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Fast simultaneous determination of BHA and TBHQ antioxidants in biodiesel by batch injection analysis using pulsed-amperometric detection

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ABSTRACT

We report the first amperometric method for the simultaneous determination of butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) in biodiesel using batch-injection analysis (BIA) coupled to pulsed-amperometry. A sequence of potential pulses was selected in order to detect TBHQ and BHA separately in a single injection step at a glassy-carbon electrode. Samples were diluted in 50% v/v hydroethanolic solution with 0.1 mol L^{-1} $HClO_4$ (supporting electrolyte) before injection using an electronic pipette. The method is highly precise (RSD < 1%, $n=10$), fast (170 injections h^{-1}), accurate (recovery between 100 and 110%), presents low detection limits (73 and 75 nmol L^{-1} BHA and TBHQ, respectively), and can be easily adapted for on-site determinations.

1. Introduction

Biodiesel is a renewable and biodegradable fuel which is being currently used in diesel engines mixed with petroleum diesel in blends up to 20% v/v. Although biodiesel is a less pollutant fuel in comparison to diesel oil, the main disadvantage of using and commercializing biodiesel is its low oxidation stability. The oxidation process leads to an increase in the viscosity of biodiesel, corrosion of engine components, and the consequent formation of gums and sediments inside the engine that plug fuel filters [1]. To overcome this drawback, the addition of antioxidants such as tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butyl-hydroxytoluene (BHT) into biodiesel to enhance its oxidation stability have been reported [2–6]. Mixtures of antioxidants have been evaluated and the combination of TBHQ and BHA has proven to be the most effective blend for increasing the oxidation stability of biodiesels [4–6]. Therefore, determination of antioxidants, and eventually, mixtures of antioxidants (BHA and TBHQ) can provide important information on the quality of biodiesel, because the concentration of antioxidants may be related to its oxidation stability.

Different approaches have been proposed to analyze complex samples such as petroleum-based fuels and biodiesels using electroanalysis because of the high electrical resistance of the matrix [7–10]. Electroanalytical methods applied to the determination of antioxidants in biodiesel with minimal sample manipulation (just dilution) have been reported [11–14]. Voltammetry at a mercury-drop electrode [11] and at a carbon-paste electrode in the presence of surfactants [12] has been proposed for TBHQ determination in biodiesel. Amperometric methods using glassy-carbon and boron-doped diamond electrodes for individual determination of TBHQ [13] and BHA [14] in biodiesel were reported, respectively.

The use of multiple-pulse amperometry (MPA) coupled to a flow-injection system (FIA) was recently demonstrated for simultaneous determinations of BHA and BHT in commercial mayonnaise (after a sample preparation step) using a single working electrode [15]. Similarly, associations of FIA with MPA have been reported for simultaneous determinations of pharmaceutical molecules [16–18] and carbohydrates [19], as well as to introduce an internal standard in FIA systems [20].

Batch injection analysis (BIA) is an alternative to FIA, and its association with MPA for simultaneous determinations has recently been presented [21]. In BIA systems, a sample plug is injected directly onto the working electrode surface in a wall-jet configuration, which is immersed in a large-volume blank solution [22]. The simplicity of operation brings advantages over FIA systems, such as elimination of pumps and valves (easily adapted for portable applications) and reduced volume of carrier solutions [23].

In the present work, we report an application of BIA using pulsed-amperometric detection in hydroethanolic medium for the rapid, direct, and simultaneous determination of the phenolic antioxidants BHA and TBHQ in biodiesel fuel samples. To our knowledge, the simultaneous determination of two antioxidants

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is only possible using voltammetric techniques [24–26], and previously has been exclusively applied to food samples after a sample preparation step [15,24–28].

