ELSEVIER

Journal of Chromatography A, 868 (2000) 127–134

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Determination of peroxides by capillary zone electrophoresis with amperometric detection

Hans-Hermann Rüttinger*, Antje Radschuweit

Institute of Pharmaceutical Chemistry, Department of Pharmacy, Martin-Luther-University, Langenbeckstrasse 4, D-06120 Halle, Germany

Received 7 May 1999; received in revised form 2 November 1999; accepted 4 November 1999

Abstract

The combination of cathodic amperometric detection with capillary zone electrophoresis is demonstrated to be a versatile method for the quantification of organic and inorganic peroxides. A gold microelectrode, polarized at −600 mV against an Ag/AgCl reference electrode, is placed at the end of the capillary. Since the electroosmotic flow purges the detector electrode from oxygen, no degassing of the detector cell or the sample is necessary. With an injection volume of ca. 1 nl, hydrogen peroxide, peroxosulfate, peroxy alkanoic acids and the hydroperoxides of linoleic acid can be detected down to 10 μmol/l. Separation of the isomeric hydroperoxides of the unsaturated fatty acids is achieved by addition of β-cyclodextrin to the electrolyte.

Keywords: Capillary zone electrophoresis; Amperometric detection; Peroxides; Lipid hydroperoxides; Persulfates

1. Introduction

Peroxides, even the simple hydrogen peroxide, are involved in a wide variety of natural and industrial processes. Hydrogen peroxide and peroxosulfates are used in etching and pickling baths especially in the production of electronics [1,4], peroxycarboxyclic acids are used as bleaching and disinfecting agents [2,3] with applications ranging from the paper industry to household washing powder, and lipid hydroperoxides have attracted medical and biochemical interest. Hydroperoxides of unsaturated fatty acids are responsible for oil and fat rancidity, but are also involved in biopathological processes known as ''oxidative stress'', and they are precursors of prostaglandins, thromboxanes and leucotrienes [5].

Since hydrogen peroxide is a byproduct of some specific enzymatic oxidation reactions its determination is an essential part of biosensors and test strips [6]. Numerous methods for the determination of peroxides are described in the literature. Most of them are based on the oxidizing properties of peroxides. Since most of the reactions suffer from kinetic hindrance, catalytic or enzymatic accelerators are used. On the other hand, different reaction rates allow to distinguish between some peroxide species. No matter what kind of analytical signal is used, e.g., from photometry [7,8], fluorimetry [9], chemiluminescence [10], amperometry [11–13] or polarography [14], a certain cross sensitivity towards other oxidants occurs. Therefore separation processes, such as high-performance liquid chromatography (HPLC) are applied [10,12,13].

In our present work we used capillary zone

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01162-0

electrophoresis (CZE) with subsequent electrochemical detection. CZE is not only a fast and very effective separation method, it has also the advantage that no stationary phase is needed, which may be attacked by the analyte. The low sensitivity associated with the usual UV detection is often considered as a major drawback of CZE. In contrast to common methods, amperometric detection does not depend on the pathlength of a light beam and has therefore a sensitivity, that is up to several orders of magnitude higher. Despite these advantages, amperometric methods have not been introduced in commercial instruments, mainly due to their complicated mechanical setup.

The majority of applications of electrochemical detectors in CZE and HPLC utilize anodical oxidation of the analyte. In reductive mode oxygen must be removed from the elute, otherwise the analytical signal is disturbed by high baseline currents. However, by purging the buffer in the source vial with argon the working electrode can be effectively shielded from ambient oxygen. The oxygen content in the sample then elutes with the electroosmotic flow (EOF). Due to high resolution in CZE the sharp oxygen peak does not influence the detection of the analyte nor compromises baseline noise. On the other hand reductive detection of peroxides is rather specific. Only few other compounds are reducible under these conditions.

