Synthetic antioxidants determination in lard and vegetable oils by the use of voltammetric methods on disk ultramicroelectrodes

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Abstract

Voltammetry on ultramicroelectrodes was used to determine antioxidants *tert*-butylhydroxyanisole (BHA), *tert*-butylhydroxytoluene (BHT) and propyl gallate (PG) in vegetable oils or lard. The present work demonstrates the advantages of using not only the conventional three electrode configuration but also the non-conventional two electrode configuration cell system, with respect to the determination of antioxidants. Different ways of performing the determinations are compared either through step by step extractive procedures or through direct measurements using standard addition. Detection limits in the range of $(0.20–1.25)\times10^{-5}$ mol dm^{-3}, $(1.00–1.25)\times10^{-5}$ mol dm^{-3} and $(0.60–1.00)\times10^{-5}$ mol were obtained for BHA and BHT in vegatable oil and PG in lard determinations, respectively, depending upon the medium composition. Relative standard deviations for antioxidants concentration were about 6–7% for most of media studied and can be compared with those reported for HPLC from collaborative studies, i.e. 4.54, 6.61 and 3.18% for BHT, BHA and PG, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Carbon fiber disk ultramicroelectrodes; Antioxidants; Lard and vegetable oils; Standard additions method

1. Introduction

Since the late 1970s investigations performed with ultramicroelectrodes (UME) have allowed a renewal in the development of the electrochemical area, contributing new insights in their use, mainly in kinetics and electroanalytical studies (Fleischmann, Pons, Rolison & Schmidt, 1987; Montenegro, Queirós & Daschbach, 1990; Wightman & Wipf, 1989).

Based on UME small size (radii commonly between 1 and 25 μm), the electrical current running through the cell is usually in the order of 10^{-9} A. Thus, the small ohmic drop related to UME appears as one of the advantages of UME use with respect to conventional size electrodes (CSE) (Bond, Fleischmann & Robinson, 1984; Fleischmann et al., 1987; Montenegro et al., 1990; Wightman & Wipf, 1989). This allows work in highly resistive media, for direct determination of analyte in an actual sample matrix or in very small sample volumes, etc, significantly enlarging the scope of analytical chemistry (Montenegro et al.). The classical three-electrode configuration (ThEC) has been employed in most of the measurements (Sawyer & Roberts, 1974), although a two-electrode configuration (TwEC) has been proposed as an inexpensive alternative with the additional advantage of increased sensitivity (Bixler et al., 1986). However, little attention has been paid to its use until now (Ceballos & Fernández, 1995).

UME have been proposed for the determination of many substances. Thus, determinations related to various substances in food (Williams, 1990), antioxidants in mineral oils (Cheek & Mowery, 1989) and synthetic antioxidants in corn oil (Ceballos & Fernández, 1995) can be mentioned. *Tert*-butylhydroxyanisole (BHA), *tert*-butylhydroxytoluene (BHT) and propylgallate (PG) are widely used antioxidants in vegetable oils and lard (Huang & Ferraro, 1992; Robards & Dilli, 1987; Valenzuela & Nieto, 1995).

Some electrochemical studies were recently conducted to assay on BHA and BHT using both glassy carbon

CSE (Clough, 1992; McBride & Evans, 1973; Yañez-Sedeño, Pingarrón & Polo Diez, 1991) a platinum and carbon fiber disk UME (Ceballos & Fernández, 1995) and a cylindrical carbon fiber UME (Aguí, Reviejo, Yañez-Sedeño & Pingarrón, 1995) in different media. A ThEC system on UME was successfully used for these purposes. The TwEC preliminary results are promising (Ceballos & Fernández).

In this work, we have tried to demonstrate the direct determination of antioxidants in vegetable oils and lards by the renewed standard addition method (Locatelli & Torsi, 1996), avoiding the steps of extraction and preconcentration of analytes. We report a comparative study of three and two electrode configuration cells using UME with carbon-fiber disks to assess the usefulness of the two-electrode configuration for analytes quantitation.

2. Experimental procedures

2.1. Instrumentation and methods

Measurements were performed in a two compartment Pyrex cell (Fernández & Sereno, 1986). Linear sweep and cyclic voltammetric experiments were conducted with both three- and two-electrode configuration. For the first case, an Elchema PS-505 potentiostat (Elchema, USA) and an EG&G PARC 175 Universal Programmer (EG&G PARC, USA) were used, while for the second case, the potentiostat was replaced by the programmer as a potential source and a Keithley 417 picoammeter (Keithley, USA) for measuring the current. In both cases, the current (i)–potential (E) curves were recorded with a HP 7035B (Hewlett-Packard, USA) or a PAR RE0150 X-Y (EG&G PARC, USA) recorder. Even when the response of the picoammeter is slow, when it is used with UME and a function generator as a potential source, renders excellent quasi steady state voltammograms when they are recorded at slow sweep rates (i.e. 5–10 mV s^{-1}). The two-electrode configuration is shown in Fig. 1.

The standard addition method was used when vegetable oil or lard were present in the solution. This method was chosen due to its advantages when interference from other substances are present (Locatelli & Torsi, 1996).

