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Electrochemical Behavior of Butylated Hydroxyanisole and Butylated Hydroxytoluene in Acetic Acid Solutions and their Voltammetric Determination in Pharmaceutical Preparations

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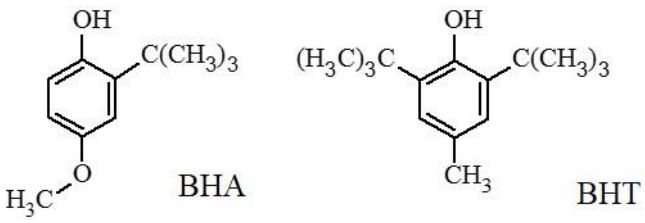
Electrochemical behavior of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) was investigated by cyclic (CV), linear sweep (LSV) and differential pulse voltammetry (DPV) in glacial acetic acid solutions in order to develop a new method for determination of these synthetic antioxidants. A glassy carbon electrode (GC) and a carbon fiber disk microelectrode (CF) were used as working electrodes. Voltammograms recorded at these electrodes show well-defined single waves or peaks attributed to the oxidation of BHA and BHT which proceeds with an irreversible exchange of two electrons. A separation of about 0.24 V between the peak potentials recorded using DPV in their binary mixtures was obtained. Wide linearity ranges were achieved: 0.03-594.62, 0.18-921.37 mg L^{-1} and low limits of detection were 0.01, 0.06 mg L^{-1} for butylated hydroxyanisole and butylated hydroxytoluene, respectively. The results obtained indicate that DPV at CF microelectrode allows sensitive, precise, accurate, and fast determination of BHA and BHT, alone or in their mixture in pharmaceuticals without the need for their separation from the matrices, and can be an analytical alternative for existing methods.

Keywords: Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Acetic acid, Microelectrodes, Voltammetry, Determination

1. INTRODUCTION

The use of antioxidants in pharmaceutical industries is important because they possess the ability to prevent or reduce the rate of an oxidative reaction of organic compounds present in prepared products [1]. The most commonly used synthetic phenolic antioxidants are: butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Their molecular structures are shown in Scheme 1. These compounds, although being antioxidants can also exhibit additional properties such as antimicrobial and against molds effect [2,3]. Two or more antioxidants working together can enhance the antioxidant

activity of one or both in a phenomenon known as synergism. This is why BHA is frequently used in combination with BHT to extend shelf life of pharmaceuticals [1,3].



Scheme 1. Chemical structures of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

The influence of these substances on the consumer safety is not totally clear. BHA and BHT could have harmful effects on human health [4,5]. By contrast, these additives have also been found to have anti-tumor and anti-mutagenic properties [6,7]. Therefore, the use of them in pharmaceutical preparations is the subject of regulation which defines the permitted amount which is 0.0075-0.1% for BHT and 0.005-0.02% (w/w) for BHA [2]. Consequently, the quality control of these antioxidants in various products is of considerable importance. For this purpose, spectrophotometric methods were first used [8-10]. Later chromatographic methods were developed, particularly high performance liquid chromatography (HPLC) [11-16], gas chromatography (GC) [16-19] and micellar electrokinetic capillary chromatography (MECC) [20,21]. The popularity of these techniques is linked to their high selectivity and sensitivity as well as to their low detection limit. On the other hand, they have many drawbacks, such as expensive instrumentation, complicated and lengthy procedures of sample preparation which includes extraction of analytes [12-21].

Electrochemical methods including voltammetric one are a promising alternative to chromatographic techniques due to their relatively low operational cost (simplicity of sample preparation, short analysis time), comparable selectivity, sensitivity, and detection limits.

Voltammetric techniques are based on the anodic oxidation of synthetic antioxidants. Due to the limited solubility in water and the presence of a hydrophobic matrix, their electrochemical properties were investigated mainly in solutions of water with methanol [22-25] or ethanol [26-28], and in mixtures of organic solvents as ethanol-acetonitrile [29], benzene-ethanol [30,31]. These solvents are mostly toxic to humans and the environment. The material of the working electrode was usually glassy carbon [22,24], carbon fiber [31], platinum [24,30], gold [29] and diamond boron doped [27]. Oxidation of phenolic compounds especially in water solutions is often accompanied by the phenomenon of blocking the surface of the electrode by polymeric products formed during this process [32]. This results in the loss of an electrode contact with the solution, which limits the reproducibility of the results. To avoid this problem, modified electrodes are increasingly used to analyze phenolic antioxidants. Surface modification often leads to increased sensitivity of the voltammetric methods by the electrocatalytic effect of modifiers [23,26]. Such sensors are made of glassy carbon modified with gold nanoparticles [25] or monolayer of choline and graphene [26], polypyrrole modified with a nickel phthalocyanine complex [23] and a carbon composite modified with $Cu_3(PO_4)_2$ immobilized in

polyester resin [28]. The unfavorable phenomenon of blocking the surface of the classical electrodes can also be reduced by appropriately selecting the composition of the solution.

From the data described in the literature it is possible to conclude that the process of the anodic oxidation of BHA and BHT in different solutions and on different electrodes is characterized as irreversible and involves with an exchange of two electrons and one proton [22,25-28].

Basing on our earlier preliminary results [33], we applied acetic acid as a medium to study electrochemical behavior of the synthetic antioxidants. Until now this solvent has not been applied to determination of these compounds. Glacial acetic acid has several interesting properties, such as a relatively wide potential window, and the ability to dissolve both hydrophobic organic compounds and their matrix as well as the necessary supporting electrolyte.

The aim of this work was to examine the anodic oxidation of BHA and BHT on carbon electrodes in solutions of glacial acetic acid in order to develop a new direct voltammetric method for determination of these compounds in pharmaceutical preparations.

