

The Electroanalytical Determination of Antioxidants in Corn Oil Using Ultramicroelectrodes

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Foram feitos estudos eletroquímicos de antioxidantes sintéticos tais como *t*-butildroxianisol (BHA) e *t*-butilhidroxitolueno (BHT). Eles foram realizados tomando como base suas oxidações sobre eletrodos de carbono vítreo de tamanho convencional e também sobre ultramicroeletrodos de fibras de carbono e de discos de platina. Também foram feitos experimentos diretos com óleo de milho comercial contendo os antioxidantes.

Os gráficos de calibração são lineares nos limites de concentração de interesse analítico para ambos, eletrodos convencionais e os ultramicroeletrodos. Entretanto, melhores linhas de base e coeficientes de correlação são obtidos para dados de ultramicroeletrodos. Os limites de detecção teórico e experimental são similares para ambos os tipos de eletrodos usados. Nenhuma interferência foi encontrada entre BHA e BHT, embora β - e γ - tocoferóis (TP) interfiram com BHA em concentrações aproximadamente idênticas de antioxidantes naturais e sintéticos.

Electrochemical studies of synthetic antioxidants such as *tert*-butylhydroxyanisole (BHA) and *tert*-butylhydroxytoluene (BHT) were carried out. They were performed taking their oxidation at glassy carbon conventional size electrodes and also at carbon fiber and platinum disk ultramicroelectrodes as a base. Direct experiments on commercial corn oil containing the antioxidants were also done.

Calibration plots were linear in the concentration ranges of analytical interest for both conventional and ultramicroelectrodes. However, better base lines and correlation coefficients were obtained for the ultramicroelectrode data. The theoretical and experimental detection limits were similar for both types of electrodes used. No interference was found between BHA and BHT, although β - and γ - tocopherols (TP) interfere with BHA at approximately identical concentrations of both natural and synthetic antioxidants.

Keywords: *ultramicroelectrodes, antioxidants, oils, electroanalysis*

Introduction

Many advantages of ultramicroelectrode (UME) properties have been proven. They permit the use of UMEs in electroanalytical methods in more demanding situations where conventional size electrodes (CSE) are unable to give an adequate response^{1,2}.

Thus, UMEs have been proposed for the determination of antioxidants in mineral oils³ and various substances in food⁴. There are also many other applications for UMEs⁵.

The food industry uses antioxidants to preserve the bromatologic quality of their products in order to prevent or retard oxidative rancidity⁶. The most widely used antioxidants in the vegetable oil industry are those based on

phenolic compounds, such as tert-butylhydroxyanisole (BHA) and tert-butylhydroxytoluene (BHT)^{7,8}.

Analytical techniques such as spectrophotometry and chromatography appear to be the most frequently used for the determination of antioxidants⁷. Only a few works in the literature report the use of electroanalytical methods, in spite of their high sensitivity, time efficiency low cost of, analysis, and rather simple equipment⁹⁻¹¹. The use of UMEs along with electroanalytical techniques should allow *in situ* determinations through the use of adequate solvents which prevent the extraction procedure¹². This possibility of using UMEs to perform electroanalysis of antioxidants in food-stuffs has recently been demonstrated^{12,13}. In most cases, the conventional three electrode configuration has been used to obtain experimental data¹⁴. However, the two electrode configuration with UMEs simplifies the method of electroanalysis and also permits a lower detection limit¹⁵.

In this work we show the results of electrochemical determinations of BHA and BHT using Glassy Carbon (G.C.) CSE and platinum and carbon fiber disk UMEs in different media.

Experimental

Instrumentation

Linear sweep and cyclic voltammetric studies were performed in conventional electrochemical cells with two or three compartments^{14,16}. Two or three electrode configuration were also used^{15,16}. In all cases the systems were enclosed in a solid wall galvanized iron Faraday cage.