An electrical signal that appears as an elongated wave was observed in the potential range of oxidation of natural antioxidants when working with UME in solvent-free oils. However, in-situ antioxidant determinations (i.e. measurements in solvent-free oils) were not performed because they are not convenient from a practical point of view, i.e., difficulties in the construction of the calibration curves and the preparation of standards for the standard addition method.

2.2. Electrodes and material

The working electrode was a carbon fiber disk UME of approximately 33 μm in diameter. Its construction and pretreatment have been described elsewhere (Ceballos & Fernández, 1995; Zón, Moressi, Sereno & Fernández, 1994). It was pretreated before each voltammetric run as previously described (Ceballos & Fernández). 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 TwEC. For the ThEC system, the counter electrode was a platinum foil with a relatively large area (approx. 2 cm^2). Both, the reference and counter electrodes were adjusted in the cell as described (Fernández & Sereno, 1986; Zón, Fernández, Sereno & Silber, 1987).

Analytical grade BHA, BHT, PG and Ferrocene (Fc) supplied by Sigma Chemical Co. (Buenos Aires, Argentine) were used as received. Acetonitrile (ACN) and acetone (Ac) were Sintorgan (Buenos Aires, Argentine) HPLC grade. Benzene (Bz) and ethanol (EtOH) were Sintorgan (Buenos Aires, Argentine) UV–visible and Merck p.a. (Buenos Aires, Argentine), respectively. The solvents were filtered through 3 or 4 Å molecular sieves for 48 h prior to use and were then used without further purification.

Sulfuric acid (Merck p.a., Buenos Aires, Argentine) was used as received. NaClO$_4$ (Merck p.a.) was purified

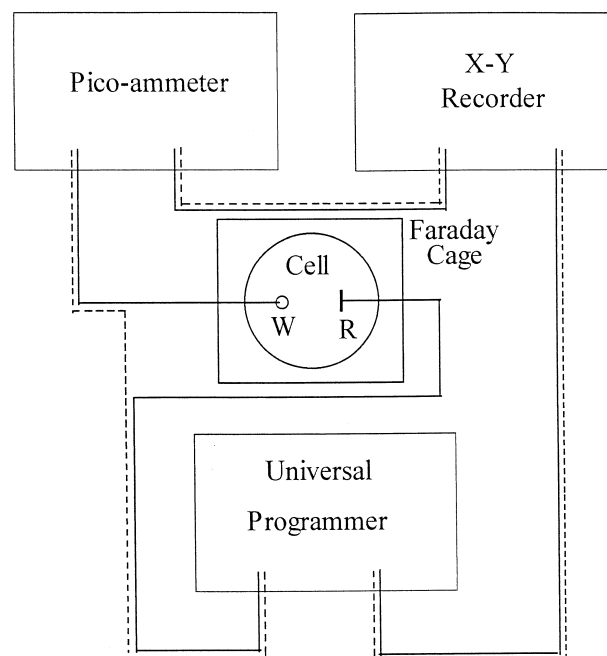Fig. 1. Block diagram corresponding to a two electrodes configuration system for the measurement of low currents associated with ultramicroelectrodes: W, working electrode (UME); R, reference/counter electrode.

as described elsewhere (Fernández, 1978). $(C_2H_5)_4NF_4B$ (TEATFB) (Fluka, Buenos Aires, Argentine, electrochemical grade) was dried under vacuum at 60°C. These three substances were used as supporting electrolytes. H_2SO_4 was replaced by TEATFB when working with BHT in Bz:EtOH (1:2), to extend the potential range of the solvent system to more positive potentials.

Commercial sunflower oil and lard with no antioxidant added (according to the manufacturer label) were obtained from a local supermarket. When necessary, binary solvent mixtures were used to dissolve the oil or lard for the direct determination of the antioxidant/s added in our laboratory to the actual samples.

The electroactive reagent concentrations were in the range of the experimental detection limit (see below) up to about 200 ppm and that of the supporting electrolytes was 0.1 M.

2.3. Density and viscosity measurements

Densities were determined by using picnometers of 10 ml capacity with accuracy greater than 1%. Viscosities were obtained through Ostwald-Cannon-Fenske viscometers at room temperature by the usual method (Levine, 1991) with an accuracy of no less than 2%.

3. Results and discussion

3.1. Ferrocene

The ferrocene (Fc)/Ferrocinum (Fc^+) redox system was chosen to check initially the ThEC and TwEC systems. The Fc oxidation in ACN was chosen as the probe system because it is well known that it comforms to a one electron reversible process (Bond et al., 1984; Zón et al., 1994). Thus, experiments of cyclic voltammetry at slow sweep rates on carbon fiber disks UME in ACN / 0.1M $NaClO_4$ gave, for the oxidation of Fc at a given concentration, comparable current (i)–potential (E) curves for TwEC as well as for ThEC. A small shift of half-wave potential due to the different reference electrodes in both systems was observed, as shown in Fig. 2, but the limiting currents (i_L) were almost identical. The log analysis, i.e. E vs log (i_L–i/i), of both i–E curves (Zón et al., 1994) gives equal slopes and intercepts within experimental error. Finally, a plot of the limiting current obtained for the TwEC against that for the ThEC, at different given Fc concentrations, gave a slope very close to one (Fig. 3). These preliminary tests served to check both electrode configurations before experiments in more complicated systems, such as those represented by the oxidation of phenolic antioxidants in different media.

