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A new voltammetric method for the simultaneous determination of the antioxidants TBHQ and BHA in biodiesel using multi-walled carbon nanotube screen-printed electrodes

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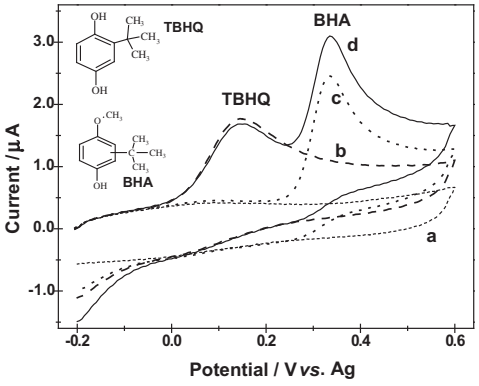
HIGHLIGHTS

- Use of modified screen-printed electrodes to simultaneous detection of antioxidants.
- Methodology for simultaneous determination of TBHQ and BHA in micelar medium.
- The electrochemical system was easily adapted for on-site analysis.

GRAPHICAL ABSTRACT

Cyclic voltammograms obtained on SPE–MWCNT surface for, (a) blank: BR buffer 0.04 mol L^{-1} (pH 2.0) containing 2.0% of methanol; (b) TBHQ 2.0×10^{-5} mol L^{-1}; (c) BHA 2.0×10^{-5} mol L^{-1}; (d) mixture of TBHQ and BHA in the concentration of 2.0×10^{-5} mol L^{-1}. Scan rate of 150 mV s^{-1}.



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ABSTRACT

An electroanalytical method for the simultaneous detection and quantification of *tert*-butylhydroquinone (TBHQ) and butyl hydroxyanisole (BHA) in biodiesel was developed using a voltammetric technique and screen-printed electrodes. The supporting electrolyte solution was composed of Britton–Robinson buffer (0.04 mol L^{-1}) containing methanol (2.0%) and the cationic surfactant cetyltrimethylammonium bromide (CTAB). In the presence of CTAB, the peak current intensity and voltammetric resolution increased significantly for both studied antioxidants. Using the optimized conditions, the method presented a linear response in the concentration range of 5.0×10^{-7}–1.0×10^{-5} mol L^{-1} for TBHQ ($r = 0.999$) and BHA ($r = 0.999$) with detection limits of 3.40×10^{-7} mol L^{-1} and 1.76×10^{-7} mol L^{-1}, respectively. The proposed method was successfully applied for the quantification of TBHQ and BHA in biodiesel samples after a simple and rapid dilution with recoveries from 97.90% to 110.0% and 91.90% to 101.0%, respectively. The obtained results were satisfactory when compared with those obtained using high-performance liquid chromatography (HPLC). Additionally, the proposed method can be easily adapted for on-site analysis.

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1. Introduction

Biodiesel, being a renewable energy source that is biodegradable and simple to use, is a potential substitute for petroleum-derived diesel and can be used in compression–ignition diesel engines with little or no modification [1–5]. Furthermore, it is environmentally friendly due to its lower levels of carbon monoxide emissions, sulfur compounds, polyaromatics, particulate matter and unburned hydr ocarbons [6–8]. However, because of its chemical structure biodiesel is highly susceptible to oxidation in the presence of air, leading to the formation of corrosive acids and deposits that can damage diesel engine components during storage [2,5,8–13].

Stability is a criterion for evaluating the quality of biofuel and is affected by storage conditions, including exposure to air, light, high temperatures, hydroperoxides and the presence of extraneous contaminants. Poor stability can have catalytic effects on oxidation, making biodiesel unstable [10–13]. Therefore, resistance to degradation is an essential requirement for the successful development and viability of alternative fuels, such as biodiesel [2,5,8–13]. For this reason, several policies have been established by governments in Europe (EN 14214) and the United States (ASTM D 6751) for biodiesel quality [2,8–12]. The European standard, EN 14112, recommends that stability be measured using the Rancimat induction period method, which allows investigation of the effectiveness of antioxidants [8–13]. Consequently, the addition of antioxidants at appropriate concentrations protects the product during storage [8–17]. Studies have shown that the synergistic effect of a combined formulation containing *tert*-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) (500 mg L^{-1} for each antioxidant) provided greater oxidative stability for biodiesel [14,15]. Therefore, analytical methods must be able determine the presence of these antioxidants in biodiesel because they can be used to distinguish the quality of the product [18–32].

In the literature, there are various methods for antioxidant detection in different oil samples that use techniques such as high-performance liquid chromatography (HPLC) coupled with different detection systems [19,20], including gas chromatography [21], micellar electrokinetic capillary chromatography [22] and voltammetry [23–32]. However, there are relatively few studies on the determination of antioxidants in biodiesel, among which HPLC with UV/Vis detection [19] and voltammetric [26,27] and amperometric [28,31,33] methods stand out.

