Cyclic Voltammetry of Lipophilic Compounds in Oil: Direct Determination of Lipid Peroxide with a Carbon Past Electrode

Ok-Sun Kim, Keiko Maekawa and Kousuke Kusuda*
Department of Environmental Food Science, Osaka City University, Osaka 558, Japan

ABSTRACT: In preliminary experiments, we demonstrated that a cyclic voltammogram of a lipophilic compound can be conveniently obtained with the past electrode that was made from graphite powder and an oil in which the compound is dissolved. This finding was applied to the direct determination of lipid peroxides. Oxygenation of lipids at 120°C was followed by the cathodic current created by the reduction of the peroxides. The validity of the method was investigated by comparison with conventional methods (peroxide value, thiobarbituric acid, and weight gain). The amount of peroxides in an oil and its cathodic current show a linear relation in the initial stages of peroxidation (*ca.* 12 h). This electrochemical method was applied successfully to the evaluation of the antioxidant activity of butylated hydroxytoluene in linseed oil.

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KEY WORDS: Amperometric analysis, antioxidant, carbon paste electrode, CV in oil, cyclic voltammetry, electrochemical determination, lipid peroxide, peroxylipid, redox in oil, reduction of peroxides.

Carbon paste electrodes (CPEs) have often been used conveniently for cyclic voltammetry (CV) because of their reproducible fresh surfaces. Such electrodes are made from graphite powder and a redox-inactive oily material, which is not soluble in aqueous electrolytic solutions. These materials should be of high purity because impurities disturb the background CV curves. For example, graphite powder is often contaminated with metallic compounds. The pasting oil can accommodate various kinds of lipophilic compounds as a solute. These facts suggest that CV performed with a CPE made from the oil in which another compound is dissolved should reflect the redox behavior of that compound.

Based on this line of reasoning, the CV of a compound that dissolves in a lipid was investigated. We found that CV of a redox-active compound in an oil can be performed with an oil–CPE in a conventional aqueous electrolytic solution. Also, the peroxides produced in an oil can be electrochemically reduced on the surface of the oil–CPE (1).

Peroxylipids in foods have attracted wide attention because of their adverse effects (2–5). Many methods have been developed to determine lipid peroxides on the basis of chemical reaction and physical measurement. Progress of air oxygenation of lipids has been studied by measuring the consumption of gaseous oxygen or the weight gain of the oxygenated sample. Measurements of peroxide value (POV), acid value (AV), carbonyl, and thiobarbituric acid (TBA) values are popular chemical methods (5,6). Although these chemical methods are simple in operation and provide reliable quantitative results, they usually need large amounts of sample. Separation analyses based on high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and column chromatography are used for identification and preparation of peroxides. Methods employing ultraviolet (UV), infrared (IR), electron spin resonance, and chemiluminescence spectra are based on the physical properties of lipid peroxides or intermediates of lipid peroxidation (5).

Recently, coulometric determination of POV with a porous carbon felt electrode was applied to peroxides of linoleic acid (7). Rapid coulometry has been developed for the determination of POV with a similar electrode system and ferricyanide as a mediator (8). Electrochemical detection of peroxylipids has been proposed for HPLC (9,10). Each of the chemical and physical methods has its own set of advantages.

However, CV of a compound dissolved in a lipid has not been reported. Kuwana and French (11) reported CV of organic compounds that are insoluble in aqueous electrolytic solutions. CV was carried out with a CPE whose paste contained the insoluble organic compounds. Successful CV curves resulted because electrochemical oxidation or reduction of such insoluble organic compounds creates ions that can be dissolved and electrolyzed in an aqueous phase. They did not consider that the organic compounds might dissolve in the pasting oil of the CPE. However, there is the possibility that the organic compounds are partially dissolved in the oil of the CPE.

As an application of CV to a compound that dissolves in an oil, we report the determination of peroxylipids that are

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*To whom correspondence should be addressed at Department of Environmental Food Science, Osaka City University, 459 Sugimoto, Sumiyoshi-ku, Osaka 558, Japan.

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produced in oils. We have demonstrated that the cathodic current at −0.60 V in CV, performed with the oil–CPE, semiquantitatively correlates with the amount of peroxides.

