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Spectrometric detection systems for gas chromatography (GC) based on gas phase ultraviolet absorbance and fluorescence measurements have been developed and evaluated in this research. Polynuclear aromatic compounds (PNAs) are selectively detected by these systems. Limits of detection for PNAs range from 4 ng to 90 ng for absorbance determinations, and from 3 ng to 400 ng for fluorescence determinations made at a fixed waveband. The spacial design and optical components have been carefully selected to be compatible with high-temperature gas-phase measurements (ca. 250°C) while preserving the resolution of the chromatographic separation and enhancing the sensitivity of the multimode spectrometric determinations.

Vapor-phase determinations of GC eluates are made directly as they leave the GC column in specially designed, heated flow-through detector cells with low volume and dead space. A long pathlength cell of 167 mm was used to enhance the sensitivity of the absorbance measurements. High-temperature, quartz fiber optic bundles transmit light to and from the detector cell, thus thermally isolating optical components.

There are two configurations for these GC detection systems, each advantageous for either quantitative or qualitative determinations, respectively. In the first, waveband selection is accomplished via optical filters. These simple and compact systems are particularly suited to precise and sensitive determinations of polynuclear aromatic compounds. The sensitivity and selectivity of the absorbance and fluorescence measurements vary with individual compounds. These differences also provide some qualitative information about eluates.

The second detection system configuration provides repetitive UV spectral information of GC eluates. This computer-controlled system scans a modified Czerny-Turner monochromator and can acquire up to two 62-nm spectra of eluates per second. Spectral information thus obtained may be used for identification purposes and to locate unresolved GC eluates. After compounds have been separated and identified, fixed waveband spectrometric data acquired at an absorption maximum may be used to determine concentrations.

SELECTIVE DETECTION FOR GAS CHROMATOGRAPHY BY ULTRAVIOLET SPECTROMETRY

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To my son, Aaron Coghlan,
who brings love and joy into each of my days
and helps me remember my priorities

To my friend, Robin Hardesty,
for her understanding support and comraderie,
and for constantly rearranging her life
to care for Aaron
in response to my erratic time schedules

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CONTRIBUTION OF AUTHORS

Debra L. Van Engelen	Developed ideas and instrumentation; implemented experiments to test ideas and instrumentation; evaluated experimental results and developed conclusions; presented results and conclusions in manuscript format.
Lawrence C. Thomas	Major Professor. Contributed many ideas, facilities, and a research environment
Edward H. Piepmeier	Major Professor. Contributed many ideas, facilities, and a research environment
Anthony K. Adams	Contributed ideas and collaborated on the development of the fluorescence measurements and the optical filter/optical filter detection systems

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SELECTIVE DETECTION FOR GAS CHROMATOGRAPHY BY ULTRAVIOLET SPECTROMETRY

I. INTRODUCTION

Gas chromatography (GC) and ultraviolet (UV) spectrometry are powerful analytical techniques which have each been used for the analyses of mixtures of polynuclear aromatic compounds (PNAs). Both techniques have the capacity to provide qualitative and quantitative information to help identify and determine the concentration of individual PNAs from a sample containing other aromatic compounds and potential interferants. However, due to the inherent limitations of each of these individual methods, analyses of complex samples of PNAs can be very time-consuming and expensive.

Capillary GC methods can be used to separate PNAs within analytical samples into individual eluates, provided the sample has been pretreated to reduce the number of compounds present. Identification and measurement of separated eluting compounds depend upon the selectivity and sensitivity provided by the GC detection system. For non-selective detection systems, extensive sample clean-up is often necessary and detection systems which provide qualitative information are often complex and costly.

The most commonly used GC detectors for the measurement of PNAs, such as the flame ionization (FID) or electron capture (ECD) detectors, can be very sensitive and yield precise quantitative measurements. These detectors are quite useful in applications for which individual sample eluates are sufficiently resolved by the GC separation, and the identity of the

components is either known or not required. These highly sensitive "universal" detectors become limited in applications for which complex samples cannot be resolved into individual components, or if the identification of GC eluates is required. In these cases, extensive sample preparation procedures may be needed to simplify the sample and reduce the number of overlapping chromatographic peaks. Even with elaborate sample preparation, auxiliary methods such as infrared (IR) or mass spectrometry (MS), are often used to properly identify eluates.

Conventional ambient-temperature UV absorption or fluorescence spectra are characteristic of classes of PNAs. However, because they possess little resolved vibronic information, they often do not allow for determinations of individual compounds in a mixture. Highly selective, high-resolution spectrometric techniques which obtain spectra under extreme conditions, such as Shpol'skii or matrix isolation fluorescence, may be used to identify individual PNAs in a mixture without the benefit of complete chromatographic separation. However, the sophisticated instrumentation required is usually cumbersome to use and incompatible with routine analysis as commercial instruments are not yet available.

Chromatographic detectors based on the absorbance or emission of electromagnetic radiation by the compounds in the sample have the combined advantages of chromatographic separation and spectrometric selectivity and sensitivity. Depending on the absorptions or emissions measured, the spectrometric detection may have enhanced sensitivity for specific functional groups or for particular classes of compounds. This enhanced selectivity depends upon the absorptivities of particular compounds at the wavelengths used. Thus, character-

istic absorbances may be used to selectively detect a few analyte peaks amongst many unresolved chromatographic eluates from a complex sample. The good selectivity of spectrometric detection may also be combined with other information such as GC retention time for purposes of compound identificaton. Therefore, the overall selectivity of the GC separation/spectrometric detection system may reduce the number of sample handling and purification steps required for analyses, or the need for supplemental analyses.

The selectivity of information provided by spectrometric detection systems may be enhanced by increasing the number of wavebands monitored, up to acquiring complete spectra over a wavelength region. For instance, the system may monitor several wavelengths simultaneously or scan wavelengths in a sequential manner. Therefore, measurements based on spectral information at several wavelengths combined with chromatographic separations may decrease the uncertainties in compound identification and help measure unresolved chromatographic peaks.

Another method which may greatly increase the amount of qualitative information available about individual eluates in a sample is the combination of selective data obtained from one detector with that obtained from another detector of substantially different selectivity. Because spectrometric detectors are usually nondestructive, they allow for both tandem or concurrent measurements made by different detector systems of varying sensitivities and selectivities.

An advantage of GC detectors based on the absorbance of light is that they may be designed to be relatively insensitive to changes in GC carrier gas flow rate, pressure and temperature, or not respond to column bleed. The result is a stable chromatogram baseline

even for temperature programmed applications. Moreover, solvent peaks may be eliminated if the detector is also insensitive to the injection solvent used.

The focus of this thesis work has been to design and develop detection systems for gas chromatography based upon ultraviolet spectrophotometry, thus taking advantage of the inherent selectivity and nondestructive nature of these measurements. These new detection systems overcome some of the interfacing and sensitivity limitations previously described for spectrophotometric GC detectors. Good sensitivities and limits of detection have been achieved, to low nanogram levels, through careful selection of wavebands, optical components, and photodetectors. The detector systems described herein have been designed to minimize chromatographic band broadening due to the detector, and effluents are detected in the gas phase directly as they elute from the column. Moreover, optical components have been thermally isolated through the use of optical fiber bundles. These improvements have resulted in ultraviolet spectrometric detectors that have good powers of detection while maintaining the temporal resolution of the GC separation.

In addition to carefully designing the UV absorption detection systems for making precise and sensitive measurements, PNA selectivities were also enhanced in several of these studies through the use of simultaneous UV fluorescence measurements. The combined absorbance/fluorescence system studies were also extended to provide computer-controlled, multiparametric measurements of both UV absorbance and fluorescence excitation spectra. These multidimensional measurements are useful for purposes of compound identifications and quantitative analyses.

The ultraviolet absorbance GC detectors are designed to selectively detect aromatic compounds. Advantages of the systems are demonstrated via analyses of polynuclear aromatic compounds (PNAs), which are generally considered toxicants of environmental concern.

A. Ultraviolet Spectroscopy of Organic Molecules

Ultraviolet (UV) absorption bands involve transitions from many overlapping electronic, vibrational, and rotational energy levels. This often results in broad spectral bands with little vibrational structure [1]. The ultraviolet region of the electromagnetic spectrum may be divided into two parts: the near ultraviolet region from about 180 nm to 350 nm, and the far (vacuum) ultraviolet region below 180 nm [2].

In the vacuum ultraviolet occur very high energy transitions which usually involve the ionization of molecules (i.e. Rydberg bands). Saturated hydrocarbons are usually transparent down to about 170 nm. In this region, they exhibit $\sigma \rightarrow \sigma^*$ transitions of slightly less energy than the strong absorption bands that result in ionization. Substituted saturated hydrocarbons containing non-bonding electrons, such as alcohols and ethers, exhibit an absorption band at slightly longer wavelengths up to 190 nm due to $n \rightarrow \sigma^*$ transitions. Many other molecules such as water, ammonia, and oxygen, absorb the high energy light in the far ultraviolet region.

Olefins, except ethylene, exhibit broad absorbance bands in the region from 175 nm to 195 nm with high molar absorptivities (e.g. $\epsilon = 10^4$ L cm^{-1} mol^{-1}). These are attributed to $\pi \rightarrow \pi^*$ transitions from their double bonds. Conjugated dienes exhibit broad, intense absorption bands around 215 nm [1,2].

Other chromophores which contain double bonds such as the carbonyl or nitro groups, absorb in the near ultraviolet region. Substituent groups on unsaturated compounds which contain non-bonded electrons such as alcohols, amines, or halogens act as auxophores. Although these substituents do not absorb in the near ultraviolet themselves, they cause shifts in absorbance maxima and also increase the absorptivity of chromaphores through resonance or inductive effects [1,2].

Aromatic compounds have three absorption bands in the UV region between 180 nm and 410 nm which result from $\pi \rightarrow \pi^*$ transitions. As the number of condensed rings increases, these three bands shift to longer wavelengths. Clar [3] devised a classification scheme for these three transitions and called them α, p or β bands. These aromatic $\pi \rightarrow \pi^*$ bands are summarized below and examples are given in Table 1. The following general observations are useful for the spectroscopy of PNAs, but exceptions exist where bands are missing or the order is changed [4]:

i) α-bands are weak absorbing transitions, $\epsilon = 10^2 - 10^3$ L $cm^{-1}mol^{-1}$, which occur at the longest PNA absorption wavelength and often show complicated vibrational structure.

ii) p-bands are moderately intense, $\epsilon = 10^4$ L cm^{-1} mol^{-1}, and occur between the α- and β-bands, usually having a very regular vibrational structure.

iii) β-bands are very strong transitions, $\epsilon = 10^5$ L cm^{-1} mol^{-1}, at shorter wavelengths than α- and p-bands and have little vibrational structure.

The spectrometric detectors described herein use UV light in the region from 210 nm to 310 nm. The absorption bands detected for polynuclear aromatic compounds may be either α, p, or β-bands, depending upon the size

Table 1. Wavelength maxima (nm) for absorption bands of aromatic hydrocarbons due to $\pi \rightarrow \pi^*$ transitions [5]

Compound	β-bands	p-bands	α-bands
benzene	183	207	264
naphthalene	221	289	315
anthracene	255	379	---
2,3 benzanthracene	274	471	---
pentacene	310	576	428
phenanthrene	255	295	345
chrysene	267	319	360
1,2 benzanthracene	290	359	385

and substitution of the compounds. These detectors, of course, may also detect other chromaphores which absorb in the near ultraviolet.

Many PNAs also exhibit fluorescence. These large, relatively rigid molecules have few low-energy vibrations. Therefore, less absorbed energy is converted to vibrational energy by internal conversion allowing fluorescence to be significant [1,2,4]. However, certain substituents such as the nitro group will quench fluorescence by predissociation.

1. Influence of Molecular Environment upon Spectra

Most published UV absorbance and fluorescence spectra of aromatic molecules have been made for analytes in solution, in which the solvent molecules influence the environment surrounding the molecules of interest. In liquid solution, the absorption and fluorescence spectra are usually shifted to lower energies. There is also usually an increase in absorption intensity, and spectra are more diffuse than vapor phase spectra [4]. Figure 1 illustrates some of these phenomena for benzene in the vapor phase, and in a condensed phase cyclohexane solution. The influence of the solvent increases with increased strength of solvent-solute interactions. For aromatic compounds in solution, the bathochromic shift (to longer wavelengths) and vibrational band broadening are more pronounced in aromatic or polar solvents.

Absorption and fluorescence excitation spectra usually have wavelength dependence and essentially the same vibrational structure [4,6]. At low pressures, fluorescence transitions from the second singlet state (S^2) may be observed because of reduced numbers of collisions and loss of energy by internal conversion

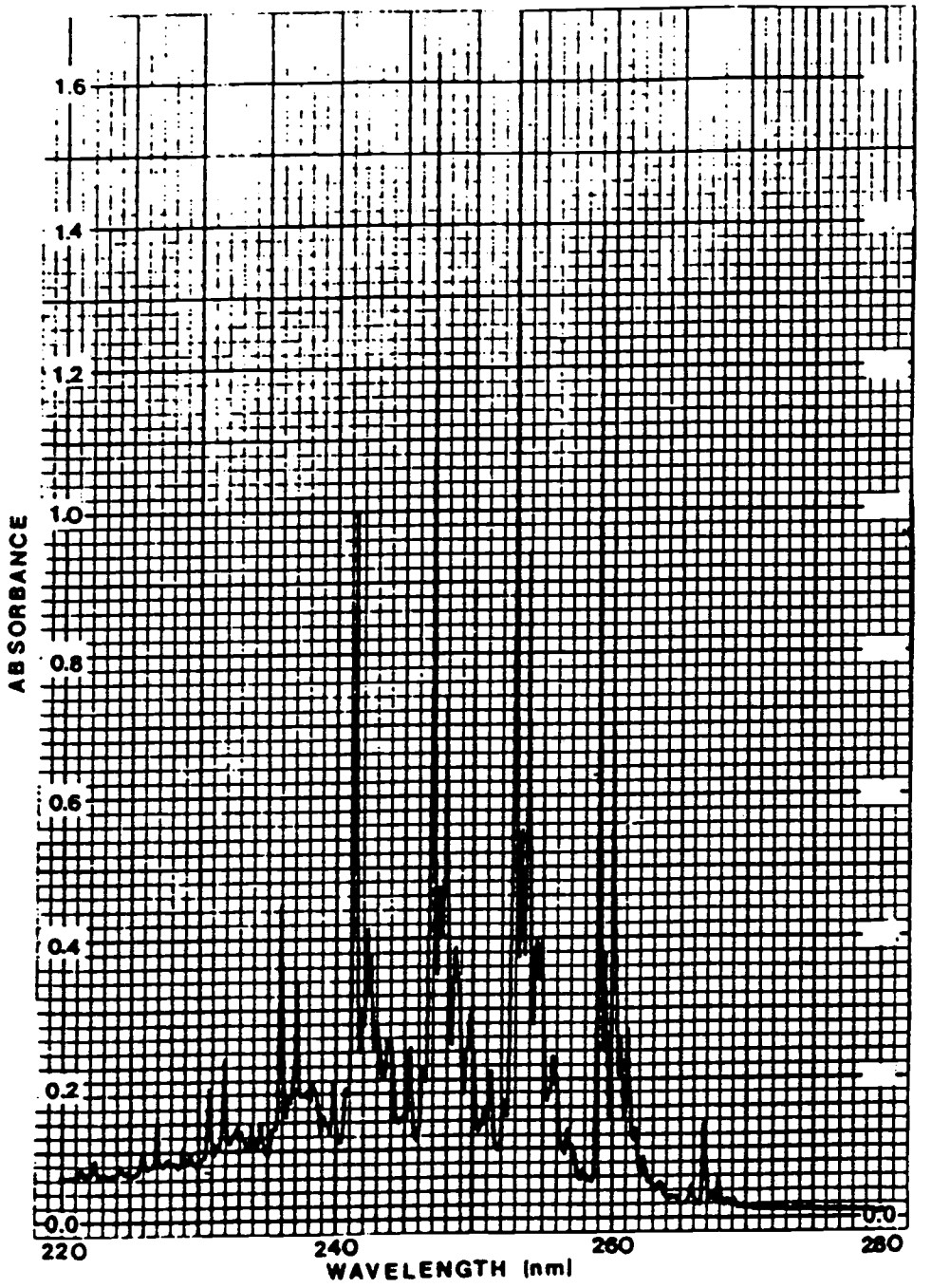

Figure 1. α-band spectra of benzene a) vapor; Slitwidth = 0.05 mm; Reciprocal linear dispersion, Rd = 0.6 nm/mm at 200 nm, 1.5 nm/mm at 250 nm, and 3.0 nm/mm at 300 nm; Rd is the range of wavelengths spread over a unit distance in the focal plane of the monochromator

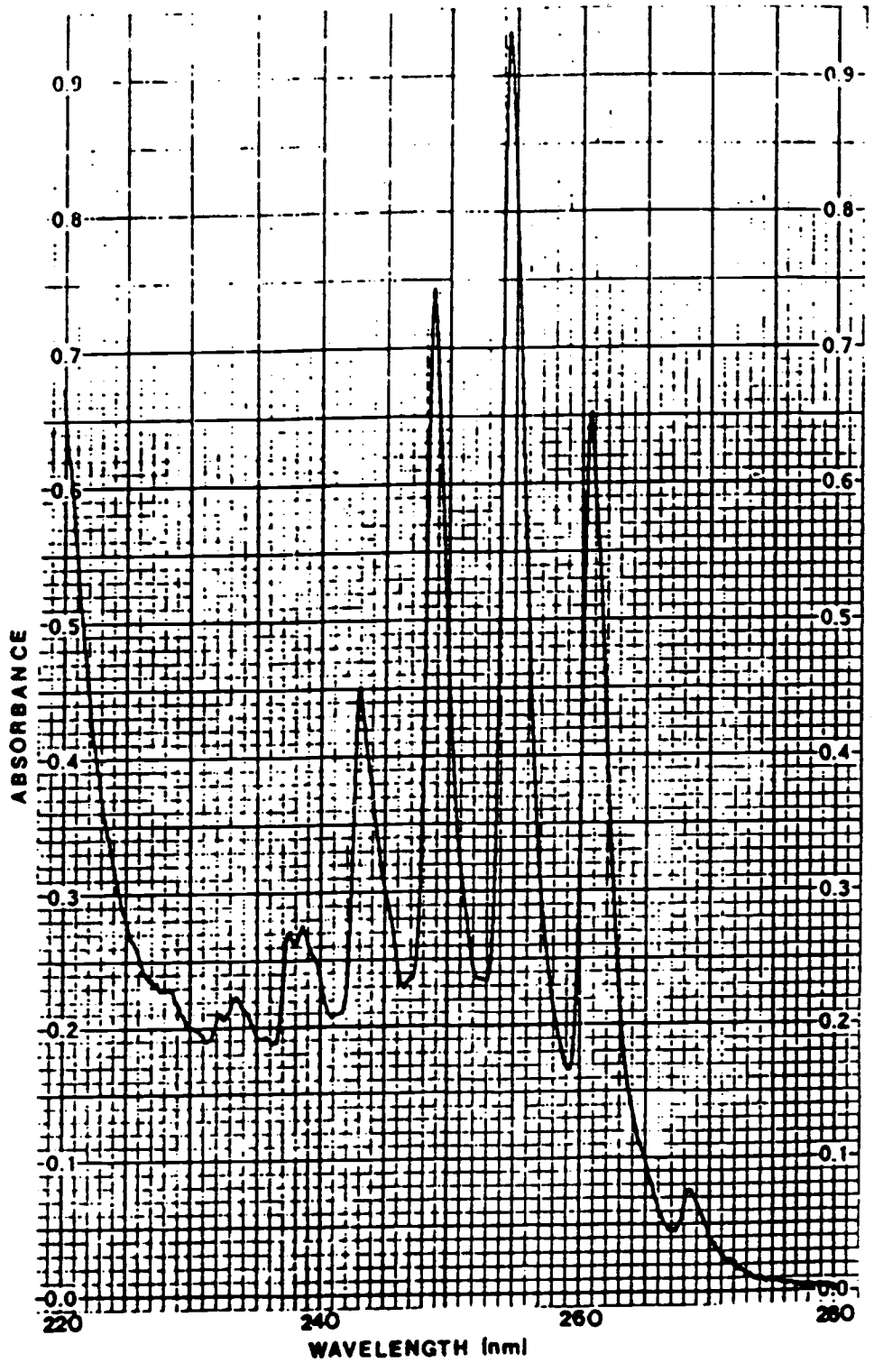

Figure 1. (cont.) b) condensed phase benzene in a solution of cyclohexane

[4,6]. Transitions from the S_2 state occur primarily with higher energy excitation radiation and the relative magnitude of the absorption bands in the fluorescence excitation spectra shift accordingly. In general, broadening of the absorption peaks is expected with increased temperature and pressure as the kinetic energies and collisional frequencies of molecules increase.

The fluoresecence and excitation spectra of naphthalene [7], anthracene [8], pyrene [6,9] and other PNAs [10] in the vapor phase have been studied at low pressure as a function of excitation energy. As the excitation radiation is shifted from a range of about 350 nm down to 210 nm, the resultant emission spectra increase intensities at longer wavelengths and exhibit essentially a complete loss of vibrational structure. The fluorescence emission bands of these large molecules have full-widths at half maximum (FWHM) of about 50 nm when excited by wavelengths 250 nm or shorter [6-10].

The intensity of the fluorescence excitation spectrum of anthracene at low pressure (0.10 mm) is markedly reduced as the temperature is increased from 170° to 300°C [7]. This is due to an increase in the probablity of radiationless transitions which compete with fluorescence emission at these temperatures and wavelengths. Similarly, a strong, temperature-dependent quenching of fluorescence has been observed for other aromatic compounds [10].

When PNAs are in a matrix of a second gas such as oxygen or nitric oxide, fluorescence intensity is quenched. At low pressures, other non-quenching gas matrices may increase the quantum yield of aromatic vapors [4,12]. Foreign gases also produce a red-shift with increasing partial pressure in both the absorption and fluorescence spectra [12,13]. The fluorescence

properties of PNAs have been studied at different temperatures and in different gas matrices by laser-induced fluorescence [14,15].

B. Polynuclear Aromatic Compounds (PNAs)

Polynuclear aromatic compounds (PNAs) were chosen as models for these UV spectrometric GC detection systems, in part, due to their absorption and fluorescence characterstics in the near ultraviolet region. In addition, the development of these analytical techniques for the identification and measurement of PNAs have important potential applications. Determinations of PNAs have been the focus of considerable effort by the scientific community in recent years because of their adverse impact on the environment and health.

Many PNAs have known or suspected carcinogenic and mutagenic capabilities in biological systems and are ubiquitously present in the environment [16,17]. PNAs have always been produced by natural sources in the environment, including forest and prairie fires, volcanic activity and synthesis by some plants and microrganisms [16]. They are also formed through the process of carbonization which synthesizes oil and coal within the earth's surface over millions of years at low temperatures, around 200°C [17]. Contemporary concern about PNAs results from increasing levels of xenobiotic PNAs in the environment due to combustion processes in industrial societies. Analyses of drilling core samples taken from a lake in Europe indicate a five-fold increase in the levels of carcinogenic PNAs from the turn of the century until 1965 [18]. Many other studies corroborate this increase [16,17].

Complete combustion or pyrolysis of organic matter produces only carbon dioxide and water. However, in

solids and liquids, it is impossible to avoid oxygen deficiency within microregions of the combusting material. Incomplete combustion results in production of a wide variety of chemical species including PNAs. Formation of PNAs occurs predominately at lower combustion temperatures (e.g. 1000°C) and primarily via fractions containing two-carbon chain structures which yield unsubstituted base components [17]. Typically a combustion process may yield approximately one hundred different PNAs including some heterocyclics containing oxygen, nitrogen, and sulfur. The combustion profile (i.e. number and relative abundance of PNAs) depends more upon the combustion temperature rather than the type of material burned [17]. However, some materials produce greater total amounts of PNAs than others.

Although many PNAs many be formed during combustion processes, only a small fraction of these are carcinogenic or mutagenic. Moreover, the biological activities of these compounds is very complex and difficult to assess. Although many PNAs are innocuous by themselves, it has been shown that some PNAs may be biologically activated by enzymatic reactions to epoxides and other metabolites which are carcinogenic and mutagenic [19]. In addition, PNAs and PNA metabolites may undergo further reaction or decomposition by photochemical oxidation to produce other carcinogenic products. There is evidence that some PNAs may react with nitrogen dioxide in the atmosphere to form mutagenic nitro derivatives [20]. High mutagenic activity has been associated with oxygen and nitrogen substituted aromatic compounds [21].

In global terms, 75% of the annual production of PNAs comes from forest fires and agricultural burning [16]. However, this figure is not necessarily indicative of the types or toxicities of the PNAs produced.

For example, benzo(a)pyrene is a known carcinogen which is primarily produced by the burning of fossil fuels by electric power plants and for domestic heating [16,17]. Other major sources of PNAs include refuse burning, coke production, emissions from internal combustion engines and jet aircraft exhaust [16,17]. Automobile emissions contribute to pollution in heavily-populated areas, especially those with frequent temperature inversions. PNAs produced by cigarettes are formed at lower temperatures and consist mainly of alkyl substituted aromatics [16,17].

Benzo(a)pyrene (BaP) occurrence in the environment has been extensively studied over the past twenty years and many estimates of the total quantities of hazardous PNAs produced are based on these measurements [21]. The estimated annual release of BaP from fossil fuel combustion is 4.6 million kilograms [22]. The concentrations in environmental samples vary depending upon the distances from known sources. For example, in the United States, BaP levels in air range from 0.01 to 1.9 $\mu g\ m^{-3}$ in non-urban areas up to 0.1 to 61.0 $\mu g\ m^{-3}$ in urban areas [16].

Typical BaP concentrations in soil are around 1000 $\mu g\ kg^{-1}$, but may be as high as 100,000 $\mu g\ kg^{-1}$ near known sources of BaP production [16]. Usually, the concentrations found in vegetation are lower than the soil in which it is growing [16]. The typical range for BaP in vegetation is from 0.1 to 150 $\mu g\ kg^{-1}$. Plants with large leaf surface areas may have very high PNA levels, and washing removes no more than 25% of the contamination [16]. Kale plants from various areas in West Germany had BaP levels of 1 to 134 $\mu g\ kg^{-1}$. Water sampled from various areas in West Germany had BaP concentrations from 1 to 10 $ng\ L^{-1}$. Smoked meats

usually have concentrations in the range 0.2 to 0.9 μg kg^{-1} [16,17].

Measurements of total PNAs or BaP alone are not sufficient to determine their toxicologic hazard to biological systems. BaP relative abundance may vary widely, representing from 1-20% of the total number of carcinogenic PNAs [21]. Also, PNAs vary widely in their carcinogenic or mutagenic activity, even in isomeric pairs. For instance, although benzo(a)pyrene is a known carcinogen, benzo(e)pyrene has not been shown to have carcinogenic activity. Thus, previous studies which have not distinguished between closely related PNAs or isomers do not indicate the true environmental and biological implications in their results [21].

1. Methods of Determining Polynuclear Aromatic Compounds (PNAs)

Due to the potential risks to human health and other biological systems, PNAs are routinely measured in environmental samples by the Environmental Protection Agency (EPA) and other laboratories which monitor levels of toxic substances in the environment. The EPA has established 129 compounds as priority pollutants of water and uses critical concentrations of these compounds as a basis for judging water quality. Sixteen of these priority pollutants are PNAs. These compounds range in size from naphthalene with two fused benzene rings and a molecular weight of 136, up to compounds with five fused rings and molecular weights of 279 [23].

Numerous and varied methods have been developed for PNA determinations in very complex samples. Usually these methods require several sample clean-up steps to separate PNAs from potentially interfering compounds. Often a contaminated sample will contain μg L^{-1} (ppb)

levels of PNAs and mg L^{-1} (ppm) levels of interfering compounds. Thus, separation of PNAs from potential interferants in a mixture may be difficult due to their wide range of volatilities and solubilities.

Methods for determining PNAs in complex samples usually include a sample collection procedure, extraction and sample enrichment steps, and an analytical measurement of the PNAs in the simplified and concentrated analytical sample. Sometimes the PNAs in the analytical sample are measured by a multicomponent procedure which is highly selective (i.e. specific) for individual compounds, but usually the quantitation of individual PNAs is carried out in conjunction with a chromatographic separation. Currently, the most popular methods for determining PNAs in analytical samples are separation by capillary gas chromatography followed by detection with a flame ionization detector (FID) or a mass spectrometer (MS). The FID is used primarily for precise quantitative measurements while the mass spectrometer provides reliable qualitative information, with limited data quality for quantitative measurements.

The EPA's recommended protocols for monitoring PNAs are representative of most techniques [24]. The protocols are intended to allow for determinations of PNAs with limits of detection approximating 10 ppb levels. The method consists of three major parts: (1) methylene chloride liquid-liquid extraction at pH 11, (2) Kuderna-Danish (K-D) concentration via selective volatilization, and (3) GC/MS measurements. Precision studies have been performed for different parts of this method [25]. These studies indicate the largest variations in the precision of results are caused by the K-D step. Moreover, higher molecular weight compounds had lower recoveries for the overall method than did lower molecular weight PNAs.

a. Sample Preparation

In this portion of this introduction are presented some of the wide variety of analytical procedures used for determination and identification of PNAs. This is not intended to be an exhaustive listing, but rather a representative survey to indicate the state-of-the-art.

(1) Sample Collection

In any method of chemical analysis, collection of a representative sample is as important as any other part of the total analytical procedure, although is it often given inadequate consideration. When PNAs are produced as combustion products, they are often in hot, highly reactive matrices which may be changed by different methods of collection. Moreover, they have a variety of volatilities and may react with heteroatomic species in the combustion atmosphere such as nitrogen dioxide, ozone, or sulfur trioxide.

PNAs on particles are collected by either high-efficiency glass-fiber filters or by electrostatic precipitation. Although it has been generally believed that PNAs are largely adsorbed onto particulate matter, some studies indicate that some may have considerable vapor pressures [26]. Thus, simple filtering by particulate collectors is not an entirely adequate method for collecting PNA samples from air. For example, if particles are collected beneath a flowing gas stream over a long period of time, PNAs may actually be vaporized from the particulate matter [18]. Vapor phase PNAs may also be collected on heated polymer resins or by collection in cold condensors [21].

PNAs in liquid samples and extracts may be oxidized and are particularly susceptible to oxidation when they

are in contact with large surface areas (e.g. on filters or chromatographic columns). In order to reduce effects of this problem, it is important to avoid exposure to light, to work under inert atmospheres, and freeze samples and extracts when stored.

(2) Extraction Techniques

The most common initial sample clean-up procedures used for PNA samples are Soxhlet extraction (6-8 hours), ultrasonic enhanced solvent extraction, or vacuum sublimation. Although one technique may be preferred for a particular compound or matrix, these methods all give comparable degrees of extraction of PNAs [21]. The extraction efficiencies of anthracene and benzo(a)pyrene from soil have been studied using either the Soxhlet or Polytron homogenizer (a high-velocity mixer/shearer) techniques [27]; they yield similar results.

The choice of organic solvent is critical to the efficacy of the extraction and solvent selection depends largely on the sample matrix. Some matrices such as fats and oils are completely soluble in an organic solvent and present little problem. The choice of solvent becomes more difficult when PNAs are included in large complex particles such as animal cells or are adsorbed onto soot particles. Polar solvents such as alcohols, acetone, or aromatic alcohol mixtures may be more effective in breaking the charge-transfer complexes of PNAs adsorbed upon a surface. If the sample matrix is similar to the PNAs, aromatic hydrocarbons such as benzene or toluene are effective extracting agents. Methylene chloride is a useful solvent for biological materials or PNAs in water samples [17,21]. Sometimes complete saponification of the matrix with alcoholic KOH is necessary prior to extraction in complex samples such

as foodstuffs [17]. A study has been made which compares several extraction systems and the effects of extraction conditions [28].

(3) Sample Enrichment Procedures

PNA enrichment procedures ideally separate polynuclear aromatic compounds from all other substances in the sample matrix and yield analytical samples which are compatible in both form and concentration with the analytical method which is subsequently used for measurement. Enrichment procedures concentrate as well as isolate. Physiochemical properties which may be exploited to separate PNAs from interfering compounds include their hydrophobic character, aromaticity, size, volatility, adsorptive properties and/or possibly Lewis acid or base reactions in the case of heterocyclic PNAs. Typically these steps are the most difficult and time-consuming part of an analytical method. The method by which final determinations of PNAs are made will require fewer enrichment steps if it is very selective and sensitive for PNAs.

Solvent extractions may be used for PNA sample enrichment [17,21,27]. Fractional distillation has also been used as an initial step to separate fractions in samples such as complex coal liquids [29]. Kuderna-Danish and other evaporative techniques are popular methods for concentrating extracts.

Thin layer chromatography on both normal and reverse-phase coatings may be used to clean-up several samples at once. However, it has the disadvantages of low capacity and sometimes causing degradation of the samples [21].

Several liquid chromatography (LC) methods are commonly used to isolate and concentrate PNAs in

analytical samples prior to separation and analysis by gas chromatography, usually with mass spectrometry detection (GC/MS). Silica gel or alumina columns are often used for rough separations [29-35] although they can have poor recoveries and reproducibilities [21]. Repeated enrichment on different adsorptive columns was used to determine dibenzofurans and dioxins at part-per-trillion levels in soil samples [36].

Other purification procedures have used either gel filtration chromatography (GFC) on Sephadex LH-20 [35,37,39], amino bonded-phase LC columns [38,40], or high-pressure liquid chromatography (HPLC) with Sephasorb HP, or normal or reverse-phase columns [41,42] to fractionate PNA samples. Cation exchange chromatography may be used to isolate nitrogen or sulfur heterocyclic PNAs [43,44].

b. Measurement and Identification of PNAs in Analytical Samples

After the sample preparation procedures are completed, the next task is to measure and identify selected PNAs from a mixture which may contain up to several hundred multiring aromatic compounds as well as other potentially interfering compounds. If an analytical measurement exists which is very selective (i.e. specific) for individual PNAs analytes, or if the sample clean-up is effective and only a few PNAs and interfering compounds are present, individual compounds may be measured within the mixture using multicomponent or multiparametric analyses. However, analytical samples of PNAs are usually quite complex and available measurement methods lack the specificity to determine individual PNAs in the sample matrix. Therefore, measurement and/or identification of PNAs in mixtures

almost always includes a chromatographic separation followed by eluate measurements to provide quantitative and qualitative data via a suitable detection system.

The efficiency of a chromatographic separation is described by the Height Equivalent to a Theoretical Plate (HETP or H),

$$H = (L/5.54)(w_{(1/2)}/t'_R)$$

where L is the column length, the base, $w_{(1/2)}$ is the width at half height and t'_R is the retention time minus the transit time of the mobile phase [45].

A chromatographic method with an H-value of 20,000 plates is often sufficient to separate most PNAs in mixtures, although best results are obtained with much greater than 25,000 plates [17]. Capillary wall-coated open-tubular (WCOT) columns are usually employed to separate PNAs in mixtures because resolving powers of 70,000 to 600,000 theoretical plates are easily realized [17,45]. High-pressure liquid chromatography (HPLC) separations typically have between 5000 and 15,000 plates with maximum efficiencies around 20,000. Packed GC columns have similar plate heights and efficiencies as HPLC but longer columns, greater then 10 m, may have values greater than 20,000.

(1) Multicomponent and Multidimensional Analyses

Multicomponent analysis is the determination of individual compounds (components) within a mixture of compounds. Multidimensional analysis includes data from the measurement of two or more analytical signals generated by the same sample. Multidimensional measurements are useful for analyzing multicomponent mixtures of PNAs.

In order to simultaneously measure PNAs in a mixture, analytical signals must be used which are

sufficiently selective for individual analyte PNAs. Room temperature UV absorption and fluorescence spectra for PNAs are broad band spectra and, thereby, have too much overlap and too little vibrational structure to be useful for simultaneous multicomponent analysis. However, by careful selection of wavelengths and conditions (e.g. low temperature), spectra may be produced which are sufficiently selective to permit the determination of individual PNA components.

In solidified n-alkane solutions at low temperatures (i.e. 70 K), fluorescence emission bands have typical bandwidths less than 1 nm [46]. This Shpol'skii effect can produce high-resolution spectra which are characteristic of individual PNAs. These spectra may, therefore, be used to analyze multicomponent samples containing several PNAs [47-51].

Vapor phase mixtures in a large excess of inert matrix gas such as nitrogen may be condensed at temperatures less than 20 K. This process is called matrix isolation (MI) and, likewise, produces isolated PNA molecules which can be determined via their narrow bandwidth fluorescence emissions [52-54]. If the matrix is formed by an organic solvent, even narrower fluorescence bandwidths are sometimes obtained [55]. Similarly, rotationally cooled laser-induced fluorescence (LIF) produces spectra of PNAs which show vibrational structure [56]. Matrix isolation samples of PNAs may also be analyzed by Fourier Transform Infrared (FTIR) spectroscopy [57,58]. Fluorescence line narrowing spectroscopy uses a narrow-line laser to excite molecules in low concentrations at 4.2 K. This method has been used to identifiy PNA metabolites and DNA adducts of PNAs directly from a mixture [59].

Another method which has been used to analyze a four-component mixture of PNAs is to sinusoidally

modulate the fluorescence excitation and subsequently demodulate the phase-delayed emission. The extent of the phase-modulated delay depends upon the emitting species [60].

Room temperature phosphorescence of PNAs has been observed for individual PNAs when a sample is adsorbed onto a solid surface such as filter paper or silica gel [61,62].

Most multicomponent techniques use an analytical calculation method which was originally introduced by Sternberg et al. [63]. In this method, multicomponent data are analyzed in algebraic matrix form by a least squares method. Although, additional matrix manipulations have added to the capabilities of this technique, these calculations are practically limited to systems of four or fewer components [64-72].

This type of least squares technique has been used to simultaneously quantitate four-component mixtures of PNAs by their UV absorption spectra over carefully selected wavelength ranges. The data from the normal, first and second derivative spectra were used [73].

A three component mixture of vapor phase PNAs was measured by laser induced fluorescence. Calibration plots were made at five emission wavelengths and the data were analyzed by a least squares technique [74].

Matrix calculations for multicomponent analyses have been most extensively studied using multi-dimensional data obtained with a video fluorometer. A video fluorometer is a computer-controlled, spectral imaging instrument which acquires a fluorescence excitation-emission matrix (EEM) in as little as 16.7 ms. The EEM is a three-dimensional matrix whose entries correspond to the fluorescence intensity as a function of both excitation and emission wavelength [75-78]. This multidimensional imaging technique has

also been applied to phosphorimetry of PNAs [79,80].

Multicomponent analyses via EEM measurements have been advantageously applied to PNAs separated by HPLC. Although HPLC often lacks the separation capability to completely resolve eluting PNA peaks from a complex mixture, it will separate the mixture into chromatographic eluates which typically contain from one to four PNA components. HPLC effluents have been passed through the video fluorometer and quantitatively analyzed by employing EEM matrix calculations [81].

Other researchers have developed computational methods based on factor analysis and reiterative least squares analysis to resolve overlapping liquid chromatography peaks [82-84]. Repetitive UV absorption spectra are obtained for HPLC effluents by a diode array spectrometer. If the theoretical or tested chromatographic or spectral peak shapes are known, the number of components within a peak may be determined. These methods have been demonstrated through analyses of PNA mixtures [85].

Other simultaneous, multidimensional determinations of mixtures of PNAs have been made using measurements of fluorescence, photoacoustic and two-photon photoionization from liquid samples [86-88].

(2) High-Pressure Liquid Chromatography (HPLC)

Although high-pressure liquid chromatography (HPLC) may not have sufficient separation efficiency to resolve very complex mixtures of PNAs, it may be used if extensive sample enrichment procedures are performed or if the detection system is sufficiently selective for analyte PNAs. HPLC may also have some advantages over gas chromatography for certain mixtures, for example, if

the PNAs have a wide range of volatilities or lack thermal stability.

The most commonly used detectors for HPLC determinations of PNAs are based on UV absorbance or fluorescence measurements. These detectors are fairly selective for PNAs although certain interfering compounds may either fluoresce , phosphoresce, or quench fluorescence [21]. Chromatographic separations and measurements of up to 20 PNAs have been performed on HPLC columns with UV spectrometric detection [30, 89-93]. Additional selectivity may be obtained by monitoring the UV absorbance at two or more wavelengths [31,40,82,94]. Data from PNA separations by HPLC with UV absorbance detection have been combined with data from GC/MS systems to provide reliable identifica tions of PNAs in airborne particulates [41,95].

Other HPLC detectors which have been used to measure PNA samples include photoionization [96] and flame ionization [97], electrochemical detection with a glassy carbon working electrode [98], and mass spectrometry [100].

(3) Gas Chromatography (GC)

Often, analytical samples of Polynuclear Aromatic Compounds are very complex and the most commonly used method for their separation is capillary gas chromatography (GC) on wall-coated open-tubular (WCOT) columns due to their excellent efficiency of separation. Capillary columns which are 10 to 30 m long are usually sufficient for most samples containing 50 to 100 PNAs. Packed column GC may also be used with either simplified PNA mixtures or with highly selective detectors which do not detect coeluting non-analyte compounds. The resolution between isomers such as anthracene and

phenanthrene or benzo(a)pyrene and benzo(e)pyrene is commonly used to assess the effectiveness of a column.

The extent to which the stationary phase coats either the solid support or inner wall of the column is very important in GC separations of PNAs. PNAs are strongly adsorbed upon silica surfaces and with small samples of a nanogram or less, irreversible adsorptive losses and peak tailing may occur if the surface has not been properly inactivated [100]. Columns should be checked occasionally with special samples of known concentration to determine their sample limits of detection [100].

The GC stationary phases most commonly used for PNA separations are either non-polar coatings such as SE-30, OV-1, OV-101, or SP-2100, or slightly polar coatings such as SE-52 or SE-54. Polar stationary phases are not often used because of thermal instability, incomplete coating, and poor column efficiencies [21].

Retention indices computed with respect to n-alkanes are not particularly useful for predicting retention times or elution orders of PNAs on different GC columns. Therefore, a retention index system has been developed based upon PNA standards [101] with naphthalene having an index of 200, anthracene having an index of 300, and so forth. Factors which may be used to predict retention times and elution orders among PNAs are relative molecular mass, boiling points, connectivities [102], and shape.

Numerous studies have been made of the separation of PNAs with different GC stationary phases. In recent studies, the factors affecting retention times of polynuclear aromatic hydrocarbons have been studied for OV-101, SE-52, and OV-17 GC coatings [103]. The separation and retention indices of nitrated PNAs have been determined on a low-polarity SE-30 column [104] and on a

methylphenylsilicone, SE-52 column [105]. A mathematical model has been developed to describe the relationship between retention characteristics on a DB-5 column and the structure of chlorinated dibenzofurans [106]. A new biphenylpolysiloxane stationary phase was found to be highly selective for the separation of sulfur-containing heterocyclics [107].

c. Gas Chromatography Detectors used in PNA Analyses

The most commonly used GC detector for sensitive determinations of PNAs is the flame ionization detector (FID). Mass spectrometer (MS) detectors are often used when compound identification is necessary. The electron capture detector (ECD) may be even more sensitive for organic compounds than the FID but gives greatly different sensitivities for different PNAs. Other selective GC detectors which have been used for PNA analyses include special nitrogen sensitive detectors, the flame photometric detector (FPD), the Hall electrolytic conductivity detector (HECD), and the photoionization detector (PID). Selective spectrometric GC detectors based on the absorption of infrared or ultraviolet light have been used to detect PNAs, as well as fluorometric detectors. In the research described herein, spectrometric GC detection systems based on UV absorption and fluorescence have been developed.

The flame ionization detector (FID) is a fairly nonselective, or universal, detector which is routinely used for quantitative analysis of carbon-containing analytes [42,104,108-110]. The GC effluent is burned in a hydrogen/oxygen flame where carbon cations are produced. The ions pass between two electrodes with a potential difference of approximately 400 volts between them. The signal measured is current between the

electrodes, which is limited by migration of cations, C^+, to the cathode. The FID response varies in organic compounds, mainly depending upon the number of oxidizable carbon atoms [45]. Aromatic compounds have lower FID sensitivities than aliphatic compounds and typical limits of detection for PNAs are in the sub-nanogram range [100]. FID response factors for different PNAs have been determined [109].

Mass spectrometer GC detectors (GC/MS) are also widely used for PNA analyses [25,29,30-36,42,95,106,111-122]. The primary advantage of the mass spectrometer is that it may provide reliable analyte identifications. It's main limitations are that GC peaks must be resolved, it cannot differentiate between isomers, and it is rarely possible to identify the positions of ring substituents [21]. The MS types which are usually interfaced to a GC have been either magnetic sector or quadrupole mass filters. The sophistication of these MS detectors varies widely. Usually, only low resolution spectra are required and identification is often based mainly on the parent peak.

The elèctron capture detector (ECD) may sometimes be the most sensitive detector available for PNA compounds. The detector has two electrodes with GC effluent flowing between. One electrode contains a radioisotope which emits electrons which in turn produce secondary electrons in the GC carrier gas. Organic compounds containing electronegative groups, capture these secondary electrons which results in a reduction of current. Limits of detection may be as low as a femtogram for the ECD, but usually are no lower than a picogram for PNAs. The ECD has been used to measure PNAs [120,123], but the ECD is much more sensitive to some PNAs than others. This selectivity due to variable

sensitivities may be used for identificaton purposes. ECD/FID response ratios have been measured and were useful for distinguishing between isomeric PNAs [124].

Many nitrogen-containing PNAs are highly carcinogenic and mutagenic. Very sensitive detection down to 10^{-13} g s^{-1} of nitrogen-containing heterocyclic PNAs has been achieved through the use of a nitrogen-sensitive thermionic emission detector (TED), also called the alkali flame ionization detector [109, 125]. This detector uses a fuel-poor hydrogen plasma which primarily ionizes nitrogen or phosphorus containing compounds. A heated nonvolatile rubidium silicate bead is used to provide thermal energy.

Another GC detector which is selective for nitro-substituted compounds is the thermal energy analyzer (TEA) or nitric oxide selective pyrolysis/chemiluminescence detector. GC effluent enters a catalytic pyrolyzer which releases NO_2 from nitro-substituted organic compounds. The NO_2 is converted to the nitrosyl radical (NO), trapped in a cold trap, and is then reacted with ozone in the reaction chamber at reduced pressure to produce near-infrared chemiluminescence. The emitted light is detected by a photomultiplier tube. This detector may have limits of detection in the low picogram range and has been used to measure nitro-substituted aromatics, including some PNAs [126-128].

Another detector which is selective for PNAs containing a specific heteroatom is the flame photometric detector (FPD). The FPD is essentially a flame emission photometer and has been used to selectively detect sulfur-containing heterocyclic PNAs [107,129]. A serious limitation of this detector is that the emission signal is greatly reduced by any coeluting interferants.

The Hall electrolytic conductivity detector is used to detect sulfur and nitrogen containing compounds

[130]. Eluting heterocyclic compounds are pyrolyzed into ionizable species inside a nickel reaction tube. The resulting ionic products are then measured in a liquid stream flowing through a conductivity cell.

Another sensitive GC detector which has been used to monitor PNAs is the photoionization detector (PID) [131]. Radiation from UV lamps at 120 nm in the vacuum UV are used to ionize molecules, and the resultant current between two electrodes is monitored. When lamps of high energy such as 11.7 eV are used, this detector functions essentially as a universal detector. However, the PID may be selective for compounds with lower ionization potentials by using lower energy UV lamps. This detector is about an order of magnitude more sensitive than the FID and limits of detection may extend to the low picogram range.

(1) Spectrometric GC Detectors

Selective GC detectors based on infrared (IR) or ultraviolet (UV) absorption, or fluorescence, may be highly selective and also sensitive depending upon the spectral resolution and wavelengths chosen. The highly selective nature of these detectors means that only the compounds of interest may be detected even in the presence of other overlapping but undetected chromatographic eluates. This characteristic may be used to substantially reduce the amount of sample preparation necessary. The spectra of compounds may often be used to distinguish between isomers and identify the position of substitution in PNAs. In addition, the sensitivity of spectrometric detectors may allow identification and quantitation of GC effluents with one measurement, rather than requiring a different measurement to provide each type of information. The UV spectrometric GC

detection systems in this thesis are simple, compact and yet have limits of detection that are comparable to those of more sophisticated systems.

(a) Absorbance Detectors--Infrared

Infrared absorbance measurements of GC effluents may detect single functional groups or provide highly characteristic vibrational spectra of compounds. However, conventional dispersive IR instruments have not been extensively used as GC detectors for gas chromatography because of their low sensitivity (i.e., limits of detection greater that a microgram) and slow scan speed. Some applications of conventional IR monitor a single functional group [132]. Other applications have used GC/IR for the analysis of acids and bases from shale oil [133].

In recent years, the development of Fourier transform infrared instruments (FTIR) has greatly enhanced the usefulness of infrared absorbance GC detectors. In an FTIR spectrometer, all wavelengths are measured simultaneously, which greatly reduces the time required to acquire spectra and also improves the signal-to-noise (S/N) ratio. Also, the aperture of the interferometer may be quite large, thus increasing the light thoughput and resulting in greater sensitivity [134,135]. Other important developments which have improved sensitivities of FTIR detectors include the introduction of a more sensitive IR photodetector, the narrow-range mercury cadmium telluride (MCT) photodetector, and the construction of gold-coated borosilicate glass light pipes [136]. Limits of detection for GC/FTIR instruments are now routinely below the microgram level and may extend to the low nanogram level for some compounds.

Factors which affect the GC/FTIR signal have been studied, such as lightpipe dimensions [137-138], temperature [139], and optical considerations [135]. Recently, GC/FTIR has been used to analyze PNAs in coal combustion products [140], polymer pyrolysis products [141], and hazardous waste samples [142].

(b) Absorbance Detectors--Ultraviolet

Ultraviolet absorption GC detectors may be used to selectively detect aromatic compounds and compounds which contain certain chromophores such as the carbonyl group. Although UV spectra in the near ultraviolet region do not have a great deal of fine structure, they often have some distinctive features, particularly in the vapor phase. In addition, the positions of wavelength maxima depend roughly upon the number of fused aromatic rings in the compound. The molar absorptivities of absorption bands in the near and far UV are often of the order of 10^5 L $cm^{-1}mol^{-1}$ which can be one thousand times greater than those in the infrared. Therefore, good sensitivities and limits of detection are possible with thoughtful instrumental design and careful selection of wavelengths.

In 1962, Kaye first interfaced a gas chromatograph with a spectrophotometer [143]. Heated, flow-through cells with pathlengths of 1.0, 5.0 or 10.0 cm were used and maintained at the same temperature as the GC column. Actual temperature ranges used were not reported in the published work. GC effluents were transferred to the spectrophotometer through a heated 1/8-inch copper tube. Along this section of tube was a cold trap and, also, a bypass valve for the collection of GC peaks. Adsorptive losses of sample occured in the copper tube and adsorbed sample was washed through the cell with the solvent after each GC injection.

This first UV absorbance detector was used to measure naphthalene at 210.8 nm, which corresponds to an absorption maxima for this compound. The limit of detection for naphthalene at this wavelength was 40 ng and the linear range was over four orders of magnitude.

At wavelengths below 170.0 nm, almost all organic compounds exhibit very high absorptivities. Kaye's spectrophotometer was modified and flushed with nitrogen to function in this wavelength region. Studies were made in the far UV of olefins, and several normal and branched alkanes with the GC/UV system. The sensitivity of the UV absorption measurement was superior to a thermal conductivity detector (TCD).

The selectivity for different classes of organic compounds was demonstrated by fixed wavelength monitoring at wavelengths from 165.0 nm to 240.0 nm. Also, repetitive wavelength scans between 165.0 nm and 220.0 nm were made throughout the separation of a gasoline sample. The scan time was 20 seconds. The scanning detector was subsequently modified to provide repetitive 6-s scans between 160.0 nm and 210.0 nm [144]. Again, heated 1/8-in I.D. copper transfer lines were used to transfer the GC eluates to the spectrophotometer. This instrument had a spectral bandpass of 0.084 nm. The system was demonstrated by the separation of seven low molecular weight organic compounds including benzene.

Another early spectrometric detector for GC eluates was described by Merritt et al. This system was made specifically to monitor benzene and toluene at 235.7 nm in a process stream [145]. The process stream was sampled with a rotary disc liquid-sampling valve, and this aliquot was then separated on a stream-heated, packed column GC at 180° C. The GC eluates were transfered to the UV analyzer via unheated, pressurized

transfer lines. The ultraviolet analyzer had a low pressure mercury vapor lamp source which was split into reference and sample beams and was detected by vacuum phototubes. The 1/8-inch I.D. stainless steel cell was heated to 60°C and had a 8-cm pathlength.

Further developments of gas phase UV absorbance detectors for GC did not appear in the literature until about fifteen years later when Novotny et al. coupled a wide bore capillary GC column (32 m x 0.7 mm I.D.) to a commercially available UV-visible absorbance detector for HPLC [146]. The mode of transfer of GC effluent to the HPLC detector was not described. A 50-μL stainless steel cell was used. The temperature of this cell was not reported. Helium purge gas was added in a mixing tee between the GC column and detector cell to overcome dead-volume problems.

The limit of detection for naphthalene when monitored at 212 nm was 300 pg (S/N=3). Under identical conditions, the detection limit was 20 pg using the FID detector. Various applications were presented to demonstrate the detector including separations of lead-free gasoline and normal human male urine. Also, chromophores were prepared by chemical reactions of non-absorbing species so that they could be measured by the detector. This UV absorbance detector shows large differences in sensitivity and selectivity when monitored at either 220 nm or 260 nm. At 220 nm, the detector is much less selective and much more sensitive. At 260 nm, the reverse is true.

Recently, a UV absorption detector for packed column gas chromatography was reported which features a long pathlength absorption cell that reflects UV light within the cell [147]. The 2-mm I.D. cell is made of quartz with the inner surface plated with silver. It has an adjustable pathlength from 2 to 4 cm. The UV

source is a low pressure mercury lamp and the UV radiation is filtered by a Rb glass filter and focused on the cell by a crytal lens. The light is detected by a GD-5 phototube. The limits of detection for low molecular weight aromatic compounds are in the low nanogram range for this detector.

Other researchers have interfaced a packed GC column with a diode array spectrometer via heated transfer lines [148]. The GC column temperature was 90°C and the transfer lines and cell were heated to 70°C. The 1.5-cm I.D. detector cell had a large volume of 30 mL and a 12.5-cm pathlength. Helium make-up gas was added to the detector cell at a rate of 150 mL min^{-1}. The spectral region between 200 and 800 nm was repetitively imaged every three seconds or a separation was monitored at one wavelength. The sensitivities for this GC/UV system were determined for benzene and toluene. Limits of detection for these compounds were about 0.5 μg at the optimum wavelength for each compound which is comparable to the thermal conductivity detector. The spectra produced in this manner can readily distinguish between the three isomers of xylene.

(c) Fluorescence Detectors

Since many polynuclear aromatic compounds exhibit fluorescence and lower molecular weight aromatics do not, UV fluorescence detectors for gas chromatography provide a very selective and sensitive means of measurement for PNAs. Furthermore, by carefully choosing the fluorescence excitation and emission wavelengths, selective detection of one PNA is possible in unresolved chromatographic peaks of similar PNA compounds. This allows for selective detection even

among PNAs which fluoresce. Fluorescence may produce a more sensitive measurement than UV absorbance, especially in aromatics with three or more fused aromatic rings.

UV absorbance and fluorescence measurements have a number of advantages in common due to the nature of the spectrometric measurement. For instance, usually neither temperature nor flowrate changes produce baseline drift. By choosing a transparent solvent, solvent peaks are not detected. Column bleed from the stationary phase is usually not detected. Any carrier gas may be used as long as it does not quench fluorescence [149]. And, of course, spectral measurements may provide qualitative information characteristic of individual compounds.

