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CATALYTIC-VOLTAMMETRIC DETERMINATION OF THE ANTIOXIDANT TERT-BUTYLHYDROXYANISOLE (BHA) AT A NICKEL PHTHALOCYANINE MODIFIED CARBON PASTE ELECTRODE

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Summary—The voltammetric behaviour of the antioxidant tert-butylhydroxyanisole (BHA), at a carbon paste electrode modified with the electron mediator nickel phthalocyanine, is described, and a method for the determination of this antioxidant, based on its oxidation on the modified electrode, is proposed. Cyclic voltammograms showed a well-defined oxidation peak for BHA slightly shifted towards less positive potentials with respect to that obtained at the plain carbon paste electrode. The peak current measured at the modified electrode is considerably higher than that obtained at the unmodified electrode. A modifier percentage of 2%, a methanol percentage of 2% and a 0.1 mol/l $HClO_4$ medium were chosen as working conditions. The i_p *vs* $v^{1/2}$ plot obtained by linear sweep voltammetry showed a linear relationship over the whole scan rate range studied (5–2000 mV/sec) which is typical of a diffusion-controlled current. Using differential pulse voltammetry at $\Delta E = 50$ mV, linear calibration graphs were obtained in the concentration ranges 1.0–30.0, 0.10–1.0 and 0.02–0.10 mg/l BHA. The detection limit was 0.0036 mg/l (2.0×10^{-8} mol/l). Interferences from other substances commonly present in commercial antioxidant mixtures were tested. The developed method was applied to the determination of BHA in spiked potato flakes.

Antioxidants are a large group of chemicals extensively used in the food and pharmaceutical industries. Biological effects, toxicological aspects and detection, estimation and evaluation of antioxidants used as food additives have been recently treated in the book edited by Hudson.[1] In particular, phenolic antioxidants are currently added to food products in order to prevent oxidative rancidity. The chemistry of the degradation of phenolic antioxidants during autoxidation of fats and oils, the degradation of mixed antioxidants and the synergistic effects of compounds that potentiate the activity of antioxidants have been reviewed.[1] One of the most common among these substances is BHA (*t*-butylhydroxyanisole) which is also employed as preservative, alone or together with BHT (*t*-butylhydroxytoluene) in dehydrated food products.[2]

Methods for the determination of phenolic antioxidants including BHA have been reviewed.[3] HPLC with different detection systems,[4–8] GC[9–11] and TLC[12,13] are the most widely used techniques. However, electrochemical methods are relatively scarce in this area. Differential pulse voltammetry at stationary[14] and rotating[15] electrodes, as well as BHA preconcentration at a carbon paste electrode[16] have been reported. Finally, flow-injection methods for the determination of BHA and BHT based on their oxidation at a glassy carbon electrode have been described.[17]

On the other hand, we have recently shown that the use of a carbon paste electrode modified with the electron mediator nickel phthalocyanine offers substantial analytical advantages with respect to conventional electrodes for the determination of BHT.[18] The suitability of electrodes modified with metal phthalocyanines as catalytic modifiers for analytical purposes has been extensively demonstrated by Baldwin's group.[19–22] The aim of this work is to investigate the voltammetric behaviour of BHA at a carbon paste electrode modified with nickel phthalocyanine in order to develop a method for the determination of this antioxidant based on its oxidation at the modified electrode. This method has been applied to the determination of BHA in spiked potato flakes, food in which this antioxidant may be present.[2]

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EXPERIMENTAL

Apparatus

An EG & G PAR 273 potentiostat equipped with the 270 Electrochemical Analysis software was used. A P-Selecta Ultrasons ultrasonic bath, a P-Selecta Meditronic centrifuge, a W 2000 Heidolph rotary vacuum evaporator and a Griffin flask shaker were also used.

Electrodes and electrochemical cell

Modified carbon paste electrodes were prepared by homogenizing graphite powder (ultra F purity, Dicoex) with an appropriate amount of metal phthalocyanine (MePC) to give a mixture that was 2% MePC by weight, adding 0.4 ml of paraffin oil (Fluka) and 5.0 ml of chloroform per gram of mixture. Then, the paste was stirred in the ultrasonic bath for 3 min. Next, the chloroform was evaporated by passing an argon stream through the paste for 5 min, and the paste was allowed to dry overnight at room temperature. The paste was packed firmly into a Metrohm 6.0807.000 carbon paste electrode (diameter of active zone 3.0 mm) with a Metrohm 6.1241.020 electrode holder, and the surface was smoothed on a sheet of computer paper for 1 min. After each experiment the surface was renewed by removing the top of the used paste and refilling with fresh paste.

