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Voltammetric Determination of BHT Antioxidant at Gold Electrode in Biodiesel

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Abstract
The voltammetric determination of synthetic antioxidant 2,6-di-*tert*-butyl-4-methylphenole (BHT) was studied using linear-sweep voltammetry (LSV) and cyclic voltammetry (CV) with a gold electrode and performed in isopropanol media containing either $0.1 \, mol \, L^{-1} \, H_2SO_4$ or $0.1 \, mol \, L^{-1} \, LiClO_4$ as supporting electrolyte. The results obtained have revealed that the most reliable detection was acquired in acidic media (isopropanol–H_2SO_4) whereas the use of isopropanol–$LiClO_4$ solution exhibited poorer reproducibility due to possible passivation of the electrode. Real samples of biodiesel mixture were analyzed without any special sample treatment or separation and results were compared with those obtained by FTIR-spectroscopy.

Keywords: BHT, Voltammetry, Gold electrode, Biodiesel, Antioxidant

1 Introduction

At present, the significance of biodiesel production is rising up. As a material, biodiesel is not toxic, undergoes biodegradability and does not contain sulfur or even aromates, which may help to decrease air-born release, supporting also renewable resources. Furthermore, addition of biodiesel into the diesel is now obligatory according to the EU rules, therefore, motor vehicles with diesel engine have to use such mixed diesel fuel [1].

On the contrary, one of the disadvantages of biodiesel is its low oxidation stability in comparison with diesel oil [2–5]. Hence, for increase of biodiesel quality, various antioxidants are being added [6], permitting the extended lifetime of biodiesel, as well as of its mixtures, which plays an important role with respect to the storage. Several antioxidants; e.g., tocopheroles, sterols, etc., are naturally present in seed oils. When biodiesel is made of seed oil, the level of antioxidants notably drops during the production and refining of biodiesel and thus, synthetic antioxidants have to be reloaded in biodiesel and its mixtures. The most common used synthetic antioxidants are: butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), *tert*-butylhydroquinone (TBHQ), propyl gallate (PG), and pyrogallol (PA) [7]. Several studies have been made dealing with the effect of each synthetic antioxidant on the oxidation stability and focused on its improvement in biodiesel and its mixtures [7–11].

For determination of antioxidants in biodiesel or lubricants, infrared spectroscopy [12] and liquid chromatography [8] are often used, but both techniques are quite expensive or demanding rather complicated sample preparation prior to analysis; in other words, they are not suitable for field analysis.

Electroanalytical methods represent a relatively cheap and effective alternative with portable instrumentation with further possibility for miniaturization. In general, voltammetric determination of common fabricated synthetic antioxidants has been the subject of interest in various studies; e.g., S. N. Robledo et al. [13] were working on the determination of four antioxidants namely on BHA, BHT, TBHQ, and PG in olive oil. In this case, square-wave voltammetry (SWV) with platinum ultramicroelectrode in the supporting electrolyte containing acetonitrile (AcN) and $0.1 \, M \, (C_4H_9)_4N \, PF_6$ were used. The analysis was done either directly in oil matrix dissolved in a mixture of benzene-ethanol (1:2) in the presence of $0.1 \, M$ or $1 \, M \, H_2SO_4$ or the antioxidants were isolated with double extraction using AcN.

Although platinum electrode is usually preferred some other electrodes have also been employed in the determination of BHA, BHT and TBHQ antioxidants. Among carbon-based ones, the proper choice can be the glassy carbon electrode (GCE) [14–18] as documented by analysis of mayonnaise, where the antioxidants were extracted by methanol (MeOH) [14]. Other types of carbon-based

electrodes for the determination of antioxidants are applicable as well. Such electrodes are, e.g., boron doped diamond electrode [19] or a carbon composite electrode modified with Cu(II)-phosphate immobilized into a polyester resin [20]. In addition, when the mixture of antioxidants is determined the problem with signal overlapping of individual antioxidant signals can occur, thus for the quantitation chemometric approach is desired, see [17].