2. EXPERIMENTAL

2.1. Reagents

The chemicals used were as follows: butylated hydroxyanisole (BHA), ≥98% (Fluka, Switzerland), butylated hydroxytoluene (BHT), >99.0% (Sigma, Germany), sodium acetate, CH_3COONa (AcNa), anhydrous, ≥99.5% (Fluka, Netherlands), sodium perchlorate, $NaClO_4$, anhydrous, p.a. (Sigma-Aldrich, USA). Acetonitrile (AN), p.a. anhydride, glacial acetic acid (HAc), p.a. ACS (each Merck, Germany) or their mixture were used as solvents in all electrochemical experiments. Methanol and acetonitrile with HPLC grade, all purchased from VWR (USA), were used in chromatographic analysis. All reagents and solvents were of high purity and used as received.

Pharmaceutical samples for humans and animals including Daktarin (McNeil Products Ltd, United Kingdom), Imazol (Spirig Pharma Europe GmbH, Germany), Hylosept (Axxon, Poland), Fypyst (KRKA, d.d., Novo Mesto, Slovenia), Tonimer (Istituto Ganassini, Italy), Advocate (Bayer Animal Health GmbH, Germany) and Emofix (D.M.G. Italia s.r.l., Italy) were purchased from local pharmacies or veterinary clinics. They were in the form of solutions, creams or ointments.

2.2. Apparatus

Cyclic (CV), differential pulse (DPV) and linear sweep (LSV) voltammetric experiments were performed using a Model M161E electrochemical analyzer connected with a Model M162 preamplifier, co-operating with the software EALab 2.1. (mtm-anko, Poland). A three-electrode system was used, which consisted of a carbon fiber (CF) disk microelectrode of 35.4 μm diameter (BASi, USA) or a glassy carbon (GC) macroelectrode of 3 mm diameter, A = 0.071 cm^2 (Mineral, Poland) as working electrodes, a platinum wire auxiliary electrode (BASi, USA) and Ag/AgCl (1 mol L^{-1} NaCl) reference electrode (Mineral, Poland). The surface of the working electrodes was polished

using 0.05 µm alumina powder on a polishing cloth (BASi, USA), rinsed with deionized water and dried before use. To avoid electrical interferences, the electrochemical cell was enclosed in a grounded Faraday cage. The pH measurements were performed on a CX-732 multifunction computer meter equipped with a glass indicator electrode and Ag/AgCl reference electrode (Elmetron, Poland).

All experiments were carried out at room temperature (25 ± 1º C).

HPLC measurements were performed on a Model 210 Varian ProStar instrument (USA) with UV-Vis detector set at 280 nm. A C-18 (250 mm × 4.6 mm i.d.) chromatographic column was used. The data were acquired by a version 6.30 LC Workstation.

2.3. Electrochemical measurements

DPV and LSV experiments were performed on a CF microelectrode in acetic acid containing 20% acetonitrile (v/v) and 0.1 mol L^{-1} sodium perchlorate. The optimized conditions for DPV were: pulse amplitude 20 mV, pulse width 80 ms and scan rate 20 mV s^{-1}. In order to achieve steady-state conditions, LSV experiments were done at a slow potential scan rate of 6.25 mV s^{-1}. CV measurements were carried out using a GC macroelectrode in the range of a potential scan rate from 6.25 to 200 mV s^{-1}. As the currents recorded on GC are relatively high in comparison to those observed on a microelectrode, the quantity of supporting electrolyte was increased up to 0.5 mol L^{-1}. The relatively high concentration of $NaClO_4$ increased conductivity of the solutions, and thus decreased the ohmic potential drop, *IR*. This composition of the solution has proved to be a good medium for investigation voltammetric behavior of synthetic antioxidants and their determination, because of its ability to dissolve both analytes, and their matrix. This mixture of solvents also avoids the passivation of the surface of the working electrode and ensures very good reproducibility of the voltammetric curves.

Calibrations were based on respective DPV curves of BHA and BHT oxidation on CF microelectrode between 3 and 2000 mg L^{-1} (50 different concentrations).

Determinations of synthetic antioxidants in the test solutions and in pharmaceuticals were conducted with the use of a multiple standard addition method. The solutions of BHA and BHT in concentration of about 200 mg L^{-1} and with volume of 50 µL were used as standards. Their concentrations in the solutions were determined with the use of a respective calibration curve.

The solutions of pharmaceuticals were prepared by their dissolving in 25 mL volumetric flask in the same solvent which was described above. The amount of the drug depended on the concentrations of the BHA and BHT. It has been certified that the optimum peak current for their quantitative analysis, should be in the range from about 0.2 to 0.3 nA. The content of the flasks were sonicated for 15 min, and if needed, they were filtered through filtration paper. The samples were directly analyzed without any extraction steps. All stock solutions were stored in a dark and cool place.

2.4. HPLC measurements

BHA and BHT were also determined using the reference HPLC method using a procedure proposed by Saad [14] with some modifications. A mixture of methanol and acetonitrile (50/50, v/v)

was used as the mobile phase at a flow rate of 1.0 mL min^{-1}, and the injection volume was 20 µL. Stock standard solutions containing BHA or BHT were prepared in the mobile phase. Calibration plots were constructed based on the antioxidants peak areas versus concentration. These curves were used for determination of BHA and BHT in real-life products.

