Use of a New Interface Cell for Off-Column CE-EC Determination of Catecholamine Neurotransmitters

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Analytical methods with high sensitivity and high selectivity for the determination of neurotransmitters continue to be important in cases such as monitoring the pharmacodynamics of neuroactive compounds. The basal extracellular levels of some neurotransmitters and their metabolites are low. Since microdialysis is widely used to obtain samples of brain extracellular fluid, the sample volume available for analysis may be only a few microliters. The small injection volume requirements of capillary electrophoresis (CE) coupled with the limits of detection offered by electrochemical detection (EC) make CE-EC a powerful tool for analysis of neurotransmitters in microdialysis samples. Here we evaluate a new prototype CE-EC interface design that uses standard BAS electrodes and a bare fracture decoupler.

Development of amperometric detection systems for liquid chromatography (LC) has been one of the most active areas of electroanalytical research in the past two decades (1). Even more recently, electrochemical (EC) detection has become a popular technique for use with capillary electrophoresis (CE) separations (2-17). It offers high sensitivity and good selectivity for a wide range of electroactive analytes. The more widely used and accepted UV absorption technique suffers from lower sensitivity due to the very short pathlength across the capillary. Laser-induced fluorescence detection offers sensitivity comparable to electrochemistry, however it can be very expensive to implement and is limited in terms of the applicable analytes.

The main disadvantage of electrochemical detection is the lack of a commercially available detection cell. Researchers in this area use laboratory-made cells usually with specially designed micro-wire electrodes and micro-positioners for capillary/electrode alignment (4-9). Susan Lunte and coworkers have described several integrated capillary mounted electrode systems for CE-EC analysis (10-14). Matysik, et al., have also described the use of a microband electrode array for end-column detection in non-aqueous CE (15). However for routine analysis, these types of systems can be very tedious and time consuming to use. Michael and coworkers have very recently reported the use of a new CE-EC detection cell with a hard mounted micro-positioner to improve sturdiness and transparent cell for ease in electrode positioning (16). There are also many different electrochemical flow cells designed for LC analysis, however relatively large dead volumes and low electroosmotic based flow rates render them useless for CE. Kok and coworkers have reported the use of a BAS UniJet cell, designed for microbore column LC, for CE-EC detection (17). Their system included a custom made 1-mm glassy carbon electrode and a palladium decoupler for off-column detection.

In this study, we report the use of a new prototype CE-EC interface and detection cell that uses standard BAS electrodes and a bare fracture decoupler. Discussion will include comparison of electrode materials for the detection of catecholamine neurotransmitters.

