Hydrogen Peroxide

- Hydrogen peroxide in health care products (toothpaste)
- Fast and sensitive HPAE-PAD method
- SenCell™ with Au working electrode
- ‘Green’ method

Summary

Hydrogen peroxide is a strong oxidizing agent and disinfectant used in a wide variety of applications, such as chemical synthesis, propellant (space rockets), water treatment, food processing, cosmetics, personal and health care products [1].

In this application note an analytical method is presented for the measurement of hydrogen peroxide in liquid samples using the DECADE Elite electrochemical detector and SenCell. The method is based on separation by High Performance Anion Exchange Chromatography followed by Pulsed Amperometric Detection (HPAE-PAD) on a gold working electrode. The use of a narrow-bore HPAE column with 4µm particle size, allowed the separation of hydrogen peroxide in less than 3 min in combination with a four-fold reduction of mobile phase usage.

A commercially available whitening toothpaste, containing peroxide as bleaching agent, was analyzed as an example to demonstrate the applicability of the method to real samples.
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Introduction

Hydrogen peroxide (H$_2$O$_2$) is a small molecule consisting of two hydroxy groups joined by a covalent oxygen-oxygen single bond. Pure hydrogen peroxide is a transparent liquid with a slightly pale blue color. Hydrogen peroxide is unstable and slowly decomposes into oxygen gas and water with the evolution of heat. The decomposition is accelerated in the presence of UV light. Although non-flammable, at higher concentrations it can cause spontaneous combustion when it comes in contact with organic material. Hydrogen peroxide is a strong oxidizing agent and disinfectant used in a wide variety of applications, such as chemical synthesis, propellant (space rockets), explosives, water treatment, food processing, paper industry, cosmetics, personal and health care products.

A range of different LC methods are available to measure hydrogen peroxide in various matrices. These methods are based on derivatization followed by either conductivity [2], UV [3] and fluorescence detection [4-6]. Derivatization is necessary due to the lack of a chromophore in hydrogen peroxide. Ion chromatography in combination with electrochemical detection is the method of choice. It combines good selectivity with sensitive detection [6-8]. Moreover, hydrogen peroxide can be directly detected using electrochemical detection without the need of derivatization in both DC and PAD mode. Several HPAE methods for quantification of hydrogen peroxide are reported in literature based on conventional anion-exchange columns with 6.5—10 µm particle sizes [7,8].

In this application note a ‘green’ HPAE-PAD method is presented based on a new type of anion-exchange column with 4µm particle size for the fast and sensitive analysis of hydrogen peroxide. A commercially available whitening toothpaste, containing peroxide as bleaching agent, was analyzed as an example to demonstrate the applicability of the method to real samples. The analysis was performed using an ALEXYS HPAE-PAD analyzer with a P6.1L pump and AS 110 autosampler (see figure 1).

Method

The LC-EC conditions are listed in table 1. In figure 2 an example chromatogram is shown of a hydrogen peroxide standard.

![Figure 1. ALEXYS HPAEC-PAD analyzer based on the DECADE Elite electrochemical detector with SenCell for the analysis of peroxide.](image)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC system</td>
<td>ALEXYS HPAEC-PAD analyzer</td>
</tr>
<tr>
<td>Detector</td>
<td>Antec DECADE Elite electrochemical detector</td>
</tr>
<tr>
<td>Columns</td>
<td>CarboPac PA20-Fast-4µm column, 100 x 2.0 mm ID</td>
</tr>
<tr>
<td></td>
<td>CarboPac PA20-Fast-4µm column, 30 x 2.0 mm ID</td>
</tr>
<tr>
<td></td>
<td>BorateTrap Inline Trap Column, 50 x 4.0 mm ID</td>
</tr>
<tr>
<td>Mobile phase (MP)</td>
<td>50 mM KOH blanketed with Helium 5.0</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Injection</td>
<td>1 µL (partial loopfill)</td>
</tr>
<tr>
<td>Temperature</td>
<td>35°C for separation and detection</td>
</tr>
<tr>
<td>Flow cell</td>
<td>SenCell with Au WE, stainless steel AE and HyREF, AST 2</td>
</tr>
<tr>
<td>Potential waveform</td>
<td>E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V</td>
</tr>
<tr>
<td>(4-step)</td>
<td>ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s</td>
</tr>
<tr>
<td>I-cell</td>
<td>about 0.2— 0.4 µA</td>
</tr>
<tr>
<td>ADF</td>
<td>0.1 Hz</td>
</tr>
<tr>
<td>Range</td>
<td>0.05, 5 and 50 µA/V</td>
</tr>
</tbody>
</table>
Separation