2. Material and methods

2.1. Reagents and biodiesel samples

All solutions were prepared with deionized water ($R \geq 18\ M\Omega\ cm$) obtained from a Direct-Q3 water purification system (Millipore, Bedford, MA, USA). Analytical grade perchloric acid (70% m/v), acetic acid (65% m/v), phosphoric acid (85% m/v), sodium acetate and potassium nitrate were obtained from Vetec (Rio de Janeiro, Brazil), and were used without further purification. Butylated hydroxyanisole (BHA, 98.5% m/m) was purchased from Synth (Diadema, Brazil) and tert-butylhydroquinone (TBHQ, 97% m/m) from Acros Organics (USA). Working standard solutions were prepared immediately before use by appropriate dilution of the stock solution. A standard stock solution containing BHA and TBHQ (both at 54 g L^{-1}) was prepared in ethanol. Methylic biodiesel samples produced from soybean oil were obtained from a local factory. Different amounts of BHA and TBHQ were added to the biodiesel samples just after its acquisition.

2.2. Instrumentation

All electrochemical recordings were performed using a μ-Autolab Type III potentiostat (Metrohm Autolab B. V., Utrecht, The Netherlands) controlled by GPES4.9.007 software. The three-electrode configuration included a glassy carbon (Ø=1.5 mm, CH instrument, Austin, TX, USA) working electrode, a platinum wire as a counter electrode, and a miniaturized Ag/AgCl/saturated KCl electrode [29] as a reference electrode.

2.3. Procedures

Cleaning of the glassy carbon electrode was performed mechanically on a felt-polishing pad using an alumina powder suspension (0.3 μm) followed by a copious rinsing with deionized water. This procedure was performed only once at the beginning of the workday.

Injections of standard solutions or diluted samples were carried out using an Eppendorf electronic micropipette (Multipette® stream), which permits injections from 10 to 1000 μL (using a 1 mL Combitip®) at a programmable dispensing rate (from 28 to 250 μL s^{-1}). A homemade BIA cell developed by our research group was used for this work [30]. The BIA cell consists of a 180-mL glass cylinder (internal diameter=7 cm) and two polyethylene covers, which were firmly fitted on the top and bottom of the cylinder. The top cover contained 3 holes for the counter and reference electrodes and for the micropipette tip (external diameter=6.06 mm). The micropipette tip was firmly introduced into the hole (diameter=6.1 mm) in the center of the cover in such a way that the injection procedure was highly precise. The bottom cover contained a single hole (precisely located at the center of the cover) in which the working glassy-carbon electrode (GCE) was inserted. The micropipette tip was positioned approximately 2 mm from the working electrode surface in a wall-jet configuration.

BHA and TBHQ (or mixtures of BHA and TBHQ) standard solutions used to construct analytical curves were prepared in 50% (v/v) hydroethanolic solution containing 0.1 mol L^{-1} $HClO_4$ (final electrolyte concentration).

Biodiesel samples were diluted 40-fold in ethanol and then 100-fold diluted in the 50% (v/v) hydroethanolic electrolyte (containing 0.1 mol L^{-1} $HClO_4$) before injection by the electronic pipette. The same hydroethanolic electrolyte (v=180 mL) was added to the BIA cell.

Hydrodynamic voltammograms of BHA and TBHQ were obtained separately by application of ten sequential potential pulses (from +0.3 to +1.2 V, 100 ms each) for triplicate injections of standard solutions through the BIA system using the multiple-pulse amperometric technique. The same technique was used for simultaneous amperometric detection of BHA and TBHQ, applying 0.6 V (for 100 ms) and 1.0 V (for 100 ms) continuously (vs. Ag/AgCl/ saturated KCl). The sequence of two pulses (0.6 and 1.0 V) was applied continuously, and the current was sampled once at the end of each potential pulse. Thus, the current was sampled in each amperogram every 200 ms (total time of the potential waveform). As each transient signal in the proposed BIA system spent 8 s to return to baseline, the current was sampled around 40 times during each current peak.

All electrochemical measurements were performed at room temperature, in the presence of dissolved oxygen.

2.4. HPLC analysis

HPLC measurements were performed using a Shimadzu LC-10VP chromatograph equipped with a UV/VIS detector (SPD-10AV), LC column (Lychrispher 100 A° RP18-C18, 250 × 4.6 mm, 5 μm), column oven (CTO-20A), degasser (DGU-20A5), small auto-injector, and pump (LC-10AD-VP). The mobile phase was composed of acetonitrile and water (75:25 v/v) at pH 2.1 (adjusted with phosphoric acid), and the flow rate was 1.0 mL min^{-1}. The detector was fixed at 280 nm. The retention times were 3.1 and 3.9 min for TBHQ and BHA, respectively. Aliquots of biodiesel samples were diluted in the mobile phase before injection.

