in subsequent experiments, values of \( n = 3 \), \( n = 8 \), and \( n = 1 \) were adopted.

### Table 2

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Linear range (mol L(^{-1}))</th>
<th>LOD (mol L(^{-1}))</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum disc</td>
<td>1.5 ( \times 10^{-5})–3.8 ( \times 10^{-5})</td>
<td>1.5 ( \times 10^{-6})</td>
<td>Rifkin and Evans (1976)</td>
</tr>
<tr>
<td>Modified-carbon paste</td>
<td>6 ( \times 10^{-7})–4 ( \times 10^{-6})</td>
<td>Not given</td>
<td>Cookeas and Efstathiou (1992)</td>
</tr>
<tr>
<td>Cylindrical carbon fibre</td>
<td>1 ( \times 10^{-5})–7 ( \times 10^{-4})</td>
<td>4.2 ( \times 10^{-6})</td>
<td>Agii et al. (1999)</td>
</tr>
<tr>
<td>PVC-graphite</td>
<td>6.6 ( \times 10^{-5})–9.2 ( \times 10^{-5})</td>
<td>2.9 ( \times 10^{-7})</td>
<td>Luque et al. (2000)</td>
</tr>
<tr>
<td>Glassy carbon</td>
<td>5 ( \times 10^{-7})–3 ( \times 10^{-4})</td>
<td>1.6 ( \times 10^{-5})</td>
<td>Hardcastle et al. (2001)</td>
</tr>
<tr>
<td>Graphite screen-printed</td>
<td>5 ( \times 10^{-6})–4 ( \times 10^{-4})</td>
<td>4 ( \times 10^{-7})</td>
<td>Bettazzi et al. (2006)</td>
</tr>
<tr>
<td>Modified-carbon paste</td>
<td>2 ( \times 10^{-5})–5 ( \times 10^{-5})</td>
<td>4 ( \times 10^{-8})</td>
<td>Zheng et al. (2010)</td>
</tr>
<tr>
<td>BDD</td>
<td>3.3 ( \times 10^{-6})–9.8 ( \times 10^{-5})</td>
<td>1.6 ( \times 10^{-7})</td>
<td>This work</td>
</tr>
</tbody>
</table>
period in refrigerator by monitoring the vanillin concentration by SWV. The obtained results presented no significant differences in the peak currents and potentials, which indicates that the BDD electrode has good stability demonstrating that vanillin solutions were stable for this period.

In order to evaluate the selectivity of the proposed method, the influence of selected potential interfering compounds such as inorganic ions and organic compounds, which may accompany vanillin in real samples, was examined by analysing synthetic sample solutions containing 2.5 μg mL⁻¹ vanillin and various excess amounts of these species. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% for the determination of vanillin. At a 100-fold excess, NO₃⁻, SO₄²⁻, K⁺, Na⁺, Ca²⁺, Mg²⁺, Cu²⁺, Zn²⁺, Fe³⁺, glucose, fructose, and sucrose did not significantly influence the height of the vanillin peak currents. Interference due to some related phenolic compounds which may be present together with vanillin in different types of samples (food and perfumery items) was also tested. In the presence of chlorogenic acid, caffeic acid and ferulic acid, it was observed negligible changes in the oxidation peak current intensity of vanillin even when an interferent-to-analyte ratio of 1:1 was used. However, the exposure of same concentration level of vanillin acid and p-coumaric acid resulted in peak widening because their oxidation peaks overlapped with that of vanillin.

Commercial samples of pudding powder containing vanillin were analysed in order to evaluate the validity of the herein proposed method. Following the sample preparation stated in Section 2.4, the voltammetric procedure, under the experimental conditions described in Section 2.3, was carried out and quantifications were performed by means of calibration curve method. For the sample preparation, the time of extraction with ethanol was optimised by sonicating the sample in contact with the organic solvent during periods of time in 15 ± 120 min range. Practically constant stripping currents were obtained from 90 min, and consequently, this time was used for extracting the vanillin from the samples. A typical voltammogram of a sample of pudding powder is shown in Fig. 4 (dashed line). To ascertain the observed oxidation peak at +1.21 V as vanillin, the sample was spiked with certain amounts of pure vanillin and the corresponding SW voltammograms were recorded (Fig. 4, solid line). All curves were constructed in triplicate. As can be seen from the figure, this peak height increases as adding vanillin standard solution which indicates that the peak observed in the sample is assigned to the oxidation of vanillin. The matrix of the sample was found to cause a slight influence on the peak potential. The peak potentials shifted towards more positive values than those obtained pure electrolyte. It was found a concentration of 2.97 μg mL⁻¹ of vanillin in the measurement cell. Taking into account the successive dilutions of the sample, it was calculated that the amount of vanillin present in the original extract is 148.5 μg mL⁻¹ i.e., 742.5 μg vanillin per gram of sample.

