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Electrochemical oxidation and electroanalytical determination of xylitol at a boron-doped diamond electrode

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ABSTRACT

Xylitol is a reduced sugar with anticariogenic properties used by insulin-dependent diabetics, and which has attracted great attention of the pharmaceutical, cosmetics, food and dental industries. The detection of xylitol in different matrices is generally based on separation techniques. Alternatively, in this paper, the application of a boron-doped diamond (BDD) electrode allied to differing voltammetric techniques is presented to study the electrochemical behavior of xylitol, and to develop an analytical methodology for its determination in mouthwash. Xylitol undergoes two oxidation steps in an irreversible diffusion-controlled process ($D = 5.05 \times 10^{-5}$ cm^2 s^{-1}). Differential pulse voltammetry studies revealed that the oxidation mechanism for peaks P_1 ($3.4 \leq pH \leq 8.0$), and P_2 ($6.0 \leq pH \leq 9.0$) involves transfer of 1 H^+/1e^-, and 1e^- alone, respectively. The oxidation process P_1 is mediated by the $^{\bullet}OH$ generated at the BDD hydrogen-terminated surface. The maximum peak current was obtained at a pH of 7.0, and the electroanalytical method developed, (employing square wave voltammetry) yielded low detection (1.3×10^{-6} mol L^{-1}), and quantification (4.5×10^{-6} mol L^{-1}) limits, associated with good levels of repeatability (4.7%), and reproducibility (5.3%); thus demonstrating the viability of the methodology for detection of xylitol in biological samples containing low concentrations.

1. Introduction

The biggest challenge in preventive dentistry is tooth-surface biofilm control (this for both natural and synthetic dental surfaces). Bacterial populations in the oral cavity are the primary causes of many problems, such as cavity formation, bad breath, hypersensitivity, gum bleeding, periodontitis, and tooth loss [1–3]. These problems are associated with excessive consumption of sucrose in human diets, and the absence of proper oral hygiene [4]. To prevent these problems, and to maintain good oral health, the use of antibacterial agents [1,3–5], such as mouthwash, and toothpaste, and both natural and artificial sweeteners, are frequently employed. Due to its high sweetening ability, its anticariogenic properties, and its potential for use by insulin-dependent diabetics [4–7], xylitol has attracted much attention in the pharmaceutical, cosmetics, food, and dental industries.

Xylitol has also become a product of great economic interest, it is approved by the US Food and Drug Administration (FDA), and current world production exceeds 10,000 t per year. This is directed mainly towards food, pharmaceutical, cosmetic, and oral hygiene uses [7,8].

Xylitol (1,2,3,4,5-pentahydroxypentanol—molecular weight: 152.2 g mol^{-1}), with the structural formula shown in Fig. 1, is a reduced sugar, derived from xylose hydrogenation [4–8].

This polyalcohol (also known as a sugar alcohol, or polyhydric alcohol) is a hydrogenated form of a carbohydrate, whose carbonyl group (aldehyde, or ketone reducing sugar) has been reduced to a primary or secondary hydroxyl group (hence the alcohol) [9]. It is naturally found in fruits, legumes, vegetables and wild mushrooms in small amounts [4–6], but can be artificially produced thru chemical or biotechnological processes [4,7,8]. In the late 1960s, dental studies showed the beneficial effects of xylitol, when replacing sucrose, for disease prevention [5,6,10]. The sugar inhibits the growth of various bacteria, including *Streptococcus mutans*, thus reducing the biofilm thickness [4,5]. Hence, it is used in the prevention of tooth decay [4,10,11]. However, the consumption of large amounts of this sugar can produce side effects, including osmotic diarrhea, flatulence, and gastrointestinal pain. The usual recommended daily maximums of xylitol are 60–70 g for adults (10–30 g per intake), and 50 g for children (10 g per intake) [12].

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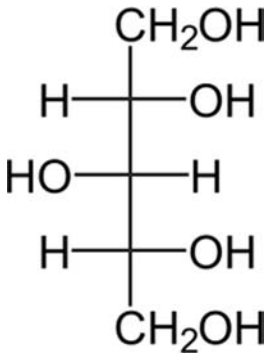

Fig. 1. Xylitol structural formula.

Xylitol has been determined by both enzymatic, and chemical methods [4,13,14]. Chemical detection of xylitol in different matrices is generally based on separation techniques, coupled with characterization [4]. Various methods, such as colorimetric [15,16], chromatographic [13,17–22], flow injection analysis [23,24], capillary electrophoresis [25] and isotachophoresis [26] have been applied to the determination of xylitol. However, electroanalytical techniques are a promising alternative for the determination of organic molecules in complex matrices, because they deliver lower cost and analysis time, high selectivity, and high sensitivity. Electroanalytical techniques have not been largely used for the determination of xylitol. However, electrochemical studies devoted to the use of xylitol as a fuel in fuel cell systems [27], and applications such as amperometric detectors [13,24,25] can be found in the literature.

The electrochemical oxidation of xylitol on Pt (111) [28], and on differing platinum single crystal electrodes [29], in acid medium (0.1 mol L^{-1} $HClO_4$) was studied using cyclic voltammetry (CV), which implies that xylitol oxidation would occur in the range of +0.4 V to 1.1 V (*versus* the reversible hydrogen electrode—E_{RHE}). In all cases, the current density decreased between the first and second cycle. This deactivation might have been due to modification of the electrode surface structure, and/or to poisoning, through accumulation of adsorbed species.