An early GC fluorescence detector solvated GC effluents into a flowing liquid alcohol stream which subsequently entered in a flow-through cell in a commercially available spectrofluorometer [150]. Pesticides, PNAs, and methylenedioxyphenyl compounds were determined and limits of detection extended to the low nanogram range with this detector. However, this fluorescence detector was cumbersome, and extraction efficiencies could vary.

Later fluorescence detectors for GC have made measurements in the gas phase which requires less interfacing instrumentation than the previously described detector. The most common approach has been to transfer GC effluents along heated transfer lines to an intact commercial fluorometer with a suitable flow-through detector cell [151-156]. These detectors have been used to detect PNAs with limits of detection in the low to sub-nanogram range. Lower detection limits had been expected, but the high temperatures required to vaporize these large molecules decreases the

fluorescence intensity [151]. Fluorescence was also found to be dependent upon the carrier and decreased in the order N_2 > He > CO_2 > H_2.

One GC fluorescence detection system was used to make fast-scanned spectra at a scan rate of 150 nm s^{-1} in a commercial fluorometer [153]. Scans were made over a range from 200 nm to 800 nm in 5 s.

Instruments which select the fluorescence excitation and emission wavebands by optical filters have been used to determine PNAs [156]. Filter waveband selection allows a larger light throughput in applications where spectral information is not required. This in turn may increase the number of fluorescent compounds detected and improve detection limits.

Recently, gas phase fluorescence detection of PNAs separated by packed column GC was described by Thomas and Adams in a modified flame photometric detector (FPD) [157]. Excitation radiation from a deuterium source and monochromator was transfered to the heated FPD detector cell via quartz optical fiber bundles. Fluoresced light was filtered by optical filters and detected by a photomultiplier tube. The cell itself had a volume of 6 cm^3 and make-up gas was added to the column effluent to reduce the carrier gas throughput time and avoid mixing in the cell. Limits of detection for this simple, compact system were in the low to sub-nanogram range. Other advantages of this detector are that it detects gas phase effluents directly as they elute from the column with no heated transfer lines and provides sensitive detection without the need for bulky and sophisticated spectrometric apparatus.

Studies have been carried out to determine instrumental effects for fluorometric GC detectors on the limits of detection of gas phase GC eluates [158]. Improvement of sensitivities could be achieved according

to this study through the use of highly intense stable sources and efficient monochromators with low light levels. Improvements in the gas phase detection of PNAs were made by using laser excitation.

Gas phase fluorescence spectra have broad bands with few distinguishing features for compound identification. However, if a gas stream is expanded through a nozzle into a vacuum, it will be rotationally cooled and produce high-resolution vapor phase spectra. Fluorescence of PNAs in supersonic jets with a laser excitation source has been used in preliminary experiments to detect GC effluents [159]. The limit of detection for substituted naphthalenes was in the low nanogram range using a continuous-flow nozzle with a pulsed excitation laser. It has been suggested that a thousand fold improvement in the limits of detection would occur if a pulsed nozzle was synchronized with the laser [160].

The GC spectrometric detection systems which are described herein are based upon concurrent absorption and fluorescence measurements. Unlike many earlier spectrometric GC detection systems which interfaced a commercially available spectrometer with a GC, this system has been designed specifically as a GC detector. The main advantage of this is that the integrity of the GC separation is not compromised by heated transfer lines and dead-volumes within the cell. The GC eluates are detected in the gas phase directly as they elute from the column.

This detection system is simple and compact yet the sensitivities and limits of detection are comparable to those of much more sophisticated systems which include lasers or Fourier transform spectrometers. The good powers of detection of these GC detectors are primarily achieved through the spacial design and compatibility of

the optical components, rather than their superior quality. Therefore, the expense of these detectors is not prohibitive. In addition, the system is easy to use and durable enough for routine analysis.

Spectrometric determinations in the UV are sensitive and precise. The combined selectivities of the absorption and fluorescence measurements also enhance the amount of qualitative information available. In this computer-controlled, fast-scanning GC detection system, the spectral information may assist identification of unknown eluates. Again, the detector is specifically designed for detecting GC eluates and acquires up to two 50-nm spectra per second. Therefore, numerous spectra may be acquired during the elution of a chromatographic band.

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II. INSTRUMENTAL

A. Overview

Two basic designs for the UV spectrometric detection systems were developed and studied in this research. Aside from various minor modifications, the main differences between these two systems are the type of wavelength isolation device, the ability either to monitor a fixed waveband or scan spectra over a wavelength region, and the manner in which data are collected.

Some of the basic components, such as a broadband deuterium lamp, are common to both systems. Ultraviolet light is transported to the detector cell via fiber optic bundles. The light which is transmitted through the cell travels through another fiber optic bundle to the absorbance PMT. Eluting compounds flow directly into the heated detector cell as they leave the GC column.

In one system, the wavebands from the source excitation radiation are selected by an ultraviolet bandpass optical filter. A schematic representation of this system is shown in Figure 22. The results of measurements made with this configuration are discussed in Chapter IV. This fixed waveband detector is particularly suited for the selective measurement of trace amounts of aromatic compounds separated by GC. In addition, for PNAs with similar GC retention times, the relative sensitivities of the absorbance and fluorescence measurements may suggest the identities of unknown GC eluates.

In the other system design for UV absorbance detection, the waveband which irradiates the sample cell is isolated by a grating monochromator. The monochroma-

tor may be used to isolate a fixed waveband and monitor eluting GC peaks. It may also be rapidly scanned to provide spectral information about effluents, which is helpful for identification of sample components or for enhancing quantitative measurements. Several wavelength scans can be made during a typical peak elution. In addition, this system enhances the selectivity of the spectrometric measurements by sequentially measuring many different narrower wavelength bands. The scanning of the monochromator is accomplished by a stepper motor mechanism controlled by a Digital Equipment Corporation (DEC) LSI-11 computer (Maynard, Massachusetts). This system is represented in Figure 2 and includes special interfacing for these studies.

In each of these configurations, there is a UV broad bandpass optical filter (described later) between the absorption cell containing the sample peak and photomultiplier tube (PMT) which detects the transmitted light. As stated previously, some of the compounds fluoresce when they absorb light, and this filtering of the transmitted light is used to keep fluoresced light from reaching this photodetector. A second PMT is used when concurrent fluorscence measurements are made. One or more optical filters are used to the intensity of reduce scattered light detected by the fluorescence PMT.

All separations referenced in this work were made on a Tracor Instruments 565 Gas Chromatograph (Austin, Texas) used in either isothermal or temperature programmed modes. The injection port was maintained at 300 C. Prepurified nitrogen carrier gas (oxygen content less than 10 ppm) was used for all separations and the carrier gas flow was controlled and indicated by a flow controller and rotameter on the GC. The gas flow components are mounted inside a controlled temperature chamber which helps to minimize fluctuations with

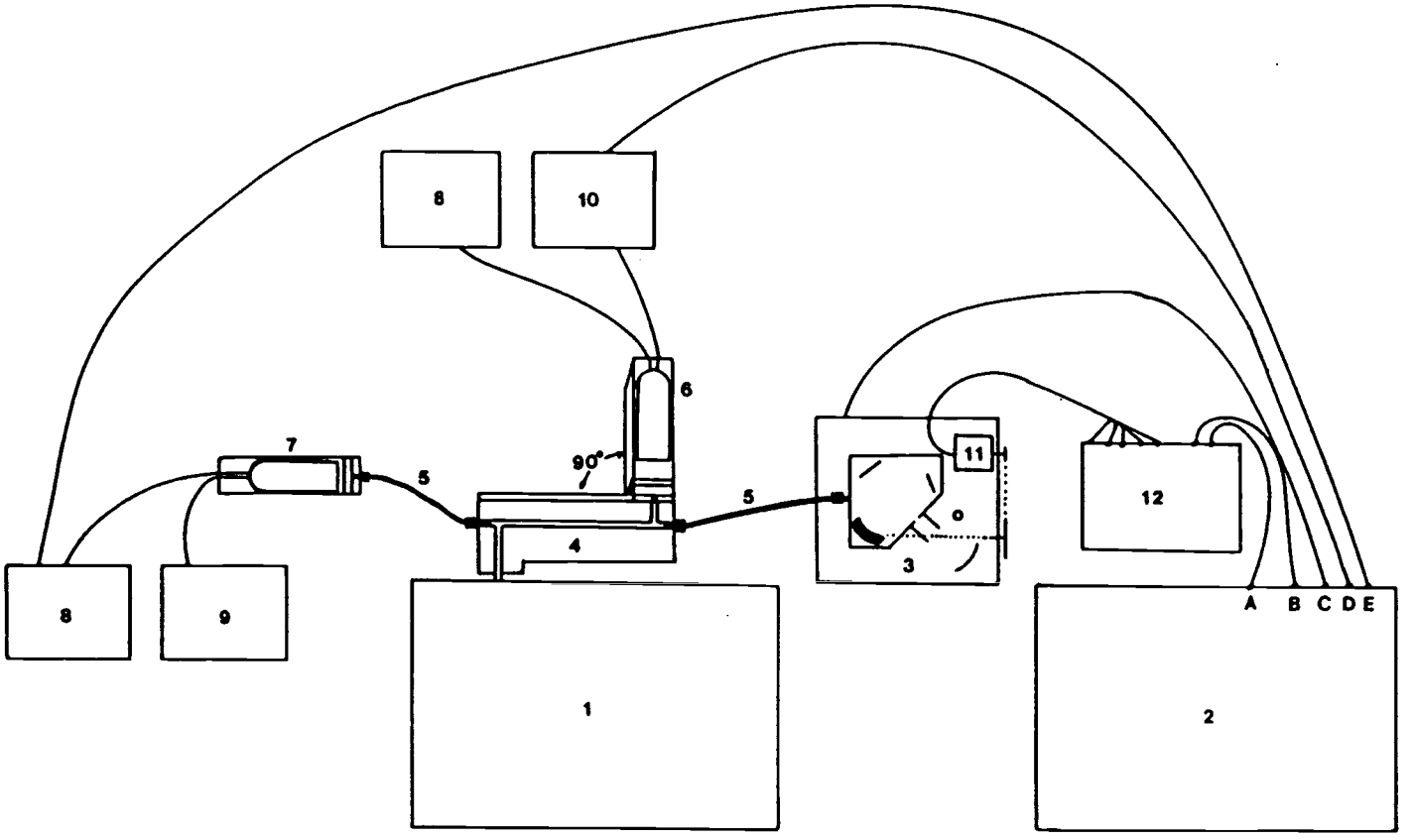


Figure 2. Schematic representation of the ultraviolet absorbance-fluorescence GC detector in the monochromator/optical filter configuration; 1 = GC; 2 = DEC LSI-11 computer; 3 = source wavelength selection module; 4 = long pathlength detector cell; 5 = fiber optic bundles; 6 = 320-nm longpass filter and fluorescence PMT; 7 = 220-nm wide bandpass filter and absorbance PMT; 8 = high voltage power supplies; 9 = absorbance signal electronics; 10 = fluorescence signal electronics; 11 = stepper motor; 12 = interfacing electronics between stepper motor and computer

changes in room temperature. The nitrogen carrier gas was filtered by a refillable hydrocarbon trap from American Scientific Products (Redmond, Washington).

A glass packed column was used for the separations with dimensions of 2 m length and 2 mm I.D. The packing material was 3% OV-101 on 100/120 mesh Supelcoport purchased from Supelco, Inc (Bellefonte, Pennsylvania). The column packing material near the injection port was replaced occasionally when it showed discoloration.

Other instrumentation used is described in the next four sections of this chapter. In the section on optical components are descriptions of the light source, grating monochromator and the associated scanning mechanisms, optical filters, and fiber optic bundles. The detector cells are described in the second section. The third deals with signal processing electronics, power supplies, and related electronics for the photomultiplier tubes. The computer and interfacing electronics are described in the last section.

B. Optical Components

1. Source

The radiation source for the absorbance and fluorescence measurements is a deuterium discharge (UV) lamp from a Tracor Instruments Model 970A Variable Wavelength Absorbance HPLC Detector. An indicator light on the exterior of the electronics module for this detector shows if the lamp is selected and functional (drawing current). The useful spectral range of the lamp is from 190-390 nm with a switch for selecting a UV lamp power of either 30 watts or 60 watts [161].

When the UV lamp is turned on, a high voltage (approximately 250 volts) is applied across the tube

filament by the High Voltage Striker in order to ionize the gas and strike an arc across the tube (Figure 3). When the lamp begins conducting current, the Lamp-on Comparator senses voltages from both the Current Sense Resistor and V(threshold) and removes the Trigger from the One Shot. Then the filament voltage and the High Voltage Striker are removed and the voltage is maintained at about 90 volts.

2. Optical Filter/Optical Filter System

In the optical filter/optical filter configuration (Figure 4) the light from the deuterium source is focussed by a front-surface mirror through a 260-nm bandpass optical filter onto the tip of a high-temperature, quartz fiber optics bundle. The filter and fiber optics bundle are secured by an aluminum holder with adjustable set screws which hold the fiber optics bundle in place. The fiber optics bundle is positioned so that its tip intercepts the focal point of the condensing mirror.

Figure 5 shows the transmittance vs. wavelength for the 260-nm broad bandpass optical filter (ARC Model No. 260-B) purchased from Acton Research Corporation (Acton, Massachusetts) This one inch diameter round filter, used to select the source radiation, has a maximum transmittance of 35.7% at 269 nm and a bandwidth at half height of 45 nm. The filter is coated on its front surface with layers of substances which absorb all but the desired waveband.

Also shown in Figure 5 are the transmittance characteristics of the 240-nm broad bandpass optical filter (ARC Model No. 240-B). This shorter wavelength filter is placed directly in front of the photocathode of the absorbance PMT to exclude fluoresced light. The

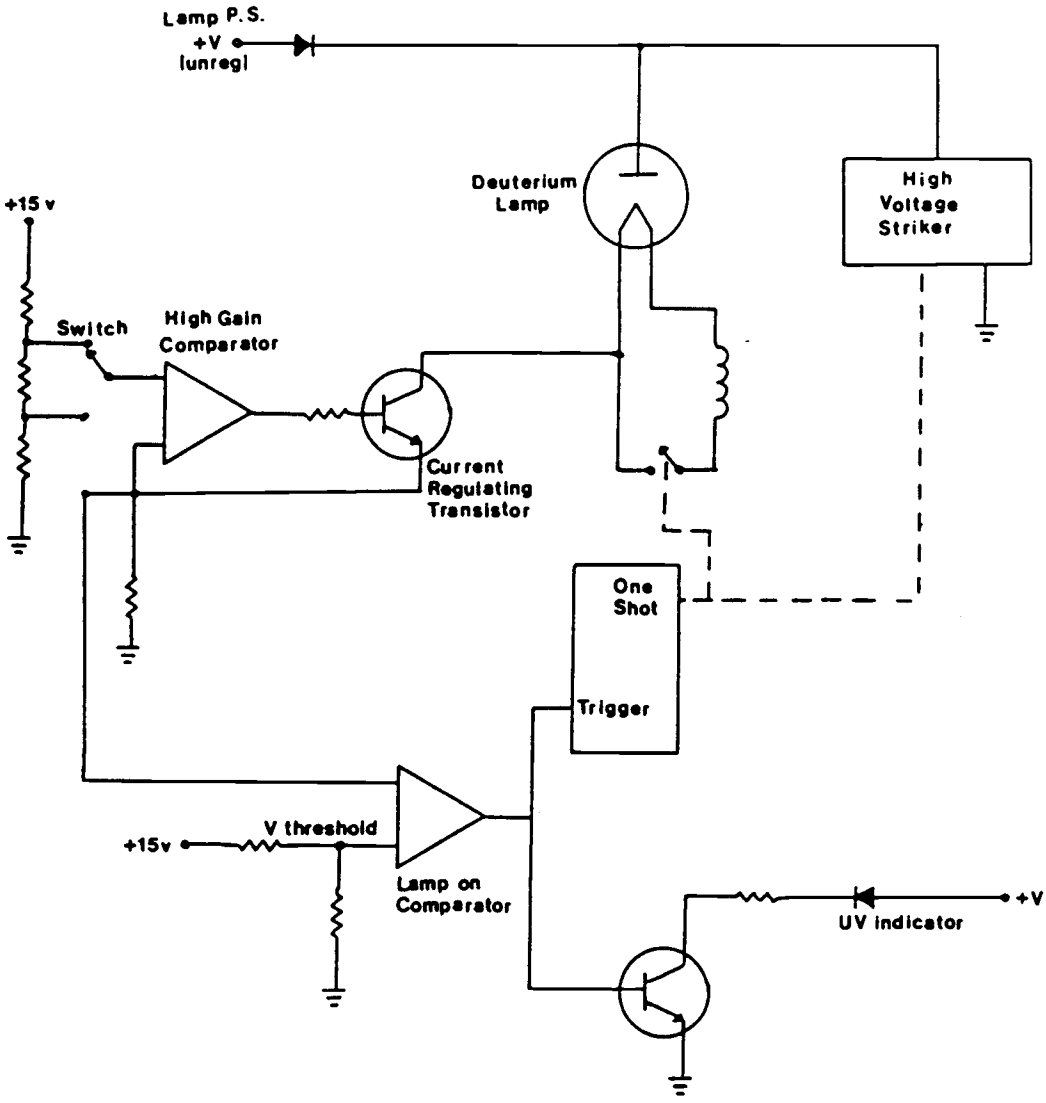


Figure 3. Functional diagram of the UV lamp power supply from the Tracor Model 970A Variable Wavelength Absorbance Detector

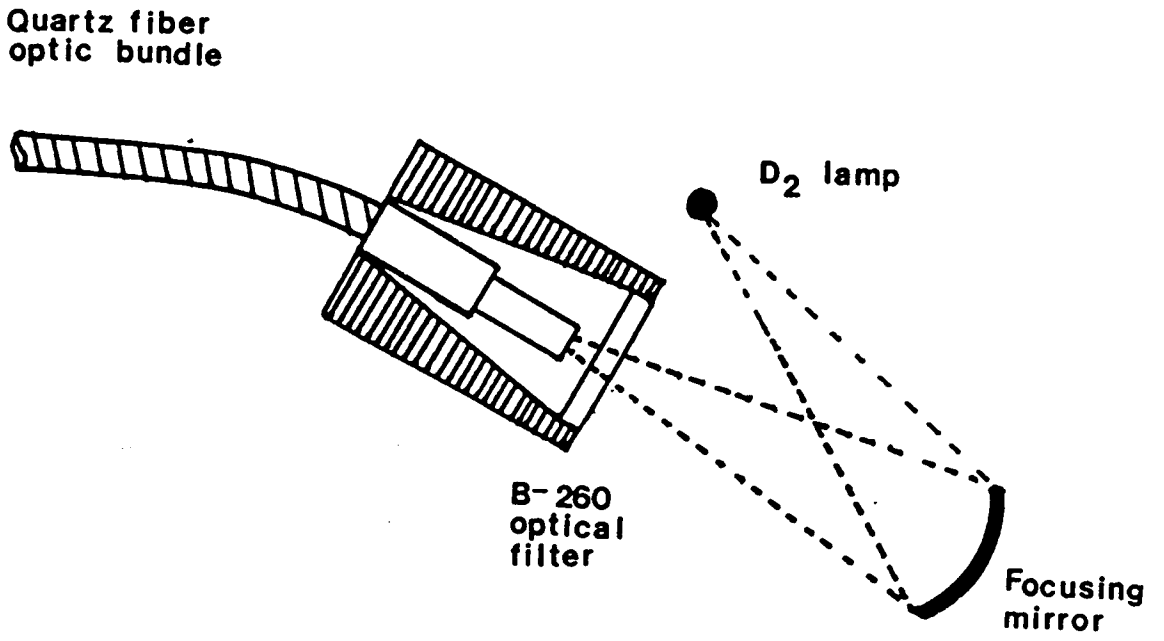


Figure 4. Source wavelength selection module in the optical filter configuration.

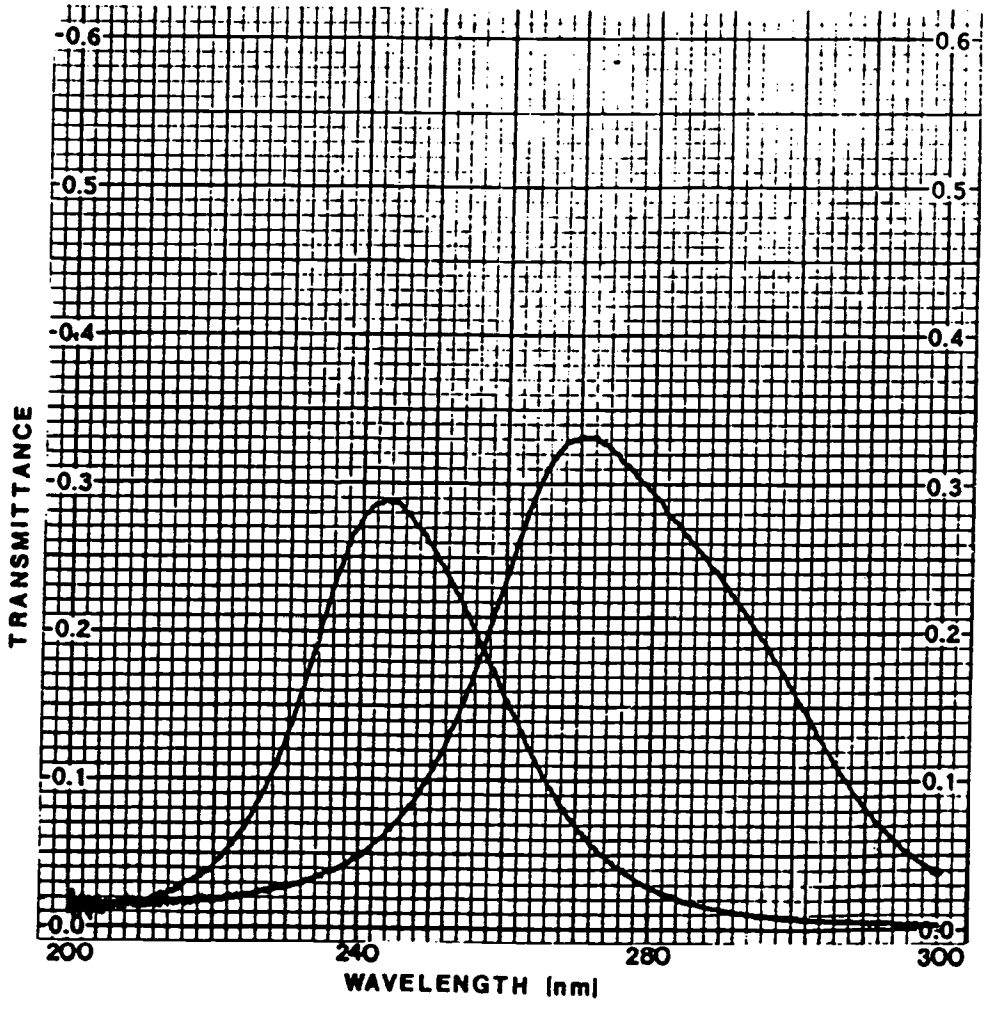


Figure 5. Transmittance vs. wavelength for the filters used in the optical filter/optical filter configuration: 260-nm broad bandpass and 240-nm broad bandpass

filter has a maximum transmittance of 30.5% at 239 nm and a 30 nm bandwidth at half maximum. The combined transmittance for concurrent use of both of these filters has a maximum of 254 nm and a width of approximately 20 nm. Both filters are at room temperature.

3. Monochromator/Optical Filter System

In the monochromator/optical filter configuration, light from the deuterium source is focussed by the focussing mirror onto the entrance slit of an aberration corrected, modified Czerny Turner monochromator (Figure 6). The monochromator has a grating ruling of 1200 line mm^{-1} and a 6-nm bandpass.

a. Scanning Mechanism

The mechanism for scanning the monochromator is shown in Figure 7 and scans approximately 100 nm per revolution of the rotating grating adjustment shaft. The shaft is turned by a 24-step, four-phase stepper motor from Herbach and Rademan, Inc. (Philadelphia, Pennsylvania). The stepper motor is connected to the grating adjustment shaft outside of the optical module housing by an adjustable position mounting. It is mounted on a specially made cover for the housing of the optical module. This cover is sturdier and less prone to vibration than the original cover. The scanning mechanism must be lubricated carefully, aligned, and the tightness of the drive belt carefully adjusted for the scanning system to run smoothly while making many rapid repetitive scans during long GC runs. Improper maintainance may result in irregularities in the stepping action of the stepper motor during scans.

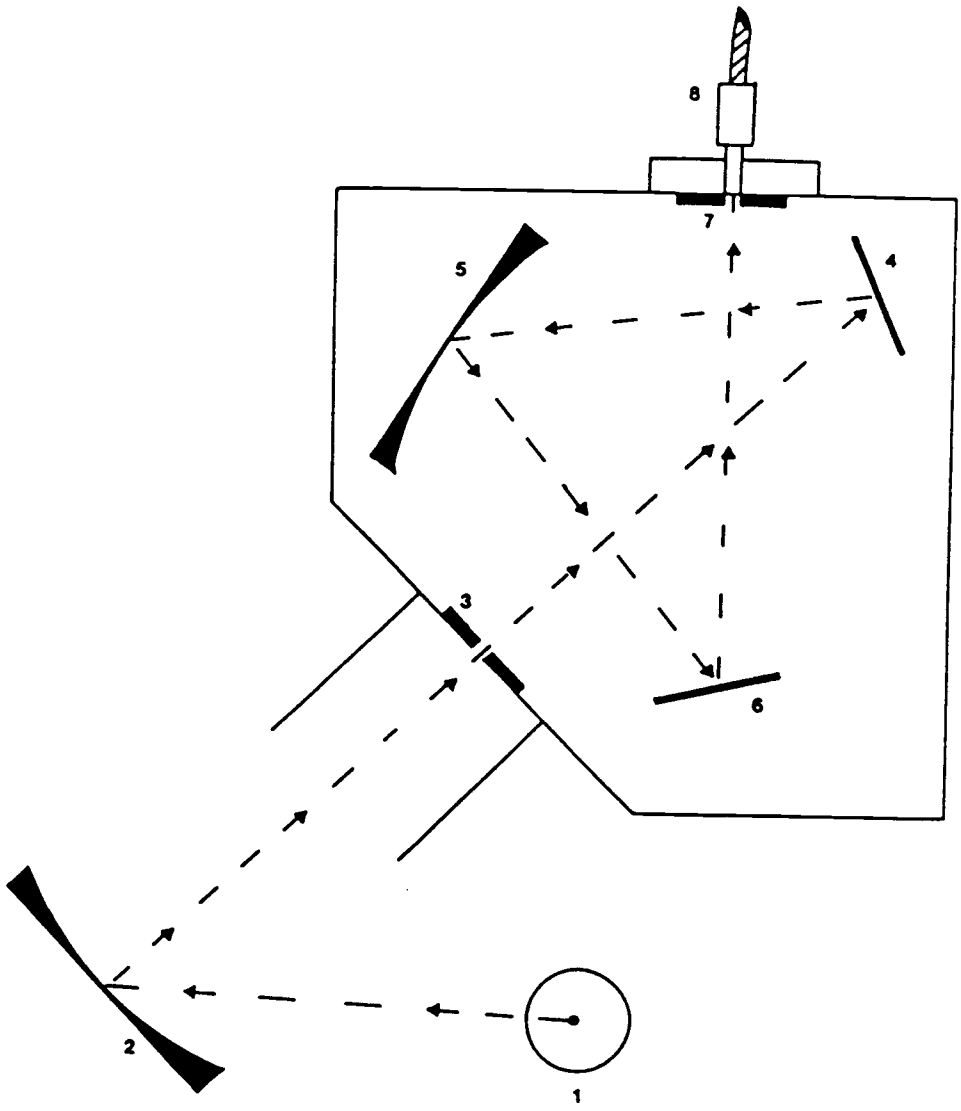


Figure 6. Source wavelength selection module in the monochromator configuration; 1 = Deuterium lamp; 2 = focussing mirror; 3 = entrance slit; 4 = mirror; 5 = grating; 6 = mirror; 7 = exit slit; 8 = quartz fiber optic bundle

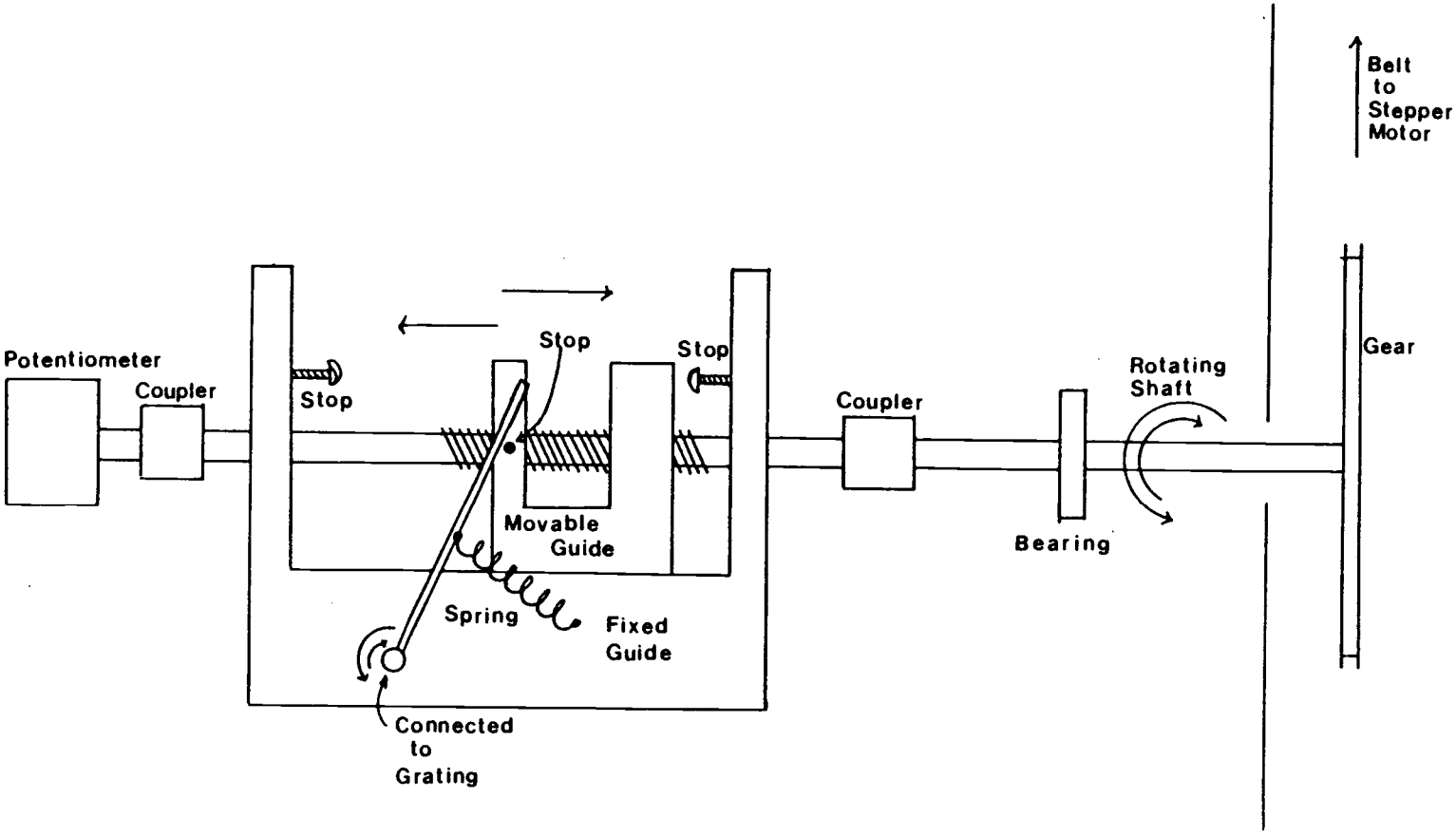


Figure 7. Mechanism for scanning the monochromator

The stepper motor is connected to the rotating grating adjustment shaft which controls the waveband selected by the monochromator. The no-slip gears purchased from PIC Design Corporation (Middlebury, Connecticut) on the stepper motor and rotating shaft are in a 1:4 ratio with a no-slip positive drive belt connecting the two, resulting in 96 steps of the stepper motor per revolution of the monochromator shaft.

The other end of the rotating shaft is connected to the knob of a ten-turn 10-KΩ potentiometer between the Potentiometer Reference Voltage of 1 volt and ground. The output of this potentiometer is directly proportional to the position of the shaft and is attached to a connection marked RCDR λ on the back of the electronics console. It provides a 1.0 mV nm^{-1} output and is based on the shaft position. This signal is amplified by a factor of eleven with a FET operational amplifier (TL081) follower-with-gain circuit.

b. Filters

Since the monochromator is scanned to provide spectral information, the 240-nm broad bandpass optical filter described above was replaced by a 220-nm wide bandpass filter (ARC Model No. 220-W) with a full-width at half-maximum (FWHM) of 84.0 nm and a maximum transmittance of 55.0% at 220 nm (Figure 8). If measurements indicated that a significant amount of fluoresced light was reaching the absorbance PMT, the 220-nm filter could be replaced by a second 240-nm broad bandpass optical filter (also ARC Model No. 240-B) with a greater width at half-maximum than the 240-nm filter described above. This filter has a maximum transmittance of 38.3% at 236 nm and a bandwidth of 42.5 nm. These open-face filters have a 1 inch diameter with front-surface coating.

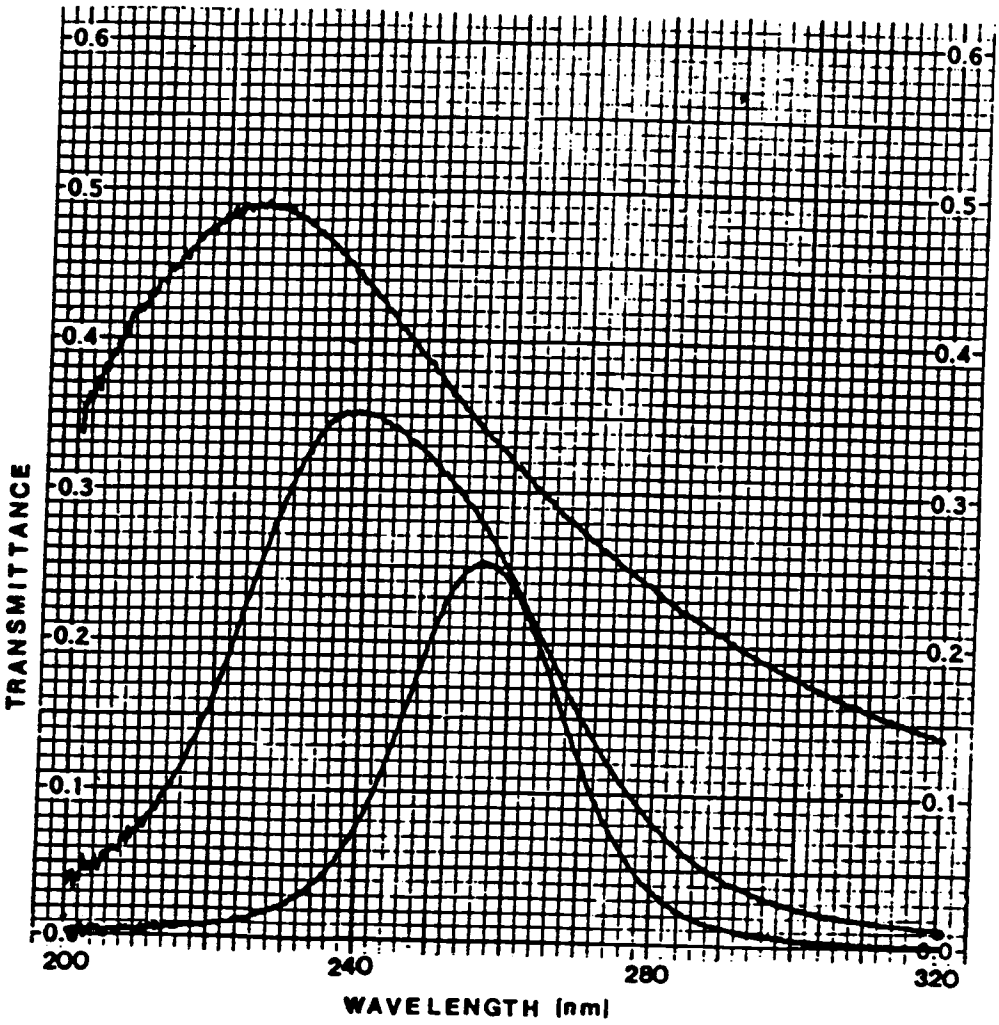


Figure 8. Transmittance vs. wavelength for the optical filters in the monochromator/optical filter configuration: 240-nm broad bandpass, 220-nm wide bandpass, and 254-nm narrow bandpass

A 254-nm narrow bandpass optical filter (ARC Model 254-N) was used to calibrate the monochromator wavelength. This filter has a peak transmittance of 26.5% at 254 nm and a bandwidth of 25 nm (Figure 8). A more complete description of these measurements is found in Chapter V.

Optical filters are placed between the detector cell and the PMT used to make fluorescence measurements in order to minimize detection of scattered light. In the optical filter/optical filter detection system described in Chapter IV, the fluorescence emission was filtered by both a 380-nm bandpass filter and a 340-nm longpass cutoff filter from Acton Research Corporation. In the monochromator/optical filter configuration, only the longpass filter was used.

4. Fiber Optic Bundles

In all detector designs, ultraviolet light is transmitted from the Source Wavelength Selection Module to the detector cell and from the detector cell to the absorbance PMT by fiber optic bundles. The purpose of the fiber optic bundles is to thermally isolate the radiation source, optical filters and the PMTs from the detector, which is typically heated to 250°C. The spectrometric components can thus be kept at room temperature, which helps reduce the background due to temperature dependent PMT noise. Also, some components such as the filters may be damaged by excess heat.

The transmittance characteristics of the optical fibers result from total internal reflection of light. For total internal reflection to occur at an interface between two optically transparent materials, the incident ray of light must come from the more dense material at an angle greater than the critical angle.

Such a reflection results in a light loss of less than 0.001 percent per reflection. Light is transmitted down the fiber at a constant angle relative to the fiber axis unless it is scattered by imperfections in the fiber or interface [162].

Each fiber accepts light from an input acceptance cone which is described by an aperature angle, α . The numerical aperture, N.A., is the sine of the aperture angle (N.A. = sin α). The light gathering capacity is numerically equal to the square of the numerical aperture. The full aperature angle at half intensity, 2α, is dependent on wavelength, particularly in the UV region [163].

Ideally, the emergence angle of the light from the fiber will be the same as the acceptance angle. In actual practice, the angle will be widened by diffraction at the ends, bending, and surface roughness [162]. The fiber optics bundles used in these instruments were wound with stiff wire to secure them and align the fibers so that they maintain the same bending angles. This also helps reduce the amount of vibration in the bundles from the GC and scanning system.

The source radiation is transmitted via a 1 m x 3 mm Welch-Allyn (Skaneateles Falls, New York) high-temperature fiber optics bundle. A 1-m optical fiber for UV light has a full aperature angle, 2α, of $22^{\circ} \pm 5$ at 254 nm [163]. The fiber optics bundle between the detection cell and absorbance PMT is a 250 mm x 1 mm high-temperature fused-silica fiber optics bundle from Maxlight Fiber Optic Division of Raychem Corporation (Phoenix, Arizona). The bundle purchased was specified by the manufacturer to have a 33.0% transmittance at 254 nm.

The percent transmittance vs. wavelength (nm) curves for these two fiber optics bundles are shown in

Figure 25. This figure summarizes the optical characteristics of components used in the optical filter/optical filter detection system.

C. Detector Cells

Two compact, heated detector cells were designed for the measurement of UV absorbance and fluorescence of GC effluents (see Figures 23 and 27 in Chapter III). The gas-phase sample components flow directly from the GC into the cell in order to minimize chromatographic band broadening. This eliminates the transfer line interface between the chromatographic and spectrophotometric systems [143]. In most instances, the cell was heated to 250°C to ensure the vaporization of large polynuclear aromatic compound (PNA) molecules.

1. Short Pathlength Cell

The first cell design is shown in Figure 23. The cell was milled from a solid aluminum block and has outer dimensions of 3.25 in x 2.00 in x 0.50 in. Two cylinderical holes are bored into the side of the cell where they do not intersect the gas flow path. These are used to hold a heater and a thermocouple from the Tracor GC. This allows the temperature of the cell to be set and monitored using the control electronics and digital readout of the GC.

The cell is designed to fit directly onto the end of the glass GC column using Swaglock fittings and either teflon or graphite ferrules to prevent gas leaks (see Figure 24). The effluent gas flows through the the cell in a "Z" pattern, and spectroscopic measurements are made in the middle horizontal section. The inlet and exhaust paths have diameters of 0.15 in. The

horizontal section, where spectroscopic measurements are made, is lined with a fused silica quartz tube, 4 mm I.D. The exhaust gases flow into a tube filled with glass fibers upon which the larger PNAs adsorb and are then vented into the hood.

The volume of this horizontal section is 200 μL. It is important that the cell volume be no greater than the volume eluted over a duration approximating the full-width at half-maximum of a GC peak in order to reduce chromatographic band broadening and loss of separation resolution [137]. For the GC separations detected by this system, the narrowest chromatographic peals have a full-width at half-height corresponding to approximately 4 mL effluent.

The source fiber optics bundle fits snugly into an opening at the exhaust end of the horizontal flow path. The fiber optics bundle which collects transmitted light for the absorbance measurement fits into a similar opening at the GC effluent entrance end. Both bundles are wrapped with Teflon (PTFE) tape or o-rings prior to insertion to prevent gas leaks.

Fluorescence measurements may be made concurrently in the same cell via a side window in the aluminum (see Figure 23). The PMT housing for a Flame Photometric Detector (FPD) from Tracor Instruments (Austin, Texas) is fastened onto the back side of the cell to make this measurement.

Usually the cell is wrapped in several layers of aluminum foil. This provides thermal insulation and acts as a light baffle for room light.

2. Long Pathlength Cell

The second detector cell is very similar in design to the first except that the pathlength for the

absorbance measurement has been lengthened by a factor of ten. This cell is shown in Figure 27. Again, the cell was milled from a solid aluminum block and the cell has a "Z" flowpath. The cell has outer dimensions of 8.00 in x 3.50 in x 0.50 in and must be supported in order to avoid placing too much strain on the GC column.

Preliminary measurements were made to determine the length of 1 mm I.D. glass tube which would transmit sufficient light for the absorbance measurement. As the pathlength of the cell is increased, the total intensity of the transmitted light, as measured by the difference between the dark current and zero absorbance signal, diminishes approximately to the inverse square of the distance. The dark current signal fluctuations are the major component of the noise at the low light intensities used in this system (discussed in more detail below). Therefore, the magnitude of the noise fluctuations remain essentially constant as compared to the reduction of signal intensity at longer cell pathlengths. A compromise much be achieved between the longest possible absorbance pathlength and sufficient transmitted light intensity to provide a good signal-to-noise ratio.

The absorbance pathlength in the long pathlength cell is 167 mm and has a volume of 320 µL. The first 151 mm of the horizontal gas flow section is lined with a glass tube, 1 mm I.D. The diameter of this section is kept small in order to significantly increase the absorbance pathlength without a large increase in the total cell volume, thus preserving chromatographic resolution.

The long, small-diameter pathlength in this cell requires very good alignment of the tips of the source and transmittance fiber optics bundles. To this end, very tight fitting PTFE sleeves were milled to hold the

tips of the bundles securely in place. These sleeves have grooves for o-rings to make them gas tight and light tight when they are pushed into the milled ends of the cell (see Figure 27).

After traversing the narrow-diameter, glass-lined section, the gases from the GC flow into a short, larger diameter section for the fluorescence measurements. This section has a 16 mm pathlength and is lined with a 4 mm I.D. fused silica tube. As above, the fluorescence measurement is made through a side window. This section is positioned at the end of the cell, near the illumination fiber optics bundle to provide more intense excitation radiation. Since light emerges from the fiber optic bundle at a dispersed angle, the light intensity diminishes geometrically along the path of the cell.

D. Photomultiplier Tubes and Signal Electronics

The UV radiation which reaches the detector cell from the deuterium source has a relatively low intensity due to combined intensity losses in the waveband selection devices, other optical components, (e.g. fiber optic bundles and mirrors), and the long distances traversed. Both the transmitted and fluoresced light are also filtered prior to detection, further reducing the measured intensity. In addition, the sample masses in a peak range from sub-microgram to sub-nanogram, of which only a fraction is present in the detector cell at any one time. Thus only a small fraction of the low intensity light is absorbed. As a result, very sensitive and stable photodetection systems are required in order to measure the minute signal changes which occur in this GC detection system. As part of this system, high-gain photomultiplier tubes (PMT) with

excellent stability connected to stable power supplies were chosen for measuring these small changes in light intensity.

1. Absorbance Measurements

a. Photomultiplier Tube (PMT)

A head-on PMT, with the photocathode close to the window, is used to measure the transmitted light for the absorbance signal. The light transmitted through the detector cell reaches the absorbance PMT via a fused-silica fiber optics bundle and, as previously discussed, the light exits the fibers in a more widely dispersed angle than it enters. As a result of this, the photocathode of the PMT is placed in very close proximity to the tip of the fiber optics bundle.

The PMT chosen for the absorbance measurement was a R1464 high-gain, head-on PMT from Hamamastu Corporation (San Jose, California) with a multialkali photocathode and a wide spectral response range (185-850 nm). The ratings and manufacturer's specifications for the PMT are given in Table 2 [165].

The absorbance PMT has an envelope made of UV transmitting glass and has outer dimensions of 18.6 mm x 88 mm (excluding the pin base) and the photocathode has a minimum diameter of 15.0 mm. A head-on type of PMT has a semi-transparent photocathode deposited on the inner surface of the entrance window and has better uniformity and lower noise than a side-on type PMT. The multialkali (Na-K-Sb-Cs) photocathode has a sensitive response over a wide spectral range and is typically used for broad-band spectrophotometers and in photon counting applications. The R1464 PMT has 10 dynode stages in a linear focused arrangement which is

Table 2. Ratings and Tested Performance of the Hamamatsu R1464 PMT Used to Measure Transmitted Light (serial number VA2084)

Spectral Response	
Range (nm)	185-850
Peak Wavelength (nm)	420
Photocathode Material	Multialkali
Window Material	UV glass
Maximum Ratings	
Anode to Cathode Voltage (Vdc)	1250
Anode to Last Dynode Voltage (Vdc)	250
Average Anode Current (mA)	0.01
Cathode Luminous Sensitivity (μA/lm)	
minimum	80
typical	120
tested*	136
Anode Luminous Sensitivity (A/lm)	
minimum	30
typical	120
tested*	260
Anode Dark Current (nA)	
maximum	30
typical	10
tested*	8
Rise Time (ns)	2.5
Current Amplification (Gain)	
typical	1.0×10^6

* tested by Hamamatsu on 8/12/83 at 25°C; Light Source: Tungsten Filament Lamp operated at 2856 K; Anode to Cathode Supply Voltage of 1000 V.

compatible with applications which require a fast time response [164].

b. PMT Housing

Figure 9 shows a diagram of the PMT housing which was designed and constructed to hold the PMT because commercially available PMT housings tended to be too bulky to accommodate the spacial arrangements required for this photodetection system. The 240-nm broad bandpass filter is placed flush to the end of the PMT and the tip of the fiber optics bundle is placed adjacent to the filter. The result is a distance of approximately 4 mm traversed by the UV light leaving the optical fibers until it falls on the photocathode of the PMT. This close proximity reduces light losses due to dispersion.

The PMT housing is constructed of wood with aluminum end plates. It is painted black and all joints sealed with black tape. The inner dimensions are 2.5 cm x 2.5 cm x 16.0 cm, which are sufficient to hold the PMT, socket (Hamamatsu D-type socket assembly, E678-12D), optical filter, and fiber optics bundle tip. The PMT is held firmly in place by Teflon o-rings. The entire housing is wrapped in aluminum foil and the aluminum endpieces and foil are connected to ground.

c. Power Supplies

The output signal from the PMT is very susceptible to fluctuations in the bias voltage between the photocathode and photoanode. Thus, a regulated and stable power supply is required. The power supply voltage for the absorbance signal PMT was provided by a Hamamatsu modular high-voltage power supply (C1309-02).

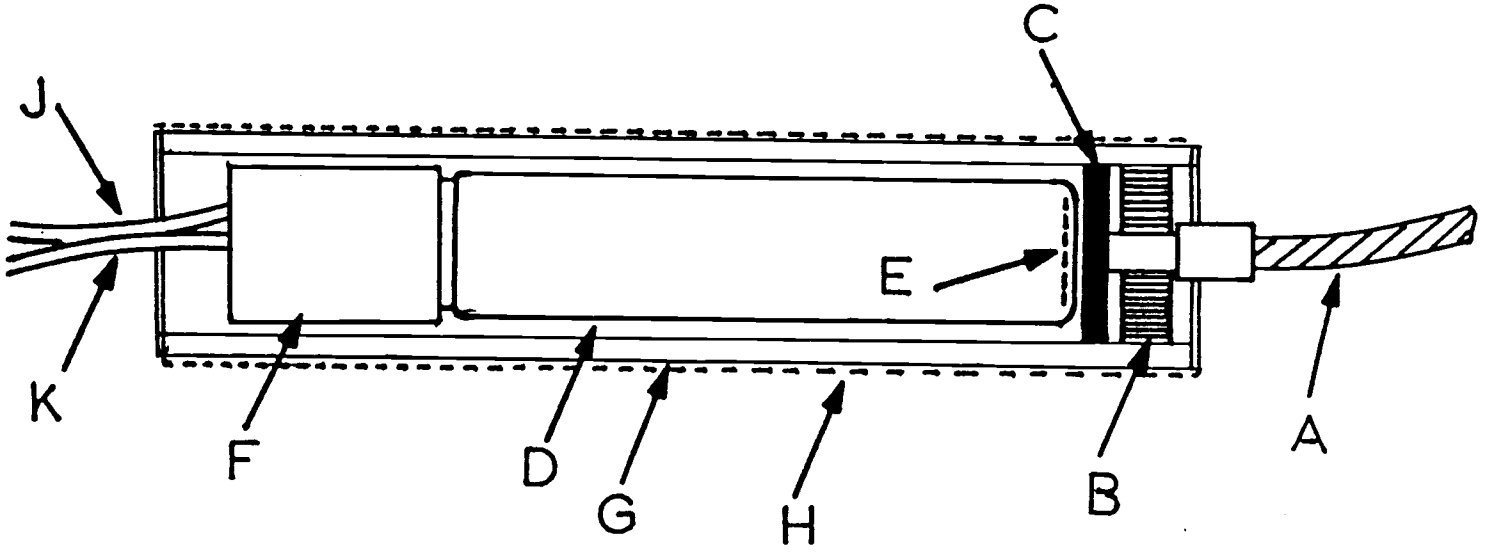


Figure 9. Diagram of the photomultiplier tube and housing used for the absorbance measurement; A = fused silica fiber optic bundle; B = support for fiber optic bundle tip; C = optical filter; D = PMT; E = multialkali photocathode; F = PMT socket; G = insulating material (wood); H = grounded shield (aluminum); J = high voltage cable; K = signal cable (shielded)

This power supply is enclosed in silicone rubber and operates with a +15 Vdc, 170 mA input voltage as shown in Figure 10. The high- voltage power supply has a maximum output current of 0.7 mA and a maximum of 100 mV p-p ripple at maximum voltage output of -1100 V. The output voltage for the high-voltage power supply is resistance programmed with a ten-turn, 10-KΩ potentiometer and is shown as a function of potentiometer dial setting in Figure 11. Connection is made from this power supply to the photocathode of the PMT through a high-voltage BNC cable.

The low voltage power supply providing power for the high voltage power supply has +15 V, -15 V, and +5 V with a maximum output current of 800 mA. It is also used as a power supply for the signal modifying electronics. Large capacitors (e.g. 0.68 mF) are connected between the output voltages of this power supply and ground to smooth any voltage ripple.

d. Signal Electronics

The signal electronics used for absorbance measurements are also shown in Figure 10. The current from the photoanode of the PMT is connected to the signal processing electronics via a shielded cable. It is converted to a voltage by a FET operational amplifier (TL081) current-to-voltage circuit with a 1.0 MΩ feedback resistor. To minimize the effect of cable capacitance, either a 0.1 μF or 0.01 μF electrolytic capacitor may be chosen by a 2-way switch placed in parallel with the feedback resistor resulting in either a 0.1-s or 0.01-s time constant, respectively. The ± 15 V power supply voltages for the FET operational amplifier are de-coupled with 4.7 μF electrolytic capacitors, as are all other power supply voltages for

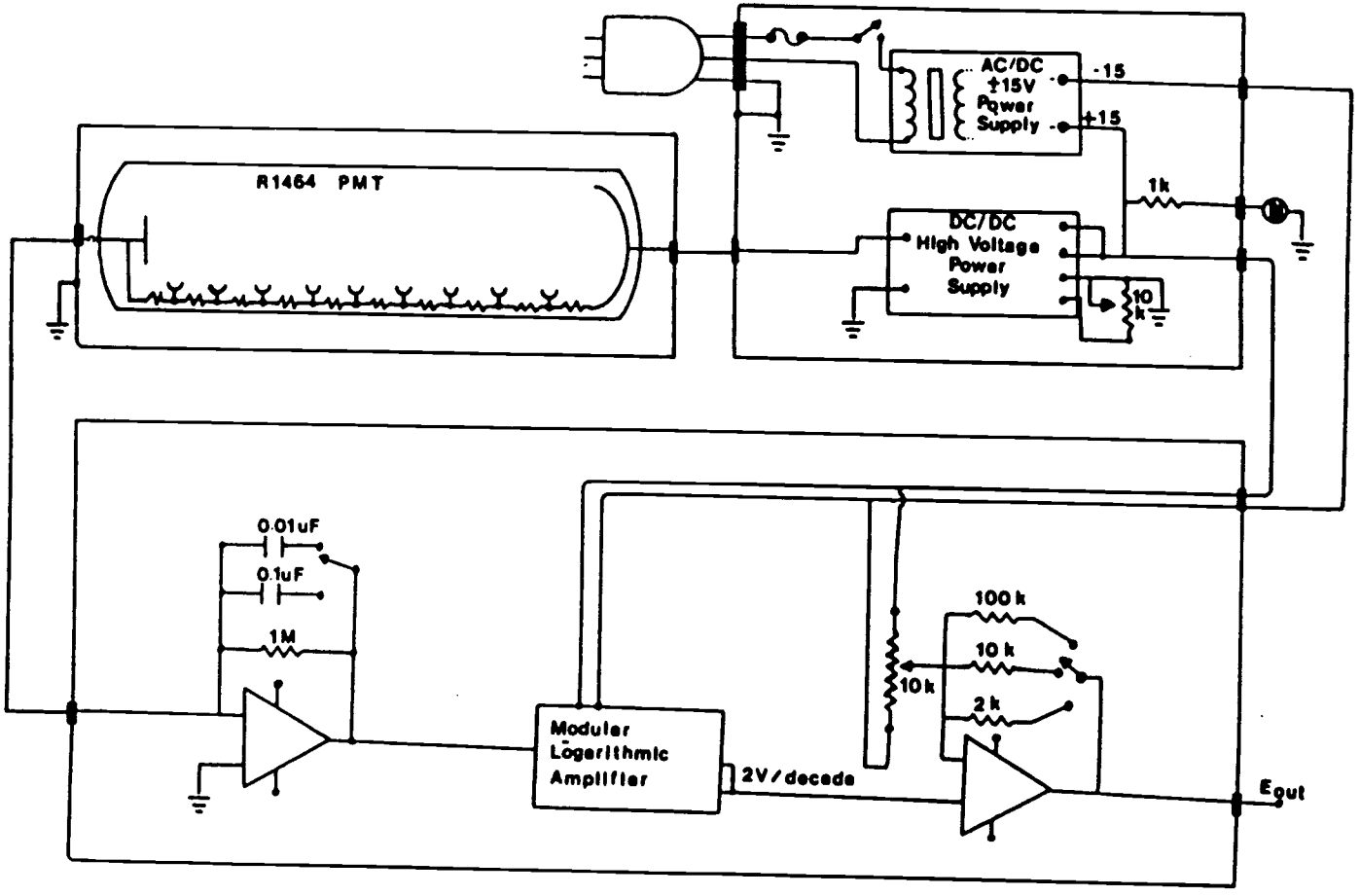


Figure 10. Diagram of the power supplies, PMT, and signal electronics for the absorbance measurement

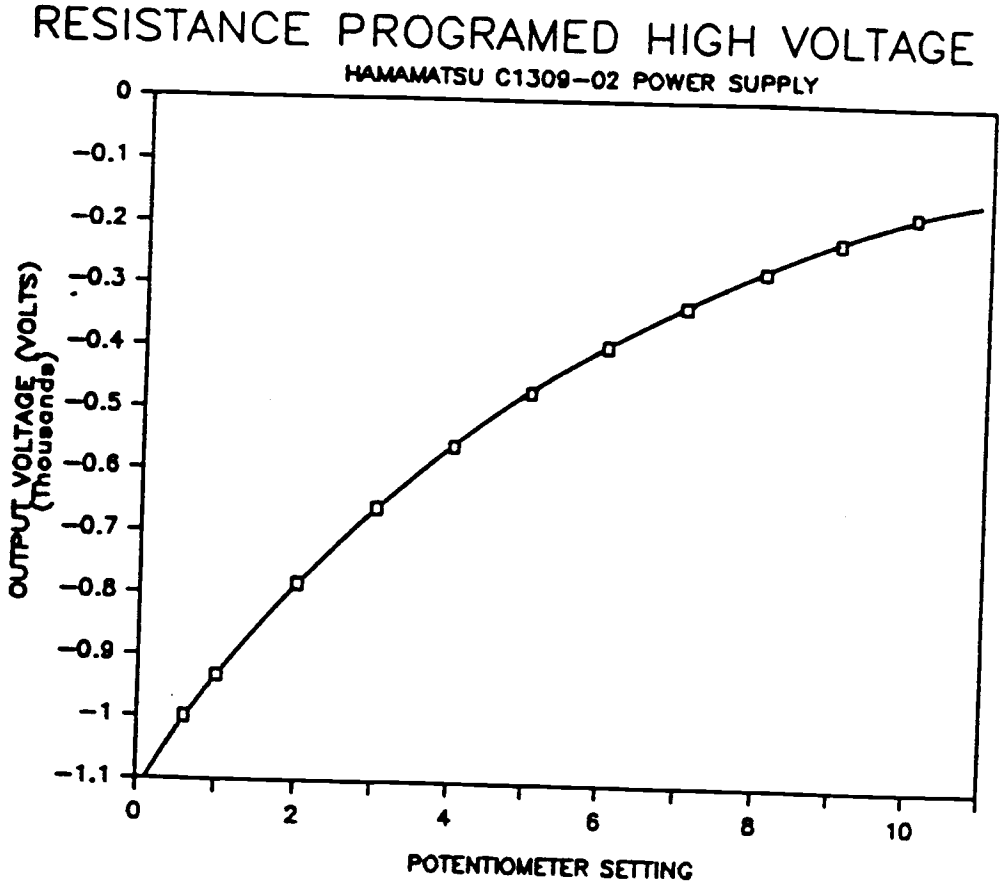


Figure 11. Output voltage for the high-voltage power supply for the absorbance PMT vs. the potentiometer dial setting

the intergrated circuits (IC) used for signal processing.

