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LORRANNE MARINS-GONÇALVES

**Investigation of the electrochemical behavior and voltammetric
determination of furfural in food samples using a solid amalgam
electrode**

Patos de Minas - MG

2024

LORRANNE MARINS-GONÇALVES

**Investigation of the electrochemical behavior and voltammetric
determination of furfural in food samples using a solid amalgam
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Orientador(a): Djenaine de Souza

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“Aquele que tem um porquê para viver pode enfrentar quase todos os como.”

Friedrich Nietzsche

“Não sou nada, nunca serei nada, não posso querer ser nada. À parte isso, tenho em mim todos os sonhos do mundo.”

Fernando Pessoa

RESUMO

Contaminantes de processo são subprodutos químicos indesejáveis que surgem como consequência de etapas de processamento de alimentos, tais como aquecimento, secagem e fermentação. Esses contaminantes podem estar presentes em alimentos produzidos artesanalmente, e em alimentos industrializados, e sua presença é relacionada aos processos necessários e essenciais para o seu preparo, sendo consideravelmente difícil evitar sua presença, sendo então recomendado, ao menos, o controle de sua concentração na composição final. Embora nem todos os contaminantes do processo sejam tóxicos, sendo alguns, inclusive, considerados congêneres em certas concentrações, certos tipos foram associados a possíveis problemas de saúde. O furfural, também conhecido como 2-furaldeído, é um destes contaminante de processo, formado por reações de hidrólise de pentoses durante o processamento e armazenamento de alimentos. Sua toxicidade se deve à sua capacidade de absorção pela pele, cavidade oral, contato dérmico e inalação, tornando necessário controlar sua ingestão diária ou regular sua presença em alimentos. A exposição aguda ao furfural pode causar danos ao fígado e aos rins, bem como promover o desenvolvimento de tumores e mutações. A detecção de furfural em alimentos processados normalmente requer o uso de técnicas cromatográficas, que envolvem etapas de pré-concentração que podem ser demoradas e caras, além de gerar resíduos tóxicos. As técnicas eletroanalíticas surgiram como alternativas favoráveis devido aos seus resultados rápidos, simplicidade operacional e boa relação de custo-benefício. Estas técnicas apresentam alta sensibilidade e podem ser facilmente empregados para a determinação do furfural, mesmo em amostras complexas, como alimentos processados. Neste estudo específico, uma técnica eletroanalítica foi empregada para explorar o comportamento eletroquímico do furfural e determinar suas concentrações em alimentos industrializados. As reações de redução e oxidação sobre eletrodo de amálgama sólido de prata polida (p-AgSAE) foram investigadas usando voltametria de onda quadrada (SWV). O furfural exibiu dois picos voltamétricos para a reação de redução e três picos para reação de oxidação, ambos em uma solução básica. A intensidade e a posição desses picos foram influenciadas pela composição do eletrólito de suporte. As reações de oxidação apresentaram melhor reprodutibilidade e sensibilidade dos sinais, levando à otimização experimental e voltamétrica. As condições ideais incluíram o uso de um tampão Brinton-Robinson de $0,04 \text{ mol L}^{-1}$ (pH 12,0), uma frequência potencial de pulso de 100 s^{-1} , uma amplitude de pulso de 50 mV e um incremento de varredura de 2 mV. Dentro da faixa de potencial de -1,8 V a 0 V, o processo redox eletroquímico do furfural ocorreu em três etapas, resultando na formação rápida e reversível de um radical aniônico que poderia se envolver na dimerização e reações químicas subsequentes. Parâmetros analíticos como faixa de linearidade, equação das curvas analíticas, coeficientes de determinação, limites de detecção e quantificação, eficiência de recuperação e desvio padrão relativo para experimentos intradiários e interdiários foram avaliados usando modelagem matemática apropriada. Os dados indicaram que o procedimento proposto é adequado para a análise do furfural em amostras de alimentos industrializados. A metodologia proposta foi aplicada em amostras de bebidas alcoólicas (cachaça, conhaque, rum, whisky e aperitivos), bebidas não alcoólicas (sucos de uva e laranja, pasteurizados), méis de

diferentes floradas, papinhas infantis e derivados láteos (farinha láctea, leite fermentado e leite em pó).

Palavras-chave: Contaminantes de processo, furaldeído, voltametria de onda quadrada, eletrodo sólido de amálgama de prata, alimentos industrializados, produtos.

ABSTRACT

Process contaminants are undesirable chemical by-products that arise because of food processing steps such as heating, drying and fermentation. These contaminants can be present in both artisanal produced and industrialized foods, and their presence is related to the processes necessary and essential for their preparation, making it considerably difficult to avoid their presence, so it is recommended to at least control their concentration in the final composition. Although not all process contaminants are toxic, and some are even considered congeners in certain concentrations, certain types have been associated with possible health problems. Furfural, also known as 2-furaldehyde, is one such process contaminant, formed by pentose hydrolysis reactions during food processing and storage. Its toxicity is due to its ability to be absorbed through the skin, oral cavity, dermal contact, and inhalation, making it necessary to control its daily intake or regulate its presence in food. Acute exposure to furfural can cause liver and kidney damage, as well as promoting the development of tumors and mutations. Detecting furfural in processed foods usually requires the use of chromatographic techniques, which involve pre-concentration steps that can be time-consuming and expensive, as well as generating toxic residues. Electroanalytical methods have emerged as favorable alternatives due to their rapid results, operational simplicity, and good cost-benefit ratio. These methods have high sensitivity and can be easily used to determine furfural, even in complex samples such as processed foods. In this specific study, an electroanalytical technique was developed to explore the electrochemical behavior of furfural and determine its electroanalytical properties. The reduction and oxidation reactions at the polished solid silver amalgam electrode (p-AgSAE) were investigated using square wave voltammetry (SWV). Furfural exhibited two voltammetric peaks for reduction and three peaks for oxidation in a basic solution. The intensity and position of these peaks were influenced by the composition of the support electrolyte. The oxidation reactions showed the finest reproducibility and sensitivity of the signals, leading to experimental and voltammetric optimization. The optimum conditions included the use of a 0.04 mol L⁻¹ Britton-Robinson buffer (pH 12.0), a pulse potential frequency of 100 s⁻¹, a pulse amplitude of 50 mV and a scan increment of 2 mV. Within the potential range of -1.8 V to 0 V, the electrochemical redox process of furfural occurred in three steps, resulting in the rapid and reversible formation of an anion radical that could be involved in dimerization and subsequent chemical reactions. Analytical parameters such as linearity range, equation of analytical curves, coefficients of determination, detection and quantification limits, recovery efficiency and relative standard deviation for intraday and interday experiments were evaluated using appropriate mathematical modeling. The data indicated that the proposed procedure is suitable for the analysis of furfural in processed food samples. The proposed methodology was applied to samples of alcoholic beverages (cachaça, cognac, rum, whisky, and aperitifs soft drinks), non-alcoholic beverages (pasteurized grape and orange juices), honey from different flowers, baby food, and dairy products (dairy flour, fermented milk, and powdered milk).

Keywords: Process contaminants, furaldehyde, voltammetry, industrialized foods, beverages, baby foods.

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Capítulo II - Electroanalytical methodology in the detection of process contaminants: quantification of furfural in foodstuffs case

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DIAGRAMA CONCEITUAL DO TRABALHO

“Investigation of the electrochemical behavior and voltammetric determination of furfural in food samples using a solid amalgam electrode”

Por quê?

O furfural é um contaminante de processos e indicador de qualidade do processamento de alimentos comumente formado em etapas de processamento que envolvam calor, como operações de secagem e fermentação, armazenamento indevido ou prolongado, em alimentos ricos em pentoses e ácidos orgânicos, como é o caso de méis, cafés, bebidas alcóolicas, dentre outros alimentos. Como sua formação é intrínseca do processo pelo qual o alimentos é produzido, ou mesmo, da composição do alimento, como é o caso dos méis, a formação desse composto é quase inevitável, sendo necessário então o desenvolvimento de abordagens que possibilitem o controle de concentração da molécula em alimentos, uma vez que há instrumentos legislativos que exigem esse controle, e o furfural que pode ser considerado apenas um congênere do alimento, em altas concentrações pode acarretar em toxicidade moderada.

Quem já fez?

Diversos trabalhos foram propostos visando a detecção e controle de furfural em alimentos. Em sua grande maioria trabalhos que utilizam de metodologias com etapas de preparo de amostra morosas, de alto custo e com geração de resíduos tóxicos, como é o caso das metodologias baseadas em cromatografia. Na literatura, há propostas do uso da cromatografia para a análise de furfural em queijos [1], produtos lácteos [2], méis [3,4], bebidas [5], dentre outros alimentos. Além da cromatografia a literatura também mostra diversas aplicações recentes para a detecção de furfural em alimentos utilizando técnicas espectroscópicas, como por exemplo, para a análise em bebidas [6] e méis [7,8]. A eletroanalítica possui poucos estudos que se propuseram a detectar o furfural em alimentos. O trabalho mais recente aplicando a técnica se baseia na eletrocatalise e analisa

o furfural em diversas amostras, dentre elas, alguns alimentos como suco de laranja, leite bovino, méis, coquetéis a base de frutas, dentre outras amostras [9].

Hipóteses

- i. É possível detectar furfural empregando técnicas voltamétricas e um eletrodo sólido de amálgama de prata (p-AgSAE).
- ii. O furfural apresenta eletroatividade e possibilita sua detecção e estudo do comportamento eletroquímico usando o eletrodo p-AgSAE.
- iii. É possível realizar análises de furfural em alimentos usando metodologias eletroanalíticas e o eletrodo de trabalho p-AgSAE.

Metodologia experimental

- i. Avaliação de parâmetros experimentais, usando diferentes eletrólitos de suporte e concentrações de analitos para a otimização experimental
- ii. Avaliação de parâmetros da técnica voltamétrica voltametria de onda quadrada, através da variação da frequência, amplitude e incremento e observação do comportamento dos resultados experimentais
- iii. Determinação dos parâmetros analíticos
- iv. Aplicação da metodologia desenvolvida em amostras de alimentos

Respostas

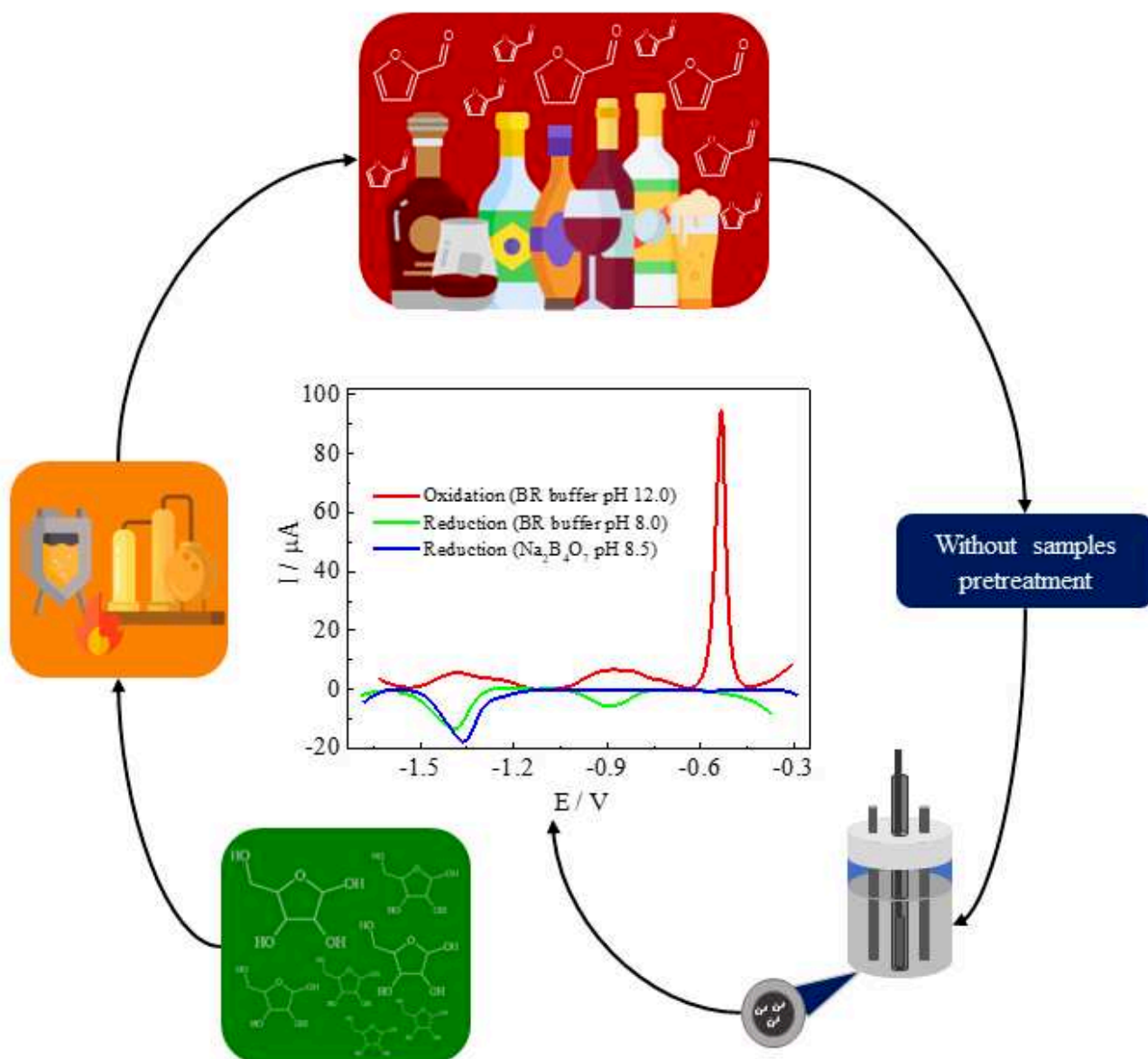
- i. O emprego das técnicas eletroanalíticas aliados ao uso do eletrodo de trabalho p-AgSAE possibilitaram a elucidação de reações eletroquímicas do furfural no sistema de estudo.
- ii. Um mecanismo de reação que beneficia a detecção de furfural no sistema otimizado neste estudo é proposto.

- iii.** Foi proposto o emprego de modelagem matemática para permitir a definição de parâmetros analíticos e quantificação de furfural no sistema otimizado, uma vez que se observou comportamento não-linear nas curvas de calibração e recuperação do analito.
- iv.** A metodologia desenvolvida foi aplicada para a determinação de furfural em alimentos complexos, com o mínimo de etapas de preparo de amostras e com parâmetros analíticos satisfatórios.

CAPÍTULO I

“Electroanalytical approach for micromolar determination of furfural in foods using solid amalgam electrode”

Graphical Abstract



Electroanalytical approach for micromolar determination of furfural in foods using solid amalgam electrode

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Highlights

- Furfural detection was proposed using a polished silver solid amalgam electrode
- In basic medium furfural, presented oxidation and reduction reactions on p-AgSAE
- Oxidation allowed in two little voltammetric peaks and a very well-defined peak
- Experimental and voltammetric parameters were optimized by square wave voltammetry
- A simple, fast and robustness detection was done in alcoholic beverage samples

Abstract

In this work it developed an electroanalytical procedure for electrochemical behavior study and electroanalytical determination of furfural, a chemical compound derived from the hydrolysis of pentoses during the heating, processing, and storage steps of the foodstuffs. For this, were evaluated reduction and oxidation reactions on polished silver solid amalgam electrode (p-AgSAE) using square wave voltammetry (SWV). In basic medium, furfural presented two voltammetric peaks for reduction (scan potential from 0 to -1.8 V_{vs} Ag/AgCl), already in oxidation (scan potential from -1.8 to 0 V_{vs} Ag/AgCl) was observed the presence of three voltammetric peaks, in both, the intensities and positions were related to the composition of supporting electrolyte. In oxidation reactions was observed better reproducibility and sensibility in the signals, and for this, the experimental and voltammetric optimization was performed, allowing as suitable conditions 0.04 mol L⁻¹ Britton-Robinson buffer (pH 12.0), with a pulse potential frequency of 100 s⁻¹, pulse amplitude of 50 mV, and scan increment of 2 mV. In the potential interval from -1.8 V to 0 V, the electrochemical redox process of furfural occurred in three steps. In the first one (peak 1), the furfural was oxidized to cation radical in a reversible redox process involving one electron transfer, followed by a chemical reaction with an acetate ion from supporting electrolyte. The product immediately losses one electron (peak 2) resulting in the reactant for the last electrochemical process (peak 3), that involved the reversible and quick anion radical formation, which can be involved in a dimerization reaction, in a following chemical reaction. Analytical parameters (linearity range, equation of the analytical curves, coefficients determination, detection and quantification limits, recovery efficiency, and relative standard deviation for intraday and interday experiments) were evaluated using suitable mathematical modeling. The data indicated that the proposed procedure is suitable for furfural analysis in alcoholic beverage samples.

Keywords

Furfural; Furfuraldehyde; Electroanalysis; Chemical Methods of Analysis; Voltammetric Sensors; Food Analysis.

1 Introduction

In the last two decades, modern electroanalytical techniques have been widely employed in the identification and quantification of organic and inorganic compounds with environmental, biological, and industrial interest, including food industries. Electroanalytical techniques involving potential pulse applications, such as differential pulse and square wave voltammetry, are powerful analytical tools due to result in high sensitivity, simplified operationality, and environmental friendliness due to low waste generation, as well as their rapid response, portability, and low cost, compared to other more traditional analytical techniques [10]. The applicability, versatility, and success of electroanalysis are mainly due to the wide variety of working electrodes from different materials, such as carbon-based [11], metal-based [12], and chemically modified electrodes [13], under which the electrochemical reaction occurs, allowing the study of the redox reaction of the analyte of interest from the consequent peak current response obtained, or from the interaction between the analyte and the electrode surface allowing changes in the voltammetric responses [14].

Due to their advantages, electroanalytical techniques have been applied in food analysis, including food chemical composition analysis, the detection of food additives [15], and mainly chemical contaminants such as pesticides [16], mycotoxins [17], and some compounds produced during the processing steps [18], which present high relevance due to be related to food safety to consumers and an indicator of adequate quality of processing of foodstuffs.

Among them, furfural, also known as 2-furaldehyde, shown in the insert of Figure 1, is a chemical compound derived from the hydrolysis of pentoses during the heating, processing, and storage steps of the foodstuffs [19–21]. Furthermore, furfural is one of the main products of carbohydrate hydrolysis reactions under heating, with the caramelization and "Maillard reaction" pathways being the most common to lead to the formation of furanic compounds in foodstuffs [20,22,23]. For this reason, it is often used as a quality indicator of various foodstuffs, such as honey [3,4], alcoholic beverages [5,6], coffee [24], bakery products [25,26], and dairy products [23,27,28].

Chemically, furfural is a molecule that has an intermediate state of oxidation between the organic acid and the corresponding alcohol [29], and for this, it can be used in the synthesis of several other compounds of interest to various chemical industrial segments, including the process industry for second-generation ethanol production,

biodiesel and the cellulose products industry [30–32]. Furthermore, furfural levels are also used as indicators of the proper functioning of equipment, such as power transformers, where high concentrations of furfural found in the insulating oil can indicate problems in the functioning of the system [33].

In addition to the importance of assessing adequate quality control in foodstuffs production, furfural detection is necessary because if ingested in high concentrations, furfural can be toxic to the human body, and acute exposure can result in liver and nephrological damage, as well as causing tumors and mutations [34]. As human contamination by furfural can originate mainly from ingesting foodstuffs, adequate identification and quantification of furfural levels in these samples is very important. For this, the foodstuffs regulatory institutions worldwide indicate the use of chromatographic techniques of separation and detection, which present excellent sensitivity and selectivity for the furan analysis, even in complex samples [35]. However, these techniques require pretreatment and pre-concentration steps, resulting in a more expensive and extended execution time analysis and generation of toxic waste.

