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DEVELOPMENT OF HIGH PRESSURE LIQUID CHROMATOGRAPHIC TECHNIQUES

NEW MEXICO UNIVERSITY

PREPARED FOR
AIR FORCE WEAPONS LABORATORY

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DEVELOPMENT OF HIGH PRESSURE LIQUID CHROMATOGRAPHIC TECHNIQUES

Edward A. Walters

Civil Engineering Research Facility
University of New Mexico
Albuquerque, NM

May 1976

Final Report

Approved for public release; distribution unlimited.

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AIR FORCE WEAPONS LABORATORY
Air Force Systems Command
Kirtland Air Force Base, NM 87117

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This is an interim report on a continuing study of the application of High Pressure Liquid Chromatographic (HPLC) techniques to water pollution problems. Reported herein are developmental data on three new detection systems; an ultrasonic velocity detector, a beta-induced luminescence detector, and a specific solid-state silver-ion detector. Preliminary results of work on separations in natural waters and in sewage effluents using HPLC techniques and an extensive literature survey on HPLC are also included.

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PREFACE

The original principal investigator, Dr. Nicholas E. Vanderborgh, left the University of New Mexico in January 1975 and supervision of the project was assumed by Dr. Edward A. Walters who has subsequently been responsible for the experimental direction, data analysis, interpretation, and presentation of results.

The electronics assistance of Fred Husher and the assistance and cooperation of Major Michael G. MacNaughton, Air Force Task Officer, are gratefully acknowledged.

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SECTION 1
INTRODUCTION

BACKGROUND

The Air Force as a result of its extensive maintenance activities discharges wastes which contain both metals and organics. Although total metals analysis is possible using atomic absorption and other instrumental and wet chemical techniques, the actual speciation of the compounds is unobtainable at present.

Knowledge concerning the types and concentrations of trace organics and metals in natural waters has become exceedingly more important in recent years as more data are gathered on their effect on humans, aquatic organisms, and plant life. Organics and organometallics even at the parts-per-billion level have been shown to exert a significant biotoxic and phytotoxic effect in fresh and marine waters.

The analysis of trace organics and metal organic complexes in the environment without disturbing the equilibrium which naturally exists is not possible with the present technology. All present techniques involve indirect measures of the speciation of the different elements. Extraction using organic solvents has been the most prevalent method of separating inorganic and organically bound metals into their separate fractions; however, this technique while applicable to gross differentiation of these two components does not give the actual species which exist in either fraction. Analysis by gas chromatography is useful only for those compounds which are volatile. Anodic stripping voltametry, an electrochemical technique, is also useful for determination of complexed metals which do not interfere with the electrodes and still gives limited data about complex solutions which contain organics and metals in both the free and complexed forms.

High Pressure Liquid Chromatography (HPLC) is a relatively new technique which has tremendous potential for analysis and identification of aqueous pollutants. Whereas with gas chromatography the compounds need to be volatile, with HPLC this is not necessary. Neither is it necessary to perform extractions of the solutions and both metals and organics can be analyzed at the same time. Although HPLC is beginning to be used more extensively particularly in the analysis of drugs, to date there have been few reported uses of this technique in the identification and measurement of pollutants.

One of the most serious shortcomings of HPLC is the lack of detectors which would measure elution peaks from the liquid chromatograph with the same broad applicability as does the flame ionization detector in gas chromatography. The most common detectors available are the ultraviolet absorption and refractive index detectors. The ultraviolet detectors usually operate at the mercury line 253.7 nm or 280 nm and are useful only for those compounds which contain chromophores which absorb at these wavelengths. The refractive index detector lacks the sensitivity of the absorption detector and also exhibits a strong temperature dependence. What is needed in order to make HPLC a more generally applicable routine analysis are detectors which are general in their response to numerous elements or compounds.

OBJECTIVE

The objective of this research and development was to investigate HPLC techniques for the identification and measurement of organics and metals in natural waters. Specifically, this effort was concerned with the procedures, column materials, and detectors needed to establish reliable and accurate identification of trace organics and metal ions present in natural waters as a consequence of industrial discharges from Air Force installations.

SCOPE

The work reported herein deals with preliminary attempts to use HPLC for identification and quantification of metals and organics in aqueous systems, and the design and construction of three new potential detectors. Before beginning the laboratory work, a thorough survey was made of the literature on HPLC with particular attention directed toward applications to the identification of components of aqueous systems.

SECTION 2
LITERATURE SURVEY

A thorough search of all literature pertaining to HPLC was conducted. This was accomplished at the UNM library with the assistance of the Technology Application Center (NASA) which permitted a search through the Federal Documentation Center. The results of this search are included in appendix A. From this search it is quite evident that HPLC is growing rapidly, although the absence of documentation on the use of this technique to determine metal ion complexes is amazing. Apparently, many of these tasks are being performed by atomic absorption spectroscopy. However, atomic absorption simply gives a total metal concentration and offers no clues as to the chemical form of the metal before the determination. The literature search brought the realization that little material on HPLC appeared before 1972, and since that time much of the material of interest appeared in three journals; viz., *Journal of Chromatographic Science*, *Journal of Chromatography*, and *Analytical Chemistry*. Thus, much of the cited literature comes from these journals.

In addition a more general search on the subject of column chromatography was performed by the Technology Application Center. This search produced several thousand references, most of which deal with gas, not liquid, chromatography. These references cover all citations included in *Chemical Abstracts* during the period 1 January 1969 to 3 June 1974, as well as the abstracts. Because of bulk, this material is not included in this report, but it is available in the UNM General Library under the title *Column Chromatography*, LSS-100.

SECTION 3
DETECTOR DEVELOPMENT

ULTRASONIC VELOCITY

As an ultrasonic wave passes through a fluid medium, the phase, θ, is shifted according to the expression

$$\theta = 2\pi ft$$

where f is the frequency of the wave and t is the time required for the wave to pass through the fluid. This is related to the velocity, V, of the wave in the medium through

$$\theta = \frac{2\pi fr}{V}$$

where r is the distance traveled. The phase shift over this distance is

$$\frac{d\theta}{dr} = \frac{\omega}{V}$$

where $\omega = 2\pi f$. For a finite distance and a finite phase shift, the velocity can be calculated from

$$V = \omega \frac{\Delta r}{\Delta\theta}$$

If Δr is constant, the phase shift, $\Delta\theta$, gives the velocity of the wave. This velocity is highly dependent upon the type of fluid and thus may be used to detect changes in fluid composition. This principle has been employed in detectors for gas chromatography.

Unfortunately, however, phase shifts commonly occur when pulses traverse electronic circuits and so tedious calibrations are essential. Therefore, this research was oriented toward establishing the potential of a differential technique for velocity measurement by a phase shift which would obviate any electronic contributions by employing the same circuits for both channels.

Feasibility of a three-transducer, phase-shift concept was demonstrated and the work was described at *The Second Rocky Mountain Regional Meeting of the American Chemical Society* on 8 July, 1974 and in a paper (ref. 1). A detector with a large volume (approximately 20 mℓ) was employed in this feasibility test. However, a detector with a much smaller volume (approximately 5 mℓ) was constructed, and a detection system which would be compatible with an HPLC device and would also provide interfacing with a small computer was designed and constructed. In this detection system (fig. 1) the gated oscillator generates a continuous wave at 1 MHz. This wave is fed to one of two transmitters depending upon the state of the control logic. Three transducers are contained in the detector (fig. 2). The transmitters drive two of these transducers which are separated from the third, receiving transducer, by different fixed distances. The signal from the receiving transducer is amplified and beat against a local oscillator to lower the frequency to near 25 kHz. This signal is then sent to the phase meter and the phase shift is converted to a voltage which is read directly on a digital panel meter. Provisions are made for attaching a recorder or a computer. Schematics of the present system* are shown in figure 3.

BETA-INDUCED LUMINESCENCE

Again, a technique known to function as a detector in gas chromatographic systems was expanded upon in an attempt to construct an HPLC detector based on the concept of beta-ray-induced (quenched) luminescence. This detection system is based on the concept that as beta particles (products of the radioactive decay of tritium) slow down in passing through a medium, they transfer energy to the molecules encountered. Although beta rays from tritium are relatively soft (1.8 MeV), they have sufficient energy to cause excitation and/or ionization of many molecules. These excited molecules emit light as they decay to their ground states or as they recombine to form new products. This light can be detected with the customary photomultiplier system, and thus it can be used

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1. Chen, Chuan, and Vanderborgh, Nicholas E., "A Three-Transducer Differential Phase-Shift Method for the Measurement of Ultrasonic Velocity in Liquids," *Review of Scientific Instruments*, (to be published).

*Recent changes will be indicated in the final report.