The output voltage from the current-to-voltage converter is then connected to an Analog Devices (Norwood, Massachusetts) 759N fast-response (200 kHz bandwidth at 1 μA), dc modular logarithmic amplifier. This complete, self-contained module has the advantages of 2% conformance to ideal logarithmic operation over four decades of voltage operation (1 mV to 10 V), an internal reference of 10 μA (or 0.1 V) and automatic temperature compensation. The ideal transfer function of this circuit is given by

$$E(out) = - K \log (E(sig) / E(ref))$$

The scale factor, K, may be chosen to be 2, 1, or 2/3 volt/decade by an external jumper and was set at 2 in these detection systems unless otherwise indicated. Since the circuit takes the base ten logarithm of the transmittance measurement, the resulting chromatographic peak heights and areas are proportional to absorbance and, therefore, concentration.

In both detector configurations, the limiting noise source for the absorbance and fluorescence measurements appears to be dark current noise, as the noise level is not increased, or only slightly increased, when the lamp is turned on. Although the electronics adds a small component to the noise, the largest increase in the magnitude of the noise occurs when the bias voltage is applied to the PMTs. In the monochromator/optical filter configuration the peak-to-peak noise of the absorbance measurement at 270 nm has a magnitude of approximately 1.6 mV which corresponds to 7.8×10^{-4} absorbance units. At wavelengths shorter than about 240 nm, the transmittance of the optics decreases and the magnitude of the noise becomes greater as compared to the absorbance signal. The noise level on the

fluorescence channel increases at illumination wavelengths longer than approximately 350 nm due to lamp flicker noise from scattered light. However, in measurements of GC eluates, the longest illumination wavelength used was 310 nm.

In order to test the linearity of this circuit over a wide range of light intensities, measurements were made of the output voltage from the current-to-voltage converter as compared to the subsequent voltage output from the logarithmic amplifier (see Figure 12). The light intensity was varied from infinite absorbance (dark current) to zero absorbance by turning off and on the deuterium lamp (HI power). The light intensity reaching the PMT could be varied for intermediate measurements simply by adjusting the wavelength of the monochromator in the monochromator/optical filter configuration.

As shown by the graph of input vs. output voltage from the circuit, the output signal is fairly linear with logarithmic output voltages of approximately 3.5 or less. This corresponds to an input voltage of approximately 2 mV. At input voltages below this, the logarithmic amplifier circuit becomes very noisy and non-linear. Therefore, when very low light levels are used, such as in the monochromator/optical filter configuration, the bias voltage of the PMT must be increased to provide a signal voltage within the linear working range of the logarithmic amplifier.

Plots of data such as shown in Figure 12 may be used to convert output voltages to absorbance units. To do this, the voltage corresponding to an absorbance of zero (100% T) is measured. Then, assuming linearity, the voltage corresponding to an absorbance of 0.301 (50% T) is calculated, based on the slope of the measured logarithmic output voltages. Then the ratio of

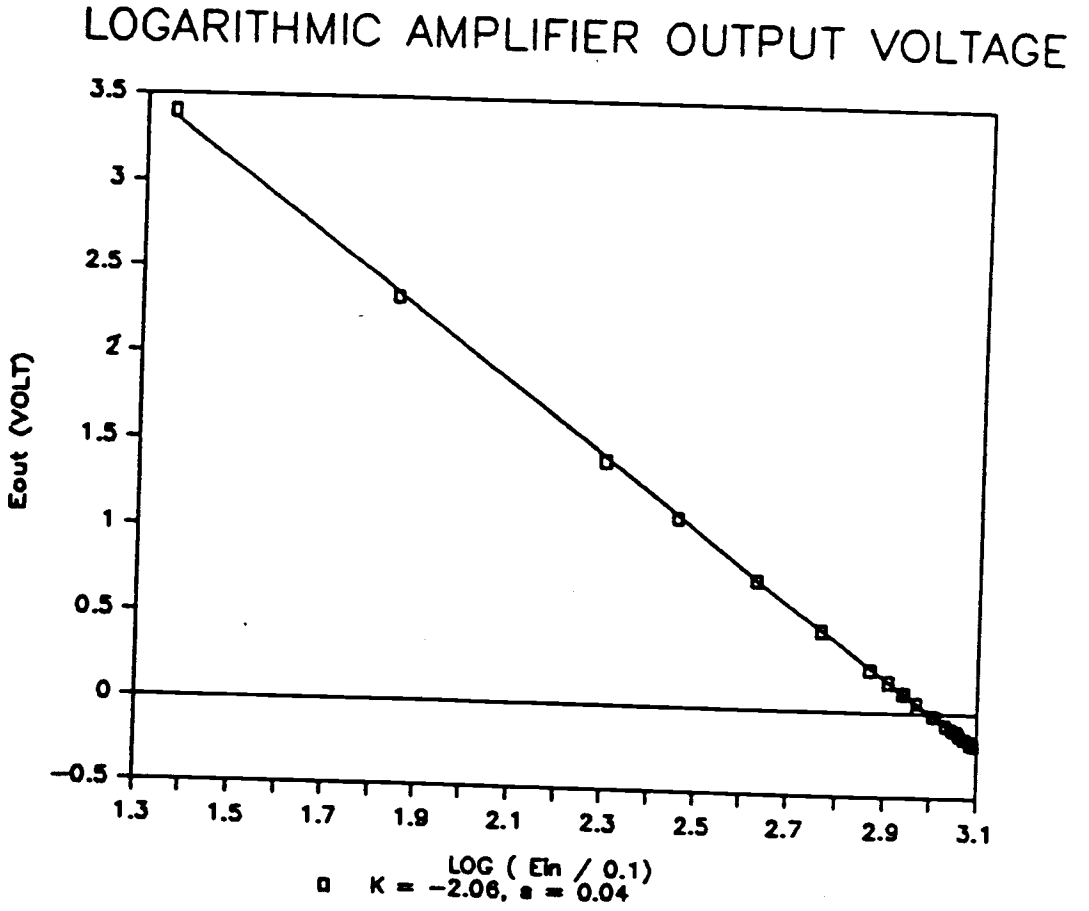


Figure 12. Output voltage for the modular logarithmic amplifier circuit as a function of input voltage

the difference in absorbance (i.e. from 0 to 0.301) and the difference of output voltage for these two points is used to calculate a conversion factor. This factor has units of microvolts per absorbance units and the result of this determination resulted in calculated conversion factor of 4.86×10^{-7} a.u. μV^{-1}.

After the transmittance signal voltage has been converted electronically to its base ten logarithm, the signal voltage is amplified by an FET operational amplifier (TL081) follower with gain circuit (see Figure 10). A three-way switch allows the gain to be set at x1.2, x2.0, or x11.0. The PMT voltage is adjusted and the the signal is amplified to yield a maximum output signal due to absorbance from a sample of approximately 1.0 V when the measurement is recorded on a digital integrator or 10.0 V when interfaced to the DEC LSI-11 computer. These output voltage ranges for the sample signal are approximately one-fifth or less of the total absorbance range from zero to infinity.

The follower-with-gain circuit is referenced to an offset voltage. This voltage can be varied between -15 to +15 volts with a ten-turn 10 KΩ potentiometer by an external dial.

The low-voltage power supply is normally the common ground for the high-voltage power supply and absorbance signal electronics. However, when the absorbance signal is measured by the integrator of the Hewlett Packard HP5880 GC, the signal ground is taken from the integrator to avoid creating ground loops. The analog-to-digital converter (ADC) channels for the DEC PDP-11 computer are not grounded by the computer, so common ground is connected to them via the shield of the signal cable. The metallic housings for the power supplies, absorbance signal electronics, and PMT are grounded in common to reduce noise due to external stray capacitance.

2. Fluorescence Measurements

The PMT and signal electronics used for the simultaneous fluorescence measurements are described as part of other research projects (see Contributions of Authors) and are summarized briefly herein.

In the optical filter/optical filter configuration, the fluorescence measurement is made using the PMT and PMT housing from a Flame Photometric Detector from Tracor Instruments and the Tracor 565 GC electronics. The PMT is a Hamamatsu R268 head-on PMT with a bialkali photocathode, 11-dynode stages, and current amplification of 2.1×10^6 with a cathode-to-anode voltage of -1000 V. This PMT has a spectral response range from 300 nm to 650 nm and uses Hamamatsu socket E678-14C. The -600-V constant high voltage power supply and Signal Processor Module are also provided by the Tracor 565 GC. A Hewlett Packard HP3390A digital integrator is used to record and measure the signals of fluorescence eluates.

The PMT housing is attached directly to the side of the heated detector cell (see Figure 23). This necessitates a distance of approximately 15 cm between the fused silica side window of the cell and the photocathode of the PMT in order to avoid a large background signal due to thermal emissions of the PMT. The housing is constructed with a fluted aluminum heat-sink between the heated detector cell and the PMT.

In the computer-interfaced, scanning configuration, the PMT housing for the fluorescence measurement is slightly shorter and the Model R268 PMT is replaced with a Model R1464 PMT and stable power supplies similar to those used for the absorbance measurements. This substitution allows a variable PMT bias voltage and reduced fluctuations in the background signal. The signal electronics for the scanning system were

constructed with a OA-2 Op-Amp Designer board from E and L Instruments, Inc. (Derby, Connecticut). The current from the photoanode is converted to a voltage with a FET operational amplifier (TL081) current-to-voltage circuit. This circuit has 22 MΩ feedback resistor with 0.01 μF capacitor connected in parallel yielding a time constant of 0.22 s. The signal is amplified by an operational amplifier follower- with-gain circuit set for an 11-fold amplification and referenced to the signal ground. The large feedback resistors in this circuit make it susceptible to external stray capacitance, so a grounded faraday cage is placed around the fluorescence signal electronics.

E. Computer Hardware and Interfacing Electronics

In the scanning configuration, the fixed waveband system is expanded to provide repetitive fast scans of the illumination wavelength to yield both absorbance and fluorescence excitation spectra. This detection system is interfaced to a Digital Equipment Corporation (DEC) LSI-11 computer.

For the spectrometric GC detection system, the waveband selection device is a modified Czerny-Turner grating monochromator. The fast scanning of the monochromator is accomplished via a 24-step stepper motor which is connected through gears and a drive belt to the rotating shaft of the grating. The stepper motor is controlled by the DEC computer, via a special electronic interface. The interfacing electronics between the DEC LSI-11 computer and the stepper motor are shown in Figure 13.

The stepper motor provides inexpensive and reproducible mechanical positioning in instrument's grating control because it rotates by precise angular

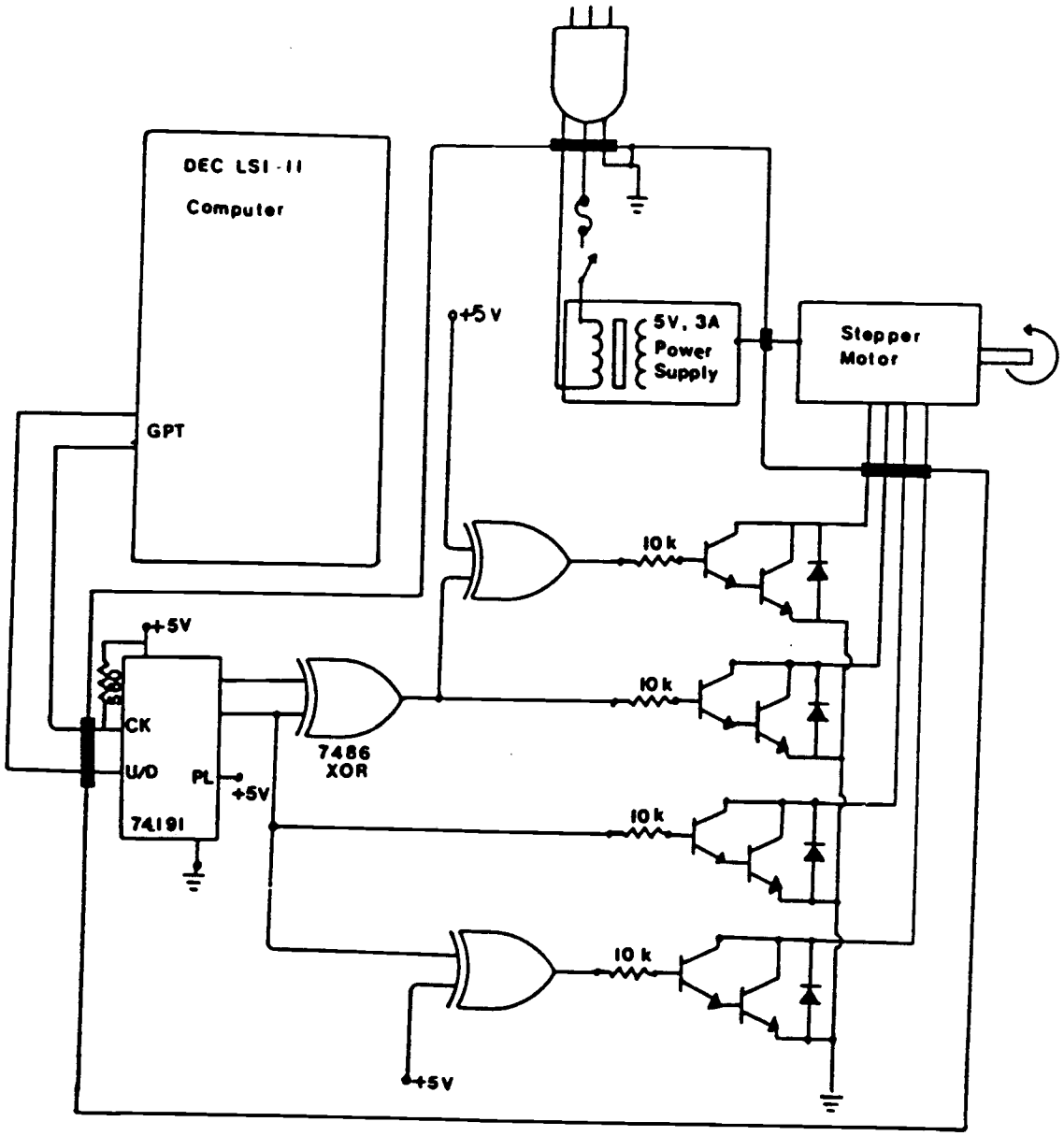


Figure 13. Interfacing electronics between the DEC LSI-11 computer and the stepper motor

increments for each step and is locked in place between steps. By interfacing the motor with a microcomputer, the scan rates, ranges and direction of the monochromator can be readily varied. A previously published circuit diagram was used as a model for the interfacing electronics between the stepper motor and the microcomputer [165]. Other such interfacing circuit diagrams are available [166].

The stepper motor consists of a rotor which is a permanent magnet with six alternating north and south poles around its circumference. This rotor is mounted on a shaft. The rotor position is determined by activation of electromagnetic poles which are driven in a specified four-step sequence. The rotor aligns magnetically with the energized stationary poles. To rotate the rotor, the polarity of the outer poles is sequentially changed by selectively providing a current through them. The motor has a 6-pole rotor and thus requires 24 steps per revolution [167]. The sequencing of the steps is controlled by a digital signal. The phasing of this signal on the four input leads is shown in Figure 14.

The power supply for the motor is a +5 Vdc, 3 A Open-Frame from Cougar D.C. Power Supply Company (Camarillo, California). Approximately 0.87 A/coil or 1.74 A/step total current is required to drive the motor. Another modular power supply from Polytron Devices, Inc. (Paterson, New Jersey) with $\pm$15 Vdc, +5 Vdc (output voltage 1% error maximum), and a current limited 250 mA output is used for the integrated circuits (see Figure 13) so that their supply voltage will not be affected by the stepper motor supply current surges.

Timed pulses and either high or low logic voltage levels (5 V or 0 V) are produced by the computer hard-

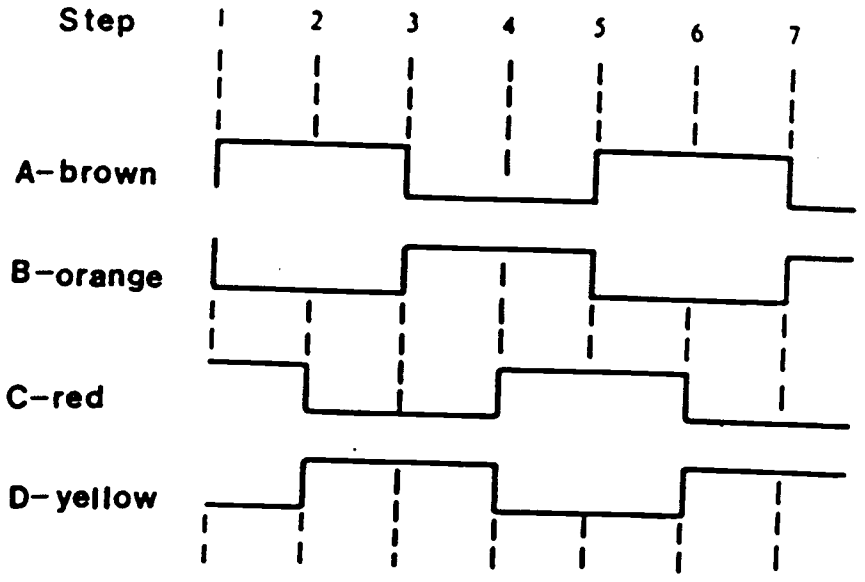


Figure 14. Phasing of the digital signal for the four input leads of the stepper motor

ware as described below. These are used to control a high-speed 74191 Up/Down Binary Counter. The timed logic pulses go to pin CK, Clock Pulse Input with an active rising edge and the logic level goes to pin U/D, the Up/Down Count Control Input. This allows the computer to control the direction and rate of counting. The clock pulse is connected to the +5 Vdc Open-Frame Cougar power supply for the motor through a 560Ω resistor as neither the computer nor the 74191 counter generate a high logic voltage (5 V) at this point. Pin PL, the Asynchronous Parallel Load Input with an active low voltage, must also be connected to 5 V to deactivate it.

The 74191 Up/Down Binary Counter provides a binary count waveform on flip-flops. Q_0 and Q_1, which are connected to the input connectors of Exclusive-Or gates as shown in Figure 13, produce the sequencing of the digital signal required for stepping by the motor. There are four Exclusive-Or gates on each 7486 chip and the logic table for these gates is:

Input Variables		Output
A	B	A + B
0	0	0
0	1	1
1	0	1
1	1	0

The sequenced digital signals generated are each connected through a 10 KΩ, 1/4-watt resistor to the base of a TIP120 NPN Darlington Pair Transistor, with a heat sink attached. The transistors are thereby used as switches to alternately provide current to the four leads of the stepper motor. The TIP120 is a general-purpose amplifier useful for low-speed switching and begins to falter at switching speeds of 200 Hz in this

circuit. A reversed biased diode (1N914) between the transistor emitter and collector provides protection from reverse voltage surges.

The LSI-11 microcomputer uses a 16-bit addressing system and has eight Central Processing Unit (CPU) registers including a Program Counter and Stack Pointer. The other six registers are General Purpose Registers which may be used in programming [168].

The LSI-11 computer contains 64 kilobytes of memory with approximately 40 kilobytes available for user programs and data. The computer uses 8-inch single-sided, double-density diskettes. Each diskette contains 974 blocks of data storage space, with each block containing 512 bytes.

The hardware boards used in this system include the M7270, Type 94V0 CPU and memory boards from Digital Equipment Corporation, a DLV-11J four-port serial (RS-232C) board, a disk drive card from Sigma Information Systems, Inc., a ADAC Model 1601GPT, General Purpose Timer, and a Data Translation DT1761 Data Acquisition System.

The LSI-11 uses a DEC RT-11 operating system which includes a Keyboard Monitor (KMON) and a DEC Keypad Editor. The editor is used to write programs which can be subsequently assembled or compliled by the CPU. The programs developed for this GC detection system are described in detail in the next section.

The timed pulses from the computer are produced by a Model 1601 GPT, General Purpose Timer from ADAC Corporation (Wasburn, Massachusetts). This card interfaces directly with the DEC LSI-11 computer and contains a 16-bit programmable counter. The crystal controlled clock rate is selected via software and may be set from 60 Hz up to 1 MHz. There are four modes of operation-two single count and two recurring count modes. In this

research, MODE 1 was used as a recurring count to activate the 74191 Up/Down Counter.

The Model 1601 timer is controlled by two registers on the interface card (see Figure 15). The Control Status Register (TIMCSR) is located at address 170420 (octal) and the Counter Input (Output) Buffer Register (TIMBUF) at address 170422 (octal). Bits 0-8 of the TIMCSR are loaded through software to set the clock rate, mode, and control the clock. The 16-bit TIMBUF stores the program-loaded count. The count in this buffer decrements at the clock rate and produces an output voltage (10 μs active low pulse) when it reaches zero. In the recurring count mode, the count is automatically reloaded in TIMBUF after each pulse [169].

The high or low logic voltage for the 74191 Up/Down Binary Counter is produced by a DT1761 Data Acquistion System from Data Translation Corporation (Marlboro, Massachusetts). This interface board is primarily designed for analog-to-digital (ADC) and digital-to-analog (DAC) conversions. In addition, however, bits 13 and 12 of the D/A Data Buffer Register (DACBR) produce four logical output voltages, called Mode Out voltages. One of these outputs is used to provide either a high or low logic level voltage to the 74191 chip to control whether it counts up or down.

The DT1761 card is one of a series of 12-bit ADC data acquisition systems designed for use with the DEC LSI-11 series of microcomputers. The DT1761 is an analog input/output (I/O) system with eight, multiplex-controlled, differential input ADC channels (sample and hold). Each multiplexer channel has a 100 MΩ input impedance. It also contains two DAC channels and a programmable input gain. The A/D module on the board is Model DT5710B-D, with a 7.0 μs A/D conversion time and a

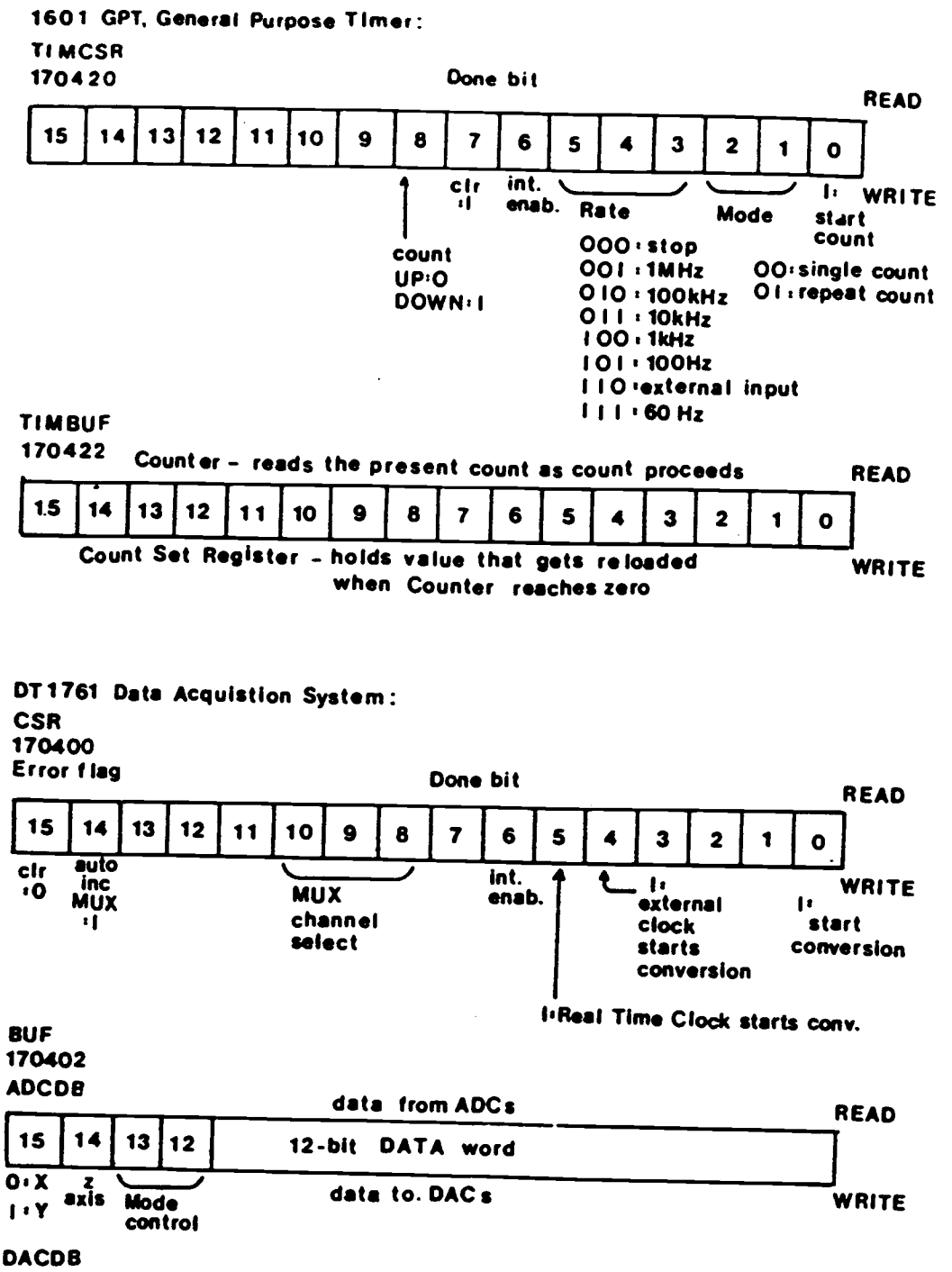


Figure 15. The two registers which control the functions of the 1601 GPT, General Purpose Timer, and the three control registers for the DT1761 Data Acquisition System

maximum number of 100,000 samples per second with a 10.0 μs throughput time.

The ADC and DAC ranges and data notation may be selected by changing the jumper connections on the card. The possible ranges are unipolar 0-5 V or 0-10 V, or bipolar ±5 V and ±10 V. The data notation modes are binary, offset binary (with negative data ranges), or two's complement.

For the ultraviolet spectrometric GC detection system, a unipolar range of 0-10 V was chosen with binary data notation. All signals in this system should be positive and using a unipolar range gives one more bit of resolution (i.e. no sign bit). A 12-bit ADC of a 10 V signal gives a resolution of 10/4096 = 0.0024 V.

The DT1761 has three interface registers that are controlled by software (see Figure 15). The Control Status Register (CSR) is located at address 170400 (octal). This register contains an ADC start bit, a done bit, multiplex (MUX) channel select, auto-increment of MUX select, a real-time clock, an external trigger enable, and a gain select. The ADC and DAC Data Buffer Registers (ADCDB and DACDB, respectively) are both located at address 177002 (octal) which contains a 12-bit data word, and a X-Y select for the two DACs [170].

Three multiplexer channels (CH 0-2) were used to make analog-to-digital conversions of the absorbance signal, fluorescence signal, and the voltage proportional to the monochromator position, i.e. wavelength. Also, the absorbance signal was output using the X-DAC and the fluorescence signal was output on the Y-DAC so that they could be monitored during a chromatographic run on oscilloscopes (the scanned signals changed too rapidly to be monitored with a regular strip chart recorder). The external connections used on the computer are summarized in Table 3.

Table 3. External Connections on the DEC LSI-11 microcomputer

Label on BNC on LSI-11	Grounded to computer?	External Connection
DAC	Y	oscilloscope for absorbance signal
Y-DAC	Y	oscilloscope for fluorescence signal
ADC CH 0	N	absorbance signal input voltage, 0-10 V
ADC CH 1	N	fluorescence signal input voltage, 0-10 V
ADC CH 2	N	"wavelength" input voltage, 0-10 V
MOD 2 (level out from DT1761 board)	Y	74191 Binary Counter, pin U/D
Pulse Out (pulse from 1601 GPT)	Y	74191 Binary Counter, pin CP

F. Computer Control Software

1. DEC LSI-11 Programs

Programs were written for the UV wavelength scanning GC detection system on the DEC LSI-11 microcomputer in Fortran IV and RT-11 Macro assembly language. These programs provide the scanning of the monochromator via control of the stepper motor and initiate and store analog-to-digital conversions for the absorbance, fluorescence, and "wavelength" voltage signals. These data acquisition programs also allow the user to select control parameters for the GC detector, store results of background spectra and noise measurements, store data files on floppy disks, and label the stored data files. A flowchart showing an overview of the data acquisition programs for repetitive spectra of GC effluents is shown in Figure 16.

When spectrometric detection data have been collected and stored on a disk, the files are transferred to an IBM-PC compatible computer for three-dimensional axonometric plotting and additional data analyses. Fortran and Macro programs retrieve the data from the LSI-11 files, re-organize it so that it is in the proper format for the IBM-PC files, and send data it to the CRT screen. A program from Freeware (Tiberon, California) named PCTALK allows the IBM-PC computer to act as a monitor for the LSI-11 and simultaneously store any characters typed on the monitor in a PC file. The data reduction methods used on the IBM-PC are described in Chapter V.

Detailed descriptions of the software developed for controlling and monitoring the UV spectrometric GC detection system are included below and hardcopies for the programs are in Appendix C, Computer Programs.

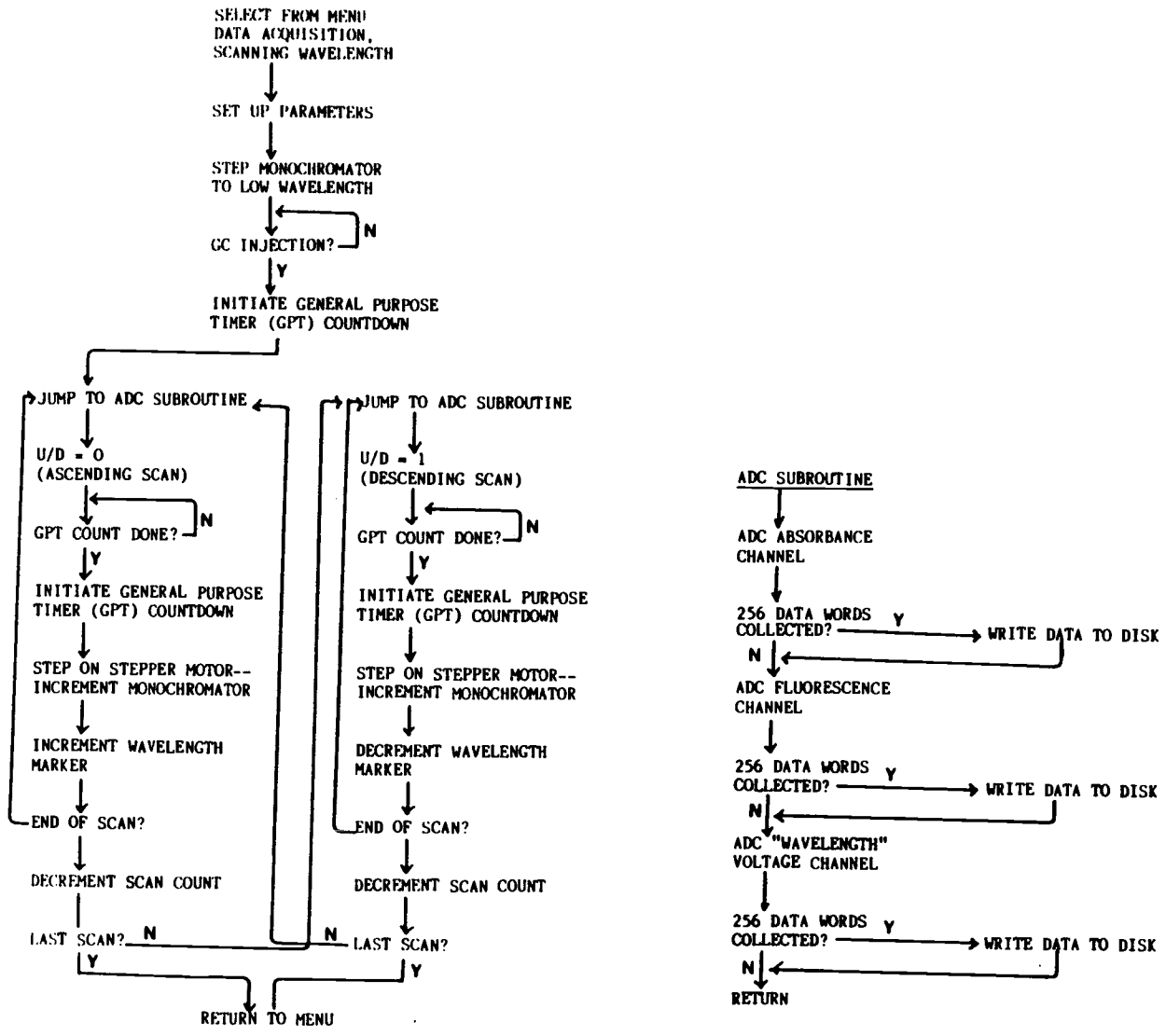


Figure 16. Overview of DEC LSI-11 data acquisition programs

a. SIMULT Fortran Program

The Fortran program named SIMULT.FOR is the main Menu program for the data acquisition from the spectrometric detection system. This program is designed to allow the operator to select control parameters such as scan ranges, stepping rates, and data accumulation durations. SIMULT.FOR also does several calculations on the collected data in Fortran IV. This program has four subprograms, formatted in Macro assembly language: SETUP.MAC, ADCSPE.MAC, NOISE.MAC, and ADCHRO.MAC. These subprograms are used to perform a variety of timed functions, make analog-to-digital conversions in response to the signal voltages, and create data files on the floppy diskettes. The flowchart for SIMULT.FOR program is shown in Figure 17.

The initial instructions of the SIMULT.FOR program designate variables as logical, real, or integers, dimensions of arrays are set, and variables which may be used repeatedly are set to zero. A title is displayed on the monitor as well as a Menu with three choices: a) make a GC run collecting spectral information (scanning wavelength), b) make a GC run at a fixed source radiation wavelength, or c) quit. The user may select the choice.

If the GC detection mode chosen allows for spectral information to be collected, the user is prompted to select the minimum and maximum wavelengths to be scanned over a maximum range of 50 nm. The operator then provides the wavelength currently selected by the monochromator, which is normally displayed by a LED on the light module housing. Wavelength scans are designed to begin at the low wavelength in this system. Thus, a variable indicating the scan direction, UD, is set via the computer program to indicate whether the monochroma-

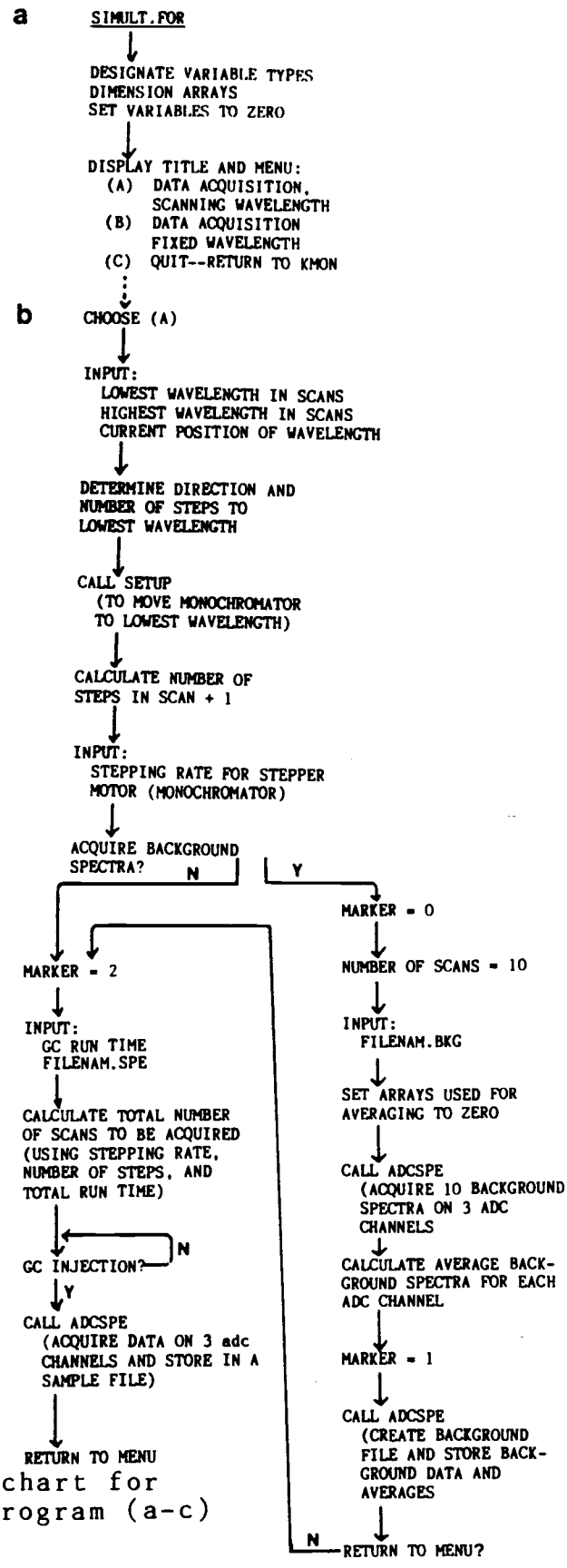


Figure 17. Flowchart for SIMULT Fortran Program (a-c)

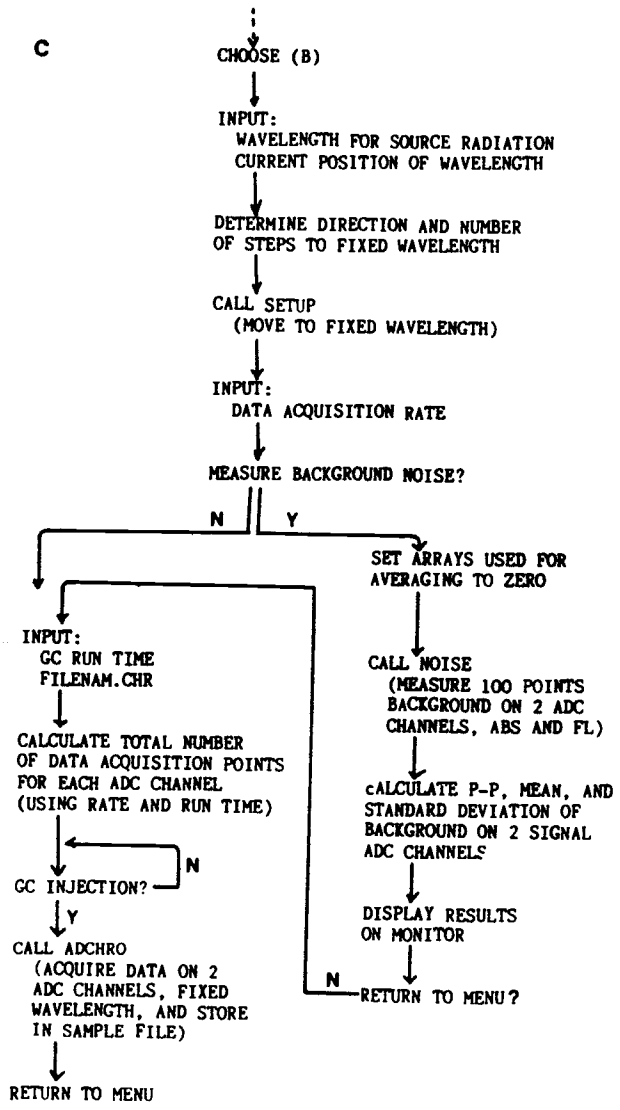


Figure 17 (cont.)

tor is currently above or below the selected starting wavelength and the number of stepper motor steps required to adjust the monochromator to the starting wavelength is calculated.

The number of steps plus one in a scan is then calculated because the monochromator will scan both toward the longer wavelengths and then toward shorter wavelengths. The user also selects the stepping rate of the stepper motor, to a maximum rate of 100 Hz. A variable called Count is calculated as 100 divided by the rate. This variable is used by the General Purpose Timer to adjust the pulse rate produced and provided to the control electronics for the stepper motor. The clock is automatically set at 100 Hz by the ADCSPE Macro subprogram.

SIMULT.FOR then calls the Macro subprogram SETUP to which it assigns the variables containing the number and direction of steps required to reach the starting wavelength for scanning. The subprogram SETUP.MAC controls the stepper motor and adjusts the monochromator to this wavelength, as described below in the next section.

The user is prompted to select whether background spectra are to be collected. If so, the number of scans is set to ten, a variable called MARKER is set to indicate a background run, and other variables and arrays used to calculate the averaged background spectra are set to zero.

In order to collect background spectral data, SIMULT.FOR calls the Macro subroutine ADCSPE. Many variables and arrays are passed back and forth between these two programs. Some are designated in SIMULT.FOR and others are assigned by the ADCSPE.MAC subroutine. The ADCSPE.MAC subroutine is called for three purposes by the SIMULT.FOR program: a) to acquire background

spectra, b) to label and store a background file, and c) to acquire sample spectra during a GC separation and store it in a labeled file. Not all variables passed between the two programs are used in all cases and the variable MARKER indicates which function the Macro program should perform.

The ADCSPE.MAC subroutine collects five scans toward longer wavelengths and five scans toward shorter wavelengths of the background absorbance, fluorescence and "wavelength" voltage signals at the selected scan speed and wavelength range, which are the same as those used for as the subsequent chromatographic sample. The background data are stored in a three-dimensional array. This filled array is passed back to SIMULT.FOR when ADCSPE.MAC returns control to the main program. The SIMULT.FOR program then averages the ten scans. Conversions between real and integer variables are necessary to perform some calculations on the integer data received from the ADC.

After the averaged background spectra have been determined, ADCSPE.MAC is again called to create a file containing the averaged and raw background spectral data. The previously selected file name should have a .BKG suffix. The first block (256 words) of all files created by this system contains an ASCII label. In the second block are stored the values of variables which describe the run parameters, followed by the averaged background spectra from the three ADC channels. In the third and subsequent blocks are stored the actual values produced by the ADC channels for the ten individual spectra. Further details on file arrangement are decribed below in the section on ADCSPE.MAC.

When control returns to the main Fortran program, the operator may opt to return to the Menu. Otherwise, he is prompted to select the duration of data

acquisition, the GC run time. Using the rate of stepper motor control pulses and the number of steps in a scan, the scan time is calculated. Then the GC run time is converted to units of scan time and the total number of scans is determined for the entire GC separation. The ADCSPE.MAC subprogram counts the number of scans completed and stops acquiring data when this number is reached.

At this point, a GC injection may be made. The Return key is pushed at the time of the injection to call the ADCSPE.MAC subprogram and begin data collection during a GC separation of a sample. If "Q" is input from the keyboard instead, the program will return to the Menu.

Again, many variables are passed to the Macro subprogram as previously discussed. During collection of data from a sample, a data file is opened immediately on the disk and the signal data acquired from the three ADC channels is written to the disk after each 256 points. This corresponds to one block of data on an 8-inch floppy diskette. It is necessary to write data to the disk often due to the limited memory available in the DEC LSI-11. At the completion of the run, after all the data have been collected and stored, an ASCII label is entered by the user and stored in the first block of the file. In the second block is stored information about the run parameters and file size, followed by the most recent array of averaged background spectra. Control then returns to SIMULT.FOR and the Menu is displayed on the monitor again.

If a fixed wavelength detection mode is selected from the Menu, the operator inputs the current wavelength and the wavelength to be used as the source radiation from the keyboard. Again, the SETUP.MAC subprogram is used to move the monochromator to the

selected wavelength. Following this, the data acquisition rate for the two ADC channels (absorbance and fluorescence, only) is selected by the operator and used to calculate the count for the GPT in the same manner as described above.

The user is prompted to select whether or not a determination of the background signal should be made for the two ADC signal channels at the fixed wavelength. If so, all variables and arrays to be used for calculations are re-set to zero. Then the subprogram NOISE.MAC is called. This program acquires 100 background noise measurements on each of the two channels and stores the values in a two-dimensional array.

When the subprogram NOISE.MAC returns control to the SIMULT.FOR main program,the values in this array are used to calculate the means, standard deviations, and low and high noise values for the absorbance and fluorescence channels. This information is displayed on the monitor in a labeled message. This is useful for monitoring the absorbance and fluorescence signals at a selected wavelength prior to any GC run, with either fixed or scanned illumination wavelength. After the background signal determination, the user may opt to return to the Menu or continue on to make a GC sample determination with fixed illumination wavelength spectrometric detection.

If a fixed wavelength determination is selected, the user enters the GC run time, and file name from the keyboard, and inputs "Return" at the time of injection (or "Q" to return to the Menu). The ADC data acquisition and storage of data in files on a disk are executed by a Macro subprogram called ADCHRO. ADCHRO.MAC counts the total number of points to be collected at the selected data acquisition rate and

automatically stops collecting data at the end of the GC separation (GC run time). Again, the acquired ADC values are stored beginning in the third block of the disk file. The first block of the file is designated for ASCII labeling information which is entered by the operator after the completion of the GC separation. The second block stores run parameters, file size information, and background signal information, as well as the raw background noise data for the absorbance and fluorescence ADC channels.

b. SETUP Macro Subprogram

The Macro subprogram SETUP is designed to move the grating monochromator from the current wavelength to either the minimum scanned wavelength or fixed wavelength for the source illumination radiation for the spectrometric GC detector. The wavelengths required are entered by the operator during execution of the SIMULT.FOR main program. The direction and number of steps to the selected wavelength are calculated and passed as variables to SETUP.MAC. The SETUP.MAC subprogram is called at two places in SIMULT.FOR, each prior to a GC run. The flowchart for the SETUP.FOR subprogram is diagramed in Figure 18.

The initial instructions of the SETUP.MAC subprogram designate the title and the addresses of the Control Status Register (CSR=170400 (octal)) and Buffer (BUF=CSR + 2) for the Data Translation DT1761 Data Acquisition board, and the Control Status Register (TIMCSR=170420 (octal)) and Buffer (TIMBUF=CSR + 2) for the ADAC 1601GPT General Purpose Timer. These Registers and Buffers are indicated at the beginning of each of the Macro data acquisition subprograms. The bit assignments for each are shown in Figure 15.

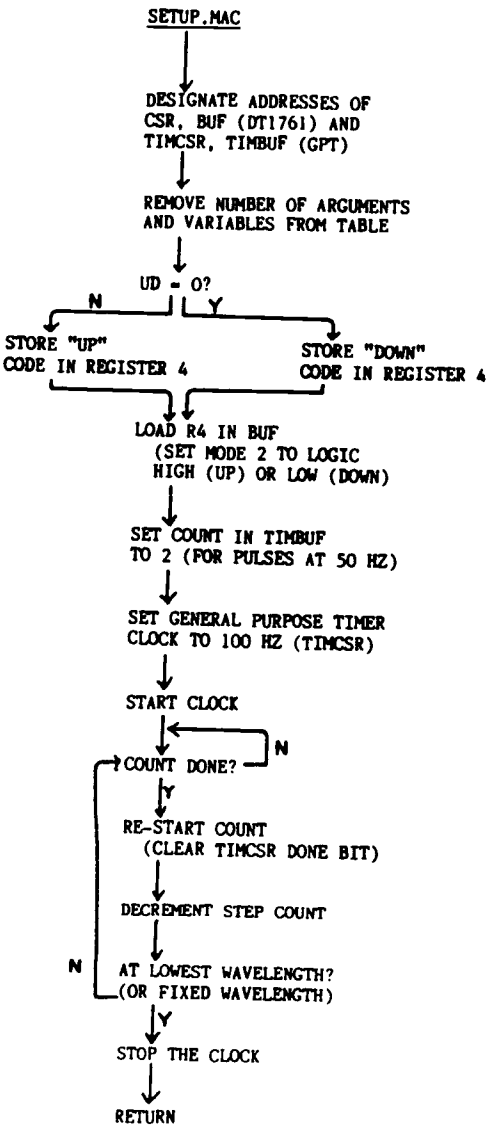


Figure 18. Flowchart for SETUP Macro Subprogram

Whenever variable assignments are passed from a Fortran main program to a Macro subprogram, they are stored in a table created by the Fortran program at the time of the Call statement. The last item stored in this table is the number of arguments. In order to use the values of these variables in the Macro assembly language subprogram, they must be removed from the table in the same order as they are listed in the Call statement and either stored in the LSI-11 Registers or assigned to variables in the Macro program. The up/down marker, UD, and total number of stepper motor pulses required to reach the selected wavelength are thus removed from the variable transfer table and stored in the R0 and R1 Registers respectively. At the end of the program, the current values of the variables will automatically be returned and assigned to the appropriate variables in the Fortran main program when a Return statement is executed.

The next sequence of statements in SETUP.MAC determines the direction indicated by the up/down marker. The appropriate 16-bit word (#030000 for down or #010000 for up (octal)) is loaded in R4 and also written to the DAC buffer so that the Mode Out 2 connection to the stepper motor electronics will produces a high or low logic voltage, thus establishing the direction of movement of the stepper motor.

A clock count of #2 (decimal) is loaded into to the general purpose timer's TIMBUF. The clock is set to a frequency of 100 Hz in the recurring count mode by loading a #656 (octal) to the TIMCSR. With the clock set at 100 Hz and the count in the TIMBUF at 2, the GPT will send out pulses at a rate of 50 Hz. To start the clock, a #1 is written to Bit 0 of the TIMCSR. The program then monitors the Done bit (Bit 7). When the count is done, this bit is cleared by writing a #1 to it.

Whenever a count is completed by the GPT, a high logic voltage pulse is produced on the Pulse out BNC which is connected to the stepper motor electronics. The program counts the number of pulses and stops the clock after the required number of steps on the stepper motor has been made. To stop the clock on the GPT, a #0 is written to Bit 0 of the TIMCSR. At this point, control returns to the main Fortran program, SIMULT.FOR.

c. ADCSPE Macro Subprogram

ADCSPE is a long Macro subprogram that has a variety of functions (see Figure 19). Among these are controlling the direction, range and rate of scanning of the monochromator, making analog-to-digital conversions of the absorbance, fluorescence and "wavelength" voltage channels, and providing an output voltage of the absorbance and fluorescence signals on the X- and Y-DAC. ADCSPE.MAC also creates data files, stores data in a disk file during a GC separation, and stores background spectra and a label at the beginning each data file after the completion of a GC sample determination. The assignments for the detector and run parameters and filename address are received as variables via a CALL statement from the SIMULT.FOR main program.

The first part of the program has a title, calls certain system MACRO routines, designates the Control Status Register and Buffer addresses for the DT1761 and GPT, and retrieves the variables assignments and array addresses from the transfer table.