Some recent studies have reported the use of electroanalytical techniques in the detection of furfural in sugarcane bagasse [36], insulating oil [33], wastewater, foodstuffs, and pharmaceutical syrups [9,37]. Although the suitable sensitivity obtained in these researches, commonly chemically modified electrodes or static mercury drop electrodes are used, which can cause difficulties in the reproducibility of the voltammetric signals and results in the generation of toxic waste, respectively.

Meanwhile, to our knowledge, no work showing the use of polished silver solid amalgam electrode (p-AgSAE) has been reported for the electroanalytical determination of any furaldehydes. For this reason, this work aimed to develop an electroanalytical procedure for investigating the electrochemical behavior and for the electroanalytical determination of furfural in alcoholic beverage samples using p-AgSAE allied to square wave voltammetry (SWV).

SWV is a pulse potential application technique that allows greater analytical sensitivity than other electroanalytical techniques and has been applied in various fields such as food [38], medicine [39,40], and the environment [41]. Furthermore, SWV data supplies information about the electrochemical behavior employing mathematical models developed considering different redox mechanisms, leading to valuable information concerning the characteristics of the charge transfer processes, chemical kinetics, and formation of intermediary compounds [42,43]. This information can be applied to

improvements in industrial processes, evaluation of stability and toxicity of intermediary and metabolites, and in optimization of the experimental and voltammetric parameters. Moreover, p-AgSAE presents electrochemical characteristics like traditional mercury-based electrodes, with considerable minimization in the toxic waste generated since the mercury is present in a metal alloy, providing a working electrode that is mechanically stable and long-lasting. Furthermore, the preparation of p-AgSAE has been previously described, indicating the suitable proportion between mercury and silver and the procedures for activation and renewal of the electrode surface via a simple electrochemical regeneration [44–47].

2 Experimental

2.1. Instrumentation and Reagents

All electroanalytical experiments were taken with a potentiostat from Metrohm Eco-Chemie μ Autolab model, controlled by a personal computer and the Nova 2.11 software. For the preparation of solutions, an analytical Shimadzu balance (model AUY 220) and a Gehaka pHmeter (model PG 2000) equipped with a 3.00 mol L⁻¹ Ag/AgCl/KCl-glass electrode was employed for mass measurements and pH adjustments, respectively. Processing of foodstuffs samples was performed in a Phoenix Luferco tube shaker vortex (model AP59) operated at 3.800 rotation per minute, and all solutions were prepared with water purified by a Gehaka model OS20 LX system (18.2 M Ω cm at 25 °C).

2.2. Reagents and solutions

All reactants employed presented analytical-reagent grade and were commercially acquired and used without further purification. The furfural standard, with a purity of 98.5%, was purchased from Sigma-Aldrich® (CAS 98-01-1). The stock solution of 2.96x10⁻² mol L⁻¹ of furfural was prepared daily by dissolving a suitable quantity of it in pure acetonitrile and subsequently used in the dilution to obtain the desired concentration, which was used in the SWV experiments. Both solutions were then stored in a dark flask and kept in a refrigerator to prevent degradation.

Britton-Robinson (BR), Clark-Lubs (CL), and tetraborate solutions were prepared and used as supporting electrolytes. A 0.04 mol L⁻¹ BR buffer was formulated with phosphoric acid, acetic acid, and boric acid to cover a broad pH range from 2.0 to 12.0, adjustment using a 1.00 mol L⁻¹ sodium hydroxide solution [48]. 0.01 mol L⁻¹ of CL buffer was prepared by combining boric acid and potassium chloride, according to the methodology reported by Bower and Bates [49], in which the pH adjustment was performed using a 1.00 mol L⁻¹ solution of sodium hydroxide chloride to cover a wide pH range from 8.0 to 10.0. Sodium tetraborate (Na₂B₄O₇) was prepared by dilution of decahydrate sodium tetraborate in deionized water to obtain solutions with different concentrations (0.01, 0.025, 0.05, 0.1 mol L⁻¹) to reach pHs from 8.0 to 9.0.

2.3. Electrochemical cell

The electrochemical cell consisted of a glass vessel containing three electrical conductors (working, auxiliary, and reference electrodes) and an ionic conductor composed of buffer solutions with predetermined pH values and furfural standard solution. The auxiliary electrode was a platinum wire, the reference electrode was an Ag/AgCl electrode in KCl 3.00 mol L⁻¹, and the working electrode was a custom-made p-AgSAE.

p-AgSAE was prepared as previously reported [45,47] by use of silver powder (99.9% purity, particle size: 5-9 μm, from Sigma-Aldrich®, São Paulo, Brazil) and mercury (99.99% purity, from Synth®, São Paulo, Brazil) in a ration the 30% of silver and 70% of mercury, considering the mass ratio (Ag/Hg; m/m). These quantities were weighed and rigorously homogenized until a mixture with the characteristics of a plastic mass was formed, which was inserted into a Pyrex® glass capillary tube with an inner diameter of about 0.30 mm and pressed in for full compaction and the formation of a well-defined disk. After 24 hours, a complete amalgamation occurred, and electric contact was provided by a copper wire affixed with silver adhesive to the paste. The resulting disks were polished with an emery paper (up to 2000 grit sandpaper, 3M), resulting in a compact surface, lacking mercury or mercury oxide, and offered advantages such as ease of surface renewal, low charge transfer resistance, and good reproducibility. Notably, it did not produce mercury waste during electrochemical analysis.

Before analysis, the p-AgSAE was polished with fine sandpaper and electrochemically activated by cyclic voltammetry experiments in a 0.20 mol L⁻¹ sodium chloride solution, with a potential scan from 0.0 to -2.2 V during 100 cycles. This pretreatment was essential to decrease the resistance in the charge transfer and increase the analytical responses. Moreover, between successive SWV experiments, a fixed potential of -2.20 V was applied for 30 seconds in the same working solution to remove the redox reaction products adsorbed under the p-AgSAE surface.

2.4. Experimental and voltammetric optimization

Preliminary electroactivity tests of furfural under p-AgSAE were performed in supporting electrolytes (BR, CL, and tetraborate) with pH values adjusted for a range from 8.0 to 10. For this, 10 mL of each electrolyte plus three successive additions of furfural standard solution, which resulted in concentrations of 1.10x10⁻⁴, 2.20x10⁻⁴, and 3.23x10⁻⁴ mol L⁻¹ of furfural, were added in the electrochemical cell. The solution was deoxygenated for 10 minutes, previously optimized in experiments for time of deoxygenation optimization. SWV experiments for each furfural concentration were performed in the scan potential from 0.0 V to -1.8 V using SWV with parameters of 100 s⁻¹ for frequency (f), -50 mV for pulse amplitude (a), and -2 mV for scan potential increment (ΔE_s).

After this, the BR buffer was selected as a supporting electrolyte, and SWV experiments were individually performed using solutions with pH values adjusted from 4.0 to 12. In addition to cathodic potential scans (from 0.0 to -1.8 V), anodic scan potentials (from -1.8 to 0.0 V) were evaluated using various pH values and similar voltammetric parameters previously employed.

Finally, the voltammetric parameters of SWV were individually optimized. For this, SWV experiments were carried out following the fixation of two voltammetric parameters and allowing the variation in the other. So, the f parameter was varied from 10 to 300 s⁻¹, a from -5 to -50 mV, and ΔE_s from -2 to -10 mV. All parameters were optimized adequately concerning the maximum peak current value (I_p) related to the sensitivity, and peak potential (E_p) and the maximum half-peak width ($W_{1/2}$) related to selectivity, and data were used in the electrochemical behavior study and voltammetric and experimental optimization.

2.5. Analytical parameters

Analytical curves were constructed in pure electrolyte using the standard addition method. The furfural concentration increased in the electrochemical cell, establishing a strong non-linear behavior between I_p and E_p values and demanding a non-linear model to study these dynamics.

So, as the calibration curve presented a sigmoidal profile the Hill equation [50–52] was used for the linearization and calculus of analytical parameters. Initially, all currents values were standardized for adequation in the imposed restrictions by the linear form of the Hill equation:

$$Y = \frac{I_p}{I_{pmax}} \quad \text{Equation 1}$$

where I_{pmax} is the maximum value of I_p and this is enough to put $0 < Y < 1$. The *cftool* Matlab toolbox from MatalabTM was used in the mathematical treatments with Hill equation and the non-linear least square method used was Levenberg-Marquardt [50,52,53] algorithm in the data linearization.

So, following the linearization data, all analytical parameters were calculated. Limits of detection (LOD) and quantification (LOQ) were determined according to International Union of Pure and Applied Chemistry (IUPAC) guidelines [54], in which the standard deviation (S_b) related to values of intersection of the previously linearized average analytical curve, and the slope of the analytical curves (s) were used, according to:

$$LOD = \frac{3.3S_b}{s} \quad \text{Equation 2}$$

$$LOQ = \frac{10S_b}{s} \quad \text{Equation 3}$$

The accuracy of the methodology was assessed through recovery experiments, adding in the sample a known amount of furfural followed by further additions of furfural standard solution to the supporting electrolytes. The recovery curves were constructed in triplicate, and the linear relationships were obtained similarly to those performed for analytical curves. Recovery efficiencies (%R) were calculated based on the percentage relationship between the added and found furfural concentrations following:

$$\%R = \frac{[furfural]_{found}}{[furfural]_{added}} \times 100 \quad \text{Equation 4}$$

The precision of the methodology was evaluated through reproducibility and repeatability experiments. Reproducibility was assessed using five standard furfural solutions on five days (*interday* precision), and repeatability was determined by ten successive measurements of a standard furfural solution and analyzing the resulting signals (*intraday* precision). Relative standard deviations (RSD) were calculated for repeatability measurements based on the percentage relationship between the standard deviation and the mean of the peak current values obtained in *intraday* and *interday* measurements.

2.6. Application of methodology

The efficiency of the proposed electroanalytical methodology was evaluated using SWV experiments in fermented and distilled alcoholic beverages, such as cognac, whiskeys, *cachaças*, rums, and aperitif-type drinks. These samples were obtained from the local markets in Patos de Minas, Minas Gerais, Brazil, and used to evaluate the influence of their composition in the furfural voltammograms.

So, an aliquot of 1 mL of each sample was added to the electrochemical cell containing 10 mL of supporting electrolyte, which were vigorously homogenized. After this, SWV experiments were performed without any sample pretreatment. Then, the standard addition method was applied similarly to the electrolyte to construct the recovery curves before fortification (artificially contaminated) and without fortification. Recovery curves were performed in triplicate, and mean recovery values were calculated using the same methodology effectuated in the pure electrolyte.

3 Results and discussion

3.1. Electroactivity tests

Preliminary electroactivity tests were performed to evaluate the redox potential of furfural under p-AgSAE in 2.81×10^{-4} mol L⁻¹ of furfural plus 0.025 mol L⁻¹ of sodium tetraborate with pH 8.0 or 0.02 mol L⁻¹ CL buffer with pH 10.0 or 0.04 mol L⁻¹ BR buffer with pH 12.0. For this, scan potential was carried out from 0.0 to -1.8 V (cathodic scan or reduction) and from -1.8 to 0.0 V (anodic scan or oxidation), as shown in Figure 1a and Figure 1b, respectively. The choices of these pH values were due to previous

literature reports that indicated that electrochemical reduction is favored in the basic medium [9,33,36,37].

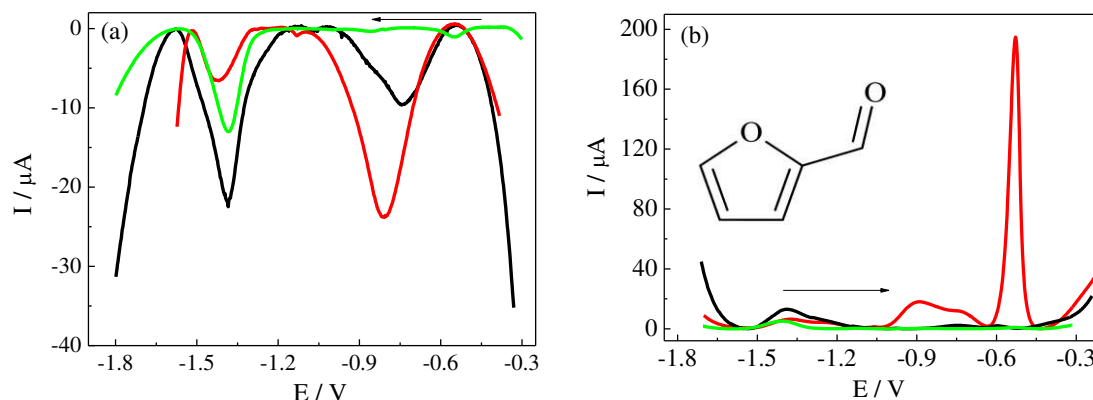


Figure 1: Square wave voltammograms for $2.81 \times 10^{-4} \text{ mol L}^{-1}$ furfural using a cathodic scan from 0.0 to -1.8 V (a) and anodic scan from -1.8 to 0.0 V (b). All experiments were performed under p-AgSAE using 0.04 mol L^{-1} of BR pH 12.0 (—), 0.02 mol L^{-1} of CL pH 8.0 (—), and 0.25 mol L^{-1} of sodium tetraborate (—) solutions. All experiments were performed using $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$, $\Delta E_s = 2 \text{ mV}$.

As shown in Figure 1a, furfural presented two well-defined voltammetric peaks in all buffer solutions, with I_p and E_p values dependent on the composition of the supporting electrolyte. In the BR buffer, the peaks appeared at close potential values, around -0.74 V and -1.38 V, like those observed in the medium of the CL buffer, where the peaks appeared around -0.81 V and -1.44 V. Already in the sodium tetraborate solution, a small peak occurred around -0.55 V; the most intense peak was around -1.38 V. On the other hand, in the cathodic scan potential, the less negative peak is called peak 1, and the more cathodic peak is called peak 2. In all supporting electrolytes, peak 2 presented values similar to those previously observed using solid electrodes, such as glassy carbon modified by nickel nanoparticles [36].

The voltammetric signals obtained on p-AgSAE were compared to similar literature reports in which other electrodes were employed to evaluate the furfural reduction reaction. Using a dropping mercury electrode (DME), Kumbhat *et al.* [37] observed only one well-defined voltammetric peak with potential shifting to negative values with the increase of pH. Shamsipur *et al.* [9] already used furfural and *in situ* derivatization with trimethylaminoacetohydrazide chloride (Girard's reagent T) to obtain furfural reduction waves around -1.4 V in a basic medium. Wang *et al.* [33] also observed

one well-defined voltammetric peak around -1.3 V in a basic medium considering the use of mercury film electrode deposited under glassy carbon. These comparisons with literature reports indicated the applicability of p-AgSAE in furfural detection without using chemically modified electrodes or without the waste toxic from mercury use.

Furthermore, literature reports did not find information about the oxidation reaction of furfural in the potential range evaluated in this work. However, as shown in Figure 1b, in CL buffer pH 10.0 and sodium tetraborate pH 8.0 the presence of two voltammetric peaks, with little I_p values and with E_p around -1.4 V were observed. Already, in the BR buffer pH 12.0 were found two little voltammetric peaks, around -1.4 (peak 1) and -0.89 V (peak 2) and a very well-defined peak at -0.53 V (peak 3), with higher I_p intensities and a very low $W_{1/2}$, indicating that these voltammetric peaks will be adequate for analytical purposes, due to high intensity and excellent potential values, that can results in excellent sensitivity and selectivity, respectively.

3.2. pH optimization

SWV experiments were performed using BR buffer solutions with pH values from 2.2 to 12.0. Cathodic and anodic scan potential and it was observed that in buffer solutions with pH lower than 3.5 the I_p resulted in very low values compared to those obtained at pH higher than 4.0, in both scan potential directions, indicating the system's low sensitivity in acid media and that under p-AgSAE the electrons transfer of furfural take place with non-protonated molecule.

Considering the cathodic scan potential for pH from 4.0 to 12.0, two well-defined voltammetric peaks, which the I_p , E_p , and $W_{1/2}$ presented values strongly influenced by pH of the medium, as shown in Figure 2a.

According to the relationships between I_p and E_p with pH, shown in Figure 2b and Figure 2c for peaks 1 and 2, respectively, both voltammetric peaks presented differences in the behavior with the pH increase. For peak 1, the I_p values for cathodic scan potential were followed by a linear increase until pH 8.0, after this, I_p decreased slightly. This behavior was similar to previous reports, which evaluated the reduction of furans using chemically modified electrodes [33,36] and platinum and gold electrodes [29,55]. Already for peak 2, I_p has a lower variation in the intensities than peak 1, with maximum values in pH 12, indicating that in the redox reaction at peak 2, there is no participation of the protonation process coupled to electron transfer [56].

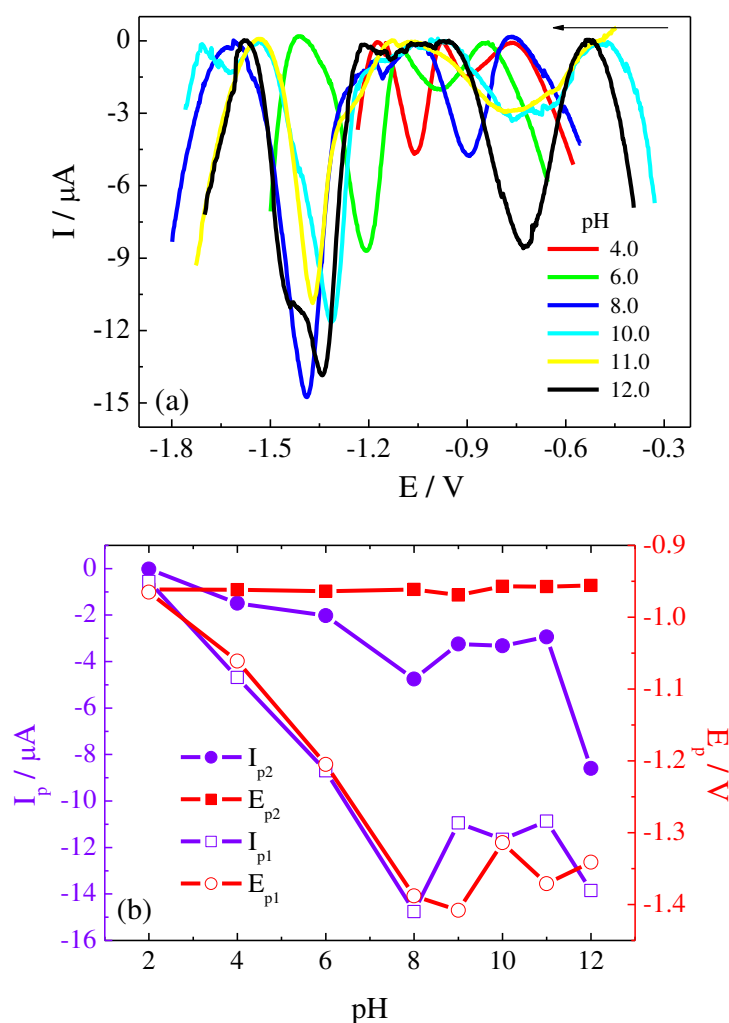


Figure 2: (a) Square wave voltammograms for 2.81×10^{-4} mol L⁻¹ of furfural reduction under p-AgSAE with scan potential from 0.0 to -1.8 V, using 0.04 mol L⁻¹ of BR with pH from 4.0 to 12.0, $f = 100$ s⁻¹, $a = -50$ mV and $\Delta E_s = 2$ mV. (b) Relationships between peak current and peak potential *versus* pH values, from SWV presented in “a” considering peak 1 and peak 2.