The New CarboPac™ PA20-Fast-4µm column (100 x 2 mm ID) Anion-Exchange column with guard (30 x 2 mm ID) was chosen for the separation of hydrogen peroxide. This column with small particle size (4 µm) and internal diameter (2 mm) combines high-resolution separation with low consumption of mobile phase. The temperature for separation was set at 35°C. The analysis is based on isocratic elution using 50 mM KOH as mobile phase. To minimize the introduction of carbonate ions in the mobile phase the eluents were carefully prepared manually using a 45% w/w KOH solution (commercially available). The diluent was DI water (resistivity >18 MΩ cm) which was sonicated and sparged with Helium 5.0 prior to use. The mobile phase should be prepared in plastic bottles instead of glass.

KOH is a strong etching agent and will react with the inner glass wall resulting in the release of silicates and borates. The appropriate amount of 45% w/w KOH solution was carefully pipetting into the diluent under gently stirring and Helium sparging to prepare the required mobile phase solutions. The bottles with mobile phase and column clean-up solution were blanket ed with Helium (0.5 bar overpressure) during the analysis to minimize the build-up of carbonate ions in the mobile phase and to assure a reproducible analysis. A column clean-up / regeneration step with 200 mM KOH can be applied in case of loss of retention to remove bound carbonate ions or late eluting compounds like oligo- and polysaccharides, which might be present in real samples. See reference [9] for an example of step-gradient elution in HPAE-PAD.

Detection

For the pulsed amperometric detection of hydrogen peroxide the Antec SenCell electrochemical flow cell is used. This novel flow cell [9] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF (Pd/H₂) reference electrode (RE) and stainless steel auxiliary electrode (AE). A 4-step potential waveform was applied as shown in figure 3. The temperature for detection was set to 35°C. The cell current was typical about 0.2—0.4 µA with these PAD settings under the specified conditions. This particular 4-step waveform with a pulse duration of 500 ms has been claimed to have as benefits: (1) a consistent long-term peak area response and (2) minimal electrode wear [10], resulting in less flow cell maintenance and system down time.

Preparation of standards and samples

Standards: a 1000 mg/L H₂O₂ stock solution was prepared by pipetting 283 µL of a 31.6 wt% H₂O₂ solution (Acros Organics, Germany) into a 100 mL volumetric flask, which was brought to volume with DI water. Working standards in the range of 0.1 mg/L to 100 mg/L were made in the same manner using 10 mL volumetric flasks using the appropriate dilution factors.

Sample preparation: Two commercial tooth paste samples were analyzed. The following sample prep procedure was used for the analysis:

Procedure:
- 100 mg of toothpaste was weighted and transferred into a 10 mL volumetric flask.
- The volumetric flask was filled with approximately 5 mL of DI water and mixed to dissolve the toothpaste.
- Subsequently, the flask was brought to volume with water.
- A part of the solution was transferred into a 5 mL plastic syringe and filtered over a 0.20 µm PES (Polyethersulfone) syringe filter (GVS life sciences, Sanford, USA).
- The filtered solution was 10 or 100 times diluted with water and transferred into a 1.5 mL glass vial, from which 1 µL was injected into the LC system and analyzed.
Results

In figure 2 an overlay is shown of two chromatograms obtained with a 1 µL injection of a 1 ppm H₂O₂ standard in water (red curve) and a water blank injection (black curve). H₂O₂ is eluting within three minutes without coeluting interferences. The H₂O₂ peak has a plate number of 45,000 plates/meter and a tailing factor of 1.3.

Linearity, Repeatability and LOD

The linearity of the H₂O₂ response was investigated in the concentration range of 0.1—10 mg/L and 10—100 mg/L, which corresponds with a molar concentration range of 2.9 µmol/L—2.9 mmol/L. In both the low and high concentration ranges the linearity was excellent with correlation coefficients better than 0.999. In fact the response was linear over the complete concentration range between 0.1 and 100 mg/L, which is significantly better than reported in ref [8].