Reference and counter electrodes were a saturated calomel and a platinum wire electrode, respectively. A double-walled Metrohm EA 876-20 cell was used as electrochemical cell.

Reagents and solutions

Nickel and cobalt phthalocyanines were obtained from Fluka whereas iron phthalocyanine was obtained from Kodak. They were used as received without further purification. A 1000 mg/l BHA (Sigma) stock solution in pure methanol (Carlo Erba) was prepared by weighing. More dilute standards were prepared by suitable dilution with pure methanol. A Britton–Robinson solution containing each component acid at 0.2 mol/l was used. All other chemicals were of analytical-reagent grade and the de-ionized water was obtained from a Milli-Q (Millipore) system.

Sample

Potato flakes (Maggi), containing sodium bisulfite (E-223) and ascorbic acid (E-300) as antioxidants, were purchased in a local supermarket. This sample was spiked with BHA from a BHA stock solution in methanol.

Procedures

The modified carbon paste electrode was placed in 50 ml of a BHA solution in 0.1 mol/l perchloric acid containing a methanol percentage of 2%. Cyclic, linear sweep and differential pulse voltammetry were used as electroanalytical techniques.

Determination of BHA in spiked commercial potato flakes

The used extraction procedure was similar to that described by King *et al.*[23] The sample was ground to fine powder with a mortar and pestle. About 1 g of sample was accurately weighed into a 40-ml centrifuge tube. Then 100 μl of a 250 mg/l BHA stock solution in methanol were added. Extraction was carried out with three 5 ml portions of 50% methanol:water. The tube was mechanically shaken for 3 min. After centrifugation at 3000 rpm for 5 min, all extracts were combined in a 100-ml vessel of the rotary vacuum evaporator and concentrated to a final volume of *ca* 2 ml. Then 25 ml of 0.2 mol/l perchloric acid were added, and the vessel was placed in the ultrasonic bath for 2 min. The solution was quantitatively transferred into a 50 ml volumetric flask diluting to the mark with water. This solution was transferred to the electrochemical cell, and the determination of BHA was carried out by applying the standard additions method which involved the addition of 25–100 μg of BHA.

RESULTS AND DISCUSSION

The oxidative voltammetric behaviour of 20 mg/l BHA at carbon paste electrodes modified with Co(II), Ni(II) and Fe(II) phthalocyanines was first tested in order to choose the most suitable electron-transfer mediator. Cyclic voltammograms obtained in 0.1 mol/l perchloric acid (Fig. 1) exhibit, in all cases, a well-defined oxidation peak for BHA. As it can be observed, both for nickel phthalocyanine (NiPC) and for cobalt phthalocyanine (CoPC), this peak is slightly shifted towards less positive potentials (around 35 mV) with respect to that obtained at the plain carbon paste electrode. Moreover no corresponding reduction peak was seen on the reverse scan. However iron phthalocyanine (FePC) practically does not cause any change in the voltammetric response. Further-

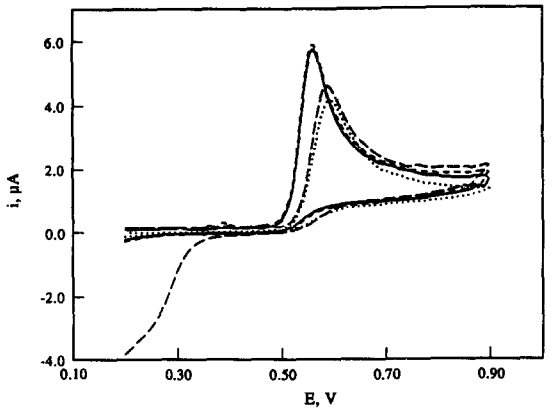

Fig. 1. Cyclic voltammograms of 20 mg/l BHA at carbon paste electrodes modified with 2% (- - -) Co(II), (—) Ni(II), and (– – –) Fe(II) phthalocyanine, (· · ·) unmodified carbon paste electrode; 0.1 mol/l $HClO_4$; 2% methanol; $v = 50$ mV/sec.

more, the oxidation peak observed at the modified electrodes occurs only in the presence of BHA.