In the work reported here we used end-column detection without junction to ground before the detection point and an unmodified gold electrode to simplify experimental setup and to get a more stable analytical signal [15]. We have to use a more negative potential for detection of H_2O_2 than that used with activated electrodes [16–18], but the analytical signal is far more independent on the kind of peroxide and electrode inactivation. The suitability of the method is demonstrated for the determination of peracetic acid as an example of washing

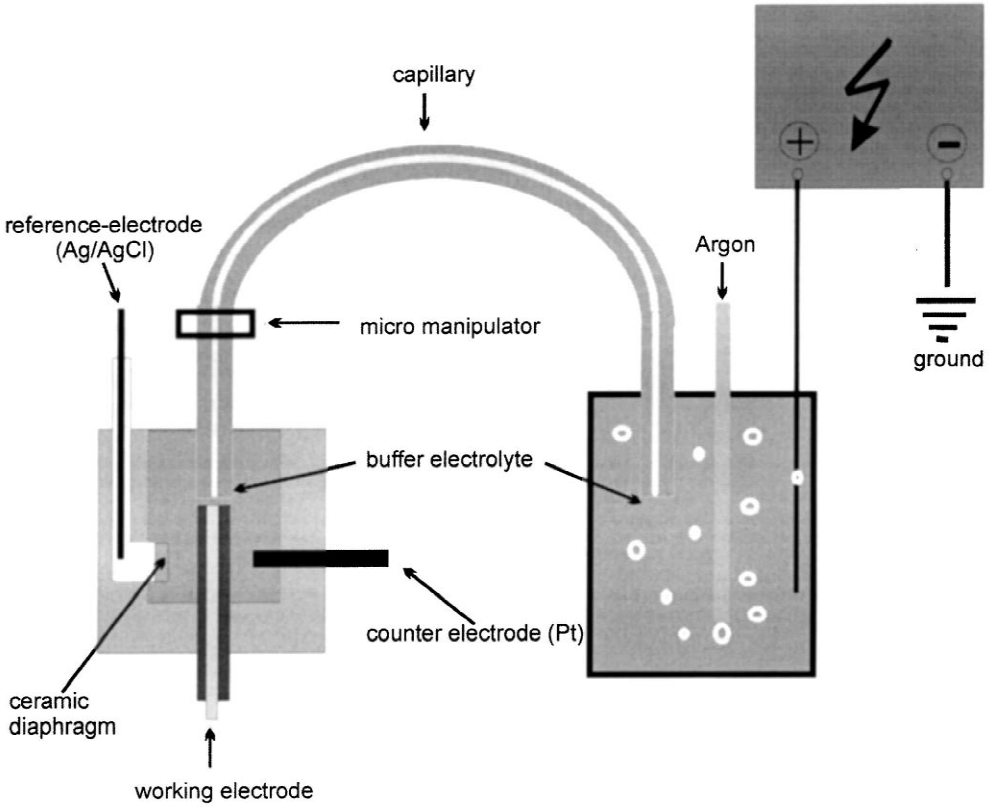

Fig. 1. Electrochemical detector.

and disinfecting solutions, peroxomono sulfate and peroxodisulfate of metal etching mixtures and hydroperoxides from linoleic, linolenic and arachidonic acid to show the biochemical impact of the methods.

2. Experimental

2.1. Apparatus

Capillary electrophoresis was performed with a fused-silica capillary of 40 cm×25 μm I.D. from Supelco (Deisenhofen, Germany). High-voltage supplies from F.u.G. (Rosenheim, Germany) type HCN 7E-35000, were used for positive voltage and negative voltages. The end of the capillary was fixed to a micromanipulator from Carl Zeiss (Jena, Germany) and adjusted to a gold microelectrode (90 μm) sealed in a glass tube. The laboratory-made electrochemical detector was equipped with a Pt-counterelectrode and an Ag/AgCl reference electrode as schematically shown in Fig. 1. An operational amplifier type CA 3140 from Harris semiconductors (RS Components, Mörfelden, Germany) served as potentiostat, and the detection current was fed to a current follower OPA 110 from Burr-Brown (Filderstadt, Germany) to achieve a gain of 1 V/μA.