Analysis of E_p values indicated that it changed towards more cathodic potentials for peak 1, reaching the most cathodic potential at pH 8.0. After this, E_p values suffered insignificant alterations. Furthermore, the relationships between E_p and pH can be represented by a straight line, Equation 5, in which the slope value obtained (-68 mV) is very close to the theoretical value predicted by Nernst Equation (-60 mV) for redox reaction involving the same number of electrons and protons [57]. For peak 2, no linear relationship between E_p and pH was observed in interval pH from 4.0 to 12.0, confirming that the electron transfer in this peak did not involve the protons.

$$I_p = -0.811 (V) - 0.068pH \quad \text{Equation 5}$$

According to some literature reports, the reduction of furfural is very complex, allowing varied products such as alcohols, hydrocarbons, or pinacols [29], which can polymerize and block the electrode surface and hinder the reproducibility in the voltammetric responses. For this, the oxidation reaction was also evaluated.

For scan potential from -1.8 to 0.0 V, considering pH variation from 4.0 to 12.0, it was observed that the increase in pH was accompanied by a slight increase in more anodic peaks (peak 1 and peak 2) and an intense increase in the peak around in -0.5 V (peak 3), as shown in Figure 3 and in Figure S1, *Supplementary data*. I_p values did not present non-linear relationships with pH; the maximum values were observed for pH 12. E_p no given alterations considerable in the pH interval evaluated, indicating the absence of protonation steps coupled to electron transfer, as previously reported by Wang *et. al* in experiments using traditional mercury electrode [33].

Successive SWV experiments were performed in BR buffer solution plus furfural to evaluate a suitable reproducibility in the voltammetric responses for oxidation and reduction reactions and the effects of the adsorption of reactants and or products process in the p-AgSAE surface since these processes could be an essential factor in the sensitivity, selectivity, and reproducibility in the voltammetric signals.

This way, for oxidation reactions, it was observed that in the BR buffer pH 12.0, the voltammetric signals for both scan potentials presented more excellent reproducibility than other buffers and resulted in a voltammetric profile with suitable intensity, good resolution, and adequate reproducibility of signals, and for this was chosen for subsequent experiments. Peak 3 presented excellent reproducibility and I_p and E_p values with excellent $W_{1/2}$, allowing reasonable selectivity in voltammetric analysis. Due to lower E_p for electron transfer, this peak can be suitable for analytical applications, mainly for analysis in complex samples, such as foodstuffs.

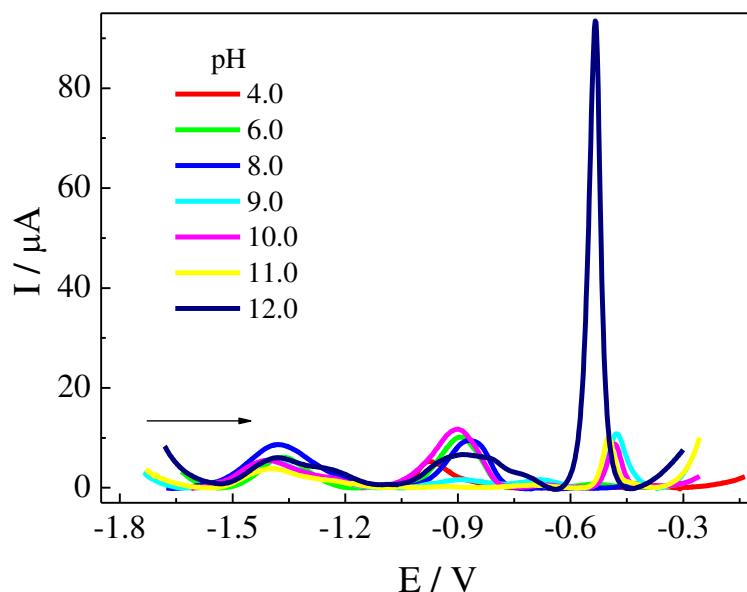


Figure 3: Square wave voltammograms for $2.81 \times 10^{-4} \text{ mol L}^{-1}$ of furfural oxidation under p-AgSAE with scan potential from -1.8 V to 0.0, using 0.04 mol L^{-1} of BR buffer solution with pH from 4.0 to 12.0, $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$ and $\Delta E_s = 2 \text{ mV}$.

However, for reduction reaction in BR buffer pH 8.0, successive experiments demonstrated a strong adsorption process of redox reaction products in the p-AgSAE surface, challenging the complete renovation of the electrodic surface, even applying electrochemical pretreatment. So, new experiments were evaluated in sodium tetraborate since this electrolyte has been reported as an excellent medium for voltammetric analysis under solid amalgam electrodes [58].

SWV experiments were performed using sodium tetraborate solution with concentrations from 0.01 to 0.1 mol L^{-1} (shown in Figure S2, *Supplementary data*), resulting in electrolytes with different ionic strengths since the pH control in this electrolyte is difficult due to disproportionation reactions. As discussed earlier, was observed the presence of a little voltammetric peak and a well-defined peak around -1.35 V with I_p values increasing with the increase in the sodium tetraborate concentration confirms that in high ionic strength occurred the maximum values of signal. As in 0.1 mol L^{-1} of sodium tetraborate, a concentration limited by the solubility of the salt, occurred a suitable reproducibility in the voltammetric signals due to minimization in the adsorption process, this medium could be selected for further voltammetric analysis.

3.3. Voltammetric optimization

After carrying out comparatives and successive analysis, considering parameters like reproducibility and sensibility in the voltammograms, the voltammetric profile from the use of BR buffer pH 12 for peak 3 (around -0.53 V) showed better results considering I_p , E_p and $W_{1/2}$, as shown in Figure 4. These voltammograms indicated that oxidation in BR buffer is more suitable for analytical purposes. The voltammetric parameters were optimized in these conditions considering the voltammetric peak at lower E_p .

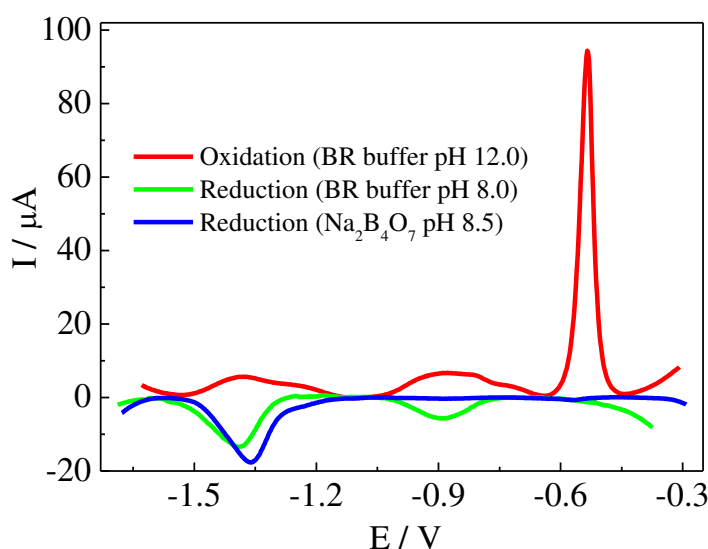


Figure 4: Square wave voltammograms for $2.81 \times 10^{-4} \text{ mol L}^{-1}$ furfural under p-AgSAE, considering oxidation in BR pH 12 (—), reduction in BR pH 8.0 (—), and reduction in $\text{Na}_2\text{B}_4\text{O}_7$ pH 8.5 (—) buffer solutions, with $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$ and $\Delta E_s = -2 \text{ mV}$.

To determine the optimum voltammetric conditions, the SWV parameters (f , a and ΔE_s) were individually evaluated, making it possible to obtain the best analytical signal in terms of I_p and E_p . Further, the relationships between each SWV parameter and I_p and E_p were used to estimate the electrochemical redox mechanism by employing the diagnostic criteria developed by Lovric and co-workers [42].

SWV responses obtained from experiments with ΔE_s varying from 2 to 10 mV indicated that in peak 1, no effects already for peak 2 and peak 3, a linear increase in the I_p occurred until 10 mV. However, due to limited points collected in the resultant voltammograms, the voltammetric profile at the maximum I_p present low definition, and for this, ΔE_s of 2 mV was set as a suitable value for analytical purposes.

The variation in the a parameter, from 5 to 50 mV, showed that for three voltammetric peaks, a linear increase in the I_p values occurred, accompanied by a little dislodgment in E_p values to cathodic potential and without considerable alterations in the $W_{1/2}$. For this, the parameter a was set as 50 mV in the further voltammetric experiments.

Finally, the parameter f was varied from 10 to 200 s^{-1} , the limit values permitted in the equipment and software used in this work. For peak 1 and 2, the increase in f promoted little effects in the I_p and E_p , compared to effects in peak 3, as shown in Figure 5. For peak 1 (around -1.4), no linear relationship between f and I_p values was observed, and the E_p remained practically constant, which, according to the theoretical model proposed by Lovrić and Komorsky-Lovrić [42,59] are indicative of reversible redox reaction with adsorption process of reactant and products. For peak 2 (around -0.9 V) occurred a linear increase in I_p when f was increased and E_p remained practically constant until 80 s^{-1} , after this, a dislodgment occurred for less anodic values, indicating that above these values, a change in redox mechanism occurs.

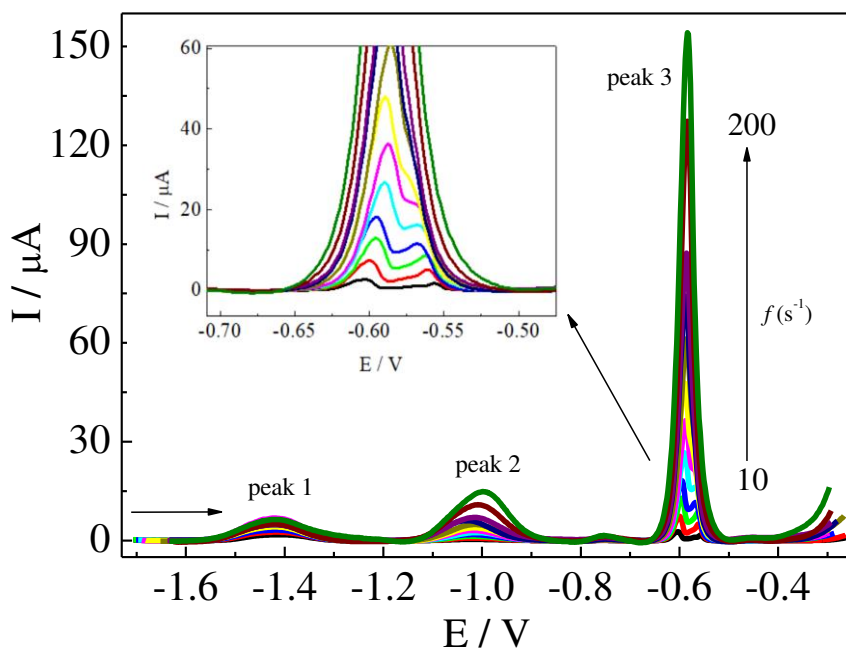


Figure 5: Square wave voltammograms for $2.81 \times 10^{-4} \text{ mol L}^{-1}$ furfural under p-AgSAE, considering oxidation in BR pH 12, with $a = -50 \text{ mV}$ and $\Delta E_s = -2 \text{ mV}$ and variation in the f values. Insert corresponds to ampliation in the peak 3.

In peak 3, two well-defined voltammetric peaks appeared for f values until 60 s^{-1} , which only became one peak for higher frequencies. According to SWV theory [42,59], these peaks in the resultant component are called as “*split in net responses*” and are a consequence of the current sampling procedure, and only occur in fast and reversible

redox reactions. This way, 100 s^{-1} was chosen as voltammetric parameters for subsequent experiments.

3.4. *Electrochemical mechanism*

Furfural is an organic molecule that can be reduced into furfurylic alcohol or oxidized into furoic acid [29], and for this, there are some reports in the literature indicating the reduction under mercury-based electrodes [9,37], chemically modified electrodes [33,36] and the oxidation using metal electrodes [55]. However, furfural can easily react with components of the medium (supporting electrolyte), producing intermediary compounds or reduce allowing a varied of products such as alcohols, hydrocarbons, and pinacols, or oxidize, resulting in secondary products coming from electro hydrodimerization [60]. These chemical or electrochemical possibilities can result in a loss of sensitivity, selectivity, and low reproducibility in the analytical methodology.

Despite the various literature reports involving the voltammetric determination of furfural, the oxidation reaction in the negative potential interval with three voltammetric peaks has not been reported, not even the use of p-AgSAE or other amalgam electrodes. For this, using the voltammetric data obtained in this work (SWV data with pH variation, data from voltammetric optimization, and the analysis of current components) jointly with electrochemical organic statements, we can propose a possible redox mechanism for the oxidation of furfural under p-AgSAE.

Forward, backward, and resultant components of current for SWV experiments were evaluated to observe the reversibility of the electron transfer, considering all voltammetric peaks, as shown in Figure 6, and better understand the redox process of peak 3. For peak 1, a redox reaction with reversible characteristics occurred due to the presence of forward and backward components with similar I_p values for both components. Furthermore, the analysis of relationships between E_p and the logarithm function of f , called the Lovrić equation [42,59], presented no linear range (not shown), confirming that the reaction in this peak is reversible with reactants and products adsorption, which explains the difficulty in reproducing this voltammetric peak.

Peak 2 appeared only in the forward component, indicating an irreversible redox reaction in this potential. Besides, as occurred a linear relationship between I_p and f values (not shown), following the theoretical model proposed by Lovrić and Komorsky-Lovrić [42,59], this behavior is typical of redox reactions involving reactant and/or product

adsorption as the rate-determining step. Furthermore, when f values were increased, can be observed that E_p varied linearly with the logarithmic value of the f according to the Lovrić equation:

$$\frac{\Delta E_p}{\Delta \log(f)} = \frac{-2.3RT}{\alpha nF} \quad \text{Equation 6}$$

where R is the gas constant; T is the temperature; α is the electron transfer coefficient; n is the number of electrons; and F is the Faraday constant. From equation 6, a plot of ΔE_p vs $\Delta \log(f)$ gives a straight-line curve. The slope of this curve, determined experimentally, was 60.08. Considering $\alpha = 0.5$, and substituting the known values of R , F , and T , the room temperature, into the right-hand side of Equation 6, and the known value of the slope, n was determined to be equal to 2.

Already for peak 3, it can be observed that the increase in f results in a decrease in the difference between the peak separations (ΔE_p) due to the potentials corresponding to the maxima of the two components are less separated and consequently causing the disappearance of the split in the net components. As the resultant current is the difference between the forward and backward component, occurs a significant increase in it, confirming the occurrence of a quick and reversible redox reaction followed by a fast surface chemical reaction, as described by criteria of diagnostic for redox reaction developed for SWV experiments [42,59]. These conclusions were confirmed by analysis of I_p in forward and backward components, where I_p forward is higher than I_p in backward components typical of reactions where α is higher than 0.5, indicating a quick reaction. From voltammograms in Figure 6, the α value was calculated according to:

$$\log\left(\frac{I_f}{I_b}\right) = 1.3205\alpha - 0.723 \quad \text{Equation 7}$$

where I_f and I_b are the I_p in forward and backward components, respectively. As the ΔE_p between split peaks is insensitive to the α , using $f = 100 \text{ s}^{-1}$ and the ration of the I_f/I_b calculated was as 1.088, the α value was calculated as 0.6. These results confirm a fast electron transfer with the formed product able to be quickly consumed by a subsequent chemical reaction.

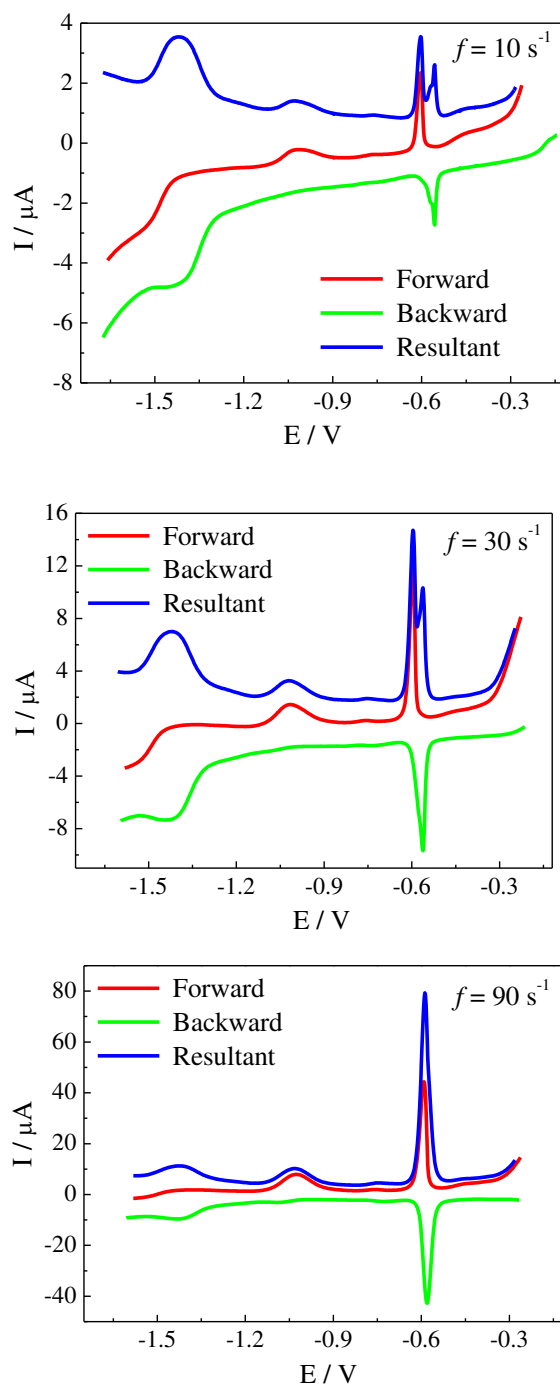


Figure 6: Square wave voltammograms for $2.81 \times 10^{-4} \text{ mol L}^{-1}$ furfural under p-AgSAE, considering oxidation in BR pH 12, with $a = -50 \text{ mV}$ and $\Delta E_s = -2 \text{ mV}$, showing the components of current (forward, backward, and resultant) for different f values.

Additionally, SWV experiments were performed using different values for initial potential scan, such as -1.8 (evaluation of three peaks), -1.0 (evaluation of peak 2 and peak 3), -0.8 (at the final potential for peak 2), and -0.7 V (only peak 3), to observe the interrelation between all voltammetric peaks, data not shown. So, it was observed that

peak 3 only occurs if the scan potential involves the interval of peak 1 and 2, indicating that the products in these peaks are the reactants for oxidation in peak 3.

So, using the abovementioned results jointly with information in literature reports and the organic electrochemical mechanism data, a possible mechanism for oxidation of furfural on p-AgSAE can be ascertained considering the reaction in three voltammetric peaks, as shown in Figure 7.