The ADCSPE.MAC subprogram is called under three conditions by the SIMULT.FOR main program and the variable MARKER is used to indicate the purpose of each call. It is called to take measurements of ten background spectra. These scans are stored in an array

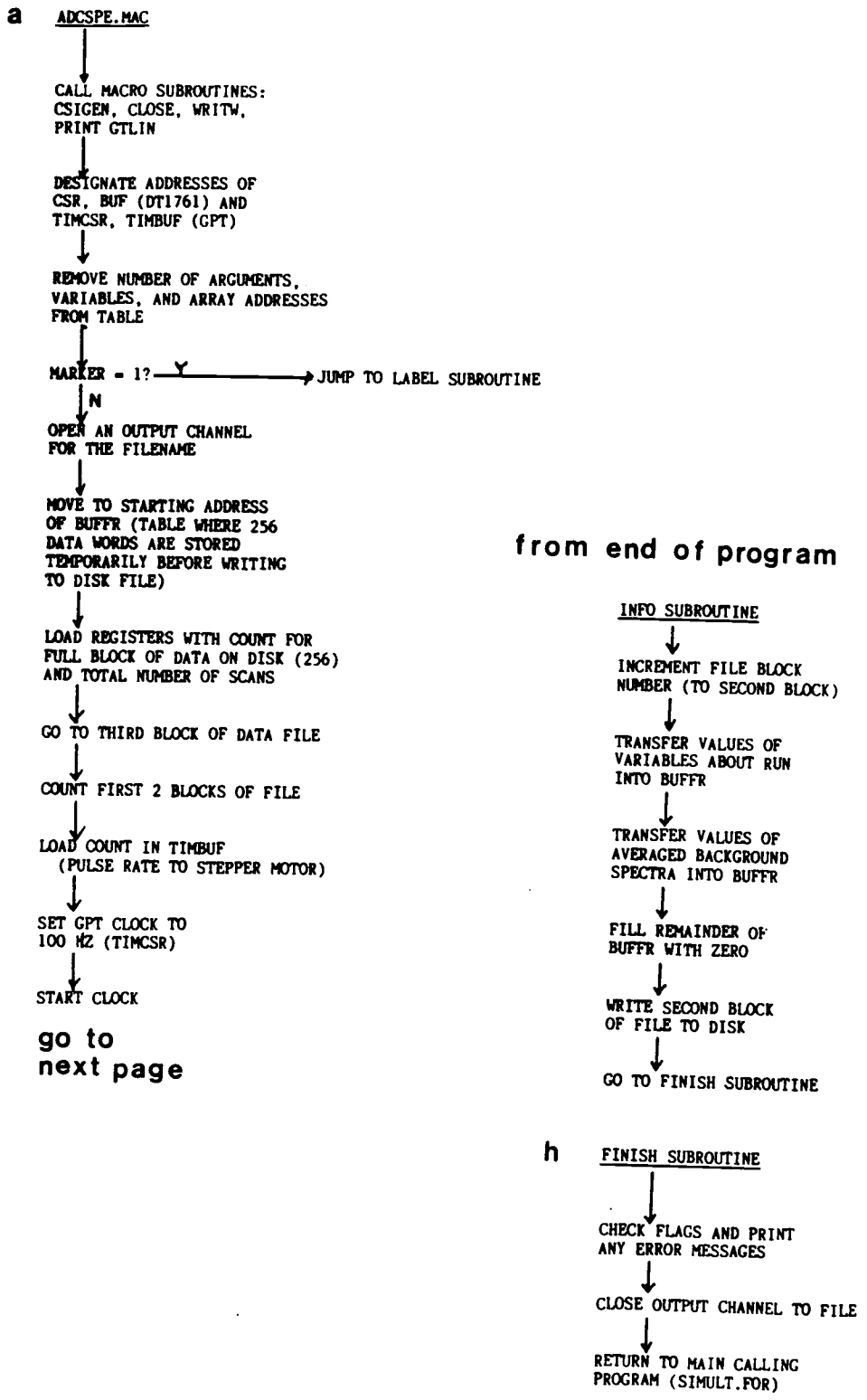


Figure 19. Flowchart for ADCSPE Macro Program (a-h)

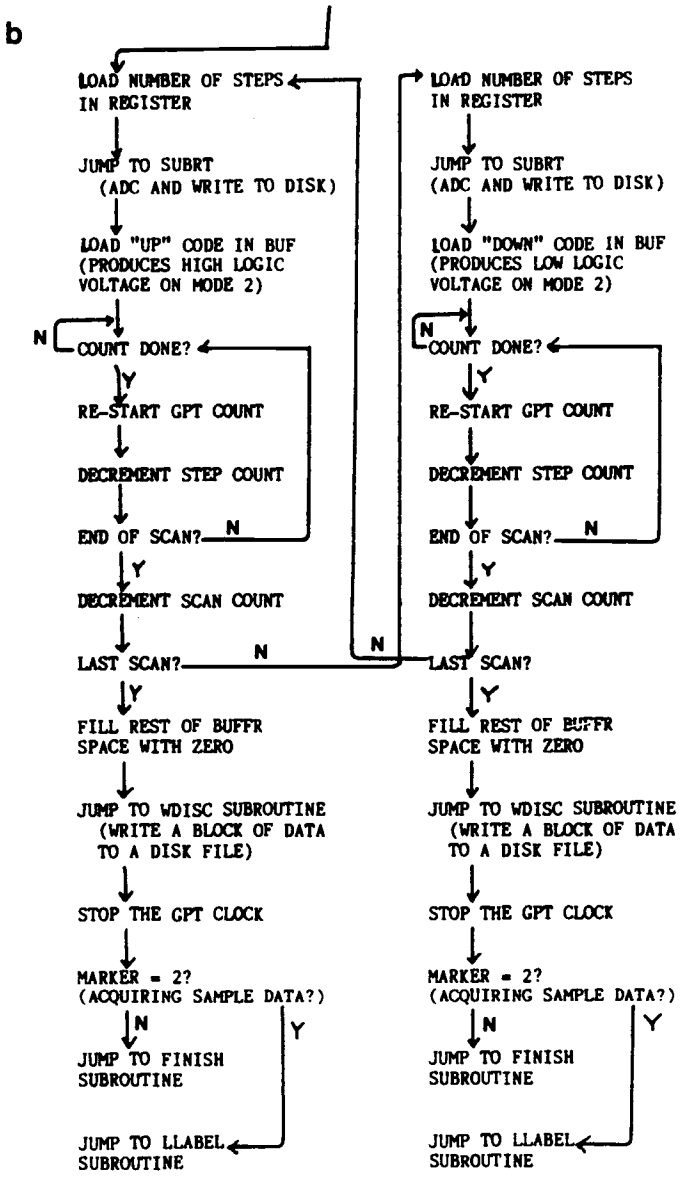


Figure 19 (cont.)

C

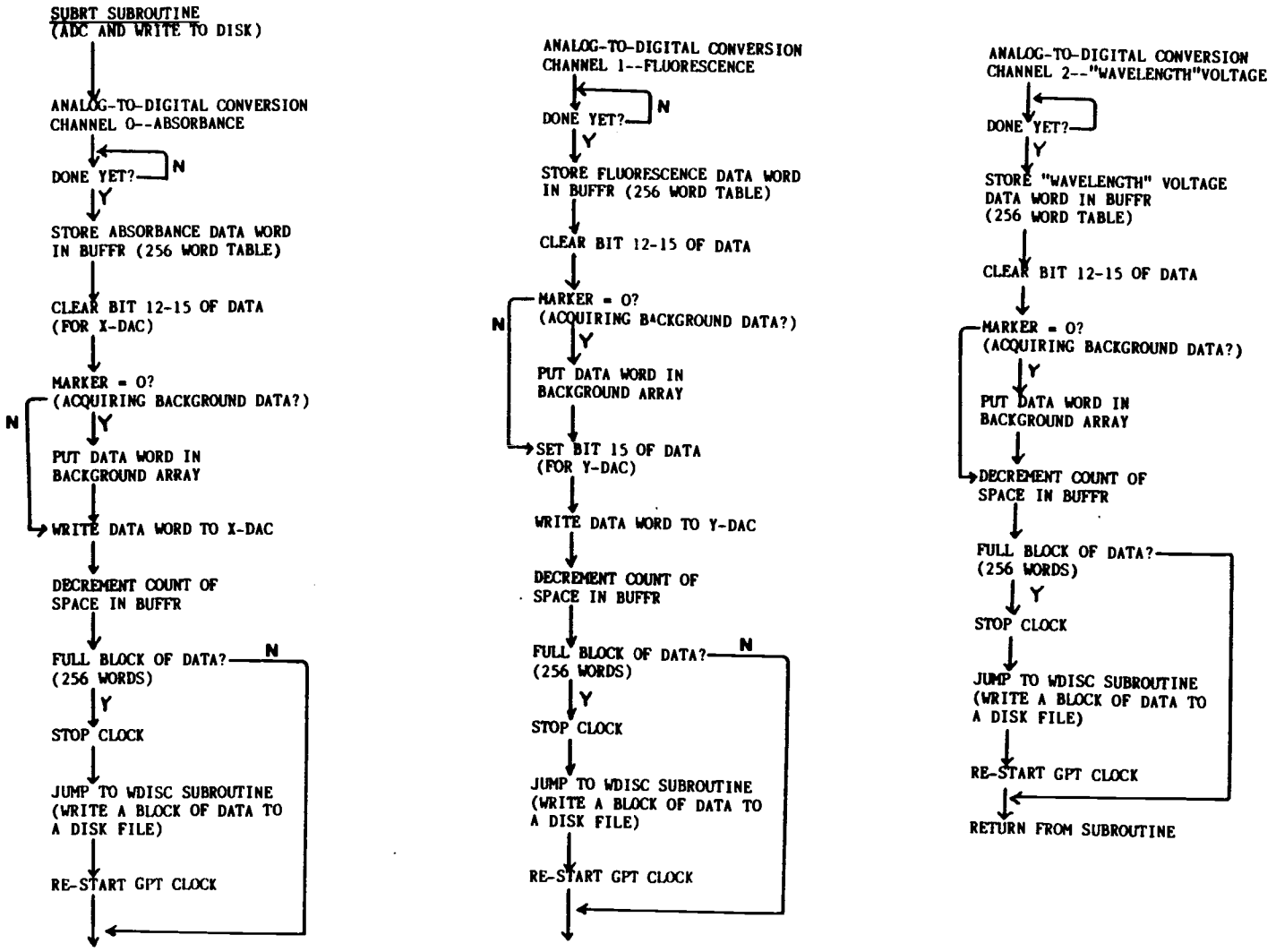


Figure 19 (cont.)

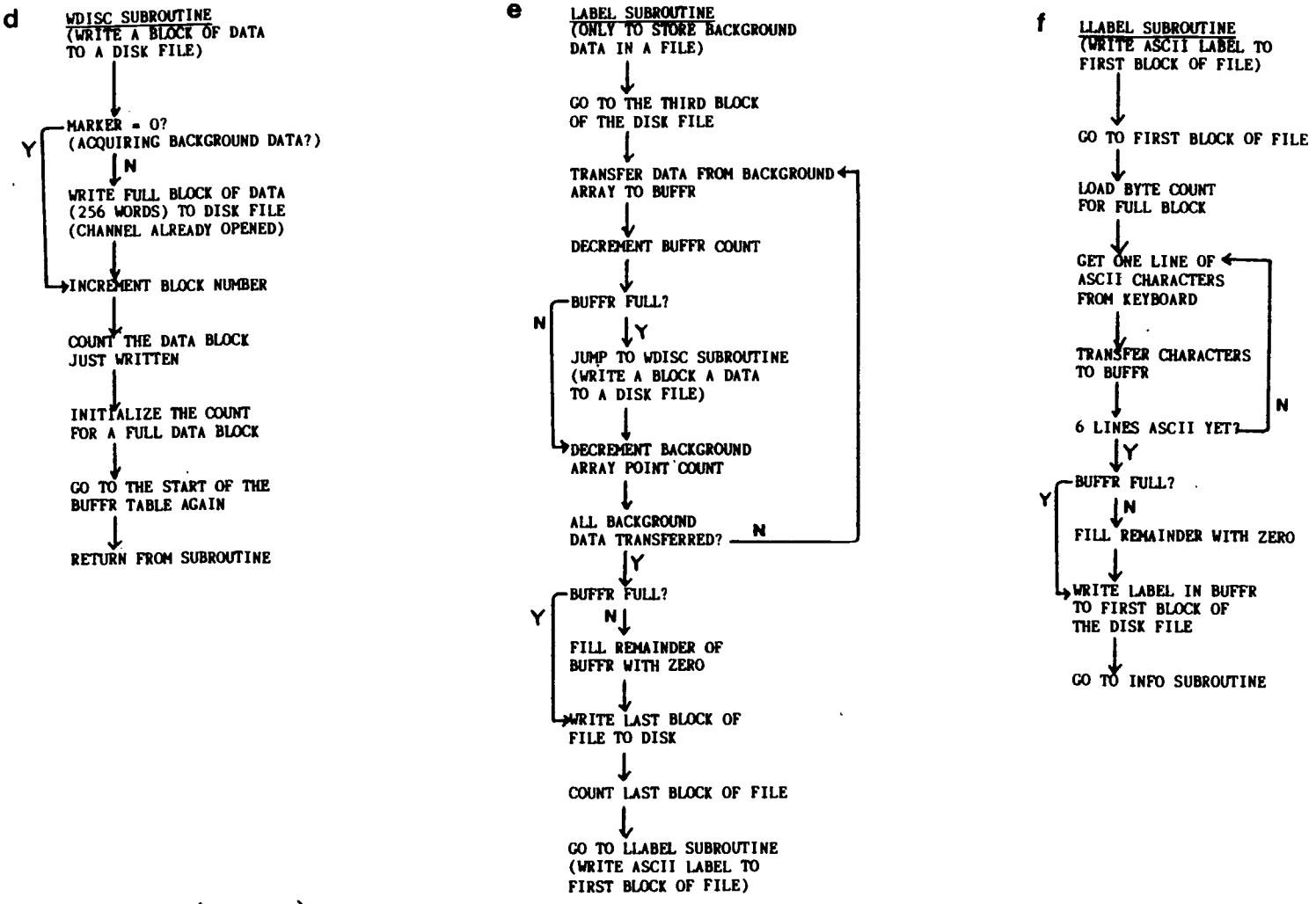


Figure 19 (cont.)

called STORE by the Macro subprogram and the filled array is returned to the SIMULT.FOR main program. This array is used to calculate the averaged background spectra for each of the three ADC channels.

The second circumstance when the ADCSPE.MAC subprogram is called by the main Fortran program is in order to write the averaged and initial data from background spectra determinations to a file on the disk. This FILENAM.BKG file will have a ASCII label in the first block and also stores information about run parameters and the averaged background spectra in the second block of the file.

The ADCSPE.MAC subprogram is also called to collect and store spectrometric data during a GC separation of a sample. In this case, a disk file with a name format of FILENAM.SPE is created immediately and the ADC data is stored in this file after each 256 acquisitions (one block of data words on an 8-in floppy diskette). In the file, the data is stored beginning in the third block, leaving the first and second blocks available for an ASCII label, run parameters, and averaged background spectra. These blocks are stored in the file by the ADCSPE.MAC subprogram after the completion of the GC separation (GC run time).

After the initialization routines in the program, ADCSPE.MAC determines if it has been called to store and label a background run. If so, it jumps to a later part of the program called LABEL. Otherwise, data will be collected on the three ADC channels.

The CSI opens an output channel for the filename stored beginning at the address FNAME. BUFFR is a block of memory consisting of 256 words which is set aside to temporarily store data collected from the three ADC channels prior to being stored in a file on the disk. Register R2 points to the current address in this buffer

and R1 is used as a count of the memory space available in BUFFR. The total number of scans, which is equivalent to the duration of data acquisitions or GC run time, is stored in R4. The address for the STORE array (background spectra) is loaded into R0. R3 is used to count the number of steps in a scan.

IOBLK is a variable which indicates the input/ output block number in the file on the disk currently being written to and BLKSAV counts the total number of blocks in a file. IOBLK is cleared and then incremented twice so that data will be stored beginning in the third block of the file.

The count, as determined in the main Fortran program, is loaded into the TIMBUF and the clock is always set at a rate of 100 Hz. Therefore, the maximum stepping rate for this system is 100 Hz, with the count set to one. UV illumination source scans are designed to always begin at the minimum scanned wavelength. Therefore, the correct 16-bit word is written to the DAC Buffer to make Mode Out 2 connection produce a high logic level (5V) to the stepper motor electronics. This will produce an ascending wavelength scan. At the completion of an ascending wavelength scan, a descending scan is initiated by loading the correct 16-bit word into the DAC Buffer to make Mode Out 2 produce a low logic voltage. Separate sections of the program which are essentially identical except for the Mode Out potential produced, control the stepper motor to scan the monochromator toward either higher wavelengths or lower wavelengths.

The GPT clock is started and the Done bit monitored in the same manner as described above in the section on the SETUP.MAC subprogram . Once a count has been completed, a pulse goes out to the stepper motor

electronics and the Done bit is cleared.

Prior to each step on the stepper motor, the program jumps to a subroutine, SUBRT, which collects one data point on each of the three ADC siganl channels. To start a conversion on Channel 0, absorbance, a #0 is written to Bit 0 of the DT1761 CSR. Bit 7, the Done bit is polled to indicate when the conversion is complete. The 12-bit data value is available in Bits 0 through 11 of the address BUF and is stored in the 256 word buffer at the address to which R2 points. Bits 12-15 of the data are cleared and then written to the DAC Buffer so the absorbance voltage may be monitored on the X-DAC connection during the GC separation with an oscilloscope. If a background spectrum is being acquired rather than sample data, the data value will also be stored in the STORE array.

To collect a data point on the fluorescence ADC channel, a #400 (octal) is written to the DT1761 CSR. This is the same as writing a #1 to Bit 8 of the CSR which selects multiplexer Channel 1. This also clears Bit 0 which automatically starts a conversion. Once the conversion is complete, the same sequence of events occurs as with the absorbance channel. The only exception to this is that the fluorescence signal is produced on the Y-DAC connection, so that it may be monitored during a GC separation by a second oscilloscope. The Y-DAC is selected by setting Bit 15 of the data word prior to loading it into the DAC buffer (BUF). This bit is cleared prior to storing the fluorescence data in a file on the disk.

The ADC on Channel 2 for the "wavelength" voltage is started by writing a #2 to the high byte of the DT1761 CSR. This writes a #1 to Bit 9. This channel is not output to a DAC.

After each data point is collected, the address that R2 points to is incremented and the memory space counter for BUFFR, R1, is decremented. R1 is checked after each ADC on each channel. If it is zero, the clock is stopped and the program jumps to another subroutine, WDISC, to write the block of data currently stored in BUFFR to the file on the disk. After the data are stored in the file, IOBLK and BLKSAV are incremented, R1 is re-loaded with a count of 256, and R2 is pointed to the first address of BUFFR again. The clock is then re-started and the program returns to continue acquiring more ADC data of the signals.

When the program jumps to the WDISC subroutine, it checks the MARKER variable to see is background spectra are currently being measured. If so, the data is not written to the file on the disk until after the averaged background spectra have been calculated by the main Fortran program.

The above sequence of collecting and writing data on each of the three ADC channels occurs prior to each step on the stepper motor. After each step, the step counter, R3, is decremented. When R3 reaches zero, the monochromator has completed either one upward or one downward wavelength scan and the count of the total number of scans to be acquired, R4, is decremented. If the scan counter has not yet reached zero at this point, the program jumps from either the ascending scan sequence of instructions to the descending scan sequence or vice versa. The number of steps in a wavelength scan is reloaded into R3 and the next scan begins.

When the count of the total number of scans, R4, reaches zero, the GC separation run time is complete. If the 256 word data buffer table, BUFFR, is not full when this occurs, the remainder of the 16-bit data words are set to zero. The program then jumps to the WDISC.

subroutine to write the last block of the file to the disk. The clock is stopped by writing a #0 to Bit 0 of the TIMCSR.

At this point, the MARKER is checked to see what type of data has just been collected. If the ADC data has been acquired of the background spectra, the program closes the output channel and returns to the SIMULT.FOR main with the filled STORE array to compute the averaged background spectra. After the averaged spectra are calculated and stored in the AVE array by SIMULT.FOR, the ADCSPE.MAC subprogram is called again. Now the MARKER variable indicates that a background file is to be created and the program jumps down to the LABEL section.

The LABEL subroutine opens an output channel and writes the background spectral data to a file on the disk. It initially increments IOBLK to the third block of the file and moves the values of the STORE array to the 256 word buffer table, BUFFR. When BUFFR is full, the WDISC subroutine is used to write the data to the disk file and increment IOBLK and BLKSAV. This is continued until all the background spectral data has been stored in the file. After the LABEL subroutine is completed, the program moves on to the LLABEL and INFO portions of ADCSPE.MAC.

If MARKER indicates that a sample GC separation has just ended when the data collection is complete, the ADCSPE.MAC subprogram jumps directly to the LLABEL subroutine without returning to SIMULT.FOR in the interim.

The LLABEL part of this program is used to store six 80-byte lines of ASCII characters which are entered by the operator from the keyboard after a prompt message. The input characters are temporarily stored in an 80-byte buffer. After each carriage return, the

program jumps to the ADDBUF subroutine which transfers each non-blank byte to the 256 word buffer. When the end of the line is encountered, the ASCII equivalent to a carriage return and line feed are added to the buffer. Then the program returns from ADDBUF to get another 80-byte input line. After six carriage returns have been received, the remainder of the buffer block is filled with zero, IOBLK is cleared to indicate the first block of the file on the disk, and the entire buffer is stored in the file.

The INFO section of the ADCSPE.MAC subprogram stores the run parameters, which are available in the variable assignments, as well as the latest averaged background spectral data, which are stored in the array at address AVE. The IOBLK is incremented to indicate the second block of the file. First, the variable values are transferred to the 256 word buffer, BUFFR, in the following order: BLKSAV, the total number of blocks in the file; COUNT, the timer count=100/the stepping rate; LOWL, the low scanned wavelength; STEP, the number of steps in a scan +1; and SCAN, the total number of scans in a run. Following this, the averaged background spectra for the three signal ADC channels are transferred from AVE to the buffer and the remainder of the unused words in BUFFR are set to zero. Then the second block of the file is written to the disk file.

Finally, the output channel is closed, any error messages are typed, and control returns to the SIMULT.FOR main program.

It should be noted that the total acquisition or GC run time based on the total number of scans is not exact although it is very reproducible under identical run parameters. The reason for this is that a small fraction of a second is lost each time the clock is stopped to write a block of data to the disk. This

error becomes greater in magnitude at stepping rates of 50 Hz or more and longer GC run times (e.g. greater than 5 min). Under these conditions, the total data acquisition time is noticiably longer than the selected GC run time by ten seconds or more.

Another factor to be aware of when making long GC separations at fast scan rates (e.g. 1-2 50-nm scans per second) is that the ADCSPE.MAC subprogram stops writing to the disk after a file reaches a size of 483 blocks (about one half of the space on an eight inch diskette). If the data from a GC separation exceeds this number of blocks, the monochromator will continue to scan, but the data values are not written to the disk and are lost. At the end of the GC run time, the program stops, writes the labeling blocks to the file, and closes the file, with no indication that loss of data has occurred. The practical importance of this limitation is that for 50-step scans (62-nm) at a stepping rate of 50 Hz, the maximum GC separation time cannot be greater then approximately 13 minutes. This problem could be overcome by closing the first file and opening another after 483 blocks have been written to the disk. However, usually the GC temperature program and column can be chosen to allow a separation to be completed quickly enough that this is not necessary.

d. NOISE Macro Subprogram

If the fixed waveband spectrometric GC detection mode is chosen from the Menu in the SIMULT.FOR program, the user may opt to make measurements of the background signal at the chosen wavelength on both the absorbance and fluorescence channels. The data acquisition for these measurements is made by a Macro subprogram called NOISE.

NOISE.MAC collects 100 ADC measurements on both of the signal channels at the same data acquisition rate as the sample GC run. The program is started with the same sequence as the previously described Macro programs with a title, call for system Macro routines, assignment of the addresses for the CSR and Buffers for the DT1761 and GPT, and getting any transferred variables assignments. In this case, these include the clock count and a two-dimensional array to hold the background noise data values.

Analog-to-digital conversions are made at the selected data acquisition rate on the absorbance and fluorescence channels. These conversions are initiated in the same manner as described above in the section on the ADCSPE.MAC subprogram. However, the background signals at one wavelength are not output to the X- and Y-DAC, nor are they stored in a file separate from the GC sample run file such as is the case with the averaged background spectra. The values are stored in the 2-dimensional array and returned to the main Fortran program where the mean, standard deviation, and high and low noise values are calculated for the absorbance and fluorescence channels. These values are displayed in a labeled message on the LSI-11 monitor.

If the user chooses to continue following this and complete a fixed-waveband GC determination, the calculated values and raw background signal data is stored in the second block of the file created on the disk.

e. ADCHRO Macro Subprogram

ADCHRO is the Macro subprogram used to collect GC data in the fixed-waveband spectrometric detection mode and store it in a file on the disk. This program is

very similar to the ADCSPE.MAC subprogram except it is shorter and simpler (see Figure 20). The primary differences are that the monochromator is not scanned during the GC run and ADC measurements are acquired on only two multiplexer channels rather than three. The GPT is used to time the data acquisition rate and in order to prevent the pulses produced by the timer on the Pulse-out connection from moving the monochromator, this BNC cable is simply disconnected from the stepper motor electronics.

Again the data is stored beginning in Block 2 (third block) of the file. The first block is subsequently filled as an ASCII labeling block. The run parameters are stored in the second block in the following order: BLKSAV, the total number of blocks in the file; COUNT, the clock count=100/the data acquisition rate; CHECKL, the fixed wavelength for the source radiation; WAVEL, the "wavelength" voltage at this monochromator position; MA, the mean background noise on the absorbance channel; SA, the standard deviation of the noise on the absorbance channel; MB, the mean value of the background noise on the fluorescence channel; SB, the standard deviation of the noise on the fluorescence channel, and ENDTIM, the total number of points to be taken until the end of the GC separation.

f. IBMPC Fortran Program

The DEC LSI-11 was chosen to interface with the scanned-wavelength UV spectrometric detection system because of the short time required to make analog-to-digital conversions (about 10 μs) and the many input and output channels available for signal and logical voltages and pulses. However, the LSI-11 has only 64

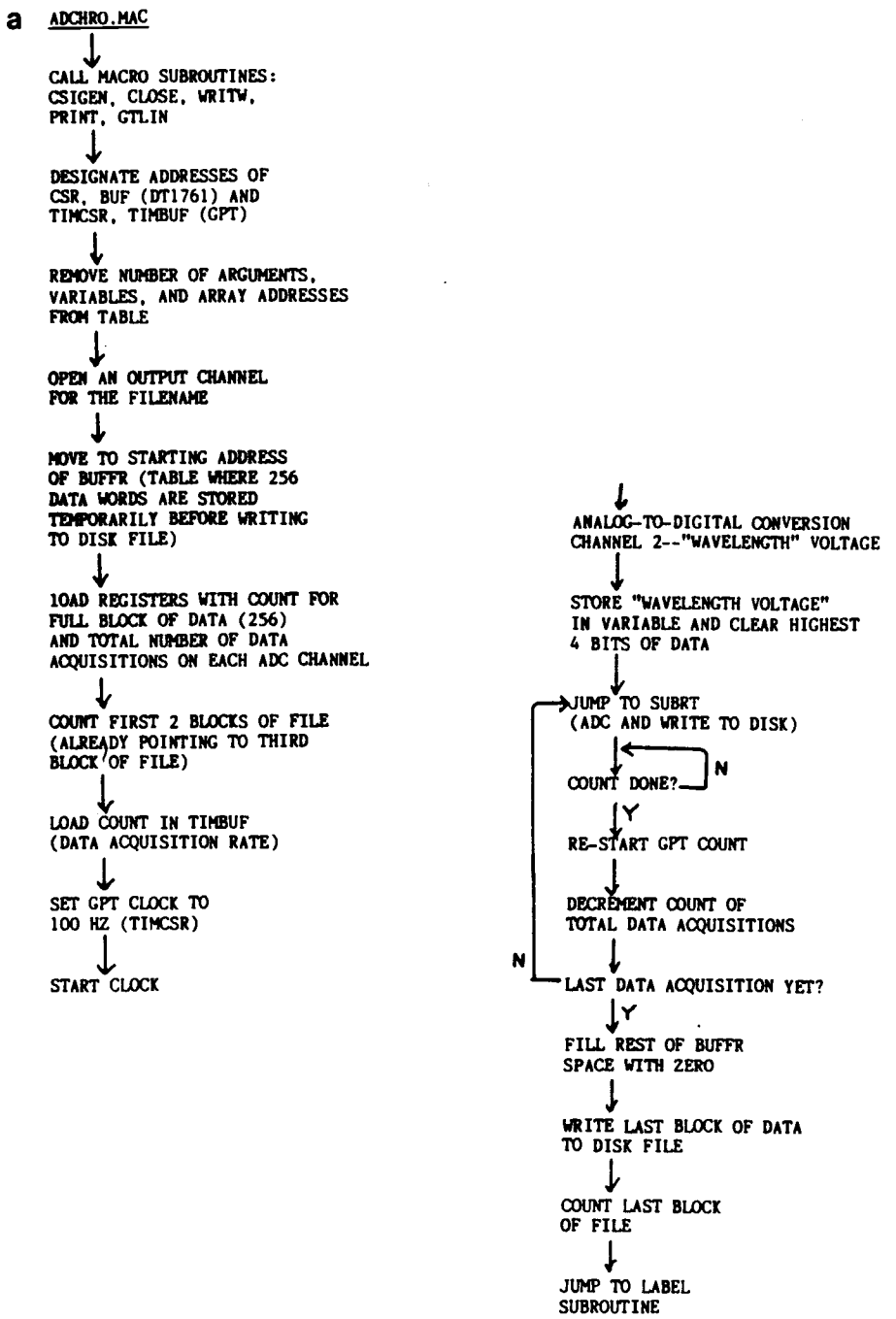
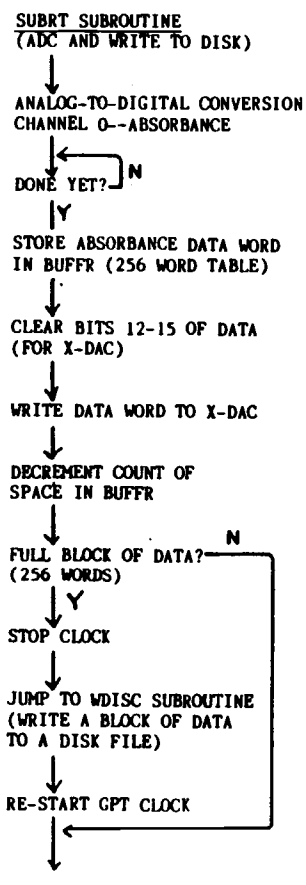
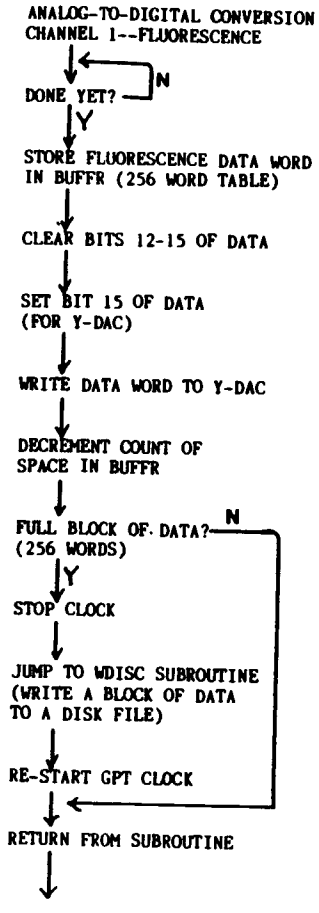


Figure 20. Flowchart for ADCHRO Macro Subprogram (a-f)

b





c

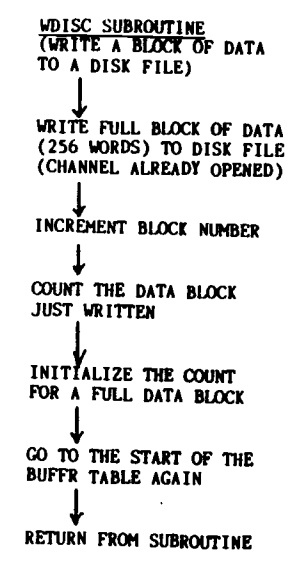
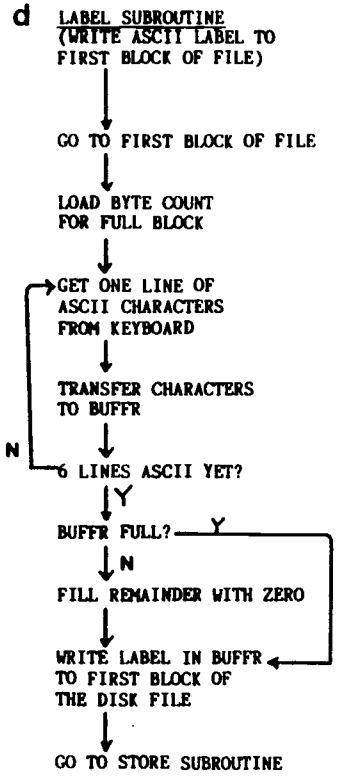
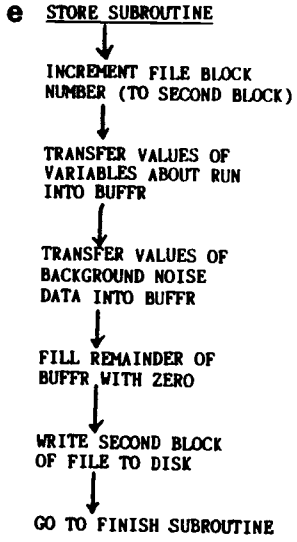


Figure 20 (cont.)





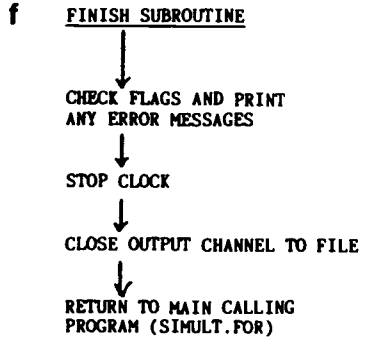


Figure 20 (cont.)

kilobytes of memory of which only approximately 40 kilobytes are available for user programs and data. Considering the large data files generated with this UV absorbance/fluorescence detector, this amount of memory was considered inadequate for data reduction and analysis.

In order to overcome the memory shortage problem, the data files stored by the LSI-11 are transferred to IBM-PC compatible computers. Actually, the data has been transferred using a Corona Personal Computer and the data analysis is performed on a Leading Edge Model M Computer. The transfer is accomplished by using the commercially available software, PCTALK. PCTALK allows the IBM-PC to act as a monitor for the LSI-11 as well as storing any characters or data printed on the "monitor" in files on 5-in IBM-PC diskettes.

The following steps are followed when setting up the system for transfer of data between the two computers. PCTALK operates at a maximum Baud rate of 1200, so the Serial Port to the monitor on the LSI-11 must be set at 1200 Baud. Then, with both computers off, the connecting cable between the LSI-11 computer and the monitor is connected from the LSI-11 to the RS-232 Serial port on the back of the IBM-PC via a RS-232 connector which exchanges the second and third pins.

After making this connection between the two computers, the IBM-PC is turned on and the PCTALK program is run. Command parameter #4 is selected for transferring data from the LSI-11. Once PCTALK is running, the LSI-11 is turned on and the IBM-PC acts as a monitor for the DEC computer. In order to begin storing an IBM file, ALT "R" is entered from the keyboard. The PCTALK program prompts the operator to enter a filename. After this, everything printed on the

IBM-PC screen (i.e. LSI-11 monitor) goes into the file until it is closed with a second ALT "R" entered from the keyboard.

A Fortran program called IBMPC is used to open a selected DEC file, re-arrange the data, convert it from binary to ACSII, and print from one to three channels of data on the LSI-11 monitor. This Fortran program has two Fortran subroutines and a Macro subprogram, GETDAT. The primary function of IBMPC.FOR is to set up files for storage on IBM-PC disks which will be convenient for later analysis. The flowchart for this program is shown in Figure 21.

At the beginning of the IBMPC.FOR program, variables are designated as integer, real, or logical and arrays are dimensioned. After a prompt, the user enters the filename of the DEC file to be transferred. It is possible to transfer from one to three ADC channels of data (i.e. absorbance, fluorescence, and/or "wavelength" voltage) although all three channels are always stored in the DEC file. The operator selects the channels to be transferred and the corresponding markers are set to one if the data from a channel has been selected. Next the user indicates whether the file contains fixed or scanned wavelength data. After these parameters are entered from the keyboard, the user is prompted to enter "ALT "R" to open the IBM-PC file.

At this point, the Macro subprogram GETDAT is called and three variables are passed to this program. The variable BLKNO indicates which block of the file to read. The variable FNAME contains the address of the array which holds the filename and HOLD is a another array of 256 words which is used to transfer the data from an entire block of the file from the Macro subprogram to the Fortran main program.

a

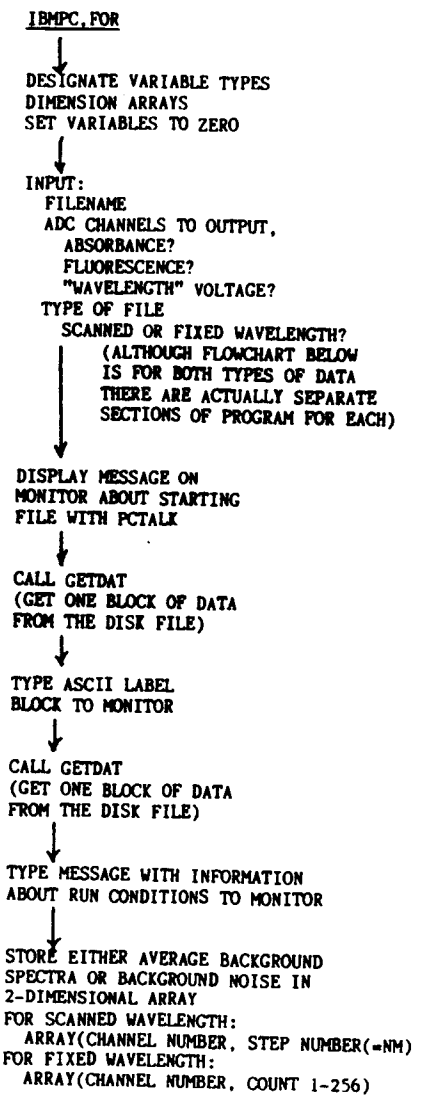


Figure 21. Flowchart for IBMPC Fortran Program (a-c)

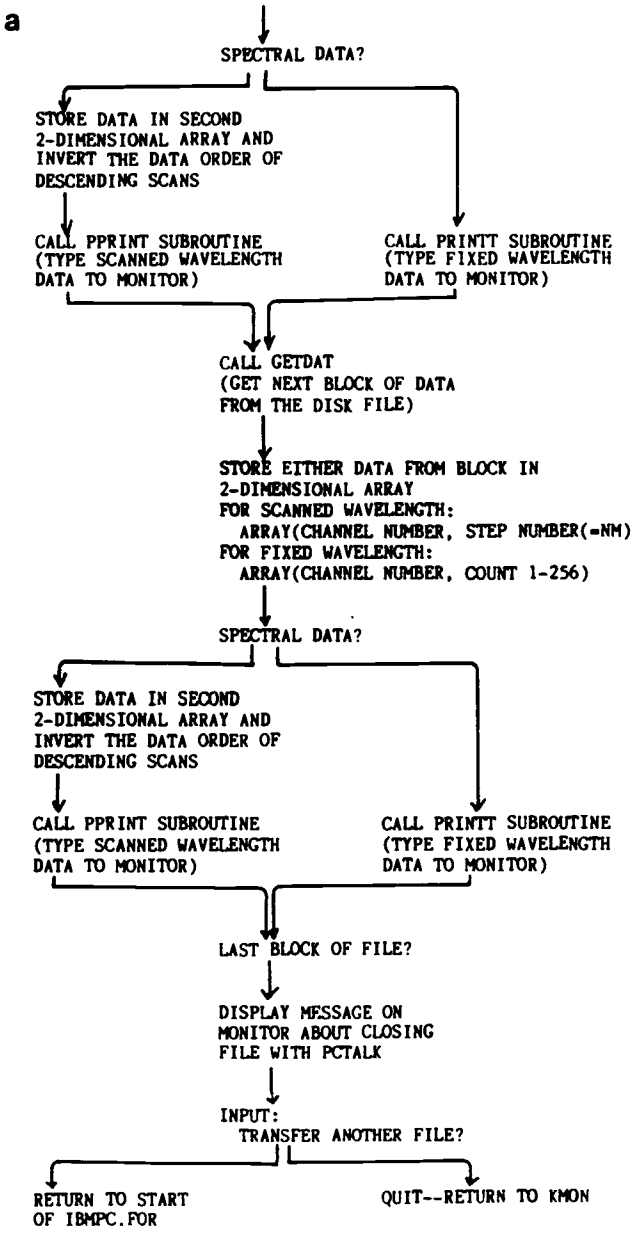
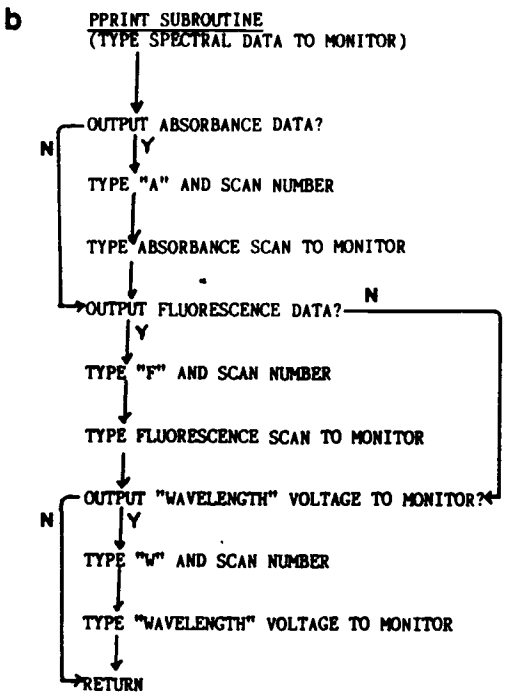


Figure 21 (cont.)



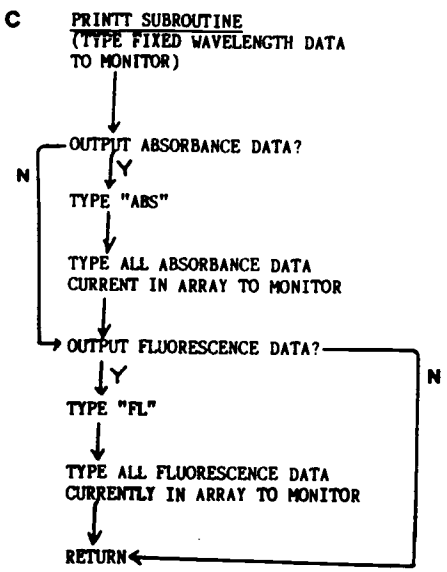


Figure 21 (cont.)

One advantage of having a Macro subprogram open the file and transfer the data in an array to a Fortran program, is that the binary data in the file is converted to ASCII automatically when it is passed to the Fortran program and printed on the monitor. This avoids complicated routines to convert binary values to ASCII values. The 12-bit binary data is contained in one 16-bit word. The maximum value of a 12-bit word is 4095. Each of these ASCII characters requires one byte of memory. Therefore, each ASCII data value fills two 16-bit words and the ASCII files stored on the IBM-PC are twice as long as the LSI-11 binary files.

The first block of data that the GETDAT.MAC subprogram returns to the Fortran main program contains the ASCII label. This is simply typed to the monitor and concurrently stored in an IBM file. The second file block retrieved from a LSI-11 file contains run parameters and either the averaged background spectra or background signal data at one wavelength, depending upon the type of file. Format statements are used to label the run parameters as they are printed on the monitor. Some of these values such as the number of steps in a scan and the number of blocks in the file are also stored in variables which will be used by IBMPC.FOR as limits for loops within the program.

If the file contains spectral data, the averaged background spectra are put into a two-dimensional array called ISTORE. The first argument of this array designates the ADC channel number and the second, the step number in the scan. In order to print the averaged background spectra on the monitor, the PPRINT subroutine is called.

The PPRINT subroutine starts by typing out the scan number (0 for the averaged background spectra). Then depending upon which markers have been previously

selected for the three ADC channels, the subroutine will sequentially print out all the elements in the scan corresponding to Channel 0 (absorbance), followed by all the array elements corresponding to Channel 1 (fluorescence) and finally those values for Channel 2, ("wavelength" voltage). If a marker has not been set to one for a channel (i.e. selected), the subroutine simply skips over that set of elements and they are not stored in the IBM-PC file.

After the labeling blocks have been stored, the GETDAT.MAC subprogram sequentially returns one block of data from the data file on the LSI-11 disk at a time in the same order as the data was collected, that is, first an absorbance signal value, then a fluorescence signal value, and finally a "wavelength" voltage value in alternating ascending and descending scans for spectral data files. The elements in the HOLD array from the GETDAT.MAC subprogram are transferred to the ISTORE two-dimensional array by the IBMPC.FOR program one scan at a time. The Fortran subroutines, PPRINT or PRINTT, type the scan number and the sequential spectral data from each of the ADC channels which have been selected for output. When the last element of the HOLD array is transferred to ISTORE, the main Fortran program calls the GETDAT.MAC subprogram to transfer the next block of data from the file.

There are two Fortran subroutines to print the spectrometric data from LSI-11 files on the monitor because the files may contain either spectral data if the monochromator was scanned during the separation or the data may have been acquired at a fixed-waveband. One subroutine, PPRINT, is used to print spectral data. This subroutine prints outs both ascending scans (odd) and descending scans (even) in ascending order. This is accomplished by reversing the order of the array

elements for the descending scans. The PRINTT subroutine is used to print out spectrometric data at a fixed-waveband.

g. GETDAT Macro Subprogram

GETDAT is the Macro assembly language subprogram which is called by IBMPC.FOR to open a data file, read one block of data from the file into an array, and return the array to the main Fortran program. The subprogram opens with a title, a call of system Macro outines, and retrieves the transferred assignments for the block number, address of the filename and address of the transfer array, HOLD. An input channel is opened for the filename, 256 words are read from the file (1 block) and the values are stored in the array, HOLD. The input channel is closed and control returns to the IBMPC.FOR main program.

III. MEASUREMENTS MADE WITH THE OPTICAL FILTER/OPTICAL FILTER CONFIGURATION

A. Detection of Gas Chromatography Eluates by Simultaneous Absorbance and Fluorecence Measurements *

* reproduced with permission from the Journal of Chromatography, Vol. 303, pages 341-349, 1984.

1. Summary

Concurrent UV-absorbance and fluorescence detection is used to measure gas chromatographic eluates in the gas phase. UV light from a deuterium source is transmitted both to and from a heated flow-through cell via high-temperature quartz fiber optic bundles. Optical filters are used for selective detection of aromatic compounds. Detection limits and sensitivities for compounds vary markedly for absorbance and fluorescence, thereby enhancing selectivity and extending the range of application for the combined techniques. Limits of detection for polycyclic aromatic compounds range between 10 to 94 ng for absorbance measurements and 9 to 238 ng for fluorescence.

2. Introduction

Polynuclear aromatic substances (PNAs) and their derivatives are spread widely throughout the environment by industrial effluents, combustion of fossil fuels by vehicles and power plants, forest and agricultural fires, and refuse burning [16]. Many of these compounds are known mutagens and suspected carcinogens [171]. Accordingly, much effort has been directed toward developing reliable analytical methods for measuring PNAs.

Analyses for PNAs in environmental or biological samples are often complicated by interfering compounds [43]. Even highly selective spectroscopic techniques such as Shpol'skii fluorescence require some sample clean-up [49], and less selective methods may require extensive sample pretreatment [31]. Instruments combining chromatographic resolution with sufficiently selective detectors may allow for less sample pretreat-

ment in PNA determinations. Thus, uncertainties in measurement due to interfering compounds may be avoided.

PNA analyses have been described which use liquid chromatography (LC) with UV-absorbance [89], fluorescence [91], or photoionization [96] detection. Detectors used for gas chromatographic (GC) analyses of PNAs include flame ionization [108], electron capture [123], mass spectrometry (MS) [35], UV absorbance [146], pyrolysis-chemiluminescence [126], and fluorescence [157].

We have previously described PNA measurements by a gas phase fluorescence detector for GC [157]. A standard flame photometric detector was modified using a UV light source, a monochromator, and a quartz fiber optic bundle. In the system described here, the detector cell includes two important modifications: (a) a second fiber optic bundle, filter, and photomultiplier tube (PMT) in a linear configuration to monitor UV absorbance, and (b) bandpass filters to select both the excitation and emission wavelength bands for fluorescence.

The additional selectivity offered by this system for multimode detection may be helpful in environmental, toxicologic, and other analyses where interferents often co-elute with analytes. Using two detection modes with different selectivities helps to avoid effects of co-eluting interferents. For example, GC-MS has been used in conjunction with LC-UV spectrometry [41,95] and LC-UV absorbance systems have been used which concurrently monitor at two different wavelengths [31]. Gas phase absorbance measurements also extends the range of detectable compounds, as compared to gas phase fluorescence.

3. Experimental

Reagents:

PNAs were obtained from Aldrich; minimum guaranteed purities are indicated in Table 4. Solutions were prepared using reagent-grade methylene chloride. Carrier gases were are least 99.5% pure.

Three PNA solutions were prepared and diluted to determine sensitivities, ranges of linearity, and limits of detection. The composition of these solutions were as follows, with concentrations indicated in parentheses ($\mu g\ \mu l^{-1}$):

Solution A. Azulene (0.09), acenaphthylene (0.36), fluorene (0.09), acridine (0.09), 2-methylanthracene (0.09), 2-nitrofluorene (0.18), triphenylene (0.09), 1,2,3,4-dibenzanthracene (0.09).

Solution B. Naphthalene (0.10), 2,3,5-trimethylnaphthalene (0.20), dibenzothiophene (0.10), phenanthridine (0.11), pyrene (0.10), 9-nitroanthracene (0.20), benzo(e)pyrene (0.10), 1,2,5,6-dibenzanthracene (0.10).

Solution C. Dibenzofuran (0.11), xanthone (0.20), 9-chlorophenanthrene (0.30), 9-bromoanthracene (0.45), 2,3-benzofluorene (0.025), benzo(a)pyrene (0.05), 13 H-dibenzo(a,i)carbazole (0.05), 9-phenylanthracene (0.10).

Apparatus:

A schematic representation of the system is shown in Fig. 22. GC separations were performed on a Tracor Instruments Model 565 gas chromatograph. Eluates flowed directly from the column into an aluminum detection cell heated to 250°C with a "Z" flow path (see Fig. 23). The 1.6-cm horizontal section of the flowpath in the cell was lined with a Suprasil quartz tube, 4 mm I.D. Light from a deuterium source of a Tracor Model 970A detector was passed via a condensing mirror through a 260-nm bandpass filter into a 1 m x 3 mm Welch Allyn high-

Table 4. Gas Phase Absorbance-Fluorescence Detection of Selected Aromatic Compounds; LR = Linear dynamic range; LOD = limits of detection; S = sensitivity.

Compound (% purity)	t_s(min)		Absorbance		Fluorescence	
		l.r.(ng)	l.o.d.(ng)	s(a.u.ng^{-1})	l.o.d.(ng)	s(μg^{-1})[a]
NAPHTHALENE (98)	0.5	50 - 500	43	0.36 x 10^{-5}	ND	-
AZULENE (99)	0.8	45 - 450	8	2.05 x 10^{-5}	ND	-
ACENAPHTHYLENE (95)	1.4	180 - 1800	97	0.18 x 10^{-5}	ND	-
OIBENZOFURAN (98)	1.8	50 - 550	46	0.39 x 10^{-5}	238	5.1
2,3,5 TRIMETHYLNAPHTHALENE	2.3	100 - 1000	64	0.30 x 10^{-5}	172	7.7
FLUORENE (98)	2.3	45 - 450	23	0.82 x 10^{-5}	220	1.7
OIBENZOTHIOPHENE (95)	4.1	49 - 500	42	0.54 x 10^{-5}	ND	-
ACRIDINE	4.4	45 - 450	41	0.57 x 10^{-5}	ND	-
PHENANTHRIOINE (98)	4.9	55 - 550	30	0.82 x 10^{-5}	ND	-
XANTHONE (99)	5.2	98 - 1000	72	0.34 x 10^{-5}	192	5.0
2-METHYLANTHRACENE (97)	5.8	45 - 450	26	1.02 x 10^{-5}	85	15.7
9-Cl-PHENANTHRENE (98)	6.5	147 - 1500	24	1.14 x 10^{-5}	91	2.2
2-NITROFLUORENE (98)	7.7	90 - 900	72	0.42 x 10^{-5}	ND	-
9-Br-ANTHRACENE (95)	7.9	220 - 2250	61	0.50 x 10^{-5}	ND	-
PYRENE (99)	8.0	49 - 500	10	3.05 x 10^{-5}	42	26.6
9-NITROANTHRACENE (97)	8.3	96 - 1000	31	1.00 x 10^{-5}	ND	-
2,3 BENZOFLUORENE	9.1	25 - 2500	42	0.79 x 10^{-5}	9	72.9
9-PHENYLANTHRACENE (98)	10.8	50 - 500	37	0.97 x 10^{-5}	130	6.3
TRIPHENYLENE (98)	11.1	45 - 450	32	1.16 x 10^{-5}	125	10.3
BENZO(a)PYRENE (98)	14.9	25 - 250	34	1.34 x 10^{-5}	16	60.9
BENZO(e)PYRENE (99)	15.0	49 - 500	41	1.09 x 10^{-5}	62	16.6
1,3 H-OIBENZO(a,i)CARBAZOLE (98)	19.1	250 - 2500	60	0.89 x 10^{-5}	22	71.7
1,2,3,4 OIBENZANTHRACENE (97)	20.1	45 - 450	21	2.66 x 10^{-5}	15	60.1
1,2,5,6 OIBENZANTHRACENE (97)	20.7	49 - 500	36	1.59 x 10^{-5}	42	28.8

[a]Sensitivity for fluorescence calculated according to a method described in reference 172

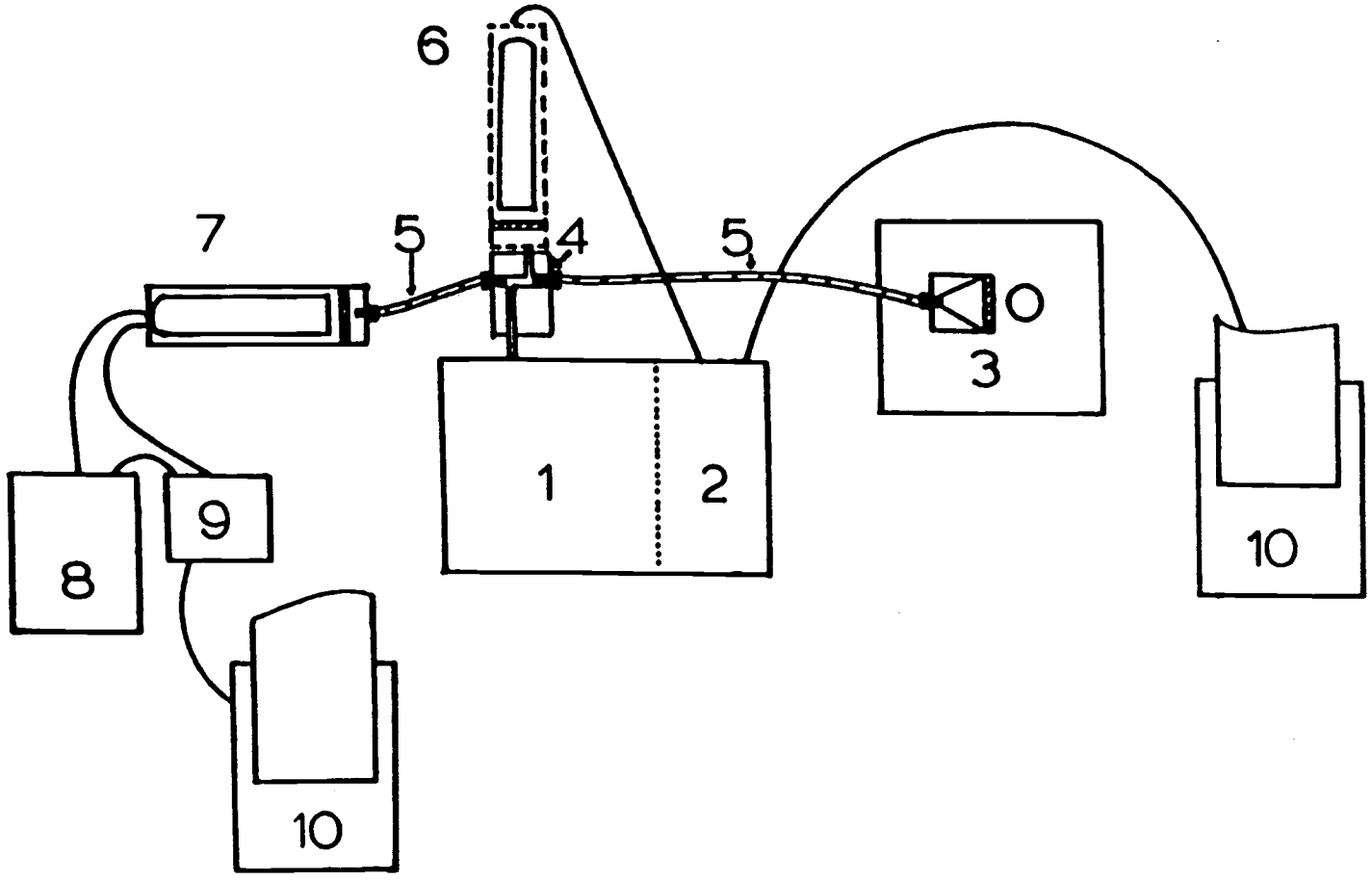


Figure 22. Schematic representation of simultaneous absorbance-fluorescence GC detection systems; 1 = GC; 2 = signal amplifier; 3 = Deuterium lamp, 260-nm bandpass filter; 4 = heated detector cell; 5 = fiber optics bundles; 6 = 340-nm longpass filter, 380-nm bandpass filter, and fluorescence PMT; 7 = 240-nm bandpass filter and absorbance PMT; 8 = high-voltage power supply; 9 = absorbance signal modifier; 10 = digital integrators

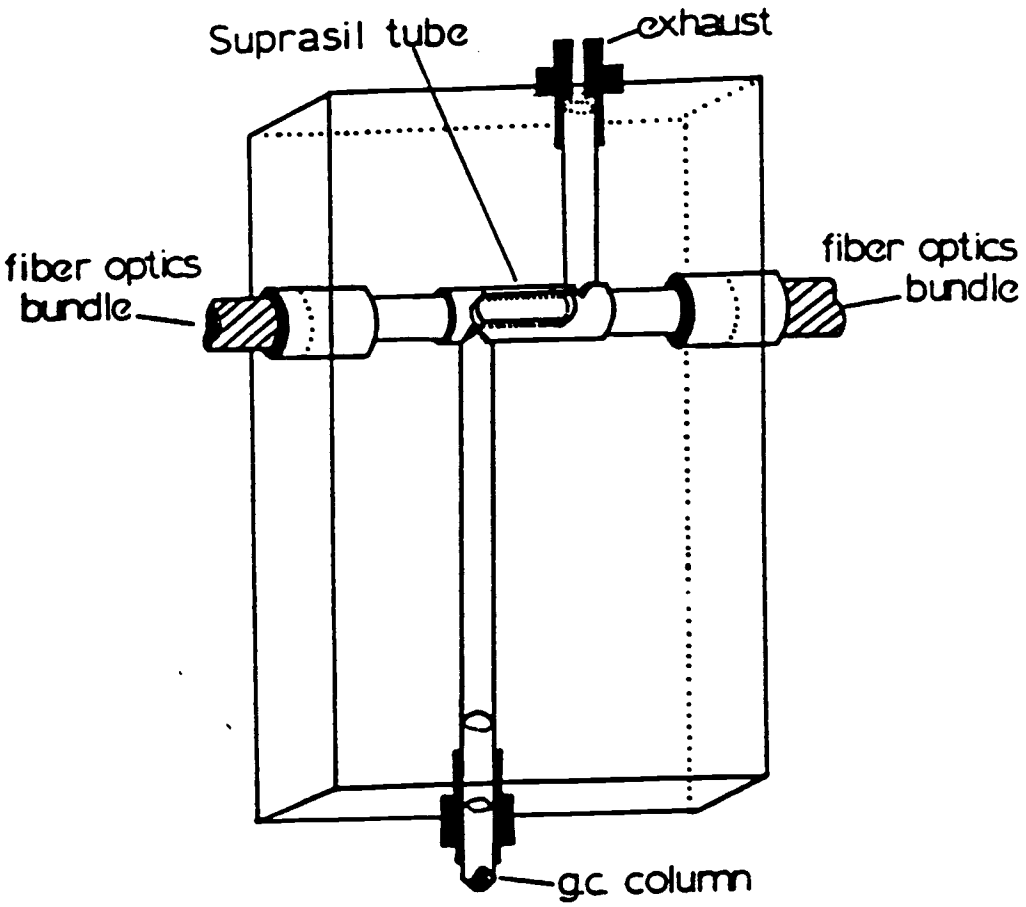


Figure 23. Heated, flow-through detector cell with volume of 200 μL and absorbance pathlength of 16 mm

temperature quartz fiber optics bundle. The other tip of the fiber optics bundle, fitted into the detector cell with o-rings and PTFE sleeves, illuminated the horizontal flow path. Light emitted from the fiber optics bundle has ca. 60° dispersion, resulting in decreasing illumination intensity along the eluate path.

