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Determination of peroxycarboxylic acids by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

A sensitive high-performance liquid chromatographic method employing reversed-phase chromatography and amperometric detection of low levels of peroxycarboxylic acids is described. Detection limits of 0.1–0.6 μM and linear dynamic ranges of at least 0.05–5 mM were obtained. As a consequence of the high sensitivity and selectivity provided by the electrochemical detector, the method is well suited for detection of various peroxycarboxylic acids even in the complex matrices represented by detergent solutions. As an illustration of the applicability of the system developed, the levels of various peroxycarboxylic acids were monitored in the course of a washing cycle performed with some commercially available detergents.

INTRODUCTION

Systems that generate peroxycarboxylic acids have become an important component in detergents over the last few decades as these highly oxidizing chemicals provide effective bleaching of a broad range of stains at low temperatures, without damaging coloured fabrics [1,2]. Owing to their aggressive nature and poor storage stability, the peroxycarboxylic acids are preferably formed *in situ* by perhydrolysis of bleach activators, *i.e.* by lysis of activated acyl compounds with hydrogen peroxide, the latter provided by peroxy salts, *e.g.* sodium perborates. The most frequently used bleach activators are tetraacetylethylenediamine (TAED) and sodium nonanoyloxybenzenesulphonate (NOBS) (Fig. 1), which form peroxyacetic and peroxynonanoic acid, respectively, upon perhydrolysis.

As bleach activators have found their way into detergents, the need for analytical methods capable of monitoring peroxycarboxylic acids in the presence of a large excess of hydrogen peroxide has emerged. The levels to be detected are in the low millimolar range and occur in the rather complex matrix of a detergent solution, comprising surfactants, builders (phosphates, zeolites), enzymes and various other components.

The analytical methods that have been described so far are largely based upon either titration or spectrophotometry. Hydrogen peroxide may be titrated initially with cerium(IV) sulphate followed by determination of the remaining peroxyacid by

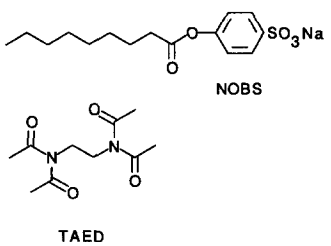

Fig. 1. Structures of TAED and NOBS.

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iodometry [3]. In another approach, the much higher reactivity of iodide with peroxyacids compared with hydrogen peroxide is utilized and liberated iodine is titrated with thiosulphate [4]. Alternatively, the liberated iodine can be determined photometrically [5]. Recently, the different rate of oxidation of methyl phenyl sulphoxide by peroxyacids and hydrogen peroxide has been utilized in a gas–liquid chromatographic method [6].

The above methods share several disadvantages. First of all, no method reported so far has been able distinguish between various peroxycarboxylic acids, which would be relevant if, for example, TAED and NOBS were combined in a detergent formulation. Second, the methods described are rather time-consuming and suffer from quite high detection limits. The aim of this study was to develop a fast and sensitive high-performance liquid chromatographic (HPLC) method allowing selective detection of various peroxycarboxylic acids and, preferably, also hydrogen peroxide, directly in washing liquors.

EXPERIMENTAL

Chemicals

All chemicals were of analytical grade and most were purchased from Merck (Darmstadt, Germany). Hydrogen peroxide was obtained either as a 30% (w/w) solution (Merck) or as a 60% (w/v) solution (BDH, UK). Peroxyacetic acid was obtained as Proxitane 4002, a solution determined to be 6.7 *M* with respect to peroxyacetic acid, 8.4 *M* with respect to acetic acid and 1.2 *M* with respect to hydrogen peroxide (and also containing, presumably, traces of strongly acidic catalyst), from Peroxide-Chemie (Germany). The surface-active agents Berol 08 and Nansa 1127 were obtained from Berol (Sweden) and Albright & Wilson (UK), respectively. The experimental lipase preparation was made in house (Novo Nordisk, Denmark). All solvents for HPLC were purchased from Rathburn (Walkerburn, UK).

Reference detergent

As a reference detergent without bleaching agents, we used a heavy-duty powder detergent with phosphate builder, containing the following ingredients (%, w/w): sodium triphosphate (36.7), sodium sulphate (42.0), sodium metasilicate (8.4), carboxymethylcellulose (1.0), EDTA (0.2), fatty alcohol ethoxylate (Berol 08, 3.8), linear alkylbenzenesulphonate (Nansa 1127, 7.8) and an experimental detergent–lipase preparation (0.1). Solutions were made by dosing 5 g/l of the detergent.

