CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Preparation And Use Of Mercury Film Electrodes

Chemical interactions of an analyte with an electrode material can alter the electrochemical characteristics of the system. In the case of a mercury film electrode, this phenomenon may be utilized to provide a unique type of electrochemical detection. The oxidation potential of mercury undergoes a shift from +0.3 - 0.4 volt to +0.0 - 0.1 volt in the presence of thiols, chelating agents, halides and similar ions. These species can therefore be quantitated in an LC eluent with amperometric detection at +0.0 - 0.1 volt vs Ag/AgCl. The method is extremely selective, and the low operating potential required for the determination offers a major advantage in sensitivity, as background oxidations are minimized.

The BAS mercury film electrode is prepared by depositing a thin film of mercury onto a gold substrate. The resulting amalgam requires rather different handling procedures than solid metal or carbon electrodes. This capsule gives you practical information on the preparation and use of this electrode.

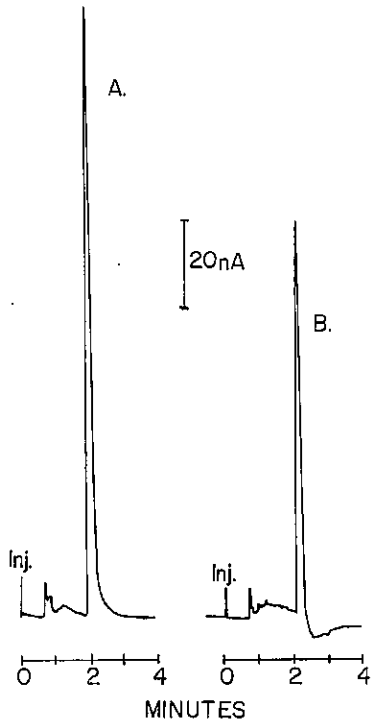

Figure 1. Chromatograms of A) correctly operating and B) incorrectly operating Hg/Au electrode. Peak represents 200 ng thiosalicyclic acid. Chromatographic conditions as in F4 at a potential of +0.1 V.

Preparation Of The Mercury Film

The mercury film is prepared according to the instructions in the BAS 200 Reference Manual or the LC-4B Operations Manual. Briefly, to the clean, dry, polished gold surface is applied a drop of mercury sufficient to cover the entire electrode surface. After 2-3 minutes, the excess mercury is removed with the side of a Pasteur pipette tip or the edge of a computer card into an appropriate waste beaker. Next, smooth the film by polishing it on a dry "kitten-ear" polishing pad as supplied by BAS. The electrode may then be installed using 2 0.005" thick Teflon (MF-1047) gaskets as the spacer. F1 describes the results which are obtained when the mercury film is too close to the auxiliary electrode block. The second gasket reduces this possibility.

There is a period of equilibration which will follow application of mercury to the gold surface. During this time, the electrode response is changing (F2), and it is not suitable for use. It is recommended that the surface be allowed to stabilize overnight prior to analytical determinations. It is not necessary to install the cell on the system for this process to occur. After overnight equilibration of the surface, a newly installed cell will require approximately 2-3 hours of "warm-up" time. This stabilization time is also necessary after an overnight shut-down of the system. For optimum performance, the detector should remain on continuously; the mobile phase may be recycled when injections are not being made. Steady-state background currents should be less than 5 nanoamperes.

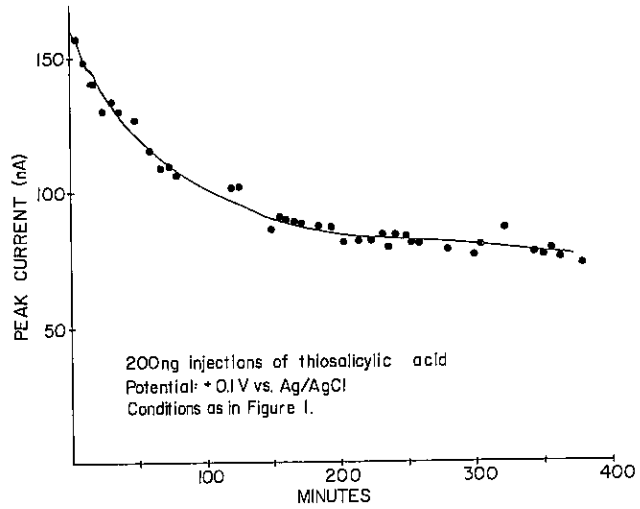

Figure 2. Hg/Au electrode response during equilibration of Hg/Au surface.