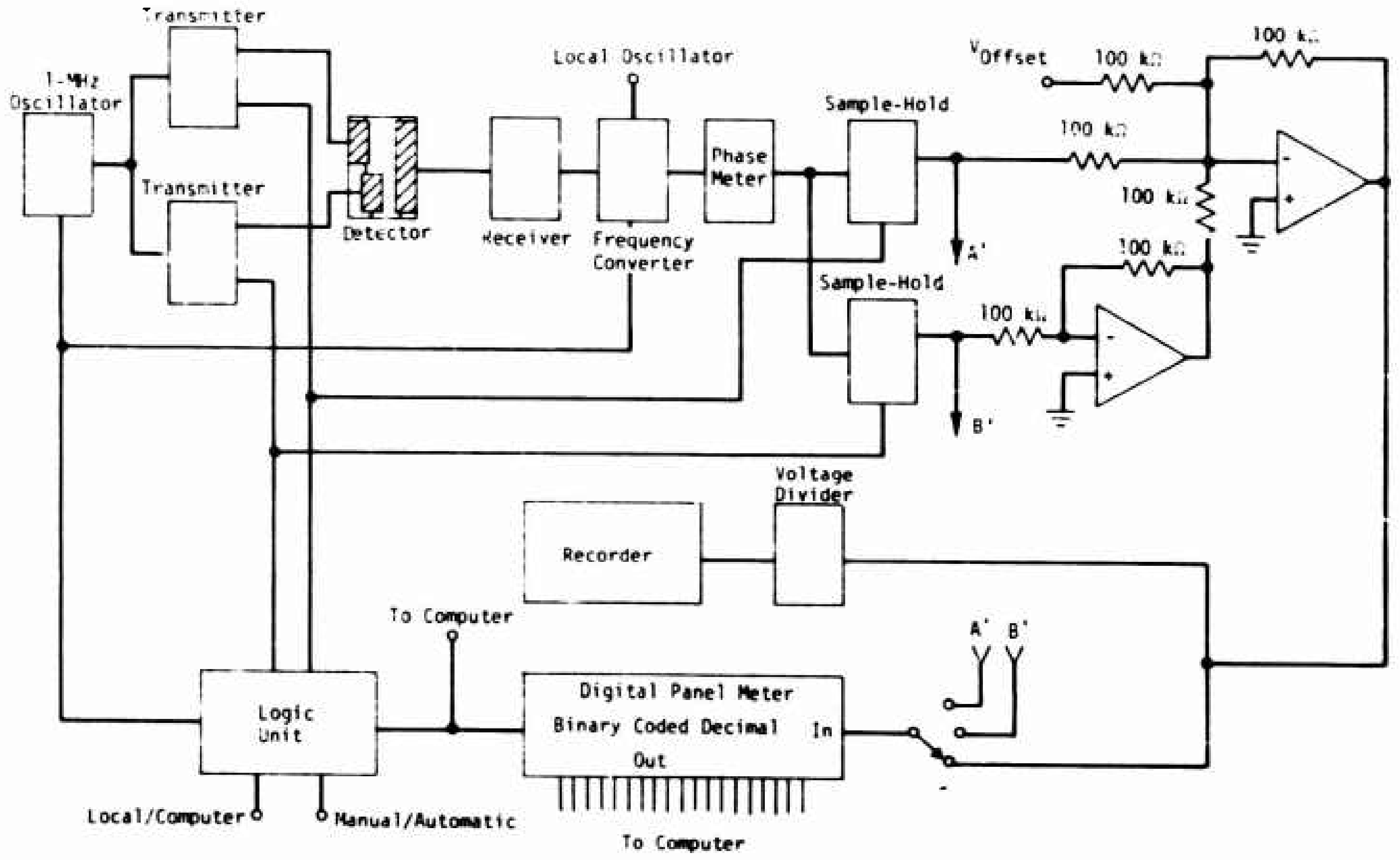


Figure 1. Ultrasonic Velocity Detection System

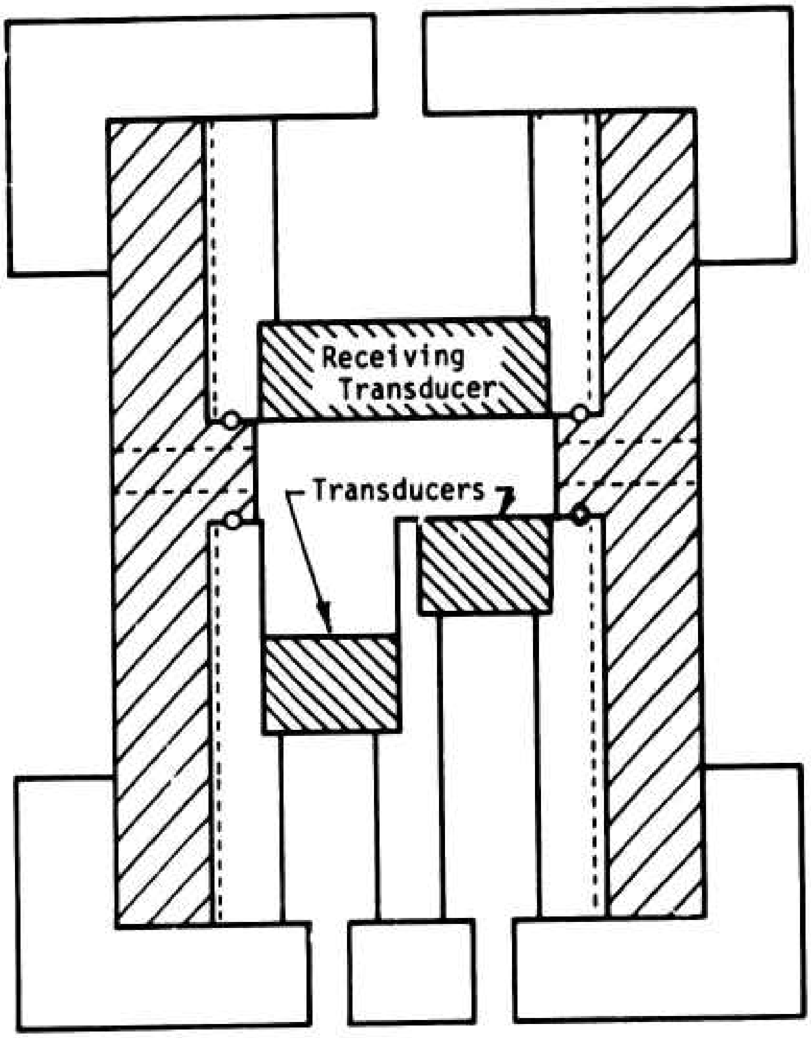
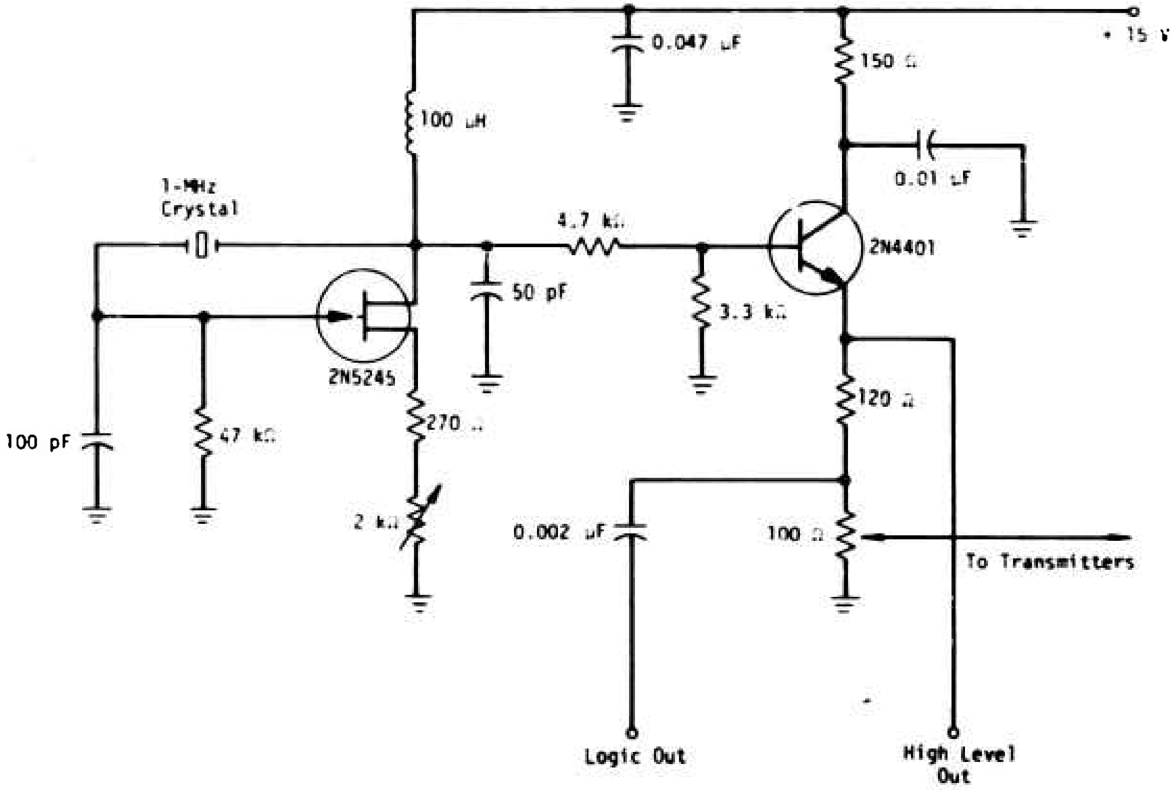


Figure 2. Ultrasonic Velocity Detector



1-MHz OSCILLATOR

Figure 3. Electronics for Ultrasonic Velocity Detection System (1 of 4)

