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## HPLC/EC Studies of Selected Explosive Components, Nitroanilines, and Nitrophenols with Dual Electrode Electrochemical Detection

### Final Report

D. L. Manning  
M. P. Maskarinec

Supported by

U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY  
Aberdeen Proving Ground, MD 21010-5401

Project Officer: Mary Ann Ryan

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National Technical Information Service  
U.S. Department of Commerce  
5285 Port Royal Road, Springfield, Virginia 22161  
NTIS price codes--Printed Copy: A03; Microfiche A01

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HPLC/EC Studies of Selected Explosive Components, Nitroanilines,  
and Nitrophenols With Dual Electrode Electrochemical Detection

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Date Published - October 1986

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## EXECUTIVE SUMMARY

Two independent electrodes in a flow stream can be configured in two ways, dual series and dual parallel operating modes. The eluant from the analytical LC column can then be monitored at two potentials simultaneously. Current ratios are constant for a given analyte and can be used to assess peak purity in real samples. This was demonstrated for selected explosives components.

Nitrophenols and nitroanilines can be detected by both reductive and oxidative high pressure liquid chromatography/electrochemical detection (HPLC/EC). A sample of pink water was found to contain major constituents TNT, RDX, and HMX at levels of 34-96 mg/L by reductive HPLC/EC. Chromatograms recorded in the oxidative mode to look for possible decomposition products revealed no prominent anodic peaks. Decomposition products which may contain -OH, -NH<sub>2</sub> or other oxidizable entities at +1.4 V were negligible for this sample.



TABLE OF CONTENTS

	<u>Page</u>
EXECUTIVE SUMMARY . . . . .	1
LIST OF TABLES . . . . .	4
LIST OF FIGURES . . . . .	5
INTRODUCTION . . . . .	7
Preparation of Mobile Phase . . . . .	7
Reagents . . . . .	7
Chemicals . . . . .	7
Apparatus . . . . .	8
Procedure . . . . .	10
RESULTS AND DISCUSSION . . . . .	12
REFERENCES . . . . .	23
DISTRIBUTION . . . . .	25

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Collection efficiencies of explosives by HPLC/EC in dual series electrode configuration . . . . .	14
2	Peak current ratios of explosives by HPLC/EC in dual-parallel electrode configuration . . . . .	15

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Block diagram for reductive electrochemical detection . . . . .	9
2	Syringe sample deoxygenation . . . . .	11
3	Single and dual electrode transducer schematic. Reference electrode (R), auxiliary electrode (A), and single, dual-parallel, and dual-series working electrodes shown . . . . .	13
4	LC/EC separation of explosives with dual-parallel electrode detection . . . . .	16
5	HPLC/EC separation of nitroanilines with dual-parallel electrode detection . . . . .	17
6	Repeat of chromatogram per right hand side of Figure 5 with glassy carbon electrode at +1.4 V vs. Ag/AgCl . . . . .	19
7	HPLC/EC separation and detection of phenols at glassy carbon electrode, +1.4 V vs. Ag/AgCl, 20 NAFs . . . . .	20
8	Reductive HPLC/EC chromatogram of pink water sample . . . . .	21
9	Oxidative HPLC/EC chromatogram for pink water . . . . .	22



## INTRODUCTION

The advantage of HPLC/EC for the separation and detection of electroactive species is well documented in the literature (1-5). It has been demonstrated that this technique offers a selective and sensitive method for the analysis of trace quantities of explosive components in aqueous solution (1,2). In an effort to further enhance the selectivity and sensitivity of the analytical method (HPLC/EC), we have investigated dual electrode electrochemical detection. This report summarizes our studies on HPLC-dual electrode detection of selected explosive components, nitroanilines, and nitrophenols.

## PROCEDURES

### Preparation of Mobile Phase

#### Reagents

0.025 M Sodium acetate, 0.025 M monochloroacetic acid. Dissolve 4.1 g sodium acetate and 4.7 g monochloroacetic acid in 2 L of distilled water.

1-Propanol, Distilled in glass, Burdick & Jackson

Mobile Phase, 1-Propanol: 0.025 M sodium acetate

0.025 M chloroacetic acid (ClHAc) (30:70 v/v). Add 300 mL 1-propanol to 1 L volumetric flask.

Dilute to mark with 0.025 M NaAc, 0.025 M ClHAc solution.

Filter through 0.45  $\mu$ m Nylon-66 filter.

Add to the 2 L flask for pump A.

(20:80 v/v) Add 200 mL 1-propanol to 1 L volumetric flask.

Dilute to mark with 0.025 M NaAc, 0.025 M ClHAc solutions.

Filter through 0.35  $\mu$ m Nylon-66 filter. Add to 2 L flask for pump B.

#### Chemicals

RDX, HMX, NG packed in water-alcohol, TNT, PETN, 2,4-DNT and 2,6-DNT (manufactured at various installations)

Cosol. Prop. Acct.

ARRADCOM Support Activity

DOVER ND 07801

o-Nitroaniline

2,4-Dimethyl-6-nitroaniline

N,N-Dimethyl-6-nitroaniline

Phenol

o-Nitrophenol

Reagent grade, standards prepared in ethanol and used without further purification.

Note: HMX = cyclotetramethylenetetranitramine, RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine, TETRYL = N-methyl-N,2,4,6-tetranitroaniline, NG = nitroglycerine, TNT = 2,4,6-trinitrotoluene, 2,6(or 2,4)-DNT = 2,6(or 4)-dinitrotoluene, PETN = pentaerythryltertranitrate.