Peroxycarboxylic acid standards

Long-chain crystalline peroxycarboxylic acids were synthesized according to Parker *et al.* [7] by oxidation of the parent carboxylic acid with 60% (w/v) hydrogen peroxide, using sulphuric acid as catalyst. The content of active peroxyacid in the preparations was determined by iodometry in a 3:2 (v/v) mixture of glacial acetic acid and chloroform, performed under nitrogen to exclude air. The contents of peroxyacetic acid and acetic acid in the above-mentioned Proxitane preparation were determined by titration with sodium hydroxide and the content of hydrogen peroxide by cerimetry. UV spectra were recorded on an 8452A diode-array spectrophotometer (Hewlett-Packard, USA).

Experiments with commercial detergents

The experiments in which the peroxycarboxylic acid evolution was followed in commercial detergents were all performed at 40°C. The detergents were dosed according to the manufacturers [5 g/l for the European detergents OMO Micro (Unilever) and Ariel Ultra (Procter & Gamble), and 1.2 g/l for the American detergent Tide With Bleach (Procter & Gamble)]. The experiments were performed in open beakers using tap water (5 l, of a total hardness equivalent to approximately 3.2 m*M* Ca^{2+}) and magnetic stirring (500 rpm). The levels of peroxyacids and hydrogen peroxide were expressed as the mean value of five repeated experiments.

Chromatographic apparatus and conditions

The chromatographic apparatus consisted of an LC-6A HPLC pump (Shimadzu, Japan) equipped with an additional external pulse damper (Shimadzu) and a Type 7125 injection valve (Rheodyne, Cotati, CA, USA). Solvents were degassed using a Type ERC-3110 degasser (Erma Optical Work, Japan). A LiChrosorb RP-18 (10 μm) reversed-phase column (250 × 4.6 mm I.D.) was used with a LiChroCART RP-18 (10 μm) guard column, both supplied by Merck. As the mobile phase, 100 m*M* phosphate buffer (adjusted to pH 6) was used for the

detection of peroxyacetic acid or 15 m*M* phosphate buffer (adjusted to pH 6)–methanol (30:70, v/v) for the detection of long-chain peroxycarboxylic acids. Sodium chloride was further added to a concentration of 5 m*M*. The mobile phases were filtered prior to use. The flow-rate was 1.5 ml/min.

The HPLC system was connected to an AMOR amperometric detector (Spark Holland, Netherlands) equipped with a platinum working electrode and a Ag/AgCl reference electrode. Cleaning of the working electrode was done either by wiping it off with a tissue moistened in acetone or ethanol or, after more severe loss in activity, by polishing it according to the manufacturer's guidelines. Of various potential settings tested, a potential of −0.27 V *versus* the reference electrode was found to be suitable for the detection of both peroxycarboxylic acids and hydrogen peroxide. At this potential the compensation current was typically 100–200 nA. In front of the amperometric detector an SPD-6A UV detector (Shimadzu) was inserted. Both detectors were linked to a Shimadzu C-R5A two-channel integrator. Peak areas were taken to represent analyte concentrations. All chromatography and detection was carried out at ambient temperature. However, the AMOR detector comprises an insulated chamber in which the amperometric cell as well as the column are mounted to reduce the influence of temperature fluctuations.

Sample preparation

Pure solutions of reference compounds were injected directly into the HPLC system. Detergent solutions were, however, filtered on a 0.45-μm Minisart disposable filter (Sartorius, Germany) prior to injection. A 20-μl injection volume was used in all experiments.