An EG&G PARC 273 potentiostat/galvanostat or an EG&G PARC 175 universal programmer were used for the three or two electrode configurations, respectively. A Keithley 417 picoammeter was used to measure the current in the second case¹⁵. In both cases the *i*-E curves were recorded by using a HP 7035B or a PAR RE0150 X-Y recorder.

Electrodes and materials

An aqueous saturated calomel electrode (SCE) and a silver wire (Alfa, 99.99%) were used as the reference and quasi-reference electrodes, respectively. The latter was also used as a counter electrode in the two electrode configuration. Otherwise, counter electrodes made of platinum foil were used for all experiments.

All working electrodes were constructed in our laboratory. A glassy carbon electrode constructed with a 3 mm carbon rod and a platinum disk with a 2 mm diameter were both sealed in teflon rods. Disk UMEs were made of platinum wires with a 3, 25 and 50 μm nominal diameters, and of carbon fiber with 6 and 33 μm nominal diameters sealed in glass tubes, as previously described¹⁷. In order to obtain reproducible results, a standard pretreatment procedure was performed on working electrodes before each

experiment. The electrodes were polished with 0.3 μm polishing alumina powder and gently sonicated for a few seconds (except for the carbon fiber ultramicroelectrodes). Then, they were wiped with a wet cloth, rinsed with distilled water and acetone, and dried in warm air.

Commercial corn oil was obtained from a local supermarket, and BHA and BHT (Sigma Chemical Co.) were used as received.

Ethanol (EtOH) (Merck, analytical reagent), acetonitrile (ACN), benzene (Bz), cyclohexane (CHx) and 1,2 dichloro ethane (DCE) (Sintorgan - HPLC) were dried on molecular sieves.

Sodium perchlorate (Koch & Light A.R.) was purified as previously reported¹⁸. Tetra-butylammonium perchlorate (TBAP) (Fluka, electrochemical grade) was dried under vacuum at 60 °C. Pure Trifluoro acetic acid (TFA) (Koch & Light Lab) and sulfuric acid (Merck, analytical reagent) were used as received.

Results and Discussion

Conventional size electrodes (CSE)

The dependence of the peak current on the antioxidant concentration was investigated for each compound in Bz/EtOH (1:2) mixtures containing 0.1 M H₂SO₄ or 0.1 M TFA, and in some cases, in Bz/EtOH/H⁺ containing commercial corn oil with added BHT. In Fig. 1A, a voltammogram obtained in an oil/acid solvent mixture on a glassy carbon electrode shows characteristic peaks for natural tocopherols (TP) (peaks 1 and 2) and BHT (peak 3). An additional peak of unknown origin is indicated as peak 4. The discharge potential of BHA is shown by dotted lines in the voltammogram (peak 5).

The most critical problem in measuring peak currents is to establish a base line⁹. Thus, for the purpose of calibration curve construction, the base line obtained after the extraction of natural antioxidants using ACN as solvent was chosen. Voltammograms obtained after several steps of the extraction process are shown in Fig. 1 (B-D). An *i*-E curve obtained in acid mixtures with no added oil is also shown (Fig. 1E). This curve does not coincide with the one obtained after the exhaustive extraction.

A thoroughly extractive procedure with ACN in corn oil supplemented with BHA and BHT consisted of adding about 30 mL of ACN to 3 mL of oil in increments of 5 mL of ACN each and sonicating for 5 min. Recovery percentages of about 95 to 97% were obtained. The results found (Fig. 1A) for the Bz/EtOH/H⁺/corn oil mixtures agree with those previously reported⁹.