EXPERIMENTAL PROCEDURES

Apparatus. UV and visible spectra were measured on a Hitachi Model 200-10 spectrophotometer with a Hitachi 200 recorder (Tokyo, Japan). IR spectra were recorded on a Nicolet 5ZDX Fourier transform infrared (FTIR) spectrometer in KBr disks. For CV, a Hokutodenko potentiostat/galvanostat Model HA 305, an arbitrary-function generator model 105 and a Yokogawa Model 3086 X-Y recorder were used. Cell design was similar to that used in previous studies (12). A saturated calomel electrode (SCE) was used as a reference. The counter electrode was a planar platinum plate with a geometric area of 1.0 cm^2. The working electrode was a CPE described below.

CPE. A nujol (oil)-CPE was made of 15.0 mg graphite powder [Shimazu (Kyoto, Japan) Grade TPS-1, 280 mesh], 3.0 mg of nujol and 1% of a redox substrate. The electrode is then indicated as a 1% redox material–CPE.

CRE. One end of a graphite rod (diameter 3 mm × 15 cm, Hitachi Spectroscopic Graphite Electrode Special Grade) was ground with sand paper (#2000) and polished with filter paper. The electrode was then cut at 4.0 mm from the polished end. The chip was contacted with nujol in a vacuum cell. The chip was then impregnated with the nujol by submitting it to three cycles of vacuum pumping and restoration to one atmosphere with air. The average amount of absorbed nujol was 6.6 mg per chip (4 mm × 3 mm). The graphite chip was fixed on the end of a glass tube (3 mm × 10 cm) by means of shrinkable Teflon. A small amount of mercury was put in the glass tube, and a copper wire (diameter 1.5 mm) was used as the electrical lead.

To produce a fresh electrode surface, the CPE or CRE was ground on sand paper (#2000), followed by polishing with filter paper. Reproducibility of the cathodic current at −0.60 V was compared for both electrodes after storing them in an aqueous electrolyte for 5–30 min. Reproducibility of the CREs was better than the reproducibility for current from the CPEs, with or without grinding on the surface.

Electrochemical oxygenation of linseed oil–CRE. A linseed oil–CRE was held in a 1 M sulfuric acid solution saturated with O_2. An oxidation potential (1.20 V) was applied to the electrode for a prescribed time. CV of the electrode was performed in an O_2-free 1 M sulfuric solution in another cell.

Photochemical oxygenation of an oil–CPE. The surface of an oil–CPE was held horizontally below a 15-Watt U-shaped low-pressure mercury lamp. After UV-irradiation at 2537 Å for the prescribed time, CV with the CPE was performed in a 1 M sulfuric acid solution saturated with N_2.

A 1/15 M phosphate buffer solution that contained 1 M sodium sulfate (pH 6.98) was used for CV. The scanning rate was 0.10 V/s unless otherwise mentioned.

Determination of POV by IR. The sample of oil (100.0 mg) was dissolved in 10.0 mL carbon tetrachloride, and the IR spectrum (resolution, 2 cm^{-1}; scan number, 200) of the solution was measured in a 0.1-cm NaCl cell. POV was calculated according to the following equations (13):

$$\text{POV} = 6107\ \text{k}/0.885\ (\text{meq./kg}) \qquad [1]$$

$$\text{k} = a/c\text{l} \qquad [2]$$

where a = (observed optical density at 3550 cm^{-1})/20, c = concentration of the oil (g/L), l = cell length.

Chemicals. Reagents and organic solvents were of extra pure grade from Wako (Osaka, Japan) and used without purification. Sulfuric acid, used in the electrochemical experiments, was of analytical grade. Ion-exchanged distilled water was used. The purities of nitrogen and oxygen gases were 99.999%.

Purification of linseed oil. Commercially available linseed oil (5.00 g) was dissolved in 30 mL purified *n*-hexane, and the solution was passed through a column of silica gel (1.2 cm × 14 cm), followed by elution with 20 mL *n*-hexane. The hexane in the eluate was evaporated under vacuum. Other oils were purified similarly by column chromatography on silica gel.

RESULTS AND DISCUSSION

Electrochemical behavior of a lipophilic compound has been measured in an organic solvent system that contained enough electrolytes (14,15). However, the study of nonpolar and high-molecular weight compounds has long been ignored by this technique because of their low solubilities in those solvent systems. Absolutely dry, pure lipid is an electric insulator and cannot be used as a solvent for electrochemical investigations. Nevertheless, lipids usually contain a small amount of water, and a thin film of lipid surface contacting an aqueous electrolytic solution is considered sufficient to establish electrical contact. We expected that small amounts of moisture in the lipid would increase the electric conductivity of the film. If this were the case, a redox-active compound in such a thin film would be detected electrochemically. We believed that a thin film with a constant thickness on an electrode could be prepared with a lipid–CPE or with a CRE.