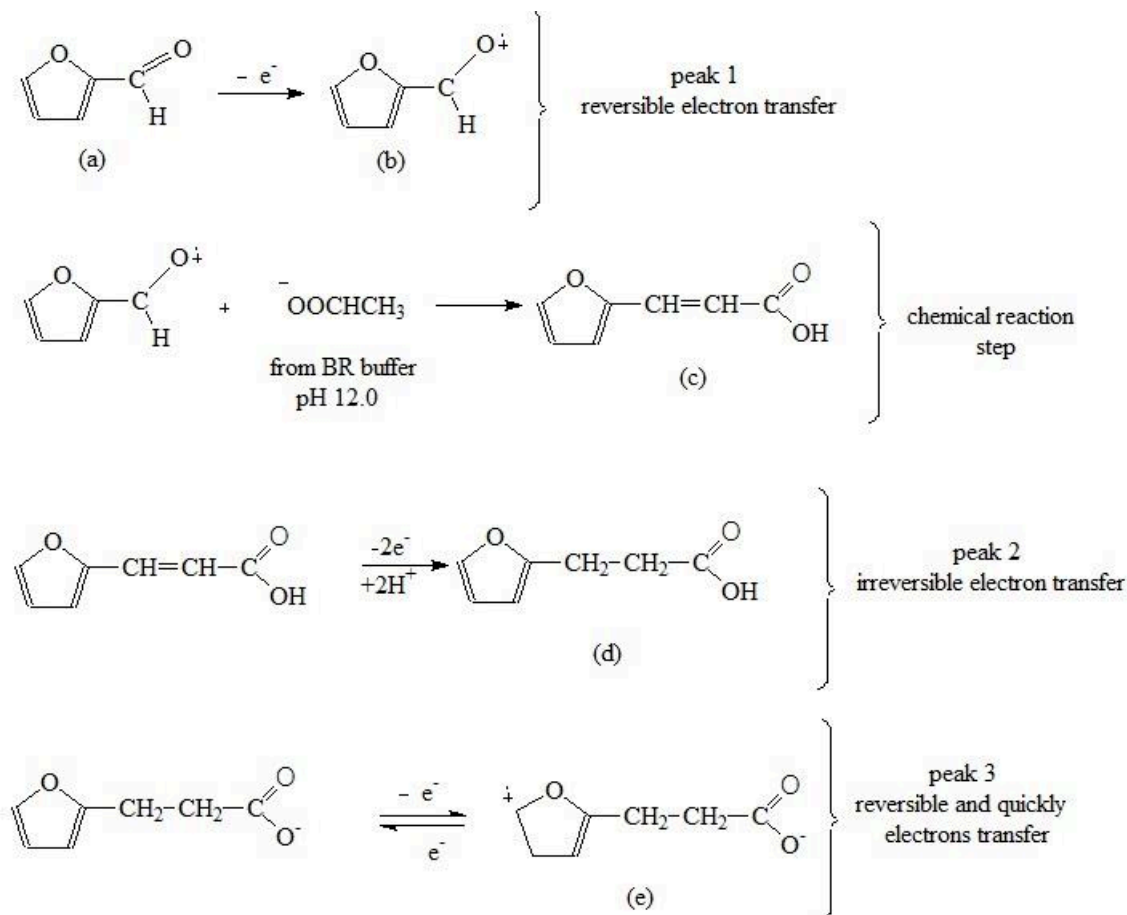


Figure 7: Proposed mechanism for oxidation process of furfural on the p-AgSAE.

Peak 1, at more negative potential values, is probably related to electron transfer reversible involving one electron, in which the furfural (a) is oxidized, resulting in a cation (b) that immediately reacts chemically with acetate anion (c, CH_3CHOO^-) from BR buffer pH 12.0 producing the furan derivative (d, or 3-(furan-2-yl)acrylic acid). These reactions justify the differences in the SWV signals in different pH values, shown in Figure 3, and previous reports about the chemical reactions of furans compounds [60].

Peak 2 probably corresponds to the irreversible oxidation of the chemical reaction product in the first step (3-(furan-2-yl)acrylic acid), involving two electrons that can be

related to the oxidation of a double bond resulting in the reactants (3-(furan-2-yl)propanoic acid) for the last oxidation observed on p-AgSAE, or peak 3.

Finally, in peak 3, as the 3-(furan-2-yl)propanoic acid molecule is predominantly in the deprotonated form, the negative charge can be dislocated, making it easier to the oxidation of double bond in the heterocyclic ring, resulting in a radical cation that, reversible and quickly can dimerize or adsorb under p-AgSAE surface.

3.5. Analytical parameters

Experimental (BR buffer pH 12.0) and voltammetric ($f = 100 \text{ s}^{-1}$, $a = 50 \text{ mV}$ and $\Delta E_s = 2 \text{ mV}$) parameters previously optimized were used in the evaluation of the analytical parameters for the determination of furfural. For this, aliquots from the stock solution were consecutively added to the electrochemical cell in a range concentration from 1.500×10^{-7} to $7.500 \times 10^{-6} \text{ mol L}^{-1}$ in potential range from -0.8 V to -0.3 V , which correspond to peak 3 due to its presented higher I_p and suitable E_p values for analytical purposes.

According to SWV responses showed in Figure 8a, I_p values increased when the furfural concentrations increased without any dislodgment in E_p or variation in $W_{1/2}$. In Figure 8b, no linear relationships between I_p and furfural concentrations were observed, and for this, these curves were built nine times, and the above-described behavior was obtained in all voltammetric results. So, three analytical curves were used to calculate the analytical parameters, and the average analytical curve obtained is shown in Figure 8b.

Analysis of the medium analytical curve, Figure 8b, and the comparison with isotherms profiles from Gilles studies [61–63] describe the relation between solute adsorption mechanisms at solid surfaces, indicating a characteristic H shape (high affinity). These curve types suggest that the solute has a high affinity by solid surfaces in low concentrations and is initially completely adsorbed. After this, the equilibrium is reached, and the plateau characterizes the final curve. Furthermore, the H shape is typical in a medium containing polymeric molecules and chemisorption of fatty acid and dyes or organic molecules that involves reactions with polymerization reactions inhibited by the absence of active adsorption sites, such as the possible proposed redox mechanism involving furfural redox reaction.

So, an adequate mathematical model for the linearization of the analytical curve was evaluated, and the most acceptable was the use of the Hill Equation as shown in

Figure 8c, which is commonly used to assess cooperativity [53]. In this phenomenon, organic molecules with multiple binding sites have an affinity for active sites for adsorption, and the binding sites for a ligand are increased, indicating positive cooperativity, or decreased, indicating negative cooperativity. So, in this work, the authors considered the aldehyde chemical group in the furfural as binding sites and the p-AgSAE surface as the active site or receptor of molecules.

The Hill Equation was used to quantify adsorption process of furfural under p-AgSAE in function of furfural concentration added, or dynamics of cooperativity or furfural adsorption process:

$$Y = \frac{Z^{n_h}}{k^{n_h} + Z^{n_h}} \quad \text{Equation 8}$$

where Y is the output responses or I_p and Z is the furfural concentration added, n_h is an interaction coefficient called by Hill coefficient, and k the half-saturation constant (quantification of the threshold concentration necessary for 50% response production).

The exponent n_h indicates the sigmoid curve steepness and is commonly used as a measure of the changing/transition of the process or saturation steps under p-AgSAE. The Hill coefficient can be interpreted as measuring the free energy of interaction between active sites. If $n_h = 1$ the Hill function reduces to the Michaelis-Menten model (when the affinity of both states towards the ligand is the same) which is a hyperbolic function and it is similar to Langmuir equation which analyzes the solute adsorption kinetic [50,62,63]. For $0 < n_h < 1$ the Hill Equation indicates negative cooperativity and $n_h > 1$ a positive cooperativity leading to a sigmoidal response [50,51,53], has showed in Figure S3, *Supplementary data*. Furthermore, the Hill model, Equation 8 and Figure S3, is a non-linear equation, and it presents a curve form like Giles isotherm classification as a Subgroup 2 (S, L, H) typical of cooperative adsorption process [63].

The parameters n_h and k can be obtained by linearized Hill Equation form given by:

$$Y (k^{n_h} + Z^{n_h}) = Z^{n_h} \quad \text{Equation 9}$$

or,

$$Y k^{n_h} = Z^{n_h} - Y Z^{n_h} \quad \text{Equation 10}$$

dividing by $k^{n_h}(1 - Y)$ it have:

$$\frac{Y}{1-Y} = \frac{Z^{n_h}}{k^{n_h}} \quad \text{Equation 11}$$

this leads to a linearized Hill equation form:

$$\ln\left(\frac{Y}{1-Y}\right) = n_h [\ln(Z) - \ln(k)] \quad \text{Equation 12}$$

The use of Equation 12 requires $Y > 0$ and $1 - Y > 0$, i.e., $0 < Y < 1$. This means that empirical data must be expressed in terms of a proportion conserving the dynamics of the process and reducing the scale, this methodology provides better conditions for the calculation of n_h and k parameters via numerical regression methods.

So, the analytical curve data were initially standarized, according to described in *Experimental Section*, in order to meet the limiting conditions for employ of Hill Equation. After standarization, in order to obtain the n_h and k coefficients, the experimental data, Figure 8b, were fitted to the polynomial model described as shown in Figure 8c. So, using the calculated coefficients, it were able to fit the data linearly by mathematically treating the normalized data according to Equation 12, as shown in Figure 8d.

Finally, based on the mathematical treatment, it was possible to obtain the linearized equation for the experimental data, as shown below:

$$\ln\left(\frac{Y}{1-Y}\right) = 1.227 [\ln(Z) - \ln(2.640 \times 10^{-7})] \quad \text{Equation 13}$$

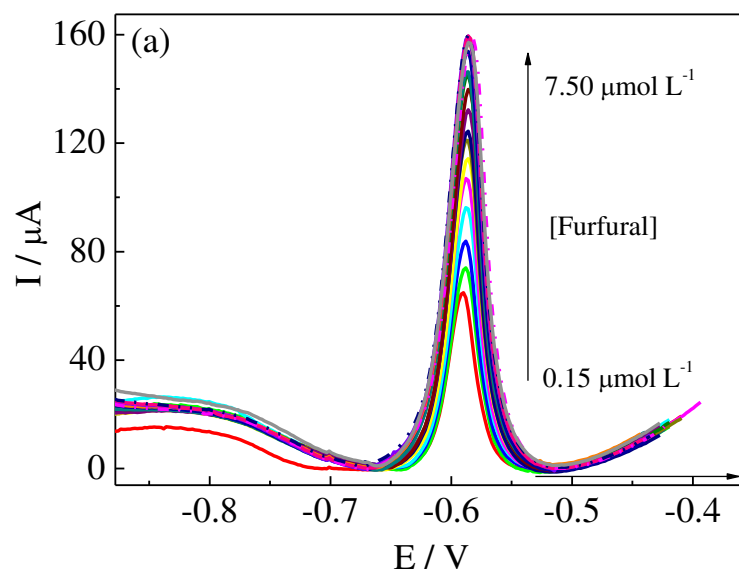
where, Y is values of I_p (μA) normalized and Z is concentration of furfural ($\mu mol L^{-1}$).

From the linearized analytical curve, the limit of detection (LOD) and limit of quantification (LOQ) for furfural oxidation on p-AgSAE was calculated, following previously optimized parameters and according to the methodology described in *Experimental Section*. Table 1 shows the LOD and LOQ calculated, the standard deviation of interception in the linearized analytical curve (S_b), the slope from the linearized analytical curve, the correlation between the linearized curve by mathematical model utilized and original analytical curves called the root mean squared error (RMSE) and by the correlation coefficient of the linearized curve (r).

Furthermore, Table 1 compares the analytical parameters obtained in this work with papers previously published using electroanalytical procedures allied with other electrodictics surfaces. So, the LOD and LOQ values obtained in our research are comparable to those obtained by Da Silva *et al.* [36], who used a carbon electrode

modified with silver nanoparticles, and higher than those obtained using static mercury drop electrode (SMDE) [9] and hanging mercury drop electrode (HMDE) [37], this is to be expected since these methodologies use highly conductive and easily renewable electrode surfaces. However, the proposed method in this work allows for intense waste reduction due to not using mercury-free. Also, it improves reproducibility and repeatability in the voltammetric signals due to the electrochemical regeneration of the electrode surface. Besides, the easily in the electrode preparation and the low cost of the materials used results in low cost analysis.

European legislation proposes controlling the concentration of furfural, along with other furans and alkylfurans, in cosmetics and foodstuffs, specifically coffee and jarred baby foods, and the maximum limits of quantification (LOQ) for these foods cannot exceed 20 $\mu\text{g/kg}$ for coffee and 5 $\mu\text{g/kg}$ for jarred baby foods. As far as we can tell, European legislation only regulates these foods regarding furan control [64]. According to Brazilian legislation, the sum of hydroxymethylfurfural and furfural in distilled alcoholic beverages must not exceed 5 mg/100 ml of anhydrous alcohol. Therefore, the proposed methodology is suitable for quality control of furfural in alcoholic beverages, since the LOD and LOQ obtained show a detection capacity below the minimum level required by the legislation [65].



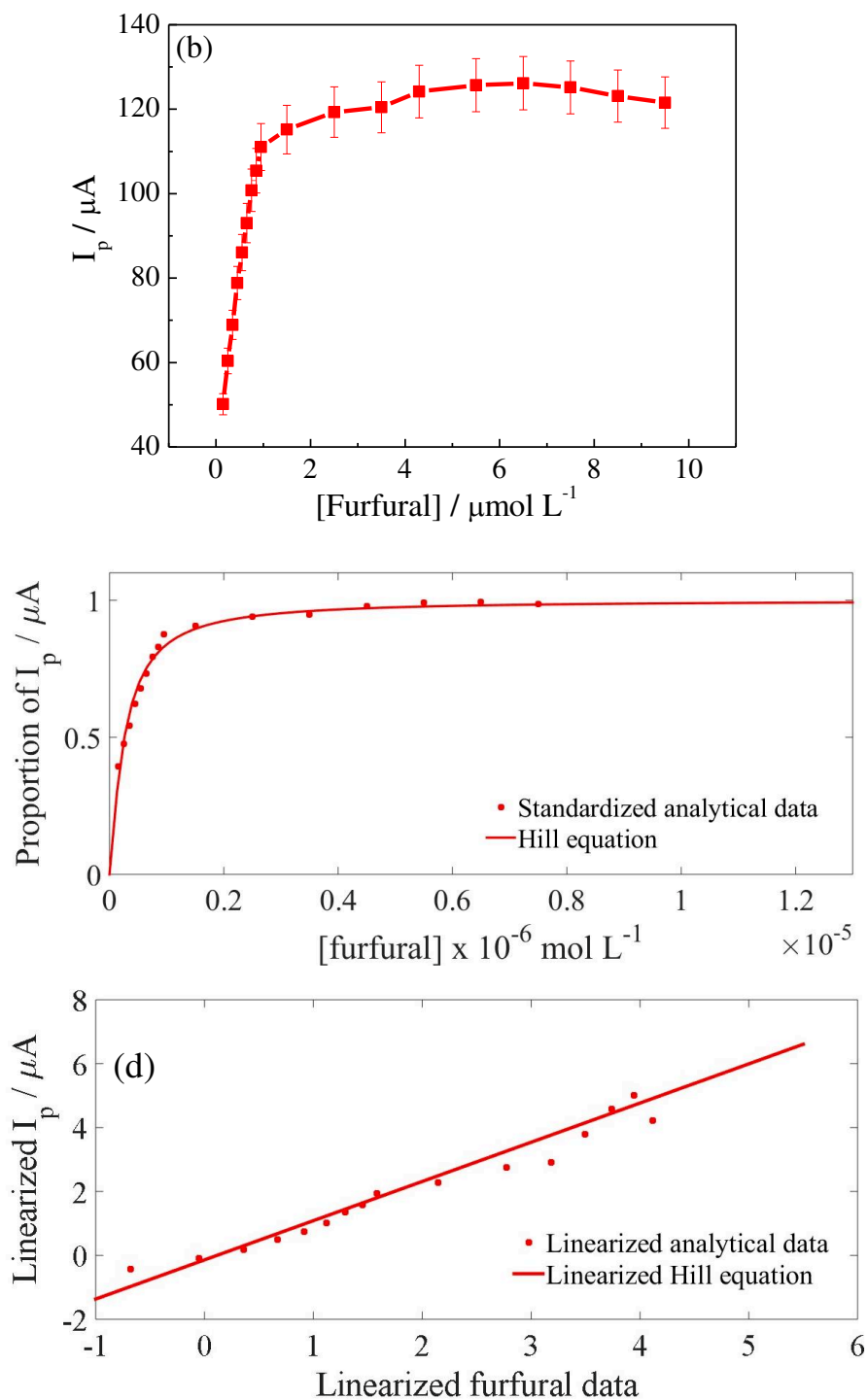


Figure 8: (a) Square wave voltammograms for oxidation of furfural using 0.04 mol L^{-1} BR pH 12 buffer solution on the p-AgSAE, $f = 150 \text{ s}^{-1}$, $a = 50 \text{ mV}$, $\Delta E_s = 2 \text{ mV}$, in the concentration range from $1.500 \times 10^{-7} \text{ mol L}^{-1}$ to $7.500 \times 10^{-6} \text{ mol L}^{-1}$. (b) Relationships between I_p and furfural concentration. (c) Normalization relationships between I_p and furfural concentration (d) Linearization

The precision of the proposed methodology was evaluated by repeatability experiments, in which the voltammetric experiments were made on five different days

(*interday* precision), and with seven successive experiments on the same day and in the same electrochemical cell (*intraday* precision), generating five and seven different measurements, respectively. In both, the experiments were performed with additions of furfural standard solution to obtain the final theoretical concentration at two different points of the linear range curve calibration, in $8.500 \times 10^{-7} \text{ mol L}^{-1}$ (point 1, low concentration) and $4.500 \times 10^{-6} \text{ mol L}^{-1}$ (point 2, high concentration), the first point being closest to the beginning of the detection range and the second nearest to the end. RSD values for *intraday* and *interday* precision were calculated as described in the *Experimental Section*, and the obtained values are shown in Table 1.

All experiments for precision evaluation were conducted using previously optimized experimental and voltammetric conditions, as previously described. The RSD calculated for interday experiments was 9.32% and 11.95% for low and high concentrations, respectively. RSD calculated for intraday experiments was already 10.31% and 4.98% for low and high concentrations, respectively. It is important to note that the RSD values calculated for *intraday* and *interday* experiments presented values around 10%, which is acceptable according to the Association of Official Analytical Chemists (AOAC) [35].

Additionally, as already discussed, furfural adsorption is favored in conditions of high concentration, allowing a blockage on the electrode surface and making it difficult to carry out experiments such as repeatability, making it difficult to renew the electrode. Similarly, even at low concentrations, mainly in the repeatability experiments, a linear increase in I_p was observed concerning the repetition of the experiment, which can also be explained by the adsorptive phenomenon that occurs in the system and the consequent difficulty in renewing the surface between measurements.

However, in general, even with the occurrence of such phenomena, the precision of the proposed methodology is adequate, and the values obtained for RSD to *interday* and *intraday* experiments are suitable for analytical applications. Thus, the use of p-AgSAE combined with the SWV technique provided an appropriate alternative method for the analytical determination of furfural due to the possibility of its use in simple and reliable determination at different range concentrations.

Table 1: Analytical parameters calculated for proposed methodology and the respective comparison with others research using other types of working electrodes and other voltammetric techniques for determination of furfural.