3.2. Synthetic antioxidants calibration curves

Typical voltammograms on UME for the oxidation of antioxidants in different media are shown in Fig. 4. Voltammograms at UME for the oxidation of BHT in an oil containing tocopherols (natural antioxidants) in Bz/EtOH/TEATFB and also for the oxidation of BHA and BHT in ACN/$NaClO_4$ solutions are shown in Fig.
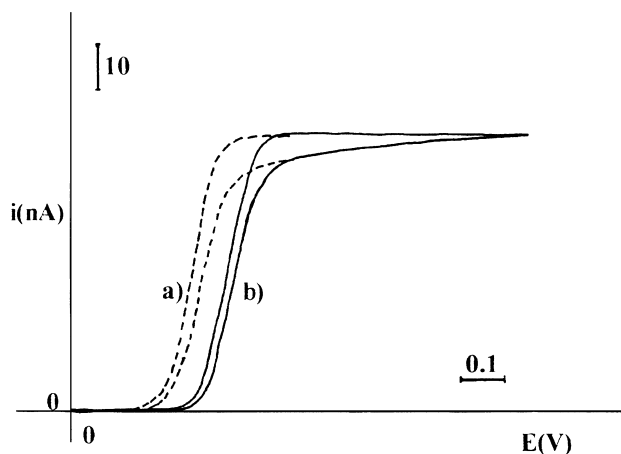

Fig. 2. Cyclic voltammograms at a 33 μm diameter carbon fiber microdisk electrode for oxidation of 4.30×10^{-3} M ferrocene in ACN/ 0.1 M $NaClO_4$: (a) TwEC; (b) ThEC ($v = 0.010$ V s^{-1}).
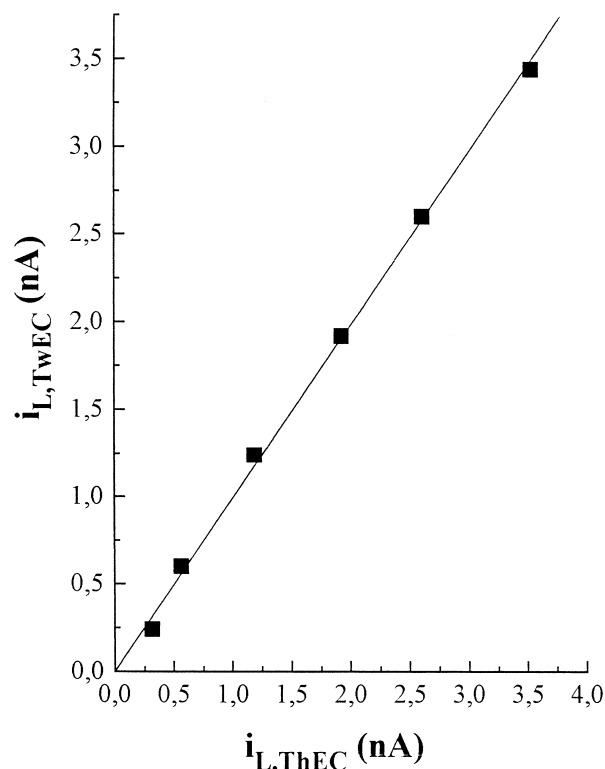