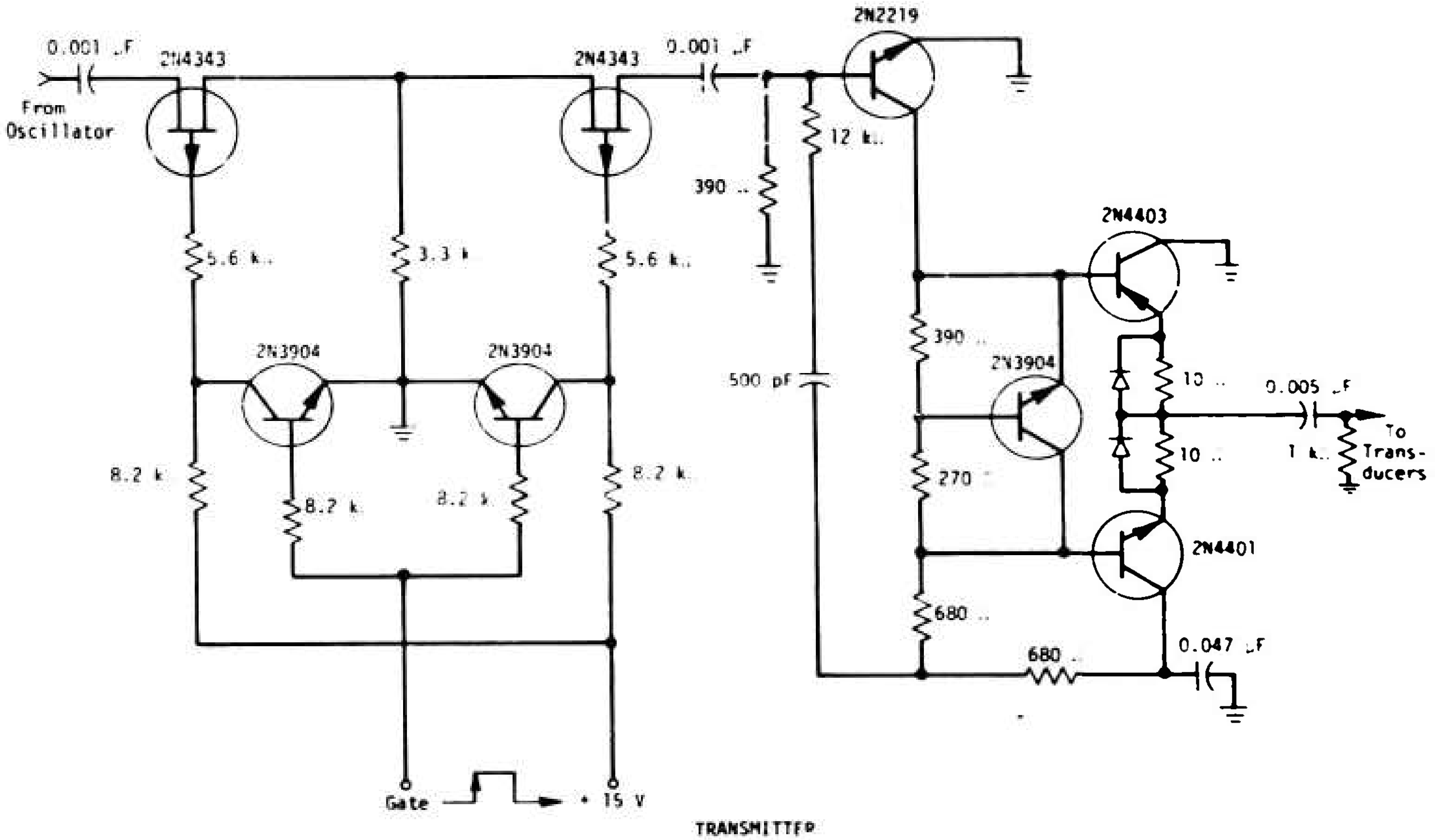


Figure 3. Electronics for Ultrasonic Velocity Detection System (2 of 4)

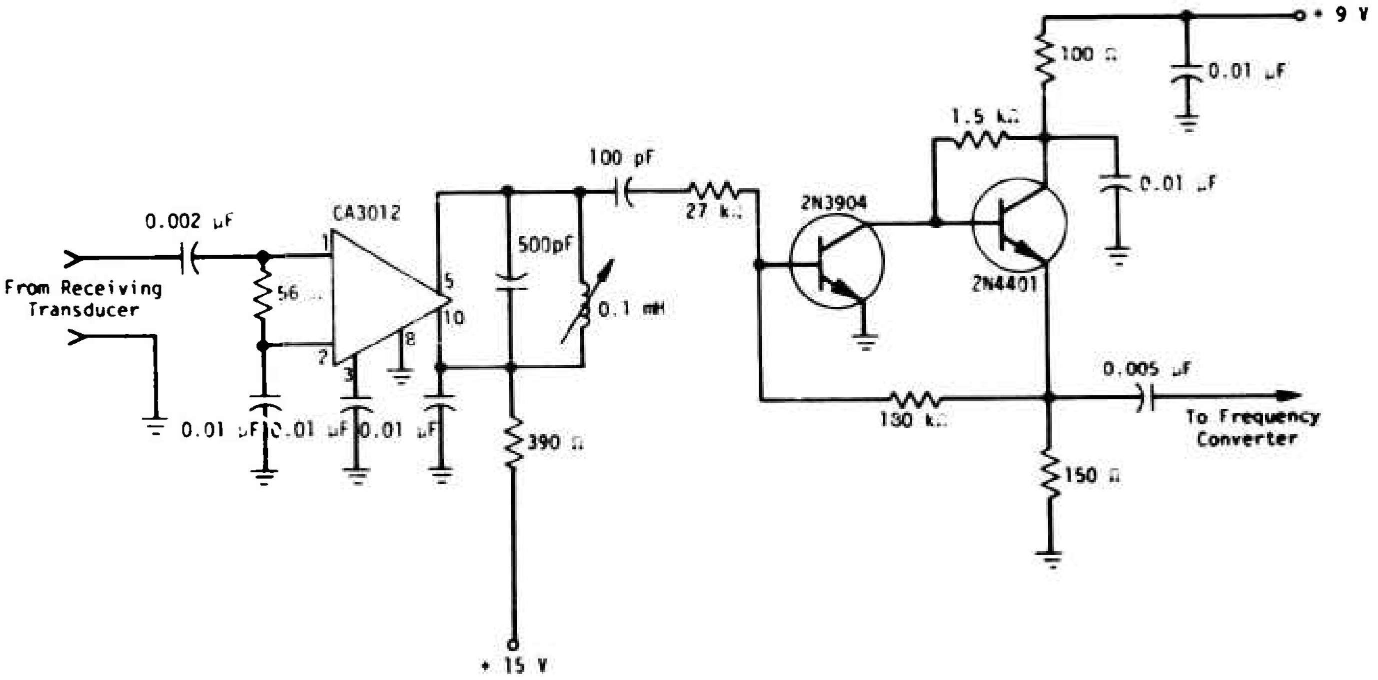


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Figure 3. Electronics for Ultrasonic Velocity Detection System (3 of 4)

