Four Channel Liquid Chromatography/Electrochemistry

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The new epsilon™ family of electrochemical detectors from BAS can control up to four working electrodes simultaneously. There are several advantages to using multiple detector electrodes. By using four different applied potentials with electrodes placed in a parallel arrangement, a hydrodynamic voltammogram can be generated quickly through acquisition of four data points for every analyte injection. This speeds method development time. In addition, co-eluting compounds in complex mixtures can be resolved on the basis of their observed half-wave potentials by using the same arrangement of electrodes, also in parallel. This article presents a few examples of four-electrode experiments performed with epsilon detectors in the BAS R&D labs during the past few months, using both radial-flow and cross-flow thin-layer configurations.

The epsilon™ Platform

BAS developed and introduced the first commercial electrochemical detector for liquid chromatography over twenty-five years ago. With this issue of *Current Separations*, BAS continues this tradition of innovation by introducing another new family of electrochemical instruments and chromatographs called epsilon. These instruments have been designed and built on an entirely new platform that takes into account the increasingly regulated environment in which analytical chemistry is performed, the rise of intranets and the Internet, and the need to detect ever decreasing amounts of more potent substances in complex media. PM-91e and PM-92e pumps have been developed with epsilon forming the basis of new families of LC systems including the BAS 200e, BAS 480e and BAS 500e.

Unlike every other participant on the instrument industry playing field, BAS has diversified *across* the user-manufacturer boundary. Over the past fifteen years, our contract research division, BAS Analytics, has provided analytical data of the highest quality to the world's leading pharmaceutical companies using state-of-the-art products from BAS, as well as other leading vendors. Unlike our competitors, we do not need to guess what instrumental features might be of interest to users (and what features are not of interest) since BAS scientists use BAS products on a daily basis in the most demanding applications.

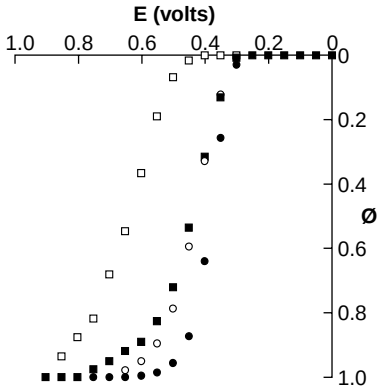
We have leveraged this combined, corporate experience to make instruments available to our external customers that truly meet the demands of modern analytical chemistry. Through optical isolation circuitry and the use of up to 24-bit resolution during data conversion, we have produced instruments that display high signal-to-noise characteristics and the ability to measure low currents in the typical laboratory environment. The epsilon instruments can be configured in 1-channel, 2-channel or 4-channel versions. These instruments are fully networkable and will be upgradable over the Internet. New techniques and features may initially be ordered *à la carte*, or added at any time when the need arises. In the coming months, we will unveil additional capabilities of the epsilon platform through our web site and through articles in *Current Separations.* In this article, we will discuss just one specific feature of the epsilon family of instruments: the ability to perform amperometric measurements using four independent working electrodes for detection in liquid chromatography experiments.

4-Electrode Hydrodynamic Voltammograms

When using an electrochemical detector in liquid chromatography (LCEC), perhaps the most important instrumental parameter controlled by the user is the potential applied between the working and reference electrode ("detector potential"). In an oxidative application, the detector

F1

A "blast from the past." Four hydrodynamic voltammograms obtained using different carbon-based working electrodes. Data were obtained over time by sequential injections. Modified from: "Choosing the Right Electrode is Important," Current Separations **1979**, *1(1), 5.*



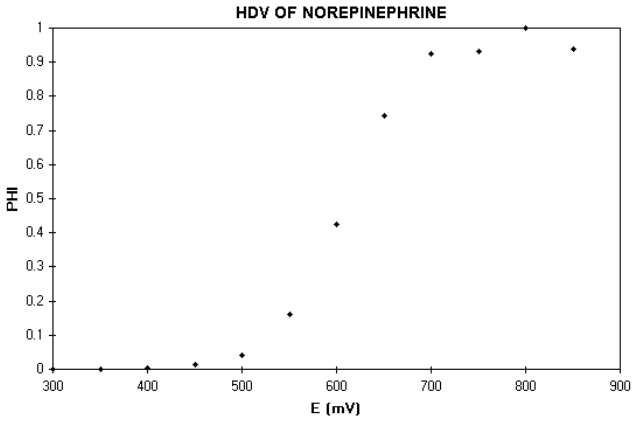
potential should be set to a value well positive of the observed half-wave potential of the analyte of interest to obtain maximum sensitivity (current per unit change in concentration).