Fig. 3. Plot of the limiting current obtained from TwEC vs ThEC at different given ferrocene concentrations. Working electrode: carbon fiber disk UME ($\varnothing = 33$ μm). ACN/0.1 M $NaClO_4$. ($v = 0.010$ Vs^{-1}).
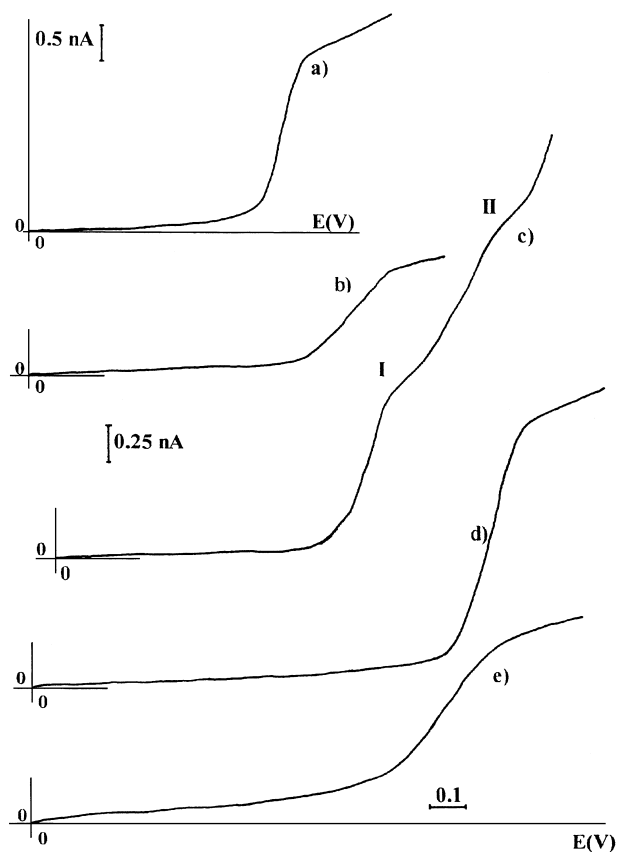
Fig. 4. Voltammograms for antioxidant oxidation using ThEC in different media. (a) 4.62×10^{-5} M PG/10.1% lard/Bz/EtOH (1:2)/0.1 M H_2SO_4; (b) 1.93×10^{-5} M BHA/ACN/0.1 M $NaClO_4$; (c) 1.04×10^{-5} M BHA (wave II)/9.09% sunflower oil/Acetone/0.1 M $NaClO_4$, (wave I corresponds to tocopherols oxidation); (d) 6.29×10^{-5} M BHT/ACN/ 0.1 M $NaClO_4$; (e) 3.08×10^{-5} M PG/ACN/0.1 M $NaClO_4$. Working electrode: carbon fiber disk UME ($\varnothing = 33$ µm). (v = 0.010 Vs^{-1}). Vertical axis: (a) 0.5 nA/div; (b)–(e) 0.25 nA/div. Horizontal axis: 0.1 V/div.
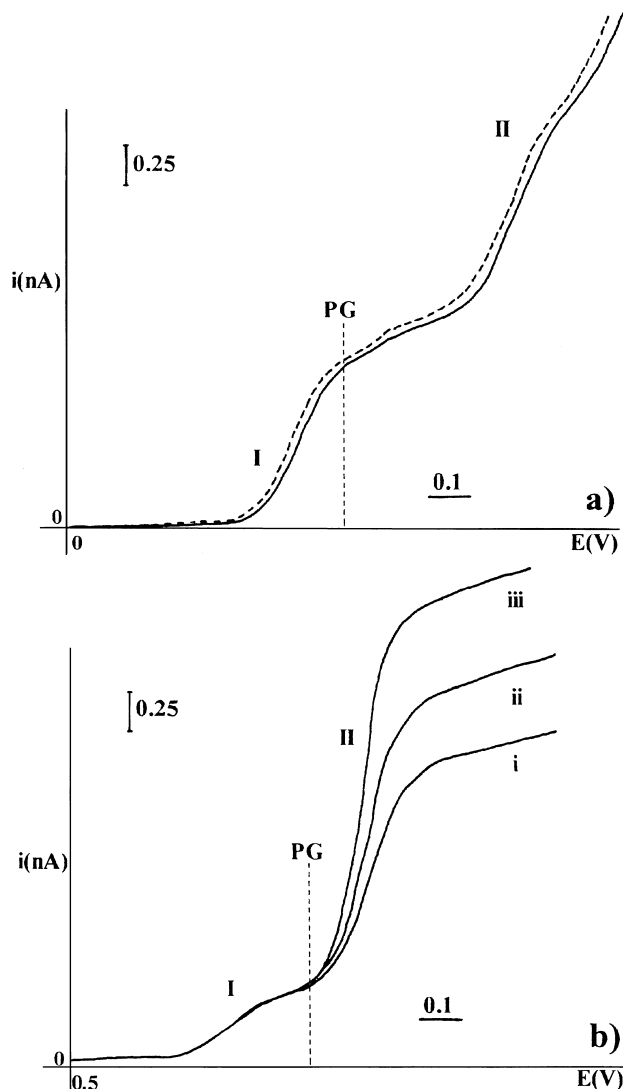

Fig. 5. Voltammograms for the oxidation of: (a) natural antioxidants (tocopherols, wave I) and BHT (wave II) added to a mixture of sunflower oil (9.09%) with Bz/EtOH (1:2)/0.1 M TEATFB, $C_{BHT} = 2.72 \times 10^{-5}$ M, obtained through ---- TwEC and — ThEC; (b) BHA (wave I) and BHT (wave II) in ACN/0.1 M $NaClO_4$, $C_{BHA} = 1.30 \times 10^{-5}$ M, $C_{BHT}=$ (i) 5.15×10^{-5} M, (ii) 6.29×10^{-5} M, (iii) 8.58×10^{-5} M. Carbon fiber disk UME ($\varnothing = 33$ µm). (v = 0.010 Vs^{-1}). TwEC system. The vertical dotted lines indicate the approximate half wave potential for the PG oxidation.

5. The anodic oxidation of tocopherols has been previously reported (Clough, 1992; McBride & Evans, 1973). The approximate half-wave potential for the oxidation of PG is also indicated in Fig. 5 by a vertical dotted line. As stated elsewhere, one of the main difficulties in the use of conventional size electrodes (CSE) for the determination of antioxidants is the proper selection of a baseline and the overlapping of anodic peaks, particularly at a low concentration of antioxidants (Ceballos & Fernández, 1995; McBride & Evans, 1973). These problems are, in principle, overcome by using UME, since the current potential responses are sigmoid shape (Montenegro et al., 1990). This leads to a better definition of the baseline and also a better resolution of the oxidation curves through measurement of limiting currents instead of peak currents, as found when working with CSE (Ceballos & Fernández; Williams, 1990).