The potential window of a CPE in a phosphate buffer solution (pH 6.96) that contains 1 M sodium sulfate is from −0.65 to 0.30 V. With the same electrode in a 1 M sulfuric acid solution, the window is from −0.40 to 0.80 V. In the range of the potential window, the redox behavior of a lipophilic compound can be measured by CV if carried out with the CPE made from nujol in which the compound is dissolved.

The range of potential windows of plant oil–CPEs in an aqueous electrolytic solution is similar to that of a nujol–CPE. Within the potential window, the CV curve obtained with a CPE showed a simple closed line, which mainly consisted of charging/discharging currents. The cathodic current created by the electrochemical reduction of lipid peroxides adds to the basic current in the negative region of the potential window. This fact suggests that the increased current indicates the concentration of the peroxides. Thus, the time course of lipid peroxidation can be traced by the cathodic currents. The relationships between the cathodic currents and the concentrations of peroxides determined by IR, POVs, and TBA values were investigated. Linear relations were found.

CV (slope 0.30 V/s, in a phosphate buffer, pH 6.98) obtained with a 1% ferrocene–CPE, as a preliminary experiment, showed clear redox peaks at 0.39 [anodic peak (Pa)] and 0.26 [cathodic peak (Pc)] V. CVs run consecutively were reproducible, indicating that a fixed amount of ferrocene is participating in electrochemical reactions. Quite similar curves could be obtained the following day with the same electrode. Although the CV peaks obtained with a nujol–CPE shifted negatively from those (Pa 0.47 V, Pc 0.35 V) obtained in acetonitrile (16), both curves were similar. The electric charges consumed in cathodic and anodic processes in CV obtained with 1% ferrocene–CPE are nearly identical. This fact indicates that the ions involved in the redox reactions remain on the electrode surface during the CV measurement.

It is worth comparing the number of coulombs consumed in CV and the surface concentration of ferrocene molecules on the CPE. The amount of ferrocene molecules located in a thin layer (depth = 20 Å) near the surface of the CPE can be calculated. The observed number of coulombs in the anodic process was more than fifty times larger than the calculated value for one electron redox of all the molecules in the surface layer. This fact indicates that the redox reaction of ferrocene takes place not only on the surface of the CPE but also in the nujol near the surface.

Rapeseed oil as a representative edible oil, highly unsaturated linseed oil, chemically pure triolein, linoleic acid, and linolenic acid were oxygenated by heating at 120°C in air. The oxygenated oils were sampled at a constant time interval. CVs (0.00 → –0.80 V) in 1 M sulfuric acid solution were carried out with oxygenated oil–CPEs. Cathodic currents began to increase around –0.40 V in the negative sweep, but did not reach a cathodic peak. This increase in current is due to electrical reduction of peroxides, Oxygenation of unsaturated oil is known to produce a complicated mixture of peroxides and their decomposition products (17). Peroxides are the most easily reducible compounds among the products because they have high oxidizing potentials. Carbonyl compounds are also electrochemically reducible, but at a more negative potential than –0.60 V (15,18). Therefore, the increase in current at –0.60 V in the negative sweep must be caused by the reduction of lipid peroxides. Consequently, the current at –0.60 V should be used as a representative value for the reduction current because the CV curve did not show a Pc in the negative sweep.

The relation between heating time and current at –0.60 V was investigated with the oils mentioned above. Among the oils, triolein showed a relatively smooth curve. Similar plots with the other oils were not smooth, indicating some unknown factors that cause irregular amounts of reducible materials or instability of the electrode surface. Therefore, triolein was employed in the following preliminary experiments.

With both a triolein–CPE and a CRE impregnated with triolein, the relation between the cathodic current at –0.60 V and the time of soaking the electrodes in 1 M sulfuric acid was studied with and without surface refreshment after each measurement (Fig. 1). Although the reproducibility of the CV for the CPEs were poorer than that for the CREs, CPEs were used in the following experiments because of their convenience.

Cathodic and anodic currents in CV measured with an oil–CPE tend to increase with time of steeping the electrode in an electrolytic solution. This is one of the factors that may have caused irregularities in CVs that were obtained with the oxygenated oil–CPEs.