#### Apparatus

The electrochemical detector was a Bioanalytical Systems (BAS) Model LC4B (17-D) dual electrode detector. The electrochemical cell was a BAS MF1006 thin layer cell assembly which consists of a gold-mercury working electrode, glassy carbon working electrode and an MF1018 stainless steel (auxiliary) top. An RE1 Ag/AgCl reference electrode is housed downstream in an RC-2A reference electrode compartment. The LC columns were 25 x 0.46 cm C<sub>18</sub> (5 μm particle size) Dupont Zorbax, Alltech Spherisorb or BAS Biophase columns. The injection valve was a Rheodyne Model 7120 fitted with a 20 μL loop and mounted vertically for sample degassing similar to the method proposed by Lloyd (4). A Linear Instruments Corp., Model 485 two-channel recorder and a Hewlett-Packard Model 3390A reporting integrator were used for data readout.

The thin layer mercury thin film electrodes were prepared following the recommendations of Bratin, et al. (2,3). Enough triply distilled mercury was placed on the highly polished gold electrode to cover the entire surface. After approximately 3 minutes, the excess mercury was removed with a straight edge. At this point, the electrode was viewed edge-on with a hand magnifying glass. If a "bulge" was noticeable, the straight edge was passed across the electrode again to remove more mercury. (Keep the mercury film as thin as possible, consistent with complete coverage of the gold surface.) The importance of achieving good amalgam formation cannot be overemphasized. Usually a new amalgam surface had to be removed with 6 M nitric acid and the electrode repolished. The old amalgam was wiped with a tissue to remove any excess mercury film. The amalgam surface was renewed in the same way as with a new electrode. (Detailed instructions for electrode maintenance are furnished with electrodes and polishing kits from Bioanalytical Systems, Inc.)

A Perkin-Elmer Series 2 liquid chromatograph was fitted with the essential dissolved solvent oxygen removal apparatus (2,3) for each pump as illustrated in Figure 1. The mobile phase vessel is a two-liter, three-neck flask fitted with a condenser, thermometer, and lines for mobile phase delivery and gas sparging. The gas sparging line from the cylinder to the mobile phase vessel is 1/8 in. OD copper tubing fitted with a Nupro Fine metering needle valve for flow regulation. The sparging line to the injection valve is 1/16 in. OD stainless steel also fitted with a Nupro Fine metering needle valve. The connections to the nitrogen saturation vials (3.5 mL screw cap) are through 12 mm Teflon-coated silicone discs. The mobile phase delivery line from the reservoir to the pump inlet is 1/8 in. OD stainless steel. From the pump outlet to

### BLOCK DIAGRAM FOR REDUCTIVE ELECTROCHEMICAL DETECTION

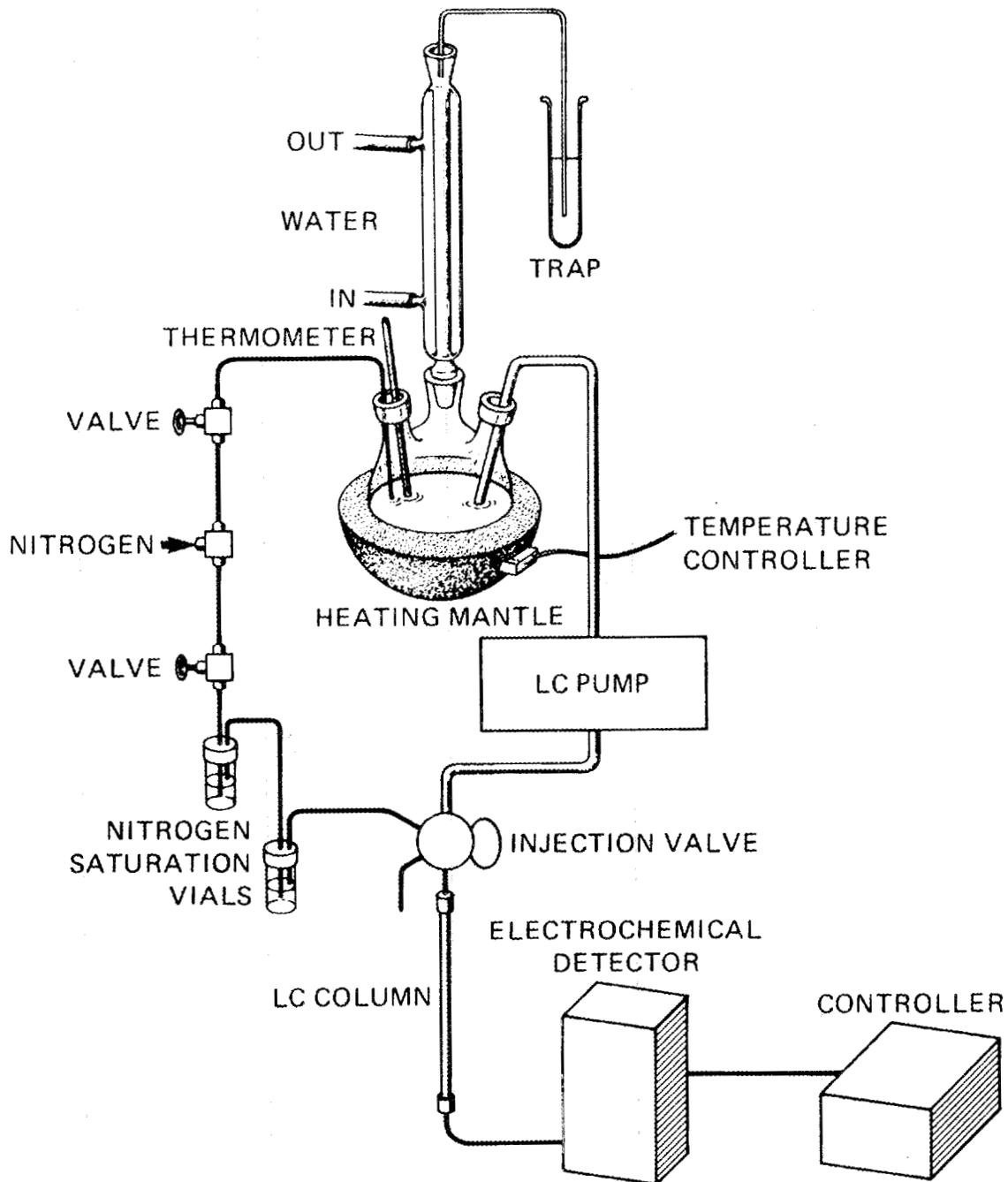


Figure 1. Block diagram for reductive electrochemical detection.

