Article

New Voltammetry-Based Technique for the Determination of Tebuthiuron in Crystal and Brown Sugar Samples

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O açúcar é amplamente consumido em todo o mundo e o Brasil é o maior produtor, consumidor e exportador deste produto. Para garantir o bom desenvolvimento e produtividade das culturas de cana de açúcar, é necessário aplicar grandes quantidades de agrotóxicos, principalmente herbicidas e pesticidas. O herbicida tebuthiuron (TBH) impede a pré e pós-emergência das plantas daninhas infestantes nas culturas de cana de açúcar. Considerando que é importante para garantir a segurança alimentar da população, este trabalho propõe um método confiável para analisar TBH em matrizes de açúcar (mascavo e cristal), usando voltametria de onda quadrada (SWV) e voltametria de pulso diferencial (DPV) em eletrodo de carbono vítreo e investigar o comportamento eletroquímico do herbicida por voltametria cíclica (CV). Os nossos resultados sugerem que TBH ou o produto da sua reação com o eletrólito de suporte é oxidado através da transferência irreversível de um elétron entre o analito e eletrodo de trabalho, a um potencial de cerca de +1,16 V *vs*. Ag|AgCl$_{sat}$ em 0,10 mol L^{-1} KOH como solução de eletrólito de suporte. Tanto DPV quanto SWV são satisfatórios para a análise quantitativa do analito. DPV é mais sensível e seletiva, com limites de detecção de 0,902, 0,815 e 0,578 mg kg^{-1}, e limite de quantificação de 0,009, 0,010 e 0,008 mg kg^{-1}, na ausência da matriz, e na presença de matriz de açúcar cristal e mascavo, respectivamente. Reprodutibilidade situou-se entre 0,53 e 13,8%, a precisão variou entre 4,14 e 15,0%, e a recuperação manteve-se entre 84,2 e 113% para DPV conduzido na ausência e na presença da matriz de açúcar cristal, respectivamente.

Sugar is widely consumed worldwide and Brazil is the largest producer, consumer, and exporter of this product. To guarantee proper development and productivity of sugar cane crops, it is necessary to apply large quantities of agrochemicals, especially herbicides and pesticides. The herbicide tebuthiuron (TBH) prevents pre- and post-emergence of infesting weed in sugarcane cultures. Considering that it is important to ensure food safety for the population, this paper proposes a reliable method to analyse TBH in sugar matrixes (brown and crystal) using square wave voltammetry (SWV) and differential pulse voltammetry (DPV) at bare glassy carbon electrode and investigate the electrochemical behavior of this herbicide by cyclic voltammetry (CV). Our results suggest that TBH or the product of its reaction with a supporting electrolyte is oxidized through irreversible transfer of one electron between the analyte and the working electrode, at a potential close to +1.16 V *vs*. Ag |AgCl$_{sat}$ in 0.10 mol L^{-1} KOH as supporting electrolyte solution. Both DPV and SWV are satisfactory for the quantitative analysis of the analyte. DPV is more sensitive and selective, with detection limits of 0.902, 0.815 and 0.578 mg kg^{-1}, and quantification limits of 0.009, 0.010 and 0.008 mg kg^{-1} in the absence of the matrix and in the presence of crystal and brown sugar matrix, respectively. Repeatability lay between 0.53 and 13.8%, precision ranged between 4.14 and 15.0%, and recovery remained between 84.2 and 113% in the case of DPV conducted in the absence of matrix and in the presence of the crystal sugar matrix, respectively.

Keywords: tebuthiuron, brown sugar, crystal sugar, voltammetric detection

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Introduction

Brazil is currently the largest producer of sugarcane, followed by India, Thailand, and Australia. Season 2010/2011 saw the production of over 620 million tons in Brazil, which is equivalent to 25% of the worldwide production.[1] According to a study conducted by UNICA in Brazil, the annual sugarcane production in this country will reach one billion tons. After 2020, the cultivated area should stabilize, but the volume of produced cane should continue to grow because new agricultural technologies will emerge, including the development of new varieties of sugarcane, efficient application of agrochemicals, and improved harvesting techniques.[1]

Sugarcane constitutes the raw material to produce various products of economic importance. For example, the sugarcane juice is used to obtain sugar, ethylic alcohol, brown sugar, molasses, and sugarcane brandy; the residue (bagasse) is burned in a boiler, generating steam and electricity, employed to make paper, biodegradable plastics, fertilizers, and animal food, just to mention a few examples.[2] Among the many products derived from sugarcane, sugar and its derivatives such as refined, crystal, and brown sugar have an important place in the Brazilian economy.