In order to gain confidence in the TwEC systems, the limiting currents obtained for the oxidation of each

antioxidant in both electrode configurations were compared. In all cases, slopes close to unity were obtained from plots of limiting currents for TwEC versus limiting currents for ThEC for given concentrations of each antioxidant. As an example, a plot of limiting current for PG oxidation on UME for TwEC against that for ThEC is shown in Fig. 6. A slope of 1.014 (0.002) was obtained for this particular case. Then, calibration curves for each antioxidant were obtained from UME measurements in solutions containing only one antioxidant in the presence, as well as in the absence, of commercial vegetable oil (or lard) in both configurations. They were constructed by measuring the limiting
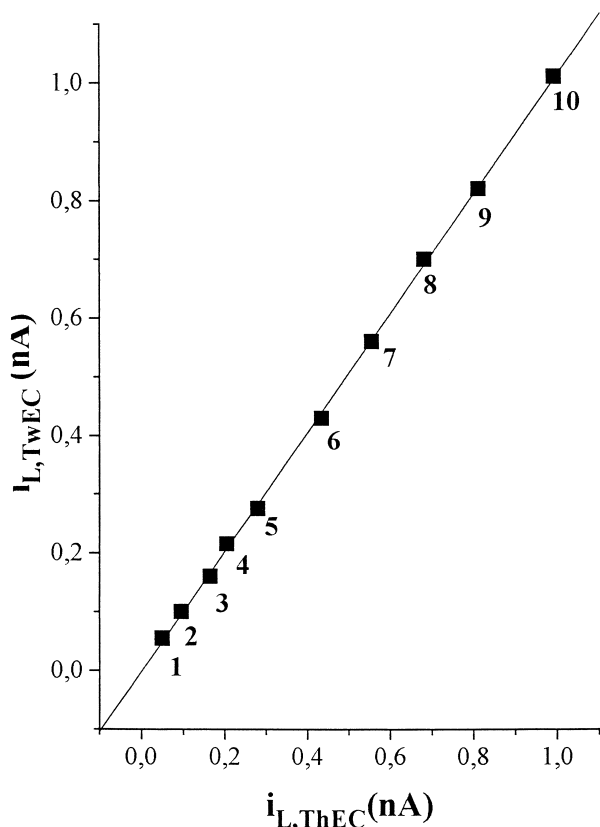
Fig. 6. Plot of $i_{L, TwEC}$ vs $i_{L, ThEC}$ for PG oxidation on carbon fiber disk UME in 10% lard /Bz/ EtOH (1:2)/0.1 M H_2SO_4. (v = 0.010 V s^{-1}.) Concentration of PG: (1) 6.05×10^{-6} M; (2) 1.21×10^{-5} M; (3) 1.81×10^{-5} M; (4) 2.42×10^{-5} M; (5) 3.02×10^{-5} M; (6) 3.63×10^{-5} M; (7) 4.84×10^{-5} M; 8) 6.05×10^{-5} M; (9) 7.26×10^{-5} M; (10) 9.68×10^{-5} M. Slope value: 1.014 (0.002). $r = 0.9998$.

currents (i_L) of the oxidation of the reagent versus its bulk concentration. This relationship is described by the equation (Montenegro et al.,1990):

$$i_L = 4nFrDC \tag{1}$$

where n is the exchanged electron number, F is the Faraday constant, r is the radius of the UME disk, D is the diffusion coefficient of the electroactive reactant and C is its concentration. Calibration curves for antioxidant determination in two/three-electrode systems with and without oil added are shown in Fig. 7. Each point of the calibration curves represents the average of i_L obtained for at least three determinations for each concentration. Also, data for antioxidants in different media are shown in Tables 1 and 2 for both electrode systems, along with theoretical and experimental detection limits for every one with the corresponding regression data. Most of the calibration curves are good straight lines (correlation coefficients, $r > 0.99$) and can be assembled, for each antioxidant in a given medium, into two groups: those that correspond to systems of two and three electrodes with no-oil added (group A),

and those that correspond to systems of two and three electrodes with oil added (group B). Lines of group A show different slopes (m) than lines of group B: in all cases, the higher slope lines correspond to the ones obtained in the absence of oil (group A). The diminution in slope implies the diminution in the sensitivity of the technique when working in the presence of lard or oil. The differences in slopes in i_L vs C plots for A and B groups were due to the difference of viscosities of both media which affected the values of diffusion coefficients of the electroactive species [see Eq. (1)]. Thus, the ratio between the slopes obtained for the systems with and with no-oil added $\left(\bar{R} = m_{no-oil}/m_{oil} \right)$ and the ratio of the respective viscosities (η) agree satisfactorily, as shown:

$$\bar{R} = \frac{m_{no-oil}}{m_{oil}} = 1.40 \pm 0.17 \qquad \frac{\eta_{oil}}{\eta_{no-oil}} = 1.33 \pm 0.03$$

The slope ratio value shown above corresponds to an average of slope ratios for the BHA/Bz/EtOH (1:2)/H$^+$ medium considering data obtained in both, the TwEC as well as ThEC (see Tables 1 and 2). Datum of viscosities ratio reported corresponds also to the same system. Average values of slope ratios for other systems are also shown in both Tables. So, it is assumed that differences in slope for i_L vs C plots for A and B groups mainly correspond to differences in viscosities of the corresponding media.