Fluorescence measurements were made through a window perpendicular to the illuminated path. Fluorescent light from this window passed through 340-nm long-pass and 380-nm bandpass filters (see Figure 24) and was detected by a head-on PMT. The distance from the side window to the photocathode of the fluorescence PMT was approximately 15 cm. Fluorescence chromatograms were recorded on a Hewlett-Packard Model 3390A digital integrator.

Light for absorbance measurements was collected in a linear configuration by a 250 mm x 1 mm Maxlight high-temperature fused-silica fiber optics bundle. Light issued from the end of this fiber optics bundle passed through a 240-nm bandpass filter (see Figure 24) to a Hamamatsu R1464 head-on PMT. The distance from the fiber optics bundle tip to the photocathode of this PMT was approximately 5 mm. The signal from the PMT was modified by Jfet operational amplifier current-to-voltage convertor with a 0.1-s time-constant, followed by an Analog Devices logarithmic amplifier to convert transmittance to absorbance. The absorbance signal was then attenuated and offset by an operational amplifier voltage follower with appropriate gain and voltage offsets. Chromatograms of absorbance were recorded on the integrator from a Hewlett-Packard 5880 gas chromatograph using an external input board.

Separations were performed on a 2 m x 2 mm I.D. glass column packed with 3% OV-101 on 100/120 Supelcoport.

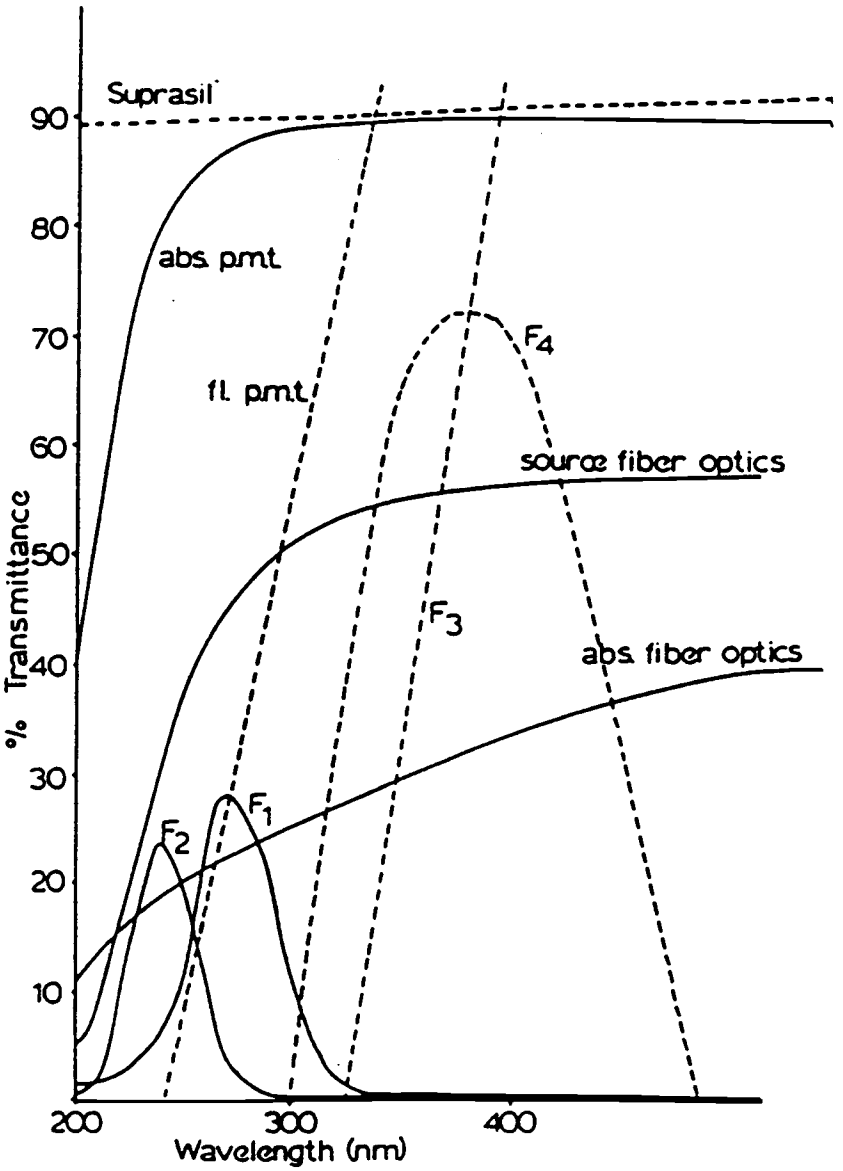


Figure 24. Percent transmittance vs. wavelength for optical components of absorbance-fluorescence GC detector; F1 = 260-nm bandpass filter (source); F2 = 240-nm bandpass filter (absorbance); F3 = 340-nm longpass filter (fluorescence); F4 = 380-nm bandpass filter (fluorescence); fl. p.m.t. = PMT window (fluorescence); abs. p.m.t. = PMT window (absorbance)

Procedure:

Injections (5 µl) were made for serial dilutions of each solution. A temperature program was used with an initial temperature of 150°C for 2 min, then changed at 10°C/min to a final temperature of 250°C for 20 min. Nitrogen carrier gas was used with a flow rate of 30 ml/min.

4. Results and Discussion

The heated flow-through detector cell (Figure 23) accepted GC eluates directly from the column, eliminating chromatographic band broadening and adsorptive losses in transfer lines from the GC to the spectrophotometric detectors. In addition, the design featured a small detector volume of 200 µl to maintain chromatographic resolution of peaks. This design resulted in a viewed volume for the fluorescence measurement of approximately one-thirtieth that of a previously described fluorescence detector [157]. However, strong fluorescent signals were obtained for many multiring PNAs.

Conversion of the transmittance signal to an absorbnce signal by the logarithmic amplifier resulted in a 20- to 30-fold improvement in detection limits. The base-ten logarithmic output of this circuit allows for a conversion of the PMT output to absorbance units for the recorded signal.

Optical filters transmitted wavebands which are characteristic for PNAs. Figure 24 shows properties of the various optical components of the system. The transmitted excitation and emission wavebands for the fluorescence measurement have maxima at 259 nm and 390 nm, respectively. The transmitted light for the absorbance measurement passes through both 260-nm and 240-nm bandpass filters to narrow the waveband yielding

a maximum transmittance of about 255 nm and a bandwidth at half height of approximately 20 nm. The arrangement of these two filters with the shorter waveband filter following the longer waveband filter, eliminates fluorescent light detection by the absorbance PMT.

Chromatograms produced by concurrent absorbance and fluorescence detection of GC eluates from PNA solution A (Figure 25) illustrate differences in selectivities of each measurement mode. Fewer compounds fluoresce than absorb, and fluorescence is not always more sensitive than absorbance for this system. The relative sensitivities depend on the excitation and emission wavelengths used, the intensity of the source, the fluorescence quantum yield of the compound, and the spatial arrangement of the detection system. For example, the chromatograms show that azulene is a strong UV absorber but shows weak fluorescence. On the other hand, 1,2,3,4-dibenzanthracene absorbs less but yields a high fluorescence. Consequently, the limit of detection for azulene is considerably better when measured by absorbance, while the fluorescence measurement is more sensitive for 1,2,3,4-dibenzanthracene.

Limits of detection (LOD), sensitivities (S) and linear dynamic ranges (LR) for 24 PNAs measured with the system are shown in Table 4. The reported dynamic range for the compounds is bounded by the highest level of analyte measured and non-linearities were not observed within the ranges tested.

All aromatic substances tested were detected by absorbance and sensitivities for these substances did not differ greatly for multiringed systems or for aromatics with a variety of functional groups. Not all of the aromatic compounds were detected by fluorescence. The best sensitivities were obtained for systems containing three or more fused rings. In addition,

Figure 25. Chromatograms of PNA solution produced by simultaneous (a) absorbance and (b) fluorescence detection; 1.26 min = 450 ng azulene; 1.97 min = 1800 ng acenaphthylene; 3.01 min = 450 ng fluorene; 5.22 min = 450 ng acridine; 6.50 min = 450 ng 2-methylanthracene; 8.41 min = 900 ng 2-nitro-fluorene; 11.71 min 450 ng triphenylene; 21.66 min = 450 ng 1,2,3,4 dibenzanthracene

certain functional groups, such as the nitro group, decreased the fluorescence signal, even for high-molecular-weight aromatics.

Selective detection of low-molecular-weight aromatic compounds by absorbance but not fluorescence was demonstrated by analysis of an extract from a cigarette filter (Figure 26). Many substances were detected by absorbance, but not by fluorescence.

Although multiring aromatic compounds are detected by both absorbance and fluorescence, the sensitivity of each measurement mode varies depending on the individual compounds eluted. These differences provide additional qualitative information and ratios of absorbance to fluorescence signals may be combined with chromatographic retention data to help identify compounds.

While limits of detection for both modes of PNA measurement in this system extend to the low nanogram levels, possibilities exist for future improvements through increased transmittance and reduced light losses. These may be achieved via changes in cell configuration and more efficient optical components.

Simultaneous absorbance and fluorescence measurement of PNAs separated by GC provides fast, non-destructive measurements with little sample pretreatment. The combination of highly selective and moderately selective detection in the same detector greatly reduces the number of interferences while expanding the number of compounds that can be identified and measured.

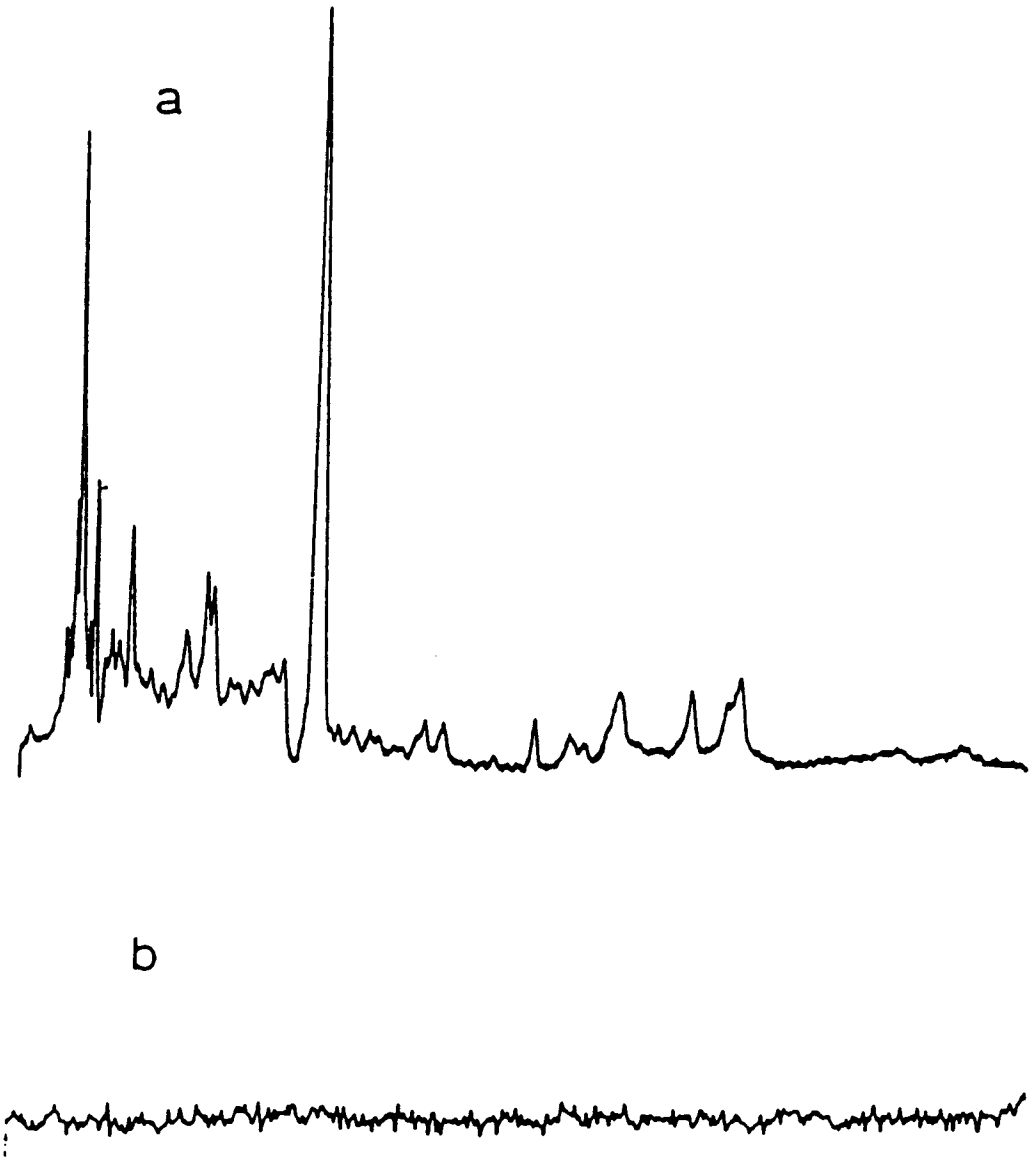


Figure 26. Chromatograms of a hexane extract of cigarette filter: (a) absorbance, (b) fluorescence. GC temperature program: 60°C, 2 min; 10°C/min; 280°C, 60 min.

B. Long Pathlength Ultraviolet Absorbance Detection for Gas Chromatography with Concurrent Fluorescence Measurement *

by
Debra L. Van Engelen, Anthony K. Adams,
and Lawrence C. Thomas

* reproduced with permission from the Journal of Chromatography, Vol. 331, pages 77-82, 1985.

1. Summary

Multimode gas-phase spectrometric measurements of gas chromatography (GC) eluates are made concurrently in a long pathlength cell. The 167-mm cell resulted in up to ten-fold improvements in limits of detection by UV absorbance relative to previous detectors. The concurrent measurement of UV fluoresence in the same cell provides highly selective detection for multi-ring polynuclear aromatics in addition to the selectivity of the absorbance measurement. Limits of detection for aromatic compounds range to low nanogram levels for the multimode detector.

2. Introduction

Although conventional ultraviolet (UV) absorbance and fluorescence detectors are routinely used with liquid chromatography, few UV spectrometric detectors have been developed for gas chromatography (GC). In previous work, we described two spectrophotometric detectors which measure gas-phase samples directly as they elute from the GC column [157,172]. Gas phase spectrometric detectors require special considerations for interfacing the separation and detection systems. Aside from bulkiness, which is often a problem, there are conflicting requirements for detector temperatures, sample mass and cell volume.

Several couplings of GC with fluorescence detection have been developed [151,157,172]. For example, a UV fluorescence detector has been developed by modification of a flame photometric GC detector to make selective and sensitive measurements of polynuclear aromatic compounds (PNAs) in the gas phase [157]. For that detector, interfacing problems were resolved by adding make-up gas

to the heated detector cell and by using quartz fiber optic bundles to thermally isolate optical components.

Long pathlengths can be helpful for GC spectrometric measurements. For example, absorbance detectors for GC have primarily utilized radiation in the IR region. These systems have the advantages that a fixed waveband may be chosen to detect a specific vibrational transition or, as in the case of Fourier transform IR, many spectra may be generated for eluates [134,135, 140]. In order to improve sensitivities, IR measurements of GC effluents are sometimes made in long light pipes of 0.5 m or more in length which are coated with metals which efficiently reflect IR light. However, long light pipes may deteriorate chromatographic resolution if the volume of the detector cell is incompatible with the chromatographic band volume [137,138]. Similarly, total reflection though long capillary cells has been used with visible light to improve absorption sensitivies for measurements in solution [173].

Ultraviolet absorbance measurements often exhibit much greater sensitivities than measurements made at longer wavelengths. The good sensitivity of UV detection makes it potentially useful for GC and compatible with trace analysis because the cell volumes and sample sizes can be small.

UV absorbance may also be quite selective for certain classes of compounds. Virtually all compounds absorb in the vacuum UV and a detector has been designed using short-wavelength UV light which is essentially a universal detector [131]. Due to the extremely high absorptivities in this region, this detector has limits of detection comparable to a flame ionization detector. At longer wavelengths, e.g. 180-240 nm, UV light is selectively absorbed primarily by unsaturated substances

and low-molecular-weight aromatic compounds. Gas-phase absorbance detectors which use radiation in this region of the spectrum have been described [143,146]. However, these systems transported GC effluents to the spectrometer via cumbersome, heated transfer lines.

At still longer wavelengths, e.g. 250-300 nm, high UV absorbance of GC effluents is limited to extended pi-conjugated compounds. Conveniently, many of the polycyclic aromatic compounds which absorb well at these longer wavelengths, also fluoresce efficiently [1]. We have previously described a dual-mode GC detector for simultaneous UV absorbance and fluorescence measurements [172]. In the system described herein, UV wavebands have been chosen which are selectively absorbed by aromatic substances with some showing strong fluorescence. The selectivity of this system, when combined with the resolving power of GC separations is advantageous for measurements of PNAs from complex environmental or biological samples. The long pathlength detector cell increases absorbance sensitivities. Considerations in the design included the low reflectivity of UV light, maintaining the cell volume compatible with the chromatographic band volumes, and stiking a compromise between the advantages of a long absorbance pathlength and a large viewed volume for the fluorescence measurement.

3. Experimental

Reagents:

PNAs were obtained from Aldrich (minimum guaranteed purities are indicated in Table 5). Solutions were prepared using reagent grade methylene chloride. Nitrogen carrier gas was 99.5% pure.

Table 5. Gas Phase Long Pathlength Absorbance-Fluorescence Detection of Selected Aromatic Compounds; LOD = limits of detection, S = sensitivity.

COMPOUND (% PURITY)		ABSORBANCE		FLUORESCENCE	
	ts(min)	LOD(ng)	S(absorbance units ng^{-1})	LOD(ng)	S(ng^{-1})[a]
Aniline (99)	0.18	25	0.12×10^{-4}	ND[b]	ND[b]
Dibenzofuran (98)	1.60	10	0.34×10^{-4}	370	0.13×10^{3}
Carbazole (96)	4.47	13	0.35×10^{-4}	65	0.77×10^{3}
2-Phenylindole (95)	6.93	16	0.35×10^{-4}	50	0.99×10^{3}
Pyrene (99)	7.54	8	0.67×10^{-4}	62	0.81×10^{3}
9-Phenylcarbazole (97)	9.47	11	0.57×10^{-4}	46	1.1×10^{3}
1,2-Benzanthracene (99)	10.78	4	1.79×10^{-4}	42	1.2×10^{3}
Benzo(e)pyrene (99)	14.18	8	0.99×10^{-4}	83	0.61×10^{3}
13 H-Dibenz(a,i)carbazole (98)	18.00	5	1.66×10^{-4}	3	1.60×10^{3}

a Sensitivity for fluorescence calculated according to a method described in Ref. 172

b Compound not detected at levels tested

A solution of aromatics was prepared and diluted to determine sensitivities, ranges of linearity, and limits of detection. The composition of this solution was as follows: aniline, 0.07 µg/µl; dibenzofuran, carbazole, 2-phenylindole, pyrene, 9-phenylcarbazole, 1,2-benzanthracene, benzo(e)pyrene, and 13 H-dibenz(a,i)carbazole, each 0.11 µg/µl.

Apparatus:

Details of the optical components and instrumentation are similar to a previously described system [172]. GC separations were performed on a Tracor Instruments Model 565 gas chromatograph. Light from a deuterium source was passed via a condensing mirror and a 260-nm bandpass optical filter onto the tip of a 1 m x 3 mm Welch Allyn high-temperature quartz fiber optic bundle. Light emitted from this fiber optic bundle illuminates the eluate flowpath through the long pathlength detector cell (see Figure 27). Fluoresced light is detected through a Suprasil quartz window perpendicular to the cell flowpath. Emitted light is filtered by 340-nm longpass and 380-nm bandpass filters prior to detection by the fluorescence photomultiplier tube (PMT) 1. Transmitted light for the absorbance signal is collected at the eluate entrance end of the cell by a 250 x 1 mm Maxlight high-temperature fused-silica fiber optic bundle. Subsequently, the light is filtered by a 240-nm bandpass filter and detected by PMT 2. The transmittance signal is converted to an absorbance signal by an electronic logrithmic amplifier.

The long UV absorbance pathlength detection cell was milled from a solid aluminum block (see Figure 27) and was heated to 250°C. The absorbance pathlength is 167 mm and has a volume of 320 µl. Upon elution from the GC column, the sample flows into a 151-mm length of the detector cell which is lined with a 1-mm I.D. glass

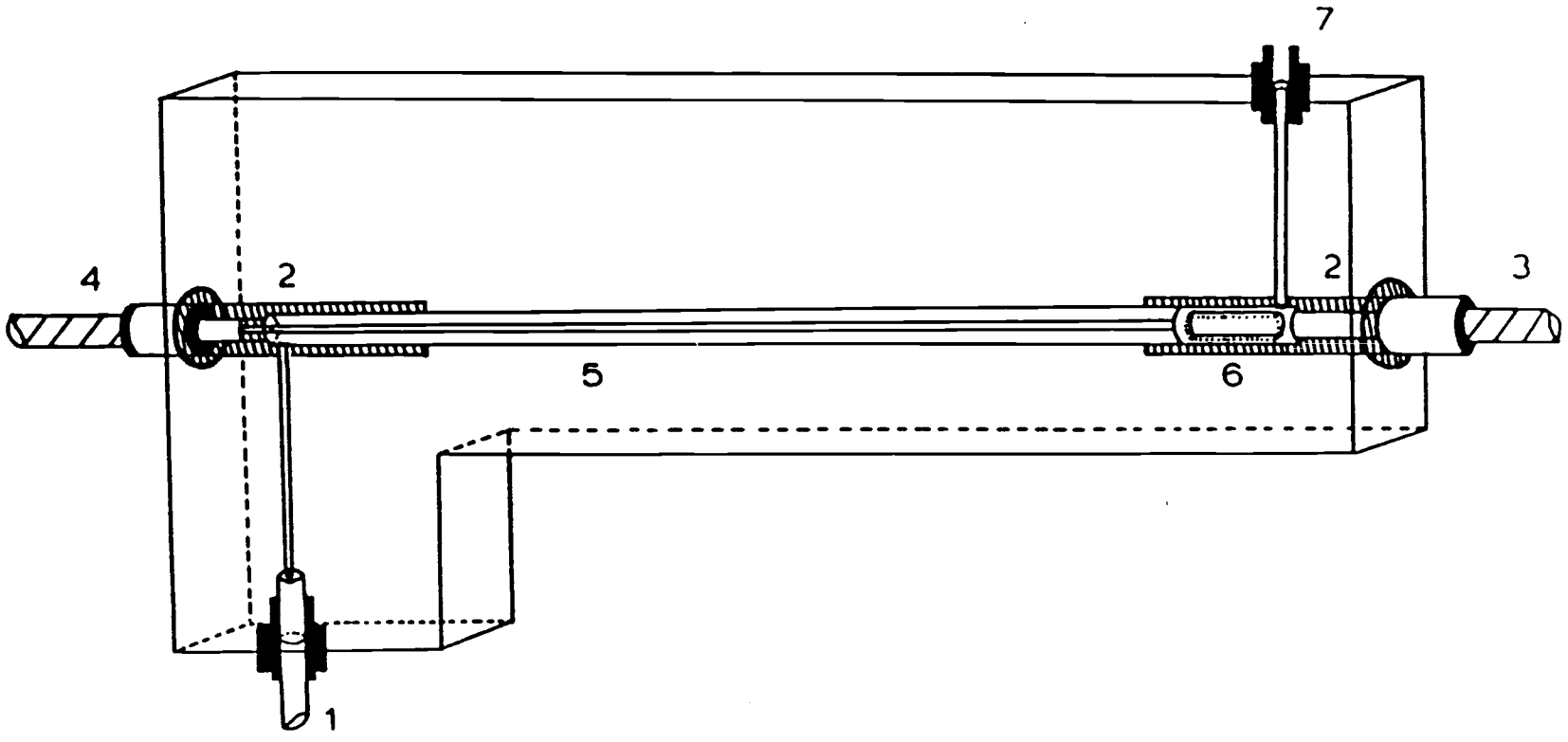


Figure 27. Heated flow-through detector cell with volume of 320 µL and absorbance pathlength of 167 mm; 1 = GC column; 2 = PTFE sleeves; 3 = source fiber optic bundle; 4 = absorbance fiber optic bundle; 5 = 1 mm I.D. glass tube; 6 = 4 mm I.D. Suprasil tube with Suprasil side window; 7 = exhaust

tube. Following this, it continues in a linear direction into a 16-mm section which is lined with a 4-mm I.D. Suprasil tube. The fluorescence measurement is made through a side-window into this larger volume cavity. The absorbance pathlength is the entire 167 mm. PTFE fittings keep the cell gas-tight and ensure the alignment of the liner tubes.

Procedure:

Dilutions of the PNA test solution were separated by GC. A temperature program was used with an initial temperature of 150°C for 2 min, then increased at 10°C/min to a final temperature of 250°C, held for 20 min. Nitrogen carrier gas was used as the carrier gas with a flow-rate of 30 ml/min.

4. Results and Discussion

Preliminary measurements were made prior to constructing the long pathlength cell to determine the length of 1-mm I.D. glass tube which would transmit sufficient UV light for adequate absorbance measurements. As the length was increased, the peak-to-peak noise remained nearly constant. However light intensity, measured by the difference between the dark current and zero absorbance signals, diminished approximately as to the inverse square of the distance. A glass tube length of ca. 150 mm was found to allow sufficient UV light transmittance, and resulted in a total cell volume of 320 µl when combined with the larger volume cavity for the fluorescence measurement. The full absorbance pathlength is 10.4 times longer than a previous design [172], yielding significant improvements in sensitivities.

Figure 28 shows chromatograms from absorbance and fluorescence measurements of aromatic eluates.

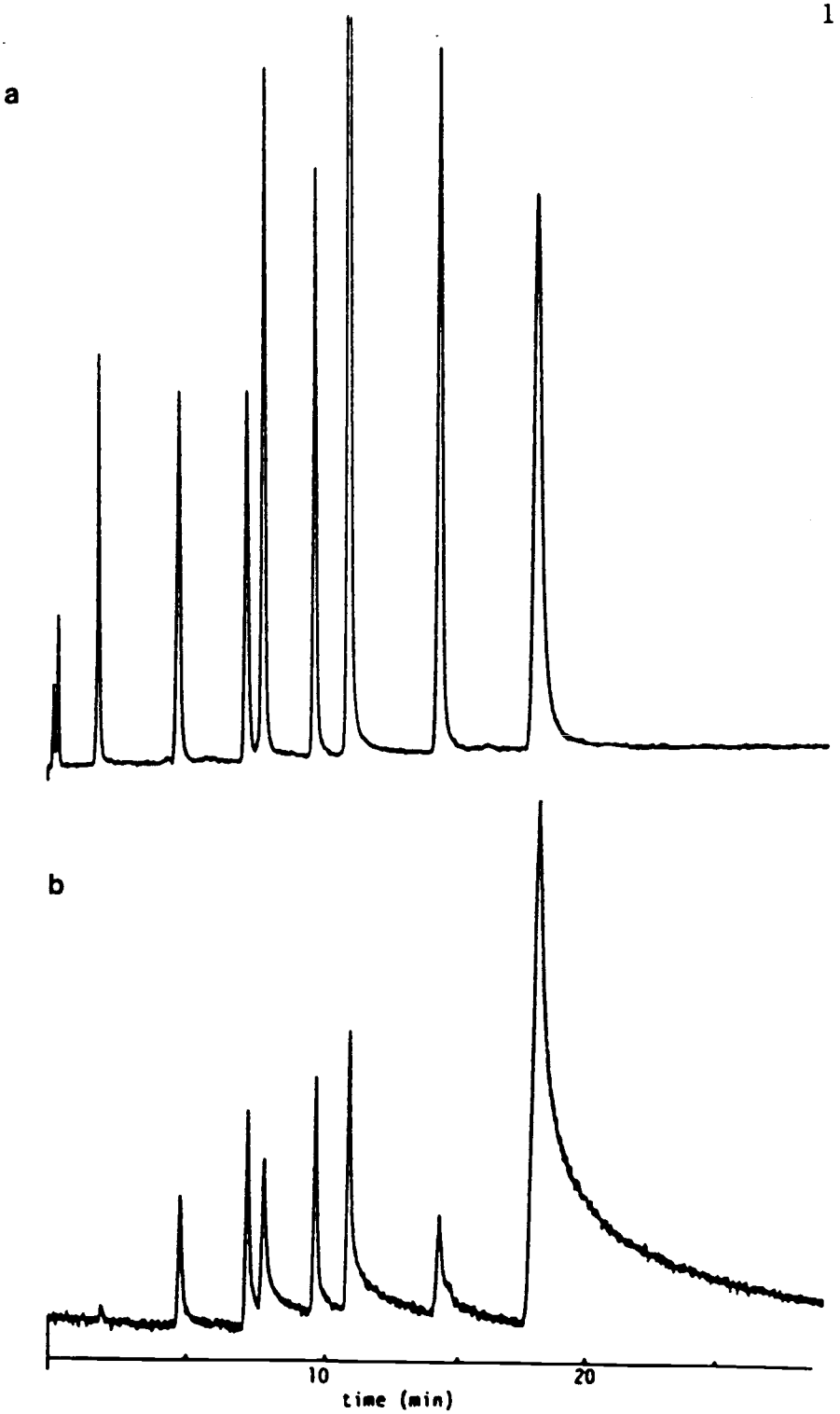


Figure 28. Chromatograms of PNA solution produced by simultaneous (a) absorbance and (b) fluorescence detection: 345 ng aniline; 550 ng each of dibenzofuran, carbazole, 2-phenylindole, pyrene, 9-phenylcarbazole, 1,2 benzanthracene, benzo(e)pyrene, 13 H-dibenz(a,i) carbazole

Sensitivities and limits of detection were determined for the nine compounds. The differences in sensitivities and selectivities effected by the two modes provide additional qualitative information relative to single measurement modes. The results of these measurements are summarized in Table 5. The range of eluted masses tested for the compounds was 13-550 ng and non-linearities were not observed within this range. The fluorescence measurements are nearly identical to the previous detector [172] and shows the greatest sensitivity for PNAs containing three or more fused rings.

The absorbance sensitivity shows an eight-fold improvement for benzo(e)pyrene and is fourteen times better for 13 H-dibenz(a,i)carbazole than the sensitivities for the shorter pathlength cell. The improvements for limits of detection are five- and ten-fold for benzo(e)pyrene and 13 H-dibenz(a,i)carbazole, respectively. Improvements for limits of detection are less dramatic than for sensitivity due to an increase in the relative background noise level accompanying decreased total transmitted light.

Figure 29 shows a GC separation of an acetone extract of used motor oil which illustrates the advantages of this dual-mode detection system. The complex sample was dissolved in acetone, filtered, and the filtrate injected on the GC column. In order to illustrate overall sensitiviies and selectivities for aromatic compounds in the sample, 400 ng of pyrene was coinjected with the sample at an elution time 13.0 min for each chromatogram. The absorbance detector responds to a solvent peak, and detectd low-molecular-weight unsaturated and aromatic compounds in addition to larger polycyclic aromatics. The fluorescence mode characteristically detects only the larger polycyclic compounds. The simplicity and speed of GC separation

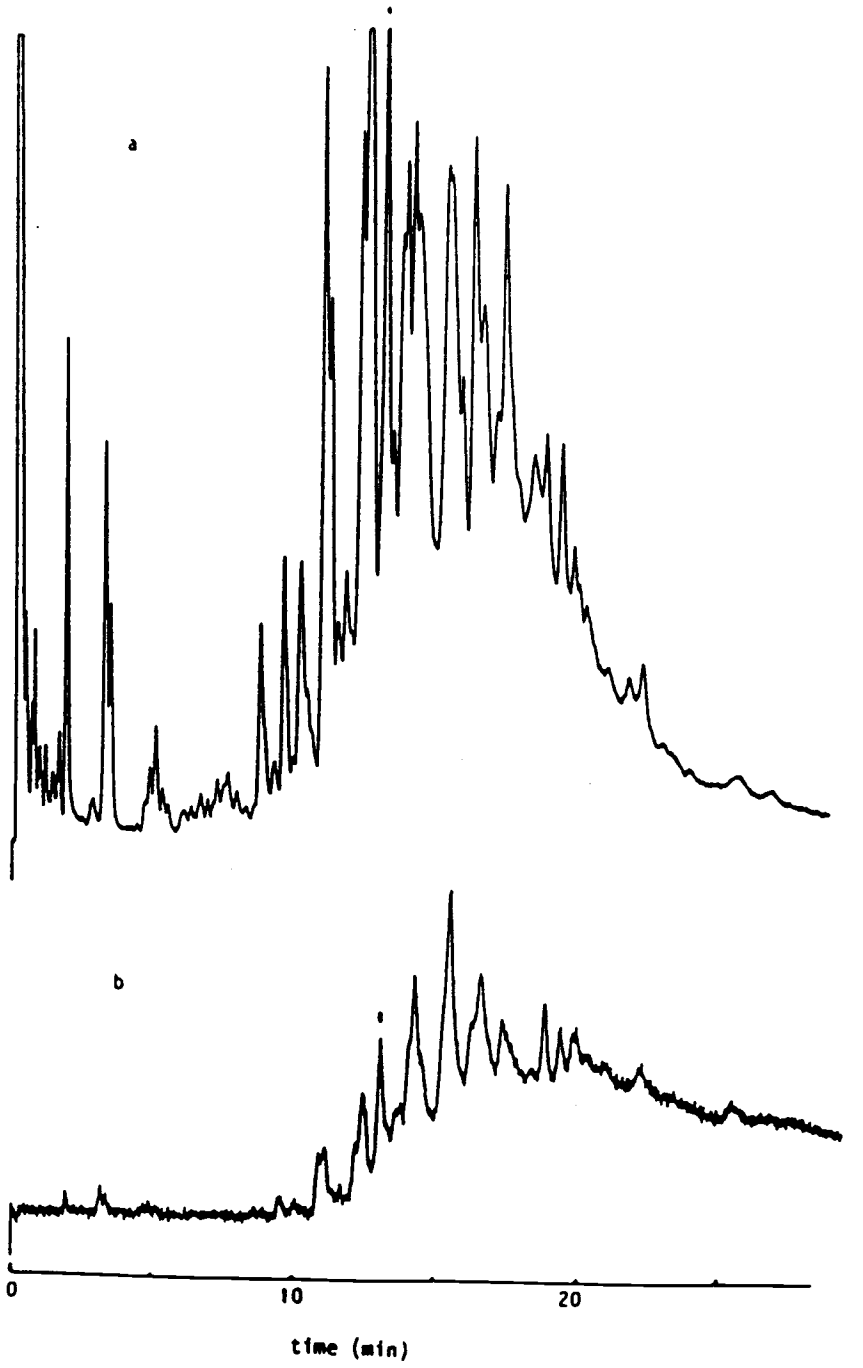


Figure 29. Chromatograms of an acetone extract of used motor oil plus 400 ng (13.0 min): (a) absorbance, (b) fluorescence. GC temperature program: 100°C, 2 min; 10°C/min; 280°C, 20 min.

with subsequent selective detection allows for quantitation of these compounds with little sample clean-up. The information available from the relative absorbance and fluorescence intensities may enhance identification of the eluted compounds.

C. Determination of Sensitivities and Limits of Detection

Multiple GC injections of mixtures of PNAs were made in order to determine sensitivities and limits of detection (LOD) of this GC-UV absorbance/fluorescence analytical system. Twelve to fifteen injections were made for each compound at levels ranging from low microgram to near the limit of detection, that is, low nanogram masses. The signal from each spectrometric channel was recorded and measured with digital integrators to measure chromatographic peak areas. The units of peak area on the integrators are microvolt-seconds (μV-s).

The sensitivity, as given by the slope of the response versus mass curve, for the absorbance channel of the detector was determined by plotting the peak area vs. mass of eluate in nanograms (a.u.-s/ng). The peak area in units of absorbance units-second (a.u.-s) was calculated as described in Chapter III. A program written by S. Hein was used to determine the best linear fit for the data using the method of least squares [174]. This program was designed to be used in conjunction with Lotus 1-2-3 (Lotus Development Corporation) and the Lotus 1-2-3 software was used to print graphical calibration plots. Such a calibration graph for 1,2 benzanthracene is shown in Figure 30. The error bars indicate the 95% confidence interval for the entire data set, that is the line as a whole. This graph is similar to those achieved for all the PNAs tested, except that triplicate injections were usually made for each mass level tested.

The fluorescence signal and, therefore, fluorescence detection sensitivity, are dependent upon the intensity of the excitation radiation as well as the

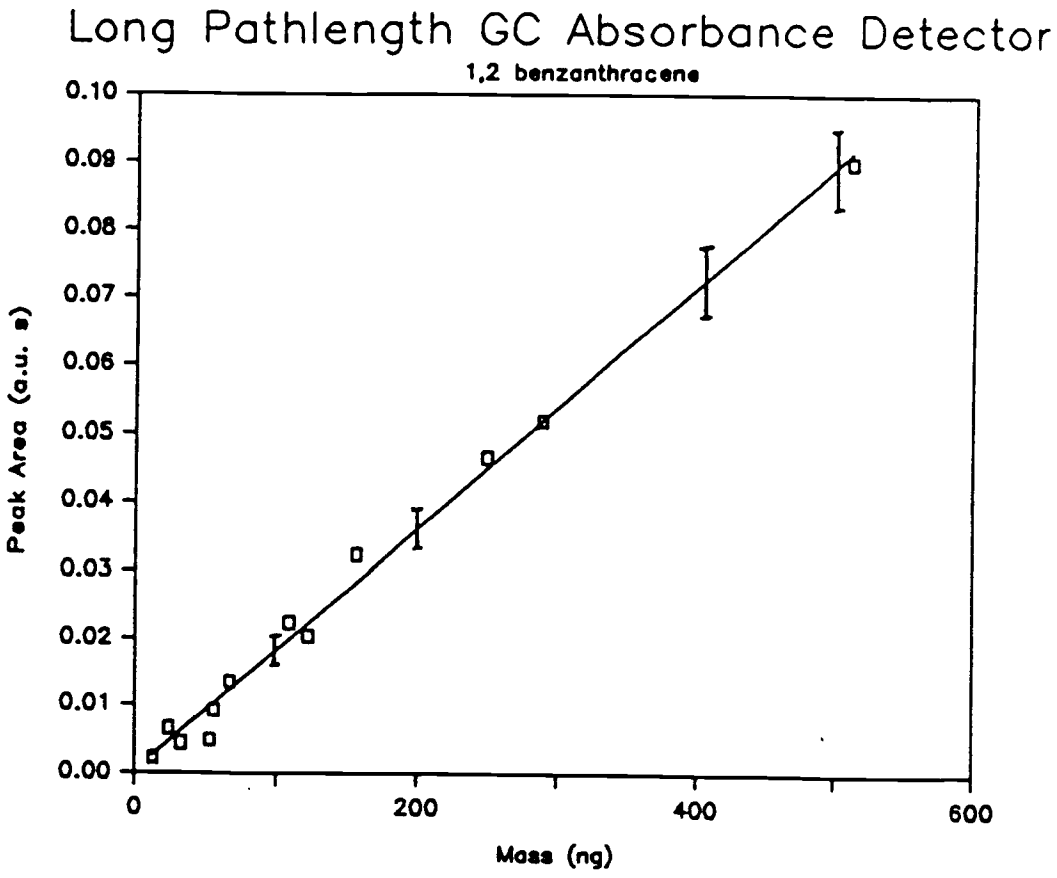


Figure 30. Calibration graph for 1,2 benzanthracene; GC peak area produced by UV absorbance detection vs. mass of sample injected. Error bars indicate the 95% confidence interval for the whole line.

fluorescence quantum yield at that wavelength [175]. Thus, both the intensity of the deuterium source and the light throughput for the detection system will affect the sensitivity for fluorescence.

In addition to the excitation radiation, the fluorescence sensitivity, unlike the absorbance, may be arbitrarily varied by increasing or decreasing the signal amplification. Of course, such amplification will also increase the background noise so that the signal-to-noise ratio for specified masses for the system could remain unchanged. However, this fact may not be reflected in the sensitivity as calculated by conventional methods, (i.e. the slope of the fluorescence signal vs. sample mass plot). This variable nature of the fluorescence sensitivity for samples separated by GC, makes comparisons between systems ambiguous or misleading.

To address this dilemma, the sensitivities for compounds measured by the fluorescence channel were calculated by a method described by L. Thomas and A. Adams [157]. This method was developed to allow for direct comparisons of sensitivities in cases in which the fluorescence signal magnitude may be arbitrarily varied by signal amplification. The response, or peak area, per nanogram is calculated relative to the response of the detector at the limit of detection. This calculation is made by the following equation:

$$[\{(Area_{(analyte)}/Area_{(LOD)}\}-1]/[Mass_{(analyte)}-Mass_{(LOD)}]$$

The limit of detection is defined by IUPAC to be "the lowest concentration (or mass) of a chemical species that can be determined to be statistically different from an analytical blank" [176]. Often, limits of detection reported for chromatographic separations and detectors are loosely defined or are not reported. Similarly, errors might be made by authors

who report the minimum detected amount of the GC detection system rather than the limit of detection for the entire analytical process including all chromatographic conditions. Also, LOD should be reported in units of amount, for example mass, rather than concentration for chromatographic separations [177].

Aside from non-uniformity in the methods for determining LOD values for chromatographic separations, a fundamental difficulty results from the fact that limits of detection become worse with increased retention time due to band broadening caused by longitudinal diffusion. This occurs even if all other conditions (e.g. sample size, column, detector) are held constant.

Methods have been reported to address problems associated with measuring and reporting LOD in chromatographic separations. One method suggests referencing all different chromatographic states to a reference chromatographic state for that particular type of chromatography (e.g. liquid, gas, etc.) [177]. Other authors have reported improvements in the chromatographic LOD values independent of band broadening effects based on a running total integration method [178].

One conventional method for determining LODs is to multiply the standard deviation of the blank by 2 or 3 and divide this by the sensitivity. In chromatography, baseline fluctuations are often the limiting factor in measuring small amounts of eluates. The peak-to-peak noise of the baseline is approximately five times the standard deviation of the blank signal if a normal distribution is assumed [176]. Accordingly, a signal of approximately three fifths the peak-to-peak noise could be detectable. However, this convention applies to

differences in signal, but integration of peak areas are not amenable to this type of measurement.

Another complicating factor which must be considered when recording a chromatographic signal with a digital integrator, is that the recorder is programmed to detect peaks of an approximate peak width. With later eluting, broadened peaks, the integrator may report one peak as several or perhaps fail to detect a low broad peak at all although one is clearly visible upon inspection. Also, there is a great deal of variation in the peak areas that are reported by the integrator for small peaks, probably due to inconsistencies in the algorithms used.

Considering these many factors, a method was developed as part of this research to determine the limits of detection for the compounds measured by the UV absorbance channel of the GC detector. For this procedure, a minimum detectable peak is judiciously defined as having a peak height approximately two times the peak-to-peak noise and a peak width selected to approximate GC peaks of concern. Multiple injections are made of compounds at or near their detection limit. The compounds are chosen to have a wide range of retention times, t(R). Then, in order to account for the effect of the digital integrator, the areas of these peaks as reported by the integrator are graphed versus their GC retention time. In this manner, a linear expression is developed between the area of a peak at the detection limit and the GC retention time. This expression depends upon the programming of the integrator as well as the other instrumental variables and, therefore, must be evaluated for different conditions used. For measurements made with the shorter pathlength cell (see Table 4), the equation for the peak

area at the detection limit is:

$$Area_{(LOD)} = 105\ t_R + 752$$

for which the units of area are converted from μV-s to a.u.-s and t_R is the retention time of the sample in minutes. The corresponding equation formula for the peak area at the LOD for the conditions used with measurements made in the long pathlength cell (see Table 5) is:

$$Area_{(LOD)} = 126\ t_R + 1163.$$

The retention time of a sample is determined for the defined system parameters and the area of a peak at the detection limit is calculated using the above equation. Then, to determine the limit of detection in nanograms, the area is divided by the sensitivity:

$$LOD\ (ng) = Area_{(LOD)}\ (a.u.-s)/\ S\ (a.u.-s/ng)$$

Although this method for LOD determination is empirical, it takes into account the effects of band broadening at longer retention times and the response of the digital integrator to different peak widths. Therefore, it should give reasonable estimates of the limits of detection for compounds measured by this GC system and UV absorbance detector.

In cases where chromatographic conditions are often changed or where the magnitude of background fluctuations change during the course of a GC separation, it may not be feasible to make multiple injections of samples near the limit of detection to derive the above formulas of minimum detectable peak area versus chromatographic retention time. For instance, in the scanned illumination wavelength GC detection system developed in this research, numerous fixed waveband separations were made at different wavelengths in order to determine sensitivities and limits of detection. The peak-to-peak noise in the baseline varied depending upon the intensity and stability of the deuterium source at

the selected waveband. In order to determine the minimum detectable peak area, not only would multiple injections of masses near the detection limit have to be made for many different retention times, but this entire procedure would have to be repeated for each illumination waveband used.

A second method was developed for determining the minimum detectable peak area at different retention times which closely approximates the results obtained by the empirical method described above. This is useful in cases where the empirical method is excessively time-consuming. With the abbreviated method, the minimum detectable peak area is defined as the magnitude of the peak-to-peak noise in the baseline adjacent to the peak of interest multiplied by the full-width at half-maximum (FWHM) of the chromatographic peak. This generalization reduces the number of injections necessary at different retention times because peak widths increase quite linearly as the retention time increases. Therefore, only a few injections representing different retention times need be made to develop a linear expression and they do not need to be made with masses near the detection limit.

After the minimum detectable peak areas have been determined, a linear expression is developed with respect to retention time as described above. For fixed waveband absorbance measurements in the monochromator/optical filter configuration of the GC spectrometric detection system, the linear expressions for peak area at the detection limit (μV-s) at various illumination wavebands are summarized in Table 6. The units of $Area_{(LOD)}$ are subsequently converted to a.u.-s.

Table 6. Peak area (μV-s) at the detection limit for different GC retention times and source wavebands for GC eluates detected by UV absorbance measurements

$$Area_{(LOD)} = m * t_R + b$$

Wavelength (nm) of the source radiation	m	b
220	2391	37134
240	1566	22964
255	1515	19495
270	1243	17681
290	965	15569

IV. MEASUREMENTS MADE WITH THE MONOCHROMATOR/OPTICAL FILTER CONFIGURATION

A. Ultraviolet Spectrometric Detection System for Gas Chromatography Based upon Repetitive Fast Spectral Scans of Absorbance with Concurrent Fluorescence Detection

by
Debra L. Van Engelen, Lawrence C. Thomas,
and Edward H. Piepmeier

for submission to Analytical Chemistry, American Chemical Society

A computer-controlled ultraviolet spectrometric detection system for gas chromatography (GC) has been developed which acquires gas-phase absorption spectra of GC eluates at rates up to two 62-nm scans per second. Concurrent fluorescence measurements enhance the selectivity of this detection system and also provide additional qualitative information. The system has been specially designed to preserve chromatographic resolution and is selective for aromatic compounds. This multi-mode GC detection system is demonstrated and evaluated through the detection of GC separations of polynuclear aromatic compounds.

Aromatic compounds, particularly polynuclear aromatic compounds (PNAs) have strong absorption bands in the ultraviolet (UV) from around 180 nm up to 350 nm. Often molar absorptivities are of the order 10^5 L cm^{-1} mol^{-1} for these absorption bands [1,2,4]. In addition, multiring PNAs are relatively rigid compounds with few low energy vibrations, and many exhibit fluorescence. These characteristics make PNAs particularly suited to sensitive UV spectrometric determinations.

Absorption bands of large molecules in the UV region result from transitions from many overlapping electronic, vibrational and rotational energy levels at normal temperatures. Consequently, the UV spectra of these compounds lack detailed structure from different energy transitions which may be present in spectra of small molecules. Nonetheless, UV absorbance and fluorescence spectra of aromatic compounds do provide useful qualitative information. Aromatic compounds usually have three absorption bands due to $-- *$ transitions in the near UV [1-4] and the position and shape of these bands may be useful for identification

purposes, particularly when combined with other qualitative information, such as concurrent fluorescence quantum measurements or chromatographic retention time.

Spectrometric detection systems based on UV absorbance or fluorescence have been shown to provide sensitive detection for aromatic compounds separated by gas chromatography (GC). Most GC detection systems measuring UV absorbance have monitored aromatic compounds at a fixed waveband [143,145-147,172,179], to provide chromatographic information, although a few have provided spectral information as well [144,148]. Kaye and Waska [144] interfaced a commercially available spectrophotometer with a GC via heated transfer lines and acquired repetitive absorbance spectra of low-molecular-weight organic compounds. This system made 6-s scans of wavelength between 160 and 210 nm. Kube et al. [148] transfered low-molecular-weight GC eluates to a 30-mL flow-through cell in a commercially available diode array spectrometer. Absorbance spectra were made by this system between 200 and 800 nm at a rate of one every 3 seconds.

Most UV fluorescence GC detectors have also monitored a fixed waveband [151,152,154-157,172,179] to produce chromatograms. Some of fluorescence detection systems are quite sophisticated and are based on laser-induced fluorescence [158] and determinations in rotationally-cooled gas streams [159]. A system has been described which acquires fluorescence spectra of GC eluates [153]. The best limits of detection for these UV absorbance or fluorescence GC detection systems have ranged from greater than a microgram down to low or sub-nanogram levels.

Aside from the inherently sensitive nature of UV spectrometric determinations, systems based upon these measurements have other advantages as GC detectors. For

instance, the good selectivity of these detectors allows aromatic GC eluates to be determined in the presence of many co-eluting but non-detected compounds. Judicious selection of the illumination waveband may even allow selective detection of some PNAs in the presence of other potential aromatic interferants. This selectivity may reduce the number of time-consuming and labor-intensive sample preparation and enrichment procedures required prior to the GC separation and spectrometric determination.

Most aliphatic compounds are transparent in the near UV and, therefore, solvent peaks can be eliminated in chromatograms produced by UV spectrometric detection. In addition, such detectors are usually insensitive to changes in temperature, pressure, and carrier gas flow rate and may also not respond to column bleed. The result is a stable chromatogram baseline, even in temperature-programmed applications. Another advantage of spectrometric GC detectores is that they are usually non-destructive and are, therefore, conducive to concurrent or tandem GC detection systems.

The approach taken by most of the above mentioned UV spectrometric GC detection systems has been to interface a GC with an essentially intact commercially-available spectrometer or HPLC detector via heated transfer lines. Such systems have demonstrated the feasibility and potential sensitivity of UV spectrometric measurements for GC detection. However, the usefulness of these systems may be limited by their bulkiness and possible deterioration of chromatographic resolution. Transfer lines cause adsorptive losses of GC eluates [143] and broadening of chromatographic peaks. Detection cell volumes which are incompatible with chromatographic band volumes, may also cause broadening and mixing of eluates within the detector

cell. The addition of make-up gases may reduce this problem in some cases [148].

Most previously described UV spectrometric detection systems for GC have also only been used for low-molecular-weight aromatic compounds with significant vapor pressures. Although PNAs absorb UV light very strongly and there has been considerable interest in their determination in environmental samples [17,21, 100], only a few spectrometric detection systems have been suitable for their measurement due to the high temperatures required (i.e. 250°C or higher) to maintain these large compounds in the vapor phase. Such temperatures could damage the commercially-available spectrometers or HPLC detectors which often have been coupled with GC instruments.