the electrochemical detector, all lines are 1/16 in. OD stainless steel. A BAS Model MF 400 flow through pulse damper was installed between the pump outlet and injection valve. The lines for mobile phase delivery and gas sparging are attached to the reservoir through gas tight rubber stoppers and extend about 2 inches inside the flask. Teflon lines (1/8 in.) are fitted to the metal tubing and extend into the mobile phase. The ends of the Teflon lines immersed in the mobile phase are fitted with stainless steel solvent inlet filters, 1/2 in. diam. x 1 in. long, 5 m pore size. The gas flow through the SS filter for sparging is a steady stream of small "micro" bubbles.

### Procedure

**Deoxygenation of Mobile Phase:** Place 2 L of filtered mobile phase into the 2 L flask shown in Figure 3. The system is made air tight by the stainless lines, thermometer and trap connections passing through tight fitting rubber stoppers. The trap at the top of the condenser is terminated in about 5 cm water which allows for a slight overpressure of inert gas inside the system. With inert gas (nitrogen or helium) slowly sparging through the mobile phase (ca. 2-4 mL/min) adjust variac or temperature controller to heat mobile phase to 60°C. Maintain these conditions for at least 24 hours. Lower temperature of mobile phase to ca. 30°C and maintain inert gas sparging at ca. 1 to 2 mL/min. An alternate and effective method of degassing the mobile phase is by purging with helium at 120 mL/min for 30 min, and then continuous sparging at 2-4 mL/min.

**Deoxygenation of Sample:** With the vertically mounted injection valve which is illustrated in Figure 2 in the inject position, insert the 1 mL syringe without plunger in the needle port with turn valve to load position. Start inert gas flow as evidenced by bubbling through the gas saturator vials and at this point mobile phase from the loop and excess sample solution from the previous injection will back-up into the syringe. Remove this solution with a disposable pipet. Add 200  $\mu$ L ethanol and allow gas to bubble through for about 10 sec to rinse syringe. Remove ethanol with disposable pipet. Place ~ 100-200  $\mu$ L sample in syringe and degas it at ~ 1 bubble/sec for about 3 min. Insert plunger just at top of syringe then turn off gas. Push in plunger to fill loop with sample, turn valve to inject, then remove and rinse syringe.

**Controller Operation:** Read and be thoroughly familiar with BAS Manual P/N MF 9003 Installation/Operations Manual for Amperometric Controller and Transducer Package.

The operation of the controller is carried out per instructions set forth in this manual.

### SYRINGE SAMPLE DEOXYGENATION

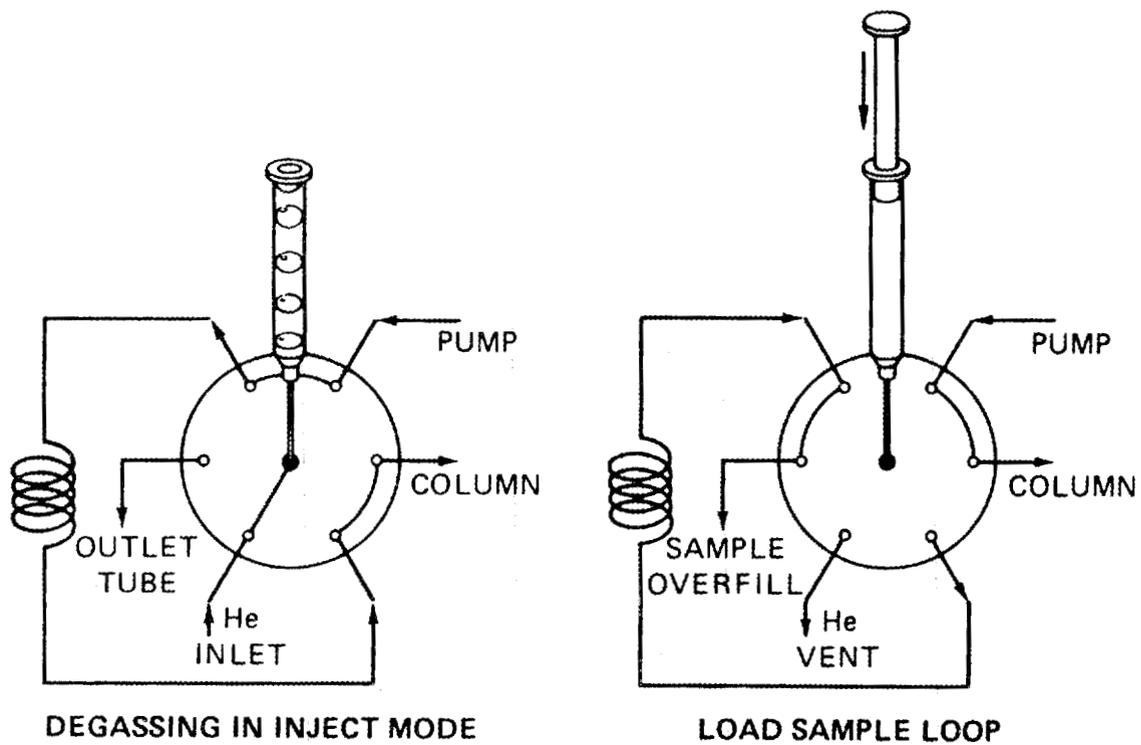


Figure 2. Syringe sample deoxygenation.

End of Day:

1. Leave voltage on working electrode.
2. Adjust flow of mobile phase to 0.1 mL/min for overnight.
3. Set recorder to stand-by.

