Capillary electrophoresis–electrochemistry microfluidic system for the determination of organic peroxides

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Abstract

A microfluidic analytical system for the separation and detection of organic peroxides, based on a microchip capillary electrophoresis device with an integrated amperometric detector, was developed. The new microsystem relies on the reductive detection of both organic acid peroxides and hydroperoxides at −700 mV (vs. Ag wire/AgCl). Factors influencing the separation and detection processes were examined and optimized. The integrated microsystem offers rapid measurements (within 130 s) of these organic-peroxide compounds, down to micromolar levels. A highly stable response for repetitive injections (RSD 0.35–3.12%; $n=12$) reflects the negligible electrode passivation. Such a ''lab-on-a-chip'' device should be attractive for on-site analysis of organic peroxides, as desired for environmental screening and industrial monitoring. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

The determination of low levels of organic peroxides is of considerable importance since these compounds are involved in a wide variety of natural and industrial processes [1,2]. In addition, there is a growing environmental interest in the identification and quantification of organic peroxides in drinking water in connection with ozonation disinfection processes [3,4]. Organic acid peroxides and hydro-peroxides may be formed in water when ozone reacts with natural organic substances and may exhibit adverse health effects [5]. To meet these needs various gas [6,7] and liquid [8–10] chromatographic procedures as well as capillary electrophoresis (CE) ones [11], have been developed for separation and detection of organic peroxides.

This paper describes a CE-microchip protocol for rapid measurements of organic peroxides. The development of microscale (chip-based) separation devices, particularly micromachined capillary electrophoresis systems, has witnessed a dramatic growth in recent years [12,13]. Such miniaturized devices represent the ability to shrink conventional ''benchtop'' analytical systems with major advantages of speed, cost, portability, solvent/sample economy, and integration. Electrochemical detection (ED) of-

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fers great promise for CE microchip systems, with features that include high sensitivity, inherent miniaturization, low-power requirements, compatibility with advanced micromachining and microfabrication technologies, and low cost [14,15]. Such detection scheme has already proven to be well suited for microchip CE measurements of toxic phenolic [16] or hydrazine [17] compounds. Amperometric flow detection has been used previously for monitoring various peroxides in conventional flow-injection [18], liquid chromatography [9,10] and capillary electrophoresis [11] systems. Both the oxidative and reductive detection modes, based on the intrinsic electroactivity of peroxide compounds, have thus been used. The adaptation electrochemical detection for on-chip measurements of organic peroxides thus represents a logical extension of its capabilities. The performance characteristics of the new CE microchip for organic peroxides are reported in the following sections.

2. Experimental

2.1. Reagents

Sodium borate, sodium hydroxide, hydrogen peroxide, *tert*.-butyl hydroperoxide, peroxyacetic acid, propionic anhydride and heptanoic anhydride were obtained from Aldrich (USA), while cumene hydroperoxide and sodium dodecyl sulfate (SDS) were received from Sigma (USA). Peroxyalkanoic acids, which are not commercially available (e.g., peroxypropionic acid and peroxyheptanoic acid), were prepared from the corresponding carboxylic anhydrides and hydrogen peroxide using a standard procedure [19]. The concentrations of the freshly prepared stock solutions were calculated based on the remaining peak of the unreacted hydrogen peroxide and the stoichiometry of the reaction [19].