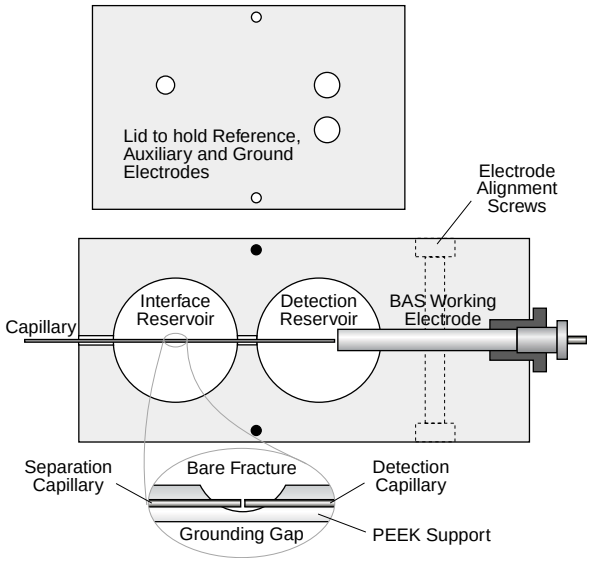
Experimental Procedures

Reagents and Solutions

The following chemicals were reagent grade or higher and used as received from Sigma Chemical Co. (St. Louis, MO): sodium borate decahydrate, catechol, serotonin (5HT), 3-methoxytyramine (3MT), dopamine (DA), norepinephrine (NE), and epinephrine (E). All water used for solutions was purified on a NANOpure system (Barnsted, Boston, MA). Borate buffer (35 mM, pH 9.3 or 9.5), made weekly by dissolving the sodium borate salt in water and adjusting pH if necessary, was used for both CE run buffer and EC electrolyte. Perchloric acid (70%, Aldrich, Milwaukee WI) was diluted to 0.1 M and used for making concentrated (~1.0 mM) catecholamine analyte solutions. Lower concentra-

F1

Schematic of New CE-EC Interface and Detection Cell.



T1

Working electrode comparison data.

Electrode	i_{bkgd} (nA)	Eq. Time (min)
3.0 mm GCE	55-60	60
1.6 mm PTE	5	15-30
1.0 mm GCE	2-3	<10

tion analyte solutions were made by pipetting a known amount of the 1.0 mM solution and diluting with water, 0.1 M $HClO_4$, or lactated Ringer's solution (USP, Baxter, Deerfield, IL). HPCE grade sodium hydroxide (0.1 M, Fluka, Milwaukee WI) was syringe-filtered through 0.2 µm nylon filters and used for capillary conditioning.

CE-EC Analysis

The CE separation system consisted of a CZE1000R high voltage supply (Spellman, Plainview, NY), a laboratory-built pressure injection system set to 10 psi, and a Plexiglas isolation box, which housed the high voltage anode reservoir. A new electrochemical detection cell (***F1***) was used for all experiments with a separation capillary (50 µm ID × 360 µm OD × 65 cm, Polymicro Technologies, Phoenix AZ) mounted with hot glue into the cell's PEEK tubing support. After mounting, a bare fracture was made 2.5 cm from the end of the capillary by scoring the polyimide coating and carefully manipulating the capillary until a crack formed.

Capillary conditioning was carried out using a BAS syringe pump (MD-1000 & MD-1001) and 1.0 mL syringe (MD-0100) set at 20 µL/min. Newly installed capillaries were initially flushed with NaOH for 30 min followed by water and run buffer for 10 min each. On subsequent days, capillaries were flushed 10 min each with NaOH, water, and run buffer. After capillary flushing, the cell reservoirs were rinsed with water and the working electrode was installed through the detection end to form a capillary wall jet configuration.

When necessary, electrode-to-capillary centering adjustments were made using the four alignment screws built into the detection cell. The cell reservoirs were filled with borate buffer and the voltage drop across the 2.5 cm detection capillary was measured with a voltmeter while 20 kV were applied across the separation capillary. With the bare fracture technique used, this voltage drop typically measured less than 1 V. If the voltage was more then 5 V the capillary fracture was further manipulated until a lower value was obtained. After testing the detection capillary, the lid was placed on the cell and the Pt grounding, Ag/AgCl reference (MF-2078), and Pt auxiliary (MW-1032) electrodes were put into their places through the lid. Amperometric detection was carried out at +0.8 V using a BAS LC-4CE with data collection and analysis via BAS DA-5 ChromGraph computer interface.

Three different working electrodes were compared. Two were standard BAS voltammetry electrodes: a 1.6 mm diameter platinum (PTE, MF-2013) and a 3.0 mm diameter glassy carbon (GCE, MF-2012) electrode; and the third was a BAS custom ordered 1 mm diameter GCE. Working electrodes were cleaned each day according to BAS instructions.