Cultivating sugarcane at large scale without using of agrochemicals is possible but not feasible in current market places.[3] Farmers use agrochemicals as the main strategy to combat and prevent agricultural plagues and ensure crop productivity. According to the Brazilian Health Surveillance Agency (ANVISA),[4] using agrochemicals also called pesticides or agrotoxics in agriculture is advantageous: the productivity and quality of the products increase and the labor and energy spent in the production process decrease.[4] However, the indiscriminate use of these substances may harm the human health and the environment. The current situation is already worrisome from the standpoint of public health, because the contact and ingestion of these compounds is hazardous. This concern tends to grow in the future, since the use of these agrochemicals is likely to rise in the coming years.

1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea (Figure 1), the common name for the active ingredient of tebuthiuron (TBH), is a urea derivative that is often employed as herbicide. According to ANVISA, its toxicological classification is II (highly toxic). Its application is recommended to prevent pre- and post-emergence of weed in sugarcane culture and grasslands.[5] It exhibits good selectivity for the crop and helps control species of great relevance. However, it is partially soluble in soil (soluble in water at a concentration of 2.5 g L^{-1}), which enhances its mobility in the medium, and also persistent, with a half-life of 12 to 15 months.[6,7]
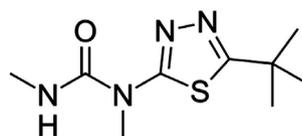

Figure 1. Molecular structure of tebuthiuron (TBH).

Traditionally, agrochemicals residues in matrixes are determined by chromatographic techniques coupled to various detectors, such as devices that capture electrons, and fluorescence, ultraviolet-visible, and flame ionization detectors. TBH is a thermally unstable compound and is usually analyzed by high-performance liquid chromatography (HPLC) or gas chromatography (GC) after derivatization.[7] Still according to ANVISA,[1] the maximum residue limit (MRL) for this herbicide in sugarcane should be 1.00 mg L^{-1}, including its derived products. Table 1 lists some relevant methods to determine TBH which have been reported in the literature and it was also compared with our results.

Table 1. Relevant analytical methodologies for the determination of TBH

Matrix	Extraction / Derivatization	Method	Recovery (%)	LOD / LOQ	Reference
Water	CH_2Cl_2	GC / thermoionic selective detector	97.2	1.3 µg L^{-1} / NR	17
	CH_2Cl_2	HPLC/detector UV 254 nm	85 - 96	0.1 / 4.0 µg L^{-1}	18
Sugarcane	HCl/MeOH (1:1) and KCl_{sat} / ethyl acetate	GC / flame photometric detector	48 - 81	0.01 mg L^{-1} / NR	19
Sugarcane bagasse	HCl:MEOH (1:1) and KCl_{sat} / ethyl acetate	GC / flame photometric detector	84 - 95	0.1 mg L^{-1} / NR	19
Molasses	0.5 mol L^{-1} HCl and KCl_{sat} / ethyl acetate	GC / flame photometric detector	58 - 94	0.01 mg L^{-1} / NR	19
Sugarcane juice	0.5 mol L^{-1} HCl and KCl_{sat} / ethyl acetate	GC / flame photometric detector	47 - 85	0.01 mg L^{-1} / NR	19
Soil	MeOH	HPLC/detector UV 254 nm	90 - 103	0.01 / 0.02 mg kg^{-1}	18
	MeOH	HPLC/detector UV 254 nm	90 - 103	0.01 / 0.02 mg kg^{-1}	20
Sugar	0.5 mol L^{-1} HCl and KCl_{sat} / ethyl acetate	GC / flame photometric detector	50 - 85	0.01 mg L^{-1} / NR	19
Brown sugar	None	Voltammetric (DPV)	84 - 114	0.578 / 0.008 mg kg^{-1}	our work
Crystal sugar	None	Voltammetric (DPV)	89 - 108	0.815 / 0.010 mg kg^{-1}	our work

*NR = Not Reported

Despite the versatility, sensitivity, and efficiency of chromatographic methods, they are expensive and time-consuming. Moreover, the instrumentation is usually high-priced and calls for spending on reagents, which increases the final cost of the analysis.[8] Furthermore, chromatographic procedures cannot be directly applied to the analysis of concentrated samples leading to column degradation which impairs performance. They can only measure residual levels in water, soils, and crops.

In recent years, many studies have been conducted to develop sufficiently selective, sensitive, precise, accurate, and inexpensive methods for the fast detection of agrochemicals in various matrixes. Among the many reported analytical techniques, we highlight the electrochemical methods, which provide rapid and reliable results while consuming small amounts of reagent and generally dismissing complicated sample preparation steps.[9]

In 1970, Hance[8] pioneered the use of electrochemical techniques to determine pesticide residues. In his work, he used polarography to investigate the electrochemical behavior of 35 herbicides in five different supporting-electrolyte solutions. He noted that 28 of the 35 pesticides were electroactive in some of the electrolytes, which allowed for construction of standard curves that were applied to the analysis of real water samples.

To the best of our knowledge, there are no reports of a quantitative voltammetric method to analyze TBH in sugar matrixes (brown and crystal). Therefore, in the present study we attempted to establish an accurate, simple, and sensitive electroanalytical method to determine TBH in sugar samples using an electroanalytical-based methodology.