Electroanalytical techniques are highly recognized as a favorable tool in the quantification of various organic and inorganic compounds. Moreover, electrochemical instrumentation is compact and portable, allowing on-site analysis and obtaining results in only minutes without preliminary sample treatment. In addition, these methods have a sensitivity comparable to HPLC methods. In the development of electrochemical devices, screen-printed electrodes can combine ease of use and practicality with simple and inexpensive fabrication. These devices are ideally used for voltammetric measurements using portable electrochemical instrumentation [34–39].

Screen-printed electrodes and carbon nanotubes are an attractive combination and present advantages such as superior chemical and mechanical properties (i.e. facilitate electron transfer between the electroactive species and the electrode), reproducibility, reduced size, ease of mass production, disposability, practicality and ability to be miniaturized to reduce consumption of sample and electrolyte [40,41]. It also has to be taken into consideration that screen-printed electrodes modified with carbon nanotubes improve electronic transfer properties and are described as useful electroanalytical tools for the development of analytical applications [40–43], resulting in high sensitivity and low detection limits as well as decreased overpotentials [44].

Another important aspect related to the development of electroanalytical methods is the use of surfactants. This method has been described in the literature as a successful strategy for overcoming adsorption interferences by protecting the electrode surface and stabilizing the electrochemical signal, enhancing the electron transfer rate and improving the detection limits [45–54]. It was shown [47,50,52–54] that surfactants could be used to stabilize voltammetric response and that this stabilization depended on the concentration and nature of the amphiphilic substance, which could influence not only the shape of the electrochemical signal but also parameters such as displacement of potential, transfer coefficients and stability of the intermediate species.

The present work therefore aims to apply multi-walled carbon nanotube modified screen-printed electrodes (SPE–MWCNT) as a working electrode to simultaneously detect TBHQ and BHA antioxidants in biodiesel samples. The work combines linear sweep voltammetry (LSV), using portable equipment, and the simplicity of screen-printed electrodes to develop a rapid and simple eletroanalytical method amenable to use on-site analysis.

2. Experimental

2.1. Chemicals, reagents and standard solutions

Butyl hydroxyanisole (BHA), 96%, and *tert*-butylhydroquinone (TBHQ), 97%, were purchased from Acros Organics (USA) and prepared by dissolving suitable amounts in ethanol (99.6%, Dinamica, Brazil). Diluted working standard solutions were prepared in ethanol by diluting the standard stock solutions (1.0×10^{-3} and 1.0×10^{-2} mol L^{-1}). All reagents were of the highest available grade and used without further purification.

Britton–Robinson (BR) buffer was used for the supporting electrolyte and was prepared by mixing acetic acid (99.7%, Acros Organics, USA), boric acid (99.9%, Acros Organics, USA), and orthophosphoric acid (85%, Acros Organics, USA) (all at 0.04 mol L^{-1}). Appropriate volumes of sodium hydroxide (0.50 mol L^{-1}, Acros Organics, USA) were used to adjust the pH.

Solutions of the non-ionic surfactant Triton X-100 (Acros Organics, USA), the anionic surfactant sodium dodecyl sulfate (95%, Sigma, USA) and the cationic surfactant cetyltrimethylammonium bromide (SERVA, Germany) were prepared in ultrapure water ($R \geqslant 18.2$ MΩ cm).

For chromatographic analysis, methanol (HPLC grade, J.T. Baker, USA) and ultrapure water were used throughout the experiment. For acidification of the aqueous mobile phase, glacial acetic acid (Suprapure, Vetec, USA) was employed.

2.2. Apparatus and electrodes

Electrochemical measurements were performed using a PalmSens portable electrochemical instrument (Palmsens BV, Houten, The Netherlands) controlled via PSLite 1.7.3 software. The working electrode consisted of a device with a planar carboxyl functionalized (SPE–MWCNT), a planar carboxyl functionalized single-walled carbon nanotube screen-printed electrode (SPE–SWCNT) and a carbon screen-printed electrode (SPE-C), all with surface areas of 12.57 mm^2. These systems were composed of printed carbon as the counter electrode and silver as the pseudo-reference electrode (DropSens, Oviedo, Spain).

The HPLC system (from Shimadzu) consisted of multichannel pumps (LC 20AT), an autosampler (SIL 20A), a degasser (DGU 20A5) and a diode array detector (DAD SPD 20A). The separation of antioxidants was performed using a reversed phase column (C-18, 150 × 4.6 mm i.d. 5 μm) from Agilent HP.

The pH adjustment was made using a combined glass electrode (BlueLine, Shott) connected to a digital pH-meter (model Tecnopon/MPA-20) and deionized water purified using a Milli-Q Plus system (Millipore). An ultrasonic cleaner (model USC 1400, Unique) was used for dissolution of the reagents and sample preparation. In addition, a micropipette (Labmate) was used for solution preparation and to transfer the reactant solution to the cell. A centrifuge (Certomat – Modelo II) was used to prepare the sample before analysis.