A voltage-to-frequency-converter VFC 110 from Burr-Brown was used for data acquisition by a micro controller MC68HC11A8 from Motorola (MCT Paul & Scherer, Berlin, Germany). All data were transfered to a personal computer through a RS 232 interface.

2.2. Reagents

Chemicals and reagents were obtained from the following commercial sources: sodium borate from Merck (Darmstadt, Germany); dodecyltrimethylammonium bromide and hydrogen peroxide from Fluka (Deisenhofen, Germany); linoleic acid, and soy bean lipoxygenase from Sigma (Deisenhofen, Germany); sodium peroxodisulfate from Laborchemie Apolda (Apolda, Germany); 13(*S*)-hydroperoxy-octadeca-9*Z*,11*E*-dienoic acid (13-HPODE) solution in ethanol also from Sigma. Peroxy alkanoic acids were prepared from the corresponding carboxylic chlorides by a standard procedure [19]. The content of the freshly prepared stock solutions was determined immediately before use by iodometric titration of the ice-cooled solution.

All samples, solutions and buffers were prepared from doubly distilled water.

2.3. Procedure

The detection cell was filled with buffer electrolyte. After cleaning the working electrode at oxidizing (+2 V) and subsequently reducing potentials (−2 V) for at least 1 min, the proper potential was applied and the capillary adjusted with the help of a stereo microscope. After filling the capillary with electrolyte with a disposable syringe, the source end of the capillary was put into the source vial, which was purged continuously by argon to remove oxygen. The EOF due to the applied voltage was used to flush the capillary. The absence of oxygen is indicated by a drastic decrease in the cathodic current at −600 mV/Ag/AgCl. The sample was then injected by application of hydrostatic pressure (gravity injection). The injection volumes were calculated according to Hagen–Poiseuilles law from time of applied pressure [20].

3. Results and discussion

3.1. Peroxycarboxylic acids

Fig. 2 shows an electropherogram of a mixture of the peroxy *n*-alkanoic acids C_1–C_4. As expected, the oxygen elutes with the EOF, followed by the anions migrating against the EOF. Hydrogen peroxide is partly negatively charged due to complexation with the borate buffer. The rapid peroxy formiate comes last to the cathodic dectection end. The detection limit for the peroxy acetate is about 10 μmol/l (3σ) with an injection volume of 0.9 nl.

Household washing solutions that usually contain perborate and bleaching activators are capable of producing peracetic acid in situ. After passing through a 0.45-μm membrane filter, to avoid clogging of the capillary, it can directly injected onto the column. Fig. 3 shows an electropherogram from a Persil® solution, a typical blend of a washing powder; for comparison a run with an original

