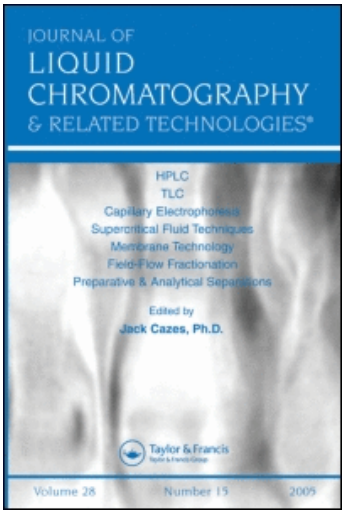

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Determination of Organic Peroxides by High Performance Liquid Chroma-Tography with Electrochemical Detection

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DETERMINATION OF ORGANIC PEROXIDES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

The application of electrochemical detection to the high performance liquid chromatographic determination of organic peroxides has been studied. The use of a buffered mobile phase was found to be critical to the successful analysis of samples containing hydroperoxides. Using amperometric detection, mixtures of peroxide containing compounds were readily determined. The sensitivity of the amperometric detector was in the one nanogram range for both benzoyl peroxide and cumene hydroperoxide. Polarographic detection was found to be a highly reproducible method for the analysis of samples containing peroxides as components of mixtures in the range of 5-2000 ng. The peroxide containing compounds determined in this manner were t-butyl hydroperoxide, cumene hydroperoxide, and 13-hydroperoxy-9(Z)-11(E)-octadecadienoic acid. The polarographic detection system was used to obtain observed half-wave potentials for the peroxides under different chromatographic conditions. These observations correlated closely with literature results on the polarography of these compounds.

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INTRODUCTION

A wide variety of techniques have been developed for the determination of organic peroxides (1). The combination of the usefulness of these compounds as free radical initiators and the hazard caused by the tendency for peroxides to form in many organic chemicals upon storage has supported this technological development. The determination of organic peroxides has also become an important biological problem. For example, it has been shown that the two primary products of arachidonic acid metabolism in mammalian cells are peroxides (2). Further, the determination of the peroxidation of lipids containing polyunsaturated fatty acids, an important medical pharmacological and biochemical phenomenon, has long been a difficult analytical problem. In fact, the commonly utilized thiobarituric acid test for lipid peroxidation does not measure the lipid peroxide content of samples at all. Rather, it is sensitive to compounds that have resulted from the decomposition of the initially formed peroxides (3).

The standard analysis of samples specifically for their peroxide content for many years has been iodometry. In this approach, the triiodide ions formed upon reduction of the peroxide by excess iodide were titrated to a visual or amperometric endpoint with a standardized thiosulfate solution (1,4). When applied to a sample containing a mixture of peroxides an analysis of this nature yielded a total peroxide value. Only a small degree of selectivity was obtainable in iodometric titrations by adjusting the reaction temperature to favor reduction of very reactive as opposed to relatively unreactive (acyl vs. dialkyl) peroxides. In addition to the lack of selectivity, standard iodometry had limited sensitivity ($\sim$100 μg benzoyl peroxide). This approach was updated recently by Hicks and Gebicki who employed spectrophotometric detection of the triiodide ion formed (5). Using this method, the detection limit for linoleic acid

hydroperoxide was reduced to 300 ng. Cathcart et al. have very recently reported on a new method for peroxide determination based on the oxidation of dichlorofluorescin to the fluorescent dichlorofluorescein by certain peroxides in the presence of hematin (6). This development reduced the detectability of linoleic acid hydroperoxide to 10 ng. While these new methods have enhanced the sensitivity of peroxide determinations, their lack of selectivity and the possibility for interference from absorbing or fluorescent compounds remain problematic.

