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Indirect pulsed electrochemical detection of aliphatic carboxylate-containing analytes following high performance anion-exchange chromatography ☆

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ABSTRACT

The mechanism of detection in pulsed electrochemical detection (PED) requires preadsorption of the analyte to the working electrode prior to its subsequent oxidation. Indirect detection is accomplished by the addition of a PED-active reagent to the mobile phase, whose signal is attenuated by an analyte that more strongly adsorbs to the electrode surface. Here, indirect PED (InPED) is applied to the determination of aliphatic carboxylate-containing compounds separated using high performance anion-exchange chromatography (HPAEC). Limits of detections of 0.05–2 ppm (10–400 pmol) are found for most analytes tested using an optimized potential-time waveform at a gold working electrode. The analytical utility of InPED is demonstrated for assays of gabapentin, biotin, proline and several over-the-counter formulations.

1. Introduction

High performance ion-exchange chromatography (HPIEC) is a powerful technique that is often applied to the separation of highly polar analytes. Although a wide range of detectors have been applied to HPIEC, conductivity is the most popular mode of detection for inorganic ions. Pulsed electrochemical detection (PED) has been popularized for the *direct* and *sensitive* detection of polar, aliphatic ions; especially carbohydrates under alkaline conditions. PED utilizes an electrocatalytic process that requires the adsorption of analyte molecules onto the surface of a noble metal electrode prior to their detection [1–3]. This requirement for analyte adsorption has led to the development of several approaches to indirect detection in PED.

Polta and Johnson [4] and later Welch and coworkers [5–8] observed that analyte adsorption could attenuate the anodic background signal arising from oxide formation on the surface of the noble metal working electrode used in PED to allow the detection of inorganic ions and penicillins, respectively. However, the extent of oxide formation is highly sensitive to numerous system parameters (e.g., pH, ionic strength, organic modifiers, and temperature), which is deleterious to system performance and reproducibility. In addition, many analytes have a Faradaic response at the potentials of oxide-formation, which further complicates accurate quantitation of even well-separated compounds. Casella et al. [9] were able to apply this mode of indirect detection in PED to electroinactive aliphatic organic acids (i.e., formic, acetic, and maleic acids) with success.

Doscotch et al. [10] developed indirect adsorption detection (IAD) that exploited the large, cathodic signal resulting from the electrochemical reduction of dissolved oxygen in the mobile phase. This signal was attenuated upon the addition of analytes (i.e., amines, halides, and sulfur-containing compounds) that could adsorb onto a noble metal electrode. IAD was found to be more tolerant to changes in pH than indirect detection based on the generation of a background signal from surface oxide formation; however, it suffered from poor inter-day precision due the inability to accurately control the amount of dissolved oxygen in the system. Also, the waveforms necessary for sensitive detection had a tendency to cause gas formation as a result of cathodic solvent breakdown. These gas bubbles sometimes lodged in the thin-layer electrochemical cell, requiring its disassembly.

LaCourse [2] presented an alternate mode of indirect detection using PED based on the controllable addition of a PED-active reagent to the mobile phase to generate a stable anodic background signal. This approach exploits the well-understood anodic response of polyols and carbohydrates, which occurs at potentials where oxide formation is minimal. In the detection mechanism, the reagent is adsorbed to the 'bare' gold electrode surface prior to

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its oxidation, with analytes that more strongly adsorb to the electrode surface (e.g., inorganic ions, amine- and sulfur-containing compounds) blocking the reagent's adsorption, resulting in the attenuation of the signal produced by the oxidation of the reagent. Because polyols and carbohydrates are only weakly-adsorbed to gold, the range of possible analytes that can be detected is increased, and this mode of action can be exploited to perform detection of compounds that have little or no electroactivity. Olson [11] applied this approach to the determination of amino acids and proteins with detection limits in the low picomole and low femtomole levels, respectively. This method, called indirect pulsed electrochemical detection (InPED), allows the use of a single, optimized waveform to detect a wide variety of compounds and depends on an easily controlled background signal.

In this paper, InPED is applied to the determination of a variety of carboxylate-containing compounds as a means to delineate the quantitative aspect of InPED following high performance anion-exchange chromatography (HPAEC). Voltammetric and chromatographic studies are used to further determine optimal waveform and system parameters. Emphasis is placed on elucidating the quantitative aspects of HPAEC-InPED, which is applied to assays of amino acids, antiepileptic drugs, and several over-the-counter formulations.