RESULTS AND DISCUSSION

Initially, we attempted to develop an HPLC system using UV detection. Peroxycarboxylic acids were found to have maximum UV absorbance at 206 nm. Accordingly, using this wavelength and the conditions described above, the detection limit was found to be around 50 μ*M* (*e.g.* 0.17 μg of peroxynonanoic acid) at a signal-to-noise ratio of 3 when analysing solutions of pure preformed peroxycarboxylic acids. However, as illustrated in Fig. 2A,

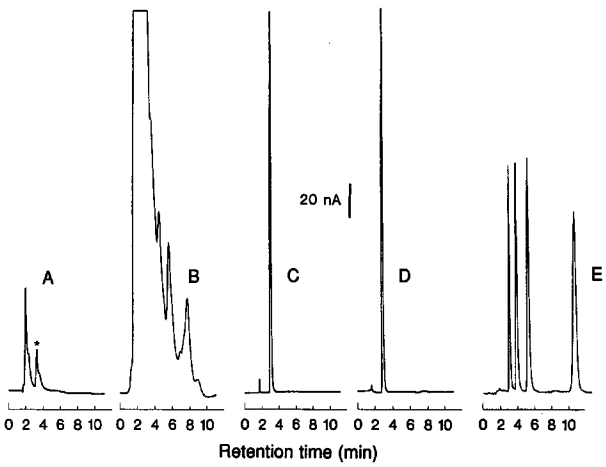

Fig. 2. Chromatograms of 0.2 m*M* solutions of peroxyacetic acid (*): (A) reference solution, UV detection; (B) detergent solution, UV detection; (C) reference solution, electrochemical detection; (D) detergent solution, electrochemical detection; (E) chromatogram of a 0.1 m*M* solution of peroxyoctanoic, peroxynonanoic, peroxydecanoic and peroxydodecanoic acids (eluted in the order given). Integrator attenuation used: UV detector, 2^2; electrochemical detector, 2^4.

when analysing a 0.2 m*M* solution of peroxyacetic acid (made from a commercial preparation further containing acetic acid, hydrogen peroxide and traces of strongly acidic catalyst), the peroxyacid proved difficult to separate from the other components even though the mobile phase was buffered at pH 6, which should keep the peroxyacid protonated (pK_a of peroxyacetic acid is *ca.* 8.3 as determined from the base titration) while dissociating other acids, *e.g.* acetic acid. Furthermore, addition of 5 g/l of the reference detergent without bleaching agent described above made detection of even higher concentrations of peroxyacetic acid impossible owing to huge absorptions of the various other components in the complex matrix (Fig. 2B).

To overcome the interference from other components in a detergent solution, reductive electrochemical detection was attempted as an alternative. This detection principle has previously been applied advantageously for detection of various organic hydroperoxides [8]. Using a platinum electrode and the conditions described above, amperometric detection fulfilled all demands with respect to sensitivity and selectivity, as illustrated by the chromato-

grams obtained when analysing the same solutions of peroxyacetic acid as described above (Fig. 2C and D). Evidently, only the peroxyacid and hydrogen peroxide (in the front of the chromatogram) are detected by the electrochemical system. Examining the chromatogram obtained after addition of the reference detergent, an identical response was found in the case of peroxyacetic acid while the signal originating from hydrogen peroxide was markedly reduced. This effect was found to be the result of decomposition caused by catalase present in the lipase preparation added to the reference detergent used.

Detection of various peroxycarboxylic acids

As gradient elution is poorly compatible with electrochemical detection, different mobile phases were applied for the detection of various peroxycarboxylic acids. The chromatogram obtained when analysing a mixture of peroxyoctanoic, peroxynonanoic, peroxydecanoic and peroxydodecanoic acids using a mobile phase containing 70% (v/v) methanol is illustrated in Fig. 2E. Obviously, hydrogen peroxide has no retention in a reversed-phase chromatographic system and is eluted in the front of a chromatogram. The system described is, accordingly, in principle not suitable for quantification of hydrogen peroxide levels. However, as the bleaching agents, as indicated in Fig. 2, are the only compounds giving detector response in a typical detergent matrix, the area of the signal in the front of the chromatogram was found to be applicable as a rough indicator of the hydrogen peroxide level in a sample, provided the concentration was in the range 1–10 m*M*.

Detection limits, linearity and repeatability of the system

The detector response was optimized by varying the potential of the working electrode *versus* the reference electrode. Fig. 3 shows the peak area–voltage curves for peroxydecanoic and peroxyacetic acids as well as hydrogen peroxide. Based on the curves, a potential of −0.27 V was chosen as a compromise. Although higher potentials would increase the detector signal, which would be particularly desirable in the case of hydrogen peroxide, this would also increase the background noise owing to deterioration of the mobile phase, indicated by the dramatic increase in compensation current on the figure. The pH dependency of the amperometric reaction was not studied in detail. However, an almost similar response was observed when analysing peroxyacetic acid using a 100 m*M* acetic buffer with a pH of 4 as mobile phase.