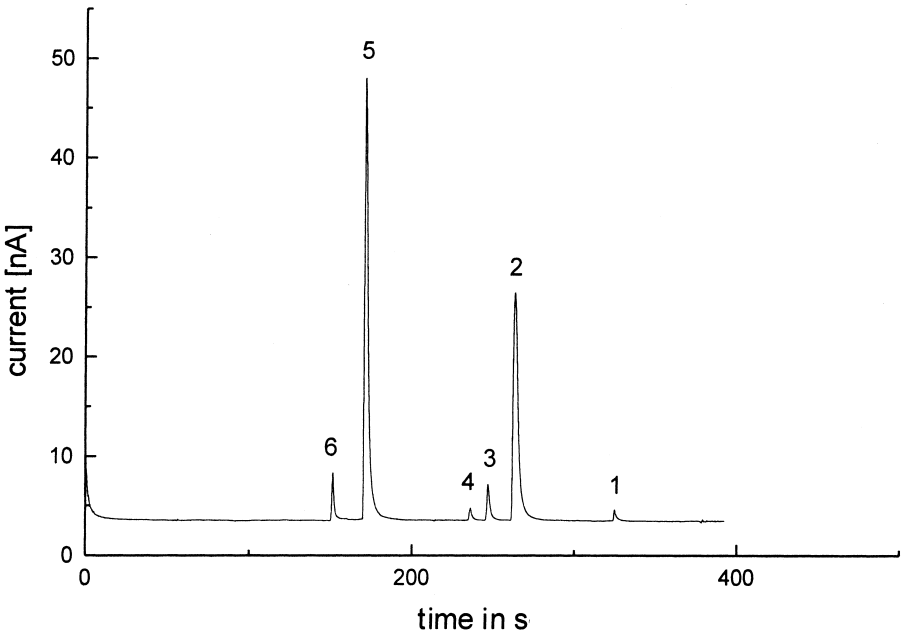

Fig. 2. Electropherogram of peroxy alkanoic acids concentrations: 1: formic (0.14 m*M*), 2: acetic (6 m*M*), 3: propanoic (0.55 m*M*), 4: *n*-butanoic (0.17 m*M*), 5: H_2O_2 (8 m*M*), 6: oxygen; conditions: injection time 10 s, injection height 19 cm, separation voltage 15 kV, electrolyte: 10 m*M* sodium tetraborate.

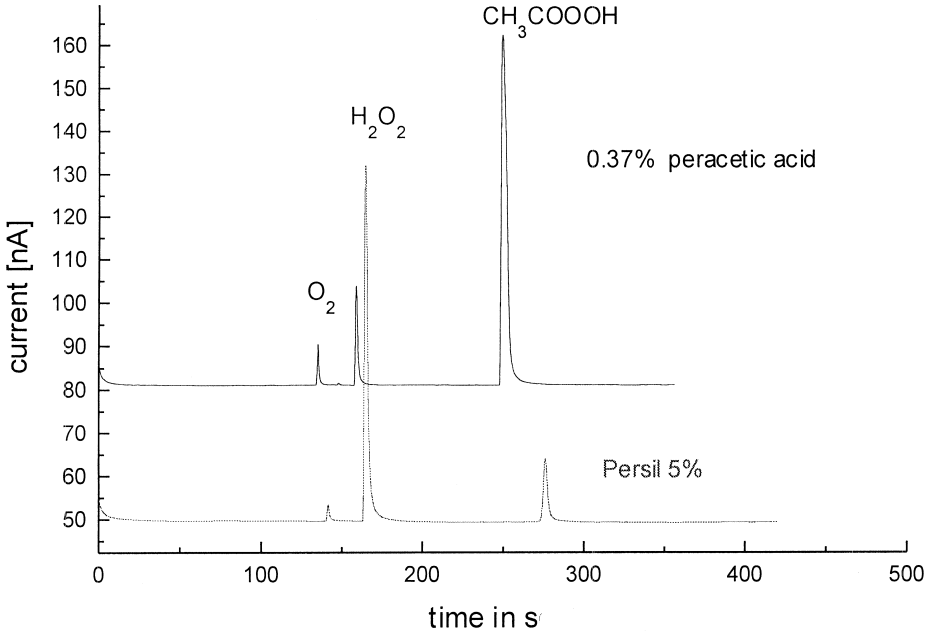

Fig. 3. Electropherogram of a washing solution containing 50 g/l Persil (a), for comparison the electropherogram of a 0.37% peracetic acid solution, containing some hydrogen peroxide is added as the upper curve; conditions as in Fig. 2.

peracetic acid solution is inserted. The detergents caused only a slight deviation of the EOF, resulting in a shift of retention times. The decomposition of the peracetic acid at 60°C in the washing solution may be easily monitored, as shown in Fig. 4

3.2. Persulfates

Peroxodisulfate is used in modern pickling baths mainly to treat copper and copper alloys. Since the activity and stability of the processing liquid strongly depend on its content of peroxomonosulfate, differentiation between the oxidizing species is highly desirable. To solve this problem we used a capillary with an EOF toward the anode. Thus we were able to determine these anions with high electrophoretic mobility. A positive charge in the capillary was achieved as usual by dynamic coating with dodecyltrimethylammonium bromide (3 m*M*) as buffer additive [20]. The elution sequence in Fig. 5 shows first peroxomonosulfate followed by peroxodisulfate while oxygen (EOF) comes last. In this way it was possible to analyze all oxidizing species in a single run.