Shutdown:

1. Set controller to stand-by.
2. Reduce voltage to zero.
3. Turn off compensation circuits.
4. Turn off pump(s).
5. Set recorder to stand-by.
6. Remove reference electrode, store in 3 M NaCl.

### RESULTS AND DISCUSSION

Two independent electrodes in a flow stream can be configured as shown in Figure 3. The dual series electrode configuration closely resembles fluorometry. A reactive intermediate is produced by some excitation function which yields a product that generates a measurable response. In the case of fluorescence, it is the emission signal; for the electrochemical detector it is the redox couple at the upstream electrode. A quantity that is a measure of the maximum amount of fluorescence from a given input intensity is the "quantum efficiency." A similar quantity for the electrochemical detector is the collection efficiency as defined by:

$$\text{Collection efficiency} = \frac{i_{\text{downstream}}}{i_{\text{upstream}}}$$

The maximum range of values are between 0.37 and 0.42 for reversible redox couples at equal surface planar electrodes in a thin layer cell. For irreversible systems, this quantity is generally much less.

The second electrode configuration is the dual parallel electrode configuration. The eluent from the LC can be monitored simultaneously at two applied potentials. This situation is analogous to UV monitoring at two wavelengths. The current ratios are constant for a given electroactive substance and can be used to assess peak purity in real samples by comparing with standards under identical conditions.

Experiments were carried out on the LC/EC measurement of explosives with the dual cell operated in the dual series electrode mode. Interest here was to observe if the sample deoxygenation step could be eliminated. This is accomplished, in principle, by operating the upstream electrode at a negative potential while the downstream electrode is maintained at a positive potential. The explosives plus oxygen eluate from the column

ELECTROCHEMICAL DETECTOR

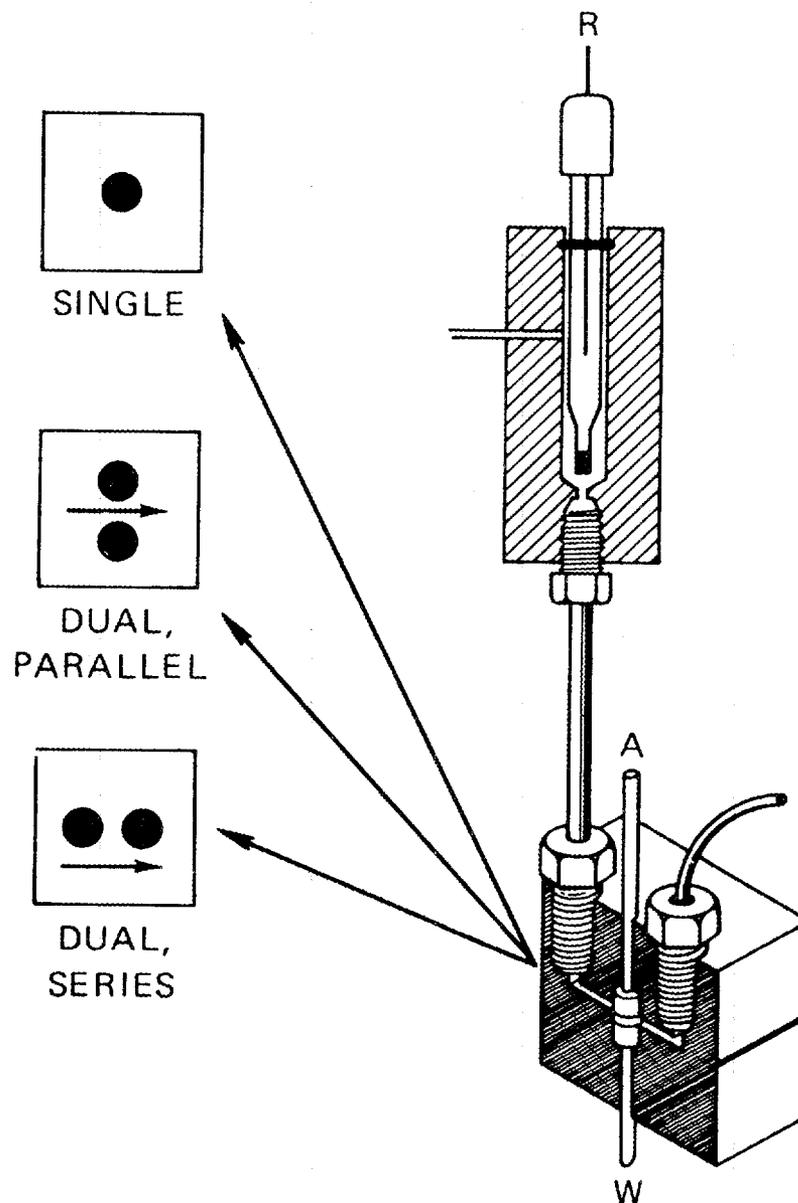


Figure 3. Single and dual electrode transducer schematic.

Reference electrode (R), auxiliary electrode (A), and single, dual-parallel, and dual-series working electrodes shown.

are reduced upstream while only the reduced form of the explosives components are reoxidized downstream. This results in an anodic electrode response. The highly irreversible nature of the oxygen electrode reaction precludes any significant downstream signal from this substance. Typical collection efficiencies are shown in Table 1.

These results are disappointing. Although downstream responses were observed, the collection efficiency was too low to be of practical value. The ideal collection efficiency for this type of flow cell and for an ideally reversible couple ranges from about 0.30 to 0.40. Our values for the explosives peaks ranged from about 0.004 to 0.008 and for oxygen approximately 0.002. This points up the highly irreversible nature of the electrode reactions of the explosives. For the remaining studies, the electrodes were placed side by side and operated in the dual parallel mode. The potentials are chosen so the  $E_1$  will be on the limiting current plateau and  $E_2$  somewhere along the reduction wave of the explosive. Simultaneous chromatograms are generated and the ratios of the peak currents ( $i_2/i_1$ ) can be utilized to assess the peak purity in real samples by comparing with standards under identical conditions. This mode of operation is analogous to UV detection at two wavelengths.

