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Note

Increased signal-to-noise ratio through grounding and the proper selection of a pump for high-performance liquid chromatography with electrochemical detection

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In recent years, high-performance liquid chromatography with electrochemical detection (HPLC–ElCD) has made a significant impact on trace analysis in various research applications. By far, however, its most elegant and extensive use has been in the detection and quantitation of tissue biogenic amines and their metabolites[1–3].

As with any analytical method, there has been a continuing search for a "limit of detection". Of prime importance in the determination of this sensitivity is the signal-to-noise (S/N) ratio. In the case of HPLC–ElCD, noise can result from power line surges, electrical interference from the use of other equipment in proximity, poorly packed electrochemical cells, inadequate grounding, mobile-phase flow-fluctuation (pump noise), incompatible mobile phase selection, and many more. Most of these sources of noise can be easily minimized or eliminated.

In our laboratory, we have found that elimination of ground loops, using a scheme of serial grounding of equipment with braided grounding cable (No. 12 AWG), has increased our S/N ratio by a factor of two. Fig. 1 shows a schematic of the grounding configuration for dual-system HPLC–ElCD.

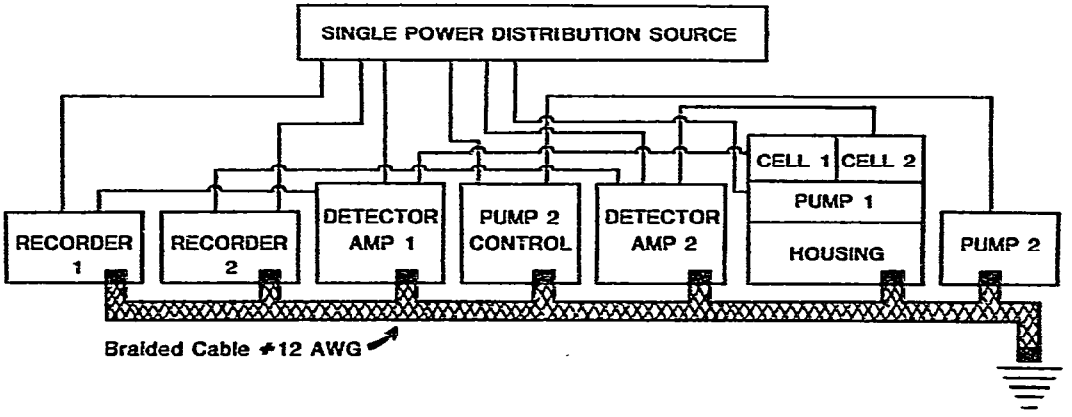

Fig. 1. Schematic of serial grounding with braided cable for dual-system HPLC–ElCD (Bioanalytical Systems, West Lafayette, IN, U.S.A.).

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The connection of braided cable to each component as well as the connection to a ground source must be bonded[4] contact. In our system the ground source is a copper pipe. Special care should be taken to make sure that the ground source chosen is truly grounded. For instance, copper water pipes in many newly constructed buildings are sometimes interrupted with plastic piping and may not be a suitable ground source. We advise that you contact the respective department in your building, such as physical facilities, and specify that you require a ground source with an impedance-to-ground as low as possible. All other ground connections are eliminated, including the ground connection normally supplied to the electrochemical cell from its corresponding detector–amplifier. In addition, the HPLC columns are grounded to the housing, and power for the entire system is supplied by a single source. Short of building a Faraday cage, this configuration affords an inexpensive and easy method for the elimination of most extraneous electrical noise.

Another major source of noise in HPLC–ElCD originates from the pump. A simplified equation for current detection in HPLC–ElCD is shown below:

$$i_d = \frac{-n \cdot F \cdot A \cdot J}{\delta}$$

where i_d = detected current; n = number of electrons involved in the electrolysis per mole of material; F = Faraday's constant; A = surface area of the working electrode; J = diffusion flux; δ = thickness of diffusion layer.

The product of the expression $n \cdot F \cdot A$ is a constant for a given chemical. The diffusion layer is the region where molecules diffuse to the electrode surface and is flow dependent. Flow surges result in changes of the thickness of the diffusion layer (δ), which then indirectly cause noise in the detected current (i_d). The need for a totally pulsationless constant-flow pump is apparent.

In our examination of various expensive and elaborate flow-feedback dual-piston reciprocating pumps, we found that at high sensitivity (0.1 nA/V, 1 V full scale) we could still see flow-surge contributions to baseline noise. We chose, therefore, to utilize a relatively inexpensive syringe pump manufactured by Instrumentation Specialties, Lincoln, NE, U.S.A.). This pump supplies truly constant flow to our HPLC-ElCD and has virtually eliminated pump noise. As a result, we have again increased our S/N ratio by a factor of two, thereby affording higher sensitivity.

HPLC–ElCD is by far the state of the art in biogenic amine trace analysis. It is unsurpassed in sensitivity and selectivity. With our HPLC-ElCD system (Bioanalytical Systems) and the modifications mentioned in this paper, we have been able routinely to measure < 50 fmole quantities of monoamine neurotransmitters in biological samples with an S/N ratio ≥ 4.

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