The samples of pharmaceutical preparations in amount of between 0.3 and 0.7 g were accurately weighed, placed in 10 mL volumetric flasks, and dissolved in a mixture of methanol/acetonitrile (1/1, v/v). The mixtures were sonicated in ultrasonic bath for 20 min. All the solutions investigated were filtered through a 0.45 µm membrane filter and degassed before use.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of BHA and BHT in acetic acid solutions

3.1.1. Cyclic voltammetry on GC electrode

In the preliminary experiments, cyclic voltammetry (CV) on GC electrode was used to investigate the electrochemical behavior of BHA and BHT in solutions containing various supporting electrolytes: sodium acetate (AcNa), and sodium perchlorate ($NaClO_4$), all prepared in acetic acid or in mixture of acetic acid/acetonitrile (Fig. 1). As can be seen, the best results were obtained in HAc containing 20% AN (v/v) and 0.5 mol L^{-1} $NaClO_4$. Voltammograms recorded in these solutions show well-defined single peaks attributed to oxidation of BHA and BHT. In comparison with the solutions containing AcNa as the supporting electrolyte, an increase in the oxidation potentials of BHA and their decreasing for BHT was observed. This may be due to a different mechanism of the overall process. In addition, the increase of the reversibility of the anodic process can be observed in the presence of $NaClO_4$. A difference of about 0.24 V between the peak potentials guarants their identification even in the binary mixtures (Fig. 1 C). The presence of AN in solutions tested ensures the increase of the peak currents and thus relatively high sensitivity of the future determinations (Fig. 1 B and C). This phenomenon can be attributed to much less viscosity of AN compared to HAc (0.341 and 1.130 mPa s, respectively [34]). The absence of cathodic peak currents corresponding to anodic ones indicates that primary products of the oxidation reaction of BHA and BHT are unstable and undergo successive irreversible homogenous reaction giving the final products which probably undergo reduction in the potential range from 0.0 to 0.1 V vs. Ag/AgCl (Fig. 1 C). This assumption is confirmed by the CV curves presented on Fig. 2. The cathodic peak current increased with increasing of the switching of potential, E_λ from anodic to cathodic and thus with increasing the time when chemical reaction occurs. In contrast to BHA, the final oxidation products of BHT are not electroactive in this medium (absence of the cathodic peak, Fig. 1).

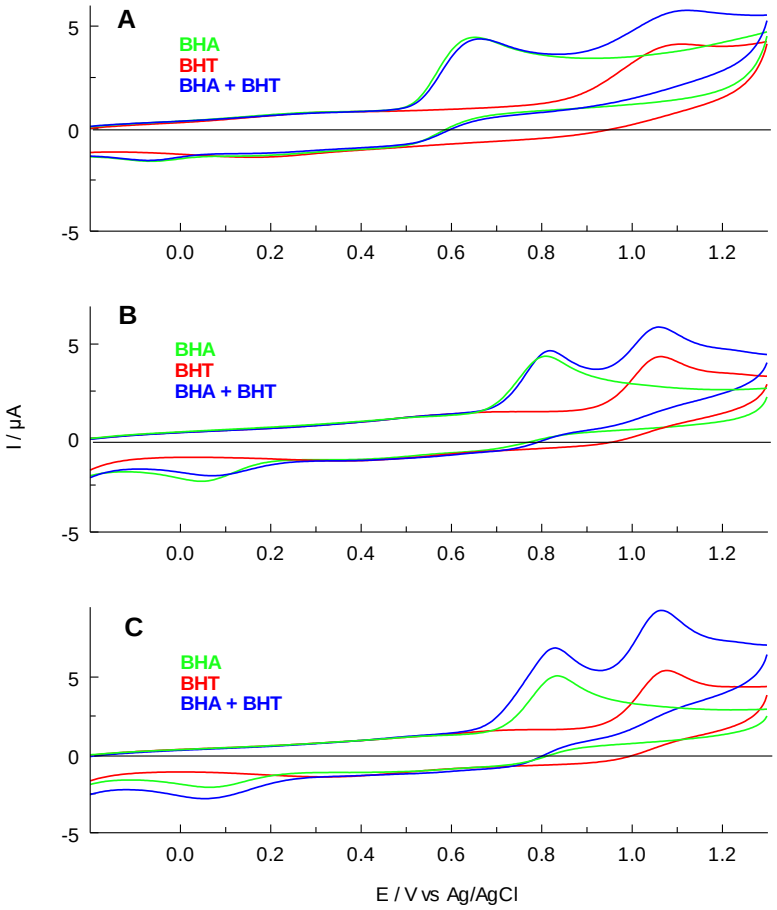


Figure 1. CV curves of BHA and BHT (each 0.27 mmol L^{-1}) and of their mixture (each 0.27 mmol L^{-1}) recorded on GC in solutions of HAc containing (**A**) 0.5 mol L^{-1} AcNa, **(B)** 0.5 mol L^{-1} $NaClO_4$, (**C**) 0.5 mol L^{-1} $NaClO_4$ and 20% AN (v/v). Scan rate 25 mV s^{-1}.

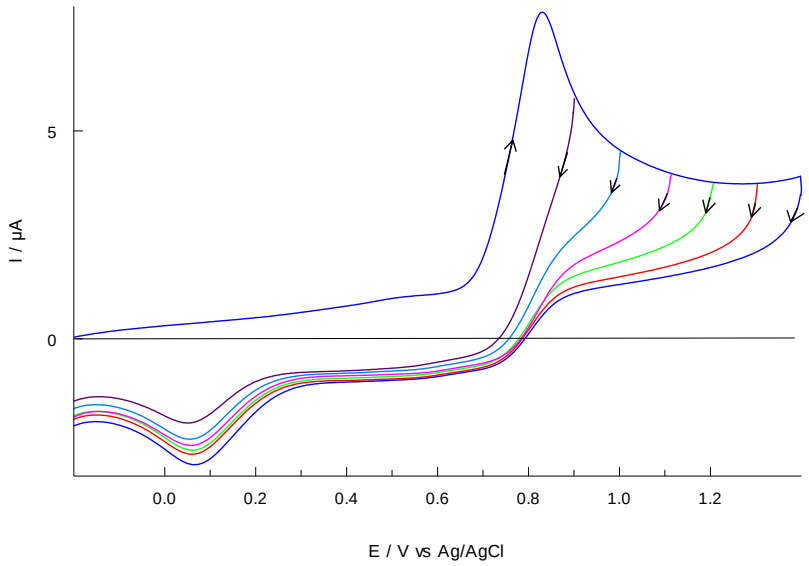


Figure 2. Cyclic voltammograms of 0.50 mmol L^{-1} BHA recorded on GC in HAc containing 20% AN (v/v) and 0.5 mol L^{-1} $NaClO_4$ at scan rate of 25 mV s^{-1}. Direction of electrode polarization was reversed from anodic to cathodic at potentials E_λ: 0.9, 1.0, 1.1, 1.2, 1.3 and 1.4 V.

