



## Short communication

## Direct amperometric determination of tert-butylhydroquinone in biodiesel

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## ABSTRACT

The direct amperometric determination of tert-butylhydroquinone (TBHQ) in biodiesel at an unmodified glassy carbon electrode is reported. A biodiesel aliquot was added into an electrochemical cell containing a 75% (v/v) ethanol–water solution under stirring (with final concentration of 50 mmol L<sup>-1</sup> HClO<sub>4</sub>). The amperometric method involved the continuous application of three sequential potential pulses to the working electrode (700 mV for 300 ms, 0 mV for 100 ms and -50 mV for 1 s). TBHQ was continuously monitored at the first (direct oxidation) and optionally at the second (reduction) potential pulse while the third potential pulse was applied for cleaning of the electrode surface. For comparison, the samples were also analyzed by high-performance liquid-chromatography and a good agreement between the results was verified. Recovery values for spiked samples were between 90% and 95% and the reproducibility of the proposed method was around 5% ( $n = 5$ ). The proposed method can be easily adapted for on-site analysis.

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## 1. Introduction

After the crude oil crisis, the investigation into alternative energy sources has become fundamental worldwide and especially in developing countries such as Brazil. Biodiesel is a renewable and biodegradable fuel and its use as a substitute for petroleum-based fuels, particularly for diesel oil, is very promising. The physical properties of biodiesel and diesel oil are similar, and for this reason the modification of diesel engines and storage infrastructure is not required. Additionally, biofuels such as biodiesel and ethanol are preferred due to the reduction of environmental pollution [1].

Biodiesel is composed of mono-alkyl esters of long-chain fatty acids derived from vegetable oils or animal fats. It is produced by a transesterification reaction in the presence of a catalyst (normally a base such as NaOH or KOH). During the production of biodiesel, glycerol is formed as the main co-product. Other molecules can be found in biodiesel such as residual alcohol, catalysts, and free fatty acids. Furthermore, the composition of biodiesel can be modified during storage and handling, for example the addition of water (absorption from air) and trace metals (corrosion of containers). These contaminant molecules can induce fuel deterioration and initiate operational and environmental problems. Therefore, the quality control of biodiesel is required to increase market acceptance of this biofuel [2,3].

A real problem related to biodiesel is its relatively low stability. The presence of significant amounts of unsaturated fatty acids derived from the raw materials typically used for obtaining biodiesel, associated with the presence of air, heat, light, and chemical species such as hydroperoxides and trace metals, accelerate oxidation processes which affect the stability parameters of biofuels. Oxidation instability can lead to changes in physical and chemical characteristics of biodiesel, such as an increase in viscosity, which results in the formation of gums and sediments. Inside the engine, deteriorated biodiesel prejudices the performance of engines by means of filter plugging, injector fouling, deposit formation in the engine combustion chamber, and corrosion of engine components [4–6].

In order to enhance the oxidation stability of biodiesel, antioxidants may be introduced into the biofuel. Synthetic antioxidants such as butylhydroxytoluene (BHT), butylhydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) were evaluated as potential antioxidants in biodiesel [5]. A better performance was verified for TBHQ [4].

Based on the electroactivity of antioxidants, electroanalysis has been used for sensing antioxidants in several samples. Bare carbon electrodes [7,8] and modified electrodes [9–12] were used for the determination of antioxidants in different food samples. For all cases, analytes were extracted prior to electrochemical measurements [9–12]. A few applications which did not require sample treatment were reported employing modified electrodes [13,14].

In this work we describe a simple and reliable analytical method for the determination of TBHQ in biodiesel based on its amperometric

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ric detection in a non-aqueous medium. The sample was introduced into the electrochemical cell containing an appropriate medium (75%, v/v ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ ) under stirring. The amperometric method involved the continuous application of three sequential potential pulses to the working electrode (700 mV for 300 ms, 0 mV for 100 ms and  $-50 \text{ mV}$  for 1 s). TBHQ contained in the biodiesel aliquot instantaneously produced an amperometric signal which was monitored at the first two potential pulses. The third potential pulse was applied for electrode cleaning (unmodified glassy carbon electrode) in order to avoid electrode fouling. The successful use of multiple-pulse amperometry was previously demonstrated for different applications [15–17].