To enhance the selectivity for the determination of organic peroxides, high performance liquid chromatography has been effectively utilized. Using ultraviolet light absorbance detection, this method has been applied to the study of the autoxidation of hydrocarbons and polyunsaturated fatty acids (7,8). While this approach has been successful in resolving closely related peroxides, the sensitivity was limited in the study of compounds not containing a chromophore in a readily accessible region of the spectrum. The method is also susceptible to interference by strongly absorbing compounds that do not contain the peroxide bond. We have recently demonstrated in a preliminary communication that an electrochemical detection system coupled to a high performance liquid chromatograph provides the potential for both enhanced sensitivity and selectivity in peroxide determinations (9). Peroxides are readily reduced electrochemically. They may, therefore, be detected in very small quantities by electronic amplification of the current obtained from this reduction. Following this basic approach, we were able to obtain analyses of samples containing as little as 10 ng of benzoyl peroxide and approximately 50 ng of t-butyl hydroperoxide. The technique was highly selective. Compounds with similar chromatographic properties but not containing the oxygen-oxygen bond did not interfere. In this paper, we report on the largely realized potential of this approach to organic peroxide detec-

tion and illustrate some of the factors that influence these determinations.

MATERIALS AND METHODS

Apparatus. The chromatograph consisted of a pump (Waters, 6000A), an injector valve (Rheodyne, 70-10) with a 20 µL loop, a column (IBM, 4.6 x 150mm, octyl or Dupont, 4.6 x 250mm, ODS) and a detector all connected by minimum hold-up volume stainless steel tubing and connectors. The detector was either a thin channel amperometric cell (BAS, TL6A, LC-4 controller) consisting of an amalgamated gold disk working electrode, a glassy carbon auxiliary electrode and a silver/silver chloride reference or a polarographic cell (Princeton Applied Research, 310, 174A Analyzer) consisting of a dropping mercury electrode, a platinum auxiliary electrode and a silver/silver chloride reference.

Reagents. Benzoyl peroxide and t-butyl hydroperoxide were obtained from Aldrich Chemical Company. Cumene hydroperoxide was obtained from the autoxidation of cumene and was purified by chromatography on silica gel. All chemicals utilized were reagent grade with the exception of the solvents used for the mobile phases which were HPLC grade. The 13-hydroperoxy-9(Z),11(E)-octadecadienoic acid was prepared and purified by a previously reported procedure (10).

Procedure. Chromatography was carried out under anaerobic conditions using the techniques presented in a published report (11). The flow rate for all experiments was 1.0mL min^{-1}. The samples were made oxygen free by purging with a stream of argon as previously reported (11). The samples were stored at -20°C and were maintained at ice bath temperature during the analyses. The peak height at a constant retention time for each component of a sample was the measure of detector response presented. The reported electrode potentials were all relative to the silver/silver chloride reference.

The polargraphic detector was used in the hanging mercury drop electrode mode in which a single newly formed, medium sized (2.9 mg) mercury drop acted as the working electrode for each chromatogram. The polargraphic analyzer was used in the sampled D.C. mode.

RESULTS AND DISCUSSION

An improved cell designed for reductive mode amperometric detection of compounds in chromatographic effluents featuring an amalgamated gold disk working electrode, all stainless steel plumbing, and optimized electrode geometry has recently become available. The new design alleviates much of the difficulty encountered in reducing the interference from atmospheric molecular oxygen in this detection system. Techniques have been developed for reducing the background current from the reduction of oxygen in the mobile phase and the sample to acceptable levels (11). In the application of this approach to the separation of mixtures of hydroperoxides, we have found that it is critical to use buffered mobile phases. An example of the analysis of a sample containing a mixture of cumene hydroperoxide, t-butyl hydroperoxide and duroquinone as internal standard is presented in Figure 1. When no buffer was used, a large negative excursion was observed preceding each hydroperoxide peak. This phenomenon was not observed for diacyl peroxides and could have been due to a decomposition of the hydroperoxides caused by active silanol groups remaining on the surface of the chromatographic medium. In support of this hypothesis, we observed that this effect was inversely related to the length of the hydrocarbon chain bonded to the silica gel even though the chromatographic media were uniformly end-capped. Figure 1 clearly illustrates that it is possible to obtain the simultaneous determination of small quantities of closely related peroxides as components of mixtures.