One way to determine half-wave potentials for the selection of detector potential(s) is to perform a cyclic voltammetry (CV) experiment for each analyte of interest. In the past, this has required access to both an electrochemical instrument (for CV) and an amperometric detector (for LCEC). One unique feature of the epsilon instrument family is that cyclic voltammetry can be performed using an epsilon LC detector (and an epsilon purchased to perform CV experiments can be used for LCEC) simply by purchasing the appropriate software package from BAS.

Another way to determine the appropriate detector potential during methods development efforts is to generate a hydrodynamic voltammogram using a flow cell and an amperometric detector. This procedure involves selecting an initial detector potential, injecting a standard solution of the analyte(s), and recording the response (height or area of the chromatographic peak observed). The detector potential is then incremented to a new value, another analyte injection made, and the response again recorded. By repeating this process, a plot of detector response (peak current) versus applied potential is gradually built up.

F2

Hydrodynamic voltammogram (HDV) obtained with only 3 injections using an epsilon four-electrode system. Experimental data from Dr. Bruce Solomon. See text for details.

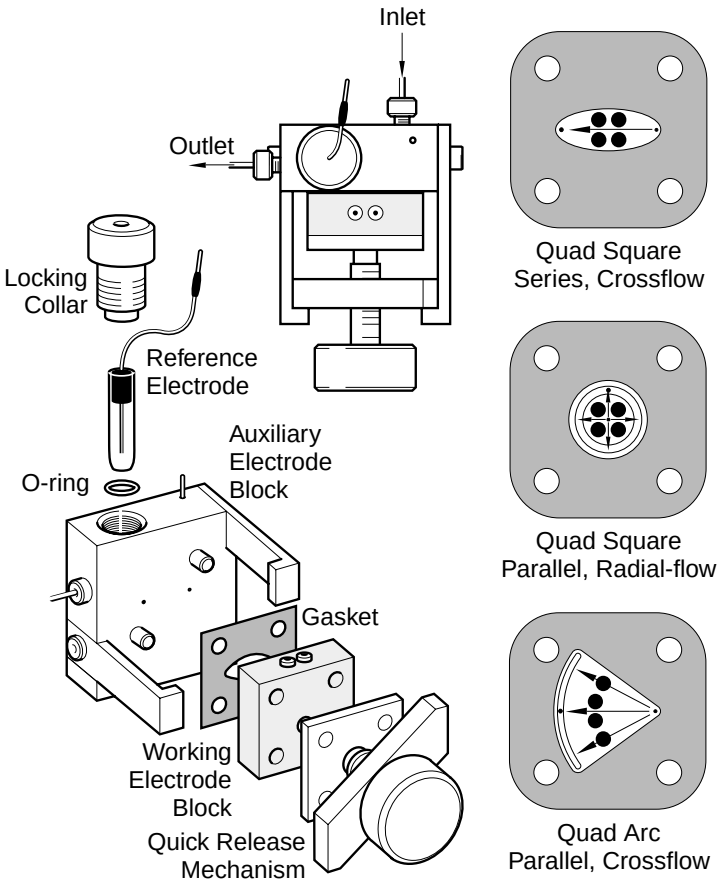


F1 is a reprint of a hydrodynamic voltammogram obtained for norepinephrine that appeared in the inaugural issue of *Current Separations* in 1979. The figure compares the response obtained using three different formulations of carbon paste and a glassy carbon electrode. The data show that the current responses recorded become maximal and (roughly) independent of the applied potential for all but one of the electrodes once a sufficiently positive potential is reached. However, as many as nineteen separate injections were made for a single electrode material, and the amount of time invested in collecting such data was considerable. Few people have the time (or patience) to do such an experiment today, when companies merge and create spin-offs seemingly as frequently as people used to publish papers in *Analytical Chemistry*!

By comparison, ***F2*** shows a hydrodynamic voltammogram for norepinephrine, generated using only three injections and an epsilon four-electrode system equipped with four