Experimental

Reagents and TBH stock solutions

All the chemicals were analytical grade and were used without any further purification. Ultrapure water obtained on a Millipore Milli-Q system (USA) was used in all the analytical and electrochemical assays and for construction of the analytical curves.

High-purity TBH (1-(5-tert-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea - Figure 1) was used as received from Sigma-Aldrich (PESTANAL, analytical standard, USA). The TBH stock solution (i.e., 10.00 g L^{-1} or 43.80 mmol L^{-1}) was prepared in methanol (Fisher, HPLC grade, USA) on a daily basis and was added to the system by direct transfer of quantitative aliquots completed to a final volume of 10.0 mL in a conventional voltammetric cell.

Sodium hydroxide (99.0%) and potassium chloride (97.0%) were purchased from SYNTH (Brazil); potassium hydroxide (85.0%), lithium chloride (99.0%), chromium chloride (III) (97.0%), potassium nitrate (99.0%), ammonium chloride (99.5%), and dimethylformamide (DMF) were obtained from VETEC (Brazil). Hydrochloric acid and nitric acid, with purity of 36.5% and 65%, respectively, were acquired from VETEC (Brazil); Carbonate buffer (pH 10) and tetrabutylammonium tetrafluoroborate (TBABF$_4$) with a purity of 95.0% were provided by ACROS Organics (Belgium) for the study of the interferents, aqueous solutions of sulfuric acid (95.0%) and sodium chloride (97.0%) furnished by VETEC (Brazil) were used.

Voltammetric measurements

Voltammetry was performed on a PGSTAT 128 N Autolab potentiostat (Eco-Chemie, Utrecht, The Netherlands) interfaced with a microcomputer operating with a General Purpose Electrochemical System (GPES) software (Version 4.9) for data acquisition. An electrochemical cell containing 0.10 mol L^{-1} KOH as supporting electrolyte and equipped with Ag|AgCl|KCl$_{sat}$ 3.0 mol L^{-1} reference electrode; a Pt wire auxiliary electrode, and a glassy carbon (GC) with geometric area of 0.071 cm^2 as working electrode was used for all the measurements. All the electrodes were acquired from Metrohm (Switzerland). Oxygen was removed by bubbling purified nitrogen gas through the solution in all the experiments. Before each voltammetric measurement, the glassy carbon surface was polished with alumina 0.3 and 0.05 μm (Buehler, USA) on an alumina polishing pad for 3 minutes, rinsed with purified water, and sonicated for 5 min in methanol.

Electrochemical detection of TBH

Three voltammetric modes were applied to detect TBH - linear sweep voltammetry (LSV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). All the measurements were carried out at 25.0 ± 0.1 °C and were obtained in triplicate (at least). The voltammetric parameters were optimized, and then the analytical curve was constructed by adding aliquots of TBH stock solution (to obtain concentrations ranging from 8.00 to 18.0 μmol L^{-1}) into the cell containing the supporting electrolyte solution. The analytical curves were obtained via linear regression least-square fit data, by plotting the current peak versus the concentration of TBH in two different situations: (*i*) addition of standard solution only and (*ii*) addition of standard solution in the presence of the

matrix. Additionally, to study the accuracy (reproducibility and repeatability) of the proposed electroanalytical method and to check interferents, recovery experiments were accomplished using commercial Brazilian crystal and brown sugar samples.

The solutions used in this assay were prepared by adding 100.0 μL of the commercial crystal or brown sugar solutions (50.0 g L^{-1}) to the electrochemical cell, to obtain a final volume of 10.00 mL.

Results and Discussion

Electrochemical behavior of TBH

First, we established which supporting electrolyte solution was suitable to determine TBH by electrolysis in acidic, neutral, buffered, basic and non-aqueous solvent (HCl, CrCl$_3$, KCl, LiCl, KNO$_3$, ammonia buffer pH 9.4, carbonate buffer pH 10, NaOH, KOH and DMF/tetrabutylammonium tetrafluoroborate). Several studies show that the change in pH can dramatically affect the voltammetric response of some compounds. According to Weber[10] the TBH displays only basic properties, it remains with the molecular species with high pH levels. There were no voltammetric responses of TBH (oxidation or reduction) in acid or neutral solutions. Moreover, the anodic scans presented in Figure 2A revealed that cyclic voltammetry offered better responses in terms of current intensity. We then compared the responses of the tested supporting electrolyte solutions with respect to the anodic current intensity (I$_{pa}$) to evaluate and select the best conditions for further study of the electrochemical behavior of TBH in the positive potential range.

Figure 2B shown the comparison of the peak currents and peak potentials obtained for TBH in each situation of supporting electrolyte solution. NaOH and KOH gave similar responses, but a slightly smaller anodic peak potential (+1.16 V) than NaOH (+1.17 V), with lower standard deviation in the replicates. Hence, we decided to use KOH as supporting electrolyte during development of the proposed electrochemical methodology.