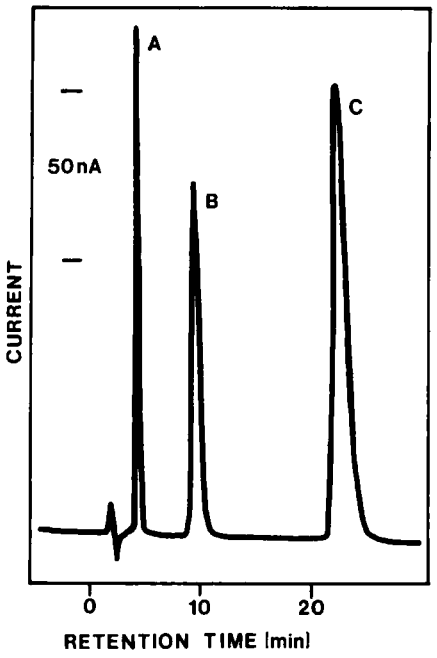

Figure 1. The simultaneous determination of A, t-butyl hydroperoxide (200 ng); B, cumene hydroperoxide (200 ng) and C, duroquinone (400 ng) using amperometric detection. Mobile phase: 55/45, methanol/sodium citrate (0.91 M, pH 6.25); Column: ODS; applied potential, -1.0V.

The optimized cell geometry has reduced the internal resistance of the amperometric detector and has resulted in an increase in sensitivity associated with a drop in the background. Examples of the sensitivity of this method for the detection of individual peroxides are presented in Figure 2. The detection limits (4X noise) for cumene hydroperoxide and benzoyl peroxide have been reduced to 1 ng and 500 pg, respectively, in this manner.

This newly developed system is not, however, without drawbacks. In an attempt to study the electrochemistry of peroxides in the chromatographic effluents by variation of the applied

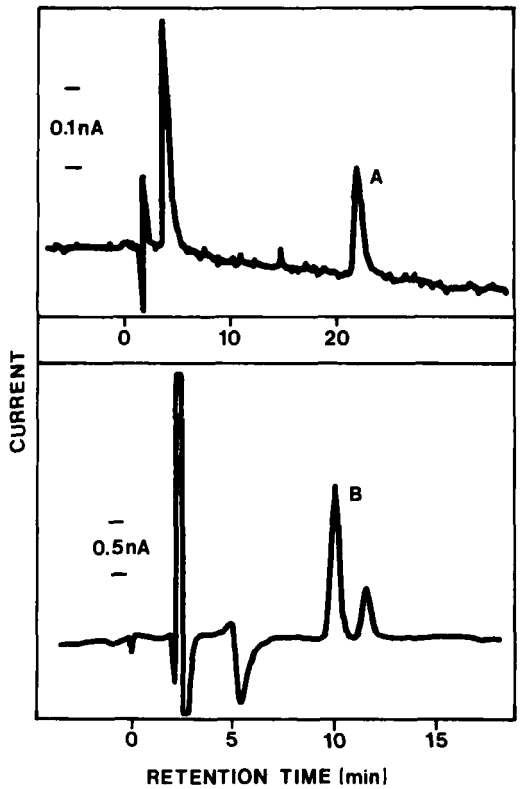

Figure 2. Top. The analysis of a sample containing 750 pg benzoyl peroxide (A) using amperometric detection. Mobile phase: 70/30, methanol/water, 0.1 M lithium perchlorate; Column: ODS; applied potential, +0.1V.
Bottom. The analysis of a sample containing 2.5 ng cumene hydroperoxide (B) using amperometric detection. A blank injection of methanol gave an identical trace except that peak B was absent. Mobile phase: 55/45 methanol/sodium citrate (0.091 M, pH 6.25); Column: ODS; applied potential, -1.0V.