We have previously developed and evaluated GC detection systems based on concurrent UV absorbance and fluorescence measurements [172,179]. Unlike other GC spectrometric detectors, these systems were developed specifically to preserve the resolution of the GC separation and allow high-temperature, gas phase measurements of PNAs. These detectors are compact and simple, which makes them appropriate for routine determinations. The improvements to these detection systems were achieved through the spacial design and careful attention to the compatibility of the optical components. Sensitivities and limits of these UV spectrometric detection systems are in the low nanogram range which is comparable to systems based on much more sophisticated instrumentation. In addition, the concurrent measurement of absorbance and fluorescence enhances the selectivity of the system.

In the UV spectrometric detection systems described herein, the above gas-phase multi-mode GC detection systems which we have previously described have been modified and expanded to provide computer-controlled,

fast-scanned UV absorption spectra. By acquiring repetitive vapor-phase, UV absorption spectra of PNAs, this detection system provides not only sensitive determinations, but also qualitative information which may be used to identify compounds. In addition, this type of data is useful for enhancing the selectivity of the system by allowing the determination of optimum wavelengths for sensitive measurement of individual compounds while reducing the response of potential interferants. And finally, spectral information may be used to recognize co-eluting PNAs in unresolved GC peaks. The ability to obtain a concurrent fluorescence measurement enhances the selectivity and potentially the sensitivity of this GC detection system, as well as provides additional information for identification in some cases.

1. Experimental Section

Instrumentation. A schematic representation of the GC detection system is shown in Figure 2 (Chapter II). GC separations were made with a Tracor Instruments Model 565 gas chromatograph equipped with a 2 m x 2 mm I.D. glass column packed with 3% OV-101 on 100/120 Supelcoport. GC eluates flow directly into a long absorbance pathlength, flow-through detector cell which has been previously described [179]. The cell was milled from a solid aluminum block and is heated to 250°C. Absorption measurements of gas-phase eluates are made through a 167-mm horizontal section of this cell with a volume of 320 µl. Along this length, there is a 151-mm long, glass-lined section with a 1-mm I.D. and then a larger volume, 16-mm long section with a 4-mm I.D. Fluorescence measurements are made through a side

window in the larger volume section which is lined with Suprasil quartz.

The detector cell is illuminated by UV light from a deuterium lamp transmitted through a 1-m x 3-mm Welch Allyn high-temperature quartz fiber optic bundle. Transmitted light for the absorbance signal is collected at the other end of the cell by a 250-mm x 1-mm Maxlight fiber optic bundle. This light is filtered through a 220-nm wide-bandpass optical filter from Acton Research Corporation prior to detection by a photomultiplier tube (PMT 1) in order to exclude fluoresced light from this photodetector. The current from PMT 1 (Model R1464 from Hamamastu Corporation) is converted to a voltage with an operational amplifier current-to-voltage converter circuit and then to its base-10 logarithm by modular logarithmic amplifier circuit. In addition, the absorbance channel has a variable gain and offset supplied by a follower-with-gain operational amplifier circuit. Single waveband determinations are recorded on the digital integrator from a Hewlett-Packard 5880 gas chromatograph using an external input board.

A second PMT (2) detects fluoresced light which has been filtered by a 320-nm longpass optical filter to reduce scattered light in this signal. The current from PMT 2 is also converted to a voltage and amplified.

UV radiation is produced by a deuterium lamp and wavebands are selected by a modified Czerny-Turner monochromator. These optical components are from a Tracor Model 970A HPLC detector. The monochromator has a 6-nm spectral bandpass, and is scanned by a rotating shaft to produce repetitive absorbance and fluorescence excitation spectra during a GC separation. The manual wavelength selection knob was removed from its shaft, which was then connected to a 24-step, 4-phase stepper motor via a 1/4 step-down precision chain drive. The

other end of the rotating shaft is fixed to the dial of a ten-turn, 10-KΩ potentiometer so that a voltage is produced which is directly proportional to its position (i.e. the position of the grating).

A LSI-11 computer from Digital Equipment Corporation is used to control the scans of the monochromator via the stepper motor and acquire and store spectral data during a GC separation. Special interfacing circuits were made between the computer and stepper motor. The stepper motor is controlled by timed pulses produced by a General Purpose Timer interface board from ADAC Corporation and logic level voltages produced by a DT1761 Data Acquisition interface board from Data Translation. The DT1761 board is also used to make analog-to-digital conversions (ADC) on 3 multiplexer-selected signal channels (absorbance, fluorescnece, and the "wavelength" voltage proportional to the grating position). These interface boards on the LSI-11 are software-controlled and this system is designed to increment the monochromator at maximum rates of 100 Hz.

Calibration. This spectrometric GC detection system scans the monochromator in both directions to produce repetitive spectra, first towards longer wavelengths and then towards shorter wavelengths. The monochromator from the Tracor 970A detector has a LED display indicating the wavelength. However, it was determined that there was an offset in the wavelength during ascending and descending scans. The transmittance profile of a 254-nm narrow-bandpass optical filter (Acton Research Corporation) was used to calibrate the repetitive scans with respect to another spectrum produced on another calibrated spectrophotometer (Cary 18). Light from the monochromator used in the GC detection system was filtered by the narrow-bandpass filter during repetitive wavelength scans from 240 to

290 nm as indicated on the LED display. Pulses were sent to the stepper motor electronics at a rate of 50 Hz so that one 50-nm spectrum was acquired per second. As usual, the signal from PMT 1 was converted to its base-10 logarithm, therefore, producing a signal directly proportional to absorbance. The digital signals stored by the LSI-11 computer were then aligned by software according to the wavelength of maximum transmittance. Figure 31 shows the aligned transmittance spectra produced by these measurements. It was determined that the LED display of wavelength accurately indicated the wavelength for descending wavelength scans ($\pm$ 1 nm), but that ascending scans (towards longer wavelengths) had a 12-nm offset towards shorter wavelengths. Therefore, a wavelength correction was made to all ascending scans during the data reduction procedures.

Reagents. PNAs were purchased from Aldrich. Solutions of PNAs were prepared using reagent-grade methylene chloride with the following composition: 0.50 µg/µL each of naphthalene and 2-nitrofluorene; 0.25 µg/µL of dibenzofuran, fluorene, carbazole, 2-methylanthracene, pyrene, and 2,3 benzofluorene.

Procedure. The above PNA solution (5 µL injections) was separated by GC using a temperature program with an initial temperature of 150°C for 2 min, then changed at 10°C min^{-1} up to a final temperature of 250°C. N_2 carrier gas was used with a flow rate of 35 mL min^{-1} in scanned wavelength determinations. Serial dilutions and multiple GC determinations were made of the PNA solution at single wavebands to determine sensitivities and limits of detection. N2 carrier gas was used with a flow rate of 28 mL min^{-1} in single waveband determinations.

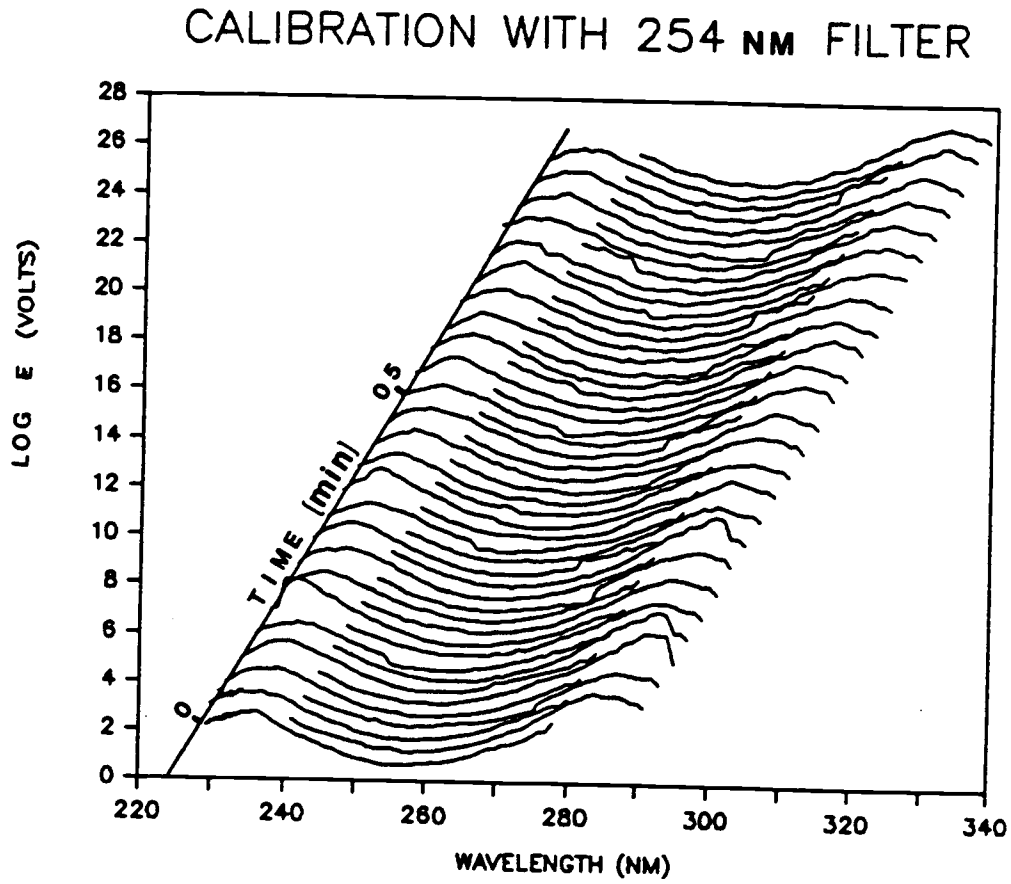


Figure 31. Data used for wavelength calibration of ascending and descending scans produced by the UV spectrometric detection system; Repetitive scans of the logarithm of the voltage produced by the PMT versus wavelength (nm) for the 254-nm narrow bandpass optical filter

Software. Programs were written in Fortran and Macro assembly language for the DEC LSI-11. This software is used to control the scans of the monochromator, acquire data from 3 ADC channels, and store data in disk files during a separation. An overview of the data acquisition sequence during a GC separation is shown in a flowchart in Figure 16 (Chapter 2). This software allows the operator to select parameters from the keyboard including spectral ranges, scan rates, and total data acquisition times. Following the completion of a run, data files may be labeled with information from the keyboard, plus other separation and run parameters and averaged background spectra for each of the 3 signal channels. Following creation of a data file on the LSI-11, the data are rearranged so that all spectra are in order of ascending wavelength. The software also is designed to allow the acquisition of fixed wavelength data to produce chromatograms at one selected waveband.

Due to the large amount of data gathered by this system and the limited memory available on the LSI-11 computer (64-K), the DEC files were transferred to an IBM-PC computer and stored in data files. Programs were written in Basic for the IBM-PC computer. These programs made further rearrangements of the data files to facilitate subsequent data reduction and analysis using Lotus 1-2-3 software. These programs also performed a 5-point least squares smoothing of the data if desired [180]. The spectral data presented in this manuscript have been smoothed.

Data reductions and plots of spectra were made on blocks of data using Lotus 1-2-3 software. Averaged background spectra were subtracted from the signal data and absorbance data were converted from voltages to absorbance units (a.u.). Lotus 1-2-3 was used to

produce three-dimensional plots of the data (signal versus wavelength versus time).

2. Results and Discussion

Scanned Waveband Detection. The UV spectrometric GC detection system described herein was designed to be particularly suited to the analyses of PNAs. Absorption spectra of PNAs have much broader bands than smaller aromatic compounds. However, the shape, position and number of bands within a selected UV region may be characteristic of individual PNAs. Other information which is provided by this system, such as GC retention time and concurrent fluorescence intensity, may further reduce uncertainties in the identities of compounds.

Repetitive fast-scanned spectra of absorbance and fluorescence excitation were made of eight PNAs separated by GC at a rate of one spectrum per second. As described previously, there is a 12-nm offset between the ascending and descending wavelength scans, so the spectra actually span 62 nm in 50 increments. Figure 32 shows one hundred repetitive absorption spectra obtained during part of this GC separation of the PNA solution, from 0.4 to 2.1 minutes. The first eluate shown is naphthalene with a retention time of 0.78 min. Dibenzofuran has a retention time of 1.62 min, but is not detected in this wavelength region. The next observable GC peak is fluorene, with a retention time of 2.05 min.

The absorption spectra of each of the PNA eluates are shown in Figures 33 through 40. The figures, which present a number of spectra acquired throughout the elution time of a peak, are three-dimensional (3-D) plots, with the time axis moving up and back with respect to the absorbance and wavelength axes. Other

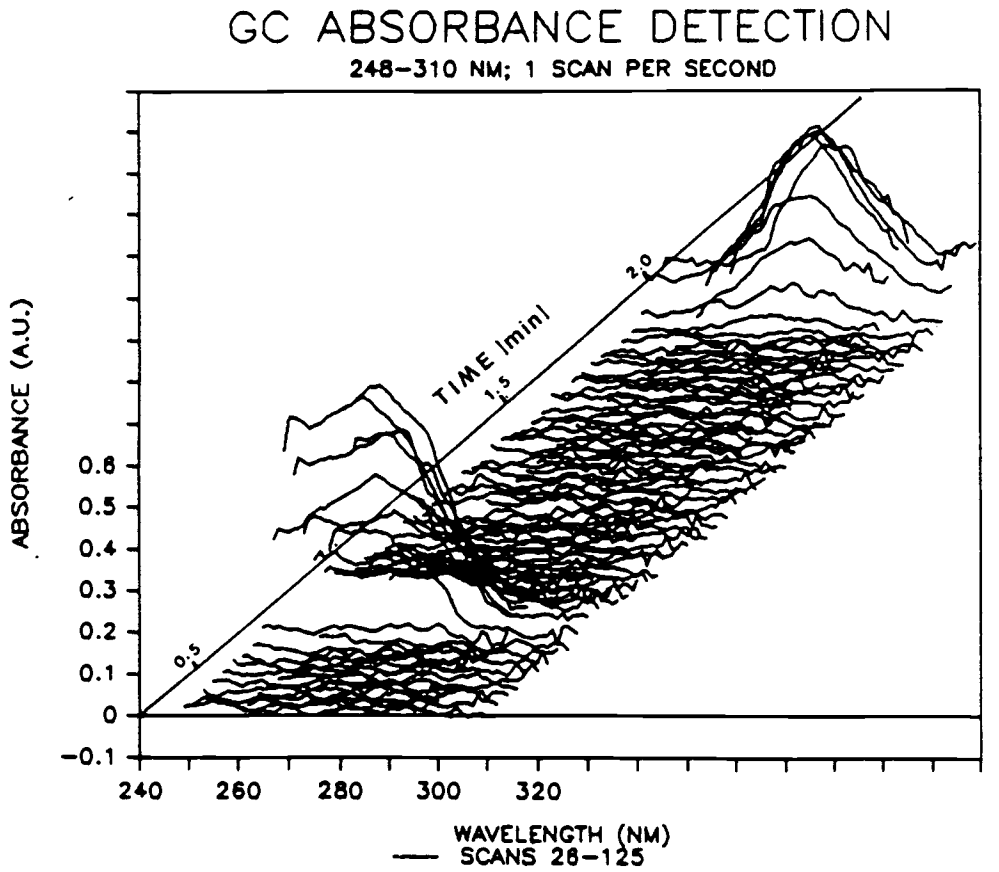


Figure 32. One hundred absorption spectra acquired by the UV spectrometric GC detection system; scans 26-125

figures, which present only one or two spectra, are superimposed with respect to GC retention time.

An enlarged 3-D view of the repetitive spectra of naphthalene is shown in Figure 33a, and Figure 33b shows the two most intense naphthalene spectral scans obtained during the elution. Naphthalene has a relatively intense absorption band at 267 nm.

As stated previously, dibenzofuran has a GC retention time of 1.62 minutes under these separation conditions. Figure 32 shows that no detectable absorption between 248 and 310 nm is observed during this time. However, when measurements were repeated on the same PNA solution separated under the same GC conditions but with wavelength scans made between 208 and 260 nm, an intense absorption band with a maximum at 218 nm was detected for dibenzofuran, Figures 34a and 34b. These three figures demonstrate the selectivity of UV absorbance measurements made at different wavebands. Absorption spectra such as these can be used to maximize or minimize the dibenzofuran sensitivity as compared to other PNAs during fixed waveband determinations.

The next three PNAs to elute during this separation are fluorene at 2.05 minutes (Figures 35a and 35b) with an absorption maximum at 281 nm, carbazole at 2.50 minutes (Figures 36a and 36b) with absorption maxima at 257 nm and 296 nm, and 2-methylanthracene at 4.58 minutes (Figures 37a and 37b) with an absorption maximum at 286 nm. The masses of each of these compounds were 1.25 μg in this separation. The sensitivities for each compound, indicated by the peak heights, vary according to the absorptivities of each absorption band in this region.

Figures 38 a, b, and c show how this GC detection system may help identify co-eluting PNAs in unresolved chromatographic peaks. 2-nitrofluorene has a GC

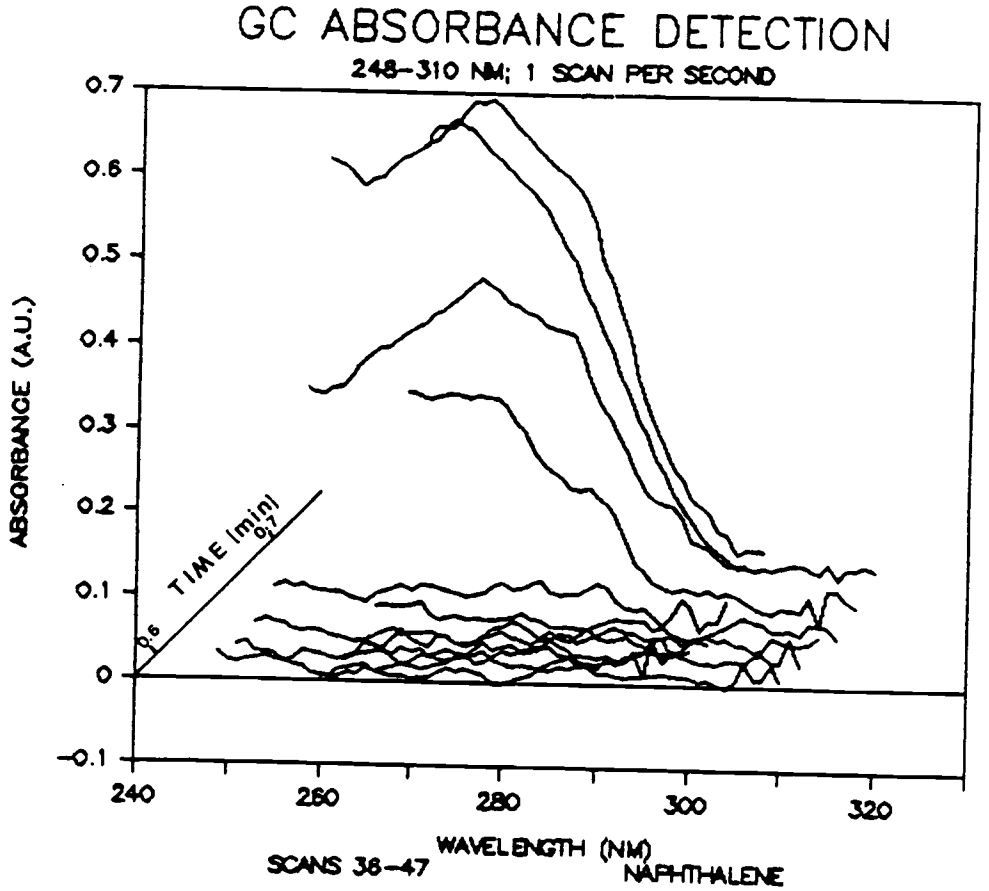


Figure 33. Absorption spectra of naphthalene from 248 to 310 nm produced during a GC separation of PNAs; (a) scans 36-47 (3-D),

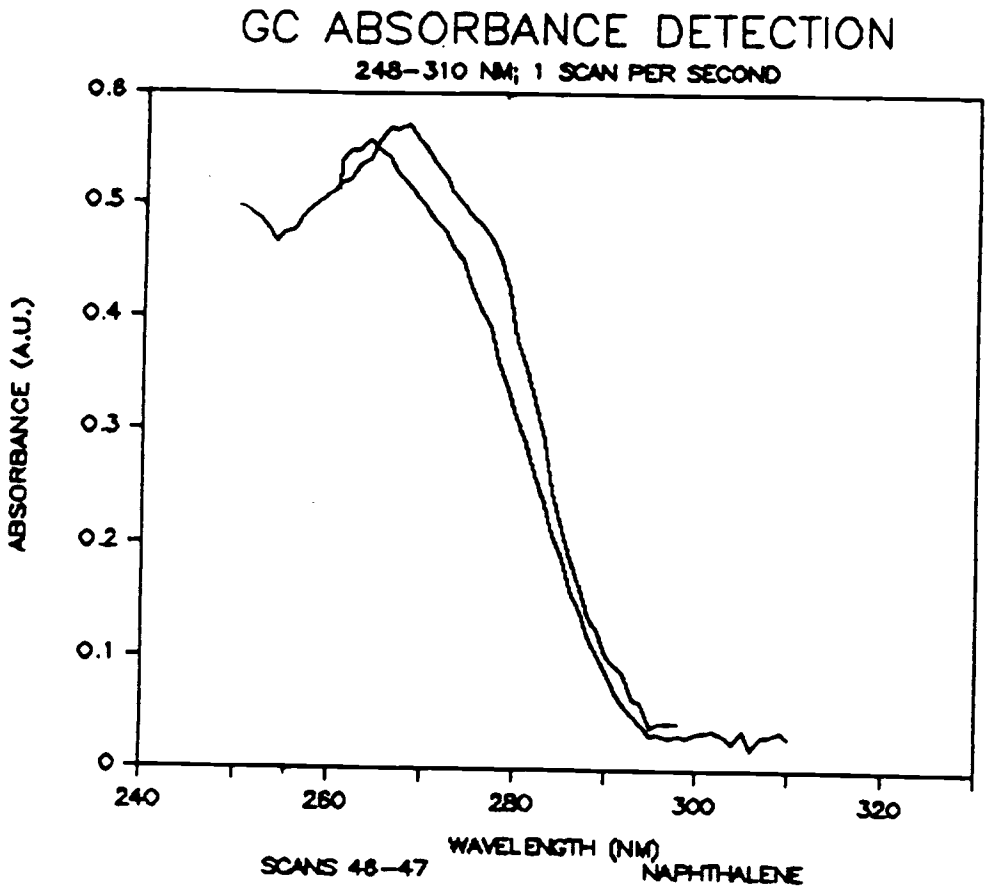


Figure 33 (cont). (b) scans 46-47 (no time axis)

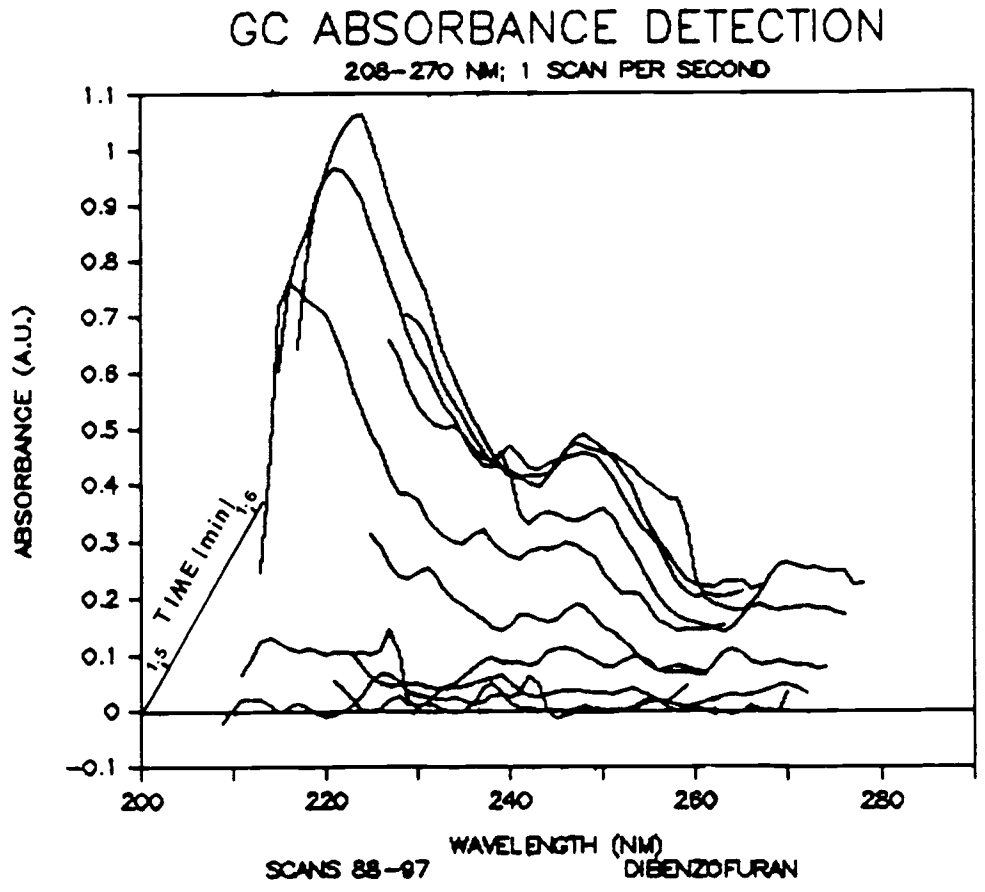


Figure 34. Absorption spectra of dibenzofuran from 208 to 270 nm produced during a GC separation of PNAs (a) scans 88-97 (3-D),

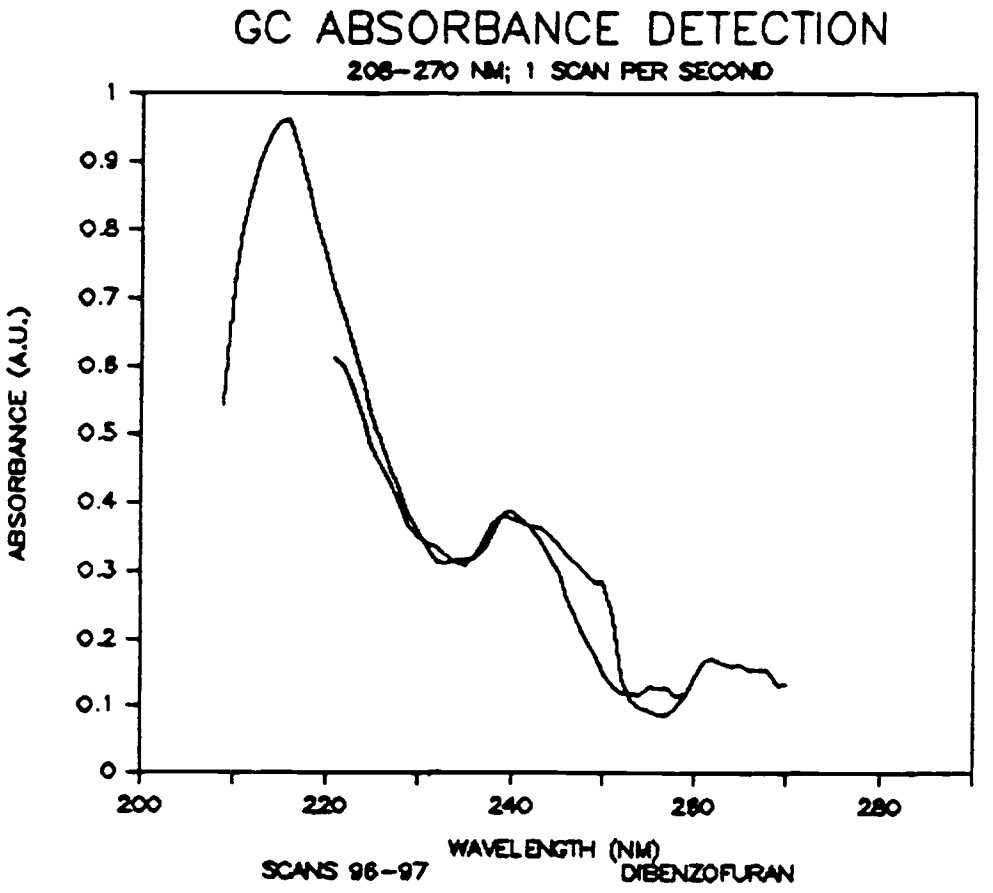


Figure 34 (cont). (b) scans 96-97 (no time axis)

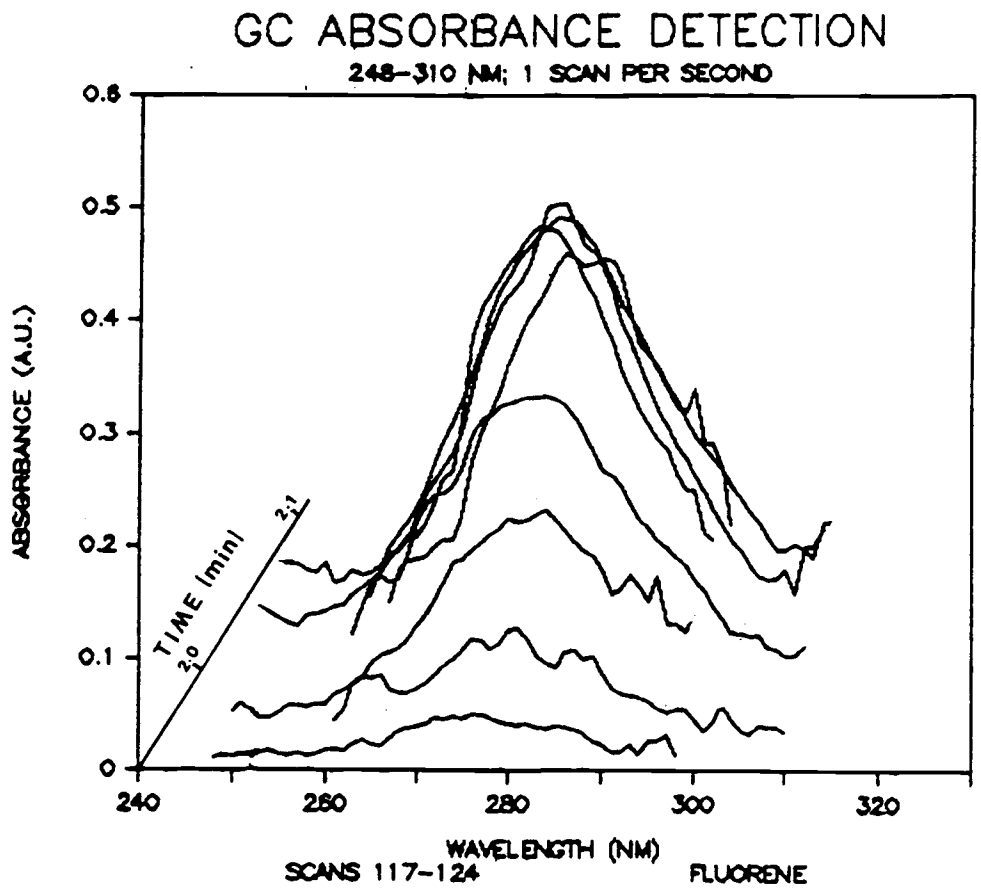


Figure 35. Absorption spectra of fluorene from 248 to 310 nm produced during a GC separation of PNAs; (a) scans 117-124 (3-D),

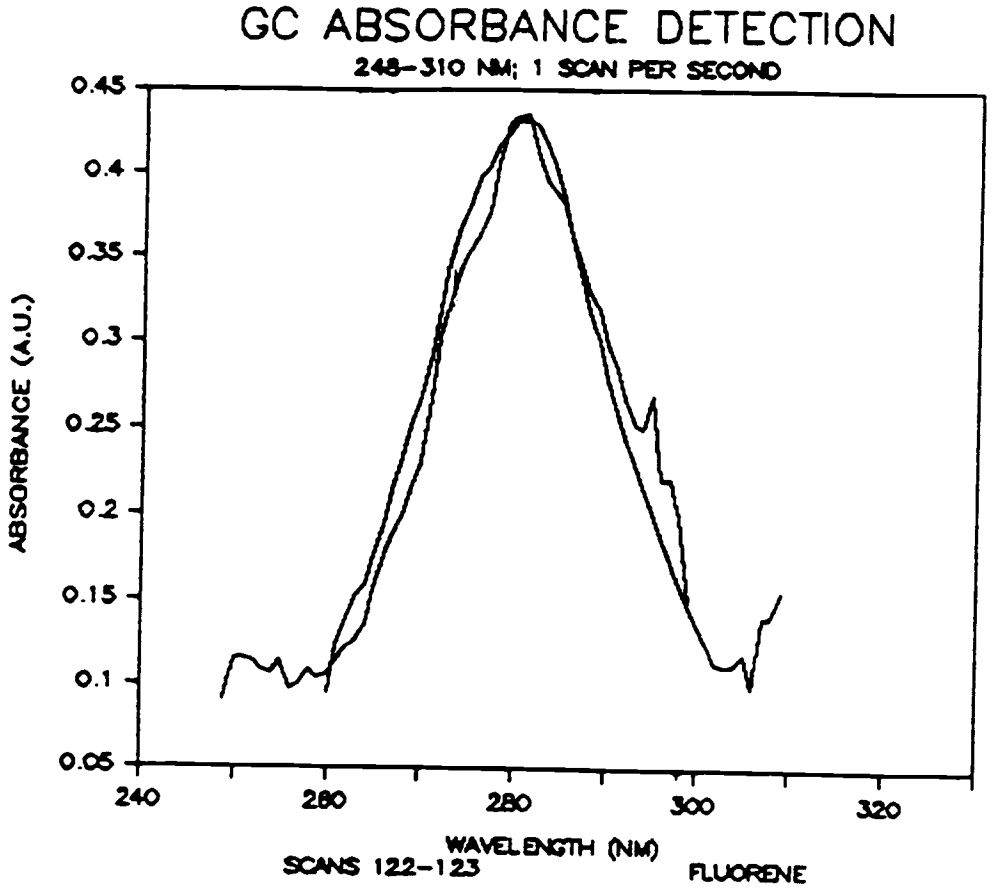


Figure 35 (cont). scans 122-123 (no time axis)

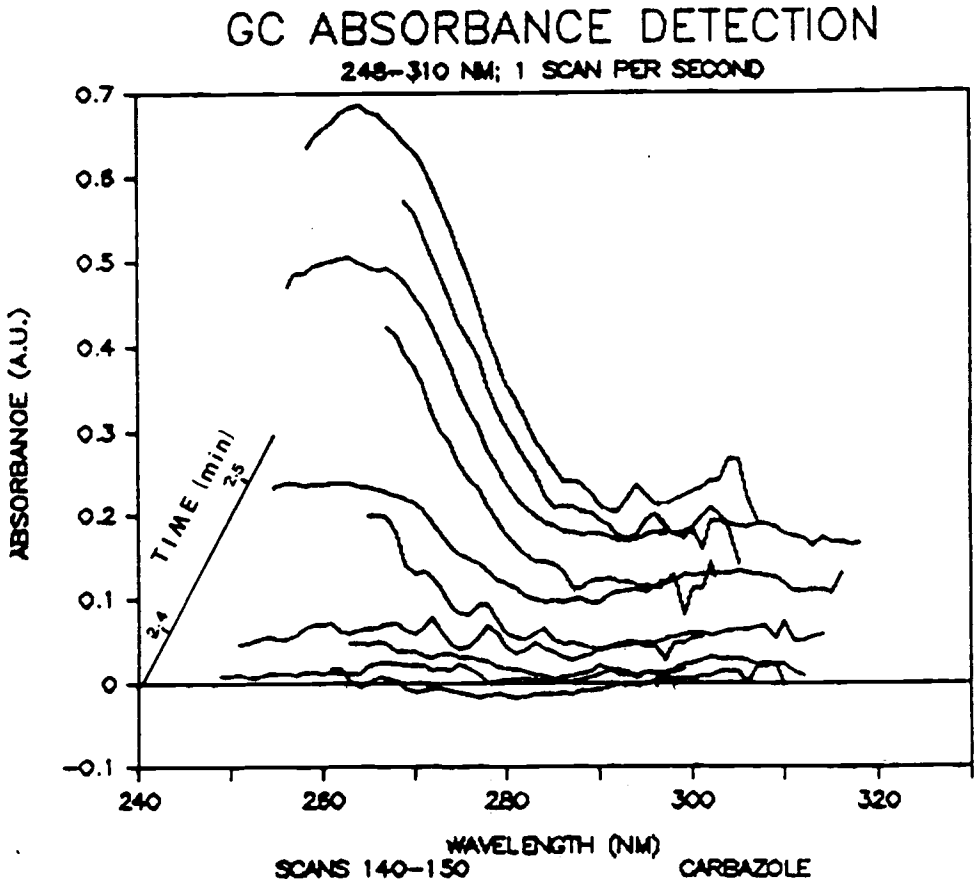


Figure 36. Absorption spectra of carbazole from 248 to 310 nm produced during a GC separation of PNAs; (a) scans 140-151 (3-D),

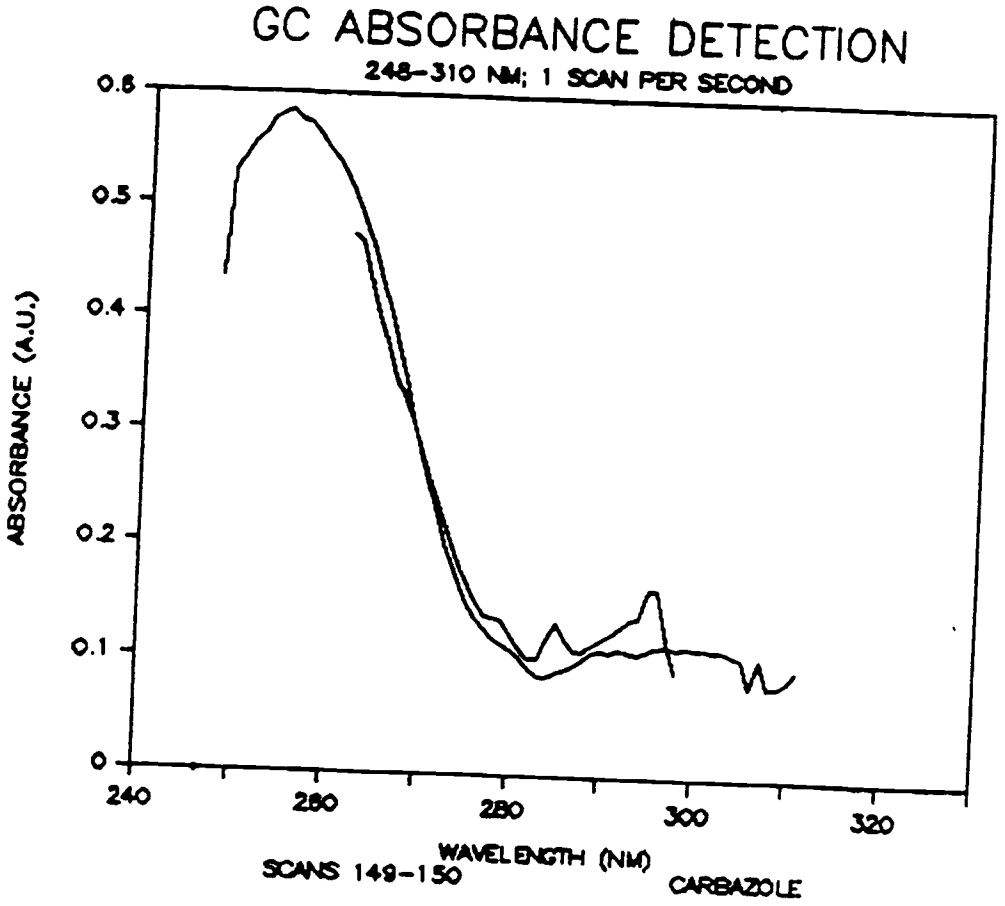


Figure 36 (cont). scans 149-150 (no time axis)

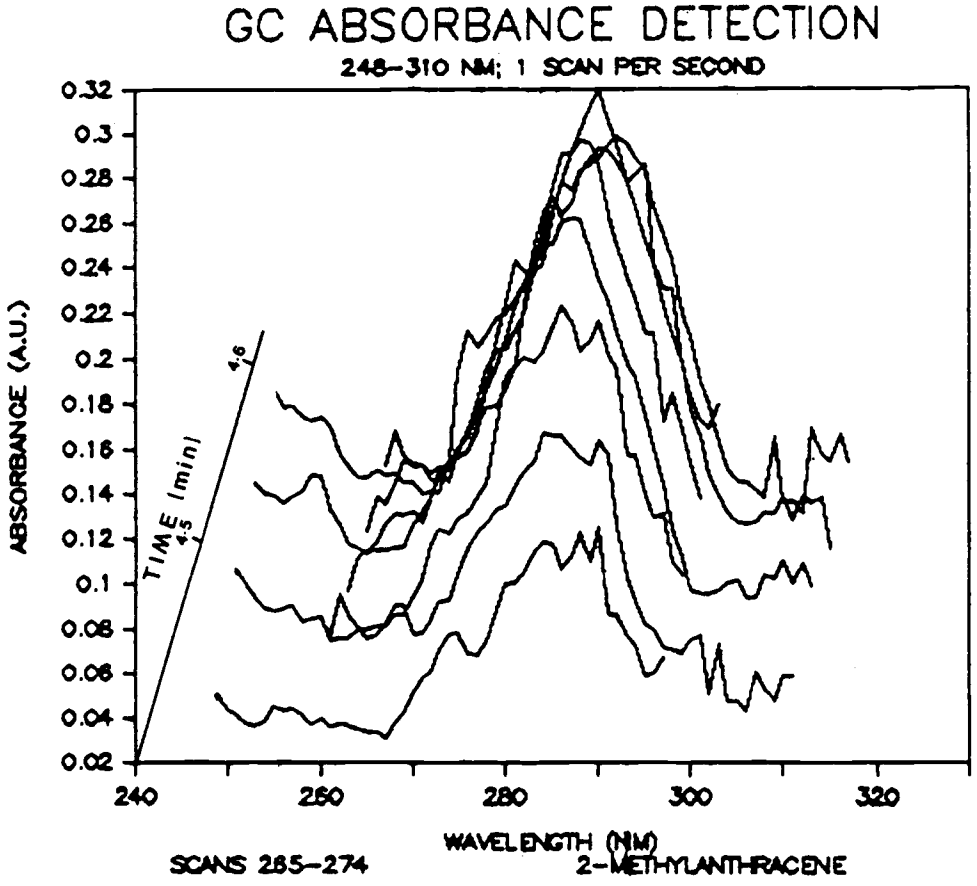


Figure 37. Absorption spectra of 2-methylanthracene from 248 to 310 nm produced during a GC separation of PNAs; (a) scans 265-274 (3-D),

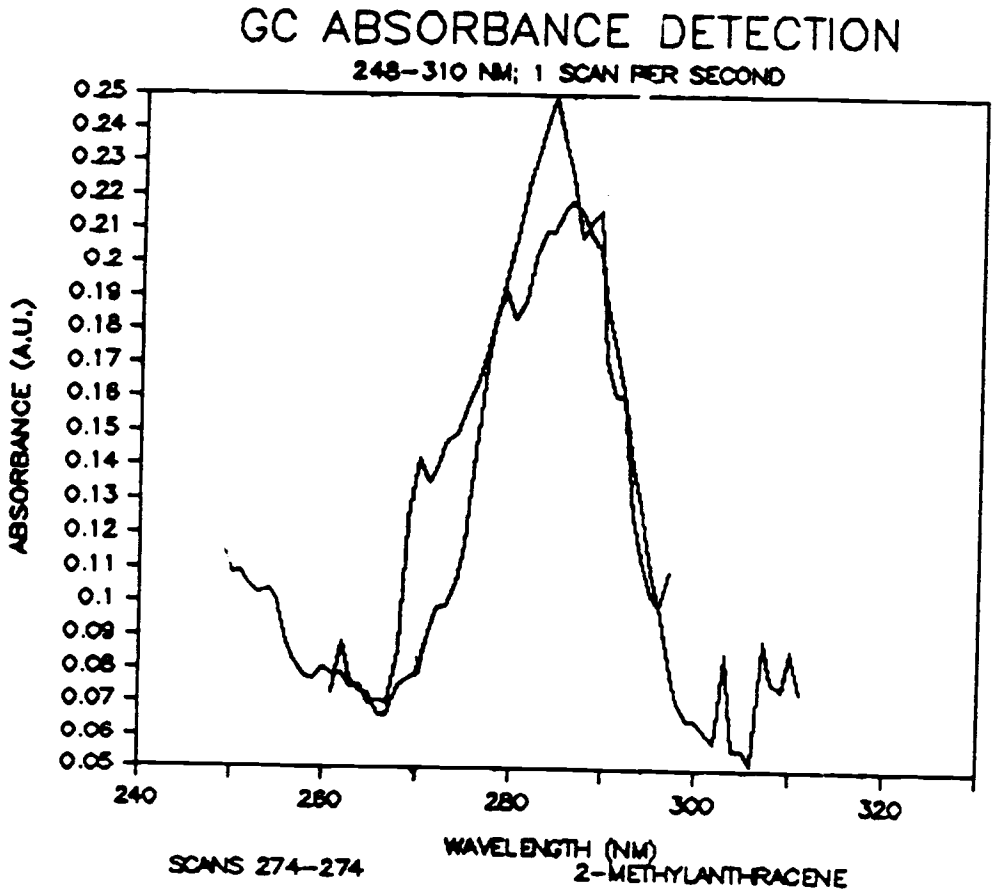


Figure 37 (cont). (b) scans 273-274 (no time axis)

retention time of 7.15 minutes and pyrene has a GC retention time of 7.23 minutes. These two PNAs are not chromatographically resolved by this packed GC column separation. However, the presence of co-eluting compounds is detected by differences in the UV absorption spectra for 2-nitrofluorene and pyrene. Figure 38a shows the absorption spectra produced with respect to time during the separation. In Figure 38b, scan number 429 is superimposed upon scan number 434, to show more clearly how the spectra change in shape during the elution of the one unresolved GC peak.

In order to identify which spectrum is produced by which PNA, 2-nitrofluorene and pyrene were each separately injected on the GC and detected by the UV spectrometric detector. Figure 39 shows one of the spectra produced for 2-nitrofluorene (scan 424 in this later determination) which has a very broad peak with a maximum around 299 nm. Figure 39 also shows one of the spectra produced in a similar manner for pyrene (scan 434 in the individual determination) superimposed upon the 2-nitrofluorene spectrum. The pyrene spectrum has a strong absorption at 255 nm and a weaker band at approximately 288 nm. When the individually made spectra are compared to the spectra of the unresolved peak in Figures 38a and 38b, it can be determined that 2-nitrofluorene is the predominate component during the earlier elution time (scan 429 in Figure 38b). As the GC peak continues to emerge from the GC column, the 2-nitrofluorene concentration diminishes while the pyrene concentration increases and spectra resembling those of pyrene are produced in the later part of the chromatographic band.

The last PNA to emerge from the GC column during this separation was 2,3 benzofluorene with a GC retention time of 8.23 minutes and absorption maxima at

258 nm and 305 nm (see Figures 40a and 40b). 2,3 benzofluorene exhibits strong fluorescence when excited in this UV region and concurrent fluorescence excitation spectra were recorded. Figure 40c shows one of these fluorescence spectra.

Although the wavelength dependence of vapor-phase absorption and fluorescence excitation spectra are expected to be nearly identical [4], the intensities of the fluorescence excitation spectra vary markedly among different PNAs. Therefore, the ratio of the absorbance to fluorescence intensity (i.e. fluorescence quantum yield) may be highly characteristic of individual PNAs. Not all the compounds separated here exhibit sufficient fluorescence intensity to be detected above the background fluctations of the fluorescence signal in this GC detection system and the presence or absence of detectable concurrent fluorescence with the absorbance measurement for each compound was used as an identifying characteristic.

The results of these measurements are summarized in Table 7. Clearly, the GC retention time, the wavelength or wavelengths of maximum absorption, and the presence or absence of detectable concurrent fluorescence are characteristic among these eight compounds. In fact, many other PNAs could be distinguished from each other given this amount of qualitative information. Since the system is selective for aromatic compounds and also detects very few other chromophores in this wavelength region, overlapping spectra or chromatographic peaks from interferants of this nature should rarely present a problem.