Although the decrease of the overpotential for the electrochemical oxidation of BHA using NiPC and CoPC modified electrodes is quite moderate, this is not an essential point, in this case, because the separation among the oxidation current of BHA and the electroactivity limit is enough so as to prevent problems arising when electrochemical detection was to be done, for example, after chromatography. Nevertheless, the peak current measured at NiPC and CoPC modified electrodes is considerably higher than that obtained at the carbon paste electrode, which will give rise to an increase in sensitivity. NiPC was chosen as electron-transfer mediator in order to compare results with those reported for BHT in a former paper.[18]

Optimization of working conditions

Both, the effect of pH and the methanol percentage on the electrochemical response of BHA, were examined using differential pulse voltammetry (dpv) at $\Delta E = 50$ mV. Voltammograms were registered at the modified electrode with a NiPC percentage of 2%.

Figure 2 shows the influence of pH on E_p and i_p for 20 mg/l BHA in a 2% methanol–0.05 mol/l Britton–Robinson buffer medium. Only one peak was observed between pH 2.0 and 6.0, as well as in a 2% methanol–0.1 mol/l $HClO_4$ medium; however, two peaks appeared for higher pH values. This behaviour is similar to that observed for hindered phenols at conventional electrodes.[24] The peak potential of the first oxidation peak, which is also the best defined one, decreased linearly ($r = 0.996$ and slope -0.061 V) with increasing pH. The peak current also decreased considerably up to pH 7.0. On the contrary, the peak height of the second peak is slightly higher as pH increases. In a 0.1 mol/l $HClO_4$ medium, the BHA oxidation peak appeared at 0.50 V and its intensity was twice that obtained at pH 2.0. Taking into account these results, and in order to have the best sensitivity and simplicity, a 0.1 mol/l $HClO_4$ solution was chosen as working medium for subsequent experiments.

The effect of the methanol percentage on the

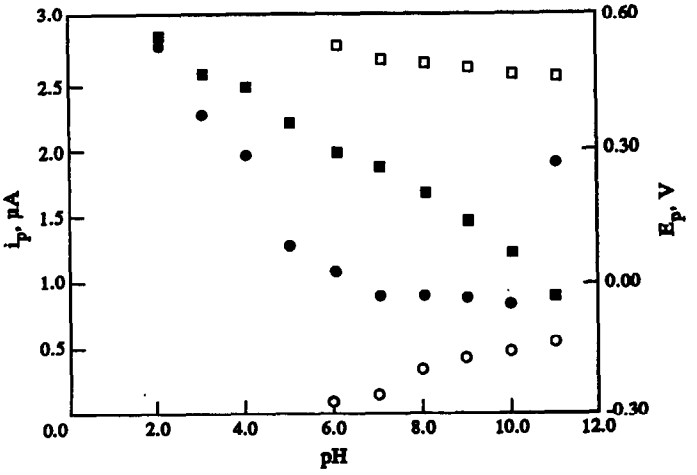

Fig. 2. Effect of pH on (□) peak potential and (○) peak current for differential pulse voltammetry at a nickel phthalocyanine modified carbon paste electrode. Solid symbols are for the first peak and open symbols for the second peak; 20 mg/l BHA; 2% methanol; 0.05 mol/l Britton–Robinson buffer; $\Delta E = 50$ mV; $v = 10$ mV/sec.

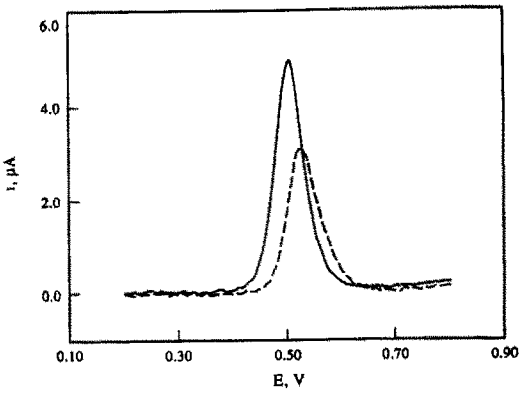

Fig. 3. Differential pulse voltammograms of 20 mg/l BHA at a (—) 2% NiPC modified electrode; (– – –) unmodified carbon paste electrode; 0.1 mol/l $HClO_4$; 2% methanol; $\Delta E = 50$ mV; $v = 10$ mV/sec.

peak current of 20 mg/l BHA in 0.1 mol/l $HClO_4$ was examined over the range 2–40% (V/V). A linear decrease of i_p values was observed as the methanol percentage increased, which can be due to a lowering of the diffusion coefficient with changing ionic strength and viscosity of the medium.[25] Consequently, the lowest methanol percentage (2%) was chosen for further studies.