Cyclic voltammetry in ACN containing 0.1 M NaClO₄ on platinum CSE was performed for the purpose of comparison. No substantial differences were found in relation to the other medium, *i.e.* the same peaks and almost the same difference in peak potentials were observed. Other

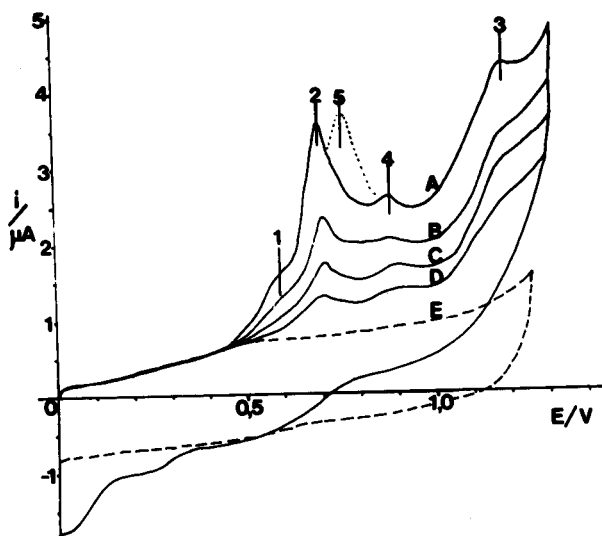


Figure 1. Voltammograms of corn oil in Bz/EtOH/0.1 M H₂SO₄ mixture obtained on G.C.CSEs. Area = 0.071 cm². Effect of extraction of antioxidants with acetonitrile. Volume (mL) of ACN per mL of oil: A) 0; B) 15; C) 35; D) 50. Curve E corresponds to the solvent mixture with no added oil. $v = 0.025 \text{ V s}^{-1}$.

solvents such as CH_x and DCE, or mixtures similar to the one described above were disregarded, since the reproducibility was found to be poor or the oil solubility low. Therefore, the Bz/EtOH/H⁺ solvent mixture appears to be the best among those checked.

The use of TFA makes it possible to obtain a positive potential limit wider than the corresponding one when using H₂SO₄. Lower concentrations (0.01 M) of the supporting electrolyte might be used with UMEs^{1,17} to allow the determination of BHT with a higher confidence level.

The calibration plots were obtained in the presence of the corn oil/Bz/EtOH/H⁺ mixture after the extraction of the natural antioxidant, as well as in its absence (ACN), for BHA and BHA/BHT, respectively. Regression parameters and detection limits are summarized in Table 1. Theoretical detection limits (d.l) were calculated using the relationship $d.l = s.t/k$, where s is the standard deviation for the appropriate constant at the 95% confidence level for $n-2$ degrees of freedom, n is the number of data points, and k is the slope of the least-square straight line¹⁹. About twelve data points over the range 0 - $1.10^{-4} \text{ mol dm}^{-3}$ were used in the calculations. Experimental detection limits were also determined. Both are presented in Table 1 for comparison. In most cases, these limits agree quite well, although the experimental ones are slightly higher than the theoretical ones. Values for BHT regression parameters in Bz/EtOH/H⁺ are not shown in Table 1 because they show a very poor correlation (correlation coefficient = 0.423). As, BHT determination on G.C.CSE in this mixture is not possible.

Ultramicroelectrodes (UME)

The fundamental difficulties in the use of G.C.CSE for the determination of antioxidants were the proper selection of a base line and the superposition of anodic peaks, a hard problem to solve when facing a low concentration of antioxidant.

Empirical criteria have usually been adopted for the selection of the base line⁹, and an alternative was described above, but the problem has not fully been overcome. The use of UMEs appears to be a promising solution to the difficulties mentioned above. This is based on the concept that the i - E responses on UMEs are sigmoideal in nature, which permits a better definition of the base line, and even a better resolution of oxidation waves due to the presence

Table 1. Regression parameters and detection limits for antioxidants calibration data in Bz/EtOH/H⁺ and ACN on glassy carbon CSEs and platinum and carbon fiber UMEs.