Table 1. Collection efficiencies of explosives by HPLC/EC in dual series electrode configuration

Explosive	Collection efficiency ( $i_{\text{downstream}}/i_{\text{upstream}}$ )
HMX	0.0070
RDX	0.0080
Tetryl	0.0050
NG	0.0070
TNT	0.0044
2,6-DNT	0.0060
2,4-DNT	0.0060
PETN	0.0075

Au/Hg @ - 1.0 V vs Ag/AgCl.  
Glassy carbon @ + 1.0 V vs Ag/AgCl.

Representative peak current ratios for the explosives of interest are tabulated in Table 2.

Table 2. Peak current ratios of explosives by HPLC/EC in dual-parallel electrode configuration

Munition	E, glassy carbon, V vs. Ag/AgCl			
	-0.55	-0.60	-0.65	-0.70
	R	R	R	R
HMX	0	0	0	0
RDX	0	0	0.10	0.32
Tetryl	0.58	0.55	0.75	0.78
NG	0	0	0.11	0.80
TNT	0.31	0.38	0.75	0.95
2,6-DNT	0.09	0.08	0.55	0.94
2,4-DNT	0.15	0.22	0.53	0.91
PETN	0	0	0.15	0.83

$i_1$  = current at Au/Hg -1.0 V vs. Ag/AgCl.

$i_2$  = current at glassy carbon.

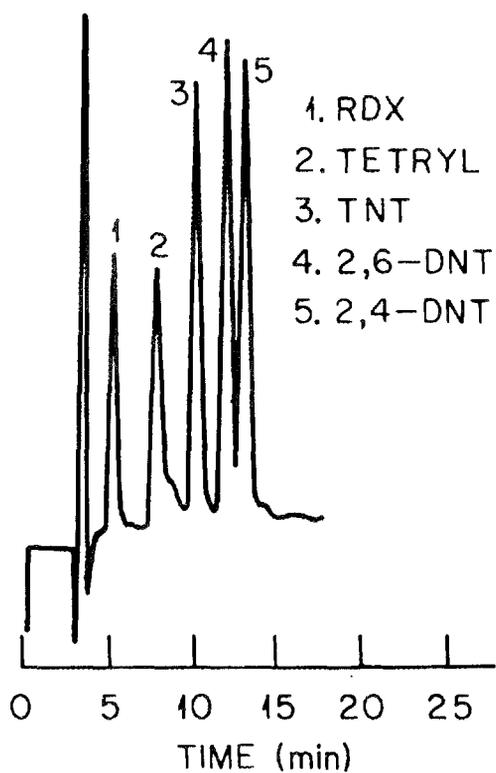
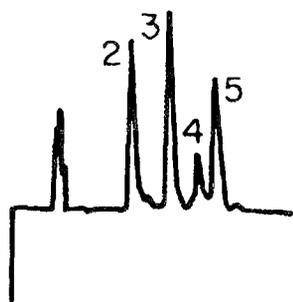
$R = i_2/i_1$ .

The Au/Hg electrode was operated at -1 V vs. Ag/AgCl reference electrode and the glassy carbon electrode was varied from -0.55 to -0.70 V vs. Ag/AgCl reference. Tetryl, being the easiest explosive to reduce, is seen at both electrodes and showed the least variation in current ratios over the voltages tested. HMX, being the most difficult to reduce, is only observed at the Au/Hg electrode. The other explosives lie somewhere in between. It is difficult to operate the glassy carbon electrode much beyond -0.8 V due to its low hydrogen overvoltage. It would not be desirable in any case to have both electrodes at or near the limiting current plateau of the explosives. A typical chromatogram for the LC/EC separation of five selected explosives is shown in Figure 4. The components were well resolved and only RDX was not observed at both electrodes. For a real sample, if "RDX" peaks were seen at both electrodes, then the peak purity would most certainly be in doubt. This information would not be available using a single trace at the Au/Hg electrode.

Another class of compounds of interest are decomposition products from the explosives. These may include such substances as nitroanilines or nitrosamines and possibly nitrophenols. Such substances contain electroactive groups that are both oxidizable and reducible. We have investigated a limited number of nitroanilines and nitrophenols with the electrochemical cell in the dual parallel electrode configuration.