For most of the above discussed electroanalytical determinations, an extraction (usually with MeOH, EtOH, AcN, etc.) of antioxidants from the matrix is inevitable. For example, Kauffmann [18] presents extraction-based sample treatment for analysis of amine and phenolic antioxidants in motor and turbine oil. The oil sample was extracted in a solution of supporting electrolyte (acetonic solution of sodium perchlorate or aqueous ethanolic solution of potassium hydroxide) in the presence of sand and whereas soluble species, together with antioxidants, were extracted into the solution and further analyzed, the suspension adhered to the sand. Concerning the above-mentioned determinations, mainly linear-sweep voltammetry (LSV), differential pulse voltammetry (DPV), and square-wave voltammetry (SWV) were the techniques of choice [17–21].

Concerning a determination of antioxidants in biodiesel several papers were published, e.g. Alfonso de Araujo et al. introduced determination of TBHQ in biodiesel in the presence of surfactants [22,23] at either carbon paste electrode [22] or mercury drop electrode [23]. Moreover further techniques were developed by Tormin et al. reporting on amperometric determination of TBHQ antioxidant using flow-injection analysis (FIA) in system of ethanol–HClO$_4$ [24] and in addition to this batch injection analysis (BIA) for determination of BHA and TBHQ antioxidants was introduced [25,26].

In this work, we report on the determination of BHT in biodiesel at a gold electrode, further elaborating our previous method [21] which is beneficial for its effective resolution of both aminic and phenolic antioxidants. Herein, after advanced optimization, the main attention is paid to the experimental testing of the method in analyses of selected real samples.

2 Experimental

2.1 Chemicals and Reagents

All chemicals used were of analytical reagent grade and purchased from Penta or Sigma Aldrich. Stock solution of BHT (4 g L^{-1}) was prepared by dissolving of appropriate amount of BHT (AppliChem; CAS:128-37-0) in 96% solution of ethanol. The real samples of mixed biodiesel were prepared by dissolving of appropriate amount of biodiesel in isopropanol.

Throughout the experimental work, the solutions were prepared or diluted using doubly deionised water (18 MΩ cm) obtained by passing through a Milli-Q Millipore laboratory purification system (Millipore, USA) and

purged with argon (purity: 99.99%; Linde Technoplyn, Prague, Czech Republic) prior to analysis.

2.2 Apparatus and Accessories

Voltammetric analyses of 2,6-di-*tert*-butyl-4-methylphenole (BHT) were performed using electrochemical analyzer EP 100 VA (HSC Servis; Bratislava, Slovak Republic) in a three-electrode cell which had comprised the gold disc (AuDE, 2 mm in diameter, HSC servis, Bratislava, Slovak Republic) as working electrode, Ag|AgCl| 3 M KCl as the reference, and a Pt-plate (3×5 mm) as the counter electrode.

For studies of the reaction mechanism the potentiostat PGSTAT 128N (AUTOLAB, Metrohm Autolab B. V., Utrecht, The Netherlands) operated via NOVA 1.7 software of the same manufacturer was used. A three-electrode cell consisting of a gold disc (AuDE, 2 mm in diameter) as working electrode, a saturated calomel electrode (SCE) as the reference separated from solution via a salt bridge containing supporting electrolyte. As the counter electrode, a Pt-plate completed the cell. Between the individual analyses, the active part of the electrode system was kept under water.

As reference method for analysis of real samples infrared spectroscopy was used. The analysis itself was carried out using a FTIR spectrometer Nicolet iS10 (Thermo-Scientific) in the spectral range of wave number 600–4000 cm^{-1}, with a resolution of 4 cm^{-1} and with a scan number of 64, using the Attenuated Total Reflectance technique (ATR).

2.3 Procedure

The determination of BHT was performed within the concentration range from 5.5 mg L^{-1} to 56.5 mg L^{-1} in isopropanol using 0.1 mol L^{-1} H$_2$SO$_4$, 0.01 mol L^{-1} and 0.1 mol L^{-1} LiClO$_4$ as supporting electrolytes. The oxidation of BHT was observed by LSV method within the potential range from +0.4 V to +1.3 V at a scan rate of 40 mV s^{-1}. Prior to each measurement the electrode was conditioned by applying a potential of −1 V for 2 s.