2.2. Apparatus

The glass chip was fabricated by Micralyne (Model MC-BF4-001, Edmonton, Canada) by means of wet chemical etching and thermal bonding techniques. The 88 mm×16 mm chip consisted of four-way injection cross, with 74 mm long separation

channel and side arms of 5 mm long each. The original waste reservoir was cut off, leaving the channel outlet at the end side of the chip, thus facilitating the end-column amperometric detection [20]. The channels were 50 μm wide and 20 μm deep. A Plexiglas holder was fabricated for holding the separation chip and housing the detector and reservoirs, see Fig. 1. A short pipette tip was inserted into each of the three holes on the glass chip for solution contact between the channel on the chip and corresponding reservoir on the chip holder. The amperometric detector was placed in the waste reservoir (at the channel outlet side), and for detection of organic acid peroxides consisted of a platinum wire counter (N), an Ag wire/AgCl reference (O), and a gold disk working (P) electrode. These wire (reference and counter) electrodes were inserted through holes drilled in plexiglas holder. The working electrode, housed in the plastic screw, was placed opposite to the channel outlet, at 50 μm distance controlled under microscope. Prior to its daily use, the gold working electrode was polished 10 min with 3 μm alumina and 10 min with 0.05 μm alumina to achieve reproducible response [21]. Platinum wires, inserted into the individual reservoirs (M) served as contacts to the high-voltage supply. The laboratory-made high-voltage power
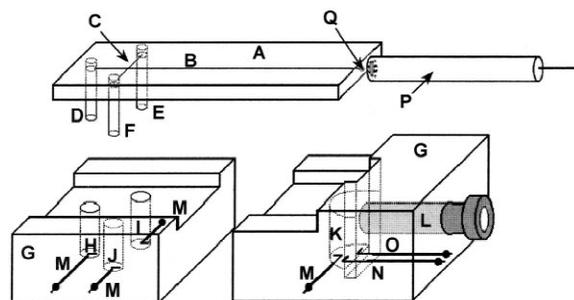

Fig. 1. Capillary electrophoretic system with electrochemical detection. (A) Glass microchip, (B) separation channel, (C) injection channel, (D) pipette tip for buffer reservoir, (E) pipette tip for sample reservoir, (F) pipette tip for reservoir not used, (G) Plexiglas body, (H) buffer reservoir, (I) sample reservoir, (J) reservoir not used, (K) detection reservoir, (L) plastic screw, (M) high-voltage power electrodes, (N) counter electrode, (O) reference electrode, (P) gold working electrode, (Q) channel outlet. Chip, chip holder and disk electrode are separated for clarity. Dimensions not in scale.

supply had an adjustable voltage range between 0 and +5000 V (0 and +633 V/cm).

The detection of cumene hydroperoxide and *tert*.-butyl peroxide was performed using a thick-film carbon electrode. Details of the integration of CE glass chip/thick-film detection electrode microsystem were described previously [20]. The thick-film carbon electrodes were printed with a semiautomatic printer (Model TF 100, MPM, Franklin, MA, USA) using an Acheson ink (Electrodag 440B; Acheson Colloids, Ontario, CA, USA).

2.3. Electrophoresis procedure

The channels of the glass chip were treated before use by rinsing with 0.1 *M* NaOH and deionized water for 20 and 5 min, respectively. The electrophoresis buffer consisted of a borate buffer (10 m*M*, pH 9.2) for determination of organic acid peroxides, and of a borate buffer (10 m*M*, pH 9.2; containing 25 m*M* SDS) for the determination of *tert*.-butyl hydroperoxide and cumene hydroperoxide. The run buffer and unused reservoirs (Fig. 1; H, J) were filled with electrophoresis run buffer solution, while the sample reservoir (Fig. 1; I) was filled with a mixture of samples. After an initial loading of the sample in the injection channel, the sample was injected by applying field +253 V/cm (organic acid peroxides) or +190 V/cm (*tert*.-butyl and cumene hydroperoxides) between the sample reservoir (I) and the grounded detection reservoir (K) for 3 s. This drove the sample ''plug'' into the separation channel through the intersection. By switching the high-voltage contacts, the separation field strength, was subsequently applied to the run buffer reservoir (H), with the detection reservoir (K) grounded and all other reservoirs floating for the separation of the organic acid peroxides. Separation field strengths of +253 and +190 V/cm were usually applied for the separation of organic acid peroxides and *tert*.-butyl hydroperoxide/cumene hydroperoxide, respectively.