Boron-doped diamond (BDD) electrodes are very attractive (for their many potential applications), and due to their interesting properties, and they are significantly different from conventional electrodes, *e.g.*, glassy carbon (GC), gold (Au), and platinum (Pt) electrodes [30,31]. The main properties of BDD electrodes are their very low and stable background current, corrosion stability in very aggressive media, extreme electrochemical stability (low adsorption of contaminants), high response sensitivity, and a very wide working potential window, which can be larger than 3.5 V [30–36]. These properties make it useful in electroanalysis, especially for the determination of organic substances; (adenosine, ascorbic acid, caffeine, carbamate pesticides, chlorophenols, cysteine, histamine, indoles, nucleic acids, tetracycline antibiotics, and xanthine among others), and inorganic substances; (azide anion, hydrogen peroxide, nitrates, nitrites, dissolved oxygen, and the metal ions Pb^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+}) [35,36].

This study describes the application of a BDD electrode allied to CV, linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) to the study of xylitol's electrochemical behavior, and the development of a methodology for its analytical determination in mouthwash samples using SWV.

2. Experimental

2.1. Apparatus and reagents

All voltammetric experiments were carried out using an Eco Chemie, μAutolab® Type II, potentiostat coupled to a Metrohm, 663 VA Stand®, three-electrode module, and a 3 mL single-compartment electrochemical cell. A platinum wire with Ag/AgCl (3 mol L^{-1}, KCl) were employed as counter and reference electrodes. GC (⌀=3 mm), carbon paste (CP, ⌀=3 mm), Au (⌀=2 mm), Pt (⌀=2 mm), and BDD (surface area of 0.36 cm^2) were used as working electrodes. BDD film electrodes (pieces of 1.2 cm × 1.2 cm) were prepared in the Centre Suisse d'Electronique et de Microtechnique SA (CSEM), Neuchâtel, Switzerland, using a hot filament chemical vapor deposition technique with a filament temperature between 2440 and 2560 °C and a gaseous mixture containing methane, H_2 and trimethylboron, having a final boron content of the order of 8000 ppm [33].

Xylitol (99.5%) and all the other chemicals were analytical grade and purchased from Sigma-Aldrich. The solutions and subsequent dilutions were prepared daily with deionized water in a Millipore Milli-Q System (conductivity ≤ 0.1 μS cm^{-1}). Stock solutions of xylitol (10 mmol L^{-1}) were prepared in water. Buffer solutions 0.1 mol L^{-1} were prepared and employed as supporting electrolyte following the procedure described by Oliveira et al. [37]: HCl/KCl (pH 2.2); HAc/NaAc (pH: 3.4, 4.2 and 5.4); NaH_2PO_4/Na_2HPO_4 (pH 6.0, 7.0 and 8.0); borax/NaOH (pH 9.0 and 10.2) and NaOH/KCl (pH 11.8). In addition, a 0.1 mol L^{-1} sulfuric acid solution (pH 1.0) was also used.

2.2. Electrode preparation and measurement procedure

Prior to each experiment the BDD electrode was submitted to an anodic treatment (+3.0 V) for 120 s followed by a cathode treatment (−3.0 V) for 240 s using a 0.5 mol L^{-1} aqueous H_2SO_4 solution as the supporting electrolyte [34]. For each pretreatment a different electrochemical cell was used without stirring the solution. Afterward, the BDD electrode surface was rinsed with ultrapure water. This procedure was repeated daily before voltammetric measurements and between measurements at different values of pH in order to obtain reliable and reproducible results, in view of possible structural changes on the BDD surface resulting from the superficial loss hydrogen caused by BDD surface oxidation by oxygen from the air [34].

All the voltammetric experiments were performed at room temperature. LSV and CV used scans rates (ν) of 25–100 mV s^{-1}; DPV used a pulse (a) of 50 mV amplitude, a modulation time of 70 ms, and ν of 10 mV s^{-1}; SWV used a frequency (f) of 25 s^{-1}, scan increment (ΔE_s) of 2 mV, scan rate of 50 mV s^{-1}, with the a at 50 mV.

2.3. Recovery

Recovery [38] experiments were carried out in order to evaluate the performance of the method by measuring xylitol concentrations in two commercial mouthwash samples from differing fabrication lots acquired in the city of João Pessoa (PB, Brazil).

The mouthwash samples were first diluted by addition of water at 1:200 (v/v) and then analyzed. Sample (25 μL) was added to an electrochemical cell containing 3 mL of the support electrolyte, and recovery curves were obtained for the sample spiked with three aliquots of 25 μL of 1.0×10^{-3} mol L^{-1} xylitol solution using the standard addition method. Each sample was evaluated in triplicate.

3. Results and discussion

3.1. Electrochemical oxidation of xylitol

3.1.1. Choice of the working electrode

The electroactivity of fresh 164 μmol L^{-1} xylitol solutions, in 0.1 mol L^{-1} phosphate buffer (pH 7.0) was studied using SW voltammograms, recorded on different working electrodes, Fig. 2.

