

## Note

# Electrochemical detection system for supercritical fluid chromatography

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The possibility of extending electrochemical detection to supercritical fluid chromatography (SFC) would greatly broaden the range and scope of analysis currently being carried out by this technique. Electrochemical measurements in supercritical media have, at least in principle, the potential for improving detection limits, since diffusion coefficients in supercritical fluids are approximately an order of magnitude greater than those found in liquids. This advantage until recently has been offset by the highly resistive nature of supercritical fluids. The recent development of ultramicroelectrodes with their inherently low residual ohmic effects and reduced requirement for added supporting electrolyte make them ideal candidates for use as electrodes in supercritical fluid chromatography electrochemical detection systems.

Voltammetric behaviour changes dramatically as the size of an electrode approaches submicrometer dimensions. Wightman [1] first reported on the unique properties of these electrodes which include reduced  $iR$  drop ( $i$  = current;  $R$  = resistance), increased mass transport to and from the electrode surface and a rapid response time. Many of the properties that distinguish microelectrodes from conventional sized electrodes overcome some of the difficulties presented by electrochemical measurements in a supercritical flowing stream. Supercritical mobile phases have the advantage of providing greatly increased diffusion rates to the electrode surface but have the disadvantage of being non-polar and highly resistive. Recently Bard and co-workers, using macroelectrodes, have investigated electrochemical processes in polar supercritical fluids including ammonia [2], water [3] and acetonitrile [4]. The high critical temperatures of both acetonitrile and water and the highly corrosive nature of ammonia make them inappropriate as mobile phases for SFC at this time. Supercritical carbon dioxide, a non-polar solvent, is the most commonly used mobile phase for SFC because of its moderate critical pressure and temperature. However, electrochemical measurements in supercritical carbon dioxide using conventional electrodes are difficult at best due to the non-conductive, non-polar nature of the media. Philips *et al.* [5] first demonstrated that voltammetry with surface-modified

microelectrodes is possible in near-critical carbon dioxide with small amounts of supporting electrolyte.

A two-electrode amperometric detector design using a platinum microelectrode working electrode based on the wall-jet principle has been developed. The electrochemical cell has been investigated as a detection system for SFC and the factors influencing its performance discussed.

## EXPERIMENTAL

### *Electrochemical cell*

A microelectrode made from 10- $\mu$ m diameter platinum wire sealed in narrow-bore glass capillary was obtained from BioAnalytical Systems (BAS, West Lafayette, IN, U.S.A.). The glass capillary was of narrower diameter (3.2 mm) than those commercially sold and the electrode body was filled with epoxy resin to provide mechanical support. Two centimetres of the electrode body were ground to accommodate the cell compartment and to provide a rough surface for sealing. The preliminary design employs a high-pressure stainless-steel tee-union as the electrochemical cell (Fig. 1). The inlet and outlet ports are 1/16 in. diameter with a 1/8 in. diameter third port for the working electrode. The column eluent enters the cell through a fused-silica capillary threaded into the Swagelok union positioned directly above the working electrode in a wall-jet geometry. The restrictor, placed through the side arm, is well removed from the working electrode to avoid decompression near the electrode which may cause precipitation of either the analyte or electrolyte modifier [6]. Graphite ferrules were used to seal all components. The cell body served as the quasi-reference electrode. Cyclic voltammogram were recorded with a BAS CV 27 voltammograph and an Hewlett-Packard (HP, Palo Alto, CA, U.S.A.) 7090A plotter. For the hydrodynamic studies, the potential was controlled by a BAS amperometric detector LC-4 and the current was recorded on a HP3390 integrator. The cell was housed in a BAS Faraday cage.

### *Chromatographic system*

A HP Model 5890 gas chromatograph was equipped with a microgradient system syringe pump (Applied Biosystems, Santa Clara, CA, U.S.A.) to pressurize and pump the supercritical fluid mobile phase. Samples were introduced onto the column via a Valco injection valve with a 1.0- $\mu$ l sample rotor. Separations were performed on a Rexchrom 300  $\text{\AA}$  C<sub>18</sub>, 5  $\mu$ m, 100  $\times$  2.1 mm I.D. column (Chromatographic Sciences Co., St. Laurent, Canada). A Kratos Model 773 UV-absorbance detector (Applied Biosystems) set at 250 nm was used in series with the electrochemical cell. A fused-silica integral restrictor, constructed in-house, maintained the supercritical conditions.