Results and Discussion

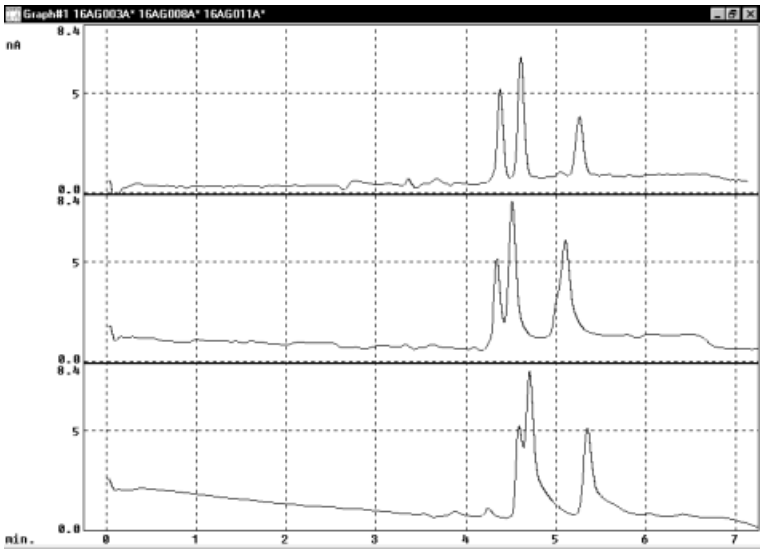
Electrochemical optimization of the new detection cell, which consisted mainly of electrode positioning, was carried out using injections of 10µM catechol solutions. Optimized signal was checked as the analyte peaks migrated to the electrode surface and adjustment in position was made as necessary. Because of the large surface area compared to capillary outlet size, the use of the standard BAS electrodes resulted in very few adjustments. Capillary centering and optimization was necessary every time the system was assembled using the 1.0 mm GCE.

There was very little difference in the determination of catechol using the three different electrode systems. The major differences in the electrodes consisted of their background electrochemical responses and equilibration times. As can be seen in ***T1***, the 3.0 mm GCE required the longest equilibration time (1 hr) and gave the largest background current (in the 55-65 nA range). This required the use of a 50-60 nA manual offset to bring background currents down to levels equivalent to those seen at the 1.6 mm PTE (*viz.*, in the 5 nA range). The 1.0 mm GCE yielded the best overall results with background currents in the 2-3 nA range and equilibration achieved in less then 10 minutes.

Separations of 1.5 s injections of catecholamine mixtures were carried out in pH 9.5 borate buffer at a separation voltage of 25 kV. Comparison of electropherograms using the three different electrode systems (***F2***) shows a third major difference

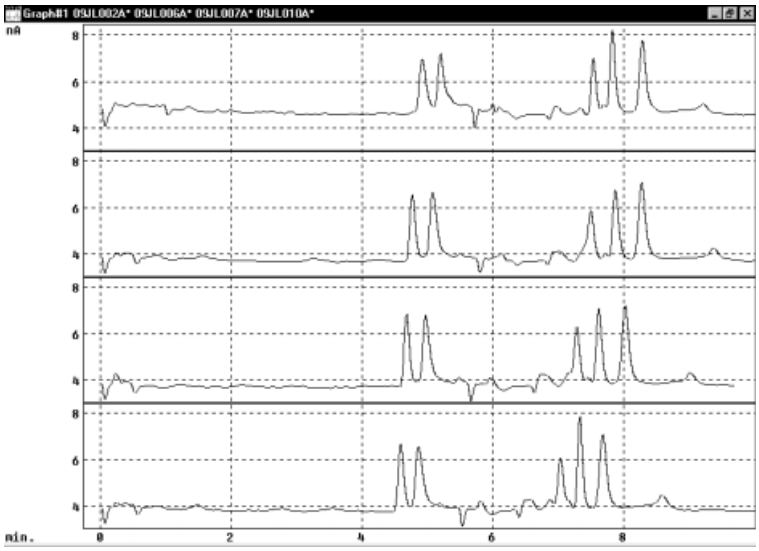
F2

Typical electropherograms of 1.5 s injections of a catecholamine mixture (10 µM DA, 20 µM E, and 10 µM NE) at three different working electrode systems. Top to bottom: 1.0 mm GCE, 1.6 mm PTE, and 3.0 mm GCE.