In the forward scan, from 0.0 to +1.4 V *vs.* EAg/AgCl, only one electrochemical oxidation process for xylitol was identified in the voltammograms taken with the Au electrode, Fig. 2A (solid curve) by peak P1 in Ep1 ≈ +0.80 V. Since the oxidation of xylitol takes place at potentials where the formation of Au oxides does, its analytical study is invalidated because the composition of the electrode surface is variable during the experiments (changes in potential produce different amounts of oxides on the surface) [39,40]. The peak observed in background voltammograms (dotted curve) of this electrode confirms this. No electrochemical response was observed on GC, CP, Pt (figure not shown), as well as, on an un-polarized BDD (Fig. 2B) electrode in the same potential range as used in Fig. 2A. Otherwise, Matos et al. [29] observed oxidation reactions of xylitol (10.0 mmol L^{-1}), around +0.8 V using a Pt single crystal electrode in 0.1 mol L^{-1} $HClO_4$ (pH 1.0). The authors add that Pt is the best catalyst, in acid medium, whereas gold is almost inactive [29]. Conversely, in alkaline medium, Au is usually a very active catalyst, at least for electro-oxidation of alcohols at higher potentials [29].

Alternatively, the wide working potential window of the BDD electrode allowed the use of a higher positive potential in the forward scan, from +1.4 V to +2.4 V *vs.* $E_{Ag/AgCl}$, Fig. 2C. The oxidation peak P_1 (solid curve) is shifted towards higher positive potentials ($E_{p1} \approx +1.8$ V) in comparison with the Au electrode (Fig. 2A) and a new peak P_2 appears at $E_{p2} \approx +2.1$ V.

The BDD electrode was either cathodically or anodically pre-treated, and its response was assessed in a 0.1 mol L^{-1} phosphate buffer (pH 7.0) solution using SWV, Fig. 2D, to obtain an improved electrochemical response for the determination of xylitol. The BDD electrode polarization effect depends on the BDD surface termination (cathodic: hydrogen-terminated; anodic: oxygen-terminated), this increases the electrochemical response of xylitol considerably when compared to the un-polarized electrode (solid curve). The choice of a BDD cathodically polarized electrode, instead of the BDD anodically pretreated electrode for analytical studies, is based on the following features: onset, and peak oxidation potentials are shifted towards more negative potentials making the voltammetric xylitol measurements more sensitive and selective. In addition, the SW voltammograms taken in 0.1 mol L^{-1} phosphate buffer (pH 7.0) solution on a BDD un-polarized or anodically polarized electrode exhibit considerable variation in background current. Thus, the cathodic pretreatment was performed daily before starting the voltammetric measurements, for electrodes previously pre-treated anodically to clean the electrode surfaces, as already pointed out [34].

3.1.2. *Cyclic and linear sweep voltammetry*

Initially, CV experiments carried out in a N_2 saturated 625 μmol L^{-1} xylitol solution in 0.1 mol L^{-1} phosphate buffer (pH 7.0) on a BDD cathodically polarized electrode from 0.0 to −2.0 V *vs.* $E_{Ag/AgCl}$ (figure not shown), did not show analytical signal in this potential range.

Afterwards, CVs were carried out scanning from 0.0 to +2.4 V *vs.* $E_{Ag/AgCl}$. Two oxidation processes were observed, Fig. 3A, at $E_{p1} \approx +1.8$ V and at $E_{p2} \approx +2.1$ which are in agreement with peaks P_1 and P_2 observed in the SW voltammograms of Fig. 2C. Inverting the scan direction, corresponding to the reduction of oxidation products formed on the BDD electrode surface, no peak was observed, indicating an irreversible process, which is in agreement with the studies developed by Matos et al. [28,29] using Pt single crystal, and polycrystalline Pt electrodes for identification of xylitol (10 mmol L^{-1}) in acid medium.

The current decrease for the peaks P_1 and P_2 observed while recording successive scans (Fig. 3A) was due to xylitol adsorption and/or its non-electroative oxidation products, and/or to competitive superficial reactions on the BDD electrode surface in the potential range studied, which may have caused fouling and inactivation of the available electrode surface area. The effect of scan rate on the potential, and current of both peaks P_1 and P_2 was investigated by increasing the scan rate from 25 to 100 mV s^{-1} by LSV in a potential range of +0.0 to +2.4 V (*vs.* $E_{Ag/AgCl}$). The LSV voltammograms of peak P_1 were chosen for presentation (inset Fig. 3B).