Sample analysis

Two commercial toothpaste samples were analyzed using this method:

- Whitening toothpaste containing peroxide* and baking soda
- Normal toothpaste with mint taste

* the amount of peroxide is undisclosed by the manufacturer

The sample preparation of the toothpaste samples is simple and described in the previous section. The chromatograms of the toothpaste samples are shown in figure 5 together with a chromatogram of a 80 mg/L H₂O₂ standard for reference and identification.

The Limit of Detection (LOD) was determined based on the response of the 0.1 mg/L H₂O₂ standard with the detector set at the 50 nA/V range. The LOD was calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 12 segments of 0.5 min). The noise was calculated based on a 6 minute section of the baseline during a run (after elution of H₂O₂ starting at t = 4 min). A LOD of 1.8 µg/L (53 nmol/L, 1.8 pmol on-column) was found, demonstrating the outstanding sensitivity of the method.

Sample analysis

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- Whitening toothpaste containing peroxide* and baking soda
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The sample preparation of the toothpaste samples is simple and described in the previous section. The chromatograms of the toothpaste samples are shown in figure 5 together with a chromatogram of a 80 mg/L H₂O₂ standard for reference and identification.

It is evident from figure 5 that the whitening toothpaste contained hydrogen peroxide. The concentration of H₂O₂ in the worked-up sample was calculated using the 10-100 mg/L calibration curve and was 83 mg/L. This amount corresponds with
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8 mg H₂O₂/gram toothpaste (0.8 wt%). The control sample (normal toothpaste with mint taste) did not contain any measurable amount of peroxide. However, the chromatogram of the control sample showed several other unidentified compound peaks. These peaks/compounds did not interfere or coelute with H₂O₂. One of the ingredients mentioned on the content label of the control sample is sorbitol. Sorbitol is a sugar alcohol with a sweet taste which is often used as sweetener. Toothpaste manufacturers add it to toothpastes to create a sweet flavor without leading to tooth decay. Due to the fact that oral bacteria can not metabolize sorbitol, it is not converted to cavity-causing acids and, therefore, the teeth are protected. Sorbitol is a fast eluting sugar alcohol. Although no sorbitol standard was injected for identification, it is very likely that the large peak at 1.3 min is due to sorbitol.

References

7. A. Takahashi et al., Determination of Hydrogen Peroxide by HPLC with Cation-exchange resin gel column and electrochemical detector, Analytical Sciences, 15 (1999), 481-483
8. S. Bhardwa et al., Hydrogen peroxide detection by ion chromatography and electrochemical detection, Thermo Application note 70967 (2014)
9. Antec Scientific, Analysis of Lactose and isomers in Lactose-free labelled dairy product, application note 220.009

Conclusion

The ALEXYS HPAEC-PAD system based on the DECADE Elite detector, SenCell flow cell and a narrow-bore ‘fast-4µm’ HPAE column, offers a simple and sensitive analysis solution for the quantification of hydrogen peroxide with minimal sample preparation. The presented HPAEC-PAD method allows fast separation, within 3 min, of hydrogen peroxide followed by direct PAD detection without the need for derivatization. A four-fold reduction of mobile phase usage was achieved by using a 2 mm ID column instead of a standard bore version (4 mm ID). The method was successfully applied for the analysis of hydrogen peroxide in a commercial whitening toothpaste sample.
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Ordering information

<table>
<thead>
<tr>
<th>Item Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>180.0055W</td>
<td>ALEXYS HPAEC-PAD Analyzer (isocratic, step-gradient applications) including DECADE Elite SCC and SenCell 2 mm Au HyREF</td>
</tr>
<tr>
<td>250.1175*</td>
<td>BorateTrap Inline Trap Column, 50 x 4.0 mm ID (047078)</td>
</tr>
<tr>
<td>250.1185*</td>
<td>CarboPac PA20-Fast-4µm anal. column, 100 x 2.0 mm ID (302749)</td>
</tr>
<tr>
<td>250.1184*</td>
<td>CarboPac PA20-Fast-4µm guard column, 30 x 2.0 mm ID (302750)</td>
</tr>
</tbody>
</table>

*) Columns are products of Thermo Fisher Scientific Inc. (USA), between parenthesis the Thermo pn’s are shown for reference.

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the DECADE Elite detector. Dependent on the type of sample and interferences optimization of the sample preparation and method may be necessary. The actual performance may be affected by factors beyond Antec’s control. Specifications mentioned in this application note are subject to change without further notice.

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