In order to check whether the anodic oxidation of BHA and BHT occurring on a GC electrode is diffusion or adsorption controlled, the scan rate studies were made. The current of the anodic peaks for all these antioxidants increased with the increase of the potential scan rate, v (Fig. 3 for BHT as example). According to Randles-Sevcik equation, linear dependencies of the peak currents, I_p on $v^{1/2}$ were obtained (inset in Fig. 3) indicating that the anodic oxidation of these compounds is diffusion controlled. This is confirmed by the linear relationships between I_p and v in a logarithmic scale with the values of a slope near to 0.5: $\log(I_p / \mu A) = 1.34 + 0.47\log(v / V\ s^{-1})$ and $\log(I_p / \mu A) = 1.36 + 0.49\log(v / V\ s^{-1})$ for BHA and BHT, respectively (data not shown). The increase in peak potentials with increasing of the potential scan rate (Fig. 3) indicates that the investigated heterogeneous electron transfer processes are irreversible. This is confirmed by the diagnostic criterion based on the difference between peak potential, E_p and the potential at the half height of the peak, $E_{p/2}$ ($E_p - E_{p/2} = 2.218\ (RT/nF) = 0.0565/n$ V at 298 K [35]). The obtained values of this parameter differ from expected for an electrode reaction which proceeds with a reversible exchange of one or two electrons (0.078 – 0.090 V and 0.066 – 0.074 mV at v = 6.25 - 100 mV s^{-1} for BHA and BHT, respectively). The literature data [22,25-28] and our previous results obtained on a platinum electrode in acetic acid solutions [33] indicate that the anodic oxidation of BHA and BHT involves with an irreversible exchange of two electrons.

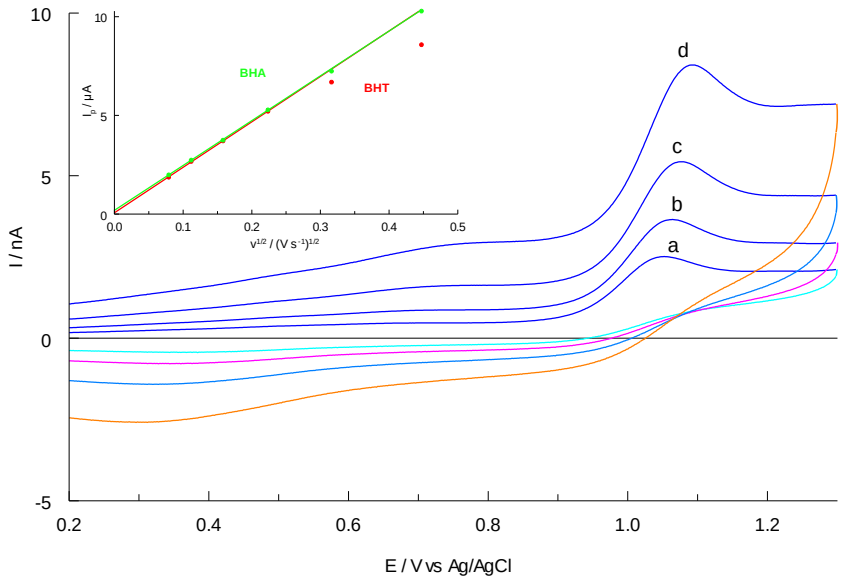


Figure 3. CV curves recorded on GC in solutions containing 0.27 mmol L^{-1} BHT at different scan rates: (a) 6.25, (b) 12.5, (c) 25 and (d) 50 mV s^{-1}. Other components of the solutions as in Fig. 2. Inset: relationship between anodic peak currents, I_p and square root of scan rate, $v^{1/2}$ for BHT and BHA.

3.1.2. Voltammetry on CF microelectrode

Investigations on the anodic oxidation of synthetic antioxidants in a chosen medium were also carried out using the linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) on a

carbon fiber (CF) disk microelectrode (Fig. 4). The use of a microelectrode allows to lower the concentration of the supporting electrolyte with no *IR* effect, and to increase the solubility of hydrophobic compounds and their matrix present in real samples. This is important from practical point of view. These electrodes are used in voltammetric experiments carried out in solvents with a low dielectric constant (ε <10) [36]. Acetic acid is one of them (ε = 6.17 at 25°C [34]). Figure 4A presents LSV voltammetric curves recorded in solutions containing BHA, BHT and their mixture. Well-defined single anodic waves attributed to the oxidation of BHA and BHT are observed with half-wave potentials, $E_{1/2}$ = 0.832 and 1.082 V, respectively. When a direction of polarization is reversed from anodic to cathodic, a small hysteresis is observed. This indicates that the anodic oxidation of synthetic antioxidants proceeds on partially covered surface of the CF microelectrode. Because the electrode reactions are diffusion controlled (sect. 3.1.1), adsorption processes can be attributed to the final oxidation products. Linear relationships between steady-state limiting currents and radius of microelectrode (data not shown) confirm this conclusion. In order to ensure the reproducibility of LSV curves, the surface of the working electrode needed to be polished between measurements. This procedure was not necessary when DPV was applied.