During ATR analysis the oil sample was placed onto the ATR ZnSe 45 crystal and the IR spectrum was collected in the range from 600 cm^{-1} to 4000 cm^{-1}. The sample was then removed from the crystal surface and the accessory was ready to collect additional spectra. Determination of BHT concentration was based on evaluation of the characteristic peak at 3650 cm^{-1} as recommended in the User Manual of FTIR spectrometer Nicolet iS10.

2.4 Samples

Various samples were analyzed: (i) model sample **1**: 27.8 mg L^{-1} of BHT in supporting electrolyte (isopropanol containing 0.1 M H$_2$SO$_4$); (ii) model sample **2**: prepared by adding 524 mg of BHT into one liter of biodiesel; and

(iii) four real samples of biodiesel containing a certain amount of BHT were obtained from a refinery (Paramo a.s., Pardubice, Czech Republic). All samples herein presented as biodiesel were mixtures of standard diesel fuel containing 30% (w/w) fatty acid methyl esters (rapeseed oil methyl ester).

3 Results and Discussion

As shown in our approach [21], voltammetric determination of antioxidants can be sufficiently sensitive, allowing us the direct determination without any special sample separation and, via the proper choice of the supporting electrolyte, ensuring that the sample matrix is completely dissolved.

3.1 Determination of BHT in Isopropanol Containing H$_2$SO$_4$

One of the principal results of the previous study [21] was that phenolic antioxidants (e.g. BHT) are oxidizable in acidic media, where they are more stable and thus less vulnerable to undesirable interactions with other species in the sample. For electrochemical oxidation of BHT, the mixed media of 0.1 M H$_2$SO$_4$ + isopropanol were chosen, where the solvent used had assured higher solubility of target analyte, as well as other components of real sample (biodiesel). As depicted by Figure 1, the peak height is linearly proportional to the concentration, which also resulted from statistical evaluation of the individual voltammograms. A dependence was obtained, $h = (0.022 \pm 0.001)c + 0.029 \pm 0.024$ (where h is in μA and c in mg L^{-1}), whereas the standard deviation for slope of calibration curve was 3.8×10^{-4} and for intercept 9.8×10^{-3}. According to the statistical evaluation [27], a linear calibration model was confirmed, and from further analysis the limit of quantitation 4 mg L^{-1} and limit of detection 3 mg L^{-1} were evaluated.

The recovery of BHT determination was tested by repeated determination of solution of 27.4 mg L^{-1} BHT in isopropanol. The obtained results show that the recovery of the individual determinations does not exceed 110% (all results exhibit positive errors). The reason could be connected to evaluation of individual voltammograms. The average (28.64 mg L^{-1}) for $n = 5$ was within 95% interval of reliability and differs by +4.5% from the real value. The standard deviation is 0.95 mg L^{-1}.

3.2 Voltammetric Determination of BHT in Isopropanol Containing LiClO$_4$

In the past, electrochemical oxidations of BHT were performed on platinum electrode in AcN containing LiClO$_4$ as the supporting electrolyte [15]. Such a type of electrolyte (0.1 mol L^{-1} LiClO$_4$) was also tested as an alternative of supporting electrolyte being used in presented work; see Figure 2.
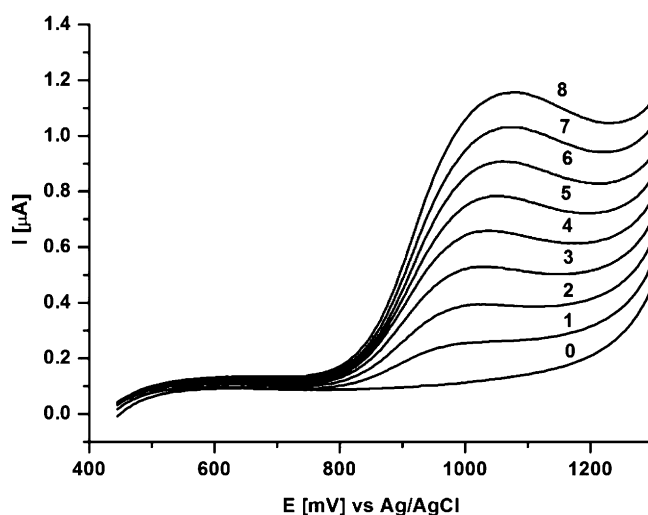