In most cases, no significant differences were found for the slopes of lines belonging to two and three electrodes systems in either the oil-free or oil-added solutions. This study was performed by statistically comparing the slopes of each calibration curve, in all cases calculated by the least square method under the considerations of the null hypothesis using the parameter t at the 95% confidence level, unless otherwise indicated (Davies, 1993; Laitinen & Harris, 1960). Values of t were calculated from):

$$t = \frac{|m_1 - m_2|}{\sqrt{\frac{(n_1 - 1)s_1^2 - (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \tag{2}$$

where m_1 and m_2 are the slopes of calibration curves calculated by the least square approach for TwEC and ThEC, respectively, either for oil added or no-oil added solutions. n_1, s_1, n_2 and s_2 are the number of data points and standard deviations of TwEC and ThEC determinations, respectively, and $N = n_1 + n_2 - 2$ is the number of degrees of freedom (Laitinen & Harris, 1960). Thus, as can be seen in Tables 1 and 2, statistical comparison of slopes of calibration curves (Locatelli & Torsi, 1996) obtained from TwEC and ThEC gives calculated t values (t_1, column 6), for experiments in the absence, as
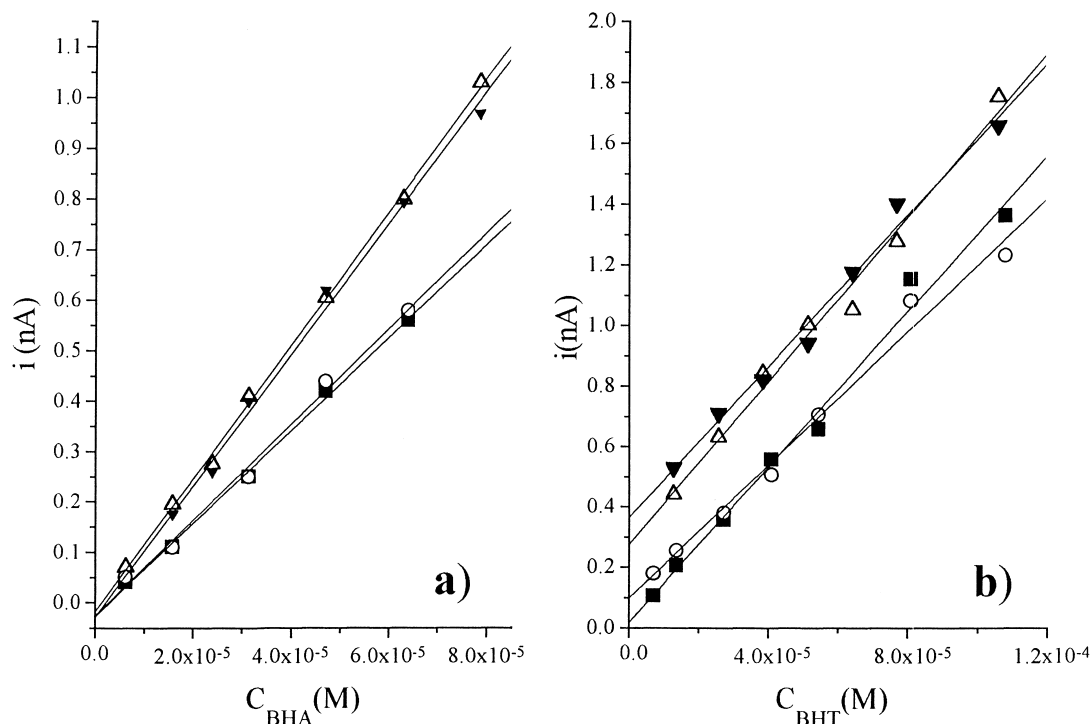
Fig. 7. Calibration curves for the oxidation of BHA and BHT in different media on carbon fiber disk UME for TwEC and ThEC in the presence as well as in the absence of oil added. (a) BHA/Bz/EtOH/0.1 M H_2SO_4. ($\bigcirc$) 9.09% oil/ThEC, ($\blacksquare$) 9.09% oil/TwEC, ($\triangle$) no-oil/ThEC, ($\blacktriangledown$) no-oil/TwEC; (b) BHT/Bz/EtOH/0.1 M TEATFB. ($\bigcirc$) 9.09% oil/ThEC, ($\blacksquare$) 9.09% oil/TwEC, ($\triangle$) no-oil/ThEC, ($\blacktriangledown$) no-oil/TwEC.