E.M. (a)	Solvent	AOx (b)	Oil (c)	Slope (d)	Intercept (e)	C.C. (f)	T.D.L. (g)	E.D.L. (h)
Carbon Fiber UME	Bz/EtOH/H ⁺	BHA	YES	$(6.93 \pm 0.23)E3$	0.022 ± 0.0091	0.994	$8.38E-6$	$1.34E-5$
		BHA	NO	$(4.55 \pm 0.05)E3$	0	0.999	$5.34E-6$	$1.06E-5$
		BHT	YES	$(8.64 \pm 0.53)E3$	0.22 ± 0.022	0.982	$1.14E-5$	$1.23E-5$
		BHT	NO	$(4.72 \pm 0.18)E3$	0.021 ± 0.0079	0.993	$9.68E-6$	$1.04E-5$
Glassy Carbon (CSE)	Bz/EtOH/H ⁺	BHA	YES-SBL(i)	$(1.48 \pm 0.11)E4$	0.30 ± 0.041	0.976	$1.6E-6$	$1.79E-5$
		BHA	YES-CBL(j)	$(1.65 \pm 0.12)E4$	0.29 ± 0.046	0.976	$1.6E-6$	$1.79E-5$
Platinum UME	Acetonitrile/0.1M NaClO ₄	BHA	NO	$(4.67 \pm 0.13)E4$	0	0.996	$1.64E-5$	$1.78E-5$
		BHT	NO	$(4.01 \pm 0.13)E4$	0	0.994	$1.46E-5$	$1.56E-5$
Glassy Carbon (CSE)	Acetonitrile/0.1M NaClO ₄	BHA	NO	$(2.35 \pm 0.02)E4$	0	0.999	$4.05E-6$	$5.05E-6$
		BHT	NO	$(2.39 \pm 0.03)E4$	0.016 ± 0.008	0.999	$2.256E-6$	$4.05E-6$

Errors shown are standard deviations. a) E.M: Electrode material; b) AOx: antioxidant; c) YES: in presence of oil; NO: in the absence of oil d) SLOPE: in nA/M or $\mu\text{A/M}$ for UME or CSE, respectively; e) INTERCEPT: in nA or μA for UME or CSE, respectively; f) C.C: correlation coefficient; g) T.D.L.: theoretical detection limit in mol/dm³; h) E.D.L.: Experimental detection limit in mol/dm³; i) SLB: straight base line; j) CLB: curved base line constructed following the trend of the i - E curve.

of limiting currents instead of peak currents, as is found when working with CSEs⁵. Another advantage of UMEs is the possibility of using lower supporting electrolyte concentrations than with CSEs^{5,17}. Thus, platinum and carbon fiber disk UMEs were used to perform cyclic voltammetry at slow sweep rates (*i.e.* $v = 0.01 \text{ V s}^{-1}$) with the intention of determining the feasibility of this technique for quantifying antioxidants in vegetable oil. Figures 2a and 2b show the typical voltammetric responses of BHA and BHT on Pt and C.F. UME, respectively. In Fig. 2a, the *i*-E curve was obtained from ACN used to exhaustively extract the natural as well as the synthetic antioxidants added to an oil sample. In Fig. 2b the response shown corresponds to a solution of BHA and BHT in the Bz/EtOH/H⁺ solvent mixture. The waves for the discharge of each antioxidant, and even for the tocopherols, are indicated. As can be seen, a good limiting current definition and good base lines are obtained in comparison with peaks and base lines obtained with CSEs. Calibration plots for Pt UME responses in ACN are shown in Fig. 3 for a 0-1.10⁻⁴ mol dm⁻³ range in BHA and BHT. Values for regression parameters and theoretical and experimental detection limits are presented in Table 1. The data correspond to Pt and carbon fiber UMEs in ACN/0.1 M NaClO₄ and Bz/EtOH/H⁺, respectively. As can be observed, detection limits are lower by about half an order of magnitude for G.C CSEs than for Pt UMEs in ACN, although an opposite trend appears in Bz/EtOH/H⁺ media. An important difference between G.C.CSE and UME performance comes from the sensitivity (slope) values. In both media, the sensitivity of UMEs is higher than that for G.C.CSEs. The use of TFA in Bz/EtOH/H⁺ mixtures per-