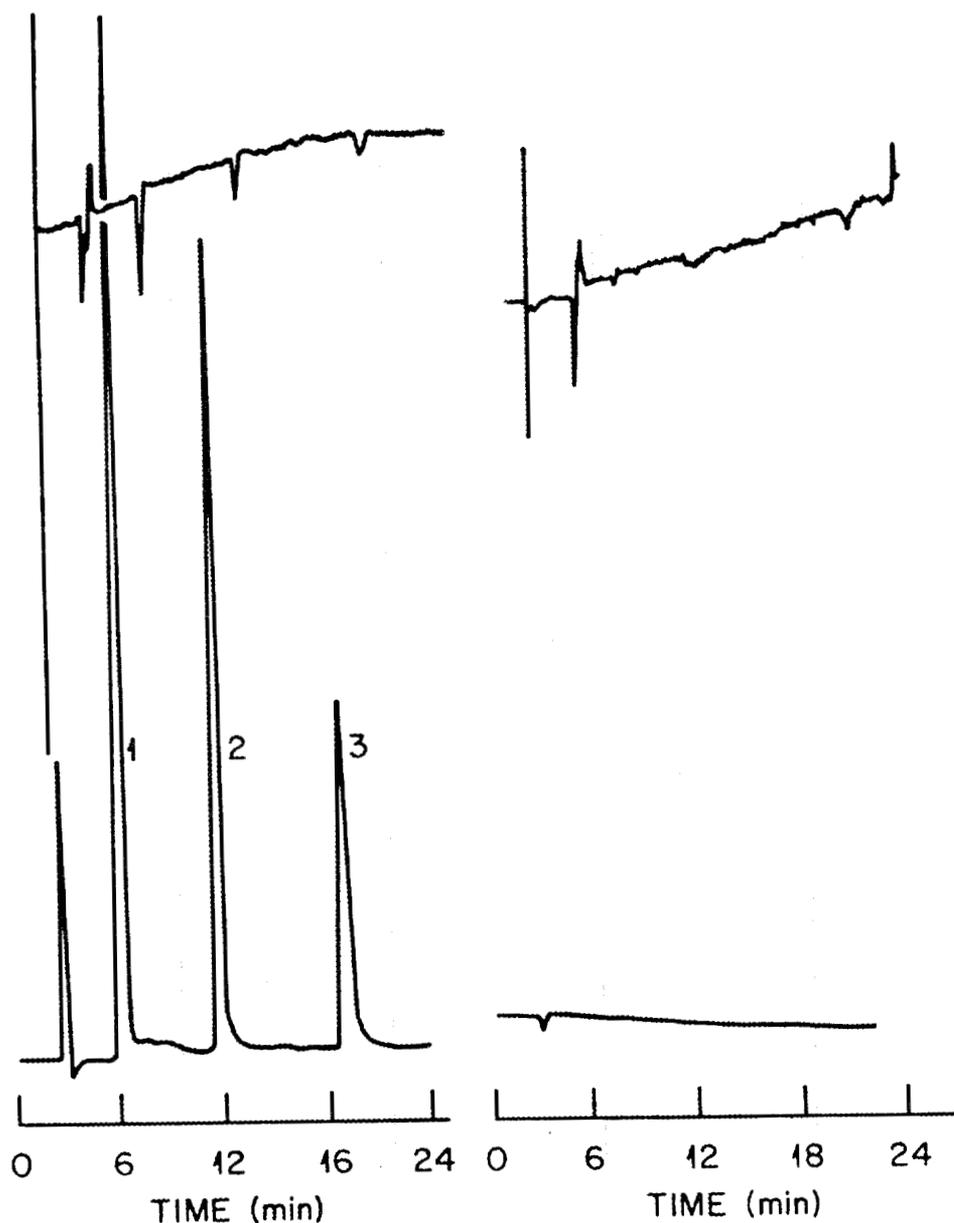
Chromatograms showing the separation and simultaneous reduction and oxidation of selected nitroanilines are shown in Figure 5. The upper

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Concentration, 2  $\mu\text{g/mL}$  each munition  
Column, biophase  $\text{C}_{18}$  (5  $\mu$ )  
Mobile phase, N-prop: .025 M NaAc (30:70)  
.025 M ClHAc  
Flow rate, 1 mL/min.  
Injection Vol., 20  $\mu\text{L}$   
Upper, glassy carbon @ -0.6 V vs. Ag/AgCl  
Lower, Au/Hg @ -1 V vs. Ag/AgCl

Figure 4. LC/EC separation of explosives with dual-parallel electrode detection.



1. O-nitroaniline, 3.2  $\mu\text{g/mL}$
2. 2,3 dimethyl-6-nitroaniline, 1.2  $\mu\text{g/mL}$
3. N,N dimethyl-6-nitroaniline, 1.5  $\mu\text{g/mL}$

Lower trace: Au/Hg @ -1 V vs. Ag/AgCl 100 NAFs  
 Upper trace: Glassy carbon @ +1 V vs. Ag/AgCl 10 NAFs  
 Right: Repeat with Au/Hg electrode off  
 Other conditions per Figure 4.

Figure 5. HPLC/EC separation of nitroanilines with dual-parallel electrode detection.

trace of the left-hand chromatogram shows small downstream signals which correspond to the large peaks resulting from the reduction of the nitro group. By repeating the scan with Au/Hg electrode "OFF" as shown on the right, only one peak was observed which corresponds to the oxidation of N,N-dimethyl-6-nitroaniline. This suggests that at a large potential difference between the electrodes (two volts in this case), there is apparently sufficient "crosstalk" to cause the electrodes to act the same as in the dual series mode. To overcome this difficulty, it is best to repeat the experiment and record the anodic voltammogram with the Au/Hg electrode "OFF" ( $\sim -0.005$  V vs. Ag/AgCl). It is also noted from Figure 5 that the glassy carbon electrode positioned at +1 V vs. Ag/AgCl reference electrode is not sufficient to oxidize the aniline-NH<sub>2</sub> group. Shown in Figure 6 is a repeat of the chromatogram with the glassy carbon electrode at +1.4 V vs. Ag/AgCl. Three well-defined peaks are observed. The Au/Hg electrode was "OFF." It is interesting to note that the relative sensitivities of the -NO<sub>2</sub> reduction and -NH<sub>2</sub> oxidation for the three anilines are reversed (i.e., N,N dimethyl-6-nitroaniline produces the least cathodic signal and the largest anodic signal).

The HPLC/EC separation and detection of phenol and nitrophenol is shown in Figure 7. Two well-defined peaks were obtained which correspond to the oxidation of the -OH group.

Good sensitivities were observed for the anodic detection of the anilines and phenols. As little as 1  $\mu\text{g/mL}$  could easily be detected. This means that in a pink water sample that contains as much as 100 mg/L TNT, decomposition products containing -OH and -NH<sub>2</sub> groups could be detected that amounted to  $\sim 1\%$  or less of the TNT. One sample of TNT pink water that approximates these conditions was analyzed. The chromatogram is shown in Figure 8. The pink water contained 29 mg/L HMX, 39 mg/L RDX, and 72 mg/L TNT. The anodic HPLC/EC chromatogram is shown in Figure 9. As noted from Figure 9, no prominent anodic peaks were observed. Contamination by impurities containing -OH or -NH<sub>2</sub> groups or any other electroxidative entity which is reactive at +1.4 V is negligible for this sample. Perhaps, these types of impurities or decomposition products are not a problem. It will require further studies on different sample types to bear this out. Efforts are being made to better define and acquire some known explosives decomposition products.