Fixing the parameters defined above, we tested three types of working electrodes: glassy carbon (GC), gold (Au) and boron-doped diamond (BDD) using CV. First we recorded the voltammogram for the blank; then, we conducted voltammetric readings at least three times, using 1.00 mmol L^{-1} of TBH. We chose to use the glassy carbon electrode to develop the methodology, because it provided sharper, lower values of peak potential, and better repeatability (RSD lower than 2%) between voltammetric measurements as compared with the other electrodes.
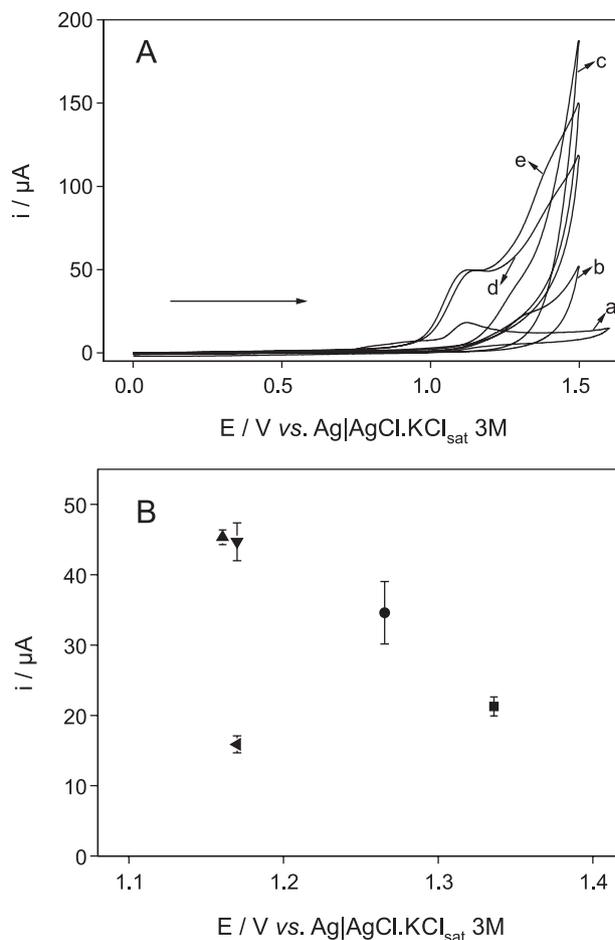

Figure 2. Cyclic voltammograms using (A) BF$_4$TBA/DMF (a), carbonate buffer (b), ammonia buffer (c), NaOH (d), and KOH (e) as the supporting electrolyte solution in the analysis of 1.00 mmol L^{-1} TBH and (B) relationship between the peak current and peak potential using BF$_4$TBA/DMF (◄), ammonia buffer (■), carbonate buffer (●), NaOH (▼), and KOH (▲). Conditions: ν = 50 mV s^{-1}.

We investigated the electrochemical behavior of TBH by CV on a glassy carbon electrode in the potential range +0.50 V to +1.35 V, using a KOH supporting electrolyte 0.10 mol L^{-1}. We obtained a well-defined anodic peak near +1.16 V using a scan rate (ν) of 50 mV s^{-1}. We attributed this peak to the oxidation of the herbicide (Figure 2). The absence of a cathodic peak in the reverse scan suggested that TBH oxidation involved an irreversible electron transfer or that the electrodic process was followed by coupled chemical reactions.

We verified linear relationships between the anodic current peak (I$_{pa}$) and the square root of the scan rate (ν$^{1/2}$) for the anodic peak obtained at +1.16 V. This indicated that the mass transport of the organic compound occurred by diffusion. If the applied potential is large enough, the electron transfer kinetics will increase to the point where the current is under diffusion control, and I$_{pa}$ is linear with ν$^{1/2}$, too even in the case of irreversible systems.

The current function ($i_p/v^{1/2}$) shown in Figure 3 remains virtually constant for all the anodic peaks registered under different scan rates, evidencing an irreversible electron transfer process (which means complicated charge transfer reactions).[11]
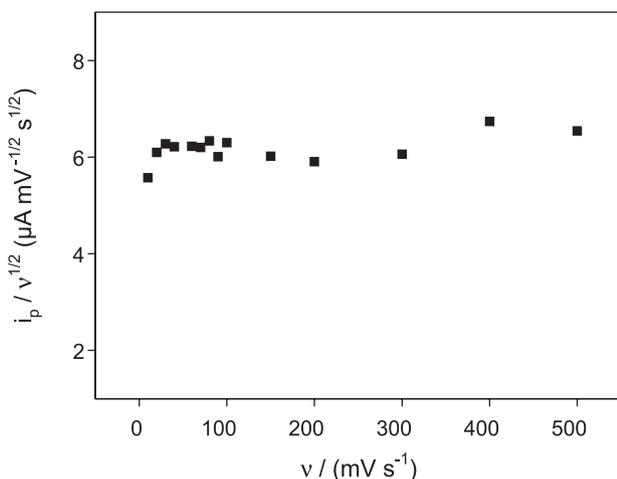

Figure 3. Variation of the current function ($i_p/v^{1/2}$) in relation to the potential scan rate for a solution of 1.00 mmol L^{-1} TBH in 0.10 mol L^{-1} KOH using GCE.