It should be noted that the vanillin peak height is considerably decreased by the sample components presented at high amounts in the sample solution (e.g., starch). This inhibition effect is caused by the working electrode surface blockage due to adsorption of these non-electroactive compounds. However, in the case of our study, the high dilution of the sample reduced this problem. In order to know whether the ingredients present in the sample of pudding powder show any interference with the analysis, the validity of the proposed methodology for vanillin quantification was also performed by plotting the recovery curves. To do so, the appropriate volume of standard vanillin solutions prepared in supporting electrolyte was added to the matrices of commercial food product and the voltammetric responses were evaluated. Recovery of vanillin was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure vanillin. The results are summarised in Table 3. It was found that vanillin amount can be quantitatively recovered by the proposed method, being thus a guarantee of the accuracy of the voltammetric determination of vanillin in the food samples.

Table 3

<table>
<thead>
<tr>
<th>Vanillin added (μg mL⁻¹)</th>
<th>Level determined (μg mL⁻¹)</th>
<th>Recovery (%) ± RSD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>6.62</td>
<td>91.25 ± 2.11</td>
<td>2.11</td>
</tr>
<tr>
<td>8.0</td>
<td>9.86</td>
<td>86.13 ± 2.57</td>
<td>2.57</td>
</tr>
<tr>
<td>12.0</td>
<td>13.62</td>
<td>88.75 ± 2.43</td>
<td>2.43</td>
</tr>
</tbody>
</table>

*a Average of three experiments.
*b RSD = Relative standard deviation.

Fig. 4. The stripping voltammograms obtained for the determination of vanillin in sample of pudding powder. A 50 times diluted the sample (dashed line) and sample spiked at a vanillin levels of (a) 4 μg mL⁻¹, (b) 8 μg mL⁻¹, (c) 12 μg mL⁻¹. Other operating conditions as indicated in Fig. 3.

4. Conclusions

An anodically pre-treated BDD electrode was used in combination with the SW-AdSV technique to develop a novel and alternative electroanalytical method for vanillin determination. The important point that should be highlighted here is the anti-fouling properties of BDD electrode pre-treated with a fast and simple cleaning procedure, which allow the use of the electrode for a long time with the same response.

The herein proposed method with a more improved LOD value (0.024 μg mL⁻¹) shows clear advantages in terms of sensitivity with respect to already reported various analytical methods, such as liquid chromatography-mass spectrometry (0.073 μg mL⁻¹) (de Jager et al., 2007), chemiluminometry (0.045 μg mL⁻¹) (Timothoeu-Potamia & Calokerinos, 2007), modified piezoelectric detection system (0.173 μg mL⁻¹) (Avila et al., 2007), capillary electrophoresis (1.6 μg mL⁻¹) (Ohashi et al., 2007) and UV–vis spectrophotometry which is chemometric approach (0.49 μg mL⁻¹) (Ni et al., 2005). Additionally, the other advantages of this voltammetric methodology such as simplicity, cheapness and rapidity were demonstrated by the successful application in the samples of commercial pudding powder after a simple preparation of samples.

The selectivity of such determination could be limited by interactions of other electroactive compounds chemically similar to vanillin which may be present together with vanillin in different types of samples. In this case, the proposed method may be used...
by coupling HPLC as the preparatory technique for the more sensitive and individual detection of vanillin. On the other hand, this is not a case of some commercial vanilla flavoured products in which these interfering compounds are not contained.

References


Talanta, 72, 1362–1369.