3. Results and discussion

Previous investigations of the electrochemistry of phenolic antioxidants (such as BHA and TBHQ) using different carbonaceous materials (glassy-carbon and BDD electrodes) have demonstrated that acidic media provide the best performance for electrochemical oxidation [13–15,25–27]. The electrochemical oxidation of both BHA and TBHQ involves a two-electron process generating a respective quinone intermediate [25–27]. The electrode processes for both molecules were found to be controlled by mass transport [26,27]. It was also noted that a hydroethanolic solution containing at least 50% (v/v) ethanol provided accurate responses for individual determinations of the phenolic antioxidants because of the low solubility of BHA and TBHQ in water, and the poor immiscibility of biodiesel samples with aqueous solutions [13,14]. For this reason, a hydroethanolic solution containing 50% (v/v) ethanol with 0.1 mol L^{-1} $HClO_4$ (final concentration) was used as a supporting electrolyte in this work.

In order to identify the potential pulses to perform simultaneous determinations of BHA and TBHQ, hydrodynamic voltammograms were first obtained separately for BHA and TBHQusing multiple-pulse amperometry with the BIA system. Ten sequential potential pulses of 100 ms each (0.30; 0.40; 0.50; 0.60; 0.70; 0.80; 0.90; 1.00; 1.10 and 1.20 V) were applied continuously. The current at each potential pulse was monitored continuously during three injections of 30 μmol L^{-1} BHA (●). The same procedure was performed during triplicate injections of 30 μmol L^{-1} TBHQ (▲). The average current peak (n=3) at each potential pulse was measured and used to construct a hydrodynamic voltammogram for the electrochemical oxidation of BHA and TBHQ (Fig. 1). A similar strategy was used

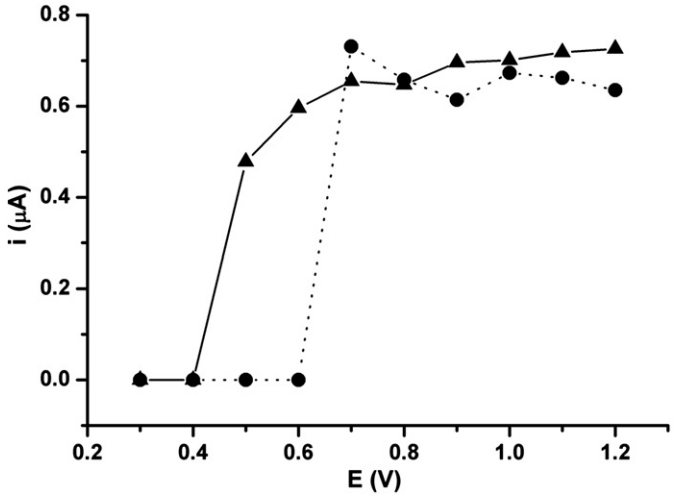

Fig. 1. Hydrodynamic voltammograms obtained by plotting peak current values as a function of the corresponding applied potential pulses. The solutions contained TBHQ (▲ 30 μmol L^{-1}) or BHA (● 30 μmol L^{-1}). Potential pulse: 0.6 and 1.0 V for 100 ms each; supporting electrolyte: 50% (v/v) hydroethanolic solution with 0.1 mol L^{-1} $HClO_4$; dispensing rate: 160 μL s^{-1}; injected volume: 100 μL.