Still regarding CV, the peak current reduced significantly after the second sweep, but remained unchanged after the 10th cycle (with no visible anodic peak potential). This phenomenon may be due to adsorption of TBH or its redox products at the electrode surface, which culminates in fouling behavior of the GCE electrode. Therefore, for analytical purposes, we recorded the voltammogram corresponding to the first cycle of +1.16 V.

Finally, according to Brett[12] one can calculate the theoretical number of electrons transferred in the redox process using experimental DPV data and the equation below:

$$W_{1/2} = \frac{3.52RT}{nF} \qquad (1)$$

where n is the number of transferred electrons, F is the Faraday constant (96.485 C mol^{-1}), T is the temperature in Kelvin (298 K), R is the general perfect gas constant perfect (8.314 J K^{-1} mol^{-1}), and W$_{1/2}$ is the width at half peak height for the electrochemical process (obtained experimentally). Using this equation, we calculated n as 0.90. Hence, one electron was transferred from the analyte to the surface of the glassy carbon electrode during TBH oxidation.

TBH anodic mechanism

Brett,[12] Lund,[13] and Zanoni[14] studied the electrochemical behavior of some related anilide herbicides (*N*-substituted amides) by cyclic and square wave voltammetry using a glassy carbon electrode. The results we obtained here agree with the substitution pattern and number of substituents on the nitrogen atom of the amide, confirming that oxidation occurs at the nitrogen. Analysis of the results showed that the electronic nature and steric hindrance of the substituents, especially their orientations toward the heterocyclic ring, determined their effects on the oxidation potential.

Optimization of SWV and DPV conditions for TBH analysis

Figure 4 illustrates the evaluation of the optimal parameters for the SWV and DPV techniques regarding TBH analysis. Lower TBH concentrations led to sharper and better defined peak as well as smaller background current, as compared with cyclic and linear sweep voltammetry, which resulted in improved resolution. Hence, it was possible to apply these techniques to the quantitative analysis of TBH.
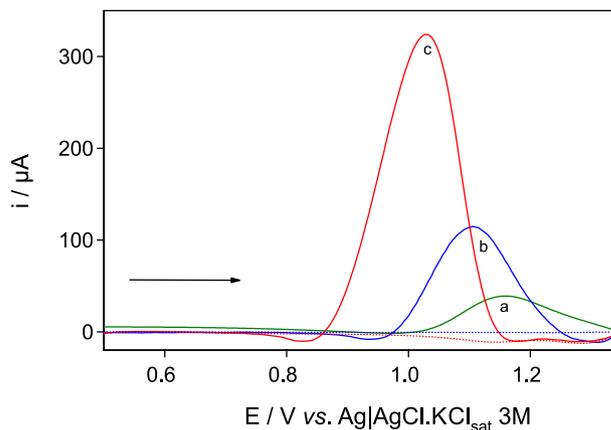

Figure 4. Comparison between the (a) LSV, (b) SWV, and (c) DPV experiments in optimized conditions for 1.00 mmol L^{-1} TBH at GCE in 0.10 mol L^{-1} KOH.

We initially tested three SWV parameters using GCE: amplitude, potential step, and frequency; we carried out all the measurements using univariate tests. First, we varied the amplitude in the 10-100 mV range, using constant frequency and potential step. An amplitude of 75 mV yielded peak potential with no deformation and did not increase significantly peak width. Next, we evaluated the frequency in the 10-100 Hz range, using an amplitude of 75 mV at a constant step potential of 5.0 mV. According to the GPES 4.9 software version, the potential step (or potential increment) together with the frequency defines the effective scan rate for the SWV mode. A frequency of 100 Hz provided the best voltammograms for TBH analysis: the peak currents increased up to 100 Hz and remained stable thereafter, with slight reduction in peak current and no deformation in the voltammetric shape of the TBH oxidation peak.

Finally, by fixing the amplitude at 75 mV and the frequency at 100 Hz, we investigated the effect of potential step increment in the 1-15 mV range. Potential steps greater than 5.0 mV resulted in constant TBH current peak height until 15 mV. Therefore, the optimal conditions for TBH analysis at the GCE were amplitude of 75 mV, frequency of 100 Hz, and potential step of 5.0 mV, which corresponded to an effective scan rate of 100 mV s^{-1}.