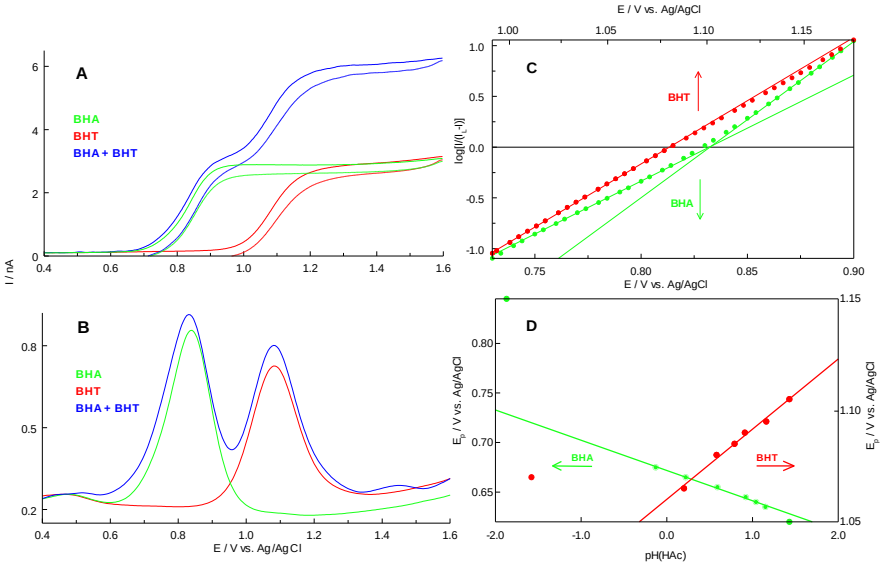


Figure 4. Anodic oxidation curves of BHA and BHT (each 0.27 mmol L^{-1}) and their mixture (each 0.27 mmol L^{-1}) recorded by (**A**) LSV and (**B**) DPV at CF microelectrode in HAc containing 20% AN (v/v) and 0.1 mol L^{-1} $NaClO_4$. (**C**) Semi-logarithmic analysis of the LSV curves. (**D**) Relationship between peak potential, E_p and pH(HAc) for BHA and BHT.

Figure 4(B) presents typical differential pulse voltammograms of the anodic oxidation of BHA, BHT and their mixture obtained at a CF. As can be seen, the oxidation of the analytes proceeds in a single stage giving well-defined peaks at 0.840 and 1.080 V (Table 1). The peak separation between these analytes is achieved to be 0.24 V, which is large enough for their potential recognition. The same conclusions were drawn from CV curves recorded on GC (Fig. 1C). Very good reproducibility of the DPV curves indicates that this technique minimizes the blocking of the electrode surface by the oxidation products.

To check the reversibility of the electrode reactions of BHA and BHT, a semi-logarithmic analysis of the LSV curves was made (Fig. 4(C) according to the equation [35]:

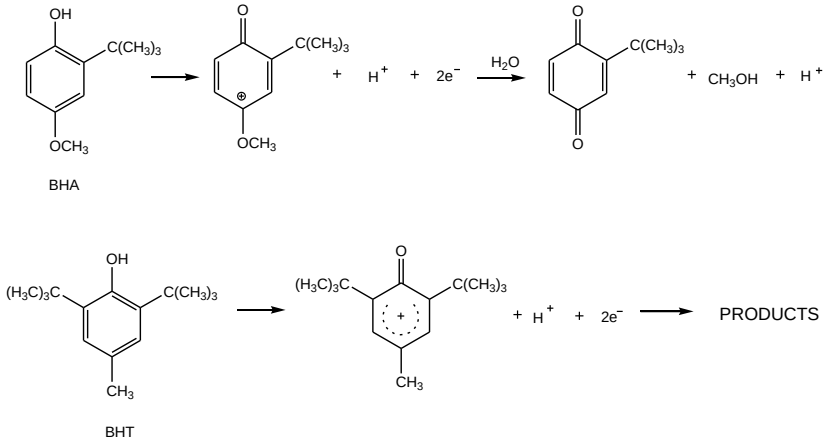
$$E = E_{1/2} + \left(2.303RT / nF\right)\log\left[I / \left(I_L - I\right)\right] \quad (1)$$

where E (V) is the electrode potential, $E_{1/2}$ (V) is the half-wave potential, n is the number of electrons involved in the electrode reaction, I (A) is the current at a given potential E, I_L (A) is the steady-state limiting current. Other symbols have their usual meanings. When the electrode reaction is reversible, the slope, S of the linear plot of E vs. log[I/(I_L-I)] should have a value of 0.0591/n V at 25^{o}C. The semi-logarithmic analysis of the wave for the anodic oxidation of BHT (Fig. 5(C) is linear (r = 0.9999) with a small deviation at higher potentials. The slope of this relationship is 0.0872 V and differs from the theoretical value. This means that the oxidation process of BHT can be related to a totally irreversible exchange of two electrons. In contrast to this, a semi-logarithmic analysis of the LSV curve for BHA consists of two linear parts (Fig 5(C). The slope of the first part (0.0958V) can correspond to the irreversible exchange of the first electron. The second part of this relationship can be related to a quasireversible exchange of a second electron (S = 0.0650V). Because the rate of the whole process depends on the slowest stage, the total BHA oxidation process can be considered irreversible. The irreversibility of the electrode reactions confirms the Tomes criterion [37]. The differences between potentials corresponding to ¾ ($E_{3/4}$) and ¼ ($E_{1/4}$) of the steady-state current recorded by LSV are 0.0810 for BHA and 0.0792 V for BHT and they differ much from the value of 0.0564/n V, predicted for the one-electron reversible electrode process.