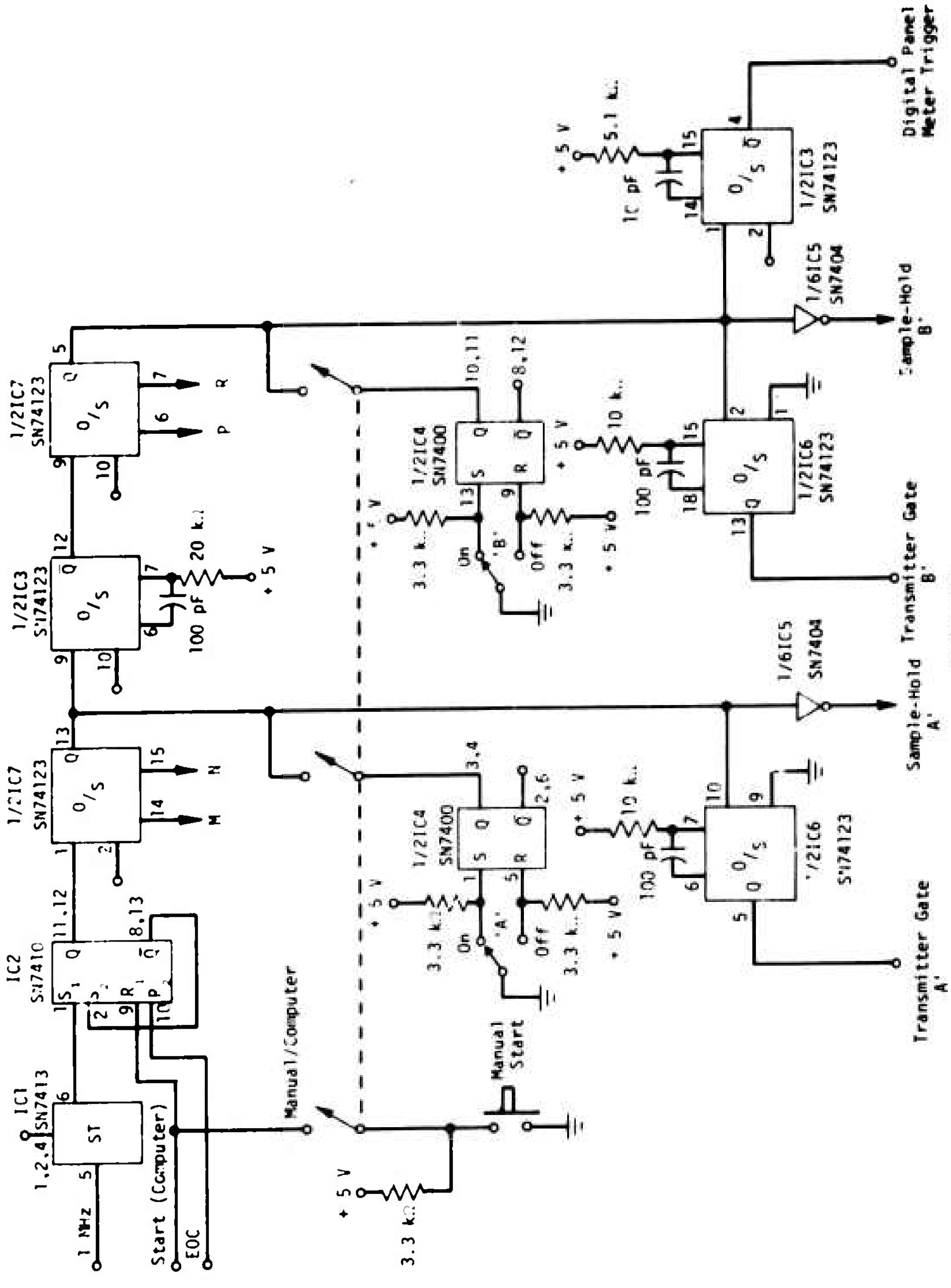


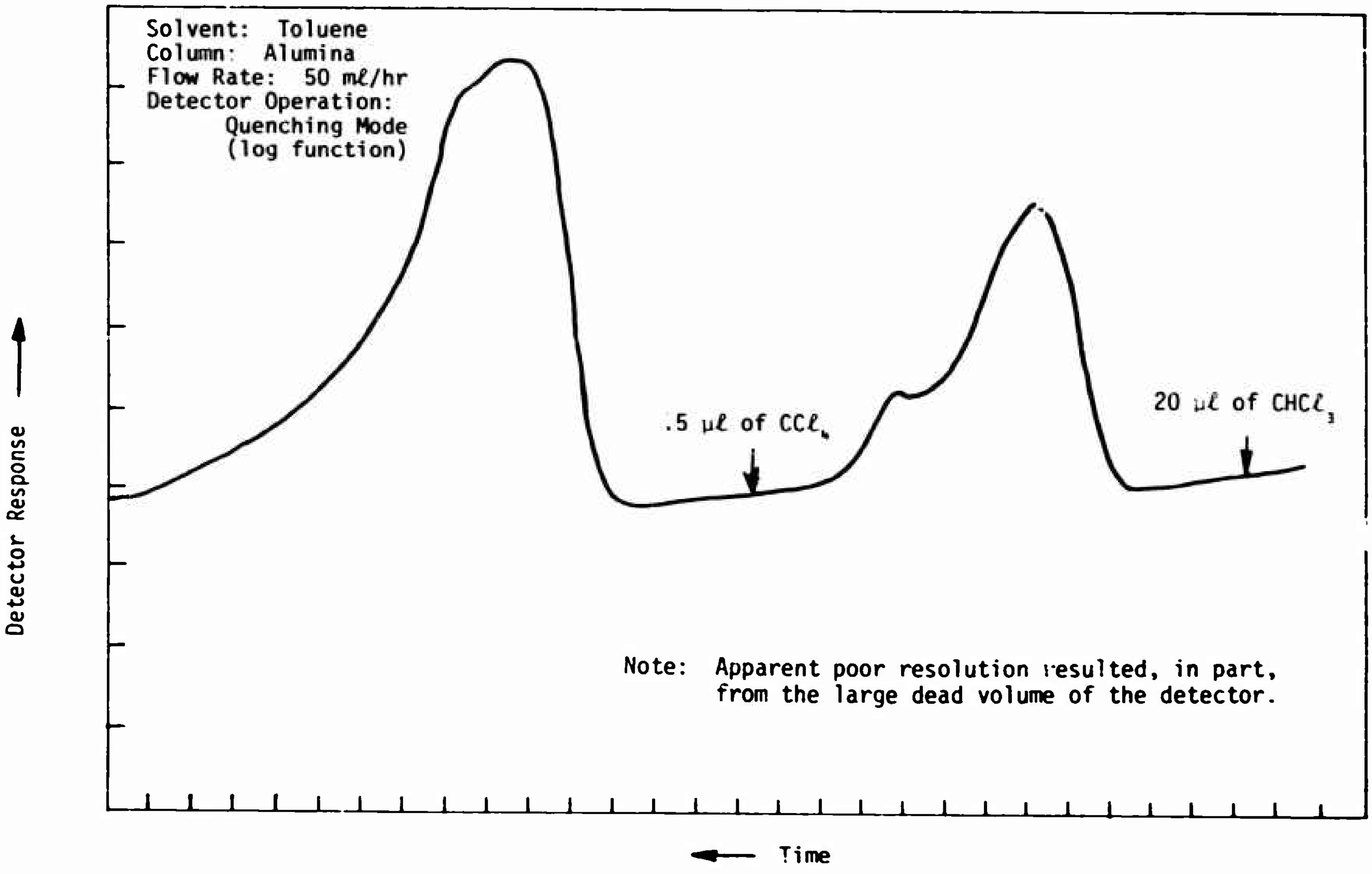
Figure 3. Electronics for Ultrasonic Velocity Detection System (4 of 4)

to determine the quantity of material in the path of the beta rays. In addition, the wavelength at which maximum fluorescence occurs is species dependent so that some distinction can be made between, say, solvent and solute.

Two detectors, a prototype and a second-generation model, were developed and tested. The prototype detector was designed to test the feasibility of the beta-induced luminescence concept in application to HPLC. This detector consists of a tritiated titanium foil over which the solvent/solute systems may pass. Luminescence is observed on an RCA 4440 photomultiplier tube whose output goes to a strip-chart recorder. The volume of the detector is relatively large ($>$ 100 μℓ). The system was tested in the dynamic sense by placing the detector at the outlet end of a 1/8-in-diameter, 5-cm-long column packed with alumina. The column was driven by a low pressure pump (~ 40 psi).

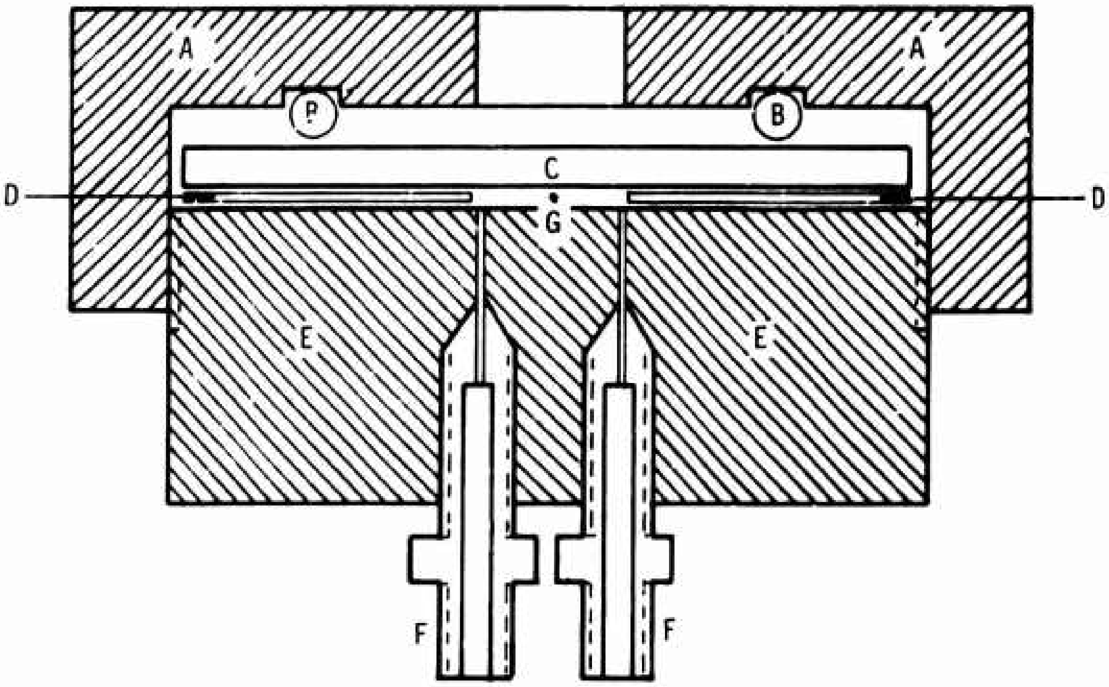
The most satisfactory performance was obtained in the quenching mode. In this mode fluorescence from the solvent, which is typically an aromatic liquid such as toluene, is quenched by the solute. The most efficient quenching, and most sensitive response, was obtained with halogenated solutes. The response of this chromatographic detection system to 20 μℓ of chloroform (CH_3Cl) and 15 μℓ of carbon tetrachloride (CCl_4) is shown in figure 4.