potential, it was found that reproducible values for responses could not be obtained. This effect appeared to be related to alteration of the working electrode in the amperometric detector as a result of peroxide reduction. The mechanism for peroxide reduction at solid electrodes is not well understood (12). From our observations, it appears that the mercury surface on the electrode participates in the electrochemical reaction to the extent that the detector response is altered. Such effects are well known for the electrochemistry of peroxides at solid metal electrodes (12). This alteration was slight for any set of experiments run sequentially over a period of 4-8 hours at a single potential, using low concentrations of peroxides. Reproducibility was lost when the applied potential was varied or the experiments were carried out intermittently over an extended period of time. These observations called for frequent dismantling of the cell and resurfacing of the electrode. In an effort to circumvent some of the problems resulting from variation of the electrode, a commercial polarographic detector has been evaluated for the detection of organic peroxides. In this technique, a drop of mercury that can be conveniently and reproducibly renewed acts as the working electrode. The detector can be operated either as a dropping mercury electrode by replacing the drop as often as every 0.5 sec or as a hanging mercury drop electrode by replacing the drop after each chromatogram. A description of the optimization of this system for the determination of nitroaromatic compounds has been recently reported (13).

The polarographic detector was found to be an effective and reproducible approach to the determination of organic peroxides. An example of the application of this technique to the analysis of a sample consisting of a mixture of peroxides is presented in Figure 3. The virtue of this system was its reliability. The relative standard deviation for both sequential analyses of

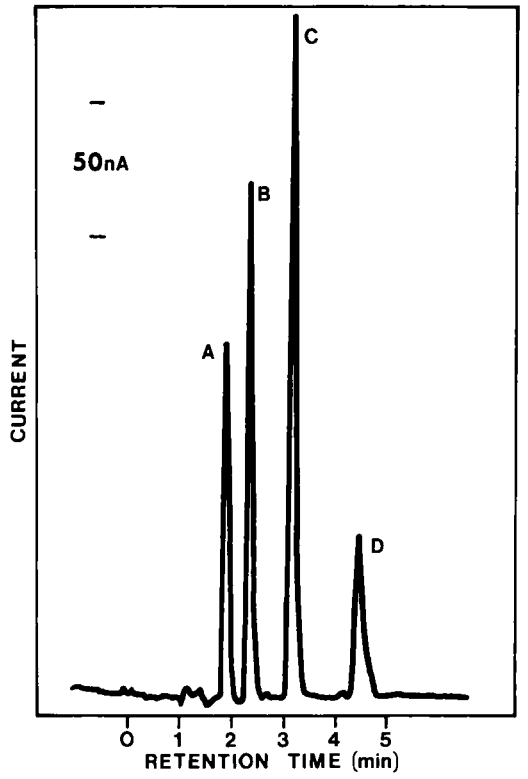

Figure 3. The simultaneous determination of A, t-butyl hydroperoxide (500 ng); B, cumene hydroperoxide (250 ng); C, duroquinone (250 ng) and D, 13-hydroperoxy-9(Z),11(E)-octadecadienoic acid (500 ng) using polarographic detection. Mobile phase: 66/32/2, acetonitrile/sodium borate (0.156M)/acetic acid; Column: C8; applied potential, -1.0V.

samples and the day-to-day reproducibility with intervening shutdown of the detector and freshly prepared samples averaged less than 5%. Because of the reliability of the measurements, it was possible to use the polarographic detector to determine observed halfwave potentials for the peroxides under the chromatographic conditions. Examples of the kind of data obtained for cumene hydroperoxide and t-butyl hydroperoxide are shown in Figure 4.