In order to check the participation of protons in the electrode reactions, the influence of pH on the on peak potentials, E_p of the DPV curves was examined. The relative changes of pH in HAc solutions were made by the increased concentration of sodium acetate. It is well known that acetate anions are the strongest base in this medium. Thus, their increased concentration must cause an increase in the pH. The constant ionic strenght was maintained by adding different amounts of $NaClO_4$. The total electrolyte concentration was always 0.10 mol L^{-1}. Since the pH scale in acetic acid (from -7 to 7.5 [34]) differs from that characteristic for water, the abbreviation pH(HAc) was used. According to Porras and Kenndler [38] these values can be named as apparent pH and can be used to define relative acidities in this non-aqueous medium. As can be seen from Fig. 4(D), the oxidation peak potential for BHA shifts toward less positive values, and for BHT in oposite direction. This difference can be due to a different mechanism of the electrode processes (proton as a product or substrate of overall electrode processes). The dependencies between the peak potentials, E_p and pH(HAc) presented on Fig. 4(D) are linear and can be expressed by the equations: E_p (V) = 0.67 - 0.030 pH(HAc) (r = 0.997) and E_p (V) = 1.06 + 0.031 pH(HAc) (r = 0.995) for BHA and BHT, respectively. The slopes of these relationships are half the expected theoretical value of 0.0591 V/pH characteristic for an equal numbers of protons and electrons involved in the electrode reaction. The results described indicate that the anodic oxidation of synthetic antioxidants proceeds with an exchange of one proton and two electrons (Scheme 2).

The results obtained show that oxidation of BHA and BHT in acetic acid solutions proceeds according to the E_iC_i mechanism. The irreversible electrode processes (E_i) run within the hydroxyl group and are accompanied by the exchange of two electrons and one proton. The products of these electrode reactions are corresponding cations which are chemically unstable and undergo subsequent

irreversible homogeneous reactions (C_i). The final products of these processes are partially adsorbed on the surface of a working electrode. These products of BHA oxidation probably undergo reduction in the potential range from 0.0 to 0.1 V vs. Ag/AgCl (Fig. 2). In contrast to BHA, the final oxidation products of BHT are not electroactive in this medium (absence of the cathodic peak, Fig. 1). The proposed mechanisms are consistent with obtained in water-alcohol mixtures [22,25-28].



Scheme 2. Postulated mechanism of the anodic oxidation of BHA and BHT.

3.2. Voltammetric Determination of BHA and BHT in pharmaceutical preparations

3.2.1. Validation of the method

Table 1. Peak potentials, E_P and characteristic of BHA and BHT calibration plots (I_P (nA) = a_0 + a_1c (mg L^{-1})) using DPV technique on CF microelectrode

Parameter	BHA	BHT
E_P / V vs. Ag/AgCl	0.840	1.080
Linearity range / mg L^{-1}	0.03 – 594.62	0.18 – 921.37
Correlation coefficient, *r*	0.9999	0.9999
[1)] $a_0 \pm S_a$ / nA	-0.0375±0.02	-0.005492±0.016
[2)] $a_1 \pm S_b$ / nA mg^{-1} L	0.01705±9.2×10^{-5}	0.009749±7.1×10^{-5}
[3)] *LOD* / mg L^{-1}	0.01	0.06
[4)] *LOQ* / mg L^{-1}	0.05	0.30
Repeatability of peak current (%*RSD*, *n* = 10)	0.8	0.6
Reproducibility of peak current (%*RSD*, *n* = 5)	1.2	1.1

[1,2)]Standard error of intercept and of slope, respectively, [3)]3.29σ_B/a_1 (σ_B denotes standard deviation of blank), [4)]5×*LOD* [39]

Basing on the obtained results we proposed the direct voltammetric method of determination of BHA and BHT in pharmaceuticals. A mixture of acetic acid and acetonitrile (20%, v/v) containing 0.1 mol L^{-1} $NaClO_4$ was used as an optimal medium. Very good results were achieved by the use of DPV technique. The proposed analytical method is based on a close relationship between the peak current and analyte concentration in the test sample. In case of each antioxidant an increase in the peak current with increasing of the concentration was observed. Voltammograms of the standards allowed to construct calibration curves and to determine the basic analytical parameters of the method (Table 1).

The developed method is characterized by a wide linearity range, low limits of detection (*LOD*) and quantification (*LOQ*). These parameters confirm the satisfactory sensitivity and utility of the method. An important advantage of this procedure is very good reproducibility and repeatability of the peak current and peak potentials. The peak potentials characteristic for BHA and BHT were stable in the linear ranges of calibration curves. The repeatability of the peak currents recorded in solutions containing 8 mg L^{-1} of these analytes was satisfactory, and *RSD* not exceeded 0.8% and 0.6% (n = 10) for BHA and BHT, respectively. The reproducibility of the results was evaluated by measuring of the peak currents with the use of the same solutions for a period of 5 days, and obtained *RSD* values were not greater than 2.6% (Table 1). Comparison of the analytical parameters characterising the developed method with literature data is presented in Table 2. A mixture of acetic acid and acetonitrile provides the broadest linearity range and lower [22,23,31] or comparable [25-28] detection limits for analytes.

Table 2. Comparison of the linearity range (*LR*) and limit of detection (*LOD*) for BHA and BHT obtained in this work with other voltammetric methods.

Method, Electrode	*LR* / mg L^{-1}		*LOD* / mg L^{-1}		Reference
	BHA	BHT	BHA	BHT	
SWV, [1)]MCCE/$Cu_3(PO_4)_2$	0.06-7.39	0.08-9.04	0.01	0.02	[28]
CV, [2)]Pt/PPy/NiPcTs	36.05-180.25	lack	18.7	lack	[23]
DPV, [3)]graphene/Ch/GCE	0.11-36.05	lack	0.03	lack	[26]
LSV, [4)]AuNPs/GCE	0.10-1.50	0.20-2.20	0.04	0.08	[25]
SWV, [5)]BDD	0.11-1.80	0.13-2.20	0.02	0.06	[27]
LSV, GCE	0.5-15	0.5-8.0	0.19	0.15	[22]
LSV, CF	lack	lack	1.35	2.76	[31]
DPV, CF	**0.03-594.62**	**0.18-921.37**	**0.01**	**0.06**	**This work**

Electrodes modified: [1)]carbon composite modified with $Cu_3(PO_4)_2$ immobilized in polyester resin, [2)]polypyrrole modified with a nickel phthalocyanine complex, [3)]glassy carbon modified with monolayer of choline and graphene, [4)]glassy carbon modified with gold nanoparticles, [5)]boron-doped diamond electrode.