Based on the foregoing preliminary experiments, a second-generation detector was designed and constructed. In designing this detector, the theoretical consideration that the intensity of the fluorescence is proportional to the activity of the radioactive source in disintegrations per second was taken into account. In addition, capture cross sections for beta particles by matter suggest that a path length of 25 μm should be sufficient to stop all the incident beta rays so that the flow thickness of the detector need not exceed this value. Thus the resulting detector has the desired small volume of 4 μℓ. This detector (fig. 5) consists of a head which houses the radioactive source and is covered by a cap through which luminescence is viewed. The sample flows transversely across the viewing region and the path length is controlled by spacers inserted between the head and the cap. All of the metal parts are stainless steel except the spacer which is brass shim stock cut to the appropriate shape; the window is quartz.



Note: Apparent poor resolution resulted, in part, from the large dead volume of the detector.

Figure 4. Chromatogram Obtained with Prototype Beta-Induced Luminescence Detector



A. Stainless Steel Cap
B. Rubber O-Ring
C. Quartz Lens
D. 50-μm Brass Spacer
E. Stainless Steel Block
F. Low Dead Volume Swagelock Fittings
G. 1-cm-Diameter Circle with 1-μm-Thick Layer of Titanium Vapor Deposited for Lattice to Hold 300 mCu of Tritium

Figure 5. Tritium Foil Beta-Induced Luminescence Detector

Tritiation of the detector head was carried out by Dr. John Crawford of Sandia Laboratories. On the surface of the head, a 1-cm-diameter, 1-μm-thick film of titanium was applied using a sputtering technique. This layer was tritiated to an activity of 300 mCu using a vacuum-alloying procedure. The block as received was rather extensively contaminated with tritium; it was carefully decontaminated under the supervision of the UNM Radiological Safety Officer. The assembled detector was satisfactory as far as radioactivity was concerned, and the typical organic solvents flowing through the detector did not leach any radioactive materials.

The light-sensitive device initially employed was an RCA 4440 photomultiplier tube (same as in the prototype experiments). However, because of the short path length (25 μm), the emitted photons were chiefly in the ultraviolet region, a region to which the RCA 4440 photomultiplier does not respond. Therefore, this tube was replaced with a 1P28 tube - a tube which responds well down into the ultraviolet region. A much more satisfactory detector was thus obtained.

As with the prototype detector, the quenching mode was the most useful. In fact, dynamic tests were made to monitor the quenched fluorescence of a standard scintillator solution. The solution was prepared by dissolving 5g of PPO and 0.3g of dimethyl POPOP in 1 ℓ of xylene. This was used as the solvent into which 1-μℓ samples of solutes were injected. A 5-cm glass column packed with beads of Poropak P was used.

A variety of test compounds was injected into the system ahead of the Porapak P column, and the detector response was recorded (fig. 6). These tests were made at a flow rate of approximately 2 mℓ/min so that the detector volume was changed about once every 0.3 sec.

Several observations can be made from these results. First, halogenated compounds give a significant response; that is, halogenated substrates are good quenchers. With the still reasonably crude second-generation detection system, it is possible to obtain a large response with 1-μg samples. Improvements in the detector should increase the sensitivity of the system by about two ardus orders of magnitude. This means that the detector used in the luminescence quenching mode should make a good detector for low levels of the halogenated

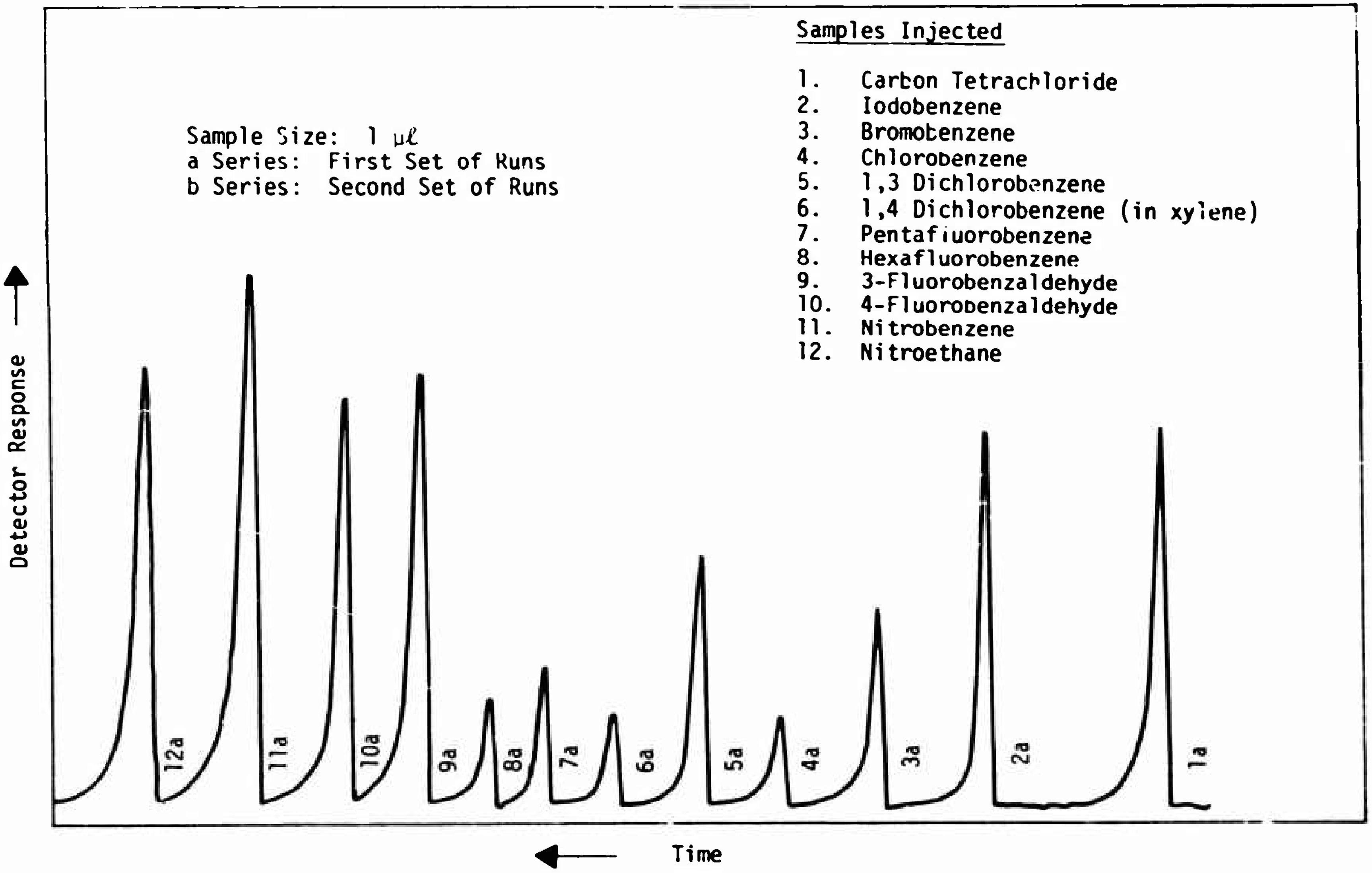


Figure 6. Chromatograms Obtained with Second-Generation Beta-Induced Luminescence Detector (1 of 2)