The tailing of the peroxodisulfate peak is caused by adsorption of this anion to the coating of the capillary wall. This can be avoided by using a simple phosphate buffer (10 m*M*, pH 3.2). This leads to very sharp peaks. The order of elution is changed (Fig. 6) but the detector electrode is not protected from oxygen, causing higher background currents, even at more positive working potentials (−200 mV). Real etching solutions containing 100 g/l $Na_2S_2O_8$, 100 g/l H_2SO_4, 20 g/l $CuSO_4$ and 200 g/l Na_2SO_4 were directly injected into the system after 1:200 dilution with distilled water. A good correlation between the etching rate and the content of the more reactive peroxomonosulfate, that is formed due to the slow hydrolysis of peroxomonosulfate in acid solutions, was found.

3.3. Lipid hydroperoxides

Unsaturated fatty acids as linoleic or linolenic and their esters readily undergo oxidation processes that may be induced photochemically or catalyzed by heavy metal ions like Fe^{2+} or Cu^{2+} or much more specifically by lipoxygenases that are produced by

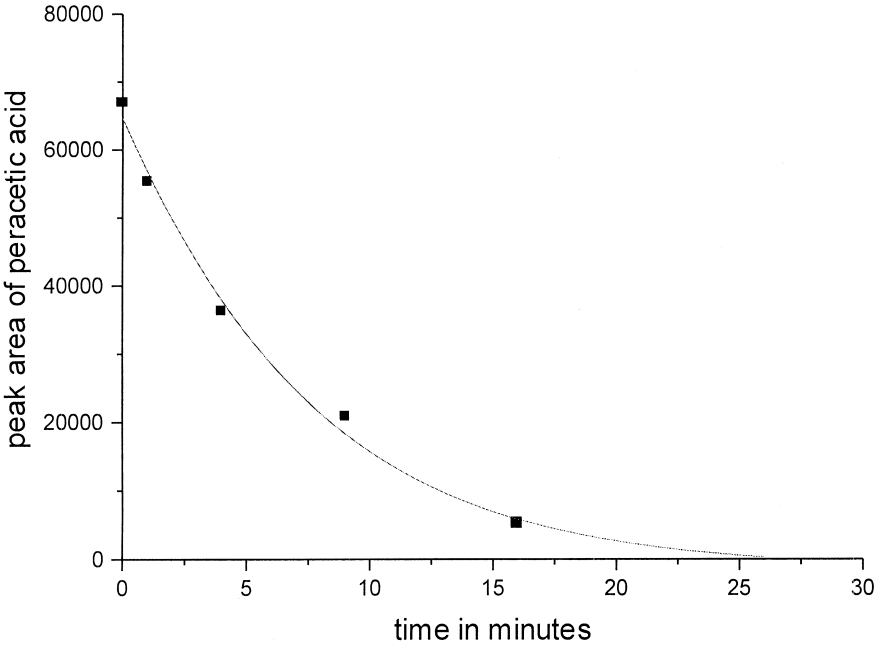

Fig. 4. Decomposition curve of peracetic acid in washing solution. 5% Persil solution in water was thermostated at 60°C, sample was injected after times given in the diagram. Separation conditions as in Fig. 2.