2.4. Amperometric detection

Amperometric detection was performed with an electrochemical analyzer 621 (CH Instruments, Austin, TX, USA) connected to a personal computer (Pentium, 100 MHz, 32 MB RAM). The elec-

tropherograms were recorded with a time resolution of 0.1 s while applying the detection potential (usually −700 mV vs. Ag wire/AgCl). Sample injections were performed after stabilization of the baseline. All experiments were performed at room temperature.

2.5. Safety considerations

The high-voltage power supply should be handled with extreme care to avoid electrical shock. Peroxides are toxic/mutagenic/explosive compounds; skin and eye contacts and accidental inhalation or ingestion should be avoided.

3. Results and discussion

Fig. 2 displays an electropherogram obtained at the gold electrode detector for a mixture of millimolar concentrations of oxygen (a), hydrogen peroxide (b), peroxyheptanoic acid (c), peroxypropionic acid (d) and peroxyacetic acid (e). The five peaks are well resolved within 120 s. Analogous measurements with conventional (fused-silica) CE capillaries usually require significantly longer (>5 min) periods [11]. As expected, oxygen elutes with the electroosmotic flow (EOF), followed by the anions migration against the EOF. The migration order of peroxycarboxylic acids reflects the charge/mass ratio. The hydrogen peroxide is partly negative charged due to complexation with the borate buffer [11]. The flat baseline and low noise level at the detection potential of −700 mV indicate an effective isolation from the high separation potential, despite the absence of a decoupling mechanism. These, along with the well-defined response peaks, indicate convenient quantitation down to the micromolar level (see data below).

The effect of separation field strength upon the behavior of the integrated CE–ED peroxide microchip was examined. As expected, increasing the separation field strength from +127 to +633 V/cm dramatically decreased the migration time for hydrogen peroxide, peroxypropanoic acid and peroxyacetic acid from 145.3 to 25.6 s, 277.4 to 48.3 s and from 319.4 to 53.9 s, respectively. The peak width (at
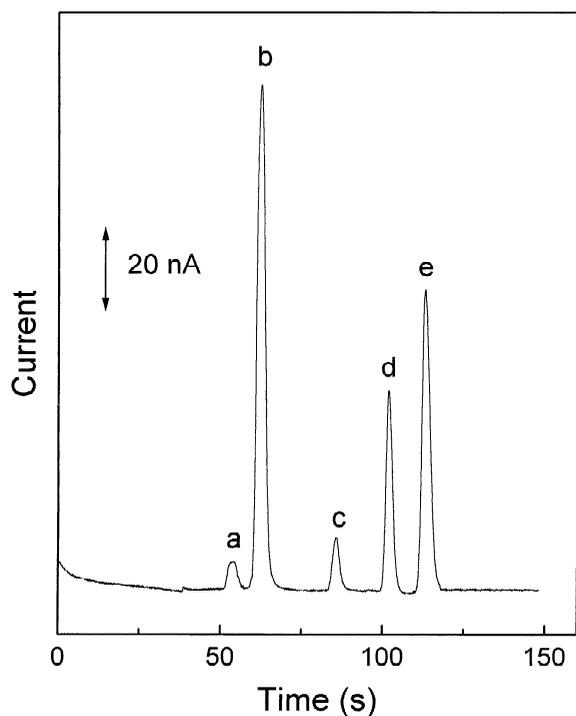
Fig. 2. Electropherograms for mixtures containing oxygen (a), 1.71 mM hydrogen peroxide (b), 4.8 mM peroxyheptanoic acid (c), 0.25 mM peroxypropionic acid (d) and 1.33 mM peroxyacetic acid (e). Conditions: separation and injection field strength, +253 V/cm; detection potential, −700 mV; gold electrode; borate buffer (10 mM, pH 9.2) as the running buffer.

half-height) decreased from 6.9 to 1.5 s, from 9.8 to 1.9 s and from 11.8 to 2.1 s, respectively. The separation field strength had a negligible effect upon the peak-to-peak background noise level (33 pA) for field strengths ranging from +127 to +506 V/cm. The noise level increased to 50 pA at separation field strength +633 V/cm. A flat baseline was observed using separation field strengths equal or lower than +253 V/cm. However, a larger initial baseline slope was observed for field strengths ranging from +316 to +633 V/cm, indicating an incomplete isolation of the detector from the high separation field strength. Most of the subsequent organic acid peroxides detection thus employed a separation field strength of +253 V/cm.