2.3. Procedure for voltammetric analysis

A 9.8 mL aliquot of the supporting electrolyte solution (BR buffer, 0.04 mol L^{-1}) and 0.2 mL of methanol were added to the electrochemical cell (adapted to accept the screen-printed carbon electrode). The solution was mixed and then left to rest for 15 s, at which point a background voltammetric curve was recorded. Afterwards, an aliquot of the surfactant solution (5.0×10^{-3} mol L^{-1}) was added to the cell with stirring for 2.0 min. The stirring was stopped, and after a 10 s rest, cyclic or linear sweep voltammograms were recorded. An aliquot of the antioxidant solution was then added to the cell, and the voltammogram was again recorded for the same SPE–MWCNT. Although SPE–MWCNT was described as disposable, it was possible to repeatedly employ the same electrode for at least 30 measurements. To reuse the electrode, the SPE–MWCNT surface was cleaned with ethanol (99.6%, Dinâmica, Brazil) and left to rest for approximately 60 s between measurements. This cleaning method did not cause loss of the peak current over time.

2.4. Preparation and analysis of biodiesel samples

Antioxidant-free samples of soybean biodiesel (produced by Laboratório de Combustíveis de Mato Grosso do Sul) were spiked with TBHQ and BHA at different ratios (Table 2) and vigorously agitated. Each spiked sample was then diluted in ethanol using calibrated flasks to achieve a final concentration of approximately 10% (biodiesel:ethanol, v/v). A 40 μL aliquot of the resulting solution was transferred into the electrochemical cell containing 9.8 mL of BR buffer (0.04 mol L^{-1}) and 0.20 mL of methanol and left to stir for 1.0 min. The resulting sample was then directly analyzed, recording linear sweep voltammograms. Then, the TBHQ and BHA present in the biodiesel samples were quantified using the standard addition method.

2.5. Chromatographic conditions

The chromatographic conditions for the determination of TBHQ and BHA using methodologies previously proposed by Tagliabue [19] and Takemoto [55] were used with some modifications. The HPLC analysis consisted of a gradient mode with methanol (solvent A) and water containing 1.0% acetic acid (solvent B) at a flow rate of 1.0 mL min^{-1} at 30 °C. The elution started with 55% of methanol between 0 and 3.0 min, 55–100% of methanol between 3.0 and 6.0 min and was maintained at 100% methanol between 6.0 and 12.0 min. Then, the proportion of methanol returned to 55% between 12.0 and 13.0 min, remaining at this composition until 18.0 min.

For chromatographic measures, appropriate volumes of the stock solutions (500 mg L^{-1}) and 500 μL of commercial antioxidant-free soy biodiesel were diluted with methanol in volumetric flasks (10 mL). Calibration curves were constructed using concentrations from 6.0×10^{-6} to 3.0×10^{-4} mol L^{-1} and from 5.5×10^{-6} to 2.8×10^{-4} mol L^{-1} for TBHQ and BHA, respectively. Prior to injection (25 μL), all samples and standards were filtered through a 0.45-μm cellulose membrane filter (Sartorius Biotec).

3. Results and discussion

3.1. Voltammetric studies of TBHQ and BHA at the SPE–MWCNT

The TBHQ and BHA antioxidants are slightly soluble in aqueous medium, but optimum results involving its solubility and voltammetric response were obtained using a mixture containing BR buffer (pH 2.0) + 2.0% of methanol. Although no significant difference in performance has been observed for methanol and ethanol, we chose methanol following a previously published method for improved antioxidant solubility [23–27,45]. Fig. 1 shows the cyclic voltammograms obtained using different kinds of screen-printed electrodes under the optimized conditions. A shift of 50 mV for BHA and 90 mV for TBHQ to less positive potentials in the recorded voltammograms using SPE–MWCNT (Fig. 1d) was observed. The peak current intensity measured using this electrode was also twice those obtained with SPE-C (Fig. 1b) and SPE–SWCNT (Fig. 1c). This voltammetric behavior indicated that the SPE–MWCNT electrode could be a simple tool for antioxidant detection and its can be exploited for new eletroanalytical techniques.