## 2. Experimental

### 2.1. Reagents and samples

High-purity deionized water ( $R \geq 18 \text{ M}\Omega \text{ cm}$ ) obtained from a Milli Q water purification system (Millipore, Bedford, MA, USA) was used for preparing all aqueous solutions. Concentrated perchloric (70%, m/v), acetic (65%, m/v) and phosphoric acids (85%, m/v) and sodium acetate (Vetec, Rio de Janeiro, Brazil) were used without further purification (analytical grade). Tert-butylhydroquinone (TBHQ) (97%, m/m) was purchased from Acros Organics (USA). High-performance-liquid-chromatography grade methanol, ethanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Working standard solutions were prepared just before use by appropriate dilution of the stock solution. The standard stock solution of TBHQ ( $50 \text{ g L}^{-1}$ ) was prepared in methanol. Biodiesel samples containing different amounts of TBHQ were obtained from local factories (the biodiesel was produced from soybean oil).

### 2.2. Instrumentation

Electrochemical recordings were carried out using a  $\mu$ -Autolab Type III potentiostat (EcoChemie, Utrecht, The Netherlands). The HPLC measurements were performed using a Shimadzu LC-10VP equipped with an UV/vis detector (SPD-10AV), a LC column (Lichrospher 100 A° RP18-C18,  $250 \text{ mm} \times 4.6 \text{ mm}$ ,  $5 \mu\text{m}$ ), a column oven (CTO-20A), a degasser (DGU-20A5), a small auto-injector and a pump (LC-10AD-VP).

### 2.3. Electrochemical cell and electrodes

A cylindrical glass cell with an internal volume of 12 mL was used for all electrochemical measurements. The reference and auxiliary electrodes were a miniaturized Ag/AgCl (saturated KCl) electrode [18] and a platinum wire, respectively. A commercial (Metrohm) glassy carbon electrode (3 mm diameter) was used as the working electrode. Cleaning of the glassy carbon electrode was performed mechanically on a felt-polishing pad using an alumina powder suspension ( $0.3 \mu\text{m}$ ) and copiously rinsing with deionized water.

### 2.4. Electrochemical measurements

The initial investigation of the electrochemical oxidation of TBHQ in a 75% (v/v) ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$  was carried out by cyclic voltammetry.

Multiple-pulse amperometry was utilized for the TBHQ determination in biodiesel. Three sequential potential pulses were continuously applied to the working electrode (700 mV for 300 ms, 0 mV for 100 ms and  $-50 \text{ mV}$  for 1 s) and the TBHQ amperometric signal was monitored at the first two potential pulses. The third

potential pulse ( $-50 \text{ mV}$  for 1 s) was executed to clean the electrode surface.

The standard addition method was applied to determine the TBHQ concentration in the biodiesel samples. Aliquots of 20–50  $\mu\text{L}$  of biodiesel were introduced into the electrochemical cell containing the optimized electrolyte (75%, v/v ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ ).

All electrochemical measurements were carried out in the presence of dissolved oxygen and at room temperature.

### 2.5. HPLC analysis

The mobile phase was composed of acetonitrile and water (75:25, v/v) with the pH value adjusted to pH 2.1 with phosphoric acid, and a flow rate of  $1.0 \text{ mL min}^{-1}$ . The detector was fixed at 280 nm. Aliquots of biodiesel samples were directly diluted in the mobile phase before injection. The TBHQ retention time was 3.5 min.

## 3. Results and discussion

The electrochemistry of phenolic antioxidants at a bare glassy carbon electrode was previously investigated and the best electrolyte composition for exploring their electrochemical oxidation process was a  $0.1 \text{ mol L}^{-1} \text{ HClO}_4$  solution with 5% (v/v) methanol [7]. In this work, the cyclic voltammetry experiments also revealed that the use of the  $\text{HClO}_4$  solution in the electrolyte provided higher current responses for the electrochemical oxidation of TBHQ. The presence of methanol or ethanol (less toxic) in the electrolyte is noteworthy since they dissolved both TBHQ (this antioxidant is only partially soluble in water) and the biodiesel aliquot. A 5% (v/v) ethanol solution was suitable for dissolving TBHQ but it was not enough to produce a single-phase solution composed of biodiesel–ethanol–water. The optimized composition of the ternary solvent system (biodiesel–ethanol–water) for keeping the single-phase solution after additions of 20–50  $\mu\text{L}$  of biodiesel was a 75% (v/v) ethanol–water solution (with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ ). A similar ternary solvent system was previously optimized and applied for the direct determination of copper in biodiesel [19].