Parameter	This Work	HDME [37]	SMDE[9]	PtE[55]	GCE/NiNPs[36]	GCE/Hg[33]
Voltammetric technique	SWV	LSV	DPV	DAP	SWV	DPV
Range concentration	$0.150 - 7.50 \times 10^{-6}$ mol L ⁻¹	$5.0 \times 10^{-4} - 0.001$ mg L ⁻¹	$400 \text{ ng g}^{-1} - 318 \text{ } \mu\text{g g}^{-1}$	$5.00 - 40.0 \text{ g L}^{-1}$	$1.00 \times 10^{-5} - 3.00 \times 10^{-4}$ mol L ⁻¹	$0 - 10.0 \text{ } \mu\text{g g}^{-1}$
R	0.979	N.R.	N.R.	N.R.	0.999	0.998
RMSE	0.029	N.A.	N.A.	N.A.	N.A.	N.A.
Intercept	18.29 μA	N.R.	N.R.	N.R.	$-3.5 \times 10^{-6} \text{ } \mu\text{A}$	N.R.
S	1.22 $\mu\text{A /mol L}^{-1}$	N.R.	0.168 nA/ ng g ⁻¹	N.R.	$-2.4 \times 10^{-2} \text{ A/mol L}^{-1}$	N.R.
S_b	$6.50 \times 10^{-7} \text{ mol L}^{-1}$	N.R.	N.R.	N.R.	N.R.	N.R.
LOD	4.50 $\mu\text{mol L}^{-1}$	5.00 nmol L ⁻¹	10.4 nmol g ⁻¹	$1.00 \times 10^{-2} \text{ mol L}^{-1}$	$6.90 \times 10^{-6} \text{ mol L}^{-1}$	$5.20 \times 10^{-8} \text{ mol g}^{-1}$
LOQ	13.64 $\mu\text{mol L}^{-1}$	N.R.	N.R.	N.R.	$2.1 \times 10^{-5} \text{ mol L}^{-1}$	N.R.
Repeatability interday (%)	10.31 for point 1	N.R.	N.R.	N.R.	4.10	N.R.
	4.98 for point 2					

Repeatability intraday (%)	9.32 for point 1	N.R.	2.8	N.R.	N.R.	N.R.
	11.95 for point 2					
Recovery (%)	97.0% (electrolyte)	99.00%	N.A.	N.R.	103.7%	215.0%

N.R.: Not reported. N.A.: Not applied. HDME: Hanging dropping mercury electrode. SMDE: Static mercury drop electrode. GCE/NiNPs: Glass carbon electrode modified with nanoparticles of nickel. GCE/Hg: Glass carbon electrode modified with electro-deposition of the mercury. PtE: Platinum electrode. LSV: Linear sweep voltammetric. DPV: Pulse differential voltammetry. SWV: Square wave voltammetry. PAD: Pulse amperometric detection

The efficiency of the proposed methodology was evaluated by recovery experiments in pure electrolytes using the SWV and p-AgSAE under optimized voltammetric parameters. For this, 0.04 mol L⁻¹ of BR buffer pH 12.0 was artificially spiked with 7.500x10⁻⁷ mol L⁻¹ of furfural (called as added concentration, [furfural]_{added}) followed by standard addition method for recovery curves preparation. The voltammetric responses were used in mathematical modeling, similar to those effectuated in analytical curves, to evaluate the percentage recovered, as described in the *Experimental Section*. For this, the graphical method was used, in which the abscissa refers to the concentration of the furfural standard added to the electrolyte, and when the recovery curve was extrapolated to this axis, the percentage recovered was obtained (called as found concentration, [furfural]_{found}). The average recovery value was 99.0%, a value in a suitable range for analytical applications, with acceptable values from 70 to 130% [66], indicating that the proposed methodology can be successfully applied in the furfural determination with good efficiency and accuracy.

3.6. Application in beverages

Furfural is a process contaminant and congener whose formation is linked to the conditions inherent in the production process of fermented and distilled beverages. Therefore, the recovery experiments in beverage samples was performed to evaluate the applicability of the proposed methodology and to observe the influence of the composition of these beverages on the voltammetric responses under p-AgSAE. Thus, recovery curves were built in *Cachaça*, rum, cognac, whisky, and aperitif type, all acquired in supermarkets in Patos de Minas city, Minas Gerais state, Brazil. All beverages were analyzed considering three commercial samples from different origins or trademarks. In each sample, the recovery curves were built in triplicate, and the average recovery curve underwent the same mathematical treatment effectuated in the analytical curve.

So, as previously described in the *Experimental Section*, a 1 mL aliquot of each beverage was mixed with 10 mL of electrolyte and enriched with 6.500x10⁻⁷ mol L⁻¹ of furfural, followed by a standard addition of furfural for recovery curve build. Three trademarks were used for each type of beverage, and all experiments were performed in triplicate, and without pretreatment of the samples, only dilution was made. The recovery

efficiencies were calculated as performed for pure electrolytes, and the values are shown in Table 2.

Table 2: Furfural concentration recovered in different types of beverages and recovery calculated using medium values from three recovery curves.

Sample		[Furfural] _{recovered} x10 ⁻⁷ mol L ⁻¹	Recovery %
Cachaça	Trademark 1	3.775 (±0.7549)	58.08
	Trademark 2	4.007 (±0.0451)	61.65
	Trademark 3	3.806 (±0.0976)	58.55
Rum	Trademark 1	4.943 (±0.4667)	76.05
	Trademark 2	4.152 (±0.3194)	63.88
	Trademark 3	4.404 (±0.1942)	67.75
Cognac	Trademark 1	5.685 (±0.4770)	87.46
	Trademark 2	6.920 (±0.6367)	106.46
	Trademark 3	7.253 (±0.0427)	111.58
Whisky	Trademark 1	6.132 (±0.5675)	94.34
	Trademark 2	6.581 (±0.3193)	101.25
	Trademark 3	6.247 (±0.094)	96.11
Aperitif type	Trademark 1	6.149 (±0.4771)	95
	Trademark 2	5.817 (±0.6192)	89.49
	Trademark 3	5.964 (±0.0371)	91.75

A search in previous papers indicated the use of electroanalytical techniques in the detection of furfural in beverage samples. Kumbhat et al. [37] used HMDE and LSV developed a methodology for low determination of furfural and other aldehydes in liquor samples. However, furfural was not found in evaluated samples, or by its absence or low sensitivity in the proposed methodology. For this, our procedure was initially applied using beverages artificially spiked by furfural, followed by standard addition method, in order to evaluate the interference of samples components in the recovered concentrations. So, the authors observed that the some of the recovery values found in this study complied with the guidelines for analyte recovery as a function of concentration for trace analysis (> 0.0000001) according to AOAC [67], with values ranging from 40 - 120%.

It is worth noting that no analysis of analyte interferents in the sample was carried out. This is an exciting analysis for future studies, especially considering other furans such as hydroxymethylfurfural [2] and even furfural reaction products [29].

4 Conclusions

This work demonstrated a simple, low cost and selective procedure for determining micromolar levels of furfural using p-AgSAE allied with the analytical advantages of SWV. The p-AgSAE showed a higher sensitivity of results for the detection of furfural were compared with the modified electrodes. The analytical parameters were calculated following suitable results for LOD and LOQ without preliminary pre-concentration steps. The electrochemical system described in this work showed a typical adsorption behavior, requiring a mathematical treatment to develop a methodology capable of determining furfural in real samples of alcoholic beverages.

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Disclosure statement

This article does not contain any studies with human or animal subjects. No potential conflict of interest was reported by the authors.

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Supplementary Data

for

Electroanalytical approach for micromolar determination of furfural in foods using solid amalgam electrode

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Figure S1: Square wave voltammograms for 2.81×10^{-4} mol L⁻¹ of furfural oxidation under p-AgSAE with scan potential from -1.8 V to 0.0, using 0.04 mol L⁻¹ of BR with pH from 4.0 to 12.0, $f = 100$ s⁻¹, $a = -50$ mV and $\Delta E_s = 2$ mV. (b) and (c) Relationships between peak current and peak potential versus pH values, from SWV presented in “a” considering peak 1 and peak 2, respectively. Page 1

Figure S2: Square wave voltammograms for 2.81×10^{-4} mol L⁻¹ furfural reduction under p-AgSAE using 0.01 (—), 0.025 (—), 0.05 (—) and 0.1 mol L⁻¹ (—) of Na₂B₄O₇ solutions under p-AgSAE, with $f = 100$ s⁻¹, $a = -50$ mV and $\Delta E_s = -2$ mV. Insert show the relationships between I_p and E_p for peak 2. Page 2

Figure 3S: Demonstration of how the exponent n_h or Hill coefficient indicates the measure of change/transition of the process studied, considering the different values and ranges of values that n_h can have. Page 3

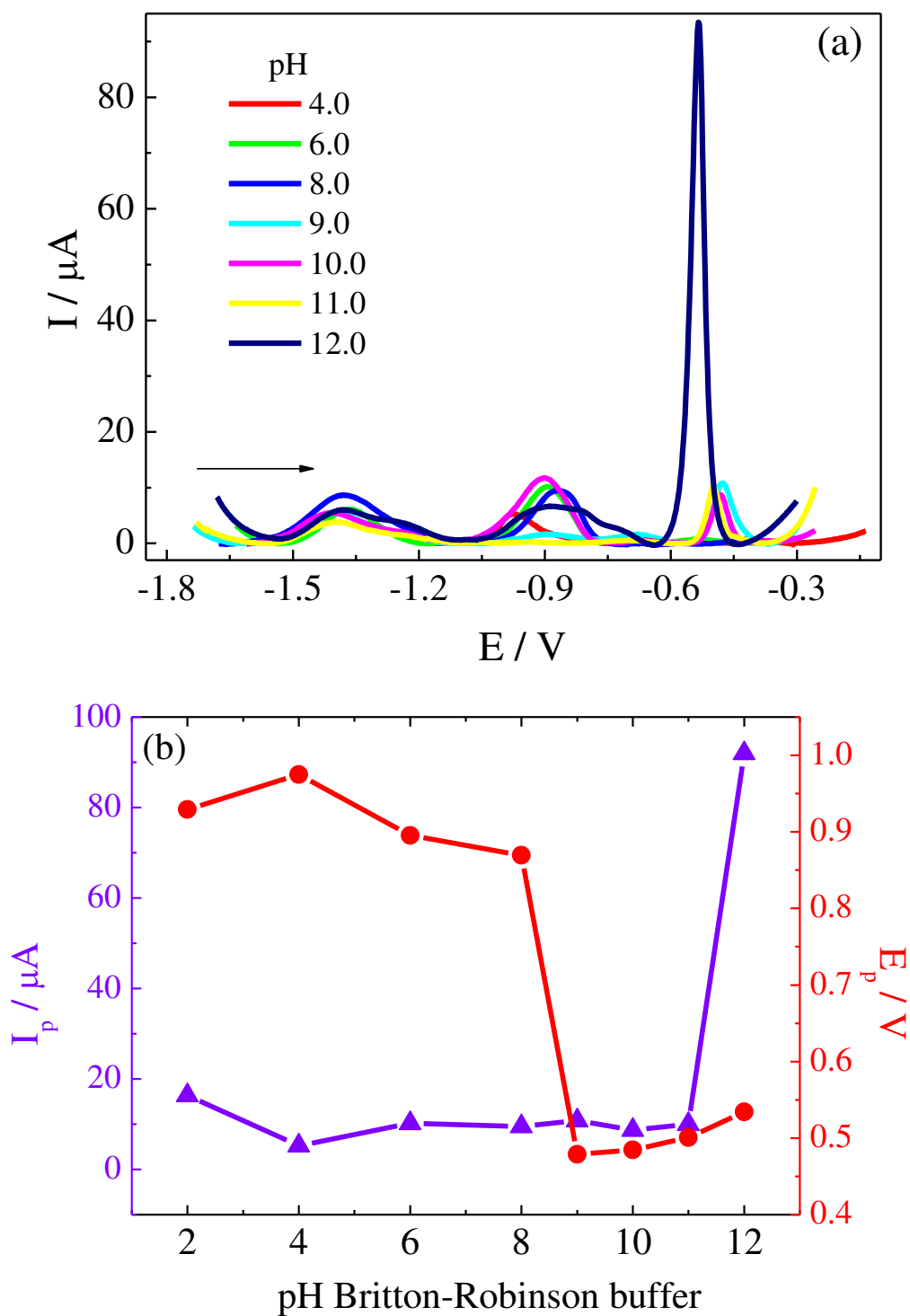


Figure S1: Square wave voltammograms for 2.81x10⁻⁴ mol L⁻¹ of furfural oxidation under p-AgSAE with scan potential from -1.8 V to 0.0, using 0.04 mol L⁻¹ of BR with pH from 4.0 to 12.0, $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$ and $\Delta E_s = 2 \text{ mV}$. (b) and (c) Relationships between peak current and peak potential versus pH values, from SWV presented in “a” considering peak 1 and peak 2, respectively.

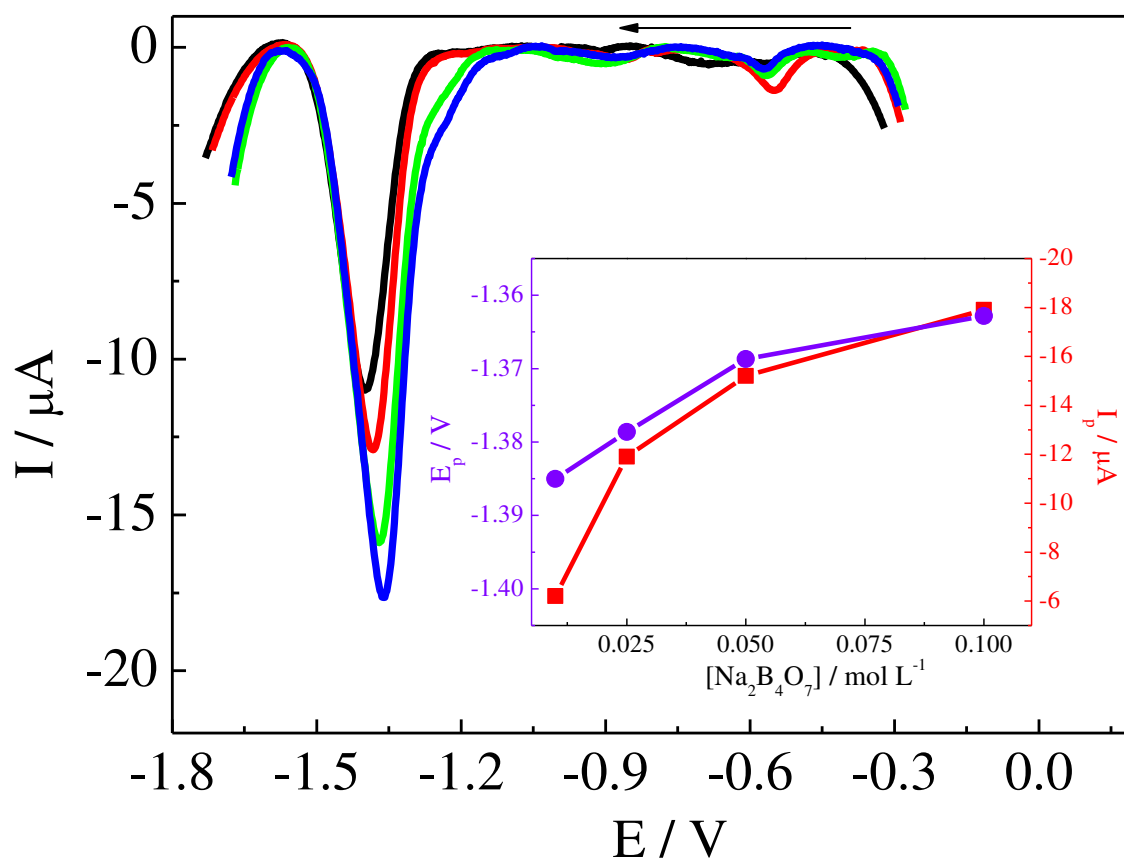


Figure S2: Square wave voltammograms for 2.81×10^{-4} mol L⁻¹ furfural reduction under p-AgSAE using 0.01 (—), 0.025 (—), 0.05 (—) and 0.1 mol L⁻¹ (—) of Na₂B₄O₇ solutions under p-AgSAE, with $f = 100$ s⁻¹, $a = -50$ mV and $\Delta E_s = -2$ mV. Insert show the relationships between I_p and E_p for peak 2

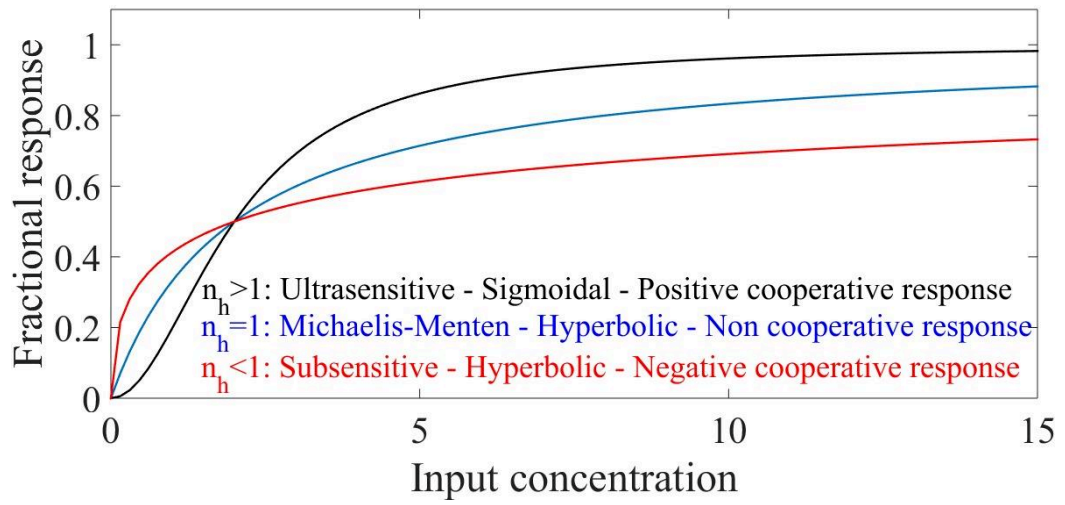


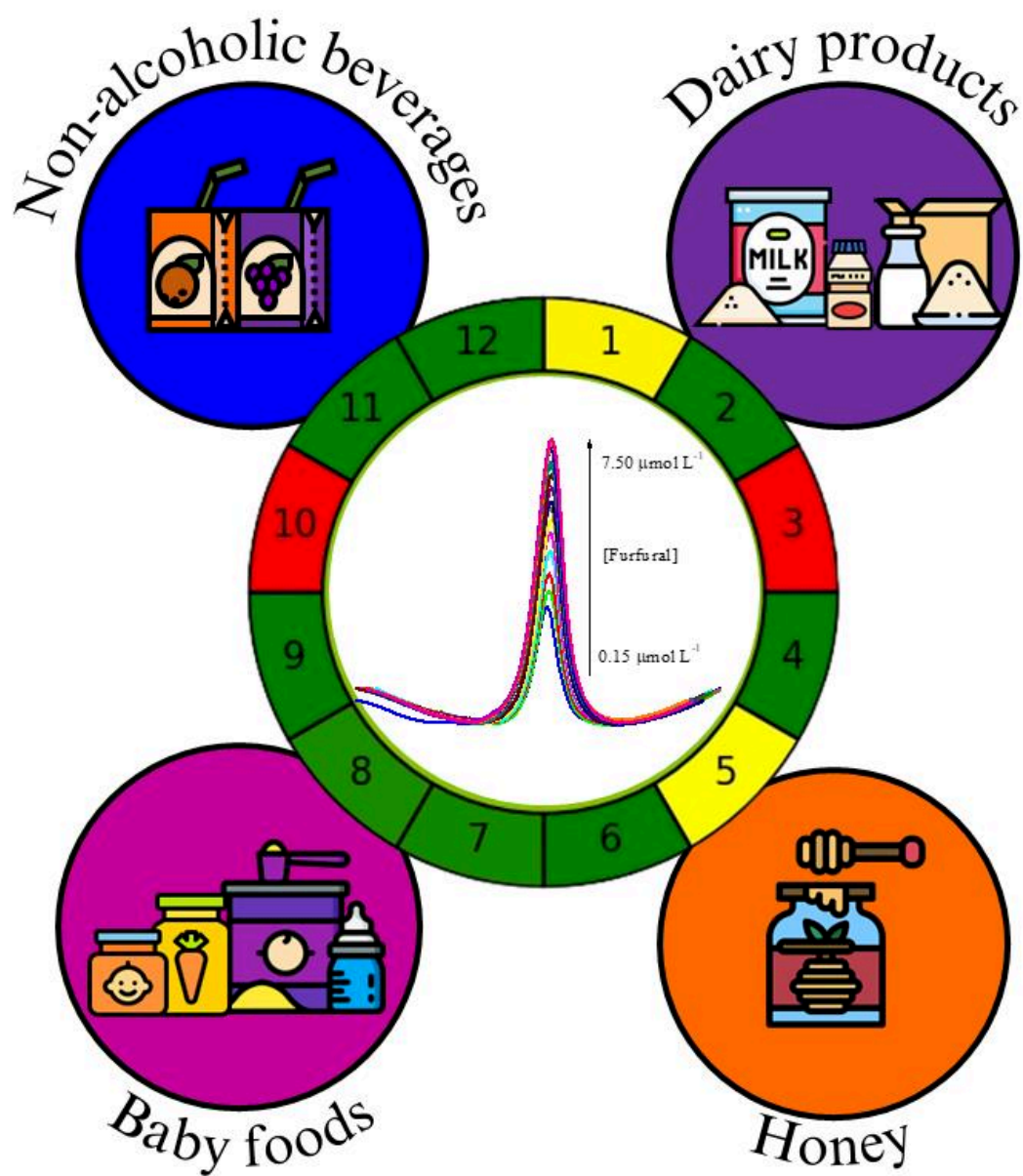
Figure 3S: Demonstration of how the exponent n_h or Hill coefficient indicates the measure of change/transition of the process studied, considering the different values and ranges of values that n_h can have.