Fig. 2 compares typical cyclic voltammograms obtained on the SPE–MWCNT surface for electrochemical oxidation of TBHQ and BHA (2.0×10^{-5} mol L^{-1}) in BR buffer (pH 2.0) containing 2.0% methanol. As observed, TBHQ and BHA exhibited well-defined anodic peaks at 0.14 V (Fig. 2b) and 0.33 V (Fig. 2c), respectively, similar to that observed previously with different electrodes [23–27,29–32]. In Fig. 2d, similar behavior for the simultaneous detection of BHA and TBHQ was observed, where electrooxidation of the antioxidants produced two well-defined anodic peaks at 0.14 and 0.33 V (vs. silver pseudo-reference from SPE–MWCNT), respectively. These results, together with electrochemical information for similar compounds [24], indicated that the first peak at 0.14 V (for TBHQ) was due to the oxidation of the hydroquinone group to quinone in a two-electron process. The second peak at 0.33 V (for BHA) was attributed to the oxidation of the hydroxyanisole group to quinine in a two-electron process. No cathodic peaks were observed in the reverse scan, indicating these processes were irreversible, as previously reported [24]. For analytical purposes, the anodic peaks at 0.14 and 0.33 V for BHA and TBHQ (Fig. 2d), respectively, had satisfactory performance. Thus, parameters affecting the voltammetric technique were optimized.

To use a straightforward array of electrodes and improve the analytical performance and detection limit of the target

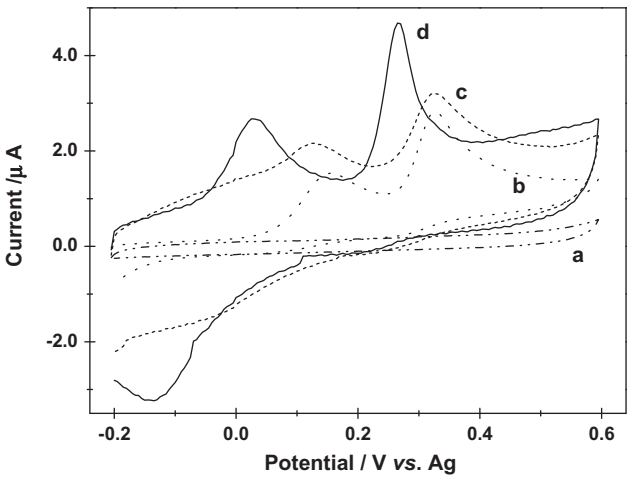

Fig. 1. Cyclic voltammograms obtained on different screen-printed electrode surfaces. (a) Blank: BR buffer 0.04 mol L^{-1} (pH 2.0) containing 2.0% methanol. A mixture of TBHQ and BHA at 3.0×10^{-5} mol L^{-1} on (b) SPE-C, (c) SPE–SWCNT and (d) SPE–MWCNT electrodes. Scan rate of 150 mV s^{-1}.

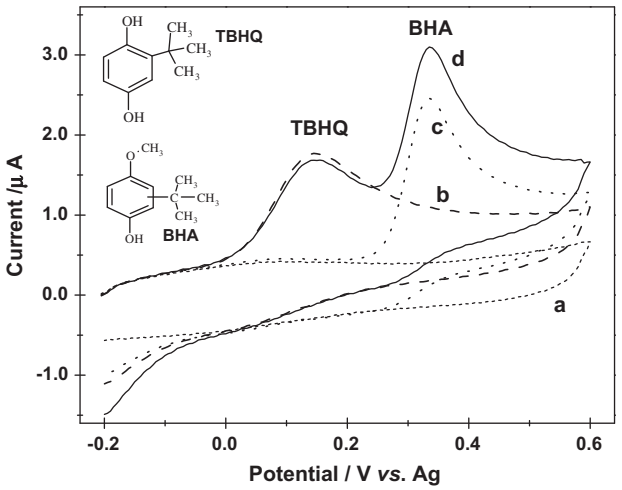

Fig. 2. Cyclic voltammograms obtained on SPE–MWCNT for (a) blank: 0.04 mol L^{-1} BR buffer (pH 2.0) containing 2.0% methanol; (b) 2.0×10^{-5} mol L^{-1} TBHQ; (c) 2.0×10^{-5} mol L^{-1} BHA; (d) mixture of TBHQ and BHA at 2.0×10^{-5} mol L^{-1}. Scan rate of 150 mV s^{-1}.

antioxidants, further experiments were carried out using differential pulse voltammetry (DPV, Fig. 3a), linear sweep voltammetry (LSV, Fig. 3b) and square-wave voltammetry (SWV, Fig. 3c) using SPE–MWCNT as the working electrode. Under the same experimental conditions, LSV showed more satisfactory analytical performance in terms of voltammetric resolution and profile, which were evaluated in the width of the peak. Therefore, because the levels of TBHQ and BHA expected in biodiesel samples are 500 mg L^{-1}, there is no need for highly sensitive techniques such as DPV or SWV. Consequently, the LSV technique was chosen for further measurements and several parameters inherent to the technique were optimized.