Fig. 1. Voltammetric determination of BHT in isopropanol containing 0.1 M H$_2$SO$_4$. Experimental conditions: LSV, concentration range of BHT: from 5.5 mg L^{-1} to 43.9 mg L^{-1}, $E_{INIT} = +0.4$ V, $E_{FIN} = +1.3$ V, scan: 40 mV s^{-1}. Curve 0 – 0 mg L^{-1}; Curve 1 – 5.5 mg L^{-1}; Curve 2 – 11.0 mg L^{-1}; Curve 3 – 16.5 mg L^{-1}; Curve 4 – 22.0 mg L^{-1}; Curve 5 – 27.4 mg L^{-1}; Curve 6 – 33.0 mg L^{-1}; Curve 7 – 38.4 mg L^{-1}; Curve 8 – 43.9 mg L^{-1}.
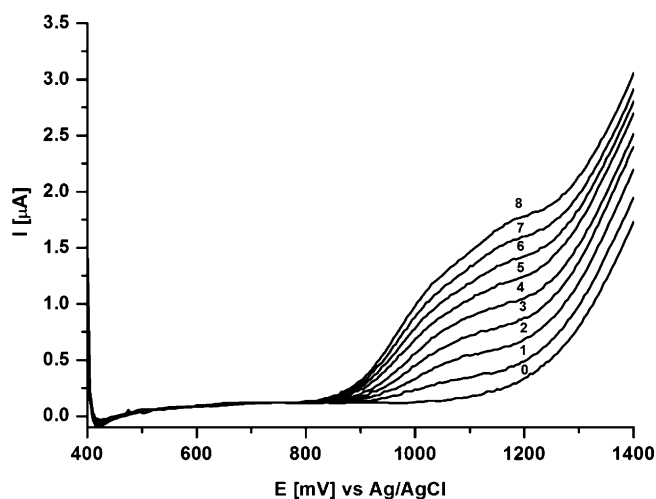

Fig. 2. Voltammetric determination of BHT in isopropanol containing 0.1 mol L^{-1} LiClO$_4$. Experimental conditions: LSV, concentration range of BHT: from 7.1 mg L^{-1} to 56.5 mg L^{-1}, $E_{INIT} = +0.4$ V, $E_{FIN} = +1.4$ V, scan rate: 40 mV s^{-1}. Curve 0 – 0 mg L^{-1}; Curve 1 – 7.1 mg L^{-1}; Curve 2 – 14.1 mg L^{-1}; Curve 3 – 21.2 mg L^{-1}; Curve 4 – 28.3 mg L^{-1}; Curve 5 – 35.3 mg L^{-1}; Curve 6 – 42.2 mg L^{-1}; Curve 7 – 49.5 mg L^{-1}; Curve 8 – 56.5 mg L^{-1}.

It is evident that the peak height of each individual curve is linearly proportional to the concentration of BHT. The corresponding dependence (analyzed according to [27]) is as follows: $h = (0.026 \pm 0.001)c + 0.002 \pm 0.036$ (where h is in μA and c in mg L^{-1}) with a standard deviation for the slope of the calibration curve of 4.59×10^{-4} and for an intercept of 1.54×10^{-2}. From the statistical

analysis, the limit of detection, $LOD = 4$ mg L^{-1} and limit of quantification, $LOQ = 5$ mg L^{-1} were estimated. As result from Figure 2, the peak potential is changing with the concentration towards more positive potential, the shift is about 200 mV within the concentration range from 7.1 mg L^{-1} to 56.5 mg L^{-1}. Based on this fact it can be stated that the potential shift is significant in comparison to determination using 0.1 M H$_2$SO$_4$ as supporting electrolyte, thus for higher concentrations it can make difficulties in evaluation of the peak height when the maximum can be nearby a decomposition of supporting electrolyte. In addition, the statistical analysis showed higher values of standard deviations of the calibration curve parameters and, also, higher values of LOD and LOQ. Therefore, the use of 0.1 M LiClO$_4$ as supporting electrolyte was found inconvenient for the determination described herein.