Table 1
Data for antioxidants determination in different media along with theoretical and experimental detection limits and relevant statistical and regression values[a]

Antioxidant	System		10^{-3} m(s)[b] (nAdm^3/mol)	n_1[c]	t_1[d]	t_2[e]	$\frac{m_{no-oil}}{m_{oil}} = R$[f]	10^5 TDL[g] (mol/dm^3)	10^5 EDL[h] (mol/dm^3)	r[i]
BHA	Bz/EtOH/H$^+$	Oil	9.51 (0.45)	5	1.195	18.9	1.39 ± 0.09	1.78	$\cong 0.75$	0.996
		No-oil	13.21 (0.22)	7	1.091			0.303		0.999
	ACN/NaClO$_4$	No-oil	24.98 (0.43)	7	0.16	–	–	0.228	$\cong 0.20$	0.999
	Acetone/NaClO$_4$	Oil	29.49 (1.04)	7	0.206	15.95	1.55 ± 0.14	1.37	$\cong 1.25$	0.997
		No-oil	45.8 (2.50)	7	2.119			1.88	$\cong 0.50$	0.992
BHT	Bz/EtOH/TFBTEA	Oil	12.78 (0.57)	7	6.057	7.36	1.23 ± 0.13	1.36	$\cong 1.25$	0.995
		No-oil	13.46 (0.07)	7	2.799			1.23		0.993
	ACN/NaClO$_4$	No-oil	25.62 (0.69)	7	1.075	–	–	0816	$\cong 1.00$	0.998
	Acetone/NaClO$_4$	Oil	9.28 (1.23)	6	0.464	–	–	2.45	$\cong 1.00$	0.960
PG	Bz/EtOH/H$^+$	Lard	11.06 (0.47)	10	1.016	–	–	1.31	$\cong 0.60$	0.993
	ACN/NaClO$_4$	No-lard	23.19 (0.41)	5	1.66	–	–	0.235	$\cong 1.00$	0.999

[a] Electrochemical system: three-electrode configuration (TLEC).
[b] m: slope of i_L vs C calibration curve; s: standard deviation.
[c] n_1: number of determinations for ThEC for a given system.
[d] t_1: t of student calculated for the corresponding slopes obtained from TwEC and ThEC for a given system.
[e] t_2: t of student calculated for the slopes obtained from oil and no-oil added solutions.
[f] R: i_L vs C slope ratio for oil and no-oil added solutions.
[g] TDL: theoretical detection limit calculated through Eq. (3) in text.
[h] EDL: experimental detection limit estimated at a 2:1 signal to noise ratio.
[i] r: correlation coefficient.

Table 2
Data for antioxidants determination in different media along with theoretical and experimental detection limits and relevant statistical and regression values[a]

Antioxidant	System		10^{-3} m(s)[b] (nAdm³/mol)	n_2[c]	t_1[d]	t_2[e]	$\frac{m_{no-oil}}{m_{oil}} = R$[f]	10^5 TDL[g] (mol/dm³)	10^5 EDL[h] (mol/dm³)	r[i]
BHA	Bz/EtOH/H$^+$	Oil	9.23 (0.27)	5	1.195	20.6	1.41 ± 0.08	1.53	≅0.75	0.998
		No-oil	13.02 (0.39)	7	1.091			1.13		0.998
	ACN/NaClO$_4$	No-oil	24.95 (0.26)	7	0.16	–	–	0.228	≅0.20	0.999
	Acetone/NaClO$_4$	Oil	29.36 (1.23)	7	0.206	13.79	1.47 ± 0.14	1.62	≅1.25	0.996
		No-oil	43.06 (2.33)	7	2.119			1.88	≅0.50	0.993
BHT	Bz/EtOH/TFBTEA	Oil	10.96 (0.56)	7	6.057	1.01	0.97 ± 0.09	1.21	≅1.25	0.993
		No-oil	12.45 (0.64)	7	2.799			1.19		0.993
	ACN/NaClO$_4$	No-oil	25.28 (0.48)	7	1.075	–	–	0.57	≅1.00	0.999
	Acetone/NaClO$_4$	Oil	8.90(1.57)	6	0.464	–	–	3.25	≅1.00	0.940
PG	Bz/EtOH/H$^+$	Lard	11.27 (0.46)	10	1.016	–	–	1.06	≅0.60	0.993
	ACN/NaClO$_4$	No-lard	22.79 (0.35)	5	1.66	–	–	0.204	≅1.00	0.999

[a] Electrochemical system: two electrode configuration (TwEC).
[b] m: slope of i_L vs C calibration curve; s: standard deviation.
[c] n_2: number of determinations for TwEC for a given system.
[d] t_1: t of student calculated for the corresponding slopes obtained from TwEC and ThEC for a given system.
[e] t_2: t of student calculated for the slopes obtained from oil and no-oil added solutions.
[f] R: i_L vs C slope ratio for oil and no-oil added solutions.
[g] TDL: theoretical detection limit calculated through Eq. (3) in text.
[h] EDL: experimental detection limit estimated at a 2:1 signal to noise ratio.
[i] r: correlation coefficient.

well as the presence, of oil (considered separately), which are lower than the corresponding theoretical ones for the appropriate degrees of freedom. Therefore, with the exception of BHT/Bz/EtOH (1:2)/oil/TEATFB, the slopes obtained either from TwEC or ThEC do not differ significantly at a 95% confidence level and, in the case of BHT/Bz/EtOH/TEATFB with no oil added, at a 99% confidence level.