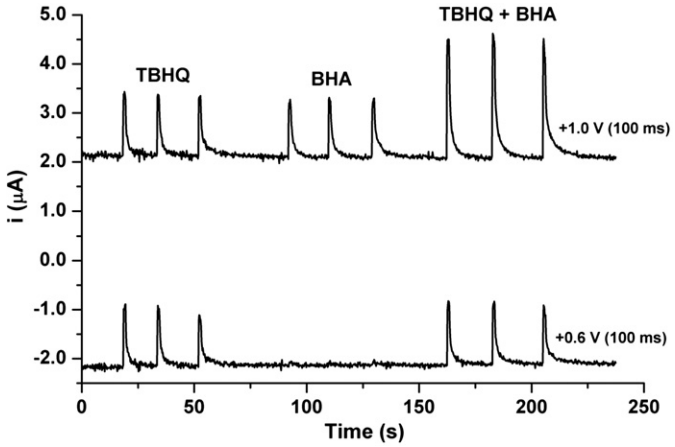

Fig. 2. Amperometric responses (n=3) of solutions containing only TBHQ (30 μmol L^{-1}), only BHA (30 μmol L^{-1}) or TBHQ+BHA (30+30 μmol L^{-1}). Potential pulse: 0.6 and 1.0 V for 100 ms each; supporting electrolyte: 50% (v/v) hydroethanolic solution with 0.1 mol L^{-1} $HClO_4$; dispensing rate: 160 μL s^{-1}; injected volume: 100 μL.

previously for simultaneous determinations of paracetamol and caffeine [18].

The hydrodynamic voltammograms revealed that TBHQ starts to oxidize at less positive potentials (0.5 V) than BHA (0.7 V) at GCE. Based on these hydrodynamic voltammograms, a potential of 0.6 V provides for a selective determination of TBHQ in the presence of BHA, and so this value was selected as the first potential pulse. Electrochemical oxidation of BHA is achieved if more positive potentials are applied; however, TBHQ is also oxidized and can be considered as an interfering molecule. A second potential pulse (1.0 V) was selected for electrochemical oxidation of BHA; the contribution from the TBHQ oxidation current could then be subtracted from the BHA response current similarly to previous studies [15–18]. Fig. 2 presents amperometric recordings obtained at 0.6 V (for 100 ms) and at 1.0 V (100 ms) for triplicate injections of 30 μmol L^{-1} TBHQ, 30 μmol L^{-1} BHA, and a mixture containing 30 μmol L^{-1} BHA+30 μmol L^{-1} TBHQ.

The amperometric recording registered at 0.6 V clearly shows that only TBHQ was oxidized at the GCE surface even in the presence of BHA, and that at 1.0 V both molecules were oxidized. The selection of the pulse length (100 ms) was based on their favorable ratio of faradaic to capacitive current in this condition. With increasing potential pulse time, the capacitive current decays exponentially whilst the faradaic current decays in function of the square root of time. However, low reproducibility of peak currents was verified when potential pulse times greater than 100 ms were applied. Further amperometric recordings were carried out using this sequence of two potential pulses.

However, selective determination of BHA depends on subtraction of the TBHQ current due to its oxidation at 1.0 V. Direct subtraction of the current response at 0.6 V (exclusive oxidation of TBHQ) from the current response at 1.0 V (oxidation of TBHQ and BHA) would be equivalent to the current response of BHA only if the current responses of TBHQ at both potentials pulses (0.6 and 1.0 V) corresponded to the same value. Since this in fact is not the case, a correction factor (*CF*) can be estimated based on the ratio of the current responses to THBQ oxidation registered at 0.6 and 1.0 V. The *CF* can be obtained by a simple injection of a solution containing only TBHQ at a concentration within the range used to obtain a calibration curve as follows:

$$CF = \frac{i_{\text{TBHQ 1.0 V}}}{i_{\text{TBHQ 0.6 V}}} \quad (1)$$

Afterwards, if a solution containing both compounds (THBQ+BHT) is injected, the current originating from BHA oxidation detected at 1.0 V can be calculated as follows:

$$i_{\text{BHA}} = i_{1.0\text{ V}} - (CF \times i_{0.6\text{ V}}) \quad (2)$$

CF values were obtained for injection of different concentrations of TBHQ (10 to 50 μmol L^{-1}) using the BIA system under optimized conditions; its average value was 1.40 ± 0.01 (n=5). The optimized BIA parameters were a dispensing rate of 160 μL s^{-1} and an injection volume of 100 μL, based on the highest analytical signal and precision. A repeatability study (Fig. 3) was conducted under these conditions following ten successive injections of a standard solution containing both BHA and TBHQ (both at 30 μmol L^{-1}).