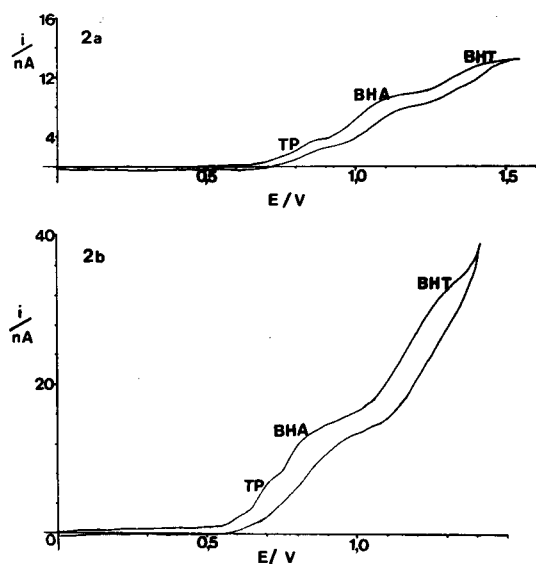


Figure 2. a) Cyclic voltammogram for the oxidation of BHA and BHT intensively extracted from oil with ACN. Working electrode: Pt UME ($\varnothing = 25 \mu\text{m}$). $v = 0.01 \text{ V s}^{-1}$; b) Voltammogram for the oxidation of BHA and BHT added to a mixture of corn oil + Bz/EtOH/0.01M H⁺. [BHA] = $8.06 \cdot 10^{-5} \text{ M}$ and [BHT] = $7.4 \cdot 10^{-5} \text{ M}$. Working electrode: carbon fiber UME ($\varnothing = 33 \mu\text{m}$). $v = 0.01 \text{ V s}^{-1}$.

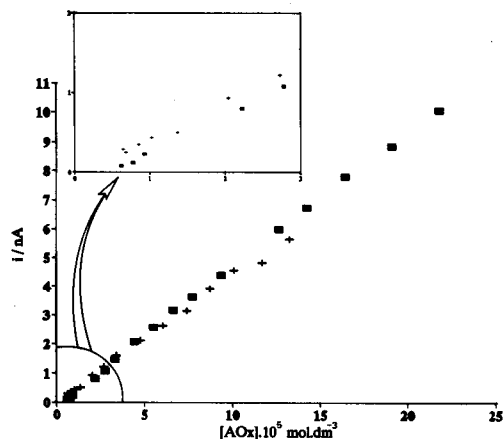


Figure 3. Calibration curves for the oxidation of BHA and BHT in ACN/0.1 M NaClO₄ on platinum UME. Antioxidant: (●) BHA; (+) BHT. Data for the inserted plot were obtained by using the two electrode format, and the units are identical to those of the main one.

mits the determination of BHT by UMEs, a determination which is rather difficult to perform by CSE, as was previously explained.

Preliminary results using a two electrode configuration (*i.e.* a Pt UME and a silver wire counter electrode) have lowered the detection limit by about three times with respect to that obtained from a three electrode configuration (see Fig. 3). From the results obtained, UME techniques appear to be a good option for the determination of antioxidants in vegetable oil samples.

Conclusion

From data obtained on CSEs and UMEs it is possible to establish that conventional electrochemical techniques constitute a fairly rapid and sensitive methodology for the analysis of antioxidants in foodstuffs.

Voltammograms obtained from conventional electrodes in a three electrode cell show peak superposition. Furthermore, an additional complication regarding the establishment of the base line arises from the fact that the BHT discharge is close to the potential limit of the supporting electrolyte-solvent system.

Extraction with ACN appears to be a successful experimental procedure for overcoming the problem.

Platinum and carbon fiber ultramicroelectrodes have experimental electrochemical responses useful for selecting a better base line. Good correlation parameters were found for calibration curves.

A two electrode configuration with ultramicroelectrodes made it possible to diminish the antioxidant detection limit.

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