A hydrodynamic voltammogram in the potential range covering the electrochemical oxidation of TBHQ was obtained by multiple-pulse amperometry. Nine sequential potential pulses of 500 ms each (200; 300; 400; 500; 600; 700; 800; 900 and 1000 mV) were continuously applied to a bare glassy carbon electrode immersed in a 75% (v/v) ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$

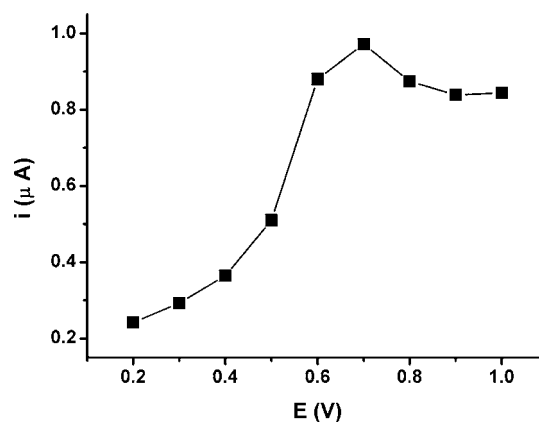
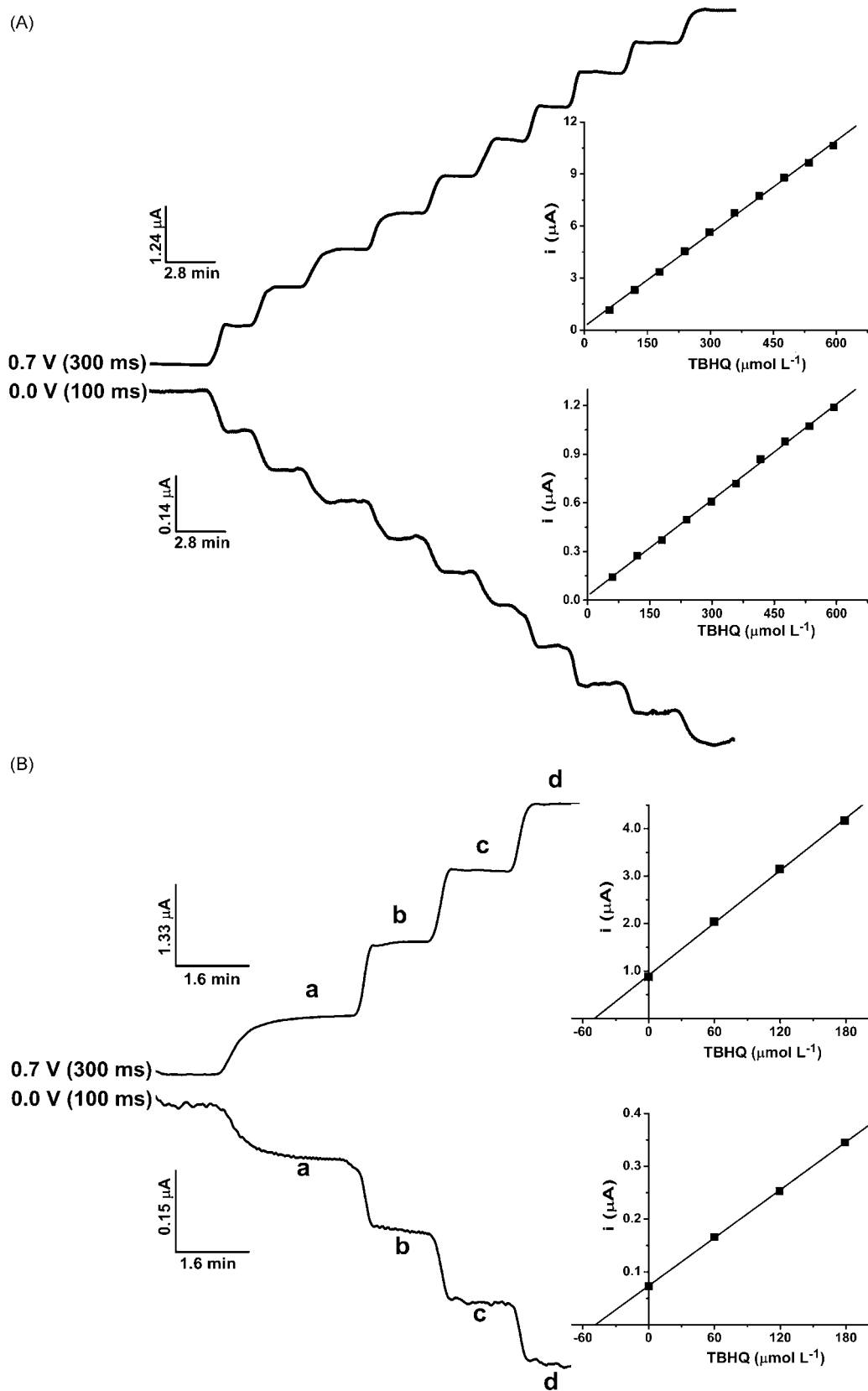