The fact that CREs are better than CPEs in reproducibility of CV current was thought to be a result of rearrangement of graphite particles on the electrode surface. If this reasoning is correct, a CPE that is made from a highly viscous oil will exhibit a more reproducible CV current because of reduced rearrangement of the graphite particles. Therefore, the relation between viscosity of pasting materials and the reproducibility of CV was investigated with nujol, a mixture of nujol and redox inactive wax (50:50), Silicon DC, Silicon QF-1, and *n*-octacosane that each contained 50% triolein. However, the background current measured with a wax–triolein CPE gradually changed with steeping time. No such current change was observed with CPEs that were made from other pasting materials. Elucidation of the causes of this phenomenon will be the subject of future study.

The progress of the thermal oxygenation of bulk triolein at 120°C was followed by POVs, weight gains (Fig. 2), and

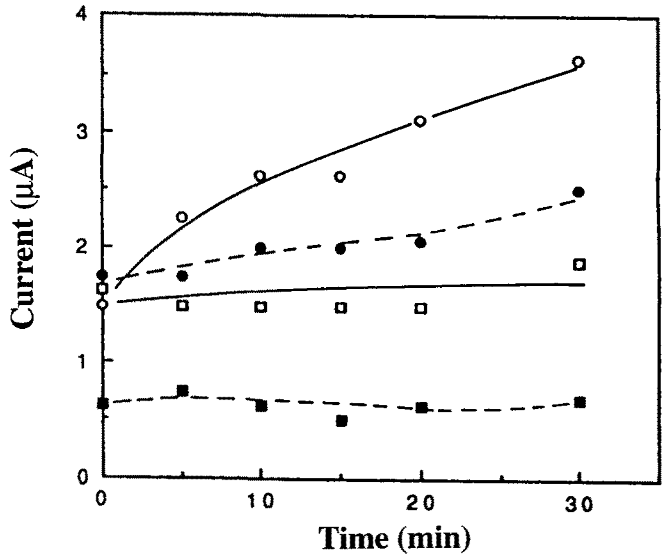

FIG. 1. Reproducibility of the cathodic current at –0.60 V in [resting potential (0.38–0.42 V) → –0.60 V; scan rate, 0.50 V/s; in 1 M sulfuric acid solution] performed on a triolein carbon paste electrode (CPE) and a carbon rod electrode (CRE) impregnated with triolein, with and without surface renewal by wiping the electrode surface after each measurement. ○, CPE; ●, CPE/erase; □, CRE; ■, CRE/wipe.

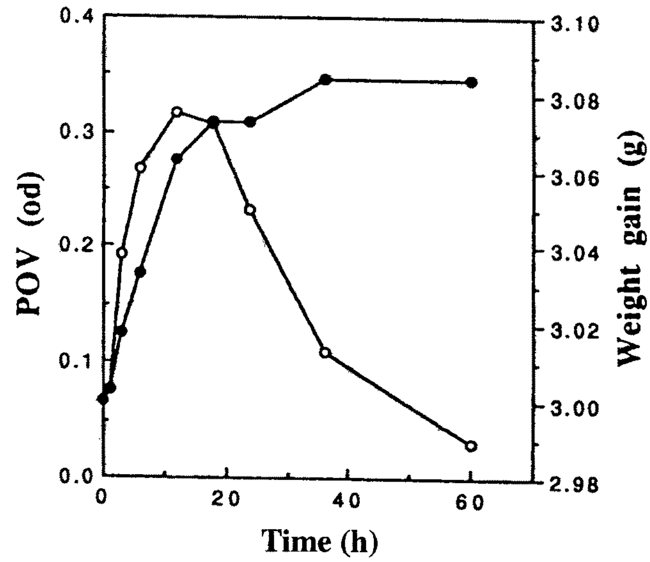

FIG. 2. The relation of peroxide value (POV), determined by ferric thiocyanate method, and weight gain (heating temperature, 120°C) of triolein vs. heating time. Each point is an average of two measurements. ○, POV; ●, weight gain.