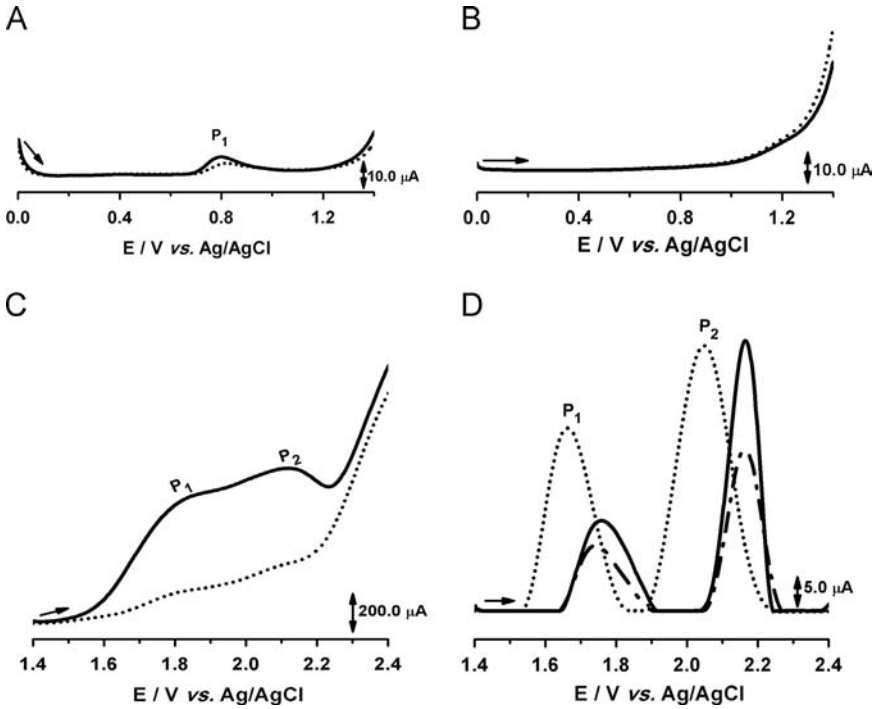

Fig. 2. SW voltammograms obtained in 164 μmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0) (solid line), and in phosphate buffer only (dotted line), at different working electrodes: (A) Au, (B) BDD un-polarized (potential range from 0.0 to 1.4 V), (C) BDD un-polarized (potential range from 1.4 to 2.4 V). (D) Background-corrected SW voltammograms of 66 μmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0) at a BDD: (-) un-polarized, (-●-) anodically polarized, and (…) cathodically polarized. Data obtained after baseline correction and electrolyte background subtraction. f=25 s^{-1}, ΔE_s=2 mV, a=50 mV.

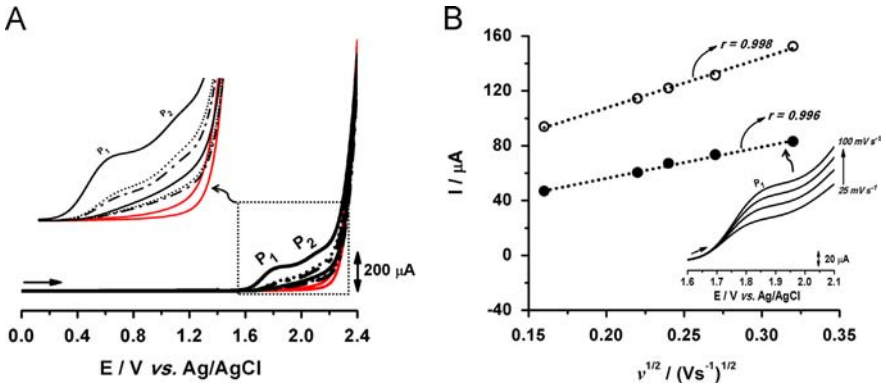

Fig. 3. (**A**) Cyclic voltammograms taken in 625 µmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0): (-) first, (…) second and (-●-) third scans and (▬) only in supporting electrolyte solution; $\nu = 50$ mV s^{-1}. (**B**) Influence of square root of the scan rate on the (●) P_1 and (○) P_2 peak current of a 625 µmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0) by LSV. Inset: LS voltammograms in different scan rates for peak P_1.

Studies show that the peak current changes linearly with scan rate according to the equation $I_{p1 \text{ or } p2} = K\nu^x$. The x values 1.0 and 0.5 are expected for the adsorption and diffusion controlled reactions, respectively [41,42]. In this study, the peak currents P_1 and P_2 increased linearly with the square root of scan rate (Fig. 3B) and follow the relationships I_{p1} (A) $= 1.05 \times 10^{-5} + 2.30 \times 10^{-4}\, \nu^{1/2}$ (V $s^{-1})^{1/2}$ and I_{p2} (A) $= 3.51 \times 10^{-5} + 3.63 \times 10^{-4}\, \nu^{1/2}$ (V $s^{-1})^{1/2}$, both with r values of around 0.997. These equations are similar to the Randles–Sevick equation for a diffusion-controlled irreversible or quasi-reversible process [43]. Therefore, the electrochemical oxidation processes for xylitol are predominantly diffusion-controlled, which is in accordance with the literature [43]. In addition, the plot of log I_{p1} and log I_{p2} *vs.* log ν (figure not shown) gave slope values of 0.41 and 0.33 for peaks P_1 and P_2. This confirms the nature of the mass transfer process, since the slope must be equal to 0.50 for diffusion-controlled mass transport processes [41].

Considering peak P_1 alone, increasing the scan rate promoted slight displacements in the peak potential to more positive values. The difference between peak potential E_{p1} and the potential at peak half height $E_{1/2,p1}$ was 77.5 mV. Since for a diffusion-controlled irreversible process $|E_{p1} - E_{1/2,p1}| = 47.7/(\alpha n_a)$, where αn_a is the product of the anodic charge transfer coefficient, and the number of electrons in the rate-determining step [43], We calculated that $\alpha n_a = 0.62$. If the value of α is assumed equal to 0.5, a value quite common for organic molecules [30], these results indicate that the oxidation of xylitol involves 1 electron per molecule, which is in agreement with the studies developed by Matos et al. [28,29], using acid medium, and Pt single and polycrystalline electrodes, as well as with the DPV studies shown below in Section 3.1.4.