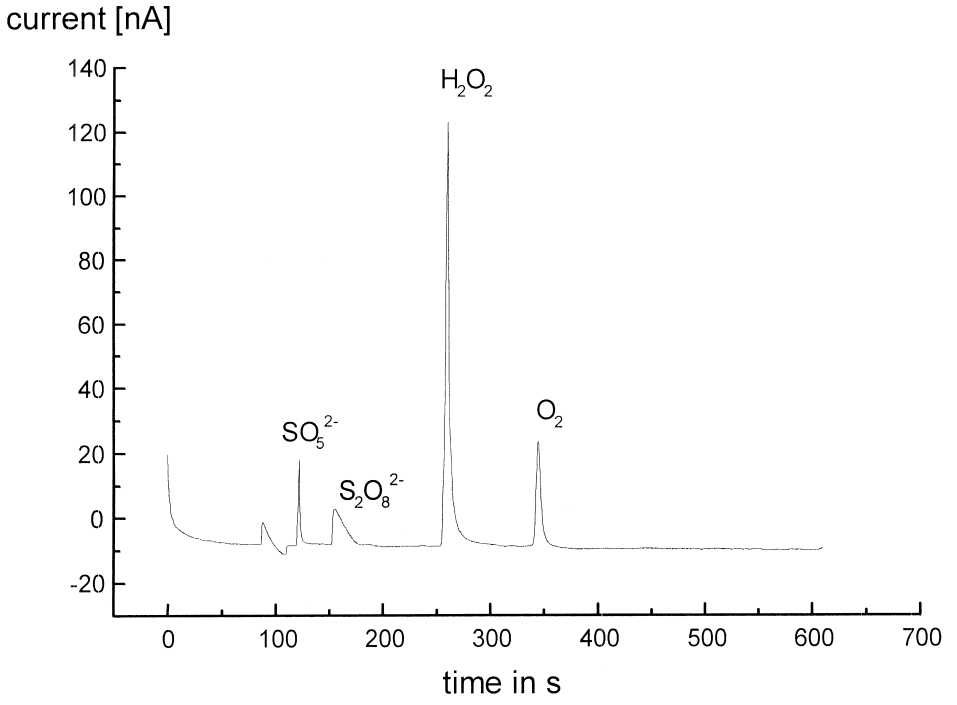

Fig. 5. Electropherogram of a mixture of peroxomonosulfate (0.5 m*M*), peroxodisulfate (1 m*M*) and hydrogen peroxide (4 m*M*); conditions: injection time 10 s, injection height 19 cm, buffer electrolyte: 10 m*M* sodium borate, 3 m*M* dodecyltrimethylammonium bromide, separation voltage 17.5 kV.

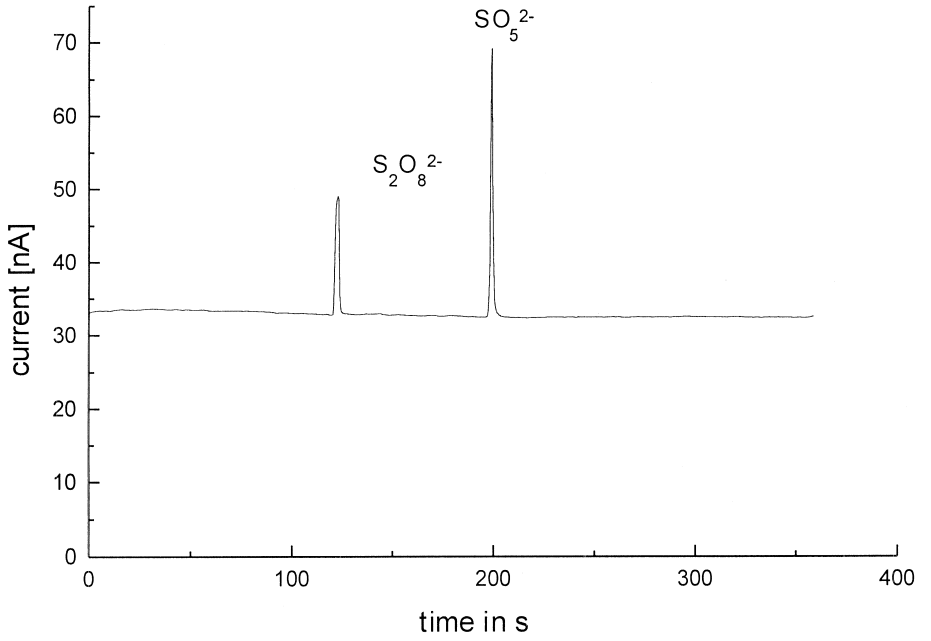

Fig. 6. Electropherogram of a mixture of peroxodisulfate 1 m*M* and peroxomonosulfate (1.3 m*M*); conditions: injection time 10 s, injection height 19 cm, buffer electrolyte: 10 m*M* phosphate buffer, pH 2.53, separation voltage 17.5 kV.