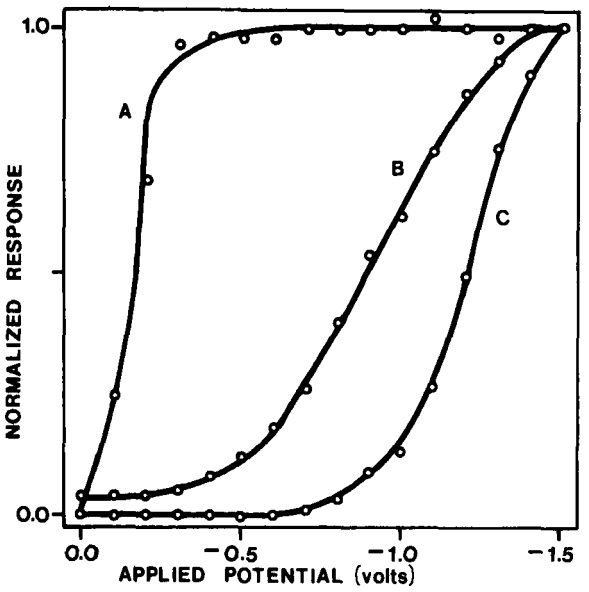

Figure 4. Response versus applied potential curves for A, duroquinone (2μg); B, cumene hydroperoxide (2μg) and C, t-butyl hydroperoxide (2μg) using polarographic detection. Mobile phase: 66/32/2, acetonitrile/sodium borate (0.156M/acetic acid; Column: C8.

The trends expected for the polarographic reductions of the two peroxides were observed. The halfwave potential for t-butyl hydroperoxide was significantly more negative than that for cumene hydroperoxide. Further, both values were much more positive when the mobile phase contained less of the nonaqueous component. In methanol/sodium citrate (0.091 M, pH 6.25), 55/45 (v/v), values of -0.2V and -0.8V were found for cumene hydroperoxide and t-butyl hydroperoxide, respectively. These observations were all similar to those reported for the polarographic determination of these compounds in solution (14).

The polarographic detector provided a satisfactory linear response between concentration and current at a fixed potential

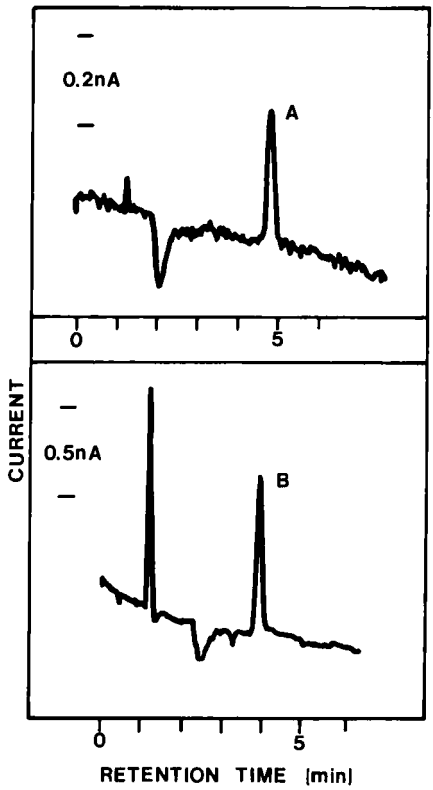

Figure 5. Top. The analysis of a sample containing 750 pg benzoyl peroxide (A) using polarographic detection. Mobile phase: 70/30, methanol/water, 0.1M lithium perchlorate; column: C8; applied potential, -0.1V.
Bottom. The analysis of a sample containing 2.5 ng cumene hydroperoxide (B) using polarographic detection. Mobile phase: 55/45, methanol/sodium citrate (0.091M, pH 6.25); Column: C8; applied potential -0.4V.

for three hydroperoxides (and one quinone) tested in the range of 5-2000 ng. The compounds with their associated coefficients of determination (r^2) were t-butyl hydroperoxide (0.999), cumene hydroperoxide (0.995), 13-hydroperoxy-9(Z),11(E)-octadecadienoic acid (0.999) and duroquinone (0.998). As supplied, the commercial polarographic detector was not as sensitive as the ampero-

metric detector. The application of the recently published modifications to the polarographic system with regard to the optimization of the parameters (13) greatly decreased this difference in sensitivity. In Figure 5, the sensitivity of the modified polarographic detector is demonstrated for samples containing benzoyl peroxide and cumene hydroperoxide. These examples show that the detection limits for the modified polarographic detector and the amperometric detector are similar.

In summary, these experiments have demonstrated that very small quantities of organic peroxides can be reproducibly determined using a modified polarographic detector for high performance liquid chromatrography, that samples containing mixtures of closely related peroxides can be reliably analyzed, and that the content of samples of low levels of individual lipid hydroperoxides can be measured. These developments represent an important step in our attempt to find a universally applicable and highly sensitive method for peroxide determinations.

ACKNOWLEDGEMENT

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