The antifouling capacity of tensoactive agents was also tested by recording successive cyclic and linear sweep voltammograms for the oxidation of the tested antioxidants in the presence and absence of anionic, neutral and cationic surfactants. According to the literature [26,27,29], both electroanalytical sensitivity and organic compound solubility can be increased using surfactants. Therefore, the effect of different surfactants on the peak current intensity, the solubility and the antifouling capacity in electrooxidation of TBHQ

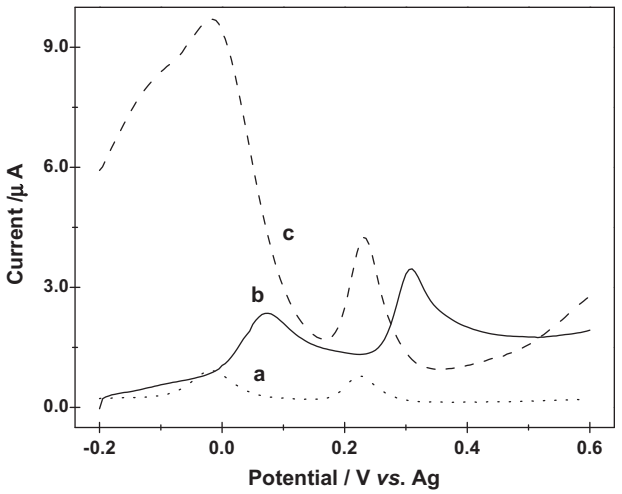

Fig. 3. (a) DPV, (b) LSV and (c) SWV obtained on a SPE–MWCNT surface in 0.04 mol L^{-1} BR buffer (pH 2.0) containing 2.0% methanol in a mixture of TBHQ and BHA at 2.0×10^{-5} mol L^{-1}. E_{step} = 3 mV, frequency = 20 Hz, amplitude = 10 mV, and scan rates of 150 mV s^{-1} (LSV) and 3 mV s^{-1} (DPV).

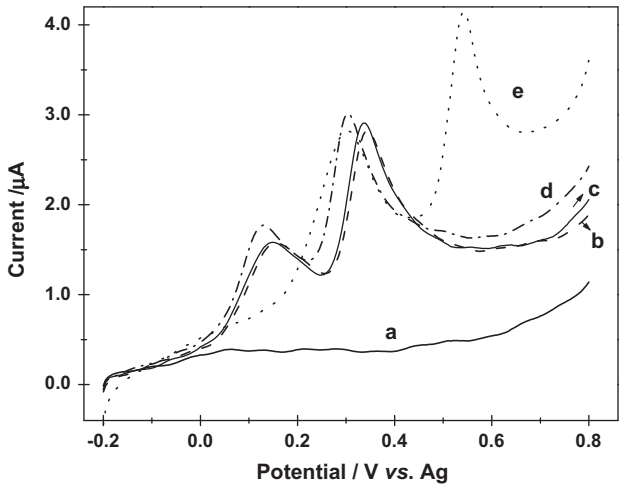

Fig. 4. Linear sweep voltammograms obtained on a SPE–MWCNT surface for (a) blank (0.04 mol L^{-1} BR buffer at pH 2.0 containing 2.0% methanol), (b) 2.0×10^{-5} mol L^{-1} TBHQ and BHA in the absence of surfactant, (c) 2.0×10^{-5} mol L^{-1} TBHQ and BHA in the presence of 1.0×10^{-4} mol L^{-1} of the surfactant TX-100; (d) 2.0×10^{-5} mol L^{-1} TBHQ and BHA in the presence of 1.0×10^{-4} mol L^{-1} of the surfactant SDS; (e) 2.0×10^{-5} mol L^{-1} TBHQ and BHA in the presence of 1.0×10^{-4} mol L^{-1} of the surfactant CTAB. Scan rate of 150 mV s^{-1}.

and BHA (2.0×10^{-5} mol L^{-1}) was monitored by recording voltammograms in BR buffer (pH 2.0) containing 2.0% methanol.

The background voltammograms recorded for the antioxidants in the presence of the neutral surfactant Triton X-100 (TX-100), the anionic surfactant sodium dodecyl sulfate (SDS, data not shown) and the cationic surfactant cetyltrimethylammonium bromide (CTAB) (Fig. 4a) did not exhibit any oxidation peaks that could interfere in the selective detection of the antioxidants of interest. At the same time, comparing the voltammograms recorded for 2.0×10^{-5} mol L^{-1} TBHQ and BHA in the absence of surfactant (Fig. 4b) and in the presence of the nonionic surfactant Triton X-100 (1.0×10^{-4} mol L^{-1}, Fig. 4c) and the anionic surfactant SDS (1.0×10^{-4} mol L^{-1}, Fig. 4d) did not significantly change the voltammetric behavior or oxidation peak intensity. As observed (Fig. 4e), a better resolution of the voltammetric behavior of the