3.3 Study on Reaction Mechanism of BHT Oxidation

Anodic oxidation of phenolic compounds has been already studied [28]. Within the presented study we wanted to compare previously published results of BHT oxidation mechanism with those obtained at the above-mentioned conditions. The basic characterization of electrochemical BHT oxidation via cyclic voltammetry (CV) revealed that the oxidation process is irreversible (see also Figure 3) and, moreover, the dependence of the peak potential on the scan rate exhibited a shift towards positive potentials, indicating kinetically controlled electrode reaction. The voltammetry with the rotating disc electrode (RDV) has shown that two electrons are involved in electrochemical oxidation of BHT, which is in agreement with the previously presented mechanism [15], where first originate intermediate which react with nucleofile (OH group) leading to the origin of p-substituted cyclohexa-2,5-dienone (see Scheme 1). A second possibility is the deprotonation of intermediate leading to form of benzylalcohol. However this possibility is less probable when using aqueous solution of sulfuric acid in isopropanol.

Within the CV measurements in isopropanol–LiClO$_4$ as supporting electrolyte it was observed the significant decrease of anodic peak current after first scan together with a slight shift of the peak potential towards less positive potential (see Figure 3). Despite the fact that cyklohexa-2,5-dienone is a relatively stable compound it is possible to explain such behavior as probable passivation of the electrode by the product of electrode reaction, where-
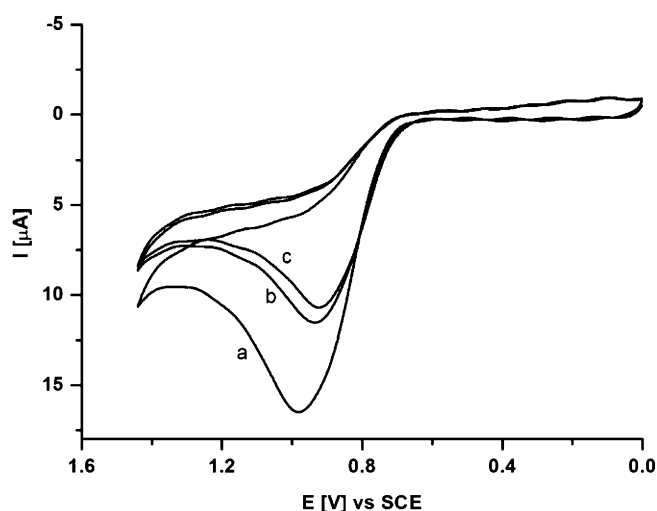
Fig. 3. Cyclic voltammetry of BHT oxidation in isopropanol containing 0.1 mol L^{-1} LiClO$_4$. Experimental conditions: CV, AuDE, concentration of BHT: 1.5×10^{-3} mol L^{-1}, $E_{INIT} = 0$ V, $E_{vertex} = +1.4$ V, scan rate: 100 mV s^{-1}; three cycles: cycle 1 (a), cycle 2 (b), cycle 3 (c).

as it tends to drop of reproducibility in such medium (isopropanol–LiClO$_4$). Contrary to this the medium of isopropanol-sulfuric acid did not exhibit such behavior and hence a significant passivation for this system, which is another benefit for the BHT determination using this method.

3.4 Determination of BHT in Model and Real Samples of Biodiesel

In case of direct determination of BHT in mixed biodiesel, it is evident that the matrix significantly affects the shape of voltammetric curves of antioxidant under study; this problem being documented on Figure 4 A, where the signal for oxidation of BHT overlaps with the background and thus, it is impossible to evaluate it properly. Since the software of EP 100 analyzer provides various mathematic operations, it is possible to solve this problem by mathematical approach, which is illustrated by Figure 4 B. The voltammetric curves were differentiated upon subtraction of the base-line (blank or supporting electrolyte, resp.). Such an approach was used for all analyses described further on.