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CAPÍTULO II

“Electroanalytical methodology in the detection of process contaminants: quantification of furfural in foodstuffs case”

Graphical Abstract



Electroanalytical methodology in the detection of process contaminants: quantification of furfural in foodstuffs case

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Highlights

- Detection of furfural by electroanalytical methodology was made in foodstuffs.
- Square wave voltammetry and solid electrodes allow detect traces of furfural.
- Mathematical modeling enabled the evaluation of analytical parameters.
- Simple foodstuff preparation steps resulted in fast and low-cost analysis.
- Furfural was analyzed in baby foods, honey, grape juice, and orange juice.
- Furfural was analyzed in powdered milk, fermented milk, and dairy flour samples.

Abstract

An electroanalytical methodology using square wave voltammetry (SWV) and polished silver solid amalgam electrode (p-AgSAE) as the working electrode was proposed for the analysis of furfural (FU) in foodstuffs, minimizing sample preparation steps. With the proposed methodology, it was possible to study the electrochemical behavior of FU on the surface of the p-AgSAE electrode. Experimental and voltammetric optimization showed good results for the detection of the FU using p-AgSAE, in an electrolytic medium of Britton-Robinson buffer pH 12.0, with a frequency of pulse potential of 100 s^{-1} , scan increment of 2 mV and pulse amplitude of 50 mV. It was possible to obtain an LOD of $4.500\text{ }\mu\text{mol L}^{-1}$ and LOQ of $13.64\text{ }\mu\text{mol L}^{-1}$, with a linear range of 1.500×10^{-7} to $7.500 \times 10^{-6}\text{ mol L}^{-1}$ and 93.00% of recovery in electrolyte. The methodology was applied to solid and liquid foodstuffs, including non-alcoholic beverages, dairy products, honeys and baby foods. The methodology allowed the analysis and detection of FU in samples with minimal preparation steps, with recoveries of between 47.00 and 89.00% (v/v) for liquid samples and 3.260×10^{-3} and $5.510 \times 10^{-3}\text{ g L}^{-1}$ (m/m) for solid samples. Greenness analyses were carried out using the developed methodology and sample preparation steps, and in both analyses the results showed that the methodology was well suited to the principles of green chemistry. This work shows that the analytical methodology developed can be used for the detection and quantification of FU in foodstuffs samples through analysis with minimal sample preparation steps and with good suitability for green chemistry requirements.

Keywords

Process contaminants; Furfural; Food Analysis; Square wave voltammetry; Foodstuffs; Food contaminants.

1. Introduction

As the world's population grows, so does the supply of food, which must be healthy, nutritious, and suitable for different types of consumers. For this reason, industrialized food or foodstuffs has seen an exponential increase in production, leading to concern about the quality and safety of the food produced and offered to the population, especially children. Statistics data indicated that around 7.5% of deaths in the world each year are associated with food-borne illnesses. Among the factors that can lead to these diseases are contamination by pesticide residues and veterinary medicines, heavy metal ions, mycotoxins, and food additives or congeners [68]. The need to prevent public health risks associated with the consumption of processed foods has led to the creation of mechanisms such as legislation to prevent fraudulent or occasional contamination and the authentication of processed foods [69].

The processes involved in the industrialization of food can lead to chemical reactions that transform the composition of the raw materials used in food production and generate contaminants and congeners in foodstuffs known as process contaminants [70]. The most commonly investigated process contaminants in foodstuffs are acrylamide, ethyl carbamate, and furans compounds, which belong to a vast group of contaminants with molecules from different chemical groups and various precursors and conditions leading to their formation [71].

Furfural, called as 2-furaldehyde, is a chemical compound derived from the hydrolysis of pentoses during the various stages of food processing, heating, and storage. It is prominently produced through carbohydrate hydrolysis reactions under heating, mainly through the caramelization and "Maillard reaction" pathways, forming furanic compounds in food products [20]. During the heating process, the Maillard reaction prompts the generation of furanic compounds, namely furfural (FU) and hydroxymethyl furfural (HMFU). Numerous studies have proposed diverse sources and mechanisms for the FU and HMFU formation. Many potential origins encompass the thermal degradation of carbohydrates or the reaction between reducing sugar and amino acids (known as the Maillard reaction), the thermal oxidation of poly-unsaturated fatty acids, and the thermal decomposition of ascorbic acid and its derivatives [72].

The toxicity of furfural arises from its capacity to be readily absorbed through various exposure routes such as the skin, oral intake, dermal contact, and inhalation. Consequently, frequent exposure to furfural can lead to detrimental effects on the liver and kidneys. Due to the possibility of human exposure to furfural through the consumption of foodstuffs, it is essential to accurately detect and measure the levels of furfural in these samples [34,73]. Thus, the identification and quantification of furfural are essential in the food processing industry,

particularly for foodstuffs products that are regularly or daily consumed, mainly by babies and children. Furthermore, furfural is a significant quality indicator for various foodstuffs items, including honey, alcoholic beverages, coffee, bakery products, and dairy products [74].

In addition to legislation that protects public health from the risks associated with foodstuffs contamination, it is also necessary to develop rapid and reliable detection methods to identify and quantify contaminants in foodstuffs and alcoholic beverages, enabling timely interventions and guaranteeing the safety of foodstuffs products [68]. The global regulatory bodies overseeing food safety recommend implementing chromatographic methods for separating and identifying furan compounds in foodstuffs [35]. These techniques offer high levels of sensitivity and specificity, mainly when dealing with complex samples. Nevertheless, the use of these methods requires additional preparatory and enrichment steps, leading to increased costs, longer analysis times, and the production of hazardous waste materials [75].

Already, voltammetric techniques have been highlighted as offering potential advantages in terms of sensitivity and detection range for the analysis and determination of various compounds in foodstuffs, such as food additives [15], pesticides (De Souza et al., 2021; Almeida & De Souza, 2023), mycotoxins [17], pharmaceutical compounds (Megale & De Souza, 2023), some compounds produced during the processing steps [18], and processing contaminants, such as FU. All foodstuffs' contaminants present high relevance due to food safety to consumers and an indicator of adequate quality of processing of foodstuffs.

Recent research demonstrated the employ of some voltammetric techniques in the identification and quantification of FU in sugarcane bagasse [36], insulating oil [33], wastewater, foodstuffs, and pharmaceutical syrups [9,37]. Despite achieving acceptable sensitivity levels, the customary employment of chemically modified electrodes or static mercury drop electrodes introduces challenges in replicating the voltammetric signals and leads to the production of hazardous waste, respectively.

So, the main of this work was to use a voltammetric procedure to identify and quantify FU in foodstuffs (baby foods, honey, grape juice, orange juice, powdered milk, and dairy flour) employing square wave voltammetry (SWV) allied to a polished silver solid amalgam electrode (p-AgSAE).

SWV was the voltammetric technique because it offers enhanced analytical sensitivity compared to alternative electroanalytical approaches [42,43]. p-AgSAE was selected as a working electrode because it exhibits electrochemical properties similar to traditional mercury-based electrodes but with a noteworthy reduction in the generation of toxic waste due to

mercury in the form of a metal alloy. It allows for a mechanically stable working electrode with prolonged durability. Furthermore, the preparation of p-AgSAE has been previously researched, with the well-defined proportion of mercury between silver and procedures, simple procedure for activating and renewing the electrode surface through a straightforward electrochemical regeneration process and elimination of the toxic waste generation [44–47].

2. Experimental

2.1. Instrumentation

Voltammetric experiments were performed in a potentiostat from the Metrohm Eco-Chemie μ Autolab model controlled by Nova 2.11 software installed in a personal computer. An analytical Shimadzu balance (model AUY 220) and an Gehaka pHmeter (model PG 2000) coupled to 3.00 mol L⁻¹ Ag/AgCl/KCl-glass electrode were used in all mass measurements and pH solution adjustments, respectively. Phoenix Luferco tube shaker vortex (model AP59) operating at a speed of 3,800 rotations per minute was used in foodstuffs preparation before voltammetric experiments. All water used in this work was previously purified by a Gehaka model OS20 LX system, with a conductivity of 18.2 M Ω cm at a temperature of 25 °C.

2.2. Reagents and solutions

In this work, all reactants utilized were commercially acquired, presented analytical-reagent grade, and were used without undergoing additional purification steps. A 0.04 mol L⁻¹ Britton-Robinson (BR) buffer prepared with phosphoric acid, acetic acid, and boric acid was used as supporting electrolytes, and pH adjustment was performed with a 1.00 mol L⁻¹ sodium hydroxide solution to cover a broad pH range from 2.0 to 12.0 [48].

FU was purchased from Sigma-Aldrich® (CAS 98-01-1) with a purity level of 98.5% and used to prepare a stock solution with a molarity of 2.96x10⁻² mol L⁻¹. This stock solution was freshly prepared each day by dissolving an appropriate amount of FU in pure acetonitrile and was subsequently diluted to achieve the desired concentration solution for use in the SWV experiments. These solutions were stored in a light-protected flask and refrigerated to ensure stability and prevent degradation. All material employed in the voltammetric experiments were preliminary washed with neutral detergent, commercial alcohol 96°GL and pure water for the removal of organic wastes.

2.3. Electrochemical cell

The electrochemical cell comprises electrical and ionic conductors immersed in a glass vessel with a Teflon[®] cap, in which electrical conductors were the working, auxiliary, and reference electrodes. The ionic conductor was BR buffer solutions with predetermined pH values. The auxiliary electrode was a platinum wire, the reference electrode was a custom-made Ag/AgCl electrode immersed in KCl solution with a concentration of 3.00 mol L⁻¹, and the working electrode was a custom-made p-AgSAE. All electrodes were inserted into glass vessel containing FU solutions plus BR buffer previously deoxygenated by bubbling N₂ (White Martins).

The p-AgSAE was prepared following previous research of De Souza and co-workers [45,47], in which silver powder (purity of 99.9%, with particle size ranging from 5 to 9 µm, obtained from Sigma-Aldrich[®]) was mechanically blended with mercury (purity of 99.99% bought from Synth[®], Brazil). The mass ratio of silver to mercury (Ag/Hg; m/m) was 30% to 70%, and it was meticulously weighed and thoroughly mixed until a cohesive mixture resembling a plastic mass was achieved. The mixture was then inserted into a Pyrex[®] glass capillary tube with an inner diameter of approximately 0.30 mm and compressed to ensure complete compaction and the formation of a well-defined disk.

After 24 hours, complete amalgamation took place, and electric contact was established by affixing a copper wire to the paste using silver adhesive. The disks obtained were polished using emery paper with a grit size of up to 2000 (3M) to obtain a polished surface free from mercury or mercury oxide, and for this, the electrode is called as p-AgSAE. This polishing process offered several advantages: easy surface renewal, low, and good reproducibility. Importantly, it did not generate any mercury waste during electrochemical analysis.

Before analysis, the p-AgSAE was electrochemically activated through cyclic voltammetry experiments with scan potential from 0.0 to -2.2 V during 100 cycles in a 0.20 mol L⁻¹ sodium chloride solution. These experiments were previously performed to reduce the charge transfer resistance and improve the analytical responses. Additionally, between successive SWV experiments, a fixed potential of -2.20 V was applied for 30 seconds in the same working solution to eliminate redox reaction products that may have been adsorbed onto the p-AgSAE surface.

2.4. Experimental and voltammetric optimization

Preliminary experiments were performed in BR buffer solutions with pH values from 2.0 to 12.0, previously deoxygenated by bubbling N₂, plus FU addition in concentration values of 1.10×10^{-4} , 2.20×10^{-4} , and 3.23×10^{-4} mol L⁻¹. SWV experiments were performed for each FU concentration, with the scan potential ranging from -1.8 V to 0.0 V, and SWV parameters used were pulse frequency (f) of 100 s⁻¹, pulse amplitude (a) of -50 mV, and a scan potential increment (ΔE_s) of -2 mV.

After pH optimization, the voltammetric parameters of SWV were individually optimized by conducting SWV experiments, in which two parameters were fixed, allowing the third parameter to vary. The f was varied from 10 to 300 s⁻¹, the a from -5 to -50 mV, and the ΔE_s from -2 to -10 mV. The optimization process ensured that the maximum peak current value (I_p), peak potential (E_p), and maximum half-peak width ($W_{1/2}$) were optimized in terms of sensitivity and selectivity.

2.5. Analytical parameters

Analytical curves were constructed for FU using the optimized SWV parameters and the standard addition method. These curves were constructed in triplicate. However, a non-linear relationship between FU concentration and I_p values was observed, allowing the necessary use of mathematical treatment for the obtention of linear curve and calculus of the analytical parameters.

Following the average analytical curve obtained, some mathematical models were tested for the obtention of a suitable model for linearization of the curve and posterior calculus of analytical parameters, which the Hill's model [51,52] was used to adapt and study the obtained data. So, preliminarily, the data were standardized to follow the mathematical restrictions imposed by the linear form of Hill's equation, as:

$$Y = \frac{I_p}{I_{pmax}} \quad \text{Equation 1}$$

where $I_{(pmax)}$ is the maximum value of I_p to place $0 < Y < 1$. The parameters for the linearization were obtained using Matlab's cftool toolbox and the non-linear least squares method used was Levenberg-Marquardt [50,51].

The linearized average curve was used in the limits of detection (LOD) and quantification (LOQ) calculus, following guidelines of AOAC International [67], which the

standard deviation (S_b) related to the intersection values of the previously linearized average analytical curve and the slope of the analytical curves (s), are used according to:

$$LOD = \frac{3.3S_b}{s} \quad \text{Equation 2}$$

$$LOQ = \frac{10S_b}{s} \quad \text{Equation 3}$$

The accuracy of the proposed methodology was evaluated by the evaluation of recovery experiments, in which a known amount of FU was added to the electrochemical cell followed by new additions of the standard FU solution to the BR buffer solution. The recovery curves were constructed in triplicates, and the linear relationships were obtained using the same mathematical modeling used in the obtention of the analytical curves. The recovery efficiencies (%R) were calculated as the percentage ratio between the FU concentrations added and the FU concentrations found:

$$\%R = \frac{[FU]_{found}}{[FU]_{added}} \times 100 \quad \text{Equation 4}$$

Experiments of *interday* and *intraday* repeatability evaluated the precision of the proposed methodology. For this, *interday* repeatability was assessed using five standard FU solutions over five days (*interday* precision) and for *intraday* repeatability, successive determinations of a standard FU solution were made and the resulting signals analyzed (*intraday* precision). Based on the percentage ratio between the standard deviation and the average of the I_p values, the relative standard deviations (RSD) were calculated for the repeatability measurements.

2.6. Application of methodology in foods

The efficiency of the proposed electroanalytical methodology was evaluated using SWV experiments in foodstuffs, such as non-alcoholic beverages, honey, dairy products, and baby foods samples. These samples were obtained from the local market in Patos de Minas city, Minas Gerais, Brazil, and used to evaluate the influence of their composition in the FU voltammograms.

2.6.1. Non-alcoholic beverages

For the analysis of non-alcoholic drinks, pasteurized juices were analyzed using the traditional pasteurization process and high-pressure pasteurization in grape and orange flavors.

Common brands obtained in Patos de Minas city supermarkets were selected, favoring brands marketed to children. So, an aliquot of 1 mL of each sample was added to the electrochemical cell containing 10 mL of supporting electrolytes, which were vigorously homogenized. SWV experiments were conducted without any pretreatment of the samples. Subsequently, the standard addition method was employed to establish recovery curves, both with and without sample fortification (artificial contamination), following a procedure similar to that used in the electrolyte. The recovery curves were built in triplicate, and the mean recovery values were determined using the identical methodology employed in the pure electrolyte.

2.6.2. Honey

The methodology was applied to different kinds of honey from different floras. For this, an aliquot of 1 mL of each sample was added to the electrochemical cell containing 10 mL of supporting electrolytes, which were vigorously homogenized. SWV experiments were immediately conducted without pretreatment. Subsequently, recovery curves were built, with prior fortification and without fortification, following a procedure similar to that utilized in the electrolyte. The recovery curves were built in triplicate, and the mean recovery values were determined using the identical methodology employed in the pure electrolyte.

2.6.3. Dairy products

For the analysis of fermented milk, 1 mL of each sample was added to the electrochemical cell containing 10 mL of support electrolyte, which was homogenized vigorously. Similarly, for the analysis of powdered milks, dairy flour and infant formula, 1 g of each sample was added to the electrochemical cell containing 10 mL of support electrolyte, which was also homogenized. After this, the SWV experiments were carried out without any pretreatment of the sample, similar to other samples. The method of addition of FU standard solution was used to build the recovery curves, prior fortification (artificially contaminated) and without fortification.

2.6.4. Baby food

For the analysis of different flavors of paste baby foods, 1 g of each sample was weighed and added to the electrochemical cell containing 10 mL of support electrolyte, which was homogenized vigorously. Similar to the analysis of the infant formula, 1 g of each sample was

added to the electrochemical cell containing 10 mL of support electrolyte and rigorously homogenized. After this, the SWV experiments were carried out, without any sample pretreatment, using the standard addition method to construct the recovery curves, prior fortification (artificially contaminated) and without fortification. All the recovery curves were carried out in triplicate, and the average recovery values were calculated using the same methodology applied to the pure electrolyte.

2.7. Greenness analysis

To assess the alignment of the analytical methodology developed and the sample preparation steps with the principles of green chemistry, a greenness analysis was carried out. The free software AGREE (Pena-Pereira et al., 2020) and AGREEPrep (Pena-Pereira et al., 2022) were used to analyze the analytical methodology and sample preparation, respectively.

3. Results and discussion

In previous research the authors developed an electroanalytical procedure for electrochemical behavior study of FU using p-AgSAE and SWV mathematical models for mechanistic approaches [76]. For this, it evaluated the reduction and oxidation reactions of FU in basic medium, which was observed the presence of two voltammetric peaks for reduction (scan potential from 0 to -1.8 vs Ag/AgCl), and three voltammetric peaks in oxidation (scan potential from -1.8 to 0 vs Ag/AgCl). Optimization of the supporting electrolyte and pH values indicated that the oxidation reaction was more favored resulting in three well-defined peaks with peak current (I_p), peak potential (E_p) and half-height width ($W_{1/2}$) suitable for analytical purposes. So, in this work, the oxidation reaction under p-AgSAE allied o SWV technique will be used in the FU detection in different types of foodstuffs samples, which the FU can be present as process contaminant.