3.2.2. Interference studies

Various substances, such as methylparaben, phenoxyethanol, disodium EDTA, benzoic acid, isopropyl, ethyl, cetyl and benzyl alcohol, ethylene glycol, phenylethanol, chloride ions and glycerine, which are commonly present together with BHA and BHT in pharmaceuticals, were tested as potential interferences in the same experimental conditions. Their concentrations in tested solutions were 10-fold higher as compared to the amount of analytes. Comparative analysis showed that only methylparaben makes it difficult to determine BHT, reducing its peak currents by about 20%. At a concentration ratio 1:1 of these compounds, the observed change was only 0.4%. The impact of the remaining interferences on signals derived from synthetic antioxidants did not exceed 4%. Thus, the developed method can be considered as specific.

3.2.3. Voltammetric analysis of BHA and BHT in real samples

Studies on the quantitative determination of BHA and BHT in pharmaceutical preparations were started with control assays. They allowed to check the reliability and accuracy of the method by comparing the true quantity of the analyte with that obtained in course of the analysis. For this purpose, solutions of known concentration were prepared: BHT (8.46 mg L^{-1}) and BHA (8.24 mg L^{-1}) in solution described above. Analytical procedure was based on the multiple standard addition method. The volume of 2.0 mL of the test solutions were transferred to a measuring cell, and DPV curves were recorded before and after the addition of 50 μL of BHA (206.0 mg L^{-1}) or BHT (211.4 mg L^{-1}) standard solutions. These concentrations of the standards allowed to obtain clear increments of analytical signals. Figure 5A presents responses obtained for BHT and the calibration plots for five independent determinations. DPV curves characteristic for BHA are identically shaped (not shown). It should be noted that all the curves recorded were very good reproducible, and no changes in their peak potentials were observed. All determinations were repeated five times. The results obtained were converted into BHT or BHA content in 1g of the preparation and statistically examined (Table 3). The average content of the analytes in control solutions only marginally differ from the true amount (recovery, R = 99.8% for BHT and 100.0% for BHA), thus the developed method can be considered accurate. The low relative standard deviations, *RSD* (0.4% and 1.0% for BHT and BHA, respectively) indicate high precision of this procedure.

Finally, BHT and BHA were determined in different commercially available pharmaceutical preparations. The analytical procedure was identical as for control determinations. DPV curves recorded at a CF disk microelectrode in the solution of a pharmaceutical preparation Fypryst which was taken as example is shown in Fig. 5(B). As can be seen, the peak corresponding to BHT oxidation can be easily identified at a potential of 1.08 V vs. Ag/AgCl which is the same as for the standard (Fig. 4B, Table 1). The additional oxidation peak at 0.84 V can be attributed to the oxidation of BHA. This signal was the basis to determination of BHA in the same preparation. The peak currents increased linearly with the addition of the standard solutions of BHA or BHT. The results obtained for all pharmaceuticals investigated are presented in Table 3. They confirm very good accuracy of the method (R values for two preparations containing known amounts of antioxidants were 96.4% - 100% and

100% for BHA and BHT, respectively) and precision (*RSD* values were no greater than 1.6% and 0.6% for BHA and BHT, respectively).