The average standard deviation was 0.98% for BHA (detected at 0.6 V) and 0.77% for the response obtained at 1.0 V which corresponded to the mixture of BHA and TBHQ. Using the optimized conditions, calibration curves for BHA and then TBHQ were obtained. Fig. 4 presents the amperometric responses for triplicate injections of solutions containing increasing concentrations of BHA and TBHQ (a–e: 10–50 μmol L^{-1}). The respective calibration curves (increasing and decreasing order) are also presented for each analyte. The current responses of BHA were calculated using

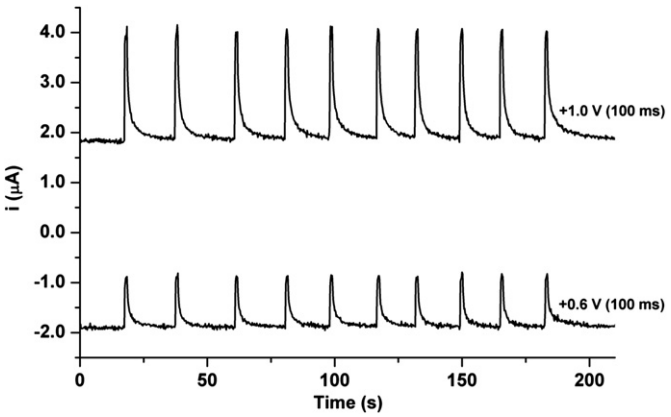

Fig. 3. Repeatability data obtained from successive injections of a solution containing 30 μmol L^{-1} both BHA and TBHQ (n=10). Potential pulse: 0.6 and 1.0 V for 100 ms each; supporting electrolyte: 50% (v/v) hydroethanolic solution with 0.1 mol L^{-1} $HClO_4$; dispensing rate: 160 μL s^{-1}; injected volume: 100 μL.

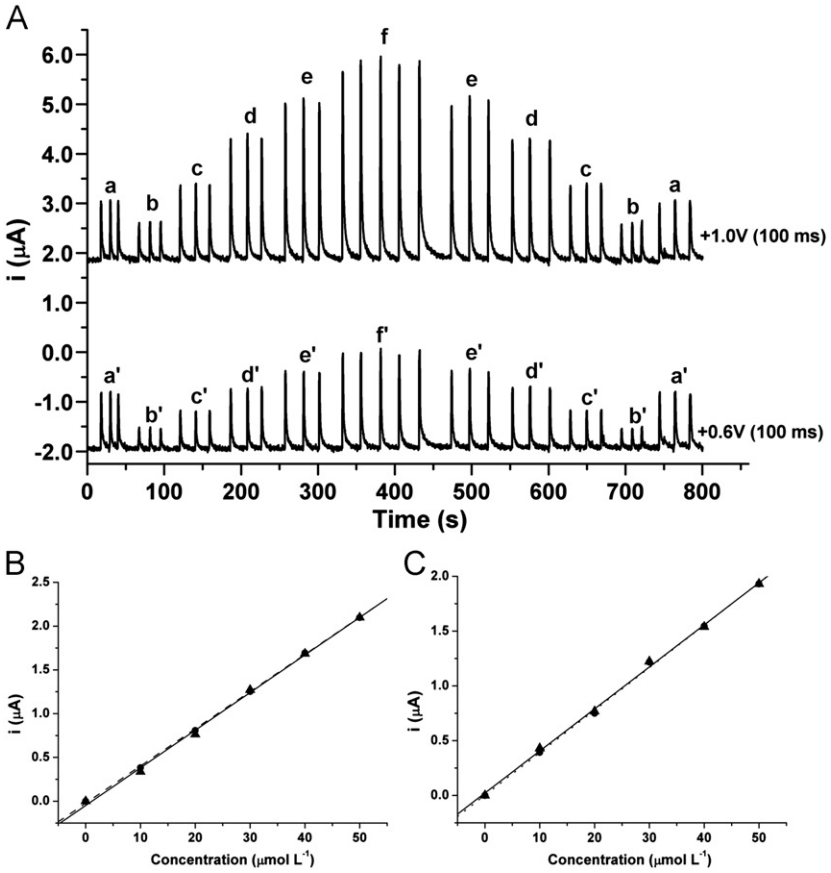

Fig. 4. A) Amperometric responses obtained after injections of a solution containing only TBHQ (a) and five solutions containing simultaneously increasing concentrations of TBHQ and BHA (b–f: 10.0 to 50.0 μmol L^{-1}). (B) Calibration curves of BHA: increasing (▲ R=0.999) and decreasing concentrations (● R=0.999). (C) Calibration curves of TBHQ: increasing (▲ R=0.998) and decreasing concentrations (● R=0.998). Other experimental conditions were as described in Fig. 3.