Yet another parameter to be optimized was the amount of sample required for analysis, which was studied via
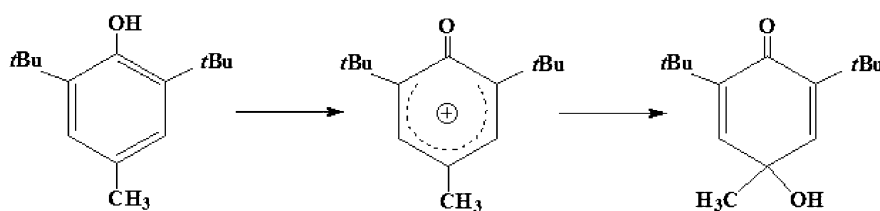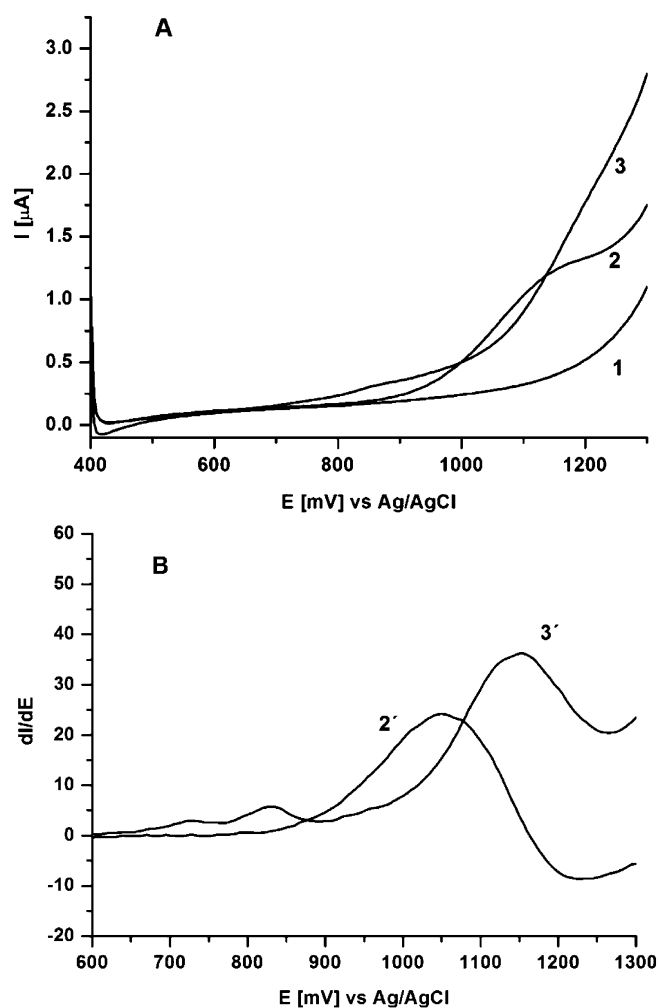
Scheme 1.

Fig. 4. Oxidation of BHT in presence/absence of biodiesel. Legend: (A) Curve 1: baseline; Curve 2: oxidation of BHT; Curve 3: oxidation of BHT in presence of biodiesel (0.5 mL). (B) Curve 2′: oxidation of BHT; and Curve 3′: oxidation of BHT in presence of biodiesel (0.5 mL) both after mathematical correction. LSV, supporting electrolyte: 0.1 M H_2SO_4 in isopropanol (15.2 mL); concentration of BHT: 70.7 mg L^{-1}; $E_{INIT} = +0.4$ V, $E_{FIN} = +1.3$ V, scan rate: 40 mV s^{-1}.

Fig. 5. Determination of BHT in model sample **2** via standard additions. Curve 1: biodiesel sample; Curves 2 and 3: additions of 50 μL and 100 μL (17.7 mg L^{-1}), respectively. LSV, 1 mL of the model sample **2** was taken for analysis; supporting electrolyte: 0.1 M H_2SO_4 in isopropanol (initial volume: 15.2 mL); $E_{INIT} = +0.4$ V, $E_{FIN} = +1.4$ V, scan rate: 40 mV s^{-1}.

Table 1. Determination of BHT in model sample **1** in presence of various amounts of biodiesel. Experimental conditions: LSV, concentration of BHT to be determined: 27.8 mg L^{-1}; supporting electrolyte: 0.1 M H_2SO_4 in isopropanol (initial volume: 15.2 mL); $E_{INIT} = +0.4$ V, $E_{FIN} = +1.3$ V, scan rate: 40 mV s^{-1}.