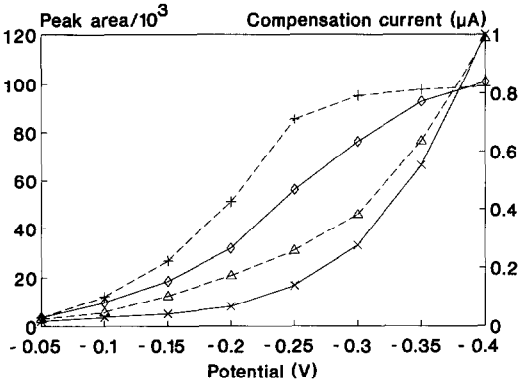

Fig. 3. Peak area–voltage curves for 0.1 m*M* peroxydecanoic acid (+), 0.1 m*M* peroxyacetic acid (◇) and 2 m*M* hydrogen peroxide (△). Compensation current of detector when measuring peroxyacetic acid or hydrogen peroxide (right *y*-axis, ×).

Using a potential of −0.27 V, the detection limit was determined to be 0.1 μ*M* (0.15 ng) in the case of peroxyacetic acid (signal-to-noise ratio of 3) and the calibration graph was linear at least in the range 0.05–5 m*M* (r^2 = 0.9999, n = 4). Using the methanol-containing mobile phase the detection limit was determined to be 0.6 μ*M* in the case of peroxynonanoic acid and a linear calibration graph was observed in the range 0.05–5 m*M* (r^2 = 0.9998, n = 4).

The repeatability of the system was found to be satisfactory as the relative standard deviation on ten analyses performed on the same day using a 0.5 m*M* solution (chosen to represent a typical concentration in a detergent solution) of peroxynonanoic acid was determined to be only 1.6%. Wear of the working electrode was very moderate under the conditions described and the system was, normally, used for several weeks without cleaning the electrode. As outlined above, the system was also used to indicate levels of hydrogen peroxide. The standard deviation on ten analyses performed on the same day using a 2.5 m*M* solution of hydrogen peroxide was found to be 2.7%. Estimation of hydrogen peroxide levels was, however, not reliable below a level of 1 m*M*

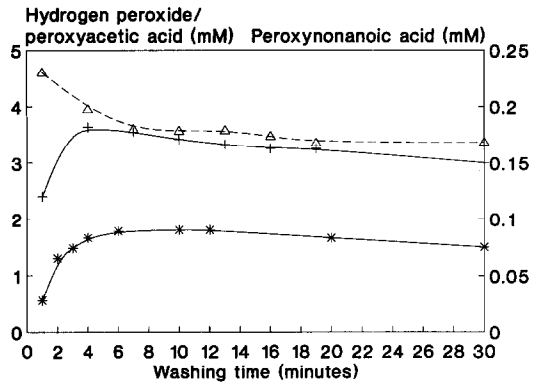

Fig. 4. Hydrogen peroxide level (△) and formation of peroxyacetic acid (+) in a washing liquor containing OMO Micro and formation of peroxynonanoic acid (*) in a washing liquor containing Tide With Bleach.

while a linear calibration curve was obtained in the range 1–10 mM (r^2 = 0.9992, n = 4).

Applicability of the method

The system is used routinely in our laboratory to study the peroxycarboxylic acid formation in the course of a washing cycle when employing various detergents. As an illustration of the applicability of the system, Fig. 4 shows the observed level of hydrogen peroxide and the formation of peroxyacetic acid in a washing liquor made using the TAED-containing detergent OMO Micro (left y-axis) and formation of peroxynonanoic acid in a solution of the NOBS-containing detergent Tide With Bleach (right y-axis). Hydrogen peroxide levels are not illustrated in the case of Tide With Bleach as they were found to be beyond the detection limit of 1 mM. Analysing a second TAED-containing detergent, Ariel Ultra, the peroxyacid formation was found to resemble closely that of OMO Micro, but reaching only roughly 2 mM peroxyacetic acid (results not shown).

CONCLUSION

Compared with the methods described previously in the literature for detecting peroxycarboxylic acids, the HPLC system developed provides a fast, highly sensitive and selective system which, furthermore, is capable of distinguishing between various peroxycarboxylic acids. However, the development of a single chromatographic system capable of detecting a broader range of peroxycarboxylic acids simultaneously could still be desirable.

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