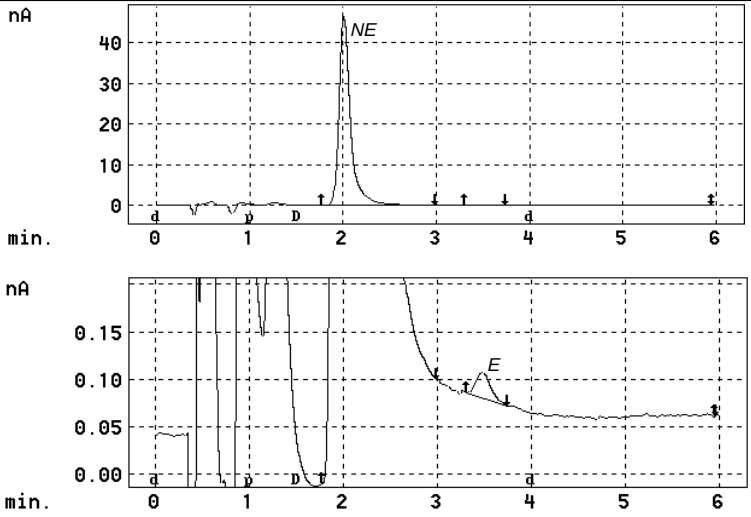
F3

Various four electrode cells used in the experiments described in this paper.



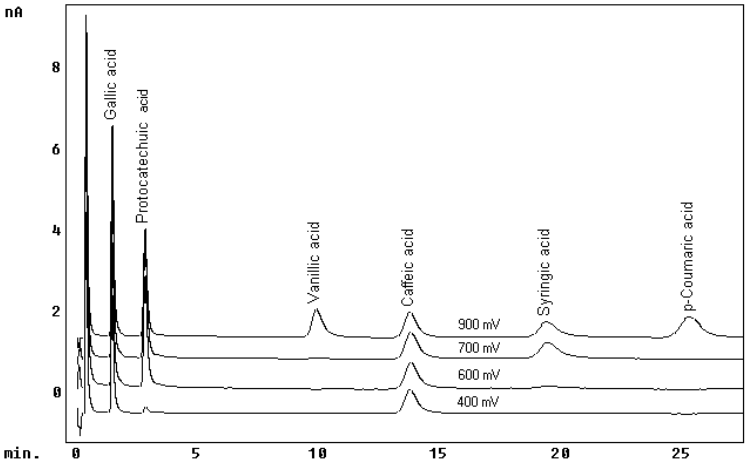
F4

Chromatogram for an injection of 5 ng norepinephrine and 5 pg epinephrine. A detector sensitivity of 100 nA full scale was employed. Top and bottom panels show the same data set displayed on two different scales. Experimental data obtained by Dr. Bruce Solomon.



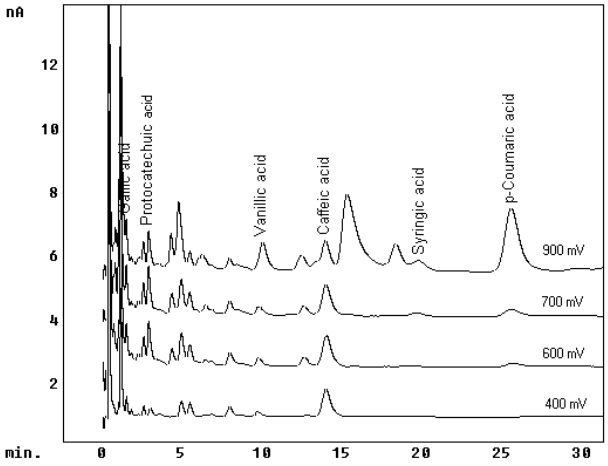
F5

Chromatogram obtained by Dr. Hong Long for a mixture of six phenolic acids.



F6

Chromatogram obtained by Dr. Hong Long for a honey extract using a four-electrode epsilon system. See text for details.



glassy carbon working electrodes in the arc configuration (see ***F3***). For the first injection, the detectors were set to 300, 350, 400, and 450 mV (vs. Ag/AgCl). The detectors were reset to 500, 550, 600, and 650 mV for the second injection, and 700, 750, 800, and 850 mV for the final injection. The electrodes were allowed to equilibrate for fifteen minutes after each change in potential. These were ten-minute chromatographic runs, so the complete HDV took only seventy-five minutes to generate, using a true (not pseudo-) reference electrode for all measurements.