A different separation mechanism was used for additional organic peroxides, namely hydroperoxides, such as *tert.*-butyl hydroperoxide and

cumene hydroperoxide. These compounds, which are neutral in the borate buffer (pH 9.2) were separated using micellar electrokinetic chromatography (MEKC). As expected, increasing the concentration of SDS from 15 to 50 mM increased the migration time of *tert.*-butyl hydroperoxide and cumene hydroperoxide from 71 to 83 s and from 89 to 150 s, respectively (not shown). These results reflect the fact that the hydrophobic cumene hydroperoxide associates with the SDS micelle stronger than the more hydrophilic *tert.*-butyl hydroperoxide. This observation is in agreement with analogous reversed-phase high-performance liquid chromatography (RP-HPLC) measurements [10]. Most of subsequent hydroperoxide work employed a SDS concentration of 25 mM, which offered a well-separated peaks and good detection sensitivity.

Fig. 3A depicts hydrodynamic voltammograms (HDVs) for the oxidation of hydrogen peroxide (a), peroxypropionic acid (b) and peroxyacetic acid (c) employing the gold working electrode. The curves were recorded pointwise by making 100 mV changes in the applied potential over the −100 to −900 mV range, using a separation field strength +253 V/cm. The three compounds display similar profiles, with a gradual increase of the response between −300 and −800 mV, and leveling off of the current thereafter.
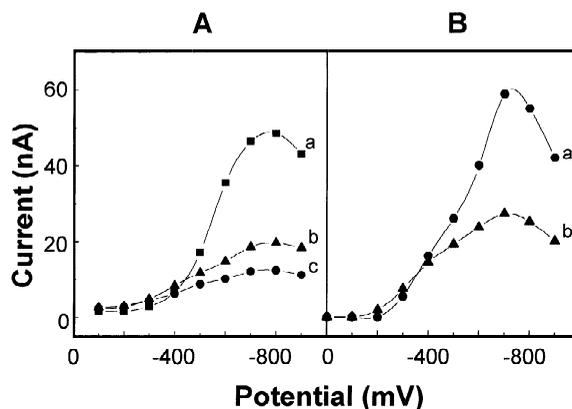

Fig. 3. Hydrodynamic voltammograms for (A) 0.7 mM hydrogen peroxide (a), 0.1 mM peroxypropionic acid (b), 0.25 mM peroxy-acetic acid (c), and (B) 1 mM *tert.*-butyl hydroperoxide (a) and 1 mM cumene hydroperoxide (b). Conditions: (A) as in Fig. 2; (B) separation and injection field strength, +190 V/cm; detection potential, −700 mV; carbon thick-film electrode; borate buffer (10 mM, pH 9.2 with 25 mM SDS) as the running buffer.

The half-wave potentials are −552 mV (a), −466 mV (b) and −431 mV (c). Hydroperoxides displayed tailed CE peaks at the gold electrode, and sharp ones at the thick-film carbon detector. Such behavior is attributed to a more favorable electron-transfer kinetics at the carbon electrode. Fig. 3B shows the hydrodynamic voltammograms of *tert*.-butyl hydroperoxide (a) and cumene hydroperoxide (b) employing this carbon detector. The waves start at −300 mV and reach a maximum value at −700 mV. Subsequent amperometric detection (of both types of organic peroxides) employed the detection potential of −700 mV that offered the most favorable signal-to-noise characteristics. A dramatic increase in the baseline current, its slope, and the corresponding noise was observed at higher potentials. Higher operation potentials would be required in connection to higher separation field strengths that may shift the voltammetric profile to the anodic direction [20].