The diffusion coefficient of 625 µmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0) was estimated at $D = 5.05 \times 10^{-5}$ cm^2 s^{-1} for a BDD electrode with an electroactive area of 0.22 cm^2 ($Fe(CN)_6^{4-/3-}$,1.0 mmol L^{-1} in 0.1 mol L^{-1} KCl following the procedure described by Hegde et al. [44]). In general, typical values of diffusional coefficients are found in the order of $1–1.5 \times 10^{-5}$ cm^2 s^{-1} for small molecules in water, and 10^{-6} cm^2 s^{-1} for small proteins in water [45].

3.1.3. Square wave voltammetry

A great advantage of the SWV technique is the possibility of determining whether the electron transfer reaction is reversible or irreversible. Peaks corresponding to both oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment, since the current is sampled in both the forward, and the backward pulses [37]. In order to clarify the reversibility of the xylitol redox processes, concomitantly with the CV (Fig. 3), and DPV (discussed hereafter) experiments, SW voltammograms were taken in 66 µmol L^{-1} xylitol in different electrolytes ($1.0 \leq$ pH ≤ 11.8). They showed similar features to the BDD electrode. The results obtained in 0.1 mol L^{-1} phosphate buffer (pH 7.0) were chosen to be presented (Fig. 4). The irreversibility, already diagnosed using the CV experiments, of both redox reactions peaks was confirmed by plotting the forward and backward components of the total current.

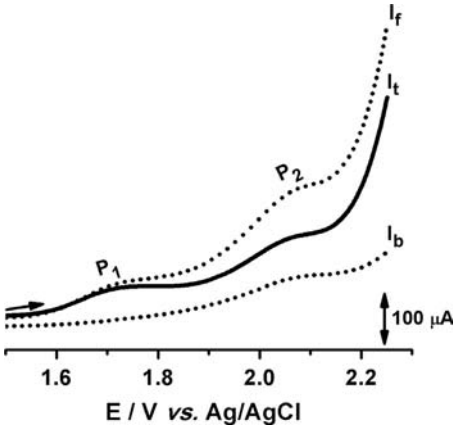

Fig. 4. SW voltammograms in 66 µmol L^{-1} xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0). $f = 25$ s^{-1}, $\Delta E_s = 2$ mV, $a = 50$ mV; It – total, If—forward and Ib—backward currents.

3.1.4. Differential pulse voltammetry and pH effect

DP voltammograms (figure not shown) were obtained in a potential range from +1.4 to +2.5 V (*vs.* $E_{Ag/AgCl}$) in 66 µmol L^{-1} xylitol solutions, and on a BDD cathodically polarized electrode in different electrolytes and in a pH range from 1.0 to 11.8 (Fig. 5). DPV, as well as SWV, LSV and CV studies show two charge transfer reactions for xylitol for the pH values between 1.0 and 9.0. However, for values of pH < 3.6, the intensity of the faradaic current contribution of •OH generated on BDD surface (effect discussed hereafter) is higher than the faradaic current of xylitol.

For pH values between 3.6 and 8.0 (Fig. 5), the peak potential E_{p1} displays a linear dependence on pH, and is shifted towards more negative values with increasing pH, as a consequence of the gradual dissociation of the hydroxyl group [46]. This indicates that the protonation of the electroactive site of xylitol affects the overall electrode reaction mechanism. Luz et al. [47] justified their investigation, considering that in water, the transference of proton from or toward an organic molecule is usually considered fast,

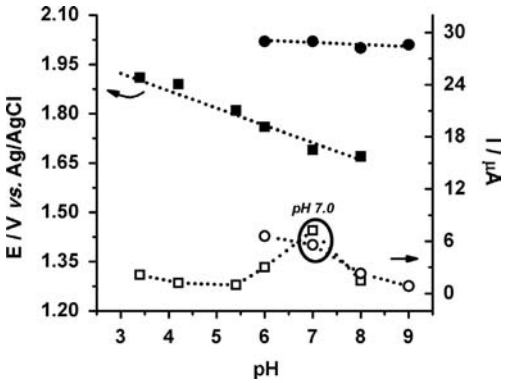

Fig. 5. Influence of pH on the (■) P_1 and (●) P_2 peak potential and (□) P_1 and (○) P_2 peak current of a 66 μmol L^{-1} xylitol solution dissolved in different buffer solutions by DPV. $v=10$ mV s^{-1}; $a=50$ mV. Data peak current obtained after baseline correction and electrolyte background subtraction.

meaning that the protons are in equilibrium in solution, and near to the electrode [48].

The linear relationship of E_{p1} *vs.* pH, (Fig. 5) revealed a slope of −58.0 mV/pH; indicating an irreversible reaction mechanism, involving the same number of protons and electrons in the process [49]. This slope is close to that expected for a monoelectronic/monoprotonic electrode reaction (59.2 mV/pH at 25 °C) [47]. Considering that the peak width at half height ($W_{1/2}$) is 117.0 mV, the oxidation process involves the transfer of $1H^+/1e^-$ for peak P_1. The $W_{1/2,p1} \approx 90.0$ mV values confirm that one electron was transferred [43].

For values of pH between 6.0 and 9.0 (Fig. 5), the peak potential E_{p2} is pH independent indicating that, in this pH range, the peak potential is not affected by the concentration of H^+, This suggests an absence of any protonation step in the oxidation mechanism. Thus, considering that $W_{1/2,p2}$ is 92.0 mV (In theory $W_{1/2,p} \approx 90.0$ mV) the charge transfer reaction mechanism would involve the transfer of $1e^-$ for peak P_2.