We also evaluated the DPV mode using GCE. The optimized parameters were scan rate of 25 mV s^{-1} (studied range: 2-25 mV s^{-1}), amplitude of 200 mV (studied range: 10-250 mV), and pulse time of 2 ms (studied range: 2-100 ms), in 0.10 mol L^{-1} KOH supporting electrolyte solution. Comparison of the voltammograms obtained for TBH oxidation using the different techniques showed that DPV furnished the best results regarding the intensity of the anodic current (Figure 5). Therefore, we registered several differential pulse voltammograms for TBH oxidation at different concentrations for quantification purposes.
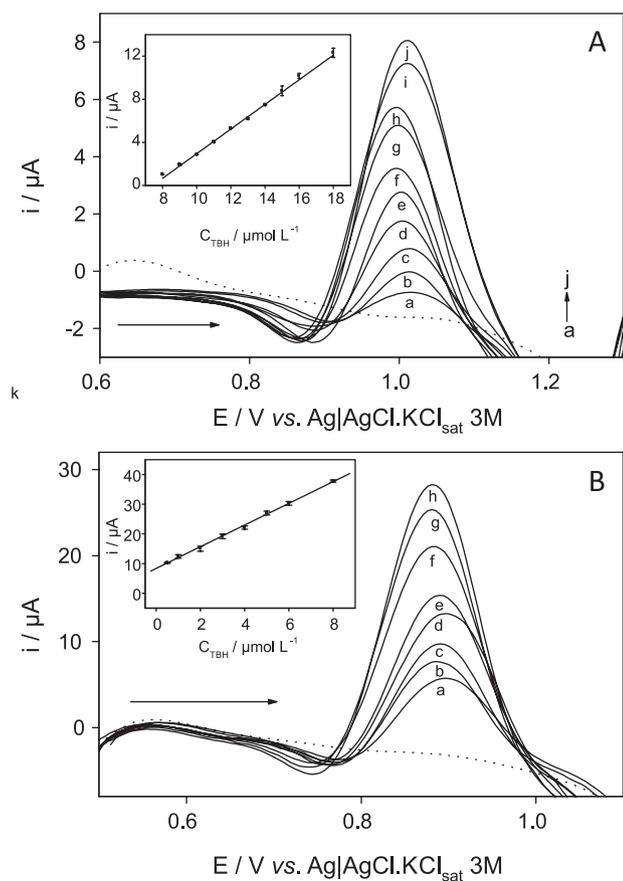
Voltammetric methodology and analytical curves

As already mentioned, in 1.00 mmol L^{-1} TBH standard solution, the current peak obtained in the DPV mode was higher than those achieved by SWV. The DPV technique yielded the best selectivity and sensitivity, as well as better-defined anodic peak at +1.03 V *versus* Ag|AgCl|KCl$_{sat}$ (Figure 5). We determined TBH by DPV (TBH concentrations ranging from 0.5 to 8.0 µmol L^{-1}) and SWV (TBH concentrations ranging from 8.0 to 18.0 µmol L^{-1}) under the optimized conditions, aiming at better electrochemical reproducibility, we then obtained analytical curves for this substance.

Figure 6 presents the analytical curves. We achieved good linear response in all the concentration range assessed analyte, which can be expressed according to the linear regression least-square fit equations listed in Table 2. We built six analytical curves using the electroanalytical method developed herein, as follows: (1) DPV and SWV
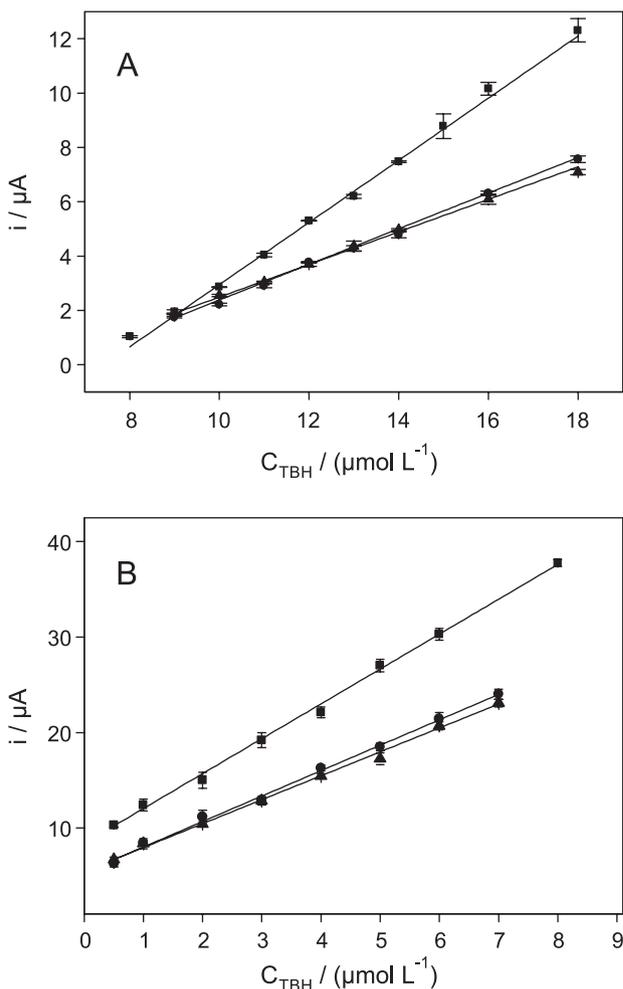
Figure 5. Analytical curves with no matrix effects for (A) SWV electrooxidation of TBH at different concentrations: blank (…); 8.0 (a), 9.0 (b), 10.0 (c), 11.0 (d), 12.0 (e), 13.0 (f), 14.0 (g), 15.0 (h), 16.0 (i), and 18.0 µmol L^{-1} (j) and (B) DPV electrooxidation of TBH at different concentrations: blank (…), 0.5 (a), 1.0 (b), 2.0 (c), 3.0 (d), 4.0 (e), 5.0 (f), 6.0 (g), and 8.0 µmol L^{-1} (h). Conditions: glassy carbon electrode in 0.10 mol L^{-1} KOH as supporting electrolyte.