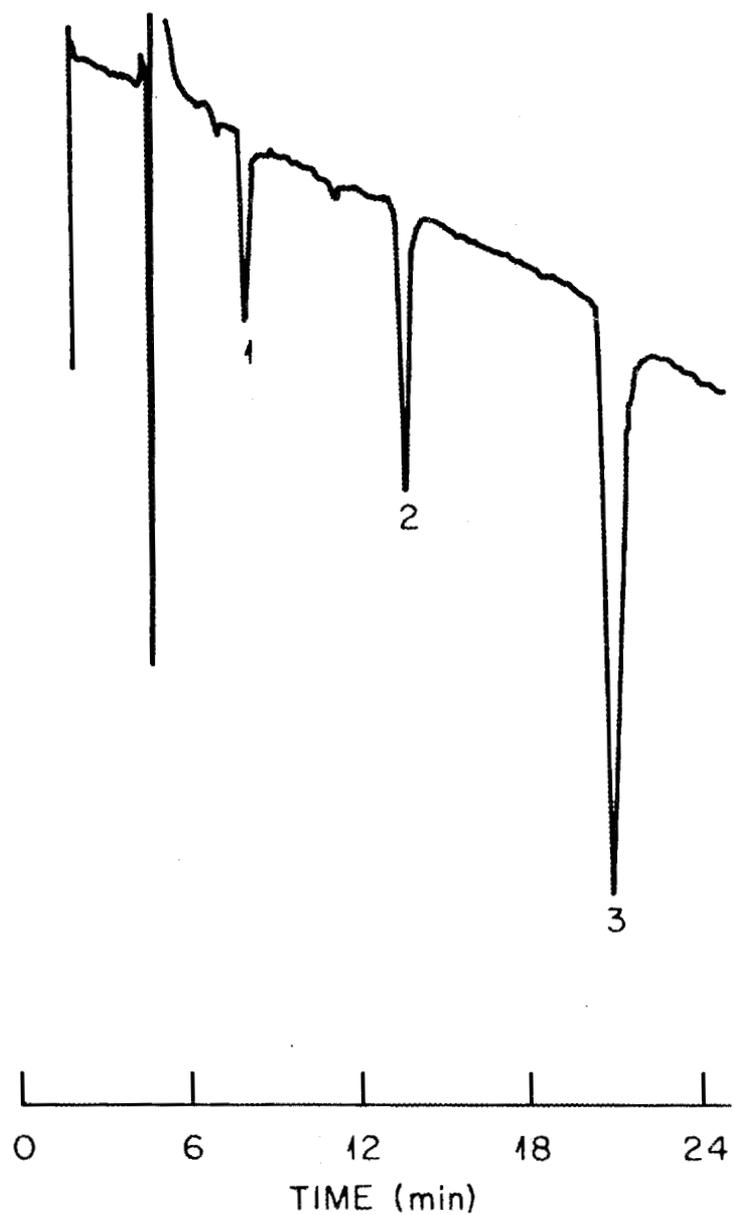
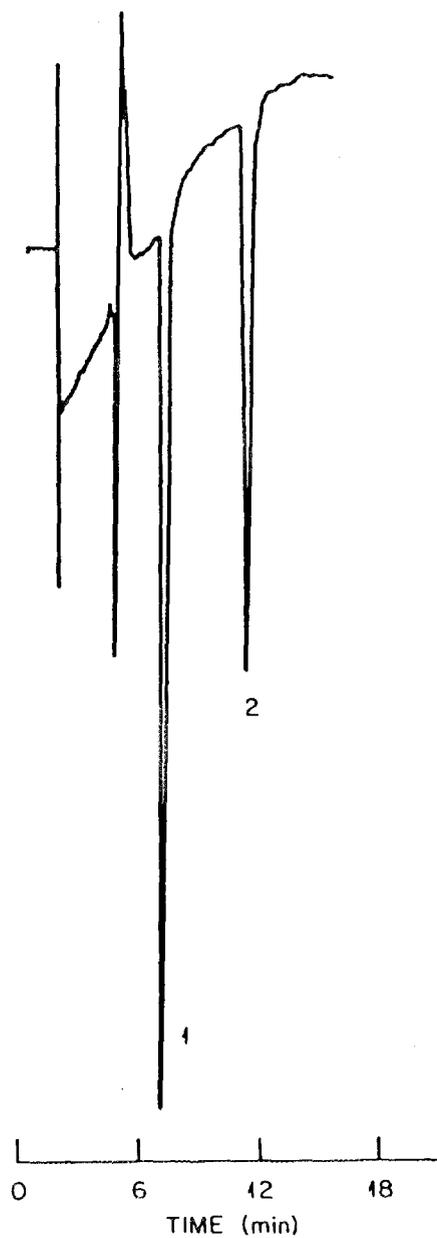


Figure 6. Repeat of chromatogram per right hand side of Figure 5 with glassy carbon electrode at +1.4 V vs. Ag/AgCl.



1. Phenol, 5  $\mu\text{g}/\text{mL}$
2. O-nitrophenol, 5  $\mu\text{g}/\text{mL}$

Other conditions per Figure 4.

Figure 7. HPLC/EC separation and detection of phenols at glassy carbon electrode, +1.4 V vs. Ag/AgCl, 20 NAFs.