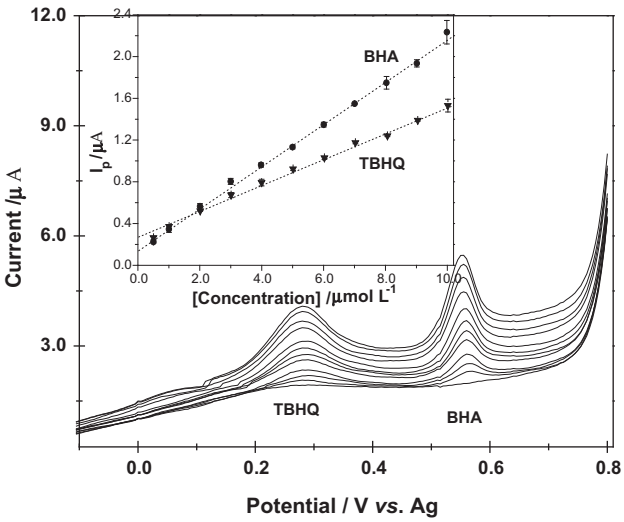

Fig. 5. Linear sweep voltammograms registered on a SPE–MWCNT surface at a scan rate of 150 mV s^{-1} for TBHQ and BHA concentrations increasing from 5.0×10^{-7} to 1.0×10^{-5} mol L^{-1}. Conditions: 0.04 mol L^{-1} BR buffer at pH 2.0 containing 2.0% methanol and 5.0×10^{-4} mol L^{-1}CTAB. Inset: analytical calibration for the dependence of the peak current on the TBHQ and BHA concentrations. Scan rate of 150 mV s^{-1}.

Table 1
Analytical parameters obtained in the calibration curves for TBHQ and BHA using the proposed method and compared with reference methods.

Parameters/methods	Linear range (mg L^{-1})	Intercept[a]	Slope[b]	R	LOD (mol L^{-1})	LOQ (mol L^{-1})
Proposed method						
TBHQ	$(0.5–10)\times10^{-6}$	2.70×10^{-7}	1.26×10^{-1}	0.999	3.41×10^{-7}	1.14×10^{-6}
BHA	$(0.5–10)\times10^{-6}$	1.73×10^{-7}	1.99×10^{-1}	0.999	1.76×10^{-7}	5.93×10^{-7}
HPLC method						
TBHQ	$(0.6–30)\times10^{-5}$	−5500	3.65×10^{9}	0.999	1.70×10^{-6}	5.50×10^{-6}
BHA	$(0.6–28)\times10^{-5}$	−17763	3.70×10^{9}	0.999	1.00×10^{-5}	4.25×10^{-5}
Reported method [33]						
TBHQ	NR	NR	NR	NR	1.90×10^{-7}	NR
BHA	NR	NR	NR	NR	4.00×10^{-8}	NR

NR: Not reported.
[a] Units: (A = ampere) for electroanalytical technique; (mAU) for the HPLC technique.
[b] Units: (A = L mol^{-1}) for electroanalytical technique; (mAU L mol^{-1}) for the HPLC technique.

Table 2
Recoveries of TBHQ and BHA from soybean biodiesel samples using the standard addition method.

Sample	Analyte	Spiked (mg L^{-1})	Found[a] (mg L^{-1}) ± sd	Recovery (%)	RSD (%)
A	TBHQ	670	69309 ± 7.10	103.6	1.00
	BHA	341	343.4 ± 3.70	100.7	
B	TBHQ	670	675.4 ± 3.60	100.8	0.50
	BHA	362	350.2 ± 4.50	96.70	
C	TBHQ	674	697.3 ± 11.0	103.5	1.60
	BHA	338	340.5 ± 6.45	98.80	
D	TBHQ	330	365.9 ± 5.85	110.0	1.65
	BHA	642	589.7 ± 8.40	91.90	
E	TBHQ	364	369.3 ± 8.25	101.5	2.20
	BHA	667	672.2 ± 7.30	100.8	
F	TBHQ	347	353.1 ± 9.50	101.8	2.70
	BHA	638	603.5 ± 13.0	94.60	
G	TBHQ	482	488.6 ± 4.50	103.0	0.90
	BHA	497	489.1 ± 11.2	98.40	
H	TBHQ	500	489.5 ± 13.1	97.90	2.65
	BHA	501	476.1 ± 5.12	95.05	
I	TBHQ	487	492.9 ± 4.60	101.2	0.95
	BHA	501	506.2 ± 4.52	101.0	

[a] Average of three determinations; sd: standard deviation for average of the three determinations; RSD: relative standard deviation for the average values.

antioxidants was observed when CTAB was added to the electrochemical cell containing 2.0×10^{-5} mol L^{-1} of the target antioxidants. This behavior indicated that the addition of surfactant could improve the detectability and solubility of antioxidants in aqueous/organic medium. Despite shifts of 160 mV for TBHQ and 200 mV for BHA to more positive potentials in the recorded voltammograms for the neutral and anionic surfactants (Fig. 4c and d), there were no other changes in the voltammetric behavior that compromised the electrochemical method. The increase in current intensity and the displacement of the potential in the presence of surfactant might be related to BCTA interacting with the surface of the electrode, facilitating interactions with the analytes [47]. The current intensity, position and baseline current were constant in all the recorded voltammograms for each new electrode and were not compromised by the proximity of the supporting electrolyte discharge. This behavior was attractive for electroanalytical purposes.