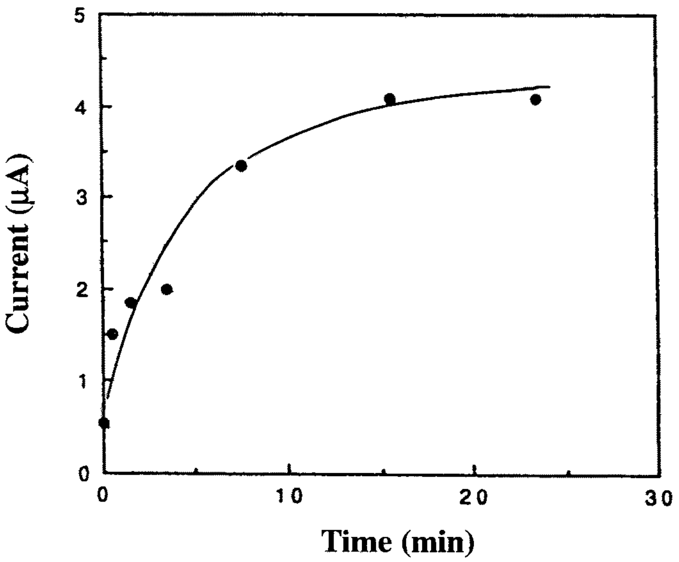

FIG. 3. The relation of the cathodic current at −0.60 V in CV (0.00 V → −0.60 V, in the absence of oxygen) performed on a electrochemically oxygenated (at 1.20 V in the presence of O_2 in 1 M sulfuric acid solution) linseed oil–carbon rod electrode vs. time (min) of electrochemical oxygenation.

TBA values (not shown). The POVs were determined by the ferric thiocyanate method (19). The curve of POV vs. oxygenation time showed a maximum at 12 h and decreased gradually. Weight gain increased rapidly at the beginning, then reached a plateau. Final weight gain from the oxygenation in air at 120°C indicates that the mole ratio of absorbed oxygen triolein is about 0.748. TBA values increased at an early stage of the oxygenation and, after reaching a maximum, decreased slowly. A plot of the concentrations of hydroperoxide determined by IR spectra vs. the currents at −0.60 V was linear. Each current measured by CV and each POV determined by IR were average values of five measurements. The correlation coefficient (r) was 0.978. A plot of the currents at −0.60 V against the POVs determined by ferric thiocyanate method was linear (r = 0.987).

Besides thermal oxygenation, electrochemical and photochemical oxygenations of oil on the surface of a CPE were traced by cathodic current at -0.60 V. Figure 3 shows the relation of the cathodic current in CV that was measured with a linseed oil–CPE vs. the time of electrochemical oxygenation at 1.20 V in an electrolytic phosphate buffer solution that was saturated with oxygen. Under the same conditions, a triolein–CPE resisted electrochemical oxygenation and did not form any reducible products. By comparison, a linolenic acid–CPE was quite unstable and produced irregular curves, even under careful treatment.

Figure 4 shows the relations between the time of photochemical oxygenations of a linseed oil-, a safflower oil-, a soybean oil-, a rapeseed oil-, a sesame oil-, a corn oil-, and a rice polishing oil–CPE, and the currents at −0.60 V in their CVs. The curves show that, at an early stage of oxygenation, an increase in the cathodic current is nearly proportional to the time of photochemical oxygenation. This observation is consistent with reports that, in the initial phase of photochemical oxygenation of an oil, formation of peroxides increases linearly with the time of oxygenation (5,20). After the initial phase, the time course of the increase in the cathodic current showed complicated behavior. One cause of this behavior may be variation in purity of the oils, especially variation of trace levels of natural antioxidative materials. In addition, higher degree of unsaturation of an oil tends to cause a large increase in cathodic current.

These experimental results indicate that the cathodic current in the CV obtained with a paste electrode can be used to determine peroxides in lipids.

As an application of this method, antioxidative activity of BHT was studied with the linseed oil–CPEs that were made from the oil containing 0.1 and 1% butylated hydroxytoluene. A linseed oil–CPE was electrochemically oxygenated at 1.20 V in an electrolytic buffer solution that was saturated with oxygen. Figure 5 shows the relation between oxygenation time and the cathodic current at −0.60 V. The plot shows a dose-response relation, thus demonstrating the practicality of this method.