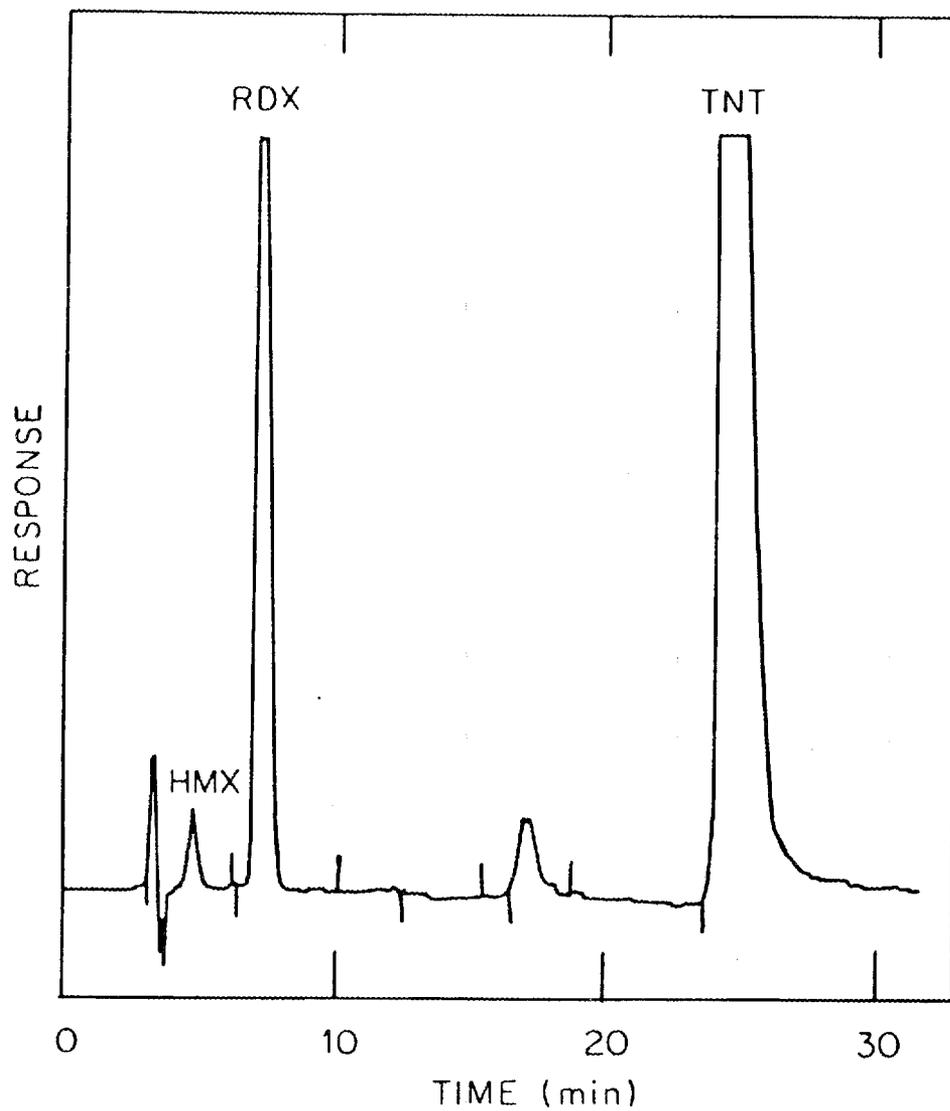
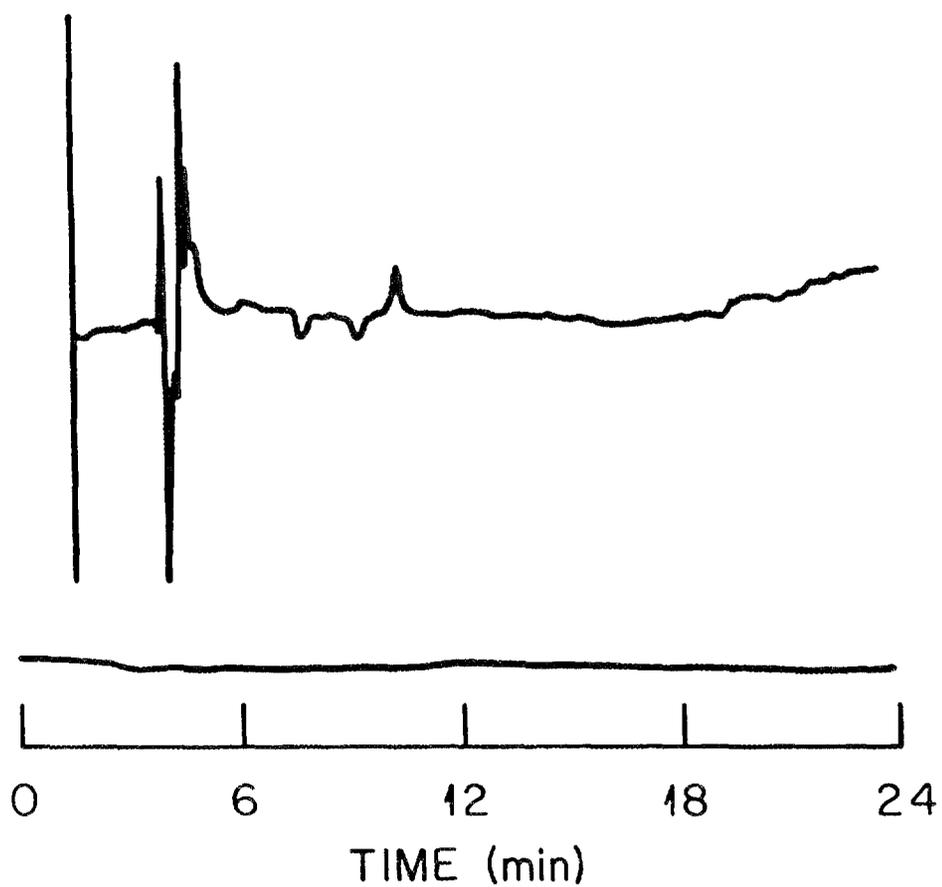


Figure 8. Reductive HPLC/EC chromatogram of pink water sample.

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Lower trace: Au/Hg electrode, OFF

Upper trace: Glassy carbon electrode @ +1.4 V vs. Ag/AgCl 10 NAFs

Figure 9. Oxidative HPLC/EC chromatogram for pink water.

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