3.1. Experimental and voltammetric optimization

The FU oxidation under p-AgSAE is favored in BR buffer solution with pH 12.0 due to the reaction at more negative potential (peak 1) results in a cation that immediately reacts with acetate anion (chemical step) contained in the BR buffer solution, with high concentration in pH 12. So, the product of this chemical reaction is irreversible oxidized (peak 2) due to electrons

transfer in the double bond, resulting in reactants for oxidation in peak 3, which results in a strong adsorption process of product in the p-AgSAE surface. These voltammetric responses are in accordance with previous reports about the chemical reactions of furans compounds [60]. Figure 1 shows the oxidation peaks of FU under p-AgSAE in BR buffer pH 12.0, with the indication of the redox reactions for each voltammetric peak [76].

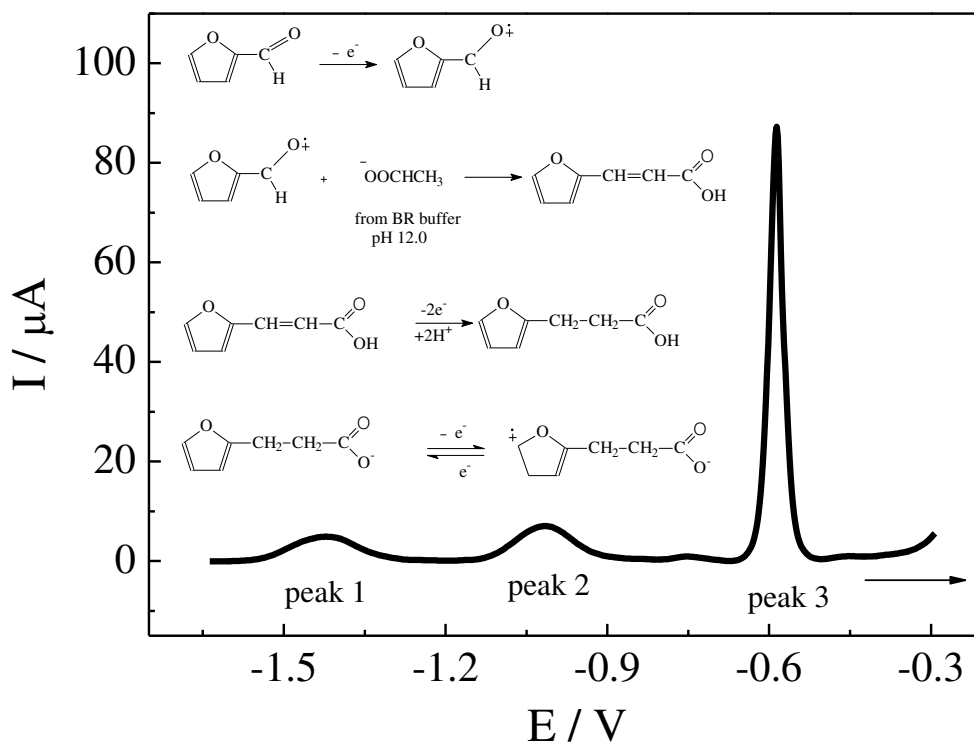


Figure 9: Square wave voltammogram for 2.81×10^{-4} mol L⁻¹ FU under p-AgSAE, in medium of 0.04 mol L⁻¹ of BR pH 12.0, with $a = -50$ mV, $\Delta E_s = -2$ mV and $f = 100$ s⁻¹. Insert corresponds to probable redox mechanism of electrons transfer considering three voltammetric peaks [76].

The adsorption of products from redox reaction under working electrodes allows in the blockage of active sites and loss of analytical sensitivity, and for this, most of the research into electroanalysis and FU involves the use of chemically modified electrodes, as described early [9,33,36,37] or the use of traditional mercury electrodes (Kumbhat et al., 2004; R. Wang et al., 2016). However, the use of p-AgSAE enables a rapid procedure for renewing the electrode surface, allowing successive measurements with good sensitivity and reproducibility [47] and without toxic waste generation, and for this was selected for use in the FU in different types of foodstuffs samples.

Furthermore, according to Figure 1, the peak 3 showed the highest I_p value and the most favorable E_p (the lower the E_p , the less interference from reactions of other compounds on the FU oxidation peak), and for this, the peak 3 was selected for the development of our research.

Previous optimization of voltammetric parameters related to use of SWV was performed. For this, each parameter (f , a and ΔE_s) was individually evaluated and the values that allowed in best analytical signal in terms of I_p and E_p were chosen, according to shown in Figure 3, where are presented the voltammograms resultant of each parameters variated and the respective relationships obtained between parameter and I_p values.

The f parameter was variated from 10 to 100 s^{-1} , and the SWV responses, Figure 2a, shown that for f values until 60 s^{-1} appeared two well-defined voltammetric peaks, which only became one peak for higher frequencies. According to SWV theory [42,59], this peak separation in low currents, called as “split in net responses”, occurs due to current sampling procedure in SWV programming and is typical of fast and reversible redox reactions, justifying the excellent sensitivity for this voltammetric peak, compared to other two peaks, shown in Figure 1. Furthermore, SWV responses in Figure 2a shown that $W_{1/2}$ and E_p values were practically constants when f were increased, confirming the suitable selectivity of the voltammetric responses. The insert in Figure 2a shown the linear relationships between I_p and f values until 100 s^{-1} , after this, no linearity was observed. This way, 100 s^{-1} was chosen as voltammetric parameters for subsequent experiments.

The a parameters was varied from 5 to 50 mV following previous values defined by SWV theory that define $50/n$ (n is the number of electrons in the redox reaction) as the suitable a value. SWV responses are shown in Figure 2a, where can be observed a linear increase in the I_p values when a was increase, accompanied by a little dislodgment in E_p values to cathodic potential and without considerable alterations in the $W_{1/2}$. For this, the parameter a was set as 50 mV in the further voltammetric experiments.

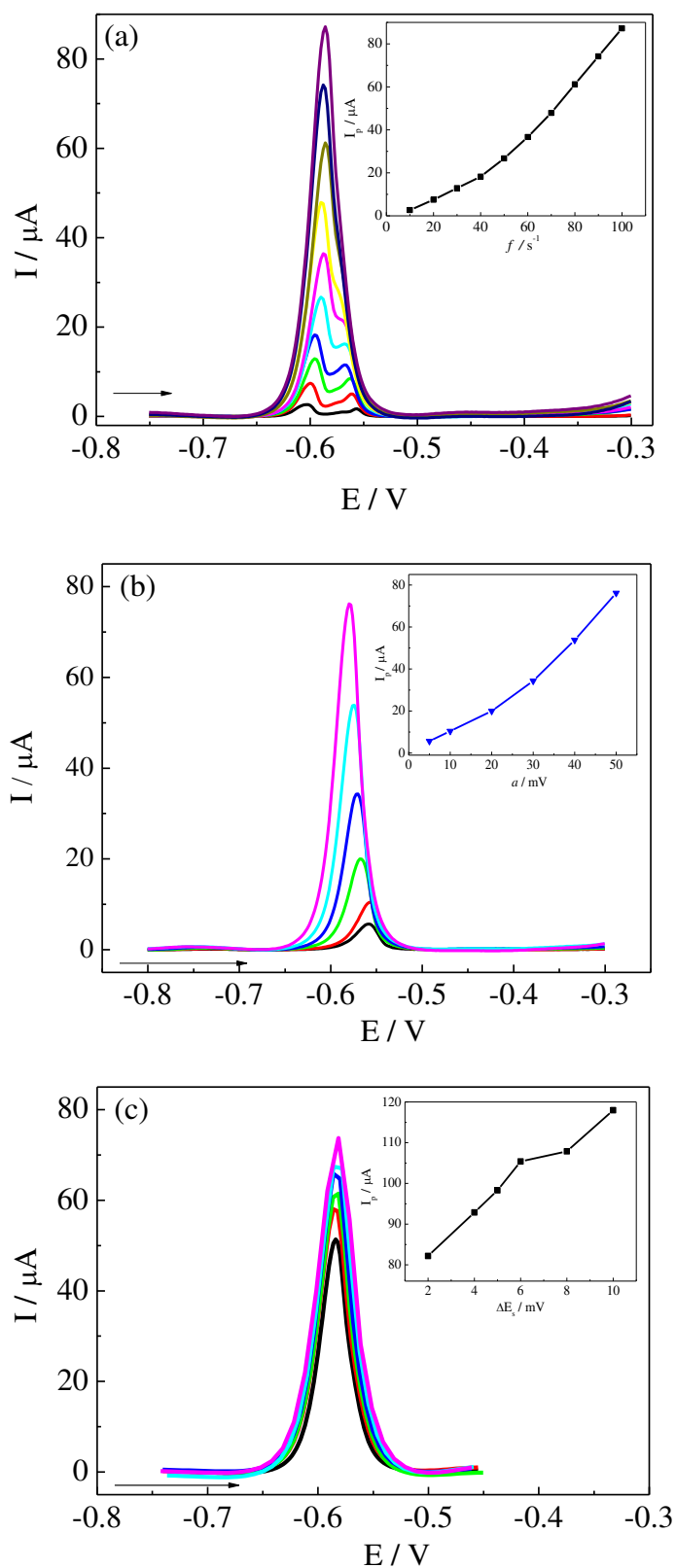


Figure 10: Square wave voltammograms for 2.81x10⁻⁴ mol L⁻¹ FU under p-AgSAE in 0.0 mol L⁻¹ of BR buffer pH 12, with $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$ and $\Delta E_s = -2 \text{ mV}$. (a) f variation from 10 to 100 s⁻¹, (b) a variation from 5 to 50 mV and (c) ΔE_s variation from 2 to

10 mV. Inserts correspond to the relationships between parameters evaluated and I_p values.

Finally, the ΔE_s was varied from 2 to 10 mV, and SWV data are shown in Figure 2c, where was observed a linear increase in the I_p until 10 mV. However, due limited points collected in the resultant voltammograms the voltammetric profile at the maximum I_p present low definition, and for this, ΔE_s of 2 mV was set as suitable value for analytical purposes.

3.2. Analytical parameters and mathematical modeling

Using 0.04 mol L⁻¹ of BR buffer pH 12.0, optimized SWV parameters ($f = 100$ s⁻¹, $a = 50$ mV and $\Delta E_s = 2$ mV) and scan potential from -0.8 V to -0.3 V, were build analytical curves for FU oxidation under p-AgSAE. For this, aliquots from the FU stock solution in a range concentration from 1.50×10^{-7} to 7.5×10^{-6} mol L⁻¹ were consecutively added to the electrochemical cell. Between each FU addition SWV responses were evaluated, as shown in Figure 3, where can be observed that I_p values were increased when the FU concentrations increased, without no dislodgment in E_p or variation in $W_{1/2}$. The resultant analytical curve (I_p vs FU concentration) was evaluated, and no linear relationships was observed, in any concentration interval. For this, curves were repeatedly built by nine times, and the average medium is shown in Figure 3b that were used in the mathematical modeling and consequently for evaluation of the analytical parameters, such as sensitivity, linearity, LOD, LOQ, precision and accuracy.

The use of mathematical modeling was necessary due to analytical parameters calculus consider a linear relationship between the parameters measured (I_p) and the concentration of compounds of interest. As the analytical curves were built nine times, and the nonlinear behavior was observed in all curves, the comparison of average analytical curve (Figure 3b) with theoretical isotherms profiles from Gilles studies [61–63], that describe the relation between solute adsorption mechanisms at solid surfaces, was performed.

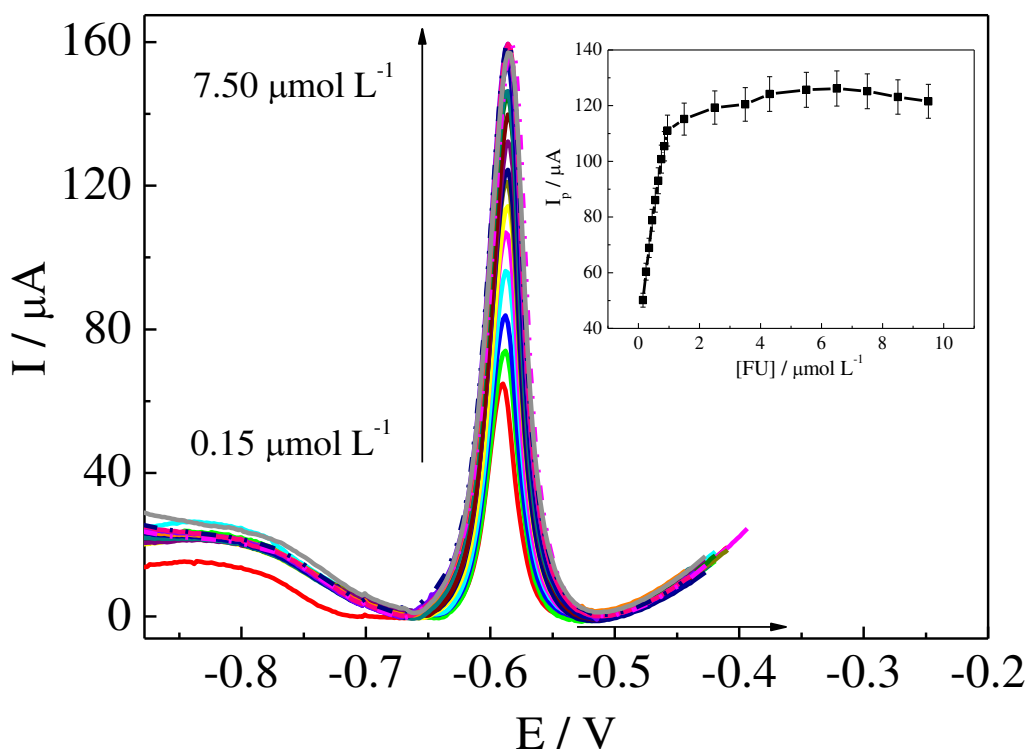


Figure 11: Square wave voltammograms for oxidation of furfural using 0.04 mol L⁻¹ BR pH 12.0 buffer solution on the p-AgSAE, $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$, $\Delta E_s = -2 \text{ mV}$, in the concentration range from $1.500 \times 10^{-7} \text{ mol L}^{-1}$ to $7.500 \times 10^{-6} \text{ mol L}^{-1}$. Insert correspond to average analytical curve resulted.

So, the average analytical curve presented characteristics H shape (high affinity), typical of the process with occurrence of high affinity of the solute (here FU), even in low concentrations that is completely adsorbed under solid surfaces (here p-AgSAE). In H shape curves, after initial adsorption the equilibrium is reached and then the final of curve is characterized by the plateau, which is common in medium organic molecules that involves reactions with strong adsorption reactions inhibited by absence of active of adsorption sites [77,78].

So, a mathematical modeling was evaluated to obtain a linearization of the average analytical curve. MatalabTM toolbox was used to select the adequate adjustment of the curve, following the shape of the H isotherm. Considering the interaction of solute under solid surfaces is convenient to use the Hill equation to measure the dynamics of cooperativity or adsorption [53], which describes the receptor molecules saturation, Y , as an effector concentration function and is given by

$$Y = \frac{Z^{n_h}}{k^{n_h} + Z^{n_h}} \quad \text{Equation 5}$$

being Z the input concentration, n_h is an interaction coefficient called by Hill coefficient, and k the half-saturation constant (quantification of the threshold concentration necessary for 50% response production). Furthermore, the exponent n_h indicates the sigmoid curve steepness and is interpreted as measuring the free energy of interaction between sites. In this research, was obtained $n_h > 1$, indicating a positive cooperativity leading to a sigmoidal response [50,51,53], has showed in insert of Figure 3.

As the Equation 5 (Hill model) is a nonlinear equation, the parameters n_h and k can be obtained by linearized Hill equation:

$$\ln\left(\frac{Y}{1-Y}\right) = n_h [\ln(Z) - \ln(k)] \quad \text{Equation 6}$$

However, the use of Equation 6 requires $Y > 0$ and $1 - Y > 0$, i.e., $0 < Y < 1$. This means that empirical data must be expressed in terms of a proportion conserving the dynamics of the process and reducing the scale, this methodology provides better conditions for the calculation of n_h and k parameters via numerical regression methods. So, before applying the experimental data, and to meet the limiting conditions for using Equation 6, the data were standardized as described in *Experimental Section*. After normalization, the experimental data were fitted to the polynomial model described by the Hill Equation and linearized by mathematically treating according to Equation 6, allowing in a suitable mathematical treatment where was possible to obtain the linearized equation for the experimental data:

$$\ln\left(\frac{Y}{1-Y}\right) = 1.227 [\ln(Z) - \ln(2.64 \times 10^{-7})] \quad \text{Equation 7}$$

in which Y is values of I_p normalized in μA , and Z is FU concentration in $\mu mol L^{-1}$.

After the linearization of average analytical curve, the LOD and LOQ were calculated as describe in *Experimental Section*. So, Table 1 presents parameters obtained, as the LOD and LOQ calculated, standard deviation of blank responses (S_b), the slope and intercept from the analytical curves, the degree of adequation between the linearization of analytical curves and the mathematical model utilized, given to root mean squared error (RMSE) and by the correlation coefficient (r).

The precision of the SWV methodology allied to p-AgSAE in FU detection was evaluated by repeatability experiments, considering *intraday* and *interday* analysis, performed using previously optimized experimental and voltametric conditions and SWV experiments in two different points of linearized average curve, in $8.500 \times 10^{-7} mol L^{-1}$ (low concentration) and $4.500 \times 10^{-6} mol L^{-1}$ (high concentration), the first point being closest to the beginning of the

detection range and the second closest to the end. Repeatability by *interday* experiments were carried out in five different days and different solutions FU, generating five different measurements. Already the repeatability by *intraday* experiments were carried out seven times in the same FU solution, generating seven different measurements. The SWV voltammograms for *intraday* and *interday* experiments were used in calculations of RSD, as described in *Experimental Section*.

Calculated RSD for *intraday* experiments was 9.32% and 11.95%, for first and second point evaluated, respectively. Already RSD obtained for *interday* experiments was 10.31% and 4.98% for points with $8.500 \times 10^{-7} \text{ mol L}^{-1}$ and $4.500 \times 10^{-6} \text{ mol L}^{-1}$ of concentration of furfural, respectively. It is important to note that the high concentration of analyte, when evaluating the second point, both for *intraday* and *interday* experiments, may have an influence on the RSD values obtained, both above of 10%, acceptable values according to the Association of Official Analytical Chemists (AOAC) [35]. Mainly because, as already discussed for this method, in conditions of high concentration, the adsorption of FU is favored and causes a blockage on the electrode surface, making it difficult to carry out experiments such as repeatability, as it makes it difficult to renew the electrode. Similarly, even at low concentrations as in the first point evaluated, mainly in the *intraday* experiments, a linear increase in I_p was observed in relation to the repetition of the experiment, this can also be explained by the adsorptive phenomenon that occurs in the system and which was previously described, and the consequent difficulty in renewing the surface between measurements.

So, the RSD values calculated in this research indicated that the precision of the proposed methodology is adequate, mainly considering the occurrence of strong adsorption of FU under p-AgSAE, due to RSD values calculated for *interday* and *intraday* presented around values below or close of 10%.

Accuracy of the proposed methodology was evaluated by recovery experiments, performed in triplicated, and the results obtained for analysis conducted in electrolyte are shown in Table 1. The pure electrolyte, composed by 0.04 mol L^{-1} of BR buffer pH 12.0 and it was spiked $7.500 \times 10^{-7} \text{ mol L}^{-1}$ of FU solution (called as added concentration, $[FU]_{\text{added}}$). In sequence, was made additions of the FU standard solution to observe and quantify the amounts of FU increased. With the I_p values for each addition of FU, the mathematical treatment of the recovery curve was carried out in the same way as the analytical curves. With linearized curve, recovery curve equation was obtained, and the interception and slope of this curve were used in the calculus of the recovered FU concentration (called as found concentration, $[FU]_{\text{found}}$).