Figure 3 shows dp voltammograms at the unmodified and modified electrodes using the above mentioned working conditions. As expected, the peak potential is shifted towards less positive potentials and the peak current is substantially increased when working with the NiPC modified electrode.

Characteristics of the oxidation process

The effect of the potential scan rate, in the range 5–2000 mV/sec, on the oxidation signal appearing in the presence of BHA at the modified electrode was studied by linear sweep voltammetry. As can be observed in Fig. 4, the i_p *vs* $v^{1/2}$ plot shows a linear relationship ($r = 0.999$) over the whole scan rate range studied, which is typical of a diffusion-controlled current. Moreover, the peak potential is shifted to more positive values as the scan rate increased indicating the irreversibility of the electrodic process.

The oxidation peak at the modified electrode is thought to correspond to the oxidation of the own BHA because of the closeness of the signal to that obtained at a plain carbon paste electrode (Figs 1 and 3). This oxidation probably involves a two electron exchange to form the corresponding phenoxonium ion as observed for substituted phenols.[24] The increase of sensitivity observed when working with the modified electrode, as well as the shift of the response towards less positive potentials, suggest an increase of the heterogeneous electron transfer rate for the BHA oxidation.

Analytical characteristics of the method developed by dpv

Using differential pulse voltammetry at $\Delta E = 50$ mV, the ranges of linearity obtained for the i_p *vs* BHA concentration plots were: 1.0–30 mg/l ($r = 0.999$, slope 0.26 ± 0.01 μAl/mg and intercept 0.06 ± 0.1 μA), 0.10–1.0 mg/l ($r = 0.999$, slope 0.26 ± 0.01 μAl/mg and intercept -0.002 ± 0.04 μA) and 0.02–0.10 mg/l ($r = 0.999$, slope 0.21 ± 0.01 μAl/mg and intercept 0.004 ± 0.008 μA). The relative standard deviation, for a concentration level of 0.04 mg/l BHA and $n = 10$, is 3.1%. Determination (10 s) and detection limits ($3\ s_b/m$), where m is the slope of the lowest calibration graph and s_b is the standard deviation ($n = 10$) of the signals from 0.04 mg/l BHA, were 0.012 and 0.0036 mg/l (6.6×10^{-8} and 2.0×10^{-8} mol/l), respectively. This detection limit is similar to that expected using preconcentration at a carbon paste electrode.[16] However, the phthalocyanine modified electrode would be easier to be used, for example as an indicator electrode in flowing systems, because preconcentration is not needed.

Interferences

Various substances commonly present in commercial antioxidant mixtures, such as tert-butylhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), propyl gallate (PG), ascorbic acid, sodium bisulfite and citric acid,

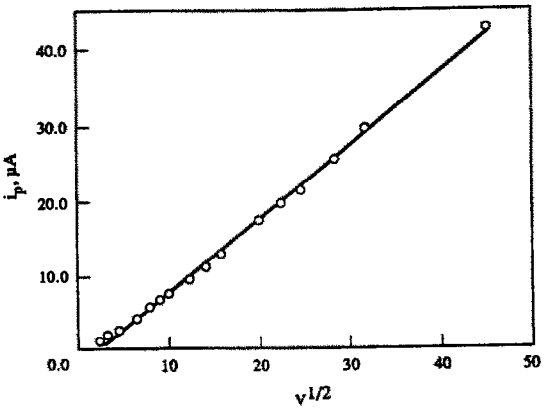

Fig. 4. Graph of i_p *vs* $v^{1/2}$ for linear sweep voltammetry at a 2% NiPC modified carbon paste electrode; 20 mg/l BHA; 2% methanol; 0.1 mol/l $HClO_4$.

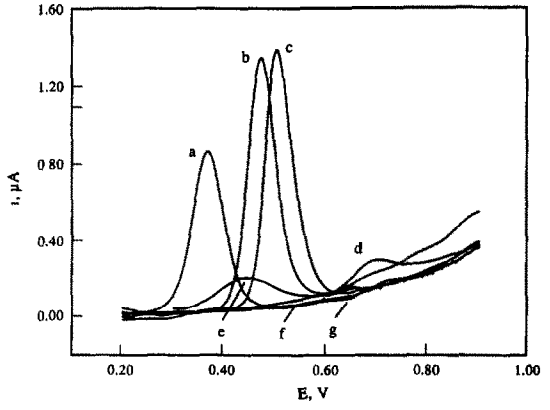

Fig. 5. Differential pulse voltammograms at a 2% NiPC modified carbon paste electrode for 5 mg/l (a) TBHQ, (b) PG, (c) BHA, (d) BHT, (e) ascorbic acid, (f) sodium bisulfite, and (g) citric acid; 0.1 mol/l $HClO_4$; 2% methanol; $\Delta E = 50$ mV; $v = 10$ mV/sec.