Eq. (2). The analytical frequency estimated from this amperometric recording was 170 h^{-1}.

The calibration curves were found to be linear for both BHA (R=0.999 for increasing and decreasing concentrations) and TBHQ (R=0.998 for increasing and decreasing concentrations), as shown in Fig. 4 *B* and *C*, respectively, confirming the absence of a memory effect. The slope values were slightly higher for BHA (0.0444 and 0.0432 μA L $μmol^{-1}$ for increasing and decreasing concentrations) than TBHQ (0.0377 and 0.0387 μA L $μmol^{-1}$ for increasing and decreasing concentrations). The linear dynamic range could be widened under the optimized conditions from 1 to 1000 μmol L^{-1} for both BHA and TBHQ maintaining correlation coefficients of 0.996 and 0.999, respectively. The detection limits under the optimized conditions were estimated to be 73 and 75 nmol L^{-1} for BHA and TBHQ, respectively with a signal-to-noise ratio of S/N=3.

The accuracy of the proposed BIA method was first evaluated by recovery tests using samples spiked with BHA and TBHQ. Recovery values for BHA and TBHQ were in the range of 102–104% and 100–110% (n=3), respectively. Next, the proposed method was applied for simultaneous determinations of BHA and TBHQ in biodiesel samples containing different amounts of BHA and TBHQ. The samples were also analyzed by HPLC for comparison, and all the results are presented in Table 1.

All results obtained by the proposed BIA method were in agreement with those obtained by HPLC. At the 95% confidence level, the calculated t values (paired Students t-Test) were smaller than the critical value (2.78, n=3), which indicates that there were no significant differences between the results.

Table 1
Concentrations of BHA and TBHQ obtained by the proposed BIA method and by HPLC (mg g^{-1} of sample) and the respective standard deviation values (n=3).

Samples	BIA (mg g^{-1})		HPLC (mg g^{-1})	
	TBHQ	BHA	TBHQ	BHA
Biodiesel 1	4.2 ± 0.1	9.2 ± 0.4	4.3 ± 0.3	9.9 ± 0.4
Biodiesel 2	7.9 ± 0.4	19.6 ± 0.8	8.6 ± 0.4	21.2 ± 0.8
Biodiesel 3	12.6 ± 0.7	31.0 ± 0.8	13.3 ± 0.7	30.2 ± 0.8

Electrochemical oxidation of phenolic compounds such as BHA and TBHQ, as well as electroanalysis of oily samples, could severely contribute to electrode passivation. Nevertheless, the results indicate that electrode passivation did not occur during electrochemical experiments with standards and samples using a bare GCE. A significant contribution in comparison to previous studies [10–14] is the possibility of simultaneous determinations of antioxidants in biodiesel, which can be found as binary mixtures due to the synergistic effects of multiple antioxidants on the oxidation stability of biodiesels [4–6].

4. Conclusions

We have demonstrated a pioneering application of BIA with pulsed-amperometric detection for simultaneous determinations of two antioxidants in biodiesel. Samples only required dilution in electrolyte solution prior to analysis. Careful pre-selection of

two potential pulses using the pulsed-amperometric technique associated with BIA permitted the simultaneous detection of BHA and TBHQ with elevated accuracy (confirmed by HPLC analysis and recovery tests), precision (RSD $< 1\%$, $n=10$), sensitivity (LDs < 100 nmol L^{-1}) and speed (a frequency of 170 injections h^{-1}). Furthermore, the proposed method can be easily adapted to monitor the content of other mixtures of antioxidants in biodiesel, and has the potential to be applied for on-site analysis.

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