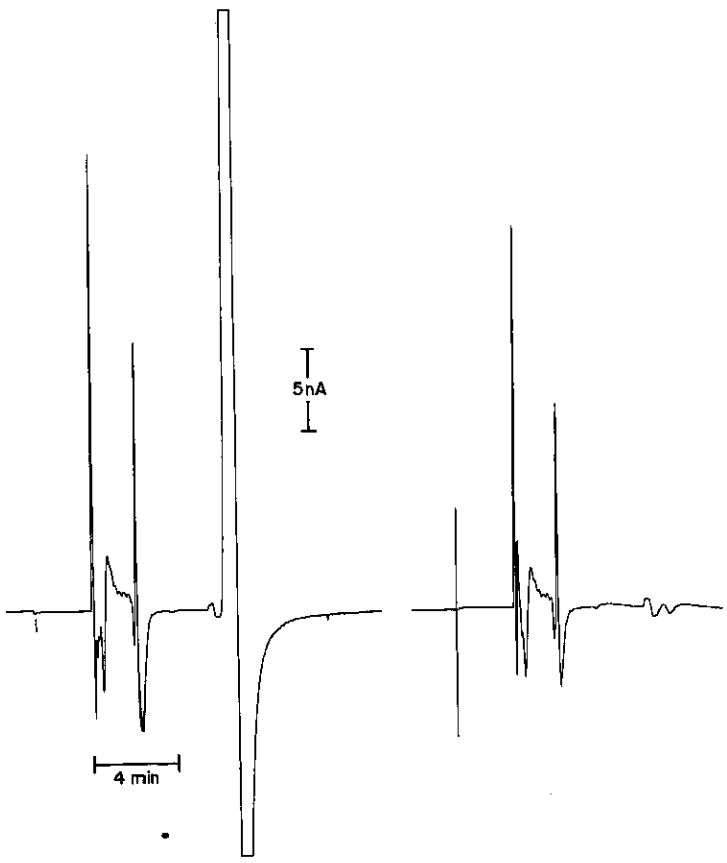

Figure 3. Blank injections demonstrating effect of sample deoxygenated. A) Air-equilibrated sample. B) Helium sparged.

Oxygen Removal

For satisfactory results on the Hg/Au electrode, the liquid chromatographic system must be capable of excluding oxygen from the mobile phase. Without this process, two problems occur: (1) dissolved oxygen oxidizes thiols on-column, leading to poor reproducibility, and (2) reduction of dissolved oxygen causes a high negative background current. This capability is automatically built into the BAS 200. For the BAS 400 system or LC-4B detectors, consult the manual.

It is usually not necessary to deoxygenate the samples for work at 0.0 to +0.15 volts. However, the introduction of sample oxygen into a thoroughly deoxygenated system can yield troublesome system peaks (F3). The large dip may or may not be a problem, depending on the analytes and the assay sensitivity.

Voltammetry

The hydrodynamic voltammogram (HDV) generated by 200 ng injections of N-acetylcysteine is shown in F4. From the HDV, +0.1 volt was selected as the optimum operating potential for detection of this compound. In general, we have observed that a voltage range of +0.10 to +0.15 V is appropriate for most thiol compounds.

Lifetimes

Under continuous operation at the conditions described above, the lifetime of a Hg/Au surface averages 2-3 weeks. When resurfacing becomes necessary, a gradual decrease in response will be observed over several days, and the electrode surface will have a gold tinge. Actual operating conditions may, of course, cause the usable lifetime of a given surface to vary from lab to lab. When resurfacing, we usually simply reapply mercury to the old amalgam surface without repolishing. This may slightly increase the random noise at high sensitivity, and should be checked for appropriateness to a given application.

