Enhanced Selectivity with Series Dual Electrodes

Catecholamines in Brain and Urine

The major advantage of LCEC with series dual electrode detection is the enhanced selectivity it provides. The electrodes are arranged so eluent passes first over one electrode and then the second. The products of the upstream reaction may be detected at the downstream electrode. Since a reversible chemical reaction is required, added selectivity is imposed by the system. For example, if the outputs of both electrodes are monitored, a response for each electrochemically active species is observed upstream; however, only the solutes which are "reversible" give an appreciable response downstream. The downstream chromatogram is therefore significantly less complicated. It should be noted that reactions which appear electrochemically irreversible on slowly-scanned voltammograms may still give an appreciable response downstream. Uric acid can be used to illustrate this point[1]. The EC mechanism for uric acid involves electrochemical oxidation followed by hydrolysis yielding products which are not electrochemically active. However, on the LC time scale we can still detect the product of the electrochemical oxidation, since the transit time between electrodes is only a few milliseconds. The collection efficiency, defined as current downstream divided by current upstream, can be used to assess peak purity and differentiate between electroactive functional groups.

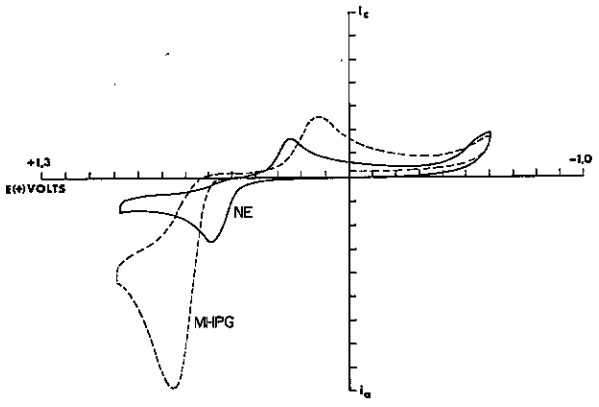

Figure 1. Cyclic voltammogram of NE and MHPG. Conditions: 1 x 10^{-3} M solutions (run separately) in 8% CH_3CN/92% 0.15 M monochloroacetate buffer, pH 3.0 with 0.7 mM EDTA and 0.86 mM sodium octyl sulfate; glassy carbon electrode; scan rate 100 mV sec^{-1}, current sensitivity 50 µA per vertical scale division.

Conditions

Liquid Chromatograph: Bioanalytical Systems Inc., LC-304, BAS 400, or BAS 200 with dual glassy carbon working electrodes.
Mobile Phase: 8% acetonitrile/92% 0.15 M monochloroacetate buffer, pH 3.0 with 0.86 mM sodium octyl sulfate and 0.7 mM EDTA.
Flow Rate: 1.6 mL/min
Stationary Phase: Biophase ODS 5 µm (p/n MF6017) 250 mm x 4.6 mm
Temperature: Ambient
Detector Potential: W_1 at +850 mV, W_2 at -150 mV (vs. Ag/AgCl/3 M NaCl)
Detector Sensitivity: 10 or 20 nA fs.
Volume injected: 100 µL.

Results

The use of series dual electrodes was useful for eliminating an electrochemical interference when determining seven neurochemicals in brain tissue[2]. Theoretically, quantitation should be possible at either electrode. However, when comparing the concentrations calculated at each electrode, discrepancies appeared in some of the norepinephreine (NE) concentrations, while those of 3,4- dihydroxyphenylacetic acid (DOPAC) and dopamine (DA) agreed satisfactorily (T1). Whenever the NE concentrations did not coincide, the collection efficiency for NE was too low, indicating a less easily oxidized interference at the upstream electrode. This, along with very slight differences in the retention time for the NE "peak" at each electrode, prompted further investigation of the interference, which was eventually identified as 3-methoxy-4-hydroxyphenylglycol

Table 1.

CONCENTRATION
(ng/mL)

	NE		DOPAC		DA	
SAMPLE	850 Mv	-150 mV	850 mV	-150 mV	850 mV	-150 mV
1	42	7.7	47	46	308	302
2	27	*	38	38	252	249
3	39	*	34	34	221	224
4	39	38	*	*	12	*
5	10	*	*	*	*	*
6	30	*	34	32	180	176
7	27	28	*	*	*	*
8	31	33	*	*	7.2	*
9	42	37	*	*	6.8	*
10	34	*	28.9	27	213	202

*The peak height fell below 1% of full scale (20 nA)

(MHPG). In certain brain homogenates, both NE and its metabolite MHPG are present. Both may be oxidized at +850 mV, as discerned by cyclic voltammetry (F1). However, the reverse reaction for NE occurs to a greater extent than that for MHPG (better collection efficiency). Using this electrode configuration with the upstream electrode operated at +850 mV and the downstream at -150 mV, both NE and MHPG are detected upstream, and primarily NE is detected downstream. The upstream electrode is responsive to all electroactive solutes while the downstream electrode excludes all but catechols (F2). The instrument in essence has provided a chromatogram equivalent to that obtained by an off-line aluminum oxide extraction cleanup step. The void volume response is subdued as well. An antioxidant such as ascorbate would be eliminated in the downstream chromatogram since it oxidizes irreversibly according to an EC mechanism.