plants, bacteria and animals [5]. The formation of peroxidized arachidonic acid in animal organisms is the beginning of the oxidative burst, and the peroxides from arachidonic acid are the precursors of highly biologically active compounds such as prostaglandins. Since the oxidation of the above-mentioned acids leads to the conjugation of the initially isolated double bonds, in most cases the formation of the peroxides is monitored by UV spectroscopy. UV detection is possible for CZE, too [20]. We found that electrochemical detection is much more sensitive and specific. The detection limit (3σ) for 13-HPODE was 6 μM with an injection volume of 2.5 nl.

In a 10 m*M* borate buffer the hydroperoxides of the fatty acids are fully dissociated and, since the differences in the mobilities are small, they cannot be separated under these conditions. The micellar electrokinetic chromatography (MEKC) method with a Brij 35 and sodium dodecyl sulfate (SDS)-containing buffer at pH 4.5 that was successfully applied in combination with UV detection by Schmitz et al. [21,22] was not suitable for amperometric detection. It seems that the electrode is completely deactivated by the detergents.

The addition of β-cyclodextrins to the buffer electrolyte opened a further means of discriminating between the different hydroperoxy isomers.

Fig. 7 shows an electropherogram of a mixture of isomeric hydroperoxy linoleic acids prepared by photosensitized oxidation of linoleic acid. Assignment of the peaks to the corresponding structure was done by addition of the enzymatically produced 13-HPODE and comparison of the isomeric distribution in Ref. [21].

The complexation of the hydroperoxy-octadecadienoic acids with the uncharged β-cyclodextrin lowers the electrophoretic mobility of the anions. From the dependence of the electrophoretic mobility on the concentration of β-cyclodextrin the complexation constant *K* and the stoichiometric factor *m* of the complex has been calculated for 13-HPODE by parameter optimization as described in Ref. [23].

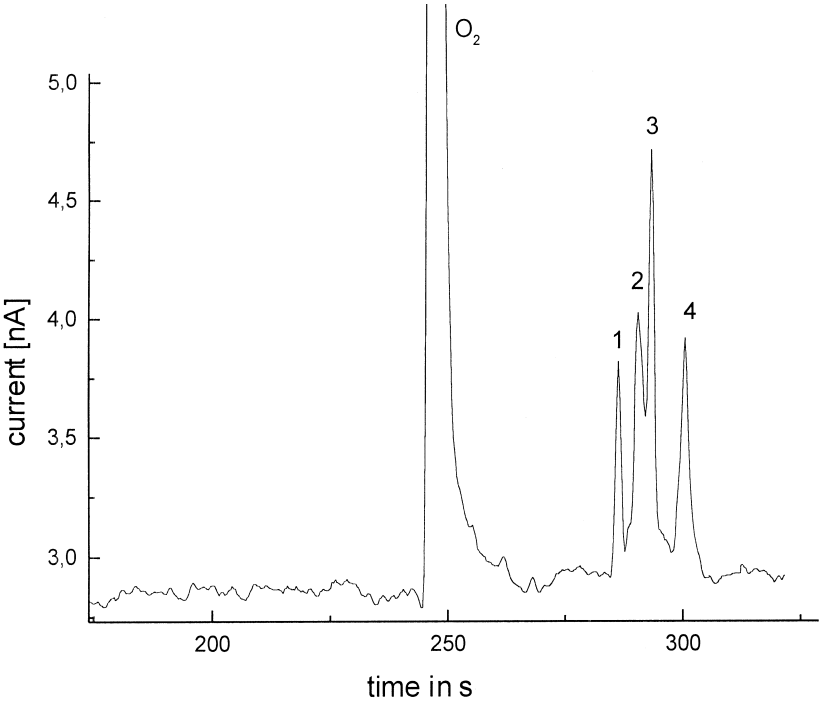

Fig. 7. Mixture of isomeric hydroperoxides from photochemically oxidized linoleic acid. Conditions: injection time 15 s, injection height 19 cm, separation voltage 17.5 kV, electrolyte: 10 m*M* sodium tetraborate, 1 m*M* β-cyclodextrin. 1: 13-Hydroperoxy-octadeca-9*Z*,11*E*-dienoic acid, 70 μM; 3: 9-hydroperoxy-octadeca-10*E*,11*Z*-dienoic acid HPODE; 2, 4: 10- or 12-HPODE.