Slopes calculated for BHT/Bz/EtOH/ TEATFB medium for both TwEC and ThEC in oil added solution differ significantly from the theoretical values even at the 99.5% confidence level. This might be due to the difficulties in assigning a baseline for measuring i_L, because the oxidation of BHT is close to the potential limit of the supporting electrolyte–solvent system, even though this limit was extended by the presence of TEATFB instead of H_2SO_4 (Ceballos & Fernández, 1995). Perhaps the same reason might explain the lower slope value for BHT/acetone/oil/NaClO$_4$ medium (see Tables 1 and 2) as compared with the other slope values. A higher relative error (about 20%) and a rather poor regression is also obtained for this determination. Anyway, it must be emphasized that BHT still may be determined by UME, more easily than by glassy carbon CSE, as stated elsewhere (Ceballos & Fernández).

When comparing the slopes obtained for a given electrode configuration system in no-oil and oil-added solutions, significant differences were found, as expected, because of the differences in viscosities of both solutions. This is seen from the values of calculated t (t_2, column 7

of both tables) for all the systems studied, with the exception of BHT oxidation in TwEC. The reason for this last case is not clear yet.

Theoretical detection limits (TDL) for antioxidants determination were calculated from the i_L vs antioxidant concentration plots by Eq. (3):

$$TDL = 3\frac{s}{m} \qquad (3)$$

where s and m have already been defined (Molina, Zón & Fernández, 1997; Yañez-Sedeño et al., 1991). They were calculated for every calibration curve and are shown in column 9 of Tables 1 and 2. Experimental detection limits (EDL) were determined as the minimal antioxidant concentration value for a current signal to noise ratio of 2:1. These values are shown in column 10 of Tables 1 and 2. As can be observed, they agree quite closely, in general, with the corresponding TDL and also with detection limits previously reported for electrodes made of different material and size (Ceballos & Fernández, 1995). Perhaps, particular attention should be paid to the slope values of calibration curves, because they are a measure of the technique's sensitivity. As stated above, higher sensitivity is obtained from measurements in oil-free solutions (see Tables 1 and 2, column 4), independent of the employed solvents. Taking into account the nature of the solution, it appears that for the determination of BHA the order of sensitivity is acetone/NaClO$_4$ > ACN/NaClO$_4$ > Bz/EtOH/H$^+$, either

in oil-free or in oil-added solutions. A similar trend is observed for the other antioxidants, with the exception of BHT in acetone (see column 4, Tables 1 and 2). Most of the concentration determinations had a precision, expressed as relative standard deviations, of about 6–7% for most of the studied media, which was considered good given the complexity of the system. Punctually, the relative standard deviation raises to about 20% for Bz/EtOH medium and BHT/acetone, as mentioned above. From these considerations, it can be assumed that at least three alternatives are possible for antioxidant determination on UME by slow sweep rate linear scan voltammetry: (a) Determination in ACN medium after extractive processes. This is perhaps the best medium because its electrochemistry does not present great problems but it is time consuming because of the several previous extractive steps (with the corresponding risk that some material may be lost) (Ceballos & Fernández, 1995). (b) Determination in acetone medium appears as the most sensitive (at least for BHA) with the additional advantage that it can dissolve the oil and the measurement can be accomplished directly in the oil/acetone solution. Also, acetone is less toxic than benzene. The disadvantage of this medium is that the determination must be performed at relatively low temperature (about 6–7°C) because of its low vapor pressure. This disadvantage could be overcome with a simple thermostated water bath. (c) Finally, measurements in Bz/EtOH (1:2), even though they show the least sensitivity and greater relative standard deviation, are done in a medium where the oil can be dissolved and the determinations can be performed directly by the standard additions method. However, the disadvantage of the toxicity of the lighter solvent still remains.

Our results allow the use of either the TwEC or ThEC to measure and quantify antioxidants in free or oil-added media (through standard additions) on UME making electrochemistry technique faster and even cheaper (by using fundamentally TwEC) than other usual analytical methods (i.e. HPLC; Cunniff, 1997). As a consequence of this, the analysis time and instrumental cost are diminished, keeping selectivity and sensitivity comparable to some other traditional analytical techniques (Table 3).

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Table 3
Comparison of antioxidant concentrations with an ultramicroelectrode and HPLC[a]

Sample	10^5 HPLC concentration (mol dm^{-3})[b]	10^5 Ultramicroelectrode concentration (mol dm^{-3})[c]	System
BHA			
1	(10.6 ± 0.7)	(10.0 ± 0.7)	ACN/NaClO$_4$/no-oil
2	(15 ± 1)	(12 ± 3)	Bz/EtOH/H$^+$/oil
BHT			
1	(12.4 ± 0.6)	(13.5 ± 0.8)	ACN/NaClO$_4$/no-oil
2	(6.7 ± 0.3)	(5.1 ± 0.9)	Bz/EtOH/TFBTEA/oil
PG			
1	(6.4 ± 0.2)	(6.5 ± 0.5)	ACN/NaClO$_4$/no-lard

[a] Electrochemical system: TwEC.
[b] Cunniff (1997).
[c] Errors indicated in column were calculated from $(s_1^2 + s_2^2)^{1/2}$, where s_1 represents the standard deviation of several repetitive measurements on the same solution and s_2 is the standard error estimated from error propagation of the corresponding calibration curve (Laitinen & Harris, 1960).

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