Recovery percentage was calculated as described in *Experimental Section*, and 99.0% was obtained, a value considered excellent for analytical purposes, since 70 to 130% are acceptable values for a suitable analytical methodology [66].

Thus, the use of p-AgSAE combined with the SWV technique provided a suitable alternative method for the analytical determination of FU due to the possibility of its use in simple and reliable determination at different concentrations.

Table 3: Analytical parameters calculated for the determination of FU in 0.04 mol L⁻¹ of BR buffer pH 12.0, using SWV allied to p-AgSAE.

Parameter	Value
Concentration range	1.500x10 ⁻⁷ – 7.500x10 ⁻⁶ mol L ⁻¹
r	0.979
RMSE	0.029
Intercept	18.29 μ A
s	1.22 μ A /mol L ⁻¹
S _b	6.500x10 ⁻⁷ mol L ⁻¹
LOD	4.500 μ mol L ⁻¹
LOQ	13.64 μ mol L ⁻¹
Repeatability <i>interday</i> (%)	10.31 for point 1
	4.98 for point 2
Repeatability <i>intraday</i> (%)	9.32 for point 1
	11.95 for point 2
Recovery (%)	99.00

3.3. Application in foodstuffs samples

To ensure the applicability of the proposed methodology using SWV allied to p-AgSAE in the FU detection in foodstuffs, some commercial samples were used in construction of the recovery curves. For each foodstuff samples, recovery curves were performed in triplicate and the average recovery curves samples were used in the evaluation of the influence of samples components of each foodstuff in the voltammetric responses. So, similar procedure effectuated for supporting electrolyte was performed in non-alcoholic beverages, honey, dairy products, and baby foods samples. For each sample, an aliquot of 1.0 g or 1.0 mL was added in the electrochemical cell containing 10 mL of BR buffer and enriched with 6.500x10⁻⁷ mol

L^{-1} of FU. SWV experiments were performed, allowing in recovery curves with profile like observed in analytical curve, as shown in Figure 4, where are shown the SWV responses for recovery curves of FU in fermented milk plus the average recovery curve obtained.

Similar to other observed in the analysis of other foodstuffs samples, the addition of fermented milk in BR buffer no shown any other voltammetric peak, indicating that the samples components no interfere in SWV responses for FU oxidation, and the FU addition allows the increase in voltammetric responses only FU, indicating the robustness of the proposed methodology using p-AgSAE.

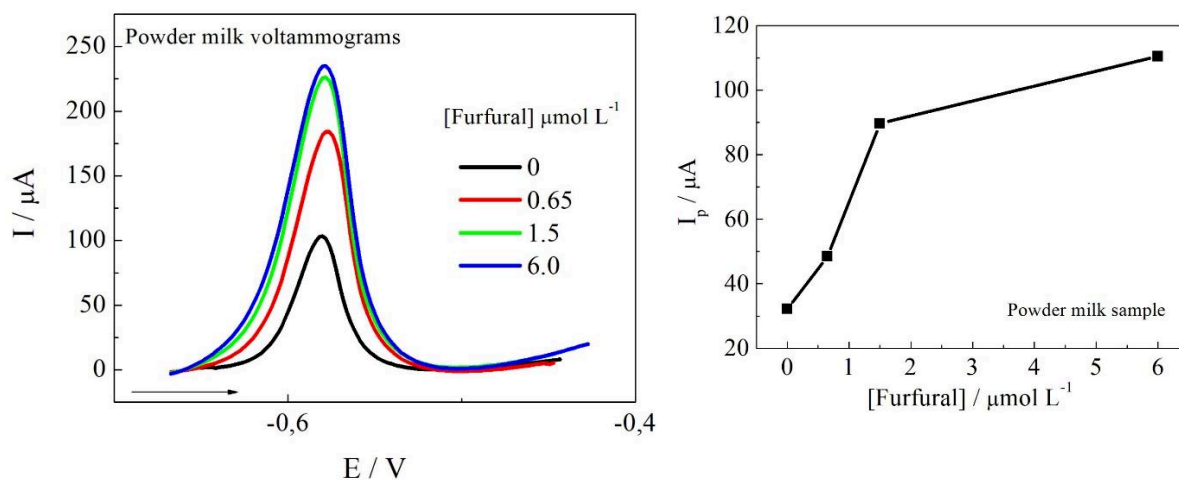


Figure 4: Square wave voltammograms for oxidation of furfural using 0.04 mol L^{-1} BR pH 12.0 buffer solution on the p-AgSAE, $f = 100 \text{ s}^{-1}$, $a = -50 \text{ mV}$, $\Delta E_s = -2 \text{ mV}$, with addition of 1.0 mL of fermented milk artificially spiked by $6.500 \times 10^{-7} \text{ mol L}^{-1}$ followed by standard addition of FU in the concentration range from $6.500 \times 10^{-7} \text{ mol L}^{-1}$ to $6.000 \times 10^{-6} \text{ mol L}^{-1}$. Insert correspond to average analytical curve resulted.

To verify the application of the proposed methodology in non-alcoholic beverages samples, including all interferents presents in real samples, recovery analyses were carried out on samples artificially enriched with FU in commercial trademark of high pressure pasteurized grape juice, grape mixed juice, grape soft drink, high pressure pasteurized orange juice, orange juice pasteurized and orange soft drink, called as here as non-alcoholic beverages.

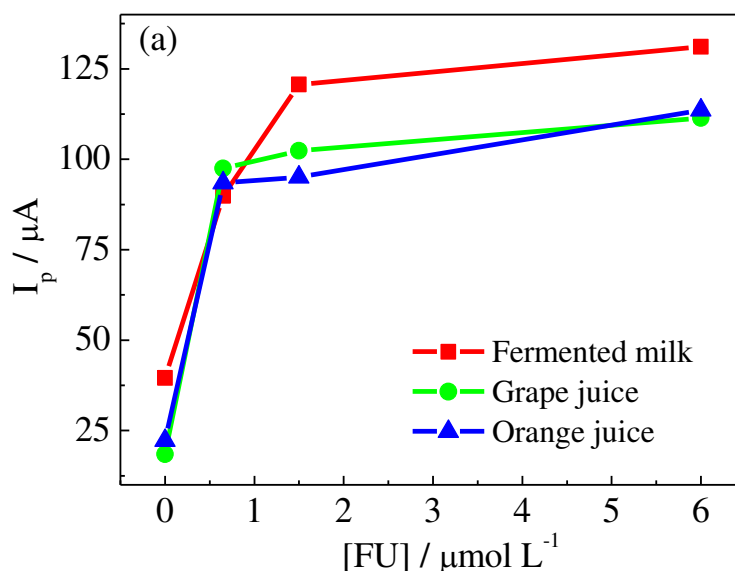
The honey samples were evaluated considering different blossoms (Eucalyptus honey, Aroeira honey and Coffee blossom honey). Honey present high organic acids concentration, and mainly pentoses, which can be converted in furans in environment temperature or during storage.

Dairy products present high scientific relevance due to extreme nutritional importance and be abundant source of high-quality proteins, including various amino acids essential in the

growth and development of humans [79]. During the processing of dairy products, the heating can result in FU production, allowing in contaminations of industrialized dairy products. For this, the control of quality of dairy products is very important in the industrial process. This way, the procedure developed in this research was applied in the FU detection in fermented milk, powder milk, milk flour and infant formula. For each sample, were analyzed three trademarks obtained in the supermarkets from Patos de Minas City and in each sample were built three recovery curves.

Baby foods contain high concentrations of sugar and proteins, and the processing or storage steps can allow in FU formation, and consequently contaminate an important source of nutrition for babies. For this, commercial baby foods (Carrot flavour, Pear and spinach flavour, Carrot and meat flavour, Apple and banana flavour, Pear, grape and apple flavour, Apple, carrot and sweet potato) were alkalized in order to evaluate the influence of their composition in the FU voltammetric responses under p-AgSAE.

The foodstuffs samples were used without any preparation, extraction, or purification step, considering the presence of all interferents compounds present in each sample. Recovery curves were built for fermented milk, grape and orange juice, honey, infant formula, power milk and baby foods, and some recovery curves are shown in Figure 5, where can be observed the sigmoidal profile similar to observed in analytical curves. For all foodstuffs samples, the recovery curves (Figure 5) were mathematically treated using the same mathematical modeling used in the analytical curve linearization. So, the FU concentration recovered ($[FU]_{\text{recovered}}$) was calculated and the values obtained are shown in Table 2. The recovery percentage obtained for all samples presented values suitable for analytical procedure, mainly, considering that any pretreatment or extraction process was applied in the samples, before SWV analysis.



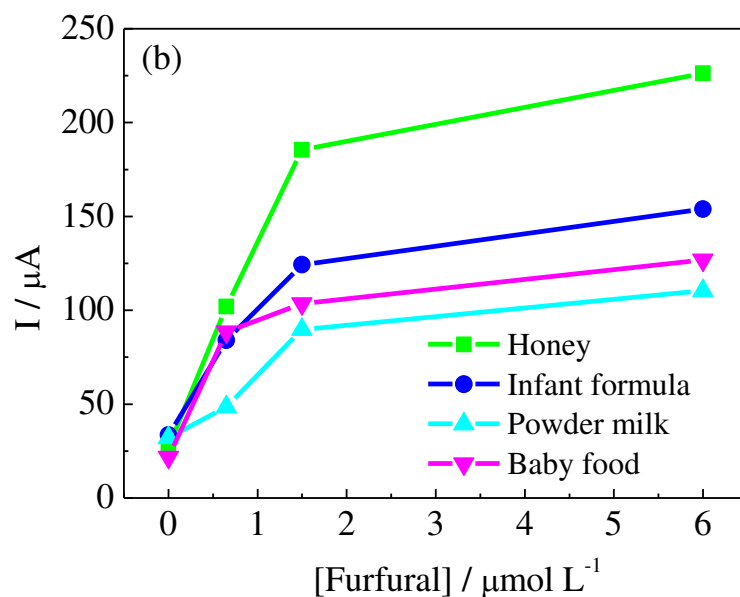


Figure 5: Analytical parameters obtained in different types of food samples for recovery of $6.5 \times 10^{-7} \text{ mol L}^{-1}$ of furfural in BR buffer pH 12 on p-AgSAE combined with SWV. All data were evaluated using medium values from three analytical and recovery curves.

Table 4: Analytical parameters obtained in foodstuffs samples for recovery of $6.500 \times 10^{-7} \text{ mol L}^{-1}$ of FU in BR buffer pH 12.0 on p-AgSAE combined with SWV. All data were evaluated using medium values from three and recovery curves.

Grape juice			
Sample	[Furfural] _{recovered} $\mu\text{mol L}^{-1}$	Recovery (%)	Bias
High pressure pasteurized grape juice	0.373	57	-4.26
Grape mixed juice	0.422	63	-3.50
Grape soft drink	0.413	64	-3.64
Orange juice			
Sample	[Furfural] _{recovered} $\mu\text{mol L}^{-1}$	Recovery (%)	Bias
High pressure pasteurized orange juice	0.398	58	-4.18
Orange juice pasteurized	0.573	89	-1.11
Orange soft drink	0.559	86	-1.40

Fermented milk			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery (%)	Bias
Trademarket 1	0.301	47	-0.54
Trademarket 2	0.351	54	+0,46
Trademarket 3	0.299	48	-0,54
Powder milk			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery g L ⁻¹ (m/m)	Bias
Trademarket 1	0.371	0.00351	-0.43
Trademarket 2	0.390	0.00375	-0.40
Trademarket 3	0.389	0.00374	-0.40
Dairy flour			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery g L ⁻¹ (m/m)	Bias
Trademarket 1	0.432	0.000415	-0.93
Trademarket 2	0.428	0.004112	-0.34
Honey			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery g L ⁻¹ (m/m)	Bias
Eucalyptus honey	0.418	0.00406	-0.35
Aroeira honey	0.411	0.00394	-0.36
Coffee blossom honey	0.415	0.00398	-0.35
Infant formula			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery g L ⁻¹ (m/m)	Bias
Trademarket 1	0.354	0.00340	-0.46
Trademarket 2	0.340	0.00326	-0.48
Trademarket 3	0.358	0.00343	-0.45
Baby food			
Sample	[Furfural] _{recovered} μmol L ⁻¹	Recovery g L ⁻¹ (m/m)	Bias

Carrot flavour	0.382	0.00467	-0.412
Pear and spinach flavour	0.363	0.00348	-0.441
Carrot and meat flavour	0.378	0.00463	-0.418
Apple and banana flavour	0.381	0.00366	+0.413
Pear, grape and apple flavour	0.393	0.00467	+0.410
Apple, carrot and sweet potato	0.366	0.00551	-0.436

3.4. Evaluation of the Greenness of proposed methodology

The greenness analysis method has been gaining ground in the development of chemical analysis methodologies since the advent of green chemistry. Greenness analysis, or green chemistry parameter analysis, is an important method for verifying the characteristics of a chemical process in order to check whether it adheres to the principles of green chemistry, i.e. it is a theoretical analysis that aims to evaluate the ecology of chemical methods and processes. There are various metrics that can be used to analyze the greenness of a method. Among the most commonly adopted metrics are NEMI, Analytical Eco-Scale, GAPI, AGREE, among others [80]. Some of these metrics have computerized tools that help assess the level of greenness of an analytical methodology. For this work, the free software Analytical Greenness AGREE [81] and AGREE Prep [82] were used for the greenness analysis of the analysis methodology and sample preparation, respectively, developed for this study.

The Analytical Greenness calculator AGREE is a computational tool that uses the twelve principles of green chemistry to establish a scale from 1 to 0, where 1 is the rating given to an essentially green method and 0 to an analytical chemical process that is completely harmful to the environment, to carefully evaluate chemical analytical methodologies. The calculator first establishes a value from 1 to 0 for each of the principles of green chemistry, and then unifies these values, still following the 1 to 0 scale, to provide a direct and comprehensive assessment of the greenness of the method evaluated. To graphically represent the assessment made on each of the criteria, the performance of the analytical method on each principle is reflected using a red-yellow-green color scale, while the weight of each principle is reflected in the width of the corresponding segment [81]. Thus, at the end of the analysis, by answering how the method is positioned according to the principles of green chemistry, it is possible to obtain a value, which fits the evaluation metric and which ranks the evaluated

method, with relative simplicity of the output to ensure that the result of the evaluation is easily readable [80].

The twelve principles of green chemistry used as evaluation criteria in the AGREE analytical calculator are (1) direct analytical techniques should be applied to avoid sample treatment, (2) minimum sample size and minimum number of samples are objectives, (3) *in situ* measurements should be performed, (4) integration of analytical processes and operations saves energy and reduces reagent use, (5) automated and miniaturized methods should be selected, (6) derivatization should be avoided, (7) the production of a large volume of analytical waste should be avoided and proper management of analytical waste should be ensured, (8) multi-analytical or multi-parameter methods are preferred to methods that use one analyte at a time, (9) energy use should be minimized, (10) reagents obtained from renewable sources should be preferred, (11) toxic reagents should be eliminated or replaced, (12) operator safety should be increased [81].

As shown in Figure 6a, according to the analysis carried out using AGREE, the analytical methodology for the detection and quantification of FU in foodstuffs samples has a score of 0.74, with the greatest limitations being criteria 1, 3, 5 and 10, which relate to the automation of the method, the possibility of direct online or *in-situ* analysis, and the analysis of multi-analytes.

The free software AGREEPrep is a very similar tool to AGREE, but it provides an assessment based on the principles of green sample preparation. The criteria evaluated, also using a metric ranging from 0 to 1, with 1 being the highest point on the scale, and also using a colour scale representation, are the ten principles of green sample preparation, namely (1) favor *in situ* sample preparation, (2) use safer solvents and reagents, (3) target sustainable, reusable and renewable materials, (4) minimize waste, (5) minimize quantities of samples, chemicals and materials, (6) maximize yield of the sample, (7) integrate steps and promote automation, (8) minimize energy consumption, (9) choose the most environmentally friendly post-sample preparation setup possible for analysis and (10) ensure safe procedures for the operator [82].

For the evaluation of sample preparation, as shown in Figure 6b, the value assigned to this study's methodology is relatively good, standing at 0.83 on a scale of 0 to 1. The limitations of the methodology, once again, lie in the lack of automation of the sample preparation and analysis stages, the lack of the possibility of carrying out analyses without any type of intervention on the sample and the limitation of the technique in integrating different sample preparation stages, automating the process, factors assessed in criteria 1, 3 and 7.

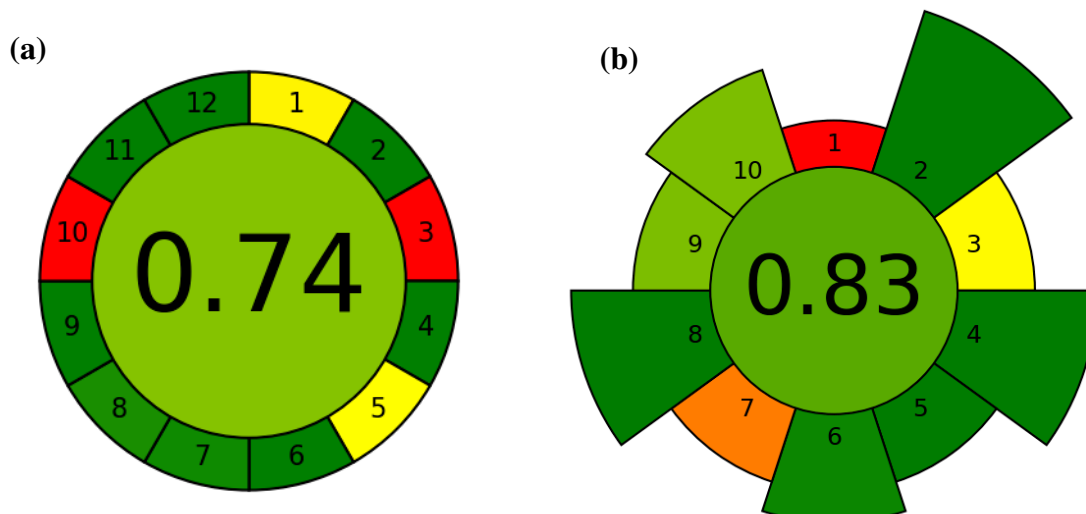


Figure 6: Pictograms representing the analytical methodology foodstuffs and the sample preparation foodstuffs analysis. In (a) the analytical methodology pictogram shows the color indications, on a scale where green represents value 1 and red 0, for each of the green chemistry principles for chemical analysis from 1 to 12. In (b) in a similar way, the sample preparation pictogram presents the color periodicities, on a scale where green represents the value 1 and red 0, for each of the principles of green chemistry for the sample preparation steps from 1 to 10.

3. Conclusions

In this work, a simple, low-cost, fast methodology with fewer sample handling steps for the determination of FU in foodstuffs was presented. The use SWV combined with p-AgSAE proved to be effective for detection at micromolar levels of FU in complex samples, even in a methodology where interferences are not minimized. Mathematical modeling based on Hill's mathematical model was used to define the analytical parameters and recovery calculations. The methodology was applied to samples of dairy foods, non-alcoholic beverages, honeys from different flowers and baby foods. Among the samples evaluated in this study, it should be noted that there is current legislation for the control of furfural quantification in baby foods by the European Food Safety Authority (EFSA), where the methodologies capable of carrying out the analysis must have an LOQ of less than 5 $\mu\text{g/kg}$ [64], since the LOQ for this work was equal to 1.28 $\mu\text{g/kg}$, it is possible to carry out quality control using the methodology developed.

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Disclosure statement

This article does not contain any studies with human or animal subjects. No potential conflict of interest was reported by the authors.

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