Single waveband detection. This UV spectrometric detection system may operate in either a scanned or fixed wavelength mode. One of the primary advantages of acquiring spectral data of GC eluates is that this type

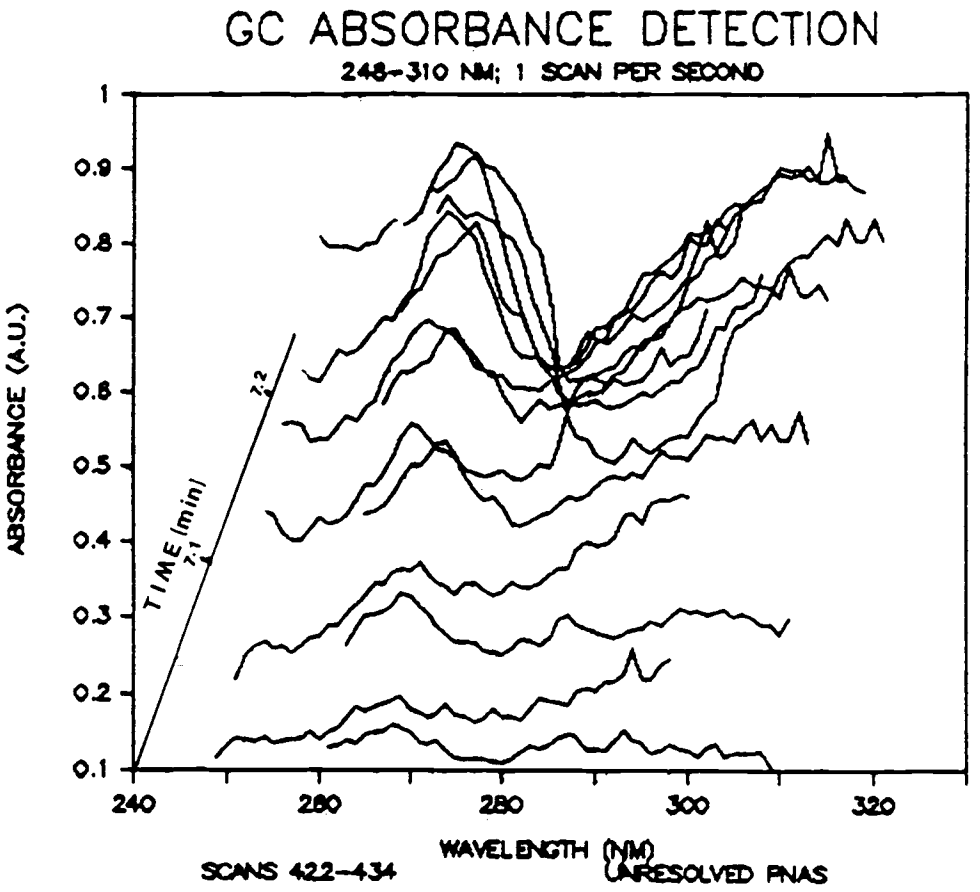


Figure 38. Absorption spectra of 2-nitrofluorene and pyrene from 248 to 310 nm produced during a GC separation (unresolved peak); (a) scans 422-434 (3-D),

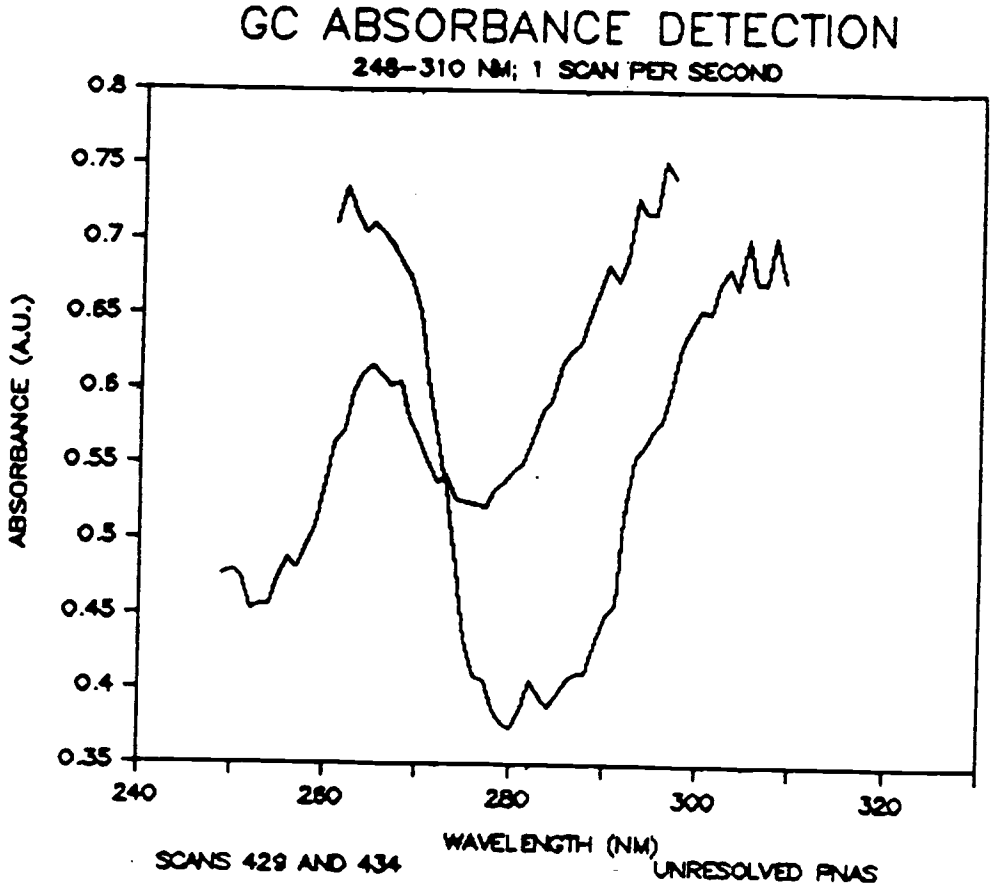


Figure 38 (cont). (b) scans 429 and 434 (no time axis)

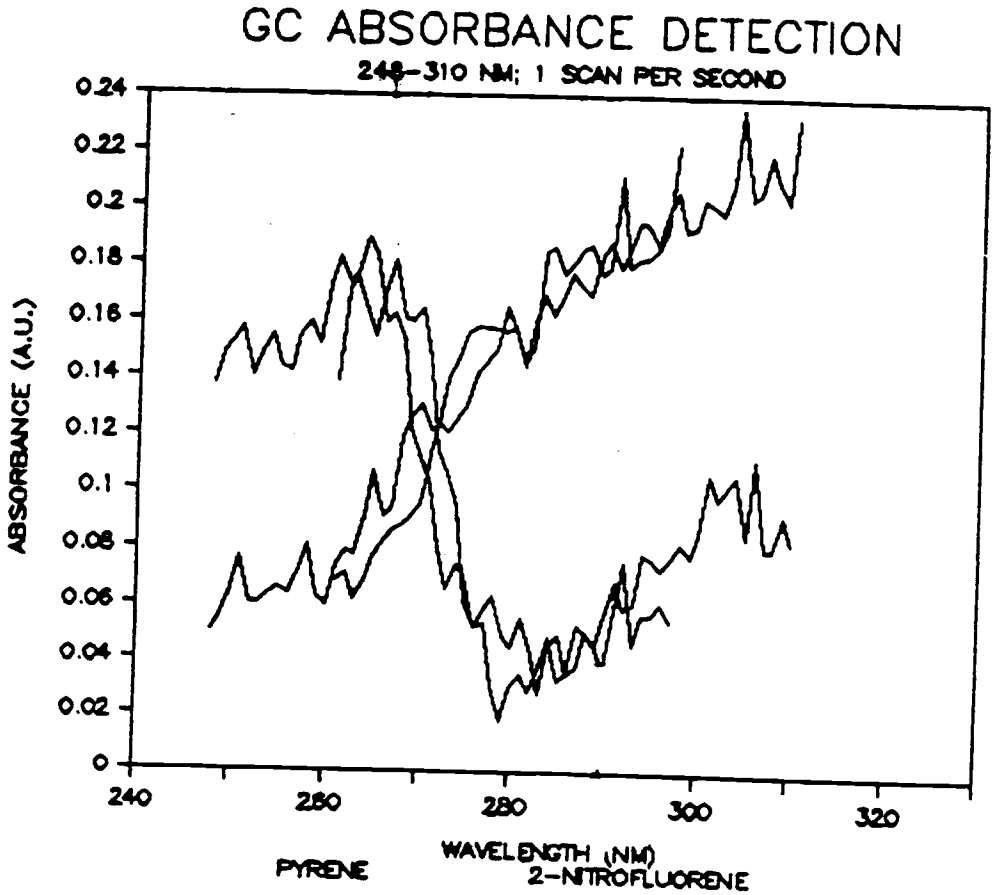


Figure 39. Individual absorption spectra of 2-nitrofluorene (scan 427-428) and pyrene (scan 434-435) from 248 to 310 nm produced by GC detection (no time axis)

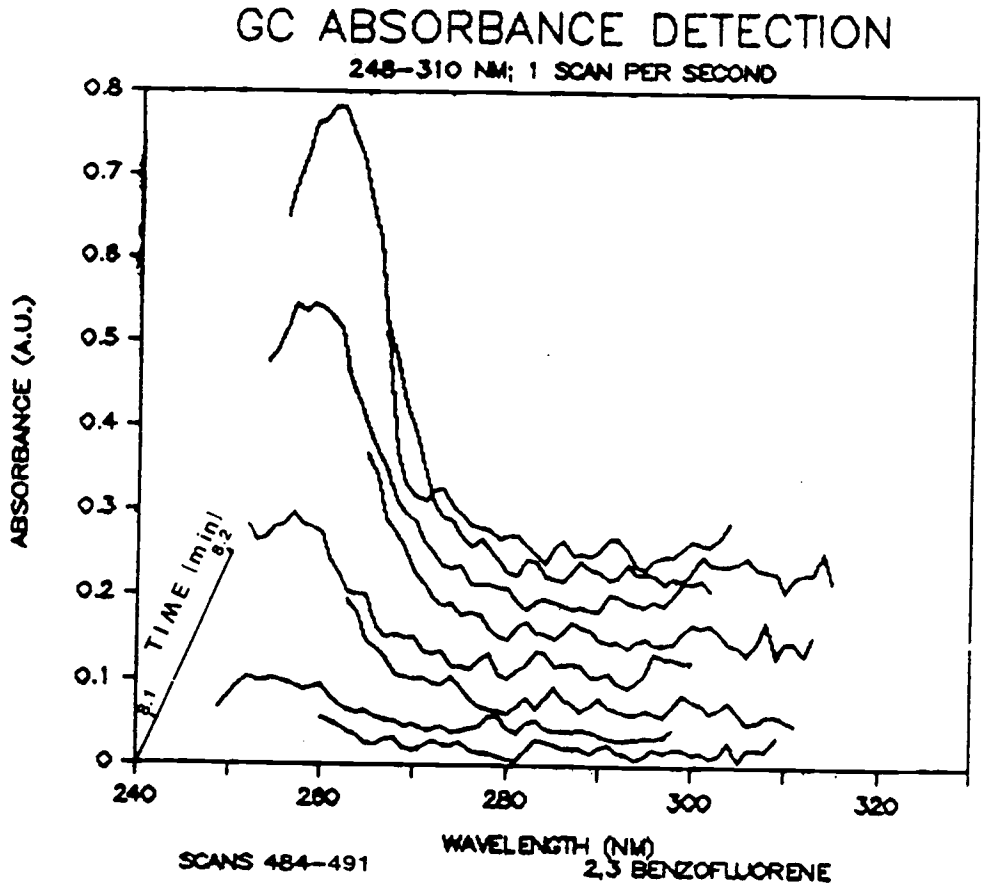


Figure 40. Absorption spectra of 2,3 benzofluorene from 248 to 310 nm produced during a GC separation of PNAs; (a) scans 484-491 (3-D),

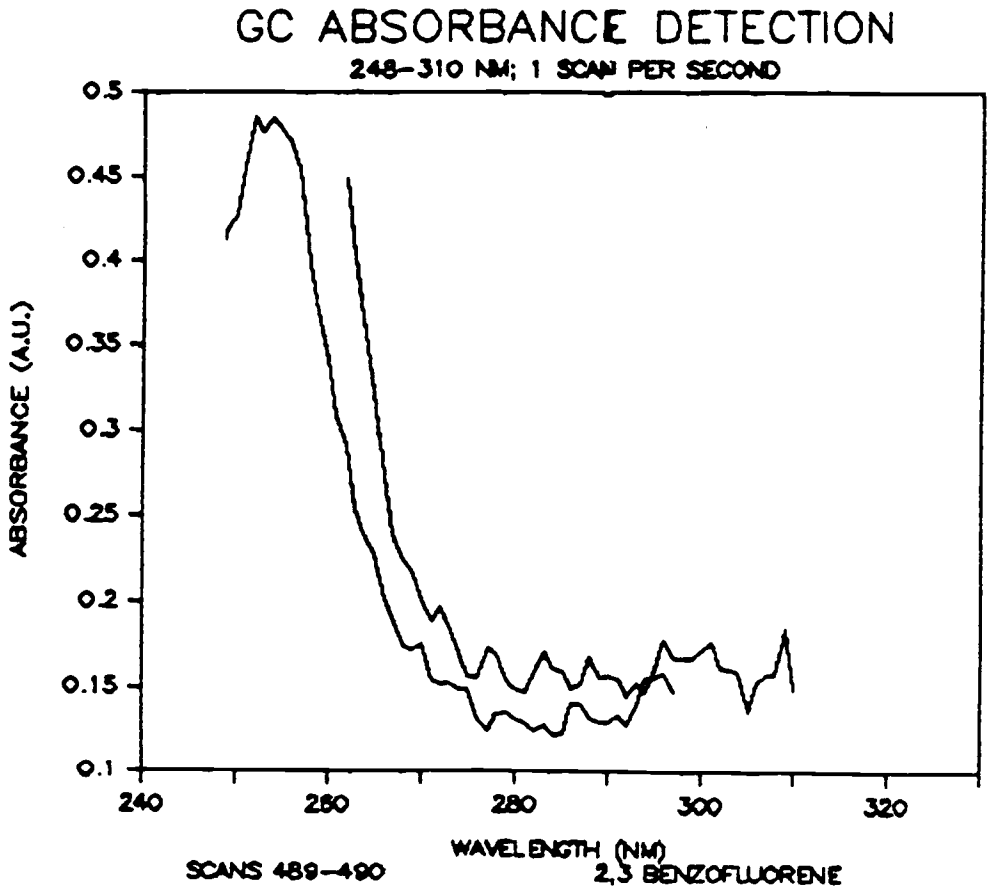


Figure 40 (cont). (b) scan 489-490 (no time axis),

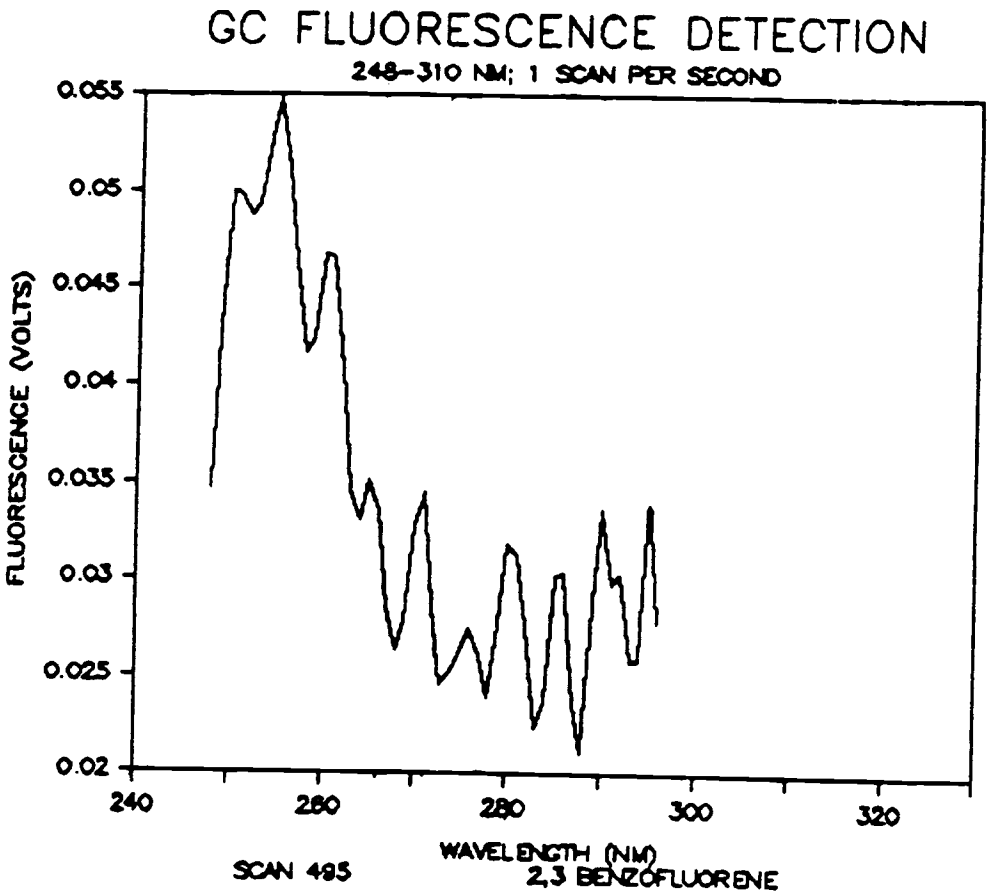


Figure 40 (cont). fluorescence excitation spectrum concurrently with absorption spectra during a GC separation of PNAs, scan 495

Table 7. GC retention time and wavelength(s) of maximum UV absorption (± 3 nm) between 248 nm and 310 nm for selected PNAs as determined by spectrometric detection of GC eluates

Compound	Retention Time (min)	Wavelength(s) of maximum Absorption (nm)	Fluorescence Detected
1. naphthalene	0.78	270	-
2. dibenzofuran	1.62	---	-
3. fluorene	2.05	280	-
4. carbazole	2.50	255 (295)	+
5. 2-methylanthracene	4.58	285	-
6. 2-nitrofluorene	7.15	300	-
7. pyrene	7.23	260 (300)	-
8. 2,3 benzofluorene	8.23	255 (300)	+

of qualitative information may be useful for compound identification. On the other hand, this detection system has less background fluctuation when operated with a fixed illumination waveband which may be advantageous for trace analysis. However, spectra obtained from scanned waveband GC detection assists in the choice of the best illumination wavebands for single waveband determinations.

To demonstrate the variable selectivity which is obtainable in GC detection systems based upon UV spectrometric measurements, chromatographic determinations of the above PNA mixture were made with the UV detector's source radiation fixed at one waveband during the separation. Chromatograms were monitored at illumination wavelengths of 220, 240, 255, 270 and 290 nm. The results of these determinations for the absorbance measurement are shown in Figure 41. The masses of each of the PNAs were the same as those injected during the separation described above in which spectral data was collected (i.e. 1.25 μg for all PNAs except naphthalene and 2-nitrofluorene which were 2.50 μg). The sensitivities of each of the PNAs vary at different wavelengths and some PNAs are not detected at some wavelengths. This characteristic may be used to selectively detect certain PNAs of interest while minimizing the response of other PNAs which are possible interferants.

Sensitivities and limits of detection were determined by serial dilutions of the above PNA solution and multiple GC injections for the absorbance measurements at the five fixed wavebands described above and are summarized in Table 8. The absorbance sensitivities, in GC peak area per nanogram, at the wavelength of maximum absorption for each compound (determined here only at 220, 240, 255, 270 or 290 nm) ranged from

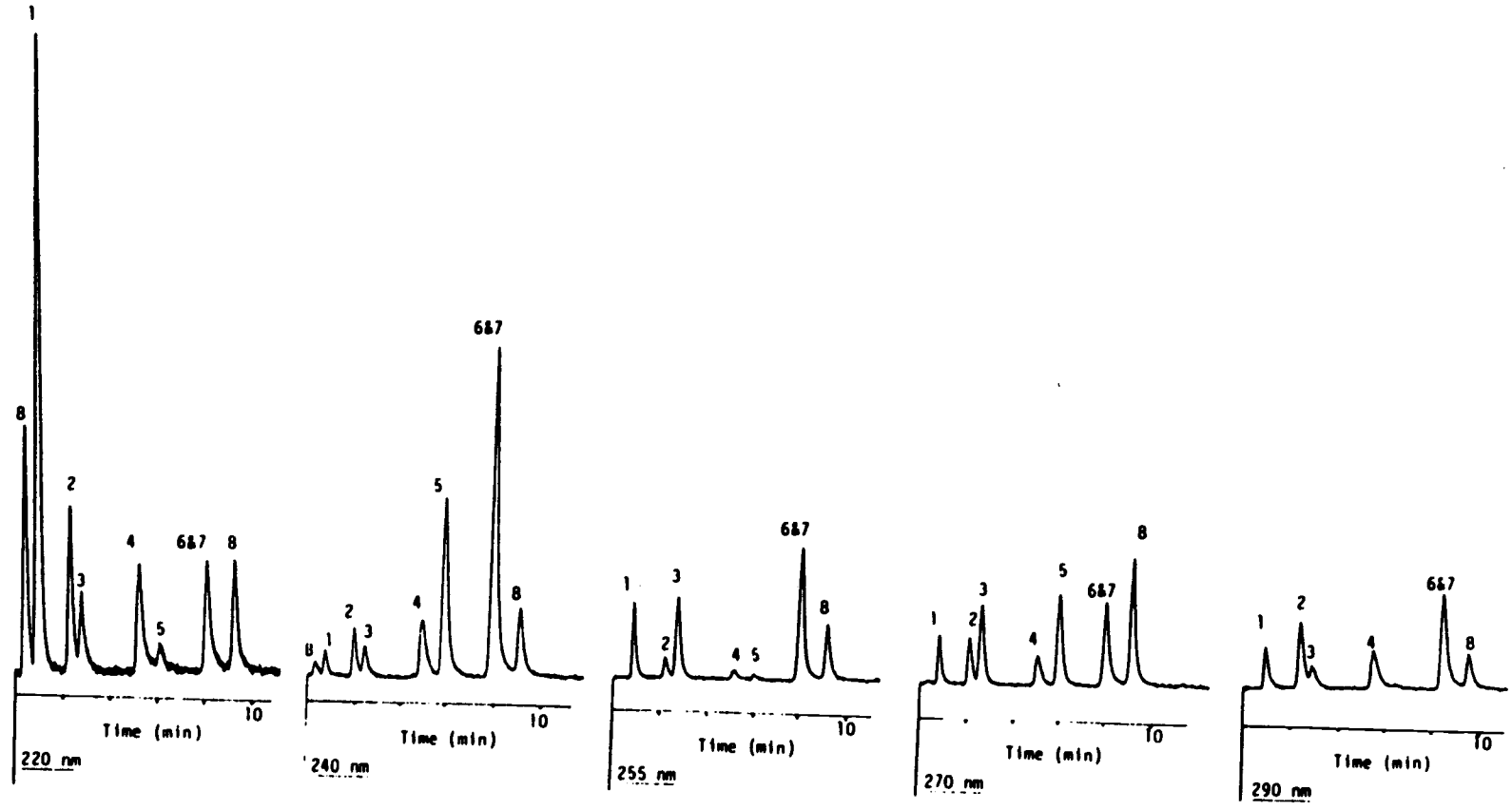


Figure 41. Chromatograms of GC separations of PNAs detected by UV absorbance at fixed illumination wavelengths of 220 nm, 240 nm, 255 nm, 270 nm and 290 nm 1 = naphthalene, 2 = dibenzofuran, 3 = fluorene, 4 = carbazole, 5 = 2-methyl-anthracene, 6 = 2-nitrofluorene, 7 = pyrene, 8 = 2,3 benzofluorene

Table 8. Gas Phase UV Absorbance Detection of Selected Aromatic Compounds at the Waveband of Maximum Absorption from among Five Selected Wavelengths LOD = limit of detection; S = sensitivity

Compound	t'R (min)	Wavelength (nm)	LOD (ng)	S (a.u.-s ng^{-1})
1. naphthalene	0.85	220	30	11.8×10^{-4}
2. dibenzofuran	2.22	220	50	8.2×10^{4}
3. fluorene	2.70	270	40	5.1×10^{-4}
4. carbazole	5.16	220	50	9.3×10^{-4}
5. 2-methylanthracene	6.09	240	30	11.6×10^{-4}
6. 2-nitrofluorene	8.07	290	70	3.2×10^{-4}
7. pyrene	8.28	240	40	8.4×10^{-4}
8. 2,3 benzofluorene	9.32	255	40	7.1×10^{-4}

3.2×10^{-4} a.u.-s ng^{-1} for 2-nitrofluorene at 290 nm, up to 11.8×10^{-4} a.u.-s ng^{-1} for naphthalene at 220 nm. These sensitivities are slightly improved over previous detectors developed in this laboratory of similar design to this one [172,179], which used optical filters rather than a monochromator for waveband selection.

Limits of detection at the maximum absorption wavebands for this GC detection system were worse for some PNAs than those for our previously described detectors with optical filter waveband selection [172,179] and better for others. The improvements were due to the ability to measure individual PNAs at their wavelength of maximum absorption with the monochromator system. In the previously described optical filter detection systems, only some of the PNAs showed good sensitivities in the illumination waveband (i.e. from 240 to 260 nm). On the other hand, the monochromator allows considerably less light through the cell than the bandpass optical filters in the other spectrometric system. The result of this is that the total signal is reduced relative to the background fluctuations. Therefore, some limits of detection for these PNAs are not as good when detected by this system at a single waveband as those that can be achieved by the optical filter GC detection systems. The limits of detection for these eight PNAs range from approximately 30 ng up to 70 ng at the wavelength of maximum absorption.

The minimum detectable peak area was defined as the magnitude of the peak-to-peak fluctuations in the baseline adjacent to the peak of interest times the full-width at half-maximum (FWHM). Thus, the minimum detectable peak area based upon this definition increases with GC retention time because of the corresponding increase in peak width with retention time while the baseline fluctuations remain the same.

The limit of detection for naphthalene is 30 ng at 212 nm as detected by absorbance in this system. This may be compared to the detection limits for other previously described GC detection systems which are also capable of providing absorption spectra. For another detection system which acquired scanned-wavelength spectra, the detection limit for naphthalene at 211 nm was 40 ng [143,144]. A previously reported GC detector which made imaged absorption spectra with a photodiode array, was not designed to detect multiring aromatics due to the lower temperatures used (ca. 70°C) in the transfer lines and cell. However, this system was reported to have limits of detection of approximately 0.5 μg for substituted benzene compounds [148].

The low light throughput of this UV spectrophotometric GC detection system has a profound effect on the intensity of the fluorescence measurement. Although the limits of detection for some PNAs are comparable or better by fluorescence than absorbance in the optical filter systems, the fluorescence limits of detection are approximately five times worse than the absorbance for this UV detection system. However, the fluorescence is more selective than the absorbance and this information may be useful.

Figure 42 shows chromatograms produced by fluorescence at fixed excitation wavebands of 220, 240, 255, 270 and 290 nm. The fluorescence signal has a large amplification, and baseline drift results from the electronics. The masses of the PNAs are five times greater than the masses shown in Figure 41 which was monitored by absorbance. However, the chromatograms produced by fluorescence demonstrate the additional selectivity offered by the concurrent fluorescence measurement. For example, only one GC eluate is detected with 220-nm excitation radiation, four GC peaks

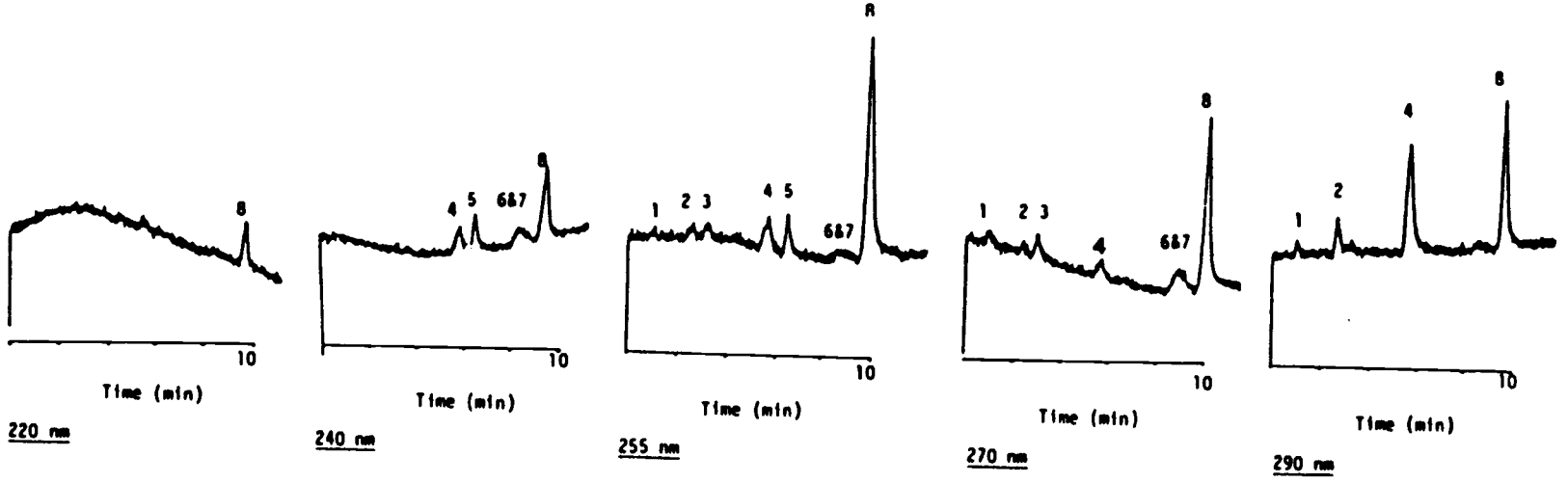


Figure 42. Chromatograms of GC separations of PNAs detected by UV fluorescence at fixed excitation wavelengths of 220 nm, 240 nm, 255 nm, 270 nm and 290 nm; 1 = naphthalene, 2 = dibenzofuran, 3 = fluorene, 4 = carbazole, 5 = 2-methylanthracene, 6 = 2-nitrofluorene, 7 = pyrene, 8 = 2,3 benzofluorene

are recorded with the source at either 240 nm or 270 nm, and five or six separate GC peaks are recorded in the other two chromatograms. As stated previously, this increases the amount of qualitative information provided by this detection system over the absorbance measurement alone.

Although this UV spectrometric detection system for gas chromatography has good sensitivity for determinations of PNAs at one waveband, the primary advantage of this system is the spectral information it provides. The absorption spectra may help identify unknown PNAs, determine the best wavelength for selective detection of individual PNAs, and help locate co-eluting compounds. The concurrent fluorescence measurement enhances the selectivity of the system and provides some additional qualitative information.

B. Data Reduction and Analysis Methods

This section provides details that are not in the manuscript for publication.

The UV spectrometric detection system for has chromatography (GC) developed in this research acquires spectral data of GC eluates at rates up to two 50-nm spectra per second. If data are collected on three analog-to-digital (ADC) channels, the system will collect 300 16-bit binary values per second. This is more than a block of data (i.e. 256 words) on an 8-in floppy diskette for the LSI-11 computer. As stated in Chapter II, data files made by this system are limited to 483 blocks on an 8-in diskette. Therefore, at the maximum data acquisition rate, the total data acquisition time is limited to just over 6 minutes.

It was determined that if pulses were sent from the computer to the stepper motor at a rate of 50 Hz rather than 100 Hz, at least eight spectra were usually acquired by the system during the elution time of an early eluting GC peak. This amount of data is sufficient to characterize the GC peak, while extending the maximum total data acquisition time to about 12 minutes.

Even with this reduction in the rate of data collection, DEC files created by this GC detection system often exceed 400 blocks of binary data (approximately 10^5 16-bit words). The DEC LSI-11 has 64 kilobytes of memory, which was considered insufficient to process this large amount of data. Therefore, the DEC files were transferred to an IBM-PC compatible computer with 640 kilobytes of memory using PCTALK software. This transfer of data is described in detail in Chapter II.

As the data files (now in ASCII format) are transferred to and stored by the IBM-PC computer, the files may be broken into smaller files, each consisting

of 200 wavelength scans per ADC channel. The scans are numbered and displayed on the monitor as they are transferred. After every 200th scan, the transfer is halted by a "CTRL S" from the keyboard and the first file is closed by a command to PCTALK. Another file is then opened on a new disk and data transfer is resumed by a "CTRL Q" from the keyboard.

These original IBM-PC files consist of a long column of data, alternating one scan each from each of the three ADC channel selected for transfer. There is a 12-nm wavelength offset for ascending scans (towards shorter wavelengths) which are always numbered with odd numbers in this system.

The long IBM-PC files are subsequently broken into short data files by the Basic program, CONVERT, which is described in detail in a later section. Usually these short files consist of either one set of odd scans or one set of even scans in a linear arrangement (i.e. scan 2-absorbance; scan 2-fluorescence; scan 2-"wavelength" voltage). All wavelength scans have been rearranged during transfer so that they are stored in order of increasing wavelength. These short files have a name consisting of a 4-letter common name plus numbers in sequential order. CONVERT.BAS also has a sequence which smoothes the data by a 5-point least square smooth [180], if the operator selects this option.

Following this, the short files consisting either of one set of odd scans or one set of even scans, are sequentially imported into a Lotus 1-2-3 spread sheet by a Macro called NEXTFILE written by E.H. Piepmeier. This Macro is described in detail below. Its function is to import the short data files into adjacent columns on a Lotus spread sheet. Usually 100 scans are imported into the spread sheet at one time. This Macro is usually modified to import a series of even scans and to skip

one column between each scan. Then the odd scans over the same data range are imported into the blank columns, except that the second set of columns are positioned so that the odd scans would be in the column prior to and 12 rows up from the following even scan. The result of this is a block of data for each channel with sequential wavelength scans which are also aligned so that each row corrsponds to the calibrated wavelength.

Ranges of the blocks containing 100 odd and even scans are then extracted and stored in Lotus 1-2-3 files for later use. Usually these extracted files are configured to contain 25-50 scans of data from one ADC channel. The position of the rectangular range is shifted and extracted into files until all the data has been stored. Then the next 100 short files are sequentially imported and the whole process is repeated until all the data from a GC separation are stored in Lotus 1-2-3 spread sheet files.

Each of these blocks of data in spread sheet files are subsequently processed in the following manner. Since there is a 12-nm wavelength offset in the scans, each scan of data has 12 data values from another ADC channel either at the bottom or top of each column for odd or even scans, respectively. The values in these cells are all set to the Lotus 1-2-3 function @NA. Then, the average background spectra for either the odd or even numbered scans are subtracted from the appropriate columns of sample data. The digital data are then converted to voltages by dividing by 4095 (the maximum digital value from a 12-bit ADC) and multiplying by 10 (the ADC board is jumpered for a voltage range of 0-10 V). The absorbance values are converted to absorbance units (a.u.) by a conversion factor of 0.486 a.u. V^{-1}. The derivation of this factor is described in Chapter II.

The data blocks which have been converted to either absorbance units or voltages are extracted into Lotus spread sheet data files, usually replacing the files of unprocessed data to conserve file storage space.

1. Plotting 3-D Data Using Lotus 1-2-3

Three-dimensional plots of data (signal versus wavelength versus time) are made of the blocks of data stored in Lotus spread sheet files using a method developed by E.H. Piepmeier. In this method, a block of Y-values, that is, signals produced during odd and even numbered wavelength scans, is plotted versus a block of X-values, that is, wavelength. In order to make the X-value block, the values of the actual wavelength range of the scans are entered into the first column of the block (e.g. 248-310 nm). Then each successive adjacent column is filled with these wavelength values multiplied by a progressively incremented offset (incremented in the horizonal direction only). The X-OFFSET used to multiply each column was 1.0 in the plots shown previously in this chapter.

The block of Y-values is made in a different manner. First, a file, containing a block of signal data of odd and even wavelength scans from one signal channel, is entered on the spread sheet. Then each successive data value in this range, beginning in the upper left-hand corner, is multiplied by a Y-OFFSET and SLOPE (in both a horizontal and vertical direction). These values are stored in a second range of calculated Y-values. For the plots of absorbance data shown earlier in this chapter, the Y-OFFSET was 0.01 and the SLOPE was zero.

The cells directly below each column in both the X-values and calculated Y-values blocks are filled with

@NA. A XY graph is created by Lotus 1-2-3 which is formatted for lines only (symbols are omitted). The X-data range is the X-value block described above including the row of cells filled with @NA. The calculated Y-value block, including the @NA row, is plotted as one data range only. The result is that the spectra produced by each successive scan are plotted as if they were all one Lotus 1-2-3 line, with each scan moved up and over on the page. The @NA cells are added between each scan so that a line will not be drawn from the end of one scan to the beginning of the next.

It was determined that spectral data plotted in this manner sometimes becomes too complex and difficult to read if the spectra obtained during the elution of an entire chromatographic peak are plotted. In these cases, the plots are more easily visualized if data from only the front half of the eluting GC peak are plotted.

2. IBM-PC Programs

Two programs were written for the IBM-PC computer to assist in the analysis of data acquired during a GC separation by the UV spectrometric detection system. Both of these programs were written primarily by E.H. Piepmeier, although some modifications were later made to them. Hardcopies of each of these programs are in Appendix B.

The Basic program CONVERT takes data from a long file and stores it in short files on a second disk drive. The names of the short files created have a 4-character name in common and then are numbered sequentially. The operator determines some of the dimensions and layout of the files. The operator may also opt to have the data smoothed by a 5-point least square fit [180].

The program CONVERT.BAS opens with a series of interactive statements in which the operator designates the disk drive of the input file, the disk drive of the output files, the input file name, the first 4 characters of which will be used in the output files names, and the input file extension. The output files have the same the same extension as the input file. The operator then enters information about the data contained in the file such as the number of steps in a scan (up to 50 for spectral data or 256 for fixed waveband data), the number of ADC channels (i.e. 1-3), whether odd and even scans are to put in separate blocks, and whether a 5-point smooth of the data is to be made. If an odd scan and the following even scan are made into one file rather than two, they will be effectively separated into two blocks of data when the short files are imported into a Lotus spread sheet. The above information about the data is given in a label at the beginning of each data file. If the operator does not know this information, the long file, beginning with this label, may be imported into a Lotus 1-2-3 spread sheet prior to running the CONVERT.BAS program.

Once the limits of the short files have been set by the operator as described above, CONVERT.BAS opens the input file, opens a numbered output file, and transfers the specified amount of data to the file. Output file number 00 contains the ASCII label with information about the data in the file. Output file number 0, contains the averaged background data. The sequentially numbered files created after this each contain the specified amount of data. If the data is to be smoothed, the program jumps to a subroutine which smooths the data prior to its storage in a disk file.

Each 5.25-in floppy diskette may contain only a limited number of files. Therefore, each time 100 short

files have been made, the user is prompted to replace the output diskette. Following this, the program proceeds.

After the short files with the same name plus sequential numbers have been made for the GC data, these files are imported into adjacent columns of a Lotus 1-2-3 spread sheet by a Lotus Macro named NEXTFILE. The Macro is imported into the spread sheet, the range of the Macro is named, and the Macro begins running with an "ALT N" entered from the keyboard. The operator is then prompted to enter the 4-character common name of the files which is stored in C_NAME (underline is a valid and useful character in Lotus 1-2-3 cell names), and also the first (C_F) and last (C_LAST) numbers of the files to be imported. Then the operator is prompted to move the cursor to the first cell to be copied into. This cell address to be copied into is temporarily named C_HERE.

The first file number, in cell C_F, is copied into cell C_NUMB. Then the cursor goes to C_NUMB and the contents are edited and changed from a number to a label (ASCII string). The cursor then goes to C_HERE and deletes that named range. A file of numbers (i.e. the short data file) is imported into the spread sheet beginning at the cell where the cursor is stationed at this point. The name of the imported file is given by C_NAME + C_NUMB (which is a label at this point).

After the file has been imported, the cursor is moved right to the next column. If the short files are entered into every other column as described above, this line of the Macro is change from {Right} to {Right}{Right} before running the Macro. The cell where the cursor is at now will be named C_HERE if the program proceeds through the next loop.

At this point, C_F is incremented. If only even or

odd scans are to be imported into every other column of the spread sheet, C_F is incremented twice. If C_F is less than or equal to C_LAST, the Macro loops and the data file import process is repeated for the next short file and the next column. Otherwise, the Macro stops.

If odd and even scans are to be imported in such a manner as to align the calibrated wavelength in rows across the spread sheet, short files containing either one set of odd or one set of even scans are imported into every other column by the NEXTFILE Macro. After the even numbered series of short data files have been imported, the cursor is moved to the column left of the first imported column of data. It is positioned so that it is 12 rows above the beginning of the even scans. Then the odd numbered files are imported in the blank columns. This will place an odd scan in the column before and 12 rows up from the following even scan, thus putting the scans in order and aligning the wavelengths for each scan.

V. CONCLUSIONS

This research has resulted in the development of ultraviolet (UV) spectrometric detection systems for gas chromatography (GC). Spectrometric determinations in the near UV are sensitive and selective for aromatic compounds. The detection systems determine gas-phase GC eluates by multimode concurrent measurements of absorbance and fluorescence. While both measurements are selective, the fluorescence is highly selective for measurements of polynuclear aromatic compounds (PNAs) of three or more fused benzene rings. The absorbance measurement detects both low-molecular-weight and high-molecular-weight compounds with sensitivities which vary between individual compounds.

Spectrometric detectors offer certain advantages over other detection systems. Determinations can usually be made in which there is no detection of a solvent peak or column bleed in temperature programmed applications. These detection systems are also relatively insensitive to changes in temperature, pressure, and carrier gas flow rate. Also, UV absorbance and fluorescence determinations in the near UV are usually non-destructive so that tandem and concurrent measurements may by made.

The UV spectrometric detection systems developed and studied in this research have other advantages. GC eluates flow directly into a heated flow-through detection cell, thus eliminating heated transfer lines and the adsorptive losses and chromatographic band broadening which results from such transfer. The volumes of the detector cells are also small with a minimum of dead space, which helps to preserve the resolution of the GC separation. Light transfer to and from the detector cell is made through quartz fiber optic bundles so that

optical components such as photomultiplier tubes are thermally isolated.

One configuration of this system monitors UV absorbance and fluorescence of GC eluates at a fixed waveband selected by optical filters. The filters allow good UV radiation throughput for the system and sensitive determinations down to low nanogram levels were made of PNAs by both the absorbance and fluorescence channels. The sensitivity of the two spectrometric measurements varied markedly from one aromatic compound to another, thereby extending the range of applications while enhancing the selectivity of the system. These variations in sensitivities between absorbance and fluorescence also provide a certain amount of qualitative information about eluting compounds.

Two heated flow-through detector cells were designed for the system. The first has a volume of 200 μL and an absorbance pathlength of 16 mm. The second cell was designed in such a way as to increase the absorbance pathlength without greatly increasing the volume of the cell. The long absorbance pathlength cell is 167 mm long with a volume of 320 μL. The longer pathlength improves the limits of detection and sensitivities of the the absorbance measurements by approximately a factor of ten. In each of these cell, a concurrent fluorescence measurement is made through a side-window in the cell. Photodetection is made by high-gain photomultiplier tubes (PMT) for both spectrometric measurements.

In a second configuration of the UV spectrometric GC detection system, waveband selection is made by a monochromator and repetitive fast-scanned UV spectra are made of GC eluates. The monochromator is scanned via a stepper motor which is controlled by a LSI-11 computer.

The computer also makes analog-to-digital conversions (ADC) of absorbance, fluorescence, and a voltage which is proportional to the wavelength and stores this data in disk files. The data are later transferred to an IBM-PC computer for reduction and analysis.

The spectral data obtained by this system may be used in the identification of GC eluates. Qualitative information includes wavelength of maximum absorption, GC retention time and whether or not concurrent fluorescence is observed. The spectra obtained by this GC detection system may also be used to select a fixed illumination waveband to monitor GC eluates which will be selective for PNAs of interest while reducing or eliminating the response of other potential interferants. Thus the selectivity of determinations may be varied and controlled. Another advantage of the collection of spectral data is that differences in spectra between PNAs may be used to identify unresolved GC eluates.

VI. RECOMMENDATIONS FOR FUTURE STUDIES

The fast-scanning configuration of the ultraviolet (UV) spectrometric detection system for gas chromatography (GC) produces a great deal of data in a very short period of time. It is, therefore, strongly recommended that one computer, equipped with a hard disk, be used to control the functions of the spectrometric detector, acquire and store signal data, and perform subsequent analysis of the data. Transfer of data between computers and storage of data in files on floppy diskettes is very time-consuming and seriously limits the ease of making determinations with this system.

In the monochromator/optical filter configuration, there are several changes which could be made to improve the signal-to-noise (S/N) ratios and, thus improve the limits of detection. The intensity of the source radiation within the detector cell is much less in this configuration than in the optical filter/optical filter configuration. To increase the illumination intensity, the fiber optic bundle to the source and wavelength selection module could be shortened, which would increase its light throughput. The source intensity could also be increased by replacing the deuterium lamp with by a xenon lamp. This, in turn, may increase the fluorescence intensity. With a more intense source, the total signal voltages would be increased allowing the bias voltages on both photomultiplier (PMT) tubes to be reduced, thereby, lowering the magnitude of the background fluctuations. If the instability of the xenon lamp were to cause problems, the source and signals could be modulated. Another suggested improvement for the fluorescence signal is to cool the

PMT, thus reducing background fluctuations due to thermal emissions.

If the above improvements in the S/N were made, the spectrometric detection system could be readily adapted to provide sensitive determinations of aromatic compounds separated by capillary GC columns. The only other modification required would be the to reduce the size of the detector cell to accommodate the smaller sample size and volume of chromatographic bands.

The spectra of aromatic compounds, including PNAs, possess distinguishing characteristics which were not preserved in the spectra recorded by the GC spectrometric detection system described herein. This is due to the large spectral bandpass, that is 6 nm, of the monochromator used. To confirm that losses in spectral structure were due to the bandpass of the monochromator and not due to broadening at the elevated temperature of the detector cell, spectra were made at room temperature of benzene vapor by the GC detection system. These spectra also showed no vibrational structure which could be used for identification purposes. If is therefore recommended that a higher-resolution wavelength isolation device (i.e. monochromator with a narrower spectral bandpass) would improve the quality and uniqueness of the spectra obtained by this detection system, which in turn would reduce uncertainties in compound identification.

This UV spectrometric detection system could also be re-configured to use a diode array to detect the absorbance signal and an intensified diode array or vidicon photodetector to detect the fluorescence signal. This would increase the acquisition rate for spectra which would be required if the system were to be used to provide multi-wavelength detection for capillary GC. It could also improve the spectral resolution over the

present system. In addition, an imaging detection system would allow the data to be analyzed by least squares techniques such as those described in Chapter 1.

If the resolution and quality of spectra acquired by this UV detector were improved, the system may be used to study effects such as changes in temperature, pressure and carrier gases upon the spectra of aromatic compounds. Studies could also be performed at reduced pressures by applying a vacuum at the outlet end of the detector cell. This reduced pressure may also help move compounds through the GC column. At reduced pressures, the fluorescence spectra may show increased fine structure or additional transitions.

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VIII. APPENDICES

APPENDIX A

LIST OF ABBREVIATIONS

ϵ	molar absorptivity (L cm^{-1} mol^{-1})
AC	alternating current
A/D	analog-to-digital
ADC	analog-to-digital converter
ADCDC	BA/D Data Buffer Register
Amp	amplifier
$Area_{(analyte)}$	GC peak area of the analyte
$Area_{(LOD)}$	GC peak area of the analyte at the limit of detection
a.u.	absorbance units
BaP	benzo(a)pyrene
CK	clock pulse
CPU	Central Processing Unit
CSR	Control Status Register
D	deuterium
D/C	digital-to-analog
DAC	digital-to analog converter
DACDB	D/A Data Buffer Register
DC	direct current
DEC	Digital Equipment Corporation
E	voltage
$E_{(ref)}$	reference voltage
$E_{(out)}$	output voltage
$E_{(sig)}$	signal voltage

ECD	electron capture detector
EEM	excitation-emission matrix
EPA	Environmental Protection Agency
FET	field effect transistor
FID	flame ionization detector
FLN	fluorescence line narrowing
FPD	flame photometric detector
FTIR	Fourier transform infrared
FWHM	full-width at half-maximum
GC	gas chromatography
GFC	gel filtration chromatography
GPT	General Purpose Timer
H	height equivalent of a theoretical plate
HECD	Hall electrolytic conductivity detector
HETP	height equivalent of a theoretical plate
HPLC	high-performance liquid chromatography
i	current
IC	integrated circuit
I.D.	inside diameter
I/O	input/output
IR	infrared
K	scale factor for 759N logarithmic amplifier
K-D	Kuderna-Danish
L	chromatographic column length
LC	liquid chromatography
LED	light emitting diode

LIF	laser induced fluorescence
LR	linear range
LOD	limit of detection
$Mass_{(analyte)}$	mass of the analyte
$Mass_{(LOD)}$	mass of the analyte at the limit of detection
MI	matrix isolation
MS	mass spectrometry or mass spectrometer
MUX	multiplexer
N.A.	numerical aperature
p-p	peak-to-peak
PID	photoionization detector
PMT	photomultiplier tube
PNA	Polynuclear Aromatic Compound
ppb	$\mu g\ L^{-1}$ or $g\ kg^{-1}$
ppm	$mg\ L^{-1}$ or $mg\ kg^{-1}$
pptr	$ng\ L^{-1}$ or $ng\ kg^{-1}$
PTFE	Teflon
Rd	reciprocal linear dispersion
S	sensitivity
S/N	signal-to-noise
S_2	second singlet state
t'_R	retention time minus the transit time of the mobile phase
%T	percent transmittance
TCD	thermal conductivity detector

TEA	thermal energy analyzer or nitric oxide selective pyrolysis/chemiluminescence detector
TED flame	thermionic emission detector or alkali ionization detector
TIMBUF	General Purpose Timer Control Status Register Buffer
TIMCSR	General Purpose Timer Control Status Register
U/D	Up/Down count control input
UV	ultraviolet
Vdc	volts-direct current
$w_{(1/2)}$	peak width at half height
WCOT	wall-coated open-tubular (capillary GC column)
α	aperture angle
2α	full aperature at half intensity
λ	wavelength