Figure 6. Analytical curves obtained by (A) SWV in the absence of matrix (■), presence of brown sugar matrix (●), and presence of crystal sugar (▲). (B) DPV in the absence of matrix (■), presence of brown sugar matrix (●), and presence of crystal sugar matrix (▲) using glassy carbon electrode and 0.10 mol L^{-1} KOH as supporting electrolyte.

Table 2. Linear regression least-square fit data of the analytical curves for quantitative determination of TBH using the DPV and SWV methods

Technique	Matrix	Equation of the calibration curve	Correlation coefficient (r)
SWV	Absence	$I_p(A) = -8.493 \times 10^{-6} + 1.144\ C_{TBH}$ (µmol L^{-1})	0.994
	Crystal sugar	$I_p(A) = -3.501 \times 10^{-6} + 0.599\ C_{TBH}$ (µmol L^{-1})	0.998
	Brown sugar	$I_p(A) = -4.173 \times 10^{-6} + 0.656\ C_{TBH}$ (µmol L^{-1})	0.995
DPV	Absence	$I_p(A) = 8.383 \times 10^{-6} + 3.652\ C_{TBH}$ (µmol L^{-1})	0.999
	Crystal sugar	$I_p(A) = 5.434 \times 10^{-6} + 2.506\ C_{TBH}$ (µmol L^{-1})	0.999
	Brown sugar	$I_p(A) = 5.339 \times 10^{-6} + 2.663\ C_{TBH}$ (µmol L^{-1})	0.997

Table 3. Limits of detection and quantification and other analytical parameters

Technique	Matrix	b	$s_{y/x}$ ($\times 10^{-7}$)	LOD (µmol L^{-1})	LOD (mg L^{-1})	LOD (mg kg^{-1})	LOQ (µmol L^{-1})	LOQ (mg L^{-1})	LOQ (mg kg^{-1})
SWV	Absence	1.144	3.441	0.902	0.206	4.12	3.001	0.685	0.101
	Cristal sugar	0.599	3.559	1.782	0.407	3.89	5.941	1.356	0.175
	Brown sugar	0.656	3.329	1.522	0.347	1.71	5.075	1.158	0.133
DPV	Absence	3.653	1.447	0.119	0.027	0.902	0.396	0.090	0.009
	Cristal sugar	2.506	1.190	0.142	0.032	0.815	0.475	0.108	0.010
	Brown sugar	2.663	1.111	0.125	0.028	0.578	0.417	0.095	0.008

for no matrix, i.e., by using only the standard TBH in supporting electrolyte solution; (2) DPV and SWV for the crystal sugar sample; and (3) DPV and SWV for the brown sugar sample, to verify the effect on the electrochemical response of TBH. In all these assays we used the same concentration range of TBH employed to construct the first curve during the development of the analytical proposal.

We obtained better limits of detection (LOD) and quantification (LOQ) for all the analytical curves in the absence of the matrix (crystal or brown sugar) as compared with the results achieved in the presence of the matrix. Therefore, the intensity of the peak current was more sensitive to TBH concentration in the absence of sugar. The four analytical curves shown in Figure 6 evidence this slightly different sensitivity after fast comparison of the six slopes obtained for TBH analysis, as indicated in Table 2.

We determined the limits of detection (LOD) and quantification (LOQ) for TBH (Table 3) using the equation LOD = 3 × $s_{y/x}$ / b and LOQ = 10 × $s_{y/x}$ / b,[15] where $s_{y/x}$ and b are the estimated standard deviation of the blank (n = 12) and the slope of the analytical curve, respectively, with a 95% (K = 3) confidence level.[16] These results attest to the analytical potentiality of the DPV and SWV technique to determine TBH in commercial crystal and brown sugar samples that lie below the MRL established by the Brazilian legislation.