were tested by dpv at the NiPC modified electrode in order to check whether they interfere with the BHA oxidation peak. Under the experimental conditions used for the BHA determination, BHT, TBHQ, PG and ascorbic acid exhibit well-defined oxidation peaks at 0.70, 0.34, 0.45 and 0.44 V, respectively. However, sodium bisulfite and citric acid showed no oxidation peak in the potential range scanned (Fig. 5). The closeness among the peak potentials for PG and ascorbic acid with respect to that of BHA (0.50 V) gave rise to only one overall oxidation peak when voltammograms of mixtures of PG and BHA and of ascorbic acid and BHA were registered. On the contrary, two well separated oxidation peaks were obtained for mixtures of BHA with BHT or TBHQ. In order to establish the degree of interference of each tested compound, voltammograms of solutions containing 5 mg/l BHA and different concentrations of the interferent were registered. As expected, citric acid and sodium bisulfite did not interfere even at a 100:1 interferent:BHA ratio. The presence of TBHQ affects the BHA signal for a TBHQ:BHA ratio of 25:1 or higher (a relative error of 6.2% was obtained for that ratio); this is because high TBHQ contents gave rise to a very high TBHQ peak whose descending part overlaps the BHA peak yielding an increase of its height. BHT does not interfere for a BHT:BHA 1:1 ratio, and at higher BHT concentrations this antioxidant precipitates in the working medium used. Finally, PG and ascorbic acid interfere (relative errors higher than 5%) for interferent:BHA ratios higher than 1:1.

Determination of BHA in spiked commercial potato flakes

Recovery studies of BHA in commercial potato flakes not containing this antioxidant were carried out by applying the procedure described under the Experimental section. First, the commercial potato flakes were tested in order to check the absence of BHA. Thus, the described procedure was applied to a 1-g blank sample. The differential pulse voltammogram at the modified electrode from this blank sample solution is shown in Fig. 6. As can be observed, a signal, which may correspond to the oxidation of the ascorbic acid contained in the sample, was obtained. Therefore, the intensity of the BHA analytical response in the recovery studies must be measured against the blank background current.

Potato flakes samples were spiked with 25 μg of BHA/g sample. Following the described procedure, the final BHA content in the analytical solution was 0.5 mg/l. The standard additions method was used to determine the concentration of BHA (Fig. 6).

The experimental mean concentration for five determinations was 0.47 ± 0.01 mg/l BHA with a mean recovery of $94 \pm 2\%$ (23.5 ± 0.5 μg/g of potato flakes) for a significance level of 0.05. These results demonstrate the validity of the proposed method for the determination of BHA in samples of this kind.

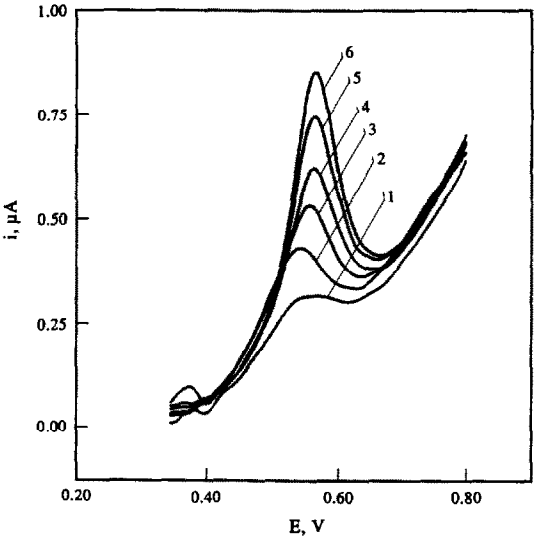

Fig. 6. Differential pulse voltammograms at a 2% NiPC modified carbon paste electrode for a commercial potato flakes solution. (1) blank solution (in the absence of BHA); (2) sample spiked with 25 μg of BHA; (3)–(6) successive additions of 25 μg BHA.

CONCLUSION

The NiPC modified electrode can be used for the sensitive determination of the antioxidant BHA in commercial food samples. Furthermore, the selectivity of the proposed method is rather good when mixtures of antioxidants are present. On the other hand, the NiPC modified carbon paste electrode seems to be suitable as indicator electrode in flowing systems when electrochemical detection of BHA wants to be done.

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