APPENDIX B

COMPUTER PROGRAMS

```
C      THIS FORTRAN PROGRAM CONTAINS THE MASTER MENU FOR
C      FOR SIMULTANEOUS U.V. ABS/FL G.C. DETECTION
C      DATA ACQUISITION ONLY
C
       INTEGER AARRAY(2,100),LOWL,HIGHL,NOWL,CHECKL,STEP,MOVE
      1,UD,ENDTIM,RATE,COUNT,SCAN,MA,MB,SA,SB,STORE(1650),MARKER
      2,BKSV,AVE(3,51),PTS
       REAL SCTIM,LONA,LONB,HINA,HINB,E,F,AV(3,51),ST(3,51,10),RR(1650)
       LOGICAL *1 CHOICE
       DIMENSION FNAME(4)
       DATA STORE/1650*0/,AVE/153*0/,AV/153*00.00/,ST/1650*00.00/
       DATA AARRAY/200*0/,MA/0/,MB/0/,SA/0/,SB/0/,LONA/0.0/,LONB/0.0/
      1,HINA/0.0/,HINB/0.0/,U/0.0/,Q/0.0/,W/0.0/,S/0.0/
C
C      DISPLAY TITLE
10     TYPE 100
C
C      PRINT MENU
20     TYPE 102
       ACCEPT 103,CHOICE
C
       IF (CHOICE.EQ.'A') GO TO 30
       IF (CHOICE.EQ.'B') GO TO 40
       IF (CHOICE.EQ.'Q') STOP
       GO TO 20
C
C      ACQUIRE NEW SPECTRAL DATA,SCANNING WAVELENGTH
C      INPUT PARAMETERS
30     TYPE 108
       TYPE 109
       ACCEPT *,LOWL
       TYPE 110
       ACCEPT *,HIGHL
       TYPE 111
       ACCEPT *,NOWL
       IF (LOWL-NOWL) 31,33,32
31     MOVE=NOWL-LOWL
       UD=0
       GO TO 34
32     MOVE=LOWL-NOWL
       UD=1
34     CALL SETUP(UD,MOVE)
33     STEP=HIGHL-LOWL+1
C      INPUT MONOCHROMATOR STEPPING RATE
       TYPE 121
       ACCEPT *,RATE
       COUNT=100/RATE
C
C
C      ACQUIRE AND STORE BACKGROUND SPECTRUM (X 10)
       TYPE 115
       ACCEPT 103,CHOICE
       IF (CHOICE.EQ.'N') GO TO 38
       IF (CHOICE.EQ.'Y') GO TO 37
       GO TO 65
37     MARKER=0
       SCAN=10
C      GET A FILE NAME
       TYPE 104
       ACCEPT 106,FNAME
       PTS=30*STEP
C
```

```
      DO 1100 I=1,51
      DO 1105 J=1,3
        AVE(J,I)=0
        AV(J,I)=0.0
1105  CONTINUE
1100  CONTINUE
      DO 1101 L=1,10
      DO 1102 K=1,STEP
      DO 1103 J=1,3
        ST(J,K,L)=0.0
1103  CONTINUE
1102  CONTINUE
1101  CONTINUE
      DO 1104 I=1,1650
        STORE(I)=0
        RR(I)=0.0
1104  CONTINUE
      CALL ADCSPE(COUNT,LOWL,STEP,SCAN,MARKER,BKSV,STORE,AVE,FNAME,PTS)
C
C     CALCULATE AVERAGE BKGND SPECTRA
      DO 1200 I=1,PTS
        RR(I)=FLOAT(STORE(I))
1200  CONTINUE
      M=1
      I=1
      DO 1210 L=1,10
        IF (M.EQ.1) GO TO 200
        IF (M.EQ.0) GO TO 205
C
200     DO 1220 K=1,STEP
        DO 1230 J=1,3
          ST(J,K,L)=RR(I)
          I=I+1
1230    CONTINUE
1220    CONTINUE
        M=0
        GO TO 1210
C
205     DO 1240 K=STEP,1,-1
        DO 1250 J=1,3
          ST(J,K,L)=RR(I)
          I=I+1
1250    CONTINUE
1240    CONTINUE
        M=1
        GO TO 1210
C
1210  CONTINUE
C
      DO 1260 L=1,10
      DO 1270 K=1,STEP
      DO 1280 J=1,3
        AV(J,K)=AV(J,K)+ST(J,K,L)
1280  CONTINUE
1270  CONTINUE
1260  CONTINUE
C
      DO 1290 K=1,STEP
      DO 1300 J=1,3
        AV(J,K)=AV(J,K)/10
        AVE(J,K)=IFIX(AV(J,K))
1300  CONTINUE
1290  CONTINUE
C
C     WRITE BACKGROUND SPECTRA AND AVERAGED BKGND TO DISC
      MARKER=1
      CALL ADCSPE(COUNT,LOWL,STEP,SCAN,MARKER,BKSV,STORE,AVE,FNAME,PTS)
C
```

```
C       WRITE BACKGROUND SPECTRA AND AVERAGED BKGND TO DISC
        MARKER=1
        CALL ADCSPE(COUNT,LOWL,STEP,SCAN,MARKER,BKSV,STORE,AVE,FNAME,PTS)
C
C       RETURN TO MENU?
        TYPE 123
        ACCEPT 103,CHOICE
        IF(CHOICE.EQ.'Y') GO TO 10
C
C       SET UP RUN TIME AND THEN WAIT FOR INJECTION
38      MARKER=2
        TYPE 114
        ACCEPT *,ENDTIM
        B=FLOAT(STEP)
        C=FLOAT(RATE)
        SCTIM=B/C
        D=FLOAT(ENDTIM)
        A=(D*60)/SCTIM
        SCAN=IFIX(A)
C       GET A FILE NAME
        TYPE 104
        ACCEPT 106,FNAME
        TYPE 112
        ACCEPT 103,CHOICE
        IF (CHOICE.EQ.'Q') GO TO 10
C
        CALL ADCSPE(COUNT,LOWL,STEP,SCAN,MARKER,BKSV,STORE,AVE,FNAME,PTS)
C
C       GO TO MENU
        GO TO 10
C
C       ACQUIRE NEW CHROMATOGRAPHIC DATA, FIXED WAVELENGTH
C       INPUT PARAMETERS
40      TYPE 108
        TYPE 111
        ACCEPT *,NOWL
        TYPE 113
        ACCEPT *,CHECKL
        IF (CHECKL-NOWL) 43,46,44
43      MOVE=NOWL-CHECKL
        UD=0
        GO TO 45
44      MOVE=CHECKL-NOWL
        UD=1
C
45      CALL SETUP(UD,MOVE)
C       INPUT DATA ACQUISITION RATE
46      TYPE 122
        ACCEPT *,RATE
        COUNT=100/RATE
        TYPE 124
C
C       CHECK BACKGROUND NOISE
        TYPE 117
        ACCEPT 103,CHOICE
        IF (CHOICE.EQ.'N') GO TO 48
        IF (CHOICE.EQ.'Y') GO TO 47
        GO TO 65
C
47      DO 900 I=1,100
        DO 901 J=1,2
          AARRAY(J,I)=0
901     CONTINUE
900     CONTINUE
        MA=0
        MB=0
        SA=0
        SB=0
```

```
      LONA=4096.0
      LONB=4096.0
      HINA=0.0
      HINB=0.0
      U=0.0
      Q=0.0
      W=0.0
      S=0.0
      CALL NOISE (COUNT,AARRAY)
C     CALCULATE MEAN, P-P NOISE AND STD. DEV
      DO 1000 J=1,100
      T=FLOAT(AARRAY(1,J))
      IF(T.GT.HINA) HINA=T
      IF(T.LE.LONA) LONA=T
      U=U+T
      P=FLOAT(AARRAY(2,J))
      IF(P.GT.HINB) HINB=P
      IF(P.LE.LONB) LONB=P
      Q=Q+P
1000  CONTINUE
      E=U/100
      MA=IFIX(E)
      F=Q/100
      MB=IFIX(F)
      DO 1001 J=1,100
      T=FLOAT(AARRAY(1,J))
      V=(T-MA)**2
      W=W+V
      P=FLOAT(AARRAY(2,J))
      R=(P-MB)**2
      S=S+R
1001  CONTINUE
      E=SQRT(W/99)
      SA=IFIX(E)
      F=SQRT(S/99)
      SB=IFIX(F)
      TYPE 116,CHECKL,MA,SA,LONA,HINA,MB,SB,LONB,HINB
C     RETURN TO MENU?
      TYPE 123
      ACCEPT 103,CHOICE
      IF(CHOICE.EQ.'Y') GO TO 10
C
C     SET UP RUN TIME AND WAIT FOR INJECTION
48    TYPE 114
      ACCEPT *,ENDTIM
      ENDTIM=ENDTIM*60*RATE
C     GET A FILE NAME
      TYPE 104
      ACCEPT 106,FNAME
      TYPE 112
      ACCEPT 103,CHOICE
      IF (CHOICE.EQ.'Q') GO TO 10
C
      CALL ADCHRO (COUNT,CHECKL,ENDTIM,FNAME,MA,SA,MB,SB,AARRAY)
C
C     GO TO MENU
      GO TO 10
C
C     ESCAPE FOR ERRORS
65    TYPE 107
C     GO TO MENU
      GO TO 10
C
```

```
C     FORMAT STATEMENTS
C
100   FORMAT('1SIMULTANEOUS ULTRAVIOLET ABSORBANCE/FLUORESCENCE'
     1,/,' DETECTION FOR GAS CHROMATOGRAPHY',//)
102   FORMAT (' SELECT MODE BY CHOOSING APPROPRIATE CHARACTER:',/
     1,/,' A=ACQUIRE NEW SPECTRAL DATA, SCANNING WAVELENGTH'
     2,/,' B=ACQUIRE NEW CHROMATOGRAPHIC DATA, FIXED WAVELENGTH'
     3,/,' Q=QUIT',//)
103   FORMAT (A1)
104   FORMAT ('0BE SURE THAT A NEW FORMATTED DISC IS IN RIGHT DRIVE'
     1,/,' THEN ENTER THE I/O FILE--EXT CODES:'
     2,/,' BKG=BACKGROUND SPECTRUM'
     3,/,' SPE=SPECTRAL DATA'
     4,/,' CHR=CHROMATOGRAPHIC DATA, FIXED WAVELENGTH'
     5,/,' (DK:FILENM.EXT=) MUST HAVE =!',$)
105   FORMAT ('0ENTER THE INPUT FILE NAME (DK:FILENM.EXT)',$)
106   FORMAT (4A4)
107   FORMAT ('0ERROR IN INPUT')
108   FORMAT ('0CHECK SOURCE, BOTH PMTS, STEPPER MOTOR, GC--'
     1,/,' ARE THEY ALL READY FOR A RUN?'
     2,/,'      ABSORBANCE ON CHANNEL 0; FLUORESCENCE ON CHANNEL 1;'
     3,/,'      WAVELENGTH POSITION ON CHANNEL 2; U/D ON MODE 2;'
     4,/,'      CLOCK PULSE ON PULSE OUT;'
     5,/,'      ABS. SCOPE ON X-DAC; FL. SCOPE ON Y-DAC'//)
109   FORMAT ('0LOW SCANNING WAVELENGTH IN NM? ',$)
110   FORMAT ('0HIGH SCANNING WAVELENGTH IN NM? (MAX. SCAN 50 NM) ',$)
111   FORMAT ('0INPUT CURRENT WAVELENGTH IN NM ',$)
112   FORMAT ('0HIT RETURN AT INJECT--OR "Q" TO QUIT')
113   FORMAT ('0INPUT WAVELENGTH IN NM FOR SOURCE RADIATION ',$)
114   FORMAT ('0INPUT RUN TIME, IN MINUTES ',$)
115   FORMAT ('0MEASURE BACKGROUND SPECTRUM? (Y OR N) '$)
116   FORMAT ('1BACKGROUND NOISE AT',I4,' NM (0-4096 FOR 0-10 V):'
     1,/,'          ABSORBANCE:      MEAN=',I8,
     2,/,'                           STANDARD DEVIATION=',I8,
     3,/,'                           RANGE=',F16.2,' TO',F16.2,
     4,/,'          FLUORESCENCE:    MEAN=',I8,
     5,/,'                           STANDARD DEVIATION=',I8,
     6,/,'                           RANGE=',F16.2,' TO',F16.2)
117   FORMAT ('0MEASURE BACKGROUND NOISE? (Y OR N) '$)
118   FORMAT ('0TRANSMITTANCE NOISE'/)
119   FORMAT ('0FLUORESCENCE NOISE'/)
121   FORMAT ('0MONOCHROMATOR STEPPING RATE IN HZ?(MAX 100 HZ)= '$)
122   FORMAT ('0DATA ACQUISITION RATE IN HZ?(MAX 100 HZ)= '$)
123   FORMAT ('0RETURN TO MENU?(Y OR N) '$)
124   FORMAT ('0DISCONNECT STEPPER MOTOR FROM CLOCK PULSE')
C
      END
```

```
.TITLE SETUP
;
;PROGRAM TO MOVE MONOCHROMATOR TO DESIRED WAVELENGTH
;
CSR=170400
BUF=CSR+2
TIMCSR=170420
TIMBUF=TIMCSR+2
;
SETUP:: TST     (R5)+           ;IGNORE # ARGUMENTS
        MOV     @(R5)+,R0       ;MOVE UP/DOWN TO R0
        MOV     @(R5)+,R1       ;MOVE # STEPS TO R1
        CMP     #0,R0           ;IS UP/DOWN 0?
        BNE     THERE           ;IF NOT, JUMP TO THERE
        MOV     #030000,R4      ;DOWN CODE TO R4
        BR      PULSE
THERE:  MOV     #010000,R4      ;UP CODE TO R4
PULSE:  MOV     R4,@#BUF        ;UP/DOWN CODE TO SET MODE 2
        MOV     #2.,@#TIMBUF    ;SET COUNT TO 2 (50 HZ)
        MOV     #656,@#TIMCSR   ;SET 100 HZ RATE
        BISB    #1,@#TIMCSR     ;START CLOCK
LOOK:   TSTB    @#TIMCSR        ;COUNT DONE?
        BPL     LOOK            ;IF NOT, WAIT
        BISB    #200,@#TIMCSR   ;CLEAR DONE BIT
        DEC     R1              ;DECREMENT STEP COUNT
        BNE     LOOK
        MOV     #0,@#TIMCSR     ;STOP THE CLOCK
;
        RETURN
;
        .END    SETUP
```

```
.TITLE ADCSPE

        .MCALL  .CSIGEN,.CLOSE,.WRITW,.PRINT,.GTLIN
;
;PROGRAM TO PROVIDE PULSES FOR STEPPING MONOCHROMATOR,
;       ADC OF ABS. AND FL. AND WAVELENGTH POSITION,
;       AND WRITE DATA TO DISC

CSR=170400
BUF=CSR+2
TIMCSR=170420
TIMBUF=TIMCSR+2

ADCSPE::TST     (R5)+                   ;IGNORE # ARGUMENTS
        MOV     @(R5)+,@#COUNT          ;MOVE CLOCK COUNT TO MEMORY
        MOV     @(R5)+,@#LOWL           ;MOVE LOW WAVELENGTH TO MEMORY
        MOV     @(R5)+,@#STEP           ;MOVE # STEPS/SCAN TO MEMORY
        MOV     @(R5)+,@#SCAN           ;MOVE # SCANS TO MEMORY
        MOV     @(R5)+,@#MARKER         ;MOVE FLAG TO MEMORY
        MOV     @(R5)+,@#BLKSAV         ;MOVE TOTAL # BLKS TO MEMORY
        MOV     (R5)+,@#STORE           ;MOVE ARRAY FOR BKGND TO MEMORY
        MOV     (R5)+,@#AVE             ;MOVE AVERAGED BKGND ADDRESS TO MEM.
        MOV     (R5)+,@#FNAME           ;MOVE FILENAME ADDRESS TO MEMORY
        MOV     @(R5)+,@#PTS            ;MOVE # PTS IN BKGND TO MEMORY
;
        CMP     #1,@#MARKER             ;IS FLAG 1?
        BNE     4$                      ;IF NOT, CONTINUE
        JMP     LABEL                   ;STORE AVERAGED BKGND ONLY
4$:     .CSIGEN #DSPAC,#DEXT,@#FNAME    ;GET CSI TO OPEN AN OUTPUT
                                        ;CHANNEL FOR THE FILENAME
        MOV     #BUFFR,R2               ;STARTING ADDRESS FOR TABLE IN R2
        MOV     #256.,R1                ;COUNT FOR FULL BLOCK
        MOV     @#SCAN,R4               ;MOVE # SCANS TO R4
        MOV     @#STORE,R0              ;BKGND ARRAY ADDRESS AT R0
        CLR     IOBLK
        INC     IOBLK                   ;GO TO THIRD BLOCK
        INC     IOBLK
        MOV     #2,@#BLKSAV             ;COUNT THE LABEL BLOCK
        MOV     @#COUNT,@#TIMBUF        ;SET COUNT FOR CLOCK
        MOV     #656,@#TIMCSR           ;SET 100 HZ CLOCK RATE
        BISB    #1,@#TIMCSR             ;START CLOCK

HERE:   MOV     @#STEP,R3               ;MOVE # WAVELENGTH STEPS TO R3
REPEAT: JSR     PC,SUBRT                ;JUMP TO CONVERSION/WRITE SUBROUTINE
        MOV     #010000,@#BUF           ;UP CODE TO MOD 2
LOOK:   TSTB    @#TIMCSR                ;COUNT DONE?
        BPL     LOOK                    ;IF NOT, WAIT
        BISB    #200,@#TIMCSR           ;CLEAR DONE BIT (RE-START CLOCK)
        DEC     R3                      ;DECREMENT STEP COUNTER
        BNE     REPEAT                  ;CONTINUE UPWARD SCAN
        DEC     R4                      ;DECREMENT SCAN COUNTER
        BNE     THERE                   ;ON TO NEXT SCAN
        CMP     #0,R1                   ;AT END OF BLOCK?
        BEQ     18$                     ;IF SO, GO TO 18$
17$:    MOV     #0,(R2)+                ;FILL REST OF BLK WITH 0
        DEC     R1                      ;DECREMENT WORD COUNT
        BNE     17$                     ;
18$:    JSR     PC,WDISC                ;WRITE LAST BLOCK TO DISC
        MOV     #0,@#TIMCSR             ;STOP CLOCK
        CMP     #2,@#MARKER             ;CHECK FLAG
        BEQ     20$                     ;IF SAMPLE RUN, CONT. WITH LABEL
        JMP     FINISH                  ;IF BKGND, RETURN TO FORTRAN
20$:    JMP     LLABEL                  ;PROG. TOO LONG TO USE BR INST.HERE
```

```
THERE:  MOV     @#STEP,R3       ;MOVE # WAVELENGTH STEPS TO R3
OVER:   JSR     PC,SUBRT        ;JUMP TO CONVERSION WRITE SUBROUTINE
        MOV     #030000,@#BUF   ;DOWN CODE TO MOD 2
LLOOK:  TSTB    @#TIMCSR        ;COUNT DONE?
        BPL     LLOOK           ;IF NOT, WAIT
        BISB    #200,@#TIMCSR   ;CLEAR DONE BIT (RE-START CLOCK)
        DEC     R3              ;DECREMENT STEP COUNTER
        BNE     OVER            ;CONTINUE DOWNWARD SCAN
        DEC     R4              ;DECREMENT SCAN COUNTER
        BNE     HERE            ;ON TO NEXT SCAN
        CMP     #0,R1           ;AT END OF BLOCK?
        BEQ     15$             ;IF SO, GO TO 15$
16$:    MOV     #0,(R2)+        ;FILL REST OF BLK WITH 0
        DEC     R1              ;DECREMENT WORD COUNT
        BNE     16$             ;
15$:    JSR     PC,WDISC        ;WRITE LAST BLOCK TO DISC
        MOV     #0,@#TIMCSR     ;STOP CLOCK
        CMP     #2,@#MARKER     ;CHECK FLAG
        BEQ     21$             ;IF SAMPLE RUN, CONT. WITH LABEL
        JMP     FINISH          ;IF BKGND, RETURN TO FORTRAN
21$:    JMP     LLABEL          ;PROG. TOO LONG TO USE BR INST.HERE

SUBRT:  MOV     #0,@#CSR        ;START CONVERSION, CH 0, ABS
TEST:   TSTB    @#CSR           ;DONE YET?
        BPL     TEST            ;IF NOT, WAIT
        MOV     @#BUF,(R2)      ;PUT DATA INTO TABLE
        BIC     #170000,(R2)    ;CLEAR BITS 12-15 OF DATA
        CMP     #0,@#MARKER     ;ACQUIRING BKGND SPECTRA?
        BNE     5$              ;IF NOT, CONTINUE
        MOV     (R2),(R0)+      ;PUT VALUE IN BKGND ARRAY
5$:     MOV     (R2)+,@#BUF     ;WRITE VALUE TO X-DAC
        DEC     R1              ;DECREMENT SPACE IN BLOCK
        BNE     CONT            ;IF NOT FULL, GET MORE DATA
        MOV     #0,@#TIMCSR     ;STOP CLOCK
        JSR     PC,WDISC        ;GO TO DISC WRITING SUBROUTINE
        MOV     @#COUNT,@#TIMBUF ;SET COUNT FOR CLOCK
        MOV     #656,@#TIMCSR   ;SET 100 HZ CLOCK RATE
        BISB    #1,@#TIMCSR     ;START CLOCK
CONT:   MOV     #400,@#CSR      ;START CONVERSION, CH 1, FL
TTEST:  TSTB    @#CSR           ;DONE YET?
        BPL     TTEST           ;IF NOT, WAIT
        MOV     @#BUF,(R2)      ;PUT DATA INTO TABLE
        BIC     #170000,(R2)    ;CLEAR BITS 12-15 OF DATA
        CMP     #0,@#MARKER     ;ACQUIRING BKGND SPECTRA?
        BNE     6$              ;IF NOT, CONTINUE
        MOV     (R2),(R0)+      ;PUT VALUE IN BKGND ARRAY
6$:     BIS     #100000,(R2)    ;SET BIT 15 OF DATA
        MOV     (R2),@#BUF      ;WRITE VALUE TO Y-DAC
        BIC     #100000,(R2)+   ;CLEAR BIT 15
        DEC     R1              ;DECREMENT SPACE IN BLOCK
        BNE     CONTT           ;IF NOT FULL, GET MORE DATA
        MOV     #0,@#TIMCSR     ;STOP CLOCK
        JSR     PC,WDISC        ;GO TO DISC WRITING SUBROUTINE
        MOV     @#COUNT,@#TIMBUF ;SET COUNT FOR CLOCK
        MOV     #656,@#TIMCSR   ;SET 100 HZ CLOCK RATE
        BISB    #1,@#TIMCSR     ;START CLOCK
CONTT:  MOVB    #2,@#CSR+1      ;START CONVERSION, CH 2, WAVELENGTH
TESTT:  TSTB    @#CSR           ;DONE YET?
        BPL     TESTT           ;IF NOT, WAIT
        MOV     @#BUF,(R2)      ;PUT DATA INTO TABLE
        BIC     #170000,(R2)    ;CLEAR BITS 12-15 OF DATA
        CMP     #0,@#MARKER     ;ACQUIRING BKGND SPECTRA?
        BNE     7$              ;IF NOT, CONTINUE
        MOV     (R2),(R0)+      ;PUT VALUE IN BKGND ARRAY
7$:     BIC     #100000,(R2)+   ;DUMMY CLR TO INC R2 AFTER BNE
        DEC     R1              ;DECREMENT SPACE IN BLOCK
        BNE     CCONT           ;IF NOT FULL, CONTINUE
        MOV     #0,@#TIMCSR     ;STOP CLOCK
        JSR     PC,WDISC        ;GO TO DISC WRITING SUBROUTINE
        MOV     @#COUNT,@#TIMBUF ;SET COUNT FOR CLOCK
        MOV     #656,@#TIMCSR   ;SET 100 HZ CLOCK RATE
        BISB    #1,@#TIMCSR
CCONT:  RTS     PC
```

```
WDISC:  CMP     #0,@#MARKER             ;IS THIS A BKGND RUN?
        BEQ     19$                     ;IF SO, DON'T WRITE YET
        MOV     #LIST,R5                ;POINT R5 TO ARGUMENT BLOCK AREA
        .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC
19$:    INC     IOBLK                   ;GO TO NEXT BLOCK
        INC     @#BLKSAV                ;INCREMENT THE BLOCK COUNT
        MOV     #256.,R1                ;RE-LOAD COUNT FOR FULL BLOCK
        MOV     #BUFFR,R2               ;GO TO START OF TABLE
        RTS     PC
;
;ADD SAMPLE LABELING INFORMATION TO INITIAL BLOCK OF FILE (6 LINES)
;(BLOCK 0 IS ALL ASCII)
LABEL:  .CSIGEN #DSPAC,#DEXT,@#FNAME    ;GET CSI TO OPEN AN OUTPUT
                                        ;CHANNEL FOR THE FILENAME
        CLR     IOBLK                   ;START AT FIRST BLOCK
        INC     IOBLK                   ;INCREMENT BLK #
        INC     IOBLK                   ;INCREMENT BLK #
        MOV     #2,@#BLKSAV             ;COUNT FIRST TWO BLOCKS
;NOW STORE THE BACKGROUND DATA
;
        MOV     #BUFFR,R2               ;GO TO START OF TABLE
        MOV     #256.,R1                ;MOVE WORD COUNT TO R1
        MOV     @#STORE,R4              ;MOVE BACKGND DATA ARRAY ADDRESS TO R4
        MOV     @#PTS,R3                ;MOVE PTS COUNT TO R3
11$:    MOV     (R4)+,(R2)+             ;PUT DATA INTO BUFFER
        DEC     R3                      ;DECREMENT POINTS COUNT
        BEQ     12$                     ;IF ALL PTS STORED, GO TO 12$
        DEC     R1                      ;DECREMENT WORD COUNT
        BNE     11$                     ;CONT. UNTIL END OF BLK
        JSR     PC,WDISC                ;WRITE BLK OF DATA TO DISC
        BR      11$                     ;CONT STORING DATA
12$:    DEC     R1                      ;DECREMENT WORD COUNT
        BNE     13$                     ;CONT. IF NOT END OF BLOCK
        JSR     PC,WDISC                ;WRITE BLK OF DATA TO DISC
        BR      LLABEL
13$:    MOV     #0,(R2)+                ;FILL LAST BLOCK WITH 0
        DEC     R1                      ;DECREMENT WORD COUNT
        BNE     13$
        MOV     #LIST,R5                ;WRITE LAST BLK TO DISC
        .WRITW  R5,#0                   ;
        INC     @#BLKSAV                ;AND COUNT IT
;
LLABEL: CLR     IOBLK                   ;GO TO FIRST BLOCK
        MOV     #512.,R1                ;BYTE COUNT FOR FULL BLOCK
        MOV     #BUFFR,R2               ;GO TO START OF TABLE
        .GTLIN  #LINBUF,#MSG1           ;LINE 1
        JSR     PC, ADDBUF              ;PUT IN OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 2
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 3
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 4
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 5
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 6
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
ZERO:   MOVB    #0,(R2)+                ;FILL REST OF FIRST BLOCK WITH 0
        DEC     R1                      ;DECREMENT COUNTER
        BNE     ZERO                    ;CONTINUE UNTIL BLOCK FULL
        MOV     #LIST,R5                ;POINT R5 TO ARGUMENT BLOCK AREA
        .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC
        BR      INFO

ADDBUF: MOV     #LINBUF,R0              ;SET R0 TO BEGINNING OF THE INPUT LINE
1$:     TSTB    (R0)                    ;END OF LINE?
        BEQ     2$                      ;IF SO, ADD <CR> AND <LF>
        MOVB    (R0)+,(R2)+             ;PUT CHARACTER IN OUTPUT BUFFER
        DEC     R1                      ;DECREMENT BLOCK SPACE COUNTER
        BEQ     FULL                    ;BLOCK FULL?
        BR      1$                      ;GO GET NEXT CHARACTER
2$:     MOVB    #15,(R2)+               ;ADD THE <CR>
```

```
          DEC     R1                      ;COUNT IT
          BEQ     FULL                    ;BLOCK FULL?
          MOVB    #12,(R2)+               ;ADD THE <LF>
          DEC     R1                      ;COUNT IT
          BEQ     FULL                    ;BLOCK FULL?
          BR      OUT
FULL:     .PRINT  #MSG2                   ;PRINT MESSAGE ABOUT FULL BLOCK
OUT:      RTS     PC                      ;RETURN BACK TO CALLING PROGRAM
;
;STORE INFO ABOUT FILE SIZE, SAMPLING RATE,AND BACKGROUND SPECTRA
;(BLOCK 1, BINARY; DATA STARTS IN BLOCK 2 (THIRD BLOCK))
INFO:     INC     IOBLK                   ;GO TO FIRST BLOCK
          MOV     #BUFFR,R2               ;GO TO START OF TABLE
          MOV     #256.,R1                ;MOVE WORD COUNT TO R1
          MOV     @#BLKSAV,(R2)+          ;SAVE TOTAL # BLOCKS IN FILE
          DEC     R1                      ;DECREMENT WORD COUNT
          MOV     @#COUNT,(R2)+           ;SAVE CLOCK COUNT IN BUFFER
          DEC     R1                      ;DECREMENT WORD COUNT
          MOV     @#LOWL,(R2)+            ;SAVE LOW WAVELENGTH IN FILE
          DEC     R1                      ;DECREMENT WORD COUNT
          MOV     @#STEP,(R2)+            ;SAVE # STEPS IN A SCAN
          DEC     R1                      ;DECREMENT WORD COUNT
          MOV     @#SCAN,(R2)+            ;SAVE TOTAL # SCANS
          MOV     @#AVE,R0                ;PT R0 TO AVERAGED BKGND ARRAY
          MOV     @#STEP,R3               ;R3 FOR STEP COUNT
          MOV     #3,R4                   ;R4 FOR CHANNEL COUNT
8$:       MOV     (R0)+,(R2)+             ;BKGND. ARRAY INTO BUFFER
          DEC     R1                      ;DECREMENT WORD COUNT
          BEQ     9$                      ;GO TO WRITE, IF BLK FULL
          DEC     R3                      ;DECREMENT STEP COUNT
          BNE     8$                      ;CONT THRU SCAN
          MOV     @#STEP,R3               ;RE-LOAD STEP COUNT
          DEC     R4                      ;DECREMENT CHANNEL COUNT
          BNE     8$                      ;CONT UNTIL DONE
3$:       MOV     #0,(R2)+                ;ZERO REST OF BLOCK
          DEC     R1                      ;DECREMENT COUNTER
          BNE     3$                      ;END OF BLOCK?
9$:       MOV     #LIST,R5                ;PT. R5 TO ARGUMENT BLK AREA
          .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC, CH 0
          BR      FINISH
;
FINISH:   BCC     DONE                    ;IF CARRY FLAG SET, THERE WAS AN ERROR
          .PRINT  #WRERR                  ;PRINT ERROR MESSAGE IF SET
DONE:     .EVEN
          .CLOSE  #0                      ;CLOSE OUTPUT CHANNEL SELECTED
          RETURN

COUNT:    .BLKW   1
LOWL:     .BLKW   1
STEP:     .BLKW   1
SCAN:     .BLKW   1
BLKSAV:   .BLKW   1
MARKER:   .BLKW   1
STORE:    .BLKW   1650.
AVE:      .BLKW   1
FNAME:    .BLKW   10
PTS:      .BLKW   1
LINBUF:   .BLKW   40.
DEXT:     .WORD   0,0,0,0               ;NO DEFAULT EXTENSIONS FOR FILES
LIST:     .WORD   0                     ;THIS IS THE AREA TO USE FOR THE
                                        ;FILE WRITING PARAMETERS
IOBLK:    .WORD   0                     ;BLOCK NUMBER
          .WORD   BUFFR                 ;I/O BUFFER ADDRESS
          .WORD   256.                  ;WORD COUNT
          .WORD   0
BUFFR:    .BLKW   256.                  ;DATA GOES HERE
WRERR:    .ASCIZ  /DISC WRITING ERROR/  ;ERROR MESSAGE, IF NEEDED
MSG1:     .ASCIZ  /ENTER SAMPLE INFO, 6 LINES (OR <CR>)/
MSG2:     .ASCIZ  /LABELING BLOCK FULL/
          .EVEN
DSPAC:.                                 ;SAFE TO PUT HANDLER HERE AT END
          .END    ADCSPE
```

```
.TITLE NOISE
        .MCALL  .CSIGEN,.CLOSE,.WRITW,.PRINT
;PROGRAM TO FIND MEAN, STD. DEVIATION, AND P-P NOISE
;IN BASELINE AT PRE-SELECTED WAVELENGTH
;
CSR=17[illegible]20                     ;ADC CONTROL STATUS REGISTER
BUF=CSR+2                               ;ADC DATA BUFFER
TIMCSR=170420                           ;TIMER CONTROL STATUS REGISTER
TIMBUF= TIMCSR+2                        ;TIMER BUFFER

NOISE:: TST     (R5)+                   ;IGNORE # ARGUMENTS
        MOV     @(R5)+,@#COUNT          ;MOVE CLOCK COUNT TO MEMORY
        MOV     (R5)+,@#AARRAY          ;MOVE ADDRESS OF AARRAY TO MEMORY
        MOV     @#AARRAY,R2             ;MOVE ARRAY ADDRESS TO R2
;
;ADC 100 NOISE POINTS, BOTH CHANNELS
        MOV     #100.,R1                ;SET R1 FOR COUNTER
        MOV     @#COUNT,@#TIMBUF        ;LOAD COUNT INTO COUNT SET REG.
        MOV     #643,@#TIMCSR           ;INITIATE COUNTING
POLL:   TSTB    @#TIMCSR                ;IS COUNT DONE?
        BPL     POLL                    ;IF NOT, WAIT
        BISB    #200,@#TIMCSR           ;CLEAR DONE BIT
        MOV     #0,@#CSR                ;START A CONVERSION, MUX 0
TEST:   TSTB    @#CSR                   ;DONE YET?
        BPL     TEST                    ;IF NOT,POLL
        MOV     @#BUF,(R2)              ;PUT DATA INTO TABLE
        BIC     #170000,(R2)+           ;CLEAR HIGHEST 4 BITS DATA
        MOV     #400,@#CSR              ;START A CONVERSION, MUX 1
WAIT:   TSTB    @#CSR                   ;DONE YET?
        BPL     WAIT                    ;IF NOT,POLL
        MOV     @#BUF,(R2)              ;PUT DATA INTO TABLE
        BIC     #170000,(R2)+           ;CLEAR HIGHEST 4 BITS OF DATA
        DEC     R1                      ;DECREMENT COUNTER
        BNE     POLL                    ;IF NOT, GET MORE DATA
        MOV     #0,@#TIMCSR             ;STOP THE CLOCK
        RETURN


COUNT:  .BLKW   1
AARRAY: .BLKW   1

        .END    NOISE
```

```
.TITLE ADCHRO

        .MCALL   .CSIGEN,.CLOSE,.WRITW,.PRINT,.GTLIN,.READW
;
;PROGRAM TO TIME DATA ACQUISTION,
;       ADC OF ABS. AND FL. AND WAVELENGTH POSITION,
;       AND WRITE DATA TO DISC

CSR=170400
BUF=CSR+2
TIMCSR=170420
TIMBUF=TIMCSR+2

ADCHRO::TST     (R5)+                   ;IGNORE # ARGUMENTS
        MOV     @(R5)+,@#COUNT          ;MOVE CLOCK COUNT TO MEMORY
        MOV     @(R5)+,@#CHECKL         ;MOVE WAVELENGTH TO MEMORY
        MOV     @(R5)+,@#ENDTIM         ;MOVE # POINTS TO MEMORY
        MOV     (R5)+,@#FNAME           ;MOVE FILENAME ADDRESS TO MEMORY
        MOV     @(R5)+,@#MA             ;STORE ADDRESS ABS. NOISE MEAN
        MOV     @(R5)+,@#SA             ;STORE ADDRESS ABS. NOISE STD. DEV.
        MOV     @(R5)+,@#MB             ;STORE ADDRESS FL. NOISE MEAN
        MOV     @(R5)+,@#SB             ;STORE ADDRESS FL. NOISE STD. DEV.
        MOV     (R5)+,@#AARRAY          ;STORE NOISE ARRAY ADDRESS

        .CSIGEN #DSPAC,#DEXT,@#FNAME    ;GET CSI TO OPEN AN OUTPUT
                                        ;CHANNEL FOR THE FILENAME
        MOV     #BUFFR,R2               ;STARTING ADDRESS FOR TABLE IN R2
        MOV     #256.,R1                ;COUNT FOR FULL BLOCK
        MOV     @#ENDTIM,R4             ;MOVE # POINTS TO R4
        MOV     #2,@#BLKSAV             ;COUNT LABEL & BKGND NOISE BLOCKS
        MOV     @#COUNT,@#TIMBUF        ;SET COUNT FOR CLOCK
        MOV     #656,@#TIMCSR           ;SET 100 HZ CLOCK RATE
        BISB    #1,@#TIMCSR             ;START CLOCK
        MOV     #1000,@#CSR             ;START CONVERSION, CH 2, WAVELENGTH
TESTT:  TSTB    @#CSR                   ;DONE YET?
        BPL     TESTT                   ;IF NOT, WAIT
        MOV     @#BUF,@#WAVEL           ;PUT WAVELENGTH VOLTAGE INTO STORAGE
        BIC     #170000,@#WAVEL         ;CLEAR HIGHEST 4 BITS OF DATA

REPEAT: JSR     PC,SUBRT                ;JUMP TO CONVERSION/WRITE SUBROUTINE
LOOK:   TSTB    @#TIMCSR                ;COUNT DONE?
        BPL     LOOK                    ;IF NOT, WAIT
        BISB    #200,@#TIMCSR           ;CLEAR DONE BIT (RE-START CLOCK)
        DEC     R4                      ;DECREMENT # POINTS COUNTER
        BNE     REPEAT                  ;LAST POINT YET?
        CMP     #0,R1                   ;END OF LAST BLOCK?
        BEQ     4$                      ;IF SO, WRITE TO DISC
5$:     MOV     #0,(R2)+                ;ELSE FILL BLOCK WITH 0
        DEC     R1                      ;DECREMENT WORD COUNT
        BNE     5$                      ;CONTINUE UNTIL DONE
4$:     JSR     PC,WDISC                ;WRITE PARTIAL BLOCK TO DISC
        INC     @#BLKSAV                ;INCREMENT BLK COUNT
        BR      LABEL

SUBRT:  MOV     #0,@#CSR                ;START CONVERSION, CH 0, ABS
TEST:   TSTB    @#CSR                   ;DONE YET?
        BPL     TEST                    ;IF NOT, WAIT
        MOV     @#BUF,(R2)              ;PUT DATA INTO TABLE
        BIC     #170000,(R2)            ;CLEAR HIGHEST 4 BITS OF DATA
        MOV     (R2)+,@#BUF             ;WRITE VALUE TO X-DAC
        DEC     R1                      ;DECREMENT SPACE IN BLOCK
        BNE     CONT                    ;IF NOT FULL, GET MORE DATA
        JSR     PC,WDISC                ;GO TO DISC WRITING SUBROUTINE
CONT:   MOV     #400,@#CSR              ;START CONVERSION, CH 1, FL
```

```
TTEST:  TSTB    @#CSR                   ;DONE YET?
        BPL     TTEST                   ;IF NOT, WAIT
        MOV     @#BUF,(R2)              ;PUT DATA INTO TABLE
        BIC     #070000,(R2)            ;CLEAR BITS 12-14 OF DATA
        BIS     #100000,(R2)            ;SET BIT 15 OF DATA
        MOV     (R2),@#BUF              ;WRITE VALUE TO Y-DAC
        BIC     #100000,(R2)+           ;CLEAR BIT 15
        DEC     R1                      ;DECREMENT SPACE IN BLOCK
        BNE     CCONT                   ;IF NOT FULL, CONTINUE
        JSR     PC,WDISC                ;GO TO DISC WRITING SUBROUTINE
CCONT:  RTS     PC                      ;RETURN BACK TO CALLING PROGRAM

WDISC:  MOV     #LIST,R5                ;POINT R5 TO ARGUMENT BLOCK AREA
        .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC
        INC     IOBLK                   ;GO TO NEXT BLOCK
        INC     @#BLKSAV                ;INCREMENT BLOCK COUNT
        MOV     #256.,R1                ;RE-LOAD COUNT FOR FULL BLOCK
        MOV     #BUFFR,R2               ;GO TO START OF TABLE
        RTS     PC
;
;ADD SAMPLE LABELING INFORMATION TO INITIAL BLOCK OF FILE (6 LINES)
;(BLOCK 0 IS ALL IN ASCII)
LABEL:  MOV     #0,IOBLK                ;GO TO FIRST BLOCK
        MOV     #512.,R1                ;BYTE COUNT FOR FULL BLOCK
        MOV     #BUFFR,R2               ;GO TO START OF TABLE
        .GTLIN  #LINBUF,#MSG1           ;LINE 1
        JSR     PC,ADDBUF               ;PUT IN OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 2
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 3
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 4
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 5
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
        .GTLIN  #LINBUF                 ;LINE 6
        JSR     PC,ADDBUF               ;ADD TO OUTPUT BUFFER
ZERO:   MOVB    #0,(R2)+                ;FILL REST OF BLOCK WITH 0
        DEC     R1                      ;DECREMENT COUNTER
        BNE     ZERO                    ;CONTINUE UNTIL BLOCK FULL
        MOV     #LIST,R5                ;POINT R5 TO ARGUMENT BLOCK AREA
        .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC, CHANNEL 0
        INC     IOBLK                   ;GO TO NEXT BLOCK
        BR      STORE

ADDBUF: MOV     #LINBUF,R0              ;SET R0 TO BEGINNING OF THE INPUT LINE
1$:     TSTB    (R0)                    ;END OF LINE?
        BEQ     2$                      ;IF SO, ADD <CR> AND <LF>
        MOVB    (R0)+,(R2)+             ;PUT CHARACTER IN OUTPUT BUFFER
        DEC     R1                      ;DECREMENT SPACE COUNTER
        BEQ     FULL                    ;BLOCK FULL?
        BR      1$                      ;GO GET NEXT CHARACTER
2$:     MOVB    #15,(R2)+               ;ADD THE <CR>
        DEC     R1                      ;COUNT IT
        BEQ     FULL                    ;BLOCK FULL?
        MOVB    #12,(R2)+               ;ADD THE <LF>
        DEC     R1                      ;COUNT IT
        BEQ     FULL                    ;BLOCK FULL?
        BR      OUT
FULL:   .PRINT  #MSG2                   ;PRINT MESSAGE ABOUT FULL BLOCK
OUT:    RTS     PC                      ;RETURN BACK TO CALLING PROGRAM
;
```

```
;STORE INFO ABOUT FILE SIZE, SAMPLING RATE, AND BACKGROUND NOISE,BINARY
;(DATA STARTS IN THIRD BLOCK (BLOCK 2), BINARY)
STORE:  MOV     #BUFFR,R2               ;GO TO START OF TABLE
        MOV     @#BLKSAV,(R2)+          ;PUT TOTAL # BLOCKS IN OUTPUT BUFFER
        MOV     @#COUNT,(R2)+           ;CLOCK COUNT IN BUFFER
        MOV     @#CHECKL,(R2)+          ;WAVELENGTH IN BUFFER
        MOV     @#WAVEL,(R2)+           ;WAVELENGTH VOLTAGE IN BUFFER
        MOV     @#MA,(R2)+              ;ABS. MEAN IN BUFFER
        MOV     @#SA,(R2)+              ;ABS. STD. DEV. IN BUFFER
        MOV     @#MB,(R2)+              ;FL. MEAN IN BUFFER
        MOV     @#SB,(R2)+              ;FL. STD. DEV. IN BUFFER
        MOV     @#ENDTIM,(R2)+          ;#DATA POINTS IN BUFFER
        MOV     @#AARRAY,R0             ;MOVE NOISE ARRAY ADDRESS TO R0
        MOV     #200.,R1                ;MOVE ARRAY COUNT TO R1
SHIFT:  MOV     (R0)+,(R2)+             ;MOVE CONTENTS OF ARRAY TO BUFFER
        DEC     R1                      ;DECREMENT R1
        BNE     SHIFT                   ;CONTINUE TO END OF ARRAY
        MOV     #47.,R1                 ;COUNT FOR REST OF BLOCK
3$:     MOV     #0,(R2)+                ;ZERO REST OF BLOCK
        DEC     R1                      ;DECREMENT COUNTER
        BNE     3$                      ;END OF BLOCK?
        MOV     #LIST,R5                ;PT. R5 TO ARGUMENT BLK AREA
        .WRITW  R5,#0                   ;WRITE FULL BLOCK TO DISC, CH 0
        BR      FINISH

FINISH: BCC     DONE                    ;IF CARRY FLAG SET, THERE WAS AN ERROR
        MOV     #WRERR,R0               ;IF ERROR, POINT R0 TO MESSAGE
        .PRINT                          ;PRINT MESSAGE R0 POINTS TO
        CLR     R0                      ;CLEAR R0
DONE:   .EVEN
        MOV     #0,@#TIMCSR             ;STOP CLOCK
        .CLOSE  #0                      ;CLOSE OUTPUT CHANNEL SELECTED
        RETURN

COUNT:  .BLKW   1
CHECKL: .BLKW   1
ENDTIM: .BLKW   1
BLKSAV: .BLKW   1
WAVEL:  .BLKW   1
MA:     .BLKW   1
SA:     .BLKW   2
MB:     .BLKW   2
SB:     .BLKW   2
AARRAY: .BLKW   1
FNAME:  .BLKW   10
LINBUF: .BLKW   40
DEXT:   .WORD   0,0,0,0                 ;NO DEFAULT EXTENSIONS FOR FILES
LIST:   .WORD   0                       ;THIS IS THE AREA TO USE FOR THE
                                        ;FILE WRITING PARAMETERS
IOBLK:  .WORD   2                       ;BLOCK NUMBER
        .WORD   BUFFR                   ;I/O BUFFER ADDRESS
        .WORD   256.                    ;WORD COUNT
        .WORD   0
BUFFR:  .BLKW   256.                    ;DATA GOES HERE
WRERR:  .ASCIZ  /DISC WRITING ERROR/    ;ERROR MESSAGE, IF NEEDED
MSG1:   .ASCIZ  /ENTER SAMPLE INFO, 6 LINES (OR <CR>)/
MSG2:   .ASCIZ  /LABELING BLOCK FULL/
        .EVEN
DSPAC:.                                 ;SAFE TO PUT HANDLER HERE AT END
        .END    ADCHRO
```

```
CCCCCCC
C      IBMPC.FOR
C      THIS PROGRAM READS DATA FILES GENERATED BY SIMULT.FOR
C      AND PRINTS THEM OUT TO PCTALK AT AN IBM-PC
C
       INTEGER ZERO,BLKSAV,COUNT,LAMBDA,RATE,ENDTIM,STEP,ISTORE(3,51)
      1,KEEP(2,256),SCAN,VOLTL,MA,SA,MB,SB,S,A,F,W,PTS,HOLD(256),BLKNO
       DIMENSION FNAME(4)
       LOGICAL *1 CHOICE
C
C      GET A FILENAME
10     TYPE 102
       ACCEPT 100, FNAME
       S=0
       A=0
       F=0
       W=0
C
C      GET INFO ABOUT WHICH ADC CHANNELS TO OUTPUT
       TYPE 105
       ACCEPT 103, CHOICE
       IF (CHOICE.EQ.'Y') A=1
       TYPE 106
       ACCEPT 103, CHOICE
       IF (CHOICE.EQ.'Y') F=1
       TYPE 107
       ACCEPT 103, CHOICE
       IF (CHOICE.EQ.'Y') W=1
C
C      WHAT TYPE OF FILE IS IT? (FIXED OR SCANNED WAVELENGTH)
20     TYPE 104
       ACCEPT 103, CHOICE
       IF (CHOICE.EQ.'A')GO TO 30
       IF (CHOICE.EQ.'B')GO TO 40
       IF (CHOICE.EQ.'Q')STOP
       GO TO 20
C
C      START CREATING FILE WITH PC TALK HERE
30     TYPE 117
       ACCEPT 103,CHOICE
       IF(CHOICE.EQ.'Q')GO TO 10
C      OUTPUT THE LABELING BLOCK (BLOCK 0)
       BLKNO=0
       CALL GETDAT(BLKNO,FNAME,HOLD)
C      PRINT OUT MESSAGE FILE HERE
       TYPE 101, (HOLD(I), I=1,240)
C
C      READ AND OUTPUT FILE WITH SPECTRAL DATA
       BLKNO=1
       CALL GETDAT(BLKNO,FNAME,HOLD)
       BLKSAV=HOLD(1)
       COUNT=HOLD(2)
       LAMBDA=HOLD(3)
       STEP=HOLD(4)
       SCAN=HOLD(5)
       RATE=100/COUNT
       SCTIM=STEP/RATE
       ENDTIM=SCAN*SCTIM/60
       TYPE 108, BLKSAV,RATE,LAMBDA,STEP,SCAN,ENDTIM
       TYPE 109
C      OUTPUT BACKGROUND DATA--AVERAGE OF 10 SCANS (BLOCK 1)
       I=6
       DO 1002 K=1,STEP
       DO 1003 J=1,3
         ISTORE(J,K)=HOLD(I)
         I=I+1
         IF(I.EQ.256)GO TO 34
1003   CONTINUE
1002   CONTINUE
34     CALL PPRINT(S,A,F,W,STEP,ISTORE)
```

```
C     OUTPUT BACKGROUND DATA--AVERAGE OF 10 SCANS (BLOCK 1)
      I=6
      DO 1002 K=1,STEP
      DO 1003 J=1,3
        ISTORE(J,K)=HOLD(I)
        I=I+1
        IF(I.EQ.256)GO TO 34
1003  CONTINUE
1002  CONTINUE
34    CALL PPRINT(S,A,F,W,STEP,ISTORE)
C
C     OUTPUT GC DATA, SCANNING WAVELENGTH
      BLKNO=2
      CALL GETDAT(BLKNO,FNAME,HOLD)
      I=1
      M=1
      DO 1008 L=1,SCAN
        S=L
        IF (M.EQ.1)GO TO 200
        IF (M.EQ.0)GO TO 201
C
200   DO 1009 K=I,STEP
      DO 1010 J=I,3
        ISTORE(J,K)=HOLD(I)
        I=I+1
        IF(I.LE.256)GO TO 1010
        BLKNO=BLKNO+1
        IF(BLKNO.GT.BLKSAV)GO TO 202
        CALL GETDAT(BLKNO,FNAME,HOLD)
        I=1
1010  CONTINUE
1009  CONTINUE
      M=0
      GO TO 202
C
201   DO 1011 K=STEP,1,-1
      DO 1012 J=1,3
        ISTORE(J,K)=HOLD(I)
        I=I+1
        IF(I.LE.256)GO TO 1012
        BLKNO=BLKNO+1
        IF(BLKNO.GT.BLKSAV)GO TO 202
        CALL GETDAT(BLKNO,FNAME,HOLD)
        I=1
1012  CONTINUE
1011  CONTINUE
      M=1
202   CALL PPRINT(S,A,F,W,STEP,ISTORE)
      IF(BLKNO.GT.BLKSAV)GO TO 203
C
1008  CONTINUE
C
C     DO IT ALL AGAIN?
203   TYPE 118
      TYPE 113
      ACCEPT 103, CHOICE
      IF (CHOICE.EQ.'Y')GO TO 10
      IF (CHOICE.EQ.'N')STOP
      GO TO 45
C
C     READ AND OUT FILE WITH FIXED WAVELENGTH GC DATA
C     START CREATING FILE WITH PC TALK HERE
40    TYPE 117
      ACCEPT 103,CHOICE
      IF(CHOICE.EQ.'Q')GO TO 10
C     OUTPUT THE LABELING BLOCK
      BLKNO=0
      CALL GETDAT(BLKNO,FNAME,HOLD)
C     PRINT OUT MESSAGE FILE HERE
      TYPE 101, (HOLD(I), I=1,240)
```

```
      BLKNO=1
      CALL GETDAT(BLKNO,FNAME,HOLD)
      BLKSAV=HOLD(1)
      COUNT=HOLD(2)
      LAMBDA=HOLD(3)
      VOLTL=HOLD(4)
      MA=HOLD(5)
      SA=HOLD(6)
      MB=HOLD(7)
      SB=HOLD(8)
      ENDTIM=HOLD(9)
      RATE=100/COUNT
      PTS=ENDTIM
      ENDTIM=PTS/(60*RATE)
      TYPE 114,BLKSAV,RATE,LAMBDA,VOLTL,ENDTIM,MA,SA,MB,SB
C
C     READ AND OUTPUT BACKGROUND NOISE DATA (100 PTS EACH CHANNEL)
      I=10
      DO 2000 K=1,100
      DO 2001 J=1,2
        KEEP(J,K)=HOLD(I)
        I=I+1
        IF(I.EQ.256)GO TO 46
2001  CONTINUE
2000  CONTINUE
46    L=100
      CALL PRINTT(L,A,F,KEEP)
      L=256
C
C     READ AND OUTPUT GC DATA, FIXED WAVELENGTH
      BLKNO=BLKNO+1
      IF(BLKNO.GT.BLKSAV)GO TO 45
      CALL GETDAT(BLKNO,FNAME,HOLD)
      I=1
43    DO 2005 K=1,256
      DO 2006 J=1,2
        KEEP(J,K)=HOLD(I)
        I=I+1
        IF(I.LE.256)GO TO 47
        BLKNO=BLKNO+1
        IF(BLKNO.GT.BLKSAV)GO TO 44
        CALL GETDAT(BLKNO,FNAME,HOLD)
        I=1
47      PTS=PTS-1
        IF (PTS.EQ.0)GO TO 44
2006  CONTINUE
2005  CONTINUE
44    CALL PRINTT(L,A,F,KEEP)
      IF (PTS.EQ.0)GO TO 45
      IF (BLKNO.GT.BLKSAV)GO TO 45
      GO TO 43
C
C     DO IT ALL AGAIN?
45    TYPE 118
      TYPE 113
      ACCEPT 103, CHOICE
      IF (CHOICE.EQ.'Y')GO TO 10
      IF (CHOICE.EQ.'N')STOP
      GO TO 45
C
```

```
C      FORMAT STATEMENTS
100    FORMAT (4A4)
101    FORMAT (80A2)
102    FORMAT ('0ENTER THE INPUT FILENAME (DK:FILENM.EXT)',$)
103    FORMAT (A1)
104    FORMAT ('0ENTER CHARACTER FOR TYPE OF FILE:',/
      1,/,' A=SPECTRAL GC DATA, SCANNING WAVELENGTH'
      2,/,' B=GC DATA, FIXED WAVELENGTH'
      3,/,' Q=QUIT')
105    FORMAT ('0OUTPUT ABSORBANCE DATA? (Y OR N)',$)
106    FORMAT ('0OUTPUT FLUORESCENCE DATA? (Y OR N)',$)
107    FORMAT ('0OUTPUT WAVELENGTH "VOLTAGE" DATA? (Y OR N)',$)
108    FORMAT ('      NO. OF BLKS IN FILE=',I8,
      1,'     STEPPING RATE=',I5,
      2,/,'     LOW SCANNING WAVELENGTH=',I5,
      3,'     NO. OF STEPS IN SCAN=',I5,
      4,/,'     NO. OF SCANS=',I8,
      5,'     GC RUN TIME=',I5,'MIN')
109    FORMAT ('0SCAN 0 IS BACKGROUND SPECTRAL DATA (OR NOISE)')
113    FORMAT ('0TRANSFER ANOTHER FILE? (Y OR N)',$)
114    FORMAT ('      NO. OF BLKS IN FILE=',I8,
      1,'     DATA ACQUISITION RATE=',I5,
      2,/,'     FIXED WAVELENGTH=',I5,
      3,'     W "VOLTAGE"',I5,
      4,'     GC RUN TIME=',I5,'MIN',
      5,/,' ABS NOISE MEAN (100 PTS)=',I5,' ABS ST DV=',I5,
      6,' ; FL MEAN=',I5,' FL ST DV=',I5)
117    FORMAT ('0HIT ALT R NOW TO START CREATING FILE WITH PCTALK'
      1,' FILENAME FOR LOTUS SHOULD HAVE .PRN EXTENSION'
      2,' *****OR HIT "Q",<CR> TO START OVER*****')
118    FORMAT ('0HIT ALT R TO CLOSE FILE MADE BY PCTALK')
C
       END
C
```

```
C     PRINT SUBROUTINE FOR SPECTRAL DATA
      SUBROUTINE PPRINT(S,A,F,W,STEP,ISTORE)
      INTEGER S,A,F,W,STEP,ISTORE(3,51)
      IF (A.EQ.0)GO TO 31
      TYPE 110,S
      DO 3004 I=1,STEP
        TYPE *,ISTORE(1,I)
3004  CONTINUE
31    IF (F.EQ.0)GO TO 32
      TYPE 111,S
      DO 3005 I=1,STEP
        TYPE *,ISTORE(2,I)
3005  CONTINUE
32    IF (W.EQ.0)GO TO 33
      TYPE 112,S
      DO 3006 I=1,STEP
        TYPE *,ISTORE(3,I)
3006  CONTINUE
33    RETURN
110   FORMAT (' "A',I5)
111   FORMAT (' "F',I5)
112   FORMAT (' "W',I5)
      END
C
C     PRINT SUBROUTINE FOR GC DATA, FIXED WAVELENGTH
      SUBROUTINE PRINTT(L,A,F,KEEP)
      INTEGER L,A,F,KEEP(2,256)
      IF (A.EQ.0)GO TO 41
      TYPE 115
      DO 4003 I=1,L
        TYPE *,KEEP(1,I)
4003  CONTINUE
41    IF (F.EQ.0)GO TO 42
      TYPE 116
      DO 4004 I=1,L
        TYPE *,KEEP(2,I)
4004  CONTINUE
115   FORMAT (' "ABS')
116   FORMAT (' "FL')
42    RETURN
      END
```

```
.TITLE GETDAT

        .MCALL  .CSIGEN,.READW,.CLOSE,.PRINT

GETDAT::TST     (R5)+
        MOV     @(R5)+,@#BLKNO          ;MOVE BLOCK NO. TO MEMORY
        MOV     (R5)+,@#FNAME           ;MOVE FILENAME ADDRESS TO MEMORY
        MOV     (R5)+,@#HOLD            ;MOVE TRANSFER ARRAY ADDRESS TO MEM.
;
        .CSIGEN #DSPAC,#DEXT,@#FNAME    ;GET CSI TO OPEN AN OUTPUT CHANNEL
                                        ;FOR THE FILENAME
        MOV     @#BLKNO,R4              ;MOVE BLOCK NO. TO R4
        CLR     IOBLK                   ;GO TO THE FIRST BLOCK
        CMP     #0,R4                   ;FETCHING FIRST BLOCK?
        BEQ     CONT                    ;IF SO, GO TO CONT
1$:     INC     IOBLK                   ;INCREMENT BLOCK NUMBER
        DEC     R4                      ;DECREMENT R4
        BNE     1$                      ;CONT UNTIL AT NEEDED BLOCK
;
CONT:   MOV     #LIST,R5                ;PT R5 TO ARGUMENT BLK AREA
        .READW  R5,#3                   ;READ FULL BLOCK FROM FILE
;
        MOV     #BUFFR,R2               ;START OF BUFFR TABLE TO R2
        MOV     @#HOLD,R0               ;START OF TRANSFER ARRAY TO R0
        MOV     #256.,R4                ;WORD COUNT TO R4
OVER:   MOV     (R2)+,(R0)+             ;BUFFER INTO ARRAY
        DEC     R4                      ;DECREMENT WORD COUNT
        BNE     OVER                    ;REPEAT UNTIL DONE
;
        BCC     DONE                    ;IF CARRY CLR, NO READ ERROR
        .PRINT  #RDERR                  ;ELSE, PRINT ERROR MESSAGE
DONE:   .EVEN
        .CLOSE  #3                      ;CLOSE INPUT CHANNEL
        RETURN
;
BLKNO:  .BLKW   1
FNAME:  .BLKW   10
HOLD:   .BLKW   1
DEXT:   .WORD   0,0,0,0
LIST:   .WORD   0
IOBLK:  .WORD   0
        .WORD   BUFFR
        .WORD   256.
        .WORD   0
BUFFR:  .BLKW   256.
RDERR:  .ASCIZ  /READ ERROR/
        .EVEN
DSPAC:.
        .END    GETDAT
```

```
1 ' SAVE"B:CONVERT.BAS
100 'PROGRAM TO MAKE LONG DATA FILE INTO SHORT FILES
110 DIM Y(2,3,260)
120 '
130 CLS
135 EXTEN$=".PRN"
140 PRINT"INPUT FILE IS ON DISK DRIVE (A,B,C): ";
150 DISKIN$=INKEY$: IF DISKIN$="" GOTO 150
160 PRINT DISKIN$
170 IF DISKIN$=>"A" THEN IF DISKIN$<"D" THEN 200
180 IF DISKIN$=>"a" THEN IF DISKIN$<"d" THEN 200
190 GOTO 140
200 DISKIN$=DISKIN$+":"
202 PRINT: PRINT"OUTPUT FILES GO ON DISK DRIVE (A,B,C): ";
210 DISKOUT$=INKEY$: IF DISKOUT$="" GOTO 210
220 PRINT DISKOUT$
230 IF DISKOUT$=>"a" THEN IF DISKOUT$<"d" THEN 300
240 IF DISKOOT$=>"A" THEN IF DISKOUT$<"D" THEN 300
250 GOTO 200
300 DISKOUT$=DISKOUT$+":"
310 '
320 '
330 PRINT:PRINT"ENTER THE FILE NAME (OMIT '.' EXTENSION):";:INPUT FILEIN$
340 IF LEN(FILEIN$)>4 THEN PRINT"ONLY THE FIRST 4 FILE NAME CHARACTERS WILL BE U
SED ON OUTPUT FILES"
344 PRINT:PRINT"ENTER THE INPUT FILE '.' EXTENSION (E.G., .PRN):";:INPUT EXTENSI
ON$
346 IF LEFT$(EXTENSION$,1)<>"." THEN EXTENSION$="."+EXTENSION$
350 PRINT"OUTPUT FILES WILL HAVE A ";EXTEN$;" EXTENSION"
360 PRINT:PRINT"ENTER THE NUMBER OF STEPS IN A SCAN"
361 PRINT"MAXIMUM OF 50 FOR SCANNED WAVELENGTH (B OR S FILES)"
362 PRINT"ENTER 256 FOR FIXED WAVELENGTH (C FILES)"
363 INPUT N
364 PRINT "ENTER THE NUMBER OF ADC CHANNELS (1-3)"
365 INPUT P
366 M=(N+1)*P
370 T=1
371 PRINT "SEPARATE BLOCKS FOR ODD AND EVEN SCANS FOR LOTUS? (Y OR N)"
372 INPUT SEP$
373 IF SEP$="Y" THEN T=2
374 IF SEP$="y" THEN T=2
390 PRINT "SMOOTH DATA BY A 5 POINT SMOOTH? (Y OR N)";: INPUT SMOOTH$
395 IF SMOOTH$="N" GOTO 425
396 IF SMOOTH$="n" GOTO 425
400 '
410 '
420 '
```

```
421 IF LEN(FILEIN$)>4 THEN FILEOUT$="X"+LEFT$(FILEIN$,4): GOTO 430
422 FILEOUT$="X"+FILEIN$: GOTO 430
423 PRINT "OUTPUT FILE NAMES WHICH HAVE BEEN SMOOTHED WILL BEGIN WITH AN 'X'"
424 GOTO 430
425 IF LEN(FILEIN$)>4 THEN FILEOUT$=LEFT$(FILEIN$,4):  GOTO 430
426 FILEOUT$=FILEIN$
430 OPEN DISKIN$+FILEIN$+EXTENSION$ FOR INPUT AS #1
440 OPEN DISKOUT$+FILEOUT$+"00.PRN" FOR OUTPUT AS #2
500 '
510 '
520 'READ 12 ASCII HEADING LINES
530 FOR I=1 TO 12
540 LINE INPUT #1,A$
550 PRINT #2,A$
560 NEXT I
570 CLOSE #2
580 PRINT"HEADING FILE ";DISKOUT$+FILEOUT$;"00.PRN HAS BEEN MADE"
590 '
600 '
640 J$="0"
650 OPEN DISKOUT$+FILEOUT$+J$+".PRN" FOR OUTPUT AS #2
700 '
710 '
730 FOR I= 1 TO M
740 LINE INPUT #1,A$
750 PRINT #2,A$
760 NEXT I
770 CLOSE #2
780 PRINT"100%T DATA FILE HAS BEEN MADE"
790 '
800 '
820 R=100
830 J=1
840 J$=STR$(J)
845 J$=RIGHT$(J$,LEN(J$)-1)
850 OPEN DISKOUT$+FILEOUT$+J$+".PRN" FOR OUTPUT AS #2
857 '
858 '
859 'TO MAKE SEPARATE BLOCKS FOR ODD OR EVEN SCANS
860 FOR I=1 TO T
869 '  A,F OR W
870 FOR K=1 TO P
874 '      SCAN LABEL
875       IF EOF(1) THEN PRINT J-1; " DATA FILES HAVE BEEN MADE":CLOSE:END
876       LINE INPUT #1,A$
880       FOR L=1 TO N+1
890          IF EOF(1) THEN PRINT J-1;" DATA FILES HAVE BEEN MADE":CLOSE:END
900          LINE INPUT #1,A$
910          Y(I,K,L)=VAL(A$)
920       NEXT L
930  NEXT K
940 NEXT I
```

```
950 IF SMOOTH$="N" THEN GOTO 990
960 IF SMOOTH$="n" THEN GOTO 990
970 'GO TO SMOOTHING SUBROUTINE
980 GOSUB 1500
990 FOR I=1 TO T
1000 FOR K=1 TO P
1005     PRINT #2,A$
1010      FOR L=1 TO N+1
1020       A$=STR$(Y(I,K,L))
1030       PRINT #2,A$
1040     NEXT L
1050  NEXT K
1060 NEXT I
1070 CLOSE #2
1080 J=J+1
1090 IF J<=R THEN GOTO 840
1100 IF J>R THEN PRINT "OUTPUT FILE ";R;" REACHED"
1110. PRINT "REPLACE DISK IN OUTPUT DRIVE ";DISKOUT$
1120 PRINT "THEN INPUT ANY KEY WHEN FINISHED"
1121 INPUT WT$
1130 R=R+100
1140 GOTO 840
1460 '
1470 '
1480 '
1490 'GOSUB TO 5 POINT QUADRATIC-CUBIC SMOOTH Y(I,K,L)
1500 DATA -3,12,17,35
1510 READ Q1,Q2,Q3,Q4
1520 RESTORE
1530 Q=N-2
1550 FOR I=1 TO T
1560 FOR K=1 TO P
1570       FOR L=3 TO Q
1580         P1=Q1*(Y(I,K,L-2)+Y(I,K,L+2))
1581         P2=Q2*(Y(I,K,L-1)+Y(I,K,L+1))
1582         Y(I,K,L)=(P1+P2+Q3*Y(I,K,L))/Q4
1590      NEXT L
1600    NEXT K
1610 NEXT I
1620 RETURN
2000 END
```

```
Alt-N   Macro from worksheet named "NEXTFILE".
Written by E. H. Piepmeier, (C) 1985.

/Range Name Delete the name C_HERE if it is in your worksheet or
or the worksheet below it will be ruined.

This macro imports .PRN files that have the same name but different
numbers (e.g., FILE1.PRN, FILE2.PRN, etc.) and puts them in
adjacent columns.

After copying this macro into your worksheet using /FCCE
position the cursor in the cell containing \F and use /RNLR . PgDn PgDn
Return, to name the cells to the right of these labels.
Start the macro with Alt-N.

\N        /xlEnter name of file (omit number): ~C_NAME~
          /xnEnter number of first file: ~C_F~
          /xnEnter number of last file: ~C_LAST~
          /xmC_MENU~
C_MENU    PRESS RETURN. THEN MOVE CURSOR TO FIRST
          CELL TO BE COPIED INTO. PRESS RETURN AGAIN.
          {?}
C_START   /rncC_HERE~~
          /cC_F~C_NUMB~
          {Goto}C_NUMB~{Edit}{Home}'~
          {Goto}C_HERE~
          /rndC_HERE~
          /fin
C_NAME    TEST
C_NUMB    4
          ~
          {Right}
          /dfC_F~C_F+1~~~
          /xi(C_F<C_LAST+1)~/xgC_START~
          /xq

C_F             5
C_LAST          4
```

APPENDIX C

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Our ref. RM/LL Your ref. Amsterdam, February 17th, 1986

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Re: "Detection and gas chromatography eluates by simultaneous absorbance and fluorescence measurements", J. Chromatogr., 303 (1984) 341-349

and

"Long pathlength ultraviolet absorbance detection for gas chromatography with concurrent fluorescence measurement", J. Chromatogr., 331 (1985) 77-82

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