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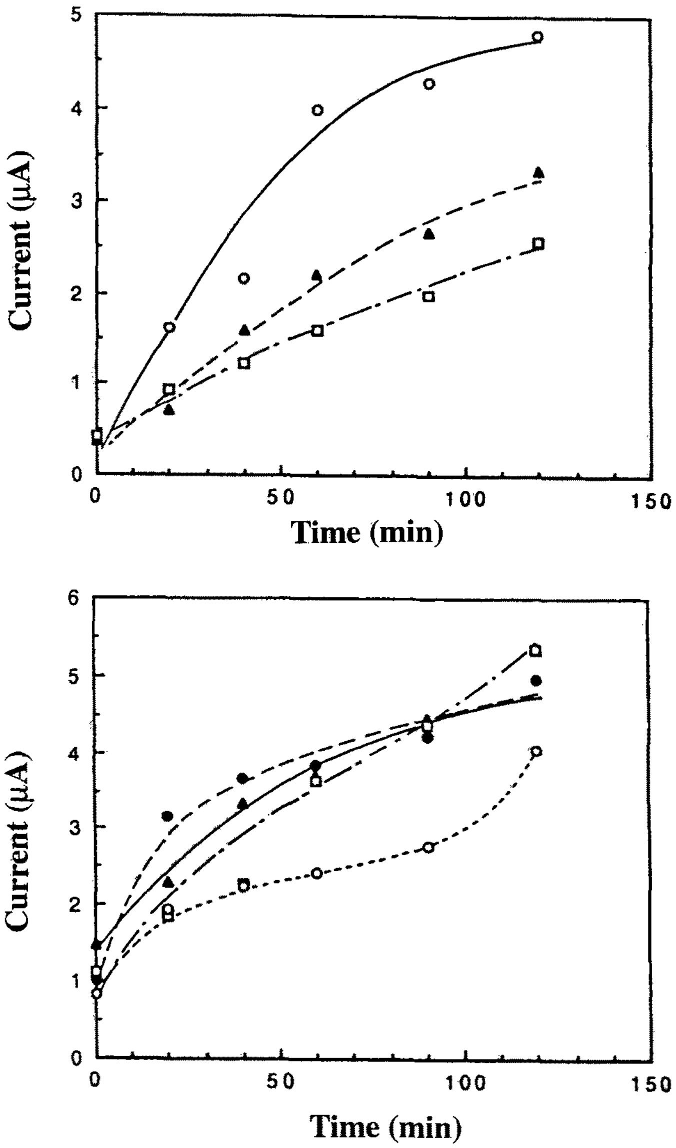

FIG. 4. Plots of the cathodic current at −0.60 V in cyclic voltammetry (0.00 → −0.60 V, in a 1 M sulfuric acid solution) performed on various kinds of oil–carbon paste electrode, which were oxidized photochemically in air, vs. time of photochemical oxygenation. (A) ○, linseed oil; ▲, safflower oil; and □, soybean oil. (B) ▲, rapeseed oil; ○, sesame oil; □, corn oil; and ○, rice polishing oil.

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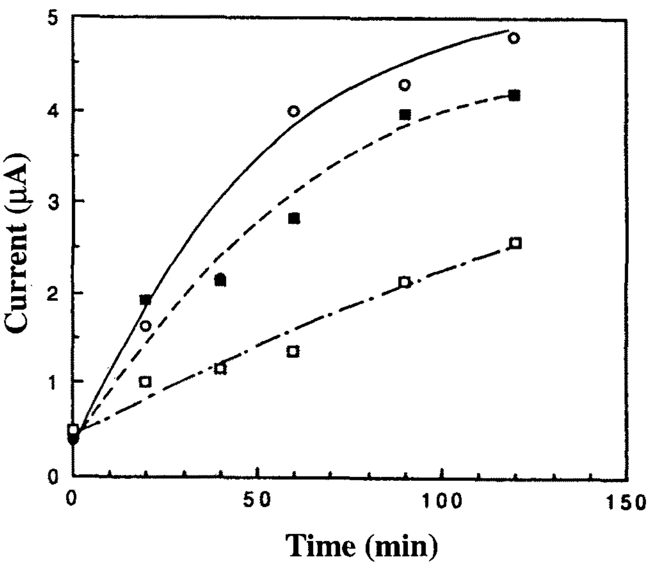

FIG. 5. Increase of the cathodic current at −0.60 V in cyclic voltammetry (0.00 → −0.60 V, in a 1 M sulfuric acid solution) on a linseed oil–carbon past electrode (CPE) that contained various amount of butylated hydroxytoluene (BHT). Each point is an average of three measurements. ○, Linseed oil CPE; ■, 0.1% BHT linseed oil CPE; and □, 1% BHT linseed oil–CPE.

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