The highly selective mode of detection lends itself to less involved isolation steps prior to the instrumental analysis. The technique has its limits, however. For example, this approach could be exploited in the determination of urinary catecholamines. Presently, samples are passed through a cation exchange resin to isolate the amines followed by adsorption of the

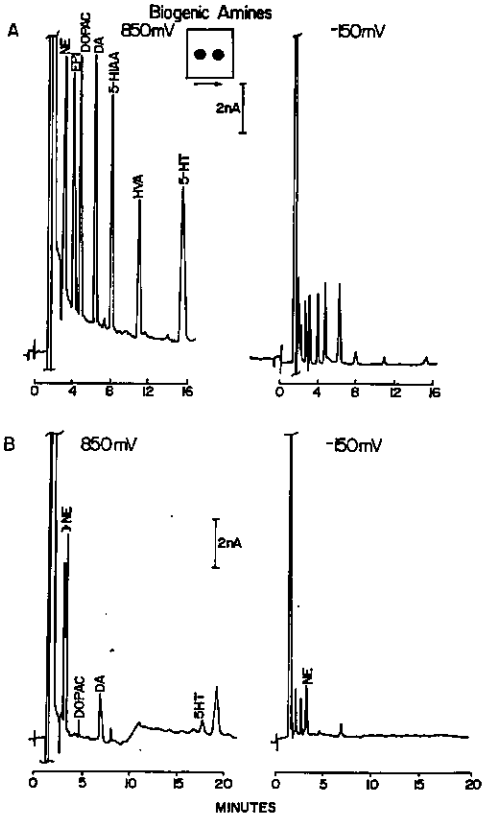

Figure 2. Chromatogram of (A) biogenic amine standard, 4 to 7 ng of each compound, and (B) brain tissue homogenate illustrating the coelution of MHPG and NE.

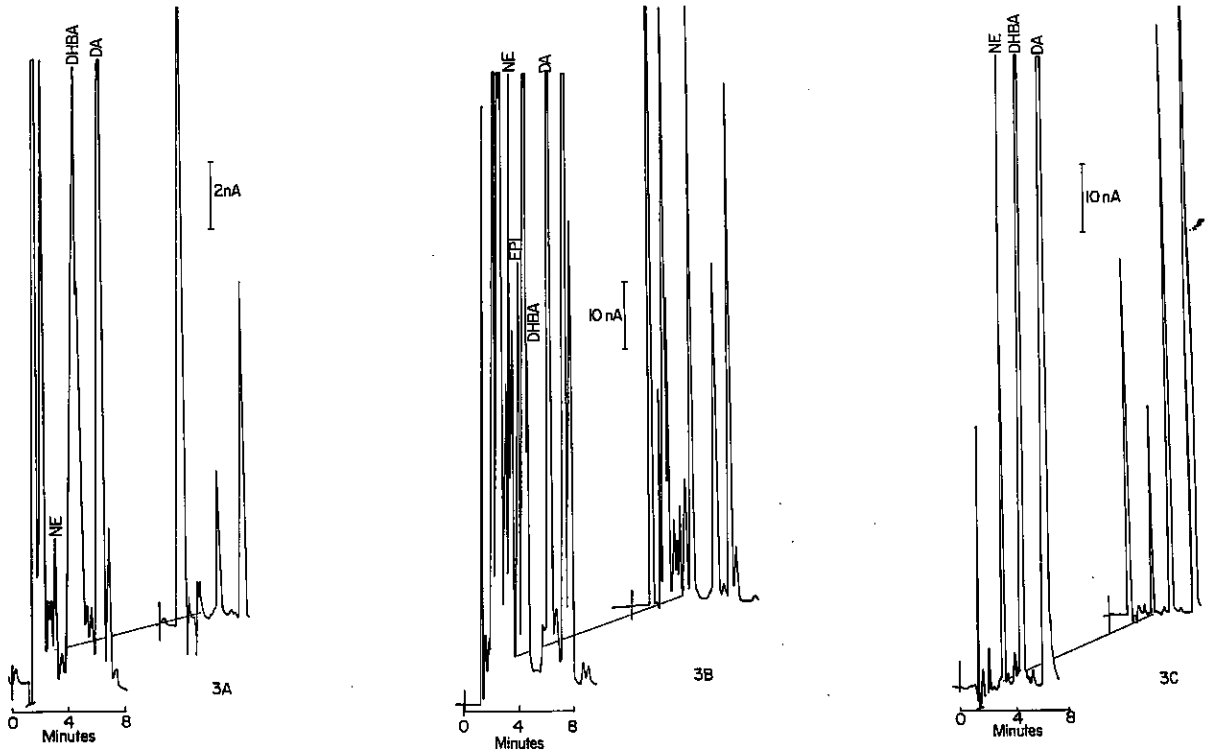

Figure 3. Dual series electrode chromatogram of urine with (A) ion exchange only, (B) aluminum oxide only, and (C) both aluminum oxide and ion exchange.

catechols onto aluminum oxide. Finally, the catecholamines are desorbed in a small volume of acid and injected onto the analytical column. To determine if one of these steps could be eliminated, urine samples were subjected to only the cation exchange resin (F3A), aluminum oxide (F3B), or both steps sequentially (F3C) to isolate the catechols. Each extract was then chromatographed. Due to the large number of oxidizable urine components, the selectivity of the series configuration could not replace the sample clean-up steps.

In summary, the series dual electrode configuration permits the exclusion of electrochemically irreversible interferences, the further assessment of LCEC peak purity, and differentiation between various electroactive functional groups.

References

1. R. E. Shoup, Current Separations, 4(1982) 52.

2. G. S. Mayer and R. E. Shoup, J. Chromatogr., 255(1983) 533-544.