Experimental data were best fitted with $m = 1.2 \pm 0.2$ and $K = 3.65 \pm 1.2$ $(10^{-3}$ mol$)^m$.

4. Conclusions

The combination of CZE with reductive amperometric detection provides a powerful tool for the determination of various peroxides. It could be shown that applications in different fields, from industrial processes of metal etching and textile and paper bleaching and disinfecting to lipid peroxidation processes, are feasible with a minimum of sample preparation steps.

References

[1] H. Matschiner, W. Thiele, in: Proceedings of BMBF Conference on Recycling of Pickling Baths, UTECH, Berlin, 1998, pp. 39–51.
[2] P.S. Malchesky, Artif. Organs 17 (1993) 147–152.
[3] H. Krüssmann, J. Bohnen, Tenside Surfact. Deterg. 31 (1994) 229–232.
[4] D. Suznjevíc, S. Blagojevic, D. Vucelic, P. Zuman, Electroanalysis 9 (1997) 861–864.
[5] M. Comparti, in: G. Poli, E. Albano, M.U. Diazani (Eds.), Free Radicals: From Basic Science to Medicine, Birkhäuser, Basel, 1993.
[6] S. Gamburzev, P. Atanasov, A.L. Ghindilis, E. Wilkins, A. Kaisheva, I. Iliev, Sensors Actuators, B Chem. 43 (1997) 70–77.
[7] D. Göckeritz, F. Friedrich, M. Yahya, Pharmazie 50 (1995) 437.
[8] M.L. Lunar, S. Rubio, D. Perez-Bendito, Analyst 118 (1993) 1209.
[9] R. Carthcart, E. Schwiers, B.N. Ames, Methods Enzymol. 105 (1984) 352.
[10] Y. Yamamoto, Methods Enzymol. 233 (1994) 319.
[11] M. Somasundrum, K. Kirtikara, M. Tanticharoen, Anal. Chim. Acta 319 (1996) 59–70.
[12] W. Korytowski, P.G. Geiger, A.W. Girotti, J. Chromatogr. B 670 (1995) 189–198.
[13] H. Arai, S. Mohri, T. Suzuki, K. Takama, J. Terao, Biosci. Biotechnol. Biochem. 61 (1997) 191–193.
[14] M. Schulz, K.H. Schwarz, Z. Chem. 7 (1967) 176.
[15] L.A. Holland, S.M. Lunte, Anal. Proc. 35 (1998) 1H.
[16] Y. Mishima, J. Motonaka, K. Maruyama, S. Ikeda, Anal. Chim. Acta 358 (1998) 291–296.
[17] J.-J. Sun, H.-Q. Fang, H.-Y. Chen, Analyst 123 (1998) 1365–1368.
[18] T. Ferri, A. Poscia, R. Santucci, Bioelectrochem. Bioenerg. 45 (1998) 221–226.
[19] M. Dankowski, G. Prescher, in: Houben-Weyl, Methoden der Organischen Chemie, Vol. E13, Georg Thieme, Stuttgart, New York, 1988, p. 767.
[20] H. Engelhardt, W. Beck, Th. Schmitt, in: Kapillarelektrophorese – Methoden u. Möglichkeiten, 1st Edition, F. Vieweg, Braunschweig, Wiesbaden, 1994, p. 94.
[21] O. Schmitz, S. Gäb, J. Chromatogr. A 767 (1997) 249.
[22] O. Schmitz, D. Melchior, W. Schuhmann, S. Gäb, J. Chromatogr. A 814 (1998) 261.
[23] M.A. Schwarz, R.H.H. Neubert, H.H. Rüttinger, J. Chromatogr. A 745 (1996) 135.