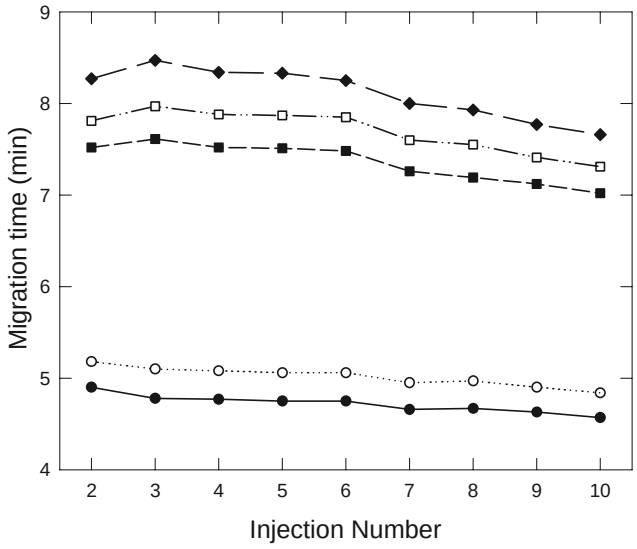
F3

Representative electropherograms of 1.5 s injections of a 10 µM neurotransmitter mixture (5HT, 3MT, DA, E, and NE) showing effects of consecutive injections. Injection numbers from top to bottom: 2, 6, 7, and 10.



F4

Migration time plot of nine consecutive 1.5 s injections of a 10 µM neurotransmitter mixture. ● Peak 1: 5HT, ❍ Peak 2: 3MT, ■ Peak 3: DA, ❑ Peak 4: E, and ♦ Peak 5: NE.



among the electrodes and a problem with using wall-jet off-capillary detection with CE. Ignoring the differences in resolution in the electropherograms, which are more likely due to CE conditions than the electrodes, the notable difference is the peak tailing. This is especially evident with use of the larger electrodes and most likely results from diffusion of the analytes across the electrode surface after exiting the capillary. Analytical parameters for serial dilutions of DA with lactated Ringer's solution were determined using the 1.0 mm GCE. A linear range was found between the measured concentrations of 50 nM and 100 µM and the detection limit was found to be 50 nM (S/N = 2) using a 3.0 s injection.

Experiments were also carried out to determine the effects of consecutive injections without capillary reconditioning. This is important for possible future use of this system for online analysis of microdialysis samples. By not having to dismantle the online system or switch to conditioning chemicals, we can avoid the interruption time between samples and thus reduce overall analysis time. This would also allow better applicability of the online system for monitoring pharmacokinetic or pharmacodynamic profiles. Representative electropherograms (***F3***) and a migration time plot (***F4***) from this consecutive injection study show shifting migration times in the later injections. Any number of factors that affect the electro-osmotic flow could cause this trend. The important thing to note is that the resolution does not appear to be affected over this number of injections. These data suggest that at least five and possibly up to ten consecutive injections may be made without reconditioning the capillary. However, this experiment should be repeated with an online system to provide a definitive test.

Conclusion

Separation and analysis of catecholamine mixtures was carried using a new CE-EC interface and detection cell. Standard BAS PTE and GCE showed good electrochemical response in the off-capillary wall-jet configuration. However, their longer equilibration times and larger background currents were limitations. The use of the 1.0 mm GCE gave the best overall analytical response with equilibration in less than 10 minutes, background currents in the 2-3 nA range, and much lower occurrence of peak tailing. Limits of detection for DA are shown to be 50 nM with the use of 1.0 mm GCE. Test of system stability showed that at least 5 consecutive injections could be made on the system without capillary reconditioning. Work is continuing toward improvement in detection limits and use of the detection cell for analysis of other neurotransmitter standard mixtures and rat brain microdialysis samples. Future plans also include use of the cell with an online microdialysis sampling system.

Acknowledgements

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