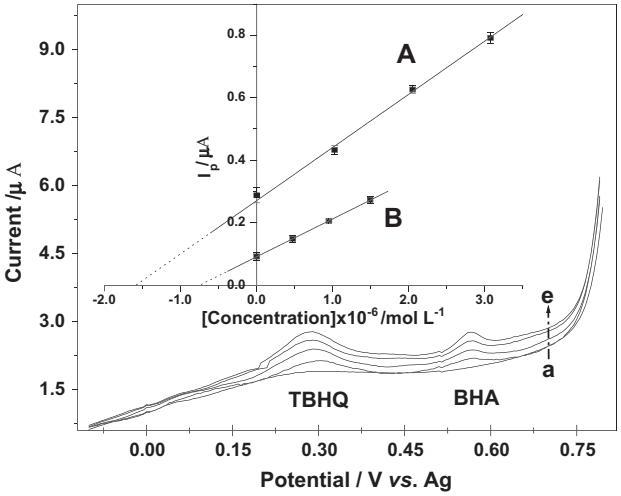

Fig. 6. Linear sweep voltammograms registered on a SPE–MWCNT surface using the LSV technique at a scan rate of 150 mV s^{-1} for the determination of TBHQ and BHA. (a) Antioxidant-free sample of soybean biodiesel, (b) biodiesel sample containing 670 mg L^{-1} of TBHQ and 341 mg L^{-1} of BHA and (c–e) successive additions (30 μL) of TBHQ (694 mg L^{-1}) and BHA (349 mg L^{-1}). Other conditions were as described in Fig. 5.

3.2. Effect of pH

The effect of pH on the voltammetric response was monitored using peak current (I_p) and peak potential (E_p) in linear sweep voltammograms of TBHQ (4.91×10^{-5} mol L^{-1}) and BHA (5.35×10^{-5} mol L^{-1}) using individual SPE–MWCNT electrodes and an electrolyte solution containing 0.04 mol L^{-1} BR buffer with 2.0% methanol in the presence of CTAB (1.05×10^{-4} mol L^{-1}) at pH values from 2.0 to 10. The peak current and peak potential were strongly pH-dependent for both studied antioxidants. Accordingly,

Table 3
Recoveries for TBHQ and BHA from soybean biodiesel samples analyzed using an HPLC method.

Sample	Biodiesel A		Biodiesel B		Biodiesel C	
Analytes	TBHQ	BHA	TBHQ	BHA	TBHQ	BHA
Spiked (mg L^{-1})	513	503	666	333	333	666
Found[a] (mg L^{-1}) ± sd	518 ± 0.88	502 ± 0.60	629 ± 0.44	321 ± 0.26	309 ± 0.40	661 ± 1.98
Recovery (%)	101.0	99.80	94.40	96.40	93.00	99.30
RSD (%)	0.17	0.12	0.07	0.08	0.13	0.30

[a] Average of three determinations; sd: standard deviation for the average of three determinations; RSD: relative standard deviation for the average values.

the peak current intensity for TBHQ became significant only at pH values of 2.0 and 8.0. For BHA, the relationship between $\boldsymbol{I_p}$ and pH was more complicated. Initially, the maximum peak current intensity was obtained at pH values lower than 3.0, but then the intensity decreased continuously as the pH rose to 6.0. Next, the peak current increased again as the pH increased up to 10. Although the peak current intensity for TBHQ was higher at pH 4.0, the intensity for BHA was markedly decreased at this pH value. Thus, comparing the peak current values, shapes and reproducibility for the recorded voltammograms, the most favorable conditions for simultaneously detection of the antioxidants was obtained with Britton–Robinson buffer at pH 2.0 containing 2.0% methanol and 1.05×10^{-4} mol L^{-1} CTAB.

The plot of $\boldsymbol{E_p}$ vs. pH for TBHQ and BHA showed that the peak potential ($\boldsymbol{E_p}$) was shifted in the negative direction when the pH increased, indicating that the process was influenced by protonation [53] even in the presence of the cationic surfactant. A linear relationship was observed for TBHQ and BHA following the equations $\boldsymbol{E_p}$ = 0.63–0.05 pH (r = −0.998) and $\boldsymbol{E_p}$ = 0.40–0.06 pH (r = −0.992), respectively. These slopes were in agreement with the theoretical value (59 mV/pH), indicating that the two oxidation processes of TBHQ and BHA occurred with the involvement of two electrons and two protons [24].