In situ electrochemical generation of hydroxyl radicals (•OH) on a BDD surface (potential range of 0.0 to +2.5 V), in different electrolytes, was shown by Enache et al. [50]. This was also presented by Oliveira et al. [51]. Enache et al. [50] pointed out that electrochemical generation of •OH is associated with an oxidation peak at +2.1 V, and shifts to smaller values with increasing pH. This peak current disappeared at pH 9.0, the process is related to water oxidation, and involves the transfer of $1H^+/1e^-$. In the region where water oxidation produces •OH the hydrogen-terminated surface may also be oxidized by chemical reaction with •OH [52]. In addition, the •OH generated at the BDD surface is non-selective [51], and can act as mediator (powerful oxidizing agent) in the oxidation of organics like xylitol [50,51].

In summary, the xylitol peaks P_1 with pH between (3.4 and 8.0), and P_2 with pH between (6.0 and 9.0) are irreversible oxidation processes, and involve the transfer of $1H^+/1e^-$ and $1e^-$, alone, respectively. The oxidation process P_1 is mediated by the •OH generated at the BDD hydrogen-terminated surface. Xylitol is a symmetric molecule constituted of 5-carbon atoms and 5-hydroxyl groups in its structure (Fig. 1) the hydroxyl groups can be electro-oxidized; so it is necessary to know at which position of the groups the oxidation takes place. In theory, primary alcohols are more reactive than secondary alcohols, because of the positioning of the alcohol group, while tertiary alcohols are almost unreactive [29]. However, the oxidation of xylitol, by enzymatic reaction, can occur at carbon 2 or 4 yielding D- or L-xylulose, respectively [53]. Therefore, based in our electrochemical data, the first step of xylitol oxidation could involve the oxidation of the hydroxyl group at carbon 2 or 4 by the •OH generated at the BDD, thus forming a thermodynamically unstable radical (xylitol-radical), which stabilizes in D- or L-xylulose. This compound can be oxidized to form the cyclic structure α or β-D-xylulofuranose, constituting the second step of oxidation. In this report, as •OH radicals are non-selective [51], this is only one likely mechanism for oxidation of xylitol.

On the other hand, Gowda and Nandibewoor [54] observed the formation of 2,3,4,5-tetrahydroxypentanoic acid as main oxidation product of the oxidation of xylitol using the oxidant Ag (III) periodate complex in aqueous alkaline medium. However, proposing an accurate oxidation mechanism to xylitol would need further studies to identify the intermediates of the oxidation reaction by using techniques like chromatography with mass detector or differential electrochemical mass spectrometry.

Successive DP voltammograms of xylitol at pH values of 4.2, 7.0, and 8.0 were recorded. No other peaks were observed in the second scan suggesting that xylitol oxidation does not form electroactive products.

The plots of the peak current variation for P_1 and P_2 (including pH) (Fig. 5) show that both are detected simultaneously phosphate buffer (pH values 6.0–8.0) alone. The maximum peak current was obtained at a pH of 7.0. This justifies the choice of the phosphate buffer (pH 7.0) as the supporting electrolyte for future electroanalytical studies, for allowing a good compromise between sensitivity and analytical response. However, for future electroanalytical studies the P_1 process is more selective.

3.2. Analytical determination of xylitol by SWV

In this study, the irreversible electrochemical oxidation of xylitol was studied using SWV (Fig. 4), and DPV (Fig. 5) with scan rates of 50 mV s^{-1}, and 10 mV s^{-1}, respectively. Considering the data obtained at pH 7.0, the peak in current, taken using SWV is six fold higher than that obtained with DPV, and justifies the use of SWV in further analytical studies carried out in this report.

3.2.1. Optimization of SWV conditions

Generally, the scanning parameters f, ΔE_s and a strongly influence the peak current (intensity), and the selectivity (half-peak width) in SWV, thus determining the sensitivity of the technique. In order to determine the influence of the parameters on the electrochemical oxidation of xylitol for peak potential and current, univariate studies were carried out at the initial conditions of the experiments, (Section 2.2, taking 66 μmol L^{-1} xylitol solution in 0.1 mol L^{-1} phosphate buffer (pH 7.0) at a BDD cathodically polarized electrode). The optimized values of the SWV parameters obtained for the determination of xylitol are presented in Table 1.

Frequency is for SWV like scan rate is for CV [43,55]. The peak current P_1 displays a linear dependence on the square root of the frequency up to 10.5 beats per second (slope of 5.8 μA $(s^{-1})^{-1/2}$); suggesting an irreversible oxidation process predominantly controlled by diffusion, which is in accordance with the literature [28] and with the studies carried out by LSV in this report. For frequency values of 11–25 s^{-1}, the peak current decreases, possibly due to slow diffusion of xylitol to/or from the BDD surface.

Table 1
Investigated SWV parameters and their optimum values obtained for the determination of xylitol.

Parameters	Studied range	Optimum value
Frequency (s^{-1})	8–25	10
Scan increment (mV)	2–5	4
Pulse amplitude (mV)	25–50	50

The potential E_{p1} is slightly shifted to more positive values with increasing of f. The linear relationship (Ep_1 *vs.* log f, figure not shown) revealed a slope of 160.0 mV dec^{-1}. For an irreversible process [56], the peak potential varies linearly with the logarithm and is equal to $59/\alpha n$, where αn was defined in Section 3.1.2, it can be calculated that $\alpha n = 0.37$. If the value of α is assumed to be equal to 0.5, a value quite common for organic molecules [30], the oxidation of xylitol involves 1 electron per molecule, which is in agreement to the literature [28,29] and with the studies of LSV and DPV in this report.