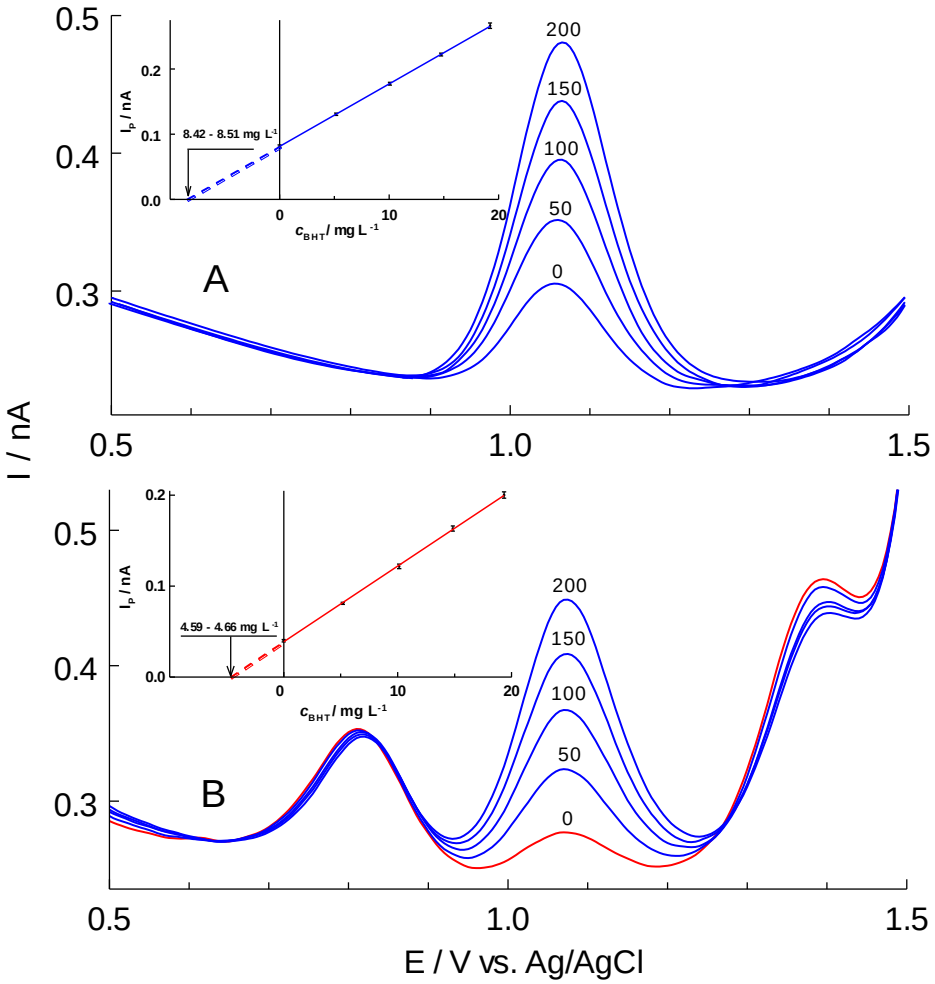


Figure 5. DPV curves recorded (**A**) in control solution of BHT (8.46 mg L^{-1}) and (**B**) in solution containing pharmaceutical Fypryst (50 mg mL^{-1}) and after additions of BHT standard solution (c = 211.4 mg L^{-1}, the volumes in µL are given at curves). Insets: calibration curves in the standard addition method for the control and pharmaceutical preparation, respectively.

The results obtained with the use of the developed voltammetric method were compared with these from HPLC. The statistically examined results are presented in Table 3. As can be seen, precision of the results for BHA obtained by HPLC (*RSD* values were not greater than 2.2% and 5.3% for BHA and BHT, respectively) is worse than these of DPV. However, the comparison of the results using the *F*-test indicates that precision of these methods is comparable only for BHA. Significant differences are observed for BHT (calculated *F*-values exceed tabulated one). The accuracy of both methods (see *R* values for preparations with known amount of synthetic antioxidants, and comparison of calculated and tabulated *t*-values, Table 3) is comparable. The exception is formulation Imazol. Developed DPV method gives the result in accordance with the summary of a product characteristic. In addition, it was impossible to determine the content of BHA in formulation Fypryst by HPLC. This

is probably a result of not separated signals characteristic for butylated hydroxyanisole and for one of the excipients with similar polarity.

Table 3. Results of the BHA and BHT determination in control and in pharmaceuticals by DPV compared with a reference HPLC method.

Sample	[1)]Amount labeled / mg g^{-1}	DPV			HPLC			*t*-test [5)](2.31)	*F*-test [5)](6.39)
		[2)]Amount found / mg g^{-1}	[3)]*R* / %	[4)]*RSD* / % (n = 5)	[2)]Amount found / mg g^{-1}	[3)]*R* / %	[4)]*RSD* / % (n = 5)		
BHA									
Control	0.412	0.412 ± 0.004	100.0	1.0	–				
Daktarin	lack	0.045 ± 0.001	–	1.2	0.044 ± 0.002	–	2.2	1.96	3.31
Imazol	0.50	0.50 ± 0.01	100.0	0.9	0.45 ± 0.01	90.0	0.9	19.28	1.10
Hylosept	lack	0.052 ± 0.001	–	1.0	–	-	-	-	-
Fypryst	0.195	0.188 ± 0.004	96.4	1.6	interferences	-	-	-	-
Tonimer	lack	0.145± 0.001	–	0.2	0.146 ± 0.001	–	0.7	2.04	4.93
BHT									
Control	0.423	0.422 ± 0.004	99.8	0.4	–				
Advocate	0.89	0.89 ± 0.01	100.0	0.6	0.89 ± 0.03	100.0	2.2	0.33	12.44
Emofix	lack	0.81 ± 0.01	–	0.6	0.77 ± 0.04	-	3.8	3.06	41.53
Fypryst	0.093	0.093 ±0.001	100.0	0.5	0.092 ± 0.006	98.9	5.3	0.44	123.46

[1)]based on the Summary of Product Characteristics, [2)]$x = \bar{x} \pm t_{0.95} s_{\bar{x}}$ for n=5 and $t_{0.95}$=2.776 (tabulated), $s_{\bar{x}}$ - denote standard deviation of mean, [3)]Recovery, $R = (\bar{x}$ / concentration involved) × 100%, [4)]relative standard deviation, [5)]values in parenthesis are tabulated *t* and *F* at $P = 0.05$, $n = 5$.

A significant advantage of the voltammetric method is the matrix effect reduction for complex samples like pharmaceuticals. Because the results of determinations obtained with the use of the developed voltammetric method are close with these declared by manufacturers, this procedure can be considered credible and accurate.

4. CONCLUSIONS

Obtained results indicate that a mixture of acetic acid and acetonitrile (20%, v/v) containing sodium perchlorate as supporting electrolyte is a suitable medium for investigations of the electrochemical properties of synthetic antioxidants and their determination. The process of the anodic oxidation of BHA and BHT is characterized as diffusion controlled, irreversible and is accompanied by an exchange of two electrons and one proton. Based on these electrode reactions, a voltammetric method with the use of DPV on carbon fiber disk microelectrode (CF) was developed for the determination of BHA and BHT in pharmaceutical preparations. A separation of about 0.24 V between

their peak potentials guarantees their determination even in binary mixtures. The characteristic features of this procedure are wide linearity ranges: 0.03-594.62, 0.18-921.37 mg L^{-1}, and low limits of detection: 0.01, 0.06 mg L^{-1} for BHA and BHT, respectively. The satisfactory recovery values for BHA (96.4-100.0%), and for BHT (99.8-100.0%) confirm its utility. An important advantage of this method is limiting the stages of samples preparation for their dissolution in an applied medium, and if necessary, filtration. The proposed method proves to be rapid, simple, sensitive, precise, accurate and warrants very good reproducibility of the results. Consequently, this new voltammatric procedure can be a useful tool for a quality control analysis of the pharmaceutical preparations containing the BHA and BHT.

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