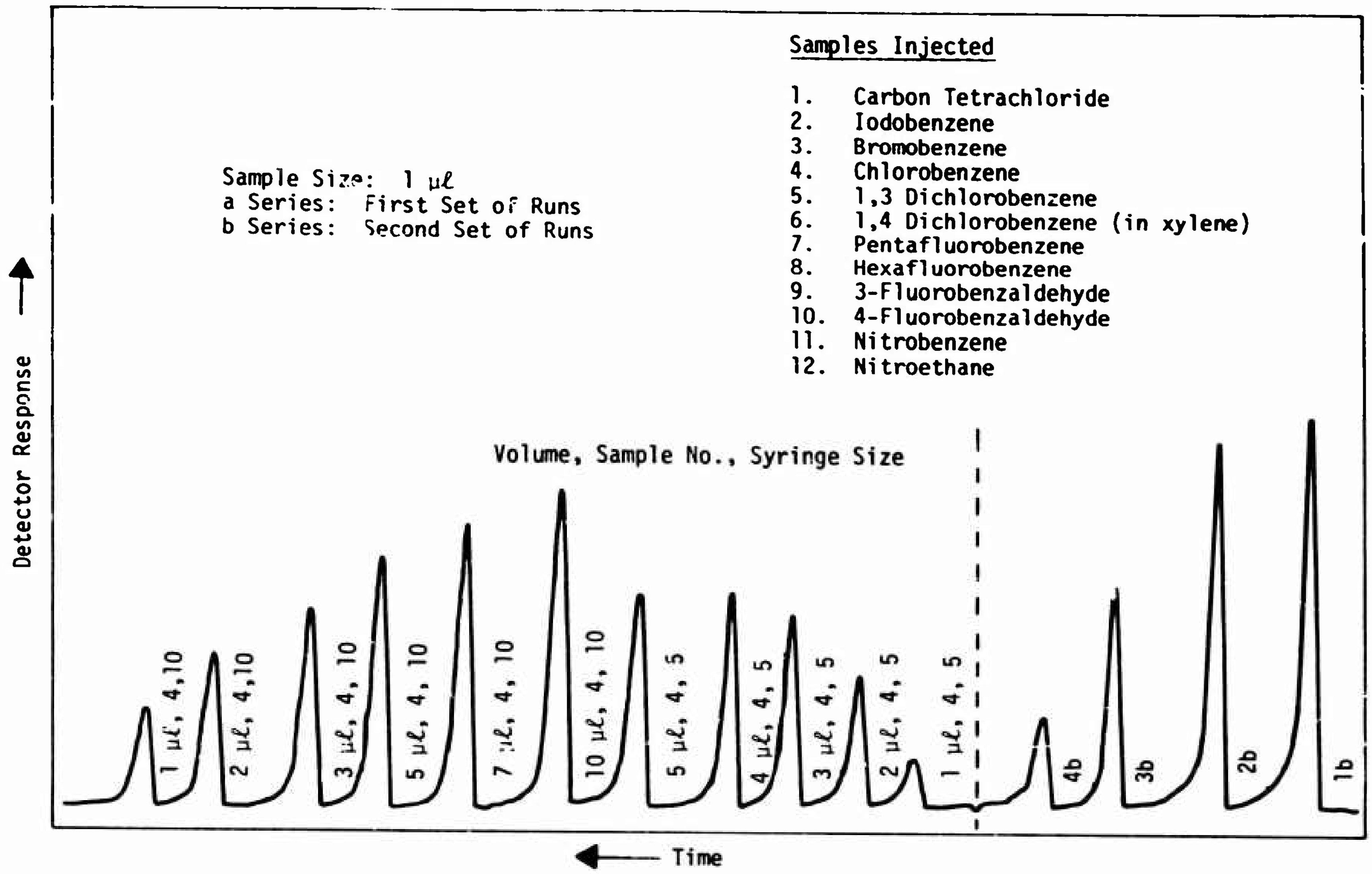


Figure 6. Chromatograms Obtained with Second-Generation Beta-Induced Luminescence Detector (2 of 2)

pesticides. Second, the response is dependent upon the chemical species employed. The response of a number of compounds relative to nitrobenzene is given in table 1. Although this means that quantification of the chromatograms requires determination of quenching factors for each substrate, it also provides a somewhat more straightforward means of qualitative identification. Third, compounds containing nitro groups are the strongest quenchers; they are typically a factor of two more effective than even the halogenated hydrocarbons. Again, good response was found in all cases at or below the 1-μg level. This is significant because, when compared to the detector volume, it is evident that solutes which fill far less than a single detector volume can be detected.

It seems reasonable that increasing the path length, while keeping the volume constant, will permit experimentation in both the luminescence and luminescence quenching modes. However, altering the path length of this detector is exceedingly inconvenient, so a third-generation detector with a variable path length is being designed.

ELECTROCHEMICAL FLOWTHROUGH

The goal in the electrochemical aspect of this project was to explore both general and specific electrochemical flowthrough detectors for metal ions. As a result of some very interesting pilot studies involving a solid-state, silver-ion conductor, work was concentrated on a specific silver-ion detector. A compound with the formula $Ag_6I_4WO_4$ is an ionic conductor at room temperature. Conductivity is due exclusively to the migration of Ag^+ through the crystal lattice. Efforts were directed toward developing a specific silver-ion electrochemical detector based on this observation. Various approaches were used.

Silver iodide-tungstate was prepared in the following manner. Silver tungstate was prepared by precipitation from equimolar solutions of sodium tungsten oxide (Na_2WO_4) and silver nitrate ($AgNO_3$). The precipitate was filtered, washed, and dried in a vacuum at 120°C. Silver iodide (AgI) was prepared and dried in the same way. Stoichiometric amounts of silver tungsten oxide ($AgWO_4$) and AgI were carefully blended and annealed in a dark furnace at 400°C. The fused material was then reduced to a powder by grinding.

Table 1. Response of Beta-Induced Luminescence Detection System to Nitrogen- and Halogen-Containing Organic Compounds

Liquid Sample	Relative Height $\left[\frac{\text{Intensity (sample)}}{\text{Intensity (nitrobenzene)}}\right]$
nitrobenzene	1.00
m-nitrotoluene	0.98
o-nitrobenzene	0.96
o-fluorobenzaldehyde	0.75
nitroethane	0.73
p-fluorobenzaldehyde	0.67
iodobenzene	0.60
o-dichlorobenzene	0.40
2-chloro-6-methoxypyridine	0.38
quinoline	0.38
bromobenzene	0.37
pentafluorobenzene	0.23
chlorobenzene	0.19
4-picoline	0.19
hexafluorene	0.18
p-dichlorobenzene	0.13
N-methylpiperazine	-0.03

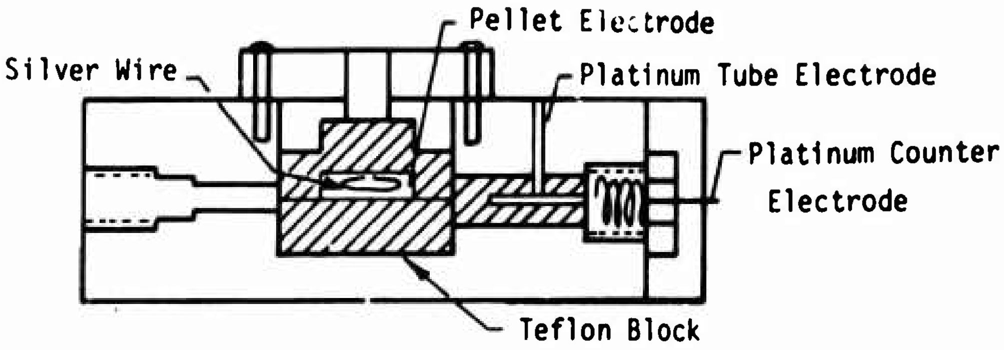
Note: All samples consisted of 4 µℓ of the liquid; retention time ranged between 8 and 12 min; and peaks were broad due to slow elution.

In one configuration, pellets of $Ag_6I_4WO_4$ were prepared by compressing the powder in a press to 20,000 psi. A pellet was incorporated in the detector as shown in figure 7. The saturated calomel electrode was protected by a saturated potassium nitrate (KNO_3) second junction. Maintaining a constant reference solution and varying silver-ion activity in the test solutions under potentiometric conditions resulted in a Nernstian response with a dynamic range of more than four orders of magnitude. When used to measure potential changes caused by the flow of trace amounts of Ag^+ past the detecting crystal, it gave a sub-micromolar response.

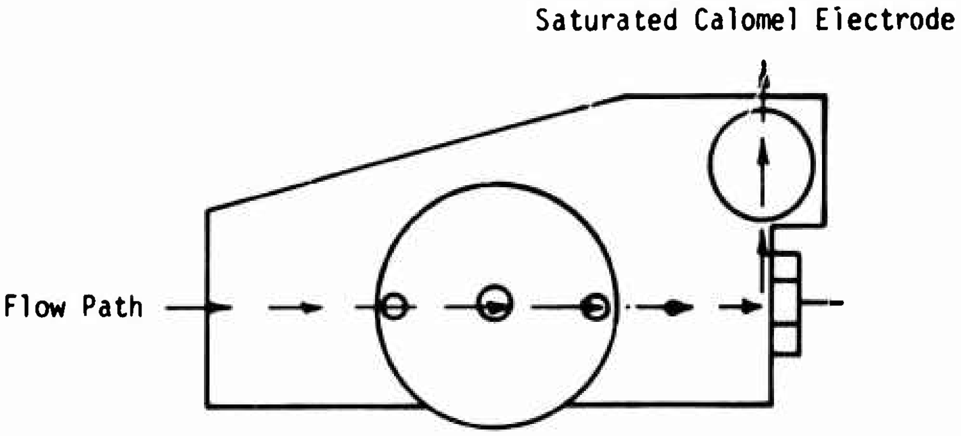
A second concept currently under investigation is the use of a $Ag_6I_4WO_4$ pellet as a membrane capable of transporting only Ag^+ (fig. 8).The idea is to impose a potential upon the electrode in such a way that conduction of Ag^+ across the membrane is impossible unless Ag^+ occurs on the test side, at which point a large drop in resistance across the membrane occurs. This drop, detected by standard instrumentation, measures the Ag^+ present in the flowing stream. The electronics for this system are shown in figure 9. The potential is set up across the detector, and the current flowing when Ag^+ is present on the test side is measured as a voltage at the output of one of the 118J operational amplifiers.

Finally, a three-electrode system was constructed. The detector is a working electrode held at a specific, but variable, potential in order to respond to electroactive components as they pass by. The conventional potentiostatic system is used with an auxiliary electrode and a calomel reference electrode. The detector and the amperometric and potentiometric mode circuits are shown in figure 10. A preliminary specific silver-ion working electrode was constructed by mixing $Ag_6I_4WO_4$ powder in just enough Duco cement to thoroughly wet the powder, dipping a silver wire into the mixture, drying the electrode, and repeating the process until an even, thin layer of electrode material was built up.