Fig. 1. Hydrodynamic voltammogram of  $60 \mu\text{mol L}^{-1}$  TBHQ obtained using multiple-pulse amperometry. Electrolyte: 75% (v/v) ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ .



**Fig. 2.** (A) Multiple-pulse amperometric calibration data obtained upon ten successive  $60 \mu\text{mol L}^{-1}$  additions of TBHQ; (B) multiple-pulse amperometric recordings for (a)  $20 \mu\text{L}$  biodiesel and (b–d) three successive  $60 \mu\text{mol L}^{-1}$  additions of TBHQ. Sequence of potential pulses:  $700 \text{ mV}$  for  $300 \text{ ms}$ ,  $0 \text{ mV}$  for  $100 \text{ ms}$  and  $-50 \text{ mV}$  for  $1 \text{ s}$ . Current-time responses were recorded at  $700 \text{ mV}$  (oxidation) and at  $0 \text{ mV}$  (reduction). Insets show the corresponding calibration plots. Electrolyte as in Fig. 1.

under stirring. The current at each potential pulse was continuously monitored. After the addition of  $60 \mu\text{mol L}^{-1}$  TBHQ to the electrochemical cell, the respective current variation at each potential pulse was measured and used to construct the hydrodynamic voltammogram for the electrochemical oxidation of TBHQ (Fig. 1).

Based on the hydrodynamic voltammogram, the potential of 700 mV was selected for the electrochemical oxidation of TBHQ during the amperometric recordings.

Constant-potential amperometry (700 mV) was performed for consecutive additions of TBHQ standard solutions to the electrochemical cell under stirring in order to obtain a calibration curve. A constant decrease of the oxidation current in such a way that no current increase was verified after the fifth injection was observed (data not shown). This result agrees with a previously published study and the explanation given was related to electrode fouling due to the adsorption of the electrogenerated species during oxidation of the phenolic compounds [14]. Because of this, instead of using the modified electrodes [9–12,14] which are normally applied to overcome problems associated with electrode fouling, multiple-pulse amperometry was utilized in this work to avoid passivation of the bare glassy carbon electrode.

A sequence of three potential pulses was optimized: (a) 700 mV for 300 ms, (b) 0 mV for 100 ms and (c)  $-50$  mV for 1 s. At the first potential pulse, TBHQ was oxidized generating the tert-butylquinone species [9]. At the second potential pulse, the oxidized species generated in the first potential pulse was electrochemically reduced at the electrode surface. The optimized potential for the reduction process was 0 mV at which the highest cathodic current was obtained. The second potential pulse could be relevant if an interfering compound, which undergoes electrochemical oxidation at the same potential of TBHQ, is also present in the sample. In such a case, the determination of TBHQ would be based on the electrochemical reduction of the oxidized species generated during the first potential pulse, which improves selectivity for the determination of TBHQ, as described in a previous study [15]. The third potential pulse was applied for cleaning the electrode surface of oxidized species electrochemically generated during the first potential pulse which cause electrode fouling. For this reason, a longer time (1 s) was necessary.

Fig. 2(A) presents the multiple-pulse amperometric responses recorded at 700 mV (oxidation) and at 0 mV (reduction) obtained for increasing concentrations of  $60 \mu\text{mol L}^{-1}$  TBHQ and the respective calibration curves (inset). The current was not monitored at the third potential. This experiment was performed in a 75% (v/v) ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ .

A linear behavior with a good correlation coefficient (0.999) was observed from 60 to  $600 \mu\text{mol L}^{-1}$  TBHQ at both potential pulses. The cleaning potential pulse ( $-50$  mV for 1 s) contributed to the enhancement of the linear analytical range by avoiding electrode fouling, which typically occurs during the oxidation process of phenolic compounds [14]. Therefore, the TBHQ concentration could be calculated using either anodic or cathodic processes. Under the same conditions, aliquots of biodiesel samples free of antioxidants were added to the electrochemical cell and multiple-pulse amperometric measurements were recorded. No current variation was verified at either of the potential pulses. Additionally, the current baseline at both potentials did not undergo any variation which could be caused by the biodiesel matrix. Further additions of TBHQ into the same system were carried out and typical current responses were verified at both potentials, which meant that the electrode had responded perfectly to the TBHQ additions.

Similar experiments were executed for the addition of an aliquot of biodiesel spiked with TBHQ (recovery test). Fig. 2(B) shows the multiple-pulse amperometric responses recorded at 700 mV (oxidation) and at 0 mV (reduction) obtained for the sample addition (a) plus three additions of  $60 \mu\text{mol L}^{-1}$  TBHQ (b–d). The respective

**Table 1**

Concentrations of TBHQ obtained by the proposed amperometric method and by HPLC ( $\text{mg g}^{-1}$  of sample) and the respective RSD values ( $n=3$ ).

Samples	Amperometry ( $\text{mg g}^{-1}$ )	HPLC ( $\text{mg g}^{-1}$ )
Biodiesel 1	$2.24 \pm 0.12$	$2.28 \pm 0.08$
Biodiesel 2	$2.57 \pm 0.09$	$2.45 \pm 0.09$
Biodiesel 3	$2.64 \pm 0.13$	$2.61 \pm 0.12$
Biodiesel 4	$2.63 \pm 0.13$	$2.66 \pm 0.13$

curves for both potential pulses are also presented (inset). The standard addition method was used to obtain TBHQ concentrations for each experiment ( $n=3$ ) and the recovery value was calculated from these results.

A linear behavior was verified for the standard addition analysis of the spiked biodiesel ( $R > 0.995$ ). Recovery values between 90% and 95% were obtained. The current increase for the addition of the spiked biodiesel sample (a) exhibited a different profile from the following standard TBHQ additions (b–d). It required a longer time to re-establish the baseline current in comparison with additions of standard TBHQ solutions. This effect was most likely due to the additional time needed to extract the TBHQ from the biodiesel aliquot to the ethanol–water electrolyte and for further electrochemical oxidation of the antioxidant. The same behavior was verified during the cathodic process. Despite the longer time needed to obtain the stable current value, the recovery values attested the satisfactory performance of such a protocol.

The optimized protocol for TBHQ determination was used for the analysis of biodiesel samples containing the antioxidant. For comparison, the samples were also analyzed by HPLC. These results are presented in Table 1.

All of the results obtained by the proposed method were in agreement with those obtained by HPLC at the 95% confidence level. The detection limit under optimized conditions was estimated in  $5 \mu\text{mol L}^{-1}$  (with a signal-to-noise ratio of  $S/N=3$ ), which corresponds to  $100 \mu\text{g}$  of TBHQ per g of biodiesel considering the approximate 200-fold dilution ( $50 \mu\text{L}$  of biodiesel in 10 mL of electrolyte). The reproducibility of the proposed method was around 5% ( $n=5$ ).

#### 4. Conclusions

A simple, precise, fast and accurate analytical method for the direct determination of TBHQ in biodiesel was developed. The protocol involves the insertion of an aliquot of biodiesel directly into the electrochemical cell containing an appropriate electrolyte (75%, v/v ethanol–water solution with  $0.05 \text{ mol L}^{-1} \text{ HClO}_4$ ) and the application of multiple-pulse amperometry. It should be emphasized that the potential pulse method not only increases the selectivity but also allows direct TBHQ determination in biodiesel using an unmodified glassy carbon electrode. The content of antioxidants such as TBHQ in biodiesel can be assessed on-site using the proposed electroanalytical method since portable potentiostats are commercially available.

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