The new CE–ED microchip protocol offers a well-defined concentration dependence. Calibration plots for hydrogen peroxide and peroxyacetic acid were highly linear over 100–700 μM range with sensitivities of 71.3 and 55.8 nA/mM and intercepts of −1.48 and −2.17 nA for hydrogen peroxide and peroxyacetic acid, respectively (correlation coefficients, 0.999 and 0.998, respectively) (not shown). The very favorable signal-to-noise characteristics observed for a mixture containing 100 μM of hydrogen peroxide, peroxyacetic acid and peroxypropionic acid indicate detection limits at the low (10–30 μM) micromolar range. (Note that such an estimate assumes a linear response and a stochiometric preparation in the case of peroxypropionic acid). The signal-to-noise characteristics of a mixture containing 50 μM of *tert*.-butyl hydroperoxide and cumene hydroperoxide indicated low detection limits of 8.2 μM and 19.2 μM respectively (S/N=3; not shown).

The high sensitivity and speed of the CE device–electrochemical detector system are coupled to a very good reproducibility. A series of 12 repetitive injections of a mixture containing 1.71 mM hydrogen peroxide, 0.25 mM peroxypropionic acid and 1.33 mM peroxyacetic acid resulted in relative standard deviations (RSDs) of 0.35, 1.33 and 1.62%, respectively, for the current response, and of 0.49, 0.77 and 0.86%, respectively, in the migration times
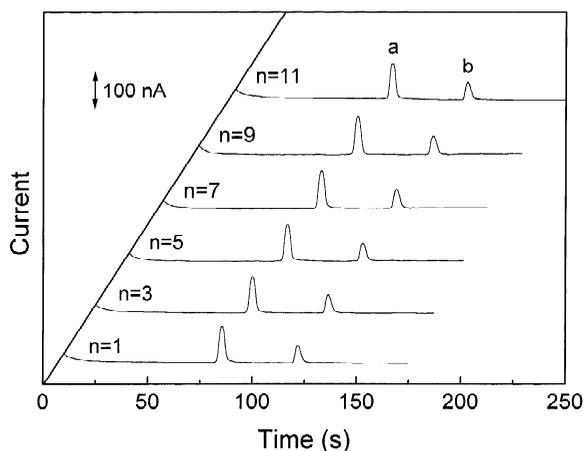

Fig. 4. Precision of repetitive electropherograms for a mixture containing 1 mM *tert*.-butyl hydroperoxide and cumene hydroperoxide. The alternatively recorded electropherograms are shown. Other conditions as in Fig. 3B.

(using the same detector). Good precision is indicated also in Fig. 4 for analogous runs using a mixture containing 1 mM *tert*.-butyl hydroperoxide and 1 mM cumene hydroperoxide, this series resulted in RSDs of 2.02 and 3.12%, respectively, in the current response, and of 0.70 and 0.79%, respectively, in the migration times (using the same detector strip). Apparently, such peroxide detection is not susceptible to surface passivation effects.

4. Conclusions

This work has demonstrated the utility of capillary electrophoresis microfluidic device with integrated amperometric detection for the separation and detection of organic peroxides. The negligible sample consumption and waste production, low power requirements and small size of CE microchip devices hold a great promise for on-site monitoring of organic peroxides, including environmental screening or industrial tests and potentially for space exploration/life detection. Combining it with the miniaturization advantage of electrochemical detection offers great promise for introducing truly ''lab-on-a-chip'' devices. Work is in progress to develop similar protocols for fatty-acid peroxides (that can potentially be used to determine whether life de-

veloped on other planetary bodies). Preliminary results of this effort are encouraging.

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