Tests made with both static and flowing systems confirmed a high response to Ag^+ and no response to a large number of other substances including CH_3OH, Cr^{+3}, Na^+. K^+, and NO_3^-. Chromatograms of the Ag^+ response are shown in figure 11. Sample sizes varied from 1 to 4 $\mu\ell$ of 5×10^{-4} m $AgNO_3$, or approximately 1 μg. Recorder sensitivity was not at its highest, and the detecting circuit was rather crudely breadboarded. However, improvements in construction should increase the sensitivity, perhaps into the low nanogram region.



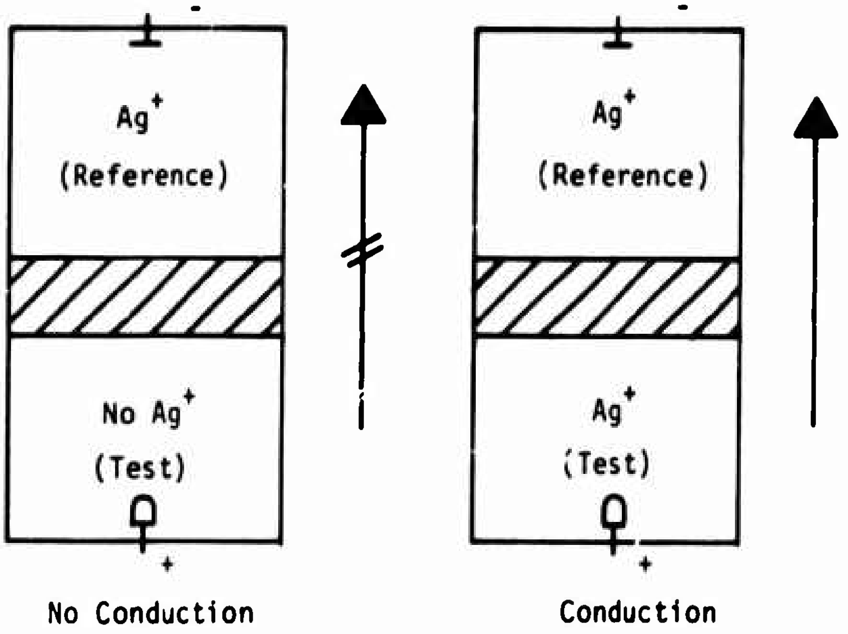
Side View



Top View

Note: This device permits simultaneous solid-electrolyte and voltammetric detection. (Initial studies were on the silver ion.)

Figure 7. Electrochemical Flowthrough Detector



▨ Crystal of Room-Temperature, Ionic-Conducting Material

Figure 8. Solid-State Conduction System for Silver-Ion Detection

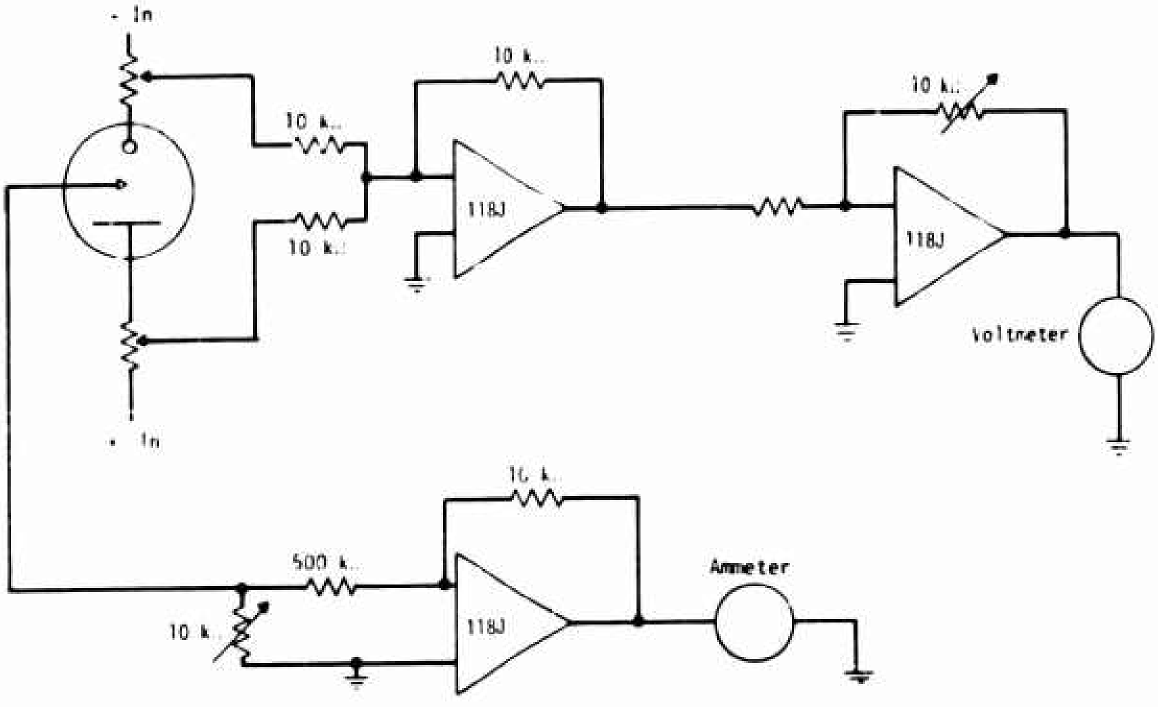
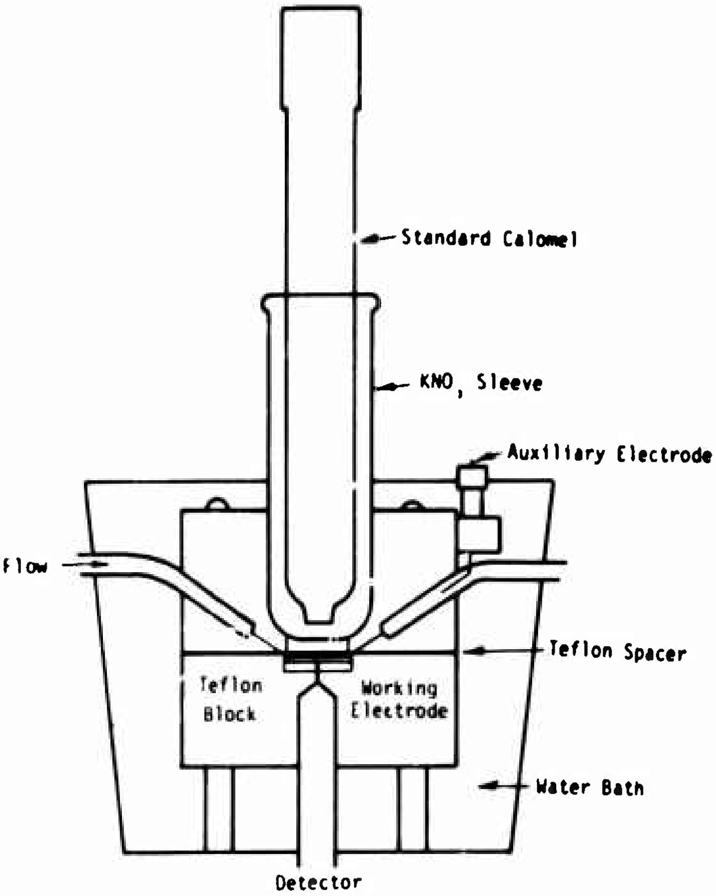
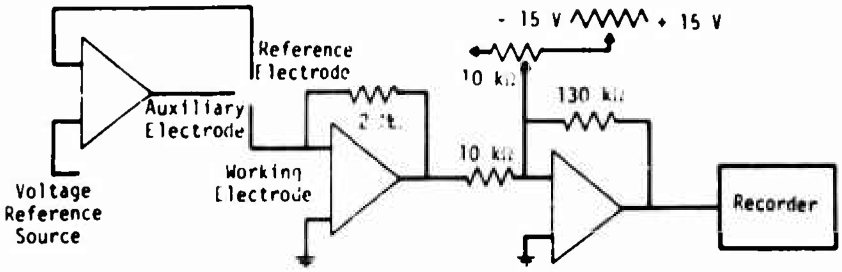
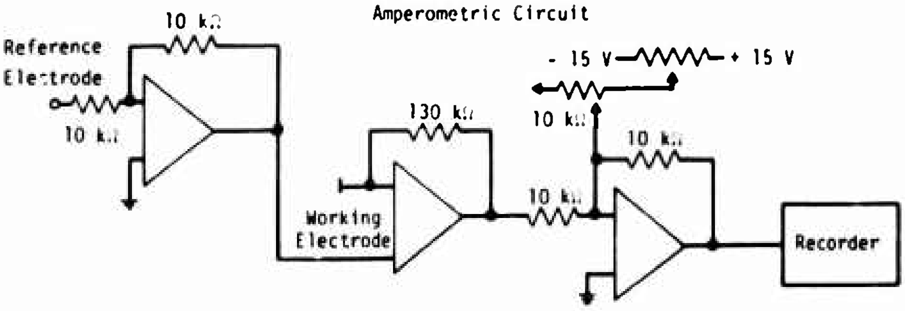