Linearity

The linearity of the electrode response for N-acetylcysteine over the range of 2 to 200 nanograms injected (0.1 to 1.2 nmoles) is demonstrated by F5.

Related BAS Publications
Thiols

CAPSULE 194. Measuring Penicillamine in Plasma and Urine.

Disulfides and Thiols

CAPSULE 195. Chromatography of Biological Thiols and Disulfides.

CAPSULE 235. Oxidized and Reduced Glutathione.

CAPSULE 234. Captopril and Captopril Disulfide in Plasma and Urine.

CAPSULE 171. Detection of Thiols and Disulfides With Dual-Series LCEC.

CAPSULE 196. Determination of Thiols and Disulfides in Urine.

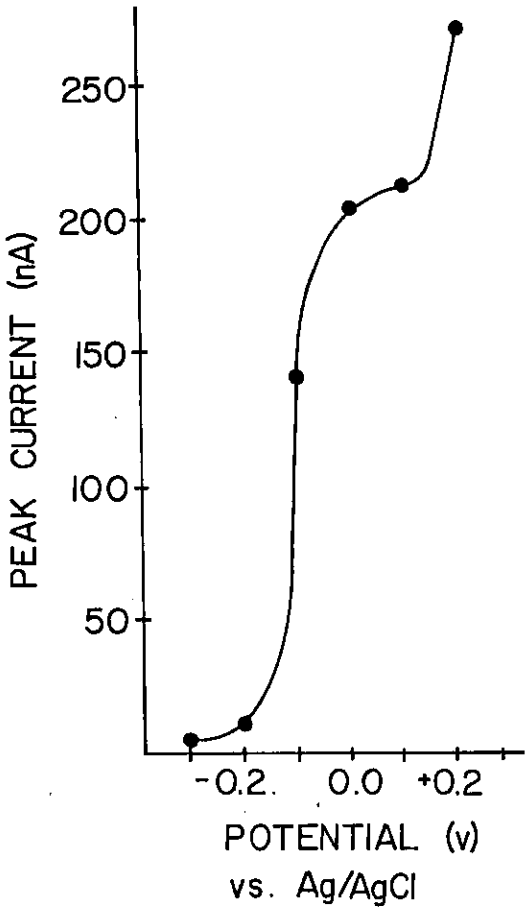

Figure 4. Hydrodynamic voltammogram of N-acetylcysteine. 200 ng injections were made, using a Biophase ODS 5 µm column and a mobile phase of 40% CH_3CN: 60% 0.1 M monochloroacetate buffer, pH 3.0.

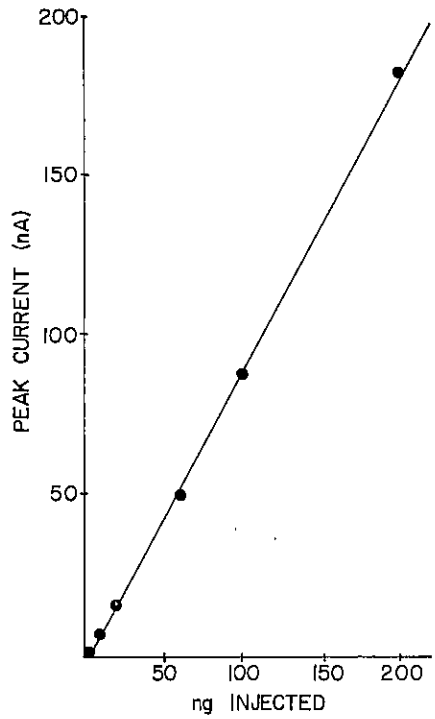

Figure 5. Linearity of Hg/Au electrode response for N- acetylcysteine at +0.1 V. A Biophase ODS 5 µm column (P/N MF-6017, 250 x 4.6 mm) was used, with a mobile phase of 5% CH_3OH: 95% 0.1 M monochloroacetate buffer, pH 3.0.