Amount of biodiesel sample (mL)	Found (mg L^{-1})	Error (%)
0.5	28.3	+2.0
1.0	28.4	+2.3
1.5	36.3	+31.0
2.0	38.3	+38.0
2.5	40.8	+47.0

analyses of BHT model samples in the presence of various quantity of biodiesel. Results were evaluated using the standard addition method, when two aliquots were applied. The data obtained are summarized in Table 1, evidencing that the most appropriate amount of the sample for analysis is about 1 mL or sligtly less, whereas higher amounts are already less suitable due to the increasing effect of the matrix.

The approach elaborated for the direct voltammetric determination of BHT in biodiesel at gold electrode was further tested by means of replicated analyses with second model sample.

Such determination of model sample **2** is documented in Figure 5, where the standard addition method had provided fairly reproducible results. Thus, it was confirmed that the suggested method ensures a reliable determina-
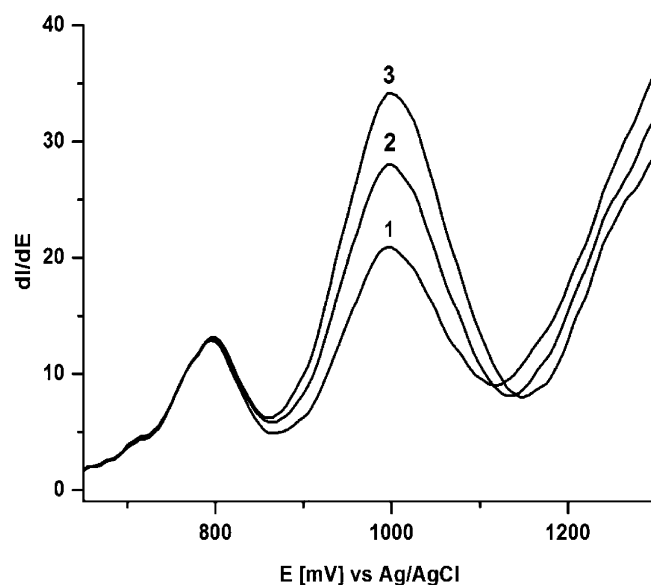
tion of BHT. Five replicate determinations ($n = 5$) gave average value of 519.8 mg L^{-1} with standard deviation 4.8 mg L^{-1}; 95 % interval of reliability with lower limit 513.7 mg L^{-1} and upper limit 525.7 mg L^{-1}.

After verification of results, the method was finally applied to the determination of BHT in four different samples of biodiesel (obtained from a refinery), where the content of antioxidant corresponds to the respective technological procedure. The resultant data are then summarized in Table 2, documenting that the content of BHT in samples #1–3 was in good agreement with the value(s) declared by manufacturer. In sample #4, the value was about 8 % lower than the content declared by manufacturer, which could be caused by the age of the sample or by an error in the technology itself. Additionally, the respective results were verified by means of FTIR spectroscopy reference determination (see Table 2).

Table 2. Concentrations of BHT in real samples of biodiesel obtained by the voltammetric method and by FTIR spectroscopy.

Sample #	Declared by manufacturer (mg L^{-1})	Voltammetry (mg L^{-1})	FTIR spectroscopy (mg L^{-1})
1	500	518 ± 4	531 ± 8
2	500	509 ± 3	486 ± 8
3	1000	1050 ± 6	1060 ± 9
4	1000	923 ± 1	911 ± 6

4 Conclusions

In this article, a recently developed method for the determination of BHT antioxidant in biodiesel samples has been further elaborated and thoroughly examined in analysis of model as well as real samples. For these purposes, the working gold electrode chosen has offered reproducible response.

At first, the mechanism of BHT electrochemical oxidation was examined in more detail and the observations and results obtained confronted with the literature. Second, it was confirmed that the analysis of BHT can be performed directly, without necessity of sample treatment or extraction, which was inevitable in previous methods (see [14,18]). Furthermore, the medium of choice was re-optimized, when the combination of isopropanol and 0.1 M H$_2$SO$_4$ was found the most suitable to ensure stable and reproducible response towards BHT.

Finally, the analysis of real samples has shown that the content of BHT in biodiesel is sufficiently high and hence, it is not necessary to use special procedures for trace analysis; e.g. combination with highly sophisticated electroanalytical instrumentation. In conclusion, the method presented seems to be convenient and fully recommendable for routine analysis in laboratories of refineries and similar industrial service units.

Acknowledgement

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