3.3. Influence of CTAB concentration

The influence of the surfactant CTAB on the peak current intensity and voltammetric behavior was investigated by monitoring the oxidation of TBHQ (2.0×10^{-5} mol L^{-1}) and BHA (1.44×10^{-5} mol L^{-1}) at various concentrations from 0 to 2.2×10^{-3} mol L^{-1}. This surfactant concentration range was chosen from values below and above the critical micelle concentration (CMC), which in aqueous solutions is approximately 9.2×10^{-4} mol L^{-1} [53]. Increasing CTAB concentration increased the anodic current peak for both target antioxidants, with the maximum anodic currents at the concentration of 5.0×10^{-4} mol L^{-1}. This was followed by a dramatic decrease in peak intensity at higher concentrations. The results indicated that up to 5.0×10^{-4} mol L^{-1} of surfactant could achieve the critical micellar concentration (CMC) for micellar aggregates. Consequently, the surfactant was able to affect the diffusion coefficient and electron transfer, influencing the mass transport of electroactive substances to the electrode surface. As a result, a gradual decrease in the peak current was observed. Under these conditions, the concentration of 5.0×10^{-4} mol L^{-1}, for CTAB, was selected as optimal for TBHQ and BHA detection, which provided improved analytical applicability and the best compromise for current intensity and voltammetric resolution.

3.4. Analytical curve

After defining the optimum experimental conditions for electroanalytical study of TBHQ and BHA, voltammograms were recorded at concentrations from 5.00×10^{-7} mol L^{-1} to 1.00×10^{-5} mol L^{-1} using SPE–MWCNT. The voltammograms were obtained in the presence of antioxidant-free biodiesel samples in B-R buffer (pH 2.0) with 2.0% methanol and 5.0×10^{-4} mol L^{-1} CTAB (Fig. 5). The increase in oxidation peak current was proportional to the increase in concentration for both TBHQ (Fig. 5, inset A) and BHA (Fig. 5, inset B). The limits of detection (LOD) and quantification (LOQ) were estimated using the statistical relations $3 \times \mathbf{Sd}/\boldsymbol{m}$ and $10 \times \mathbf{Sd}/\boldsymbol{m}$, where **Sd** is the standard deviation of the peak current for the blank (measured at the same potential of the target antioxidants) and $\boldsymbol{m}$ is the slope of the analytical curve [56]. The LOD and LOQ were 3.41×10^{-7} mol L^{-1} and 1.14×10^{-6} mol L^{-1}, respectively, for TBHQ, and 1.76×10^{-7} mol L^{-1} mol L^{-1} and 5.93×10^{-7} mol L^{-1}, respectively, for BHA, which clearly indicated the sensitivity and demonstrated that the reported method could be employed outside the laboratory. The LOD when compared with reported methods [33] was satisfactory, considering that the proposed methodology was applied to on-site analysis and was well below the amount of antioxidants in biodiesel samples (Table 1).

3.5. Analytical application

To investigate application to the antioxidants TBHQ and BHA, matrix effects were evaluated using addition-recovery experiments carried out on a biodiesel sample treated in accordance with the procedure described in the experimental section. Initially, biodiesel samples did not exhibit any oxidation signals for the target antioxidants. Next, the biodiesel samples were spiked with different TBHQ and BHA concentrations determined according to the literature studying synergism of these antioxidants [15] and NO_x emission reduction [6]. Fig. 6 depicts typical linear sweep voltammograms for the blank sample without antioxidants (Fig. 6a) and for antioxidant detection after applying the standard addition method (Fig. 6, curves b–e). These voltammograms revealed that matrix effects were absent and that the analytical signal exhibited a well-defined peak for both antioxidant oxidations on the SPE–MWCNT surface. Recoveries ranging from 97.9% to 103.6% for TBHQ and 91.9% to 101% for BHA were obtained using the proposed method (Table 2), indicating satisfactory precision and accuracy. The results obtained with the proposed method demonstrated that the electrochemical technique using SPE–MWCNT could be successfully employed for TBHQ and BHA determination in biodiesel samples in a fast, simple manner without pre-treatment. Furthermore, comparing the obtained results with those obtained using the reference method (Table 3), no significant difference between the reference and the electroanalytical methods was observed. Finally, the statistical calculations for the obtained results were satisfactory, indicating that the electroanalytical method could be applied to the analysis of antioxidants in biodiesel samples.

4. Conclusion

In this work, we showed that the developed electrochemical technique can be used for simultaneous detection of TBHQ and BHA and that it can be successfully applied to quantify these antioxidants in biodiesel samples without pre-treatment. The inclusion

of the cationic surfactant CTAB improved the electroanalytical response for both antioxidants and allowed their detection in spiked biodiesel samples at low concentrations. The satisfactory analytical performance obtained for the proposed methodology was verified by the acceptable recovery rates and the statistical calculations relating the experimental results to HPLC methods. The method is fast and sufficiently sensitive to simultaneously detect and quantify both antioxidants, it can be implemented with simpler, less expensive analytical instrumentation, and it can be performed on-site for routine quality control.

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