Electronics Optimized for Demanding Analytical Problems

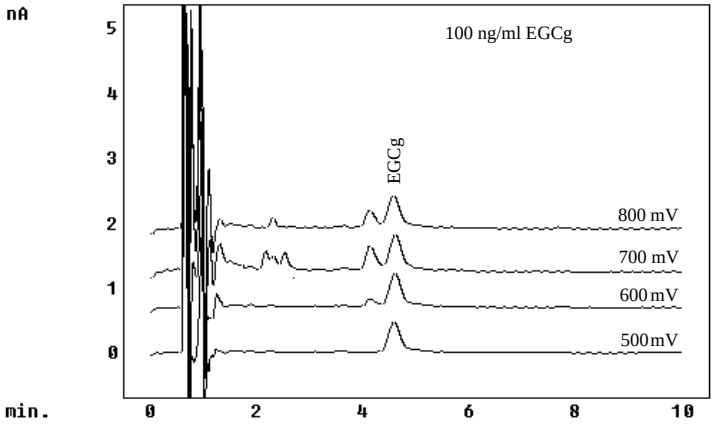
By virtue of optical isolation of the detector circuitry as well as high-resolution analog-to-digital conversion, the epsilon family of LC detectors has been optimized for the most demanding analytical situations. ***F4*** shows two chromatograms obtained for a mixture of 5 ng of norepinephrine (NE) and 5 pg of epinephrine (E). The detector sensitivity was selected to a relatively low gain setting (*viz.*, 100 nA full scale) in order to keep the peak for NE on scale. In the upper panel, the peak due to E cannot be discerned. However, by using the zoom function in the BAS epsilon ChromGraph software, the scale can be adjusted to reveal and quantitate the E peak (bottom panel). Note that it was *not* necessary to select a higher gain setting and to re-inject the sample. The data are of such high signal-to-noise quality that the display only needs to be re-scaled to examine the E peak. This sort of situation commonly arises in pharmaceutical analysis, where an active ingredient must be quantified along with an impurity.

Multi-Potential Detection: An Additional Separation Dimension

The ability to acquire chromatographic data using four parallel detectors set at different potentials provides an additional separation dimension (oxidation potential) that is orthogonal to the time axis (retention time). That is, the applied potential can be used to resolve different compounds in a mixture in a way analogous to the use of retention times to discriminate between compounds. Of course, all four working electrodes need not be constructed of the same material; thus, selectivity on the basis of kinetic and/or surface catalytic effects is also possible. For

F7

Chromatogram obtained by Dr. Yongxin Zhu showing quantitation of the anti-cancer green tea catechin EGCg in an extract of spiked rat plasma.



example, one or more of the electrodes might be coated with our "wired" peroxidase (osmium redox) polymer and compared to a bare electrode, placed either in parallel or series.

Separation of a synthetic mixture of phenolic acids is shown in ***F5***. In this case, the square array configuration of glassy carbon electrodes shown in ***F3*** was used, with a radial solution-flow pattern. The column effluent entered the flow cell in the center and then spread out through 360º over the four electrodes, which were poised at +400, 600, 700 and 900 mV vs. Ag/AgCl reference. Gallic and caffeic acids are oxidized at the electrode held at +400 mV, since these compounds have the least positive half-wave potentials. By contrast, p-coumaric acid is detected only at the electrode with the most positive (+900 mV) potential.

One of the virtues of this approach may be seen in ***F6***, which is a chromatogram obtained for an extract of a sample of honey obtained at a local supermarket. The more complex matrix gives rise to additional peaks, as well as a substantial signal near the void peak, presumably due to elution of oxidizable polar compounds. Gallic acid, the earliest analyte peak, may be easily quantified in the chromatogram for the electrode held at +400 mV, whereas later eluting compounds such as syringic and p-coumaric acids are only detected (and hence quantifiable) at electrodes held at more positive potentials. Note, however, that the gallic acid peak obtained at the electrode held at +900 mV is not resolved from other compounds eluting near the void peak. (These additional compounds are not detected at +400 mV.) Thus, there is no single detector potential that could be used to quantify, for example, both gallic and syringic acids when using these particular separation conditions (10% methanol in phosphate buffer at a flow rate of 0.7 mL/min). The four-electrode approach permits quantitation of all six phenolic acids from a single injection.

A final example is presented in ***F7***, which shows four-electrode chromatograms for the catechin compound (-)-epigallocatechin gallate (EGCg) in an extract of rat plasma. In this case, an endogenous compound present in the rat plasma with a similar retention time to EGCg complicates quantitation when a detector potential of +800 mV is used. An uncomplicated peak is observed at the electrode held at +500 mV.

EGCg is one member of a family of natural products isolated from green tea leaves shown to have remarkable anticancer properties. These compounds exhibit a range of oxidation potentials and are present in plasma extracts at differing concentrations, depending on the route of administration. This complicates selection of a detector potential before the start of a new study. Such difficulties are avoided with the four-electrode approach. The BAS R&D team has recently undertaken a major project in collaboration with researchers at Purdue University to study the bioavailability and pharmacokinetics of these unique compounds. These bioanalytical studies involve the use of both epsilon technology and our new *Culex*^{TM} automated blood sampling instrument, also discussed in this issue of *Current Separations*.