2. Materials and methods

2.1. Instrumentation

2.1.1. Pulsed voltammetry

All equipment used for pulsed voltammetry (PV) was purchased from Pine Instrument Company (Grove City, PA). PV experiments were conducted using a 3.0 mm diameter gold working rotating disk electrode (RDE) that was rotated at 900 RPM by an analytical rotator coupled to a model AFRDE4 Bipotentiostat and a model AFMSRX speed control device. The RDE was positioned in the center of a glass voltammetry cell with two arms separated from the main body of the vessel by coarse glass frits. The three electrode system was completed by a platinum wire auxiliary electrode and an Accumet Ag/AgCl single-junction reference electrode (Fisher Scientific, Pittsburgh, PA). A computer with ASYST scientific software (Asyst Software Technologies, Inc., Rochester, NY) was used to generate the triple-potential PED waveform and to collect data. One cycle of the waveform consisted of a detection potential (E_1) that was held for a period (t_1) of 450 ms followed by positive and negative potential pulses at +800 mV for 180 ms and −300 mV for 360 ms, respectively. The current was sampled during the detection step starting at 240 ms for a period of 200 ms. For the PV experiment, three cycles of the waveform were averaged at each E_1 condition, which was scanned from −800 mV to +800 mV in 10 mV increments.

2.1.2. Chromatography

A DX-500 Chromatography System (Dionex, Sunnyvale, CA) was used to perform all chromatographic experiments. Mobile phase was stored under helium (99.997% purity) at a pressure of 8–10 psi in a pressurizable reservoir and was isocratically pumped at a flow rate of 1.00 mL/min by a GP40 Gradient Pump. A GM-3 gradient mixer was added to the system by placing it between the pump head and the injection valve when required. Sample injection was performed either manually by a pneumatically-controlled PEEK™ (Rheodyne, Cotati, CA) injection valve or automatically by an AS3500 Autosampler (Dionex). A 25 µL injection loop was used in both cases, with the autosampler set to inject in the full loop setting. Separations were performed on a CarboPac PA1 analytical column (10 µm, 4.0 × 250 mm; Dionex), which was located after either an IonPac NG1 or a CarboPac PA10 guard column. A LC30 Chromatography Oven (Dionex) was maintained at a temperature of 30 °C and used to house the gradient mixer, injector, guard and analytical columns, and the electrochemical cell. An ED40 Electrochemical Detector (Dionex) in conjunction with the electrochemical cell was used to perform all detection. The cell contained the 1.0 mm diameter gold working electrode and a combination pH/reference electrode, with the cell body acting as the auxiliary electrode. All experiments were performed using the Ag/AgCl-only option of the combination electrode as the reference. Table 1 displays the quadruple-potential waveform used to detect these carbohydrates by direct PED following HPAEC using a mobile phase of 0.10 M NaOH. PeakNet Chromatography Software (version 5.21, Dionex) was used to analyze and collect data.

Table 1
Optimized quadruple-potential PED waveform used for all experiments.

Step	Potential (mV)	Time (ms)	Integration
E_1	100	0	
	100	200	Begin
	100	400	End
E_2	−1500	410	
	−1500	420	
E_3	700	430	
E_4	−100	450	
	−100	950	

2.2. Materials

All water used to prepare solutions was obtained from a Millipore Elix-3 electrodeionization station coupled to a Milli-Q A10 water purification system (Millipore, Billerica, MA). NaOH solutions were prepared by diluting 50% (wt/wt) NaOH (Fisher Scientific). All polyols (sorbitol and gluconic acid), amino acids (lysine, methionine, isoleucine, and proline), and standards of norvaline, vigabatrin, gabapentin, and biotin were purchased from Sigma-Aldrich (St. Louis, MO). Two brands of biotin tablet (Spring Valley – Nature's Bounty, Inc., Bohemia, NY and Origin – Target Corporation, Minneapolis, MN) and one type of proline capsule (Solgar Vitamin and Herb, Leonia, NJ) were used in application work.

2.3. Procedures

2.3.1. Pulsed voltammetry

50 mL of 0.10 M NaOH was always used as the supporting electrolyte for all PV experiments and a voltammogram of this solution was always collected at the beginning of a set of experiments to use for background subtraction. A 50 mM stock solution of sorbitol and a 39.9 mM stock solution of lysine (corrected for HCl contained in the lysine standard) were prepared by dissolving the respective analyte standard in water. A micropipette (Gilson, Middleton, WI) was used to add sorbitol or lysine into the PV cell to obtain the desired concentration, making sure that the total volume change in the cell was less than 1% to avoid dilution effects.

2.3.2. Selection of the background reagent for HPAEC using PED

A *ca.* 100 ppm stock solution of sorbitol and gluconic acid was made by diluting solid analyte in water and in turn was used to prepare solutions ranging in concentration from *ca.* 0.1–25 ppm. These solutions were used in experiments to generate calibration

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