### *Chemicals*

Ferrocene and tetrabutylammonium tetrafluoroborate (Aldrich, WI, U.S.A.) were used as received. The acetonitrile and tetrabutylammonium tetrafluoroborate-acetonitrile-doped supercritical carbon dioxide were obtained from Scott Specialty Gases (Plumsteadville, PA, U.S.A.). The solubility of the salt supercritical carbon dioxide-acetonitrile mixture was verified before shipping [7].

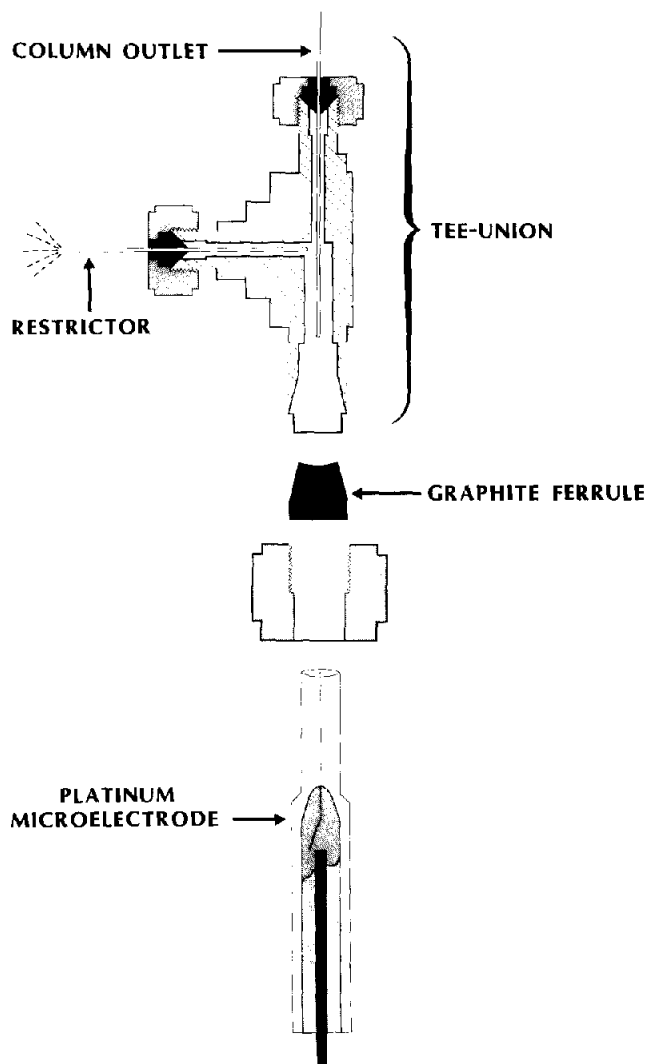


Fig. 1. High-pressure electrochemical cell with a platinum microdisc electrode.

## RESULTS AND DISCUSSION

Operation of an electrochemical detector in a flowing stream of supercritical media places strict requirements on both the electrode system and the cell. The electrochemical cell must be placed before fluid decompression in order to make measurements while the mobile phase is in the supercritical state. This means that under conventional SFC operating conditions the detector must withstand pressures up to 5000 p.s.i.

The current response of amperometric detectors employing conventional electrodes in laminar flow streams is proportional to  $1/3$  power of the flow-rate [8]. In

liquid chromatographic systems where analyses are conducted under conditions of constant flow, this does not present a problem. However in SFC, pressure or density are controlled as opposed to flow; this results in varying flow-rates throughout the chromatographic run. This precludes the use of conventional-sized electrodes in SFC detectors. The small diffusion layer at a microelectrode is predominantly within the stagnant layer next to the electrode surface which eliminates current flow-rate dependencies.