The scan rate in SWV is the product of the f and ΔE_s [55]. The peak current displays a linear dependence on ΔE_s up to 4 mV. Meanwhile, for $\Delta E_s = 5$ mV a widening of the peak occurs, thus diminishing the resolution of the analysis. The potential E_{p1} is slightly shifted to more positive values with increasing of ΔE_s.

The influence of a on peak current intensities was also considered, and the results obtained demonstrated that the peak current increased linearly up to 50 mV, following the relationship $I_{p1}(A) = 4.22 \times 10^{-6} + 6.98 \times 10^{-8} a$ (mV), without any observed shift in peak potential or in the half-peak width. Using the slope of this relationship, an approximate calculation of the surface concentration (Γ) of the adsorbed species is given by $I_{p1} = (5 \pm 1) \times 10^2\, q\alpha n^2 F f\, \Delta E_s\, \Gamma a$, where q is the electrode area, F is the Faraday constant, and the other terms have already been defined [56,57]. For this calculation, the value for Γ was 2.7×10^{-16} mol cm^{-2}.

In the optimized conditions, pre-concentration studies of a 66 μmol L^{-1} xylitol solution were performed in open circuit, and no current gain was observed through the range of the deposition time of 30–600 s.

3.2.2. Analytical curve

Fig. 6 displays the SWV responses and the respective analytical curve for xylitol in the optimized experimental conditions.

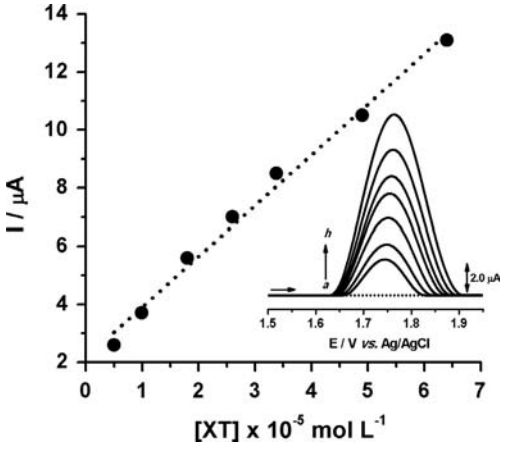

Fig. 6. Analytical curve for xylitol. Inset: SW voltammograms for additions of xylitol in 0.1 mol L^{-1} phosphate buffer (pH 7.0). (a) 0, (b) 5.0, (c) 10.0, (d) 18.0, (e) 26.0, (f) 33.8, (g) 49.1, and (h) 64.0 μmol L^{-1}. $f = 10$ s^{-1}, $\Delta E_s = 4$ mV, $a = 50$ mV.

A half-wave potential ($E_{1/2}$) around +1.75 V was identified for the xylitol peak, and a good linear relationship between peak current and concentration was verified for the concentration range of 5.0–64 μmol L^{-1} ($r^2 = 0.990$ for $n = 7$, and I_p (μA) = 2.2 (±0.2) + 1.7×10^5 ($\pm 0.07 \times 10^5$) [xylitol] (mol L^{-1})). A limit of detection (LOD) of 0.2 ppm (1.3×10^{-6} mol L^{-1}), and a limit of quantification (LOQ) of 0.7 ppm (4.5×10^{-6} mol L^{-1}) were estimated as kS_d/b, where $k = 3$ for LOD, and $k = 10$ for LOQ, where S_d is the standard deviation of the blank signal ($n = 10$), and b is the slope (sensitivity) of the analytical curve [58].

Table 2 compares the performance of the proposed SWV approaches to methods reported in the literature for xylitol determination. The proposed method is generally more sensitive than other methods; however, shows similar results when compared with electrophoresis with amperometric detection methods. Despite their generally satisfactory sensitivities, the chromatographic and electrophoretic methods have some drawbacks: they are laborious, expensive, require skilled labor, and involve several steps, biochemical tests, and sophisticated equipment. The proposed approach is has more advantages than either previously reported analytical methods, or the electroanalytical ones.

The precision of the proposed method was evaluated in terms of repeatability and reproducibility. A relative standard deviation of 4.7% (10 successive measurements), and 5.3% (5 different measurements) for a 1.0×10^{-5} mol L^{-1} (1.5 ppm) xylitol solution demonstrated good repeatability and reproducibility of the method, respectively, this, particularly if it is considered that the BDD electrode was not polarized between measurements. These values are also in agreement with the values established by the Association of Official Analytical Chemists (AOAC) International guidelines for single laboratory validation of chemical methods, for dietary supplements and botanicals, *i.e.,* acceptable values of repeatability (rsd = 8%), and reproducibility (rsd = 16%), both in the absence of inter-laboratory studies and with an analyte concentration of 1 ppm [59].