Figure 9. Electronics for Electrochemical Three-Electrode Detector





Amperometric Circuit



Potentiometric Circuit

Figure 10. Electrochemical Three-Electrode Detection System

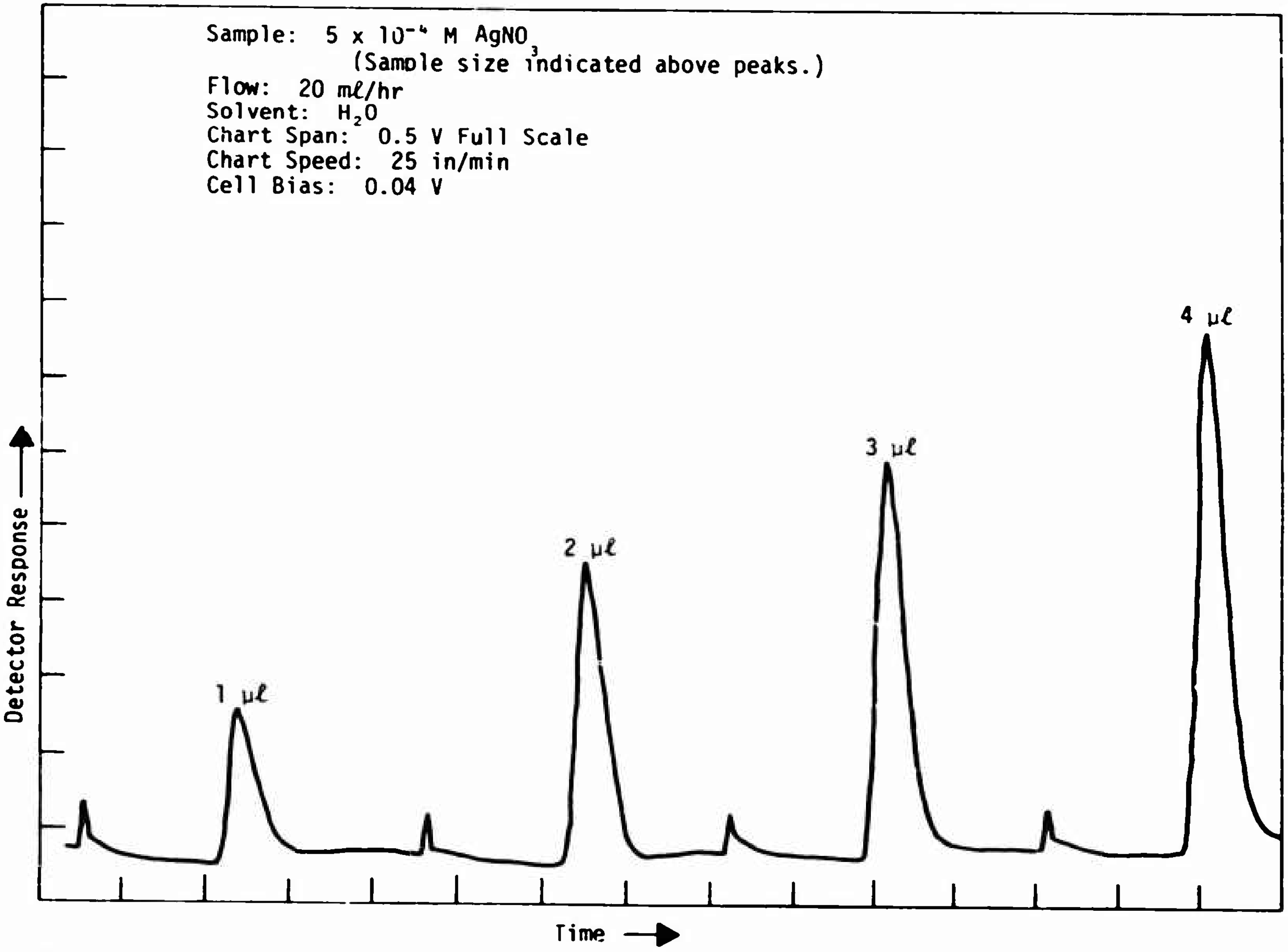


Figure 11. Chromatograms Obtained with Electrochemical Three-Electrode Detector

SECTION 4

COLUMN AND CHROMATOGRAPHIC SYSTEM DEVELOPMENT

A Varian 4100 HPLC system was transferred from AFWL and installed in the CERF laboratory. This system was tested and calibrated with nitroanilines in aqueous methanol solutions (70 pct by volume methanol) on a silica column.

Some preliminary experiments directed toward exploring the potential for separations of metal ions by the HPLC technique were performed. The concept being tested is the formation of complexes with complexing agents such as EDTA which have strong absorbances in the ultraviolet region so that the appearance of metal-complexed EDTA can be ascertained with the standard ultraviolet absorbance detector as it emerges from a liquid chromatographic column. Experiments with 0.75 $\mu\ell$ of 0.01 m EDTA injected into the column and chromatographed gave the response shown in figure 12. This figure shows the response to 7.5×10^{-9} moles of EDTA. The peak could be attenuated by a factor of 50 and still be discernible over the baseline. Next, solutions of $AgNO_3$ in an excess of EDTA were chromatographed; a clear separation of the silver-EDTA complex from EDTA was observed. This points to the potential of using this method to separate metal ions in the conventional HPLC system.

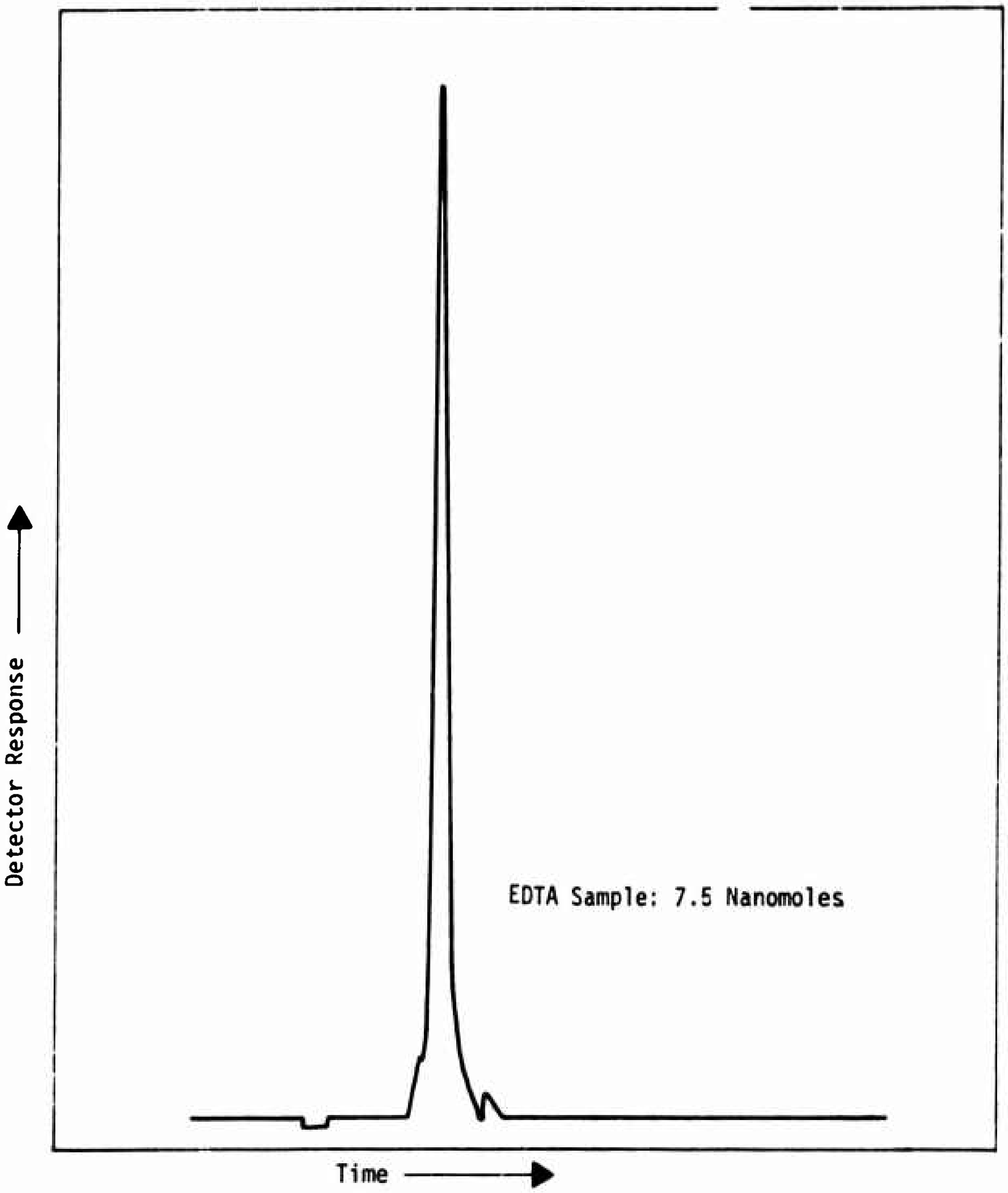


Figure 12. Chromatograms Obtained for EDTA Using Varian 4100 HPLC

APPENDIX
LITERATURE SURVEY LISTING

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