Voltammetric data obtained with a two-electrode system in resistive media cannot be directly compared to that obtained in conventional solutions with a three-electrode system. For this reason initial cell characterizations were carried out using ferrocene since (i) this redox couple has been widely used in the characterization of electrochemical systems, thus providing means for comparison and (ii) ferrocene is soluble in supercritical carbon dioxide [9]. Cyclic voltammograms of ferrocene in acetonitrile and supporting electrolyte were carried out using the 10- $\mu\text{m}$  platinum working electrode in a conventional three-electrode mode. A sigmoidal-shaped cyclic voltammogram with a half-wave potential of 0.42 V was obtained. This established the performance of the electrode under normal operating conditions. The platinum working electrode was then incorporated into the flow cell, the flow cell was then filled with the same ferrocene-acetonitrile-supporting electrolyte mixture and a cyclic voltammogram carried out under static conditions using a two-electrode mode, where the cell body is used as a quasi-reference electrode. This established the performance of the working electrode in the cell operating in the two-electrode mode. The cyclic voltammogram has retained its characteristic sigmoidal shape but the half-wave potential has shifted negatively by 140 mV. This is due to the lack of a true reference electrode. The electrode performance was then evaluated under more realistic operating conditions though still in the static mode. A solution of ferrocene-acetonitrile-supporting electrolyte (50  $\mu\text{l}$ ) was placed in the cell, the system then sealed and pressurised with carbon dioxide from a syringe pump to 2000 p.s.i. at 40°C. Cyclic voltammograms were recorded with the cell inverted such that measurements were obtained in the supercritical fluid as opposed to in undissolved acetonitrile-supporting electrolyte mixture. Again the sigmoidal shape of the cyclic voltammogram is maintained but the half-wave potential shifted more negatively by about 220 mV (Fig. 2), again due to the lack of a true reference electrode. This established the performance of the detector using carbon dioxide-acetonitrile-supporting electrolyte mixtures under supercritical but static conditions.

The next evaluation was to establish its performance in a flowing stream under normal operating conditions. Due to interference from injection solvents and turbulence generated by the injection process itself it was necessary to develop a chromatographic procedure so that the ferrocene could be separated from these void volume effects. Separations were achieved on a reversed-phase column at 40°C using a variety of mobile phases from simple carbon dioxide to mixtures of carbon dioxide-acetonitrile-tetrabutylammonium tetrafluoroborate. An ultraviolet absorbance detector was positioned before the electrochemical detector, to monitor the retention time of the ferrocene. Ferrocene-acetonitrile solutions were then injected onto the column and the detector response monitored under a variety of operating conditions. No faradaic response was obtained from the electrochemical detector at applied potentials up to 1.2 V with simple carbon dioxide mobile phases. Acetonitrile

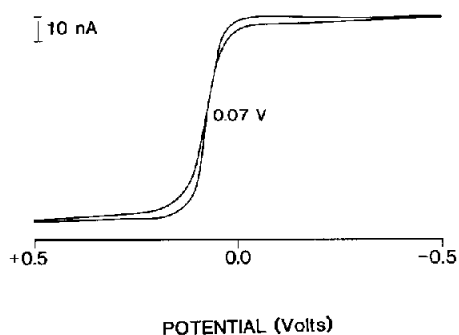


Fig. 2. Cyclic voltammogram for 5.0 mM solution of ferrocene in acetonitrile with 0.1 M tetrabutylammonium tetrafluoroborate. Working electrode: platinum microelectrode. Scan rate: 100 mV/s. Electrochemical cell, stainless-steel quasi-reference electrode, pressurized at 2000 p.s.i. with  $\text{CO}_2$  at  $40^\circ\text{C}$ .