3.2.3. Effect of interferents and practical application

Xylitol, as well as mannitol and sorbitol, has an anti-caries effect, and these sweeteners have become increasingly used in chewing gum, toothpaste, confections, and mouthwash [3–6]. So, mannitol and sorbitol could be possible interferents in the xylitol voltammetric determination in mouthwash samples. We evaluated the selectivity of the proposed method and the electrochemical behavior of these substances (3.3×10^{-5} mol L^{-1}; standard solution individual and/or mixed) by using square wave voltammograms recorded from 1.5 to 2.0 V *vs.* $E_{Ag/AgCl}$, in 0.1 mol L^{-1} phosphate buffer (pH 7.0) and at a cathodically polarized BDD electrode. All these substances undergo electrochemical oxidation at the BDD surface, *i.e.*, sorbitol ($E_p = 1.698$ V; $I_p = 3.1$ μA), xylitol ($E_p = 1.677$ V; $I_p = 5.2$ μA), mannitol ($E_p = 1.694$ V; $I_p = 2$ μA), and a mixture of polyols ($E_p = 1.706$ V; $I_p = 10$ μA); thus suggesting that these substances can interfere in the direct electrochemical determination of xylitol.

Table 2
Comparison between the results of published methodologies for xylitol determination with the results from this study.

Technique	Sample	LR[a] (mol L^{-1})	LOD (mol L^{-1})	Ref.
Electrophorese-amperometry	Gums	$(5–1000) \times 10^{-5}$	5.0×10^{-6}	[25]
Enzymatic	Serum	$(0.4–1.2) \times 10^{-3}$	0.1×10^{-3}	[13]
Cyclic voltammetry	Not specified	$(1–300) \times 10^{-3}$	Not specified	[28]
HPLC-light scattering	Medicinal plants	$(1.1–8.3) \times 10^{-3}$	2.4×10^{-4}	[22]
HPLC-light scattering	Medicinal plants	$(6.6–65.7) \times 10^{-4}$	Not specified	[21]
Isotachophoresis-conductometry	Pharmaceutical formulations	$(3.3–32.8) \times 10^{-4}$	Not specified	[26]
Square wave voltammetry	Mouthwash	$(5–64) \times 10^{-6}$	1.3×10^{-6}	This work

[a] LR: linear range

Table 3
Results for the recovery study of xylitol in different mouthwash products.

Sample	Xylitol (10^{-6} mol L^{-1})		Recovery[b] (%)	Rsd[a] (%)
	Added	Found[a]		
Mouthwash (adult)	8.2	6.3	77	0.9
Mouthwash 1 (kids)	8.2	5.7	70	1.8
Mouthwash 2 (kids)	8.2	4.5	55	4.4
Mouthwash (adult)	16.2	14.3	88	0.4
Mouthwash 1 (kids)	16.2	13.8	85	0.8
Mouthwash 2 (kids)	16.2	12.5	77	1.6
Mouthwash (adult)	24.2	22.5	92	0.3
Mouthwash 1 (kids)	24.2	21.8	90	0.5
Mouthwash 2 (kids)	24.2	20.5	85	1.0

[a] $n=3$.
[b] (100 × found/added).

The method was applied to xylitol in three batches of mouthwash commercial formulations (Table 3), with two mouthwash kids samples contained no sorbitol and mannitol. The BDD electrode responded efficiently to the incremental xylitol concentrations. Recoveries of around 86.0 ± 0.5%, 82.0 ± 1.0% and 72.0 ± 2.3% were verified for the three different concentrations of xylitol added to the real samples studied (Table 3). According to AOAC International, an acceptable recovery value is a function of both the concentration, and the purpose of the analysis. Recovery limits of 75–120% and 80–115% are expected for analyte concentration of 1 and 10 ppm (or 6.6×10^{-6} and 66×10^{-6} mol L^{-1} for xylitol), respectively [59]. The mean recovery percentage for different concentrations of xylitol showed no significant excipient interferences. However, all mouthwash samples with a xylitol concentration of 8.2×10^{-6} mol L^{-1}showed recovery values below that recommended by the AOAC International, in particular the 55% value (mouthwash 2 (kids)). We rationalize this low value, considering that the xylitol concentration added is near the quantification limit of the method (LOQ=4.5×10^{-6} mol L^{-1}). Thus, the recovery values taken using 16.2×10^{-6} mol L^{-1}, and 24.2×10^{-6} mol L^{-1} offer the possibility of analytical xylitol determinations for quality control in biologically critical matrices. Furthermore, this method can be successfully applied to xylitol containing formulations without the presence of sorbitol and/or mannitol.

4. Conclusions

Xylitol is an oxygenated organic molecule, and its electrochemical oxidation depends on both the pH and the electrode material. This behavior was investigated using cyclic, linear sweep, square wave, and differential pulse voltammetries on a BDD electrode (cathodically polarized). Xylitol undergoes two oxidation steps in an irreversible diffusion-controlled process ($D=5.05\times10^{-5}$ cm^2 s^{-1}). Differential pulse voltammetry studies revealed that the oxidation mechanism for the peaks P_1 (pH of 3.4–8.0), and P_2 (pH of 6.0–9.0) involve the transfer of $1H^+/1e^-$, and $1e^-$ alone, respectively.

An electroanalytical method employing SWV was developed for xylitol in pH 7.0 with a low LOD (1.3×10^{-6} mol L^{-1}), and an LOQ of 4.5×10^{-6} mol L^{-1} associated with a good level of repeatability (4.7%), and reproducibility (5.3%). This method was successfully applied for the determination of xylitol in mouthwash samples with recoveries of around 90%. The combination of both the high sensitivity of the SWV technique, coupled with the unique properties of the BDD electrode establish the viability of the developed methodology for low concentration detection of xylitol in biological samples.

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