Finally, we obtained satisfactory precision for the developed technique: repeatability of the current peak and peak potential expressed as the percentage coefficient of variation (CV) of several independent determinations on three samples of each crystal and brown sugar matrixes in different concentration levels over the same day gave CV lower than 13% in all cases (triplicate experiments, at least), as shown in Table 4. Likewise, the reproducibility of CV of the current peak and peak potential as a result of eight independent determinations on two different samples over five days (in triplicate) was lower than 5%.[16] In terms of accuracy, expressed as relative error, CV was in the order of 3-6%.[16]

Determination of TBH in crystal and brown sugar samples by DPV and SWV

We analyzed commercial crystal and brown sugar samples under the same conditions as those employed to construct the analytical curves in 0.10 mol L^{-1} KOH using GCE. To assess the applicability of the proposed voltammetric-based method to the analysis of sugar samples, we studied three samples of crystal sugar and three samples of brown sugar. We recorded voltammograms in sample solutions containing the supporting electrolyte and various aliquots of the sugar matrix solutions and detected no anodic peak in any of the six samples, which demonstrated that detectable residues of TBH did not exist in these matrixes.

The procedures for TBH analysis followed the standard addition method carried out after addition of known amounts of TBH to various samples (four consecutive additions to a final concentration of 1.99, 2.99, 3.98 and

Table 4. Repeatability, expressed by the coefficient of variation (CV), for the various concentrations and several brown and crystal sugar samples analyzed in the apparent recovery test using DPV

Crystal sugar matrix	Concentration added / (μmol L^{-1})			
	1.99	2.99	3.98	4.97
	Coefficient of variation / %			
Sample A	2.09	0.53	2.53	3.52
Sample B	8.88	6.35	13.5	4.29
Sample C	5.25	13.8	7.89	7.51
Brown sugar matrix	Concentration added / (μmol L^{-1})			
	1.99	2.99	3.98	4.97
	Coefficient of variation / %			
Sample A	11.7	3.99	3.69	3.10
Sample B	11.1	6.26	7.81	5.93
Sample C	6.02	12.8	8.59	8.65

4.97 μmol L^{-1}) containing proposed contaminated samples. The results clearly demonstrated a linear relationship for all the samples evaluated by DPV. The electrochemical response was also satisfactory. We also accomplished a recovery test for TBH concentrations ranging of 1.99, 2.99, 3.98, and 4.97 μmol L^{-1} using DPV. The recoveries from different samples lay in the 84 to 113% range (Table 5). On the basis of these recovery experiments, we concluded that deviations in the recovery values were due to random errors, since values were higher and lower than expected, demonstrating that this methodology is not biased or does not incur systematic errors.

Table 5. Results of the recovery tests for TBH using different samples of crystal and brown sugar and DPV

Crystal sugar matrix	Concentration added / (μmol L^{-1})			
	1.99	2.99	3.98	4.97
	Recovery / %			
Sample A	89.5	90.8	109	93.9
Sample B	108	91.3	94.2	97.5
Sample C	103	104	92.3	91.4
Brown sugar matrix	Concentration added / (μmol L^{-1})			
	1.99	2.99	3.98	4.97
	Recovery / %			
Sample A	84.2	95.2	102	104
Sample B	114	102	97.7	99.9
Sample C	99.8	105	104	97.6

Under the optimum experimental conditions, we investigated the effects of potential interferents such as H_2SO_4 and NaCl on the voltammetric response of 2.0 μmol L^{-1} TBH . The experimental results showed that these compounds did not interfere in the voltammetric

signal of TBH for quantitative analysis purposes, since there was no attenuation or change in the voltammetric signal of the original TBH 2.0 μmol L^{-1} solution. We did not examine other interfering compounds because they do not remain in solution at pH values as high as that of the medium under study (e.g., iron, calcium, magnesium, and phosphate salts).

Conclusions

We successfully employed a methodology involving unmodified GCE to analyze TBH in a 0.10 mol L^{-1} KOH supporting electrolyte solution. The GCE carbon surface is highly sensitive to TBH oxidation, as characterized by the enhanced peak current, a probable result of amide bonds. Oxidation peak potential at about +1.03 V is suitable for analysis, and the peak current has a linear relationship with TBH concentrations over a certain range, under the three selected matrix conditions. This sensor can be used to determine the analyte at concentrations as low as 0.090 mg L^{-1} (0.396 μmol L^{-1}) by voltammetry, with good reproducibility and repeatability. The unmodified electrode can also be used to determine TBH in commercial crystal and brown sugar samples. The proposed method is accurate and fast; the reagents and apparatus are simple. In addition, the results obtained during TBH analysis in spiked sugar samples and data from the study about interferents demonstrate the potential applicability of this electroanalytical method for the analysis of real samples.

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