modifier was then added to the carbon dioxide up to 1.6 mole% at 0.4-mole% intervals. Again no faradaic response was detected at applied potentials ranging from 0.0 to 1.2 V. Tetrabutylammonium tetrafluoroborate (TBATFB) at 0.05 mole% was then added to the acetonitrile-modified supercritical carbon dioxide and a faradaic response was observed from the electrochemical detector. Fig. 3 represents a chromatogram obtained for a 10- $\mu\text{g}$  on-column injection of ferrocene in acetonitrile using a 1.6 mole% acetonitrile and 0.05 mole% TBATFB modified supercritical carbon dioxide mobile phase under isobaric conditions at 2200 p.s.i. and  $40^\circ\text{C}$ . The addition of the electrolyte has either increased the conductivity of the mobile phase or, as predicted by Nichaus *et al.* [10], the electrolyte has formed a conducting layer on the electrode surfaces. The latter is unlikely as *in situ* formation of a conducting layer would tend to give irreproducible current responses, at least initially, and this was not observed. The high solubility of TBATFB in acetonitrile and the low concentration of the electrolyte in the mobile phase also precludes the precipitation of the salt at the electrode surface.

Formal redox potential tables or potentials obtained from cyclic voltammograms obtained under static conditions are insufficient for determining the optimal operating potential for detecting the same species in a flowing stream. This is due to the increased  $iR$  drop in these systems. The optimum operating potential for the detection

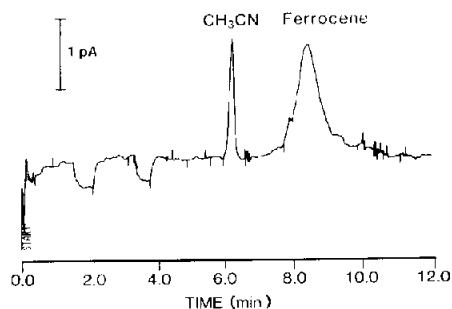


Fig. 3. Response of electrochemical detector for 10  $\mu\text{g}$  of ferrocene injected on column. Applied potential set at 0.8 V. For chromatographic conditions refer to text.

of ferrocene was established by injecting 10  $\mu\text{g}$  of ferrocene on column and measuring the current response at applied potentials between 0.0 and 1.1 V. The reduced capacitive currents at microelectrodes lead to rapid equilibration after changes in the applied potential. Unlike liquid chromatographic systems, chromatograms were recorded within 15 min of a change in applied potential. A hydrodynamic voltammogram plotted for ferrocene in this system shows an optimal applied potential of 0.9 V (Fig. 4). Beyond this potential there is a drop in the current response that may be due to increased  $iR$  drop which produces a reduction in the effective applied potential. The relative standard deviation calculated for three replicate injections at each applied potential is less than 5% indicating good reproducibility for this electrochemical detection system. Although it is necessary to add an electrolyte to the mobile phase for detection, anticipated problems of precipitation of the tetraalkylammonium salt at the restrictor were not encountered.

The results reported above have shown the feasibility of developing an electrochemical detection strategy for SFC. According to two recent reports [11,12], voltammetry in supercritical carbon dioxide is possible only with a thin film of conducting phase, either in the form of an ion-exchange polymer or a molten salt layer, on both the surfaces of the working and quasi-reference electrodes. We have demonstrated that this conducting phase is not necessary and that electroactive species can be detected at a bare platinum microdisc working electrode of an SFC-electrochemical detection system.

#### ACKNOWLEDGEMENT

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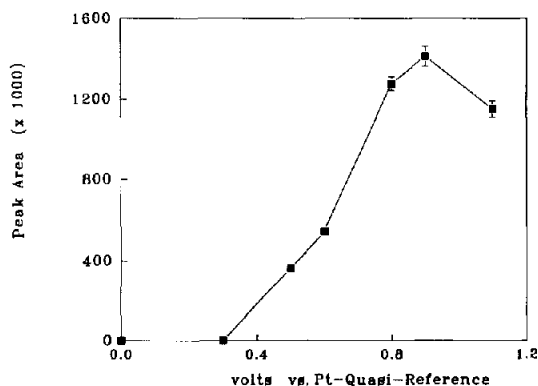


Fig. 4. Hydrodynamic voltammogram for 10  $\mu\text{g}$  of ferrocene injected on column. Refer to text for operating conditions.

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